Current Practices and Controversies in Assisted Reproduction

Report of a meeting on
"Medical, Ethical and Social Aspects of Assisted Reproduction"
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Contents

3. Recent medical developments and unresolved issues in ART

Gamete source, manipulation and disposition

Embryo selection methods and criteria

Multiple pregnancies and multiple births

4. Social and psychological issues in infertility and ART

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Foreword

The last quarter of the 20th century has witnessed several major advances in reproductive medicine. One of the most widely publicised, celebrated and, at the time, controversial medical landmarks in this area was the birth, in 1978, of the first human baby resulting from *in vitro* fertilization (IVF). Since then, IVF has become a routine and widely accepted treatment for infertility. However, IVF is but one of many procedures in the increasingly complex and sophisticated field of biomedicine known as assisted reproduction. Since 1978, nearly one million babies have been born worldwide as the result of assisted reproductive technology (ART) of one form or another. It has been estimated that in some European countries up to five per cent of all births are now due to ART. It is clear that ART has made a significant impact on the lives of many infertile and subfertile couples. However, it has also been the source of great disappointment to those couples for whom ART has proven unsuccessful and to many more infertile people around the world who have no access to these technologies.

It is commonly accepted that infertility affects more than 80 million people worldwide. In general, one in ten couples experiences primary or secondary infertility, but infertility rates vary amongst countries from less than 5% to more than 30%. Most of those who suffer from infertility live in developing countries where infertility services in general, and ART in particular, are not available. Malaria, tuberculosis and infection with the human immunodeficiency virus (HIV) among other diseases which have significant morbidity and mortality and adversely affect developing country economies, are, justifiably, the centre of public health attention. In many developing countries, infertility is the result of genital tract infection which includes sexually transmitted infections (STIs), postpartum or postabortal infection and pelvic tuberculosis or schistosomiasis. Tubal blockage is responsible for infertility in up to two-thirds of infertile nulliparous women in sub-Saharan Africa and between one-quarter and one-third of infertile women in developed and developing countries, respectively. It is often argued that the solution to the problem of infertility in developing countries can only be found in prevention of infertility through prevention of STIs and unsafe abortion. Therefore, the use of ART to manage infertility is a contested issue in the context of the cause of the problem, the attitude to overpopulation and the availability of scarce health resources in developing countries. Even in developed countries, however, where infertility patients stand a better chance of receiving infertility treatment, access to ART is limited. The generally high cost of ART procedures and national policies regarding accessibility and reimbursement leave many infertile people without the option of treatment.

Although infertility may not be a public health priority in many countries, it is a central issue in the lives of the individuals who suffer from it. It is a source of social and psychological suffering for both men and women and can place great pressures on the relationship within the couple. While the role and status of women in society should not be defined solely by their reproductive capacity, in some societies womanhood is defined through motherhood. In these situations, the personal suffering of the infertile woman is exacerbated and can lead to unstable marriage, domestic violence, stigmatization and even ostracism. Although peer and social pressures to have children vary from country to country, what remains common in all is the desperate need of infertile people to give birth to a healthy child. Many infertility consumer groups consider this to be a human right based on the following note from the UN Declaration of Human Rights, Article 16.1:

> Men and women of full age, without any limitation due to race, nationality or religion, have the right to marry and to found a family.

In the past decade, developments in the field of ART have intensified the hopes and the wishes of infertile people to resolve their infertility and have resulted in an increasing demand for such services in both developed and developing countries. While developments in ART have evolved rapidly, so have the ethical, social and political controversies which surround nearly all aspects of ART. Few other areas in medicine have posed so many social and ethical questions and have attracted so much public attention as ART.

International concerns about ART and its social and ethical implications were raised at the 52nd World Health Assembly in 1999, which requested the World Health Organization (WHO) to review recent developments in the field of ART as well as their social and ethical implications. In response to this request, the WHO Department of Reproductive Health and Research convened a meeting on the medical, ethical and social aspects of assisted reproduction on 17–21 September 2001.

WHO had previously dealt with the issue of ART in the context of other work on the subject of infertility. In 1990, a similar meeting was convened by WHO which resulted in a technical report entitled *Recent advances in medically assisted conception* (Technical Report Series No. 820, 1992). At that time, the emphasis was on technical aspects of ART. The meeting in September 2001 was organized to provide a forum for interdisciplinary discussion involving as many interested parties as possible. More than 40 participants from 22 countries took part in the meeting; they included clinicians, embryologists, social scientists, ethicists and consumer representatives. The objectives of the meeting were (a) to review and assess recent developments in ART; (b) to identify unresolved issues in the field; and (c) to provide recommendations for future research.

To permit in-depth analysis and discussion, the meeting did not cover the whole field of ART. Instead, it focused on a selected number of topics that either were too recent in their development to have been discussed at the previous WHO meeting in this area, or considered to present particularly difficult and pressing problems at the present time. These topics included national and international surveillance of ART, intracytoplasmic sperm injection (ICSI), manipulation and cryopreservation of gametes, multiple pregnancy, techniques of ovarian stimulation, preimplantation genetic diagnosis, psychosocial issues, ethical aspects in relation to the individual, to the couple and to the offspring, as well as issues of equitable access, the role of consumers and the place of ART in developing countries. The issue of cloning was not reviewed and discussed as it has been the subject of previous meetings held by WHO. Also, artificial insemination was not considered since it was covered in-depth by the *WHO Technical Report* published in 1992 and an update was not deemed necessary.

Background papers were commissioned from invited speakers and peer-reviewed prior to the meeting. The papers were briefly presented with emphasis being given to discussion and the delineation of recommendations for practice and future research. All of the meeting sessions were plenary to allow full interaction among the different disciplines. A wide variety of views and approaches was presented in an intense, dynamic and rich debate.

This publication comprises 6 sections, consisting of the background papers and the recommendations agreed to by the meeting participants. The papers are grouped by subject in an order similar to that of the meeting programme but also in a way that the different views are juxtaposed or complementary. Despite the diverse opinions on ART voiced at the meeting, there were many points upon which consensus was reached. These are reflected in the corresponding sets of recommendations, which appear in the last section of this report. These recommendations include some that are directed towards those responsible for health policy, some for those who are concerned with clinical practice and some to the research community. The meeting also agreed on a list of working definitions for ART for the purposes of the meeting's discussions. These definitions are included as a glossary in the report for consistency and clarity. However, it needs to be emphasized that these are not definitions that have been adopted by WHO, but they can be used to facilitate the standardization of language in the field and in national and international registries.

Over the past years, WHO has received several requests from developing countries for advice on how to handle the introduction of ART in their, often resource-poor, settings. On the other hand, the number of ART clinics in such settings is increasing and infertility consumer groups have become an established and outspoken force in several developing

countries. At the same time, there has been considerable international debate on related topics such as stem cell research, claims for successful human cloning and vigorous condemnations thereof. Inevitably, ART has ramifications beyond the treatment of infertility as it is related to the current research on molecular reproductive biology of fertilization and implantation, as well as on stem cell and cloning research. Progress in this area can only be achieved through informed debate among all those involved in ART. This, the most recent WHO meeting in this area, provided one opportunity for such debate. The meeting and this report are not meant to provide solutions to the many problems encountered in the complex subject of assisted reproduction. Rather, they are intended to stimulate further debate among all those interested in the scientific, social and ethical aspects of ART and to provide some guidance and clarification for ongoing discussion.

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Glossary

Aspiration cycle: initiated ART cycle in which one or more follicles are punctured and aspirated irrespective of whether or not oocytes are retrieved.

Assisted hatching: an *in vitro* procedure in which the zona pellucida of an embryo (usually at 8-cell stage or a blastocyst) is perforated by chemical, mechanical or laser-assisted methods to assist separation of the blastocyst from the zona pellucida.

Assisted reproductive technology (ART): all treatments or procedures that include the *in vitro* handling of human oocytes and sperm or embryos for the purpose of establishing a pregnancy. This includes, but is not limited to, *in vitro* fertilization and transcervical embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, oocyte and embryo donation and gestational surrogacy. ART does not include assisted insemination (artificial insemination) using sperm from either a woman's partner or sperm donor.

Birth defect: Structural, functional or developmental abnormalities present at birth or later in life, due to genetic or nongenetic factors acting before birth.

Blastocyst: an embryo with a fluid-filled blastocele cavity (usually developing by five or six days after fertilization).

Cancelled cycle: an ART cycle in which ovarian stimulation or monitoring has been carried out with the intent of undergoing ART but which did not proceed to follicular aspiration, or in the case of a thawed embryo, to transfer.

Clinical abortion: an abortion of a clinical pregnancy which takes place between the diagnosis of pregnancy and 20 completed weeks' gestational age.

Clinical pregnancy: evidence of pregnancy by clinical or ultrasound parameters (ultrasound visualization of a gestational sac). It includes ectopic pregnancy. Multiple gestational sacs in one patient are counted as one clinical pregnancy.

Clinical pregnancy rate: number of clinical pregnancies expressed per 100 initiated cycles, aspiration cycles or embryo transfer cycles. When clinical pregnancy rates are given, the denominator (initiated, aspirated or embryo transfer cycles) must be specified.

Controlled ovarian hyperstimulation (COH): medical treatment to induce the development of multiple ovarian follicles to obtain multiple oocytes at follicular aspiration.

Cryopreservation: freezing and storage of gametes, zygotes or embryos.

Delivery rate: number of deliveries expressed per 100 initiated cycles, aspiration cycles or embryo transfer cycles. When delivery rates are given, the denominator (initiated, aspirated or embryo transfer cycles) must be specified. It includes deliveries that resulted in a live birth and/or stillbirth. The delivery of a singleton, twin or other multiple pregnancy is registered as one delivery.

Early neonatal death: death occurring within the first seven days after delivery.

Ectopic pregnancy: a pregnancy in which implantation takes place outside the uterine cavity.

Embryo: product of conception from the time of fertilization to the end of the embryonic stage eight weeks after fertilization (the term "pre-embryo" or dividing conceptus has been replaced by embryo).

Embryo donation: the transfer of an embryo resulting from gametes that did not originate from the recipient and/or her partner.

Embryo transfer (ET): procedure in which embryo(s) are placed in the uterus or fallopian tube.

Embryo transfer cycle: ART cycle in which one or more embryos are transferred into the uterus or fallopian tube.

Fertilization: the penetration of the ovum by the spermatozoon and fusion of genetic materials resulting in the development of a zygote.

Fetus: the product of conception starting from completion of embryonic development (at eight completed weeks after fertilization) until birth or abortion.

Full-term birth: a birth that takes place at 37 or more completed weeks of gestational age. This includes both live births and stillbirths.

Gamete intrafallopian transfer (GIFT): ART procedure in which both gametes (oocytes and sperm) are transferred to the fallopian tubes.

Gestational age: age of an embryo or fetus calculated by adding 14 days (2 weeks) to the number of completed weeks since fertilization.

Gestational carrier: a woman in whom a pregnancy resulted from fertilization with third-party sperm and oocytes. She carries the pregnancy with the intention or agreement that the offspring will be parented by one or both of the persons that produced the gametes.

Gestational sac: a fluid-filled structure containing an embryo that develops early in pregnancy usually within the uterus.

Hatching: it is the process that precedes implantation by which an embryo at the blastocyst stage separates from the zona pellucida.

Host uterus: see gestational carrier.

Implantation: the attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) which starts five to seven days following fertilization.

***In vitro* fertilization (IVF):** an ART procedure which involves *extracorporeal* fertilization.

Infertility: failure to conceive after at least one year of unprotected *coitus.*

Initiated cycles: ART treatment cycles in which the woman receives ovarian stimulation, or monitoring in the case of spontaneous cycles, irrespective of whether or not follicular aspiration is attempted.

Intracytoplasmatic (intracytoplasmic) sperm injection (ICSI): IVF procedure in which a single spermatozoon is injected through the zona pellucida into the oocyte.

Live birth: a birth in which a fetus is delivered with signs of life after complete expulsion or extraction from its mother, beyond 20 completed weeks of gestational age. (Live births are counted as birth events, e.g. a twin or triplet live birth is counted as one birth event.)

Live-birth delivery rate: number of live-birth deliveries expressed per 100 initiated cycles, aspiration cycles or embryo transfer cycles. When delivery rates are given, the denominator (initiated, aspirated or embryo transfer cycles) must be specified. It includes deliveries that resulted in at least one live birth. The delivery of a singleton, twin or other multiple birth is registered as one delivery.

Malformation rate: includes all structural, functional, genetic and chromosomal abnormalities identified in aborted tissue or diagnosed before or subsequent to birth.

Medically assisted conception: conception brought about by noncoital conjunction of the gametes. Includes ART procedures and intrauterine, intracervical and intravaginal insemination with semen of husband/partner or donor.

Micromanipulation (also referred to as **assisted fertilization):** the use of special micromanipulative technol-

ogy that allows operative procedures to be performed on the oocyte, sperm or embryo**.**

Microscopic epididymal sperm aspiration (MESA): procedure in which spermatozoa are obtained from the epididymis, by either aspiration or surgical excision.

Missed abortion: a clinical abortion where the products of conception are not expelled spontaneously from the uterus.

Neonatal death: death within 28 days of birth.

Newborns or infants born: the number of live births plus stillbirths.

Oocyte donation: an ART procedure performed with third-party oocytes.

Preclinical abortion: an abortion that takes place before clinical or ultrasound evidence of pregnancy.

Preclinical pregnancy (biochemical pregnancy): evidence of conception based only on biochemical data in the serum or urine before ultrasound evidence of a gestational sac.

Preimplantation genetic diagnosis (PGD): screening of cells from preimplantation embryos for the detection of genetic and/or chromosomal disorders before embryo transfer.

Preterm birth: a birth which takes place after at least 20, but less than 37, completed weeks of gestation. This includes both live births and stillbirths. Births are counted as birth events (e.g. a twin or triplet live birth is counted as one birth event).

Recipient: in an ART cycle refers to the woman who receives an oocyte or an embryo from another woman.

Spontaneous abortion: spontaneous loss of a clinical pregnancy before 20 completed weeks of gestation or, if gestational age is unknown, a weight of 500 g or less.

Stillbirth: a birth in which the fetus does not exhibit any signs of life when completely removed or expelled from the birth canal at or above 20 completed weeks of gestation. Stillbirths are counted as birth events (e.g. a twin or triplet stillbirth is counted as one birth event).

Surrogate mother: see gestational carrier.

Testicular sperm aspiration (TESA): procedure in which spermatozoa are obtained directly from the testicle, by either aspiration or surgical excision of testicular tissue.

Zygote intrafallopian transfer (ZIFT): procedure in which the zygote, in its pronuclear stage of development, is transferred into the fallopian tube.

Zygote: is the diploid cell, resulting from the fertilization of an oocyte by a spermatozoon, which subsequently develops into an embryo.

This glossary was generated by the International Working Group for Registers on Assisted Reproduction and was further developed and used during this meeting.

Introduction

Current challenges in assisted reproduction

MAHMOUD F. FATHALLA

> And God blessed them, and God said unto them, Be fruitful, and multiply, and replenish the earth and subdue it.
>
> —Holy Bible: Genesis 1:28 *(1)*

Our human species is not exactly known for its willingness to comply with divine instruction. But when God said unto them "Be fruitful and multiply", they were more than eager to comply. They took the task to heart. They turned a duty into a pleasure. The majority of couples had no problem. A minority, however, were distressed because of delay or inability to conceive and bring forth children. Medicine tried to help them to conceive naturally. There remained, however, a group who could not reproduce naturally without assistance.

When the first baby conceived *in vitro* was born *(2)*, a completely new frontier was opened up in reproductive medicine, and new hope was given to infertile couples. The new technology brought happiness and harmony to many families.

Since 1978, the field of assisted reproductive technology (ART) has witnessed spectacular scientific advances and additional medical applications. With the introduction of intracytoplasmic sperm injection (ICSI) *(3)*, ART can now help infertile couples with a male factor, a condition for which results of traditional treatment have not been satisfactory. The potential of ART is now not limited to infertile couples. It can help fertile couples as well to conceive healthy children through the application of the new technologies of preimplantation genetic diagnosis and embryo selection *(4)*. Further into the future, ART allowed a much better understanding of the early stages of human development and differentiation, and opened up a new field of stem cell research, bringing a new hope for the treatment of certain serious diseases, for which no effective treatment is currently available *(5)*.

With all these advances, challenges are still to be faced. There are challenges which scientists have to deal with. There are challenges to the health services. There are challenges for which the health profession, as a group, has to take responsibility. And last but not least, society has to cope with new challenges brought to the forefront by this technological revolution and its social implications.

One challenge that cuts across all others is how to make ART more widely available and affordable for all who need it, particularly in developing countries.

We live in a world of rich cultural diversity. This has to be taken into account when these challenges are discussed.

Scientific challenges

Tremendous scientific progress has been made in the

field of assisted reproduction. Important challenges still remain. Three of these challenges are worthy of special mention.

Improving success rates

Success rates have so far been fairly constant, at around 25% live births per cycle, until the age of 34 years, when there is steep decline *(6)*. This success rate sounds good enough. But it also means a failure rate of around 75%, that is distressing for those who went through the financial and heavy psychological cost of the procedure.

Can we bring happiness to more couples? Or, can we have better predictors of the outcome, to save those unfortunate couples from going through the heavy burden of the procedure if they have no chance of success?

There is room for improving the success rates of assisted reproduction. Success rates have been improved by simply increasing the chances for implantation through transfer of more than one embryo to the uterus. The drawback is an increased risk of multiple gestation. The introduction of better criteria for embryo assessment and selection, before transfer, is likely to improve implantation rates. This will allow wider use of elective single embryo transfer. Recent advances in molecular biology are also bringing new insights into our understanding of the complex and delicate mechanism of implantation, which again may increase the success rate of implantation *(7)*.

Shortage of gametes

ART depends on the availability of an adequate number of healthy gametes to allow successful manipulation. Three approaches are currently being pursued: increasing the production, cryopreservation and donation from a third party. The applicability and success have been different for female and male gametes. Oocytes are scarce and are more difficult to obtain. But the ovary can be hyperstimulated to yield enough ova. Sperm is normally more than plentiful in fertile men, but can be very scarce in infertile men. There is, however, no way to increase the supply from infertile men, though sperm can now be obtained directly from the epididymis or testis. The relative ease and the success of cryopreservation of sperm and embryos contrast markedly with the problems associated with freezing of oocytes. Sperm donation has been in use for decades. Oocyte donation is possible, but is more difficult and raises different concerns. Shortage of gametes is still a challenge in ART. More research is needed to develop innovative approaches.

Multiple gestation

Improving success rates and overcoming shortage of gametes are important challenges on which scientists need to work. The pressing problem that challenges scientists is, however, the prevalence of multiple gestation, twins and higher-order births, as a result of assisted reproduction.

A world collaborative report on *in vitro* fertilization (IVF) recorded a multiple birth rate of 29%, the majority of which are twins *(8)*. The large scale Swedish retrospective cohort study reported a 20-fold increased risk of multiple pregnancy following ART compared with the general population *(9)*. With increasing resort to and utilization of assisted reproduction, we may soon be facing a public health problem.

Regardless of whether they originate from assisted or nonassisted reproduction, multiple gestation is associated with an increased risk of preterm delivery, low birth weight, congenital malformations, fetal and infant deaths and long-term morbidity and mortality as survivors *(10)*.

The risks of multiple gestation are often expressed in terms of perinatal and infant morbidity and mortality. The impact on the woman often takes second place or is completely ignored. First, there are the complications of pregnancy and labour that are more frequently encountered with multiple gestation. Second, there is the psychosocial impact (including the impact of multifetal pregnancy reduction) and the heavy burden on the mother of caring for more than one immature baby. Third, perinatal morbidity and mortality should be appropriately counted as part of the burden of disease on women, rather than on the child, as it is often counted. It is women who have made a major investment of themselves in the pregnancy, and who have the responsibility of caring for a disabled baby.

Controlled ovarian hyperstimulation and intrauterine insemination (IUI) and transferring multiple embryos account for the high incidence of multiple gestation. Controlled ovarian hyperstimulation with IUI is associated with the higher orders of multiple pregnancy. Transferring multiple embryos is a standard practice to increase the chances of success.

Monozygotic twinning has been reported to be higher following assisted reproduction *(11)*.

Health service challenges

The introduction of assisted reproduction in mainstream medical practice posed a number of challenges to health care services, particularly in relatively resource-poor settings. Three such challenges face health administrators and policy-makers: deciding on what resources can be allocated to ART services, defining who can have access to such services, and striking the right balance between investment in prevention and in cure.

Equitable allocation of resources

> And when Rachel saw that she bare Jacob no children, Rachel envied her sister; and said unto Jacob, Give me children or else I die.—Holy Bible: Genesis: 30:1 *(1)*

Infertility is not a disease. In fact, in many cases of infertility no evidence of any disease may be found. Per se, infertility does not threaten life or endanger physical health. But this is not how we define health. Health, as defined in the constitution of the World Health Organization, is not merely the absence of disease or infirmity. It is a state of complete physical, mental and social well-being. Patients do perceive the suffering from infertility as very real.

With the population problem on our hands, is it appropriate that we continue to worry about the problem of infertility? The answer is that we should worry even more. The adoption of a small family norm, through voluntary infertility, which is a desired target at country and at global levels, makes the issue of involuntary infertility more pressing. If couples are urged to postpone and to widely space pregnancies, it is imperative that they should be helped to achieve a pregnancy when they so decide, in the more limited time they have available.

Resources are finite. Allocation of relatively scarce resources is difficult. There is always an opportunity cost. If resources are used for one purpose, the opportunity is lost for using the same resources to deal with another important disease condition. The World Bank attempted to prioritize health problems on the basis of a quantitative assessment of the disability-adjusted life years (DALYs) lost as a result of the health problem *(12)*. The problem is in the definition of disability. The World Bank study tried to quantify disability from a disease condition by multiplying the expected duration of the condition (to remission or to death) by a severity weight that measured the severity of the disability in comparison with loss of life. Diseases were grouped into six classes of severity of disability. The World Bank report *Investing in health* published tables quantifying the burden of disease in females by cause. In a list of 96 causes, infertility is conspicuous by its absence.

If DALYs are substituted with QUALYs (quality-adjusted life years), the priority ranking of health problems will differ. If ranking is based, not just on the basis of productivity loss but on how people perceive the disability, infertility will rank high on the list. The physical and psychological burden the infertile couples are willing to go through, and the financial cost couples are willing to pay if they can afford it, attest to the high ranking of infertility as a perceived burden of disease.

Access to services

> The States Parties to the present Convention recognize the right of everyone to the enjoyment of the highest attainable standard of physical and mental health.
>
> The States Parties to the present Convention recognize the right of everyone . . . to enjoy the benefits of scientific progress and its applications.
>
> —International Covenant on Economic, Social and Cultural Rights, Article 12. 1; 15.1.b *(13)*

The Human Rights Treaty, the International Covenant on Economic, Social and Cultural Rights (known as the Economic Covenant) *(13)*, adopted by the United Nations General Assembly in December 1966, and entered into force in January 1976, recognized the right of *everyone* to enjoy the benefits of scientific progress and its applications, and the right of *everyone* to enjoy the highest standard of physical and mental health.

In its general comment 14, the United Nations Committee on Economic, Social and Cultural Rights expanded on the right to health, stating that: "The right to health is not to be understood as a right to be *healthy*. The right to health contains both freedoms and entitlements" *(14)*.

Entitlements depend on the availability of resources from public funding. There is a legitimate need to rationalize the allocation of scarce resources. The important point is that resources are allocated with equity and transparency, with more to those who are more in need.

Freedoms in the pursuit of the right to health means that people are not denied access to services. In the area of assisted reproduction, access to services is often restricted, legally or medically, in one way or another. This raises human rights concerns. Any restrictions on access have to be justifiable and defensible, and have to be shown as not violating people's human right to health.

In some countries, access to ART is often limited to certain procedures or to certain consumers. The following procedures are subject to restrictions in certain countries: sperm donation, ovum donation, embryo donation and surrogacy. Restriction may be absolute or related to commercialization of these procedures. Preimplantation genetic manipulation and selection is also subject to certain restrictions.

Certain consumers are denied access to assisted reproduction for widely different reasons. These include single women, lesbians and homosexual couples. A concept of fitness to parent is sometimes invoked, including no outstanding criminal charges, no history of an offense that was sexual or violent in nature, and no disease or disability which could interfere with the capacity to parent. Assisted reproduction has been denied to women with HIV infection, on the basis of concern over the life expectancy of the infected parent and the risk of viral transmission to the offspring. This has recently been challenged *(15)*. The best interest of the child is often made as an argument. The implication is that it may be in the best interest of the child not to be born at all.

Because of the inconsistency of restrictive regulations in different countries, the situation invites what may be called "reproductive tourism". This has been a topic that attracted media attention in Europe, such as the following recent extensive report in *L'Hebdo* magazine *(16)*:

> La quête du bébé a tout prix
> En Europe, le paysage des pratiques et des lois sur la PMA (Procréation médicalement assistée) est bien contrasté. Cette situation encourage, de façon modeste, un tourisme procréatif des pays les plus réglementés vers les pays les plus libéraux.

Balance between preventive and curative services

The allocation of resources does not only depend on the magnitude and severity of a health problem. It also depends on the availability of cost-effective interventions. Health services need to balance the utilization of their resources between preventive and curative services. Preventive services may be more cost-effective and benefit more people than expensive curative services. In a sense, assisted reproduction is not a curative treatment. The couple remains infertile after the success of the procedure. Prevention of infertility, particularly infection-related infertility, should not be ignored in the enthusiasm for assisted reproduction. Prevention of sexually transmitted infections, particularly among adolescents, may save their fertility potential. Sexually active adolescent girls are appropriately advised to use the condom if they want to have a child in the future. It should be recognized, however, that infertility is not always preventable. Preventive services will also not help the many couples who already suffer from the problem.

Professional challenges

The introduction of assisted reproduction as a novel technology poses a number of significant challenges for the health profession as a whole. The profession is challenged to ensure transparency about the outcome and risks of these new procedures. The profession is challenged to assume the role of self-regulation and to provide quality control. The profession has a responsibility to put proper surveillance in place as a follow-up after the introduction of these completely novel technologies. Last, but not least, the health profession is challenged to partner with consumers, and not to deal with them as passive recipients.

Transparency

As with many other novel technologies in medicine, unjustified claims can be made by enthusiasts of new procedures. This does not mean that they may present false data. However, data can be presented in ways that can be misleading to potential clients. Randomized, double-blind, controlled trials are difficult to design in this field except to compare certain modifications or changes in the procedure. One of the

professional challenges is to ensure that benefits and risks are presented in an objective and transparent way that allows potential consumers to make informed decisions.

Success of assisted reproduction procedures has variously been reported as a positive beta-hCG test 14 days after treatment, ultrasound diagnosis of a gestation sac, pulsating heart on ultrasound examination, or a take-home baby. Success has been expressed per transfer or per cycle commenced.

A standardized way of presenting results is often advocated, but it can also be misleading if used to compare the performance in different centres without taking into consideration variables that may influence the outcome. The selection of clients influences the success rate. Success can be expected to be higher in younger women. Success is also generally higher in the first cycles, and so may be higher in centres receiving more new clients. The procedure used also influences the success rate. Ovarian hyperstimulation and transfer of more than one embryo carry a higher success rate, but also a higher risk of multiple pregnancy.

When results of different centres are compiled and published by professional bodies, lack of anonymity may influence centres to project their published results in a more positive light. Anonymity and collective analysis and presentation of the results is an alternative that would still be helpful to potential clients.

Self-regulation

Medical and surgical procedures are more difficult to regulate than new drugs or new devices. New drugs and devices are subjected to vigorous testing in animals and in human volunteers before they are finally approved for general medical use, for specific indications and under specific instructions.

The challenge to the profession is to take over this responsibility. The experience with Reproductive Technology Accreditation Committees in Australia has demonstrated that self-regulation can work. Committees accredit practitioners who are qualified to perform the procedures and have adequate facilities to perform them. They also try to ensure that the accredited centres maintain the quality of their services and regularly report their results in an objective, unbiased way.

Benefits of self-regulation include its flexibility. It can respond to new developments in a rapidly advancing field. If the profession does not fulfil this responsibility, it is likely that law-makers or policy-makers may be persuaded by the public to impose regulation, which may restrict access and hinder progress.

Surveillance

Although scientists, regulatory authorities and service providers all do their best to ensure that drugs and devices are safe for the consumers before they are introduced, postmarketing surveillance has become an established practice and a professional responsibility. It is only through surveillance that *rare* events and *distant* events can be discovered. Methodologies for surveillance are well established. They include systematic reporting of adverse effects, case–control studies and longitudinal cohort studies in which subjects and matched controls are followed for years.

The same principles should apply to new medical procedures. In the field of assisted reproduction, surveillance presents the profession with new and different challenges, in terms of methodology and in terms of targets. The methodology should not be limited to medical surveillance, but has to include psychosocial studies. Also, the follow-up has to be long term, and not just short term. The targets for surveillance should include not only the child, but also the woman and the family.

The child

The follow-up of babies is not to be confined to the short-term health consequences of mortality, morbidity and clinically recognizable congenital anomalies. Follow-up is needed to provide assurance of psychological and mental development. Follow-up is needed to continue for the period of adolescent development. Surveillance is still needed to ensure potential future fertility. The direct injection of a single spermatozoon into the mature oocyte eliminates the process of natural sperm selection. Of particular concern is the future fertility of babies born to infertile men after this ICSI procedure. The finding that hypospadias is more frequent in ICSI children may be related to paternal subfertility with a genetic background *(17)*.

Women

Controlled ovarian hyperstimulation is a standard component of assisted reproduction, to ensure an adequate supply of oocytes. Women exposed to ovarian hyperstimulation need to be followed up to ensure that they will not be exposed to an increased risk of epithelial ovarian cancer. Incessant ovulation has been postulated as a factor in the pathogenesis of common epithelial ovarian tumours *(18)*. The surface epithelium of the ovary is subjected to trauma at every occurrence of ovulation. The repair process involves repeated cell division with an increased chance for DNA damage. The protective effect of oral contraceptive use lent support to this hypothesis. Bamford and Steele *(19)* reported the first case of ovarian carcinoma in a patient receiving gonadotrophin therapy. Some studies have shown that infertile women using infertility drugs are three times more at risk for invasive ovarian cancer than women without history of infertility *(20)*. A cohort study reported a relative risk of 11.1 (confidence interval [CI] 1.5 to 82.3) for women who received clomiphene for 12 or more monthly cycles *(21)*. It may also be noted that assisted reproduction not only involves excessive ovulation, but also repeated minor trauma for ovum pick-up.

The jury is still out on the risk of ovarian cancer in women who are superovulated. Some studies have been reassuring *(22,23)*. Large prospective and retrospective studies will be necessary before the risk can be excluded *(24)*.

Families

Families need to be followed up for the impact of assisted reproduction, particularly if assisted reproduction involves a third party in the way of gamete or embryo donation. Questions can be raised as to whether parenting in these families is different from parenting in families with natural reproduction. Surveillance should include the quality of parenting, family functioning and child psychological development *(25)*.

Surveillance, particularly its psychosocial component, needs to be carried out in different regions of the world. It is quite possible that the psychosocial outcome of assisted reproduction may be influenced by the cultural context in which the technologies are used.

Consumers as partners and not passive recipients

Dealing with consumers as partners and not as passive recipients is not always easy for the medical profession. The profession has to adopt new attitudes, and has to abandon a lot of strongly entrenched patronizing attitudes. Partnership between consumers and providers is needed at three levels. At the level of the individual patient–doctor relationship, it means the active participation of the infertile couple in making the decisions about treatment. To make informed decisions, consumers are entitled to factual and objective information about all alternatives. They should know the chances of success, and the risks involved.

At the level of the profession as a group, there is a need for a constructive dialogue with consumer groups in which both sides can listen to each other and talk to each other. Mutual trust is crucial to build. Consumer groups can be represented on appropriate scientific and professional committees. The WHO Special Programme of Research, Development and Research Training in Human Reproduction realized the need for this dialogue and convened, in collaboration with the International Women's Health Coalition, a meeting that included both women's health advocates and scientists. The report of the meeting was published under the title *Creating common ground (26)*. This was followed by a series of regional meetings, and by institutionalizing the participation of consumers in the advisory bodies of the Programme.

At a third and equally important level, there is power in partnership and this power can be used in advocacy. In the late 1980s, a coalition of consumers and physicians successfully lobbied the Australian federal government for recognition of fertility as a medical condition and for reimbursement for ART treatment.

Social challenges

Assisted reproduction introduced a number of challenges with which society has to cope. Three challenges need to be addressed. The first challenge is rather generic: the public trust in science. The second challenge is specific to human reproduction: ethical and cultural concerns. The third challenge is that assisted reproduction has brought the issue of gender to the forefront, and society is being

challenged to re-think the way it views the role of women.

Public trust in science

In recent years, a question often crops up in the public mind and in public media: Is science a solution or a problem?

There are probably more scientists alive and working today than all scientists who have lived throughout human history. Scientists never imagined that they would be able to explore the new frontiers of science that have now opened up. On the positive side, the promise of science to improve the quality of life has never been so great. On the negative side, there are unmistakable signs of public mistrust. The area of assisted reproduction is one such area, where science is rapidly advancing and public mistrust is also growing. Scientists are suspected of trying to play God, and to mess in what has always been God's act: procreation.

Public mistrust in science can result in giving science a bad image, can endanger the flow of public funding for science, and is likely to raise pressure to enact restrictive regulation, which impedes the utilization of the fruits of science or impedes the further progress of science.

What can scientists do to help build and maintain public trust in science?

First, scientists need to engage the public in an informed debate. This involves more than just making scientific information freely available. The role of scientists is no longer to preach enlightenment to the ignorant masses. The role of scientists is to present the case objectively to an enlightened citizen jury to allow them to make an informed judgement. Scientists must accept that they are no more qualified than the general public to make value judgements as to the uses to which science shall be put.

Second, scientists should avoid giving the public a perception of being arrogant. Time and again, the limits of science are being exposed. With the recent crisis of the bovine spongiform encephalopathy (mad cow disease), people may feel justified if they question whether they can really trust scientists with their supper.

Third, scientists should be careful in communicating scientific data to the public media. The media, in its presentation of science, aims first to engage and entertain, and only second to inform. Scientists should resist the temptation of publicity.

Cultural and ethical concerns

No society, primitive or advanced, no culture, no religion, and no legal code has been neutral about reproductive life. Even in societies which do not interfere with fertile individuals making reproductive decisions, infertile couples are often subject to public attention and scrutiny. In addition, assisted reproduction has brought new issues to the forefront. Societal institutions have been slow to cope with the new developments.

Cultural and ethical concerns cannot be lumped all in one basket. First, there is what may be called "moral panic" reaction. On a completely different level, there are concerns based on ideology and religious belief. Third, there are concerns based on utilitarian considerations.

The term "moral panic" describes the reaction when traditional moral beliefs about the family and reproduction appear to be threatened by perceived dangers. The reaction is often unjustified or exaggerated. This may be a transient phase before the procedures get accepted. The reaction tends to move from horrified negation to negation without horror, then gradual understanding and finally a slow but final acceptance. Included in this moral panic is the fear of a "slippery path"—if science is not reined in now, it will cross red lines in the future.

Concerns based on ideology and religious belief include the status of the embryo as a person, with interests and rights, in the Catholic religion. They include also the sanctity of the family's genetic lineage in Islamic faith. Ideological concerns are entitled to respect, but cannot be forced on others who do not uphold them.

Ethical concerns, based on utilitarian principles, are about what is best for society and what is in the best interests of the child. Different conclusions can be reached through different ethical approaches and are generally tolerated. One example is the concern about donation of human gametes and embryos. People may accept such procedures if there are no commercial transactions involved, or if payment is not considered for a commodity but for a service transaction.

In principle, societies have had to cope with two revolutionary concepts in human reproduction: separation of sex from reproduction, and reproduction with involvement of a third party.

Separation of sex from reproduction

Sex without reproduction

The controversy about contraception may now be largely something of the past, but the days are still remembered when there was a moral outrage and strong opposition on ideological grounds. When Margaret Sanger, her sister Ethel, and a social worker Fania, opened the first contraceptive clinic in Brooklyn, the clinic was soon raided and the three women arrested *(27)*. Released on bail, they promptly reopened the clinic and were arrested again and charged with maintaining a public nuisance. The Roman Catholic Church still rejects any acts that separate the procreative aspects of human intercourse from the unitive love-making aspect of the sexual act. But contraception has become a way of life. In the world, as a whole, it has been estimated that about 58% of couples in the reproductive age group are currently using contraception, to separate the sex act from the act of procreation, with a range between 55% in less developed regions and 70% in more developed regions *(28)*.

Reproduction without sex

The other side of the coin, reproduction without sex, is relatively more recent, with the introduction of assisted reproduction. There was less moral outrage probably because it responded to a limited need of infertile couples, it did not contradict pronatalist attitudes, and particularly that it allowed infertile men, and not only women to reproduce. Controversy arose with the expansion of applications that allowed reproduction outside the social frame of a traditionally married man and woman.

Reproduction with the involvement of a third party

> And she said, Behold my maid Bil-hah, go in unto her, and she shall bear upon my knees, that I may also have children by her.
>
> —Holy Bible: Genesis 30:3 *(1)*

Gamete and embryo donation raised more ethical and cultural concerns. The act of reproduction is an act of a couple; a third party is considered one too many. Partial surrogacy is mentioned in the old testament. Illicit relationships of married partners exist and have been more tolerated than assisted reproduction with donation of gametes.

Gender issues

Sex is biologically determined; gender is a social construct. Gender is related to how we are perceived and expected to think and act as women and men because of the way society is organized, not because of our biological differences.

Responsibility for infertility is commonly shared by the couple. Analysis of data compiled in a large WHO multinational study showed that a major factor in the female with no demonstrable cause in the male was diagnosed in only 12.8% of cases, and a major factor in the male with no demonstrable cause in the female was diagnosed in only 7.5% of cases *(29)*. For biological and social reasons, however, the burden of infertility is unequally shared.

The psychological and social burden of infertility in most societies is much heavier on the woman. A woman's status is often identified with her fertility, and failure to have children can be seen as a social disgrace or a cause for divorce. The suffering of the infertile woman can be very real.

The burden and risks of assisted reproduction are by no means equally shared between men and women. Women bear the physical and psychological burden. The incidence of severe ovarian hyperstimulation syndrome has been estimated at 0.2%–1% of all assisted conception cycles *(30)*. The burden of assisted reproduction for male infertility falls largely on the woman. It has been reported that 27% of ART in the UK is carried out for severe male infertility *(31)*.

The noble task of reproducing our species has not brought societal awards to women. On the contrary, it has often led to their subordination. Women in society are subject to both pronatalist and paternalist attitudes. The introduction of assisted reproduction is a challenge to societies to re-examine these attitudes.

Pronatalist attitudes

> But first we must ask: what is a woman? 'Tota mulier in utero', says one, 'woman is a womb'.—Simone de Beauvoir *(32)*

Women have been identified with their role as mothers. A woman is reduced to a mobile womb walking on two

legs, and with a human face on top. In many societies women are coerced to be mothers. The Declaration of Nicolae Ceausescu that the fetus is the socialist property of the whole society, that giving birth is a patriotic duty, determining the fate of the country, and that those who refuse to have children are deserters, escaping the law of natural continuity, may have been an extreme example of coerced motherhood *(33)*. But coerced motherhood or "compulsory" childbearing, broadly defined, is a major problem in the world today. It does not take place by directly forcing women to be mothers. Women are coerced into childbearing when they are denied the choice, when they are denied the means to avoid unwanted pregnancy, and when society makes children the only goods a woman can deliver and is expected to deliver. In many societies in the world today, women are left with no choice in life except to pursue a reproductive career. It is a valid question to ask whether women resort to assisted reproduction of their own free will, or whether they are being coerced by society to be mothers.

Paternalist attitudes

A woman can claim as her own her head, her hair, her hands, her arms, her upper body, her legs and her feet. She cannot claim the same right to the remaining area of her body, which appears to belong more to certain males of the species, moralists, politicians, lawyers and others, all of whom try to decide on how the area is best utilized.

The paternalistic attitude towards women restricts their reproductive freedom in natural reproduction. Women in need of assisted reproduction are even more subject to paternalistic subjective decisions. Decisions, which women can make and should be able to make, are often made on their behalf by others. Because of the subordination of women, societies may see fit to make decisions on who should be mothers and who should not. This does not have a parallel about who should be a father and who should not. Egg donation is treated differently from sperm donation. A postmenopausal woman is treated differently from an elderly man, or a man with a disease which may shorten the lifespan.

The challenges and the reward

The challenges to assisted reproduction cannot be underestimated. But the reward is also great. What reward can be better than a happy mother, a proud father, a healthy child and a harmonious family?

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Section 1

Infertility and assisted reproductive technologies in the developing world

Infertility and social suffering: the case of ART in developing countries

ABDALLAH S. DAAR, ZARA MERALI

Introduction

While the prevalence of infertility in developing countries is difficult to assess given inconsistencies in defining infertility, between 8% and 12% of couples around the world have difficulty conceiving a child at some point in their lives, "thus affecting 50 to 80 million people"*(1)*. In some areas, particularly in sub-Saharan Africa, up to one-third of couples are infertile *(2)*. Throughout the world, the core prevalence of infertility is about 5%, attributable to anatomical, genetic, endocrinological and immunological problems *(3)*. Between countries and regions, infertility rates vary dramatically, corresponding to the incidence of preventable conditions that lead to infertility. While women's infertility is the greater focus of research, health care attention, and social blame, male infertility is the cause or contributing factor to infertility in approximately half of infertile couples *(2)*.

Infertility in developing countries raises distinct and complex problems beyond those well known to developed nations. The effects of infertility and the concomitant need for its health care management relate to the cultural realities of specific regions.

While the relevance and need for assisted reproductive technologies (ART) may be readily established, some challenge their use in developing nations. This criticism is levelled on two grounds. First, given the overpopulation problem in many developing countries, it is argued that overfertility, rather than infertility, should be the focus of family planning programmes. Second, treating infertility through expensive ART cannot be justified in low resource settings where other more pressing needs must be given priority.

From an analysis of the suffering that arises from infertility, these criticisms of the use of ART in developing countries can be rebutted. Infertility in developing countries is pervasive and a serious concern. Further, there is evidence that the infertility rates that are generally quoted are, in fact, underestimates. The consequences of infertility in developing countries range from severe economic deprivation, to social isolation, to murder and suicide. It is suggested that the overpopulation and limited resource arguments falsely target ART and lack a more comprehensive understanding of the public health, social, psychological, economic, political and moral issues that are involved.

Infertility is a major problem in developing countries

In poor resource areas and in developing countries, the need for infertility treatment in general, and ART in particular, is great. These rates vary dramatically between and within most countries. In particular,

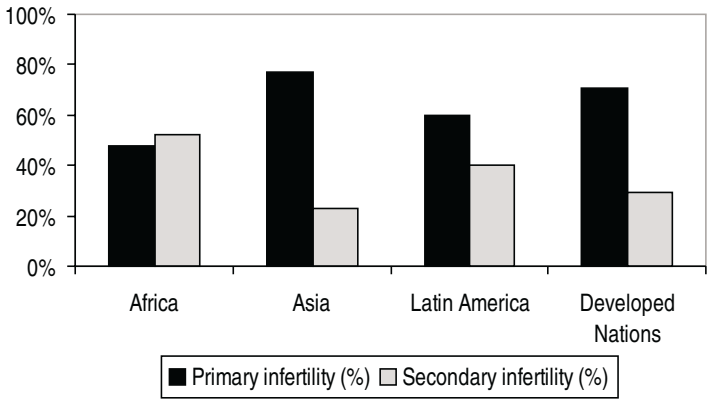

Figure 1. Comparison of primary and secondary infertlity according to regions
Source: Cates *et al.* (1985) *(4)*

Africa records some of the highest infertility rates in the world, mainly secondary infertility (Figure 1).

Secondary infertility is especially problematic in Africa and Latin America. Noninfection-related infertility is difficult to resolve without even some simple form of ART. The impact of sexually transmitted diseases (STDs) or reproductive tract infections that are frequently the cause of secondary infertility may be devastating. For example, Swinton *et al. (5)* argue that a 20% incidence of untreated gonorrhoea in sexually active adults may lead to a 50% reduction in population growth due to the resulting infertility.

More so than in Africa, there is a lack of data on the problem of infertility in Asia. Analysing infertility in Asia is further complicated by first, the diverse cultural, ethnic, and religious groups represented throughout the region; and second, the wide discrepancies in public policy. In Asia, as with Africa, infertility is treated as an ancillary issue to over-population. India, in particular, has paid little attention to infertility given that it is wrongly assumed to be a condition that does not threaten life. Rather, India's policy has focused on managing population size.

Notwithstanding the high rates of infertility in developing countries, there are substantial reasons to think that these rates are underestimates. Many analyses of the prevalence of infertility in developing countries are based on fertility data. The WHO 1991 study *(1)* on infertility underscores the inherent limitations in demographic studies that provide only an indirect indicator of primary infertility and an imprecise indicator of secondary infertility. Moreover, divergent cultural understandings of motherhood or severe social stigmatization associated with infertility bias data, creating a subcategory of *hidden infertility.* The methods used in collecting, recording and analysing data on infertility in contexts where severe social harms persist around infertility should be critically examined.

Infertility has severe social consequences in developing countries

Infertility in developing countries extends beyond the loss of human potential and unrealized self. The experience of infertility causes harsh, poignant and unique difficulties: economic hardship, social stigma and blame, social isolation and alienation, guilt, fear, loss of social status, helplessness and, in some cases, violence *(6–13)*. Many families in developing countries depend on children for economic survival. Without children, men and women may starve to death, especially in old age. In some communities, infertile people are ostracized as they are perceived to be unlucky or the source of evil, or they become the object of public humiliation and shame. Some, even, choose suicide over the torturous life and mental anguish caused by infertility. In other communities infertile men and women are often denied proper death rites. For women in developing countries, infertility may occasion life-threatening physical as well as psychological violence *(11,12)*. Childless women are generally blamed for their infertility, despite the fact that male factor causes contribute to at least half of the cases of infertility around the world. In developing countries, especially, motherhood is often the only way for women to enhance their status within the family and community.

In Asia, being childless has more negative social,

cultural and emotional repercussions for women than, perhaps, any other non life-threatening condition. An infertility study in Andhra Pradesh, India, reported that approximately 70% of women who experienced infertility would be punished for their "failure" through physical violence. Nearly 20% of the women reported that their husbands used severe violence as a result of their childlessness *(12)*.

Much less published research is available on the consequences of infertility in Latin America. From what we do know, communal families are commonplace. That is, children are integral to families' prestige, honour, and happiness. Children are often cared for by multiple, extended family members, which makes having children central to family life. Thus couples suffer extreme emotional and psychological stress over infertility. In certain parts of Latin America, including Mexico, children are an important part of the families' economic prosperity and survival. The role of children for economic survival is a recurring theme throughout developing countries. There is a lack of research on the consequences of infertility for women in Latin America. However, anecdotal sources indicate that violence in relation to infertility is not common in Latin America.

The World Health Organization defines health as "a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity." By this definition, infertility is a major cause of diminished health in developing countries. Indeed, few health conditions could more profoundly or pervasively affect a person's well-being in many developing countries than infertility. For when someone presents with infertility, he or she is often denied voice or advocacy, he or she does not have recourse to therapy, and he or she is blamed for the infertility. Couple infertility is used against the afflicted couple, or exclusively against the woman, as a justification for social isolation, abuse, violence, indignities, physical and mental torture, and sometimes murder. Infertility not only infringes upon the human right to health, it often leads to a violation of the most basic moral protections that are intrinsic to humanity itself.

We propose a continuum to categorize the harm inflicted by infertility and to demonstrate the increasing severity of social suffering (Figure 2). This continuum illuminates the multiplicity of harms that follow infertility, and it underscores the severity of social suffering characteristic of infertile people in developing countries.

In developed countries, the consequences of infertility rarely extend beyond level two; in developing countries, at least in Asia and Africa, the consequences of infertility are infrequently as mild as level three. Thus the very nature, severity and impact of infertility harms in developing countries differ significantly from those found in developed nations. A significant difference between the two contexts is the availability of and access to infertility treatments, especially ART.

Infertility must become a social priority

While accurate figures are difficult to obtain, there is little doubt that access to ART is extremely limited in all developing nations. Accurate information on the per capita availability and regional accessibility of infertility treatments needs to be collected. Even access to infertility information is severely limited in developing countries. While access to ART is extremely rare, the cost is even more prohibitive. However, there are differences between regions that suggest promising alternatives for improving the access and affordability of ART.

Assisted reproductive technologies are embedded in a global background of high fertility, overpopulation, and population control. According to the theory of *economic modernization (14)*, many low-resource nations fail to achieve development based on their population size. In a demographic discourse, the overly populous character of developing nations equate with poor performance and retarded development. Hence, there is a failure to make infertility a research or treatment priority and acknowledge it as a public health issue.

Moreover, the overpopulation discourse and lack of social focus on infertility exacerbates women's inequitable treatment in developing countries. As Ginsburg and Rapp *(15)* have observed, women's bodies are frequently the locus through which social, economic, and political power is exercised. Fertility and reproduction represent mechanisms through which what Foucault *(16)* calls *biopower* is exercised. The lack of medical discourse on infertility and ART exacerbates the harms of infertility. It contains the experience of infertility as a private harm for which individuals, usually women, are to blame. It contributes to the narrow health approach of family planning clinics that often falsely disconnect STD management, maternal and child health facilities, and the more

(i) The distinction between mild, moderate, severe and very severe physical violence is based on the frequency of beatings by the husband, cuts and bruises, abusive emotional or verbal behaviour of the husband toward the wife, intimidation resulting in fear of the husband, familial or community physical or emotional violence, and sexual abuse (including refusal of sexual relations or rape) by the husband toward the wife. The greater the number of indicators of violence, the greater the degree of violence recorded (adapted with revisions from Unisa 1999 *(12)*; see also Papreen 2000 *(11)*).

(ii) The distinction between economic stress, serious economic hardship and severe economic deprivation marks the presence and extent of loss of income due to loss of child labour, loss of job availability due to social alienation, inability to operate familial business (for example, farms) if applicable, GDP per capita relative to others in the country, self-sufficiency, financial indebtedness to others (see Bergstrom 1992 *(6)* for some limited examples).

(iii) The distinction between social alienation and social isolation culminating in loss of social status marks the presence, frequency or extent of social avoidance (not speaking to persons, avoiding persons, not being invited to social gatherings); disrespectful and humiliating social treatment even within one's own social class (for example, being referred to by derogatory names); social assumption that the couple (or in some cases the woman only) afflicted with infertility is evil or cursed, familial mocking or blame, social blame for social harms (for example, woman blamed for health epidemic arising in the community because she is infertile); overall denigration of social status (quality of lifestyle, respect and honours of society) (see Bergstrom 1992 *(6)*; Griel 1997 *(9)*; Gerrits 1997 *(8)* for some examples).

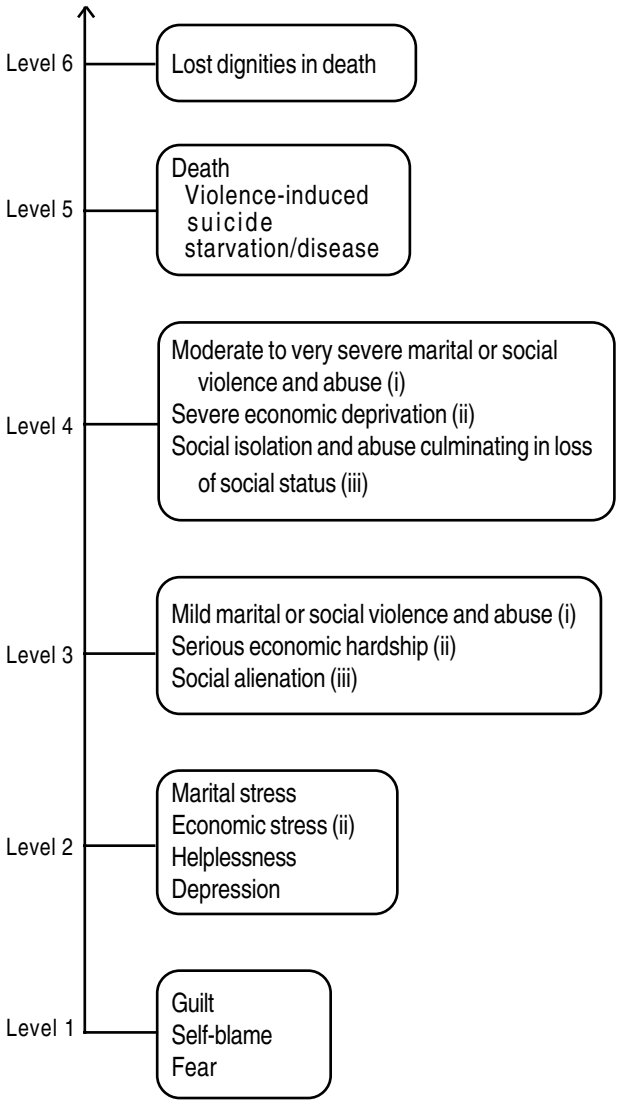

Figure 2. Continuum of the consequences of infertility

comprehensive objectives of the WHO definition of health.

The central difficulty associated with infertility in developing countries is that infertility transforms from an acute, private agony into a harsh, public stigma with complex and devastating consequences. Even if infertility were so narrow in its impact, a harm to individuals rather than to society, it would be no less a serious public health concern. Given the pervasiveness of infertility and the seriousness of its harms, infertility is a substantive public health problem. According to the WHO definition of health, it could be argued that the emotional and psychological harms associated with infertility are health harms. Denying psychological harm as a serious health problem is a classic manoeuvre that has been the cause of pervasive health care discrimination in most countries. Moreover, infertility does transform a potentially private, individualized health problem into social suffering. Infertility has the potential to disrupt peace, exacerbate poverty, and devastate communities. The harms caused by infertility are pervasive, socially embedded and serious, precisely because infertility interacts with a complex network of social relationships, social expectations and social needs.

Infertility in developing countries translates into

an ailing body, an unfulfilled human identity, and disturbed social relations that have direct social, political, and economic impact. Accordingly, developing countries need to re-evaluate social and health care research and treatment priorities to reflect the impact of infertility in their societies.

Reply to the overpopulation and limited resources arguments

Two key arguments are frequently used to challenge the development of new reproductive technologies in developing countries. First, the argument from overpopulation suggests that an overpopulated country should not prioritize infertility management, for the overpopulation poses a demographic problem for the country and for the global community.

The primary response to this argument is that individuals should be able to reproduce "if, when and as often as they wish," as it was stated in the definition of reproductive health adopted by the United Nation's 1994 International Conference on Population and Development. References in both the *Universal Declaration of Human Rights* and the *Convention on the Elimination of All Discrimination Against Women* may also be interpreted to argue for a right to access to infertility treatment through ART. For, if infertile persons do not have access to ART because this would "contribute" to overpopulation, why save lives in developing countries using medical technologies, as this too would have an "overpopulation effect"? If it is thought that it is justified to employ medical technologies to prevent suffering, why is it not justified to use medical technologies to alleviate suffering from infertility? Distinguishing the cases requires the assumption that the harms of disease necessitate medical technology in a way that the harms of infertility do not.

Some think that infertile people should adopt children, given the number of children in developing countries that are available for adoption. Many regional studies indicate a lack of availability of such children and the social customs that resist giving children up for adoption. In Cameroon, letting go of a family's children may be shameful and bring disrepute to an extended family. Children belong in the care of extended families. Moreover, Asian families report an intense fear that adoptive children will not reciprocate parental love and security *(12)*. The argument that infertile people should adopt instead of having their own biological children, in the context of the overpopulation problem, is rather weak. For why should fertile people not have one less child and adopt one instead, for example? Proposing adoption as the only alternative for infertile couples, in the context of global overpopulation, denies the importance of reproductive autonomy and it distributes social responsibility for overpopulation unjustly upon the infertile.

Macklin *(17)* has observed that "a far more effective and ethically desirable way of curtailing population growth lies in education of women in developing countries, along with other modes of development shown to be determinants of lowering population." Denying infertility treatments including ART to infertile persons is simply ill-considered population control policy. A more effective means to manage population size is to assure a right to education that virtually all developing countries have committed to when the UN General Assembly adopted the *Universal Declaration of Human Rights*.

The second argument, the limited resources argument, suggests that developing countries should not allocate resources for expensive technology that can benefit only a few. Proponents maintain that a country's resources should be directed toward the prevention of infertility *(18)*. While the affordability of ART is a problem that needs to be assessed in the specific context of a country's needs and economic conditions, it cannot be assumed that ART is unfeasible. Rather, given the social suffering and public health harms associated with infertility, research should be directed towards finding effective, low-cost solutions to infertility and this exploration should extend to ART. Macklin contends that it is not at all obvious that infertility treatments should not be a key health care priority, especially when their pervasive and grave consequences are considered *(17)*. The idea that infertility treatment is not a health care priority is based on the fallacious assumption that it does not have devastating, material and life-threatening consequences. Indeed, since the consequences of infertility are so severe in developing countries, infertility treatment should assume an even higher priority in developing countries than it does in developed countries.

Sen *(19)* has persuasively argued that rights cannot be justifiably denied on the basis of governments' generalized comments on the scarcity of economic resources. The same kind of argument has been used to deny women's rights around the world, a right to education, and even development rights to

developing nations. As Sen *(19)* observes, a careful examination of governmental budgets in both developing and developed nations frequently reveals mismanagement of funds, rather than an inability to finance social and economic rights.

Many studies have established that *in vitro* fertilization (IVF) may be cost-effective and feasible in developing nations *(19–29)*. In some instances ART may be the only way to treat infertility, even if prevention programmes are successful. Often, affluent people have the means to travel to developed countries for treatments; however, the costs may set them back financially while a developing nation's scarce foreign currency reserves are expended *(30)*. Others, who cannot afford the costs of a treatment abroad, might be obliged to sell off their remaining property, often with severe personal consequences. Finally, if governments cite scarce resources as the justification for not funding ART, this is a cause for governments to think creatively about infertility solutions, rather than a justification for rejecting all ART advances and development. The Colombian Profamilia Programme is a good example of the implementation of ART in developing countries. It demonstrates how govern-ment support for nonprofit organizations may be vital in providing affordable access to infertility care *(2)*. Cooperative public and private partnerships have the potential to make infertility care affordable and to make access more just.

The potential for public–private partnerships

Public–private partnerships (PPPs) are joint ventures that may: (i) bring in technical resources (technology, people, knowledge) at lower costs; (ii) promote efficiencies in the public sector; (iii) attract foreign investors and capital; (iv) introduce and develop effective processes, practices and standards for infertility treatment in developing countries; and (v) instil greater accountability and responsibility. A framework that ensures knowledge transfer through PPPs would enable domestic drug companies to manufacture their own infertility drugs, especially for those drugs whose patents have expired. Furthermore, public–private clinics may be a source of revenue that may be used to support other health care objectives. For example, Indian clinics have been successful in attracting business from people coming from wealthier countries.

Developing nations often have a cost-competitive advantage due to inventiveness under adverse conditions, longer working hours, cheaper labour, involvement of volunteers, etc. The cost of ART may be further reduced, if research confirms that similar ART success rates are achievable with less expensive stimulation protocols.

Finally, as demand for ART increases and supply expands, costs will decrease due to economies of scale and increased competition. Developing countries are well positioned to advance their health care technologies relative to other technologies, because these are often less dependent on multinational corporations. Medical knowledge is readily available and transferable across borders. Developing countries have substantial social capital in the field of health care through existing NGOs and skilled, native practitioners. This affords the public sector stronger negotiating power and greater autonomy in PPPs.

Protecting reproductive rights and meeting health care needs are not inimical objectives. Government policy on ART will necessarily reflect its societal values, religious commitments if any, traditional ways of life, and economic conditions. Having the programmes and infrastructure for existing reproductive technologies may facilitate the introduction of other reproductive and genetic advances that may prove indispensable to better health care in the future.

Conclusion

Evidence supports the conclusion that there is a compelling need for infertility treatment beyond prevention. In many instances, ART are the last hope or the only means to achieve a child for couples. There is a heightened need for ART in developing countries. While developing countries have generally not established adequate infertility programmes, mainly due to arguments based on overpopulation and cost, some notable exceptions *(2)* raise hope of successful and just implementation of ART, perhaps through public–private partnerships. A failure to even consider examining low-cost models of ART will be to conceive of developing countries as perpetually developing, rather than developed, with respect to public health.

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ART in developing countries with particular reference to sub-Saharan Africa

OSATO F. GIWA-OSAGIE

Introduction

It is a common saying that "prevention is better/ cheaper than cure". This saying is universally applicable in the field of infertility. The ability to conceive, conception itself, and successful pregnancy, delivery, and infant survival, are of special importance in Africa where the cultures of the peoples place a premium on childbearing and -rearing. The causes of infertility in general, and tubal and male infertility, in particular, are mainly preventable. The treatment of tubal infertility and of most forms of male infertility increasingly involves the use of assisted reproductive technology (ART). ART is far more expensive than strategies and actions required to prevent infertility. Of adult couples in African countries it is estimated that 10%–25% are subfertile and of these subfertile couples female factors account for about 55%, male factors for 30%– 40% of causes, while 5%–15% of causes are unexplained. Tubo-peritoneal factors are the single most common cause of infertility in African couples followed by oligo-azoospermia from whatever cause *(1–3)*.

The most common cause of infertility in Africa is infection *(2–5)* of which the two sexually transmitted infections (STIs), gonorrhoea and chlamydia are the main culprits in both males and females. Delayed diagnosis of STIs, lack of diagnosis, incomplete therapy, no therapy, or inappropriate therapy compound the problems of STIs in Africa. After STIs, infections during or after abortion and during and after childbirth represent the next major cause of female infertility in Africa. The latter explains the preponderance of secondary infertility over primary infertility in Africa *(1,2)*.

In comparison, the developed countries of Europe and North America have more endocrine causes of infertility and have better facilities for the diagnosis and appropriate therapy of STIs and can therefore be expected to have better prognosis in infertility management. In Africa, there are more tubal factors, more irreversible oligo- or azoospermia and less resources for the management of infertility due to economic, political, capacity-building factors and the severity of disease.

The focus of this paper is sub-Saharan Africa. However, the Republic of South Africa has been excluded as the level of scientific activity there is more akin to the developed countries than the realities in developing countries. Information for this review was obtained through personal enquiry from health personnel in the countries of sub-Saharan Africa, from publications in scientific journals, and conference abstracts, and news media and from site visits. Requests for information were made to doctors and relevant health personnel in the following countries: Cameroon, Ghana, Guinea, Kenya, Nigeria, Senegal, Sierra Leone, Togo, Uganda and Zimbabwe. In the

case of Cameroon, Ghana and Nigeria, information was sought from identified practitioners of ART. Responses, which varied in content and value, were received from Sierra Leone, Senegal, Ghana, Nigeria, Cameroon and Zimbabwe. Some of those who have declared in the media that they practice ART and have had ART successes did not make their statistics available. In spite of these difficulties, it was still possible to gain an insight into what is being practised in the field of ART in sub-Saharan Africa through indirect sources and a few direct sources.

Objectives

The goal of this paper was to obtain the following information relating to ART in the subregion:

- Is ART being practised?
- Where and by whom?
- What methods of ART are available?
- Cost of ART per cycle;
- Statistics and results of ART;
- Sources of equipment and consumables;
- Technical collaboration, if any;
- Assessment of opinion on relevance, need, affordability and accessibility of ART.

Types of ART practised

Virtually all forms of ART are now available in the subregion namely: artificial insemination by husband (AIH); donor insemination (DI); *in vitro* fertilization (IVF); gamete intrafallopian transfer (GIFT); zygote intrafallopian transfer (ZIFT); intracytoplasmic sperm injection (ICSI); embryo freezing and embryo donation; surrogate motherhood.

The most widely practised methods are AIH and DI, and IVF *(6–8)*. Oocyte donation is also available *(9)*. The least widely available methods are ICSI and embryo freezing. Adjunctive methods such as laser assisted hatching and implantation are not yet available.

Table 1 shows the methods of ART available per country listed. From information available from various sources, the countries that have centre(s) offering donor semen insemination, IVF and related ART are Cameroon, Ghana, Nigeria, Togo and Zimbabwe. Other countries, such as the Benin Republic, Kenya and Sierra Leone have physicians who offer AIH.

ART was available usually in a sustainable manner in private clinics rather than in the public sector. The usual pattern was for the practitioner(s) to have started the procedure in the public sector and then moved to the private sector for want of funding in the public sector. A classic example was the experience of the Lagos University Teaching Hospital team of Giwa-Osagie, Ashiru and Abisogun who produced documented pregnancies through IVF in 1984, 1986 *(10)* and a live birth in 1989. There was no public sector funding and the service transferred to the private sector. The successes from IVF at the Lagos University Team were the first in West, East and Central Africa then. Now all the ART units doing IVF in West, Central and East Africa are in the private sector or incorporated as foundations. This has serious implications for accessibility and equity. The sources of consumables, equipment and technical collaboration were influenced by the country in which the principal clinician or embryologist had his training in ART. Equipment and consumables for AIH, DI and IVF were purchased from the United Kingdom, Germany, Holland, France, Sweden, USA and Australia, in order of frequency. All

Table 1. Methods of ART available in various countries in sub-Saharan Africa (excluding South Africa)

Country	AIH/DI	IVF	GIFT/ZIFT	Oocyte donation	Egg sharing	Embryo freezing	TESA	ICSI
Nigeria	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ghana	Yes	Yes	N/A	N/A	N/A	Yes	Yes	Yes
Cameroon	Yes	Yes	N/A	N/A	N/A	N/A	N/A	N/A
Togo	Yes	Yes	No	No	N/A	N/A	N/A	N/A
Senegal	Yes	Yes	N/A	N/A	N/A	N/A	N/A	N/A
Benin Republic	Yes	No	No	No	No	No	No	No
Zimbabwe	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Kenya	Yes	No	No	No	No	No	No	No
Sierra Leone	Yes	No	No	No	No	No	No	No

N/A: information not available

Table 2. ART in sub-Saharan Africa: technical collaboration

Location of ART units	Location of technical collaborators
Lagos, Nigeria	UK
Abuja, Nigeria	UK/Denmark
Tema, Ghana	Germany
Tema, Ghana	USA/UK
Lome, Togo	France
Yaoundé, Cameroon	France
Douala, Cameroon	France
Harare, Zimbabwe	Australia/South Africa/UK

ART practitioners used gonadotrophin-releasing hormone analogues mostly on a long-protocol, for downregulation followed by gonadotrophin stimulation. The ultrasound scanners were from Austria, Germany, Japan and the USA. All centres recovered oocytes transvaginally under ultrasound guidance while some still performed laparoscopic recovery for a few cases that were difficult to access transvaginally. The indications for IVF were tubal factor infertility, which accounted for over 60% of cases in most series, followed by male factor infertility and combined tubal and male factors. Unexplained infertility and endometriosis were not so common indications and accounted for less than 20% of indications for IVF.

The countries with which there is/was technical collaboration are listed in Table 2. The main countries are France, Germany, the UK and the USA. There was very little intra-African collaboration and most ART practitioners in Africa were not aware of the activities of similar practitioners in the subregion although the ART centre in Lome, Togo had some discussions with one centre in Tema, Ghana. This lack of communication led to many claims at being the "first" to achieve various milestones in the subregion.

Table 3 summarizes ART activities in established ART units in Nigeria. There is close cooperation between some centres and ART centres in South Africa, the UK, Australia and Denmark. In one centre in Lagos, the egg recovery, embryology and embryo transfers, and ICSI are done at intervals when a team from the UK comes to work at the centre. The centre in Harare, Zimbabwe, has visits from ART specialists from Australia, the UK and South Africa to carry out quality checks and updates at intervals. One centre in Lagos, another in Abuja and one in Tema, Ghana have had varying degrees of collaboration during their start-up phase with centres in the UK, Denmark and Germany. The embryologists of two of the three centres in Lagos, the centres in Abuja, Tema and Harare are all indigenes of the respective countries. The results of AIH and donor semen insemination are summarized in Table 3. Tables 4 and 5 summarize ART statistics from three units in Nigeria and the unit in Zimbabwe *(9,11–13)*.

Similar information on ART procedures including DI was requested from the ART centres in Ghana and Cameroon. Most centres transferred embryos at the 4–8-cell stage, although there is an increasing tendency recently to transfer blastocyts. Three centres

Table 3. AIH and DI in some countries in sub-Saharan Africa

	AIH			DI		
	Total cycles	Cycles per annum	Pregnancy rate (%)	Total cycles	Cycles per annum	Pregnancy rate(%)
Giwa-Osagie *et al.* (1985) Lagos *(6)*				35	–	71*
Giwa-Osagie *et al.* (1995–2000) Lagos	830	138	22.8	1543	257	58.2
Wada *et al.* (1999) Abuja *(12)*	32		25	–	–	
Robertson *et al.* (2000) Harare		100	22†	–	20	30†
Fraser B (2000) Freetown				20	–	70‡

* Cumulative pregnancy rate at 8 months
† Pregnancy rate after 3 cycles
‡ Cumulative pregnancy rate

Table 4. ART activities in established units in Nigeria and Zimbabwe

	AIH/DI	IVF	ZIFT/GIFT	Embryo freezing	Oocyte donation	ICSI
Lagos						
1. Advanced Fertility Clinic	Yes	Yes	Yes	Yes	Yes	No
2. Providence Hospital	Yes	Yes	No	No	Yes	No
3. The Bridge Clinic	Yes	Yes	No	No	Yes	Yes
Abuja						
4. Nisa-Premier Hospital	Yes	Yes	Yes	Yes	Yes	Yes
Harare						
5. Alfacare	Yes	Yes	No	Yes	Yes	Yes

Table 5. Statistics of IVF in some centres in sub-Saharan Africa

Centres	Cycle/annum	Pregnancy rate/ OR	Pregnancy rate/ ET (%)	Multiple pregnancy rate (%)	Take-home baby/ ET (%)
Alfacare, Harare (2001)	50		28 (frozen embryos)		15.8
Nisa-Premier *(13),* Abuja (1999)	50	15	21	16.6	14
Advanced Fertility Clinic, Lagos (1999–2000)	50	16	21	14.3	14.1
Bridge Clinic, Lagos (1999–2000)	159	19	21.6	36	
Providence Hospital, Lagos (1999)	10 (oocyte donation)		40		10

transferred no more than three embryos at a time. All centres provide progesterone support after egg recovery. Embryo freezing is available in two centres in Nigeria while oocyte donation is available in four centres in Nigeria. One centre in Lagos has practised surrogate motherhood but the overall experience of this is little. The cost of IVF and related procedures varies from the equivalent of US$ 1200 to US$ 4000 per IVF cycle including drugs, with two centres in Nigeria and one centre in Tema, Ghana stating that a cycle costs about US$ 2500 at their centre. Two centres in Nigeria engaged in egg-sharing schemes and charged between US$ 1200 and US$ 1800 per person for egg-sharing cycles. The IVF centre in Zimbabwe charges US$ 2500 per cycle of IVF, or US$ 3500 per cycle including drugs.

The regulation of ART

In all the countries surveyed, there is no state regulation of ART. ART practitioners in the subregion, however, stated that they have a voluntary adherence to guidelines set by the American Society of Reproductive Medicine, the British Human Fertilisation and Embryology Authority or the equivalent body in France or Germany. Because of the interaction and collaboration of the African centres with scientists from Europe, South Africa, Australia and America, the African centres voluntarily abide with accepted guidelines from those countries. The centre in Harare states that it has regular visits from top scientists from the UK, Australia and the USA to guide it and update it on quality control, ethics and new advances. Most of the countries do not have national ethics committees.

The relevance of ART

WHO has enunciated and propagated a holistic definition of health, which includes physical, mental and social well-being and not merely the absence of disease or infirmity. Reproduction is vital for species survival. Infertility causes major marital, family and social disruption in Africa. Africa has more of the type

of infertility that can be solved by ART procedures than any other part of the world. ART is therefore relevant in Africa. Sub-Saharan Africa, however, has deficits of capacity, finances and communication, which need to be addressed in a relevant and cost-conscious manner. In one centre in Nigeria, it was estimated that 30% to 40% of infertile patients with tubal disease need ART procedures *(14)*. Both in terms of the numbers of people that could benefit from ART and the cultural place of childbearing in sub-Saharan Africa, ART is relevant.

The need for ART

The need for ART in sub-Saharan Africa is related to its relevance there. The need for ART far exceeds the availability of facilities and expertise, thereby creating a large gap between need and availability and uptake. In Nigeria alone there are at least 12 million infertile persons, and tubal factors, severe oligospermia and azoospermia account for the majority of the causes of infertility. As of 2000, the total number of IVF cycles performed in Nigeria was less than 600 cycles per annum. One can only conclude from this that the need is not being met at the present.

Affordability of ART

One way to assess the affordability is to consider it in relation to minimum wage. In Nigeria, a cycle of IVF costs about US$ 2000–US$ 2700 (250 000 to 350 000 Naira). The minimum wage in Nigeria is US$ 52–US$ 60 per month. A National Health Insurance Scheme is just about to start. A few companies and large federal government parastatals are prepared to pay for only part of ART costs such as drugs, or oocyte recovery and embryo culture, but not for everything. This means that most people who need ART fund it themselves. ART is therefore not currently affordable for the average Nigerian. The same is true for all of West Africa. Because of the premium placed on childbearing, it is common to find that members of the extended family and friends contribute to make up the cost of an ART cycle.

Accessibility of ART

Access to health care in most countries of sub-Saharan Africa is not ideal and is mostly inequitable. Access to ART is no different. ART centres are usually in the major or capital cities such as Abuja, Dakar, Douala, Harare, Lagos, Lome, Tema/Accra and Yaoundé. These cities are the centres of economic and academic activity where the medical specialists concentrate and where there are patients who can afford to pay for medical services. Access is limited by economic as well as physical access factors. There is also an educational factor *(6)*. Many persons in the subregion confuse artificial insemination with IVF and related procedures. There are groups within the subregion who object to artificial insemination and other ART procedures on religious, ethnic or cultural grounds. Access to ART can be improved in a number of ways. Continuing increase in the level of basic education and literacy in the subregion will enhance communication and population awareness of reproductive health, including ART. The introduction and correct implementation of social/health insurance will enable health services to be more readily available to those who need them. In many countries in the subregion, there is a tendency to look at the private sector as an opponent or rival of the public sector. Collaboration between the public and private sector in health care delivery will increase access to health care for the populace. The government, through its health institutions, can reduce the costs of health care including ART by bulk purchase of drugs, consumables and equipment. Private hospitals can also do bulk purchase to enable them to cut their costs and reduce costs to the patients. In a subregion that is short in facilities, expertise and capacity, all of which have been worsened by "brain drain" to Arabian Gulf States, Europe and the United States of America, centres of excellence should be established in some states within the subregion. This will assist the provision of accessible, cost-effective health care and ART. The costs of setting up these centres can be shared between the countries, private sectors, individuals and international agencies such as WHO, the Rockefeller Foundation, the Ford Foundation, the Bill Gates Foundation. These centres could offer research and service at region-realistic prices. Baseline studies will establish the best location of these international centres of medical excellence.

Conclusion

The communication gap in Africa was clearly demon-

strated in the preparation of this paper. It was difficult—even with the aid of facsimile and e-mail communication—to collate available evidence. Many medical practitioners were not aware of the activities of their colleagues in neighbouring countries, but were usually up-to-date about events in the developed countries of the world. The fact that there are well-documented successes through ART in sub-Saharan Africa confirms that it is possible to transfer technology from more developed countries *(7,9–11,13)*. The reluctance of some ART centres in sub-Saharan Africa to share their ART statistics even when contacted directly in person or by mail may be because their results are not as good as those from the developed countries. If this is so, it is unfortunate because such disparities in results should encourage collaboration within Africa as well as with countries outside Africa. My deduction from available evidence is that the IVF clinical pregnancy rates of most of the ART centres in sub-Saharan Africa are possibly between 10% and 20% of oocyte retrievals, with probably 10%–20% spontaneous abortion rates, and take-home baby rates of 5%–15%. These rates can be improved by taking account of uterine factors such as uterine synechiae and fibroids and treating them before IVF attempts and by considering transferring up to four embryos rather than just two or three embryos. International agencies such as WHO and the various national governments can assist by encouraging and funding collaborative intraregional as well as north–south research to improve the results of infertility management and to adapt available technology to local conditions and finances. The countries with ART centres should be encouraged and assisted to develop appropriate regulations and guidelines on ART. The subregional ART centres should be encouraged and empowered to have collaborative meetings and to share their statistics and expertise. Such collaboration would ensure that all the ART centres state their results in the same manner and encourage the standardization of procedures and equipment.

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Section 2

Infertility and assisted reproductive technologies from a regional perspective

Assisted reproductive technology in Latin America: some ethical and sociocultural issues

FLORENCIA LUNA

Introduction

Latin American countries differ in many ways including size, natural resources (for example, Brazil and Uruguay), and the configuration of their populations (indigenous population, of African origins, and multiple ethnic backgrounds mainly of European immigrants). Such differences have influenced the formation of the many cultures in Latin America. Hence, all generalizations will have to be subject to such limits. Latin America is not a homogeneous region; countries are diverse and have a cultural and sociological individuality. Despite their differences, however, Latin American countries share certain features: wide gaps between poor and wealthy people, and the high prevalence of Catholicism. Even though the Catholic Church may differ from country to country, religion does have a great impact on reproductive issues. And assisted reproductive technology (ART) is no exception.

In this paper, some ethical and social issues of ART in Latin America, particularly those influenced by the culture, religion or particularities of the region are presented. However, it should be recognized that it is very difficult to avoid concerns that are of a universal nature or that are also present in other regions of the world.

Availability of ART in the region

It is a fact that in the majority of Latin American countries, ART is not a high priority when compared with larger public health issues, particularly prevention of easily treated diseases.

ART lacks priority in public policies. In general in Latin America, ART is not provided in public hospitals. The few centres treating infertility problems offer very basic treatments. For instance, in Argentina, only diagnosis of infertility is provided in public hospitals but no treatment is offered[i]. In Brazil, few public hospitals or centres offer ART, the Outpatient Clinic of UNICAMP[ii] does not cryopreserve embryos or gametes and the cost of the drugs must be paid by the patients. This is costly and ranges from US$ 750 to US$ 2000 (personal communication with Maria Yolanda Makuch). In Argentina, some of the private fertility centres do have foundations that help people with scarce resources. However, their impact is minimal.

The poor, then, cannot access these techniques. This situation should not be considered unique to Latin America; these services are not provided in many other countries either. In the USA, for example, there are no publicly funded infertility treatments and most private insurance companies do not cover them.

i The same point was made by Haroldo López from Guatemala. There is no IVF in hospitals.

ii UNICAMP (Universidade Estadual de Campinas) is the State University of Campinas, in Saõ Paulo, Brazil.

However, in Latin America this situation is surrounded by a pervasive feeling of lack of justice and discrimination.

In this vein, ART is perceived as a luxury for wealthy couples, and not as a service for anyone wanting a son or daughter. A frequently voiced prejudice is that poor women have a lot of children; they do not need these techniques[iii]. Infertility is viewed as a problem of the wealthy, which further reflects the big socioeconomic gaps among the populations of this region; on the one hand, a wealthy population with access to highly sophisticated medical technology and, on the other hand, large numbers of people who cannot even cover their basic needs, let alone have access to expensive infertility treatment.

A first issue that explains this feeling of lack of justice is that even if these techniques are restricted to the upper and middle classes with access to them through private centres, the deprived is the population in need. It is this population that is most likely to have sexually transmitted diseases (STD) and reproductive tract infections (RTI) and therefore a higher incidence of infertility *(1,2)*. Makuch quotes a Brazilian study where 42% of women who consulted for infertility had tubal obstruction because of reproductive tract infection. Illegal abortions practised in deficient conditions may also leave this population with infertility problems. In addition, some working conditions or contact with pollutants may alter the quality and quantity of sperm. These are jobs often done by people with little education. It has been reported that indigenous people from Guatemala, after having been exposed to certain kinds of fertilizers, seemed to have problems of azoospermia (personal communication with Haroldo López). Similar data on secondary infertility have also been reported.

A second reason that may play a certain role in the perception of injustice is related to the idea of a right to health care. This is especially strong in Argentina and Brazil. In the latter country, the notion of right to health care is present in many different fields *(3)*. One example is Brazil's position on the protection of research subjects, or the use of generics for AIDS treatment[iv] *(4)*.

Many Latin American countries used to enjoy a tradition of public health systems. In Argentina, even some very costly treatments such as organ transplantations, are carried out in public hospitals[v]. This tradition of publicly supported health care is not the case in all countries, for example, the USA, where the lack of ART services is not so strongly perceived as unfair as in other countries where this tradition has existed. In some of the Latin American countries the ideal is in line with European countries and their strong support for public health systems. In addition, this tradition and ideal of a welfare system in health is accompanied by at least a decade of deterioration in the public health system[vi]. This gives rise to a feeling of resentment and of an increasing injustice in health care.

Regulations

Legal regulations

Another issue related to the above is that in many Latin American countries, ART is not well regulated and may vary widely. The 1998 Surveillance Study *(5)* only considers the situation in Mexico and Brazil as having

iii Discrimination is reinforced by the population control that has occurred recently in Latin America by which poor women were sterilized. These recent facts leave the impression that this group does not deserve help to achieve fertility. "Poor women are not allowed to have children." This memory of past abuses is still present and shapes the perceptions of discrimination.

iv When the Declaration of Helsinki was discussed and a double standard was proposed regarding the treatment to be provided to research subjects; the National Council of Health (Conselho Nacional de Saude) issued a declaration (Resolucao N 301, 3-16-00) asserting the maintenance of the "best proven criterion". It argued against lowering standards to the detriment of developing countries. A similar attitude was taken by Brazil regarding the excessive HIV drug prices. Considering the importance and growth of this pandemic in the country, Brazil is aggressively negotiating generic drugs with international pharmaceutical companies in order to be able to give them freely to all the population in need. During the past seven years it has provided free medication to 100 000 patients.

v Not only are costly life-saving treatments given, but also "non-vital" treatments such as plastic surgeries. This may raise other concerns, such as what the criterion is for distributing scarce resources (a point I cannot undertake here). It also raises such questions as: Why is ART not provided while plastic surgery is?

vi This is another global process, and many resource-poor countries are feeling the impact of these kinds of international policies.

legislation[vii]. In these countries ART is offered to married couples or to those in stable relationships. Embryo cryopreservation is allowed *(5)* but there is no provision for embryo donation, even if sperm or oocyte donation is allowed. Brazil accepts surrogacy only if a relative is willing to undergo this procedure.

In startling contrast, Costa Rica forbids assisted reproduction through a Constitutional Amendment. It only allows homologous insemination. As a consequence, patients seek treatment in other countries in the region (personal communication with Carlos Valerio). Aside from the doubtful rationale for such a prohibition, an additional problem is that this prohibition makes these techniques even more expensive and promotes "reproductive tourism". I will not go into each country's legal situation or the conditions in which such laws are passed (whether they were the product of a public deliberation process or the imposition of some group).

Informal regulations

Countries with an "informal" kind of regulation (that is, without legal regulations) suffer other kinds of problems. The first ethical or sociocultural problems faced—at the private practice level—are the difficulties in setting limits. Despite consensus documents by the scientific community, this remains a pressing issue[viii]. What are the limits on medical interventions? Should physicians provide ART to single women? What about lesbian or homosexual couples? Should there be an age limit for ART? Should these techniques be offered to postmenopausal women?

The lack of a law fixing the permissible or the impermissible puts pressure on the providers. They have to decide without an external rule that sets limits. In addition, in societies that are influenced by the Catholic Church, whatever minimal intervention falls outside the standard may easily be criticized (single women, postmenopausal women, etc.). Strict adherence to Catholicism goes against the gamut of these techniques. However, Catholics who are not so strict may still have problems with some of the techniques and for whom they may be provided (for example, single, lesbian or postmenopausal women). Hence providers sometimes have to decide what should be done on a case-by-case basis and these decisions are ostensibly very difficult. This fosters an attitude of self-restraint in the physicians themselves. They want to be able to exercise their profession without troubling the *status quo*. In this sense, I will try to show later on that there is a subtle influence of religion on how ART is practised.

There is a widely felt need for regulation. However, legislation is not sought because of the fear of very restrictive laws, given the prevalence of the Catholic lobby. However, religion should not be a problem for the practice of ART if it would be maintained within the religious boundaries. Religious people should, then, follow the teachings and dogma of their faith. The problem arises when there is pressure to impose a religious dogma in the legislation of a secular country; as all the Latin American countries are. In this sense, it is understandable that Costa Rica's model of total prohibition raises apprehension. In the case of Argentina, some projected laws were so restrictive that it would have been nearly impossible to implement these techniques[ix].

Even if there is no legal regulation, there is a scientific consensus in the region. An example is the consensus on ethical and legal aspects agreed upon by a Latin American network, constituted in 1994 in Chile (Reñaca) in order to provide guidance to legislators, health authorities, women's organizations and the general public *(7)*. Supposedly, individual clinicians agree to abide by it. Some of the consensus points are:

- ART are to be provided to infertile heterosexual couples. They are unacceptable for single women who do not seek a heterosexual partner or who do not want to have recourse to intercourse (coitus)

vii This report considers three categories of countries. Countries with legislation (such as Brazil and Mexico), countries with guidelines (here Argentina is included), and countries without legislation or guidelines. Those mentioned are the only three Latin American countries analysed in this report. Interestingly, there is not yet a law in Brazil. In a recent article about the situation of ART in Brazil, the author mentions a CMF Resolution 1358/92 issued by the Federal Medical Council. This does not have the force of a law; it directs recommendations to the medical profession. It also mentions other three law projects—in 1997, Law Project N 3638, Law Project N 2855 and in 1999, Law Project N 90 *(6)*.

viii These consensus documents are not always respected by all the infertility centres. They do not bind them. And there are known cases of surrogacy, lesbian couples, postmenopausal women that use these technologies.

ix Argentina only has guidelines proposed by the Argentine Society of Fertility and Sterility.

as an expression of sexuality[x] *(7)*.

- Every cryopreservation programme *must be linked to a programme of donation or adoption of embryos* that makes it possible to find a mother for those embryos that will not be transferred to their progenitors[xi] *(7)*.

Research on embryos is acceptable only when it is foreseen that the embryos will not be affected as a result of the research. *Even if the progenitors authorize research that entails a mortal danger, the right to life of the embryo should take priority and be safeguarded by the medical team*[xii] *(7)*.

These issues are highly controversial and civil society may disagree. Some of these clauses implicitly grant a status to embryos that may be problematic. For example, they exclude discarding unwanted embryos. Some women who seek these techniques would prefer to discard unwanted embryos rather than submit to a requirement that the embryos are donated to another couple.

Religious impact

Religion is mostly perceived as a barrier to some of the uses of ART. Only the most conservative religious views endorse this position. Representatives of this extreme view are practising Catholics who oppose any of these techniques for the artificiality they imply (even the case of insemination).

One of the reasons proffered is the inseparable connection between the unitive and procreative meaning of marriage *(8–10)*. Persons are not allowed to voluntarily dissociate these two. Some consequences of this position are:

—Assisted reproduction is unacceptable because it promotes procreation in the absence of sex.

—The use of donor gametes constitutes the interference of a third party in the holiness of marriage.

—The right to life from the moment of conception bans embryo cryopreservation, manipulation or their voluntary disposal. The biological identity of a new human being is already established in the zygote resulting from fertilization[xiii].

To this absolute and radical refusal of ART, it is interesting to point out the practice of a "double moral standard". One thing is what religion says and another is what people do. The great majority of the population coming from a Catholic upbringing (84.4% claim to be Catholic) do not follow Catholic teachings. This phenomenon is not unique to ART; in contraception the attitudes are identical. For example, abortion laws are highly restrictive; however, illegal abortions are carried out continuously. In Argentina, between 350 000 to 500 000 illegal abortions are performed every year. The same attitude seems to operate in ART too. Zegers-Hochschild points out that:

> Specific instructions directed to legislators, health providers and patients, containing these and other directives [the teachings of the Catholic Church], have been vastly disseminated. Hence, it is difficult to understand how contraception, artificial insemination with donor sperm and assisted reproduction can be so widely performed in the Latin American region. During 1995, 7000 cycles of assisted reproduction including 351 transfer cycles with donated oocytes were performed in 59 centers throughout Latin America *(8)*.

These facts speak of hypocrisy and a double moral standard. They also speak of a lack of real deliberation and consultation with society in the legislative arena. They show a lack of tolerance and respect for beliefs

x My translation of: "… no se considera aceptable la aplicación de dichos procedimientos [técnicas de reproducción asistida] en mujeres solteras que no deseen tener una pareja heterosexual o que teniéndola no desean recurrir al coito como expresión de sexualidad" (p. 10).

xi My translation and my emphasis of: "Todo programa de criopreservación debe ir ligado a un programa de donación o adopción de concepti que permita encontrar una madre para aquellos concepti que no serán transferidos a sus progenitoras" (p. 16).

xii My translation and my emphasis of: "La investigación en conceptus es aceptable sólo cuando la indemnidad de éste no se ve afectada como consecuencia de la investigación. […] De esta manera, aunque los progenitores autorizaran una investigación que conlleve un peligro vital, debe primar el derecho a la vida y ser salvaguardado por el equipo médico" (p. 21).

xiii This third point prohibits only manipulation or destruction of embryos, not their creation and implantation. But taken together with the first consequence which considers the artificiality, it gives a substantial opposition to all of these techniques.

and practices other than the religious ones.

Although many Constitutions uphold the separation of State and Church, in the majority of the Latin American countries this is not respected. Strong political pressure and lobbying from the religious organizations exist. And, as Zegers-Hochschild again rightly points out: "In Latin America, legislators (especially from the right wing of politics) have preferred to legislate in literal conformity to principles emanating from moral teachings of the Catholic Church" *(9)*.

Specific problems

Each technique presents a different challenge. I will only focus on some specific issues that not only pose ethical quandaries but that are also related to some social issues particular to Latin America. There is an implicit "priority" of embryos subtly seen in some attitudes and practices in the region by:

- reluctance to cryopreserve embryos when there is no law forbidding it;
- obligation to donate embryos;
- use of certain "terminology"; and
- the paradox of preimplantation genetic diagnosis (PGD).

The consideration of the embryo in the "Latin American culture" shapes and limits the options a woman or a couple may be offered. The first issue is the limits or harms that may be involved in order to protect embryos against the welfare of the women.

Religious background seems to play a role in the provision of treatment, as well as in treatment-seeking behaviour. Even though the above consensus document argues for the importance and beneficial aspects of cryopreservation *(7)*, Argentine ART centres limit the quantity of embryos to be frozen (personal communication with directors of Argentine Fertility Centers). There is an ongoing trend by which centres are cryopreserving fewer embryos. Hence, women may have to be submitted more frequently to hormones and medication with the inconvenience and harm this may entail.

A second issue is that no option for discarding supernumerary embryos is offered and the only alternative available—when a person does not want to transfer the remaining embryos into herself—is to donate embryos to another couple. This, however, is a compulsive donation which can also have disturbing psychological effects. It may prove to be especially painful when the donor cannot achieve pregnancy.

A third consideration arises with the practice of embryo donation and its current denomination as prenatal adoption. Terminology carries weight, it implies a particular way of understanding facts. The term prenatal adoption is deceptive and conveys the idea of an actual adoption. It is not a neutral term, much less in a region where the embryo is sometimes more protected than women. I will not enter into the controversy regarding the ontological or ethical status of embryos. However, with this denomination, embryos are considered as orphans, an analogy that leads to paradoxes. If we were to accept that embryos are persons, the whole process of cryopreservation would, at least, be odd—how could we freeze persons? Moreover, *in vitro* fertilization (IVF) could be seen as a massacre, due to the loss of embryos when transferring them to the woman's uterus; and the same practice of giving them up in adoption (with the obvious intention of protecting them) may imply their death and destruction.

Finally, the last issue is PGD. This is related to the health status of the embryo and the prohibition of abortion. While the International Federation of Fertility Societies encourages screening for serious diseases which would be a potential threat to the potential child's health *(5)*, the majority of Latin American countries do not accept either discarding of embryos or abortion.

The nonacceptance of discarding embryos while genetic screening is permissible and available creates a paradox. In the case of Argentina, Chile and other Latin American countries, embryos which are diagnosed with genetic abnormalities have to be transferred because they cannot be discarded. ART centres in Argentina report that they do not discard embryos[xiv]. This leads to a situation where a woman at risk must accept the embryo transfer and pregnancy knowing that the offspring may suffer from a serious

xiv In Brazil, the CMF resolution 1358/92 makes several recommendations although it does not have legal force. It states that diagnosis in the pre-embryos is allowed only for purposes of their viability or the investigation of hereditary diseases. However, the discarding of embryos is forbidden by CMF and two of the law projects (personal communication with Dirceu Guilhem).

or even fatal illness.

Thus, either PGD should be banned, harming the couple and the future offspring, especially if they are undergoing these procedures in order to avoid transmitting a genetic disease; or else, PGD is undertaken and embryos with genetic problems are discarded. In the latter case, the majority of fertility centres in Latin America deny that this is what happens. This is another example of hypocrisy and double standards and can possibly harm the couple or the offspring.

In such cases the responsibility of the physician and the couple or woman are at stake. They are consciously bringing into the world a baby with a severe disability or illness. A number of thorny questions arise: Can we knowingly seek a pregnancy resulting in a handicapped or severely ill offspring? What are our responsibilities towards this future human being? Should we avoid bringing what has been called "evitable suffering" into the world *(11)*? Arguing that only healthy embryos and fetuses should be allowed to survive is challenged by the disability groups, who argue that abortion of defective fetuses or refusal to implant abnormal embryos discriminates against those with disabilities. These radical proposals seem too extreme. If they consider preventive measures as discriminatory then they could also object to warnings on alcoholic beverages, about the risks of birth defects *(12)*. The disability groups implicitly suggest that it is wrong to interfere with the natural order of things, an extreme view that would object to any medical treatment.

Taking into consideration the different perspectives (radical and controversial), one could argue that evaluation of the moral responsibility of knowingly bringing into existence a severely handicapped child and the final decision about it should be made by the couple. But this would imply offering the possibility of discarding severely damaged embryos, a claimed nonexisting option in Latin America.

Other issues

Although not only related to the situation of Latin America, two more issues of critical importance are: (i) secrecy in gamete or embryo donation; and (ii) the widespread use of intracytoplasmic sperm injection (ICSI), although follow-up of ICSI children into adolescence and adulthood is not yet possible.

Secrecy and gamete donation

Secrecy is particularly important in ART. Despite the widespread use of ART, it is often associated with feelings of shame. Such feelings often lead the ART parents to avoid open discussion about their infertility treatments[xv]. Secrecy regarding the origins of the child in cases of gamete donation is controversial[xvi]. Zegers-Hochschild illustrates this point:

> In countries like Chile, less than 10% of couples with babies born as a result of ART are willing to express publicly their views on this form of technology which enabled them to become parents. Furthermore, none of the couples that have become parents with the assistance of donor gametes have told their children. It is evident from this sad reality that, in a way, these couples live a certain form of isolation and feel the need for keeping secrecy for the rest of their lives *(8)*.

Even more, this can be problematic for the child, not only because of the secrecy in the family, or because later on the child might find out the information that was withheld, but also because of the genetic difference between the child and the parents[xvii] *(14)*. This is particularly important in the cases of some genetic diseases, where knowledge of family history is important for preventive measures to be taken by the offspring *(14)*. Moreover, with the advancement

xv This kind of analysis is present from different backgrounds. Daniels, from New Zealand, points out that depriving the child of this information is considered as a protection. And he points out that "This desire to give protection is, I believe, based on adults' discomfort with infertility, the practice of semen, oocyte and embryo donation and their belief that there is something 'wrong', 'stigmatizing' or 'marginalizing' about being conceived in this way"*(13)*.

xvi There are different degrees of openness: the fact of a donor intervention shared with the child; the identity of the donor may be told to the child. The latter depends on the legislation or on the possibility of getting such information.

xvii Family therapy experience indicates that family relationships are damaged when they are based on deception, and that such deceptions lead to stress and anxiety. Psychological problems are also mentioned. In addition, finding out the truth about one's genetic parents later in life may erode the trust in parents (those who kept this information). It may come out in psychologically disturbing situations, around a crisis period: in the middle of a fight, after a divorce or the death of the "supposed" parent. Unexpected disclosure to the child in such contexts may be very damaging *(14)*.

of genetics and the popularization of DNA tests, secrecy about genetic origins seems an "old-fashioned" attitude that will need to be reconsidered in the near future.

Whether the child should have access to information about his or her origins and if so, at what point is one of the controversies still present in the world's ethical and legal arena[xviii]. There are still conflicting positions between those who oppose the rights and interests of the gamete donors to stay anonymous and the rights and interests of the offspring to know their origins.

The ethical debate can be framed in relation to the notion of "best interest of the child" *(14)*. One of the difficulties is the lack of agreement on how this concept should be interpreted and what it means. Those who argue in favour of secrecy of the information and anonymity of the provider defend the view that this information will be damaging to the identity of the child and to family relationships *(15,16)*. This is the position most endorsed by the medical communities around the world, including the one in Latin America[xix] *(17)*. Opponents of this practice contend that there is growing evidence to the contrary. They quote studies showing a number of offspring claiming that they have been psychosocially damaged by the secrets that have been part of their families *(14,18)*. Still other important issues such as the right to information about oneself is at stake. Findings from adoption studies are usually used to provide empirical support *(14)*. However, it should be recognized that this issue is not particular to Latin America. Here the consensus document strongly defends anonymity, which as mentioned earlier, is the main position of physicians *(7)*.

ICSI

ICSI has been widely used in ART. However, there are serious problems voiced by feminists *(19)*, ethicists and the scientific society. These concerns are raised by certain Latin American physicians too *(20)*.

In the Surveillance Study the following comment was made: "It has been suggested that ICSI should completely replace conventional IVF as even better pregnancy rates might be expected if normal spermatozoa are injected" *(5)*.

The same study reports: "ICSI is a more expensive technique, it is time-consuming, requires more equipment and extra skills, and in addition to the invasive nature of the procedure, it does not give better results" (*5)*.

These are not conclusive reasons for avoiding this technique. It might be less convenient, but the truly disquieting facts appear in the summary of the same report: "Follow-up studies of children show no increase over controls in congenital anomalies diagnosable at birth. There is a special concern about the reproductive potential of male offspring conceived using ICSI. There are unknown risks (e.g. cystic fibrosis and microdeletions in the Y chromosomes) but there may be others" *(5)*. And as Jacques Cohen points out: "Despite recent animal work, the ICSI technique was essentially developed through its clinical application in the human." Until now there are some studies with children up to 9 years of age and there are still concerns not only regarding those future adults but also multigenerational problems.

The Surveillance Study recommends that this information be disclosed and that informed consent of participants be obtained. However, it seems highly questionable that this procedure continues to be implemented without having clear results about the health and reproductive consequences of the procedure on the offspring in the medium- and long term. Its lack of certainty and its possibility of harm merits more caution and the continuation of well-designed, controlled experimentation. Here again, questions arise about the ethical permissibility to knowingly harm or risk the future offspring.

xviii For example, Sweden (1984), Austria (1992) and the State of Victoria in Australia (1995) have adopted legislation allowing the offspring access to this information; on the contrary, Norway (1987) and Spain (1996) have legislated for anonymity *(14)*.

xix As Daniels points out "…the emphasis on not sharing information was established by doctors. There were understandable reasons for this—DI being regarded as akin to adultery, the social attitudes surrounding sexuality and the legal status of the donors and offspring to mention the most obvious" *(17)*. In the case of Latin America, Reñaca's document explicitly says that donors' identity should not be disclosed, but leaves it to parental responsibility to disclose gamete donation. In Brazil, the CMF Resolution protects medical secrecy for the donor's identity.

Other sociological aspects

Gender issues

In a report for public discussion in the Parliament, Silvina Ramos, an Argentine sociologist, pointed out:

> In a social and cultural context where motherhood is seen as necessary for the personal realization of women, the finding of infertility is a devastating experience: the infertility experience is seen as a vital crisis.... Consider that infertility in our culture is a social stigma… *(21)*.

This quote perfectly depicts the importance of motherhood for women in Latin America.

A recent work of Luisa Barón reinforces the previous point. Baron analyses the psychological aspects of the female partner in cases of male infertility. The study looked at 100 couples with male infertility and under-35 women with no evident pathology who took part in the ICSI (89/100) and donor insemination (11/100) programme. What was interesting was the point about sharing emotions with others: 84% of the patients *did not talk to their husbands because they feared they would hurt their feelings;* 81% *did not talk with their families to protect their partner's image*. But, at the same time, the study also reports women's aggressive attitudes towards partners *(22)*.

This study reveals how hard it is to express these emotions, how women hide this problem and blame themselves. It also shows how "macho" attitudes exist not only in males but are reinforced by the women's attitudes. This also indirectly shows the social pressure of being a mother and the difficulties of undergoing these techniques.

The feminist voice

Criticism of these techniques is widely endorsed by feminists working in this field. Particularly important are these visions in Brazil and also, but with less force, in Argentina. Feminists are very suspect of these methods. Instead of focusing only on the impact they may have on the individual's private life, they provide a bigger picture of the problem. They focus on the political, social and economic consequences that these technologies have. One of the points they make is the lack of long-term experimentation and the quick application to patients.

> Changes in the technique are very quick and modifications of new protocols without enough time for the clear evaluation of long-term risks can be observed, which show the experimental nature of these methods *(19)*.

They fear long-term consequences or side-effects from these techniques in the woman or in the child. They have also criticized the use of women as a means of achieving pregnancy when the male is the cause of the problem, mainly because the procedures are more invasive of the woman's body. An example of this appears in Barón's study, previously presented. The female partners had no demonstrable cause of infertility; however, 89% underwent ICSI, a more invasive procedure for the woman than donor insemination.

Feminists have also expressed fear about what happens in the laboratories, especially in countries where there is no legal regulation, quality control or a licensing system for clinics *(6,19,23)*.

What is extremely interesting is that for almost "opposite reasons" the very progressive views of feminists, who endorse respect for women, support abortion, and fight for reproductive law enforcement, agree with the conservative views of the Catholic Church, which as a religious authority objects to all ART.

Possible accessibility

Upper- and middle-class infertile couples have access to ART through private centres, yet such treatment remains inaccessible to those who cannot afford the costs, despite the fact that they are in need. For example, it is this population that is most likely to have STD and RTI *(1,24)*. Daar also mentions that secondary infertility in Latin America is 40%.

While recognizing the complexities of a fair distribution of resources in a public system, solutions are always complex and controversial. There are two possible approaches from an ethical perspective regarding ART. The first one is a full coverage criterion, which represents a goal or ideal that should be tried to be applied. It endorses the right to health care, and argues for a comprehensive health care package including ART. It justifies these practices from the perspective of justice in health care. Society has an ethical obligation to ensure equitable access to health care for all *(25)*. This approach emphasizes

the importance of having a child, the suffering and tragic situation that an infertile couple faces. ART should be included as part of the individual's moral right to reproductive freedom. From this perspective, a negative right (i.e. the noninterference of the State in the reproductive choice of the couple or woman) is not enough. Such a right only allows the choice or freedom of wealthy people. This position argues that a positive right is needed. This positive right gives the possibility to all populations in need to have access to these techniques[xx] *(26)*. Arguments from a moral stance are the right of self-determination, contribution to the well-being of the woman and the couple, and equality *(25)*. This position defends a wide coverage of these services, although it need not be unlimited. It may recognize certain limits on reproductive freedom, as well as on provision (for example, the number of attempts of a certain technique).

However, serious consideration should be given to a second answer which recognizes the scarcity of resources in these countries. This approach does not emphasize so much the provision of these costly procedures, but rather the prevention of infertility to avoid further detriment to poor women who have already been excluded from treatment. This second approach points to the costs of these procedures, the lack of resources in the region and the percentage of sterility that arises from a lack of services in the area of reproductive health. In this sense, it stresses the need for fertility regulation including the use of contraceptives and prevention of unwanted pregnancies or unsafe, illegal abortions. It attacks some of the causes of infertility: poor prevention and lack of effective care in the area of STD; failure to provide support for behaviours that reduce the incidence of infertility-causing diseases (not only by reinforcing these services, but also by increasing global education).

Although this second approach does not argue for total coverage, and gives prevention priority over IVF or ICSI, it targets the needy. They are the ones who are most likely to have infertility problems due to lack of sex education, lack of contraceptive devices, and untreated STD. Within this framework, proposals may differ on how much ART should be covered, depending on need and available resources.

This second approach also means a commitment to a more comprehensive answer to the problem: considering ART in Latin America separately from basic reproductive health seems to be an irrational approach. If contraceptive services are denied, if STD are not adequately treated, if IVF embryos are worshipped, if abortions of genetically abnormal fetuses are forbidden, and the focus is only on providing ART, at least one important point is missed. ART cannot be maintained as a purely scientific objective technique that circumvents and tries to prevent the pressure certain ideologies pose without any connection to the major suffering of women, or as an endeavour that uses euphemisms to avoid pseudo-moral or religious criticisms. It is not healthy to maintain such a hypocritical stance. It is, rather, advisable to implement an integral and comprehensive approach to ART, including reproductive health of women and men as a priority. Such an approach could be achieved through a broader range of clinics covering not only ART but other reproductive health services, through capacity building, emphasis on gender issues, and also with the involvement of consumers.

Conclusions

Even if ART is widely practised throughout Latin America, it still presents many challenges. Some of the main difficulties are related to the particularly sensitive issue of the moral and ontological status of the embryo.

Although sociocultural and religious values shape the ways of providing ART in Latin America, it remains remarkable that all those involved do not engage in a dialogue: religious views are based on dogma or authority; physicians maintain their own scientific stance; feminist concerns are marginalized; patients and human rights advocates are unheeded. Our common history of authoritarianism and dictatorships may still be shaping this process. This lack of dialogue, thus, may need more years of democracy, tolerance and the real exercise of open deliberation before the incredibly difficult dilemmas ART poses in Latin America can be worked out.

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xx As I suggested at the beginning of the section, it should be acknowledged that positive rights in the reproductive area are controversial while reproductive freedom as a negative right is usually the accepted approach.

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Attitudes and cultural perspectives on infertility and its alleviation in the Middle East area

GAMAL I. SEROUR

Introduction

Medical ethics are based on the moral, religious and philosophical ideals, and principles of the society in which they are practised. It is therefore not surprising to find that what is ethical in one society might not be ethical in another. It is mandatory for practising physicians and critics of conduct to be aware of such backgrounds before they make their judgement on different medical practice decisions *(1)*. The ethical attitude of the individual, whether a patient or a treating physician, is often coloured by the attitude of the society, which reflects the interest of theologians, demographers, family planning administrators, physicians, policy-makers, sociologists, economists and legislators. Responsible policy-makers in the medical profession in each country have to decide on what is ethically acceptable in their own country, guided by international guidelines which should be tailored to suit their own society. Truly ethical conduct consists of personal searching for relevant values that lead to an ethically inspired decision.

In a part of the world such as the Middle East, where three major religions, namely Judaism, Christianity and Islam emerged, religion still means a lot and greatly influences behaviours, attitudes, practices and policy-making *(2)*. This explains why, though many other parts of the world witnessed the secularization of medical ethics and its loss of religious influence during the past two decades, the Middle East area did not experience these changes to a similar scale.

As infertility and its treatment are related to procreation and the preservation of humankind, the attitudes and cultural perspectives regarding it and its alleviation are extremely sensitive issues for the Middle Eastern people. Prevention of infertility and its relief are of particular significance in the Middle East area because a woman's social status, her dignity and self-esteem are closely related to her procreation potential in the family and in the society as a whole. Childbirth and rearing are regarded as family commitments and not just biological and social functions *(3)*.

There are different modalities available for the treatment of infertility in both the female and male partners, depending upon the cause of infertility. Some of these modalities have been practised for hundreds of years and were never of ethical concern; medical therapy, hormonal therapy, corrective and reconstructive surgery for female or male infertility are some of these modalities. The general ethical principles, which govern the medical practices in general, are also applicable to all these lines of treatment. All these modalities of treatment were not of a major ethical concern because they did not separate the bonding of the sexual act from the process of conception. Reproduction was only possible when both partners had sexual intercourse mostly within the frame of

marriage for months or years after undergoing these modalities of treatment *(4)*.

With the advent of assisted reproductive technologies (ART) since the birth of the first test-tube baby Louise Brown in the UK in 1978 *(5)*, it became possible to separate the bonding from the reproductive aspects of sex. ART, whether *in vivo* or *in vitro*, enabled women to conceive without having sex. This challenged the age-old ideas and provoked discussion. Furthermore, ART made it possible for the involvement of a third party in the process of reproduction, whether by providing an egg, a sperm, an embryo or a uterus. The use of ART also made it possible for scientists, for the first time, to be able to handle human gametes and human embryos in the laboratory.

There are several factors which affect the attitudes and cultural perspectives of members of the society towards ART. Some of the important factors will be dealt with in this text with special reference to the ethical issues and principles involved in providing and utilizing such technology for the treatment of infertility.

Religious and cultural factors

It was not surprising that all these new modalities of ART created and provoked debate, disagreement and controversy among all societies and religious sectors all over the world, including the Middle East area.

Infertility and its remedy is allowed and encouraged for all sectors in the Middle East. For Muslims and Christians it is essential, as it involves preservation of procreation and humankind *(6,7)*.

The Jewish attitude to infertility treatment is based on the fact that the first commandment from God to Adam was "Be fruitful and multiply". This is expressed in a Talmudic saying from the second century, which says "Any man who has no children is considered a dead man." This attitude arises from the Bible itself and refers to the words of Rachel, who was barren, "Give me children or else I die" *(8)*.

Though ART as an additional line of treatment of infertility would have been expected to be welcomed in the Middle East area, the instalment of ART centres in many countries in the area was delayed until the mid-1980s due to several factors, the most important of which were religious and cultural factors.

The handling of human gametes in the laboratory and the involvement of a third party in the process of conception created the most bizarre and inconsistent attitudes in many countries all over the world and particularly in the Middle East area *(6,7,9,10)*. It was not until the Fatwas from Al-Azhar in 1980 *(6)*, the Islamic Fikh Council in Mecca in 1984 *(10)*, and the Church of Alexandria in 1989 *(7)* that the procedure gained popularity and became widely acceptable to the medical profession, patients, religious leaders and policy-makers in most countries of the area. These bodies and organizations issued guidelines which were adopted by the National Medical Councils and Ministries of Health in the various countries and controlled the practices of ART centres.

The guidelines encouraged couples to seek infertility treatment including ART, as long as there was medical indication for its use. However, it made it clear that gametes of a third person should not be used and the fertilized eggs should be transferred to the uterus of the wife whose eggs they were within a valid marriage contract. No egg donation, sperm donation, embryo donation or surrogacy was allowed. There were little differences among these guidelines, mostly on the issue of surrogacy. While the Islamic Fikh Council of Mecca and the Church of Alexandria previously allowed surrogacy to be performed on the second wife of the same husband or a friend of the family, respectively, both councils soon after denied surrogacy and their guidelines became identical with those of Al-Azhar. The promulgation of these guidelines was just a response to the needs of the community, and the discussions and debates which arose in the area, as well as the problems anticipated with the practice of surrogacy with special reference to the cultural and religious background of the people in the area.

These guidelines and legislation played a major role in comforting patients and physicians. In the eighties, seeking infertility treatment was associated with secrecy, feelings of shame, doubt and even sometimes guilt, but in the nineties such feelings were replaced by openness about seeking infertility treatment and ART in particular. This was confirmed in several studies conducted at the infertility and *in vitro* fertilization (IVF) centres in Egypt *(11)*. The introduction of intracytoplasmic sperm injection (ICSI) for male infertility played a role in the change of attitudes of many couples to ART. This happened because men or husbands in the structure of the family in the area are usually the more influential members in decision-making. When the female factor was the cause of infertility, not uncommonly, husbands would be reluctant to seek medical advice early in marriage,

especially when the treatment was not conventional. Husbands were very reluctant to participate in or to agree to their wives undergoing ART for the treatment of female factor infertility. Also, the fact that polygamy is a possible solution for husbands to father children if the wife was the cause of infertility made many husbands reluctant to agree to undergo ART, especially in the rural areas. However, when ICSI was introduced it offered great hope to many infertile men, especially those with severe oligoasthenospermia or azoospermia. Husbands became very enthusiastic about ART and they took the initiative and encouraged their wives to undergo this new modality of treatment once it became available. The objections and resistance of male partners to undergo ART almost disappeared. This encouraged many couples in the area to make use of ART for the treatment of their infertility.

More recent debates and conferences addressed new practices of ART, such as selective fetal reduction, preimplantation genetic diagnosis (PGD), cryopreservation of human gametes and embryos, surrogacy and sex selection. Guidelines on these practices were published and made known to the public and helped patients and physicians to formulate their attitude towards these new practices *(12–17*). These guidelines were basically consistent with the previous guidelines. They also discussed and formulated guidelines on new practices in the field of ART in the light of new knowledge which became available. They were published by different authorities in the area, including the organization of Islamic Medicine in Kuwait, the International Islamic Center for Population Studies and Research, Al-Azhar University, Egypt and by other scholars and scientists in international journals and textbooks.

Cost

Another factor which hindered early establishment of ART centres in many countries of the area was the high cost of such centres, as many of these countries have limited health resources. In the eighties, the establishment of an ART centre with accepted international standards would cost about US$ 400 000–US$ 500 000 *(18)*. This relatively high cost limited the instalment of such centres in governmental institutions. Most of the centres in the majority of the countries in the area are in the private sector. The cost to the patient is still quite high in relation to the average family income. Indeed, this is a particularly distressing factor for both the patient and the physician, as ART is not supported by the health authorities in many countries in the area. Many organizations, companies and government authorities cover medical expenses of their employees but not those expenses related to infertility treatment. Policy-makers do not consider infertility to be a disease. Consequently, infertile patients have had to cover all expenses for their infertility treatment in most countries in the area. In one study in Egypt, it was shown that only 63% of those patients who needed ART seen at a private infertility centre could afford to have the procedure done. Only 40% of those who did not get pregnant after the first attempt at ART could afford to have the procedure repeated *(19)*.

The ethical principle of justice implies that all people should have equitable access to health care services. However, because of the rapidly increasing cost of medical technology and advanced health care services in different fields of medicine, the question of resource allocation becomes a pressing and sometimes a decisive one. If the country has adequate resources and can provide basic health services as well as advanced health care services, there is no problem. In countries where resources are limited and basic health services are lacking, implementation of advanced health care services, though it could benefit a certain sector of the population, could be unjust, because it deprives a major sector of the population of basic health services. In this context, there is a collision between the principles of justice and equity *(20*).

The principle of liberty guarantees a right to freedom of action, including the right to have access to health care services such as ART. However, in many countries, resources are scarce and many needy patients cannot exercise such a right of freedom of action. By contrast, rich members of the society not uncommonly have access either to private centres in the country or to the desired services in one of the developed countries. This certainly violates the principle of justice, which requires that everyone has equitable access to necessary goods and services.

A mechanism should be found in these countries to allow the needy to have access to such expensive centres. A suggested solution for such situations may be donations to provide support for the treatment of the poor and needy at these centres. The donations may be provided by those rich members of society who have had successful treatment in these centres,

by pharmaceutical companies which profit from these centres, and possibly by funding from research projects conducted in these centres *(20)*. Provision of services at governmental and university institutions, establishment of charitable projects and appropriate collaboration with drug companies and other commercial enterprises committed to causes of social justice are some alternative mechanisms. Arrangements might also be considered by which private ART centres, perhaps as a condition of state licensure, would be required to offer a proportion of their services at no cost or very low cost to needy recipients. For instance, governmental or other subsidies might be paid to private ART centres where public facilities are few, or the fees charged by private centres to those with adequate means to pay might include a surcharge to fund services for patients unable to pay the full, or any, fees.

Accessibility of ART

Concerns of equitable access to ART go beyond economic equity. The professional skills and sophisticated equipment required to establish an ART centre has resulted in only a small number of such facilities in most countries and these are largely concentrated in major private centres in major cities. Residents of rural areas often find services they can afford geographically inaccessible. The challenge of taking ART services to rural areas appears almost insuperable. This is particularly the case because undergoing ART treatment entails several visits by the patient to the ART centre, which may extend over a four- to six-week period for each treatment cycle. More should be done to prevent infertility in rural areas. In countries where more than 70% of all births are attended by traditional birth attendants (*Dayas*), aseptic precautions are not always observed, resulting in a high incidence of pelvic infections and secondary infertility. Furthermore, harmful practices, whether by the patients or the doctors, have been shown to result in a high incidence of iatrogenic infertility *(21)*. Improvement in the standard of health care services and health education, especially in the rural areas, would prevent a substantial number of cases of infertility. Clinical care of treatable infertility should be promoted, but equitable provision of ART to overcome irreversible fertility among rural and remote populations present a continuing challenge *(17)*. Until the early nineties, only a few ART centres existed in a few countries of the area. Patients had to travel from one country to another or, if they could afford the expense, they had to travel to one of the European countries or to the USA. Even in the same country, couples sometimes had to travel long distances to the capital of the country to undergo the procedure and had to stay away from home for some time. This created unacceptability of the procedure for many couples, even though they could afford to have the procedure done. However, today ART centres are available in almost all countries of the region and in many countries there is more than one centre, depending upon the size of the country and its population. Such accessibility played a role in changing the attitude of many couples in these countries regarding the procedure.

Techniques and complications

When the successful outcome of ART was first published in 1978, the procedure involved laparoscopic retrieval of the oocyte in a natural cycle *(5)*. Most of the patients at that time had had several laparotomies which made oocyte retrieval a difficult procedure, needed a lot of experience and was a procedure associated with risks to the patients. Also, both the patients and the treating teams had to suffer from long, tedious monitoring and working hours. These problems, coupled with the relatively low live-birth rate following the procedure, had a negative effect on the attitude of patients towards the procedure and several medical associations and obstetrics and gynaecology societies in many countries in the area questioned the whole procedure and its cost-effectiveness. WHO called an expert scientific group meeting on ART for evaluation of the whole issue *(22)*. However, improvements in ovarian stimulation protocols, simplification of the technique and monitoring of the procedure, and the introduction of cryopreservation significantly improved the results *(23)*. ART is today considered to be an effective management of infertility and has an acceptable incidence of complications. Complications rarely endanger the life of the patient *(24,25)*. This had a positive effect on the attitudes of the patients towards ART. However, when such treatment is offered, the indications should be definitive. Patients should be monitored properly and measures should be taken to minimize the incidence of complications *(24,25)*.

Acceptable guidelines and legislations on ART in the Middle East area

Today ART guidelines for Christians and Muslims in the area of the Middle East are almost the same. A few differences existed in the eighties but with the final modifications of these guidelines by various religious authorities they became almost identical *(2)*. However, Jewish guidelines and legislations are different.

There is near unanimity in that the use of semen from the husband for artitificial insemination by husband (AIH) is permissible if no other method is possible for the wife to become pregnant. Masturbation should be avoided if at all possible, and coitus interruptus or the use of a condom seem to be the preferred methods for semen collection.

Artificial donor insemination (AID) is not accepted by most rabbinical authorities. All Jewish legal experts agree that AID using the semen of a Jewish donor is forbidden. Some rabbinical authorities permit AID when the donor is a non-Jew. The attitude favoured by Jewish religious authorities with regard to IVF and embryo transfer (ET) is based on the commandment of procreation in the Bible. Many rabbinical authorities permit the collection of semen either by coitus interruptus or in a perforated condom. In the case of ovum donation or embryo donation, there is a divisible partnership–ownership of the egg and the environment in which the embryo is conceived. Jewish law states that the child is related to the one who finished its formation; the one who gave birth. The religious law decrees that only the offspring of a Jewish mother may be regarded as a Jew. Freezing of sperm and pre-embryo is permitted in Judaism only when all other measures have been taken to ensure that the father's identity will not be lost. The Jewish religion does not forbid the practice of surrogacy *(8)*.

In the field of embryo research, the Biblical and Talmudic law holds that the full status of a human being is not present at the moment of fertilization, but is acquired after a period of postimplantation development. An important feature of Jewish thinking in this area is that embryos outside the womb, analogous to gametes, have no legal status unless parental intent gives them life potential by implantation and pregnancy. An embryo made for IVF treatment and maintained *in vitro* without the potential for implantation could therefore be donated and used for therapeutic research. This would be in line with life-saving duty, which is strong in Judaism *(26)*.

For Muslims and Christians, since marriage is a contract between the wife and husband during the span of their marriage, no third party intrudes into the marital functions of sex and procreation. A third party is not acceptable, whether he or she is providing a sperm, an egg, an embryo or a uterus *(6,7,12,13,19)*.

If the marriage contract has come to an end because of divorce, or death of the husband, artificial reproduction cannot be performed on the female partner even using sperm cells from the former husband.

At an International Workshop on "Ethical Implications of the Use of ART for Treatment of Infertility Update", organized by the International Islamic Center for Population Studies and Research, Al-Azhar University in November 2000, there was a vigorous, principled debate on whether a couple's preserved embryo could properly be implanted in a wife after her husband's death. The strict view was that marriage ends at death, and procuring pregnancy in an unmarried woman is forbidden by religious laws; for instance, on children's rights to be reared by two parents, and on inheritance. After due time, the widow might remarry, but could not then bear a child that was not her new husband's. An opposing view, advanced as reflecting both Islamic compassion and women's interests as widows, was that a woman left alone through early widowhood would be well and tolerably served by bearing her deceased husband's child, through her enjoying companionship, discharge of religious duties of childbearing, and later support. Unable itself to resolve the conflicting views, the workshop recommended that the question be forwarded to the Islamic Research Council regarding whether an ART centre could agree to a widow's request for thawing and implantation of an embryo created while her husband was alive *(17)*.

The Grand Mufti of Egypt stated that permission had been given once for embryo implantation after the husband's death, based on the circumstances of the particular case (personal communication). The Grand Mufti made it very clear that permission was given in that particular case for several reasons combined together. The husband had agreed to the procedure of ART including ET when he voluntarily came to the IVF and ET centre and delivered his semen sample. He was informed by the IVF centre that his sperms had successfully fertilized the oocytes from his wife. He informed the centre that he would bring his wife on the day of ET to have the procedure done. The husband's parents witnessed all these arrangements

and reported to the Grand Mufti that the man died in an accident on his way to the centre with his wife to have the procedure completed. All members of the family strongly supported the transfer of the cryo-preserved embryos and all guaranteed that there would be no heritage problems in the family. Although permission for implantation was granted in this particular case, it should not be generalized, and each case should be considered on its own merits *(27)*.

Surrogate motherhood

Although it was allowed in the past *(7,10)*, the present status in the region is that surrogacy is forbidden. Very recently an intensive debate started in Egypt on the issue of surrogacy. Many scholars, lawyers and physicians from Egypt and the other Arab countries participated in this debate. For the first time, supporters of surrogacy declared their views on this issue and strongly defended it. They defended it on the basis of response to a patient's demand and to satisfy the urge for motherhood in a patient who has had her uterus previously removed because of multiple fibroids. The Islamic Research Council held a special meeting in April 2001 to discuss this issue. The Council published its statement which condemned surrogacy on the basis of third party intrusion into procreation. However, there was no unanimous agreement from its members on the statement *(28)*.

Multifetal pregnancy reduction

Multifetal pregnancy reduction is only allowed if the prospect of carrying the pregnancy to viability is very small. It is also allowed if the life or health of the mother is in jeopardy *(3,13,29)*. The couple should be carefully counselled on this issue and the final decision should be left to the couple. The physician should obtain the voluntary informed consent of the couple before performing multifetal pregnancy reduction for high-order multiple pregnancy. The high-order multiple pregnancy is reduced to two. The procedure is a must for quadruplets and higher-order multiples. In the case of triplets, the issue is not very clear and the choice of the couple is the determining factor in such situations *(30)*.

Sex selection

Preimplantation genetic diagnosis for sex selection for the prevention of sex-linked diseases is allowed. However, sex selection for the mere choice of sex is generally not acceptable *(20)*. Some had recently advocated the use of PGD for social reasons and restoration of sex balance in the family *(31)*. However, this policy is not supported by either Muslim or Christian scholars in the region.

The Cairo Workshop of 2000 recognized the importance of PGD, but was guarded about its use on nonmedical grounds, such as sex selection or family balancing, considering that each case should be treated on its own merits. The medical application of PGD was seen as marking progress in the field of ART, and as a welcome alternative to prenatal diagnosis that results in abortion. Muslims have not accepted the opinion of the Roman Catholic Church adopted in 1969 that human life be considered to begin at conception, but adhere to the view that human life requiring protection commences some weeks, perhaps two weeks or so, from conception and uterine implantation. Accordingly, decisions not to attempt implantation of embryos produced *in vitro* on grounds that they show serious chromosomal or genetic anomalies, such as aneuploidy, cystic fibrosis, muscular dystrophy or haemophilia, are acceptable. PGD is encouraged, where feasible, as an option to avoid clinical pregnancy terminations of couples at exceptionally high risk *(32)*.

More contentious is nonmedical PGD, particularly for purposes of sex selection. Sex selection technologies have been condemned on the ground that their application is to discriminate against female embryos and fetuses, thereby perpetuating prejudice against the girl child *(33)* and social devaluation of women. For instance, the Convention on Human Rights and Biomedicine of the Council of Europe provides that "The use of techniques of medically assisted procreation shall not be allowed for the purpose of choosing a future child's sex, except where serious hereditary sex-related disease is to be avoided" *(34)*. The Workshop endorsed the condemnation of such discrimination and devaluation, but considered that universal prohibition would itself risk prejudice to women in many present societies, especially while births of sons remain central to women's well-being. Family balancing was considered acceptable, for instance, where a wife had borne three or four daughters and it was in her and her family's best

interests that another pregnancy should be her last. Employing PGD to ensure the birth of a son might then be approved, to satisfy a sense of religious or family obligation and to save the woman from increasingly risk-laden pregnancies. The Workshop considered that an application for PGD for sex selection should be disfavoured in principle, but resolved on its particular merits *(32)*.

Pregnancy in the postmenopausal period appeals to egalitarians as it is just for old women to have children since older men have always been able to father children. However, the issue is not that simple. Men are not directly involved in the process of pregnancy, childbirth and, to a great extent, in the process of mothering the newly born child, at least in the first few months of life. These reproductive processes may carry an increased maternal risk to mothers, especially if pregnancy occurs in the late fifties or sixties *(3)*. Also, postmenopausal pregnancy may increase the chances of the children becoming orphans at a young age. Postmenopausal pregnancy, at least at present, involves egg donation, and a possible increased maternal risk. Pregnancy in the postmenopause using the wife's frozen–thawed oocytes or embryos overcome the problems of mixing genes *(3)*.

The Cairo Workshop of 2000 was able to agree on the recommendation of postmenopausal pregnancy when it is necessary. Its agreement marked a point of development, since earlier the possibility of post-menopausal pregnancy was considered dependent on ovum donation, which was disapproved of in principle at the 1991 Conference *(13)*. The 1997 Workshop similarly found that "pregnancy […] after menopause is extremely dangerous for both mother and child, also involving a third party and, accordingly, is unacceptable in the Muslim world" *(15)*. Neither the 1991 nor the 1997 meetings took account of the prospect of cryopreservation and *in vitro* growth, if necessary, of a woman's own ovum for IVF and postmenopausal implantation. The Cairo Workshop in 2000 considered this as a still remote but feasible prospect, and shaped its recommendation accordingly *(17)*.

The Workshop considered the special care necessary for the safe induction and completion of pregnancy in a woman who was of advanced, or beyond normal, childbearing years, and of the easier case where premature menopause affects a woman who would otherwise be of suitable maternal age. The Workshop took account of children's needs of parents likely to survive at least into their mid-adolescence. It accordingly recommended that research efforts be concentrated on the prevention of premature menopause and that attempts at postmenopausal pregnancy be permissible in exceptional cases justified by the maintenance of integrity of a child's genetic parentage, the pressing nature of the circumstances, the relative safety to mother and child, and parental capacity to discharge childbearing responsibilities *(17)*.

Gene therapy

Gene therapy includes somatic cell gene therapy, germ-line gene therapy, enhancement genetic engineering (whether somatic cell enhancement or germ-line enhancement), and eugenic genetic engineering. Genetic manipulation is desirable to remedy genetic defects *(3,16)*. Serious ethical questions begin to arise in borderline cases, when the aim of genetic manipulation shifts from therapy to the creation of new human types. Though there was a general approval of somatic cell gene therapy, ethics has not been able to solve the dilemmas of germ-line gene therapy *(3,16,20)*.

Allied with stem cell research is the prospect of gene therapy *(35)*. Progress in somatic cell gene therapy, which alters the genes only of a treated patient, has suffered recent setbacks. Germ-line gene therapy, which would affect all future generations of a patient's offspring, remains little short of being universally condemned and prohibited. The Cairo Workshop in 2000 recognized that genetic alteration of embryos before their cells have reached differentiation—that is while they are still totipotent—would constitute germline manipulation. The Workshop found that little would be added to reiterate prevailing condemnation, and offered less of a recommendation than an observation. The Workshop stated that gene therapy is a developing area that may be used with ART in the future. It is critical that its use be clearly beneficial, focused on alleviating human suffering *(17)*.

The focus on therapeutic applications would exclude purely cosmetic uses and goals of enhancement of nonpathological conditions. Alleviation of genetic diseases and pathological conditions alone would exclude such applications as to make people who would be within the normal range of physique, capacity, and aptitude, taller, stronger, more likely to

achieve athletic success or to be more intelligent or artistically sensitive or gifted. The background concept was that gene therapy might be legitimate, not to promote advantage or privilege, but to redeem genetically or otherwise physiologically inherited disadvantage *(17)*. Though gene therapy and handling human embryos at the early phase of embryonic development would be acceptable for Muslims and Jews until two weeks or more after fertilization, the issue is rather complicated for the Christian sector of the area.

Some branches of Christian thought (in the Protestant tradition) regard full human status as something which is acquired gradually, and which might therefore not be present in the early embryo. Protestant theology, however, is very diverse, and it is more difficult to find a single source of authority on this issue to which reference might be made. It is, in fact, part of the Protestant ethos that moral questions are determined by the individual conscience, and there is, therefore, room for a variety of stances on this point. Protestant thought, therefore, may accept that this is an issue on which Christians may have very differing views, with these differing views being compatible with Christian beliefs. However, one must not forget that there are many fundamentalist Protestants, whose views are as strict as those of the Catholic Church on issues in human reproduction.

The most strongly argued opposition to the use of embryos for therapeutic or research purposes is to be found within the Roman Catholic tradition. In the Catholic view, a human being comes into existence at the time of fertilization, and the embryo is therefore considered as a human individual having the right to its own life. An individual embryo should therefore be given the opportunity to develop into a mature human being. It is an implication of this position that it is necessary to strictly control the fertilization of ova *in vitro*, and it is impermissible to use supernumerary embryos for therapeutic purposes. This is because the life of that embryo is sacred and it cannot be ended by any human agency.

This brief statement of some of the major religious positions on the use of embryos reveals a stark contrast between religious notions of the status of the embryo. If the question is defined as the embryo's moral status, the various religious traditions are prone to take opposite positions. If it is defined in a broader perspective, then there may be room for some agreement *(26)*.

Uterine transplant

The Cairo Workshop in 2000 recommended that research in uterine transplantation in animals could go forward. However, if and when it should prove to be safe and effective for possible use in humans, within approved transplantation guidelines, further consideration of the use of this procedure should be referred to the Islamic Research Council for discussion. Other organizations and authorities, such as the Church of Alexandria, may have to do the same. An issue would be whether such transplantation would violate the prohibition against third-party involvement in a married couple's reproduction. Involvement might not be as personal as gamete donation or surrogate motherhood, but may not simply be analogous to, for instance, anonymous kidney donation that enables a person to survive and become a parent, due to the influence the uterine environment may have on the child's biological development and personality *(17)*.

Less novel but worthy of serious attention are conditioning issues of donors' competence, free and informed consent to total hysterectomy, their tissue compatibility with potential recipients, and their childbearing or postmenopausal status. A menopausal uterus can function normally under hormonal stimulation and, once transplanted into a recipient without rejection, could receive an ovum released by the recipient and fertilized by her husband in the normal way. A mother might thereby donate a uterus to her daughter to allow birth of her grandchild. The Workshop was aware of an apparently abusive and disastrous pioneering attempt at uterine transplantation in Saudi Arabia *(36)*, illustrating the potential for exploitation of donors, and also considered recipients' indications for the procedure. These would include congenital absence of the uterus, extreme uterine hypoplasia, previous medically compelled hysterectomy, destruction of the endometrium by infection such as tuberculosis, and excessive curettage following dilatation and curettage.

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Social and ethical aspects of assisted conception in anglophone sub-Saharan Africa

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Scope

This paper focuses on the anglophone countries of sub-Saharan Africa. The sources of information are the Gambia, Ghana, Kenya, Nigeria, Sierra Leone and Zimbabwe. Information has been gathered from visits to these countries, correspondence with colleagues in these countries, from the media, publications, and discussions with informed persons.

Sociocultural context

In this subregion there are multiple ethnic groups and languages. In Nigeria alone, there are over 50 ethnic groups and languages. However, in the subregion, English is the main language of official communication and the countries share a colonial past. There are distinct cultures and practices that affect the perception of fertility and inheritance. In matrilineal societies such as among the Akan of Ghana, the intense pressure to have male children is not as obvious as in patrilineal societies, since inheritance and child-ownership is on the mother's or sister's side. In patrilineal societies, the vast majority of West Africa including the Ga and the Ewe of Ghana and virtually all of Nigeria *(1),* it is particularly important to have male children. In these patrilineal cultures the male offspring inherits the estate and maintains the family name. In sub-Saharan Africa, polygamy is still widely practised. Several countries in the subregion in West, Central, East and Southern Africa have significant Muslim populations in which a man is allowed by his religion to have up to four wives. The traditional African societies also allow polygamy, and the people have practised polygamy *(1).* This combination of antecedents has produced an environment in which polygamy is common and is tolerated, if not encouraged, in modern times. Polygamy produces competition between wives for mating rights, pregnancy, childbearing and great pressure to produce male heirs in patrilineal societies. Polygamy also results in an increase in the average number of children per man and therefore the average size of the family unit, compared with a nuclear family. Concerns about child survival serve as a push factor for the desire for more pregnancies and children. In this sociocultural context, therefore, it is not surprising that the accepted international definition of infertility may not be satisfactory. A multiparous woman in a poly-gamous family may consider herself a prime candidate for fertility enhancing therapy because she has no male children or may seek treatment after less than 12 months of unprotected sexual intercourse.

Causes of infertility

Several of the social and cultural practices in the subregion contribute to infertility. Genital scarification or mutilation, and vaginal incisions offer portals of entry for genital infections, which can, and do, damage the fallopian tubes. Child marriages and pregnancies, which often lead to complications of labour and the puerperium, as well as labour and delivery in unhygienic circumstances, cause secondary infertility. Unsafe abortions in adolescents and young adults are a major cause of secondary infertility in Africa. The rotation of mating dates between wives in a polygamous marriage easily leads to intercourse at nonfertile times as well as infrequent individual female exposure to sexual intercourse, which may be a cause of infertility *(2)*. In sub-Saharan Africa, the male usually controls the finances and so determines where his spouse(s) go for medical treatment.

It is also quite common for the male partner to confuse sexual potency with normal male fertility, which can result in him refusing further investigation of himself or his wife if the practitioner insists on him being properly investigated. A man who contracts a sexually transmitted infection (STI) can easily infect all his wives and partners. In some cases he may ensure that he and his favourite partners get properly treated but abandon his less favoured partners whom he then refuses to have sexual intercourse with. Such neglected female partners may have inappropriate treatment just because they do not know any better or they cannot afford appropriate therapy.

The mother-in-law and peers play an important role in shaping health-seeking behaviour. They may direct the infertile couple to a practitioner they know or to one of the many fringe churches *(3)* or traditional medicine practitioners. In traditional African society, a presumptive diagnosis of male or female infertility can be made by giving the man a second wife who is younger than his first wife. If she fails to be pregnant, the man is presumed to be the cause of infertility. The same thought process allows a diagnosis to be made if the woman in an infertile marriage marries another man who already has children and becomes pregnant or fails to become pregnant. A distinction is made between potency and fertility and, in many ethnic groups, the words for impotence and failure to make a partner pregnant are distinct and different. There is, however, no attempt to relate failure to achieve pregnancy to the content of the semen. In most cultures of the subregion, amenorrhoea which is not due to pregnancy or lactation, is seen as being unnatural and is viewed with seriousness as is pregnancy that is prolonged beyond 10 months.

Treatment of infertility

The older relatives or peers are usually the first ports of call for the couple with infertility. It is therefore obvious that the level of reproductive health information available to peers and relatives affects the health-seeking behaviour of couples. Traditional African society recognizes the role of native doctors, herbalists and spiritualists in everyday matters including the diagnosis of the causes and management of infertility. These cultures also accept a role for traditional gods and goddesses. In most parts of South Western Nigeria and among some ethnic groups along the West Coast of Africa, the sea goddess, Olokun, is believed to be the giver of fertility, wealth and the good things of life. The role of native doctors, herbalists, spiritualists and the new churches may be said to be that of fertility counsellors and psychotherapists in Western medical interpretation *(3)*.

In the traditional society of many ethnic groups in the subregion, male infertility was treated through surrogate fatherhood and, within the family, by natural insemination. When the family is convinced that their son is infertile, his brother or another very close relation is allowed to have intercourse with the wife to bear children for the infertile brother/relation. Before such a relationship starts, the wife would have been counselled by her husband and/or a close relation such as an elderly uncle/father or mother-in-law. The matter is never openly discussed outside the family and the biological father of the children will not claim the children. In modern scientific medicine this is akin to donor semen insemination using a known related donor. The rationale for the choice is to ensure that the offspring is from the man's family gene pool. In traditional society it was easy to ensure that information did not flow beyond the village boundary. In some cultures in this subregion also, an infertile woman would "marry" a younger wife to bear children for her husband. This practice still occurs among some Ibos of Eastern Nigeria and among some Edos of South Western Nigeria *(4)*. Quite often this younger wife is a relation—sometimes a cousin or sister—of the older infertile wife. The older wife and the younger wife, their husband and their children all

live together. In some cases the younger wife lives in the village while her older children live with their father and the older wife and are brought up as the children of the older wife.

In virtually all cultures in this region it is accepted, and it is a common saying, that it is only a woman that knows the father of her child(ren). However, if she has had multiple sexual partners, she would not know who the father is. The traditional Edos of Nigeria attempt to solve this riddle of paternity by deciding that if a woman has multiple partners in a pregnancy cycle, the man who had intercourse first with the woman in the cycle in which she became pregnant is the father of that child *(4)*. Yet, the concept of any pregnancy during a marriage belonging to a woman's husband is strong in many cultures. Among some Ibos of Eastern Nigeria, any child conceived by a woman while still married to a man, even if separated, is recognized as the child of the man. This is even extended to any children borne by the woman after the man's death if the woman's new partner has not returned the dowry paid by the dead man. It is therefore clear that the approaches to fertility, infertility and paternity within the cultures of sub-Saharan Africa are fairly versatile. Traditional therapies for female infertility include: manual transabdominal massage of the uterine fundus—this technique is common among the Ijaws of the Niger Delta area of Nigeria; transvaginal repositioning of the uterus for presumed retroversion; and application of herbs to the vagina. All these therapies are supposed to aid the outflow of impurities from the uterus. Sacrifices of poultry, goats or rams may also be performed to the gods/goddesses that may be annoyed or to invite their favour so that pregnancy may occur. Gifts or sacrifices may also be made to ancestral spirits while followers of various religions, including Christians *(3)*, often fast, apply anointed oil to their bodies or drink what they describe as "holy water" to hasten pregnancy. This is the background in which modern assisted conception has been brought to sub-Saharan Africa.

Modern assisted conception

Natural, within-the-family insemination is a familiar process in some cultures. Therefore, artificial insemination is not that strange, but the idea of a third party, unknown donor of sperms is not welcome because of fears of heredity, disease and mental illness in the donor. On the other hand, educated couples prefer unknown donors to family or related donors. Among women, the concept of donor eggs and embryos is very new, but indications in those requiring it is that they accept it when there is no choice and many such women prefer a related donor. Is the woman more tolerant of the whole process of donor gametes because she carries the embryo while the man is an outsider even when he has donated the semen? The vast majority of couples keep insemination to themselves and do not tell people about it. The main concerns are about the appearance of the baby, the mental health of the baby and the absence of antisocial behaviour such as stealing and drunkenness. *In vitro* fertilization (IVF) is accepted when there is no other choice of method and is looked upon as being more acceptable than donor insemination or oocyte donation. Surrogate motherhood is not spoken about—the main fear is the risk of the carrier not giving up the baby or developing a relationship with the sperm donor.

The past two decades of the 20th century have seen the interplay of traditional and modern concepts regarding infertility in Africa. Evidence supports the conclusion that traditional African society recognized that infertility could be due to female or male factors and that the society had its own remedies, however scientifically imperfect, for infertility. The advent of western education, urbanization and the rural to urban migration has had an impact on the perception and management of infertility in Africa.

Ethical aspects of assisted reproductive technologies (ART)

The ethics of ART in Africa is influenced by the cultures of the peoples and the status of men, women and children in the societies.

Traditional family relationships

The child is valued as the embodiment of the family genes into the future. He is trained accordingly and part of this process involves the child helping with family activities and work at home, or in the farm in rural societies. As the child grows into adulthood it is expected to carry the adult responsibilities of his gender and family. The child is therefore a valued member of the family. The woman's role relates to the vital role of ensuring continuation of the family, tribe, and race through procreation *(1)*. The woman brings

up the children, manages family domestic matters as well as farming in the rural areas, and trading in the city. She is expected to be faithful and obedient. As she gets older, bears children, and enters the menopause, she is accorded increased societal value. Now free from menstruation, the menopausal woman in many African societies can participate in family and ethnic activities with almost the same status as male elders. In matrilineal societies, the older female is usually given more status and respect than her male relations in family matters, although the civil society is still male dominated. These family relationships are important in understanding how some ethical issues are considered and how decisions on them may be reached.

Consent

Consent is a crucial part of deciding the ethical status of any procedure such as assisted conception. Consent, or lack of it, also has vital legal implications. The prevailing body of universal ethics requires that informed consent be obtained before procedures are carried out. Where an underage person requires ART, informed consent would have to be obtained from the junior's parent(s) or guardian(s). This was the undisputed ethical and legal position until about 20 years ago. In the last decade of the 20th century, some societies—particularly in Western Europe and North America—placed substantial premium on the ability of mature juniors, who are under the legal age of consent, to consent to procedures such as family planning and related procedures. In sub-Saharan Africa, the under-age person is deemed to be firmly under the care of her parents, guardians, adult siblings or close relations who have to give consent to any procedure. If ART were to be necessary in juniors, therefore, these adults named above would be the ones to give consent!

The legal right of the adult female to give consent for procedures, including ART, is recognized. If she is married, the adult female would be encouraged, and be expected to obtain her partner's consent, which would be mandatory if his gametes are required for insemination or other ART procedures. In the African context, it would be disastrous for a man to find out that his partner had undergone ART procedures without his knowledge and consent. A few women would like to undergo procedures without telling their husbands. These cases are usually due to stresses existing in the marital relationships, or between the woman and her in-laws. We have been studying some of these matters in our fertility practice and some preliminary results of our surveys in Lagos may be instructive. Of 150 couples (male/female partners) 100% chose not to tell anybody about their treatment if it was donor insemination, 70% would tell nobody of it, irrespective of whether the semen used was husband or donor semen; 30% of the women would have preferred their male spouses not to be told they were receiving donor semen; while 80% of men whose wives required donor insemination would have preferred this not to be revealed to their female partner. In only three of 267 consecutive pregnancies by donor insemination did the patients reveal this fact to anybody. Among the first 15 IVF babies in Lagos, only six parents were happy to reveal this fact to other persons once the babies were born. Of 30 couples who rejected donor insemination as therapy, 17 accepted insemination with mixed (donor/husband) semen. These preliminary urban-based experiences indicate an evolution in the attitude of urban Africans to ART. With a growing population of educated females and males, there should be more openness in the discussion and acceptance of ART *(5)*.

The status of babies born from ART

Since most couples who conceive with ART do not announce this fact, there is no reason to expect such babies to be viewed differently from those conceived naturally. The fact that the mothers are seen to be pregnant before delivery further consolidates the position of the offspring. African parents of children conceived with the use of donor semen do not declare this and do not adopt the offspring. The offspring is registered and brought up as a biological, normal offspring in every way. Were this not so, there would be a problem if a male heir in a patrilineal family were to be from donated sperms, as his adversaries could argue that he was not a genetic and rightful heir. Because of this potential legal and sociocultural problem, most practitioners of ART in Nigeria are reluctant to keep long-term records of their donors. Without records it would be impossible, therefore, to establish the aforementioned case, although DNA analysis would provide enough evidence to ascertain or disprove paternity. This matter should also be viewed in the context of the traditional society in which some ethnic groups accept that a son should inherit and procreate with the young widow of his father, and that a fertile brother can save the face of

the family by impregnating the wife of his infertile brother. It would be interesting to observe how the shrinking world with information technology and more education will affect the social, cultural, and ethical aspects of infertility and family life in sub-Saharan Africa.

ART regulation

In all the states reviewed there is no state regulation of ART. This must be because the governments of the subregion think that ART is not being practised to any significant extent yet. This status of benign neglect suits those who prefer the field of ART to be kept out of the public arena. However, with frequent media reports of successes in the field of ART in the sub-region, that position is likely to change in the not-distant future.

Conclusion

Assisted conception is new to Africa. Certain forms of it, such as artificial insemination , were already being practised in traditional societies. The first IVF baby in West Africa was delivered at the Lagos University Teaching Hospital in 1989. By the year 2001, there are now assisted conception centres in Abuja, Dakar, Douala, Harare, Lagos, Lome, Tema and Yaoundé, which carry out human IVF, while the technique of artificial insemination is even more widespread in Africa. In spite of this, the techniques are still not available widely enough, considering the population that requires the procedures. Virtually all the countries that now have ART centres have no legislation regulating the procedure. There is a need for encouragement of South-to-South collaboration for the evolution of region-specific adaptations which may take into consideration the diverse circumstances, including the cultures of sub-Saharan Africa. International agencies such as WHO need to enable these processes to take place.

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ART and African sociocultural practices: worldview, belief and value systems with particular reference to francophone Africa

GODFREY B. TANGWA

Introduction

Although it is often controversial or misleading to make generalizations about Africa, one of the safest and less controversial of such generalizations is that human procreation is highly valued in African cultures. This should not, of course, be interpreted to mean that there are parts of the world or cultures where procreation is not valued. Procreation is a value for human beings in general and within all human cultures. But the ways and manner in which this value is manifested and expressed differs from place to place, from culture to culture, and these differences can be used as a rough gauge of the extent or magnitude to which the value is affirmed or upheld against competing values. There is no part of Africa where children are not greatly valued and where, as a consequence, large families do not exist or polygamy is not practised.

Children are so highly valued in Africa that procreation is everywhere considered the main purpose of marriage and the main cause of, if not justification for, polygamy and other forms of marriage which may be considered more or less strange from the perspective of other cultures. Conversely, childlessness remains the main cause of divorce, as a childless marriage is considered to be equivalent to no marriage at all. The idea of "illegitimate child" or "bastard" is one that could make no sense and had no application in traditional Africa because of the very high value placed on children. In Cameroon, it is very common for the parents of a girl who is approaching her thirties as a childless spinster to urge her to try and get a child by all means "before it becomes too late". If afterwards she finds a husband, her parents are usually only too happy to keep her premarital child, who often, in any case, bears the father's name.

Some consequences of the great love for the offspring

All population control policies, family planning strategies, birth control plans and all recommendable procedures that obstruct the direct link between sexual intercourse and possible conception have had to reckon with very resilient attitudes in Africa, arising ultimately from a worldview and value system in which children and, consequently, conception are greatly valued. The failure of the condom, for example, to serve as a method of prevention against the deadly HIV/AIDS infection in some parts of Africa to the same extent as elsewhere in the world could be linked directly to this fact, that the high value placed on children and procreation has been transferred to the sexual act as basically an act of fecundation. It is very significant to note that, in cases of certain cancers, many Africans prefer death to having any of their

body parts directly connected with reproduction surgically removed.

In traditional as well as modern Africa, there is, perhaps, no category of patient that the healer counsels more frequently than the infertile woman and the impotent man. These are medical conditions that very few Africans are willing to accept with resignation as long as the last possible healer has not been consulted and the last possible method or product tried. Also, everywhere in Africa there is some form or other of the maternal cult, which considers motherhood the plenitude and crown of womanhood, while a childless man may often be bracketed with the children, a very degrading thing in a culture in which great respect is accorded to age and status. Again, on the approach of death, a childless person is particularly terrified because, while death is considered a transition into the realm of the ancestors, the living-dead, life, well-being and prosperity in that realm is believed to depend on the reciprocal interaction between the progeny and the ancestors, between the living kin and the living-dead *(1)*.

Therefore, assisted reproduction of almost any putative type would, *prima facie*, be of great interest in Africa and assisted reproductive technologies (ART) could not fail to generate great interest and even excitement in Africa. Nevertheless, ART in Africa is fraught with a number of problems and contradictions. But, before considering these, the situation in Cameroon and francophone Africa will be briefly presented.

ART in Cameroon, the Central African sub-Region and francophone Africa

In the francophone countries of central Africa as well as the rest of francophone Africa, as indeed in sub-Saharan Africa in general, the sociocultural system, attitudes and practices make infertility and childlessness in general a highly undesirable condition and, more or less, a metaphysical curse. The social importance attached to fertility cults *(1)*, to birth, naming and initiation rituals, to marriage, death and burial ceremonies, flow from a logic of, and accord well only with, generalized fecundity and procreativity. Any assistance towards fertility is therefore highly valued and sought.

In francophone Africa, Cameroon has a leading position in the domain of medically assisted reproduction and two centres for *in vitro* fertilization and embryo transfer (IVF-ET) exist in Yaoundé and Douala, although they each present very different attitudes, results and outlook. Other francophone African countries, notably Algeria, Benin, Burkina Faso, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Mali, etc. have all had some of their patients coming to Cameroon or are currently trying to learn from Cameroon and are in the process of setting up their own facilities for medically assisted reproduction.

The *Centre de Chirurgie Endoscopique et Reproduction Humaine Chantal Biya* (CCERH) was inaugurated in Yaoundé on 6 March 1998. Conceived as a part of the *Fondation Chantal Biya*, the CCERH is presently hosted by the Department of Obstetrics, Gynaecology and Human Reproduction of the Yaoundé General Hospital.

In 1999 (1–3 December), about 750 delegates from all parts of the world gathered in Yaoundé for the "First Pan-African Congress on Endoscopic Surgery in Gynaecology". According to press reports *(2)*, all the Congress participants were unanimous that gynaecology in Africa in the year 2000 would be marked by great technological progress, particularly in endoscopic surgery. During this conference, which was held at the Yaoundé Conference Centre (Palais des Congres), an exhibition on endoscopic surgical operations was performed at the CCERH and transmitted live by the Cameroon Radio and Television (CRTV) to Congress participants.

The *Centre de Techniques de Pointe en Gynecologie-Obstetrique* (CTPGO), Douala, went operational in the middle of 1996. The Centre, which works in collaboration with Dr Guy Cassuto's *Laboratoire DROUOT* (Paris), is run by a team of four gynaecologists and two biologists.

Since 1997, the CTPGO has attempted IVF-ET on a total of about 200 women, of which 45 have been successful, giving a percentage success rate of about 19% as against 25% reported in the industrialized world. The average age of the couples seeking to undergo the procedure in Douala is about 45 years, a factor on which the Centre partly blames the high rate of failure. The main indication for treatment has been problems related to the fallopian tubes of the woman (91%). A number of potential patients have presented seeking IVF-ET with sperm other than that of a husband but have, so far, been turned away on the grounds that the Centre is still too young, has no facilities for gamete storage, and that the ethical problems involved here are more complex and require

more careful consideration. Since 1997, the per patient cost of the treatment in Douala has progressively been reduced from 1.5 million CFA francs (about US$ 2500) through 1.2 million CFA francs (US$ 2000) to 1 million CFA francs (about US$ 1700) today. The Centre claims that all its babies are doing fine and that it is following up on several of them systematically.

Evaluation

Although medically assisted reproduction generally, and IVF-ET particularly, generates a lot of interest in this part of the world, for reasons some of which have already been evoked, it also raises many questions and problems. It can be said that, for good or ill, ART has a place and a future in Africa. But one of the problems connected with it has to do with its cost. At a cost of 1 million CFA francs (about US$ 1700) in Cameroon, for instance (said to be only about 25% of the cost elsewhere), this health care procedure is affordable for only a very small minority of the small, though by no means negligible, number of couples who stand in need of it anywhere in Africa. Infertility is commonly understood to be indicated if, after 12 months of regular normal sexual practice without contraception, a couple has not succeeded in achieving pregnancy *(3)*. Between 75% and 80% of all couples are said to achieve pregnancy within these circumstances and, if the waiting period is extended to 24 months, around 90% of all couples succeed in achieving pregnancy. In the absence of any reliable statistics, there is no reason to suppose that the incidence of infertility in Africa is much more or much less than the global average which has been estimated at 10% *(4)*. It is not, therefore, a health care service that public health authorities could justifiably try to promote in the face of other urgent health and reproductive health problems facing a greater proportion of the population. It is thus most suitable for only the private sector, where pure economic considerations, the profit motive and promotional advertisement will be the main determinants of developments.

ART, whose medium- and long-term impact is still to be assessed, is liable to subvert and damage traditional African sociocultural ideas, attitudes, customs and practices that have hitherto adequately handled and cushioned the problem of infertility. In most parts of Africa, biological parenthood is de-emphasized to the advantage of social parenthood. Because of this, infertile couples, frequently under a veil of ignorance, can solve their problem not with the help of technology but through a social network. The possibilities are many and varied: a brother or other relative discretely fathering a child for another, a sister more or less openly begetting a child for another with the latter's husband, a wife "marrying" another wife[i] to beget children for her with her husband, begging, giving and receiving a child as a special gift, adopting children, assimilating children into families without prior intention or further calculations, etc.

Although some sort of counselling would usually be given to couples seeking ART, the psychological effects of undergoing the procedures, especially on the unsuccessful cases, need to be very carefully assessed. And, although ART children are said to be not any different from naturally conceived children and to be generally doing fine, only careful and long-term study and follow-up can determine their psychological if not physiological status *vis-à-vis* children conceived and born more normally. For now, the fact that these children are "test-tube babies" is concealed from them and neighbours, but, sooner or later, it will become known.

Furthermore, infertility might have a genetic origin and the resort to ART to solve this problem means that the genetic defect involved is sustained and perpetuated in the population in such a way that resort to technology will be the only option for an ever-

i The practice is quite common among several African communities, whereby a married woman who is unable to beget children of her own arranges to marry another woman, on whom she may personally pay the dowry, so that the latter could beget children with the former's husband, and such children are considered as her (the "woman-husband's") children. This approach is practiced among the Igbo of Nigeria *(7)*. Marriage in Africa is a very complex institution with a diverse variety of interesting variations from community to community. While the variation described above was not practiced among the Nso' of the Bamenda highlands of Cameroon, some "Queen Mothers" such as the famous Yaa wo Faa *(8)* could marry other women who begot children (with any men of their own choice) and such children were considered as the Yaa's children. That is how some Nso' people today pass for second-degree princes and princesses (won-wonntoh) when, in fact, they have no drop of royal blood in them. The bottom line is that, in many parts of Africa, biological parenthood is downplayed in favour of social parenthood and that marriage is considered as being more of a family, lineage and community affair than a contract between individuals *(9)*.

increasing number of persons within that population. This prospect needs to be carefully weighed against the present benefits of providing satisfaction to infertile couples or unconventional couples seeking to become parents.

The providers of ART in Africa presently operate within a legal and ethical vacuum, as the legal systems of most African countries have not yet caught up with the rapid developments in the field of reproductive health, as indeed in many other domains, while precise ethical guidance is not yet available. There are, therefore, real dangers of abuse and the possibility of unregulated experimentation without any fear of consequences or repercussions. Providers are thus left with only their own personal moral sensibilities and sensitivity or their consciences as the guiding lights for their actions and acts within the field. But ART is certainly an area where personal morality and good intentions alone, while necessary, are far from sufficient. The domain belongs to public morality and needs both clear ethical guidelines and appropriate legislation.

Above all, the wider implications of ART—its mechanization of reproductive processes, its severance of the link between "love-making" and "baby-making", its dispelling of the mystery of procreation through human mastery, its de-mystification of motherhood, so central to the status of women in African cultures, its reduction of reproduction to production and the human control and obsession with quality implied, etc.—would be hard to harmonize with the most general of African metaphysical and religious conceptions and beliefs, value systems, ideational and ideological thrusts, customs and consolidated practices.

Conclusion

There seems to be some contradiction about the worldwide concern with, interest in and promotion of ART that needs recognizing. How can this concern and interest be reconciled with the global emphasis on, and campaigns for, population reduction, whose success can be seen in the downward trend of population growth, especially in the industrialized world? In describing the advantages of endoscopic surgery (see above), Professor Maurice Antoine Bruhat ironically remarked that the greatest challenge of medicine in the third millennium would be the control of reproduction because it is no longer desirable to continue breeding at random and filling the earth with great numbers of people. Concern with population reduction would seem more consistent with encouraging the infertile to courageously accept their condition rather than trying to do everything to help them overcome it. Before asking the fertile to voluntarily refrain from procreating in the interest of population control, would it not be logical first to ask the infertile to voluntarily spare themselves the trouble, cost and pain of medically assisted reproduction? As a fertile person, to become aware of the trouble and pain infertile persons take just to get a child, is to realize just how lucky one is to be fertile. Such a realization is not likely to be a help towards the prospect of voluntarily refraining from procreating, which alone can eventually help to reduce the population growth rate of poor or impoverished African countries or communities to manageable proportions.

Although at the individual personal level African people's attitudes towards ART may be determined by their *technophobia* or *technophilia*, it is clear that, at the level of society or culture in general, technology as such is not what counts but rather the *uses* to which it is put, its general implications and the surrounding packaging with which it comes. In my natal language, Lamnso', there is the saying: "*wan dze wan a dze lim nyuy"* (a child is a child, the handiwork of God), which connotes the unconditional acceptance and love of a neonate, irrespective of its individual and particular characteristics *(5)*. This, incidentally, has the implication, among others, that the ART child would be as welcome and as loved as any other child conceived and born in any other manner. The moral implications of this saying are very important in view of the innumerable differences and enormous variety with which individual babies/human beings come from the hand of God or Nature.

In view of this, one of the deeper and more important implications of ART is that it is inevitably concerned with quality control *(6)* and that this concern has the direct implication that we can no longer say that a child is a child, the handiwork of God, but rather, in this case, a deliberate work of human hands or, more precisely, of human technologists. Fletcher's work just cited, which attempts to discuss the ethics of increasing human control of the reproductive domain, is a paradigm of optimism and big thinking about reproduction technology. The work is full of such expressions as: "quality control", "controlled human baby-making", "increasing the

quality of the babies we make", "human reconstruction of humans", "biological manufacture of human beings to exact specifications", "discarding the surplus", etc. In the African perspective, within the context that I have tried to describe, suggesting that one putative baby is of "better quality" than another would be considered outrageous, if not completely meaningless.

Part of the "packaging" of ART, as it comes to other peoples and cultures from the industrialized western world, that would not sit well with African ideas and attitudes, is its almost overt connection with business and commercialization, patenting and marketing, talk about quality control, shopping and advertising, and all the media publicity that goes with these. This packaging can and needs to be modified or changed if ART is to be firmly planted in the background soil of traditional African culture, customs and practices. Within that background, the category of mystery, the God-metaphor and the constant affirmation of human limitations and fallibility are very important and cannot be easily discarded or ignored.

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Sociocultural attitudes towards infertility and assisted reproduction in India

ANJALI WIDGE

Introduction

It is estimated that about 8%–10% of couples experience some sort of infertility in their reproductive lives *(1)*. In India, primary and secondary infertility figures, as given in WHO studies, are 3% and 8%, respectively *(2,3)*. Evidence from a village-level study in the state of Maharashtra in India puts the level of infertility at 6%–7% *(4)*. According to the recent National Family Health Survey in India, 3.8% of women between the ages of 40 and 44 years have not had any children and 3.5% of currently married women are declared infecund *(5)*.

As in many developing countries, in India too infertility treatment is not part of the reproductive health services offered. There is no public health programme that focuses on infertility in the Indian context, though the International Conference on Population and Development (ICPD) programme of action states that reproductive health services should include prevention and appropriate treatment of infertility *(6)*. The Ninth Five-Year Plan (1997–2002) document of the Government of India has included infertility in the comprehensive reproductive and child health package.

The recent AIDS epidemic has raised an interest in understanding sexually transmitted diseases (STD); however, the interventions focused on reproductive tract infections (RTI) and STD are not adequate to deal with the whole gamut of infertility problems. The knowledge that contraceptives are being rejected for fear of infertility has also contributed to the recent interest in the topic *(7)*.

According to Jejeebhoy, there is a paucity of studies in India exploring the perceptions and experience of infertility, even though there has been an adequate exploration of priorities for social science research on infertility *(8–10)*. Rigorous social science research in the context of infertility, its causes and consequences and a focus on programme needs have been suggested. According to her, the little evidence on the levels and patterns of infertility in South Asia comes mostly from censuses and surveys. The particular factors that may be associated causally with infertility require a qualitative focus, such as the study in Egypt on culturally specific determinants of infertility *(10)*. There is also very sparse research available on the sociocultural and behavioural correlates of infertility in South Asia. Sexually transmitted diseases, maternal health factors, poor health and nutritional status of women which could lead to fetal wastage, age (adolescent sterility and infertility among premenopausal women), lifestyle-related infertility, previous contraceptive use, marriage patterns, occupational patterns, availability and accessibility of reproductive health services, levels of education, economic status and women's autonomy are recognizable correlates of infertility *(8)*.

The new reproductive health package of the Government of India envisages, besides other services, the provision of infertility services through the health care delivery system. Besides being a sequelum of sexually transmitted infections, infertility could also be an outcome of poor obstetric and gynaecological practice *(11)*. Inadequate research is available on these causes of infertility. For example, RTI can be acquired through lack of access to clean water, unsterile procedures during childbirth or abortion and other bad medical practices. Services for infertility and RTI are available only at district and subdistrict hospitals and some community health centres. The social factors that may cause infertility such as poor health care, nutritional status, contraceptive methods or environmental factors were not considered important earlier *(11)*.

Objectives

There are only a few studies in India that have focused on the sociocultural context of fertility-seeking practices in relation to infertility and childlessness. Even fewer studies have focused on the social implications of infertility and assisted reproduction. This paper summarizes some of the key findings of most of these studies. It also provides an overview of some of the literature on how people's beliefs, values, and sociocultural norms shape the way in which they deal with infertility and how they determine treatment-seeking behaviour, including the use of assisted reproductive technologies (ART), in the Indian context. As it is critical to understand the way in which motherhood and female identity is constructed in a society, literature on the social construction of motherhood and female identity in India is also reviewed.

Background

The sociopolitical context

In India, infertility appears to be an unimportant question for policy-makers and women's groups alike. As an example, none of the programmes of the national reproductive health policy has focused on implementing preventive and curative services for infertility treatment. Infertility seems to be a relatively unimportant issue, as it affects only a few couples in an "overpopulated" country. Among them, even fewer couples, those of a certain class group, are able to access ART. These are the primary reasons that the voice of the childless woman is absent in the feminist and policy debates surrounding reproductive technology in India. Hence, neither the issue of infertility nor the implications of ART are given any value. However, irrespective of the number, or the class of women who are affected by infertility, or have access to infertility treatment, the infertile or the childless woman is a focus for patriarchal power and this has major social repercussions for the woman. Structural inequalities too cannot be ignored, as even childless women who are not at the lower end of the social strata are situated in the patriarchal Indian family.

The issues surrounding pregnancy, childbirth and motherhood are very complex in all societies. There is a huge stigma attached to being infertile/childless and childlessness has negative implications in Indian society, especially for the woman. Fertility defines womanhood and womanhood is defined by a woman's capacity to "mother". Since it is the woman who becomes pregnant and gives birth, society puts pressure on her to "mother" even though the male may be the one who is infertile. There is no space for infertile couples as part of the governing definition of the family, which burdens the woman even more. In most societies, including India, men need children to have heirs and to prove their masculinity. The system of patriarchal descent, patrilocal residence (residence with or near the patrileneal relatives of the husband), property inheritance, lineage and caste are responsible for the extreme importance given to fertility in Indian society *(12)*. Due to social pressures exerted by such systems, women go through all kinds of treatments to have a child. Women want to have children (sons, in the Indian context), because it brings them power in real terms, and also because, for many, it is the only power-base they have from which they negotiate the terms of their existence.

Social/cultural construction of motherhood in India

The identity of a woman in India is formed in relation to the values, meanings and symbols of Indian society. Her self is affected by the cultural world outside. The meanings and values of the cultural identity are internalized. The ideology of motherhood differs according to the sociocultural context, ethnicity, and class. In India, which is mostly a

patriarchal society, motherhood has connotations of respect and power. Here, the "mother goddess" as mother is highly revered, but real-life mothers are respected to a very limited extent, and that respect is usually for mothers of sons. This can vary depending on factors such as gender relations, class and caste. A woman is considered "complete" or "real" only when she becomes a mother. She proves her womanhood in this way and feels secure in her marriage because it is believed to bond the marital relationship. As a mother she feels she has accomplished what she was supposed to do as an adult woman.

The ideology of motherhood is related to the way families are structured on kinship practices and depends on the variations in them. Ideas about womanhood and motherhood are linked to family and marriage. Family organization and marriage are important to understand reproduction and motherhood. Kinship is also important in understanding inheritance, rights over children, authority and responsibility of members of the family or kin-group. The way the patriarchal family is structured is one of the major causes of inequality between men and women and of the understanding that motherhood is one of the major roles of a woman in society.

The mothering role of women is reproduced in society irrespective of whether women become mothers or not and the ideology of motherhood is dependent on the way a society constructs it. Motherhood is seen to be positively significant in many traditional societies, since women's reproductive capacity is something which women consider their source of power, and as defining their identity and status. It is also considered a resource for women who are denied the experience. This is true of childless women who centre their whole life on the fact that they cannot become mothers or bear children and have to pay a social cost for it. The ideology of motherhood in Indian society explains why fertility is so important. Feminine identity is defined by the ideology of motherhood, being fertile is important and infertility is a huge problem. Though the control of fertility might be a problem for the state, yet infertility is very important in the cultural context as kinship and family ties depend on the progeny.

Throughout India, marriages take place early and are arranged by parents, with partners chosen within a caste, subcaste or a superior caste, but not within very close kin. The man is usually a stranger to the bride, which puts her in a very vulnerable position. The bride is expected to bring enough dowry, be efficient in housework, be respect-ful and serve elders and the husband, besides also bear sons. In a joint family, living with the in-laws, the bride has further lack of control over her life, and despite being productive, usually has no control over resources.

The bride's major role is mothering and taking care of the house. Only when she becomes a mother of a son does she feel completely at home in her husband's house. The notion of purity is very strong and control over her sexuality begins at puberty in her parent's home, and continues after marriage. After marriage the bride is controlled by the patri-kin and because of its patri-local nature, feels isolated among strangers. Even when the family becomes a (semi) nuclear unit with only the husband, wife and children, it continues to have economic and ritual ties with the patri-family. Therefore, for the bride/wife, the situation remains almost unchanged. She attains some freedom only when she becomes a mother or when her mother-in-law becomes old.

The patriarchal, patri-local and the patrilineal nature of the family exists even today. Marriage is a relationship between two families rather than between two individuals but certain aspects of patriarchy are common to most patri-kin groups, irrespective of whether they are from the north or the south, especially when it comes to women's overall status in the family. Joint families, whatever type they might be, define property relations and regulate marriage and inheritance. Nuclear families seem to be more advantageous to women as they might give them relative freedom. Though it had been said that modernization would bring about nuclear families and subsequently various other changes such as freedom of marital choice, increase in divorce, disappearance of dowry, etc. this change has been minimal. Some rules of patrilineality are relaxed, but to a limited extent, as marital choice is rarely free and dowry exists. There are inequalities within the family in terms of division of labour and distribution of resources.

Adoption is encouraged only within the family so that property stays within the same group. As relation by blood is so important, illegitimate and adopted children are not accepted easily. Earlier, the most important reason for males to have more than one wife was the desire for a male child. Even now, in spite of the law against polygamy, there are some men who have two wives, because they could not have a child with their first wife.

Sociocultural context and consequences of infertility/childlessness in Indian society

Since a woman is defined by her fertility, she internalizes the motherhood role to the extent that if she is infertile, she feels worthless. Then she proceeds to do all she can to reverse the situation. The experience of infertility/childlessness is usually marked by anxiety and fear, societal pressures to conceive and social stigmatization, and various trials of various treatments. Infertility is a major problem in the context of important domains of social life such as kinship, inheritance, marriage and divorce patterns. It is political in the sense that it is in the overpopulated state's interest to control fertility and not be concerned about infertility. It is a threat to a woman's identity, status and economic insecurity, to a man's procreativity and to lineage, familial and community continuity. Infertility has very often been compared to bereavement and can be a wrecking experience for both the woman and the man. It may lead to identity dilemmas, lowered self-esteem, frustration and a sense of powerlessness *(13)*.

There are many reasons for the importance given to biological children in society. It is assumed that the desire to have children is normal and parenthood is part of the natural order of things. Some childless women might not be that enthusiastic about motherhood but want a child to satisfy their in-laws or husband, or experience pregnancy, childbirth or parenthood. Some are under external pressures to have children (as in India). For some, it makes them feel part of daily life and for some couples a child is like an achievement. For some men, having a child is proving their sexual potency. It is important for women, because for them there is a link between femininity and fertility. Motherhood also gives women a female adult identity and a reputation of a responsible human being. Children provide emotional satisfaction, make life interesting and provide a reason for living. People also want children because it is almost like a biological need, as they want to see a part of themselves in their child. Some want to be able to spend the wealth they have acquired or achieved on someone, and a biological offspring is the best person to spend it on. Having a child for some couples affirms their love for each other as a child is seen as a binding factor. A child is also looked upon as someone who helps an urban middle-class housewife spend her time, since the child occupies her and gives her status in society, she also has something to talk about with other women. A poor woman has children for economic reasons too. The more children she has, the more earnings there are for the family as a whole. So children are precious resources for her, as she usually cannot send them to school.

Among the studies/research that have focused on the sociocultural context and social isolation issues resulting from childlessness are those conducted by Jindal and Gupta *(14)*, Singh and Dhaliwal *(15)*, Neff *(16)*, Patel *(17)*, Iyengar and Iyengar *(18)*, Prakasamma *(20)*, Unisa *(19)*, Widge *(21)* and Mulgaonkar *(22)*.

According to Das Gupta, Chen and Krishnan, children in Indian society are looked at as a source of labour, income, happiness and security in old age. The perceptions of women's roles and attitudes may be shifting, especially in the upper and middle classes, but procreation still remains an important factor in the socioeconomic well-being of most Indian women *(23)*. The Indian tradition demands that all marriages must result in children, preferably male ones. In the patrilineal Indian society there is a strong desire for a son to continue the family line and perform religious rituals for the salvation of departed souls.

Jindal and Gupta, through their study, reiterated that in India the social pressure to become parents is even more because of the joint family system and the influence of elders. If the couple is infertile, there is loss of status and prestige. Among the women that they studied, social problems increased with the duration of marriage or duration of infertility, while these decreased with increase in age, education and income of the husband. The problems were inversely related to education and economic independence. Insistence on a male child was responsible for such problems in both primary and secondary infertility cases when the first offspring was female. Poverty and illiteracy emerged as important socioeconomic determinants of these problems *(14)*.

Patel's anthropological study of fertility behaviour in a Rajasthan village illustrates that the graduation in status through motherhood is so marked that barrenness is a dreaded condition. Parenthood confers honour on a couple and a person's image and respectability gets enhanced with every additional child's birth and survival. Childbirth lends stability and security to the bride's relationship with members of the household. As far as she can prove her fertility in about three years, even the birth of a daughter is welcome although a son is always better. Children symbolize prosperity and happiness. A household

without children is unfortunate and unbearably desolate, "one that pounces on one like a glutton" (*khali ghar khavane daude*). The importance of sons is very obvious in all caste groups as they have a number of functions to perform towards the discharge of kinship and ritual obligations. It is a matter of serious concern if a woman does not bear a child for 4–5 years. Barrenness is held as a curse. Besides being inauspicious for auspicious occasions, she is insulted and is under constant pressure and faces innuendoes during quarrels and disputes. Rarely is male fertility questioned whereas her fecundity is doubted. Even though men are not oppressed due to their childlessness, they do face some humiliation. Childless widows do not have the status and emotional moorings of widows with children but among some castes, childless widows are more likely to get remarried *(17)*.

Neff explored the social construction of infertility among the matrilineal Nayars of South India. The research shows that it is the duty of matrilineal kin to attend to the family god of fertility and to the needs of females of the matrilineage to see that they foster progeny in the kin group's best interests. When this responsibility is violated, powerful forms of negative consequences may transpire for all lineage members, in the idiom of curses of family fertility gods[i] *(16)*.

According to Prakasamma, procreation, continuity, perpetuation through the progeny and the need for self-preservation form the social construction of infertility in India. The study revealed that infertility threatens the social acceptability of a woman, her legitimate role of a wife, her marital stability, security, bonding and her role in the family and community. The childless woman is not considered feminine and suffers from low self-worth and blame. The cycle of denial–treatment–frustration–resignation leads to emotional strain. If there is a case of a second marriage, there is loss of entitlement *(20)*.

Mukhopadhyay and Garimella conducted a study on reproductive choice that revealed a high rate of morbidity among the women, apart from inadequate services and supplies and a near-total absence of male support or sensitivity towards female health problems. Women carried the burden of infertility alone and they had a nagging fear of desertion by their husbands *(24)*. The blame for sterility lies entirely with the female partner, conclude Devi *et. al*., who studied the social factors contributing to sterility in the state of Manipur *(25)*.

Recent research by Gerrits, Unisa, Widge and Mulgaonkar has also highlighted the consequences of childlessness *(19,21,22,26)*. The women who were interviewed for the study conducted by Widge felt that motherhood is still the most important goal for a woman. The blood-bond between mother and child overtakes the one with the husband. Maternal instinct in most cases in the study was perceived to be an individual urge. The woman is usually blamed for childlessness while men hesitate to even get tested. Childlessness also brings exclusion from the social nexus of mothers and couples with children. People are usually insensitive to this problem and sometimes make pointed references to it. Moreover, children seem to be the central point of discussion for mothers, so a childless mother feels excluded and her childlessness becomes more obvious. Most couples that are childless centre their lives on trying to conceive or coping with not being able to. Friends are not very supportive but are less interfering than relatives/ extended family, as the latter can make the couple feel excluded. The couples who were interviewed felt that it is very important to have a son, as lineage, kinship, dowry and old age concerns are prime. The motherhood role is internalized by women. The study conducted by Widge reiterates the connection between fertility–womanhood–motherhood in Indian society *(21)*.

According to Iyengar and Iyengar, one of the social consequences of infertility in the study they conducted in Southern Rajasthan is the practice of *nata* (by which a person can take on a new spouse) that is prevalent in this area. About 20% of childless women were affected by this practice but, interestingly, about 15% of the women had taken another spouse[ii] *(18,27)*.

i In the ritual of *pampin tullal* performed to propitiate these gods, concepts of fertility are extended to include other "auspicious" forms of prosperity. In the ritual, unattached Nayar women serve as proxy for the well-being of the matrilineage. These unattached women—infertile, unmarried, "separated", and widowed—are, for the natal kin group, symbolic virgins (*kanya*), the life force (*sakti*) of which lineage members seek to harness for their well-being.

ii In Ladakh (a high-altitude region of the Himalayas in North India), there is evidence for low rates of marriage among women that may be attributable to the practice of polyandry (one woman with more than one husband). Wiley presents an analysis of 1981 Indian Census data that documents low natural fertility in Ladakh. Age-specific fertility rates derived from the number of current births are also unexpectedly low. The most likely explanations for low marital fertility include sterility

Unisa suggests that early marriage, early initiation of cohabitation and early spontaneous abortions may be the reasons for some of the higher rates of infertility in the state of Andhra Pradesh where she carried out her study. According to her, the rate of childlessness was 5% among currently married women aged 20 years or more. Cross-cousin marriages and close blood relative marriages are common in South India, which could be a determinant of infertility. In this study, a large number of women had married a close relative. Although the extended family system is the norm in India, a majority of women in this study lived in nuclear families. Unisa feels that childless women are kept purposely from celebrations of newborn children and celebrations of first pregnancies, as their presence is considered inauspicious. Many people expressed the opinion that a childless couple should also not bless a newly married couple as that might result in the newly married couple's childlessness. After a few years of marriage, a childless woman avoids ceremonies. Some of them were called *godralu* (a Telugu term with a negative connotation meaning a woman without eggs). Actual and anticipated rude comments at social functions forced many women in this study into becoming social recluses. About 20% of these women considered themselves to be a source of bad luck. Unisa pointed out that the women themselves had low self-esteem as a result of these negative social attitudes *(19)*.

Mulgaonkar reiterates through her study in Mumbai that women are usually blamed more than men are for their childlessness *(22)*. This has been substantiated by Dhaliwal *et al. (28)*, Jejeebhoy *(8)*, Jindal *et al. (14)* and Unisa *(19)*. Most women felt that they were blamed and stigmatized for their childlessness. Men in the study attributed their infertility to fate and God's will. They felt that there was a right time for motherhood and fatherhood and that it was too late for women to become mothers after they were 25 years old. These concerns related to lack of adult identity (male and female), also proving their capability of motherhood, status in the family, the stigma of late motherhood and lack of security in old age. Children are valued by women because they continue the patrilineal family line and helped them establish a relationship with the elders in the family. Many women felt that it was important to have the blessings of elders to have a child. Some women who were interviewed felt they had to do more domestic work as a result of their childlessness and some felt that they had more time, as they did not have children to take care of. Some complained that their health was affected due to the stress they went through because of their childlessness *(22)*.

Many studies have pointed out the negative image childless women have of themselves, implying a role failure *(14,21,23,25,26,29,30,31)*. The motherhood role brings fulfilment. The importance of parenthood in Indian society reinforces the feeling of inadequacy of the childless couple. During religious and social functions, couples interviewed by Mulgaonkar reported that they were the butt of rude comments, were made to feel inadequate, suffered from lack of attention, and were questioned about their childlessness. Some were excluded from such functions. Some also felt that they were given unnecessary and sometimes wrong advice during these occasions. But many of the men did not have such experiences though a few of them did and they expressed their desire by interacting with other's children *(22)*.

Psychosocial consequences

Feelings of losing control over their body and their life are expressed by some women who are childless. For many women, conception becomes a preoccupation resulting in anxiety, despair, depression and various other psychological problems. This adds to the problems that already exist. They feel sad, disappointed and exhausted by the intensity of their emotions because this problem has taken over their lives *(21,32)*. Some women who are infertile feel constantly preoccupied with their body, waiting for a sign of something going wrong or right. There is anxiety with the onset of menstruation and it is viewed not as a sign of femininity, but as a failure. There is concern whether the pregnancy will continue or not, or preoccupation with the cause of infertility, or with unexplained infertility. In-depth interviews conducted with infertile couples undergoing treatment have revealed that women express disappointment at failing expectations and that treatments allow hope but also defer final acceptance of infertility. Since this experience damages self-esteem it might have

from STD, high rates of fetal loss, and possibly nutritional constraints on ovarian hormone status. There are also high rates of primary and secondary sterility within marriage, resulting in low completed parities for postreproductive age married women.

repercussions on other relationships of the woman. In India, women often complain of being ridiculed by their in-laws for not being able to conceive. They feel rejected by their partners because they are made to feel incomplete and the threat of someone else coming on the scene looms large *(21)*.

One of the few psychosocial studies of infertile couples in India revealed that infertility is a life crisis and a stressful experience with invisible losses, especially for women *(33)*. They experience marital and psychological instability, and stress and strain, including deterioration in the quality of life. The crisis is long lasting and not much is known about the strategies adopted by infertile couples to cope with their childlessness. In the study, 77% of couples had alterations in sexual response (reduction of sexual happiness), which also has implications for the marital relationship. About 40% of the males blamed their spouse whereas 36% of the wives tended to blame themselves for childlessness, irrespective of whose problem it was.

The study revealed that relatives were more sympathetic towards men than women, as was also suggested by another study *(34)* that stated that more wives than husbands reported insensitive behaviour from their neighbours and friends towards their childlessness. Twenty per cent of the wives received threats of divorce and women were considered inauspicious for religious and ceremonial rites; and 40% of women were socially ostracized. Women suffered this indignity more than men; however, in-laws were more sympathetic as compared with relatives. Women felt hopelessness and despair, resulting in suicidal tendencies five times more than men. Many felt a tremendous sense of guilt, blame and loss of self-esteem.

Mulgaonkar also focused on the psychosocial consequences for couples and her study revealed that couples felt depressed and grieved, had lack of hope, loss of relationships with friends and relatives, doubts about their bodily functions and sexual competence, loss of health due to the specific treatment, feelings of jealousy, anger, guilt, lack of social security and support, fear of extinction of family lineage, addiction to bad habits of chewing tobacco, smoking and consumption of alcohol by some women and men as an effect of childlessness *(22)*. Another study also revealed that infertile couples had sexual problems as a result of their childlessness and were unhappy about their sexual lives *(35)*.

The couples coped with infertility by involving themselves in religious practices such as praying and visiting religious places, by caring for others in the family, fostering relatives' children and some by being involved in social organizations. Some couples also expressed the positive freeing aspects of childlessness.

Women feel responsible for being childless and if something goes wrong during a pregnancy they tend to blame themselves and feel guilty. This guilt, though self-imposed, has an obvious relation to the societal belief that women are completely responsible for reproduction and are to blame if something goes wrong. Women are blamed if they are childless for 4–5 years, and they are considered inauspicious (*bhanjhri)*. They are often considered to be possessed by a witch (*daakan) (18)*.

The study by Dhaliwal and Dhall emphasized that there is a great social stigma attached to childless women in Indian society, regardless of the medical cause *(28)*. Even identifying infertility is sometimes difficult *(15)*. Women face the blame for it and also personal and social consequences, such as personal grief, frustration, economic deprivation, ostracism, violence and marital disruption, including divorce. This has also been substantiated by some other studies *(17–22,32)*.

Son preference

In India, there is not only societal pressure to bear a child but to bear a male child who will continue the name and legacy of the family and provide physical and financial security to the parents in old age. In a patrilineal society like India's, where property is inherited by male heirs and passed on in the male line (even though legally women have a share), male children are desired. It is common to have another wife if the first one cannot conceive, therefore male infertility is not considered as problematic as female infertility. For the woman, there is always the insecurity that the husband will remarry. Therefore, bearing a child (a son in this case) gives a woman greater negotiating space in the household. But if the couple is childless, the woman is held responsible, as she is the one who gives birth. She is not left guilt-free even when it is proved that conception is not taking place because of a fertility problem in the male. The in-laws usually support their son and blame the daughter-in-law. Nevertheless, it is a blow to the male ego if he discovers that he is impotent or infertile. It does not fit into his concept of malehood and virility.

Due to preference for sons, India has an unfortunate history of female infanticide and sex-selective abortions[iii]. With access to sex-selective abortion after ultrasound and sex pre-selection methods, the situation has become worse. The focus for the Indian woman in a patriarchal context is not so much the experience of pregnancy or childbirth but the enormous social pressure and the feeling of security she gets as a result of having a child, especially a male child. Jindal and Gupta's study revealed that when a couple's previous offspring was female, social pressure was the same as in cases of primary infertility *(14)*.

Marital/family disharmony and domestic violence as a consequence of childlessness in India

A childless woman not only faces problems with her in-laws because of her childlessness but also in her marriage. The relationship between husband and wife is sometimes strained, there are very few men who would be supportive of their wife if she had an infertility problem; but if the husband has the problem, the wife is mostly supportive and even tries to cover up and take the blame. Infertility also results in the consequence of low self-esteem, as in some cases there are threats to the woman of another marriage or divorce.

When a bride gets married she is not seen just as a reproducer of sons but is also expected to contribute to productive labour. If the woman happens to have any flaws (such as infertility) she may be ill-treated, as were some women in the study conducted by Widge. Besides the threat of the spouse marrying again, there are threats regarding the woman's claim to property. Widge reiterated through her study that when women get married they have a sense of a lack of identity in the house they marry into. They are often victims of violence and abuse and have nobody to talk to or share their pain with. The childless woman is considered inauspicious and feels unworthy and unwanted. The woman also wants to become a mother to feel complete and, as a mother, besides other things, she has greater access to resources. If the couple remains childless, it mostly has a negative impact on their marriage, though some husbands are supportive and defend their wives against family pressure or criticism. There is a feeling that the extended family takes advantage of the situation of childlessness *(21)*.

Feelings of inferiority and self-defect in childless women are exacerbated by the in-laws and husband. Disharmony with the in-laws and husband has been reported by 34% and 16% of infertile women in the study conducted by Jindal and Gupta in North India *(14)*. The pressure varies from taunting, to abusing, to violence and threats of abandonment and remarriage. Ignorance regarding male infertility was the main cause for this but husbands were more sympathetic when they were aware of a male factor in their infertility or had one or more living children.

In the study conducted by Desai, Shrinivasan and Hazra, none of the males were threatened with divorce from their wives but about 20% of women received such threats. About 15% of both men and women considered remarriage. About 10% of the women also faced physical assault. Extramarital liaisons were also reported *(33)*.

According to Patel, in the community that she studied, if a woman cannot produce a child her husband can divorce her or remarry, but not all childless women have to face divorce or a second marriage as bride price here makes a second marriage very expensive *(17)*. Singh, Dhaliwal and Kaur also found instances of threats of second marriage, maltreatment of the childless woman within the family, taunting and physical assault by husband. The study concluded that grave social stigma continues to be attached to infertility and is largely borne by women *(34)*.

In Unisa's study sample, only 4% of the women said that their husbands wanted a divorce in order to have children, but in 12% of the sample, the husband already had more than one wife and 16% of the women reported that they felt that their husbands wanted a second wife. A few husbands were also having relationships with other women. Two-thirds of the women reported a harmonious marital relationship with no threats to their marriage. Unisa pointed out that the harmonious relationship among these couples may be due to the awareness that infertility was treatable in many cases and that women alone were not responsible for this condition. But two-thirds of the women experienced violence from their husbands

iii The recent Census of India 2001 has revealed a marginal increase in the national sex ratios but very low sex ratios in some states, even in relatively developed states such as Gujarat and Maharashtra. This low sex ratio is also being attributed to practices such as sex selection and female infanticide/feticide.

and out of these, 13% felt that the reasons were partly due to childlessness. Infertility did spur some men to physical abuse or to take another wife. The level of violence decreased as the level of education increased *(19)*. The incidence of physical violence as a result of childlessness had also been reported in a study in North India *(34)*.

Some women in the study conducted by Mulgaonkar felt that the sole purpose of their marriage was to have children but others, who felt that this was only one of the reasons to get married, felt that they could do without children *(22)*.

Treatment-seeking behaviour of infertile/ childless couples in India

Though there are many consequences of infertility, the obvious commonly expressed consequences include fertility-seeking behaviour which includes treatment mostly from traditional healers *(36,37)*. More recent studies have also identified allopathy as one of the more popular treatments that are sought besides traditional treatments *(19,22)*. Although infertility treatment is available in government hospitals, there is often poor coordination between gynaecologists, infertility specialists, surgeons and laboratory technicians. Couples who can afford the cost of ART such as *in vitro* fertilization (IVF) are using them increasingly *(21)*.

A WHO multicentre study revealed that, in Chandigarh (India), couples with primary infertility had more interest in treatment as compared to those with secondary infertility *(37)*. Seventy-five per cent of couples had a duration of infertility of more than two years prior to embarking on investigations, while more than a third came with a duration of more than four years. Women usually initiated the first contact with a physician. The reasons for couples delaying seeking medical advice were because of the fear of a final definitive diagnosis and partly because of the dread of the emotional stress and physical discomfort of the tests they would have to undergo. Some also felt that in seeking medical attention they were admitting failure in their efforts to conceive.

Many follow-up studies of infertile couples have shown that 12%–16% of couples conceived over a period of 2–3 years. In one such study, 38% reported pregnancy before treatment was even started, 27% while investigations were being carried out and only 35% completed treatment before onset of pregnancy *(28)*.

The treatment-seeking behaviour of couples in the study conducted by Mulgaonkar depended on the socioeconomic status of the couple, and the decision for seeking treatment involved other people besides the couple. Most couples sought treatment within the first year of their marriage. A few couples took *ayurvedic* and homeopathic treatment, though almost all followed religious practices. Most couples believed that childlessness was a disease and that they would have to spend a lot of time to undergo treatment at the cost of participation in other activities *(22)*.

In the study conducted by Unisa, one-quarter of infertile women never sought help, for the most part because of the high cost (43%) or because they felt it was unnecessary (41%). Some women reported a lack of information regarding treatment and nonavailability of treatment nearby. Some were not able to get permission from elders for treatment. In the patriarchal context, the problem of infertility was initially considered to be the woman's problem and the husbands were generally uncooperative. But in this sample, in many of the cases, the husbands went with the wife and accepted treatment when required. These women, on an average, began allopathic treatment[iv] and visits to holy places after three years of marriage. Some even began seeking treatment after a year of marriage as a result of pressure, or their own impatience. Many women sought allopathic treatment first (73%) and then tried other options which includes other sorts of treatment (*ayurvedic*, homeopathic and *unani*), prayer, rituals and traditional treatments when allopathy did not work and many cut their treatment short because they could not afford its high costs. Although 63% visited at least one holy place or spiritual healer (these are based on religious belief but are also readily available alternatives), surprisingly, most did so only after modern medical treatment failed. Strong beliefs and varied religious practices were reflected from the in-depth case studies, including many visits to specific temples of some Hindu goddesses. Some women continued to seek allopathic treatment for 25 years and some who took religious help did not give up for up to 32 years. A strong desire to have their own biological child is evident from this behaviour *(19)*.

iv These include treatment from state health services and private doctors and hospitals; the use of ART is not mentioned specifically by Unisa.

Seventy-six per cent of couples in the study conducted by Singh, Dhaliwal and Kaur consulted private and government modern medicine practitioners, TBAs (traditional birth attendants) and faith healers. But only 25% of the men underwent a semen test. More couples with primary infertility than with secondary fertility expressed the desire for a child. The couples also incurred considerable expense for treatment *(34)*.

The study conducted by Iyengar and Iyengar revealed that most couples wait for many years before seeking treatment, even though many women feel that they have a problem. Newly married women seemed keener to seek treatment perhaps out of the anxiety of a new relationship. Those women who had sought treatment visited either a *bhopa* (faith healer), *vaid* (*ayurvedic* doctor) or an allopathic doctor. The ones who went to a doctor were not investigated completely. Male participation in infertility management was limited as it was difficult to convince men to undergo an examination. As revealed from one case in this study, one of the husbands resented that he had been asked to be examined and that his fertility had been doubted and refused to cooperate. Beliefs about women being possessed by evil spirits, combined with traditional practices in a patriarchal society and migration by men also affects the extent of utilization of services even if they are available *(18)*.

The study conducted to understand the emotional aspects of infertility by Desai, Shrinivasan and Hazra concluded that most couples performed religio-magical practices to cure their infertility. Abstaining from visiting a place where a delivery has taken place, *tantric* rites, wearing charms and visiting astrologers were some of the practices adopted by couples *(33)*. Even in the community studied by Patel, women are usually expected to undergo indigenous treatments and various rituals are observed and vows made to local deities *(17)*.

Assisted reproductive technologies: social, physical and psychological costs

There are social, cultural and family pressures that impinge on couples (who can afford it) to use advanced technology such as IVF, gamete intra-fallopian transfer (GIFT) and intracytoplasmic sperm injection (ICSI). It has been suggested that some childless women go through procedures such as IVF repeatedly, although they believe that it is not beneficial, is invasive and has risks of physical and psychological damage. They do, however, repeat the treatments due to a sense of being responsible for reproductive failure and because of the constant valorization of motherhood as a woman's most important role. The repeated use of this technology is also encouraged by physicians as it is commercial and profit-making *(38–42)*.

The ambiguity towards the experience with IVF cycles has been revealed by many studies. However, it is believed that IVF focuses exclusively on biological reproduction and curtails any potential for the redefinition of parenthood or infertility. In so doing, it reinforces the notion of the "natural" bond between a mother and her biological children as well as reinforces the idea that the only desirable structure of social relations between adults and young children is the nuclear family or indeed one's own biological children *(43)*.

Seeking ART in the Indian context

In Indian society, where fertility is valued to the extent that womanhood is defined as motherhood, ART give hope to the infertile even though only a few can afford it. Couples that come from the higher socioeconomic group, in the search to have their own biological child, can now have a child through high technology options like IVF. In India where there is a stigma against infertility and childlessness, this is perceived as a great scientific achievement. Most couples who opt for treatment are very apprehensive about the societal reaction to their childlessness. As in the West, the need for a child in India is not so much of a biological need but is a social one. Having a child is the next immediate step after marriage for a woman. She is valued most for her fertility, if she is not fertile, she is not a "complete" woman *(21)*.

According to Srinivasan, overestimates of infertility help justify the industry's and the medical practitioners' existence, but in a large country with a large population it is a substantial number and requires attention. IVF and other ART are promoted today for all forms of infertility. The Institute for Research in Reproduction (IRR) began work on an IVF programme in 1982 to provide subsidised IVF. The Indian Council for Medical Research (ICMR) suggested that this promotion would also help in couples accepting sterilization if they knew that they could have a child through IVF and would indirectly help the family planning programme *(44)*. Besides, the

services in the private sector are mostly market driven. According to an infertility specialist in Delhi, as private specialists invest a lot in acquiring hi-tech equipment, the costs of service are therefore high (personal communication). According to another infertility specialist, IVF clinics are mushrooming in India. There were 107 IVF centres in India in February 1999, out of which 60 are active. Sperm banks also exist in metropolitan cities *(45)*. According to the Ministry of Health and Family Welfare in India, there are about 60 centres in India offering ART *(46)*. A few centres also exist in the public sector. An evaluation of the public sector ART centres is proposed, following which the existing ones may be strengthened with trained and interested staff *(47)*. The mushrooming of private centres combined with the lack of public sector centres has created a situation of exploitation of helpless, childless couples. The first case of surrogate motherhood in the country, which received considerable media coverage in October 1997, has also brought the need for ART guidelines to the forefront. Recently the ICMR finalized ethical guidelines for ART, but how they will be implemented remains to be seen[v] *(48)*.

In a study of 24 couples who underwent artificial insemination (AI) in 1976, more than half the couples wanted to undergo the procedure to have a child, some wanted to conceal their infertility and some wanted it because they preferred it to adoption, as they wanted a child of their own *(49)*. In another study conducted about 25 years later, only very few women and men agreed (among those whose childlessness was due to the husband) to undergo artificial insemination by donor (AID) *(22)*. In another earlier study, there was a very marginal acceptance of AID by couples in addition to poor facilities for it *(14)*, but according to interviews conducted about four years ago by Gupta, there is a tremendous demand for AID in India, which is offered by private medical practitioners. According to her, one reason for seeking AI is to increase the chances of having a male child. Despite the demand, there are only a handful of sperm banks in India. Some of these do not follow WHO guidelines and supply fresh semen to gynaecologists. The interviews also revealed that some doctors also conduct microscopic tuboplasty, an operation to surgically reverse tubectomy, but the services that are increasingly offered are IVF, GIFT, etc. IVF is encouraged by such doctors as treatment for infertility. Research on IVF also offers opportunities to identify and study factors contributing to infertility. Some doctors offered guarded criticism of these ART but some were clearly in favour of low-cost, low-tech methods *(32)*.

As far as the success rate of IVF is concerned, the ICMR reports an average take-home baby rate of 20%–30% per cycle, but that has not been substantiated by any studies *(50)*. The best clinics in India claim a 30%–40% success rate but it seems to be much lower in reality *(21,32)*. Moreover, this is the pregnancy rate and not the birth rate. Most clinics claim a higher success rate than 5%–10%, which is usually the rate reported by clinics in the West.

Some interviews with infertility specialists conducted by Srinivasan *(44)* revealed that though doctors quote a success rate of 30%–40%, it is usually the rate of biochemical pregnancies. However, the take-home baby rate is not more than 15%. During the course of another study conducted at ART clinics in New Delhi, none of the couples had a baby during the course of the study and only one couple had a baby through intrauterine insemination (IUI). Most women in the study underwent many unsuccessful IVF cycles and had been through various unfortunate experiences with this technology. In spite of these experiences, some did not give up. Among the couples interviewed only one couple had adopted a baby girl from an agency after three unsuccessful attempts at IVF *(21)*.

The experience of childless women using IVF

Couples undergoing IVF, especially women, go through a very discomforting process of examination and treatment. They do not have easy access to information and usually select doctors through trial and error. Most believe that the doctors have a very definite profit motive and sometimes mislead patients. It is difficult for most couples to understand procedures and what the doctors tell them is usually not enough. Most women understand the complexity of the procedure only after experiencing it. For most couples, the physical and psychological effects of the treatment are hard to deal with and it is more difficult if complications arise. Some of the women interviewed

v Medical professionals, pharmaceutical giants, private insurance companies, sperm donors/banks, egg donors have a commercial interest in ART. The fact that this technology has commercial potential and is unregulated could lead to a plethora of unethical practices.

during the study at ART clinics in New Delhi had experienced complications during some procedures.

These women felt that their lives were centred on reproduction and the IVF cycles and that they could not take their thoughts away from pregnancy. Most couples perceived this treatment as a blessing for infertile couples and those who could afford it, said they would like to try all kinds of treatment available before they gave up on having a biological child. The women felt that IVF increased their financial dependence on their husbands, which already exists, if the woman is a housewife. Adoption is usually not an alternative or is the last one for most couples.

Some couples had reservations about using donor sperms or eggs to have a child. In such cases, donor eggs were preferred from within the family. Donor sperms of unknown origin were usually acceptable but it depended on the couple. The lineage principle was still very important and some women did not want to use donor sperm or did not want their husbands to know that donor sperm was being used for fear that they would not allow it *(21)*.

ART are very expensive and only a few can afford them. They are not offered in government hospitals, though some public sector companies reimburse some percentage of the costs. Recently, the ICMR suggested that besides preventive measures, it is essential to reduce the costs of ART so that all couples have access to them *(50)*. It has also been suggested that as ART are expensive, the option of adoption should be offered and there should be a shift to preventive services *(51)*.

Specialists of IVF interviewed for the study by Widge presented the technology as fairly successful and unproblematic. They believe that infertility is either on the rise or is being reported more than before. They felt that social issues need to be addressed, as they are the cause of a lot of stress associated with infertility and that, in Indian society, infertility is a medical, social and psychological problem. One of the specialists pointed out that the only way there could be a change in social attitudes towards the infertile is through technology. She finds that women tend to be more depressed than men because of infertility as they have to deal with many other pressures. According to another specialist, some couples are stressed, others are able to cope better, some have plenty of support, and others face family pressure. But they felt that lineage is a major concern for most couples who undergo IVF and that most women feel the pressure of motherhood. They mentioned marital breakdowns and some cases of bigamy due to stress associated with infertility. They also felt that there is not much resistance among couples to using donated material because of their desperation to have a child *(21)*.

According to another infertility specialist, infertility treatment could be stressful for the patient and counselling is important. Most clinics do not have a counselling facility for couples before and during IVF procedures. There are no laws regarding the (mis)use of ART or use of donor eggs and sperm in India and these doctors feel that just a signed contract or a waiver form is enough. They believe that regulation should suit the needs of everyone. Implementing ethical guidelines would mean that the doctors would have to look critically at issues of informed consent[vi], screening donors, legal issues and the quality of provided services.

Son preference and ART

There are also dangers of IVF perpetuating the already prevalent unethical practice of sex-selective abortions of female fetuses through methods such as pre-implantation diagnosis. IVF specialists offer this as a choice to the couple and do not believe that it amounts to perpetuating male domination and discrimination against the girl child. One of the specialists interviewed for the study admitted to offering sex selection, which is legal, and a choice for couples. This amounts to blatant perpetuation of male domination and discrimination against the girl child, as most of the children selected would be males. One of the important and disturbing implications of IVF in the Indian context is the fact that a related technology can be, and is being, misused for sex-preselection and the existing law does not deal with it.

Attitudes towards adoption

Adoption was traditionally seen as a last resort for the infertile couple. Though now perceived as an acceptable option very few couples actually adopt a child *(8,19,21,22)*. Adoption within the family is encouraged more than adoption of any child,

vi Interviews with these specialists revealed that the concept of informed consent does not exist and there are not too many limits these specialists would set for themselves in terms of how far they would go with the use or misuse of ART.

especially of unknown origin. But it is resisted for a long time and used as a last resort for most as it does not get societal legitimacy. Some women do not want to adopt because they have feelings of inadequacy and want to prove their fertility. Some women felt that independent decisions regarding adoption are difficult to take in a joint family. As lineage is so important, some couples prefer to adopt from within the family or a child is especially conceived for them from within the family. Surrogacy is uncommon in India, though it is quite common within families where a brother or a sister gives up a child for adoption or has a child specifically for the childless couple. There are variations in what is seen as natural and what is tolerable in different cultures *(21)*.

Earlier, most of the childless couples studied had a negative attitude towards adoption *(14)* and only a male child offered by a blood relative was acceptable for adoption by the majority of couples. Possibilities of adoption are few because of the strongly rooted desire of biological progeny *(18)*. Couples usually say that they would like to consider it as an option but few actually adopt a child *(15,19)*. In Mulgaonkar's study most couples (80%) did not accept the idea of adopting a child for various reasons *(22)*.

Conclusion

Motherhood in Indian society defines a woman's identity even before marriage because the preparations for a self-sacrificing life begin long before she is married. It is still the most important goal for a woman. The blood-bond between mother and child overtakes the one with the husband. If she cannot reproduce, due to whatever reasons, she is the subject of ridicule and abuse. Children, especially males, give the woman status within the patriarchal family (be it joint or nuclear), define her identity, give her psychological and emotional security and strengthen kinship bonds. But this responsibility of women to mother in India excludes them from public life and most importantly from power and authority. Infertile women often complain of being ridiculed by their in-laws for not being able to conceive. If the couple remains childless, it sometimes results in violence and at times has a negative impact on their marriage. Even if the woman's husband is infertile, she has to bear the social and psychological consequences. There is also the threat of divorce or debarment from property.

Women/couples seek treatment from traditional healers but also from the modern systems of medicine. Some couples use AI and ART. Since IVF has been introduced in India it has given desperate couples great hope and a chance to have their own baby instead of going through endless treatments without any hope. In the search to have one's own biological child, couples that belong to the higher socioeconomic groups can now have a child. In the present context of consumerism and market-oriented technologies, the private health care sector and the pharmaceutical and genetic engineering companies use the slogan of "help for the infertile", but it is the companies that stand to gain. There are hardly any subsidised clinics in India and the government hospitals do not offer these advanced technologies. Preventive and curative services for infertility are not a priority.

Furthermore, the moral, ethical and social issues raised by ART are unresolved. The problems posed for parenthood, when the legal parent of a child born to a woman who is neither its genetic nor social mother have not been confronted in India. The issue of commercial surrogacy has not been debated in India and emerged only recently when a case was reported.

These technologies are offered as a choice. However, in a country such as India, where modern and/or hi-tech infertility treatment depends on the couple's/woman's ability to pay, there is not much choice. Moreover, due to the lack of regulation and laws there are concerns about the lack of professionalism and the safety of the treatments offered.

The total absence of monitoring and self-regulation can lead to the misuse of ART and related technologies. India, unfortunately, has a history of female infanticide and sex-selective abortions of female fetuses. The new law against sex-selective technologies now includes technologies like preimplantation diagnosis, but it remains to be seen if it is a deterrent. Legal battles regarding ART and/or surrogate motherhood have been going on in the West, but it will not be long before similar problems are faced by the Indian society.

A set of rational and consistent policies to manage ART is required. There should be a focus on non-technological solutions such as preventive measures for infertility, adoption of children of all sexes, raising consciousness to reduce the social pressures for biological parenthood and on protesting against perverse uses of ART.

The reality of the childless woman is complex. Experiences of infertile women with reproductive medicine are not very pleasant but they still want to

use the technology, sometimes because it helps them negotiate their position in the patriarchal family. Also, an overemphasis on the negative impact of these technologies distracts attention from the politics and organization of health care in general, from the legal system, from political struggles over the nature of sexuality, parenthood and the family, and from the impact of the varied material and cultural circumstances in which people create their material lives.

The problem of women's health in India has to be looked at within a general context of poverty, class and gender inequalities and unequal access to resources. The health and well-being of women are important as a value. The politics and organization of health care in general, the legal system, the nature of sexuality, parenthood and the family and the present economic structures all are important related issues that impinge on a childless woman.

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Sociocultural dimensions of infertility and assisted reproduction in the Far East

REN-ZONG QIU

Introduction

The way in which people deal with infertility and the use of assisted reproductive technologies (ART) are at least partly affected by the values and sociocultural norms of the community in which they live. It is important to know how sociocultural traditions, beliefs and values define infertility and shape treatment-seeking behaviour as well as views about ART. This paper addresses the sociocultural and ethical dimensions of infertility and ART in the Far East and how they are embedded in the influential traditions of Confucianism, Taoism, and Buddhism.

Perceptions of sex and reproduction in the Chinese culture

During the five millennia of her history, China has developed her own unique culture influenced primarily by the teachings of Confucianism, Taoism and Buddhism. Although these philosophies appear to have similarities in their cosmologies, how they view sex and reproduction varies. For example, Taoists believe in a spiritual sphere in which people will transcend all mundane dichotomies between truth and falsity, virtue and vice, beauty and ugliness, poor and rich, noble and mean, happiness and misery, etc. In this context they view sex only as a means to promote health and longevity. Chinese Buddhists seek to discard *karma* and reach the *nirvana* and view any desire in sex or reproduction as unacceptable, if not sinful.

In the cosmology of the Chinese culture the universe consists of *qi*, a vital energy or a physico-psychological entity, and all things or beings in the universe are nothing but the different patterns of *qi*. *Qi* exists in two forms: *yin* and *yang*. The *yin–yang* dualistic theory emerged from the worship of sex and reproduction. An analogy was made between human birth (micro world) and creation of the universe (macro world). Intercourse or interaction between *yin* (female) and *yang* (male) in the universe created all entities that eventually come from the "mysterious gate". It is reflected in the sentences of *Dao De Jing*, such as "the mystery of mysteries is the gate of all wonders", and "the gate of mysterious female is the root of the world" *(1)*. In the Chinese culture, the universe is an organism which is capable of continuous reproduction, transformation and creation. Chinese philosophers had argued that sex is one of the integral elements of the human nature. "Eating and sex are human natures" *(2)*, or "Eating, drinking and sex are the greatest desires of human life" *(3)*.

Yin–yang dualism was developed in the *Book of changes*, although these two concepts were put forward in about 600 BC. The *yang* represents the heaven (and the male), and the *yin* the earth (and the

female). This dualism was accepted by both Confucians and Taoists. According to the *Book of changes*, interaction between these two kinds of vital energy in the universe is the basis of life. "All things are created by the intercourse between male and female", "If there had been no intercourse between heaven and earth, there would not have been all things in the world"*(4)*.

Sex

Sperm is called *jing zi* in the Chinese language, and contains *jing* or *yuan qi* (vital energy) which means the essence of life and health. Male sperm is limited, but women's vaginal secretion contains *qi* and blood which are inexhaustible. Sex has dual goals, one is for reproduction or extending ancestors' life, the other is for health.

Taoists believe that there are some elements in the female body which are indispensable for immortality and vice versa, so sex is indispensable for human life and health. Confucians believe that the function of sex is reproduction. They maintain that reproduction is the only purpose of sexual intercourse. They also believe that the primary role of the woman in society is to bear children. But Taoists and Confucians shared the idea that men's *yang* can be nurtured by women's *yin*, while women become healthy by absorbing *yang*. However, it is not clear how or why *yang* and its *jing* can be enhanced by *yin* and *yin* nourished by *yang*.

Ancient Chinese thought that it is the fifth day after menstruation when women can conceive because on that day women's *yin* abounds most. So men should ejaculate on that day. Before and after this date, men should restrain themselves from ejaculation in order to keep their *jing* and absorb women's *yin* as much as possible. Thus they can use women's *yin* to enhance their *yang* which will rise up to the brain along the spine (*bu jing huan nao)*. So if a man can keep strong *jing* or *yang* when he has intercourse with a woman on the fifth day after her menstruation when *ying* in her abounds most, then she will become pregnant and give birth to a healthy son. Due to strong sexist views at the time, ancient Chinese writers never mentioned giving birth to a girl. If a man ejaculates at each time of intercourse with any woman, his *jing* or *yang* will be exhausted, he will not be able to have a child, and he will even fall ill or may die prematurely *(5,6)*.

Few philosophers discussed sex for the purposes of pleasure. One of them was Bai Xingjian (?–826), the brother of a famous poet Bai Juyi in the Tang Dynasty, who wrote that sex is the greatest joy in human life *(5,6)*. Sex for pleasure and sex for health are compatible. In Chinese traditional medicine, a healthy life entails a life with pleasure. However, for Confucians a healthy life entails a life with virtue or morality based on Confucian ethical principles. Therefore, the order of priorities in the purposes of sex should be: reproduction, health and pleasure.

In Confucian texts and tradition, infertility is always blamed on women. However, in traditional Chinese medical texts it is recognized that there are three elements that control reproduction: a man's *shen* (kidney), woman's *tiankui* and the *chong-ren* channels. It should be noted that in such texts the words kidney, heart, lung, liver and spleen not only refer to organs, but are also used symbolically and refer to certain functions. The kidney keeps *jing qi* and controls growth, development and reproduction. *Jing* is the root of the human body *(7)*. *Jing* is partly inherited from the parents, and partly generated from water and food. *Jing qi* has the ability to promote the growth and development of the human body and also controls the ability to reproduce. During adolescence, *jing qi* in the kidney or kidney *qi* increasingly flourishes, and in ageing kidney *qi* is in decline. If an infant is short of *jing qi*, it will suffer from hypoplasia or dysplasia. If a young man is short of *jing qi*, he will suffer from impotence, infertility or premature ageing.

Tiankui is called "shapeless water" which controls menstruation (*8)*. Ancient physicians believed that a good order of menstruation is indispensable for reproduction, and if *tiankui*, as a variant of *yin* is abnormal, it will lead to disorders of menstruation.

Chong-ren channels are branches of the *jingluo* system which come from the head to the kidney via the liver; their function is to enhance *shen*. If they are obstructed, the *shen* will be weakened and reproduction will be affected.

Explanations of infertility

In traditional Chinese medical texts, two different words are used to describe infertility. Man's infertility is called *buyu* which means the "inability of insemination", and woman's infertility is called *buyun* which means "inability to be pregnant".

Writings from all periods of Chinese history

referred to the problem of infertility and tried to give some explanations for its causes. Wan Quan described the abnormal structure of the female reproductive organs (the vagina in particular) as a cause of infertility *(9)*. Sun Simiao, the author of *The sincerity of great physicians* (the equivalent of the Chinese Hippocratic Oath), pointed out that a closed uterus makes a woman unable to conceive, and that a man who suffers from any serious diseases that can make the *jing* and *qi* cold will become infertile *(10)*. Other causes of infertility included: eating some poisonous herbs, inbreeding, excessive sex, suffering from serious diseases, etc. *(3,11–13)*.

Moreover, infertility was believed to have moral causes. People with Confucian or Buddhist beliefs considered infertility as a retribution for wrongdoing either by the man, the woman or even their ancestors who might have led an immoral life. According to Buddhist beliefs, if a man does something wrong in his current life, as a retribution he will have no offspring in his next life.

Confucian morality

In Chinese culture, Confucianism is dominant and it has profound and wide influence in the Far East, especially in the Republic of Korea, Japan, Singapore, but less so in Malaysia, the Philippines, Thailand and Viet Nam. Confucianism is mainly the philosophy and ethics of morality, which teaches one how to be a human. According to Confucianism, humans are born biologically, but not morally. Therefore, one has to learn how to be a human in the moral sense. Confucian ethics are embodied in social conventions and institutions.

The central concept of Confucianism is *ren* (humaneness), which means love and care. There are a number of ethical principles in Confucian ethics which embody *ren* and regulate interpersonal relationships, such as filial piety (*xiao*) for children–parents, relationship, fraternity (*ti*) for brotherhood and sisterhood, trustworthiness (*xin*) for friendship, kindheartedness, and benevolence (*ci*) for doctor–patient relationships, etc. However, among these principles, filial piety serves as the basis for the others. According to Confucius the best way to learn *ren* is from near to far: "If you learn how to care about your own parents and sisters/brothers, then you can learn how to care about others" (*The analects of Confucius*). This idea is still very strong in China.

Filial piety requires that people extend the life of their ancestors, and make their family unlimitedly continuous from generation to generation. However, only a son, not a daughter, can do that. Some traditions in Japan seem to share this idea *(14)*. According to Mencius:

> There are three vices that violate the principle of filial piety, and the biggest is being without an offspring *(2)*.

In Confucianism there are some strong sexist views. For example, Confucians emphasize the concept of *san cong* (three types of obedience) for women, that is obedience to one's father before marriage, obedience to the husband after marriage, and obedience to the son after the husband's death. In this context, a husband can divorce his wife using infertility or a serious disease as a reason *(5)*.

For Confucians, a person is not an independent entity. On the contrary, a person exists only in an interdependent relationship with other persons, especially with other members of her/his family or community. So a person is a relational person, or person-in-relation. This concept implies that when making a moral judgement and behaving accordingly, the context in which the moral agent exists has to be taken into account. While the context is taken into account, values which may be in conflict should be balanced as well as all possible consequences resulting from the various options.

Attitudes towards infertility

The Chinese attitudes towards intervention in reproduction have been influenced by cultural values and morality as well as by the perceptions of sex and reproduction.

Value of reproduction and family

In oriental cultures, reproduction is one of the highest values linked with the significant value of the family. This is clearly stated in the Confucian classic *The book of rites*:

> Gentlemen (*jun zi*) [should] pay great attention to the marriage that unites two families into one in order to serve ancestors in the temple of family and extend the family to

> future generations. Man and woman are different, so there is affection between husband and wife, then there is kinship between father and son, then there is political relation between king and his subjects *(3)*.

When talking about the value of family, Japanese scholars emphasized the spiritual tie within or between family members *(14)*. Actually, the spiritual tie is based on blood tie and conjugation. According to Confucianism, conjugation between a husband and wife is the basis of the family and country. Any nonconjugal reproduction will weaken the blood tie between the members of a family and lead to its instability. And the value on reproduction and family is closely related with morality.

Attitudes towards infertility and assisted reproduction

The desirable product of reproduction is a healthy baby. In Chinese culture healthy birth is of an imperative value. The Confucianist, Xun Zi (next to Confucius and Mencius) argued that "Birth is the beginning of a person, and death is the end of a person. If you have a good beginning and good end, you fulfill the human Tao." A good beginning and good end have been interpreted as healthy birth and peaceful dying; both very important values in the Chinese culture as well as for other cultures in the Far East. Chinese attitudes to infertility and assisted reproduction are influenced by three values: life-preserving or health-promoting, reproduction and pleasure. Of these three values, priority is usually given to reproduction, and pleasure comes last. Confucianism rejects pleasure as a value of sex, despite the fact that in practice almost everybody pursues pleasure. Preservation of life or promotion of health are desirable because they are in compliance with filial piety: life and body are imparted by the parents, so the offspring has the responsibility of preserving it and keeping it healthy. Excessive sexual activity is believed to lead to the shortage and weakening of *jing* or *yang* and then to infertility.

Living in such a pronatalist society, people who experience infertility while maintaining cultural traditions are often under psychological and social pressure. In a traditional family, the wife has a lower status and an infertile wife has an even lower status with the risk of being divorced. The infertile husband feels responsible for not continuing the biological lineage. The infertile couple worry that they may be stigmatized in the community. As a result, they will make every effort to seek treatment for their infertility.

In general, any intervention in natural reproduction is not desirable because it disturbs the *dao* of nature. However, disturbing the *dao* of nature is more acceptable than being without an offspring.

Artificial insemination by donor (AID)

In a Confucian society, infertility is blamed on the woman and the husband is permitted to marry a second wife or borrow a wife. In the case of AID, the husband is in a dilemma as he has to make a choice between sex without reproduction or reproduction without sex. A traditional Chinese will choose the latter and that is why AID is well accepted in China.

A couple in Shanghai married for many years but childless went to a clinic for help. The physician diagnosed that the husband could not ejaculate and suggested that they use AID. The wife gave birth to a boy. They were back home joyfully. But the husband's parents thought that the child did not look like their son, and suspected that their daughter-in-law had had an affair. After the couple explained to them that they had used AID, the grandparents still refused to accept the child. The result was the couple divorced and the wife brought the child back to her mother's home *(15)*.

In another case, all family members agreed that the infertile son's wife could have sex with his brother for the purposes of reproduction. In this case, the values conflict, but more weight is put on having offspring than fidelity.

Another problem is that Chinese men are reluctant to donate sperm because they think sperm contain *jing* or *yuan qi* which are indispensable for their health and life. As a result, medical scientists and health administrators are concerned about incest since the availability of sperm donors is disproportional to the demand for AID and therefore in some cases sperm from one donor is used for large numbers of inseminations.

In vitro fertilization, cryopreservation and transfer

In vitro fertilization (IVF) is acceptable when the gametes of the couple are used. Sperm and embryo cryopreservation and embryo transfer are not

objected to. However, there are concerns about whether the environment in which the sperm or the embryo are preserved is rich with vital energy (*jing* or *yuan qi*), although there is no way to determine vital energy and its possible effect on ART procedures.

Intracytoplasmic sperm injection

The Chinese culture would not favour intracytoplasmic sperm injection (ICSI) because in the husband's immature or possibly damaged spermatozoa *yang* or *jing* or *yuan qi* will be deficient, and this may lead to the birth of an unhealthy or congenitally defective child. Despite this belief, some infertile couples would probably take the risk of undergoing ICSI, as this would give them the opportunity to have a child genetically linked to the father.

Surrogate motherhood

Surrogate motherhood is controversial. In the Hong Kong Special Administrative Region of China, the government promulgated a regulation in which commercialization of surrogate motherhood and nongenetic surrogacy (in cases of homosexuals, single parents and unmarried individuals) are banned. The regulation provides that the surrogate mother cannot be forced to give the child to its adoptive parents, because during gestation a mother-to-child relationship was developed. However, some scholars *(16)* suggested a comprehensive ban on surrogate motherhood on the basis of an argument of "do no harm". They argued that unlike sperm or oocyte donation, surrogate motherhood is not confidential and that might eventually harm the adoptive family. Moreover, the strong ties between the surrogate mother and the future child may eventually create problems for the surrogate mother, adoptive parents and the child. Their suggestion was adopted by the Chinese Ministry of Health which prohibited surrogate motherhood in the recently published *(17) Measures of administration of human assisted reproductive technologies* (Article 22). Some experts have already opposed this prohibition and have proposed the permission of noncommercial surrogacy.

Commercialization

In many traditional cultures of the Far East the human body is sacred. Commercialization of body parts will be considered as an insult to human dignity. However, the human embryo is not viewed as a person (although there is a time limit, for example, for some research on embryos more than 14 days should be banned). Yet, in *Measures of administration of human assisted reproductive technologies* (Article 22), and the *Measures of administration of human sperm bank (17)* the Chinese Ministry of Health also prohibits buying and selling of gametes, zygotes and embryos. The prevailing Chinese viewpoint is that even though gametes, zygotes, embryos and fetuses do not have the moral status of a person, they should still not be treated as commodities.

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Section 3

Recent medical developments and unresolved issues in assisted reproductive technologies

Gamete source and manipulation

HERMAN TOURNAYE

Introduction

According to the World Health Organization *(1)*, 51.2% of couples are infertile because of a male factor and 39% of these men present with an abnormal semen analysis for idiopathic reasons. Obviously, no specific treatment is available for these men. The second largest category (23%) is men presenting with a varicocele. While varicocelectomy is the specific treatment for this condition, meta-analysis of randomized controlled studies has failed to show any benefit of this approach *(2)*. The third largest category is men suffering from male accessory gland infection (12%). But again this condition has been under debate and reports on its impact on male fertility have been inconclusive *(3)*.

Because many men have either no demonstrable cause for their abnormal semen profile or suffer from conditions for which treatment is debatable, less than 20% of men with reproductive failure have potentially treatable conditions for which a rational or proven effective treatment is available *(4)*. Furthermore, meta-analysis has shown that empirical treatments for unexplained male infertility not only confer no benefit but may also have side-effects *(5,6)*.

As improving the fertility status of one partner may result in an increased fertility status of the couple as a whole, in men with borderline semen profiles, any improvement in the female fertility status will have an important impact on the couple's fecundity.

When treatment of the female partner has failed, techniques of assisted reproduction are usually employed. Their common rationale is to enhance the probability of fertilization by bringing the spermatozoa closer to the oocyte(s). The choice of the most appropriate assisted reproduction treatment for the individual couple is often based on the quality of the ejaculate or the source of the gametes.

Male gametes and assisted reproduction

Ejaculated spermatozoa

Ejaculated spermatozoa may be used either for intrauterine insemination (IUI), conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

The objective of IUI is to bypass the filtering effect of the cervical mucus by placing thousands of motile spermatozoa directly into the uterine cavity by means of a catheter. After the semen is washed, it is centrifuged over a density gradient or the sperm is left to swim up in a medium. In this way, motile spermatozoa can be selected and their motility characteristics can be enhanced. Finally, a few hundreds or thousands to even a few million motile spermatozoa can be loaded into an insemination catheter with a volume not exceeding 0.5 ml in order to prevent uterine

contractions. The numbers of spermatozoa in the ejaculate together with the female fertility status and the timing of insemination, are the most important factors in the success of treatment by IUI.

Apart from IUI, other techniques of artificial insemination have been described. In "intrafallopian insemination", washed spermatozoa are placed directly into the fallopian tube on the side of the dominant ovarian follicle, preferentially under sonographic guidance. A variant of this technique is "fallopian sperm perfusion" (FSP) in which a suspension containing 10–100 x 10^6 motile spermatozoa is slowly flushed through the uterine cavity and into the fallopian tubes. In "direct intraperitoneal insemination" (DIPI), washed motile spermatozoa are introduced in the pouch of Douglas around the moment of ovulation by transvaginal puncture using a fine butterfly-type needle. A last variant is the "intrafollicular insemination" in which a small volume of a suspension containing washed spermatozoa is put into a preovulatory follicle using transvaginal puncture. According to several controlled studies, these alternative insemination techniques do not appear to be superior to standard IUI *(7,8)*.

The objective of IVF is to bring the spermatozoa even closer to the oocyte than is the case with IUI. In IVF, gamete transport, gamete interaction and fertilization take place outside the woman's body. Since this technique involves several steps, each with their specific limitations in terms of success, IVF needs a relatively large number of oocytes and spermatozoa. The female partner undergoes ovarian stimulation to increase the number of oocytes available for insemination *in vitro*.

IVF was introduced in the late 1970s for the treatment of women with problems of oocyte and sperm transfer caused by tubal dysfunction. Because only a few thousands of motile spermatozoa were needed to obtain fertilization, IVF was also proposed as a treatment of infertility due to oligoasthenoteratozoospermia. While in normozoospermic males, 60%–70% of the oocytes will be fertilized normally after *in vitro* insemination, this percentage may drop to less than 50% when oligoasthenoteratozoospermia is present. Not only is the fertilization rate significantly decreased, but complete fertilization failure of all oocytes may occur in up to one out of four couples *(9)*. The cumulative conception rates and cumulative live birth rates have been reported to be 28.0% (95% confidence interval [CI] 19.1%–40.0%) and 17.8% (95% CI 11.5%–26.9%), respectively, after three IVF treatments and even after five IVF treatment cycles, these figures do not improve *(10)*.

Several alternative techniques to *in vitro* fertilization-embryo transfer (IVF-ET) have been introduced. Gamete intrafallopian transfer (GIFT) and zygote intrafallopian transfer (ZIFT) are among the most popular. In these techniques, oocytes and spermatozoa or fertilized oocytes are transferred into the fallopian tube, respectively. However, meta-analyses of randomized controlled trials (RCTs) on these alternative techniques do not show any benefit over classical IVF-ET *(11,12)*, especially in cases of male infertility in which fertilization is the major bottleneck. Considering the increased risk of side-effects from laparoscopic transfer in GIFT and ZIFT and the lack of any evidence that their results are superior to the standard IVF technique, these alternative techniques should not be used as primary treatment options.

In contrast to IVF, ICSI uses only a single spermatozoon to inseminate an oocyte. This spermatozoon is injected into the cytoplasm of the oocyte by means of a micromanipulator, thus bypassing capacitation and hyperactivation, recognition of specific zona pellucida receptors, acrosome reaction and penetration of the zona pellucida and oolemma. Once introduced into the oocyte, the nucleus of the spermatozoon will decondense and form the male pronucleus.

Theoretically, ICSI needs only a genetically functional paternal genome, a functional microtubule-organizing centre (the paternally inherited sperm centrosome), and an oocyte-activating factor. The first successful application of this invasive insemination technique was reported in 1992 *(13)* and since then it has been used extensively worldwide for the treatment of male infertility.

ICSI is highly efficient in cases of severe male infertility that would otherwise be untreatable. The success of ICSI, however, has led to its overuse. An obvious reason for this is the avoidance of complete fertilization failures. The risk of fertilization failure after conventional IVF with normozoospermic semen has indeed been a frustrating event ever since the introduction of IVF. Unexplained failure of fertilization after IVF in normozoospermic males is observed in about 10% of IVF cycles. In contrast, fertilization failure after ICSI occurs in less than 3% of cycles and is recurrent in only 15% of cases *(14,15)*.

In a meta-analysis of RCTs dealing with "borderline semen parameters", more oocytes were found fertilized after ICSI than after conventional IVF: the

relative risk (RR) (calculated on a per-oocyte basis) was 2.2 with a 95% CI of 1.6–3.0 in favour of ICSI. The RR (calculated on a per cycle basis) for a complete fertilization failure was 10.8 (95% CI 3.1–38.1) and the number needed to treat (NNT) was 2.6 (95% CI of 1.5–10.1). However, when only studies using "high insemination concentration" IVF were considered, the RR for an oocyte to become fertilized *in vitro* was 1.1 (95% CI 0.7–1.7), again in favour of ICSI. The NNT was 4.1 with a 95% CI of 1.4–4.9 *(16)*. This meta-analysis clearly shows that ICSI significantly improved the probability of fertilization in couples suffering from male infertility. However, given the wide confidence intervals and the ongoing debate regarding the safety of ICSI, proposing ICSI to all couples with borderline semen values may not be the best first-line treatment option. Several authors have proposed a split IVF/ICSI management scheme when performing assisted reproductive technology (ART) in couples with male infertility, while others prefer to perform at least one IVF procedure before starting ICSI. In practice, the choice between the "straight ICSI" approach, the "first-IVF" approach and the "split IVF/ICSI" approach will depend mainly on differences in defining the "borderline semen profile", insemination procedures, patient selection, centre performance, reimbursement policies and even patient preference.

After five cycles of ICSI, a cumulative live birth rate of 60% has been reported in patients less than 38 years old having their first course of ICSI treatment *(17)*. Fertilization failure after ICSI using ejaculated spermatozoa is rare (2.8% of ICSI cycles) and is due mainly to defective oocyte activation. From a clinical viewpoint, fertilization failure after ICSI is related to a sperm factor in half of the cases, including the absence of motile spermatozoa or the injection of spermatozoa with severely abnormal morphology *(14)*.

Ejaculated spermatozoa from anejaculatory patients

When no ejaculate can be produced, several strategies exist to obtain spermatozoa for assisted reproduction.

Retrograde ejaculation

In retrograde ejaculation, ejaculation does occur, but is directed into the bladder. Here, spermatozoa are exposed both to hypo-osmotic stress and to a low pH. When specific treatments are not available or indicated, spermatozoa may be recovered from postmasturbatory urine and subsequently used for assisted reproduction. The urine osmolality should be adjusted and alkalinization should be performed to improve the quality of the spermatozoa. The number and the motility pattern of the spermatozoa recovered will determine whether they may be used for IUI, IVF or ICSI *(18)*.

Anejaculation

Anejaculation can be part of a general problem of erectile dysfunction. While there are several treatment approaches possible, including psychotherapy or medical treatment, assisted reproduction may be used in some cases.

Acute ejaculation occurs relatively frequently even in patients undergoing ART. The production of an ejaculate is for many patients a stressful event which may induce the problem of anejaculation. Administration of sildenafil citrate may overcome the problem of an unexpected, acute erectile dysfunction *(19)*.

Most patients with a chronic anejaculatory problem from a neuropathy or spinal cord injury who need assisted reproduction, will undergo either penile vibrostimulation (PVS)*(20)* or electrostimulation *(21,22)* in order to obtain an ejaculate. Vibro-stimulators are applied to the penis to induce ejaculation. The best results are obtained using high-amplitude penile vibrostimulators which deliver an amplitude of at least 2.5 mm. Only vibrators intended for medical use will deliver this amplitude. To obtain ejaculation with PVS, both the cauda and conus of the spinal cord must be intact up to the S-2 level. Ejaculation rates in spinal cord injured are around 60%, while in idiopathic anejaculation this figure is about 70% *(18)*. Spermatozoa obtained by penile vibro-stimulation can be used for either IUI or IVF, depending on the quality of the sample obtained.

As an alternative, or when penile vibrostimulation fails, electrostimulation can be performed. In this technique, ejaculation is induced by introducing an electrode in the rectum and using electrical pulses to stimulate the nerves and muscles involved in ejaculation. Overall, in different categories of patients, electroejaculation is successful in about 90% of patients *(18)*. Again, according to the semen quality, insemination, IVF or ICSI may be performed.

Nonejaculated spermatozoa

Epididymal spermatozoa

In patients with obstructive azoospermia, fertility can be restored by surgical correction, i.e. vasoepididymostomy, vasovasostomy or perurethral resection. When surgery has failed or is not indicated, as with patients with congenital bilateral absence of the vas deferens, for example, spermatozoa can be aspirated from the epididymis and used for assisted reproduction. Spermatozoa can be recovered by microsurgical epididymal sperm aspiration (MESA) or percutaneous epididymal sperm aspiration (PESA). Before 1992 and the introduction of ICSI, the first pregnancies with ART using epididymal sperm were achieved with conventional IVF *(23,24)*. However, the success rate of fertilization after conventional IVF with epididymal sperm was poor. The most important factors related to the success of IVF with epididymal spermatozoa are sperm maturity and the number of motile sperm retrieved; often only a few spermatozoa showing progressive motility are obtained after epididymal aspiration. ICSI, on the other hand, yields high fertilization and pregnancy rates when epididymal spermatozoa are used *(25)*. Review of the literature from different studies shows that the fertilization rates reported after ICSI using epididymal sperm ranges from 39% to 65%, with clinical pregnancy rates ranging from 12% to 39% per ICSI cycle *(26)*. These variations are most probably due to differences in the age of the female partners and in the number of treatment cycles included in the data. Epididymal sperm may be cryopreserved and used for ICSI after thawing. Several reports show similar outcomes after ICSI using either fresh or frozen-thawed epididymal spermatozoa *(27–32)*.

Testicular spermatozoa

When no motile spermatozoa can be retrieved because of epididymal fibrosis, testicular sperm retrieval may then be an alternative. Testicular sperm can be retrieved by different techniques *(33)*: testicular sperm extraction or TESE (this refers to open excisional testicular biopsy), testicular sperm aspiration or TESA (refers to any method by which testicular sperm are aspirated percutaneously) or fine-needle aspiration (refers to any aspiration technique using needles of 21 gauge or thinner).

Most azoospermic patients suffer from primary testicular failure or nonobstructive azoospermia. However, in a few cases, nonobstructive azoospermia may be the result of hypogonadotrophic hypogonadism. These patients need hormonal stimulation by follicle stimulating hormone (FSH) and luteinizing hormone (LH) or pulsatile gonadotrophin releasing hormone (GnRH) to restore their fertility and they do not, in the first instance, need assisted reproduction.

Most patients with primary testicular dysfunction resulting in azoospermia show a Sertoli cell-only pattern in their testicular histology. Others have maturation arrest or have sclerosed and/or atrophied seminiferous tubules. Notwithstanding these findings, a few seminiferous tubules may still show occasional foci of active spermatogenesis. The classification proposed by Levin *(34)* has proven useful when dealing with men suffering from nonobstructive azoospermia undergoing multiple testicular biopsies. When the main prevailing histopathology is germ cell aplasia (Sertoli cell-only syndrome), but some tubules do show active spermatogenesis, the terminology of incomplete Sertoli cell-only syndrome is used. The same goes for maturation arrest: when full spermatogenesis is present in a few tubules, the condition is referred to as incomplete maturation arrest. Hypospermatogenesis is an ill-defined condition with active spermatogenesis in all tubules present, but with only a few spermatozoa developing. Many men with hypospermatogenesis may have an obstruction as the main cause of their azoospermia, rather than a severe testicular dysfunction. The diagnosis of "nonobstructive azoospermia" should be made according to the histopathological findings, rather than on the basis of only clinical indicators such as FSH levels or testicular size.

The sperm recovery rates in men with nonobstructive azoospermia varies between 40% and 70% according to the recovery technique used *(35–40)*. Differences in recovery rates may also be due to differences in the patient populations studied. Some studies refer to nonobstructive azoospermia when no histological diagnosis is obtained or they tend to include a majority of patients showing hypospermatogenesis. In the latter category of patients spermatozoa will always be found *(39)*.

Often, testicular spermatozoa are recovered only after examining and preparing multiple excisional biopsies *(38)*. Even in about half the patients with a nonmosaic 47,XXY Klinefelter syndrome, mature spermatozoa can be recovered from multiple testicular biopsies *(38,39)*. Multiple biopsies can be taken through several small incisions into the tunica

albuginea or by one large opening through the tunica which provides a maximal exposure of the testicular mass of seminiferous tubules. With the latter approach, using microsurgical inspection at a 40–80 times magnification, the more distended tubules can be selected for excision *(38)*.

As for epididymal spermatozoa, cryopreservation of testicular sperm may be a method to avoid repeated surgery and even unnecessary controlled ovarian hyperstimulation in the female partner of a male with nonobstructive azoospermia due to primary testicular failure. At present, however, the results are inconclusive as to whether the outcome after ICSI with frozen-thawed testicular spermatozoa is comparable to ICSI using fresh spermatozoa. Some authors report similar outcomes *(36,41)*, while others report a decrease in success rates *(42–44)*.

Other sources of nonejaculated spermatozoa

There have been reports on the recovery of sperm from the vas deferens *(45,46)*. This technique has been reported to yield many spermatozoa and they may be especially useful in patients with anejaculation in whom vibrostimulation or electroejaculation has failed. However, both vasal sperm retrieval and retrograde epididymal sperm collection may result in the collection of more dysfunctional senescent sperm or sperm with damaged DNA, since in patients with obstruction the most distal sperm are the most senescent.

Immature gametes

Spermatogenetic cells

When no testicular spermatozoa can be recovered, more immature testicular spermatogenetic cells can be used for ICSI *(47)*. There have been several reports on the use of spermatids to treat male infertility. Both spermatids from ejaculates and from testicular biopsies have been used. However, only one group has reported fertilization and subsequent pregnancies with ejaculated spermatids *(48)*. Most pregnancies reported after spermatid injection are pregnancies obtained with elongated and round spermatids from testicular biopsies, often derived from patients with normal spermatogenesis *(49)*. The number of pregnancies reported so far is still limited.

A pregnancy has been reported after ICSI with secondary spermatocytes, a spermatogenetic cell with a diploid DNA content undergoing the second meiotic division *(50)*.

If azoospermia is the result of an arrest in spermatogenesis at the primary spermatocyte state, then only diploid cells are available and ICSI is not feasible. Yet, a recent paper reported ICSI with spermatids obtained after *in vitro* culture of primary spermatocytes. Two out of five men with premeiotic arrest of spermatogenesis developed spermatids *in vitro* which were used for ICSI and a set of twins was born *(51)*. So far, these results have not been confirmed by others.

Testicular tissue

The seminiferous tubules contain spermatogonia, cells that have the capacity to renew themselves and to initiate meiosis at puberty. Only a small proportion of spermatogonia—the stem cells, have the potential to renew themselves. Recently, research in mice has shown that spermatogenesis can be re-initiated after transplanting testicular stem cells *(52)*. It was also shown that the transplanted mice could reproduce *in vivo* after transplantation. These experiments were also performed using stem cells that had been frozen and thawed *(53)*. These experiments, extrapolated to a human model, may have important applications. When an adult male undergoes sterilizing chemotherapy, spermatozoa can be frozen to circumvent sterility after treatment. However, such treatment is not possible before puberty since no active spermatogenesis is present. Here, testicular tissue containing stem cells may be cryopreserved before any cytotoxic treatment. These stem cells can then be thawed when their donor has reached adulthood and can be reintroduced into his empty seminiferous tubules (autologous transplantation). Other applications may be even more futuristic; it has been stated that it may become possible to develop human testicular stem cells to maturity in surrogate animals after transplantation. So far, preliminary experiments have not been successful. Transplantation of testicular stem cells from hamsters, rabbits and dogs has failed to initiate spermatogenesis in recipient mice, probably because the phylogenetic gap between these species is too wide. However, the phylogenetic gap between great apes and humans is smaller than that between rats and mice, a model in which xenotransplantation has worked.

Oocytes and ovarian tissue

The female partner of an infertile couple requiring ART needs to undergo ovarian hyperstimulation to recover a larger number of oocytes. In most procedures, some loss of oocytes occurs at each stage—oocyte retrieval, fertilization, embryonic development *in vitro*, and implantation. Therefore, attention has been directed towards the possibility of the *in vitro* maturation of oocytes which may circumvent the need for superovulation and the use of expensive drugs. It may also reduce the risks related to superovulation. However, oocyte retrieval will still be necessary. At present, the use of immature oocytes for ART has been limited and some pregnancies have been reported in patients undergoing short ovarian stimulation followed by a short *in vitro* maturation of the so-called immature oocytes. The definition of "immature oocytes" can be diverse. In most studies, oocytes in their final stage of maturation are further matured *in vitro*, reducing ovarian stimulation by a few days *(54)*.

In vitro maturation may be useful where cryopreservation is employed. Cryopreservation of unfertilized mature oocytes (metaphase-II oocytes) has so far not been successful *(55)*. Results of ART are very variable and although pregnancy rates ranging from 1% to 10% per thawed oocyte have been reported, oocyte survival and function is limited even in those series of studies that focused on selected patients. When expressed per frozen oocyte, the success rate in terms of a viable pregnancy is around 1% per oocyte. Better results have been reported in studies using oocytes that survived the thawing procedure.

Cryopreservation of immature oocytes may, however, become more successful, as these oocytes may be less sensitive to damage during freezing or thawing. These oocytes, which are embedded in the ovarian cortex, cannot be retrieved by ovarian puncture and have to be recovered directly from the ovarian cortex of an excised ovary. Collection of immature oocytes will probably not be an issue for patients undergoing fertility treatment, but may become an important issue for young girls who need to have sterilizing chemo- or radiotherapy. If immature oocytes can be retrieved from the ovarian cortex and can be successfully cryopreserved, this may provide a means to overcome this problem *(56)*.

Cryopreservation of immature oocytes is still in an experimental phase and the techniques for freezing of ovarian tissue or the isolated follicles need to be optimized *(57)*. When ovarian cortex or the isolated primordial follicles are frozen, an 80% survival rate can be obtained. However, these oocytes need to mature subsequently to obtain their capacity to be fertilized. Currently, two possibilities are being investigated: either the cortex or the primordial follicles are matured *in vitro* in order to obtain metaphase-II oocytes which can than be used for assisted reproduction, or the ovarian cortex is autotransplanted into the recipient in the natural site (peritoneal cavity) or in another site ensuring easier retrieval of oocytes once *in vivo* maturation occurs. In the mouse and sheep, live young have been obtained after transplantation. In the human, only transplantation to the peritoneal cavity or transplantation under the skin of the forearm has been performed with limited results *(58)*.

Artificial oocyte activation

When a spermatozoon fertilizes an oocyte, it liberates a factor which induces the release of calcium from the oocyte. Since this release happens in a pulsatile fashion, this factor has been named oscilline. Oscilline is not present in round spermatids and, therefore, when ICSI is performed with these immature gametes, oocyte activation is deficient *(50)*. This problem can be bypassed by artificially activating the oocyte using an electrical current to increase the inflow of calcium over the cell membrane, or by using a chemical such as a calcium ionophore, which causes an increase in intracellular calcium that triggers further steps of fertilization.

This factor may be deficient also in men with absent spermatozoal acrosomes (globozoospermia or round-headed spermatozoa) *(59)*. Again, artificial activation may help their spermatozoa to fertilize an oocyte successfully with ICSI.

However, the use of a calcium ionophore has raised a lot of concern since this compound is a suspected mutagen and has not been approved for human use (see below).

Ooplasmic transfer

In 1997, Cohen and co-workers introduced an ooplasmic transfer procedure *(60)*. With this technique, small amounts of cytoplasm of donor eggs (5%–15%) are injected into oocytes that are assumed to be deficient in factors important for further embryonic development. The approach is empirical since it supposes that embryonic failure results from

these deficiencies in the oocyte's cytoplasm. The injected cytoplasm may contain mRNAs, proteins, mitochondria and other unknown factors or organelles. Recently it has been reported that offspring born after ART using these "upgraded" oocytes show mitochondrial DNA heteroplasmy *(61)*. About 30 babies have been born worldwide following the application of this approach in selected patients *(61)*. No controlled study has so far been reported establishing the benefits of this technique.

Indications and contraindications, benefits and eligibility of patients

Ejaculated spermatozoa as a source of gametes

Spermatozoa for IUI and IVF

IUI is indicated in men with idiopathic oligozoospermia, asthenozoospermia, teratozoospermia or a combination of these conditions. The technique may also be useful in case of male infertility due to retrograde ejaculation, anejaculation or hypospadias. IUI is also possible in the treatment of cervical factor infertility, immunological infertility and unexplained infertility.

Before intrauterine instillation, the ejaculate has to be washed to prevent uterine contractions induced by the presence of prostaglandins in the semen but washing may increase the generation of reactive oxygen species in the sample, which may adversely affect sperm function *(62)*. Using additional procedures, motile spermatozoa can be selected and their motility enhanced. In this way, a few hundreds or thousands to even a few million motile spermatozoa are concentrated in a small insemination volume not exceeding 0.5 ml. Thus ten times more spermatozoa may reach the uterine cavity as a result of using this technique compared to the normal physiology of sperm migration. However, there is an important qualitative difference: after *in vitro* sperm selection techniques, another, less functional, population of spermatozoa will be selected than after selection *in vivo* by the cervical mucus. For IUI, at least one million progressively motile spermatozoa should be obtained after preparation, otherwise the technique is not very successful *(63)*.

Commonly used methods for sperm preparation are the "swim-up" procedure and discontinuous density gradient centrifugation. While the swim-up procedure can be applied only on high-quality samples, density gradient centrifugation is used on semen samples of lower quality in order to recover a reasonable number of motile sperm. The swim-up procedure is not used any more, as density gradient centrifugation results in higher sperm recoveries and even better IVF rates in certain categories of patients. Moreover, the swim-up procedure has a higher risk of generating reactive oxygen species.

Two independent meta-analyses have been published investigating the benefits of IUI *(63,64)*. In overall comparisons between IUI and natural intercourse and/or intracervical insemination, IUI has been shown to be effective in male factor infertility. In the larger meta-analysis by Cohlen, a significant increase in fertility was found as a result of using IUI compared with natural intercourse, with or without ovarian stimulation.

Cohlen *et al. (63)* also performed a meta-analysis of four randomized controlled trials comparing IUI in natural cycles versus cycles with controlled ovarian hyperstimulation. The pregnancy rate was 12.6% in stimulated cycles versus 7.3% in unstimulated cycles. Although there was a tendency for controlled ovarian hyperstimulation to increase fertility (OR 1.80) the difference was not significant (95% CI 0.98–3.3). In the studies using controlled ovarian hyperstimulation under review, the multiple pregnancy rate was 5.5% (all twins) and the incidence of developing the ovarian hyperstimulation syndrome (OHSS) was 0.8%.

IVF is indicated for infertility caused by tubal blockage, severe endometriosis (not manageable by surgery or after failure of surgery), polycystic ovarian syndrome with uncontrollable ovulation induction, the presence of antisperm antibodies in the male or female partner and oligoasthenoteratozoospermia in the male partner. IVF may also be indicated in some couples with unexplained infertility. However, for many of these indications alternative treatments may exist and therefore, the risks and benefits of each treatment strategy should be compared.

The lower limits for sperm numbers compatible with fertilization after conventional IVF are poorly defined. In most centres, a cut-off value for the number of progressively motile spermatozoa after semen preparation is defined so as to allow conventional IVF. A minimum total number of rapid (type A, >25 μm/s) and slow (type B, 5–25 μm/s) progressively motile spermatozoa together is generally used *(65)*. Payne *et al. (66)* used one of the lowest cut-off values

of 200 000 motile spermatozoa obtained after a mini-Percoll preparation, but most programmes will prefer 500 000 or more type A+B spermatozoa for conventional IVF.

Apart from the numbers, sperm morphology according to the strict Tygerberg criteria is one of the most frequently used single semen parameters to screen for conventional IVF. A literature review showed that sperm morphology analysis according to the strict criteria represents the most effective indicator of male fertility potential in IVF. Fertilization failure will occur in most patients where there are fewer than 5% morphologically normal spermatozoa in the ejaculates *(67)*. Increasing the number of motile spermatozoa at insemination has been proposed to improve fertilization in cases with borderline semen characteristics, including those with sperm morphology below the 5% value *(68,69)*. A recent review shows that in the studies where these high insemination concentrations were used, the probability of fertilization after IVF was better than that after ICSI *(16)*.

Spermatozoa for ICSI

With ICSI, the spermatozoon is injected directly into the ooplasm and therefore specific gamete interaction defects may be bypassed, including sperm dysfunction at the level of the zona pellucida, zona penetration or fusion with the oolemma. Since most patients with recurrent failure of fertilization after conventional IVF present one or more of these disorders, ICSI may successfully alleviate these forms of infertility. Patients with severe oligozoospermia may also benefit from ICSI even when conventional insemination *in vitro* is impossible. Basically, as long as the same number of motile spermatozoa can be recovered as there are oocytes to be injected, ICSI can be performed with ejaculated spermatozoa. Therefore, an extensive search of the ejaculate should be performed. ICSI is also successful when dealing with absolute teratozoospermia. Patients with immunological infertility may also benefit from ICSI since antisperm antibodies do not interfere with the fertilization process after ICSI *(70)*. When no progressive motile ejaculated spermatozoa can be obtained for insemination *in vitro*, again fertilization can be achieved by ICSI. ICSI has also proved to be a valuable technique using frozen-thawed sperm from cancer patients: even if sperm quality is impaired, ICSI offers superior results and uses a minimal volume of cryostored sperm *(71)*.

However, fertilization failure after ICSI may still occur. Injecting immotile spermatozoa decreases the ICSI fertilization rate *(70)*. Therefore, an extensive search of the centrifuged semen pellet must be done to recover motile spermatozoa and the collection and examination of a second ejaculate may be necessary. When the second semen sample or its centrifuged deposit does not contain motile spermatozoa, immotile but live spermatozoa may be selected by a hypo-osmotic swelling test (HOS test). This test is, however, not useful in patients with 100% immotile sperm due to axonemal structural defects such as immotile cilia syndrome. Patients with the immotile cilia syndrome are sterile because their spermatozoa lack motility, hindering natural gamete-interaction. Pregnancies have been reported after subzonal insemination and ICSI using these spermatozoa *(72,73)*; however, results after ICSI with these spermatozoa remain poor and unpredictable, even after selection by an HOS test *(74)*. The reason why ICSI results are so poor in these patients is not clear. Many of the ultrastructural defects may have concomitant defects affecting other microtubular structures such as the sperm centrosome and deficiencies at this level may be involved in fertilization failure or poor embryonic development *(75,76)*.

In cases of necrozoospermia, viable spermatozoa can still be recovered by a testicular recovery procedure *(77)*. Another category of patients with deficient gamete interaction is those suffering from globozoospermia or round-headed sperm syndrome. In contrast to the immotile cilia syndrome, this is a well-delineated condition in which spermatozoa are devoid of the acrosome and postacrosomal sheath. They cannot attach themselves to and penetrate the zona pellucida, neither can they attach themselves to or penetrate the oolemma of the oocyte. Again, since ICSI may circumvent such gamete interaction defects, patients suffering from globozoospermia may benefit from the direct injection of their spermatozoa in the oocyte cytoplasm. A few pregnancies have been reported after ICSI with round-headed spermatozoa *(78,79)*, but the general results after ICSI with spermatozoa with no acrosome are poor. In those patients with ICSI failure, oocyte activation may be deficient *(59)*. Artificial oocyte activation with a calcium ionophore has been reported to overcome this problem *(80)*.

Epididymal spermatozoa as a gamete source

Epididymal spermatozoa may be used for ICSI in patients with congenital bilateral absence of the vas deferens, Young syndrome, failed reconstructive vasal surgery, azoospermia resulting from bilateral herniorrhaphy with accidental vas deferens occlusion and obstructions at the level of both ejaculatory ducts. Epididymal sperm retrieval methods have been proposed as a means by which spermatozoa may be obtained in men with anejaculatory infertility. However, given the efficiency of assisted ejaculation in these men, surgical methods are only to be considered when penile vibrostimulation or electro-ejaculation has failed. Furthermore, it is preferable in such patients to refrain from epididymal sperm aspiration techniques because of their higher risk of subsequent iatrogenic obstruction *(81)*.

Epididymal spermatozoa can be retrieved using an operating microscope at a magnification of 40 to 80 times (MESA) *(24)*. An alternative technique is PESA. The latter technique has been described in detail by Craft and co-workers *(82)*. By means of a percutaneous puncture using a 19-gauge needle, epididymal sperm may be aspirated blindly from the epididymis under local or regional anaesthesia. Its relatively noninvasive character is the most important advantage of PESA; it can be easily performed on an outpatient basis, it is quick and simple when compared to MESA and is more cost-effective. It has been argued also that there may be fewer complications after PESA than after MESA *(83)*. The sperm recovery rate reported after PESA is comparable to that after MESA *(84)*. Both MESA and PESA may be repeated in the same patient several times with good results in terms of sperm recovery.

The main criticism of the PESA technique is that blind percutaneous puncture may cause inadvertent damage to the fine epididymal structures and produce uncontrolled bleeding causing postoperative fibrosis *(81)*. Therefore, MESA may be the preferred method to retrieve epididymal sperm in patients with obstructive azoospermia in whom surgical repair still remains an option. If no surgical correction is feasible, PESA is to be preferred. The epididymal spermatozoa that are obtained can be easily cryopreserved for later use without jeopardizing the outcome after ICSI *(29)*. If, however, previous investigations have shown that microsurgical reconstruction is not possible, then PESA may be performed since epididymal damage and fibrosis is not an important issue where reconstruction is not possible. After MESA or PESA, epididymal sperm may not always be obtained *(85)*. In this case, recovery of testicular spermatozoa may be attempted.

Testicular spermatozoa as a gamete source

All the indications for using epididymal spermatozoa in ART are valid for testicular spermatozoa. Testicular sperm recovery is also indicated in men with absolute necrozoospermia and has been proposed for anejaculatory patients as an alternative for penile vibrostimulation or electro-ejaculation. However, it is preferable to refer these patients, especially those with spinal cord injuries, to specialized services where assisted ejaculation can be performed. Vibro- or electrostimulation are noninvasive techniques which may be performed without any anaesthesia in paraplegic men. Since scrotal haematoma may take a long time to heal in such men, surgical sperm retrieval techniques are indicated only where these non-invasive techniques fail to produce an ejaculate that may be used for ICSI. Even in these cases, vas deferens aspiration may be preferable because of its low risk of iatrogenic obstruction *(46)*. The ejaculates, even when oligoasthenoteratozoospermic, can be cryopreserved for later use. Testicular sperm retrieval must be considered only when primary testicular failure or obstruction is present in an anejaculatory patient or when techniques of assisted ejaculation have failed to produce an ejaculate that can be used for ICSI. An additional but important indication for testicular sperm recovery is azoospermia in patients with primary testicular failure.

Testicular spermatozoa may be obtained either by open testicular biopsy or by fine-needle aspiration of the testis. Both methods are similar in terms of outcome *(33)*; however, the numbers of sperm obtained after open biopsies are much higher. For this reason, open testicular biopsy may be preferred whenever cryopreservation is desired. Alternative methods of testicular aspiration have been described yielding higher numbers of spermatozoa *(86,87)*. In these aspiration techniques, needles with a larger diameter are used in order to obtain tissue cylinders. Compared to fine-needle aspiration, these alternative methods are less patient-friendly and need local or regional anaesthesia. Sometimes they may need to be combined with a small incision in the scrotal skin. Their main advantage is that cryopreservation is easy and efficient because of the higher numbers of sperm obtained.

In patients with normal spermatogenesis and obstructive azoospermia, all these techniques provide a 100% sperm recovery rate *(88)*. In patients with testicular failure showing different degrees of maturation arrest, germ cell aplasia or tubular atrophy and sclerosis, spermatozoal recovery rates are around 50% with excisional techniques.

TESA has been proposed in order to obtain testicular spermatozoa from patients with non-obstructive azoospermia. However, several prospective controlled studies have shown that the retrieval rate is significantly lower than with excisional biopsies *(33,89,90)*.

Furthermore, in patients with a history of cryptorchidism, testicular aspiration is contraindicated. These patients have a higher risk of developing a testicular cancer from carcinoma *in situ* cells and an excisional biopsy must therefore be performed in order to check for carcinoma *in situ (91)*.

The recovery of testicular spermatozoa in these difficult cases is highly dependent not only on the surgical technique used but also on the procedures used in the laboratory to prepare the tissue samples. Sperm recovery may be facilitated by using erythrocyte-lysing buffer *(92)* and enzymatic digestion *(93)*.

It is important to keep in mind that about 50% of patients suffering from azoospermia due to primary testicular dysfunction may not benefit from the combination of ICSI and testicular sperm recovery because no spermatozoa can be recovered from the testicular mass even after extensive biopsies. Unfortunately, parameters such as testicular volume, preliminary semen analysis or serum FSH are not able to predict successful sperm recovery *(12,94)*. Inhibin-B, which is a marker for premeiotic germ cell proliferation, may be a better predictor in combination with serum FSH for testicular sperm recovery, although the limited evidence available on this at present is contradictory *(95,96)*. In a small subgroup of patients suffering from azoospermia because of a microdeletion at the Y chromosome, it was reported that no spermatozoa can be recovered when a deletion is present in the AZF-B region *(97)*.

Another limitation of the efficiency of the combination of ICSI and TESE is the decreased overall success rate when compared to patients with normal spermatogenesis. Implantation rates do not exceed 10% per embryo, especially in patients showing maturation arrest with focal spermatogenesis *(33)*. The reasons for this finding are currently unclear, but may be associated with deficient meiosis *(98)*. However, no data are currently available showing that a higher proportion of embryos obtained in these patients is indeed aneuploidic.

Immature testicular gametes as a source

Recovery of immature testicular gametes has been proposed as a means of overcoming infertility in azoospermic men where no spermatozoa could be recovered from the testicular tissue. Pregnancies have been reported after both the use of elongated spermatids and round spermatids from testicular biopsies or from ejaculates *(49)*. Although this approach was introduced more than five years ago, the number of pregnancies reported after the use of spermatids is still limited. Differentiating an elongated spermatid in its final stage of spermiation from a testicular spermatozoon is not easy and requires a detailed microscopic examination *(99)*. It has even been assumed that in many so-called failed spermatid injections, other testicular cells were actually injected *(100)*. Most pregnancies reported after spermatid injection are pregnancies obtained with elongated spermatids recovered from testicular biopsies from men with obstructive azoospermia showing normal spermatogenesis! It may thus be assumed that spermatid ICSI is not a very successful approach for treating infertility in azoospermic men with a primary testicular dysfunction.

There is much debate concerning the pregnancies reported after an adapted ICSI procedure using secondary spermatocytes *(50)* and with spermatids obtained after a short *in vitro* culture of primary spermatocytes *(47)*, as no other groups have been able to reproduce these results.

Banking testicular spermatogonia may be indicated for boys undergoing chemotherapy or extensive radiotherapy. A study by Kliesch *et al. (101)* demonstrated that adolescent patients 14–17 years of age are good candidates for semen banking, even though they may not have completed their puberty. From these results, it appears that semen cryopreservation has a role to play, even for adolescents. Although testicular tissue or testicular cell suspensions from prepubescent males may be cryopreserved, this must be considered an experimental procedure at present.

Oocytes and ovarian tissue as a gamete source

The use of immature oocytes and ovarian tissue is still experimental. Patients who may benefit from *in vitro* maturation of oocytes in their last stages of maturation are those who are at risk for developing OHSS. However, many of these patients will suffer from polycystic ovarian syndrome, and in these patients, *in vitro* maturation has not been very successful so far.

Patients who may benefit the most from this technology are women or girls who need to undergo sterilizing treatments such as chemotherapy or bilateral ovariectomy. Since cryopreservation of mature oocytes has not been successful to date, research has focused increasingly on banking immature oocytes. Although the technology is still under development, many ovaries have been cryopreserved before starting sterilizing treatments.

Those needing unilateral ovariectomy by laparoscopy may have their follicles banked after removal from the excised ovarian cortex or embedded in thin slices of ovarian cortex. The latter approach still leaves the opportunity to autotransplant the slices, while follicle banking only leaves the possibility of subsequent *in vitro* maturation.

Patients at risk of having malignant cells in their ovaries, either from the primary tumour or from metastases, may bank their ovarian tissue but, at present, only with a view of future *in vitro* maturation.

Another category of patients from whom ovaries are being banked are patients with Turner syndrome (45,XO karyotype). These patients have a progressive but accelerated loss of primordial follicles and banking their ovarian issue may be a means of preserving their future fertility. However, this approach, although experimental, may raise concerns regarding the risks for congenital malformations in the offspring.

Artificial oocyte activation

Defective oocyte activation is an infrequent cause for failed fertilization after ICSI. According to recent research using mouse oocytes and human spermatozoa, artificial oocyte activation may overcome repeated fertilization failures after ICSI *(102)*.

When round-headed acrosome-less spermatozoa are used, fertilization after ICSI is poor and unpredictable because of insufficient oocyte activation. Artificial oocyte activation with a calcium ionophore has been reported to overcome this problem *(80)*. Artificial oocyte activation is necessary in order to obtain fertilization after microinjection of round spermatids.

Ooplasmic transfer

The clinical indications for ooplasmic transfer are far from clear. There is no method as yet to detect cytoplasmic deficiencies in oocytes and ooplasmic transfer has so far been applied in selected patients with a suspicion of ooplasm deficiencies because of poor early embryonic *in vitro* development after IVF or ICSI. The technique should be considered as empirical and experimental.

Risks

Ejaculated spermatozoa as a gamete source

When ejaculated spermatozoa are used for IUI or conventional IVF, the natural barriers controlling fertilization are still functional. The main risk with these techniques is the transmission of infectious agents. For many of these infectious agents an effective treatment is available. In contrast, the use of spermatozoa from men with human immunodeficiency virus (HIV) or hepatitis C infection has raised considerable debate since there are no vaccines to provide protection against these infections that can be given to their sexual partners.

Although at present it is still unclear whether the viral genome may be incorporated into the male gamete, it is assumed that the white blood cells within an ejaculate are the main source of infection. The sperm washing procedure may eliminate many viral particles *(103)*.

There have been many pregnancies resulting from IUI or IVF using the spermatozoa of HIV-positive males without any case of transmission of the infection to the female partner being reported *(104)*. These male partners have an undetectably low viral load and are under strict medical control. Often a man's semen is pooled after several ejaculations and the viral load is assessed on the frozen pool of semen. When no viral contamination can be detected, the risk of transmission may be low in using frozen-thawed semen from these men for ART.

Despite the low risk of transmission of the HIV virus in these circumstances, the published series are still too small to conclude that this approach will

indeed be safe. At best it may be concluded that this strategy offers an important risk reduction. Because of the low numbers of spermatozoa used, it has been argued that ICSI should be used with semen from HIV-positive or hepatitis C-positive men to limit the risk for transmission of the infection to their partners. However, when the viral genome is incorporated into the sperm's genome, the transmission risk is real. In this case, preimplantation genetic screening may offer a further risk reduction.

For ICSI, apart from the risks related to the source of the gametes used, the main important, but yet unproven, concern is the introduction of contaminating foreign material into the oocyte's cytoplasm during ICSI. The use of polyvinylpyrrolidone (PVP) to slow down sperm movement may constitute a risk due to endotoxins. Although sound evidence is as yet unavailable to support this, PVP has been withdrawn from the market and replaced by products controlled for endotoxin contamination. Microbiological contamination of semen is common but washing and further semen preparation may eliminate many of the microbiological contaminants. On the basis of the current literature, this hazard is assumed to be minimal or even nonexistent for bacteria *(105)* but for viruses other than hepatitis C or HIV such as the human papillomavirus (HPV), this issue is less clear *(106)*. Theoretically, prions may be introduced during an ICSI procedure *(107)*. And although the technique is of a debatable reproducibility, it has been shown that exogenous DNA could be integrated during ICSI in the mouse, producing transgenic offspring *(108)*. More recently, foreign DNA was successfully introduced into nonhuman primate oocytes during ICSI, however, without producing transgenic offspring *(109)*. Therefore, it has been proposed that human spermatozoa should be exposed to a sequence of "sanitizing" treatments before ICSI *(109)*.

An important risk related to the gamete source for ICSI is the status of the sperm genome. ICSI may be performed with senescent ejaculated spermatozoa that may have accumulated DNA strand breakages from oxidative damage, ageing or extended epididymal storage *(110)*. Because of the genetic concerns related to DNA strand breakage and the fact that spermatozoa with oxidative damage may successfully fertilize by ICSI *(111)*, it is preferable to use viable testicular sperm rather than nonvital dysfunctional spermatozoa from the ejaculate *(77)*. The ultimate selection of the spermatozoon to be injected in ICSI is made subjectively through the light microscope, selecting a suitable motile spermatozoon on sight. Although no adverse effects in terms of outcome have been reported after microinjection of spermatozoa from patients with morphologically abnormal spermatozoa, most of these studies have not correlated sperm morphology with ICSI outcome. Only a few studies, comprising a limited number of cases, have reported on the outcome following injection of morphologically abnormal sperm *(112,113)*. Both of these studies showed a reduced fertilization rate when abnormal sperm were used for ICSI. These findings are not surprising since an increase in chromosomal aberrations has been observed, in spermatozoa from oligozoospermic individuals, using fluorescence *in situ* hybridization (FISH)*(98,114–116)*.

Furthermore, up to 3% of patients with oligozoospermia are reported to have autosomal karyotype abnormalities while the incidence of sex chromosome abnormalities in azoospermic men is reported to be 14% *(116,117)*. The incidence of karyotype abnormalities may be inversely related to the number of spermatozoa in the ejaculate *(117)*. Both azoospermic and oligozoospermic patients must therefore be karyotyped before any ICSI treatment to prevent possible transmission of aneuploidy to the offspring.

This increase in aneuploidy rate in infertile men will probably at least partly explain the increase in sex chromosome abnormalities in the offspring of ICSI patients *(119)*. Abnormal sperm decondensation after ICSI may also contribute to the problem of increased de novo sex chromosome aneuploidy *(120)*.

Finally, a distinct subpopulation of men who benefit from ICSI treatment may have deletions at the long arm of the Y chromosome *(121)*. These Yq deletions will be passed on to the next male generation by ICSI *(122,123)*. Autosomal Y homologues or other unknown recessive gene defects may exist and many genes related to spermatogenesis may be interlinked in gene networks. Apart from Yq deletions, mutations in the androgen receptors, including an increased number of CAG repeats in the androgen receptor gene, have been reported to cause male infertility *(124,125)*. At present, all of the inheritance patterns of male infertility are far from having been elucidated but more evidence is arising that hereditary traits may be involved in many candidate patients for ICSI *(126)*.

Globozoospermia and immotile cilia syndromes are now increasingly assumed to be hereditary disorders. Apart from reproductive disorders, ICSI candidate patients may have a slightly higher prevalence of

potentially heritable nonreproductive disorders as well *(127)*.

In patients with less than five million spermatozoa in their ejaculate, a cytogenetic analysis and Y-deletion screening should be performed before embarking on an ICSI treatment *(128)*. Notwithstanding genetic counselling, many patients may still prefer to use their own gametes, hence taking a risk of transferring hereditary male infertility to their sons. There have been several cytogenetic studies performed in ICSI candidate patients that have shown that female partners of those males with severe oligozoospermia necessitating ICSI may themselves have increased aneuploidy rates (more than 1% after excluding mosaicism) *(129–132)*. These findings are hard to explain but may be related to a bias in the selection of the patients under investigation and call for further study.

Epididymal spermatozoa as a gamete source

The risk when using epididymal spermatozoa for ICSI may be twofold. First, there is the risk related to the technique used to retrieve the spermatozoa. Blind percutaneous puncture (PESA) may cause epididymal damage and fibrosis although this is not an important issue where surgical reconstruction is not possible. Second, there are the risks related to the source of the gametes. Senescent spermatozoa with DNA strand breaks may be retrieved and used for ICSI and care must be taken to select motile epididymal spermatozoa.

Patients with congenital absence of the vas deferens are known to have mutations or deletions in the cystic fibrosis transmembrane conductance regulator (CFTR) gene *(133)*. Screening for CFTR gene mutations must be performed before embarking on ICSI in these patients. There are currently more than 600 different gene mutations known and screening for the most frequent mutations representative of a given population should be performed on the wives of men with congenital absence of the vas deferens (CBAVD). When the female partner is found to carry a mutation, preimplantation genetic diagnosis should be proposed if the male partner, too, is a carrier.

Patients with anejaculation may be exposed to specific risks related to the techniques of assisted ejaculation. If spinal cord lesions are located at the level of T-6 or higher, a risk of developing autonomous dysreflexia exists, with dangerous elevation of the blood pressure. Therefore, prophylactic administration of 10–40 mg sublingual nifedipine may be necessary.

Testicular spermatozoa as a gamete source

It is important to keep in mind that at least 50% of patients suffering from azoospermia due to primary testicular dysfunction may not benefit from testicular sperm recovery because no spermatozoa can be recovered even after extensive biopsies. Unfortunately, parameters such as testicular volume, preliminary semen analysis or serum FSH are not able to predict successful sperm recovery. Many patients may thus be at risk to undergo repeated attempts at retrieval without success.

An important issue is the adverse effects of such a procedure including testicular haematoma, oedema and postoperative testicular fibrosis, which may occur when multiple excisional testicular biopsies are taken *(134,135)*. There are concerns that this fibrosis may result in a decline in testosterone output from the testis. Although this concern is only borne out by the findings of a small series *(136)*, patients should be informed about this possible adverse effect. Less invasive methods, such as fine-needle aspiration, may cause less damage to the testicular tissue but prospective controlled studies have shown that this method is not useful in some patients since the chances for recovering spermatozoa are about three times lower than with the other techniques *(33, 36,94)*.

Possibly more promising may be microsurgical sperm retrieval *(137)*. This approach may facilitate finding the best area of the testis without removing much testicular tissue and may, as such, lower the risk of side-effects *(40)*.

Some authors have reported that scheduling the testicular recovery procedure one day before the ovum pick-up *(138)* or the use of sperm motility stimulants may facilitate the retrieval of motile spermatozoa from the tissue *(139)*. Both strategies may, however, result in additional risks. Testicular spermatozoa may be more prone to accumulate DNA strand breakage during overnight incubation than ejaculated spermatozoa. Pentoxifylline, on the other hand, may induce embryonic toxicity *(140)*.

As with men with severe oligozoospermia, men with nonobstructive azoospermia have an increased risk of aneuploidy and Yq deletions. An additional important risk related to the use of testicular spermatozoa may be an increase in aneuploidy,

especially in men with testicular failure *(98)*. This may not only reduce the implantation rates but may also cause an increase in aneuploidy in the offspring. At present, there is only limited information available on the offspring of men with testicular failure undergoing ICSI with testicular sperm and these data do not show any increase.

Immature testicular gametes as a source

Although pregnancies have been reported after the use of elongated spermatids and round spermatids from testicular biopsies or from ejaculates, the use of such immature haploid germ cells gives rise to a lot of concern and confusion *(141)*. Reports often refer to ICSI performed with testicular spermatozoa and elongated spermatids since the distinction between both cell types is not always possible after enzymatic digestion of the testicular tissue and observation of the wet preparation. A classification system has been proposed for observations made without fixation and staining *(49)*. Furthermore, it is difficult to distinguish round spermatids from diploid germ cells by their morphological appearance *(99)* and thus injection of diploid cells is not impossible *(100)*.

There are also concerns relating to genomic imprinting *(142)* as this may not be completed in these immature cells. However, in a mouse model it has been shown that genomic imprinting is completed at the spermatid stage *(143)* and that normal viable young with normal behaviour can be obtained after round spermatid injection up to the fifth generation *(144)*. Since mouse offspring obtained after injection of secondary spermatocytes were found to be fertile, it was concluded that imprinting may be completed by the second meiotic division or may even be completed within the oocyte's cytoplasm *(145)*. So far, therefore, the results from animal models in which ICSI is performed with immature germ cells do not call for concern but it is not clear whether these results can be extrapolated to the human.

Oocytes and ovarian tissue as a gamete source

The post-treatment transplantation of ovarian tissue obtained pretreatment in cancer patients, risks transplantation of malignant cells as well. This is not an issue when the immature gametes are matured and used *in vitro*. Currently, all these techniques are experimental and not very promising. The use of human immature oocytes for ART has been limited. From experimental animal models it appears that using immature oocytes matured *in vitro*, starting from primordial follicles, may not be without risks. One paper has reported the birth of one mouse after complete *in vitro* maturation and ART. The offspring, however, had severe developmental problems *(146)*.

Artificial oocyte activation in ART

Artificial oocyte activation with a calcium ionophore has been promoted to overcome fertilization failure in ICSI with acrosome-less spermatozoa and round spermatids. However, whether this is a safe approach is unknown. The calcium ionophore treatment causes a massive calcium release in the oocyte and may thus activate several secondary messenger-signalling pathways in the oocyte. In addition, calcium ionophore has been shown to be mutagenic.

Ooplasmic transfer

The number of babies born after ooplasmic transfer is limited and no scientific evidence is available showing any benefit from this method. The genetic modification of the germline introduced by this technique raises a lot of concerns. However, the advocates of this technology consider the risks to be minimal since only small amounts of foreign mitochondrial DNA are being transferred. They also refer to the finding that mitochondrial DNA heteroplasmy may occur spontaneously without apparent problems *(61)*. It has been reported, however, that the active donor mitochondria are unevenly distributed throughout the embryonic cells and subsequently between embryonic and extraembryonic tissues *(61)*. The implications of this finding remain unclear. Finally, by transferring small amounts of donor cytoplasm, theoretically, prions too may be transferred to the recipient ovum.

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Ovarian stimulation for assisted reproductive technologies

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Introduction

Within the past decade, a better understanding of the hormonal control of human folliculogenesis and a simultaneous development of DNA technologies providing new products with high purity and good clinical efficacy have allowed most physicians to use ovarian stimulation in every clinical situation where the main goal is to achieve pregnancy. Indeed, ovarian stimulation has been advocated as a common practice not only in the treatment of infertile couples with amenorrhoea or anovulation but also for couples whose infertility was related to either tubal, male or unexplained factors. For these reasons, controlled ovarian hyperstimulation (COH) has increasingly become a new tool in many situations.

The concept of controlled ovarian hyperstimulation

The concept of COH emerged from the practice of *in vitro* fertilization (IVF). Although Louise Brown was born following *in vitro* fertilization-embryo transfer (IVF-ET) in a natural cycle, it soon became clear that the pregnancy rate was greatly improved if more than one embryo was replaced in the uterus *(1,2)*. Thus, the aim of any regimen for controlled ovarian stimulation was to obtain as many follicles as possible from which good quality eggs could be collected. However, the simultaneous risks of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies have led to the adoption of a compromise between pregnancy rates and multiple follicular development, and restriction in the number of embryos transferred *(3)*. However, such a policy is not fully applicable to intrauterine insemination (IUI) and major concerns remain about the risk of multiple pregnancies when ovarian stimulation is performed in a context of *in vivo* fertilization. This critical issue will be discussed throughout this review.

Controlled ovarian stimulation protocols

Clomiphene citrate

The antiestrogenic properties of clomiphene citrate have been used and have proved to be very effective for the induction of follicular development in anovulation since 1962. However, the clinical benefit of this compound is confined to WHO Group II anovulation patients with endogenous ovarian steroidogenesis. In this situation, the ovulation and pregnancy rates achieved with this compound have justified its use as a first-line therapy in this type of anovulation. Nevertheless, some clinical and biological features may be relevant to predict the effectiveness of

clomiphene citrate to achieve ovulation or pregnancy *(4,5)*.

As administration of clomiphene citrate alone may induce a limited increase in the number of preovulatory follicles, it may be worthwhile for the management of IUI in normally ovulating patients. However, its antioestrogenic action could, theoretically, interfere with endometrial receptivity and with implantation *(6)* and, as discussed below, the rationale to use clomiphene citrate in this indication is controversial.

The use of clomiphene citrate in combination with gonadotrophins was first recommended for patients undergoing IVF. However, the effectiveness of such a regimen has been hampered by the risk of a premature spontaneous luteinizing hormone (LH) surge which occurs in about 20% of stimulated cycles and leads to IVF cancellation or impaired oocyte quality *(7)*. Therefore, gonadotrophin releasing hormone (GnRH) agonists, by preventing an untimely LH surge, have offered an effective alternative to this regimen and this approach has been used since the mid-1980s. The recent marketing authorization of GnRH antagonists, which induce an immediate suppression of endogenous LH secretion, has provided the opportunity of reconsidering the role of clomiphene citrate therapy in ovarian stimulation. The clinical effectiveness of this new regimen is now being tested.

GnRH analogues

A GnRH analogue is a peptide in which the primary structure of GnRH has been altered by the deletion or the substitution of one or more amino acids. A large number of structural analogues of GnRH have been synthesized, including both agonists and antagonists.

GnRH agonists (GnRH-a)

While GnRH agonists display a high *in vitro* biopotency, results from experimental and clinical studies have shown that repeated daily *in vivo* administration of these compounds induces a biphasic pattern of gonadotrophin secretion. Indeed, the first GnRH-a injections induce a sharp release of both gonadotrophins (the so-called "flare-up effect") followed by a state of pituitary desensitization related to both downregulation of the GnRH receptor and intracellular uncoupling. This leads to a progressive reduction in gonadotrophin synthesis which is maintained during GnRH-a administration. Both the intensity and duration of the pituitary desensitization are dose-dependent, at least for LH *(8,9)*. However, it is well recognized that, during this period of desensitization, LH radioimmunoassays do not actually reflect hormonal bioactivity. After cessation of GnRH-a administration, a refractory period to endogenous GnRH is observed, the duration of which is dependent on both the dose and the formulation of the GnRH-a.

These properties of GnRH-a have been applied in clinical practice with two main regimens of administration.

The short-term GnRH-a protocol

This regimen takes advantage of the initial rise (flare-up) of serum gonadotrophins on follicular recruitment and of the subsequent pituitary desensitization induced by daily agonist administration. Gonadotrophin administration is started in the early follicular phase. Its clinical efficacy has been previously established *(10–13)*. Several adjustments to this protocol have been suggested:

- a shorter period of GnRH-a administration, for 3 days (ultra-short protocol) *(14)* or for 7 days *(15)*, on the assumption that suppression of the endogenous LH surge may be obtained through a very short course of GnRH-a administration;
- pretreatment with progestogens during the luteal phase of the cycle preceding IVF in order to programme the patient's cycle and the timing of oocyte retrieval *(16,17)*.

The long-term GnRH-a protocol

With this regimen, both pituitary and ovarian desensitization are induced by GnRH-a administration in the early follicular or mid-luteal phase of the cycle preceding the planned IVF. Once desensitization is obtained, ovarian stimulation with gonadotrophins is started and GnRH-a injection is continued until human chorionic gonadotrophin (hCG) is administered.

GnRH-a administration is now used routinely for all patients undergoing IVF-type procedures although the early studies advocating the superiority of GnRH-a over conventional stimulation regimens were performed in patients who were poor or abnormal responders *(18)*. A meta-analysis of randomized studies has shown that GnRH-a reduces IVF cancellation rates, increases the number of oocytes recovered

and improves the clinical pregnancy rates per cycle and per embryo transfer *(19)*.

In clinical practice, the long-term protocol is the most traditional and widely used regimen, probably because it is more convenient for programming IVF. However, controversial conclusions have been drawn from studies comparing the respective effectiveness of long-term and short-term GnRH-a protocols. Some authors have reported a better outcome for patients treated with the long-term GnRH-a regimen *(20–22)*, advocating that the initial flare-up effect of the GnRH-a causes a rise in serum LH, androgens and progesterone (P) levels that might be deleterious for oocyte quality. In contrast, others have published good clinical results with the flare-up regimen *(23,24)*. Discrepancies between these results may be related to several biases in patient selection rather than to the regimen itself, since no significant difference was found in a meta-analysis of seven trials comparing both regimens *(19)*. It must be pointed out that the short-term GnRH-a regimen may provide some economic advantages in terms of reducing both agonist and gonadotrophin doses because the long protocol requires a more prolonged stimulation and administration of higher doses of exogenous gonadotrophins than the short one.

Whatever regimen is used, the long-term protocol with GnRH-a for IVF cycles does not exclude some minor concerns in clinical practice. Within the past decade, several issues have been addressed with regard to this protocol.

One of these issues concerns pituitary desensitization. The main parameters of desensitization (rapidity, intensity and duration) are critically dependent on numerous factors in the GnRH-a protocol including: which analogue is used; the time of its first administration in the cycle; the dose and duration of administration; and the formulation (see review in 25). As far as the most appropriate time of GnRH-a administration is concerned, downregulation seems to be achieved more rapidly when the GnRH-a is started in the midluteal phase. A combination of norethisterone acetate or a combination of oral contraceptive with GnRH-a has proved to be effective in preventing ovarian cyst formation *(26,27)*. Although it has been suggested that ovarian response to gonadotrophins could be reduced in patients whose pituitary downregulation is delayed *(28)*, the outcome in terms of pregnancy rates is still not clear *(29–31)*.

Another issue is related to the duration of the desensitization phase prior to ovarian stimulation. Using leuprolide acetate for 15 days prior to ovulation induction, Scott *et al. (32)* did not observe any impact of the duration of the hypoestrogenic state on the ovarian reponsiveness to gonadotrophins and the success of IVF. According to these authors, apart from the increased cost, there is no reason to believe that a patient should be stimulated as soon as pituitary desensitization and ovarian quiescence are achieved. This observation allows greater flexibility in scheduling ovulation induction cycles but seems to contradict other evidence in the literature which indicates that the implantation rate is higher in amenorrhoeic patients *(33)* as discussed below.

The slight increase in serum P levels observed at the time of hCG administration in up to 20% of stimulated cycles has caused some concern. A critical threshold P serum level of 3.1 nmol/ml on the day of hCG administration has been proposed, above which the pregnancy rate may be adversely affected (*25*). This subtle premature rise in serum P is presumed to impair endometrial receptivity rather than oocyte quality *(34–37)* and a recommendation has been made for the cryopreservation of embryos for subsequent transfer in these situations *(38)*. However, several other studies have not found a relationship between late follicular P levels and IVF outcome (25). Furthermore, the mechanisms that account for the premature elevation of P, despite suppressed endogenous gonadotrophins by GnRH-a, are still unclear. As a pituitary escape from the suppressive effect of GnRH-a is unlikely, serum P elevation could result from exposure to a large amount of exogenous gonadotrophins *(39)* through an increased follicle stimulating hormone (FSH)-induced LH receptivity *(40)*. The contribution of the adrenal gland to both P and androgen production cannot be excluded and this may be reduced by simultaneous dexamethasone administration, but it is still uncertain whether this actually improves the IVF outcome *(41,42)*. At the present time, serum P cut-off levels on the day of hCG, as a means of making a clinical decision with respect to the possible cancellation of the IVF cycle and the cryopreservation of all embryos for future transfer, should be questioned *(43)*.

GnRH antagonists

GnRH antagonists are synthetic analogues of GnRH that compete with endogenous GnRH for pituitary binding sites but are unable to induce GnRH receptor cross-linking, a process that appears to be necessary

to effect calcium ion-mediated gonadotrophin release *(44)*. Clinical advantages of GnRH antagonists over GnRH agonists are the absence of the initial stimulation of gonadotrophin release (flare-up effect) and, as a consequence, a more direct, immediate and reversible suppression of gonadotrophin secretion which allows their use without the need for a desensitization period.

To date, three generations of antagonists have been used. The first- and second-generation compounds exhibited high potency in the suppression of ovulation in rats but induce histamine release, resulting in transient systemic edema and inflammation at the injection site (first generation) or local reactions only (second generation). The third generation of GnRH antagonists has negligible histamine-release properties and comparable anti-ovulatory activity to that of the second generation.

In initial clinical studies, GnRH antagonists were used to prevent a premature LH surge during the menstrual cycle *(45,46)* and, subsequently, during ovarian stimulation for IVF. There are two regimens using GnRH antagonists.

Multiple-dose GnRH antagonist administration

In this protocol, daily injections of low-dose antagonist are given from day six of ovarian stimulation using exogenous gonadotrophins, which is when multifollicular development and estradiol secretion may trigger an endogenous LH surge *(47)*. A multiple dose-finding study performed with orgalutran, one of two available antagonists, clearly demonstrated that the optimal daily dose is 0.25 mg *(48)*. Indeed, this dosage is able to adequately prevent the endogenous LH surge before hCG administration and simultaneously maintain a residual basal LH secretion compatible with a high rate of estradiol secretion, mature oocyte collection and pregnancy. The short half-life of the antagonist with this dose requires a daily administration up to the time of hCG administration *(49)*. A similar study using 0.25 mg cetrotide has confirmed that this dose is adequate to prevent an endogenous LH surge *(50)*.

Single-dose GnRH antagonist administration

The injection of a single and large dose of GnRH antagonist in the late follicular phase has proved to be effective in postponing the spontaneous LH surge in normo-ovulatory women *(46)*. On this basis, several clinical studies have been performed using cetrotide in IVF cycles *(51,52)*. In a comparative study, a 3 mg dose was selected as a safer choice since a "protection period" of at least four days can be obtained *(53)*. When this dose was injected on day eight of the stimulated cycle, or earlier if the ovarian response was more rapid, no LH surge was observed. Moreover, in some cases where plasma LH levels were above 10 IU/L at the time of GnRH antagonist injection, the LH surge was completely blunted *(52,53)*. When ovarian stimulation needed to be prolonged over the three days following the first GnRH antagonist injection, a second large dose or additional daily 0.25 mg doses of the drug were required, due to the relatively short half-life of the antagonist *(53)*. In every situation, oocyte retrieval was performed in the absence of follicular rupture, demonstrating the effectiveness of this regimen in the prevention of the endogenous LH surge.

While the primary goal of GnRH antagonist administration—the prevention of the LH surge—was achieved in these studies, some concern remains about the overall effectiveness of the protocol. Indeed, at least in multicentre studies *(54,55)*, the number of ovarian follicles, collected oocytes and pregnancy rates tended to be lower than those obtained with the long-term GnRH-a protocol. Reasons for these differences in the effectiveness of the two protocols are still poorly understood and may be partly related to the regimen of GnRH antagonist. However, it is likely that other factors, such as the absence of ovarian quiescence before ovarian stimulation, and the regimen of exogenous gonadotrophins, also contribute to the relatively lower effectiveness of GnRH antagonist protocols. Finally, if the results of these studies are adjusted according to each centre, differences between protocol effectiveness were limited, attesting that a learning period is required to adequately control stimulation protocols employing GnRH antagonists. Furthermore, programming the IVF cycle through steroid administration during the cycle preceding IVF may not only be convenient for most centres but could also be effective in improving the size of the cohort of recruited follicles. The benefit of a programming cycle is currently under consideration.

Nevertheless, several advantages have been clearly identified with these protocols. The compliance of patients with the GnRH antagonist protocols was excellent due to the shortened exposure to GnRH analogue administration and to the good clinical

tolerance of this third generation of antagonists. Furthermore, the amount of exogenous gonadotrophins needed for ovarian stimulation was reduced as well as the occurrence of hyperstimulation syndrome *(54,55)*. Finally, the overall cost of this regimen was significantly lower than that of the GnRH-a protocol.

The new GnRH antagonists also permit the design of more gentle stimulation schemes, with the return to the use of clomiphene citrate, minimal stimulation or even natural cycles *(56,57)*.

Gonadotrophins

FSH preparations

Human menopausal gonadotrophin (hMG), extracted from the urine of menopausal women, has been used successfully for many years for ovarian stimulation. It is a mixture of both FSH and LH with low specific activity and also contains other urinary proteins *(58)* which can induce allergic reactions *(59)*.

According to the "two cells–two gonadotrophins" theory, both FSH and LH are required to achieve steroidogenesis. Therefore, for patients whose anovulation is related to hypogonadotrophic hypogonadism, hMG preparations must be used that ensure adequate estradiol production and endometrial maturation for embryo implantation.

However, although LH is necessary for thecal androgen production, the amount of LH required for follicular development and steroidogenesis is minimal. Indeed, when LH secretion is suppressed after long-term GnRH-a administration, FSH alone is sufficient to ensure adequate steroidogenesis and endometrial development. Furthermore, there has been some concern about the theoretical possibility that excessive amounts of LH could compromise successful maturation of the oocyte. Two recent meta-analyses of randomized trials, comparing hMG and FSH for IVF *(60,61)*, concluded that the clinical pregnancy rate per cycle was greater with the use of FSH than with hMG if no pituitary desensitization was undertaken. However, for patients assigned to long or short GnRH-a protocols, the difference between the efficacy of FSH and hMG treatments was less and only significant in the case of FSH *(60)*. These data strongly suggested that the choice of gonadotrophin preparations used for ovarian stimulation during IVF treatment should take into account the GnRH analogue regimen that is used. Finally, a meta-analysis performed in patients with polycystic ovary syndrome (PCOS), showed that the rate of OHSS was significantly reduced when using FSH instead of hMG *(62)*.

The need for an improved product combined with advances in purification techniques led to a highly purified human urinary FSH (u-hFSH), which contains more than 95% pure FSH with a specific activity of 9000 IU FSH/mg. Nevertheless, this preparation still has the inherent disadvantages of all urine-derived preparations which require the collection of large quantities of urine, leading to unreliable supply and, most importantly, batch to batch inconsistency.

Recombinant DNA technologies were used in the early 1990s for the production of recombinant human FSH (r-hFSH) with the insertion of alpha and beta FSH subunits into genetically engineered mammalian cells (Chinese hamster ovary [CHO] cells). The most significant advantage of this technology is that the manufacture of r-hFSH is independent of urine collection, ensuring the consistent availability of biochemically very pure FSH preparation (>99%) with minimal batch to batch variation *(63)*. The purification procedure consistently yields an FSH preparation with a very high specific activity of >10 000 IU FSH/mg and a low level of degradation or oxidation *(64)*.

Two r-hFSH preparations are currently available: follitropin alpha and follitropin beta. While the manufacturing and purification procedures for these two preparations are different *(65)*, it is uncertain to what degree these factors are clinically relevant. There is some evidence that r-hFSH preparations have clinical advantages over u-hFSH or highly purified u-hFSH. Several comparative studies have demonstrated significant advantages for r-hFSH in terms of efficacy as assessed by the number of oocytes retrieved as well as efficiency judged by FSH consumption and the duration of treatment *(66–71)*. Pregnancy rates in individual studies were marginally higher after r-hFSH than after u-hFSH *(72)* when comparable numbers of embryos were transferred. However, when pregnancies after the transfer of frozen embryos were assessed, a statistically significant difference in pregnancy rates was observed in favour of r-hFSH *(73)*. Furthermore, a meta-analysis of 12 randomized trials comparing 1556 patients receiving r-hFSH and 1319 patients receiving u-hFSH in IVF and intracytoplasmic sperm injection (ICSI) programmes *(74)* showed that the pregnancy rate per started cycle was significantly higher with r-hFSH. Thus it may be concluded that clinical practice should favour the use of r-hFSH over urinary preparations.

For these reasons, urinary products have been progressively replaced by r-hFSH preparations in Europe, despite the increased cost. The use of r-hFSH results in a higher pregnancy rate with a lower dose of drug over a shorter period of administration. Furthermore, two recent cost-effectiveness studies in the USA and the UK have shown r-hFSH to be significantly more cost-effective than u-hFSH in terms of ongoing pregnancy *(75)*. Neither social costs nor the costs of patients' time away from work and travel expenses were incorporated into the models. It is assumed that as fewer attempts are required with r-hFSH, the social, employment and travel costs would be lower than with u-hFSH. However, this requires further study.

LH preparations

Following the development of r-hFSH for use in associated reproductive technology (ART), a reliable recombinant DNA LH preparation, free from the potential problems associated with human source material, has been developed. The biological activity of this r-hLH preparation has been demonstrated in the rat seminal vesicle weight gain bioassay and in a primate model of IVF. In both cases, the biological responses to r-hLH and hMG were shown to be similar. Clinical studies were undertaken to investigate both the efficacy and the safety of r-hLH for patients with hypogonadotrophic hypogonadism *(76)*. It was found that daily injections were well tolerated and produced a dose-related effect on estradiol production and endometrial thickness. A daily injection of 75 IU r-hLH seems to be the minimal dose to achieve adequate follicular maturation.

The issue of the need for LH in normal women simultaneously treated with GnRH analogues is still to be addressed. In IVF cycles programmed with a long-term GnRH-a protocol, r-hFSH administration is sufficient to produce adequate folliculogenesis in most cases *(77)*. Preliminary studies have also shown that addition of LH (225 IU) in this situation does not improve the parameters of ovarian stimulation and the cycle outcome *(78)*. However, while it is likely that only a subset of patients would benefit from LH therapy, a study comparing different doses of r-hLH in addition to r-hFSH is needed. No data are available about the use of LH in IVF cycles treated with GnRH antagonists.

Finally, *in vitro* studies have suggested that LH could be involved in the control of follicular growth during the late follicular phase of the cycle *(79)*. Therefore, clinical studies are in progress to evaluate the potential effects of r-hLH in the reduction of the number of developing follicles in hyperstimulated cycles.

Dose of gonadotrophins

There has been no systematic investigation of the optimal dose of FSH needed for controlled ovarian stimulation. A daily dose of 150 IU or 225 IU of urinary or recombinant preparations is usually recommended with subsequent adjustments from day six or seven of stimulation, according to the ovarian response assessed by serum estradiol levels and/or ultrasound. More recently, the introduction of r-hFSH with a higher efficiency than urinary preparations has led to the comparison of several starting doses of FSH in a 100–225 IU range *(80,81)*. Although the design of these studies was not strictly comparable, it seems that 150–200 IU is the standard starting daily dose for patients with no evidence of ovarian dysfunction.

Triggering of ovulation

Human chorionic gonadotrophin

Due to its similarity to LH, urine-derived hCG (u-hCG) has been used clinically in anovulatory women for about 40 years to trigger ovulation and luteinization and to support the corpus luteum. In patients enrolled in IVF protocols which include GnRH-a, u-hCG is used as a surrogate LH surge. Despite the widespread use of u-hCG, commercial preparations suffer from the same disadvantages as other urine-derived gonadotrophins. They require the collection of large quantities of urine from which the extracted starting material is of poor quality, leading to unreliable pharmaceutical activity and unpredictable adverse immunological reactions *(82)*.

The advent of recombinant DNA technology has also permitted the development of a recombinant form of hCG (r-hCG) which is pure, and whose production is independent of urine collection. One product, from genetically engineered CHO cells, is now commercially available and preliminary studies have estimated that 250 µg r-hCG correspond to 5000 IU u-hCG. Two recent studies comparing u-hCG and r-hCG in women undergoing ovulation induction for ART, concluded that the final oocyte maturation was similar *(83)* or

better *(84)* following the administration of the recombinant preparations. Moreover, in both studies, serum P concentrations on day one and days six and seven post-hCG, and serum hCG concentrations at all post-hCG time points, were statistically higher in the group treated with r-hCG preparations. Furthermore, the incidence of adverse events was significantly higher in the u-hCG group while the incidence of injection-site reactions was significantly lower in the r-hCG group. Therefore, r-hCG seems to have significant advantages compared to u-hCG.

Recombinant LH

Although human LH produced by DNA recombinant technology has similar pharmacokinetic characteristics to the pituitary-derived hLH, r-hLH has a terminal half-life of about ten hours compared to the 30 hours of u-hCG *(85)*. This difference between the two preparations may be important in the prevention of OHSS which is likely linked to the long half-life of u-hCG.

In humans, r-hLH has been tested for triggering final follicular maturation before IVF. The first pregnancy using this approach was reported in 1996 *(86)*. More recently, a large multicentre, double-blind study has been undertaken to compare different doses of r-hLH in 258 patients enrolled in a regular IVF protocol following pituitary desensitization with GnRH-a. Each dose of r-hLH, from 5000 to 30 000 IU, appeared to induce an adequate final follicular maturation with a percentage of mature and fertilized oocytes similar to that found with u-hCG. However, a second dose of 15 000 IU of r-hCG, two days after the first injection, appeared to be required to adequately support the luteal phase. As this observation was made in patients whose low residual secretion of endogenous LH was induced by pituitary desensitization, it deserves confirmation in cycles without desensitization with treatment protocols including GnRH antagonists. This study also confirmed that the duration of the exposure to LH/hCG is a major determinant for the development of OHSS.

GnRH agonist

The endogenous LH surge induced by GnRH-a works through an indirect mechanism that relies on the patient's own pituitary response to GnRH. The GnRH-a induced LH surge has a sharper profile with a higher peak value but a shorter duration than the natural LH surge *(87)*. Consequently, in clinical practice, triggering ovulation with GnRH-a may be considered in women at risk of OHSS or multiple pregnancy and in cycles where pituitary desensitization has not been previously performed. The efficacy and safety of the LH surge induced by GnRH-a was first investigated in WHO Group II anovulatory PCOS patients *(87–90)*. These studies showed that the risks of OHSS and multiple births were not totally blunted and demonstrated a high incidence of luteal phase deficiency. Therefore, luteal phase support is likely to be required when this ovulation triggering regimen is applied. Furthermore, in normally ovulating patients whose ovarian stimulation was performed before IUI, GnRH-a administration was reported to improve the pregnancy rate and to abolish the risk of OHSS *(91)*. Finally, preliminary results have been published in patients whose endogenous LH surge was prevented by GnRH-a administration *(92)*. An LH surge was successfully elicited by administration of triptorelin (one bolus of 0.1 mg) but no pregnancy was reported. Altogether, these studies show that triggering ovulation through GnRH-a administration may be useful for patients or cycles at risk of OHSS and multiple pregnancy, but a further evaluation of the outcome of this treatment is needed.

Luteal phase support

Progesterone (P) and estradiol (E_2) play central roles in the maintenance of human reproduction. Steroid production peaks about four days after ovulation and continues at this level for about a week, falling about five days before the next menstrual period *(93)*. During this time, P is secreted in a pulsatile fashion and P production is 40-fold the maximal E_2 production. Until the luteo-placental shift occurs at about seven weeks of gestational age, the ovary's production of these hormones is critical to pregnancy maintenance *(94)*.

In stimulated cycles, the luteal phase differs from the natural one because hormone production from multiple corpora lutea is supraphysiological. Moreover, the luteal phase may be shortened in relation to a sharper decline in serum P and E_2 than in natural cycles. For this reason, the principle of luteal support was widely adopted and this policy was further reinforced with the advent of GnRH-a use in the late 1980s *(95)*. Nevertheless, the supraphysiological steroid levels following ovarian stimulation may also

have adverse effects on uterine receptivity, even when luteal length is adequate. Indeed, advanced endometrial maturation and increased uterine contractions have been observed in high-responding women *(96–97)* with a risk of a lower pregnancy rate *(98)*.

In stimulated IVF cycles, steroid production during the first week after ovum retrieval is likely to be well-timed and sufficient. Therefore, the start of exogenous steroid support does not seem to be critical. In contrast, the timing of P administration is critical in ovum donation programmes where the only source of P is exogenous. The highest pregnancy rates are likely to occur when two-day-old embryos are transferred on the fourth or fifth day of P therapy *(99)*.

While P alone is recommended in a conventional luteal support regimen, E_2 supplementation should also be considered. Indeed, even if E_2 does not directly mediate luteinization, it may be required to stimulate P receptor replenishment. For this reason, hCG administration has been advocated for its stimulating effect on E_2 and P secretion by the corpora lutea. Meta-analysis has shown that hCG injections seem to be more effective than P alone in terms of pregnancy rates in GnRH-a cycles *(100)*. However, the increased risk of OHSS following repeated hCG administration during the luteal phase may explain why the use of hCG has not been widely adopted. Furthermore, if the superiority of hCG over P is related to its ability to stimulate E_2 production, adding exogenous E_2 to P supplementation must be considered as a safer alternative. This issue has been recently addressed in a study performed in high-responding patients treated with a long-term GnRH-a protocol *(101)*. Patients who received both E_2 and P had higher implantation rates and lower spontaneous abortion rates than those who did not. Therefore, luteal supplementation with both steroids may be a better alternative than the use of repeated hCG injections, at least in patients at risk of OHSS.

Several routes of P delivery have been proposed, including oral, intramuscular and transvaginal.

Although the development of micronized formulations of natural P has resulted in preparations with improved absorption and bioavailability, the systemic P levels that can be achieved with these preparations following oral ingestion (100 mg) are too low to provide adequate endometrial support *(102)*. This may be related to a first-pass effect following oral administration of P and its extensive hepatic metabolic degradation *(103)*. Several clinical trials of oral supplementation with natural P in IVF cycles (200 mg three times daily) have confirmed the inadequacy of this route compared with the other routes of administration *(104–106)*.

Intramuscular administration of P significantly increases its bioavailability *(107)*. The usual intramuscular dose varies from 25 mg to 100 mg daily, sometimes in divided doses. Serum peak levels are well above the physiological range and endometrial maturation is "in phase"; this is associated with good clinical results *(108)*. Furthermore, a comparison of oral micronized P with intramuscular P resulted in significantly higher implantation rates with the latter treatment *(105)*. However, the intramuscular route also has several drawbacks: it is inconvenient, uncomfortable for the patient and it may produce some local side-effects, such as marked inflammation at the injection site.

The vaginal route offers several important advantages over intramuscular dosing as it avoids the first-pass hepatic metabolism and ensures sustained plasma P concentrations. Progesterone absorption is further influenced by the formulation used (tablets, suppositories, creams, oil-based solutions or, more recently, slow-release polycarbophil gel) *(109)*. Compared with intramuscular P, higher doses of vaginal P are often necessary to achieve adequate serum P levels but, using sustained-release formulations, lower doses (45–90 mg) applied once a day or even once every other day, might be effective *(104,110–111)*. As shown by endometrial biopsies performed in the midluteal phase, most endometria were in phase after the use of vaginal micronized P *(112)*, even though serum P levels were less than normal *(113)*. This suggests that vaginally administered P exerts a pronounced local effect on the endometrium, the so-called "first uterine pass effect". While comparison between vaginal and intramuscular P administration provided contradictory results *(109)*, a recent study in a donor ovum programme showed an improvement in the cycle outcome with the use of sustained-release formulations *(114)*. The vaginal route has proved to be a valuable route for drug delivery in infertility treatment.

Monitoring of treatment

The main objectives of monitoring treated cycles are to control follicular maturation, to time hCG administration and to predict the outcome of the cycle. Another purpose of ovarian monitoring is to prevent

OHSS by cancelling cycles at risk for high ovarian response or, alternatively, to detect poor ovarian response and adjust ovarian stimulation accordingly. This approach is valid for an IVF cycle and, to a lesser extent, for programming an IUI cycle.

The combination of serum E_2 determination and ultrasonography has long been accepted as the most widely used mode of follow-up during COH.

In the early development of IVF when GnRH analogues were not available, clinical studies emphasized the need for monitoring serum E_2 concentrations and different serum E_2 patterns were correlated with cycle outcomes *(115,116)*. However, the level of serum E_2, although a functional indicator of folliculogenesis, is not always correlated with follicular growth *(117)*. Due to the considerable variety of protocols used in ART cycles, no description of a common and optimal E_2 pattern is available. Nevertheless, there is some evidence that, whatever the protocol used, a plateau of plasma E_2 values for more than three days is associated with a poor outcome of the ART cycle. Conversely, measurements of plasma E_2 are helpful in predicting excessive ovarian response and in deciding subsequent doses of gonadotrophin or the cancellation of the cycle or the ET.

Serum E_2 levels were used as an early marker of ovarian responsiveness to exogenous gonadotrophins. On the fourth day of treatment, assessment of serum E_2 levels may predict the subsequent ovarian response to exogenous gonadotrophins *(118)*. Similarly, evaluation of E_2 response to the endogenous gonadotrophin flare-up induced by GnRH-a in the short-term protocol, was designed as a "lupron screening test" *(119,120)*. Both the E_2 pattern and the maximal E_2 response following the first injections of GnRH-a have been correlated with the subsequent ovarian response to COH *(121)*. These data underline that a single determination of plasma E_2 may be a helpful predictor of a poor or high ovarian response and useful for the tailoring of gonadotrophin administration.

Determination of plasma E_2 is also recommended for the assessment of whether or not pituitary desensitization induced with a long-term GnRH-a protocol is effective at the ovarian level. Indeed, as plasma LH immunometric evaluation may not adequately reflect the state of pituitary desensitization, it is commonly stated that plasma E_2 must be lower than 180 nmol/ml to ensure that ovarian activity is actually suppressed. In every situation, it is recommended that ovarian stimulation is started with FSH only when ovarian activity has been suppressed, whatever the duration of GnRH-a administration required to obtain suppression of ovarian activity.

Finally, the recent availability of GnRH antagonists in ART cycles provides the opportunity of reconsidering the role of plasma E_2 determinations during the stimulation phase of the ART cycle. Indeed, administration of a GnRH antagonist may alter the pattern of plasma E_2 response *(54)*. However, it is still not clear if a decrease in serum E_2 levels after GnRH antagonist injection is responsible for the lower pregnancy rate sometimes observed with this protocol. Plasma E_2 determination is a valuable tool for monitoring ART cycle treatment while the value of plasma LH measurement seems to be strictly limited to cycles stimulated without the addition of GnRH analogues.

Additionally, ultrasound measurement of follicular growth plays a key role in the assessment of the adequacy of follicular maturation and of the correct timing of hCG administration. In most clinical studies, triggering of ovulation by hCG is recommended when at least three large (>16 mm) follicles have been visualized on ultrasound. Furthermore, measurement of endometrial thickness through a vaginal probe allows an indirect assessment of E_2 secretion. With the extensive use of GnRH-a protocols, it has been emphasized that patient follow-up could be simplified by using only ultrasound determination of both follicular growth and endometrial maturation and this approach seems effective in triggering ovulation without reducing the pregnancy rate *(122)*. This minimal monitoring is more cost-effective and especially relevant in low-resource settings.

Clinical indications for ART

Intrauterine insemination

The rationale for performing IUI is to increase the number of highly motile spermatozoa with a high proportion of normal forms at the site of fertilization. While in the past, the whole ejaculate was placed in the uterus, new semen preparation techniques derived from IVF procedures and able to remove prostaglandins, bacteria and immunocompetent cells, have led to the recommendation that a direct transfer of motile spermatozoa after sperm preparation and concentration in a small volume of medium is to be preferred. Several studies have shown that IUI is more effective than intravaginal or intracervical insemina-

tions with unprepared semen *(123)*.

Indications for IUI include:

- physiological and psychological dysfunctions, such as hypospadias, vaginismus, retrograde ejaculation and poor erectile function;
- cervical hostility related to poor quality mucus or persistently negative postcoital tests;
- male infertility: a minimal amount of total motile sperm cells (at least one million) should be inseminated for optimal results *(124)* and it is presumed that a certain degree of normal morphology of spermatozoa is also required to achieve pregnancy. Treatment should be restricted to IUI alone, because induction of ovulation has little additional beneficial effect *(125)*;
- unexplained infertility in both the male and the female partner.

While both IUI and COH independently increase the probability of conception *(126,127)*, COH seems to be a more contributory factor than IUI by overcoming some subtle cycle disorders. In male and in unexplained infertility, it has been recommended that three to six cycles of IUI are undertaken and, if unsucessful, the couple can be offered IVF/ICSI.

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection is primarily indicated in the most severe forms of male infertility with very low sperm count, poor motility and/or high teratospermia, and in males with obstructive or idiopathic azoospermia by using epididymal or testicular sperm. ICSI is also currently used in couples who have failed to provide an embryo through routine IVF procedures. These topics are discussed elsewhere in this volume.

Eligibility of patients

Determinants of ovarian response

Age

The age of the female partner is the single most important factor determining spontaneous fertility and the effect of age is enhanced when considering the outcome of all forms of fertility treatment. Age is a particularily strong factor when the outcome of ART treatment is considered *(128,129)*. As reported in many countries *(130,131)*, pregnancy rates are fairly constant (at around 25% live births per cycle) up to the age of 34 years, when there is a steep decline. No pregnancies have been recorded among women over the age of 46 years. While many factors may contribute to the deleterious effect of age, there is compelling evidence that ovarian function is altered. Not only is the number of follicles available decreased with advancing age but oocyte quality is reduced and the incidence of aneuploidy increased. Therefore, the ageing ovary is a major concern in infertility treatment, particularly when COH must be achieved. The assessment of ovarian function is a key issue before performing COH.

Duration of infertility

While the duration of infertility is a major factor determining the likelihood of spontaneous pregnancy occurring in untreated infertile patients *(132)*, it is still uncertain whether reduced effectiveness of ovarian stimulation is related to the duration of infertility or to the age of the woman, which are clearly closely linked.

Weight

Obesity is often associated with menstrual disorders and anovulation *(133)*. As the impact of weight gain on the reproductive system is multifactorial, the respective roles of abnormalities such as hyperandrogenism, hyperinsulinism or estrogen production from adipose tissue is still a matter of debate. Furthermore, obesity itself may be a factor affecting ovarian response. Indeed, in women with no ultrasound evidence of PCOS, a higher dose of clomiphene citrate is needed to obtain ovulation *(134)* and the response to gonadotrophin induction of ovulation is inversely related to body mass index *(135)*. Moreover, PCOS patients with moderate obesity have a blunted response to gonadotrophin therapy compared with their nonobese counterparts *(136)* and require a more prolonged stimulation and a higher dosage of FSH *(137)*. It is likely that the pharmacokinetic profile of ART drugs is different in overweight compared to lean patients and may partly explain the need for higher doses. Weight reduction is beneficial in restoring ovulation or in reducing drug dosage. Weight reduction in obese patients reduces hyperandrogenism and hyperinsulinaemia, both factors influ-

encing the ovarian response to FSH. Therefore, it is presumed that obesity is responsible for the relative ovarian insensitivity to infertility treatment.

Smoking

Epidemiological studies have shown that 38% of nonsmokers conceive in their first ART cycle compared with 28% of smokers, with smokers being 3.4-fold more likely to take more than one year to conceive than nonsmokers *(138)*. Both active and passive smoking have been associated with elevated FSH concentration *(139)*. It has been postulated that impaired fertility and delayed conception among women who smoke result from interference with gametogenesis or fertilization, failure of implantation and early miscarriage *(138)*. As far as ovarian function is concerned, some studies have shown that smoking women, in particular, young women, had a higher basal and post-clomiphene citrate test serum FSH *(139–141)*. Other studies have shown a detrimental association between cigarette smoking and ovarian response to stimulation. Women who smoke required a significantly higher dosage of gonadotrophins than nonsmokers *(141–143)*. Hence smoking may further increase the cost of ovarian stimulation and this must be emphasized to the infertile couples in whom the female partner is a smoker.

Management of poor ovarian responsiveness

Diminished or poor ovarian response to COH occurs in about 9%–24% of patients *(144)* and is still a challenging issue. One of the main reasons may be related to the lack of a universally accepted definition of "poor responders".

The original definition of poor responders was based on low peak E_2 concentrations (<1000 pmol/ml) during ovarian stimulation with 150 IU hMG *(145)*. The low pregnancy rate in these patients was attributed to the low number of recruited follicles and retrieved oocytes. Later, the definition evolved with the advent of more aggressive stimulation protocols to peak serum E_2 values greater than 1700 pmol/ml and less than four dominant follicles on the day of hCG administration.

Another approach involved patients without previous experience of stimulatory ART cycles but whose diminished ovarian reserve could predict a poor response to gonadotrophins. Indeed, several tests of the functional ovarian reserves seem reliable for predicting a low response to standard protocols *(146,147)*.

These tests include:

- day three determination of basal serum FSH *(148–150)*, E_2 *(151,152)*, inhibin B *(153)* or the FSH/LH ratio *(154)*;
- dynamic tests, such as the clomiphene citrate challenge test *(155,156)*, the GnRH-a stimulation test *(119,120,157)* and the exogenous FSH ovarian reserve test *(158)*;
- imaging techniques, such as measurement of ovarian volume *(159,160)*, antral follicle count *(161,162)*, and measurement of ovarian stromal bood flow with colour Doppler *(163,164)*.

Several stimulation protocols have been proposed to improve the outcome in poor responders. These include:

- varying the dose or the day of the cycle for initiating stimulation with gonadotrophins *(165–169)*;
- pituitary desensitization with a GnRH-a long protocol followed by stimulation with a high dose of gonadotrophins *(170)*;
- initiating GnRH-a and gonadotrophins together in a short-term protocol *(171,172)*;
- cotreatment with growth hormone or growth hormone-releasing hormone *(173–176)*;
- cotreatment with estrogens or combination oral contraceptives *(177,178)*;
- using clomiphene citrate for stimulation *(179, 180)*;
- natural IVF cycles *(181)*.

However, while some of these protocols may improve the ovarian response to stimulation, none of them were able to significantly improve the pregnancy rate. Other approaches have been shown to be more successful in improving the outcome of IVF cycles. All recommend the reduction of GnRH-a or manipulation of the agonist differently, for example:

- a "micro-dose" GnRH-a flare-up regimen: after a pretreatment with oral contraceptives, patients received microdose of GnRH-a (20–40 µg of leuprolide twice daily) in the early follicular phase and started gonadotrophins on the third day *(182–184)*;

- reducing the dose of GnRH-a when ovarian desensitization is achieved has been proposed as a "mini-dose" long protocol *(185)*. Dosage of GnRH-a may be reduced by half or a fifth without risk of a premature LH surge *(186)*;
- the "stop-lupron" protocol which involves stopping GnRH-a administration with the onset of menses and stimulation with high-dose gonadotrophin therapy *(187,188)*.

With these protocols, some advantage was observed in terms of pregnancy and it is possible that they may be an effective approach in those cases in which the use of GnRH-a is presumed to be responsible for the poor ovarian response to gonadotrophins.

From these data, it is clear that the different causes of poor ovarian response should be taken into consideration when deciding what protocol is the most suitable and in what circumstances the cycle must be cancelled. As an example, in young, poor-responder patients with normal serum FSH basal values, it has been reported that a low response to gonadotrophins does not adversely affect the IVF cycle outcome *(189,190)*. Conversely, the least favourable situation is clearly that of patients with a low ovarian reserve in relation to their advanced age, or ovarian dysfunction. They do not benefit from the use of ICSI *(191)* and should be considered as candidates for oocyte donation.

Management of PCOS

This is one of the more common endocrine disorders with a very heterogeneous definition, from the single finding of polycystic ovarian morphology detected by ultrasonography to a complete form with clinical symptoms such as obesity, hyperandrogeny, cycle disorders and infertility *(192)*. It is estimated to be the major cause of anovulatory infertility (accounting for 73% of cases) and hirsutism *(193)*. There is evidence that ovarian hyperandrogenism is the main factor responsible for persistent anovulation *(194)* and it is likely that the hyperinsulinaemia usually observed in these patients may contribute to the infertility.

The strategy for ovulation induction in PCOS anovulatory women must take into account that the risks of hyperstimulation and multiple pregnancy are markedly enhanced with gonadotrophin therapy. Therefore, clomiphene citrate administration has been recommended as the first-line therapy in these patients. Indeed, clomiphene citrate induces ovulation in about 70%–85% of patients although only 40%–50% conceive *(195)*. While a large range of daily doses has been studied, it must be emphasized that an exuberant response may be observed with 50 mg in some patients and, in the USA, the maximal dose approved by the FDA is 100 mg/day for five days. The use of clomiphene citrate is currently recommended for only six months because of the putative increased risk of ovarian cancer.

Gonadotrophin therapy is indicated for women with anovulatory PCOS who have been treated with clomiphene if they have either failed to ovulate or have a response to clomiphene that is likely to reduce their chance of conception (for example, negative post-coital tests). To prevent the risks of overstimulation and multiple pregnancy, traditional protocols have been replaced by either low-dose step-up, step-down regimens or by a sequential step-up, step-down protocol. Several studies have shown that these regimens are effective and safe by reducing the number of leading and medium-sized follicles *(196–198)*. The risks of multiple pregnancy and OHSS are further reduced if ovulation is triggered with a single injection of hCG and in the absence of more than two follicles larger than 16 mm or more than four follicles larger than 14 mm. In overstimulated cycles, hCG is withheld and the patient advised to refrain from unprotected sexual intercourse. Many published series support the notion that carefully conducted ovulation induction therapy results in a good cumulative conception rate in women with PCOS (*199)*.

Different gonadotrophin preparations have been used to induce ovulation in PCOS anovulatory women. Two meta-analyses were carried out on the use of FSH versus hMG treatment. The first concluded that FSH is associated with a reduction of moderate-to-severe OHSS *(62)*. The other *(60)* showed that treatment with FSH results in a 50% higher pregnancy rate in IVF cycles. In spite of the well-known drawbacks of meta-analyses, it may be concluded that FSH is more suitable in PCOS patients to reduce the risk of OHSS, which is still a major concern with this therapy.

As hypersecretion of LH is a classical endocrine feature of PCOS and may result in reducing the conception rate *(200)*, particular attention has been paid to the possible advantages of adding GnRH-a prior to ovarian stimulation. However, prospective randomized studies have indicated that GnRH-a provide no benefit over gonadotrophin alone and do

not reduce the tendency to multifollicular development, cyst formation and OHSS *(201,202)*. For patients resistant to ovulation regimens, IVF therapy has been advocated as another modality but the persistent risk of OHSS justifies recommending careful administration of FSH.

Finally, reducing hyperinsulinaemia through the administration of insulin-sensitizing agents such as metformin, is effective in decreasing serum androgens in both obese and nonobese PCOS patients *(203)*, improving spontaneous ovulation *(204)* and the ovarian responsiveness to clomiphene citrate in obese PCOS patients *(205)*.

Risks of COH

The side-effects of COH still remain the challenging issue of ovulation induction. Some of them are more serious, OHSS being the most serious complication. Others are longer-term side-effects with a special concern regarding the risks of neoplasia and the morbidity and mortality associated with multiple births.

OHSS

The worldwide incidence of severe OHSS has been estimated at 0.2%–1% of all ART cycles *(206)* and the associated mortality at 1:45 000–1:50 000 per infertile women receiving gonadotrophins *(207)*. However, the frequency of this life-threathening situation depends on many factors such as criteria used for diagnosis, identification of patients at risk, the type of medication and the use of preventive measures. For example, after IVF, the overall incidence is reported to be 0.6%–14% *(208)*. Thus, clinicians have to balance the risks and benefits of medical intervention when considering treatment options.

While the pathogenesis of OHSS has not been completely elucidated, it is likely that the increased capillary permeability triggered by the release of ovarian vasoactive substances under hCG stimulation plays a key role in this syndrome. Factors belonging to the renin–angiotensin system, cytokines and vascular endothelial growth factor (VEGF) are also involved in this process *(209)* and awareness of these mechanisms may provide opportunities for the design of specific treatment regimens. While there is no consensus about the management of OHSS, it is agreed that prevention of this syndrome is the main objective.

Individualizing ovulation induction protocols may lead to better control of ovarian hyperstimulation. Several factors may be taken into account including a history of exaggerated response to gonadotrophins in previous cycles and ultrasonographic appearance of PCOS.

Using step-wise regimens and, if needed, early cancellation based upon serum E_2 and ultrasound findings, withholding hCG administration or cancelling oocyte pick-up have been proven to be efficient in reducing the occurrence of OHSS. However, these approaches take a heavy emotional and financial toll on patients and do not resolve the issue of the infertility. Therefore, other less drastic prevention measures have been proposed such as “coasting”.

Coasting consists of stopping gonadotrophin stimulation for one or more days and seems to be a valuable method to reduce the incidence and severity of OHSS in patients at risk *(210–212)* without compromising the cycle outcome *(213,214)*.

As the incidence and duration of severe OHSS is greatest for patients who conceive *(215)*, cryopreservation of all embryos has been proposed as an alternative way to minimize hCG exposure without cancelling oocyte retrieval *(216)*. Subsequent transfer of cryopreserved-thawed embryos in a programmed cycle with steroid substitution yields a good pregnancy rate *(217)*. However, as the obstetric outcome of pregnancies complicated by severe OHSS could be worsened *(218)*, interruption of pregnancy when life-threatening situations occur may be need to be considered.

An attempt to distinguish between early OHSS (three to seven days after hCG) related to high estrogen levels and late OHSS (12–17 days after hCG) associated with clinical pregnancy could help to better define the preventive effects of these therapeutic approaches *(219)*.

Neoplasia

Breast and ovarian neoplasia are of multifactorial etiology and infertility is one of the factors considered to increase the risk. A linkage between the drugs used in ART and breast or ovarian cancers has not yet been fully established *(220)*.

Ovarian cancer

Ovarian cancer represents the sixth most common

cancer in women and is the most fatal gynaecological malignancy with a 5-year survival rate of about 40% *(221)*. However, the incidence of ovarian cancer varies widely among countries *(222)* and a large number of identifiable factors have been associated with increasing risk of ovarian cancer, including environmental, hormonal and genetic factors. Parity and oral contraceptive use have well-documented protective effects as does tubal ligation and hysterectomy. The explanation for these protective effects possibly involves decreased ovulation, at least in older infertile women *(223)*. Conversely, infertility and "incessant ovulation" have been documented as risk factors for the development of ovarian cancer *(224)*. As a result of this consistent association, it has been hypothesized that the hyperstimulatory effects of fertility medication may be linked to the genesis of some cases of ovarian cancer. However, inability to conceive is by itself a risk factor for ovarian cancer independent of nulliparity *(225)*. Therefore, the fundamental question is whether the use of drugs for the induction of ovulation independently increases a woman's risk of ovarian cancer over and above that predicated by infertility alone or infertility in conjunction with low parity.

After the first report in 1971 of a possible relationship between incessant ovulation and ovarian cancer *(226)*, it was suggested that epithelial inclusion cysts in the ovarian surface epithelium, which occur in association with ovulation, may be the source of such neoplasms. The first case of invasive epithelial ovarian cancer associated with ovulation induction was described in 1982 *(227)* and was followed by several additional case reports, cohort and case–control studies *(228)*. Concern about the risk of ovarian cancer and the use of fertility medications was recently highlighted in a series of publications *(229, 230)* which reported that infertile women using fertility drugs are three times more at risk for invasive epithelial ovarian cancer than women without a history of infertility. Conversely, in these studies, infertile women not using fertility drugs were reported to have no increased risk. However, critics of these reports have cited selection bias, wide confidence intervals, lack of a uniform etiology of infertility and temporal incompatibility between licensing of modern fertility drugs and treatment for infertility in the subjects in these studies *(231)*. In addition, no attempt was made to control for confounding factors such as infertility itself or family history of ovarian cancer.

In a well-designed and executed large case–cohort study *(232)*, infertile women using clomiphene citrate had a threefold increased risk of developing any ovarian neoplasm but, when infertile clomiphene citrate users were compared with infertile nonusers, no statistically significant increased risk was observed. Nevertheless, the use of clomiphene citrate for more than 12 ovulatory cycles was associated with an increased risk of developing an ovarian neoplasm. However, if the relationship between clomiphene citrate use and ovarian cancer had been truly causal, a more evident dose–response relationship would have been expected.

The possibility of a relationship between infertility secondary to underlying ovarian pathology and ovarian cancer has been also suggested *(233)*. It is also possible that follicular hyperstimulation may drive an already existing epithelial ovarian neoplasm to become clinically apparent. However, evidence against this hypothesis is provided by other studies that have shown epithelial ovarian carcinomas to be insensitive to gonadotrophins *(234)*.

Finally, IVF procedures including not only ovarian hyperstimulation but also repeated minor trauma for ovum pick-up, were associated with some cases of ovarian cancer. However, comparison between stimulated and natural cycles for IVF in women could not find any excess risk for ovarian cancer in the treated group *(235)*. By contrast, a significant association was detected between a diagnosis of unexplained infertility and invasive epithelial ovarian cancer.

Breast cancer

Breast cancer is the leading cancer in women and the use of oral contraceptives and hormone replacement therapy as risk factors remain controversial. Many studies have failed to observe excess risk for breast cancer among infertile women *(236)*. Furthermore, no excess risk for breast cancer can be attributed to ovulation induction. On the contrary, some antiestrogenic agents, such as clomiphene citrate, could be considered protective because of their similarity to tamoxifen *(237)*. However, a diagnosis of breast cancer during or shortly after infertility treatment should warrant close medical follow-up.

Multiple births

The medical, social and financial risks caused by multiple birth needs to be addressed in terms of policy and practice. Iatrogenic multiple pregnancy occurs

after ovulation induction with clomiphene citrate and gonadotrophins, with reported incidence figures of 5%–10% and 16%–40%, respectively *(238)*. The most common result is twinning but the greatest relative increase consists in triplet and quadruplet pregnancies. ART has affected the rate of multiple births in two ways: first, the procedures themselves have a direct impact on the incidence of multiple pregnancy; second, the number of couples undergoing infertility treatment has increased dramatically. As far as the procedures are concerned, it is obvious that the use of more aggressive ovarian stimulation for IVF has resulted in more oocytes being collected and a greater number of embryos being transferred. Nevertheless, recent adoption by several countries of a restrictive transfer policy has led to a significant reduction in high-order multiple pregnancies *(239)*.

Paradoxically, some major concern remains regarding the policy of ovulation induction for chronic anovulation or in preparation for IUI. Indeed, as the results of IUI from natural cycles proved to be inferior to those from gonadotrophin-stimulated cycles *(240)*, ovulation induction is currently performed in normally ovulating patients. As a consequence, multiple pregnancies after ovulation induction alone or associated with IUI, have accounted for the majority of all multiple pregnancies related to infertility treatment *(241)*. This is partly due to the development of a large number of leading follicles (more than two or three) *(242)*. However, careful monitoring of stimulated cycles can reduce, but not totally eliminate, this risk. Indeed, according to an extensive retrospective study *(243)*, the peak serum E_2 concentration and the total number of follicles are independent predictors of the risk of high-order multiple pregnancy. However, as recently shown *(244)*, the number of follicles with a diameter of 12 mm or more seems to be more predictive of multiple birth than that of mature (>16 mm) follicles. Therefore, the current guidelines for ovulation induction, based upon the number of large-sized follicles, may be inadequate for reducing the incidence of high-order multiple pregnancies. Nevertheless, in clinical practice, the best way to minimize the risk of multiple pregnancies is to use much milder stimulation regimens, to carefully control follicular development with both hormonal and ultrasound assessments, and to cancel the cycle in cases of overstimulation. The ovarian stimulation policy must also take into account other parameters such as the patient's age, duration of infertility, previous parity and etiology of infertility, all factors that interfere with the potential risk of multiple pregnancy following induction of ovulation *(245)*. Development of new ovarian imaging technologies may be helpful in improving the reliability of ovarian monitoring. This strategy could limit the use of IVF procedures as an alternative way to avoid the risk of high-order multiple pregnancy *(243)*.

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Intracytoplasmic sperm injection: technical aspects

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Introduction

What is micromanipulation?

No reproductive specialist could have predicted that the emerging field of experimental micromanipulation would have such an impact on assisted reproduction in less than a decade after the first relatively simple, but elegant, applications appeared. Since then, thousands of babies have been born worldwide from micromanipulative methods aimed at alleviating male infertility, enhancing implantation and prediction of normal development in embryos derived from fertile, but genetically affected, individuals. Some existing and larger laboratories have three or more complete stations for micromanipulation. Rather than certain embryologists subspecializing in the area of micromanipulation, which was the practice in the first few years, most embryologists now aim to become proficient in one or more techniques. The main emphasis in this review will be on micromanipulation of gametes, particularly intracytoplasmic sperm injection (ICSI) and handling of embryos. Micromanipulation is practised on all stages of gametes and preimplantation embryos, since the applications are mostly diagnostic or potentially therapeutic. In this context, it becomes increasingly difficult to discuss micromanipulation as a separate subject, as these techniques have become ubiquitous laboratory tools, covering such diverse areas as ovum freezing via zygote reconstitution, cryopreservation of isolated testicular spermatozoa and cytoplasmic transfer for reversal of cytoplasmic and potential nuclear incompetence as well as the other better-known techniques. Only the procedures that are not considered experimental will be discussed below.

Intracytoplasmic sperm injection

Review and problems

ICSI has been in use for the successful treatment of male factor infertility for almost a decade. The technique involves the direct injection of a single immobilized spermatozoon into the cytoplasm of a mature oocyte. Direct sperm injection was developed in invertebrates, rodents and large animals *(1–4)*. However, few births were reported in those studies. This has led to criticism, particularly from basic scientists who refer to this as "ART before science" *(5)*. Other attempts to microsurgically fertilize ova included zona drilling and subzonal sperm insertion *(6,7)*. Though those studies led to many births in animal models, application in the human was only partially successful due to physiological differences and low rates of normal fertilization *(8)*. The advent of microsurgical fertilization is an example of the

limitations of animal modelling prior to clinical application, since early methods that appeared safe and promising in the mouse were very limited in the human, whereas the ICSI method that now appears safe in the human, seems problematic in studies involving rodents and primates *(9–11)* . The first births from ICSI were reported by Palermo and colleagues in 1992 *(12)*. Since that time, use of the technique has spread rapidly due to its relative simplicity and high rate of success in achieving apparently normal fertilization in cases of previously intractable male infertility.

Spermatozoa for ICSI are obtained through standard preparation and washing of ejaculated or electro-ejaculated semen. In the case of azoospermia, a variety of techniques for microsurgical sperm aspiration or isolation from the epididymis or testis can be used *(13,14)*. Sperm suspensions are diluted, if necessary, to an appropriate concentration with culture media and combined with media containing polyvinyl pyrrolidone (PVP) to increase viscosity and facilitate handling of the spermatozoa, though the efficacy and safety of PVP use for this purpose has been questioned *(15,16)*. Mature oocytes are obtained following standard techniques and denuded of corona cells by exposure to hyaluronidase; the use of this enzyme is considered safe, but detailed follow-up of the effects of different concentrations and alternatives on pregnancy outcome are lacking *(17,18)*. Micromanipulation for ICSI requires a standard holding pipette and a bevelled, sharpened injection pipette with an outer and inner diameter of seven and five micrometers, respectively. For injection, a single, preferentially motile and overtly morphologically normal spermatozoon is selected from the PVP drop and immobilized using the injection pipette. The effects of sperm morphology on pregnancy outcome and congenital malformation is unknown. Alternatives for immobilization are mediation by a piezo actuator, laser application and pipetting *(19)*. The efficacy and safety of immobilization has been questioned *(20)*. The severity of mechanical immobilization may increase the rate of fertilization by mature and immature spermatozoa *(21,22)*. The tail of the spermatozoon is positioned at a ninety-degree angle to the injection pipette, which is lowered and drawn across the tail. This results in plasma membrane permeability and a loss of motility, both of which have been associated with an improvement in fertilization *(23,24)*. The immobilized sperm cell is aspirated tail-first into the injection pipette and transferred to the drop containing the oocyte. The oocyte is held with the holding pipette and positioned such that the polar body is not adjacent to the "3 o'clock" entry point of the injection pipette. The spermatozoon is positioned at the bevelled terminus and the injection pipette is gently advanced through the zona pellucida and into the plasma membrane of the oocyte. The membrane will either spontaneously break due to this penetration or can be broken by gentle manipulation of the injection pipette *(22)*. When the membrane has broken, cytoplasm can exhibit flow back into the injection pipette. The sperm cell is then carefully expelled into the oocyte cytoplasm and the pipette is withdrawn, completing the procedure. It should be noted that the position of the polar body in relation to the deposited sperm may affect embryo morphology, development rate and pregnancy *(25)*.

In a recent report on over 3700 ICSI cycles, the survival rate following the ICSI procedure was 94% with a normal fertilization rate of 75% with ejaculated spermatozoa and 69.8% with surgically retrieved spermatozoa *(26)*. The clinical pregnancy rate following ICSI was 43.8%. Complete fertilization failure following ICSI is rare and has been linked to oocyte activation failure or incomplete sperm decondensation *(27,28)*. Timing of the procedure may be important and failure has been linked to the inability of embryologists to time cytoplasmic maturity (Cohen and Garrisi, unpublished observations). Down-regulation and synchronization of cohorts of follicles may avoid the need for cytoplasmic timing *(29)*. Noninvasive assays for maturity are unfortunately not available *(30)*.

Concerns over the use of ICSI fall into two main categories based around fertilization abnormalities and genetic issues. Despite recent animal work, the ICSI technique was essentially developed through its clinical application in the human. This is occasionally the case with clinical assisted reproduction techniques (ART) since the available rodent or large animal "model" systems often differ markedly from the human and are therefore inappropriate for all but the most rudimentary studies. For example, successful ICSI in the mouse was only recently developed and requires specialized piezo-manipulation systems to allow for nontraumatic membrane penetration *(31)*. It appears that reproductive specialists were particularly motivated once ICSI was applied to the human and showed such high fertilization rates; they probably based the safety of the procedure on preceding technology (partial zona dissection and subzonal sperm insertion) that used microsurgical fertilization to expose the human oocyte membrane to spermatozoa, but had low clinical

success rates. These methods had reasonably accepted standards for preclinical research in animal models *(6,7)*. Besides the physical differences between animal and human work, the fertilization process differs markedly between the mouse and human due to the persistence of a functional maternal centriole in the mouse *(32,33)*. As discussed above, some ultrastructural studies were undertaken on human oocytes following failed ICSI *(27,28)*. However, the functional processes of the ICSI technique were not carefully studied until the recent development of a successful primate model system in the rhesus macaque with fertilization and implantation rates similar to those in the human *(34–37)*.

Ultrastructural and other analyses of the events of fertilization and early development following ICSI in the macaque monkey have revealed an interesting phenomenon. Following ICSI, decondensation and remodelling of the sperm chromatin is delayed and structurally distinct, and this apparently leads to a delay in the onset of DNA synthesis in the first cell cycle *(10,11)*. The ICSI process bypasses the normal events of sperm–ovum interaction and fusion. One of the key events during this interaction is the membrane fusion and exocytosis of the sperm acrosome, a cap-like membrane that overlays the sperm head plasma membrane. Following ICSI, the intact acrosome and associated perinuclear theca must be removed and processed by the oocyte cytoplasm. These events apparently result in an extension of the normal sperm head remodelling process as well as the creation of an asymmetry in chromatin decondensation. Due to the persistence of the perinuclear theca at the base of the acrosome, access to and remodelling of the apical portion of the sperm chromatin is perturbed leading to a situation where the posterior portion of the sperm chromatin is decondensed while the apical portion remains intact. This delay in chromatin decondensation apparently leads to a delay in subsequent nuclear remodelling processes including the recruitment of nuclear pore constituents and to a delay in DNA replication (by both paternal and maternal pronuclei) and the first cell cycle following fertilization *(10,37)*. Despite these observations, made during aberrant sperm head processing, the macaque oocyte can ultimately process the unusual acrosome-related structures and complete sperm chromatin decondensation successfully. Macaque zygotes fertilized by ICSI or *in vitro* fertilization are indistinguishable at 20 hours postfertilization *(10)*. Human ICSI embryos possibly exhibit slower cleavage as well however, their performance later does not reflect this initial delay (*38,* Garrisi and Cohen. unpublished). The authors of the monkey studies speculate that the delay and asymmetry in chromatin processing seen following ICSI could potentially result in abnormal chromosome behaviour during the first cell cycle and/or disturbances to gene expression. Nonrandom positioning of chromosomes in human sperm has been described with evidence that the X chromosome exhibits a bias for the apical portion of the sperm head *(39)*. This has been of particular interest since there may be a slight increase in the incidence of sex chromosome abnormalities in either human embryos, fetuses or offspring following ICSI (see below). Also, a bovine study has indicated that perturbations to replication during early embryogenesis can lead to subsequent disturbances of gene expression *(40)*. However, a direct relationship between the unusual events observed during ICSI fertilization in the macaque and any chromosomal, genetic or epigenetic effect has yet to be demonstrated. Furthermore, it is still unclear as to what extent the situation in the macaque is duplicated under normal conditions in the human. Problems with this model and interpreting these particular findings may be related to physical differences between procedures, the consequences of captivity on physiology and behaviour, the use of exogenous hormone preparations from other species, the limited number of offspring obtained, and the restricted funds that exist for studying primate embryology.

Another genetic concern with the application of ICSI is the fact that men with nonobstructive azoospermia and extreme oligospermia are at increased risk of harbouring genetic lesions related to infertility including Y chromosome deletions *(41,42)*. While such men can successfully father children through ICSI, the genetic trait related to their infertility may be transmitted to their male offspring *(43,44)*. This phenomenon is not a congenital malformation, since those are obvious in the newborn, but a predisposition *(45,46)*.

Application criteria

While the standard *in vitro* fertilization procedure has proven efficacious in the treatment of mild male factor infertility, it has become obvious that, when semen quality is impaired, the incidence of fertilization is significantly reduced *(47,48)*. The ICSI procedure has proven to be consistently successful in achieving fertilization across a large spectrum of male factor

issues including the most severe oligozoospermia, and with ejaculates completely lacking in normal forms *(49,50)*.

One factor that seems to be directly correlated with successful fertilization following ICSI is sperm motility *(51–54)*. An analysis of ICSI cycles with failed fertilization revealed a lack of motile sperm as the most common cause *(53)*. When exclusively immotile sperm are used for ICSI, fertilization and outcome are compromised. Apparent sperm motility deficiencies can result from a variety of conditions, most of which are addressable, including infection and obstructive necrozoospermia. However, genetic causes, such as immotile cilia syndrome, can also be present resulting in spermatozoa with inherently nonfunctional flagella *(52)*. ICSI results with this patient population are particularly poor *(53)*. In the absence of a clear genetic defect, a consistent lack of motile sperm in multiple ejaculates is a rare occurrence. When only nonmotile sperm are present in multiple ejaculates, an attempt to surgically isolate motile testicular sperm is indicated. If necessary, viable immotile sperm can be selected via the hypo-osmotic swelling test for membrane integrity *(55)*, or by pipetting the tail and measuring its plasticity. There is some evidence that ICSI results with immotile epididymal or testicular sperm are somewhat better than those with immotile ejaculated sperm *(56)*.

The ICSI technique has proven to be a particularly powerful tool in the treatment of men who are overtly "azoospermic" through either a physical (obstructive) or physiological (nonobstructive) cause *(57)*. Microsurgical techniques for the retrieval of viable sperm from either the epididymis (MESA) or testis (TESE) have been combined with ICSI with consistent success *(58,59)*. It has been estimated that there is some level of spermatogenesis present in at least 60% of azoospermic men and surgical sperm isolation followed by ICSI has been successful even in cases classified as Sertoli cell-only syndrome *(12,60)*. ICSI has also been successful with cryopreserved spermatozoa derived from surgical isolation techniques, although motility issues often complicate this aspect *(60,61)*. Testicular tissue and isolated spermatozoa have a high ability to survive cryopreservation, particularly in cases of obstructive azoospermia *(62,63)*. The use of ICSI following cryopreservation of extremely dilute sperm samples has been facilitated by micromanipulation techniques for the "storing" of isolated, individual spermatozoa in evacuated zona pellucidae *(64)*. The use of such animal zonae has been met with certain criticism, as it has been postulated to work as a vehicle for pathogens. Animal zonae obtained in a sterile fashion are devoid of pathogens and passive absorbance of compounds is theoretical. There are many other compounds and solutions used in IVF and ICSI that may potentially "label" spermatozoa. Such labels may not penetrate the egg during natural conception and IVF, though there is no proof that exogenous DNA cannot enter the egg in rare conditions (perhaps this is a pathway for evolution in nature). ICSI has been postulated to be a good experimental vehicle for loading DNA onto spermatozoa and creating transgenic animals *(65,66)*. Although in these studies, the transgenesis was mediated and labels were tagged onto the spermatozoa purposefully, DNA is present in many laboratory solutions such as sera and albumins, hence the need for protein-free and sterile conditions during the operation of PCR techniques. It is unknown whether such potential agents can tag spermatozoa primed for injection during ICSI.

As mentioned previously, certain genetic risks including Y chromosome deletions and sex chromosome abnormalities are increased in individuals exhibiting extreme forms of male infertility, particularly in cases of apparent azoospermia *(12)*. Another distinct risk with surgical retrieval involves the well characterized connection between cystic fibrosis (CF) mutations and congenital bilateral aplasia of the vas deferens *(67)*. Men with idiopathic obstructive azoospermia are also at increased risk for harbouring CF mutations *(68)*.

A final treatment methodology based on ICSI is the use of immature male germ cells to achieve fertilization. Several pregnancies have been reported following the injection of what are described as round spermatids of ejaculatory or testicular origin, although the success rate is extremely low *(69,70)*. Furthermore, the requirement for using such immature spermiogenic cells is questionable since it is thought that a true maturational arrest does not occur at this stage of spermiogenesis *(71)*. Theoretically, if round spermatids are present in a biopsy or ejaculate, then later stages including elongated spermatids and mature spermatozoa should also be present. Births have also been reported following elongated spermatid injection; however, the overall success of these techniques is also much reduced from that of ICSI with mature spermatozoa *(72,73)*. Finally, concerns have been raised about the potential for epigenetic problems derived from insufficient oocyte activation

by spermatid injection and issues of nuclear maturity in these cells *(74)*. There is evidence that spermatids from azoospermic males may be deficient in oocyte-activating factors and this may partially explain the poor fertilization and development rates following spermatid injection *(75)*. Furthermore, it is not known if the haploid genomes of immature spermatogenic cells are equivalent to those of mature spermatozoa and therefore a potential exists for incorrect epigenetic programming *(69)*. We concur generally with the comments against round spermatid injection made by others *(76)*.

Risk–benefit analysis

ICSI is still the only commonsense alternative to male factor infertility treatment. There are no other alternatives either known, such as *in vitro* fertilization, or to be developed that show any promise. It is in this light that a risk–benefit analysis should be discussed. What are the risks? One concern would involve potential consequences in future generations. Even from animal models, there are no clues as to what happens to the next generation. Animal models are also hampered since there are no good models for human male infertility. The closest model is perhaps the cheetah, and obviously that species cannot be used for clinical work. The primate work discussed above shows many cell biological variations that occur after ICSI and that may be considered anomalous, but it appears that these phenomena are overcome during further development. The same research team has now found, apparently, that there are also more subtle differences between these monkeys born after ICSI showing apparently greater levels of developmental aberrations (G. Schatten, personal communication). What about monkeys as laboratory models for ICSI? They live in captivity, are usually fertile, but are not out-bred like humans. In addition, they receive hormone preparations for follicular stimulation that are not just exogenous, but prepared from blood or urine from other species. Are we to believe that none of these important criteria should be considered when assessing models for ICSI? Similar criteria can be applied to the mouse model when this becomes available for evaluating ICSI. Here again, there are important differences that are similar to the ones described for monkeys, but in addition, conventional ICSI using a piercing needle cannot be applied in the mouse. Instead, one has to apply a piezo actuator to pierce the membrane. Such piezo actuators are damaging to mouse development when used at certain settings (T.Schimmel, unpublished observations).

Considering the above, and considering the clinical evaluations of ICSI to date, the risk–benefit scale weighs heavily towards benefit. There is a slight increase in congenital anomalies, particularly concerning sex chromosome disorders and male-determined genetic diseases. In some subgroups there could be an incidence of 10% or more of Y deletions. Even considering that this is a mild congenital error, many patients show little interest in this fact.

Conclusions

ICSI is indicated as a treatment in essentially all cases of severe male factor infertility where some spermatozoa can be obtained either via ejaculation or surgical isolation. A lack of sperm motility is one factor that can compromise success and a careful evaluation and diagnosis of the cause of such motility deficits is indicated. The use of immature spermatogenic cells for ICSI has been reported although success rates are very low and this methodology remains controversial. Genetic counselling for patients with male factor infertility is strongly suggested due to general and specific risk factors present in this population. It remains to be determined whether ICSI is a replacement for IVF in patients with nonmale factor infertility, but more importantly, whether it should be a replacement for IVF.

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Intracytoplasmic sperm injection: micromanipulation in assisted fertilization

ANDRÉ VAN STEIRTEGHEM

Assisted hatching: overall outcome

Different assisted hatching (AH) procedures have been developed to help embryos escape from their zona during blastocyst expansion. These include mechanical incision of the zona, chemical zona drilling with acidic medium, laser-assisted hatching and piezo technology *(1)*. These different procedures for AH will be reviewed in this chapter.

The clinical relevance of AH within an assisted reproductive technology (ART) programme remains controversial and elusive since reports on only a very few randomized controlled trials (RCT) are available. The discussion in this chapter will be limited to these reports.

Using chemical zona drilling, Cohen and Feldberg reported in 1991 *(2)* the results of an RCT including 69 couples in the study group and 68 couples in the control group. In this study, the implantation rate of zona-drilled embryos was 28% (66/236) which was better ($p<0.05$) than the 22% implantation rate of zona-intact embryos (50/226).

In an RCT involving 137 couples in whom the female partner had normal basal serum follicle stimulating hormone (FSH) concentrations, the implantation rate of zona-drilled embryos (28%; 67/239) was not statistically different from the implantation rate of zona-intact control embryos (21%; 49/229) *(3)*.

The hypothesis as to whether or not AH could rescue poor-prognosis embryos (i.e. those showing thick zonae, low developmental rates and excessive fragmentation) was tested in 163 patients randomized in a control group and a study group in which embryos were selectively zona-drilled. The implantation rate in the study group was 25% (70/278), significantly ($p<0.05$) higher than the implantation rate of control embryos (18%; 51/285). In addition, selective AH appeared most effective in women over 38 years of age and in those with elevated basal FSH levels *(3)*.

In 1996, two prospective randomized trials demonstrated clearly that no benefit resulted from AH for an unselected group of patients undergoing embryo transfer (ET), whether using partial zona dissection (PZD) *(4)* or acidic Tyrode zona drilling *(5)*. It can be concluded that AH might be of value in increasing embryo implantation rates in humans, but only in selected cases.

The benefit of AH in the older age group remains uncertain since only one RCT has evaluated whether AH improved implantation rates and pregnancy in this age group *(6)*. Patients >36 years of age were in a study group with hatching (n=41) or a control group without hatching (n=48). No significant differences were observed in the rates of implantation (11.1% versus 11.3%), clinical pregnancy (39.0% versus 41.7%) or ongoing pregnancy (29.3% versus 35.4%) between the two groups.

A prospective but uncontrolled pilot study on 19 patients reported that high pregnancy rates per cycle/transfer (53%) and implantation rates (33%) could be achieved when enzymatic pronase treatment of the zona pellucida of Day 5 blastocysts was carried out *(7)*. This hypothesis was also tested after transfer of zona-free Day 3 embryos *(8)*. Women below the age of 40 years undergoing a first intracytoplasmic sperm injection (ICSI) cycle were randomized to receive zona-free embryos (27 women) and zona-intact embryos (25 women). The pregnancy rate was not significantly improved when the zona pellucida was removed. A second group of patients included women 40 years of age and more and/or who had had at least two previous failed *in vitro* fertilization (IVF)/ICSI attempts; they were randomized in a 3:4 ratio (30 zona-free, 41 zona-intact). Zona removal resulted in a significantly higher pregnancy rate when compared with controls (23% versus 7.3%). The authors conclude that complete removal of the zona pellucida can improve pregnancy rates in women with poor IVF/ICSI prognosis.

When reviewing the outcomes of ART, two phenomena have been identified which appear to influence monozygotic twinning, i.e. ovulation induction *(9)* and zona pellucida architecture and manipulation *(10)*. ICSI and AH are both zona-breaching procedures and the subject of monozygotic twins in the context of zona pellucida micromanipulation needs to be carefully addressed. Reviewing a large number of transfer cycles (n=3546), the Cornell group assessed the occurrence of monozygotic twins and a possible relationship with zona pellucida manipulation and did not find a different monozygotic twinning rate between zona-manipulated and zona-intact subgroups. The authors concluded, however, that studies with greater statistical power are needed *(11)*.

The association between AH and monozygotic twinning was assessed in a case–control study design by the Division of Reproductive Health of the Centers for Disease Control on a sample of IVF-ET cycles initiated in 1996 in clinics in the USA *(12)*. Cases were those pregnancies for which the number of fetal hearts observed on ultrasound exceeded the number of embryos transferred. These pregnancies were considered to contain at least one monozygotic set of twins. Cases were compared with two control groups: other multiple-gestation pregnancies (>2 fetal hearts but number of fetal hearts <number of embryos transferred); and singleton pregnancies (1 fetal heart). Women with a case pregnancy were more likely to have received embryos treated with AH procedures than were women in either control group. After appropriate adjustment for confounding variables (patient age, number of embryos transferred, prior cycles, infertility diagnosis, ICSI and whether embryos were cryopreserved in the cycle), odds ratios and 95% confidence intervals for use of AH were 3.2 (1.2 and 8.0) compared with other multiple-gestation pregnancies, and 3.8 (1.8–9.8) compared with singleton pregnancies.

ICSI

Since the birth of Louise Brown in July 1978, it has become clear that conventional IVF is an efficient ART procedure for couples with female factor infertility, especially tubal disease, and for couples with unexplained infertility *(13,14)*; however, it soon became apparent that it yielded generally poor pregnancy rates for couples with male factor infertility *(15)*. In this study, IVF results were compared in a group of couples with male factor infertility and a similar group with tubal factor infertility. In cases with male infertility, more oocytes were recovered but fewer oocytes were fertilized; fewer ETs were performed, the average number of embryos per transfer was lower and the total pregnancy rate was also lower at 13% versus 23%. From this and other studies it could be concluded that in male infertility the results of IVF were disappointing. A number of couples with severely impaired semen could not be accepted for IVF; this included of course couples with obstructive and nonobstructive azoospermia.

Several procedures of assisted fertilization were explored. The clinical results of partial zona dissection and subzonal insemination were, for both procedures, initially encouraging but with wider experience they proved to be inconsistent and rather disappointing *(16–19)*. A major turning point in assisted fertilization occurred in 1992 when our group reported the first pregnancies and births after ICSI *(20)*. ICSI involves injection using micromanipulation procedures of a single spermatozoon directly into the cytoplasm of the oocyte through the intact zona pellucida. It soon became apparent that ICSI was superior to PZD or subzonal insemination (SUZI), in that the fertilization rate was consistently better and more embryos with implantation potential were produced *(21,22)*. Since July 1992, ICSI has been the only procedure used in

our Centre when some form of assisted fertilization is necessary *(23)*.

Success rates

ICSI has become well established worldwide as an effective form of treatment for couples with male factor infertility, including azoospermia. In the last available report from the Human Fertilisation and Embryology Authority (HFEA) in the UK (1997/98), there were 9295 ICSI cycles, alongside 24 889 conventional IVF cycles, indicating that 27% of ART in the UK is carried out for severe male factor infertility. In that year, the reported birth rate was 20.7% per cycle for ICSI compared to 14.9% for IVF. However, in that same report the multiple birth rate was over 25% and the age of the female partner was a major determinant of success: for women over 40 years of age the success rate per cycle was 5% or lower while in women in their early thirties the success rate was 20%–25% *(24)*.

The results of the survey carried out by the ESHRE ICSI Task Force were recently reviewed by Tarlatzis and Bili *(25)*. In this worldwide survey over a three-year period (1993–1995), the number of centres undertaking ICSI increased from 35 to 101, and the number of ICSI cycles per year increased from 3157 to 23 932. Less than 10% of oocytes were damaged by the procedure and the normal fertilization rates obtained with ejaculated, epididymal and testicular spermatozoa for 1995 were 64%, 62% and 52%, respectively. An ET was possible in 86%–90% of couples, the viable pregnancy rate was 21% for ejaculated, 22% for epididymal, and 19% for testicular sperm, with an incidence of multiple gestations of 29%, 30% and 38%, respectively. In this survey there was no difference in the results of ICSI according to the etiology of the azoospermia.

Our own group *(26)* reviewed the outcome of the first seven years of ICSI practice (1991–1997) which involved 7374 cycles involving 74 520 metaphase-II oocytes injected with a single sperm. In only 3.1% of scheduled cycles the procedure could not be carried out because there were no oocytes or spermatozoa available for the microinjection procedure. The damage rate of oocytes after ICSI was 9.1%. The normal fertilization rate was higher after ICSI with ejaculated spermatozoa (67.0%) than after ICSI with epididymal or testicular spermatozoa (between 56.6% and 59.8%). It was extremely exceptional that none of the injected oocytes fertilized normally. The morphological quality of the embryos was also better after ICSI with ejaculated sperm but the percentages of embryos actually transferred or frozen as supernumerary embryos was similar for the four types of spermatozoa and varied between 59.6% and 65.7% of the two-pronuclear oocytes. The transfer rates and overall pregnancy rates were similar for the different types of sperm used. Reviewing the data on ICSI outcomes in a group of Belgian couples, less than 37 years of age, who had their first ICSI cycle between 1992 and 1993, the per cycle delivery rate was as high as 31% but the real cumulative pregnancy rate was 60% after 6 cycles of treatment. Again, female age had a powerful effect on success rates *(27)*.

Safety of ICSI

Since the introduction of ICSI there has been major concern about its safety and the health of the ICSI offspring. The direct injection of a single spermatozoon into a mature oocyte bypasses the natural physiological processes of normal sperm selection and raises concern over the potential risk of congenital malformations and genetic defects in children born after ICSI. When ICSI with nonejaculated spermatozoa was introduced, more concern was expressed. This concern related to the safety of the ICSI technique itself, to the chromosomal or genetic constitution of the sperm used, and to the possibility of incomplete genomic imprinting at the time of fertilization when testicular spermatozoa are used. Recent work indicated that poor quality semen contained spermatozoa with compromised DNA integrity *(28)*, but this did not compromise their fertilizing ability at ICSI *(29)*, further highlighting the importance of assessing ICSI outcome.

When ICSI was introduced in Brussels, we further enhanced our extensive follow-up procedures for ART pregnancies and children. Before starting ICSI, the couples are asked to participate in this prospective follow-up study, which includes karyotyping both partners, genetic counselling, prenatal karyotype analysis (now amended, after careful information of the different risks, to optional karyotyping in the first two years postnatally) and participation in a prospective clinical follow-up study of the children. This includes completing a standardized questionnaire and returning it to the research nurses, and—where possible—visiting the Centre for Medical Genetics with the child after birth. All couples referred for assisted conception were evaluated for possible genetic risks (personal history, family history,

medication, alcohol abuse, smoking, socioeconomic status and possible environmental or occupational risk factors). A karyotype was routinely performed for the couple. At birth, written data concerning the pregnancy outcome were obtained from the obstetrician or the paediatrician in charge. A detailed physical examination was carried out on babies born at our University Hospital. Whenever possible, babies born elsewhere were examined at two months in our Centre by a paediatrician–geneticist. Further follow-up examinations were done at 12 months and two years to assess physical, neurological and psychomotor development. At approximately two years, Bayley tests were performed in order to score the psychomotor development of the children. Further psychomotor evaluation and social functioning are scheduled at a later age. This extensive follow-up programme is a collaborative study of the Centres for Reproductive Medicine and Medical Genetics of the Vrije Universiteit Brussel *(26,30–35)*.

Prenatal diagnosis in ICSI pregnancies

The available results of prenatal diagnosis after ICSI are summarized in Table 1. Seven studies report on 2175 fetal karyotypes. There were 42 de novo chromosomal aberrations (16 sex chromosomal aneuploidies and 26 autosomal aneuploidies or structural aberrations) and 31 inherited aberrations. In our own, so far unpublished, series on 1473 fetal karyotypes, we concluded that there is a statistically significant increase in sex chromosomal aberrations and structural de novo aberrations as compared to a control neonatal population.

These results are used in our practice to inform the patients during genetic counselling about the risk of chromosomal aberrations in ICSI fetuses. Currently, in our own practice, about half of the patients choose to have amniocentesis or chorionic villus sampling. In a survey of counselling about prenatal diagnosis to 107 women pregnant as a result of ICSI, Meschede *et al*. *(42)* reported that among these patients there was a strong preference (82%) for noninvasive prenatal diagnosis (ultrasound, serum markers) while only 17% made use of amniocentesis or fetal blood sampling.

The question has been raised as to whether invasive prenatal testing involves additional risks for the patients undergoing ICSI. The pregnancy outcome was compared in 576 pregnancies after invasive prenatal diagnosis with that of 540 pregnancies without it. Amniocentesis was recommended for singleton pregnancies and chorionic villus sampling for twin pregnancies. Prenatal testing did not increase the preterm delivery rate, the low-birth-weight rate, or the very low-birth-weight rate, as compared with controls. The fetal loss rate in the prenatal diagnosis group was comparable to that of the control group *(43)*.

Congenital malformations in ICSI children

It should first of all be stressed that the data in the literature do not give a full picture of the outcome of ICSI pregnancies. In the ESHRE survey *(44)*, 94 centres from 24 countries provided data on 14 000 cycles of treatment, from oocytes collected to embryos transferred. A total of 24 centres completed the follow-up survey for the children but only two centres reported major congenital malformations. All voluntary surveys on IVF outcome are incomplete regarding such parameters as complications during pregnancy and perinatal outcome. Furthermore, the comparison between surveys is difficult since the methodology used to collect data varies from one survey to another including such points as the way in which major or

Table 1. Karyotype analyses in prenatal diagnoses after ICSI

References	Fetuses	De novo chromosomal aberrations		Inherited structural aberrations
		Sex chromosomal	Autosomal	
36	115	–	–	5
37	71	6	3	–
38	101	–	1	3
39	209	–	6	1
40	57	1	–	1
41	149	–	2	2
35	1473	9	14	19

Table 2. Liveborn children and congenital malformation rate in intracytoplasmic sperm injection children

Reference	Liveborn children				Children from multiple pregnancies (%)	Prenatal mortality	Major and minor congenital malformations
	Total	Singleton	Twins	Triplets*			
46	2059	708	980	271	60.8	16	22 major/11 minor
39	721	473	230	18	34.4	1.4%	2.2% major/1.2% minor
47	1139	736	400	3	35.4	NA	47 (4.1%) "serious"
48	2762	1861	782	119	32.6	3.0%	2.5% minor
35	2840	1499	1228	113	47.2	1.7%	3.4% major/6.3% minor

* Triplet or higher-order liveborn

minor malformations are categorized *(45)*.

Table 2 summarizes relevant reports of groups in the USA *(46)*, Denmark *(39)*, Sweden *(47)*, Australia and New Zealand *(48)*, and Belgium *(35)*.

When the number of children in these surveys are categorized in terms of originating from singleton, twin, triplet or higher-order pregnancies, it becomes evident that the overall percentage of children born who do not originate from singleton pregnancies is 44% (4244 out of 9521 children); this percentage varies from 32.6% to 60.8% according to the survey. Most of these children are from twin pregnancies. The mean percentage of children from triplet or higher-order pregnancies is 5.5% (524 out of 9521 children); this percentage varies greatly among centres, in one centre it is as high as 13.2% *(46)* and in the other centres between 2.5% and 4.3%. The problems generated by multiple births after ART arising from the practice of replacing multiple embryos has been well documented *(49,50)*. It is more than obvious that this excessive number of multiple pregnancies will result in problems appearing either immediately or later in life. It is therefore without any doubt the objective for all ART centres that the multiple pregnancy rate should be reduced substantially in the future.

As indicated in Table 2, perinatal mortality in the different surveys varies from 1% to 3%. The major and minor congenital malformation rate cannot be compared between the different surveys because of the differences in methodology used in these surveys *(45)*.

In Denmark, a national cohort study of 730 infants born after ICSI included all clinical pregnancies obtained after ICSI registered in Denmark between January 1994 and July 1997 at five public and eight private fertility clinics *(39)*. The frequency of multiple birth, caesarean section rate, gestational age, preterm birth, and birth weight were comparable with previous studies. The perinatal mortality rate was 1.37% for children born at a gestational age of 24 weeks or more. Major birth defects were reported by the parents in 2.2% of liveborn children and in 2.7% of all infants. Minor birth defects were found in nine liveborn infants (1.2%).

A Swedish study *(47)* reported the malformation rate in 1139 infants born after ICSI in two IVF clinics in Göteborg using data from the medical records, the Swedish Medical Birth Registry and the Registry of Congenital Malformations, and compared the results with all births in Sweden and also with births after conventional IVF. An identified anomaly was seen in 87 children (7.6%), 40 of which were minor. The odds ratio for ICSI children having any major or minor congenital malformation was 1.75 (95% CI 1.19–2.58) after stratification for delivery hospital, year of birth and maternal age. In a population study from Sweden on all infants born after IVF between 1992 and 1997, hypospadias was more frequent in ICSI children *(51)*. This may be related to paternal subfertility with a genetic background. The groups from Göteborg also examined the obstetric outcome of pregnancies after ICSI *(41)*. Deliveries occurred in 75.9% and early spontaneous abortion, late spontaneous abortion and ectopic pregnancy in 21.4%, 1.0% and 1.2% of pregnancies, respectively. Multiple birth occurred in 21.3% (almost all twins) of deliveries, and preterm birth occurred in 15.7% of all deliveries. Preterm birth was not related to sperm origin or quality but was related to multiple birth. The prematurity rate was 8.4%, 42.8% and 100% for singletons, twins and triplets, respectively. The perinatal mortality rate was 11.7 per 1000 born infants; 7.6% of infants had a malformation, 40 of which were minor. They concluded that the obstetric outcome of ICSI pregnancies was similar to that of conventional IVF and was not influenced by sperm origin or quality.

The current details of the major malformations in the Brussels Free University (Vrije Universiteit Brussel

VUB) survey can be summarized as follows *(35)*: major malformations were found in 18 terminated pregnancies and in eight stillbirths among a total of 49 stillbirths after 20 weeks. There was one additional malformation detected prenatally, i.e. a child with a holoprosencephaly detected at 15 weeks of pregnancy; this child died at birth. The major malformation rate was 3.4% (96/2840 live-born children), with 3.1% in children from singleton pregnancies and 3.7% in children from multiple pregnancies. Defining the total malformation rate as (affected live births + affected fetal deaths + induced abortions for malformations) divided by (live births + stillbirths), the figures are (96 + 8 + 18) divided by (2840 + 49) or 4.2% (Brussels Free University, unpublished). The major congenital malformation rate was also similar between children born after ICSI with ejaculated sperm (3.5%) and nonejaculated (epididymal or testicular) sperm (3.4%) and in children from singleton (3.1%) or multiple pregnancies (3.7%).

Further medical and developmental outcome of ICSI children

In 1998, two publications in *The Lancet* reported on the further development of ICSI children. An Australian study *(52)* compared the medical and developmental outcome at one year of 89 children conceived by ICSI with 84 children conceived by routine IVF, and with 80 children conceived naturally. Developmental assessment was done with the Bayley Scales of Infant Development from which a mental development index was derived. The incidence of major congenital or major health problems in the first year of life was similar in the three groups. The mental development index was lower for ICSI children (especially boys) at one year of age than for IVF or naturally conceived children; more ICSI children showed mildly or significantly delayed development in this test, which assesses memory, problem-solving and language skills (generally in the predominant language of the country). In the same issue of *The Lancet*, our own group *(53)* reported that at two years of age the mental development, as tested by the Bayley Mental Developmental Index, of 201 ICSI and 131 non-ICSI IVF children was similar between the ICSI and IVF groups and comparable to that for the general population. Our conclusion was that there was no indication at this point that ICSI children have slower mental development than the general population. In a later case–control study from the UK, Sutcliffe *et al.* *(54)* compared 123 ICSI children and 123 children conceived naturally when they were between 12 and 24 months old. Only singleton children were recruited; children were matched for social class, maternal educational level, region, sex and race but not maternal age. The mean mental age and the mean Griffiths quotients were comparable; only eye–hand coordination was lower in the study group. There was no difference in the numbers of major and minor malformations in the children from the study and control groups.

As suggested in a commentary in *The Lancet (55)*, further case–control studies taking into account parental background and other confounding variables, such as language spoken at home, are needed before final conclusions can be drawn in this area.

Conclusions and suggestions for further studies

As the database of ICSI offspring grows larger, the available evidence on the short-term health of these offspring is generally reassuring. The major issue remains the high prevalence of multiple pregnancies and the short- and long-term consequences of prematurity.

Several issues need to be further addressed in relation to different aspects of ICSI outcome, including the role of prenatal testing during ICSI pregnancies, the significance of malformations among terminated pregnancies and stillbirths, the outcome of ICSI pregnancies in cases where nonejaculated sperm has been used, the incidence of abnormalities in children after replacement of frozen-thawed ICSI embryos, and the long-term follow-up of ICSI children.

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Cryopreservation of oocytes and ovarian tissue

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Introduction

The concept of oocyte storage

Oocyte freezing has the potential to be an important adjunct to assisted reproductive technologies (ART) in humans and domestic animals. However, the ease and success of cryopreservation programmes for sperm *(1)* and embryos *(2)* contrast markedly with the problems associated with freezing mammalian oocytes *(3)*. The results of numerous studies suggest that the survival of human oocytes after cryopreservation can be affected by their stage of maturation, their quality or by biophysical factors resulting from the cryopreservation procedure used *(4)*. For example, the maturity, quality and size of the oocyte are particularly important characteristics affecting the outcome of cryopreservation. However, oocyte freezing has been slow to be adopted clinically as the number of oocytes which survive the freeze-thaw process is extremely variable and fewer than 1% of fertilized cryopreserved oocytes have developed to term, even when fertilized by intracytoplasmic sperm injection (ICSI).

Human oocytes can be stored as either (i) denuded individual oocytes; or (ii) cumulus-enclosed oocytes. The approach selected usually depends on the stage of nuclear maturity of the oocyte prior to cryopreservation. For example, where mature metaphase II (MII) oocytes are harvested for storage the gametes are commonly denuded prior to freezing to confirm their nuclear status. In contrast, it is physiologically far more appropriate to leave full-grown, germinal vesicle (GV) oocytes enclosed within their cumulus cells during the freezing process for optimal *in vitro* maturation (IVM) after thawing. Each approach has advantages and limitations.

Mature metaphase II oocytes

Metaphase II oocytes are collected as part of the existing practice in assisted reproduction and oocyte cryopreservation can be easily incorporated into the current ART protocols. At this stage in their developmental pathway, the oocytes have undergone nuclear and cytoplasmic maturation, the first polar body has been expelled and the chromosomes are condensed and arranged on the delicate MII spindle. Freezing of MII oocytes has been the subject of numerous early publications *(5–9)* and probably even more unreported studies. Despite this intense interest, the results in humans have been generally disappointing and, to date, only a few dozen live births using cryopreserved oocytes have been reported. Several reasons can be offered—mature human oocytes have a short fertile lifespan, they are exquisitely sensitive to chilling and have little capacity for repairing cryoinjury before fertilization. Nevertheless, a number of recent reports

Table 1. Details of the methodology, survival and pregnancy rates obtained after oocyte cryopreservation [adapted from *(12)*]

Authors/reference	Years	Type of CPAs	Freezing method	Number frozen (% survival)	Stage of freezing and method of fertilization	Pregnancies/ deliveries
Porcu *et al. (13)*	1997	PR	Slow	12 (33)	MII/ICSI	1/1
Tucker *et al. (14)*	1998	PR	Slow	311 (24)	MII/ICSI	5/3
Polak de Fried *et al. (15)*	1998	PR	Slow	10 (30)	MII/ICSI	1/1
Young *et al. (16)*	1998	PR	Slow	9 (89)	MII/ICSI	1/–
Tucker *et al. (17)*	1998	PR	Slow	13 (23)	GV/ICSI	1/1
Porcu *et al. (18)*	2000	PR	Slow	1502 (54)	MII/ICSI	16/9
Kuleshova *et al. (10)*	1999	EG+S	Vitri	17 (65)	MII/ICSI	1/1
Yoon *et al. (11)*	2000	EG+S	Vitri	90 (63)	MII/ICSI	3/1
Fabbri *et al. (19)*	2001	PR+S	Slow	1769 (54)	MII/ICSI	–

CPAs: cryoprotective agents; DM: dimethylsulfoxide; PR: 1.2-propanediol; EG: ethylene glycol; S: sucrose; Slow: slow freezing programme; Vitri: vitrification programme; GV: germinal vesicle; MII: metaphase II; ICSI: intracytoplasmic sperm injection

show improved oocyte survival and developmental potential with viable pregnancies after oocyte cryopreservation by slow freezing techniques (Table 1). Other recent studies *(10,11)* give hope of success with vitrification as an alternative to slow freezing protocols. Together these reports have regenerated clinical interest in the cryopreservation of fully grown human oocytes.

Immature germinal vesicle oocytes

An alternative approach to MII oocyte cryopreservation is the storage of GV stage oocytes. These cells can be harvested from Graafian follicles after gonadotrophin stimulation using ultrasound-guided follicular aspiration with modified needles and lower pressure *(20,21)*. At the time of recovery, GV oocytes are full-size but their chromatin remains at the diplotene stage of prophase I and they therefore do not possess a spindle apparatus. Unlike MII oocytes, immature GV oocytes require a period of maturation to induce the required nuclear and cytoplasmic changes before they are competent to undergo fertilization and support early embryo development.

Alternative approaches to oocyte freezing—ovarian tissue cryopreservation

In view of the limited success with the cryopreservation of oocytes collected from preovulatory follicles, recent research has focused on the development of an alternative strategy of storing oocytes at the earliest follicle stage—the primordial follicle. A number of options are available for primordial follicle and oocyte freezing. These include (i) storage of denuded primordial oocytes from isolated primordial follicles; (ii) storage of intact primordial follicles; and (iii) storage of thin slices of the ovarian cortex. Biopsies of ovarian cortex obtained by laparoscopy, laparotomy or oophorectomy can yield tens to hundreds of primordial and primary follicles depending on the mass of tissue and age of the patient from which the tissue was harvested *(22)*. Despite the apparent difficulties of freezing complex tissues as compared with single cells, the storage of ovarian tissue has proved surprisingly successful and a number of reports have demonstrated that human primordial follicles can survive cryopreservation after cooling to –196°C in liquid nitrogen *(23–25)*. Furthermore, the banking of primordial follicles *in situ* offers the potential to restore natural fertility by autografting when the thawed tissue is returned to the body at either orthotopic or heterotopic sites *(24, 25)*. The suitability of ovarian tissue for freezing is enhanced by the developmental plasticity of the tissue as the ovary is capable of functioning even when its complement of follicles has been severely reduced such as naturally occurs during ageing, after partial ablation or after injury. Unlike cryopreservation of isolated cells, freeze-storage of tissue presents new problems because of the complexity of tissue architecture and cryopreservation protocols must strike a balance between the optimal conditions for each different cell type. The characteristics that influence the sensitivity and suitability of each of these three stages of oocyte development for cryostorage are shown in Table 2.

Table 2. Comparison of the characteristics that influence cryosensitivity and suitability for cryostorage [reproduced with permission from *(3)*]

Material	Primordial oocyte	Full-size immature (GV stage) oocyte	Full-size mature (MII stage) oocyte
Availability	Abundant, always present	Scarce, only from antral follicles	Scarce, only at mid-cycle
Ease of collection	Easy, e.g. biopsy	Oocyte retrieval	Oocyte retrieval
Size	<50 µm	80–300 µm (species dependent)	80–300 µm (species dependent)
Support cells	Few, very small	Numerous corona/cumulus	Numerous corona/cumulus
Nuclear status	Resting prophase I, nuclear membrane	GV, has nuclear membrane	Resting MII, on temperature sensitive spindle, no nuclear membrane
Zona	No	Yes	Yes
Cortical granules	No	Central	Peripheral
Intracellular lipid	Little	May be abundant	May be abundant
Metabolic rate	Low	Low	Low
Surface:volume ratio	High	Low	Low

GV: germinal vesicle; MII: metaphase II

The principles and practice of oocyte cryopreservation

Developing protocols that optimize the survival, fertilization and developmental rates of fully grown GV and MII stage oocytes following exposure to the extreme chemical and physical stresses associated with cryopreservation has proved to be a major challenge. Cryopreservation of biological specimens causes complex changes in structure and cellular composition, and no single approach has yet proved to be universally effective. In addition, there are significant stage- and species-specific differences between freezing oocytes and embryos. A number of parameters of normal oocyte physiology have been highlighted as potential targets for injury that may result from various cryopreservation methods (Table 2). Changes in any of these parameters will contribute to loss of developmental competence of the stored oocytes through cellular injury or even to irreversible loss of viability during the cryopreservation process.

Cryopreservation protocols for oocytes and ovarian tissue can be broadly classified as "equilibrium" (slow freezing) or "nonequilibrium" (rapid freezing) according to the cooling rates and cryoprotective agents (CPAs) used. However, the basic concepts of these two protocols are the same, as they both aim to protect the cells from the effects of intracellular ice crystal formation, cellular dehydration and drastic changes in solute concentrations at both high and low temperatures. Fundamentally, oocyte cryopreservation requires that cells tolerate three nonphysiological conditions: (i) exposure to molar concentrations of CPAs; (ii) cooling to subzero temperatures; and (iii) removal or conversion of almost all liquid cell water into the solid state.

Exposure to cryoprotectants

The first and essential step in any cryopreservation protocol for isolated oocytes, follicles or samples of ovarian cortex is to equilibrate the cells with a CPA. Various compounds act as CPAs to protect cells against freezing injury. CPAs share common features as they are completely miscible with water, are nontoxic even at high concentrations, and easily permeate cell membranes. Cryoprotectants are thought to protect cells by stabilizing intracellular proteins, by reducing or eliminating lethal intracellular ice formation, and by moderating the impact of concentrated intra- and extracellular electrolytes *(26)*. One of the keys to the success of oocyte cryopreservation appears to be achieving adequate permeation of CPAs across the oocyte membrane. This necessitates optimization of protocols for each cell or tissue type as post-thaw survival of ovarian tissue is profoundly affected both by the type of cryoprotectant used and the equilibration time required for CPA uptake and removal *(27,28)*.

As first proposed by Mazur in 1963 *(26)*, one major factor that influences the response of a cell to freezing is the ratio of its surface area to volume of the cell. In

general, the larger the cell, the slower it must be cooled to survive freezing. For example, a human oocyte with a diameter of 120 µm has a volume of 9.05×10^5 µm^3 and a surface area of 4.5×10^4 µm^2. By comparison, a human spermatozoon has a volume of 28 µm^3 and a surface area of 120 µm^2 *(29)*. Thus, the flattened, paddle-shaped sperm cell has a surface area to volume ratio of 4.3, whereas the spherical oocyte has a surface area to volume ratio of only 0.05. Consequently, human oocytes require much longer to reach osmotic equilibrium when exposed to CPAs than do spermatozoa. Furthermore, optimum cooling rates for spermatozoa are much higher than those for oocytes *(30)*.

Additionally, at temperatures near 0°C, care must be take to avoid extreme fluctuations in cell volume during CPA equilibration as abrupt volume excursions *(31)* can immediately damage cells and also make them more susceptible to stress during subsequent cooling or thawing procedures. At the same time, the duration of exposure to these potentially toxic chemicals should be minimized. Cryoprotectant toxicity can be reduced by lowering the temperature of exposure, but this may necessitate a longer exposure time. Although many investigators attribute cellular damage during freezing solely to the toxicity of the CPAs, others argue that cellular damage is due entirely to osmotic shock (see below).

Cooling to subzero temperatures

When oocytes are frozen in molar concentrations of CPAs, their survival is strongly dependent on cooling rate, and the specific optimum cooling rate that yields maximum survival is dependent both on the type and concentration of the CPA. Cell survival is also equally dependent on warming rate. The optimum warming rate depends both on the CPA and its concentration, as well as on the cooling rate that preceded it. Cryobiological studies have shown that different types of cells, even when frozen in the same solution, exhibit different optimum warming rates. These facts are especially relevant to the cryopreservation of ovarian cortex, since this tissue comprises many diverse types of cells and each type has its own characteristic size, shape and permeability properties. Therefore, cooling and warming conditions that are optimum for one cell type within the ovarian cortex may be harmful to others. Because of the complexity of ovarian architecture, survival of frozen tissues is dependent not only on cooling and warming rates, but more importantly, is affected by the rate and method by which CPAs are removed from cryopreserved tissues *(32–34)*.

Osmotic events during cell cryopreservation

When aqueous solutions are frozen, water is removed in the form of ice, causing the cells to become increasingly concentrated as the temperature falls. The reverse occurs during thawing. Furthermore as the cells are frozen, they must respond osmotically to large changes in extracellular fluid concentrations. The efflux of water during slow (<2°C/min) freezing causes oocytes to undergo osmotic dehydration leading to contraction *(28)*. It has also been argued that cells can be damaged because of long exposure to high electrolyte concentrations, excessive cell dehydration and the mechanical effects of external ice. But these interpretations are not universally held.

Although cells in suspension can tolerate exposure to very high concentrations of CPAs, whether the cells survive the freeze-thaw process or not is dependent on how they are removed from the CPA solution. When frozen cells are warmed rapidly, the melting of the cell suspension is equivalent to rapid dilution of the CPA that became concentrated during the freezing process. The rapid influx of water into cells or tissues as the extracellular milieu begins to melt can cause osmotic shock at subzero temperatures *(35)*. Sensitivity to osmotic shock is therefore a function of the cell's permeability to water and solutes. This shock can be reduced by use of an osmotic buffer, such as sucrose, as a nontoxic, impermeable substance *(36,37)*. Tissues are even more sensitive to osmotic effects than cell suspensions, because cells in the interior of a piece of tissue can respond osmotically only when the neighbouring cells have also responded.

Cryopreservation protocols

Equilibrium freezing protocols for oocytes and ovarian tissue

For the most part, the cryopreservation procedures used for equilibrium freezing of oocytes and ovarian cortex are very similar to the procedures designed for cleavage stage embryos. Denuded and cumulus-enclosed oocytes are usually frozen in straws or ampoules after being equilibrated in an aqueous solution containing an optimal concentration of 1–1.5

M cryoprotectant and an osmolyte such as sucrose at a concentration of 0.1–0.5 M. The CPAs most commonly used for mature oocyte cryopreservation are propylene glycol, ethylene glycol and dimethylsulfoxide (DMSO) (Table 1). Following CPA exposure, the temperature is then slowly lowered and ice crystal growth is initiated ("seeded") in the solution. The ampoules or straws are seeded at –7°C, and cooled at 0.3–0.5°C/min to approximately –40°C, then at 10ºC/min to –150°C, and finally transferred into liquid nitrogen for storage. It has been shown that embryos and spermatozoa do not deteriorate even when stored for decades in liquid nitrogen *(38,39)*.

An effective method for the preservation of human ovarian cortex involves the equilibration of thin slices (<1 mm thick) of ovarian cortex for 30 minutes at 4ºC in freezing solution containing cryoprotectant plus sucrose *(27,28,40,41)*. This approach provides a maximal surface area for rapid CPA penetration, as evidenced by nuclear magnetic resonance spectroscopy *(28)*. Oocytes and ovarian tissues can be stored in liquid nitrogen at –196ºC for as long as required. Exceptionally, Harp *et al.(42)* and Gunasena *et al. (43)* cooled ovarian specimens at 0.5°C/min to approximately –55ºC and then plunged them into liquid nitrogen, whereas Salle *et al. (44)* cooled at 2ºC/min to approximately –140ºC.

Most procedures stipulate that oocytes or ovarian cortex are thawed rapidly by being swirled in a water bath at ~20°C or 37°C, and the CPA is progressively diluted from the tissue by repeated rinses with fresh medium.

Nonequilibrium protocols: vitrification

Vitrification refers to the physical process by which an aqueous solution forms an amorphous glassy solid, rather than crystallizing. A number of different methods have been used to vitrify human oocytes and preimplantation embryos. These approaches that have been developed for use with animal embryos and oocytes have met with varying degrees of success when used for human tissues. The methods include the use of conventional straws *(45,46)*, the open-pulled straw method *(47)*, the cryoloop *(48)* and vitrification of cells using electron microscope grids as a support *(49)*. Typically, high concentrations of CPA or mixtures of several CPAs and an extremely high cooling rate (>2000°C/min) are used to vitrify oocytes. Such conditions preclude the formation of intracellular ice crystals. The automated programmable freezers that are commonly used for slow freezing protocols are not required for vitrification and the actual time required for cooling the specimens is significantly decreased. Despite these apparent advantages, the high concentration and potential toxicity of the CPAs used in vitrification protocols can cause severe osmotic shock to the oocytes and compromise their post-thaw survival and developmental potential. Because oocytes are extremely permeable to ethylene glycol (EG), its use lessens the likelihood of osmotic shock when oocytes are diluted out of even very concentrated solutions. Therefore, EG has been used successfully to cryopreserve MII oocytes which have been used to produce term pregnancies *(10,11)*. Furthermore, the question of long-term stability of the "glassy state" of the vitrified cells, which is prone to fracture, remains to be tested under normal working conditions in the IVF laboratory (i.e. storage in liquid or vapour phase under conditions of routine access to storage tanks).

Table 3. Applications of oocyte and ovarian freezing

• Improve the efficiency of IVF
• Alternative to embryo freezing
• Oocyte preservation for patients with ovarian hyperstimulation syndrome
• Oocyte donation programme
• The treatment of congenital infertility disorders
• Prevent fertility loss through surgery
• Treatment of premature ovarian failure

Application, indications and contraindications for oocyte freezing

Cryopreservation of unfertilized oocytes or ovarian tissue is desirable to improve the efficiency of assisted reproduction as it offers the possibility of establishing an oocyte banking system. Stored oocytes could potentially be used for several purposes (Table 3).

Routine oocyte freezing would greatly benefit ART as it would provide an ethically acceptable alternative to embryo freezing. The ovarian stimulation regimens routinely adopted for *in vitro* fertilization (IVF) remain relatively imprecise as the number of preovulatory follicles that develop in response to these treatments varies greatly between individuals. As it is preferable to collect all ova from all stimulated

follicles, these procedures may result in the recovery of large numbers of oocytes from a range of antral follicle sizes at different stages of maturity. However, because of the lack of efficient procedures to cryopreserve these cells, the common clinical practice in many countries is to fertilize all the oocytes and select only two or three of the best quality embryos for immediate transfer. The majority of the remaining embryos are frozen for subsequent frozen embryo transfer cycles. Therefore, because of the heterogeneity of the stimulated follicle population, there may be a significant wastage of oocytes from a typical IVF stimulation cycle. The ability to store oocytes from controlled ovarian stimulation would allow greater flexibility in the treatment of the oocytes of different maturational stages and so maximize fertilization rates and embryo quality. Furthermore, after a period of quarantine, excess cryopreserved oocytes could be donated to other infertile women, an alternative that would help to overcome the chronic shortage of oocytes for donation.

While the efficient cryopreservation of fully grown oocytes would improve the efficiency of current ART practice, oocyte freezing could also be used to conserve the fertility of young women who are diagnosed with cancer and are too young to have either started or completed their families. For patients of reproductive age, conventional IVF and embryo freezing is often not possible because the treatment is both costly and lengthy and, where there is no male partner, donor sperm is required. What is more, the required ovarian hyperstimulation may be contraindicated in the case of steroid-dependent cancers. More importantly, it is often dangerous to delay cancer therapy in order to induce follicular development and oocyte collection. Finally, this strategy is clearly not an option for prepubertal girls.

In contrast to the preservation of mature oocytes, ovarian tissue cryopreservation has the potential to be used as a means to conserve the fertility of both young adults and prepubertal girls. In addition, high-dose chemotherapy is now being used for an increasing number of nonmalignant conditions, such as autoimmune diseases and thalassaemias. Finally, ovarian tissue freezing may become a viable option for fertility conservation in young women with a familial history of premature ovarian failure. Ovarian tissue banking has the added potential of restoring ovarian function and natural fertility in these groups by autografting the frozen-thawed tissue at either an orthotopic or heterotopic site within the body *(50)*.

The safety of mature oocyte cryopreservation

Studies using MII oocytes suggest that oocyte survival after freezing and thawing can be affected by a number of factors. Among the morphological factors, the presence or absence of the cumulus granulosa cells during the freezing process may have a direct impact on oocyte survival, post-thaw. Furthermore, sublethal damage to the cells post-thaw may well affect their developmental capabilities. Although not proven, it is thought that the cumulus cells offer some protection against sudden osmotic changes and stresses induced by the rapid influx of the cryoprotective agents during the procedures of equilibration and removal of CPA during the prefreeze and post-thaw periods *(19)*.

Mature human oocytes show great heterogeneity in the distribution and organization of cytoplasmic organelles which may influence the outcome of cryopreservation procedures (Table 4). For example, during human oocyte maturation *in vivo* the cortical granules move to the periphery and are distributed under the oolemma. Following fertilization, the granules are exocytosed and their contents alter the biochemistry and sperm-binding characteristics of the zona pellucida which induces zona hardening and provides the natural block to polyspermic fertilization. Premature zona hardening will undoubtedly compromise normal fertilization and may impair implantation of the embryo *(51,52)*. In support of this hypothesis, human oocytes stored after slow cooling have lower recorded fertilization rates after standard IVF and electron microscopy of these cells has demonstrated a reduction in the number of cortical granules *(53)*, suggesting that zona hardening has occurred in response to the freeze-thaw process. However, Gook *et al. (52)* found an abundance of cortical granules in the cytoplasm of cryopreserved oocytes. Where there is any potential risk of zona hardening as a result of cryopreservation, it can be bypassed by micromanipulation techniques such as ICSI and assisted hatching *(54,55)*.

MII oocytes are vulnerable to cryoinjury because the meiotic spindle on which the chromosomes have become aligned is acutely temperature sensitive. Transient cooling of human oocytes to 20ºC can cause irreversible disruption of the spindle apparatus while rapid depolarization occurs when the temperature is lowered to 0ºC *(56,57)*. The appropriate organization of spindle microtubules is essential for the correct alignment and segregation of chromosomes when the

Table 4. Factors associated with cooling and cryopreservation that contribute to cellular injury and death in biological systems [reproduced with permission from *(3)*]

System	Type/cause of damage
All	Intracellular ice formation, extracellular ice formation, apoptosis, toxicity, calcium imbalance, free radicals, ATP levels, general metabolism, fertilization failure, cleavage failure, pHi, parthenogenetic activation, cleavage
Membrane	Rupture, leakage, fusion, microvilli, phase transition
Chromosomes	Loss/gain, polyspermy, polygyny (failure to extrude polar body), tetraploidy
DNA	Apoptosis, fusion, rearrangements
Cytoskeleton	Microtubules dissolve, actin
Proteins/enzymes	Dehydration, loss of function
Ultrastructure	Microvilli, mitochondria, vesicles, cortical granules, zona pellucida
Zona pellucida	Hardening, fracture
Lipids	Free radicals?

PHi: intracellular PH

spindle reassembles once the temperature returns to normal. Oocyte freezing can therefore increase the incidence of aneuploidies after extrusion of the second polar body through nondisjunction of sister chromatids. This disruption of the cytoskeletal architecture may also lead to abnormal cytokinesis, retention of the second polar body and alterations in the organization and trafficking of molecules and organelles *(58)*. While deleterious effects on the cytoskeleton resulting from chilling may be avoided by cryopreservation of GV oocytes, the difficulties associated with IVM and extended culture appear to counteract the potential benefits of freezing oocytes at this stage. Furthermore, chilling reduces the developmental capability of GV oocytes, quite apart from damage to the meiotic spindle *(59)*. Consequently, few pregnancies have been achieved after IVM of human oocytes *(60,61)* and even fewer after the initial attempts to cryopreserve full-sized GV oocytes (Table 1). At present, cryopreservation of GV oocytes offers little or no advantage over freeze-storage of MII oocytes.

In addition to the cytogenetic impact of cryopreservation, there is an increased risk of parthenogenetic activation of the oocytes after thermal shock and exposure to CPAs. Although individual reports differ in the extent of activation observed *(62)*, parthenogenetic activation appears to be influenced by the type of CPA used and by the use of ICSI versus IVF to fertilize the oocytes post-thaw. Although the precise mechanism by which activation is initiated is unknown, recent evidence suggests that CPA exposure promotes the passive influx of Ca^{2+} across the plasma membrane *(63)*, possibly by stimulating the release of Ca^{2+} from storage sites in the mitochondria and endoplasmic reticulum. Furthermore, calcium fluxes are known to activate intracellular phospholipases, proteases, ATPases and endonucleases, which may result in altered plasma membrane integrity, denaturation of cystolic proteins, and chromosomal fragmentation, all of which can lead to irreversible cell injury and apoptosis.

The safety of primordial oocyte cryopreservation

Primordial oocytes appear to be less vulnerable to cryoinjury than mature oocytes as they are smaller, lack a zona pellucida and cortical granules, and are relatively metabolically quiescent and undifferentiated (Table 2). Additionally, primordial follicles are apparently more tolerant to insults such as immersion in CPA solutions and cooling to very low temperatures as their small size makes them less susceptible to damage induced by water movements into and out of the cells during freezing and thawing. Finally, primordial oocytes have more time to repair sublethal damage to organelles and other structures during their prolonged growth phase after thawing.

Although storage of slices of ovarian cortex is an attractive alternative to mature oocyte freezing, there are a number of technical problems associated with the cryopreservation of ovarian tissues compared to isolated oocytes. Tissues respond very differently to ice formation than cell suspensions. Cells in tissues are usually closely packed, and they also have

interacting connections with each other and with basement membranes. Tissues have a three-dimensional structure and are traversed by fine capillaries or other blood vessels. Changes in extracellular ice surrounding the tissue during the freezing process, and recrystallization during warming of the tissue are both hazardous. In the hands of experienced cryobiologists, morphological assessments of cryopreserved human ovarian cortex at the light microscopy *(64)* and electron microscopy *(22,65)* levels have confirmed that cellular damage in the tissue can be minimal. However, the choice of an inappropriate CPA together with poor laboratory practice can lead to extensive cellular damage which will compromise tissue viability on thawing *(22)*. The problems of achieving adequate permeation of tissue fragments with CPA can be overcome either by preparation of thin strips of tissue no more than 1–2 mm thick, which provides maximal surface area for solute penetration *(28)*, or by dissociation of the tissue into follicles or isolated cells before freezing *(66)*.

Although the cryopreservation protocols used for the banking of human primordial oocytes have not been fully optimized, they are nonetheless effective. Using the slow freezing approach, high post-thaw follicle survival rates of 84% and 74% have been recorded for human primordial follicles stored using 1.5 M ethylene glycol and DMSO, respectively, compared with survival rates of only 44% and 10% with 1.5 M propylene glycol and glycerol, respectively *(27)*. It is therefore anticipated that far greater technical problems will be encountered when the stored tissue is thawed and used to restore fertility. On the basis of animal experiments, it is likely that fertility restoration will be achieved by heterotopic or orthotopic autografting *(24,25,40,50)* once the high levels of follicle loss associated with re-perfusion injury have been reduced *(67)*. Xenografting has also been successfully used as a research tool to investigate the developmental potential of frozen-thawed human ovarian tissues *(68)*.

While it may be possible to store ovarian tissue for young cancer patients where there is any risk of reintroducing malignant cells in the tissue graft *(69)*, a far safer strategy is to culture the follicles to maturity *in vitro (61)*. Following fertilization by IVF or ICSI, embryos which are free from contamination could be transferred back to the patient. Human follicle culture is an emerging technology and encouraging new data suggest that it may soon be possible to grow follicles to antral stages after cryopreservation *(61)*. Nonetheless, a considerable amount of research effort will be needed in the future to confirm that primordial follicle cryopreservation, followed by extended culture, is a safe procedure and that it does not induce epigenetic alterations in the female gametes *(70)*. If successful, cryopreservation followed by follicle culture may eventually supersede cryostorage and grafting as a strategy to restore natural fertility, as this approach makes more economic use of scarce follicles.

Finally, the freeze storage of isolated oocytes and ovarian tissue is subject to the usual safety concerns of long-term banking of tissues in liquid nitrogen. Because of the known risks of viral transmission in nitrogen storage tanks *(71)*, it is preferable that patients should be screened for blood-borne viral diseases prior to tissue storage. The possibility of the long-term storage of ovarian tissues from paediatric patients also raises the question of how long banked ovarian tissues and oocytes should be stored. In the case of ovarian tissue storage for young cancer patients, it is important to ensure that the patient (or guardian) consents to an upper time limit for storage and also to the disposal of tissue in the event of death or mental incapacitation.

Conclusions

Oocyte cryopreservation strategies still require much developmental work to improve the survival rates and development potential of frozen-thawed oocytes. The potential ethical benefits, the convenience and lower costs which will result from successful programmes of mature or immature oocyte freezing will surely stimulate continuing efforts to improve protocols and clinical practice as this approach may well transform ART.

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Cryopreservation of human spermatozoa

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Introduction

Documented efforts to maintain the fertilizing capacity of mammalian spermatozoa for extended times have been made for at least 135 years. Having observed survival of human spermatozoa that had been cooled to –15ºC, in 1866, an Italian physician, P. Mantegazza, proposed the concept of a human sperm bank to store semen specimens (as described by Bunge *et al. (1)*). Among somewhat more recent, yet still early, formal studies of sperm cooling were those of Hammond *(2)* and Walton *(3)*. In companion investigations of rabbit spermatozoa, they showed that the fertility of spermatozoa collected from the vas deferens of bucks or from the vaginas of mated does could be extended for several days by cooling sperm samples to temperatures of 15ºC or below. Ejaculated spermatozoa were still fertile after four days at 10ºC, and spermatozoa collected from the vas were fertile even after seven days at that temperature, resulting in multiple litters of live young after artificial insemination (AI). In a seldom cited paper, Shettles *(4)* reported preservation of human spermatozoa after plunging thin-walled capillaries of undiluted semen directly into alcohol cooled to –79°C with dry ice or into liquid nitrogen at –196ºC or into liquid helium at –269ºC. Although the survival of sperm motility was low and varied significantly among eight donors, Shettles observed that motility continued for several hours after thawing. He wondered whether such spermatozoa were still capable of fertilization. Soon after, Hoagland and Pincus *(5)* reported preservation of human and rabbit spermatozoa by making films of semen on a bacteriological loop, and plunging the loops directly into liquefied gases to achieve very high cooling rates. Again, the results were highly variable, but in a few cases they observed motility rates of 60% after thawing frozen samples of human semen. They also attempted to dehydrate the cells by plasmolysis by exposing spermatozoa to various sugars before attempting to freeze them. Several aspects of these methods first described 60 years ago for sperm preservation presaged methods now being used to cryopreserve mammalian oocytes and embryos by ultrarapid cooling. These include use of a fine capillary, the "open-pulled straw" method of Vajta *et al. (6)*, and the "cryoloop" method of Lane *et al. (7)*.

Apart from these early attempts at sperm preservation, the single observation most often credited with having resulted in a reliable method of cryopreservation of cells was that of Polge, Smith and Parkes *(8)*. They found that fowl spermatozoa, suspended in a glycerol–albumen solution, survived freezing to –79ºC. They then showed that bull spermatozoa were also protected by glycerol against damage caused by freezing to –79ºC *(9)*. The birth of the first mammal resulting from AI of a female with frozen-thawed semen was reported shortly thereafter *(10)*. These

results prompted Sherman and Bunge *(11)* to study the effects on survival of human spermatozoa of various cryobiological factors, such as glycerol concentration and cooling rate. Since then, although difficult to document precise figures, it is reasonable to estimate that many tens of thousands of human pregnancies have been produced by AI with cryopreserved spermatozoa. Numerous publications have described the methods, results and variables affecting human sperm freezing *(12–15)*, as well as that of sperm cryopreservation of nonhuman primates *(16–20)*.

Clinical use of cryopreserved spermatozoa

The first human pregnancies and births resulted from AI of four women with semen samples that had been frozen and stored in dry ice at –70°C for up to six weeks *(1)*. Several years later, births resulting from AI with human spermatozoa frozen and stored for 5 months in liquid nitrogen were reported *(21)*. These successes led to the gradual clinical application of cryopreserved human spermatozoa and the establishment of human sperm banks in several countries. One of the first advocates of this approach to treating infertility by insemination with frozen semen was Sherman *(22)*, who summarized the development and implementation of the first human sperm banks. Other advocates of semen banking were David and Lansac *(23)* who described the unique organization and management principles of the sperm banks of CECOS (Centre d'Etude et de Conservation du Sperme Humain) in France. This sperm bank still operates under the guidelines that all semen donations are given voluntarily and without payment to the donor. At about the same time, Trounson *et al. (24)* summarized the experience of multiple sperm banks in Australia, and recently, Critser *(25)* reviewed the current status of semen banking in the USA.

One of the most important aspects of clinical applications of frozen spermatozoa is its efficacy compared to that of fresh semen for AI. Nowadays, because of the risk of transmission of human immunodeficiency virus (HIV), it would be unethical, if not illegal, to attempt such comparisons using fresh spermatozoa from large numbers of sperm donors. In most countries of the world today, semen samples are frozen and quarantined for at least six months so that the health status of sperm donors can be tested retrospectively, i.e., at the time when donors actually ejaculated their specimens. However, in the past, several retrospective and some prospective comparisons were conducted. For example, Steinberger and Smith *(26)* compared conceptions and births resulting from a total of 107 inseminations with fresh or frozen semen. They found that, on average, it required somewhat more cycles of insemination and sperm concentrations about 50% higher to induce pregnancy with frozen semen; nevertheless, 61% of 59 attempts with frozen semen resulted in pregnancy compared to 73% of 48 attempts with fresh semen.

Details of previous comparative studies of fresh and frozen semen are varied, and methods used to cryopreserve the spermatozoa differ substantially. Nevertheless, it is useful to consider these data because they provide important information that would now be difficult to obtain. In Table 1, the results observed in five comparative studies are shown in terms of the average probability of pregnancy after AI with fresh or frozen semen. Consideration of these data illustrates the difficulty of evaluating the efficiency of methods to cryopreserve human spermatozoa. Undoubtedly, the principal objective of these trials was to induce pregnancy in the inseminated patients, not necessarily to optimize freezing methods.

In three of these comparisons [Richter *et al. (27)*, DiMarzo *et al. (30)* and Subak *et al. (31)*], the probability of pregnancy with fresh semen was two to four times greater than with frozen semen. In the other two studies (Bordson *et al. (28)* Keel and Webster *(29)*), however, there was no apparent difference between fresh and frozen semen. But

Table 1. Average probability of pregnancy per treated cycle after artificial insemination with fresh or frozen semen

	Richter *et al.* 1984 *(27)*		Bordson *et al.* 1986 *(28)*		DiMarzo *et al.* 1990 *(30)*		Keel and Webster 1989 *(29)*		Subak *et al.* 1992 *(31)*	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
Treated cycles	676	1200	165	165	3405	371	67	209	102	96
Pregnancy probability	18.9	5.0	11.5	10.3	10.5	5.7	5.8	4.8	20.6	9.4

differences in the methods used may account for some of the apparent differences among the results. For example, in the Richter study, patients receiving fresh semen received the entire ejaculate, whereas those receiving frozen specimens received only semen that had been diluted with cryoprotectant, so that the total number of spermatozoa was less. In the Bordson study, a minimum of 40×10^6 total motile spermatozoa, whether fresh or frozen, were inseminated; in some cases, two inseminations were used *(28)*. In the Keel–Webster trial *(29)*, patients were inseminated with the use of a cervical cap, with at least 20 million motile cryopreserved spermatozoa; many of those inseminated with fresh semen were treated twice per cycle. In the DiMarzo study *(30)*, at least 30 million total spermatozoa/ml with more than 50% motility were used for fresh samples, whereas the frozen samples contained 20–40 million motile spermatozoa per insemination. In the Subak study *(31)*, about two-thirds of the patients were inseminated with the use of cervical caps, and the balance by intrauterine insemination (IUI). A confounding aspect of this trial emerged retrospectively. On average, patients inseminated with fresh semen who became pregnant had received 250 million spermatozoa, whereas those inseminated with frozen semen had received less than 50 million spermatozoa. Not surprisingly, in all of these studies, and as previously observed by Steinberger and Smith *(26)*, the higher the concentration of motile spermatozoa, the greater the likelihood of pregnancy. The overall efficiency in terms of probability of pregnancy resulting from inseminations with frozen semen found by Richter *et* al. *(27)*, DiMarzo *et al. (30)*, and Keel and Webster *(29)* were similar and were about 5%. In the analyses by Bordson *et al. (28)* and Subak *et al. (31)*, the likelihood of pregnancy was about 10%.

An even clearer picture of the difference between fresh and frozen semen emerges when the cumulative likelihood of pregnancy is plotted as a function of the sequential number of patients' menstrual cycles during which inseminations were performed. Such data for four of the comparisons cited above have been plotted in Figure 1. Despite some differences in the methods used, the probabilities of pregnancy with both fresh and cryopreserved semen observed by both Richter *et al. (27)* and by Subak *et al. (31)* were very similar. In contrast, the likelihood of pregnancy resulting from AI with frozen semen in the study by Bordson *et al. (28)* was approximately equal to that observed with fresh semen by DiMarzo *et al. (30)*.

Other clinical trials of cryopreserved semen have also been described in the literature. For example, Mahadevan and Trounson *(32)* conducted a detailed and exhaustive study of the role of various cryoprotective additives (CPA) on human sperm survival after freezing and thawing. The CPAs included glycerol, dimethyl sulfoxide and ethylene glycol, in some cases supplemented with sucrose, raffinose, or glycine. Having derived a complex defined medium without egg yolk for human semen, they determined methods that yielded high sperm survival after

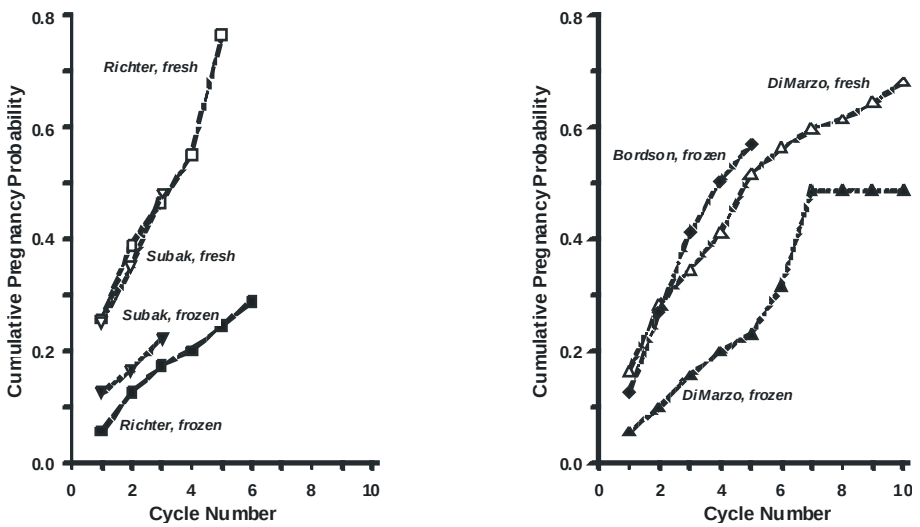

Figure 1. The cumulative probability of pregnancy as a function of the number of successive treatment cycles during which women were artificially inseminated with fresh or frozen human spermatozoa.

freezing, and then conducted a double-blind clinical trial to compare their optimized method with a standard one. Of 52 patient cycles treated with the optimized method, 50% of the women became pregnant and 24 children were born.

Another very important clinical trial was described by Federation CECOS *(33)* for the accumulated experience of French semen banks over the 15-year period from 1973 to 1987. Since the inception of the CECOS programme, more than 17 000 pregnancies were produced during that period with the use of cryopreserved semen, despite the fact that many of the women who were treated successfully had various types of infertility problems. The overall mean success rate per cycle was 8%, and the theoretical success rate was 48% after six treated cycles. These rates are quite similar to those shown in Table 1 and Figure 1 above. Although fewer than in the past, clinical investigations of the use of frozen semen continue to be published. For example, Byrd *et al. (34)* compared the use of two media for semen preservation and then performed IUI of 304 patient cycles. The likelihood of pregnancy with the standard bicarbonate-buffered solution was 9.8% for 164 cycles, whereas it was 17.5% when HEPES-buffered solution was used in the treatment of 160 cycles. In a recent study, Matilsky *et al. (35)* compared the use of two-day treatments for IUI with cryopreserved semen with those of one-day treatments. Of 180 one-day cycles, the pregnancy rate per cycle was 5.0%, whereas with 222 two-day cycles, the pregnancy rate per cycle was 17.9%. Comparison of these results with those shown above in Table 1 and Figure1 reveal that the overall efficacy of cryopreserved semen for AI can be significantly influenced not only by methods used to freeze the semen itself, but also by various clinical variables. The role of such clinical variables in treating human infertility with cryopreserved semen have recently been reviewed by Oehninger *et al. (36)*.

Sperm cryopreservation as an adjunct to assisted reproduction

When weighing the current status of cryopreservation of spermatozoa, another important issue must also be considered. The ultimate function of a spermatozoon is to fertilize an oocyte. In the past, methods of assisted reproduction achieved fertilization primarily by AI. In 1978, in a landmark publication, Steptoe and Edwards *(37)* reported the birth of the first child resulting from *in vitro* fertilization (IVF) of a human oocyte. Since then, tens of thousands of children have been born as a result of IVF in its various forms and variations (summarized, for example, in the textbook by Edwards and Brody *(38)*). Furthermore, human spermatozoa collected from fertile donors have been used for successful IVF of oocytes aspirated from infertile patients *(39)*. In this comparison of fresh versus frozen semen, equivalent fertilization rates of >90% were achieved. Those same investigators also found that it was possible to use cryopreserved spermatozoa from subfertile men for IVF. For many years, it has been common practice in the cattle industry to use cryopreserved spermatozoa for IVF of oocytes *(40)*. Because so few motile spermatozoa are required to effect fertilization of multiple oocytes by IVF, this has become a routine procedure. In the case of cryopreserved mouse spermatozoa used for IVF, it has been shown that, in principle, frozen spermatozoa from a single male mouse are sufficient to produce thousands of offspring *(41)*.

Several years ago, Palermo *et al. (42)* derived an innovative method of assisted fertilization, referred to as intracytoplasmic sperm injection (ICSI), to treat human male-factor infertility. Since then, ICSI, in which a single spermatozoon is isolated from an ejaculate and injected directly into an oocyte, has been widely used as an adjunct to assisted reproduction in the human *(43,44)*. The same procedure has been used to a lesser extent in domestic species *(45)*. Remarkably, when ICSI has been used with animals, live calves have even been produced by injection of deliberately killed spermatozoa into bovine oocytes *(46)*. Recently, Wakayama *et al. (47)* also produced live mice by ICSI with spermatozoa killed by freezing. Although these latter observations might suggest that ICSI with dead spermatozoa obviates the necessity of improving methods to cryopreserve spermatozoa, there will continue to be a very important role for frozen spermatozoa to be used for AI and conventional methods of IVF.

The procedure of ICSI is so efficient that it has become the treatment of choice to alleviate male factor infertility. Full-term human pregnancies have been produced by ICSI of spermatozoa aspirated directly from the epididymis *(48)*, and also by ICSI with frozen-thawed epididymal spermatozoa *(49)*. It has even been possible to produce pregnancies and human births by the transfer of embryos produced by ICSI with spermatids (immature spermatozoa) *(50)*. Another practical and imaginative approach to ICSI has been

described by Cohen *et al. (51)* who froze single or small numbers of human spermatozoa deliberately injected into empty zonae pellucidae obtained from human, mice or hamster oocytes. The spermatozoa that were frozen within the zonae to improve the efficiency of their recovery were used to fertilize oocytes successfully. This same method has also been used to cryopreserve small numbers of testicular spermatozoa to be used for fertilization by ICSI *(52)*. In the past few years, numerous articles have been published describing various factors that influence the efficiency of cryopreservation of human spermatozoa obtained by microsurgical aspiration of the epididymides *(53)*. Comparison of frozen-thawed spermatozoa collected either by ejaculation or by epididymal aspiration showed that a significantly higher rate of fertilization was achieved with ejaculated spermatozoa *(54)*. Somewhat higher rates of pregnancy per transfer (27%) and of births (22%) were achieved with cryopreserved ejaculated spermatozoa compared to those resulting from epididymal spermatozoa (18% and 12%, respectively). Nevertheless, the efficiency is high enough to have clinical utility. A large retrospective analysis has recently been made of results of ICSI in which fresh or frozen epididymal spermatozoa were used to fertilize totals of 1821 or 1255 oocytes, respectively *(55)*. The rates of fertilization and cleavage of injected oocytes were not significantly different between the two groups. Most importantly, rates of pregnancies and births were approximately the same: the percentage delivery per cycle with fresh spermatozoa was 17.9%, compared to 20.5% with frozen spermatozoa. A variation on this same approach has been described in which small pieces of testicular tissue have been obtained by an open biopsy procedure and frozen for later use in ICSI *(56)*. Spermatozoa have been isolated from thawed tissue, injected into oocytes, with resultant births of 29 children.

The variables of sperm cryopreservation

Hundreds of millions of cattle and hundreds of thousands of children have been born as a result of AI of females with frozen-thawed spermatozoa *(12,15,57,58)*. Measured in those terms, the procedure of sperm cryopreservation seems extremely successful. However, that is only partially correct. On average, of all human and cattle spermatozoa, the two species for which the most comprehensive data have been collected, 50% of sperm cells are damaged or destroyed by freezing and thawing, limiting the overall efficiency and efficacy of semen preservation *(13,15,59)*. For most other species, usually fewer than 50% of spermatozoa survive cryopreservation *(60)*. What makes sperm freezing even less efficient is the fact that, within a species, spermatozoa from different males exhibit widely variable responses to the same freezing conditions. Such differences among males of freezing sensitivity of their sperm are widespread among various species and have profound practical and fundamental implications. Examples of male-to-male differences have been described for spermatozoa of dogs *(61)*, horses *(60)*, cattle *(62,63)*, and rhesus monkeys *(64)*. The results are usually presented as post-thaw motility or recovery relative to prefreeze values for individual males of each species.

Analogous and significant male-to-male differences in sperm sensitivity to cryopreservation among humans have been reported by Glaub *et al. (65)*, Cohen *et al. (66)*, Taylor *et al. (67)*, Centola *et al. (68)*, Kramer *et al. (69)*; and Morris *et al. (70)*. As mentioned above, significant differences in the response of spermatozoa from different men were first noted by Shettles *(4)*, and also by Steinberger and Smith *(26)*. Analysis of prefreeze and post-thaw motility of spermatozoa from 315 men showed that the coefficients of variance for repeated ejaculates from multiple individuals was much larger than the variance for multiple ejaculates of the same male *(71)*.

A detailed comparison has been made of the motility characteristics of ejaculated spermatozoa from several individuals before and after cryopreservation *(72)*; the results of this study are presented in Figure 2. In general, freezing caused a decrease in the percentage of progressively motile spermatozoa from all men. However, as can be seen from the results in this figure, the extent of the decrease varied widely among donors. With only one exception, the coefficients of variation for a given man were smaller than the coefficients of variation among the six men. Thus, these results show that the response of spermatozoa was consistent among different ejaculates from the same individual, but that spermatozoa from different men responded differently. An important practical consequence of such differences in sperm freezing sensitivity may occur in the treatment of cancer. For many human males facing the prospect of chemotherapy or radiation therapy, the option of having their semen frozen for future use may be precluded if they happen to produce freezing-sensitive spermatozoa. In

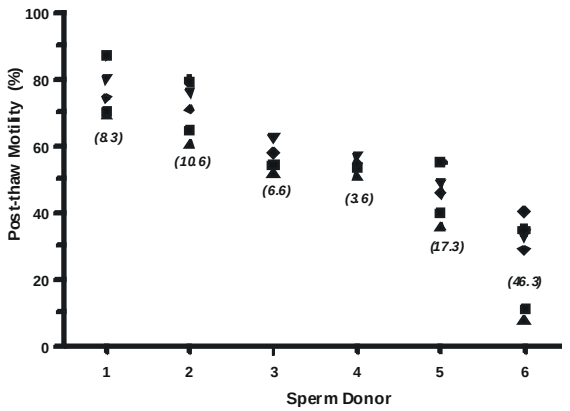

Figure 2. The post-thaw motility of frozen spermatozoa of six human donors. Each point shows the progressive motility of individual ejaculates. The figures in parentheses are the coefficients of variation (CV) for each donor. The CV between donors was 33%. The data are redrawn from McLaughlin *et al. (72)* and are used with permission of the authors.

short, sperm cryopreservation is an integral and essential procedure used routinely in animal and human reproduction. Yet, for no species, including the human, does a single freezing procedure work effectively for spermatozoa of all males of that species.

The process of sperm freezing

That there are such significant male-to-male differences in freezing sensitivity seems difficult to understand. Cryopreservation of spermatozoa by equilibrium cooling usually consists of the following steps: (i) cells in suspension are placed in a solution of a cryoprotective agent (CPA); (ii) the cells are cooled to temperatures near 0ºC; (iii) they are then cooled to subzero temperatures at a moderately low rate of about 5–10°C/min to an intermediate subzero temperature (about –75°C for spermatozoa) and then plunged into liquid nitrogen at –196°C for storage; (iv) to restore their function, cryopreserved cells are warmed and thawed and the CPA removed. Any one or all of these steps may damage cells during the entire sequence of cryopreservation. Spermatozoa, in particular, are especially sensitive to these fluctuations of temperature and of osmolalities of solutions because of the delicate nature of the acrosome, the function of which is to disassemble during fusion of the spermatozoon with the oocyte. Nevertheless, it seems reasonable to assume that all cells of a given type ought to respond similarly to the same conditions of freezing and thawing. One might theorize that the same procedure should be reliable and reproducible for spermatozoa of all males of a given species. That clearly is not the case.

To understand the responses of spermatozoa to cryopreservation, it is useful to briefly consider the basic aspects of cell cryobiology, especially as they apply to spermatozoa. These have been reviewed by many authors *(12,15,58,60,73)*. There have been innumerable studies of the very many factors that influence the survival of human spermatozoa when they are frozen and thawed. An early exhaustive analysis was that of Freund and Wiederman *(74)* who found that rates of dilution with cryoprotective solutions and of cooling from 25ºC to 5ºC before semen samples were frozen affected the ultimate motility after thawing. Other notable studies of cryopreserved human spermatozoa in which effects of CPA concentrations, methods of freezing, presence or absence of seminal plasma, suspending media, thawing rates, effect of hyaluronate in the freezing solution, and post-thaw dilution methods are those of Serafini and Marrs *(75)*, Critser *et al. (76)*, Hammitt *et al. (77)*), Centola *et al. (68)*, Verheyen *et al. (78)*, and Sbracia *et al. (79)*.

When cells suspended in an aqueous solution are frozen, the following concomitant physical and chemical changes occur: (i) the temperature is decreased; (ii) ice forms so that liquid water is removed in the form of crystals; and (iii) dissolved solutes become more concentrated. Depending on the initial composition of the solution (electrolytes and nonelectrolytes, its pH, and macromolecular supplements), when the temperature decreases sufficiently, some of the dissolved solutes become so concentrated that they begin to crystallize or solidify. These changes in solution osmolality cause the cells in suspension to respond osmotically. The capability of a given type of cell to respond osmotically to increasing solution osmolality at decreasing temperatures reflects the cell's hydraulic conductivity (L_p: its permeability to water) and the activation energy of that permeability (E_a: the effect of temperature on water permeability). Decreasing temperature decreases the rate at which cells can lose water.

Almost 40 years ago, a mathematical model was derived by Mazur *(80)* which predicted the response of cells as they were frozen. This model incorporates the various parameters described above; it predicts that a cell's L_p and E_a are the principal characteristics that determine the cooling rate at which a cell must be cooled to remain in osmotic equilibrium with increas-

ing solute concentration resulting from increased ice formation at lower subzero temperatures. As a consequence of these factors, one of the most important variables of cell cryopreservation is the rate at which it is cooled to low subzero temperatures. It has been demonstrated, with spermatozoa from several species, that sperm motility and other basic characteristics vary significantly with cooling rate and warming rate [pig *(81)*, sheep *(82)*, cattle *(83)*, summarized for other species by Leibo and Bradley *(58)*].

Examples of the important influence of cooling rate on survival of human spermatozoa are shown in Figure 3, in which data of Henry *et al. (84)* are presented. Figure 3 shows that the post-thaw motility of human spermatozoa frozen in 0.85 M glycerol varies significantly as a function of cooling rate and also of warming rate. As is true for other types of cells, survival is low at low cooling rates, increases to a maximum at the optimum rate, and then decreases with further increases in cooling rate.

The generally accepted interpretation of such inverted V-shaped survival curves is that high cooling rates damage cells by intracellular ice formation. Rapid cooling means that cells have insufficient time to dehydrate during cooling. Thus, they freeze intracellularly; intracellular ice damages the cells by disrupting their intracellular architecture. What is less clear is the mechanism responsible for damage at low cooling rates. The usual explanation is that slow cooling damages cells by exposing them for extended times during cooling to solutions the properties of which have been altered by ice formation. This effect is referred to as the "solution-effect" injury.

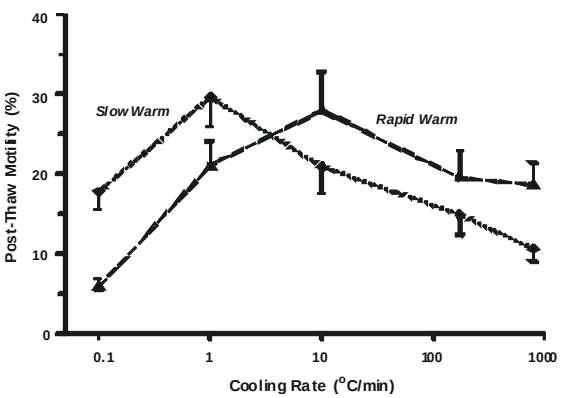

Figure 3. The post-thaw motility of frozen human spermatozoa after being cooled at each of five rates and then warmed either slowly or rapidly. The data are those of Henry *et al. (84)* and used with permission of the copyright owners and the authors.

But the data in Figure 3 illustrate one contradiction of that explanation. Consider spermatozoa cooled at 0.1°C/min. These would have been exposed to altered solutions for several minutes during the initial cooling. If warmed rapidly, about 5% survive. But if warmed slowly, 18% survive, despite the fact that slow warming would have increased the cells' exposure to altered solutions as they melted. This suggests that the usual explanation of solution-effect injury is inadequate to explain damage caused to spermatozoa by slow freezing.

Predicting freezing sensitivity

Do male-to-male differences in sperm freezing sensitivity reflect properties of membranes? Are these properties genetically determined? Several lines of evidence suggest that characteristics of sperm membranes are genetically determined. For example, it was recently reported that spermatozoa from one hybrid and two inbred strains of mice differ both in terms of sensitivity to osmotic shock and to freezing *(85)*. Fresh spermatozoa from B6D2F1, 129/J and C57BL6J mice fertilize oocytes with the same efficiency. However, after their spermatozoa are exposed to and diluted out of a CPA solution, or especially after being frozen, there are significant differences among the three strains in the viability and fertilizing ability of their spermatozoa. Nakagata and Takeshima *(86)* had previously reported analogous differences in sperm freezing sensitivity among eight inbred strains of mice. Sperm membranes may also be influenced by differences among males in their hormonal status and by the season of the year. If the answers to the questions posed above are positive, this will suggest that factors that influence freezing sensitivity of spermatozoa from species other than mice, e.g. cattle or humans, may also be genetically determined. Several observations regarding differences in permeability and hormones provide clues as to possible explanations of variable responses of spermatozoa from different individuals to the same procedure.

Permeability differences

Cells of a given type collected from different individuals may have different membrane characteristics. For example, oocytes from different females of outbred ICR mice exhibit significant differences in

their water permeability characteristics *(87)*. In contrast, oocytes from different females of an F1 hybrid strain consisting of genetically homogeneous mice (heterozygous only for the gene pairs by which the parent strains differ) do not show such differences in the same permeability properties *(88)*. Very recently, Phelps *et al*. *(89)* reported that permeability coefficients of spermatozoa from outbred ICR mice differ significantly from those of a hybrid strain (B6C3F1). Together, these data suggest that membranes of the same cell type collected from different individuals of a genetically heterogeneous species, e.g. outbred mice or humans or cattle, may have significantly different properties. These latter observations by Phelps *et al*. could explain the observations described above of Songsasen and Leibo *(85)* and of Nakagata and Takeshima *(86)* that spermatozoa from different strains of inbred mice have different freezing sensitivities.

Therefore, it seems reasonable to hypothesize that different human males, or in fact different males of all mammalian species, may produce spermatozoa of which the membranes may differ very significantly. By analogy, different males may have significantly different levels of serum cholesterol, yet still be completely healthy. If the membranes of spermatozoa from different individuals differ, then permeability characteristics of their spermatozoa may also differ. In turn, such differences may be exhibited as significant differences in chilling and/or freezing sensitivity of the spermatozoa. Support for this concept is found in recent observations of Giraud *et al. (90)* who measured the fluorescence anisotropy of spermatozoa from 20 men before and after cryopreservation to determine the fluidity of sperm membranes. Anisotropy refers to the rotational motion of the membrane probe distributed throughout the hydrophobic core of the lipid bilayer of the cell membrane. These authors found that fluorescence anisotropy varied significantly among different men, and that sperm membranes of all 20 individuals were rendered less fluid by the entire process of cryopreservation. Recently, measurements of lipid dynamics in the plasma membrane of human spermatozoa showed that lipid diffusion in the acrosome and midpiece of spermatozoa was reduced after cryopreservation compared to that of fresh cells *(91)*. Together, all of these observations suggest that a key to understanding variable responses of spermatozoa to freezing may be found in further studies of sperm membranes, especially comparisons among different men.

Part of the explanation of these differences among males may be found in the composition of sperm membranes. The lipid composition of sperm membranes is unusual *(92)*. They possess the same types of lipids as other cell membranes (phospholipids, glycolipids and sterols) but sperm membranes differ in the relative proportions of these molecular species *(93)*. In particular, a characteristic feature of sperm membranes is the extremely high proportion of ether-linked fatty acids instead of the more usual ester links. Many of the phospholipids contain a docosahexaenoic acid (DHA) side chain, which may result in increased membrane fluidity. This fatty acid has been identified as being specifically localized in the head membrane of rhesus spermatozoa *(94)*. The potential instability resulting from DHA is thought to be counteracted by the presence of sterols like cholesterol. Sperm plasma membrane lipids exist in two phases, fluid and gel. At physiological temperatures, the two forms coexist but as the temperature is lowered, a phase transition occurs in favour of the gel form *(92)*. As mentioned, this leads to a reduction in the fluidity of the membrane, which has been associated with lower sperm survival during cryopreservation *(90)*.

During the past few years, several groups have been investigating permeability characteristics of spermatozoa of various species so as to explain and to improve their survival after cryopreservation. Several of these studies have been summarized by Gao *et al*. *(15)* and by Watson *(13)*. Notable among these are determinations of permeability of human spermatozoa to CPAs and to water *(95–97)*. These studies have led to the derivation of methods to add and remove CPAs from human spermatozoa while preserving high survival of their motility and membrane integrity. In many respects, these very recent observations confirm early studies by Richardson and Sadleir *(98)*, and Mahadevan and Trounson *(32)*.

Hormone differences

Laboratory-housed macaque monkeys exhibit diurnal variations in their plasma levels of testosterone *(99)*, and males of different ages also have significantly different concentrations of androgens *(100)*. Furthermore, androgen concentrations in rhesus males, even those maintained in a controlled laboratory environment, vary significantly as a function of season as well as among each other. Others have reported similar seasonal changes in plasma levels of various hormones (e.g. testosterone, ACTH) in laboratory-

housed monkeys *(64,101–105)*. Since hormones affect the structure of the cellular cytoskeleton, such as the distribution and domains of intermediate filament proteins in spermatozoa *(106–108)*, endocrinological differences among individuals may influence susceptibility of their spermatozoa to chilling injury. Recently, seasonal and male-to-male differences in the concentration of the phospholipid, platelet-activating factor, have been found in spermatozoa of squirrel monkeys *(109)*. It seems possible that analogous differences in hormone levels may exist within and among human males.

Other factors influencing sperm banking

Genetic consequences

One important yet frequently overlooked question is whether the entire process of cryopreservation might have genetic consequences. The literature is somewhat in disagreement on this point, in that some have concluded that defective spermatozoa are eliminated by cryopreservation, because there seemed to be fewer spontaneous abortions and birth defects after inseminations with frozen semen *(110)*. In contrast, an increased frequency of trisomic births after AI with frozen semen has also been reported *(111)*. A more recent analysis of 1960 sperm karyotypes has been conducted by a method that permits direct visualization of human sperm chromosomes after heterologous fertilization of hamster oocytes. This analysis showed that there were no significant differences in the frequency of structural chromosomal anomalies before or after sperm freezing, and there was no evidence of an altered sex ratio caused by cryopreservation *(112)*.

Possible cross-contamination during long-term storage of frozen semen specimens

It has now been firmly established that cryopreserved specimens of animal spermatozoa, as well as embryos, can be stored for decades with no loss of function *(113,114)*. A recent practical study demonstrated that plastic straws commonly used for freezing semen specimens may leak their contents if the straws are not sealed properly *(115)*. Because frozen specimens may be stored for extended times, and because viruses and other microbes survive freezing in liquid nitrogen for many decades, special attention must be paid to avoid the possible transmission of infectious agents during extended storage. Some concern has been expressed about this potential for cross-contamination of samples with viruses, such as HIV and hepatitis B *(116,117)*. A transparent straw fabricated of an ionomeric resin that is designed to be heat-sealed, and does not crack or become brittle at low subzero temperatures is now available commercially. Use of this straw ought to reduce possible cross-contamination, although it does not lessen the need for vigilance and careful attention to methods used during the processing and handling of human semen specimens. An alternative that is being implemented in some sperm and embryo banks is to store cryopreserved specimens in the vapour phase above boiling liquid nitrogen at about –180ºC. This will prevent cross-contamination between samples within liquid nitrogen. However, this places the specimens in a somewhat more precarious situation, in that they may be subjected to unintended and inadvertent warming to temperatures above –130ºC, the glass transition temperature of ice. Incipient damage to the cryopreserved cells caused by recrystallization may result, but it may not become evident until months or years later when the sample is thawed. The problem is that such slight damage is cumulative; each incident of warming above –130ºC will contribute to decreasing functional survival of cryopreserved cells.

Long-term stability of frozen specimens

Once it had been established that frozen semen retained its functional ability to fertilize oocytes, it was then of interest to determine the length of time that spermatozoa could be safely stored. As mentioned above, some of the first publications on clinical trials specifically noted the length of time that specimens had been stored. There have been reports that frozen human semen decays with length of storage. For example, Freund and Wiederman *(74)* found that when semen was stored at –76ºC to –85ºC, there was a significant loss of sperm motility after 14–15 months and even more after 22–23 months. More surprisingly, Smith and Steinberger *(118)* reported that frozen human semen was stable for three years of storage, but not for longer times. Recently, two investigations have demonstrated that animal gametes are functional even after several decades of storage in liquid nitrogen. For example, frozen bull spermatozoa that had been stored for 37 years in liquid nitrogen were capable of fertilizing oocytes that subsequently

developed into blastocysts *(113)*. Cryopreserved ram semen stored for 25 years was capable of inducing full-term pregnancies in ewes *(114)*. Although there are profound ethical and legal issues that arise from this possibility, at a biological level, there seems to be no practical limit to the length of time that spermatozoa remain "alive" in liquid nitrogen.

Conclusions

Cryopreserved semen was used to produce the first human pregnancies almost 50 years ago. Tens of thousands of children have been born as a result of this rather simple procedure of assisted reproduction. Despite this unequivocal evidence of success, the procedure of human sperm freezing can hardly be considered optimum, and the fundamental mechanisms responsible for damage to spermatozoa resulting from exposure to CPAs, or from chilling, or from freezing itself remain unknown. It is not known why spermatozoa from different men respond differently to the same freezing procedure, so that semen specimens of some men exhibit high survival of sperm function, whereas specimens of others exhibit little or no survival. Regardless of functional survival, it is not known why about half of the sperm cells in ejaculates of most men are damaged or destroyed by freezing. Another extremely important aspect of sperm cryopreservation that will continue to be investigated is the capability to screen sperm donors for hereditary, as well as infectious, diseases *(119)*.

Nevertheless, at a practical level, the advent of ICSI and other methods of assisted fertilization have eliminated many of the limitations of current procedures of sperm cryopreservation. The ability to cryopreserve individual spermatozoa, or even spermatids, or testicular tissue, coupled with innovative ways to use such germ cells to fertilize oocytes has revolutionized the treatment of human infertility. It requires little foresight to predict that other, equally imaginative approaches to medically assisted reproduction will be derived in the future.

Among such approaches will almost certainly be the application and use of spermatozoa retrieved either after death or from patients in a persistent vegetative state. Live animals have been produced by IVF with spermatozoa recovered post mortem within 24 hours *(120)* and up to 7 days *(121)*. There have been reports of such unusual approaches to human reproduction, and the ethics of such procedures have been discussed *(122,123)*. Another innovative approach to sperm preservation is that of freeze-drying. This method is currently being investigated with mouse spermatozoa, and preliminary results indicate that live young can be produced by ICSI of mouse oocytes with freeze-dried spermatozoa. If successful, this method will have profound implications both at a fundamental level, as well as at a practical one.

Finally, there is yet another rather recent innovation in assisted reproduction with which sperm cryopreservation will play an increasingly important role. This procedure separates X- and Y-bearing spermatozoa, so that fertilization of oocytes can be performed to yield either female or male offspring, respectively *(124)*. The procedure has been used successfully with spermatozoa of several animal species, especially of domestic species, and has also been applied to human spermatozoa *(125,126)*. One important benefit of this method is that it offers the possibility to prevent the conception of children who will be born with or will develop sex-linked diseases. Efficient use of this procedure requires that "sexed" semen be cryopreserved. Altogether, it seems clear that there will continue to be the necessity to study sperm cryobiology, so as to improve cryopreservation of human spermatozoa.

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Gamete and embryo donation

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Introduction

The ready availability of donor sperm and the ease of artificial insemination have long enabled couples with refractory male infertility to achieve successful pregnancy. The development of *in vitro* fertilization (IVF) and related techniques has made oocyte and embryo donation another option for infertile couples.

Oocyte, spermatozoa and embryo donation are ethically and legally accepted forms of assisted conception in many countries. These treatments are also associated with the highest pregnancy and live birth rates after IVF *(1–5)*.

Sperm donation

Donor sperm have been used in the treatment of male infertility for more than one hundred years. It is recognized that pregnancy rates using frozen semen are lower than those with fresh semen *(6)* but, because of the risk of acquired immunodeficiency syndrome (AIDS), the use of frozen sperm that has been quarantined for six months is now accepted clinical practice.

Donors

Complete medical, social, sexual, family and genetic histories must be taken at the time of the initial interview and donors must have a physical examination. Donors over the age of 40 years may be accepted but only with the agreement of the recipient's physician and the couple.

Prior to cryopreservation and post-thaw cryosensitivity testing, two semen analyses must be performed using criteria established by the semen bank. Semen samples should be examined before proceeding with the evaluation of potential donors. The World Health Organization *(7)* suggests the following for normal semen analysis values:

• Volume	>2 ml
• Sperm concentration	20 million/ml or more
• Sperm motility	>50% or more with forward progression, or 25% or more with rapid progression within 60 minutes of ejaculation
• Sperm morphology	15% or more normal forms
• White blood cells	fewer than 1 million/ml
• Sperm mixed antiglobulin reaction (MAR) test	fewer than 10% spermatozoa with adherent particles

Kruger and co-workers have reported that sperm morphology is the best prognostic indicator for subsequent successful fertilization with IVF *(8)*. Many clinics have adopted strict criteria for the assessment

of sperm motility after thawing, which should be greater than 50% of the prethaw value *(9)*.

The health and fertility of the donor must be unimpeachable, and there should be no family history of genetic disease. Screening for thalassaemia in Mediterranean races, Tay–Sachs heterozygosity in donors of Jewish origin, and sickle cell disease in donors of African origin should be performed. Potential donors who are at high risk for AIDS (male homosexuals, bisexuals and intravenous drug users) should be excluded as well as individuals who have multiple sexual partners. Similarly, those individuals with history of herpes, chronic hepatitis and venereal warts should be excluded. There is no absolute method of completely ensuring couples that infectious agents will not be transmitted by donor insemination, but adequate screening should make that possibility remote. Testing should be performed when the donor is first seen and at repeat donations for

- human immunodeficiency virus (HIV)
- syphilis (RPR/TPHA): there is no need to repeat it unless clinically indicated
- hepatitis B and C antigens: and repeated six monthly
- hepatitis B antibodies
- gonorrhoea: semen or urethral cultures where indicated from the potential donor's history
- *Chlamydia trachomatis*
- cytomegalovirus IgG and IgM: every 6 months. Before the sample is released, donors should be retested. Only IgG-positive tests may be accepted.

Clinics must only use frozen semen that has been quarantined until the donor has been retested for HIV after six months and found to be seronegative; then the specimen can be released. Procedures for the precise identification of all semen samples must be established to allow appropriate tracking *(10)*.

Genetic screening is recommended for all potential donors. Furthermore, couples should be aware of the fact that in 4%–5% of pregnancies following donor insemination, the child could be born with a congenital anomaly but that this risk is comparable to rates seen in spontaneous pregnancies *(11)*.

In a sense, a woman chooses her own donor by choosing her partner *(12)*. Most women want a donor to resemble their partner as closely as possible. The resemblance is generally restricted to hair and eye colour, ethnic origin, height and blood type. Often, the reason behind this wish for resemblance is secrecy.

Considerable debate continues on the type of gamete donors to be recruited and payment of fees or expenses *(13–17)*. Traditionally, men donating sperm have been paid a nominal fee including reimbursement for their direct and indirect expenses. Several studies of sperm donors have shown clearly that the majority of men who donate sperm are young single students who are motivated predominantly by payment *(18–20)*. In the UK, the Human Fertilisation and Embryology Authority has suggested that payment can be given *(14–21)*. Following examination of various aspects of payment for donors, the Authority agreed that donation should be something entered into freely and voluntary and it was felt that any risk that the decision to donate might be influenced by financial inducement was not desirable *(14)*. This issue has been widely debated *(16,22,23)*. In the USA, men have always been paid to provide gametes. It has been suggested that donors should be paid inconvenience allowances similar to payment made to health volunteers who take part in drug and treatment trials *(23)*. The largest group from which sperm donors are recruited in the USA are students. Lyall *et al. (15)* undertook a survey designed to solicit opinion on whether sperm donors should be paid. They surveyed 717 individuals in three groups: the general public, students (potential donors) and infertility patients (potential recipients). The majority of the potential donor group was in favour of paying sperm donors, as were infertile patients. In contrast, the general public was not. Although not in favour of paying sperm donors, the general public overwhelmingly approved of the use of donated spermatozoa for the treatment of infertile couples. If such payments are withdrawn, there is a possibility that the availability of donor sperm may decline. This raises an interesting question from an ethical and social perspective: should sperm donors be paid?

Limiting donors

There is concern about limiting the use of donors so as to reduce the risk of consanguinity between the offspring of recipients of the same donor. This is a problem in small communities in which a very limited supply of donors is available. It is far less likely where there are large commercial sperm banks. It has been suggested that in a population of 800 000, limiting a single donor to no more than 25 pregnancies would avoid inadvertent consanguineous conception *(9)*. However, only 20%–30% of the pregnancies produced

through commercial donations are ever reported to the sperm banks *(24)*.

Protocols

The accurate prediction of the time of ovulation is critical to success in a donor insemination programme. Most ovulation prediction techniques are indirect for reasons of logistics, cost, and patient or programme convenience. Ovulation in the natural cycle can be monitored by several means. Progressive follicular growth and ovulation can be assessed ultrasonographically. The luteinizing hormone (LH) surge is detected via rapid radioimmunoassay of the urine or serum, as well as by a monoclonal antibody urinary test. The latter technique has gained widespread acceptance because of its reliability, availability, low cost and patient convenience. Less precise methods of timing of insemination in the natural cycle include reliance on basal body temperature or cervical mucus changes.

Intrauterine insemination (IUI) has shown clear advantages over vaginal or intracervical insemination *(25–27)*. Since the fertilizable life span of the ovum is estimated to be only 12–24 hours, the timing of the insemination procedure may critically influence success rates. There is controversy as to the optimal number of inseminations per cycle. The majority of published series have documented the efficacy of one insemination performed on the day after the LH surge *(25,28,29)*. Nevertheless, Centola *et al. (30)*, in a prospective trial, reported a fertility rate of 21% with two inseminations per cycle compared to only 6% when one insemination was performed. The procedures were performed on the day after the positive test (one insemination) or on the day of the LH surge and the following day (two inseminations).

The routine use of clomiphene citrate or any other ovulation-inducing agent in conjunction with IUI should be reserved only for women who have documented anovulation.

The success rate of donor insemination can be measured by both cycle fertility (pregnancy rate per cycle) and cumulative conception rates (number of pregnancies per insemination cycle). It has been suggested that the fertility rate using frozen, compared to fresh, semen will ultimately be almost the same, although the number of cycles required for conception with frozen specimens will be substantially greater (50% success rate within six months for frozen semen versus three months for fresh semen) *(31,32)*. Total cumulative success rates of 70%–80% have been observed with twelve months of insemination with frozen sperm. A study of 21 597 pregnancies obtained after artificial insemination with donor semen was carried out in France, from 1987 to 1994 *(33)*. It was concluded that, after donor insemination, the spontaneous abortion and tubal pregnancy rates, the birth weight and the prematurity rate were not different from that of the general population.

The presence of coexisting factors interfering with female fertility should be ruled out, such as endometriosis, tubal disease, and ovulatory disturbances. Fertility also decreases with age *(34,35)*. This consideration is an important part of the counselling process for all women considering donor insemination.

Record management

The nearly universal practice of physicians using donor insemination has been to keep confidential the identity of both donors and recipients. Records, including those pertaining to donor suitability, quality assurance, and collection, processing, storage, medical and laboratory data, must be retained for at least ten years after insemination *(10)*. This allows for donors and recipients to be tracked in the event of a medical problem in the donor or donor's family being discovered.

Psychological and social implications

Perhaps the most common question asked of the practitioner is whether the couple should inform the child of the donor insemination. It is prudent to advise the couple to explore their own feelings thoroughly and to arrive at a conclusion with which they will be comfortable. Counselling with a psychologist may be helpful in this regard. It may also prove to be helpful in addressing many issues that are not immediately apparent to couples considering donor insemination such as disclosure and secrecy of the donor's identity.

Oocyte donation

Successful oocyte donation as a treatment for infertility was first reported 17 years ago *(36)*. The practice of oocyte donation differs greatly from centre to centre and from country to country. In this procedure, the donor undergoes superovulation, as with conventional IVF. Oocyte retrieval is performed

and the donor oocytes are inseminated *in vitro* with the sperm of the partner of the recipient. The fertilized ova are either transferred to the hormonally synchronized recipient in the same cycle or cryopreserved for transfer at a later date.

Oocyte donors

Oocyte donation involves two women: the donor and the woman who wishes to be pregnant. In some fertility centres, recipient couples can choose between two types of donation: a known donor, such as a sister, a relative or a friend, or anonymous donation by a paid donor or infertile patient who volunteers to donate a proportion of their ova ("shared" egg donation). However, in other programmes, only anonymous donation is offered to couples *(37)*.

One of the major difficulties in establishing a donor oocyte programme is the limited availability of donor subjects. Although the number of potential recipients is continuously increasing, the shortage of donors is the limiting factor. Donor recruitment is a difficult endeavour, taking into account that a considerable number of potential donors may have a contraindication found on medical, genetic, or psychological testing that prohibits donation. Furthermore, in some countries, recruitment of paid donors is prohibited by law *(38–40)*.

It has been recommended that anonymous donors should be less than 35 years of age. The drawback of using oocytes from older women is the increased risk of chromosomal abnormalities. Another potential problem is that older donors produce fewer oocytes than younger women.

Ovum sharing raises several ethical and medical concerns. A "shared" ovum donation programme is limited by the number of ova available for donation and the oocyte quality, which depends largely on the donor's age and in the case of donation by an infertile woman, her infertility etiology. Historically, shared donation involved IVF patients willing to donate some of their oocytes to a recipient. In return, recipients paid the cost of ova collection. However, others *(41,42)* believe that an ethical problem might arise in cases where the recipient woman conceives and give birth to a child, while the donor herself does not conceive. With the current low-dose protocols that are now used for induction of ovulation, the ovum-sharing procedure with IVF patients does not provide a sufficient number of extra oocytes for donation. Furthermore, IVF patients wish to maximize their chances of pregnancy by using a significant number of their own oocytes and by cryopreservation of supernumerary embryos for their own future use.

Most fertility centres do not allow donors to be compensated financially, except for a reasonable reimbursement of their expenses.

Extensive and careful psychological screening of potential donors is recommended *(37,43,44)*. Furthermore, donors need to understand the boundaries of their role and need to be fully capable and free from any coercion in giving informed consent.

Screening of oocyte donors

Oocyte donors should have attained their legal majority and preferably be between the ages of 21 and 34 years. Proven fertility is desirable but not required. All prospective oocyte donors should be screened for genetic and infectious diseases in order to minimize transmission to the recipient or their offspring. The history and physical evaluation should exclude inherited disorders, and the possibility of reproductive dysfunction. The laboratory evaluation should include a blood count and blood type to identify potential Rh incompatibility. The screen for infectious diseases should, at a minimum, includes

- HIV
- syphilis (RPR/TPHA)
- hepatitis B antigen
- hepatitis B antibodies
- hepatitis C
- *Chlamydia trachomatis*

In selected cases, sickle-cell disease or thalassaemia should be ruled out.

A number of controlled ovarian hyperstimulation protocols have been developed; essentially all of them include administration of gonadotrophins (human menopausal gonadotrophin [hMG] or recombinant FSH) in conjunction with GnRH agonists. Usually, the GnRh agonist is administered as a daily dose, commencing in the midluteal phase of the donor's previous cycle. Ovarian suppression is usually completed at the onset of menses, one week later. A baseline ultrasonogram and estradiol level are obtained on the third day of the following cycle, to exclude ovarian cysts and to ensure that adequate ovarian suppression has been obtained.

Gonadotrophin therapy is initiated and monitored throughout the cycle. Human chorionic gonado-

trophin (hCG) is administered at a dose of 5000 or 10 000 IU, once appropriate follicular and hormonal criteria have been achieved. Ultrasound-guided, transvaginal oocyte recovery is performed with intravenous sedation and analgesia. After a pre-incubation interval of two to six hours, the donor oocyte is inseminated with the recipient's partner's sperm. The embryos that result are either cryopreserved or transferred to the synchronized recipient 48–72 hours after the retrieval.

It is important that both the recipient and the donor should be adequately counselled and be aware of the psychological, legal and moral implications of oocyte donation, before being accepted into the programme *(40,41,45)*. The psychological evaluation is intended to uncover any risk factors that may render a subject emotionally unsuitable for ovum donation and to ensure that there is no element of coercion in her decision to donate oocytes, particularly in the case of known donors.

The nature of any risk of developing serious complications following donation, whether they be paid ovum sharers or volunteer donors, should be fully explained.

Recipients

Both the oocyte recipient and her partner should be healthy, and there should be no physical contraindication to pregnancy. Information on donor oocyte success rates for the individual centre should be given to the couple.

Screening for infectious diseases in the patient and her partner generally include tests for HIV, hepatitis, *Chlamydia trachomatis* and syphilis. The male partner should have a semen analysis with a sperm wash and swim-up in order to rule out a coexisting male factor. Hysterosalpingography should be performed (or previous films reviewed) to evaluate any uterine abnormalities.

Debate over limiting oocyte donation to younger recipients continues. Perhaps the most controversial application of oocyte donation has been its use in circumventing age-related infertility in patients nearing or beyond the menopause. The number of women between the ages of 35 and 50 years is steadily increasing. Also, many women are electing to delay childbirth for personal, economic or professional reasons. Fertility potential decreases with advancing maternal age and it is expected that most older women will be unsuccessful in their effort to reproduce. The introduction of oocyte donation to establish pregnancy in patients with age-related infertility has allowed many older women a new opportunity of conceiving. However, there are concerns over the health of the older mother and her fetus during pregnancy and worry as to whether older women have the stamina to raise a child to adulthood.

Protocols

By definition, ovarian and endometrial events are dissociated in donor oocyte IVF cycles. Effective synchronization of embryonic and endometrial development is therefore crucial to the success of an oocyte donation programme. In most fertility clinics, the recipients undergo one or more preparatory cycles to measure endometrial response to exogenous estradiol and progesterone. Assessment of the preparatory cycles include serum steroid levels, measurement of endometrial thickness with transvaginal ultrasound and an endometrial biopsy for histological review *(46)*.

Synchronization between the donor and recipient depends on whether or not the recipient has intact ovarian function. Although in a natural cycle (patients with a normal ovarian function) frozen embryo transfer is a straightforward procedure, fresh embryo transfer, on the other hand, is difficult to achieve and may not be possible because of the problem of synchronization of donor and recipient cycles. Recipients possessing endogenous ovarian function are usually treated with an exogenous hormonal regimen, after suppression with a GnRH agonist. A variety of steroid replacement regimens have been developed, all of which are designed to approximate the pattern of hormone secretion occurring in the natural menstrual cycle. Estrogen may be administered orally or by transdermal patches. Protocols have been developed employing

transdermal patches *(46–47)*. Endometrial proliferation can be induced with fixed or increasing doses of estrogens *(48)*. This protocol has the advantages of simplicity and providing a larger window for implantation.

Progesterone replacement is initiated the day before or on the day of the donor's oocyte retrieval and is usually given as an intramuscular injection of progesterone in an oil vehicle *(48)*, vaginal suppositories *(46)*, micronized oral progesterone *(49)* or more recently, via a vaginal ring drug-release system *(50)*.

Pregnancy and live-birth rates following oocyte

donation are excellent in comparison with those achieved after transfer of the patient's own oocytes *(3)*. The reported pregnancy rates per ovum recipient vary between 20% and 67% per embryo transfer *(51–54)*.

The technique of oocyte and embryo donation makes it possible for women to be gestational mothers well beyond the age of the normal reproductive life span. Thus, women in their forties, fifties or more may become mothers as a result of oocyte donation. This scenario raises the ethical question of the potential health risk to the gestational mother of being pregnant beyond the age where this would occur in nature. The risk of pregnancy for women increases considerably with increasing maternal age *(55)*. Thorough medical screening is essential to minimize the obstetric risk in the older group of patients. Maternal complications are also increased by multiple pregnancies *(56)*; limiting the number of embryos to be transferred to two could reduce this risk *(3)*. These women should be encouraged to seek preconceptual counselling and early prenatal care, and should be counselled regarding potential obstetric complications.

Social implications

While biomedical advances in oocyte donation have preoccupied most investigators, more recently, interest in the behavioural aspects of this procedure has begun to be reflected in the literature. A number of studies have examined the attitudes of oocyte donors and recipients *(37,57–59)*. To obtain information concerning Latin American oocyte recipients' thoughts and feelings about the donation, the Latin American Network of Assisted Reproduction undertook a multicentre study *(37)*. A follow-up questionnaire was sent to 106 women who underwent oocyte donation between 1989 and 1997. The recipients were asked about their age, education, marital status and their thoughts and feelings after the treatment. In all, 81% of the recipients were Catholics and 62% had higher education. Sixty per cent of the patients who became pregnant and 64% of those who did not achieve pregnancy stated that they would be willing to repeat the treatment; 76% of the recipients intended not to disclose the type of treatment, whereas 24% were undecided. Dominant reasons for not disclosing were the fact that the child "is my child, no matter his/her origin". Responders noted several primary concerns when asked to discuss the most difficult part of an oocyte donation cycle. One recurrent early theme centred around the need to grieve the loss of the "dream child" that would never be born. Although only 37% of the recipients had psychological counselling, 67% of the women stated the need for such support before and throughout the treatment.

Embryo donation

Embryo donation is a well-established and successful form of assisted conception treatment when both partners are infertile.

Embryo donors

The large majority of embryo donors are couples who have completed their families through IVF and have had the spare embryos cryopreserved. The age of the woman at the time the oocyte was obtained for IVF should be taken into account when considering the use of donated embryos: they should be above the age of 18 and below 36 years.

The other source of donated embryos is by way of separate oocyte and sperm donation.

Screening of embryo donors

The embryo donors must sign an informed consent document indicating their permission to use their embryos for donation, relinquishing all rights to the embryo(s) and any child or children that may result from the transfer of these embryos. They should be screened for genetic and infectious diseases to prevent transmission of these to the recipient or the offspring. If embryos have been created as part of another couples' IVF treatment, the appropriate records should be reviewed in an anonymous and confidential manner to assess previous laboratory results including tests for HIV and hepatitis B and C antibodies. The recipient couple must be fully aware and informed about the results of these tests.

Screening of embryo recipients

Embryo recipients should be informed about the chances of pregnancy based on each programme's experience with the transfer of cryopreserved embryos. Recipient couples should also be made aware of the limitations of genetic and infectious disease screening and informed there can be no guarantee of a child

being born free of birth defects or illness. Because embryo donation is a new procedure, all couples should be aware of the uncertainty about the long-term outcome from the psychological or developmental standpoint.

Couples who will receive embryo donation should be fully evaluated, including medical history, physical examination and psychological counselling. Pelvic ultrasound should be performed to assess uterine size, endometrial thickness and rule out pelvic pathology such as endometrial polyps, myomas or ovarian cysts. Other tests that may be required are

- HIV antibodies
- hepatitis B and C antibodies
- syphilis antibodies
- blood typing
- cervical cytology
- rubella antibodies.

Protocols

The protocol for embryo transfer depends on whether the recipient has ovarian function or not. Recipients without ovarian function will have their embryos transferred in a hormone replacement cycle with an estrogen and progesterone preparation. Different protocols have been developed, all of which are designed to approximate the pattern of hormone secretion occurring in the natural menstrual cycle.

Recipients with ovarian function may have the embryos transferred in a natural cycle. This is generally recommended for patients with normal, regular menstrual cycles. Patients are asked to attend the clinic from Day 10 of the cycle, and from this point, follicular growth is monitored by serial ultrasound scanning and serum estradiol and LH estimations. Embryos are replaced three or four days after the LH surge has been detected. No luteal phase support is given in these cycles.

Psychological counselling

Adequate counselling should be offered to all couples who will undergo embryo donation. The recipient woman and her husband or partner should be aware that they would be the legal parents of any child *(60)*. The recipient couple should be given adequate information on the limitations and possible outcome of treatment, the likelihood of pregnancy and live birth, the possible side-effects of any medication and the risks of treatment, such as multiple pregnancy *(61)*, the extent to which genetics and infectious screening tests have been performed and the cost of treatment.

Disclosure

With ovum, sperm and embryo donation as now practised, the resulting children may never learn that they were conceived via gamete or embryo donation. If disclosure does occur, the children may want further information about their genetic origin. The decision as to whether to tell such children of their origin remains a private one for the parents.

Indications

Sperm donation

The American Society for Reproductive Medicine *(9)* indications for therapeutic donor insemination are

- the male partner has azoospermia;
- the male partner has a known hereditary or genetic disorder which cannot be overcome with pre-implantation genetic diagnosis (PGD);
- the male partner has uncorrectable ejaculatory dysfunction secondary to trauma, surgery, medication or psychological abnormalities;
- the female partner is Rh-negative and severely Rh-isoimmunized, and the male partner is Rh-positive;
- in ART where a severe male factor has been demonstrated, such as previous failure to fertilize, male immunological infertility and where ICSI is unacceptable to the couple;
- in females without male partners.

Oocyte donation

Oocyte donation has increased rapidly in popularity since it was first described *(47,62)*. Initially, the major indication for oocyte donation was premature ovarian failure, defined as hypergonadotrophic hypogonadism occurring before the age of 40 years. Oocyte donation has now become widespread throughout the world to treat a variety of reproductive disorders, such as incipient ovarian failure, recurrent IVF cycle failure, and poor response to conventional ovarian hyperstimulation. Some of these problems are age related. In general, women over 43 years who wish to

become pregnant are asked to consider oocyte donation because of the low success rates for treatment with their own oocytes *(3)*. Donor oocyte technology may also be applied in cases of inherited maternal genetic abnormalities (e.g. balanced translocations, autosomal dominant or X-linked recessive disorders) to obviate the risk of transmission to the offspring. These patients currently comprise a small proportion of candidates for donor oocyte IVF, and its indication is likely to wane with the continued development of specific gene probes and refinement of PGD.

Embryo donation

At present, there are two ways in which embryos are made available for donation**.** Unwanted additional cryopreserved embryos can be designated for donation by couples who have undergone IVF, and embryos can be created for the specific purpose of donation using donor sperm and donor ova.

There are three main indications for embryo donation:

- women with an infertile partner;
- recurrent IVF failure;
- carriers of genetic disease or chromosomal abnormalities.

Risk–benefit analysis

In the past century, sociological changes have resulted in an overall delay in the decision to become pregnant. This fact, along with the increase of therapeutic alternatives offered to infertile couples, such as oocyte and embryo donation and surrogate motherhood, has resulted in a significant increase in the number of patients of more advanced age wishing to become pregnant. Several groups have shown that women in their late fifties and older can achieve successful pregnancy after oocyte donation and that the uterus of a menopausal women can respond to steroid therapy and embryos can implant therein *(1,2,63)*. However, it has been postulated that older patients are at greater risk of having complications during their pregnancies *(64)*. Concerns do exist with respect to extending oocyte or embryo donation to older women and are based not only upon the welfare of the child but also upon beliefs that pregnancy presents a high medical risk for the elderly mother and her fetus *(55,56,65)*. It has been shown that most of the complications in pregnancy associated with older age are caused by age-related confounders such as myomata, diabetes mellitus, hypertension and multiparity *(56)*. Postmenopausal women should be considered particularly at increased risk of vascular complications during pregnancy. This risk is likely to increase progressively with the number of years that have elapsed since the onset of menopause. At present, establishing pregnancy in postmenopausal women is more an ethical than a medical issue, partly because the information that is available on pregnancy in postmenopausal women is insufficient to determine a reliable risk profile.

Advanced maternal age has been associated with adverse pregnancy outcomes such as increased perinatal mortality, preterm delivery, low birth weight and small-for-gestational-age infants.

There are concerns about increasing the inadvertent consanguinity brought about the use of the same sperm donor or shared oocyte donation. Because some parents may choose not to disclose to their children their genetic origins, it is remotely possible to have unknown consanguinity between individuals genetically related to the same oocyte or sperm donor. The risk of potential unknown consanguinity is not absent in gamete donation because donors may have their own children. This raises the responsibility of fertility clinics to limit the number of donor attempts for donor safety. Couples should be counselled about the rare possibility of unknown consanguinity, a risk that can be lessened with disclosure of the identity of the donor.

In the early years of reproductive medicine, the major focus of clinicians was the well-being of the infertile couple and the development of successful treatments was the first priority. It was in the early 1990s that more questions were raised about the long-term effects on the well-being of the children born as a result of gamete or embryo donation.

There is still no consensus on the possible association between ovarian stimulation and epithelial ovarian cancer *(66–68)*, but it is clear that this theoretical risk is a concern. Two important studies have suggested an association between exposure to fertility drugs and an increased risk of ovarian cancer. A pooled analysis of three case–control studies showed an odds ratio (OR) of 2.8 (95% CI 1.3–6.1) for ovarian cancer in infertile women treated with fertility drugs compared with women with no diagnosis of infertility or fertility drug treatment *(69)*. In 1994,

Rossing and colleagues using record linkage with a population-based cancer registry, identified an increase prevalence of ovarian cancer (invasive or borderline malignant tumours) with a standardized incidence rate of 2.5 (95% CI 1.3–4.5) in a cohort of infertile women compared with age-standardized general population rates. An increased relative risk (RR 11.1; 95%CI 1.5–82.3) was also found in women, with or without abnormalities, who had been treated with clomiphene citrate for more than one year, compared with infertile women who had not taken the drug *(66)*. For this reason, a limit on the total number of donation cycles per donor should be set, to reduce exposure to ovarian stimulation. This is an issue of importance when assessing risk-exposure.

Conclusions

Gamete and embryo donation have been shown to be safe, cost-effective, and an overwhelmingly beneficial treatment for thousands of infertile couples. A thorough understanding of the clinical science and contemporary guidelines, as well as the legal and social implications of these procedures, is required that these treatments are used safely and effectively.

There is no doubt that ART helps many infertile couples to have children. But some of these techniques, such as sperm, oocyte and embryo donation have also given rise to a wide variety of legal and ethical issues *(60)*.

Research on the consequences for children growing up in these new family structures is in its infancy. For example, although keeping the method of conception secret from young children conceived by oocyte or sperm donation does not appear to have a negative impact upon family relationship or on the children's prepubertal development, it remains to be seen whether nondisclosure leads to difficulties as these children grow up and move into adolescence.

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Embryo culture, assessment, selection and transfer

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Embryo morphology and growth rate

Preparation for mitotic division begins with two haploid pronuclei duplicating their DNA, the two pronuclei then come together and syngamy occurs at 20–34 hours after insemination followed by division at approximately 35.6 hours which results in the formation of two diploid blastomeres containing approximately half the cytoplasm of the fertilized oocyte *(1)*. Mitotic division during the preimplantation period results in a rapid increase in blastomere numbers, as there is no cell growth phase prior to mitosis. This results in daughter cells that are smaller than parent blastomeres, leading to a progressive reduction in individual blastomere volume. With the use of co-culture methods and the advent of complex and sequential culture media, the embryo quality and growth rate have improved and there are variations in the cell number and morphology of the embryo depending on the culture media and culture conditions employed.

Once the sperm has fertilized the oocyte, the centriole and microtubules arising from the sperm bring the male and female pronuclei into juxtaposition *(2)*. Pronuclear alignment occurs 16–18 hours post-insemination and failure to do so is indicative of one or more fertilization events failing to occur *(3)*. Nucleoli are visible within the respective pronuclei during this period and are responsible for rRNA synthesis, which resumes at fertilization *(4,5)*. The nucleoli or nuclear precursor bodies show various patterns of presentation which are suggestive of the chromosomal status and developmental inferiority of the embryo. Tesarik and Greco *(6)* examined the pronuclear morphogenesis in an attempt to determine embryo viability dependent on distribution and alignment of the nucleolar precursor bodies in the respective nuclei. Embryos resulting from certain distribution patterns exhibited greater viability and lower rates of embryonic arrest. This group constituted the "normal" pronuclear stage morphology, with the remaining groups described as abnormal, attributed to asynchronous pronuclear development, which the authors believe is harmful to embryo viability. Pronuclear alignment in relation to the polar body axis, positioning of the nucleoli, and cytoplasmic consistency have also been shown to affect embryo viability. Sadowy *et al. (7)* suggested that size variation between the two pronuclei may indicate chromosomal anomalies. The authors showed an increase in embryonic arrest in zygotes with pronuclei that differ by four microns from each other. A higher incidence of multinucleation and mosaicism was also observed in zygotes with dysmorphic pronuclei. Recent evidence suggests that polarity in mammalian oocytes, including human oocytes, exists and can be evidenced in the distribution of surface structures and molecules, cytoplasmic organelles, and RNA. The

animal pole of the oocyte may be estimated by the location of the first polar body, whereas after fertilization, the second polar body marks the so-called embryonic pole *(8)*. In human oocytes, there is a differential distribution of the molecules leptin and STAT3 to the cortical region of the oocytes containing the animal pole and subsequent mitotic divisions produce blastomeres with different allocations of these polarized molecules *(9)*. Prior to syngamy, pronuclei can be observed to rotate within the ooplasm, directing their axes towards the second polar body *(10)*. Theoretically, embryos that do not achieve an optimal pronuclear orientation may exhibit cleavage anomalies that may be observed as poor morphology, uneven cleavage or fragmentation. Garello *et al. (11)* demonstrated that there is no relationship between the magnitude of the angle between the first and second polar body and subsequent embryo morphology, probably due to the fact that the first polar body is capable of moving over an angle of 30° while the second polar body remains stationary. However, these authors provided some evidence in support of the hypothesis that the orientation of the pronuclei relative to the polar bodies relates to the subsequent morphological grade of the embryo *(11)*. Although the absolute magnitude of the angle between the pronuclei and polar bodies did not relate to embryo grade, the angle increased significantly with decreasing embryo quality.

Tripronuclear oocytes are usually formed by dispermic fertilization and, as a result of the formation of a tripolar spindle, the majority (62%) divide into 3 cells with severely abnormal chromosome numbers *(12)*. Some oocytes with a bipolar spindle (24%) form triploid embryos and some (14%) regain a diploid karyotype by expulsion of a set of chromosomes in a nucleated fragment *(12)*. As a consequence, it is recommended that tripronuclear oocytes are discarded.

Monopronuclear oocytes may be diploid and some will develop to a blastocyst and to term when transferred to patients *(13–15)*. As a result, it has been recommended that monopronuclear oocytes are rechecked for multiple pronuclei within a few hours *(16)* and cultured for biopsy to confirm diploidy or to the blastocyst stage for transfer.

In normally fertilized oocytes, the two pronuclei come together, the chromosomes arrange themselves along a common spindle and syngamy occurs. The centrioles pull the chromatids apart, a furrow appears between the two poles and the zygote cleaves into a two-cell embryo at a mean of 35.6 hours post-insemination *(1,17)*. Evidence from the distribution of surface markers on the human oocyte and embryo confirm observations in other mammalian embryos that the first cell division is meridional, with the polar bodies marking one pole (reviewed in *(18)*), and the second cell division involves a meridional division for one blastomere and an equatorial division for the other *(9)*. Early entry into the first cell division has been used as an indicator of embryo viability. Selection of embryos for transfer on Day 2 from the cohort that had undergone early cleavage by 25 hours postinsemination resulted in higher pregnancy rates *(19,20)*. Sakkas *et al*. *(20)* postulated this increase in viability in early cleaving embryos was due to intrinsic factors regulating cleavage within the oocyte or embryo rather than the timing of fertilization.

On Day 2, embryos are at the four-cell stage of development by 45.5–45.7 hours postsperm insemination, and three days after fertilization are at the eight-cell stage by 54.3–56.4 hours *(1,17)*. The individual cells of the embryo may be asynchronous in their cell division resulting in embryos with uneven cell numbers. Between the four-cell and the eight-cell stage, the transition from maternal to embryonic gene expression occurs; therefore, during the first 48 hours after sperm insemination the embryo primarily relies on maternal transcripts rather than its own activated genome *(21)*.

A morphological criterion used to assess embryo quality throughout human embryology is embryonic fragmentation. Fragmentation is the extrusion of the plasma membrane and subjacent cytoplasm of an embryo into the extracellular region. Fragmentation is not an *in vitro* phenomenon due to compromised embryonic development but appears to be a natural occurrence in human embryos as it is evident in *in vivo* grown embryos *(22)*. The intracellular mechanisms causing fragmentation are not fully known, although it has been speculated that the process may be caused by developmentally lethal defects or apoptotic events *(23)*. Hoover *et al. (24)* examined blastomere size and embryo fragmentation as an indicator of embryo viability and pregnancy potential. The authors found blastomere size influenced the developmental potential; however, no correlation was observed with cellular fragmentation. Contrary to this, Giorgetti *et al. (25)* suggested that the embryo developmental potential decreased significantly as the number of cytoplasmic fragments increased. Furthermore, Antczak and Van Blerkom *(9)* suggested that the amount of fragmentation an embryo has is not the

determining factor; it is what is being extruded that relates to embryo viability. These authors showed that several regulatory proteins are localized to polarized domains in the oocyte and are asymmetrically distributed to individual cells during developmental progression. A decrease or elimination of these necessary proteins by fragmentation may then lead to blastomere apoptosis. Therefore, the size and distribution of fragments may have different consequences for the developmental competence of the embryo as a whole *(26)*. Small, scattered fragments may be due to imperfect cytokinesis during successive divisions and not specific anomalies, as may be the case with greater localized fragmentation.

Embryo morphology has been associated with chromosomal abnormalities. Almeida and Bolton *(27)* showed that 63.4% of embryos that arrest between the pronucleate and the eight-cell stage are chromosomally abnormal. Embryos exhibiting irregular-shaped blastomeres and severe fragmentation are considered poor quality embryos and show a higher incidence of chromosomal abnormalities (62%) compared to embryos of good quality (22.2%) *(28)*. Fragmentation has been shown to be correlated to chromosomal mosaicism *(29)*. Slow cleaving (two to six cells on Day 3) and rapidly cleaving (nine or more cells on Day 3) embryos show a higher incidence of chromosomal aneuploidy than those embryos showing normal cleavage kinetics (seven to eight cells on Day 3) *(30)*.

On Day 2 or Day 3 an embryo may exhibit blastomeres with more than one nucleus. The incidence of embryos containing multinucleated blastomeres is not uncommon. These embryos are not necessarily degenerate and some are capable of DNA and RNA synthesis *(31)*. However, Munne and Cohen *(32)* showed that 30.4% of arrested embryos were multinucleated. Kligman *et al. (33)* found that 74.5% of multinucleated embryos are chromosomally abnormal compared to 32.3% of nonmultinucleated embryos. Multinucleated blastomeres have been speculated to result from accelerated ovulation induction response *(34)* or cytokinetic failure *(32)* and are indicative of poor development.

During Day 3 postinsemination, the cytoplasm of the embryo may become granular and tiny pits appear (cytoplasmic pitting). There is an increase in cell–cell adhesion early in the morning of Day 3 at the eight-cell stage and loss of definition between individual blastomeres of embryos that are likely to undergo compaction *(35)*. The blastomeres of the embryo undergo a rearrangement process during compaction with the establishment of cell–cell adhesions leading to communication between blastomeres. Desai *et al. (36)* speculate that cytoplasmic pitting and increased cell–cell adherence may be early markers of cytoplasmic activity and potential for embryonic activation, as embryos exhibiting these features were more likely to proceed to the next stages of preimplantation development—the morula and blastocyst stages.

The embryo is termed a morula approximately four days after fertilization when it has undergone compaction, the formation of tight junctions and gap junctions and polarization of the blastomeres, resulting in communication between blastomeres and segregation of two cellular populations of inside and outside cells *(37)*.

Once a cavity forms within the morula it is termed a blastocyst *(38)*. Cavitation involves the formation of the blastocoele, the fluid filled cavity necessary for blastocyst formation. Wiley *(39)* showed that Na^+/K^+ ATPase, located on the basolateral membrane, pumps sodium out of the cell and into the intercellular spaces and water passively follows, thereby forming the blastocoele cavity. As cavitation proceeds, the two populations of cells formed by polarization of the blastomeres during compaction become (i) the trophectoderm that forms extraembryonic tissue; and (ii) the inner cell mass that forms the embryo lineage *(39)*.

In vitro blastocyst formation occurs between Day 5 and 7 postinsemination *(40,41)*. Blastocysts that occur on the respective days do not appear morphologically different, although their growth rate differs, nor do they have significant differences in hCG secretions, the latter suggesting comparable mature trophectodermal tissue *(40)*. The blastocyst has several different appearances depending on its development *(42,43)*. When a cavity is apparent and the inner cell mass and trophectoderm are distinct, the embryo is termed an early blastocyst. The blastocyst begins to increase in size, termed expanding blastocyst, until it has fully expanded. The expansion of the blastocyst causes thinning of the zona pellucida. The zona pellucida is an acellular glycoprotein coat that surrounds oocytes and embryos and is important during fertilization and early preimplantation development as it keeps the blastomeres of the embryos together when there are no intercellular junctions. The zona pellucida thins with the growing blastocyst until it ruptures and the blastocyst begins to herniate

through the zona pellucida in a process called hatching. This occurs on approximately Day 6 or 7. When the blastocyst fully escapes from the zona pellucida it is termed a hatched blastocyst.

A morula may become vacuolated and appear to be cavitated. The vacuoles do not appear to be lined with the trophectoderm or inner cell mass *(40)*. These embryos do not hatch from the zona pellucida, degenerate with ongoing culture (Day 8 or 9) and have significantly less cells than true blastocysts *(41)*. These embryos are termed vacuolated morulae and should be distinguished from blastocysts.

Blastocysts can appear morphologically similar but may contain significantly different cell numbers *(38,41,44–47)* and have a different ability to hatch from the zona pellucida *(46)*. Blastocyst cell numbers may be correlated with embryo viability. Hardy *et al. (44)* found expanded blastocysts on Days 5, 6 and 7 had an average of 37.9, 40.3 and 80.6 trophectoderm cells and 20.4, 41.9 and 45.6 inner cell mass cells, respectively, using a simple culture medium. The authors also observed that the total cell numbers were lower in morphologically abnormal blastocysts and blastocysts arising from abnormally fertilized zygotes. Van Blerkom *(46)* found that the stage-specific differentiation of the cells and, in particular, the inner cell mass, may be a function of age of the embryo rather than the overall number of cells that constitute the embryo. The distribution of the cells between the inner cell mass and trophectoderm also affects embryo viability *(44)*.

Therefore, embryo morphology and growth rate are determinants of embryo quality. The overall embryo quality differs markedly and this variation is evident in embryos produced *in vivo (48)*. Patients generally do produce a similar embryo quality from cycle to cycle independent of maternal age. Embryo quality may be an inherent feature of the female and probably depends in part on oocyte maturity *(49)*. There is a decline in embryo quality in embryos generated from oocytes from ageing women. Janny and Menezo *(50)* speculate that this may be due to increased chromosomal abnormalities, the role of maternally inherited products from the oocyte, time of genomic activation, and the temporal pattern of gene expression during the initial embryo development. Munne *et al. (51)* have also shown that embryos that appear morphologically normal contain chromosomal abnormalities and the frequency of these abnormalities increases with maternal age.

Human embryo culture media and culture conditions

Culture conditions for human embryos have evolved over the past decade; significantly increasing the viability of *in vitro* fertilized embryos. Improvements in culture media and the physical environment in which embryos are cultured have resulted from an increased understanding of both the physiology of the embryo and the environment of the oviduct and uterus. Conventional media for culture of human embryos to Day 2 or Day 3 postinsemination were simple media such as T6 *(52)*, HTF *(53)* and EBSS *(54)* containing a serum additive. Development to the blastocyst stage, however, was limited *(55,56)* because these simple media lack important regulators of embryo development. Co-culture of human embryos with various somatic cell monolayers with more complex media and serum additives was shown to support development of embryos to the blastocyst stage *(57–60)*.

Co-culture of human embryos with somatic cells has been reported to improve blastocyst development, decrease fragmentation and/or improve pregnancy and implantation rates *(57,61–63)*, particularly for patients with repeated IVF failures *(61,64,65)*. Somatic cells may benefit embryo development by providing trophic factors and/or by modifying inhibitory media components *(66–68)*. There is not general consensus, however, that co-culture systems improve pregnancy rates *(46,60,69)*. Co-culture is time-consuming with an associated risk of pathogen transmission.

A greater understanding of both the physiological requirements of the embryo as it develops from the zygote to the blastocyst stages *in vitro* [reviewed in *(70,71)*], and the composition of oviduct and uterine fluids *(72–74)* has led to the more recent development of stage-specific or "sequential" complex media for extended culture. The first of such media was G1/G2 *(75)* which was recently modified, resulting in increased pregnancy rates after blastocyst transfer *(76,77)*. Currently, conditions employed for human embryo culture have been largely based on studies using mouse, rabbit and hamster models. Due to the restricted use of human embryos for research purposes, there is a general lack of prospective randomized clinical trial data relating to the effects of variable culture conditions on embryo viability.

The most abundant chemical constituents of

culture media (next to water) include solubilized ions. Ionic components detected in oviduct and uterine fluids *(72)* are present in formulations of both simple and complex media that support human embryo cleavage and blastulation *in vitro*, yet vary markedly in comparative concentration *(78)*. Little evidence exists as to the significance of single ions on the development of human embryos. Many reports implicate the presence and variable concentrations of most of the dissolved salts [e.g. potassium *(79,80)*] in regulation of mouse embryo growth. Only the putative inhibitory effects of inorganic phosphate ions have been investigated clinically *(81)* with contradictory pregnancy results *(82,83)*. The subtle, integrated effects of ions may be better assessed with simultaneous optimization of concentrations, e.g. KSOM medium *(84,85)*, which was recently reported to enhance human blastocyst formation *(86)*.

Irrespective of concentration, ions should be solubilized in injection-quality, filter-purified and toxicity-tested water *(87)* to minimize the risk of biological contamination and to perhaps improve long-term outcome *(88)*. Together with purified water, ionic constituents contribute largely to the osmolality of a medium (or osmotic pressure imparted by dissolved particles)—a testable physical parameter *(89)*. Media that range from 250–290 mOsmols can satisfactorily support mammalian embryo development *(90,91)*. Deviation from this range and especially above 300 mOsmols *(92,93)* can induce deleterious changes in cell volume and hydration resulting in the compensatory uptake of organic osmolytes *(94,95)*.

Protein sources in culture medium, aside from playing a major role in preventing embryo adhesion, may also act as organic osmolytes and pH buffers (reviewed in *(78)*). Filtered, heat-inactivated maternal serum, a traditional additive with undefined, variable composition *(96,97)*, was found to have no effect on *in vitro* fertilization (IVF) pregnancy rates when compared to media without protein supplementation *(98)*. Since then, several attempts have been made to compare fractionated serum proteins to whole sera with equivocal results with respect to embryo quality and development *(99–108)*. Presently, there are several risk factors associated with supplementation of culture media with patient serum such as potential viral transmission *(99)* and the negative effects on embryo development of sera from various subgroups of infertile women *(109,110)*. Other negative effects have included impaired blastocyst development *(55)*, possible trophectodermal *(111)* and mitochondrial deterioration *(112)* and metabolic perturbations *(113)*. More chemically defined alternatives to human sera include purified albumin *(99)*, recombinant serum albumin (synthesized by bacteria) *(114)* and glycosaminoglycan molecules *(115)*.

Media with conventional ionic and protein supplements vary little in their composition of the main energy substrates pyruvate, glucose and lactate which regulate mammalian embryo metabolism in a stage-specific manner *(70)*. Glucose, however, has been excluded from some media formulations *(81, 105)*. Only a small number of studies of human embryos exist that confirm the mammalian evidence suggesting that pyruvate is a requirement to support cleavage and blastulation *(116,117)*. However, there is a shift in preference from pyruvate to glucose with the onset of embryo compaction *(118,119)*. There is little evidence on the effects of energy substrates and their concentrations in IVF culture media formulations on embryo viability. Sequential media were designed to vary in concentrations of carbohydrates to more adequately reflect the difference in composition of fluid components in the oviduct and uterus *(120)*.

Amino acids are found in the fluids of the human reproductive tract *(72,73)*; however, there has been minimal research on the effects of amino acids on the human embryo. Studies in other mammalian species have found amino acids to be important regulators of embryo development [for review see *(78)*]. Glutamine (1 mM) was found to significantly increase development of human embryos to the morula and blastocyst stages and increase energy metabolism *(121)*. Taurine *(94)* and glycine *(95)* have been shown to act as osmolytes and therefore may help to minimize the stress induced by osmotic fluctuations. Interestingly, isoleucine (0.2 mM) and phenylalanine (0.1 mM), both constituents of Eagle's essential amino acids *(122)*, were found to inhibit cleavage and implantation of human embryos *(123)*. Based on mouse and hamster embryo studies, sequential media for human embryos typically contain glutamine, taurine and Eagle's nonessential amino acids for development from Day 1 to Day 3 postinsemination, and glutamine and Eagle's essential amino acids for culture to the blastocyst stage *(75)*.

Vitamins are key components of cellular metabolism and have been shown to have significant effects on embryo quality during culture of rabbit, mouse and hamster embryos *(124–126)*. Despite the presence of B-group vitamins in recently developed sequential media for the development of human

embryos from Day 3 to Day 5, there is no information as to the effects of these vitamins on the human embryo. Ascorbic acid (vitamin C) had no significant effect on embryo development or morphology up to Day 2 or 3 postinsemination *(127)*.

The addition of growth factors such as leukaemia inhibitory factor (LIF) *(128)*, epidermal growth factor (EGF) *(129)* and insulin-like growth factor-I (IGF-I) *(130)*, and the cytokine granulocyte-macrophage colony stimulating factor (GM-CSF) *(131)*, has been shown to increase development of human embryos to the blastocyst stage. Furthermore, IGF-I *(130)* and GM-CSF *(131)* stimulated development of the inner cell mass. Thus, further studies are required to determine whether growth factors and cytokines have a significant impact on embryo viability.

EDTA and transferrin have also been added to many media for their potential function as chelators of metal ions. Careful consideration should be given, however, to the addition of EDTA to media for blastocyst development as EDTA has been shown to reduce glycolytic activity and thus inhibit blastocyst development in the mouse *(132)*.

Human embryos have typically been cultured individually in large volumes of media (usually one millilitre). Studies in the mouse have found that embryo density has a significant effect on cleavage rate and blastocyst development *(133,134)*, possibly due to embryo-derived trophic factors. Culture of human embryos in groups for up to 48 hours post-insemination was associated with an increased rate of cleavage *(135,136)*; however, the effects on pregnancy rates have been variable *(135–138)*. Rijnders and Jansen *(139)* reported that culture of human embryos to the blastocyst stage in either reduced volumes of media and/or groups, had no effect on pregnancy rates; however, the culture media was not sequential and the sample size was low.

Further improvements could be made to the formulation of media for human embryo culture through determination of the effects of individual medium components on embryo development and viability. The physical conditions under which embryos are cultured also have a significant impact on viability. The majority of culture systems for human embryos use a bicarbonate/CO_2 buffered medium to maintain a physiological pH of 7.2–7.4. Embryos are typically cultured in an incubator at a gas atmosphere containing 5% CO_2 *(76,139,140)*. According to the Henderson-Hasselbalch equation, the combination of 25 mM sodium bicarbonate in the culture medium and a 5% CO_2 gas atmosphere, results in a pH of 7.45 at sea level. Thus, it would be prudent to culture embryos at a slightly elevated concentration of 6% CO_2 *(123)*, which theoretically results in a pH of 7.37. The optimal concentration of CO_2 for culture of human embryos is yet to be determined. An N-2-hydroxyethylpiperazine-N´-2-ethanesulfonic acid (HEPES) buffered medium is often used to maintain a pH of 7.3–7.4 when embryos are exposed to atmospheric oxygen tensions for an extended period of time. It is important to consider, however, that exposure to atmospheric CO_2 and a reduced concentration of HCO_3^- may affect the embryo's ability to regulate its intracellular pH, specifically acidosis, via the HCO_3^-/Cl^- exchanger *(141)*.

The oxygen tension in the reproductive tract of mammalian species has been reported to be lower than atmospheric O_2, ranging between 1.5% and 8% *(142,143)*. Recent evidence indicates a low pO_2 in the human vaginal epithelium *(144)*. Culture of mammalian embryos at low concentrations of O_2 (5%–7%) is thought to minimize the formation of embryotoxic reactive oxygen species; however, studies on human embryos are few. Culturing human embryos at a reduced oxygen tension (5%) for up to 46 hours following insemination had no effect on cleavage rates or subsequent pregnancy rates *(140)*. Culture of frozen-thawed pronuclear stage embryos for four days at a reduced oxygen tension (5%) did not affect blastocyst development but did significantly increase total cell number compared to culture at 20% O_2 *(145)*. Thus, it may be prudent to culture human embryos at a reduced oxygen tension.

Further optimization of human embryo culture systems is needed to gain a fuller understanding of the specific requirements of the human embryo for regulatory support.

Selection of embryos for transfer

One of the most difficult aspects of assisted reproductive technology (ART) is the determination of which embryos are most suitable for transfer into the uterus. Two factors requiring consideration include the choice of embryos with the best developmental competence and the risks of multiple pregnancy associated with the number of embryos transferred. The development of technological advances such as micromanipulation and a wealth of experience in embryo culturing techniques have resulted in an

increase in embryo implantation potential. Numerous criteria have been suggested to optimize the selection process. These include: the rate of embryo development; blastocyst development; pronuclei expression and nucleoli orientation; ovarian/follicular vascularity; noninvasive assessment of metabolic products of embryos during development; preimplantation genetic diagnosis; and morphological assessment. Embryo scoring techniques have been developed to aid in evaluating the potential of embryo implantation.

Morphological assessment has been employed for many years as a tool in determining which embryos display the greatest pregnancy potential *(146)*. Embryos are scored based on cell number, fragmentation pattern and extent, cytoplasmic pitting, blastomere regularity, presence of vacuoles and blastomere expansion *(36,147–151)*.

Fragmentation is one of the most common morphological features used in assessing embryo quality. Grading systems have gauged embryo quality based on the percentage of fragmentation observed within the embryo. Low implantation rates have been reported from embryos with 10%–50% fragmentation on Day 2 of development *(25,152)*. Not all fragmentation appears to be detrimental to embryo development but the pattern of fragmentation has a profound effect on the embryo's developmental potential. Large fragments formed at the two-cell to four-cell stage appear more detrimental due to the depletion of essential organelles such as mitochondria, or structures such as pinocytotic caveolae, involved in exogenous protein uptake *(26)*. The presence of small fragments does not appear to effect developmental rates to the same degree as large fragments. The formation of small fragments may represent incomplete cytokinesis. Implantation rates are similar between embryos without fragmentation and those with moderate fragmentation. Antczak *et al. (9)* compared the relationship between blastomere fragmentation and the effect on the distribution of regulatory proteins. The findings demonstrated that certain patterns of fragmentation might result in partial loss of certain regulatory proteins from specific blastomeres, resulting in compromised development if fragmentation occurs during the one-cell or two-cell stages. Correlation between fragmentation and apoptosis is not clear but fragmentation may be an initiator of apoptosis if regulatory proteins are altered.

Embryo development rates were initially used in scoring for embryo's developmental competency *(17)*. Early cleavage of fertilized oocytes to the two-cell stage is used for its prognostic value in determining embryos for transfer *(19)*. A study by Giorgetti *et al. (25)* involving single embryo transfers, concluded that the use of embryo scoring based on cleavage rate and morphology was advantageous in maximizing pregnancy rates. One of the shortcomings of early cleavage as a selection criterion relates to oocyte maturity. Immature oocytes may fertilize later than mature oocytes under standard IVF culture conditions. Oocytes selected for intracytoplasmic sperm injection (ICSI) are biased due to maturation status. Sakkas *et al. (20)* concluded that early cleavage rate was not entirely influenced by timing of fertilization but is more likely influenced by intrinsic factors within the oocyte or embryo. The expression of human leukocyte antigen G (HLA-G) has been demonstrated to correlate with mRNA expression and improve cleavage rates *(153)*. Embryos that have undergone early cleavage may be less likely to experience critically low reserves of maternal mRNA prior to embryonic genome activation *(154)*.

Cell stage at the time of transfer has become a significant factor in determining which embryos have the greatest potential for implantation. Embryonic genome activation occurs between the four-cell and eight-cell stages of preimplantation development *(21)*. Delaying embryo transfer to Day 3 of development allows a selection of embryos undergoing embryonic activation.

In an attempt to further refine selection criterion based on morphology, zygote scoring of pronuclei was investigated. Scott *et al. (155)* outlined scoring systems based on the alignment of nucleoli at the junction of the two pronuclei and the appearance of the cytoplasm. These systems have been refined over time to encompass embryo morphology and development rates and now include nucleoli alignment, appearance of cytoplasm and the incidence of blastomere multinucleation.

The scoring system, often classified as a "Z" rating, for zygote, records a number of crucial phases of development. The first record of pronuclei alignment or appearance of "touching" at 16–18 hours postinsemination relates to activation of the oocyte by the introduction of the spermatozoon. The sperm-derived centriole and the microtubules arising from it are responsible for alignment of the pronuclei. If this fails to occur, developmental potential is limited *(29)*. Pronuclei are often slightly different in size but large differences have been associated with chromosomal defects such as aneuploidy *(7)*. Another aspect of

"Z" scoring relates to size, number and distribution of nucleoli. Nucleoli are the sites where pre-rRNA is synthesized. Following fertilization, rRNA synthesis resumes and the nucleoli reform and grow. It is presumed that "Z" scoring allows observation of the resumption of rRNA synthesis *(155)*. Zygotes with three to seven even-sized nucleoli per nucleus appear to give rise to embryos with greater developmental potential. Pronuclei scoring used in association with embryo development and morphology may offer a technique for determining which embryos would benefit from prolonged *in vitro* culture *(156–158)*, particularly as the score has been related to the ability to continue development to the blastocyst stage *(3)*.

Extended embryo culture to the blastocyst stage was proposed as a possible solution to the risks of multiple pregnancy. Determination of which embryos survive to the blastocyst phase was considered the most vital criterion for selection of embryos with the greatest implantation potential. Blastocyst culture addresses the issue relating to endometrial asynchrony, uterine hostility associated with early cleavage-stage embryos, and the assumption that all embryos have equal implantation potential. Selection of blastocyst-stage embryos may allow for the transfer of a single blastocyst.

Blastocyst culture has developed significantly over the past few years but is still fraught with the inherent problem of zygote development potential *(159)*. Only half of all zygotes have the potential to develop to the blastocyst stage. Aneuploidy can be used as an explanation for approximately half of the embryos failing to develop to the blastocyst stage *(160)*. Extended culture to the blastocyst stage does not eliminate those embryos displaying chromosome abnormalities *(160)* as 40% of embryos displaying normal morphological development to blastocyst are aneuploid.

Blastocyst development occurs between Day 5 and 7 postinsemination. Menezo *et al. (161)* observed that the transfer of embryos at the compacting morula stage resulted in poor pregnancy rates. This is thought to be associated with the fragile nature of the embryo at this phase of development. Pregnancies have been noted from the transfer of blastocysts ranging from Day 5 to Day 7 of development. Shoukir *et al.(162)* suggested that "good" quality blastocysts not only displayed well expanded blastocoelic cavities and well-defined inner cell masses but also had attained this stage by Day 5 or 6. A scoring system for blastocyst development was first described by Dokras *et al. (41)*. This system uses three grades for blastocyst classification based on the timing of cavitation, cavity formation and inner cell mass definition and trophectoderm distinction. Scoring systems have undergone further refinement and now include blastocoele volume, zona thinning and blastocyst hatching *(120)*. Scholtes *et al. (163)* suggested the success of blastocyst culture techniques depends primarily on the number of oocytes retrieved and not maternal age. Others have also noted that there is a reduction in the number of blastocysts formed in cases of male infertility *(42,164,165)*.

Assessment of embryo metabolism presents another potential technique for viable embryo selection. Gardner *et al. (70)* suggested that glucose uptake and metabolism might be used to predict the embryos most suitable for transfer. The noninvasive measurement of glucose and pyruvate uptake by human embryos *(166)*, the measurement of ATP and ADP levels *(167,168)*, the expression of EGF, transforming growth factor alpha and EGF receptor *(169)*, extracellular matrix protein production *(170)*, [illegible] *(86,171)*, production of human chorionic gonadotrophin (hCG) and HLA-G and pregnancy-specific [illegible] have all been suggested as having potential value in predicting those embryos with high implantation competency. Jones *(159)* concludes that pregnancy; [illegible] chemical factor with the greatest potential to act as a prognostic indicator of blastocyst viability. The use of biochemical factors as predictive markers in embryo selection is limited to those techniques that use noninvasive assessments or measurements. Further investigation of such markers is required. Follicular vascularity has also been suggested as another tool in determining follicles containing oocytes with greater developmental potential. Nargund *et al. (173)* concluded that a statistical correlation does exist between follicular peak systolic velocity, the ability to retrieve an oocyte and morphological development. Van Blerkom *et al. (174)* suggested an association between intrafollicular oxygen content and perifollicular vascularity, which may provide a useful indicator in oocyte developmental potential. Defects in chromosomal number, spindle organization and cytoplasmic structure have been observed in embryos developing from oocytes retrieved from hypoxic follicles. Colour Doppler imaging has been used as a predictive tool in analysing which preovulatory

follicles may contain oocytes with the potential to develop to normal embryos *(175,176)*. The predictive value of perifollicular blood flow on embryo development remains to be determined but it may offer additional information useful in predicting embryos with high implantation potential.

Embryonic developmental failures have been associated with cleavage arrest and chromosomal abnormalities. Aneuploidy is commonly associated with embryo arrest *(51)*. Preimplantation genetic diagnosis offers another useful technique to eliminate chromosomally abnormal embryos with little to no developmental potential prior to transfer. Sex selection of embryos for couples with sex-linked genetic disorders offers the possibility of eliminating carrier embryos prior to transfer.

Numerous embryo scoring systems have been designed to assist in determining which embryos have the greatest developmental potential. Regardless of the many criteria proposed to aid in the selection process, no single criterion has been identified which offers a significant benefit over the others. The majority of embryo selection systems are based on a combination of criteria, including morphology, cleavage rate and embryonic genome activation. The use of blastocyst culture, first suggested as the most useful embryo selection process, does not take into consideration reduced oocyte numbers and embryo production due to maternal age and ovulatory defects. Nor does it overcome the problem of identifying embryos with potential genetic abnormalities. Racowsky *et al. (177)* suggested an embryo selection process which compensated for maternal age and etiology. This selection process incorporates the number of eight-cell embryos available for transfer on Day 3 postinsemination. Patients with greater than three morphologically "good" embryos are encouraged to undergo Day 5 (blastocyst) embryo transfer. This process not only attempts to address endometrial asynchrony but also to reduce the risks of multiple pregnancy due to the transfer of fewer embryos.

Preimplantation genetic diagnosis (PGD) is becoming a useful adjunct in embryo selection with the development of additional DNA probes, particularly in cases of advanced maternal age. DNA fingerprinting may prove to be a significant tool in finally assessing which of the simple selection criteria are beneficial. The current practice of transferring multiple embryos precludes the identification of which embryo is responsible for the pregnancy and DNA fingerprinting may provide conclusive evidence as to which embryo is responsible for each live birth. Although a long-term assessment technique, eventually embryologists may be able to quantify, retrospectively, the criteria that are essential in determining the "best" embryos for transfer.

Despite all the advances in determining embryo developmental competence, no consensus has been reached on how many embryos to transfer. Multiple pregnancy rates remain high with recommendations of three or more embryos for transfer, depending on maternal age, still being promoted *(178)*. One of the potential benefits of refining embryo selection criteria is to reduce the number of embryos for transfer and thus the high multiple pregnancy rates.

Embryo transfer

The embryo transfer procedure should be considered as important to the success of ART as embryo quality and uterine receptivity. However, embryo transfer is perhaps the least understood link in the chain of procedures. Embryos from the one-cell stage (pronucleate embryos or zygotes) to the blastocyst stage of development may be transferred to either the fallopian tube or the uterus.

The fallopian tube can be cannulated from either the fimbrial end (orthograde transfer) or the uterine end (retrograde transfer) *(179)*. The surgical techniques of laparoscopy or laparotomy (orthograde transfer), or the nonsurgical techniques of transcervical or transvaginal cannulation (retrograde transfer) under ultrasound guidance, hysteroscopic guidance or by tactile sensation, can be used to cannulate the fallopian tube *(180)*. Laparotomy is no longer needed for tubal catheterization, as the procedure can be performed successfully via laparoscopy, using video guidance *(180)*. However, laparoscopy usually requires the patient to have a general anaesthetic and the abdomen insufflated with CO_2 *(181)* with attendant risks and side-effects. For these reasons, laparoscopic intrafallopian transfer has been attempted with success under local anaesthesia, but has not received wide acceptance *(181,182)*. Laparoscopic transfer has fallen out of favour since the introduction of transvaginal ultrasound-guided oocyte retrieval, which is usually performed with no anaesthesia, with or without light sedation. Cannulation of the fallopian tube via the cervix under ultrasound guidance, hysteroscopic guidance or by tactile impression, without general anaesthesia has

been performed with success for transcervical zygote intrafallopian transfer (ZIFT), tubal embryo stage transfer (TEST), tubal embryo transfer (TET). However, complications such as uterine anomalies, a retroverted uterus, or unsuspected tubal cornual obstruction may make it impossible to regularly cannulate the fallopian tube *(183)*. Transcervical transfer to the uterus can then be undertaken but it is possible the endometrium may have already been traumatized during the initial attempts to cannulate the fallopian tube with a consequent reduction in the chance of implantation *(184)*. There are also side-effects of the procedure including the possibility of flushing the embryos from the fallopian tube if injection flow rates are too high *(185)*, tubal perforation, vasovagal faintness and pelvic discomfort in some (less than 10%) patients *(179)*.

Zygote or embryo transfer into the fallopian tube is not possible in all patients, and in order to minimize the risk of ectopic pregnancy, should only be performed when the fallopian tube is patent and when the patient has had no previous history of pelvic inflammatory disease, ectopic pregnancy, or tubal surgery *(179,184,186)*. The fallopian tube transfer procedures include pronucleate stage transfer (PROST) in which pronucleate-stage embryos are transferred to the fallopian tube, ZIFT in which pronuclear to early cleavage-stage embryos are transferred to the fallopian tube, and TET or TEST in which early cleavage-stage embryos are transferred to the fallopian tube.

PROST has been used for patients in whom the etiology of infertility is unexplained or due to moderately severe male factor, antisperm antibodies or endometriosis. Similarly, the techniques of ZIFT, TET and TEST have been used for patients with male factor or immunological infertility and, in addition to confirming successful fertilization, offer the further possibility of eliminating those zygotes that fail to undergo the first cleavage divisions.

Until recently, one of the disadvantages of transferring early zygotes was the inability to select the most viable zygotes from a large cohort for transfer. However, high pregnancy and implantation rates have recently been reported for PROST when zygotes are selected for transfer according to certain pronuclear morphological features *(155)*. Early reports comparing embryo transfer to the fallopian tube to transfer to the uterus reported significantly higher implantation, pregnancy and birth rates when embryos were transferred to the fallopian tube *(187–190)*. However, others have reported no benefit when transferring embryos to the fallopian tube instead of the uterus *(191–196)*.

The majority of embryo transfers are currently carried out by nonsurgically cannulating the uterine cavity via the cervix (transcervical transfers). However, it is also possible to transfer embryos to the uterus using surgical techniques; ultrasound-guided perurethral transvaginal embryo transfer *(197)*; or transmyometrial transfer *(198,199)*. These methods have been used for patients with nonpatent tubes when anatomical abnormalities of the uterus or severe cervical stenosis would predict that cannulation of the cervical canal would be difficult or impossible *(180)*.

Transmyometrial embryo transfer is widely practised in Asia and pregnancy rates are reported to be high *(198)*. However, in a randomized prospective trial, no benefit could be demonstrated for transmyometrial transfer over transcervical transfer and pregnancy rates were low *(199)*. This difference may be due to anatomical features of the cervix in oriental women. A more compressed cervical canal compared to those from other races is frequently observed, but further studies need to be carried out to evaluate this *(200)*.

Transcervical embryo transfer is a rapid and easy technique, and does not require analgesics or anaesthetics *(180)*. Disadvantages include the technical difficulty encountered in patients with cervical stenosis *(199)*, the risk of infection from the introduction of microorganisms into the endometrial cavity *(179)*, and release of prostaglandins that may cause myometrial contractions and loss of embryos into the fallopian tube or vagina *(179)*.

To perform an embryo transfer, preparation of the patient is required. This includes positioning the patient, introducing the speculum, cleaning the cervix and manipulating the uterus.

The dorsal lithotomy position is most commonly used for embryo transfer, especially for patients with an axial or retroverted uterus, whereas for patients with an anteverted uterus, the knee–chest position has been recommended to eliminate expulsion of embryos from the uterus *(201)*. However, there is no convincing evidence that patient position affects the outcome of embryo transfer *(202–204)* and it is recommended to choose a position most comfortable to both patient and clinician.

A bivalve speculum is then introduced gently into the vagina to expose the cervix. Manoeuvring the speculum can improve cervico-uterine alignment to

allow easier access by the catheter *(200)*. Further manipulation can be achieved by passive bladder distension which has been reported to result in significantly higher pregnancy rates than when patients have an empty bladder *(205)* and is a requirement if abdominal ultrasound monitoring of catheter placement is to be employed. The uterus can also be straightened artificially by using a tenaculum or cervical suture; however, this may stimulate uterine junctional zone contractions and lead to implantation failure *(206)* or the transportation of the embryos into the fallopian tube, increasing the risk of ectopic pregnancy *(207)*. For patients with cervical stenosis where passage of the catheter is extremely difficult, cervical dilatation can be performed. It has been recommended that cervical dilatation be performed at the time of embryo transfer *(208)* rather than at the time of oocyte retrieval *(209)* as it does not appear to affect pregnancy rates.

The vulva and vagina require no special preparation but the cervix can be cleaned by swabbing, vigorous flushing, or aspiration to remove excess mucus and there is no clear consensus as to which is the best method. Some favour complete aspiration of cervical mucus *(210)* whereas others have demonstrated improved results when the cervix is vigorously flushed with culture medium to remove mucus *(211)*. As yet, no controlled, randomized studies have been carried out to evaluate the requirement of removing mucus prior to embryo transfer.

To gain a better understanding and knowledge of each patient's anatomy, a "mock" embryo transfer can be performed. A mock transfer can take place either in a cycle prior to the treatment cycle *(201,212)* or just prior to the real embryo transfer procedure *(213)*. Performing a mock transfer offers many potential advantages: the most suitable catheter can be chosen for each patient to facilitate atraumatic transfer; the direction of the uterus can be assessed and the length of the uterus can be measured *(213)*. The main disadvantage of performing the mock transfer before the treatment cycle is that the uterus is mobile so it is possible that the direction of the uterus may be different on the day of the real embryo transfer *(213)*. Mock embryo transfer has been shown to minimize the problems associated with embryo transfer and to improve pregnancy and implantation rates *(212)*.

Medications such as anaesthesia, uterine relaxants or prophylactic antibiotics and corticosteroids have been given during the embryo transfer procedure. General anaesthesia as a routine for embryo transfer has not proven to significantly improve pregnancy rates *(202)*. However, general anaesthesia is sometimes required if the embryo transfer procedure is extremely difficult. Care should be taken as to the choice of anaesthetic agent used as it may affect results *(214)*. Tranquillizers such as diazepam have also been used to reduce patient anxiety and promote ease of transfer *(202,212)*. However, it has become common practice to withhold medication except in the case of very difficult transfers. Instead, patient reassurance by staff members familiar to her, previous experience with mock embryo transfer and a physician performing the procedure who is known to the patient, have appeared to be sufficient to achieve patient relaxation and improve ease of procedure.

Prostaglandins may adversely affect outcome following embryo transfer due to their action in stimulating uterine contractions. Prostaglandin inhibitors such as ibuprofen *(215)* or indomethacin *(201)* have been used to inhibit uterine contraction but have not had any beneficial effect on pregnancy rate *(202)*. Similarly, administration of the smooth muscle relaxant, glyceryl trinitrate, has no affect on pregnancy rate *(216)*.

Microbial contamination of the embryo transfer catheter tip is correlated with a significant reduction in pregnancy rate *(217,218)*. Prophylactic antibiotics administered at the time of oocyte retrieval significantly reduce the incidence of positive microbial cultures from embryo transfer catheter tips 48 hours after antibiotic administration *(219)*. However, no controlled, randomized studies have been undertaken to investigate the effect on pregnancy rates. One of the many protective functions of the zona pellucida surrounding the early cleavage-stage embryo is in reducing contact with microorganisms and immune cells. Zona-manipulated embryos when transferred to the uterus, are potentially at risk of exposure to these cells and for this reason, low-dose immunosuppression with corticosteroids has been advocated *(220)*. However, the effectiveness of this immunosuppression and its effect on pregnancy outcome has not been investigated in any systematic way.

There are over 50 different catheters available commercially for clinical embryo transfer and several studies have been undertaken to compare different catheters *(221–227)*. Catheters are classified according to their tip, flexibility, presence of separate outer sheath, location of the distal port (end- or side-loading), degree of stiffness and malleability, memory, thickness and length. Catheters are generally manu-

factured from nontoxic plastic materials but have different sterilization processes that may affect the relative toxicity and handling procedures *(200)*. There is little difference in concept and technology between embryo transfer catheters and so it is not surprising that there is no clear consensus as to the one catheter that is superior. However, soft embryo transfer catheters are used most frequently as they produce superior results *(221,222)* and are easy to use in all but the most difficult transfers *(222)*. The main characteristic required of a transfer catheter is the ability to manoeuvre into the uterine cavity while causing no trauma to embryos and endometrium.

There is as yet no consensus as to the depth of placement of ET catheters within the uterus. However, contact between the catheter and the uterine fundus stimulates junctional zone contractions that may be responsible for relocating intrauterine embryos, and so contribute to embryo transfer failure or ectopic gestation *(228)*. High-frequency uterine contractions on the day of embryo transfer have been found to decrease clinical and ongoing pregnancy rates as well as the implantation rate, possibly by expelling embryos from the uterine cavity *(229)*. Others have reported that there is no relationship between the site of embryo deposition in the uterus monitored by ultrasound (measured as distance from fundus) and the pregnancy outcome *(230)*. However, all embryos were deposited at least one to two millimetres from the fundus and no mention was made as to whether the fundus was touched by the catheter. Although there is no agreement as to the exact depth of placement of the catheter within the uterus, there appears to be general agreement on the avoidance of touching the uterine fundus *(231,232)*.

In order to assist more accurate catheter placement within uteri of various dimensions *(233)*, ultrasound-guided embryo transfer has been developed instead of relying on clinician "feel". Studies comparing ultrasound-guided embryo transfer to embryo transfer by clinician "feel". failed to demonstrate a significant improvement in pregnancy and implantation rates *(234,235)*, with the exception of a small subgroup of patients receiving a single embryo for transfer *(234)*. However, tactile assessment of embryo transfer catheter placement has been demonstrated to be unreliable *(236)*: in 17.4% of cases the guiding cannula was inadvertently abutting the fundal endometrium; and in 7.4% of cases abutting the internal tubal ostia. More recent studies suggest that an improvement in both the clinical pregnancy rate and the implantation rate can be achieved if ultrasound-guided embryo transfer is used *(221,237)*. Ultrasound confirmation of the retention of the fluid droplet containing the embryos at the site of catheter placement results in improved clinical pregnancy rates *(221)*.

During ultrasound-guided embryo transfer, Woolcott and Stanger *(236)* identified that the transfer catheter embedded within the endometrium in 24% of cases and deposition of the embryos beneath the endometrium (intraendometrial transfers) occurred in 22% of cases. However, this appeared to have no effect on pregnancy outcome. Intentional intraendometrial embryo transfer has been carried out under direct visualization using a CO_2-pulsed flexible hysteroscope *(238)*. A very low implantation rate resulted and it was concluded that the acidifying effect of the CO_2 on the endometrial stroma might have produced a suboptimal environment for early embryonic development.

To minimize the potential for movement and expulsion of embryos following embryo transfer, a fibrin sealant, or biological glue, has been used to attach embryos to the endometrium at the site of embryo deposition *(239,240)*. In a prospective, randomized study, no significant difference in clinical pregnancy rate or ongoing pregnancy rate, but a significant reduction in ectopic pregnancies, was found *(240)*. No follow-up studies have been reported.

At the completion of transfer, the catheter should be examined carefully by the embryologist for retained embryos. Blood, mucus or uterine tissue may attach to the end of the catheter and impede egress of the embryos from the catheter. Blood on the outside of the catheter has been related to poorer results *(241)*. Visser *et al. (242)* reported that failure to deliver embryos on the first attempt at embryo transfer resulted in a decrease in pregnancy rate and suggested that retained embryos should be transferred one day later. In contrast, no difference in the clinical pregnancy rate was found in another study when all embryos were transferred in the first attempt compared to when repeated attempts were necessary to transfer all embryos *(243)*.

Historically, bed rest for up to 24 hours following embryo transfer has been advised. Bed rest began as a precautionary measure to try to improve implantation rates, even though there is a lack of evidence as to its benefit. Immediate mobilization of the patient following embryo transfer does not, however, appear to have a detrimental effect on pregnancy outcome

(244–246). It has been shown by ultrasound that standing immediately after embryo transfer resulted in movement of embryo-associated air bubbles within the uterine cavity in only 6% of cases *(247)*. Furthermore, there was no evidence of movement of embryo-associated air outside the uterine cavity and it would appear that standing shortly after transfer plays no significant role in the final position of transferred embryos.

It is difficult to know which embryo transfer protocol to follow to ensure the highest degree of success, as many of the technical aspects have undergone very little scientific evaluation, if any. In fact, little evidence exists to support many of the choices made in this aspect of ART. It is clear, however, that an easy, atraumatic transfer is important with factors such as patient preparation, medication, mock embryo transfer, choice of catheter, transfer technique and bed rest optimized to provide improved outcomes and patient well-being.

Day of transfer

Embryos may be transferred to the patient on Day 1 postinsemination at the zygote stage of development (PROST, ZIFT), on Day 2 as two-cell to four-cell early cleavage-stage embryos (ZIFT, TEST, TET, ET), on Day 3 as six-cell to eight-cell early cleavage-stage embryos (ZIFT, TEST, TET, ET), on Day 4 as morulae (ET), or on Days 5 to 7 as blastocysts of varied morphology (ET).

In the past 10 years, the majority of clinics have opted to transfer embryos at the early cleavage stage of development to the uterus on Day 2 postinsemination, despite the fact that, *in vivo*, the embryo would not pass into the uterus until two to three days later at the morula to blastocyst stage of development *(22, 248)*. Transfer of early cleavage-stage embryos has been the preferred option as it allows confirmation of fertilization and a limited assessment of developmental potential as the embryo undergoes its first few cleavage divisions, while at the same time minimizing the potential compromise to embryo viability posed by prolonged exposure to suboptimal *in vitro* culture conditions. In fact, when *in vitro* culture conditions are significantly compromised, embryo transfer at the zygote stage of development, rather than at the early cleavage stage of development, results in much higher pregnancy rates *(249)*.

Recent advances in the formulation of embryo culture medium and culture conditions has seen a shift in interest toward transfer of later-stage embryos, particularly blastocysts, as high pregnancy and implantation rates have been reported despite the fact that fewer embryos are usually transferred *(77,120, 250–252)*. Transfer at the morula to blastocyst stage of development allows selection of embryos that have demonstrated the potential for continued development under embryonic genomic control *(21)*, although these embryos may not necessarily have a normal chromosome complement *(160)*.

More recently, morphological parameters have been established for embryos at the pronucleate or zygote stage of development that identifies embryos with a high viability *(3,6,19,20,155–158)*. It is difficult to determine which of the various options for day of transfer will result in the highest success rates as very few controlled, randomized comparisons using similar patient populations have been undertaken.

van Os *et al. (253)* were the first to demonstrate that embryo transfer could be delayed from Day 2 to Day 3 without any impact on subsequent pregnancy rates. In fact, they argued that a higher number of viable pregnancies was established following transfer of embryos on Day 3. In a later study, Dawson *et al. (254)* demonstrated that although the pregnancy rate is not different when embryos are transferred on Day 2 or Day 3, the implantation rate is significantly higher on Day 3, indicating that selection of viable embryos is improved with a further day in culture. This was further supported by the finding that fewer embryos miscarried before six weeks of gestation when embryos were transferred on Day 3 *(254)*. A recent study, using one of the newer culture medium formulations without glucose and phosphate, reported a significant improvement in both pregnancy and implantation rates when embryos were transferred on Day 3 rather than on Day 2 *(82)*. In contrast, Ertzeid *et al. (255)*, in a prospective, randomized study, showed no benefit on the implantation rate or the live birth rate in delaying transfer from Day 2 to Day 3.

Similarly, delaying embryo transfer until Day 4 postinsemination results in similar implantation and pregnancy rates to those achieved following embryo transfer on Days 2 and 3 *(256,257)*. In addition, Huisman *et al. (256)* noted that delaying transfer to Day 4 provided the ability to identify embryos with a very high implantation potential. Transfer of cavitating morula stages on Day 4 resulted in a 41% implantation rate per embryo *(256)*. It was suggested that deferring embryo transfer for a few days may provide

the possibility of selecting fewer and better quality embryos for transfer and therefore limit the incidence of multiple pregnancy *(256)*.

Initial attempts at extending culture to the blastocyst stage followed by embryo transfer on Day 5 resulted in disappointingly low pregnancy and implantation rates *(56, 257)*. However, improvements in the culture media and culture conditions for human embryo development in the past few years have resulted in the successful development of viable blastocysts (reviewed in *(159)*). Scholtes and Zeilmaker *(258)*, in a prospective randomized trial, reported no significant difference in pregnancy and implantation rates when embryo transfer was performed on either Day 3 or Day 5. However, deferring embryo transfer to Day 5 allowed the identification of embryos with very high implantation potential. Pregnancy and implantation rates were almost double that recorded for Day 3 transfers when exclusively cavitating embryos were transferred on Day 5. Gardner *et al. (77)* using culture media formulated to mimic physiological parameters reported a significantly higher implantation rate when blastocysts were transferred on Day 5 compared to early cleavage-stage embryo transfer on Day 3 (51% and 30%, respectively). They further observed that the implantation rate following blastocyst transfer was not affected by the number of blastocysts transferred, suggesting that high-order multiple pregnancies could be avoided by reducing the number of embryos transferred without a corresponding reduction in the pregnancy rate. Similarly, Milki *et al. (252)*, using similar patient populations demonstrated that the implantation and pregnancy rates following blastocyst transfer on Day 5 was higher than following transfer on Day 3. In contrast, in prospective, randomized trials using an unselected population of patients, it has been reported that there is no difference in pregnancy and implantation rates when a similar number of embryos are transferred on either Day 3, Day 4 or Day 5 *(259,260)*. Coskun *et al*. *(259)* suggested that the superiority in selection of any particular embryo stage should be shown by comparing the result of transfer of the best single embryo at any stage in a randomized trial.

Blastocyst development occurs *in vitro* between Days 5 and 7 *(40,41)*. Although several studies have reported that the rate of development to blastocyst affects viability *(162,261)*, others have suggested that in some instances, blastocysts that form as late as Day 7 may have some inherent viability as there is no difference in cumulative hCG secretion by embryos which formed blastocysts from Days 5 to 7 *(41,262)* and transfer of Day 7 blastocysts occasionally results in pregnancies *(161)*. The majority of programmes transfer blastocysts on Day 5 but some programmes have elected to delay transfer to Day 6 to allow for a further element of selection among a cohort of blastocysts *(42,76,263,264)*. Delaying transfers until a time when selection of fully expanded or hatching blastocysts is possible, rather than automatically transferring blastocysts on Day 5, may increase implantation rates by providing a further element of selection *(42)*. Transfer of cryopreserved blastocysts has revealed that pregnancies can be achieved following transfer of blastocysts on Days 5–9 following the LH peak but no pregnancy resulted from transfer of blastocysts as early as Day 4 following the LH peak *(162)*.

One of the advantages of deferring embryo transfer beyond Day 3 is the possibility of performing preimplantation genetic diagnosis. Embryo biopsy is usually performed on Day 3 and successful pregnancies have been established following transfer of biopsied embryos on Day 4 which has allowed a full day for genetic analysis by polymerase chain reaction (PCR) *(265)* or by repeated cycles of fluorescence *in situ* hybridization (FISH) *(266)*. There is also a significant advantage to extending culture to the blastocyst stage of development before performing the biopsy. At this stage, more cells can be biopsied from the extraembryonic trophectoderm for more complex and accurate preimplantation genetic analysis *(262,267,268)*. Preliminary research results on the recovery rate of biopsied human blastocysts are promising *(262,268)* but as yet the procedure has not been performed clinically.

One of the disadvantages of deferring embryo transfer to Day 5 or beyond is that the embryo transfer may be cancelled if no blastocysts develop *in vitro*. Certainly, for some groups of patients, transfer on Day 5 may not be the best option. It has been reported that sperm quality can affect the number of blastocysts developing *in vitro (42,164,165)*. In addition, several studies have reported a maternal age-related decline in the number of embryos developing to blastocysts *in vitro (50,269,270)*. This finding may be due to the progressive reduction in the number of oocytes retrieved with advancing maternal age *(50)*, as the number of oocytes retrieved is correlated to the number of blastocysts that develop *in vitro (42)*. An increase in the number of oocytes retrieved can

ameliorate the negative affect of maternal age *(163)*. Racowsky *et al*. *(177)* suggested that the number of eight-cell embryos on Day 3 should be used as the determinant for the day of transfer as this has been correlated to the number of blastocysts developing *in vitro (42)*. For patients with three of more eight-cell embryos on Day 3, transfer on Day 5 results in a high ongoing pregnancy rate with a significant reduction in the incidence of high-order multiple pregnancies *(177)*. Poor prognosis patients with no eight-cell embryos on Day 3 do not benefit from deferring embryo transfer from Day 3 to Day 5 (33% and 0% pregnancy rate, respectively) *(177)*. It has been hypothesized that the uterus is apparently able to "rescue" some of the suboptimal, slower cleaving embryos and that extending the culture time to Day 5 for these suboptimal embryos, even in optimized culture systems, reduces their viability *(177,271)*.

In conclusion, the introduction in recent years of more subjective selection criteria that are better able to predict viability has resulted in reports of high implantation rates following transfer of embryos from the zygote to the blastocyst stage of development. Although blastocyst transfer has not always resulted in an improvement in pregnancy and implantation rates in the wider population of infertile patients, it appears to be the most likely choice if the number of embryos transferred is to be reduced to one to eliminate multiple pregnancies. As there is a relationship between zygote morphology and embryo morphology at later stages *(3)*, a combination of subjective assessments throughout development may further improve the chances of selecting the single most viable blastocyst from the cohort and improve the implantation rate and the number of healthy offspring born as a result of assisted reproduction procedures.

Endometrial suitability for embryo transfer

Embryo implantation is the result of the successful interaction between the embryo and the endometrium *(272)*. Increasing evidence indicates that steroid-induced molecules acting as paracrine modulators are necessary for embryo–uterine interactions. Successful implantation, therefore, is determined both by the quality of the embryo and the receptiveness of the endometrium.

To further improve pregnancy rates, it is clear that ART would benefit substantially from being able to determine the exact timing of endometrial receptivity (the implantation window). It is generally believed that this window of opportunity occurs between Days 18 and 24 of the normal menstrual cycle *(273)*. However, this window may not be absolute and considerable interindividual variability may exist. Hence, the need to determine for each patient whether the endometrium is adequately prepared at the time of embryo transfer.

Currently available technology for the assessment of the endometrium prior to embryo transfer can be divided into microscopic assessment of endometrial biopsies and imaging techniques.

Microscopic assessment of endometrial biopsies

The major disadvantages of endometrial biopsies are their invasiveness, their negative impact on the integrity of the endometrium and their interference with the implantation process itself. These techniques should only be used in unstimulated cycles prior to ART. However, the extent to which assessments performed in a natural cycle are predictive of the quality of the endometrium in a subsequent stimulated cycle has not yet been studied. In addition, the literature is unclear about the intraindividual variation from cycle to cycle. Some uncertainty also exists as to whether one biopsy per cycle is sufficient for the assessment of receptivity.

Histological dating used in the assessment of morphological markers has shown that the timing of biopsy, the methods used for chronological standardization, and the extent of discrepancy required to define an endometrial biopsy as being "out of phase" remains unsettled *(274)*. A high inter- and intra-observer variation has further limited the clinical utility of traditional dating techniques.

The formation of pinopods, which are sponge-like smooth membrane projections that arise from the entire surface of endometrial cells lining the uterine cavity, has been related to the presumed time of blastocyst implantation *(275)*. They are detected by electron microscopy, making it a cumbersome and expensive technique for use in a clinical setting. Conventional light microscopy has been shown to be an unreliable technique for the detection of pinopods *(276)*.

There is a large body of evidence suggesting that numerous factors are involved in human implantation. The expanding group of potentially important factors includes mucins, integrins, trophinin/tastin, EGF, HB-

10 and COX-2. Most of these biomarkers are typically expressed around the time of the implantation window. However, most of these putative markers of uterine receptivity have no proven clinical relevance to date. The expression of b
studied extensively. These molecules are considered likely to correlate to uterine receptivity. While patterns of integrin expression cannot be used to accurately date the endometrium *(274)*, they may reveal subgroups of endometrial dysfunction in patients in the absence of morphological abnormalities identified at the light microscopic level *(277–279)*. Although this information may be useful when advising the patient about the cause of her infertility, there are no objective data to show that integrin expression patterns actually predict endometrial receptivity.

Imaging techniques (ultrasound and magnetic resonance imaging)

Because of their noninvasive nature, imaging techniques are ideally suited to assess the endometrium immediately prior to embryo transfer. In contrast to magnetic resonance imaging (MRI), ultrasonography is a much cheaper and more widely available imaging technique. However, the spatial resolution (degree of detail) of MRI is superior to that of ultrasonography.

Conflicting results have been reported regarding the correlation between the thickness of the endometrium and pregnancy rates following ART *(280–289)*. Measurement of endometrial thickness, or even endometrial volume, with three-dimensional ultrasound techniques does not yield better results *(290)*. This may be explained by the fact that the thickness of the endometrium has been shown to be more dependent on the time of exposure to estrogen rather than the dose of estrogen exposure *(288)*. In addition, important interindividual variation in endometrial thickness on the day of hCG administration has been noted.

The echogenicity of a tissue is a measure of its capability to reflect ultrasound waves. Some studies have shown that endometrial echogenicity in the late follicular phase predicts ART outcome *(280,283,288, 291–293)*. Others, however, have failed to find a relation between endometrial echogenicity and implantation rates *(294, 295)*. This controversy may be explained by operator-dependent variability, the use of arbitrary and heterogeneous classifications, and the lack of control for confounding factors (e.g. poor embryo quality and uterine cavity abnormalities) that influence the analysis of results. In an encouraging study, endometrial echogenicity on the day of hCG administration was assessed objectively with a computer-assisted module for the analysis of ultrasound images in a selected subset of ART patients *(289)*. In this study, echogenicity patterns were strongly correlated with implantation rates. However, the diagnostic value in an unselected population of patients remains to be determined.

Endometrial blood flow may be used as a functional marker and since the development of power Doppler sonography and, more recently, three-dimensional power Doppler sonography, it has become possible to evaluate the vessel density and perfusion in the endometrial and subendometrial layers in a quantitative way. Most studies so far have been rather small and conflicting results have frequently been reported *(296–301)*.

The junctional zone which is the myometrial layer just underlying the endometrium, has recently been described as a separate functional unit. One of the functional properties of this layer is that it produces contraction waves. Contractions can be directed towards the uterine fundus, the cervix, or they can be chaotic or opposing. These contractions have been demonstrated to be strong enough to displace embryos in the uterine cavity *(204)*. The direction and amplitude of these contraction waves are stimulated by hormonal influences *(302)* and physical stimuli (e.g. difficult transfer) *(204,206)*. One study was controlled for confounding variables and uterine contractions were assessed objectively by a computerized system *(229)*. In the selected patient population, high frequency contractions on the day of embryo transfer were found to decrease implantation and pregnancy rates. A negative correlation between uterine contraction frequency and serum progesterone concentrations was also observed, illustrating the uterine relaxant properties of progesterone.

Ultrasonography clearly has a number of major advantages (low cost, wide availability, possibilities for standardization using computer software), making it the area with the greatest potential for clinical application. However, to become generally accepted, any assessment will need to be rigorously tested for its diagnostic value. Although some tests have been shown to be quite promising in selected subpopulations of patients, these same tests need to be re-evaluated in unselected patient populations. In these

evaluations, investigators will need to report on commonly used parameters such as sensitivity, specificity, positive predictive value and negative predictive value. In particular, receiver–operator curves should be provided with each test.

A further area of research will have to focus on how the results of these endometrial assessments can assist the clinician in achieving better outcomes for the patient. Some authors have suggested that fresh embryo transfers may need to be delayed in the event of an unfavourable assessment of endometrial receptivity. It seems unlikely that many patients will accept this decision and therefore research efforts should concentrate on the development of medical interventions that may improve endometrial receptivity.

Luteal phase support

One area that has received a lot of attention is the need for luteal phase support in downregulated cycles. Since the original articles of Smitz *et al. (303)* and Wildt *et al. (304)* were published, the use of luteal support in downregulated ART cycles has been accepted as best medical practice. Various methods have been described to support the luteal phase. The use of hCG injections has been abandoned in most centres in favour of progesterone. The long half-life of hCG and its direct stimulation of the ovary contribute to the associated increased risk of ovarian hyperstimulation syndrome (OHSS) *(305)*. Progesterone can be administered in a variety of ways. The intramuscular and vaginal routes are currently the most widely adopted. Orally administered progesterone is rapidly metabolized in the gastrointestinal tract and its use has proved to be inferior *(306)*. A lot of controversy still exists as to whether the vaginal route results in better secretory endometrial transformation. This controversy stems from the fact that vaginally administered progesterone results in adequate secretory endometrial transformation, despite serum progesterone values lower than those observed after intramuscular administration, even if they are lower than those observed during the luteal phase of the natural cycle. This discrepancy is indicative of the first uterine-pass effect and therefore of a better bioavailability of progesterone in the uterus *(306)*.

Recent research has highlighted the detrimental effects of high estradiol levels on implantation *(307,308)*. Implantation rates are lower in patients who are high responders. The implantation and pregnancy rates were correlated to the peak estradiol concentration, regardless of the number of oocytes collected *(309)*. The effect of estradiol was mediated through endometrial receptivity as demonstrated in a study involving oocyte donors. The implantation rates in recipients of embryos derived from high responders were similar to normal responders *(309)*. It has been suggested that stimulation protocols aimed at reducing the follicular response may overcome the low implantation rates in high responders. Simon *et al. (309)* used the step-down protocol, originally proposed by Fauser *et al. (310)*, to successfully improve both the implantation and pregnancy rates in high responders. This involved the administration of 100 or 150 IU/day recombinant FSH starting on cycle Day 5 *(311)*. From cycle Day 8 or later, cotreatment was begun with 0.25 mg/day GnRH antagonist. No luteal support was provided. This pilot study demonstrated that IVF is feasible with a minimal stimulation approach and that luteal support may not be necessary.

This also raises the issue of whether the use of the short-acting gonadotrophin-releasing hormone (GnRH) antagonists is likely to change the need for luteal phase support. The pilot study of de Jong *et al. (311)* seems to suggest that luteal support is not necessary following GnRH antagonist administration in an IVF cycle. This finding is in direct contrast to the results from another small study that concluded that corpus luteum function may be impaired in cycles stimulated with hMG and a GnRH antagonist *(312)*.

Antiphospholipids

In a completely different area of research, mounting evidence suggests that inheritable thrombophilias, such as activated protein C resistance, Factor V Leiden mutation, or hyperhomocysteinaemia are associated with an increased risk of fetal loss and pre-eclampsia. Acquired thrombophilias, such as the antiphospholipid syndrome (APS), are also emerging as an important cause of recurrent pregnancy loss. The common pathogenic pathway is thought to be slow progressive thrombosis and infarction in the placenta. For patients with APS who have a history of thrombosis or recurrent pregnancy losses, heparin plus low-dose aspirin appears to be the regimen of choice *(313,314)*. Interestingly, antiphospholipid antibodies (APA) have also been shown to interact with syncytiotrophoblast and cytotrophoblast layers and could, therefore, theoretically affect implantation.

Several trials of treatment with heparin and aspirin in women with positive APA undergoing IVF have been completed. Although none of the studies were randomized, prospective, blinded trials there does not appear to be a significant effect of heparin–aspirin treatment on implantation rate, pregnancy rate, or ongoing pregnancy rate. Furthermore, it should be stressed that heparin–aspirin treatment may not be without complications. One maternal death has been reported associated with heparin–aspirin treatment in IVF, due to a cerebral haemorrhage in a nine-week pregnant woman carrying triplets *(315)*. Subcutaneous heparin does not cross the placenta and therefore has no adverse effects on the fetus, but potential side-effects for the mother include bleeding, thrombocytopenia and osteoporosis.

Antibiotics

Recently, there has been growing interest in the effect of infectious agents on ART pregnancy rates. The assumption is that women with specific vaginal pathogens may have an increased incidence of endometritis, which would lead to a reduced implantation rate. Although it is part of good clinical practice to treat any clinically manifest genital tract infection, it is unclear whether screening for microorganisms should be routine. One study assessed the impact of individual bacteria isolated from the vagina and the tip of the embryo transfer catheter on livebirth rates *(316)***.** It was found that different types of bacteria recovered from the embryo transfer catheter had variable effects on live-birth rates. Prophylactic doxycycline had little effect on the vaginal flora.

The issue of whether bacterial vaginosis, if present at the time of oocyte recovery, adversely affects fertilization and implantation has been investigated more closely. The prevalence of bacterial vaginosis was much higher in infertile patients undergoing ART treatment than found by others in antenatal and general gynaecological populations *(317)*. In all studies to date, no significant effect of bacterial vaginosis on fertilization and implantation rates has been demonstrated *(317–319)*. Therefore, routine screening and treatment for bacterial vaginosis before ART treatment would appear to be unwarranted.

Furthermore, antibiotic therapy may increase the likelihood of inoculation of antibiotic-resistant pathogenic bacteria from the vagina into the embryo culture system during vaginal oocyte collection *(320)*. Whether screening and treatment of bacterial vaginosis would result in a reduction in later complications during pregnancy remains an open question *(317)*.

In summary, ultrasound techniques seem to hold the greatest promise of becoming clinically useful tools to assess endometrial receptivity. Most of the ultrasound techniques still await proper validation in unselected patient populations. Further research is required to investigate how patients with a poorly prepared endometrium should be managed clinically.

Impact on offspring

Since the birth of the first IVF baby in 1978, several hundred thousand babies have been born worldwide as a result of assisted conception. Several international registers of births resulting from IVF have been established to enable assessment of the health of these children and several analyses of the data recorded in these registers have been published to date and are summarized in Table 1.

The majority of studies have demonstrated no major differences in outcomes for singleton pregnancies except perhaps for an increased incidence of premature and low-birth-weight babies. However, these findings seem to be dependent more on patient characteristics than on the ART per se, as no major differences can be found for ART children compared to the general population when patients are matched for parity, maternal age and year of delivery *(321, 322)*.

By far the greatest adverse impact on offspring born as a result of ART is as a direct result of the increased incidence of multiple pregnancy. The

Table 1. Summary of national registers of ART and outcomes

Publication reference	Register	Years
(330,338,340,372–378)	Australia and New Zealand	1979–1997
(321)	Denmark	1994–1995
(332)	Finland	1991–1993
(328,329)	France	1986–1990
(327)	Great Britain	1978–1987
(337)	Israel	1982–1989
(323)	Sweden	1982–1985
(339,379–387)	USA and Canada	1988–1997

Swedish registry study showed a 20-fold increased risk of being born as a multiple birth baby for an ART child compared with the general population *(323)*. The last world collaborative report on ART recorded a multiple birth rate of 29%, the majority of which were twins *(324)*. Multiple birth infants, regardless of whether they originate from ART or spontaneous conception, have an increased risk of preterm delivery, low birth weight, congenital malformations, fetal and infant deaths and long-term morbidity and disability as survivors *(325,326)*. There is a fivefold increase in premature delivery of children resulting from ART *(323)*. This can be explained in the main part by the incidence of multiple births but is also true for singleton births *(323,327–331)*.

There is also an increased incidence of low-birth-weight babies following ART compared to the general population, even when only singleton pregnancies are considered *(323,327–330,332)*. More recently, with the increasing use of extended culture followed by blastocyst transfer, concerns have been expressed about the potential for producing babies with high birth weights similar to the "large offspring syndrome" reported following blastocyst transfer in domestic animal species *(333,334)*. However, human infants conceived following blastocyst transfer are not significantly different in birth weight from infants conceived spontaneously *(335)* or from infants conceived following transfer of early cleavage-stage embryos *(336)*. Perinatal and infant mortality is 1.7–3 times the national average *(323,327–330,337)* and, in some ART populations, is accounted for entirely by the high percentage of multiple births *(327,328)*.

Children born as a result of ART have an increased risk of malformations compared with the general population; however, this risk can be partly explained by the high proportion of multiple births in the ART group *(323)*. An increased incidence of neural tube defects (anencephaly, hydrocephaly, spina bifida) *(323,327,328,338)*, oesophageal atresia *(323)* and transposition of the great vessels *(338)* has been reported for ART infants. In the Swedish study, ten ART infants (0.2%) had neural tube defects (anencephaly and spina bifida) compared with the expected number of three to four. Seven of these ten infants and six of seven ART infants with hydrocephalus were from sets of twins *(323)*. Similarly, in the Australian study, three of six infants born with spina bifida and two of four infants born with transposition of the great vessels were from multiple births *(338)*. Other studies have reported no increase in congenital malformations in large numbers of ART children, despite a very high incidence of multiple pregnancy *(339)*.

Multiple pregnancies are also associated with increased infant and childhood morbidity such as cerebral palsy and mental retardation, due primarily to the increased incidence of prematurity and low birth weight *(325,326)*. The Swedish study failed to identify any increase in the incidence of cancer in children resulting from ART *(323)* which is in contrast to a previous report indicating a possible increase in neuroectodermal tumours *(340)*. Most follow-up studies of children born as a result of ART have not been running for long enough to assess the risks of long-term handicap.

A follow-up study of singleton infants conceived by ART to first-time mothers for the first year following birth has shown no difference in mental, motor, speech and social development compared to a matched control from the general population *(341)*. Similarly, the mental development of children conceived by ART at the age of 12 months was normal and not different from matched controls *(342)*. However, children of multiple births score lower on average, both on physical and mental scales *(342)*. Brandes and co-workers *(342)* concluded that when an ART pregnancy is carried to term, yielding an apparently healthy infant, the infant can be expected to develop and thrive similarly to non-ART-conceived peers. Similarly, Wennerholm and co-workers *(331)* concluded that if the neonatal period is uncomplicated, subsequent growth and development of children conceived by ART will be normal. In studies of older children, no independent ART effect has been found for growth and physical outcome (when matched for plurality and weeks of gestation) *(343)* or for cognitive, behavioural and social development *(344)*.

In very large studies, the sex ratio for births resulting from ART does not differ significantly from the national ratios *(327,328)*, although it has been suggested that sex selection may be inadvertently performed in ART programmes by selecting for the faster cleaving embryos *(345)*. It has, however, been reported that the sex ratio for births resulting from blastocyst transfer, when the fastest cleaving embryos are selected for transfer, favours males and represents a significant shift in the sex ratio for births resulting from spontaneous conceptions *(335)*.

The high incidence of multiple births following assisted conception is largely due to the practice of transferring multiple embryos. This practice has arisen from the data indicating that at least for the first three

to four early cleavage-stage embryos transferred, there is a positive correlation between the number of embryos transferred and the pregnancy rate *(346)*. However, progress in the past decade has seen the introduction of improved culture media, culture conditions and selection criteria for embryo transfer and the corresponding implantation rates have increased dramatically. The improvement in implantation rates allows, for the first time, the consideration of reducing the number of embryos for transfer to two *(152,250,347–356)*, and possibly even one *(120, 149,151,357,358)*, to eliminate the risks associated with multiple pregnancy whilst still maintaining high pregnancy rates.

However, reducing the number of embryos for transfer will not entirely eliminate multiple pregnancies. Monozygotic twinning (identical twins formed from the one embryo) has been reported to be higher following assisted reproduction *(359,360)* than in the general population. Monozygous twins, and in particular monozygous, monochorionic twins (identical twins which share the same chorion), add significantly to the risks associated with multiple pregnancy and to the poorer health and survival of offspring. The incidence of monozygotic twinning has been reported to be very high in the subgroup of ART patients whose embryos have been zona manipulated, either for assisted fertilization (SUZI or ICSI) or assisted hatching (creating a small hole in the zona by mechanical, chemical or laser methods) *(361–364)*. Others, however, have reported that the frequency of monozygotic twinning is not different for patients whose embryos have been zona manipulated and those that remain zona intact *(365)*. The incidence of monozygotic twinning has also been reported to be very high following blastocyst transfer *(366,367)*.

Although the exact mechanism of monozygotic twin formation in ART is unknown, it has been ascribed to ovulation induction *(368)*, ART culture conditions *(359)*, zona architecture or micromanipulation *(364)*, or asynchrony between the uterus and embryo *(369)*. The observation that the incidence of monozygotic twinning following ovulation induction is also higher than that of the general population *(368)* suggests a role for hormonal manipulation rather than *in vitro* embryo culture conditions. Alterations in the hormonal milieu may lead to delays in oocyte or embryo transport and implantation at crucial developmental moments resulting in induction of twinning *(360)*. Alternatively, exposure to high concentrations of gonadotrophins may lead to zona hardening *(370)* resulting in impaired hatching at the blastocyst stage and retention of part of the embryo within the zona. This could result in two separate embryos forming if the inner cell mass is bisected *(371)*. Exposure to *in vitro* culture conditions rather than oviductal and uterine secretions containing lysins may similarly result in zona hardening and impaired hatching *(359)*. The higher incidence of monozygotic twinning observed following transfer of embryos that have undergone zona manipulation suggests an additional role for the architecture of the zona *(364)*. The dimensions of the artificial gap created during assisted fertilization or assisted hatching, particularly if small, may impose a physical restriction to the emerging embryo causing it to split *(364)*. The size of the artificial gap, however, varies significantly according to the method of assisted hatching and according to the degree of technical expertise of the embryologist performing the procedure. This variation may help to explain the discrepancy in the reported incidence of monozygotic twinning following assisted hatching *(365)*. Zona trapping, however, cannot be the entire explanation for the increased incidence of monozygotic twinning reported following blastocyst transfer, as the incidence remains high despite transfer of blastocysts that have had the zona chemically removed prior to transfer (G.M. Jones and A.O. Trounson, personal communication). The findings of Meintjes *et al. (369)* that the incidence of monozygotic twinning following blastocyst transfer and in patients using donor oocytes lends some support to the idea that monozygotic twinning arises due to some compromised endometrial–embryo communication and subsequent asynchrony between the uterus and endometrium.

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Preimplantation genetic diagnosis

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Introduction

The potential transmission of genetic disorders to the offspring has been a major problem for many couples when contemplating pregnancy. The risk has been greatly decreased by the evaluation of the family history or the age of the mother, and the implementation of prenatal diagnosis in those couples where the risk was increased compared to the general population.

An alternative approach that is available with assisted reproductive technology (ART) is preimplantation genetic diagnosis (PGD) by which it is possible to have the disorder screened before the corresponding embryo is transferred to the uterus of the mother. The idea of PGD emerged in the early 1960s when sexing of rabbit embryos at the blastocyst stage was attempted *(1)*. This happened even before the introduction of *in vitro* fertilization (IVF) techniques in human reproductive medicine *(2)*. In the following years, the merging of these two techniques made PGD possible as a new approach for the prevention of genetic disorders.

PGD can be applied in two different situations: it can be offered to couples at risk of having children with single-gene disorders, such as cystic fibrosis or thalassaemias; or, it can be implemented for the screening of chromosomal disorders, both numerical (i.e. aneuploidy) and structural (i.e. inversions or translocations).

The application of PGD is especially relevant in reproductive medicine as genetic factors can be associated with infertility. In addition, the identification, after chromosomal analysis, of euploid embryos and their selective transfer has a positive effect on the clinical outcome observed in patients at risk of developing aneuploid embryos. This is probably due to the fact that chromosomal abnormalities represent one of the major causes of spontaneous abortions and, possibly, implantation failures. Ovarian hyperstimulation and IVF are necessary to generate *in vitro* several embryos in order to select those that are not affected. One or two cells are generally available for genetic analysis and they are removed from embryos on Day 3 of development, or from the trophectoderm of an expanded blastocyst (the clinical use of blastocyst biopsy is still under investigation and therefore it is not taken into consideration in this review). Alternatively, polar bodies can be excised from oocytes and used to analyse disorders of maternal origin. In any case, the diagnostic method must be sensitive enough to deal with very low quantities of DNA and sophisticated to the point of giving results with the highest reliability. The polymerase chain reaction (PCR) is the technique of choice for the identification of single-gene defects. Special strategies are used to avoid contamination from exogenous DNA and to check that both alleles are amplified during the reaction. The numerical analysis of interphase

chromosomes is based on the use of specific fluorescence-labelled DNA probes in the fluorescent *in situ* hybridization (FISH) technique. The use of multiple probes permits the simultaneous screening of several chromosomes and can be completed by successive rounds of FISH using different probes.

The first clinical applications of PGD were reported in 1990 *(3,4)* with the first pregnancies obtained following blastomere biopsy and sex determination by PCR in couples at risk of having children with X-linked disorders *(3)*. Since then, hundreds of PGD cycles have been undertaken which show a growing interest in this approach even by fertile couples who agree to IVF in order to make PGD possible. The list of diseases for which PGD has been used is rapidly increasing. Theoretically, it can be performed for any condition for which gene sequencing is available. This information is necessary to design the primers needed for the selective amplification of the DNA region involved in the mutation. Similarly, FISH can be used for the screening of aneuploidies and structural chromosomal defects such as translocations. The advantages are represented by an increased implantation rate and a concomitant reduction in the incidence of spontaneous abortions due to the better viability of euploid embryos *(5,6)*. Therefore, PGD for aneuploidy is not only an alternative to the management of embryo abnormality leading to therapeutic termination of an already established pregnancy, but also a method to maximize the efficacy of ART procedures.

Special emphasis during the past few years has been given to maximizing the efficiency of single-cell analysis. Strategies aimed at the identification and exclusion of sources for misdiagnosis during PCR or FISH have been designed. This has enabled the definition of the best conditions and scoring criteria which provide the highest accuracy and reliability of PGD.

The state of the art of PGD was reported at the meeting of the International Working Group on Preimplantation Genetics during the 3rd International Symposium on Preimplantation Genetics held in Bologna in 2000 *(7)*. More than 2500 cycles had been performed worldwide at that time, resulting in approximately 600 clinical pregnancies (24%) and the birth of nearly 500 children. Seven cases of discordance between PGD and the genotype/karyotype of the corresponding fetus have been reported, establishing an accuracy rate of 98.2%. The follow-up of the first 236 infants born after PGD revealed a total of 11 malformations (4.7%). This incidence is comparable to the malformation frequency observed in the general population *(7,8)*. A comprehensive review of the results obtained over 1318 PGD cycles has been published by the ESHRE PGD consortium *(8)*.

Identification of problems with the techniques, protocols and methodology

Diagnostic methods in PGD are based on DNA technology. PCR is mostly used for the detection of single-gene mutations related to monogenic disorders, while FISH is used to screen for aneuploidy or structural chromosomal abnormalities.

The methods used to retrieve, from oocytes or embryos, the material destined to undergo PGD are the same, irrespective of the type of genetic analysis required. The biopsy procedure entails micromanipulation. Special attention has to be paid to the correct and clear labelling of the dishes used for biopsy and culture to guarantee concordance between the biopsied cell and the oocyte or embryo of origin.

As a general consideration, an accurate assessment of oocyte maturity and quality—the appearance of pronuclei and polar bodies—and a morphological evaluation of embryo development are crucial for the appropriate selection of the material for genetic analysis *(9,10)*. Therefore, a careful and expert evaluation at each stage of oocyte morphology and development is necessary to obtain the best results *(11)*.

Polar body biopsy

The majority of oocytes retrieved after induction of multiple follicular growth are at the metaphase II stage indicated by the presence of the first polar body (PB1) which is generally extruded at 36–40 hours after hCG injection. The PB1 is the byproduct of the first meiotic division and contains the counterpart of the oocyte chromosomes, which are diploid at this stage. Following fertilization, the number of chromosomes in the oocyte is reduced by half by extruding a 23-chromosome set in the second polar body (PB2).

Analysis of both polar bodies is necessary to obtain a genetic diagnosis. In the case of PGD for single-gene disorders, PB1 and PB2 must be removed sequentially and analysed separately to evaluate the possible occurrence of crossover between homologous chromosomes during meiosis. For screening of aneuploidy, both polar bodies can be removed simultaneously; the interpretation of the results is

based on the fact that PB1 is diploid and PB2 haploid *(9)*.

PB1 biopsy

Removal of the PB1 is performed by micromanipulation of the oocyte approximately four hours after oocyte retrieval. At this time, PB1 is usually detached from the oolemma. The oocyte is secured by the holding pipette with the polar body at the six or twelve o'clock position. A slit is opened in the zona pellucida by using a glass needle and the polar body aspirated with a thin, polished glass pipette. Within one hour, oocyte insemination is accomplished by intracytoplasmic sperm injection (ICSI) with the injection needle introduced through the breach already opened in the zona. ICSI is recommended, not only to decrease the risk of sperm DNA contamination, but also to avoid the high incidence of polyspermy resulting from the opening made in the zona pellucida. PB1 biopsy does not adversely affect either fertilization or cleavage. This approach could be especially advantageous in the case of female translocations detected by FISH analysis with chromosome painting *(12)*.

PB2 biopsy

The PB2 is removed by introducing the aspiration pipette through the slit previously made to remove the PB1. This step is delicate as connections may still exist between PB2 and the oocyte membrane. Biopsied oocytes are then returned to culture dishes and incubated until the time they need to be checked for cleavage.

PB1 and PB2 biopsy

The simultaneous removal of both polar bodies involves the same technique as described in the case of PB1 biopsy. This strategy reduces the time and possible stress associated with the micromanipulation procedure, and limits biopsies and genetic analyses to normally fertilized oocytes. Nevertheless, some concerns still remain about the frequent degenerative changes found in PB1 at the time of fertilization control.

In conclusion, polar body biopsy maintains embryo integrity as only byproducts of meiosis are used for the genetic analysis of the oocyte. It is also an alternative in cases where PGD is unacceptable. However, paternally-derived defects and those originating after fertilization or the first embryonic divisions cannot be diagnosed.

Blastomere biopsy

The biopsy of one or two blastomeres is generally performed 62–64 hours after insemination. This time constraint is due to the fact that compaction starts at this time. Cellular damage is very likely if the procedure is attempted when cell–cell interactions and junctions have already begun to be established. Alternatively, embryos can be washed in a divalent cation-deficient medium to disrupt cell adhesions and tight junctions *(13)*. The biopsy procedure involves an opening in the zona pellucida of approximately 20–25 µm diameter. Three different methods have been proposed.

Mechanical

A glass microneedle is used to make a series of slits in the zona pellucida. In this way, a V-shaped triangular or square flap opening is created *(9)*. This method is time-consuming and requires a very skilled operator.

Chemical

After loading acidic Tyrode's solution (pH 2.35) in a 12 mm diameter pipette, the blastomere selected for biopsy is set at the three o'clock position. The outer edge of the blastomere is brought into focus and the pipette is quickly lowered in close proximity to the embryo. The acidic solution is blown towards the zona pellucida at a point corresponding to the blastomere's position, with both the blastomere and the tip of the acidic solution pipette kept in focus. When the breach is open, the excess of acidic solution in the medium close to the embryo is aspirated by using the same acidic solution pipette. This is the most commonly used method *(8)*.

Contact laser

A nontouch microdrill is used to make an opening in the zona pellucida and there is no need to remove the embryos from the culture medium *(14)*. Although very quick and precise, not enough data are available yet about the safety of this method when used on human oocytes and embryos to make its use more common.

When opening the zona pellucida, the position of the embryo is selected so that a nucleated blastomere

is at the three o'clock position. The presence of a nucleus is fundamental for DNA analysis. Unfortunately, nuclei are not always visible and, in this case, the selection of the blastomere is mainly based on its size and cytoplasmic appearance to reduce the chance of removing anucleated fragments. A 30–40 µm diameter glass pipette is brought close to the opening in the zona pellucida. The outer edge of the blastomere and the tip of the blastomere aspiration pipette are kept in focus. Using very gentle aspiration, the blastomere is slowly brought into the pipette and then released in the medium. Extreme care is required to avoid cell membrane rupture and damage to either the biopsied cell or the surrounding blastomeres. After biopsy, the embryo is carefully washed, put in fresh medium and incubated until the time of transfer. The biopsied cells are left in the micromanipulation dish at room temperature.

In conclusion, PGD using blastomeres is advantageous due to the possibility of screening disorders of both maternal and paternal origin, and those originating after fertilization. Although the mass of the embryo is reduced, no detrimental effects on embryo viability have been reported *(15)*. In fact, data from the clinical outcome of biopsied embryos have demonstrated that approximately one-fourth of these embryos are able to implant *(5)*. On the other hand, mosaicism, which seems to be a common characteristic in human embryos generated *in vitro*, may represent a problem for genetic diagnosis performed at the cleavage stage. This complication will probably persist in the case of blastocyst biopsy as well *(16,17)*.

Chromosomal analysis

The cells obtained by the biopsy procedures described above are put in hypotonic solution, fixed on a glass slide with methanol and glacial acetic acid, dehydrated in increasing concentrations of ethanol and incubated with the hybridization panel.

The process of fixation is critical for the final diagnosis. The most common problems are:

- loss of the cell during fixation. Constant observation under the microscope is necessary during fixation; the location of the fixed nucleus must be defined by encircling with a diamond pen.
- residual cytoplasm. All cytoplasm must be dissolved completely so that it does not interfere with the interpretation of the fluorescent signals. Fixative must be added when the cell starts to flatten out and before the complete drying of the hypotonic solution.
- reduced spreading of the chromatin. The correct timing for adding the fixative is critical to obtain a good spreading of the chromatin. An inverse correlation has been demonstrated between the diameter of the fixed nucleus and the occurrence of overlapping signals *(18)*.
- loss of micronuclei. Following rupture of the cytoplasmic membrane, the addition of more fixative can frequently cause loss of micronuclei (and chromosomes) which is easily detected by a high incidence of false monosomies *(19)*.

Following dehydration of the fixed nuclei, the fluorescence-labelled probes are added for multicolour FISH to take place. Attention must be paid to (i) adding the probe on the same side of the glass slide where the nuclei are fixed; (ii) avoiding any scratches by not touching the surface of the glass slide next to the fixed nuclei with the dispensing pipette; and (iii) avoiding the formation of air bubbles, especially when the coverslip is added.

A simultaneous denaturation of DNA probes and specimen DNA is accomplished at temperatures of 68–73°C for three to five minutes (these conditions can vary depending upon the probe mixture used and the protocols established in each laboratory). Reannealing and formation of hybrids follow by incubation at 37°C for at least three hours in the case of blastomeres, but a longer incubation is required for polar bodies. At the end of the reaction, the excess of probe (either unbound or nonspecifically bound) is removed by washing at high temperatures (71–73°C) in salt solution. Following counterstaining in antifade solution, fluorescent signals are scored under 600 times magnification with a fluorescence microscope. Criteria for the interpretation of the fluorescent signals have been described *(9,19)*. Rehybridization of the same biopsied cell with additional probes in a subsequent round of FISH is important as it expands information on a larger number of chromosomes *(20)*. The capture of the fluorescent signals on a computerized image analysis system is of help not only for storage but also for the interpretation and review of the detected signals.

The most common sources of error during this procedure are loss of micronuclei, signal overlapping, failed hybridization, inadequate scoring criteria for the interpretation of split signals and mosaicism.

Special precautions have been established to

reduce the technical errors associated with chromatin fixation, hybridization and interpretation of the correct signals. Mosaicism still remains a potential source of misdiagnosis; however, the simultaneous analysis of multiple chromosomes is of great help for the identification of complex abnormalities *(21)*. The most recent report about the accuracy of FISH results on singe cells gives a rate of 97% *(7)*.

Genetic analysis for single-gene disorders

When the biopsy procedure is completed, the removed cell is transferred under the stereoscope to a microcentrifuge tube containing lysis buffer. Lysis is then performed using one of the most commonly used procedures such as proteinase K treatment or potassium hydroxide plus dithiothreitol.

The main problem with PCR is the possibility of exogenous DNA contamination whose main sources are of maternal (cumulus cell) or paternal (sperm cell) origin. Operators can also contribute exogenous DNA. Special precautions have to be followed in both laboratories since standard sterile conditions are not sufficient to prevent DNA contamination.

In the IVF laboratory
- all disposable, medium and oil involved in the culture and micromanipulation of PGD cases are prepared from unopened, sterile packages;
- sterile gloves, masks and gowns are used at all stages;
- micromanipulation tools are exposed to ultraviolet radiation before use; and
- at the time of biopsy, only the two embryologists involved in the procedure are allowed in the laboratory to minimize the air currents associated with persons' movements.

In the genetic laboratory
- a special, completely equipped room should be exclusively dedicated to PCR for PGD cases;
- the access to the PCR laboratory is restricted during the analysis and preparatory phases;
- opening of the tubes containing specimen and reagents is reduced to a minimum; and
- all reagents are checked the day before use for DNA contamination by running a control PCR to detect the possible presence of human DNA.

Besides the positive controls necessary for the interpretation of the results, a series of negative controls are also added during the reaction to verify the presence of external DNA, including oocyte or embryo culture media. In addition, polymorphic markers are included as an additional method of identifying exogenous DNA.

Different methods are used to analyse the PCR products, heteroduplex formation and restriction digestion being the most common *(22,23)*. An alternative approach is represented by the fluorescent-PCR (F-PCR) where fluorescence-labelled primers are used. In this case, the amplification product is analysed on a fluorescence detector that is more sensitive compared to the standard gel system. F-PCR is especially valuable for the detection of point mutations where direct sequencing of the PCR product is necessary *(24)*.

The correct interpretation of PCR results can be distorted by the possible occurrence of allelic preferential amplification failure or ADO (allele drop-out). This phenomenon is not confined to single-cell PCR, but in this case, the consequences are more serious and can lead to complete misdiagnosis. Some strategies have been defined to make ADO recognizable. The most efficient entails the definition of polymorphic markers which are strongly linked to the gene under study *(7)*. These markers are generally represented by short tandem repeats (STRs; short sequences where a 2–5 base pairs per repeat unit occurs throughout the human genome) that are mainly located in intergenic regions. STRs are amplified simultaneously with the gene to which they are strongly linked in a multiplex PCR with the presence of several primers in the amplification mixture. F-PCR is the technique of choice for the simultaneous amplification of the studied gene and the corresponding informative linked markers. If two or more informative markers are available for the mutation under study, the great majority of ADOs are detected leading to an accuracy rate of approximately 97% *(9,25)*.

Indications and contraindications for PGD

The use of PGD necessarily involves ART techniques and even fertile couples need to undergo all the steps associated with ART treatment, including induction of multiple follicular growth, oocyte retrieval at 34–36 hours after the injection of hCG and *in vitro* insemination of the oocytes. As a general consideration, several embryos are required to identify those that are

affected by the disorder under study. Therefore, a good response to hyperstimulation is an important requisite, especially when considering that embryos are transferred not only on the basis of their genetic analysis but also on the basis of their morphology. This approach makes embryo selection stricter since PGD results are included as an additional selection criterion. If the incidence of affected embryos is predicted to be 25%, in the case of single-gene disorders according to Mendelian inheritance, the frequency of chromosomal imbalance will reach higher percentages in selected categories of patients *(26)*. In these cases, the pregnancy rate has been demonstrated to be directly related to the number of oocytes generated and makes the quality of response to follicular hyperstimulation a key factor for a successful PGD cycle *(27)*.

Some of the more advanced techniques in ART have made a great contribution to the outcome of PGD. Improved efficiency has been achieved using ICSI as the insemination technique, thus minimizing the risk of sperm DNA contamination. In addition, embryo cryopreservation, especially when performed at the two-pronuclear stage, provides the possibility of timing the embryo transfer.

The results reported in the past decade show that PGD is becoming a valuable choice for couples at high reproductive risk. The list of diseases for which PGD can be performed is rapidly increasing.

In the case of a diagnosis of aneuploidy, the parents' peripheral blood karyotype is necessary to select the probe panel for the appropriate chromosomes. In patients of advanced maternal age, this selection generally involves the chromosomes whose aneuploidies are more common in spontaneous abortions and live births, namely chromosomes XY, 13, 16, 18, 21 and 22. Additional probes can also be included to identify other chromosomes whose errors could have an impact on implantation. The results obtained can give useful information about the contribution of single chromosomes to successful implantation. It is theoretically possible that aneuploidies of these chromosomes which are not thought to have clinical relevance are so deleterious that implantation never occurs due to very early failure of embryo development.

When one or both parents carry an altered karyotype such as balanced translocations, the selection of probes must include those specific for the abnormality. In the case of reciprocal translocations, an appropriate combination of telomeric and centromeric probes specific for the condition under study must be identified. Preliminary testing of the selected probes on the carriers' lymphocytes is recommended in order to verify the efficiency of the system.

PGD of single-gene disorders entails a long preparatory phase beginning with an estimate of the diagnosed mutations in the couple's lymphocytes. All monogenic disorders are, in principle, detectable by PGD, if the corresponding coding region has been sequenced and the mutation identified. This step is necessary for the development of the most suitable primers. The PCR assay has to be prepared for each particular situation, with special attention being paid to the inclusion of informative linked polymorphic markers (when available) which are aimed at signalling exogenous DNA contamination and the occurrence of ADO. When the PCR protocol is complete and the PGD for the studied condition is considered feasible, all the reagents involved must be demonstrated to be DNA-free.

There are no contraindications to undertaking PGD except those that are associated with either the induction of multiple follicular growth by ovarian hyperstimulation or the establishment of a pregnancy. All couples at high reproductive risk can consider PGD as an alternative to therapeutic abortion, irrespective of their fertility status, including, for example, fertile carriers of single-gene disorders. Different considerations are needed for PGD of aneuploidy which is aimed at increasing the chances of implantation by avoiding the transfer of embryos with aneuploidy or structural chromosomal abnormalities. Carriers of reciprocal translocations are often fertile but they have a very poor prognosis as unbalanced segregation occurs at very high rates. The selection of embryos with a normal or balanced karyotype can alleviate the poor prognosis associated with this condition which is characterized by a high incidence of spontaneous abortions *(12)*.

Eligibility of patients

The use of PGD has increased considerably over the past decade. Analysis of the data collected has allowed the identification of the patient categories in which PGD is indicated.

Chromosomal abnormalities

Numerical chromosomal abnormalities are associated

with implantation failure and high rates of embryonic death. Aneuploidy derives mainly from nondisjunction events during gametogenesis and increases significantly with maternal age *(28)*. Nondisjunction can also occur after fertilization, leading to the formation of mosaic embryos where normal, monosomic, and trisomic blastomeres may coexist in combination with more complex abnormalities *(29)*. Most cases of aneuploidy are associated with implantation failure or spontaneous abortion although some (trisomy 13, 18, 21 and aneuploidy of sex chromosomes, for example) are able to develop to term. All chromosomal abnormalities, even the more complex, have been detected in the inner cell mass of human blastocysts *(16,17)*. This finding confirms that morphological criteria alone, including blastocyst development, are not sufficient to ensure the selection of euploid and viable embryos.

As reported at the meeting of the International Working Group on Preimplantation Genetics, more than 1500 cycles have been performed worldwide resulting in approximately 400 pregnancies *(7)*. The following categories of patients have been studied:

Maternal age 36 years or more

According to the largest studies reported, the percentage of chromosomally abnormal embryos is approximately 65% when the maternal age is 36 or more years, with an incidence that increases proportionately with the woman's age. A significant improvement in the clinical outcome after PGD has been shown in terms of a higher implantation rate and a decreased frequency of spontaneous abortion *(5,6)*. This is especially true for women up to the age of 42 years whose chances of pregnancy after PGD are the same as those found in younger women *(30)*. At older ages, other factors probably contribute to a poorer success rate.

Couples with ≥3 IVF failures

The occurrence of unexplained, multiple IVF failures in young patients is probably due to the combined effect of many factors. The frequency of chromosomal abnormalities exceeds 55% in this group of patients; however, the selection of those with euploid complement does not improve the clinical prognosis, and the pregnancy rate after FISH is similar to that obtained in a control group *(5)*. Haploidy/polyploidy and complex abnormalities are the most frequent chromosomal alterations detected. This finding suggests that altered mitotic divisions possibly related to centriolar defects represent the origin of these errors *(31)*. Alternatively, other chromosomes besides those screened could have an important role in the viability of the embryos *(26, 32)*.

Altered karyotype due to chromosomal mosaicism or balanced translocations

Frequent implantation failure and the high incidence of abortion are significantly reduced by the transfer of euploid embryos *(5)*. In these categories of patients, the frequency of chromosomally abnormal embryos is 62%, with monosomy and trisomy contributing 46% of the abnormalities. Carriers of reciprocal, balanced translocations are particularly likely to develop unbalanced embryos (approximately 80%). Provided that an acceptable number of normal or balanced embryos are detected, a higher rate of implantation results from FISH selected embryos *(12)*.

Epididymal or testicular sperm aspiration with ≥1 IVF failures

A very high percentage of chromosomally abnormal embryos (72%) is found in this group of patients *(33)*. The transfer of PGD-selected embryos results in a 25% clinical pregnancy rate with an implantation rate of 18.8%. This clinical outcome is similar to that observed in patients with multiple IVF failures. However, the causes of this poor prognosis are probably different as suggested by the type of abnormalities observed, with 45% due to monosomy and trisomy and, interestingly, 8.6% of the abnormal embryos having gonosomal aneuploidies. A significant involvement of the male counterpart in the etiology of these alterations is a plausible possibility.

Recurrent abortions

Preliminary data on this condition show a high rate (68%) of chromosomally abnormal embryos *(30)*. Although the preliminary results in terms of live-birth rate are promising, more data and a comparative study are necessary to evaluate the proposed validity of PGD for aneuploidy in this category of patients *(34)*.

Single-gene disorders

Presently, about 750 PGD cycles for single-gene

disorders have been performed worldwide with approximately 150 pregnancies and more than 100 healthy infants born *(7)*. PGD for 26 disorders have been reported and are listed in Annex 1. The list is expanding rapidly.

Especially innovative are the applications for dynamic mutations such as Fragile-X syndrome, Huntington disease and myotonic dystrophy, which are associated with the expansion of a trinucleotide class of repeats that are located within the coding and untranslated regions of genes *(35,36,37)*. The use of multiplex markers has notably increased the reliability of the diagnosis. New developments are under evaluation to reduce the risk of misdiagnosis, especially in the case of noninformative or partially informative mutations. With these diseases, a special concern arises about the information given to the patients if they are not aware of their status (e.g. Huntington disease).

Genetic predisposition for late-onset disorders has been reported as suitable for PGD *(7)*. Carriers of the p35 tumour-suppressor gene mutation are at higher risk of developing an inherited predisposition to cancer. PGD can be performed to select embryos without this mutation. Whether a genetic predisposition to severe disorders can be a criterion that justifies selective embryo transfer is open to discussion. PGD for HLA matching permits a pregnancy to be planned where the baby will be a potential donor for an affected brother or sister who needs bone marrow transplantation. The genetic analysis selects among the healthy embryos those with a haplotype match for the affected child *(7)*.

In conclusion, PGD of single-gene disorders is not only a technique aimed at establishing a healthy pregnancy, but also a general approach towards the prevention and therapy of genetic diseases.

Risk–benefit analysis

Oocyte and embryo biopsy are invasive procedures, and concerns have been expressed regarding the development and viability of the embryo after biopsy has been performed. Experimental studies have demonstrated that biopsy at the eight-cell stage had no detrimental effects on further growth *(15)*. More important, data from the clinical outcome of biopsied embryos showed that approximately one-fourth of them are able to implant *(5)*.

The diagnosis of genetic disorders based on a single-cell analysis demands extremely sophisticated technology and special precautions to maximize the accuracy of the procedure. Special care has been devoted to evaluate the efficiency of the technique. Considerable improvement has been achieved in the identification of possible causes of misdiagnosis, including the definition of guidelines to be followed in ART laboratories when dealing with PGD cases *(38)*.

In the case of PGD for aneuploidy, a control on the quality of the results is carried out by repeat biopsy of the nontransferred embryos and performing FISH on the blastomeres that are obtained *(19,20,39)*. The results demonstrate an *in vitro* efficiency rate of 97% that is confirmed by the *in vivo* efficiency rate (97.8%). The *in vivo* efficiency rate is calculated by the data derived from prenatal diagnosis of the established pregnancies or direct examination of the infants at birth *(7)*.

Similar rates have been reported for PGD of single-gene disorders *(7,9)*. The use of special precautions to avoid exogenous DNA contamination and ADO has dramatically reduced the main causes of misdiagnosis.

Only a few misdiagnoses have been reported worldwide (seven were reported by the International Working Group on Preimplantation Genetics, 2001); this figure establishes PGD as a safe and reliable procedure. Despite these results, conventional prenatal diagnosis still needs to be recommended to patients to confirm the PGD results by amniocentesis or chorionic villus sampling.

More recently, PGD for aneuploidy has been considered a powerful criterion for the selection of viable embryos. In this case, the higher take-home baby rate after PGD of aneuploidy *(5,6)* suggests that the cost of a successful treatment cycle with PGD is less expensive than the corresponding number of conventional ART cycles that are needed for a live birth.

On the other hand, the majority of fertile patients who decide to undergo PGD either have strong objections to therapeutic abortion after prenatal diagnosis or have previously experienced termination of affected pregnancies. Unfortunately, the stress associated with the procedure, the relatively low pregnancy rates and the high financial cost are major concerns. This is especially true in those countries that have decided not to fund assisted reproduction including PGD, despite the advantages to many couples wishing to conceive a healthy child. It has always been said that the prevention of genetic

diseases is much less expensive that the cost to support the technology needed to make the diagnosis.

Conclusions

After 10 years of PGD in reproductive medicine and the performance of approximately 2500 cycles, the main conclusions are that PGD is a reliable procedure in preventing the birth of affected children and it can be regarded as an alternative to therapeutic abortion.

PGD of aneuploidy is effective and results in a high take-home baby rate when implemented in selected categories of patients. Despite the efficiency of the technique, conventional prenatal diagnosis is still recommended.

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Annex 1. List of the single-gene disorders for which PGD has been reported

Cystic fibrosis	Marfan syndrome	Phenylketonuria
Thalassaemia	Rhesus incompatibility	Epidermolysis bullosa
Sickle-cell anaemia	Retinitis pigmentosa	Ornithine transcarbamilase deficiency
Haemophilia A and B	Multiple epiphyseal dysplasia	Fanconi anaemia
Duchenne muscular dystrophy	Alport disease	Familial adenomatous polyposis coli
X-linked hydrocephalus	Alpha$_1$-antitrypsin deficiency	Early-onset alzheimer disease
Neurofibromatosis I and II	Achondroplasia	Fragile-X syndrome
Tay–Sachs disease	Long chain hydroxyacyl CoA dehydrogenase deficiency	Huntington disease
Lesch–Nyhan syndrome		Myotonic dystrophy

Multiple pregnancy in assisted reproduction techniques

OZKAN OZTURK, ALLAN TEMPLETON

Introduction

The incidence of multiple pregnancy has increased considerably over the past two decades due to ovarian stimulation during fertility treatments *(1)*. Women undergoing *in vitro* fertilization (IVF) treatment face a 20-fold increased risk of twins and 400-fold increased risk of higher-order pregnancies *(2)*. This is related to the current practice of transferring multiple embryos into the uterus. The emphasis on pregnancy rates as a performance indicator has provided a powerful incentive for increasing the number of embryos transferred after IVF. Such a policy, however, challenges safe practice, increases perinatal mortality and morbidity *(3–5)* and imposes a steep financial burden on maternity and neonatal services *(6)*. In this context, the medical, social and economic consequences of multiple pregnancy have been the subject of much recent debate.

Clinicians and couples are under considerable pressure to maximize pregnancy rates. However, multiple pregnancy is an inadvertent and unacceptable consequence of these pressures. Although potential parents generally feel that multiple pregnancy is not an optimal outcome, their acceptance level may be high *(7)*. In fact, one survey of infertility patients demonstrates that multiple pregnancy is desired, with only half objecting to triplets and 20% deeming quadruplets acceptable *(8)*. However, it has yet to be assessed how well-informed infertility patients are as to the adverse medical, psychological, economic and social consequences of multiple gestation and whether it is really fair to expect them to solve this dilemma.

The impact of assisted conception on population demographics was highlighted in the 1994 figures from the American Society for Reproductive Medicine and Society for Assisted Reproductive Technology (ASRM/SART) Registry. It reported a 36% multiple pregnancy rate with 7% triplets or higher, constituting 55% of all the children born from IVF and gamete intrafallopian transfer (GIFT) *(9)*. From 1980 until 1997, the annual number of live-born babies from twin gestation rose by 52% (from 68 399 to 104 137), while the number of high-order multiple gestations increased by 404% (from 1377 to 6727) *(10)*. Looking at the 71 826 ART cycles performed in 1997 as a whole, including the GIFT and ZIFT procedures, 38% of all births following nondonor treatments were multiple, with 26% twins and 5% higher multiples *(11)*. Similarly, the most recent data on 80 634 ART cycles performed in 1998 show 38% multiple live births. Twins constitute 32% of these deliveries, while triplets or more are responsible for 6% *(12)*. Interpretation of the figures should take the effect of fetal reduction (MPFR) practices into consideration. These statistics from the USA underline the extent and the persistence of the multiple pregnancy problem in ART.

The most recent data from the UK on 35 363 IVF

cycles in 1998, compiled and published by the Human Fertilisation and Embryology Authority (HFEA), also show a high incidence of multiple births as a result of ART *(13,14)*. Of individual babies born from all types of IVF treatment, 47% come from multiple pregnancy, and this figure has remained unchanged during the period 1994–1999. For conventional IVF treatment, including frozen embryo transfers (ETs), the contribution of multiple live births to total live-birth events in 1998/1999 was 27%, which remains almost unchanged since 1991. The figure for fresh ICSI treatment was 27%, which also showed minimum fluctuations since 1993. Breakdown of this overall figure provides a live-birth rate of 24% for twins and 2.4% for triplets and greater. The historical data from this national database during the periods 1994–1995, 1995–1996, 1996–1997, and 1997–1998 demonstrate a fairly stable trend with 25%, 27%, 25% and 25% for twins and 4%, 3%, 3% and 3% for triplets, respectively *(15–18)*. Our own experience from Aberdeen reflects a totally different picture. Triplet births have been totally eliminated with a steady increase in the singleton birth rates since 1997. This difference from the national average is the direct result of a two-embryo transfer policy implemented as the routine. The same trend on a larger scale is also evident in Belgium and the Nordic countries, without any evidence of an overall reduction in success rates *(19)*.

The situation with respect to other treatments for infertility is not very different. Gonadotrophin stimulation is now a major cause of multiple pregnancy, particularly in the category of high-order multiple pregnancies *(20)*. Evans and his co-workers highlighted this emerging trend of escalating multiple pregnancy numbers, not only in IVF settings but also in gonadotrophin stimulation, over an eight-year period from 1986 to 1993. They further emphasized that ovulation induction was responsible for a higher percentage of quintuplets and higher-order pregnancies than ART and this was attributed to the relative lack of control over gonadotrophin stimulation. The first large study to stress this problem in the context of gonadotrophin-mediated superovulation was conducted by Guzick and colleagues, who found that superovulation was associated with a 20% incidence of pregnancy with twins and nearly 10% incidence of higher-order multiple pregnancies *(21)*. These figures were similar to those reported in the large retrospective study by Gleicher and colleagues *(22)*, whose results can be subgrouped as 20% twins, 5% triplets, 2.3% quadruplets, 1.1% quintuplets and 0.5% sextuplets. A recent review, which provided a comparative view with a wider perspective documented that 50%–72% of quadruplet and greater multiple pregnancies were related to ovulation induction in comparison to 42% in assisted reproduction and 6%–7% in spontaneous conceptions *(23)*.

Maternal complications

Multiple pregnancy is associated with greater risks for both mother and fetuses compared to singleton pregnancy. Morbidity and mortality rates are appreciably increased, an issue that is underestimated by the lay public, who tend to see the overall process as "double the joy". However, the maternal response to multiple pregnancy comprises a series of dramatic changes in all organ systems *(24,25)*. The alterations are so marked that they would be considered pathological in a nonpregnant woman. Despite this remarkable adjustment, actual complications are common and it is not an overstatement to consider multiple pregnancy as a major pathology. Commonly encountered maternal complications with multiple pregnancy are discussed in the following section.

Miscarriage

The exact risk of miscarriage in multiple pregnancy is difficult to determine because of the incompletely documented phenomenon of the "vanishing twin". Up to half the multiple pregnancies diagnosed via first-trimester ultrasound are known to deliver more than one baby *(26–28)*. This is explained by the loss of viability and subsequent resorption of fetuses with minimal or mostly no evidence of existence at term *(29)*. The resorption process of nonviable embryos is observed mainly during the first seven weeks of gestation and does not occur beyond the fourteenth week *(30)*.

Nevertheless, miscarriage, either threatened or inevitable, was reported as a common complication of multiple pregnancy *(31)*. The risk is an exponential function of the number of fetuses. First-trimester bleeding rates of 12%, 53% and 80% were observed in twins, triplets and quadruplets, respectively *(32,33)*, with a correspondingly high clinical miscarriage rate of 30% and 42% in twin and triplet pregnancies, respectively *(34)*.

Preterm labour and delivery

The incidence of preterm delivery and its clinical severity increases with the number of fetuses *in utero*. Although the background risk is known to be high in IVF pregnancies, mothers who conceive multiple pregnancy by means of IVF are at an even higher risk of morbidity *(35)*. Preterm delivery before 37 weeks' gestation was reported in 44% of all twin pregnancies compared with 6% in singleton pregnancies in a Scottish twin study *(36)*. A number of investigators have also reported similarly high rates for premature labour and delivery in multiple pregnancies: 41%–50%, 92%, and 75%–95% for twins, triplets and quadruplets, respectively *(31,32,34,37,38)*.

The maternal risks are mainly associated with prolonged hospitalization (a mean of 23±19 and 56±30 days, respectively, during pregnancy for triplets and quadruplets) *(32)* and the use of tocolytic therapy. In particular, an increased risk of pulmonary oedema was observed with beta mimetic tocolysis and steroid use in multiple pregnancies. Intravenous ritodrine infusion for longer than 24 hours and excessive fluid administration appear to be the main risk factors for the development of pulmonary oedema *(39)*.

Premature rupture of membranes, which occurs more frequently in multiple pregnancy (20% of triplet pregnancies), not only triggers preterm labour but also predisposes mothers to high risk of intra-amniotic infection *(37,38)*.

Anaemia

There are differences in the conventional definitions of anaemia based on clinical symptoms, haemoglobin levels, red cell indices or bone marrow investigations. However, it is generally accepted that the dilutional effect of the increased maternal plasma volume and the higher fetal demands for nutritional precursors predispose multiple pregnancy to a state of iron and folate deficiency anaemia. This incidence in triplet pregnancies was found to be between 27% and 58% *(33,37,38,40)*. Women with multiple pregnancy are also vulnerable to acute blood loss, mostly secondary to postpartum haemorrhage, and in need of sufficient iron and folate levels to combat the high haemopoietic demands.

Hypertension

Pregnancy-induced hypertension and (pre) eclampsia occurs more frequently in multiple pregnancies. The clinical presentation is much earlier and more severe in comparison to singleton pregnancies *(31,33,41–43)*. The risk was reported to be fivefold and tenfold higher in primigravidae and multigravidae with twin pregnancy in comparison to a singleton pregnancy *(44)*. Both monozygotic *(45)* and dizygotic *(42)* pregnancies were found to be at a greater risk of developing pregnancy-induced hypertension.

The reported incidences for pregnancy-induced hypertension were 17% for twins, 20%–39% for triplets, and 50%–67% for quadruplets, respectively *(32,34,37,40)*. Malone *et al*. observed severe pre-eclampsia in 24%; haemolysis, elevated liver function tests, and low platelets (HELLP) syndrome in 9%; and eclampsia in 2% of a study group, which comprised triplet pregnancies beyond 20 weeks' gestation *(38)*.

Polyhydramnios

Polyhydramnios may be suspected clinically in up to 12% of multiple pregnancies *(46)* and is associated with an increased risk of preterm labour. The acute form, which tends to present in the second trimester, is a complication of monozygous twin pregnancies with an incidence of 1/20 000 births, or 1/200 twin births *(47,48)*. It is caused by a feto-fetal transfusion leading to anaemia in the donor and polycythemia in the recipient twin.

Maternal risks are mainly associated with severe discomfort and the complications of bed rest, tocolysis and recurrent amniocenteses. Rare complications due to pressure effects are also reported. Obstructive acute renal failure in a patient at 34 weeks' gestation with a twin pregnancy complicated by polyhydramnios was presented in a case study. The authors emphasized the potential risk of increased uterine pressure on the ureters as a cause of significant obstructive renal failure during pregnancy *(49)*. A further case of spontaneous rupture of the renal pelvis during a twin pregnancy was also published *(50)*.

Antepartum and postpartum haemorrhage

Despite the greater surface area of the placental bed and the associated risks of placenta praevia, placental abruption and vasa praevia, clinical evidence does not provide support for an increased incidence of antepartum haemorrhage *(31,41)*. A study, which analysed the outcome of 26 sets of triplet and five sets

of quadruplet pregnancies resulting from IVF, reported an 8% and 0% incidence of third-trimester bleeding, respectively *(32)*.

However, the incidence of postpartum haemorrhage (PPH) is significantly higher due to overdistention of the uterus leading to atonia. The higher incidence of operative deliveries is also a possible cause contributing to postpartum blood loss. The reported figures for PPH range from 9% to 35% *(37,38,40)*.

Risk of an operative delivery

The likelihood of an operative delivery, whether vaginal or abdominal, is significantly higher in multiple pregnancy, mostly due to medical and patient choice, as well as intrapartum complications of malpresentation, cord accidents, incoordinate uterine action and fetal distress. Operative delivery is associated with an increased maternal risk of genital trauma, pelvic infection and haemorrhage *(40)*. Multiple pregnancies are more frequently delivered by caesarean section compared with singleton pregnancies, either as an elective procedure or an emergency *(31,51)*. Albrecht and Tomich reported a 100% caesarean section rate in their series of 57 triplet deliveries *(40)*. Nadal and his co-workers however, delivered 87% of triplet pregnancies by caesarean section *(52)*, reflecting the differences in local practices. The total twin caesarean section rate, including caesarean section for the second twin, was published as 45% in 1995–1997 by Wolff from Sweden, while caesarean section for the second twin was performed in 11% *(53)*.

Prolonged antenatal hospitalization

The traditional management of preterm labour, hypertension, polyhydramnios, and fetal growth restriction in multiple pregnancies often requires prolonged hospital admission *(31,54)*. A mean duration of 9, 25, and 56 days of hospitalization was reported for twin, triplet and quadruplet pregnancies, respectively *(32,55)*. Further to recognized complications of prolonged hospital stays, such separations from the family are often a stressful experience *(56)*.

Postnatal problems

After the initial sense of achievement of parenthood, the rearing of children in these cases is stressful and fraught with practical difficulties *(57)*. Impaired maternal bonding, social isolation, marital disharmony and depressive illnesses are all common. Even four years after delivery, mothers of triplets report fatigue, emotional stress and difficult relationships with their children *(40,58)*. Lack of adequate psychological support and financial help was a common theme among families of quadruplets *(54)*.

Given the increased perinatal mortality among multiple pregnancies, the problems of coping with the loss of babies may be an added burden in the postnatal period *(59–61)*. A wide spectrum of postnatal complications in 55 triplet pregnancies was reviewed by Malone *et al*., including endometritis in 24%, postpartum haemorrhage in 9%, pneumonia in 4%, urinary tract infection 4%, and diastasis of the rectus muscles requiring surgery in 2% *(38)*.

Increased symptoms of pregnancy

An increase in nausea and vomiting is common in multiple pregnancy and may be associated with the increased hormonal levels in such pregnancies *(62)*, although the etiology is not fully established. The extra weight of multiple pregnancy inflates certain symptoms of pregnancy such as dyspepsia, gastro-esophageal reflux, constipation, chronic backache, breathlessness, varicose veins, lower extremity and vulvar oedema. Postpartum laxity of the abdominal wall and umbilical hernias may occur frequently. Malone and his co-workers reviewed a wide spectrum of antenatal problems in triplet pregnancies. They reported acute fatty liver of pregnancy in 7%, gestational diabetes in 7%, supraventricular tachyarrhythmias in 4%, dermatoses in 4%, urinary tract infection in 4%, and acute disc prolapse requiring surgery in 2% of their study group *(38)*. The prevalence of gestational diabetes is a function of the number of fetuses, and is reported in 3% of singleton pregnancies in comparison to 5%–8% in twins, 7% in triplets, and >10% in quadruplets. Seoud *et al*., however, published the much higher figure of 39% for triplets *(34)*. One investigator found a higher incidence of gestational diabetes in pregnancies conceived after ovulation induction in comparison to spontaneous pregnancies *(63)*.

Prevention

Multifetal pregnancy reduction

Rates of perinatal mortality and morbidity are higher

in twins than in singletons, and the adverse maternal outcome rises with increasing number of multiples. Recognition of these unfavourable features of multiple pregnancy, both at the individual and societal level, has initiated the move towards the implementation of preventive measures against the creation of multiple gestations in ART.

Against this background, multifetal pregnancy reduction (MFPR) has been presented as an option to improve the pregnancy outcome of patients trying to carry a pregnancy to term. Although there are technical, moral, ethical and psychosocial concerns about reducing the number of fetuses *(64)*, this procedure is now a choice for women found to have a higher-order multiple pregnancy.

The term "multifetal pregnancy reduction" is preferred to selective reduction, as selective reduction implies a procedure in which an anomalous fetus of a multiple pregnancy is terminated with the intent of allowing the patient to deliver healthy infant(s) at term. Multifetal pregnancy reduction, however, is the elective reduction of fetuses to a smaller number in an attempt to reduce the incidence of premature delivery, which accounts for most of the morbidity and mortality associated with multiple pregnancy *(65)*. The intention is to enable the pregnancies to continue with the least harm and most benefits *(66–68)*.

A regression analysis on 274 IVF pregnancies showed that at the eighth week ultrasound, each viable fetus could be expected to reduce the duration of the pregnancy by about 3.6 weeks, and each fetus reduced medically or spontaneously could be expected to prolong the pregnancy by approximately 3.0 weeks *(69)*. In multiple gestations of four fetuses or more, the overall benefits of MFPR in increasing the duration of the pregnancy are generally recognized *(67,70)*. However, for triplet pregnancies, the place of MFPR is more controversial because of advances in obstetric and neonatal care. Lipitz *et al.* found a greater rate of total fetal loss and prematurity in triplet gestations compared to triplet pregnancies reduced to twins *(71)*. Furthermore, after reduction, the outcome of triplets was found to be comparable to that for twin pregnancies *(72)*. Likewise, Yuval *et al.* reported a significant reduction in prematurity and low birth weight following MFPR of triplets to twins *(73)*.

Data from the HFEA in 1999 highlight the prevailing trend in the UK towards the use of MFPR, particularly in triplet pregnancies (Table 1). A recent trend to minimize the risk of multiple pregnancies further was manifested as "MFPR to a single fetus". Brambati *et al.* reported a significantly better outcome of pregnancies reduced to singletons than of those reduced to twins *(74)*.

However, multifetal pregnancy reduction comes at a cost. Postprocedure pregnancy loss rates are reported as 8%–23%, depending on the starting number of fetuses, while the delivery of severely premature fetuses vary from 9% to 23% *(75)*. The importance of increasing technical experience on lower rates of pregnancy loss has been emphasized by many authors *(76–79)*. Analysis of 3513 MFPR procedures at 11 centres in five countries highlighted this trend of improved outcomes in pregnancy losses and early prematurity with time and experience. They reported a collaborative loss rate of 4.5% for triplets, 7.3% for quadruplets, and 15.4% for sextuplets or higher-order multiple pregnancies *(80)*.

Selective reduction for fetal abnormality, which is similar in technique to MFPR, was reported in all trimesters with good outcomes for the surviving fetus in >90% of cases *(81)*. Geva *et al.* as well as others published high success rates with second-trimester MFPR *(82–85)*, although the first-trimester approach still remained safer (5%–6% miscarriage rate in first-trimester versus 8%–16% in second-trimester MFPR). Differences in pregnancy loss following reduction at

Table 1. Multifetal pregnancy reduction (MFPR) in high order multiple pregnancies (UK)

	Triplet pregnancy	Triplet pregnancy undergoing MFPR	Triplet delivery	Quadruplet pregnancy	Quadruplet pregnancy undergoing MFPR	Quadruplet delivery
1992–1993	130	4 (3.0%)	85	0	0 (0%)	0
1993–1994	151	4 (2.6%)	115	3	0 (0%)	2
1994–1995	204	17 (8.3%)	130	2	1 (50%)	0
1995–1996	215	18 (8.3%)	152	6	2 (33%)	2
1996–1997	260	37 (14.2%)	160	2	0 (0%)	1

Ref.: Human Fertilisation and Embryology Authority (HFEA) data, *(15)*

different gestational ages were attributed to varying background risks of spontaneous miscarriage *(86)*. Based on these favourable reports on second-trimester fetal reduction and the anticipation of high spontaneous resorption rates during the first trimester, it was recommended that MFPR should be delayed until 12 weeks' gestation in quadruplet or higher multiple gestations. However, this was not felt to be necessary in twin and triplet gestations *(87)*.

Similarly, a large multicentre study reporting the outcome of 402 cases of selective termination in pregnancies with dizygotic multiples from four countries found a loss rate of 7% up to 24 weeks in which the final result was a singleton fetus and 13% in which the final result was twins. Loss rates ranging from 5% to 9% attributable to the reduction procedure were not statistically different *(81)*.

One of the new developments in this area is the use of chorionic villus sampling before MFPR. The aim is to minimize the risk of selecting a chromosomally abnormal fetus to proceed with the pregnancy. It has been shown that multiple pregnancies are at a higher risk of carrying at least one fetus affected by Mendelian or chromosomal anomalies. The incidence is a function of the order of multiples *(74)*. De Catte, from his small series of 32 multiple pregnancies, concluded that prenatal cytogenetic diagnosis during the first trimester prior to fetal reduction is a feasible, accurate and safe procedure, where abnormal chromosomal results indicate the fetus(es) that should be reduced *(88)*.

With the intention of improving safety and efficacy, different techniques of MFPR have been advocated, including transcervical aspiration, transabdominal intracardiac or intrathoracic injection of air, potassium chloride or hypertonic saline, as well as transvaginal intrafetal injections or mechanical destruction of the embryo *(89)*. Transabdominal fetal reduction using simultaneous transvaginal ultrasonographic guidance for selective feticide was used to combine the benefits of both the transabdominal and transvaginal approaches *(90)*. Transvaginal puncture in comparison to transcervical aspiration at eight to ten weeks of pregnancy was found to have less early complications *(73)*. The reduction of a fetus overlying the internal os by the transvaginal puncture procedure was reported with similar success rates as the transabdominally performed puncture procedures for MFPR *(91)*. In this study, the corrected pregnancy loss in 148 cases of MFPR was 11%.

Although MFPR is advocated as an effective and safe option for women with high-order multiple pregnancies, its use may still result in the loss of all fetuses and may have adverse psychological consequences for the potential parents *(92–94)*. Further, selective fetal reduction with the attendant ethical and legal issues may be unacceptable to many couples and should not be perceived or presented as a quick fix to a fundamental problem.

Number of embryos transferred

Against this background, the number of embryos transferred is of crucial importance. One logical step forward is to determine the point at which transferring an additional embryo either does not increase or actually decreases the probability of a singleton pregnancy without substantially increasing the

Table 2. Factors affecting the results of *in vitro* fertilization (IVF)

	Odds of a birth (95% CI)	*P* value	Odds of a multiple birth (95% CI)	*P* value
Maternal age	0.9 (0.9–1.0)	<0.001	0.97 (0.95–0.99)	0.013
Tubal factor infertility (versus no tubal infertility)	0.7 (0.7–0.8)	<0.001	0.8 (0.7–0.90)	<0.001
Number of previous attempts at IVF (versus none)				
1–3	0.8 (0.8–0.9)	<0.001	1.0 (0.9–1.1)	0.85
>3	0.6 (0.5–0.7)	<0.001	0.6 (0.4–0.8)	<0.001
Duration of infertility (per additional year)	0.98 (0.98–0.99)	<0.001	0.98 (0.97–0.99)	0.02
Previous live birth (versus none)				
Not IVF	1.1 (1.0–1.2)	<0.001	Not included in model	
IVF	1.6 (1.4–1.8)	<0.001	Not included in model	

Ref.: Templeton A, Morris JK, *(96)*

overall birth rates *(2)*. A number of other key determinants of success in IVF treatment, including maternal age, duration of infertility and numbers of previous IVF attempts also appear to affect the risk of multiple pregnancy *(95,96)*. It is this association which frames the current debate on minimizing the risks of multiple pregnancy without jeopardizing the likelihood of a single, healthy birth.

Analysis of a large national database provided the answer to this question *(96)*. Recognition of the fact that the number of ova fertilized and the number of embryos available for transfer was an important factor in determining the outcome led to the formulation of a guideline. Where more than four embryos were available for transfer, the live birth rate was not effected by transfer of two or three embryos, although there was a significant reduction in the multiple pregnancy rate when two embryos were transferred. This was shown to be valid for all ages up to the age of 40 years. In women 40 years of age with more than four embryos available for transfer, transferring three rather than two embryos would lead to an increase in the rate of multiple births from 23% to 27% with no obvious benefit in the overall pregnancy rate *(96)*.

This recommendation has been criticized on the basis that no allowance was made for the possibility of varying embryo quality *(97)*. The ability to select suitable embryos remains an important determinant in the outcome of IVF treatment *(96,98,99)*. However, given the limitations of current embryo grading methods, only a large randomized trial would ensure complete comparability among women with different numbers and quality of embryos transferred. Furthermore, availability of four or more embryos suitable for transfer inherently designates a higher level of ovarian reserve and a potential for better embryo quality.

A further retrospective analysis of data from 35 554 IVF procedures reported to Centers for Disease Control and Prevention in the USA in 1996 has addressed this argument, reporting that embryo quality was not related to multiple birth risk but was associated with increased live-birth rates when fewer

Table 3. Number of embryos available and treatment outcome

Eggs fertilized	Embryos transferred	Odds of a birth (95% CI)	*P value*	Odds of multiple births (95% CI)	*P value*
2	2	0.5 (0.4–0.5)	<0.001	0.5 (0.4–0.7)	<0.001
3–4	2	0.6 (0.5–0.7)	<0.001	0.7 (0.6–0.9)	0.008
3–4	3	0.7 (0.7–0.8)	<0.001	1.3 (1.1–1.4)	0.008
>4	2	1.01 (0.9–1.1)		1.0 (0.9–1.1)	
>4	3	1.0 (0.9–1.1)	0.78	1.6 (1.5–1.8)	<0.001

Ref.: Templeton A, Morris JK, *(96)*

Table 4. Number of single, twin and triplet births according to the number of ova fertilized and embryos transferred

	Number of live births				
	0	1	2	3 or more	Total number of transfers
2 ova, 2 embryos					
Number	3 918	434	82	2	
% of transfers	88	10	2	0	4 436
% of births		84	16	0.4	
>2 ova, 2 embryos					
Number	8 297	1647	586	8	
% of transfers	79	16	6	0	10 538
% of births		73	26	0.4	
>2 ova, 3 embryos					
Number	23 171	3980	1755	356	
% of transfers	79	14	6	1	29 262
% of births		65	29	6	

Ref.: Templeton A, Morris JK, *(96)*

embryos were transferred *(100)*. The authors concluded that the risk of multiple pregnancy from IVF treatment varied not only by the number of embryos transferred but also by maternal age. Among women <35 years of age, maximum live-birth rates were achieved when two embryos were transferred. However, unlike the UK study *(96)*, this retrospective cohort analysis indicated an increased live-birth rate, although not statistically significant at the 0.05 level, among women >35 years of age if more than two embryos were transferred, but with a highly significant increase in multiple birth rate from 12% to 29%.

Age is a well-documented prognostic factor in embryo quality *(101)* and treatment outcome *(95)*. Although live-birth rates drop with advancing age, its effect on multiple pregnancy rates is not strong enough to relax the recommendations on the number of embryos transferred. In this context, the research-based data have challenged the perceived sense of security regarding the low multiple pregnancy risks of women in their 40s. It was reported that although high-order multiple pregnancy rates showed a decreasing trend with diminishing implantation rates, no statistically significant effect was actually observed as maternal age increased *(102)*. This conclusion was verified by other authors *(103)*. Where more than three embryos were transferred in patients with a poorer prognosis for successful IVF treatment based on maternal age of >36 years, previous unsuccessful IVF treatment and poor embryo quality, a significant trend toward higher multiple pregnancy rate still remained *(102)*. Likewise, in their analysis of 1116 IVF cycles resulting in 242 pregnancies with 70 multiple gestations, Senoz *et al.* showed that both overall and multiple pregnancy rates were inversely correlated with age, but this trend disappeared when the data were adjusted for the number of embryos transferred *(104)*. Preutthipan *et al.* also found no significant effect of age, including >35 years, on multiple pregnancy rates *(105)*.

From the earlier studies by Staessen *et al.* *(98,99,106)* investigating the effect of the quality and the number of embryos transferred compared to multiple pregnancy risks following IVF/ICSI treatment, to the recent retrospective case–control study by Licciardi *et al. (107)*, the same trend has emerged persistently. Reducing the number of transferred embryos from three to two largely eliminates the occurrence of triplet pregnancies without altering overall pregnancy rates. The British Fertility Society and the Royal College of Obstetricians and Gynaecologists recommends units in the UK to limit themselves to two embryos per transfer *(108)*. Recently the HFEA have also recommended that only two embryos be transferred.

However, as these studies have already highlighted, such a policy still does not address the high prevalence of twin pregnancies, which are more frequent than higher-order multiples and contribute substantially to perinatal morbidity. Therefore, if the ultimate goal of IVF is the birth of a single healthy child, the way ahead must lie with elective single embryo transfer (eSET), with the promise of subsequent transfer of frozen-thawed embryos *(109)*.

Until recently, eSET was not really an option in clinical practice, for fear that overall success rates would decline too far. This presumption has been perpetuated mainly by the published results of single embryo transfer where only one embryo was available. Because no opportunity for the selection of more suitable embryos existed, the implantation potential of the only available embryo was usually poor with clinical pregnancy rates of around 10%.

Pregnancy rates reported by the French National IVF Registry for 1986–1990 ranged from 9% to 12% for single embryo transfers and incrementally increased for the transfer of two, three and four embryos with rates from 30% to 35% *(110)*. A retrospective study by Giorgetti *et al.* on 957 compulsory single embryo transfers showed that implantation rates varied from 4% to 16% per transfer in accordance with the embryo quality *(111)*. In the UK, according to 1997 HFEA figures, the pregnancy rate per cycle was 8% after single embryo transfer, 20% after transfer of two embryos, and 27% after transfer of three embryos *(3)*. Data in the Danish National IVF Registry for 1994–1995 also showed that for IVF and ICSI the birth rates per single embryo transfer was 13%, two embryos 25%, and three embryos 26% *(112)*.

Likewise, a retrospective cohort study from Finland reported a 20% pregnancy rate in 94 patients where only one embryo was available for transfer and 30% where single embryo transfer was elected by choosing the best quality embryo from those available. The cumulative pregnancy rate after frozen-thawed embryo transfers in the elective single embryo transfer group was 47% per oocyte retrieval. By comparison, the pregnancy rate for two embryo transfers was 30% per transfer and 24% of these were twin pregnancies *(113)*. Retrospective analysis of 2573 consecutive transfer cycles following either IVF or ICSI revealed that the ongoing clinical pregnancy

rates were 4%, 9% and 18%, respectively, for the groups with nonelective transfer of one, two and three embryos, compared with 22% with elective transfer of either two or three embryos. Therefore, if the transferred embryos are the only embryos available for transfer, thus eliminating any opportunity for selection of embryos, the pregnancy rates are much lower *(114)*. This point was further communicated by Coetsier during the ESHRE Campus Course 2000 based on their comparative data on elective and compulsory single versus double embryo transfers *(115)*.

Initially, elective transfer of a single embryo was evaluated in the theoretical sense *(116)***.** If 733 single embryo transfers had been done electively in a good prognostic group, defined on the basis of age, number of previous IVF cycles, number of embryos available for transfer, and quality of transferred embryos with a predetermined implantation rate of 26%, a decrease in the overall clinical pregnancy rate from 30% to 26% per initiated cycle, including both fresh and frozen-thawed embryo replacements, would have been balanced by a reduction in the multiple pregnancy rate from 28% to 15%. Although the reduction in the overall pregnancy rate would be entirely due to the reduction of multiple pregnancies, this could easily be compensated with 15% extra treatment cycles, all with a prospect of singleton gestation *(116)*.

This hypothetical exercise has also concluded that the selection of a good prognostic group for single embryo transfer should be made to keep an acceptable balance between reduction in multiple gestation and overall pregnancy rates. Although more treatments might be needed to achieve a similar live-birth rate after single embryo transfers compared with two embryo transfers, the lower twin pregnancy rate of the former approach would cause it to be more cost-effective than three embryo transfer *(117)*.

The concept of eSET in IVF practice, albeit in the context of clinical research, is not new. Much earlier than the current debate on two versus three embryo transfer, Frydman *et al.* questioned the place of alternative policies for embryo transfer in IVF programmes in 1988 and concluded that when three or fewer embryos were available following an IVF cycle, the deliberate limitation of fresh embryo transfer to one embryo followed by one or more cycles of frozen-thawed embryo transfer is not detrimental to the pregnancy rate and is less likely to be associated with multiple gestation *(118)*.

However, the first prospective randomized trial comparing elective single embryo transfer with double embryo transfer was not published until 1999 *(119)*. It was shown that by using single embryo transfer and strict embryo selection criteria in a good prognostic group of women aged <34 years and in their first IVF/ICSI treatment, an ongoing pregnancy rate similar to natural conception in fertile couples can be achieved. In this pioneering trial from Belgium, 26 single embryo transfers resulted in a 42% implantation rate, 38% ongoing pregnancy rate and one monozygotic twin pregnancy, whereas 27 double embryo transfers resulted in a 48% implantation rate, 74% ongoing pregnancy rate and six twin pregnancies. Further data from the Finnish group indicated that, when the extra pregnancies obtained from the subsequent frozen embryo replacements were added, the cumulative pregnancy rates per oocyte retrieval became 48% in elective single embryo transfers and 41% in double embryo transfers *(120)*.

The authors of the initial randomized study from Belgium later extended their research experience to comprise a series of 779 IVF/ICSI cycles *(121)*. Their findings from 111 single embryo transfers confirmed the previous outcome with similar ongoing pregnancy rates of 32% in single, and 34% in nonsingle embryo transfers. Multiple pregnancy was virtually eliminated in the previous group (<1% versus 37%). They also emphasized the importance of embryo selection in elective single embryo transfers as ongoing pregnancy rates dropped from 36% to 13% when the transferred embryo was not “top grade”.

The second prospective randomized trial addressing the same issue was presented by Tapanainen *et al.* in the ESHRE Campus Course Report *(115)*. They reported similar implantation rates both for elective single and two embryo transfers in their selected study population (31% versus 30%, respectively), likewise the cumulative pregnancy rates after fresh and cryopreserved embryo transfers were comparable in both groups (46% versus 53%, respectively). However, the price of this statistically nonsignificant difference was a 36% twin delivery rate in the two embryo transfer group as opposed to one twin delivery in the single embryo transfer group *(115)*.

In the same workshop *(115)*, De Sutter and Coetsier from Belgium further demonstrated that even after correction for the possible confounding effect of embryo quality, elective single embryo transfers achieved the same clinical pregnancy rates as elective double embryo transfers, reflecting the importance of endometrial receptivity: 42% versus 40%, respectively, in the excellent embryo group and 45% versus 43% in

the good embryo group *(115)*. This emphasized the previous observation that increasing the number of embryos for transfer to compensate for lower uterine receptivity only leads to higher multiple pregnancy rates without effecting the overall chances of pregnancy *(109)*.

Blastocyst transfer has been proposed as a possible means of restricting multiple pregnancies by replacing fewer but more competent embryos. However, this is yet to be demonstrated in clinical trials. Unresolved issues, such as criteria for selection of the most viable blastocysts, the possibility of failure to produce blastocysts in extended culture and the likelihood of fewer embryos for cryopreservation, mitigate against routine introduction of this intervention at the present time *(122)*. Furthermore, the paucity of published data on elective transfer of one blastocyst hinders attempts to provide conclusive recommendations.

Superovulation and intrauterine insemination

Unlike the case with IVF, strategies for the prevention of multiple pregnancies in superovulation (SO) treatments have not been defined. Evans and his coworkers evaluated 220 cases of triplets or higher-order pregnancies over an eight-year period and concluded that the number of cases with triplets and quadruplets as compared with quintuplets or greater showed an improvement for ART but not for ovarian stimulation *(20)*. In its published guidelines, the Royal College of Obstetricians and Gynaecologists highlights the inherent risks of administering gonadotrophins and recommends careful monitoring of stimulated cycles *(123)*. Gleicher and others have, however, reported that current guidelines for monitoring may not be enough to control the rate of multiple pregnancy *(21,22,124)*. In their large retrospective cohort study of 3347 gonadotrophin-stimulated ovarian cycles, Gleicher *et al.* found that the peak serum estradiol concentration and the total number of follicles were independent predictors of high-order multiple pregnancy *(22)*. However, the number of follicles with a diameter of 16 mm or more were not correlated with the risk of developing this complication. Since the total number of follicles is often difficult to determine by ultrasound, and since the number of large follicles did not have the predictive power to be clinically useful, sonographic assessment of the ovarian stimulation was not found to be valuable in reducing the risk of multiple pregnancy. Furthermore, peak serum estradiol concentrations (5084 pmol/L), which were much lower than their current cut-off point for cancellation (7342 pmol/L), were significantly associated with the occurrence of multiple pregnancy. Beyond this cut-off point, where the risk of high-order multiple pregnancy was significantly higher, the probability of pregnancy also started to exceed that of no pregnancy. Therefore, it becomes evident that associations between ultrasonographic and hormonal parameters of ovarian stimulation and the clinical outcome were not clear enough for clinical guidance. The authors suggested that given the limitation of current guidelines and limited scope for developing better ones, the options to minimize the risks of multiple pregnancy were either to choose a regimen with milder ovarian stimulation or to consider IVF as a substitute. While the philosophy behind the first option would lead to natural cycle intrauterine insemination (IUI) or even IVF without ovarian stimulation, the second option would require a culture change among the clinicians and couples towards one embryo transfer.

In the context of superovulation, preovulatory vaginal ultrasound-guided aspiration of supernumerary follicles has also been advocated as an effective strategy to control multiple pregnancy rates *(125)*. However, critics would argue the place of this technique, which intends to rescue the SO/IUI cycle in the presence of multiple follicles, but refuses to convert the treatment to IVF/ET, which could provide

Table 5. Pregnancy outcomes by the number of embryos transferred (including frozen embryo transfers)

System	Live births (%)	Multiple births (%)	Stillbirths and neonatal deaths (per 1000 birth events)
One-embyo transfer	8.0	2.9	8.4
Two-embryo transfer	22.8	24.7	20.8
Three-embryo transfer	22.3	31.0	22.1

Ref.: Human Fertilisation and Embryology Authority (HFEA) data, *(15)*

Table 6. Singleton and multiple pregnancy outcomes

	Stillbirths and neonatal deaths (per 1000 birth events)
Singleton pregnancy	9.9
Twin pregnancy	43.8
Triplet pregnancy	59.6

Ref.: Human Fertilisation and Embryology Authority (HFEA) data, *(15)*

a higher level of clinical control over the prevailing risks of multiple pregnancy.

Conclusions

As multiple pregnancy imposes significant risks and several adverse outcomes on both maternal and fetal health, the most important concern in any ART must be a reduction in the risks of multiple birth at least to the level of natural incidence. In the context of triplets, this can be achieved in most women by transferring no more than two embryos at one time. This will not reduce the live-birth rate but will reduce the number of children born prematurely. The perinatal mortality rate associated with IVF would then decrease (see Tables 5 and 6), as would the significant risk of disability in the survivors. Better allocation of resources would minimize the inequity and a larger population could benefit from easier access to treatment. Similarly, with the increasing recognition of twin pregnancies as an adverse outcome, transfer of a single embryo is a feasible option that virtually eliminates the possibility of twins without compromising cumulative birth rates.

A move in emphasis from pregnancy rate per cycle to cumulative live births per patient as a measure of performance will challenge current practices but have a demonstrable effect on the health of children born from assisted conception technology. The adoption of such an approach will inevitably have an effect on the way treatments are planned, financed and valued *(126)*. This implies a paradigm shift in traditional beliefs, values and attitudes of patients and physicians alike.

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Outcome of multiple pregnancy following ART: the effect on the child

ORVAR FINNSTROEM

Introduction

This report focuses on multiple pregnancies, especially twinning following assisted reproductive technology (ART) and, to a limited extent, following ovarian stimulation, and the effects on the child. Comparisons are made with singletons and with twins or triplets born after spontaneous pregnancies. Since the first child was born after *in vitro* fertilization (IVF) in l978, the number of ART children has increased steadily. At present, in the Scandinavian countries, more than 2% of all children are born after ART (all techniques) and approximately 40% of these are the result of intracytoplasmic sperm injection (ICSI) *(1)*. This high rate of ICSI treatment, as part of ART treatment, has been achieved recently, thus few reports on the fetal, neonatal and subsequent effects of ART, specifically address ICSI. At present, slightly more than 20% of ART pregnancies result, in multiple births.

Since the introduction of IVF, ART has been accompanied by an increased number of multiple births due to the transfer of two or more embryos in the majority of cases. The incidence of twin births varies from 18% to 24% in large published national series and the birth of triplets or quadruplets from 3.2% to 5.7% *(1–6)*. At present, about 45% of all live-born children following ART are twins. Recently, the first report from the European Society for Human Reproduction (ESHRE) was published, based on treatments that started in 1997 in 18 European countries *(7)*. Major differences were found regarding the proportion of multiple births and considerably higher figures than given above were reported from some countries.

Over the past two decades, multiple births, especially twins, have increased in many countries. In about a third of the cases, this increase is the result of ART treatment and in another third the result of ovarian stimulation for non-ART purposes. Increasing maternal age is the third probable explanation *(8,9)*. Between 1971 and 1997, multiple births of all orders increased in epidemic proportions in the USA, for the same reasons *(10)*. The proportion of multiple births among infant deaths has increased accordingly *(11)*. Few centres or countries have programmes for reducing multiple births following ART, namely, reducing the number of embryos transferred. In Sweden, the proportion of twins following ART has been reduced slightly, from a peak value of 41% of liveborn children in 1991 to 36% in 1997. Triplets and quadruplets have almost disappeared, following a gradual change in professional policy according to which only two embryos are transferred in the majority of cases.

The rationale for trying to reduce multiple births after ART is obvious: all types of neonatal complications as well as their sequelae have been found to be more common in children born as the result of multiple pregnancies. However, the literature that specifically

Table 1. High-ranked studies showing delivery outcome. Ranking according to the Swedish Council on Technology Assessment in Health Care report Swedish Council on Technology Assessment in Health's high-ranked studies with delivery outcome following ART

National studies	Number of deliveries	Time period	Controls
Bergh *et al.* 1999 *(1)*	4517	1982–1995	1 488 053 (all deliveries in Sweden)
Gissler *et al.* 1995 *(6)*	1015	1991–1993	National data
von Düring *et al.* 1995 *(5)*	782	1988–1991	236 635 (all deliveries in Norway)
FIVNAT 1995 *(4)*	5371	1986–1990	National data
Friedler *et al.* 1992 *(3)*	1149	1982–1989	National data
MRC working party 1990 *(2)*	1267	1978–1987	National data

addresses the effect of ART on twins and triplets compared to controls is limited.

Sources for the present review

Relevant literature up to 1998 was reviewed extensively by a working group within the Swedish Council on Technology Assessment in Health Care *(12)*. Up to 1998, 9000 references dealing with ART were available in Medline, but only about 350 of these discussed the effects on the child. The following six aspects were evaluated in all papers: selection criteria in the experimental group; quality of the control group; quality of measurements; completeness of material; sources of error; and strategy of analysis. The greatest emphasis was on population-based studies (national data), and on hospital-based studies with control groups. However, the number of population-based studies was limited. Reports based on national data are listed in Table 1. This Council report has been published only in Swedish, but forms the basis for part of the present report. An overview of the literature was published by Buitendijk in 1999 *(13)*.

A Task Force was set up in Sweden in l996 with members from the National Board of Health and Welfare, the Swedish Society for Obstetrics and Gynaecology and the Swedish Paediatric Society (see Appendix). This Task Force has been responsible for conducting a Swedish national study on the effects of ART which has a number of unique characteristics. This is one of the largest studies so far where perinatal data following ART have been compared with national data and where information on the neurological outcome in comparison with controls is available. The study group included all children born in Sweden after ART.

All individuals in Sweden have a unique identification number used by health care providers. All deliveries are reported to the Medical Birth Registry at the National Board of Health and Welfare which has been in existence since 1973. This registry contains information collected during pregnancy, delivery and the immediate postpartum period. Medical data on the outcome of delivery and on neonates, such as birth weight, duration of pregnancy and diagnosis during the neonatal period are included. The quality of the registry has been assessed and the dropout frequency is low *(14)*. Malformations, including minor ones, are reported both to the Medical Birth Registry and to the Swedish Registry of Congenital Malformations, which started in 1965. All ART pregnancies from all 14 IVF clinics are also reported to the National Board of Health and Welfare, and a special ART register has been built up.

Perinatal data

The obstetric outcome of ART babies born between 1982 and 1995 (n=5856) was compared with all babies born in the general population during the same period, by using data from the Medical Birth Registry and the Registry of Congenital Malformations. The incidence of childhood cancer was investigated through the Swedish Cancer Registry. In some of the analyses, data were stratified for birth year, maternal age, parity and duration of infertility. The results have recently been published *(1)*. In this paper, information on children born in 1996–97 has been added (n=3179). Thus, in total 9111 children are included. Data on congenital malformations for the whole cohort 1985–1997 has been published previously *(15)*.

Follow-up study

For all children born between 1982 and 1995 after an

ART pregnancy, two control children were selected from the Medical Birth Registry, stratifying for sex, year of birth and birth hospital. To control for the high frequency of twins, another set of control children were chosen—all spontaneously conceived twins—two for each ART twin. There is no national registration of disabled children in Sweden but a search was made for ART children and controls in the registries of the regional rehabilitation centres where all children with more than minor disabilities are registered. These 26 centres cover the whole country and provide all disabled children and adolescents with free medical, psychological, social and pedagogical care. Reported diagnoses were converted to ICD 10 codes and categorized into 20 major groups. In case a child had more than one diagnosis, the most important of these was chosen. At the follow-up the age of the children varied from 1.5 to 17 years.

In addition, the national register of blind children or children with severely reduced vision was searched to find children with severe visual impairment. Reduced vision was defined as visual acuity of <0.3 using both eyes, or severely reduced visual fields. The results of these follow-up investigation have been published recently *(16)*.

Review of the state of the art: results of ART studies

Unless stated otherwise, different ART techniques are grouped together.

Delivery outcome

The evaluation is based on studies with some type of control material, either national registry data or studies with matched controls.

Preterm birth and low birth weight

The percentage of children with a birth weight below 2500 g varied between 21.3% *(1)* and 36.0% *(4)*, which is approximately seven times the national level in most studies. For children with birth weight below 1500 g, this varied between 6.0% *(1)* and 9.7% *(5)*, which is seven to ten times the national figure. The number of children with a gestational age below 37 weeks ranged from 23.6% *(1)* to 29.3% *(4)*, which is a six- to sevenfold increase. Details are shown in Table 2. Thus, the risk of preterm birth or low birth weight is increased considerably after ART pregnancies. A large proportion of the increased preterm births and low birth weights is explained by the high rate of multiple births although few studies have addressed this aspect. The Swedish study showed that 11% of ART singletons and 47% of ART twins were born before 37 weeks, a twofold and ninefold increase in risk, respectively, compared with the whole population. ART twins and their controls did not differ regarding the proportion of low birth weights or preterm births.

Perinatal mortality

In all population-based studies, the perinatal mortality

Table 2. Percentage of multiple births, low-birth-weight children, and preterm children following IVF in six national studies *(12)*

Reference group		Multiple births			Birth weight		Preterm birth	
		Twins	Triplets	>3	<2500 g	<1500 g	<32 weeks	<37 weeks
Bergh *et al. (1)*	ART	24.0	3.0	0.2	21.3	6.1	6.7	23.6
	Controls	1.1	0.02	–	3.8	0.8	1.2	5.1
von Düring *et al. (5)*	ART	24.3	5.7		25.9	9.7		29.6
	Controls	1.2 (all multiple births)			3.8	1.4		6.5
FIVNAT 1995 *(4)*	ART	22.4	4.2	0.2	36.0	5.6	4.8	29.3
	Controls		2.3					
Gissler *et al. (6)*	ART	21.7	4.6	0.2	30.6	7.0		24.7
	Controls		1.1		3.9	0.8		5.0
Friedler *et al. (3)*	ART	18.7	4.6	0.3	23.8	6.3		28.6
	Controls				6.4	0.7		
MRC working party *(2)*	ART	19.0	4.0		32.0	7.0		24
	Controls	1.0 (all multiple births)			7.0	1.0		6

has been reported to be higher after ART pregnancy, varying from two *(1,2)* to three *(5)* times the average figures. A number of hospital-based studies, as a rule based on a considerably smaller number of cases, do not find an increased perinatal death rate after conventional ART *(17)* or ART with cryopreserved embryos *(18)*. Several studies find no increase or only a slight increase in the perinatal death rate for ART singletons compared to controls *(1,2,3)*, although a few do *(19)*. The perinatal mortality for singletons and twins was 11.7 and 39.7 per thousand total births in a large study in the UK *(2)*, and 11.0 and 34.0 per thousand total births in the Swedish study. When ART twins and control twins were compared, no increase in the death rate was found *(1,3,20)*. Oliviennes *et al.* compared perinatal mortality for ART twins, twins conceived after ovulation stimulation or spontaneously and found no differences *(20)*. However, recently Lambalk *et al*. *(21)* found a somewhat higher perinatal mortality in twins born after assisted reproduction compared with natural twins. Roest *et al*. *(22)* specifically analysed the results for triplets and found a higher perinatal mortality among triplets than twins, and Friedler *et al*. *(23)* found similar results for triplets born after ART or ovulation stimulation. Although spontaneous triplets had a higher survival rate, the number of spontaneous triplets was small.

Thus the higher death rate among ART children is mainly, but not exclusively, dependent on the high number of multiple births.

Few studies give results for ICSI children compared with standard ART. No differences were found in two studies of limited size regarding the number of twins, gestational age, birth weight and perinatal mortality *(19,24)*. Similarly, few studies deal specifically with the effects of cryopreservation. No harmful effects have been discovered so far *(13,18)*.

Neonatal care

Preterm infants and twins, also full-term twins, have an increased neonatal morbidity and consume more neonatal care than full-term singletons. Few authors address this question with regard to ART. More ART infants, singletons or twins, had a hospital stay exceeding seven days compared with controls *(17)*. Tallo and co-workers reported prolonged hospital stay, increased need of oxygen and ventilatory support in ART children *(25)*.

Congenital malformations

Several studies discuss the malformation ratios, with conflicting results. In a large French prospective multicentre study, the malformation incidence did not differ from that of the general population in Europe or France *(4)*. However, the risk of neural defects was increased in comparison with French national data. A UK study of 1581 children born after IVF or gamete intrafallopian transfer (GIFT) did not show an increased malformation rate, but again the risk of central nervous system malformation was 2–3 times higher *(2)*. A smaller but later study in the UK found an increased risk both for minor and major malformations *(26)*. Finally, the Swedish study showed a frequency for any type of malformation of 5.4% inART children, versus 3.9% in the general population—a 50% increase. The frequency was 4.7% among singletons and 6.3% among twins (Table 3).

The difference between ART children and controls disappeared when the following confounders were taken into consideration: year of birth; maternal age; parity; multiple birth; and length of infertility. Both major and minor malformations were more numerous in ART children which is mainly due to the large number of twins. The following specific major malformations were significantly increased: neural tube defects including anencephaly; alimentary atresia; and omphalocele. During the later time period, 1995–1997, the incidence of hypospadias was also higher *(15)*. Regarding spina bifida, three out of six were from a set of twins; all six cases of anencephaly were twins; five out of seven cases of hydrocephalus were in twins, pointing to the increased risk of these central nervous system malformations in twins. ART and control twins did not differ regarding the frequency of malformations *(1)*.

Bonduelle and co-workers from Belgium compared ICSI children with national malformation data in several studies. The number of chromosomal aberra-

Table 3. Congenital malformations in Swedish IVF children born between 1985 and 1997 (Odds ratios and 95% confidence intervals for being recorded in the Medical Birth Registry) *(15)*

	Per cent	Odds ratio	95% CI
All children, *n*=9011	5.4	1.47	1.34–1.61
Singletons, *n*=5316	4.7	1.25	1.07–1.46
Multiple births, *n*=3795	6.3	1.08	0.93–1.25

tions transferred from the father increased after ICSI performed because of male infertility but the number of major malformations did not increase *(27)*.

Thus, it is very likely that there is an increased risk (but low in absolute numbers) of congenital malformations after ART. This can partly, but not solely, be explained by the high number of twins. Severe central nervous system malformations have been seen to be more common after ART in several studies, again probably due to the high number of twins. The observed increased risk of intestinal atresia is not restricted to twins. An increased risk for hypospadias is probably related to male infertility and ICSI treatment *(15)*, as well as the observed increase in the risk of chromosomal aberrations *(27)*.

Childhood cancer

Very few studies have investigated the question of childhood cancers and ART. However, there are a few reports from small databases, which point to an increased risk of congenital and embryonal tumours *(28,29)*. Two studies based on a large ART dataset have analysed the occurrence of malignancies *(1,30)* and neither of these found an increased risk. However, since the number of malignancies is so small, it is difficult to draw conclusions at the present time.

Neurological sequelae

Limited information is available on neurological sequelae and most studies are based on small hospital databases. A few well-controlled but small studies have not detected a difference at 1 to 2 years of age concerning development or neurological sequelae between ART children and controls *(31,32)* and in one of these studies, children born following cryopreservation were compared with children born after conventional ART or controls *(32)*.

D'Souza *et al*. *(26)* examined a cohort of 278 children (150 singletons, 100 twins, 24 triplets, four quadruplets) after fresh embryo transfer and controls at the age of four years. The percentages of mild and moderate disabilities were 2.1 in ART children and 0.4 in the controls and they were all from multiple births.

The Swedish follow-up study gave the following significant differences: 1.8% of the ART children were registered at rehabilitation centres in comparison with 1.1% of controls, and 2.3% of twins. Thus the ART children had a 70% higher risk of being registered at the rehabilitation centres for any reason. They also had a fourfold risk of being investigated at the centres for suspected developmental delay (0.39% versus 0.09%) and an almost fourfold risk of having cerebral palsy, (0.55% versus 0.15%) (Table 4).

Table 4. Cerebral palsy in ART singletons and twins and their controls. (Odds ratios and 95% confidence intervals) *(16)*

Incidence	Per cent	OR (95% CI)
Controls – all	0.15	
ART children – all	0.55	3.7 (2.0–6.6)
ART singletons/controls	0.37	2.8 (1.3–5.8)
ART twins/controls	0.70	0.9 (0.4–1.8)

For other disease groups there were no significant differences. There was a slightly increased risk for singletons versus controls, even after excluding preterm and growth-retarded infants. ART twins and controls did not differ, however. On the other hand, twins showed an almost fivefold increased risk of having a cerebral palsy syndrome compared with the whole control group. For singletons, the risk was more than doubled, (Table 4). The number of triplets and quadruplets was small in this study. Nine triplets (2.7%) and one quadruplet (4.0%) were registered at the rehabilitation centres. Keith *et al*. *(10)* state that triplets have a threefold increased risk of handicap in comparison with singletons, and an almost sevenfold increased risk of having cerebral palsy, which is the same magnitude as twins in other studies *(11)*. There was higher risk of severe visual disturbances reported in the ART group compared with the controls *(16)*.

Cognitive development and behavioural deviations

Information available on this subject is limited, and very little of it concerns children born after multiple births, therefore limited conclusions can be drawn. Many studies lack control groups. This subject is extensively reviewed in the chapter "Parenting and the psychological development of the child in ART families" by S. Golombok in this volume.

In two studies, cognitive development has been compared for ART and control children, although at a young age. No differences were noted *(31,33)*. Cederblad *et al*. examined 81 children at five to seven years of age comparing the cognitive test results with general Swedish data and found no statistical

difference *(34)*. Behavioural problems were also assessed in this study and compared with normative data. Sixteen per cent in the general population but none of the ART children showed behaviour problems. Golombok *et al*. *(35)* compared ART children, children born after donor insemination, adopted children and children born after natural conception at the age of 4 to 6 years. There were about 115 children in each group. No differences between the groups were noted, but the number of dropouts was high. Olivienne *et al*. *(36)* used questionnaires and telephone interviews with ART parents of 370 children between six and ten years of age. School performance was "good" for 92% of the children. Controls were not included.

Cook *et al*. *(37)* compared parental stress and child behaviour in families with twins conceived by IVF with families having naturally conceived twins. The children were, on average, five years old at follow-up. No differences were found in parental quality and child behaviour. ART parents reported greater stress associated with parenting in this small group of families.

Different ART techniques, protocols and methodology

It has not been possible in this review to distinguish results obtained with different techniques for ART and the effect on the child, with one exception: malformations following ICSI. Among studies on the effect of ART and hormonal stimulation for non-ART purposes on the child, few deal with results other than perinatal outcome. However, in this chapter I have attempted to describe aspects of short-term and long-term outcome based on the available studies. The best information is gained from a few comparatively large population-based studies.

Conclusions

The incidence of multiple births is still very high following ART and hormonal stimulation for non-ART and there is an increased risk of preterm birth following ART, mainly due to the high frequency of multiple births.

The perinatal death rate is doubled after ART, mainly due to an increased frequency of multiple births. ART twins do not seem to have a higher risk of perinatal death than other twins. There is, however, a moderately increased risk of congenital malformations in general following ART, partly due to the increased number of twins. There is probably an increased risk of the following malformations: neural tube defects; intestinal atresia; and hypospadias (following ICSI). There is evidence supporting that ICSI probably increases the risk of chromosomal aberrations transferred from the father when the technique is used for the treatment of poor semen quality

The risk of contracting a malignant disease does not seem to be higher in ART children but there is an increased risk of neurological sequelae in ART children. The magnitude of this increase depends upon the proportion of twins and higher-order multiple births, since multiple birth per se leads to an increased risk. The results presented in this chapter are based upon data with comparatively few twins and very few higher-order multiple births and therefore the numbers are small *(16)*. Bearing this in mind, there is a fourfold increase in the number of children cared for in rehabilitation centres in Sweden and a fourfold increase in the number of children with cerebral palsy born as a result of IVF in comparison with controls. There is a small increase in the risk for singleton IVF children versus controls, but not for IVF twins versus twin controls. However, twins born after IVF, ovarian stimulation without IVF, or spontaneously have a five- to sevenfold increased risk of cerebral palsy syndrome.

Cognitive development is probably not affected in ART children and they may have fewer behavioural problems at school age than their controls.

Triplets and multiple pregnancies of higher order should no longer be acceptable following ART. There is no scientific evidence that the transfer of more than two embryos enhances the likelihood of pregnancy *(38)*. Since the high frequency of twinning explains most of the increased risks following ART, an important aim should be to reduce instances where more than one embryo is transferred. The estimated costs per successful pregnancy after transfer of one embryo only are lower, even if more treatments cycles are needed *(39)*. Reducing the multiple gestation pregnancy rate should be a high priority for assisted reproductive programmes according to the ESHRE Capri Workshop Group *(40)* and others *(22)*.

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Appendix

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Multiple birth children and their families following ART

JANE DENTON, ELIZABETH BRYAN

Introduction

Couples who have tried for many years to have one child may think they would be fortunate if they had two or even three at once—thereby creating an instant family. Their mental picture may well be of two or three healthy, happy children. They rarely think of the medical risks to the children as well as to the mother herself. Even less do such couples consider the practical, financial and emotional stresses that often result from the demands of having two or more children of the same age.

Few parents who are told they may conceive twins or triplets after infertility treatment will have any prior knowledge of what is entailed in the care and upbringing of multiple birth children. Professionals themselves, including infertility specialists, often fail to recognize the problems and stresses because they may have dealt with few such families.

In the UK, assisted conception clinics providing *in vitro* fertilization (IVF) services must be licensed by the Human Fertilisation and Embryology Authority (HFEA) and the Code of Practice *(1)* by which they must abide, specifically requires information on the risks and implications of a multiple pregnancy to be given before treatment is commenced.

Background

The incidence of twin births has been steadily increasing in all developed countries since the early 1980s *(2,3)*. The incidence of triplets has been rising much faster. In addition to the increase in the number of multiple births, there has been an even greater rise in the number of multiple birth children who survive due to the considerable recent improvements in neonatal care. The increase in multiple births is known to be largely due to the widespread use of ovulation induction and multiple embryo transfer in the treatment of subfertility. However, East Flanders in Belgium is the only region that has so far provided accurate data on the origin of all multiple births *(4)* and has done so since 1964. In most countries, as in the UK, accurate data on conception are only available for those multiple births that arise following IVF or gamete donation. A single year's survey of triplets in 1989 showed that approximately one-third were spontaneously conceived, one-third followed ovulation induction alone and the remaining third arose from IVF or genetic intrafallopian transfer (GIFT) *(5)*. More recent estimates suggest that a larger proportion of triplets arise from IVF than from ovulation induction. Such data have never been available in relation to twins.

Treatment by IVF has had statutory regulation by the HFEA in the UK since 1991. The HFEA Code of

Practice requires the number of embryos that can be transferred in one IVF cycle to be limited to three. Nevertheless, the high and increasing incidence of multiple births in the UK, as elsewhere, continues to cause concern. The multiple birth rates in the most recent *HFEA Annual Report (6)* showed 27.3% (3.3% triplets) following IVF, 6.4% with donor insemination and 26.9% with micromanipulation. As long as three or more embryos continue to be transferred, triplets will occur—and even quads. Either may include a monozygotic (MZ) pair. The MZ twinning rate is probably several times higher among pregnancies involving ovulation induction *(7)* or micromanipulation *(8)*.

Problems of multiple births

Whatever their hopes may be, no couple expects to have twins even when they have a two or three embryo transfer. Some couples, of course, would welcome the idea but even among those couples who have been warned of the risks, the diagnosis of a multiple pregnancy frequently comes as something of a shock. Pretreatment preparation is often inadequate *(9)*. After a multiple pregnancy is diagnosed, there is frequently a lack of information and advice, resulting in many couples suffering unnecessary anxiety.

Many professionals, having had little or no experience with twins themselves, are not sufficiently aware of the special needs of families who have them. For a childless couple, the practical and emotional difficulties of caring for two or more babies at the same time may be particularly difficult to imagine. A survey by the British Fertility Society and CHILD, a UK patient support group, found that 66% of respondents felt that twins would be an ideal outcome of IVF treatment *(10)*. Older women, in particular, may feel that this is the only chance to have two children. Nevertheless, it has been shown that parents do not have realistic expectations of how the birth of twins will affect their family *(11)*. Mothers are often unrealistically optimistic about the likely outcome. Many are unprepared, for example, for the impact the birth of twins can have on the relationship with their partner. The father may be genuinely surprised by how very much his help is needed with the baby care and may not understand how much the mother's attention and emotions will now be focused on the children. A preliminary study of couples who have twins following IVF indicated that they may find parenting considerably less rewarding than they had expected *(12)*.

Studies have shown that both parent satisfaction and parenting skills with IVF singleborn children are, if anything, superior to that with spontaneously conceived children. Perhaps not surprisingly, as these were all much wanted children, unlike some in the general population, but the situation may be different with twins. In a small study comparing families with IVF twins and spontaneously conceived twins, Cook *et al. (12)* found that parenting stress for both mothers and fathers was greater in the IVF group although the quality of parenting was equally good. Parental satisfaction, however, was less. This may well be due to the inevitable failure to reach the high standards of parenting they had set themselves and had for so long expected to achieve *(13)*. Colpin *et al*. found that first-time mothers of one-year-old twins with a history of infertility obtained significantly higher scores for parental competence and health and showed lower psychosocial well-being compared with naturally conceiving first-time mothers *(14)*. Even with singleborn children, IVF mothers have claimed to feel less competent than those who had conceived spontaneously *(15,16)*.

An alternative explanation for the higher stress in IVF parents of twins could be that more parents of spontaneous twins had previous experience of parenting. Studies on larger numbers are urgently needed.

Pregnancy

Both would-be parents of twins and their professional carers need to be clear that a multiple pregnancy is more likely to be associated with medical complications such as pre-eclampsia, anaemia, polyhydramnios, preterm labour and a difficult delivery. In addition, the mother is likely to suffer from tiredness, indigestion and general discomfort far earlier than with a single baby. The severity of the symptoms may mean that the mother has to stop work early, thereby using a disproportionate amount of her total maternity leave allowance before the babies are born.

The fetus in a multiple pregnancy is also at greater risk from conception onwards.

Vanishing twin

The miscarriage rate is higher than in singleton pregnancies but more common still is the survival of only one twin and the loss of one fetus (or more)—the vanishing twin syndrome. Accurate figures for the

rate of this event are not available but recent first-trimester ultrasound studies suggest that over half of twin pregnancies may end up as singleton births *(17)*. The effects of these early intrauterine deaths on the mother or the surviving twin are still not clear.

Multifetal reduction

As the number of higher multiple pregnancies has escalated, so have the number of couples who feel they should maximize the chances of having a healthy baby or twins by reducing the number of viable fetuses. A few sets of quintuplets and sextuplets, as well as many triplets and quadruplets, have been born healthy and lived happily. Nevertheless, some couples expecting higher multiples with all the risks and problems involved, see a real dilemma in which a fetal reduction will seem the least bad option. Fetal reduction is never an easy or uncontroversial solution and carries its own risk of medical and emotional complications *(18)*.

The balance of risks and advantages will be seen differently by each couple but for all there will be a sense of responsibility and much anxiety. Not surprisingly, partners themselves sometimes disagree. One or both may have deep religious objections. One, often the mother, may be distressed at the thought of disposing of a potential baby of hers whereas the father may be equally distressed by the idea of having a disabled child. Partners are almost bound to disagree to some extent, at least. There may often be a need to compromise as to what is best for them as individuals, as a couple and as a family. Both partners will need to weigh carefully and sensitively the arguments on both sides.

For many couples the overriding aim will be the safe birth of the one healthy child they originally sought. Their next biggest concern will be the health and welfare of any surviving twins or other children. Parents' concern about the physical, emotional and financial demands will vary greatly between couples and will not necessarily correlate with their socio-economic status *(19)*.

Even where the couple has decided to undergo a reduction, they may well be distressed by the seemingly (and often actual) arbitrary choice as to which fetus should live and which should die. Some parents will feel a lasting grief and guilt over the death of one or more potentially healthy children. Nevertheless, it appears that the great majority still feel that they had made the right decision *(20)*.

As yet, no studies have been reported on the long-term psychological effects on parents and the surviving children. This is partly due to it still being a fairly recent procedure. However, it may also in part be due to parents' reluctance to discuss their feelings. In contrast to studies on perinatal bereavement, a substantial number of parents decline to participate in follow-up studies, being reluctant to talk or write about their feelings and personal experience *(19)*. Several highly sensitive questions are of course involved. Any survivor could feel their own survival was at the expense of a sibling. They could also see their parents as "murderers" or their own existence as arbitrary and their own individual value as therefore impaired. Another key question is whether the parents should ever tell the survivors about the fetal reduction or keep it as a life-long secret. In the short term, at least, the surviving children appear to be developing normally and the parents rarely appear to regret their decision.

The Multiple Births Foundation (MBF) publishes a leaflet for couples faced with the option of fetal reduction and can also give more individual advice about dilemmas that are bound to be difficult and painful and to which there cannot be an ultimately painless resolution.

Perinatal mortality

Despite improvements in obstetric care, the risks to a fetus remain significantly higher throughout a multiple pregnancy and the stillbirth rate for twins is about three times that for singletons.

The perinatal mortality rate in twins is nearly five times higher than that in singletons. In triplets, it is seven times higher. The main contributor to the high death toll and rates of disability in multiple births is prematurity and its complications. The neonatal mortality rate is about ten times that of singletons *(21)*.

Parents who lose a twin or one or two of triplets and still have a surviving multiple, face special problems *(22)*. They have a constant reminder of the dead child in the surviving child—especially with MZ twins. Parents of multiple infants also lose what many of them see as a proud status and their bereavement is often underestimated by other people who may indeed tell them that they are fortunate to still have a surviving baby. These factors further inhibit the grieving process which has already been delayed by their inevitable preoccupation with the surviving child.

The loss of babies from a higher multiple set can be particularly difficult. After many years of infertility, a mother may suddenly have three, four or more live babies but then see one, two or more of them die soon after birth or die one by one over what can be many painful months. Alternatively, all the babies may miscarry before they are viable. Despite these deaths, a couple who is left with one or two babies will receive remarkably little sympathy about the death of the others.

Some couples have to cope with their grief over the death of one (or more) babies while also having to face the daily difficulties and emotional strain of caring for a disabled child at the same time. In one set of quads, conceived following GIFT, after 12 years of the parents trying for a baby, the babies were born 14 weeks early. One died after six days. The second died after six months, having never left hospital. The third was severely disabled and the fourth proved to be a bright child but very small. The strain on the family was inevitably enormous and the marriage broke down after four years.

Neonatal problems

Preterm delivery and low birth weight are the main causes of the increased morbidity and mortality in the neonatal period. The average duration of pregnancy is 37 weeks for twins, 33.5 for triplets and 31.5 for quads. Half of the quadruplets who are born weigh less than 1500 g compared to a quarter of triplets, one in ten twins and one in a hundred singletons *(23)*.

Costs

The price of multiple births can clearly be high for both the parents and for the children themselves. However, a considerable price has also to be paid by the health and social services for the extra burden imposed by the multiple pregnancy and its outcome. In the early period, especially, both the mother and the children are likely to consume far more resources than the average mother or singleborn child.

The cost of obstetric care of a multiple pregnancy is inevitably high in terms of hospital stay, extra investigations and the likelihood of operative delivery. It has been calculated that the costs of twin, triplet and quad pregnancies compared to single ones are 2.1, 4.5 and 7 times greater, respectively *(24)*.

Preterm infants, as such, add a huge burden to the neonatal services through the inevitable technical, pharmaceutical and staffing requirements. Furthermore, high multiple births often use up most or all the limited number of available neonatal intensive care cots. If the preterm delivery of triplets or quads is expected in a hospital those cots must be kept free thereby preventing the admission of ill singleton babies.

The average financial cost of neonatal care for a twin infant has been estimated to be 13 times that for a singleton, with a triplet costing 41 times and a quadruplet 77 times that of a singleborn infant. This means that, for the neonatal care alone, the infants from a quadruplet pregnancy cost 308 times more than that from a singleton pregnancy *(25)*.

Inevitably the drain on the health services continues. The babies are more likely to have special needs and these will require ongoing paediatric care and other therapies as well as special education. An idea of the costs involved can be derived from studies of low-birth-weight children in general. It was shown that the health care, up to eight years, of a low-birth-weight (<2000 g) child costs, on average, five times as much as that of a normal-birth-weight child. For a disabled low-birth-weight child the cost was 17 times greater. The total cost of health care and education up to the age of eight for a low-birth-weight child who survived with a long-term disability was four times that of a low-birth-weight child with normal development and nine times that of a normal-birth-weight control *(26)*.

In the UK and some other countries, the twins and triplets born following assisted reproductive technology (ART) in private clinics operating on a commercial basis are as likely as any others to become a practical and financial burden on the publicly funded neonatal, paediatric, educational and social services. This situation is a legitimate area for public policy debate.

Separation

In addition to the cost, higher multiples pose serious logistical problems to the neonatal services. Risks are run not only of needing to transfer several very preterm infants but of distributing the infants to two or more hospitals. This often leaves the mother in the original hospital separated from all her babies and unfit to travel. One or more of the infants may die before the mother even touches them.

Zygosity determination

In the past it was usually assumed that infants conceived following ovulation induction or multiple embryo transfer must be dizygotic (DZ). It is now recognized that although the majority of iatrogenic multiple pregnancies will be DZ twins or trizygotic triplets, the chance of MZ twinning either in twins or within a triplet set is not only equal to but greater—probably about threefold—than would be expected in the general population *(7)*.

Development

Although the development of most multiple birth children is within the normal range, for both medical and environmental reasons, these children do face a higher risk of long-term disability and learning difficulties. In addition to the risks associated with low birth weight and prematurity, the problems of having to constantly share the attention of their mother can affect their development, particularly in relation to language *(27,28)*. Twin children have also been found to have less good concentration and a higher incidence of attention deficit hyperactivity disorder *(29)*. Furthermore, depression, more commonly seen in mothers of preschool twins than in mothers of singletons, could be an additional factor *(30)* as maternal emotional well-being is known to influence development.

Disability

The parents of multiple birth children, by definition, face not only twice (or more) the risk of one of their children having a disability, but there is also a much increased risk per child. This is because many forms of disability, particularly cerebral palsy, are much more common among twins. Those with a monochorionic placenta, as occurs in two-thirds of MZ twins, will be at particular risk from intrauterine damage associated with a haemodynamic imbalance in their shared blood circulation *(31)*. Prematurity and intrauterine growth retardation also increase the risks.

Population studies have shown a threefold to sevenfold higher incidence of cerebral palsy in twins compared to singletons: it is over tenfold higher in triplets. Of course the chances of any particular multiple pregnancy producing a disabled child is much greater still *(32)*.

The care of a child with special needs always brings challenges for parents: these are increased by the difficulty of caring for other children of the same age, but with very different needs *(33)*. The parents, and often the child too, have a constant reminder in the unaffected child of how they both might—indeed should—have been. Moreover, the special status of having or being a twin is effectively lost if the twins look very different.

The healthy child may also suffer emotional disturbance. He or she may feel guilt about escaping the affliction but at the same time may constantly feel jealous of the time and attention given to his or her twin.

Siblings

A little recognized problem arising from the arrival of multiples is the effect on other children in the family, particularly on the single toddler who has been the centre of the family until suddenly displaced by an attention-attracting pair or trio. It has been shown that a sibling is likely to be more disturbed by the arrival of twins than of a single sibling and that behaviour problems in the older child are more common *(34)*.

Psychosocial stresses

It is only in the past decade that information about the lives of families with triplets and higher-order births has become available. This emerged primarily from a series of linked surveys called the United Kingdom National Study of Triplets and Higher Order Births. The population-based project was undertaken by the Office of Population Censuses and Surveys with the National Perinatal Epidemiology Unit and the Child Care and Development Group at Cambridge University. The study covered medical and social aspects, from the time of conception, of over three hundred families with higher-order birth children born in 1980 and 1982–1985 *(35)*.

The report demonstrated that the practical difficulties alone of looking after three babies at once are huge, even when all are healthy. At the most simple level, no mother can carry three babies at a time. Only with the greatest difficulty can she feed or transport them on her own. These plain facts produce all sorts of stresses and complications. Many mothers

cannot take their babies out of the home and so become housebound and isolated as a result.

The UK study repeatedly found that help for families, both statutory and private, had been inadequate in amount and often slow to arrive. Too often the parents became ill and exhausted before help was provided. A mother simply cannot look after three babies on her own—there are not enough hours in the day. A study by the Australian Multiple Births Association showed that 197.5 hours per week were required to care for six-month-old triplets and to carry out the necessary household tasks—but there are only 168 hours in a week *(36)*.

It is sometimes assumed that families with triplets become rich through commercial sponsorship. The UK study disproved this belief. Although 75% of families with triplets received some local or national press coverage, few derived financial benefit from it. Only with sets of five or more healthy babies was substantial sponsorship forthcoming and for that the price was the loss of any remaining privacy.

Few people appreciate the very great financial cost of triplets to the families compared with that of three single children. A new car and house may be needed urgently. Clothes and equipment have to be bought at once for all three. They cannot be handed down. Unlike a family that grows gradually and can plan and save, most of a triplet family's expenditure has to be immediate. Furthermore, an otherwise working mother is likely to take longer to get back to work and the father may well be torn between working extra hours and spending more time helping at home. A similar but less extreme financial situation is met by the parents of twins.

Increasingly the clinicians providing treatment for infertility are aware of the many medical and psychosocial disadvantages resulting from a twin conception, let alone that of a higher multiple. Nevertheless, it appears that the couples themselves are less able to perceive the problems. Several studies have shown that the couples are now becoming aware of the chances and of the implications of a multiple pregnancy but, despite this knowledge, many are still fully prepared to take the risk. Gleicher *et al. (37)* found that in the USA 67% of couples undergoing infertility treatment would "love to have twins" and less than 10% would have been "upset to have twins". Indeed 50% would not have been upset to conceive triplets. Similarly, Murdoch *(41)* found that in the UK, 69% considered that twins would be the ideal outcome of their treatment.

Help

All the economically advanced countries are reporting increasing numbers of children from higher-multiple births but assistance from public health and social services has generally been inadequate and very patchy. It varies greatly in quantity and quality between different countries and regions. For example, no help is automatically provided in the UK whereas in New Zealand and Belgium help is routinely offered by the state. Regular practical help is needed by nearly all families during the early years as the extended family can only rarely provide the amount needed and may well be daunted by the challenge. In some cases, grandparents have become overwhelmed by the task and moved away *(35)*.

Few, if any, countries give adequate financial and practical help from statutory sources to multiple-birth families. Many parents have to spend time and emotional energy striving to get any of the help they need.

Experiences of the Multiple Births Foundation

The MBF was established in the UK in 1988 as the first organization to offer professional support to families with twins, triplets and more, as well as information, advice and training to the many medical, educational and social work staff concerned with their care.

Initially the work focused largely on helping families, through Twins Clinics (and also special ones for higher multiples), the Telephone Advisory Service, parent evening meetings and literature. The evening meetings focus on particular topics such as prenatal preparation, language, behaviour, individuality and schooling. The Telephone Advisory Service provides booked consultations at which information, advice and support are given to parents. It is also given to adult twins, including those who have been bereaved.

One charitable organization can never itself meet the needs of all families. It is anyway much more appropriate for the family's own professional caregivers to advise locally. Therefore, the MBF is now concentrating increasingly on the education of professionals including doctors (infertility specialists, obstetricians, paediatricians and family doctors), nurses (midwives and community), social workers and teachers. It does this through a teaching programme and by offering the services described, including the

Telephone Service, as models for others to follow.

Future plans include working closely with midwives and health visitors (community nurses) to develop models of good practice that will be disseminated nationally and internationally with the MBF acting as a specialist information and advice centre.

With financial assistance from the European Union, a series of five *Guidelines* for professional caregivers have recently been completed for the care of multiple-birth families from before conception through adolescence *(38)*.

Services provided worldwide

Most European countries as well as Australia, India, Indonesia, Japan, New Zealand, Nigeria, Sri Lanka, Republic of Korea, Russian Federation and the USA now have some form of association for parents of twins and for twins themselves which provides information and mutual support. A national organization will often act as an umbrella for the local groups or clubs. In the UK the Twins and Multiple Births Association (TAMBA) also provides a Telephone Helpline and a number of subgroups including ones for bereaved families, for those with a twin with special needs, and those with "supertwins" (triplets and more). Another group provides for parents whose children were conceived following treatment for infertility. This latter group has links to infertility clinics and members are available to talk with those considering treatment which may result in multiple births. They also produce a newsletter, keeping members in touch with each other.

The financial and practical support provided for multiple-birth families from government sources varies greatly from country to country and even within a country.

Information for couples considering treatment for infertility

The Royal College of Obstetricians and Gynaecologists (RCOG) *Guidelines (39,40)* and those of the British Fertility Society give detailed information on the chances of a multiple pregnancy. The HFEA Code of Practice requires information to be given about the risks associated with multiple pregnancy and the possible practical financial and emotional impact of a multiple birth on the family and this should be distinguished from counselling *(1)*.

The HFE Act stipulates that counselling must be offered to all those seeking licensed treatments and understanding the implications of the proposed treatments is defined in the Code of Practice as part of the counselling process. Counselling is strongly recommended but couples are not obliged to accept the offer.

Fetal reduction should be discussed in advance with those whose treatment could result in the conception of triplets or higher-order births.

Written information should always be provided as well as an invitation to a further discussion. A leaflet "Multiple Pregnancy and Multiple Birth" specifically to inform couples about the risks and implications of a multiple pregnancy has been produced by the MBF *(42)*. This is freely available in all infertility clinics and should be promoted by any clinicians, including general practitioners, who are offering ovulation-inducing therapy.

A visit to a family with multiples can sometimes be more enlightening for a couple than several sessions with a counsellor! But, however careful and thorough the counselling, it can be difficult for couples themselves to make a rational decision about the number of embryos to be transferred, when they are under emotional stress in their long and painful quest for a baby. The decision should therefore be a joint one between the clinician and the couple with the final responsibility being taken by the clinician to minimize the risk of a higher multiple pregnancy.

Conclusion

For a childless couple the practical and emotional difficulties of caring for two or more babies at the same time may be particularly difficult to imagine. Whatever type of treatment is planned couples therefore need to be seen together, not only to be given factual information on the risks and implications of a multiple pregnancy, both medical and psychosocial, but to receive counselling on the possible implications for them as individuals and as a couple. This should include not only the problems associated with the pregnancy and of having a preterm and low-birthweight infant, but also the likely psychosocial costs of parenting multiples.

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Section 4

Social and psychological issues in infertility and ART

Consumer perspectives

SANDRA DILL

Introduction

Most people take for granted their ability to have a child. Some choose not to but most of those who try to have a child have no difficulty in achieving that goal. However, for between 13% and 24% of couples who would like to have a child but are not able to, it can be a very painful experience and one difficult to manage *(1–3)*.

Infertility is an extremely isolating experience. This is exacerbated because infertility and the death of a child are taboo subjects. As a society we have difficulty in dealing with these sad experiences. Infertile people need medical and social choices to help them deal with infertility. Some pursue adoption and for over 20 years, assisted reproductive technology (ART) has provided *in vitro* fertilization (IVF) and related treatments as another way of overcoming infertility and childlessness.

The limited recognition of infertility as a disease or medical condition

Governments worldwide have demonstrated a reluctance to acknowledge that infertility is a disability or medical condition (Appendix A). In most countries, infertility treatment is viewed as an elective procedure and therefore not worthy of reimbursement. In Bangladesh, despite the fact that infertility is considered a curse that brings couples bad luck, the majority is unaware of the possibility of treatment (Appendix A). The need to have access to health care is balanced against the need for governments to responsibly manage scarce resources and to distribute them justly and equitably for the good of the whole community. The challenge for consumers of infertility services is to persuade governments that infertility is a medical disability which causes suffering and, as such, is worthy of inclusion in their national health plan. This is one of the objectives of the International Consumer Support for Infertility (iCSi) network which brings patient leaders together to discuss common interests and concerns. There are also national patient associations in many countries which provide support for infertile people and advocate for access to affordable infertility treatment. The International Federation of Infertility Patient Associations (IFIPA) is another international group, whose membership is made up of national patient associations.

The emergence of patient support networks worldwide

Like any life crisis, infertility can be best understood by those who have experienced it. Therefore, infertility self-help groups play an invaluable role, as there is

comfort in speaking with someone who really understands. It can ease the feeling of isolation. Many infertility self-help groups have been established around the world since the early 1980s (Appendix A). These groups seek to provide information to infertile people, their families and friends and also to government, media and the medical and scientific community. The iCSi network is a global family of patient leaders from support associations in more than 30 countries and strives to expand the number of countries reached each year, particularly to include developing countries. New contacts have been established recently with patient leaders in Bangladesh, India and Japan.

Equity of access to ART

The United Nations Declaration of Human Rights recognizes that, "Men and women of full age, without any limitation due to race, nationality or religion, have the right to marry and found a family" *(4)*. This is supported by the European Convention on Human Rights which guarantees respect for family life and the right to found a family *(5)*.

It can be argued that these provisions create a positive right to access ART to achieve this goal, one taken for granted by fertile people in the community. For those who need medical assistance to form their families, infertility causes immense suffering. For those who finally remain without a child, infertility can be a lifelong disability.

The objective of a health system is to deliver health care to all those in need. However, in some western countries, the limitless demand for health care can often not be met due to the scarcity of resources to service it (Appendix A). This has been exacerbated by an ageing population and costly advances in technology which have exceeded our ability to pay for them. Therefore, the need for rationing or micro allocation of health resources becomes apparent. No system of allocating limited resources at the level of the individual patient can work without resorting to notions of utility. While rationing is a necessity, it is important that the system used to decide who gets health care be one that promotes equity of access between people with health needs.

The question "*Who shall be eligible for assisted reproductive technologies?*" signals the onerous task of health professionals and governments to allocate scarce resources equitably. This raises the question of what criteria can be used to distribute resources fairly.

A utilitarian perspective, which argues that justice involves trade-offs to ensure that the greatest good can be delivered, can present a conflict for the medical practitioner who seeks to act in the best interests of the patient *(6)*. Notions of utility are inevitably resorted to when practitioners make decisions to ensure that the maximum benefit can be obtained for the greatest number of those in their care.

Many criteria are used in deciding which patient will receive health care. It has been argued, as in New Zealand, that determining an initial eligible pool of patients based on substantive standards and procedural rules is preferable to the decision-making process being left to the final selection of an individual for a particular procedure *(7)*. However, this process does not remove the possibility of value judgements impacting on selection. Governments in some countries that reimburse ART treatment, such as Austria, France and the United Kingdom, impose age criteria. Israel is currently debating this issue (Appendix A).

Methods of rationing that introduce notions of utility can use medical or social criteria. The use of social criteria is necessarily subjective, arguably immoral and is contrary to the principle of individual autonomy. However, it is difficult to see how those making decisions about rationing resources can avoid such judgements. Value judgements can be made based on an individual's past and potential contribution to society or, in the case of ART, on old-fashioned prejudices masquerading as new ethical dilemmas *(8)*.

For example, there has been discussion about whether it is ethical to allow single women, lesbian or homosexual couples access to ART. Many believe that this is morally wrong, arguing that it is preferable for a child to be raised within a stable, heterosexual relationship. Whatever our personal views, those who argue that the traditional concepts of family should be maintained, fail to recognize a different reality. An Australian government statistical report found that 69% of households had no children, 32% of households comprise two persons, 19% had two or more children and 13% of households had one child. Marriage rates continue to fall, divorce occurs in more than 40% of marriages and 27% of births were to single women *(9)*. These figures demonstrate the diversity of family arrangements that can exist.

It is important to distinguish public funding from legal access to health services in situations where people without a medical condition could pay for

needed services, irrespective of the social choices that they have made.

Decisions about who will access health care resources can be complex and difficult. The scarcity of resources available to meet the needs of everyone seeking them compels health professionals and governments to make decisions about which individuals should have priority access to them and we are mindful that there is "very little distance between policy and politics" *(10)*. While this dilemma may be a practical necessity, it is important to be aware of the moral conflict and to aspire, wherever possible, to a deontological perspective such as the Kantian ideal or the biblical exhortation, of treating others as we would like to be treated if we were in their circumstances.

Success rates—how can we be sure of treatment quality?

Success, like happiness, can be different things to different people. For some, success of IVF is a confirmed pregnancy, for others it is a healthy baby nine months later and some may suggest that success is a few years down the track when your child is enrolled in medical or law school.

In considering what success means to consumers of IVF services the familiar model of the Human Fertilisation and Embryology Authority (HFEA) in the UK provides a perspective. ISSUE, the National Fertility Association in the UK, has reported on the HFEA publication, *The patients' guide*, claiming that the practice of publishing success rates of identified clinics has impacted on the range of treatments available. An example cited was of one clinic that ceased to offer natural cycles because they impacted negatively on their success rates (personal communication with ISSUE, CEO, 1998). As a result, consumers are denied access to a less invasive treatment because of the commercial impact caused by the misleading way in which success rates have been reported. While explanations are given for the way statistics are reported, they have little meaning for most consumers trying to make a decision about where they should go for treatment.

When the patrons of ISSUE were asked about the *Patients' guide (11)*, Professor Ian Cooke from the Jessop Hospital for Women in Sheffield, UK found that there was very little information about the statistical data, making it vulnerable to the media to rank clinics, ignoring all statistical ranges. He found that the data from a very large number of clinics did not differ significantly.

Doctor Peter Brinsden from the Bourn Hall Clinic in Cambridge, UK said that while the *Guide* was a valuable source of information, he was not in favour of league tables. He noted that while the HFEA did not rank clinics, the press did, and this had had unfortunate consequences for some clinics whose effectiveness had been misrepresented.

Doctor Simon Fishel from CARE at the Park Hospital in Nottingham, UK commended the section with suggested questions for prospective patients to ask clinics but he also found a real problem with the data. He questioned the fairness of the adjusted live-birth rate as there was no information about the formula used to determine this. He also identified a significant disadvantage in the way clinics could manipulate these figures by driving a certain kind of practice that may be more "successful" rather than being concerned about specific treatment that could be tailored for the individual couple. He suggested that age divisions would provide more relevant information for couples considering treatment.

How then can success be determined?

The conflict of clinics to provide the best information for their patients while presenting their units in a positive light has been discussed, as has the problem of how best to present that information *(12)*. The inadequacies of the simplistic scenario of dividing the number of pregnancies by the number of patients who underwent treatment have been improved by a method of identifying a monthly pregnancy rate and cumulating the outcomes *(13)*. This has been further developed by expressing results as a "life table analysis" which has become an accepted method of reporting results for donor insemination *(14)*, ovulation induction *(15)*, and IVF *(16)*. This provides information to couples about the prospects of success over a specified number of treatment cycles. However, many variables remain including questions about what is a pregnancy. Should success be regarded as a positive beta-hCG test 14 days after treatment? Or it could be when a fetus is visible on ultrasound—but this includes ectopic pregnancies and early miscarriages. Is success determined when a normal fetal heartbeat can be detected? Is it more realistic to express success as a live birth, often referred to by consumers as the THB or "take home baby" rate?

Success rates can seem better if expressed "per transfer" rather than "per oocyte pick-up" (per OPU) or "per cycle commenced". Other variables include

- that the probability of success is higher in the first few cycles so programmes with new patients will have higher success rates;
- younger women are more fertile;
- multiple pregnancies increase reported success rates;
- cancellation rates have a negative impact on success rates; and
- the number of embryos transferred will also impact on reported results.

When reporting success rates should clinics be identified or anonymous?

An integral function of accrediting bodies and licensing authorities is to collect results for a specific group of patients, who have a similar likelihood of having a live-birth pregnancy, to compare success rates at different units *(17)*. The use of these data to measure performance for accreditation purposes is a useful means of identifying ways to improve practice, while maintaining confidentiality. However, publishing success rates that identify clinics in league tables can weaken the quality of the information available for consumers, as the UK examples have shown.

One strength of the annual Australian Institute for Health and Welfare (AIHW) report in Australia is, arguably, its anonymity *(18)*. There is no incentive to manipulate data so consumers can be confident of its reliability. Patient associations encourage consumers to approach individual clinics, perhaps more than one, to discuss the options available for their individual needs and the clinic's ability to meet them. Because there are wide differences between patients, specific information about an individual's chances of success should be obtained from the clinicians. This approach seems preferable to selecting a clinic based on statistics that do not reveal the full picture.

At the heart of a consumer's question about a clinic's success rates is the need to know where the best chance of success can be assured. This lies in the quality of the health care delivered by the clinic approached for help. If quality is effectively monitored through an accreditation process, where the data can be rigorously scrutinized, then consumers can be confident of having the best chance of realizing their dream of having a healthy baby.

It can be argued that the self-regulation model is weak as the locus of control lies with doctors. However, a strength of the Reproductive Technology Accreditation Committee (RTAC) model in Australia is that consumers participate as equal partners. This is unique in medicine in Australia and possibly in ART practice worldwide. Any successful attempt by a health professional to inappropriately manipulate the process would destroy the credibility and effectiveness of the RTAC.

What should the role of the law be in reporting success rates?

Emerson said over 100 years ago, "the less government we have, the better—the fewer laws and the less confided power" *(19)*. As consumers, we are careful not to be seduced by the assumption that the law will necessarily protect us from harm. The adoption experience in some countries is one historical example of this where, during a 30-day cooling-off period after the birth prescribed by law, many mothers who had changed their minds about relinquishing their children were told falsely by health professionals that their child had already been adopted.

In determining how success rates can best be reported, a model which delivers the most reliable data to assist informed decision-making is preferred by consumers to one which seeks by statute to make individual clinics accountable but fails to deliver meaningful data.

In working towards a model that meets the needs of all stakeholders, consumers seek a spirit of cooperation, which will ensure transparency and quality in the delivery of infertility services. Justice Hand of the US Supreme Court said that such a spirit is one "which is not too sure that it is right, which seeks to understand the minds of other men and women and weighs their interests alongside its own—without bias" *(20)*. Such a spirit of cooperation is crucial to achieving good outcomes and ensuring public confidence in the regulation and oversight of ART.

The impact of legislation on ART treatment

While some are proponents of restrictive legislation, others have argued that there is too much legislation for ART and cite existing legal choices for women in relation to human reproduction which respect individual autonomy. These include contraception,

abortion, where it is permitted (the father has no say), tubal ligation and tubal reversals. There is only intervention when the child is at risk as in adoption *(21)*.

However, the Canadian Royal Commission took an opposing view, which claimed that, "Given rapidly expanding knowledge and rapid dissemination of technologies, immediate intervention and concerted leadership are required as citizens in provinces with insufficient regulation may suffer harm" *(22)*.

Fertile people have been free to determine their own meaning of family and to live their lives accordingly. Where there is no evidence of detriment to the child, there appears to be no need for society to interfere in these arrangements.

Adherence to the "best interests of the child" principle, while laudable, can be difficult to apply in practice. It would be difficult to argue that it would be in the best interests of a child not to be born at all. In South Australia, the Reproductive Technology Act requires that a couple seeking assisted conception must demonstrate that they have no outstanding criminal charges or a history of an offence that was sexual or violent in nature. It also states that a couple must have no disease or disability which could interfere with their capacity to parent a child. DeLacy argues that "while plausible, such requirements are extraordinary and unjust, and are likely to be both ineffective in protecting the welfare of children and harmful to individuals in the long term". She identified the assumptions on which these requirements rest. Firstly, that "a parental history of crime of violence will result in the child being exposed to violence" *(23)*. Secondly, that parents who have had a child removed from their care have been proven to be abusive or neglectful. This does not account for children removed from care for reasons other than poor parenting.

The requirement about a disability that could interfere with the capacity to parent offers no parameters with which to make that judgement. Given that reproductive medicine is called upon to intervene in situations of infertility caused by disease and disability, this presents a paradox for practitioners. This is supported by Douglas who argues that instituting a "fitness to parent" code is "difficult enough to apply in cases concerning children who are in existence, let alone those who are only a twinkle in the doctors' eye and it is open to many different assessments, depending on the person making the judgement" *(24)*. DeLacey asserts that judgements are being made about a child who does not exist when clients who do exist and to whom the practitioner owes a fiduciary duty, are being refused treatment, which may not be in their best interests, leaving a practitioner vulnerable to an accusation that she may have acted in an ethically questionable manner *(23)*.

Sometimes, specific treatments such as egg donation and surrogacy are prohibited. Surrogacy is not new. One of the earliest recorded instances of surrogacy appears in the Bible in the book of Genesis *(25)*. However, both these treatments are forbidden in some countries, such as Denmark, Germany, Norway and Switzerland, and in the state of Queensland in Australia (Appendix A).

Surrogacy is permitted under Buddhist law but questions may arise about family ties as well as legal and moral issues. While Jewish law does not forbid surrogacy, questions about the status of the child are raised when one of the women involved is not of the Jewish faith *(26)*. When traditional surrogacy is used, the resulting Jewish child belongs to the donor of the sperm but this question remains unresolved in the case of IVF surrogacy. In the case of Islam, the practice of surrogacy is not permitted. In New Zealand, the Maori culture of *whanau* (extended family) sanctions informal surrogacy arrangements. There is no evidence in the literature to suggest that in the vast majority of such arrangements there is any detrimental effect on the child or the other parties involved.

Current law in most countries recognizes the woman giving birth as the legal mother, even where she has no genetic link to the child. This leaves the genetic mother no option but to apply to adopt the child to secure legal parentage and leaves the woman who gestated the child in the position of needing to give up for adoption a child that she never intended to raise.

The Australian Capital Territory (ACT) introduced a fresh approach with the *Substitute Parent Agreements Act* 1994, making it the only jurisdiction where specific legislation has been enacted to allow noncommercial IVF surrogacy. The Act prohibits commercial surrogacy but does not prohibit the facilitation of pregnancy where there is a non-commercial agreement. Children have been born through IVF surrogacy in the ACT since 1994, with full knowledge and contact between the children and the women who gave birth to them. There has been no evidence of harm done to any party, except by inadequate legislation with unintended consequences, which left the children being raised by their biological parents but not recognized as such in law.

In 1996, the Chief Minister of the ACT introduced

the *Artificial Conception (Amendments) Bill.* Its purpose was to allow biological parents to obtain legal parentage of a child born to another woman as the result of a surrogacy arrangement. The Bill imposed five conditions, including that at least six weeks and no more than six months must have elapsed since the birth and the birth parents were required to have agreed freely and with full understanding of what was involved. Both genetic and birth couples were required to have received assessment and counselling from a service other than that which carried out the IVF procedure and the biological parents were required to be residents of the ACT.

After lengthy public discussion, the Bill was passed in August, 2000 and provided for the ACT Supreme Court to issue a parentage order to allow the biological parents to be recognized as the legal parents of the child. The effect of the Act has been to ensure that the courts, known for their conservative approach, retain control of judging what are the best interests of the child. Primarily, it provides certainty to any children born as permitted under the Act, as to his/her parentage, thus allowing their best interests to be served. Importantly, it ensures that the wishes of the gestational mother are considered in any application for a parentage order. It has also, humanely, provided closure for the biological parents who may have undergone many years of medical treatment in order to have a child and who have lived with uncertainty from the outset.

The question is whether particular legislation will necessarily protect citizens from harm and, where it is considered necessary, what degree of protection should be imposed by the law in a society where most citizens are free to make a multitude of choices about their lives or health care, including reproduction. In Australian States free from restrictive legislation, there has been no evidence that consumers or society have been disadvantaged. It can be argued that where genuine informed decision-making occurs and there is a process for legitimate ethical review, restrictive laws make little sense and in some cases deny access to appropriate treatment for some couples who have no other means of forming their families. History has demonstrated that governments can often make ill-informed, politically expedient decisions, which are not necessarily in the best interests of their constituents. Furthermore, legislation is difficult to repeal. Even the most well-intended legislation in a high-tech, rapidly evolving area such as ART, can quickly prove obsolete.

Australia is the only country in the world with unrestricted access to public reimbursement for ART treatment. Crucial to securing this coverage has been the genuine involvement of consumers in all components of regulation, legislation, accreditation, and policy development. The inclusion of a consumer representative on the Federal Council of the Fertility Society of Australia (FSA) and on the RTAC, ensures that consumers have access to reliable information about treatment outcomes, possible drug side-effects and the quality of service provided by individual clinics. Despite the initial skepticism of the government, RTAC has demonstrated that self-regulation can work. Access to government funded drugs used in treatment in Australia is provided only to those clinics which have been accredited by RTAC. The availability of counselling is a requirement of accreditation, as is provision of detailed, written information on treatment, prior to its commencement. Clinics must demonstrate compliance with guidelines laid down by the National Health and Medical Research Council, the Australian Health Ethics Committee and a code of practice, together with relevant statutes in some States. To gain approval to conduct research or undertake new treatment with ethical considerations, individual clinics must apply to their local Institutional Ethics Committee. This ensures that the concerns of the community are addressed and that the interests of consumers are protected. In those states with regulatory authorities, their personnel accompany the RTAC team on clinic site visits in order to examine the clinic's state licence renewal. Benefits of self-regulation include its flexibility as it is more able to respond to emerging scientific advances and allow for a greater degree of autonomy for consumers in the decision-making process.

It also removes the need to rush to legislation every time a new procedure becomes available. In some countries, this has resulted in strange anomalies, such as:

- allowing sperm donation but not in an IVF cycle (Norway and Sweden), or
- allowing sperm donation but not oocyte donation (Denmark and Germany), or
- recommending that use of her frozen embryo by a woman if her husband dies be disallowed but allowing that same woman to receive donor sperm (Canada, France, Germany and UK).

Consumers of ART services seek politicians with

integrity who have the courage to act fairly rather than expediently. In addition, almost one million ART children have been born worldwide. Some of them have reached voting age and will show great interest in how their elected officials value their existence.

Developing effective partnerships with providers

A significant factor in the success of negotiations with government in relation to regulation and reimbursement issues in Australia has been the commitment of consumers and providers to work in partnership to achieve common goals. This has proved to be a powerful tool in the political arena and has provided a model for similar representation in other countries. In the late 1980s, this coalition of consumers and physicians successfully lobbied the Australian federal government for recognition of infertility as a medical condition and reimbursement for ART treatment. In 1990 the Prime Minister announced the provision of reimbursement of ART procedures through Australia's national health plan. This has helped to provide equity of access to health care for infertile people in Australia. The continuing participation of consumers in public policy and the regulation of IVF clinics is a reassuring demonstration of openness by health ministers, physicians and bureaucrats in ensuring transparency and quality in the delivery of infertility services.

This paradigm shift from consumers as passive participants to partners has been difficult for IVF physicians in some countries but the political benefits for consumers and providers can be significant. These partnerships are also appropriate as they recognize that consumers of ART services must live with the consequences of policy and treatment decisions.

The challenge for consumers is to ensure that all stakeholders have confidence in our integrity, professionalism and our ability to work effectively with the medical profession, government ministers and senior public officials. This may not always be an easy task but the suffering of those who come to all of us for support, compels us to commit to nothing less.

Real costs of infertility: emotional, social, societal

Governments have argued that the costs of providing reimbursement for infertility treatment are too high but it can be argued that the financial costs are less significant than the real costs of infertility.

The Royal College of Obstetricians and Gynaecologists and the British Infertility Counselling Association found, based on papers by infertility specialists and interviews with medical, scientific and psychological experts, that infertility costs the nation in absenteeism, poor productivity and wasted resources *(27)*.

There are also social costs to consider such as marital relationships, taking time off from work, refusing promotions, strained family relationships, exclusion from inheritances or family mementos and isolation from friends. The quality of life for some infertile people can become marginal when they have difficulty coping with a friend's pregnancy, seeing babies and young children or watching television advertisements featuring babies. Events such as Christmas, Mother's Day and Father's Day can be painful reminders of other people's fertility and success and are times to be endured. Many couples do not participate in these family celebrations.

The emotional costs can be the most significant. Nicol, in examining the impact of maternal loss, found that—on average—10% of women suffered some form of reproductive loss each year. Furthermore, she found that the death of a child had an emotional and physical impact on a woman that was as significant as that caused by the death of a spouse and that with multiple losses the impact was exacerbated *(28)*. It is easy to see the implications for women who have undergone successive attempts at assisted conception.

In 1993, the London newspaper, the *Daily Mail* reported on the 15th birthday of "Bubbly Louise" (Brown), the world's first baby born through IVF *(29)*. A few pages away appeared a story headlined *Tragic teacher who longed for a baby*. Gillian Martine, a 34-year-old primary schoolteacher from Southampton and her husband Michael, after trying to conceive for some years, had been told by their doctors the heartbreaking news that they would never have a child. Depressed and discouraged, Gillian committed suicide *(30)*. On the same day, the joy of assisted parenthood and the desperation and despair of infertility were graphically contrasted. The question is not whether infertile people have a right to infertility treatment reimbursement but rather, why they should be discriminated against in being denied access to appropriate health care services.

The profound impact which infertility and

involuntary childlessness has had on millions of people worldwide, means that the global family of infertility associations will continue to lobby and represent the needs of our constituents. We will not rest until all those we represent are treated with the dignity enjoyed by others in the community. Infertile people, as citizens and taxpayers of our respective countries, seek rather to claim our right to equity of access, with fellow citizens, to affordable quality health care and appropriate recognition of ART as a standard, proven treatment for infertility.

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Appendix A

(The information in this Appendix is the positions and opinions of the organizations and individuals indicated.)

Country	Legislation, regulation, access and social issues	Reimbursement
Argentina	Concebir, Estela Chardon	
	In Argentina there is no law about infertility treatments, that is the reason why patients have no reimbursements for their treatments	No reimbursement
Australia	ACCESS Australia Infertility Network, Sandra Dill	
	While some are proponents of restrictive legislation, others have argued that there is too much legislation for ART and cite existing legal choices for women in relation to human reproduction which respect individual autonomy. For Australians, the question is whether particular legislation will necessarily protect citizens from harm and, where it is considered necessary, what degree of protection should be imposed by the law in a society where most citizens are free to make a multitude of choices about their lives or health care, including reproduction. In Australian States free from legislation, there has been no evidence that consumers or society have been disadvantaged. It can be argued that where genuine informed decision-making occurs and there is a process for legitimate ethical review, restrictive laws make little sense and in some cases deny access to appropriate treatment for some couples who have no other means of forming their families. History has demonstrated that governments can often make ill-informed, politically expedient decisions, which are not necessarily in the best interests of their constituents. Legislation is difficult to repeal. Even the most well intended legislation in a high-tech, rapidly evolving area such as ART, can quickly prove obsolete. In Australia, crucial to achieving good outcomes in the regulation and oversight of ART has been the genuine involvement of consumers in all components of regulation, legislation, accreditation and policy. The inclusion of a consumer representative on the Federal Council of the Fertility Society of Australia (FSA) and on the Reproductive Technology Accreditation Committee (RTAC), ensures that consumers have access to reliable information about treatment outcomes, possible drug side-effects and the quality of service provided by individual clinics. Despite the initial scepticism of the government, RTAC has demonstrated that self-regulation can work. Access to government-funded drugs used in treatment in Australia is provided only to those clinics which have been accredited by RTAC. The availability of counselling is a requirement of accreditation, as is provision of detailed, written information on treatment, prior to its commencement.	The Australian experience has shown that before governments are willing to reimburse from the public purse, the medical community must demonstrate that quality standards of health care are met and that the practice of assisted reproductive technology (ART) is effectively monitored. A significant factor in the success of negotiations with government in relation to regulation and reimbursement issues has been the commitment of consumers and providers to work in partnership to achieve common goals. This has proved to be a powerful tool in the political arena in Australia and has provided a model for similar representation in other countries. In the late 1980s this coalition of consumers and physicians successfully lobbied the Australian federal government for recognition of infertility as a medical condition and reimbursement for ART treatment. In 1990 the Prime Minister announced the provision of reimbursement of ART procedures through Australia's national health plan. This has helped to provide equity of access to health care for infertile people in Australia. In November 2000 the restriction of six cycles of IVF treatment in a woman's lifetime in Australia's national health scheme was removed, making this the only country in the world with unlimited government reimbursement for infertility treatment. This followed successful representation, by consumers of infertility health care and providers, to the federal Health Minister and his Department. The continuing participation of consumers in public policy and the regulation of IVF clinics is a reassuring demonstration of openness by health ministers, physicians and bureaucrats in ensuring transparency and quality in the delivery of infertility services. It is also appropriate as it recognizes that ultimately it is consumers who must live with the consequences of policy and treatment decisions.

Country	Legislation, regulation, access and social issues	Reimbursement
	Clinics must demonstrate compliance with guidelines laid down by the National Health and Medical Research Council, the Australian Health Ethics Committee and a code of practice, together with relevant statutes in some States. To gain approval to conduct research or undertake new treatment with ethical considerations, individual clinics must apply to their local Institutional Ethics Committee. This ensures that the concerns of the community are addressed and that the interests of consumers are protected. Clinics inspected by RTAC can receive full accreditation for three years or preliminary accreditation for twelve or eighteen months and there are therefore ministers, physicians periodic mechanisms for reviewing general or specific concerns. Only those clinics with RTAC accreditation are included on the ACCESS referral list. Benefits of self-regulation include its flexibility as it is more able to respond to emerging scientific advances and allow for a greater degree of autonomy for consumers in the decision-making process.	
Austria	Wuki Kiwu, Norbert Rickmann	
	An ART law was passed by the Parliament in 1991, including several restrictive provisions.	In Austria there exists reimbursement for ART costs by the social security system since 1 January 2000, when a new law came into effect, so called "*in vitro* fertilization-fund-law". For affected couples there is now a takeover of 70% of the particular costs (treatment as well as medication), which are regulated all over Austria, but the following conditions must be present: women must be younger than 40, men younger than 50, the only medical indications are male infertility or (female) dysfunction of the tubes. In our opinion, the law is very restrictive and does not address the needs of the majority of the infertile people. While the law was a significant step, further revisions are needed.
Bangladesh	Infertility Awareness Network (IAN), Mizanur Rahman	
	There is no government law governing ART in Bangladesh. However, religious law prohibits egg and sperm donation. Ninety-nine per cent of couples are unaware of these possible procedures and ART is a very new idea, in practice unknown to patients. Infertility is considered a curse and brings bad luck to couples. We need A to Z information about infertility and to focus attention on raising awareness in the media.	There is no insurance at all to reimburse couples for treatment. As the population explosion is a major problem in Bangladesh, the government would need courage to allocate funds for reimbursement of infertility treatment.

Country	Legislation, regulation, access and social issues	Reimbursement
Canada	Infertility Network, Diane Allen and Infertility Awareness Association Canada, Alison Conley	
	At present, there is no legislation governing ART; the government has indicated that it plans to ban: commercial surrogacy, the sale, purchase, barter or exchange of eggs, sperm, embryos, fetuses, genes; reasonable reimbursement for expenses will be allowed, sex selection for nonmedical reasons, use of cells from an embryo, fetus, corpse or person 18 year of age, use of sperm, eggs, embryos, fetuses for assisted reproduction or research without the consent of the donor, cloning, maintenance of an embryo outside the human body after the 14th day following fertilization, animal/human hybrids, germline alteration. The government has indicated that legislation will also establish a regulatory body to deal with: licensing of clinics and staff, information registries for donors and offspring, outcomes and success rates of clinics, surveillance systems to track the risk–benefit profiles of fertility drugs and monitor the health of patients and their children. Human rights legislation protects patients in some provinces against discrimination on the basis of marital status or sexual orientation. However, access to treatment is limited by how much money you have, as well as where you live, since clinics are located in only a few large cities; travel adds additional stress and not everyone can afford the cost or get the time off work. In Canada egg donation for postmenopausal women is rarely performed in those over 50 years of age. Egg donation is performed for women with premature ovarian failure on the condition that the recipient provides her own donor (friend or relative) and that she can cover the cost of the donor who will undergo IVF and share the eggs retrieved. An embryo bank or egg donor bank has not as yet been established in Canada.	Most infertility treatment is provided free under government-paid health insurance; in large cities, physicians/clinics often charge administrative fees. Some procedures are usually not covered (e.g. sperm wash, the cost of donor sperm). Ontario is the only province to pay for any IVF: women with both fallopian tubes completely blocked or missing (other than from sterilization) are covered for 3 complete treatment cycles. Some people have private insurance through their job which covers fertility drugs but there is usually a pre-set limit on the number of cycles, the period of time or the total cost.
Denmark	Landsforeningen for Ufrivilligt Barnløse (LFUB), Martin Kristensen	
	Egg donation is so far forbidden in Denmark. LFUB has urged politicians to vote for it, but so far without success.	The legislation allows couples to have treatment free of charge at hospitals in Denmark. The waiting time is up to two years. Private clinics have been established in recent years and many couples prefer private treatment to avoid long waiting lists.
Finland	Lapsettomien Tuki, Pirre Saario	
	There is not yet any law concerning infertility treatment in Finland and there is a wide choice of treatment available. That is why it has been possible to concentrate on the mental welfare of infertile couples.	Infertile people pay 25%–40% of infertility/IVF treatments. The National Pension Institute covers the rest. With private health insurance there are no common standards of covering infertility/IVF treatments.

Country	Legislation, regulation, access and social issues	Reimbursement
France	Association Pauline et Adrien, Chantal Ramogida	
	IVF treatment is reimbursed for women under 43 years of age. The couple must be married or living together for more than two years. Infertility treatment is not provided for single or lesbian women. Oocyte and sperm donation must be anonymous and the donor cannot be paid. Postmortem transfer is prohibited (from frozen sperm or embryos).	The Government reimburses infertility treatment up to 100% if it is done in a public hospital. In a private clinic the treatment may be more expensive and the couple then need a private insurance to cover the extra cost. Social security reimburses six artificial inseminations and four IVF treatments (meaning embryo transfer). After the birth of a baby the patient can obtain these again for a second child.
Germany	Wunschkind, Petra Thorn	
	In 1990 the Embryo Protection Act (EPA) was introduced. This Act is a penal law forbidding the following: • Full and gestational surrogacy • Oocyte donation • Transfer of more than 3 oocytes • Sex selection unless a severe hereditary disease can be avoided • Use of the sperm of a man known to be dead • Manipulation of human genetic information • Cloning, formation of chimera or hybrid Currently, a new act on assisted human reproduction supplementing the EPA is being discussed. In May 2000 the first public hearing took place debating gamete donation, preimplantation genetic diagnosis, the use of human stem cells, quality standards and patient care. Four cycles of ICSI are reimbursed by the health insurances.	Health insurances in Germany (and there is little difference between private and state insurances) reimburse the following: • 8 inseminations without hormone treatment • 6 inseminations with hormone treatment • 2 cycles of GIFT • 4 cycles of IVF Until April 1999, health insurances also reimbursed 4 cycles of ICSI. As budgets get tighter and as there are insufficient German data on the risk of malformation of children born after ICSI, currently no health insurances reimburse this treatment. However, there are several ongoing legal cases which may change this sitaution. To be eligible for reimbursement, couples have to be married or be in a de facto realtionship and the male partner must not have undergone sterilization. In addition, only the costs of treatment using the gametes of the couple are rembursed. DI is therefore not reimbursed.
Ireland	National Infertility Support and Information Group (NISIG), Helen Browne	
	Presently, there is no legislation on assisted reproduction but the Irish Government has convened a Commission to look into all aspects of ART, except funding, with a view to seeing whether legislation should be brought in or not.	Infertility treatment in Ireland is almost exclusively privately funded. Many couples borrow the money for IVF treatment but they can apply for tax refund at the end of the tax year. Under the Drug Payment Scheme, the costs are covered by the government, except for £ 42.00 per calendar month. The two private health companies in Ireland do not cover infertility treatment but do cover investigations such as testicular biopsy, laparoscopy and hysterosalpingogram. The cost of IVF treatment in Ireland is approximately £ 2000 per cycle but this does not include the fee for

Country	Legislation, regulation, access and social issues	Reimbursement
		first consultation, hormone analysis or semen analysis. Costs vary from clinic to clinic.
Israel	CHEN, Ofra Balaban-Kasztelanski	
	We are working to submit new laws and regulations regarding infertility. On our agenda is to change the regulations regarding egg donation. In Israel only a woman who is undergoing the IVF process can donate an egg. Because of this, there are 2000 women today waiting for an egg donation and the waiting time is between 6 and 12 months. The law we are submitting will allow every woman who wants to donate eggs to do so. To achieve this purpose we are lobbying the Israeli Parliament members for their support and we are doing a lot of PR work in the media.	We are campaigning so that the government will update the National Health Bill so it will include new medications and treatments. The state insurance pays IVF costs until two children have been born. However, some new male infertility treatments are not reimbursed. Current public debate is ongoing to decide at what age the state will pay for treatment. It has been suggested that the ages should be 45 years for IVF and 52 years for donor oocyte treatment.
Italy	Associazione Madre Provetta, Monica Soldano	
	A very restrictive law was passed mid-2002 in one House of the Parliament in Italy and will be debated in the Senate in order to become law.	In Italy it is possible to have reimbursement for infertility treatment.
Japan	Kidsless Party, Jahana Mari and Friends of FINNRAGE, Yukari Semba	
	• There is no legislation governing ART treatment in Japan. • Professional guidelines limit IVF to only legally married couples therefore they indirectly prohibit donor gametes and surrogacy. Donor gamete procedures will be reconsidered within two years but surrogacy will not be considered in the near future.	There is no reimbursement for ART in Japan.
Republic of Korea	Agimo, Eunhee Pack and Dr Kichul Kim	
	• Surrogacy, egg and sperm donation are allowed but commercial trade is forbidden. • Nuclear transfer and cytoplasmic transfer are viewed negatively. • IVF, GIFT, blastocyst transfer, co-culture, ICSI, assisted hatching, PGD and IVM are available.	There is no reimbursement for ART in the Republic of Korea
Latvia	No patient group, Gints Treijs, MD	
	There is no reimbursement for infertility treatment in Latvia.	Unfortunately most of the couples cannot afford IVF treatment. Therefore all three IVF centres together perform no more than 170 cycles per year, on about 1/4 or 1/5 of all couples needing this kind of treatment.

Country	Legislation, regulation, access and social issues	Reimbursement
Mexico	Asesores en Infertilidad, Yolanda Secades	
	• A special multi-institutional committee, headed by representatives of the Mexican Ministry of Health, has been formed to address issues related to ART and create a regulation. So far, they have not published any official policies or guidelines. ART clinics have a lot of latitude in their work, they just have to comply with the General Regulation for Medical Services Providers.	• ART procedures are performed at two or three public health insitutions, all of them in Mexico City. However, they do not recieve enough economic support and do not operate on a constant basis. • Reducing population growth is a priority in Mexico, so the government has no interest in supporting ART.
	• Surrogacy is not encouraged but some clinics allow it. To avoid possible complications of a legal nature or bad publicity the infertile couple has to contact and make the necessary arrangements with the surrogate mother on their own, relieving the clinic of any responsibility for such arrangements.	• There is no reimbursement or private aid for ART treatment in Mexico.
	• According to the last population census (INEGI. XII Censo General de Población y Vivienda, 2000) Mexico´s population is 97.5 million. There are around 16 million women between 20 and 39 years old and 14 million men in the same age group. If the incidence of infertility is estimated at 8%–10%, then approximately 2.4 to 4.6 millions of persons are in need of some kind of infertility treatment. It is important to note that 57% of the Mexican population do not have access to public health services.	
The Netherlands	Freya, Truuske Dijkstra-Hazelhorst	
	Almost all infertility treatments are legal. IVF laboratories in the Netherlands have to obtain a special dispensation. There are 13 clinics permitted to execute the laboratory procedure of IVF/ICSI. Approximately 25 other clinics cooperate with these 13 clinics. For MESA/TESE a moratorium has been declared, so it is not possible to get this treatment. Surrogacy on a commercial basis is prohibited by law, as well as any assistance for making contact between surrogate mothers and would-be parents.	The public health service pays for three IVF treatments; most other treatments are paid for as well. In the Netherlands, there are many different private insurance companies, each with their own range of policies. Most private insurance companies also pay for 3 IVF treatments, but charge the patient a contribution of Nlg 800 (+US$ 400). Private insurance is needed when one earns more than approximately Nlg 60 000 per year. Nearly all medication for infertility treatments is paid for by public health service as well as private insurance, except for some new preparations which have to prove to be useful first.
New Zealand	New Zealand Infertility Society, Robyn Scott	
	There are two Bills currently before the Parliamentary Law Select Committee with the aim of regulating assisted reproductive technology. There is no legislation specifically covering ART, though aspects of other legislation (e.g. the Human Tissues Act) covers aspects of ART. The two Bills are very different—one, a Private Member's Bill seeks to regulate ART through the intoduction of a Licensing	Access to publicly funded infertility treatment in NZ varies, depending on where you live. Criteria for acceptance for treatment varies widely, although each area does provide consultations and some tertiary treatment. Criteria are fixed in some areas, i.e. you must meet all criteria to access treatment, while in other areas points are awarded within both subjective

Country	Legislation, regulation, access and social issues	Reimbursement
	Board and has a number of regulatory features that the NZIS does not support. The second Bill, introduced by the former Minister of Justice is more flexible and contains 3 main parts—it seeks to place the National Ethics Committee within a proper legal framework, it sets up a number of provisions to allow for storage and access to information about donors and their offspring, and outlaws certain procedures. We are presently waiting for the opportunity to present an oral submission on the Bills, following our submissions in 1998/99.	and objective criteria—this is less absolute. Funding commitment to infertility treatment varies between 0.78 cents per capita in the Northern region to 0.37 cents in the Central region. In some areas drugs are funded as part of the treatment, while in other areas co-payment for drugs is required. In some areas there is a 3-year wait for two cycles of IVF while in other areas you may have a shorter wait, but receive one cycle of treatment. The health system is undergoing its fifth restructuring in nine years and the Health Funding Authority is soon to be replaced by 26 District Health Boards. Provision of infertility services in NZ has been reviewed comprehensively on several occasions, but the review recommendations have yet to be acted upon. Little reimbursement by private medical insurance is possible, although the Police Health Fund provides a one-off life-time payment of $ 5000 for infertility treatment. Some health care funds will cover the cost of investigations such as a laparoscopy.
Norway	Foreningen for ufrivillig barnløse (FUB), Kaja Graff Huster	
	Norway was one of the first countries to have legislation to control and regulate infertility treatment. The main outlines of the legislation are as follows: i. Infertility treatment is only given to heterosexual couples, either married or living together in a stable relationship. ii. IVF and ICSI are allowed treatments. But treatments which involve operational collection of sperm are not allowed. iii. Fertilized eggs can be stored for up to three years. iv. Sperm donation is anonymous. The couple must be married. v. Egg donation is not allowed. vi. Surrogate motherhood is not allowed vii. No research on fertilized eggs is allowed.	In Norway medical treatment in general is free, but this is only true for treatment which is offered by and within the public health system. Although IVF treatment is free in public hospitals, people often have to pay for medication. Large costs for medical treatment can be deducted from an individual's income tax but the following three conditions must be satisfied: 1. The medical condition, which is treated, must be a disease. 2. The treatment must be legal in Norway. 3. The treatment is not available in the public health system. • Many couples are going abroad for treatment because of long waiting times. • Many couples are going abroad because some treatments are not allowed and not available in Norway (PESA, TESA, MESA, egg donation). • Several couples are using alternative treatment for their infertility, spending a lot of money without getting any tax reduction or other refunds because infertility is not regarded/accepted as a disease.
Sweden	IRIS, Lena Gimbergsson	
	In Sweden, couples can get all kinds of treatment—IVF and ICSI, and insemination with donor sperm. Egg	It was a great victory for us when on 16 April 1997 the government decided that the treatment of

Country	Legislation, regulation, access and social issues	Reimbursement
	donation and sperm donation with IVF is illegal. All the questions and answers have been ready since 5 years to take a new decision about this illegal treatment. IRIS is now fighting with the government and the Social ministry to make them change this law at the earliest. In the Nordic countries, legislation on human reproduction and embryology varies from one country to another. But the case of Sweden is still unique, since we are the only country in the world to have a special law on donor insemination. Donor insemination is not allowed for single women or lesbians and can only be performed in general hospitals. Children born following donor insemination can have access to the identity of their genetic father once they reach majority.	infertility should be placed in group 3 within the National Health Maintenance Organization. This category refers to "Treatment of chronic diseases and diseases of a non-acute nature". At last, infertility was accepted as an illness! However, in real life, a beautiful legislative text does not always help. In our country the Swedish Health Maintenance Law also states that "Everybody in Sweden shall have the right to treatment under the same conditions wherever they live". But in fact, every day district and country councils break this law, as reimbursement is still uneven between different countries. Reimbursement of IVF treatment cycles can vary from 1 to 3, depending where people live. Within the public system, couples very often have to wait 2–3 years to get access to treatment. In one of our districts couples have to wait 6–8 years for treatment and then they are too old to get treatment. In the case of infertility, many people still make huge financial sacrifices to be able to pay for treatment in private clinics. The cost for one treatment in private clinics is between 15 000 to 30 000 Skr.
Switzerland	Azote Liquide, Ghila Zoutter	
	Assisted reproductive technology methods—notably homologous and heterologous artificial insemination and *in vitro* fertilization (IVF) with embryo transfer—are at present authorized in Switzerland. The donation of embryos and all other forms of substitute maternity, such as surrogate motherhood, are illegal. Transparent use and practice of assisted reproductive technology is desired.	The present situation: the Federal Insurance Tribunal was confirmed that health insurance companies have no legal obligation to reimburse the cost of ART treatments, arguing that these are not "scientifically recognized" diagnostic or therapeutic measures as defined by law and Swiss jurisprudence.
United Kingdom	ISSUE, Lydia Pollard and CHILD, Clare Brown	
	Clinics offering treatment involving the handling or storage of gametes require a licence from the HFEA which is a statutory organization. They do not decide who should access reimbursed infertility treatment/assisted conception services. The HFEA produces a Code of Practice to which clinics must adhere. They also publish an Annual Report. There are no laws on who should have access to infertility treatment. The Health Authorities (HAs) decide what funding they will provide as described above and set the eligibility criteria such as upper age limit and previous children, which also vary enormously around the UK. They therefore ultimately decide who should access NHS-funded treatment. Clinics also set their own guidelines as to whom they will treat and these do vary a little from clinic to clinic.	The situation with reimbursement in the UK has always been complicated. However, devolution in the UK resulted in Scotland becoming self-governing which has made the situation even more complicated. Before that time the UK was divided into more than 100 HAs who are each given a budget by the Department of Health (DoH), from which they purchase the health care needed by people residing in their area. There was no central guidance from the DoH on what infertility treatment they should provide. On 10 February 2000, the Department of Health in Scotland published a Framework for the provision of infertility services setting out criteria to which every Health Board in Scotland is expected to work towards implementing.

Country	Legislation, regulation, access and social issues	Reimbursement
		Subject to eligibility criteria, each couple is entitled to a maximum of 3 National Health Service (NHS)-funded assisted conception cycles. However, the situation in the rest of the UK remains the same with no central guidance and each HA making differing decisions as to the funding they provide, resulting in huge variations around the country. No HA provides sufficient levels of funding to meet demand. Therefore, patients living in an HA which does not fund infertility have to pay the total costs themselves. Private health insurance does not cover infertility treatment.
United States of America	American Infertility Association (AIA), Pamela Madsen There is no federal legislation ensuring treatment of infertility.	In the United States of America, insurance coverage for infertility varies greatly. There is no federal legislation ensuring treatment of infertility. Thirteen states have some kind of mandate that allows for limited to liberal coverage of infertility. Presently, coverage of infertility is patchy at best, and is often left in the hands of the individual employer and private insurance companies. The end result is that most couples are left paying on their own for treatment.

Gender, infertility and ART

ELLEN HARDY, MARIA YOLANDA MAKUCH

Introduction

Human sexual life had a simple and predictable outcome: a man and a woman lived together and had sexual intercourse, frequently followed by pregnancy and the birth of a healthy baby. Having children is a fundamental part of the life project of men and women and is seen as a necessary step to reach maturity and personal development, and to respond to what is their socially expected role, leading to the preservation of the human species *(1,2)*.

The outcome referred to above is not always simple and predictable; in some countries, there might be an increasing number of couples facing difficulty in reproducing. Estimated percentages of infertile women in the USA show an increase between 2000 and 2025 *(3)*. Part of the apparent increase in the number of infertile couples may be the result of better diagnosis of this condition, as until a few decades ago there were no diagnostic procedures or treatments available. On the other hand, during the past 30 to 40 years, women have had access to modern and highly effective contraceptive methods, which allow them to control their fertility, postponing their first pregnancy until an older age, when they are less fertile.

The couple's inability to produce an offspring has been described in a variety of clinical, epidemiological and demographic definitions of infertility. Infertility, when considered in the context of women and men's lives, has also been defined as an experience of "biographic disruption". This definition emphasizes the suffering and emotional conflicts of those who have this condition *(4)*. The impossibility of having a desired child becomes a loss in couples' lives: a loss that has been compared to the loss of a very dear person *(5)*. Lauritzen *(6)*, in the description of his experience as an infertile male states: "The emotion we felt most acutely was that of loss over a child we believed we would have together."

While in the past, couples who could not have a child tried to adopt one or remained childless, currently more and more sophisticated procedures are available to help them have a baby that is biologically related to both, or at least one, of the parents. Such possibilities have become available over the past few decades, when knowledge and understanding of reproductive physiology experienced a great expansion, following research in reproductive technology that resulted in new therapeutic alternatives.

The impact of infertility and assisted reproductive technology (ART) on the persons affected has to be considered within the broader context that includes social, historical, economical, political and cultural aspects. At the same time, so as to understand the actual meaning that infertility has on the life of an individual woman or man, it has to be considered within the context of gender roles.

Gender and parenthood

The term "gender" is derived from the Latin "genus". Initially it was used to distinguish sex (in the anatomical sense) from identity (in the social or psychological sense). Sex defines the anatomical organization of the difference between male and female while gender designates the feeling (social or psychological) of sexual identity *(7)*.

In a broader sense, gender is socially constructed and the term refers to the social relationships and roles that men and women have in society *(9)*. According to Matamala and Maynou *(8)*, the four principal concepts that integrate this definition, and through which the social relationships and the symbolic meaning of being a woman or a man are defined, are sexuality, reproduction, sexual division of labour and citizenship. Gender basically refers to the socially constructed difference of power between men and women.

In the early 1970s, during the period when the question of gender roles and women's rights became a more prominent issue, the new modern, effective contraceptives became widely available. The access to efficient means to control fertility gave women the power over procreation that they were demanding. The capacity to decide when to conceive affected the most fundamental role attributed to the female gender: to be a mother and to take care of the children. According to Rich *(10)*, a major fact in women's lives is their status as childbearers. Any further identity has been negated by terms such as "barren" and "childless" while the term "nonfather" does not exists as a social category.

Traditionally, motherhood has been defined biologically and the mother is the woman who delivers the child while the definition of fatherhood is social and elusive: the father is "normally" the husband of the mother *(10,11)*. However, women are more likely to define the term "mother" according to the interaction established between a woman and a child and not only in terms of biology *(11)*. Motherhood is earned first through pregnancy and childbirth and later through nurturing *(10)*.

Men, on the contrary, seem to define their relationship to a child according to their participation in conception *(10,11,12)*. The concept of fatherhood is primarily related to the ownership of children, as men are given father's rights over offspring who come from their sperm *(13)*. However, the roles of mothers and fathers are changing at the same time as categories of feminine and masculine are being revised. Modern fathers, in many societies, are increasingly involved in the upbringing of their children, a responsibility which traditionally was exclusively maternal *(14)*.

Assisted reproduction and gender

Until the 1950s, many cases of infertility were explained through an emotional rather than a biological cause. During the following 20 years the development of diagnostic techniques and laparoscopy created new possibilities of exploring women's internal anatomy and physiology. With these new resources it became possible to identify biological causes of infertility that in the past would have been considered to be of an emotional nature. In addition, better understanding of reproductive endocrinology allowed interventions in women and men's bodies to "correct" alterations of the physiological process. Thus, many of the unexplained causes that were considered emotional became treatable.

Although this scientific progress has the potential to disrupt conventional gender roles and family structure, it has not yet changed social norms related to gender roles *(15)*. Thus, women and men who are infertile feel socially inadequate in a predominantly fertile society. Society continues to require women to be mothers and to "give" children to their husbands, grandchildren to the couples' parents and continuity to a family *(16,17)*. Thus, the desire of the couple to have a child is a complex one, determined by many family expectations. In this context, the emotional consequences of infertility, which affect women and men differently, began to be understood and to become a subject of research.

Having children, becoming parents and establishing a family when a couple decides to do so is considered part of adulthood for women and men *(1,2)*. When the expected family model is not achieved, each partner and both as a couple must go through a process of psychological re-organization to cope with this new unexpected reality

When a couple is infertile, some societies still tend to blame the woman and even accuse her of having been promiscuous, having had too many abortions or of having a sexually transmitted disease *(13)*. The woman is the first to consult a doctor for a solution to the problem. Until not so long ago, women had to go through a long and painful diagnostic procedure before men were submitted to the simple procedure

of obtaining a spermiogram. In some societies, irrespective of the cause of infertility, childless women can be easily divorced or abandoned.

ART has greatly improved the chances of infertile couples having children, but they have increased the imbalance in the share of the burden of treatment, relying more heavily on women. At the same time these technologies may have even greater emotional consequences than infertility for the couple.

As reproduction technologies are based on interventions into women's bodies, they reinforce motherhood as a biological rather than a social relationship. According to Lorber *(18)*, as referred by Lasker and Borg *(19)*, infertility treatment relies on the imperative that women must be mothers and on a social structure in which "motherhood" only applies to women who have a biological relationship with a child. However, in her study of women participating in an *in vitro* fertilization (IVF) programme, Crowe *(11)* found that all the women considered social motherhood more important than the transference of genetic traits involved in biological motherhood. Simultaneously, they perceived motherhood as well as marriage as necessary social relations.

When childlessness is the result of a woman's infertility, she will need to persuade her husband to collaborate with the treatment. According to Lasker and Borg *(19)*, Lorber *(18)* considered that, a fertile woman who undergoes assisted reproduction to try and have a child with an infertile man, may be expressing love but is more likely to be making a patriarchal bargain. Consequently she feels obliged to have a baby within the constraints of monogamy, the structure of the nuclear family and the value of biological parenthood.

The social and psychological consequences of infertility for the couple have increased with the advancement of ART. While the media have occasionally been critical of some of these new technologies, it mostly tends to present them as a kind of magic bullet that will solve the infertility problem of almost everyone *(20)*. Given the increased expectations *(21)*, the higher economic and emotional cost for the couple and greater physical demand on women, the failure of ART leads to greater psychological and social consequences for the couple than the unavailability of these technologies.

The high cost of establishing an ART clinic and the known association of greater success rate with larger number of cases treated per month or per year, stimulate the expansion of the indications for the use of these technologies. While in the early days of ART, the treatment was confined to women without fallopian tubes or with tubal obstruction not eligible for surgical treatment, today ART is used for a number of conditions, including male infertility. Furthermore, it has been argued that ART could be an acceptable solution for couples with no infertility problem, but who wish to improve their chances of having a baby of a specific sex *(22)*.

In contrast with the advances in ART, their psychosocial implications have attracted little attention. There is an imbalance between the level of sophistication and the care given to every small detail of the technologies used for assisted reproduction and the attention given to the feelings, fears and anxieties of the infertile couple, the psychosocial consequences of infertility and of treatment failure, as well as to the gender difference between the effects that these technologies have.

Survey of methodological approaches used to address the issues: interdisciplinary perspectives

A survey was done of published studies carried out over the past 20 years on the psychosocial aspects of assisted reproduction and which evaluated both women and men. The methodological approaches followed by these studies were diverse.

Sample and selection of subjects

The sample size of these studies varied from 40 to 1149 couples, though sometimes not all the male members of the couples participated. Subjects were recruited in hospitals and private infertility clinics, usually consecutively. Criteria for inclusion as well as the time when subjects were invited to participate varied. For example: couples whose treatment had been successful; those who were initiating their first IVF cycle; three weeks after the IVF procedure; after the first cycle of IVF; and having completed all its phases through embryo transfer; all patients entering IVF treatment over an eight-month period; and couples who had been through at least one IVF attempt. In one study, couples were located using a modified snowball technique *(23)*.

Instruments

Data, in general, were collected through standardized interview schedules, questionnaires, scales, inventories, checklists, guides or a combination of these instruments. For only a few studies had the service staff developed the questionnaire. Many of these questionnaires were self-reporting and scales were self-rating. For one of the studies reviewed, a male and female version of a questionnaire had been developed.

Time of response

Mostly couples responded or received a package of instruments during the assessment visit to the infertility clinic. Others were provided with instruments during their final hospital visit or three weeks or more after embryo transfer. For some studies, participants were given instruction sheets, questionnaires and stamped envelopes and were asked to return them to the programme after completion or to return them by mail at the end of the current treatment cycle or when the pregnancy status was known. Other alternatives of response were to complete part of the instruments at the clinic and the rest at home. In another case, couples completed three assessments at six-month intervals. Information was also obtained through telephone interviews.

Data were elicited from subjects at different moments during the infertility diagnosis and treatment process, sometimes even within the same study. For example, in one case, women undergoing IVF were evaluated on the second day of a cycle, those who were to receive donor insemination were evaluated just before the procedure and their partners responded at home. In other studies, subjects were evaluated during their initial attendance at the clinic, before any medical investigations were begun, and seven to nine months later when in most of the cases the diagnostic tests were completed.

Data collection

A joint, hence potentially affected, interview was carried out with both partners, when the answers of one could have influenced the other. Sometimes, separate interviews followed the joint one. In only one study of 40 couples about to begin treatment, subjects were requested to keep daily records that allowed prospective data to be collected. For another study, interviews were taped and transcribed verbatim.

Answers to proposed questions through critical review of data; identification of gaps; identification of limitations

The results of the studies reviewed were organized according to the association of gender issues to different assisted reproduction variables.

Reactions to infertility

Practically every author found that both men and women who experienced infertility were more distressed than the general population. However, with almost no exception, the studies found that women reacted more strongly to infertility than men. Depression, anxiety, cognitive disturbance, lower self-esteem, greater guilt, blame, hopelessness and hostility were some of the emotional reactions described by various authors *(24–27)* but were no more common in those with unexplained infertility than in others with a specific diagnosis *(21,28–32)*. Psychiatric morbidity was also significantly more common among females than males in one study *(33)*.

In addition, women rated themselves as experiencing greater social effects of infertility than men *(21)*. Men referred to feelings of losing the power of being able to reproduce as well as worry about their partner's reaction *(32)*. One study found that men experienced extreme worry significantly more often than women did—19% of men versus 3% of women *(5)*. Both sexes' most common coping strategy was the active pursuit of treatment *(29)*.

Reactions to treatment of infertility

A comparison of couples who were initiating treatment and others who were in a repeat cycle found that about a third of husbands and wives experienced clinically elevated anxiety, regardless of stage of treatment *(30)*. While there was remarkable similarity in the type and pattern of the couple's reactions to the different stages of treatment, women were more distressed than their partners during IVF *(25,30,31)*. Many husbands expressed fears for their wives' safety during the laparoscopy, guilt that their wives were the ones who bore all the pain and discomfort, and helplessness in the face of their wives' grief when treatment was unsuccessful *(34)*. In general, the scores of the General Health Questionnaire indicated more emotional distress for women than for men in

relation to the diagnosis and treatment of infertility *(28)*.

Long-time effects of infertility

In this review, only one study evaluated distress over a period of three years of infertility treatment. During the first year, emotional strain was moderately elevated, returning to normal levels during the second year and increasing during the third *(35)*. No study was identified referring to the long-time effect of failure or success of treatment.

Response to male and female infertility

No differences were found among women in their emotional response to infertility, regardless of whether a female or a male infertility factor was present. Men with a male factor experienced more negative emotional response and a higher state of anxiety over time than those without a male factor *(36,27)*. Among women, when the cause of the reproductive difficulty was female or unexplained, depression scores decreased significantly at the follow-up assessment *(27)*.

Effect on marital relationship and sexual functioning

Stress related to fertility problems appeared to increase marital conflict *(37)*. The satisfaction in marital relationship was determined in part by the degree of stress that men and women experienced during treatment. Men's marital satisfaction was not determined by their wives becoming pregnant or by their own sexual satisfaction, but in part by the degree of stress that they experienced. Women's marital satisfaction was partly determined by their degree of sexual satisfaction regardless of the stress they experienced *(38)*. Both these studies looked at marital functioning during treatment. Other authors did not find any significant increase in psychological abnormalities or family dysfunction. Both men and women were in general well adjusted and enjoyed good stable relationships with their partners *(39,27)*. Some studies described improvement rather than deterioration of the marital relationship with infertility; subjects reported that problems related to infertility had resulted in a closer relationship with greater emotional intimacy *(40,32)*. Women described marital relationships more positively than men and men scored higher on achievement orientation *(41)*.

Results with reference to the effect of infertility over sexual function were also contradictory. Some authors found that, as a group, these infertile couples were within the normal range of sexual functioning *(37)*. Others found that infertility treatment had changed the sexual relationship of 46% of the women and 32% of the men. Two-thirds of those who reported change stated that sex had become less pleasurable *(24)*. Another study that reported changes found that men felt that infertility had a greater negative impact on their sexual relations than that observed by their wives *(30)*.

Perœption and desire for social support by gender

Women sought more social support than did men *(21,32)* and there was a tendency for them to express the desire for counselling and support *(42)*. Both men and women perceived their partners as providing the most support, followed by doctors, nurses and friends *(42,32)*. Related to support within the couple, women reported receiving greater emotional and physical care from their husbands in their relationship than their husbands did from their wives (*30)*. Significantly more men than women had not confided in anyone about their infertility problems *(32)*. Information routinely provided about the practical aspects of IVF could improve knowledge and passage through a treatment cycle *(42)*.

Effect of treatment failure

The results indicate that IVF offers hope for infertile couples and that, when unsuccessful, it can be emotionally traumatic *(34)*. Both males and females agreed that negative outcome and waiting for results during the period of time between embryo transfer and the outcome of the procedure were the most stressful aspects of IVF. Immediately after failure, both men and women presented increased levels of anxiety and depression, and appraised their life situation more negatively *(41,28)*. However, women reported more stress at different stages of the IVF procedures *(28)* and showed increased depression after failure *(41)*. Women with children had less anxiety than those without, while all men had increased anxiety, irrespective of their reproductive history *(41)*.

One study, which followed couples for two to four weeks after a positive or negative outcome of the

procedure, found that more than half of the women who failed to become pregnant reported initial disappointment and sadness. These subjects also said that they were recovering and adjusting to this fact. However, one-fourth reported feeling very distressed *(43)*. Although the study collected information from couples, only results related to the women were reported.

No studies were found evaluating the long-term consequences of treatment failure or success on the psychological and social life of the couples.

Sex selection

The desire to control the sex of children, with preference for males, has been observed throughout history, with preference for male offspring among monarchs and infanticide of newborn girls being described in various cultures *(44–46,63)*. However, until recently, most of the literature referred to selective abortion of female fetuses and not to selection of sex through ART *(47)*. Ultrasonic diagnosis of the sex of fetuses has allowed the practice of selective abortion of females, allegedly being carried out in Northern India *(48)*. In summary, the capacity to select the sex of children has evolved from infanticide to selected abortion of female fetuses *(48,49)* to preimplantation selection of embryos *(50)*.

Preconceptional sex selection has been tried for decades and recent progress in sex identification of pre-embryos before implantation and separation of X-bearing and Y-bearing spermatozoa by flow cytometry, combined with ART, appears to offer real possibilities for pre-conception determination of the sex of the fetus *(51–53)*. This has meant shifting the possibility of determining the offspring's sex away from selective abortion and infanticide. At the same time, it has raised new ethical issues resulting from the potential for social problems caused by the sexual imbalance, as has already been observed in some countries in Asia *(49)*.

The Committee for the Ethical Aspects of Human Reproduction and Women's Health of the International Federation of Gynecology and Obstetrics (FIGO), the American College of Obstetricians and Gynecologists (ACOG), and the American Society of Reproductive Medicine (ASRM) have made recommendations on how their associates should act with reference to sex selection, which refers mostly to preimplantation diagnosis and selected transfer. Such recommendations vary from complete banning of sex selection for nonmedical reasons *(50,54)*, to discouragement of its practice while not favouring its legal prohibition *(55,56)*.

These recommendations have caused debate among physicians *(22)*. Those who have a more global view of the social and cultural implications of sex selection appear to be more strongly against its practice, based on the situations in China and parts of India, which has led to a distortion of the sex ratio in those countries *(49)*. Globally, it has been estimated that 100 million women have died prematurely due to a variety of discriminations *(57,58)*. Others, with great clinical practice experience, but a more restricted western perspective, do not see a major risk in allowing couples to choose the sex of their offspring for social or personal reasons *(59,22)*. Wertz and Fletcher *(60)* already found a trend to increasing acceptance of the couples' rights to choose the sex of their children, among geneticists from all around the world. The authors conclude that the geneticists believe they are serving their clients' needs.

The rights of the parents to wish for a baby of a given sex have been defended with several arguments. Warren *(61)* presented some of the most articulate claims for sex choice. She gave three reasons to justify the parent's desire for sex selection. First, it would enhance the quality of life of the child that would supposedly be better if it is of the wanted sex than if it were of the sex unwanted by the parents. Second, it would improve the family's quality of life if it is formed with the desired balance of sex among the children. Third, it would also enhance the quality of life of the mother, as she would need to give birth to fewer children to have those of the desired sex.

Although these arguments appear rather convincing and come from a feminist writer, the three claims involve the concept that there is one sex that is more desirable than the other. Sex selection tends to perpetuate a sexist society because it implies that one sex is better than the other and would contribute to gender discrimination.

Those who defend the couples' right to choose their children's sex do not see a risk of changes in the sex ratio. They claim that couples attending infertility clinics already have two or three children of one sex and want to have at least one of the opposite sex, with no particular preference for boys or girls *(53,62)*. They argue that this is part of reproductive freedom while others claim that there is evidence for a heavy preference for males *(46,63)*.

The most striking evidence comes from the observed changes in the sex ratio at birth indicating a

reduction in the number of female births in India and China, which have been attributed to selective abortion of female fetuses diagnosed through ultrasound *(48,49)*. The differences between the various authors reflect the different cultures they refer to. For example, in Asia there is strong evidence for the preference of male offspring, while this is not necessarily the case in western countries.

Limitations of studies reviewed

Several limitations affected most of the studies reviewed. Almost every study was descriptive, convenience samples were used, and the results cannot be generalized but are valid only for the specific sample. In some studies the only selection criterion for the couples was the time availability of the nurse who carried out the interviews. Sometimes couples at their first visit were pooled together with those who had already had one or more cycles of assisted reproduction, generally IVF, in the same or in an institution different from that where the study was carried out. In most cases the couples were interviewed during the first visit or later during the treatment period. Information is lacking about the reactions of couples after the treatment had ended and when they were confronted either with failure or with a pregnancy and a child.

There seems to be a total lack of information about the possible consequences of the economic stress on the family caused by the high cost of the new ART. Sometimes the wife has had to stop working to be able to follow the requirements of several cycles of assisted reproduction.

The question of sex preference in sex selection in different cultures, factors associated with sex preference and consequences of sex selection, have been subjects mostly neglected by research.

In spite of all these limitations, there are some issues where the consistency of the results of practically every study, carried out in a variety of settings, with different methods, allows valid conclusions to be drawn. This is so for the greater emotional impact of being infertile, as well as the greater psychological consequences of the treatment, for women than men.

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Family networks and support to infertile people

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Introduction

Millions of women and men worldwide are confronted with infertility. It is estimated that between 8% and 12% of couples around the world have difficulty conceiving a child at some point in their lives *(1)*. However, the incidence of primary and secondary infertility varies enormously in each region *(2,3)*. Globally, the World Health Organization (WHO) estimates that sixty to eighty million couples experience unwanted infertility *(4)*.

Although data on the etiology of infertility vary in their accuracy from region to region, it is estimated that worldwide about 5% of infertile couples are infertile due to anatomical, genetic, endocrinological or immunological factors *(1)*. The remainder suffer from involuntary infertility related to preventable conditions including: sexually transmitted, infectious and parasitic diseases; iatrogenic health care practices; exposure to potentially toxic substances in the diet or environment; and medical neglect of precursor conditions *(5,6)*.

Recent studies show that in resource-poor countries, where children are highly valued for cultural and economic reasons, childlessness often creates enormous problems for couples; especially women who are generally blamed for the infertility. Motherhood is often the only way for women to enhance their status within their family and community. In some places, the stigma of childlessness is so great that infertile women are socially isolated and neglected. Studies from communities in Egypt *(7)*, Nigeria *(8)*, Mozambique *(9)*, the Gambia *(10)* and India *(11)* showed that infertile women are often excluded from societal events and ceremonies or may even be despised and perceived as inauspicious *(12)*. In some cases, they are feared as casting the "evil eye" on pregnant women. The psychological consequences of infertility have been well described in the literature. Infertile women often feel guilt and worthlessness leading to low self-esteem, depression and anxiety. In terms of the economic impact of childlessness, childless women and their families may feel that they have a lack of social security and support in their old age.

Conjugal relations often become stressful due to infertility. Infertile women often receive disrespectful treatment by husbands, and the husbands' families may encourage them to divorce or take a second wife. At the family and lineage level, childless women receive disrespectful treatment and maltreatment by in-laws due to the concern for their family lineage dying out. In more extreme cases, acts of violence are committed against them.

Women develop various mechanisms to cope with infertility. A significant role in such mechanisms is played by the family and other social networks, although such a role could be positive, constructive

or negative. There is limited information regarding how family and social networks support women and men to cope with the problem of infertility. Such knowledge could be useful in developing culturally sensitive programmes to help infertile women cope with their problems and improve their lives.

This paper summarizes findings from studies on the role of supporting networks in coping with infertility. The studies vary considerably in terms of design, scope, site, study population and other methodological issues. Both quantitative and qualitative research methodologies were used. In some studies, such as in Bangladesh, Mozambique and Thailand, data originate from the exploratory phase of more extended research projects, while data drawn from other research studies including those in Mexico, Nigeria and Zimbabwe were collected as part of larger, long-term (two- or three-year) studies on various reproductive health concerns. However, what these studies have in common is that they allow people confronted with infertility to speak out about their personal experiences and views regarding their infertility as well as suggest strategies to cope with the problem.

Sources of support and their impact

Family support

Most studies showed that childless couples find in their spouse, family and relatives sources of support for solutions and stress. In the Mexican society, among the Tjolabal women, if no pregnancy occurs after several months of living together as a couple, the mother, mother-in-law or another older women in the family will recommend a series of home remedies to warm the woman's body. In Pakistan, coercion by in-laws had a strong effect on women who experience infertility and was an important motive for seeking treatment *(13)*. In the family context of infertile women in the Thai society, family support for the infertile woman has both positive and negative aspects. Family members including the mother, mother-in-law, sisters and sisters-in-law may speak negatively to women about the potential of a broken marriage. The following narrative from the Thai study demonstrates this point *(14)*:

Chem: "My relatives teased me that my husband will have a new wife, for I have no baby for him, I get very hurt."

Mala: "Because I do not have a child for my husband's family, I get insulted from my mother-in-law. My husband is the only child of his parents."

Together with the negative criticism within the woman's family, family members also suggest ways to conceive, such as herbal medicine for self-treatment, sources of western treatment in clinics and hospitals and religious practices.

Sri: "I prayed to the spirit of Prince Pichai, with a pig's head. It was my parent's advice but I am still infertile."

Tang: "I choose to go to this hospital because my sister recommended it to me."

Chaem: "My mother-in-law is the one who told me to get the sperm of my brother-in-law to use in the treatment."

In Thai cases, women will usually get pressure from and be criticized by the husbands' family members or relatives if they delay or if they do not seek treatment. For example, Sri, one of the women interviewed for the study, stated:

Sri: "My husband's parents who stay in another town once visited me and they criticized me that I do not have a child. They say that when my husband and I get old, we will suffer and have hard lives. My husband's parents want to have a grandchild very badly and encourage me to see the doctor. For my husband, he does not care much. If I do not conceive, it is O.K. for him."

Mala, another woman in the Thai sudy felt that:

Mala: "The fact that I do not have a child for my husband's family is a really important matter. If I have only one child and no more it is O.K. My mother-in-law wants one because she has only one child herself. I am really mad and want to criticize my mother-in-law. For my husband, he really does not mind, it is only because of my mother-in-law's pressure. If it were not for her, I would not go through this treatment process. It is really painful and I feel very embarrassed when the doctor investigates my cervix. This time if it does not work, I will give up" *(14)*.

The advice may be encouraging but may also bring

too much pressure, stress and psychological harm to women. Society and often family blame women for not having done enough to conceive a child. Chaem, another informant, said:

Chaem: "Everybody asked me—'Why don't you consult the doctor? Why don't you pray to the spirit for having a child?' When they questioned me, I felt so bad. I don't want anybody to ask me like that, it makes me angry. Why do they think that I did nothing?"

The Thai cases show that women get more blame or more negative than positive support from their family network. Support for infertile couples includes information regarding sources of treatment or other practical solutions, often given without understanding and sharing of the emotional problems involved. Infertile women may get criticized by the family as well as by the community for being deficient, although they have struggled very hard and may have suffered physical and psychological pain from the treatments. The data from Thailand show that childless women do not get enough support from their families. In the interviews, many women expressed the feeling that no family member sympathized with them or wanted to share experiences and emotions with them.

The Thai study is only one among very few studies that look at women's lives in the context of infertility over the life course. It showed that infertility causes a prolonged state of crisis that changes throughout the life cycle. In the first stage, women receive blame, criticism and pressure for treatment. Such pressure often pushes women to seek information and medical treatment. After women have gone through several coping strategies, they may gain a new moral identity and sense of self-worth. Some turn to religious explanations of "karma" to interpret their life suffering, some redirect their energies to their work and look forward to succeeding in their careers, others give new meaning to their infertility by realizing that their karma is good and they are lucky not to have the burden of children; and still others choose to adopt a child. In addition, during the later stages of their infertility, women receive more support from their families which at this stage seem to sympathize more with the problem of infertility and to show a rather neutral attitude toward the women.

A study in the UK by Boivin *et al. (15)* showed that infertile couples relied primarily on their spouse, family and friends as sources of support when distressed, rather than on formal support resources such as psychosocial counselling, support groups organized by professional staff or the media. In the UK study, women used significantly more sources of support than men. There is a tendency for women to evaluate the discussions with family and friends as more helpful than those with their partners. Although patients were given the opportunity to list additional sources of support, none mentioned their family doctor or general practitioner. It is interesting to state that in the UK study, using a standard weighted rating, the rank order for the different sources of support from the highest to the lowest were: spouse, family/friends, media information, library books, counsellors and support groups. The rank was similar for men, except that discussion with families and friends was rated lower than media information, indicating that the men in this study relied more on nonhuman sources of support, i.e. media information, than on family and friends *(15)*.

Spouse's reaction and support

It is necessary to understand the spouse's reaction to understand the spouses' support. Childlessness strongly affects the relationship of the couple in terms of both sexual and marital life. Most studies in resource-poor countries found a similar pattern of negative reactions rather than positive support from childless women's husbands. In the Bangladesh study, it was found that women who are abandoned by their husbands due to their childlessness have to find work to survive, as the parents refuse to bear their living expenses. Deprived of education or appropriate job opportunities, some turn to commercial sex for income generation. Childless women complain about domestic violence and disrespectful treatment by husbands and family in-laws, sometimes being treated by their husbands as a servant. Others are abandoned by their husbands or end up as a second wife in a polygamous marriage *(16)*. In Mexico, the lack of children was found to be an excuse for domestic violence against the wife and for drunkenness of the husband. In southwest Nigeria, women are most often blamed for infertility with a common consequence of a couple's infertility being the expulsion of the woman from the husband's house *(8)*.

Similarly, studies in Bangladesh *(16)*, Nigeria *(17)*, Mexico *(18)* and Zimbabwe *(19)* showed that infertile women expressed fear of being divorced because they could not give their husbands a child. Men often face

social pressure from their own relatives to divorce their wives who are held responsible for the problem of infertility. The husband's relatives may also maltreat, taunt and threaten the childless women. On the contrary, in the Thai study it was found that husbands of childless women did not criticize or blame their wives, but were supportive. Many of them promised their wives they would never leave them because of the infertility problem. The differences in husband's support in Thailand, compared to Bangladesh, Nigeria, Mexico and Zimbabwe, may be due to the fact that women's social status in Thailand is relatively higher.

Nevertheless, Thai women still fear abandonment by their husbands, as do the infertile women in the study carried out in Mozambique. Many of the Mozambican infertile women had been married and divorced earlier. However, past divorce of infertile women in Mozambique was often not related to their infertility *(9)*.

Studies in resource-poor countries revealed that women have an active fertility-seeking behaviour in both the medical and traditional health systems, but they seek help alone. There is a lack of male involvement in solving the infertility problem even in cases of male factor infertility. For example, all infertile women in the Bangladesh study *(16)* sought medical treatment, but their husbands did not. This is the case even if a woman had clearly stated evidence of her husband's impotence. In Mexico *(18)*, the Tjolabal women stated that when weakness or coldness of the man's body is diagnosed, he frequently refuses to accept such treatments because it puts his masculinity in doubt. On the contrary, when Tjolabal women do not become pregnant after several months of living together as a couple, a series of treatments is given to warm the women's body and men cooperate in this treatment as assigned by mothers, mothers-in-law, or other older women. In the Gambia, very few women bring their husband along for formal treatment and the health care providers do not usually ask for this *(20)*. The situation is similar in the Thai society; women alone are usually blamed for the reproductive failure and must carry the burden of solving the problem through a therapeutic process that is sometimes traumatic and often unsuccessful. The woman is the first one to get tested when she "fails" to conceive. This is the case even though testing the man's sperm is easier, less invasive and less costly. Medical doctors do not request the husband's presence at the beginning of the treatment process. Women themselves also follow this dominant ideology without being aware that infertility treatment should be the treatment of the couple. The following narrative from the Thai study illustrates the point:

Pern: "My husband never got checked up but he is healthy and fertile because he got another woman pregnant when he lived in Libya. This woman had to go for an abortion because she wanted to have a new husband. I believe that the problem comes from me. It is about my operation [tumour at the side of her cervix]."

Srikarn: "I never blame my husband, I think the problem comes from my side, although I know that he has a problem also. I used to warn him about his smoking and drinking habit but I do not blame him … he told me that I already give them [sperms] to you but you cannot keep them yourself. From the sperm count, his sperms have two heads and no tail. The doctor told me that the problem comes from both of us. But I think the problem comes from my side. I have a 'bad' cervix, I travel a lot, I got an infection and abnormal discharge and have an inflammation of the ovary tube. Later I had to have an appendix operation…. To travel often makes me have frequent reproductive tract infections and it is chronic."

However, in the Thai case the situation is relatively better than in Bangladesh. In studies in India *(21)* as well as in Thailand, it appeared that husbands were supportive of their infertile wives. Some Thai men accompanied their wives to the infertility clinic for treatment or diagnosis, although this is not very common. The degree of active involvement is generally not great. Some husbands accompany their wives and sit outside the patient's room waiting without participating in any process of treatment. Most Thai men generally cooperate with the tests unless their masculine identity is threatened. For example, if the man has been asked to give semen too often, he will refuse to cooperate. The case of Srikarn reflects this point:

Srikarn: "When I ask him for his sperm several times, he will get upset, he told me that he felt like a mouse in an experiment. The first few times that I asked for his semen, he cooperated very well, but later when I asked him again and again, he said no to me, he said he has feelings, he is upset and feels humiliated, I was mad when my husband

refused to cooperate but I feel sympathetic to him. He told me that even if I had a child for him, he can still get a minor wife."

In western countries, infertility seems to be a couple's problem for which both men and women will seek treatment together, and male involvement in the treatment does not appear to be a problem.

Community support

Community support for infertile couples is not given much attention in studies on infertility in developing countries. Childless women and men are found to be stigmatized because of their infertility. Exclusion from certain social activities and ceremonies was observed for infertile Macua women in Mozambique, while childless men in Dhaka, Bangladesh, are not treated as equal to other men in their society. For women, being childless is worse, as the studied communities offer women very few or no alternative roles to that of mother. However, considerable variation is found among regions. For example, among the matrilineal Macua in the north of Mozambique, men are quite often blamed for infertility, which can lead to divorce initiated by the women or their relatives. An interesting example of this was described by Bharadwaj *(21)*. In a multi-sited research study among couples visiting infertility clinics in India, he found that—contrary to the popular belief—men are no less affected by the stigma of infertility than women, and the fear of being considered impotent was found central to the anxiety that some men experience. Miall *(22)* also found that males are seen as more stigmatized by infertility than females, who generally receive sympathy. Couples perceived both the infertility and the clinical management of it as stigmatizing conditions. Therefore, several of his respondents accepted donor egg or sperm only if it was kept secret, because in this way others would consider their offspring as biological, while adoption would make their infertility most visible for the public, and was therefore seen as a more problematic and stigmatizing option. Differences between countries and regions regarding social isolation and rejection of childless women seem to be, among others, influenced by the specific kinship systems, family and conjugal ties, by moral and legal rules and religious customs. Generally speaking, a relatively low status of women compared with men is associated with a strong negative response to infertile women.

None of the studies reported the community's mechanisms of support to infertile couples. The only known examples of community assistance for women who are childless because they have lost many children come from the Gambia and Senegal. In the Gambia, it is pointed out that community members of the Mandinkas and Jolas have organized an association named the Kanyaleng group. The group recruits women who have lost many children or who have never given birth. The social significance of the Kanyaleng has been described as enhancing the survival of children through different rites and behaviour *(10)*.

Media and written documentation as a source of support

A study in the UK by Boivin *et al. (15)* also revealed that media and written documents are other important support resources for infertile couples, especially childless men. In this study, the couples sought support from written documentation on the emotional aspects of infertility provided in clinics, as well as such information provided through the media. This mode of intervening with patients remains unexplored despite the fact that many patients have requested more written psychosocial information in past surveys. In this study, almost 50% of women had used written information provided at the clinic, newspaper articles and/or television documentaries on the emotional aspects of infertility as a way of coping with this medical problem and/or its treatment. While documentation and information in the media would seem unlikely to provide some aspects of support (e.g. comfort), these may fulfil other important support functions. For example, television documentaries on couples undergoing *in vitro* fertilization (IVF) were watched by many of the patients in the study, who commented that they felt reassured in discovering that they were not the only ones having difficulty coping with infertility. In addition, many felt that such documentaries or articles helped their families and friends better understand the impact that infertility was having on their lives. This finding suggests that the importance of psychosocial documentation, whether developed by the clinic or provided by the media, should not be underestimated because it has already been used by patients and can be a cost-effective way for the clinic to provide psychosocial services to patients.

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Parenting and the psychological development of the child in ART families

SUSAN GOLOMBOK

Introduction

Advances in assisted reproduction procedures have had a fundamental impact on the way in which mothers and fathers may be related to their children. As Einwohner *(1)* has pointed out, it is now possible for a child to have five parents: an egg donor, a sperm donor, a birth mother who hosts the pregnancy and the two social parents whom the child knows as "mum" and "dad". In addition, a small but growing number of lesbian and single heterosexual women are opting for assisted reproduction, particularly donor insemination (DI), to allow them to conceive a child without the involvement of a male partner. Children in these families grow up without a father right from the start, and many children in lesbian families are raised by two mothers. This paper will examine research on parenting and the psychological development of children in assisted reproduction families with particular attention to the issues and concerns that have been raised by creating families in this way.

IVF families

Concerns about parenting in IVF families

It may seem that having a genetically related child by *in vitro* fertilization (IVF) is just the same as having a child by natural conception; all that is different is the process of conception. However, there are a number of reasons why having a child by IVF may result in a rather different experience for parents than having a child in the usual way. One very apparent difference is the higher incidence of multiple births, preterm births and low-birth-weight infants following IVF *(2–10)*. Whereas only 1% of natural births involve twins, triplets or more, this is true of more than one-quarter of births resulting from IVF. Parents who have multiple births not only have to cope with two or more infants born at once but also with infants who may have greater needs as a result of prematurity and low birth weight *(11)*. The impact of these factors on parenting and child development must be considered separately from the impact of IVF per se. Many, but not all, of the empirical investigations described below have focused on families with a singleton child born as a result of IVF to avoid the confounding effect of a multiple birth.

It has also been suggested that the stress associated with the experience of infertility and its treatment, often lasting for many years, may result in parenting difficulties when a long-awaited baby is eventually born. According to Burns *(12)*, the stresses produced by infertility are likely to result in dysfunctional patterns of parenting. She argued that parents who had difficulty in conceiving may overprotect and emotionally overinvest in their much longed-for child.

Other authors have also suggested that those who become parents after a period of infertility may be overprotective of their children, or may have unrealistic expectations of them, or of themselves as parents, due to the difficulties they experienced in their attempt to give birth *(13–16)*. Psychological disorders and marital difficulties have also been predicted for those who become parents following IVF *(14)*.

Research on parenting in IVF families

In a prospective study of IVF families in Australia, psychological adjustment to first-time motherhood was assessed when the baby was 4 months old *(17)*. Sixty-five IVF families were compared with 62 families with no history of infertility. The aim of the study was to determine whether mothers who conceived by IVF differed from the matched comparison group of natural conception mothers with respect to adjustment to early parenthood, specific adjustment to the maternal role, and quality of the parent–child relationship. There were no differences between the two groups of mothers for anxiety, postnatal depression, marital adjustment or feelings of attachment toward the infant. In addition, an observational assessment showed no difference in maternal behaviour between the two groups of mothers. However, the IVF mothers reported lower self-esteem and lower maternal self-efficacy. The authors concluded that the overall findings were reassuring for parents who conceive by IVF, and that the specific differences identified between the IVF and the natural conception mothers may be explained by the IVF mothers judging themselves too harshly.

These families were followed up when the babies were one year old *(18,19)*. At that time, the two groups of parents did not differ on measures of anxiety, depression or social support, or on measures more specific to parenthood such as attachment to the child, attitudes to childrearing, separation anxiety, interaction during play and parenting stress. There was, however, a nonsignificant trend towards the IVF mothers reporting lower self-esteem and less parenting competence than the natural conception mothers. In addition, although there were no group differences in maternal protectiveness, the IVF mothers saw their children as significantly more vulnerable and "special". The IVF fathers reported significantly lower self-esteem and marital satisfaction but no less competence in parenting. It was concluded that the IVF parents' adjustment to parenthood when their child was one year old was similar to that of the natural conception families. However, there were minor differences among the IVF parents that reflected heightened child-focused concern and less confidence in parenting for mothers, less satisfaction with the marriage for fathers, and vulnerable self-esteem for both parents. The authors emphasized that although the IVF mothers continued to have higher levels of concern over child well-being and notions of child "specialness", their concerns did not appear to be associated with more protective parenting compared with mothers who had conceived naturally. In addition, these parenting attitudes did not seem to translate into differences or impairments in the quality of mother–child interaction. They did suggest, however, that the IVF mothers' concerns may cause them to be preoccupied with the child and exclude their husbands, thereby contributing to the fathers' lower marital satisfaction and self-esteem.

In France, Raoul-Duval *et al. (20)* studied 33 families with an IVF child in comparison with matched groups of 33 families with a history of infertility (who conceived their child through ovarian stimulation), and 33 families with a naturally conceived child. The families were first seen in the hospital after delivery, and then followed up at home after nine months, 18 months and three years. There were no significant differences among the three family types in the incidence of either maternal depression or problems in the relationship between the mother and the child at any of the assessment periods. It should be noted, however, that by the time of the three-year-old assessment, only 39% of the natural conception mothers and 33% of the mothers with a history of infertility remained in the study, although a higher proportion of IVF families (76%) was retained. As the authors point out, if the other types of family were lost due to difficulties in family functioning, this would give greater weight to the conclusion that the IVF families were not at risk.

Twenty couples in the UK who had conceived by IVF were compared, when their babies were between 15 and 27 months old, with a matched group of 20 couples who had conceived without medical assistance *(21)*. There were no differences between the two groups of parents for emotional health or marital adjustment, and their scores were closely comparable to general population norms. The IVF parents reported significantly more positive feelings toward their babies than the natural conception parents, and rated themselves as feeling significantly less tied down.

However, the IVF parents reported being more protective toward their child than the natural conception parents. It may be relevant that these parents were the first to give birth to an IVF child in this area of the country. It should also be noted that these findings were based on questionnaire data only.

IVF families with children between 24 and 30 months of age in Belgium were studied by Colpin *et al. (22)* using both self-report questionnaires and observational measures of mother–child interaction. Thirty-one families with an IVF child were compared with 31 natural conception families with no history of infertility. No differences were identified between the IVF families and the natural conception families for any of the measures of the parents' psychological functioning or the mother's relationship with her child. The only difference to emerge related to the subgroup of IVF mothers who were employed; those who were employed were less encouraging of their child's autonomy during problem-solving than both the nonemployed IVF mothers and the employed natural conception mothers. A possible explanation for this finding put forward by the authors was that it may be more difficult for IVF mothers to resume work outside the home than it is for natural conception mothers, and that they may compensate by inhibiting their child from being autonomous. Certainly, the IVF mothers who resumed work were more likely to have done so for financial reasons than the mothers of naturally conceived children. Nevertheless, an alternative explanation is that this finding represented a chance effect resulting from the large number of group comparisons carried out.

In a study of IVF families with two-year-old to four-year-old children in the Netherlands, van Balen *(94)* included a comparison group of formerly infertile parents with a naturally conceived child to control for the experience of infertility, in addition to a comparison group of natural conception families with no history of infertility. Forty-five IVF parents, 35 formerly infertile parents and 35 fertile parents completed questionnaires on parenting behaviour and on their child. The IVF mothers and the previously infertile mothers differed from the natural conception mothers with respect to emotional involvement with their child. They reported experiencing more pleasure in their child and stronger feelings toward their child than mothers with no history of infertility. The IVF and the previously infertile mothers also reported greater parental competence than the fertile mothers. There were no differences regarding the mothers' reports of parental concern, parental expectations or parental burden. Fathers did not differ on any of the measures. It seems, therefore, that where differences existed the findings of this study pointed to positive outcomes for mothers of two-year-old to four-year-old children with a history of infertility, whether or not the child was conceived by IVF. It is important to point out that these results are based on self-report questionnaires and the response rate was low (ranging from 69% for the IVF mothers through 52% for the previously infertile mothers to 35% for the fertile mothers). An advantage of the study, however, was the inclusion of a comparison group of previously infertile couples who did not conceive by IVF.

The European Study of Assisted Reproduction Families examined the quality of parenting in families created as a result of both IVF and DI, in comparison with families with a naturally conceived child and adoptive families. In the first phase of the study, conducted when the children were between four and eight years of age, representative samples in the UK of 41 families with a child conceived by IVF and 45 families with a child conceived by DI were compared with 43 families with a naturally conceived child and 55 families with a child adopted in the first six months of life *(23)*. The findings relating to the DI families are presented below. It was found that parents with a child conceived by IVF obtained significantly higher ratings for mother's warmth to the child, mother's emotional involvement with the child, and both mother–child and father–child interaction, than the natural conception parents. The adoptive parents' ratings on these variables were closely comparable to those of the IVF parents. In line with these findings, mothers and fathers of naturally conceived children reported significantly higher levels of stress associated with parenting. Thus, the findings of this study indicated that the quality of parenting in families with a child conceived by IVF was superior to that shown by families with a naturally conceived child. Where parents in the different family types differed with respect to anxiety, depression, or marital satisfaction, this reflected greater difficulties among the natural conception parents.

In the second phase of the research, the study was expanded to include an additional country from northern Europe (the Netherlands) and two countries from southern Europe (Spain and Italy) to increase the sample size and to allow the examination of the influence of culturally determined attitudes toward assisted reproduction on the functioning of families

that have resulted from these techniques. Identical procedures were employed in the Netherlands, Spain and Italy as had been used in the UK. The inclusion of these three countries brought the total number of IVF, DI, adoptive and naturally conceived families, respectively, to 116, 111, 115, and 120 (see below for findings regarding DI families). The results of the extended study confirmed the findings of the original investigation *(24)*. Mothers of children conceived by IVF were found to express greater warmth to their child, to be more emotionally involved with their child, to interact more with their child and to report less stress associated with parenting than the comparison group who conceived their child naturally. In addition, IVF fathers were reported by mothers to interact with their child more than fathers with a naturally conceived child.

The families were followed up as the children approached adolescence *(25,26)*. One hundred and two of the IVF families, 102 of the adoptive families and 102 of the natural conception families were assessed as near as possible to the child's 12th birthday, representing response rates of 92%, 91%, and 93%, respectively, excluding those who could not be traced. The focus of the study was on parent–child relationships with an emphasis on warmth and control, two aspects of parenting that are considered important for the psychological adjustment of the adolescent child. The IVF parents generally continued to have good relationships with their children characterized by a combination of affection and appropriate control. The few differences found between the IVF families and the other family types reflected more positive functioning among the IVF families, with the possible exception of over-involvement with their children of a small proportion of IVF mothers and fathers.

In the first study to be conducted in a nonWestern culture, Hahn and DiPietro *(16)* compared 54 IVF families with 59 natural conception families when the target child was between three and seven years of age. The quality of parenting was generally found to be similar for the two types of family, although IVF mothers showed greater protectiveness, but not restrictiveness, of their children. The children's teachers, who were unaware of the nature of the child's conception, did not report the IVF mothers to be more protective or more intrusive in their parenting behaviour, but did rate them as more affectionate towards their children than the natural conception parents. The expectation that mothers would be more protective and indulgent of boys than girls due to the higher value placed on sons was not supported by the findings of this study.

Research on children in IVF families

Cognitive development

The early studies of the cognitive development of IVF children did not employ comparison groups. However, the children were administered standardized measures of cognitive development for which normative data were available. In a study of 33 children in Australia, the Bayley Scales of Infant Development were administered to one- to three-year olds *(13,28)*. These scales produce a mental development index (MDI) and a physical development index (PDI), each with an average score of 100. For the IVF children, the scores were within the normal range with a mean MDI of 111 and a mean PDI of 105. With one exception, those with the lowest scores had been born prematurely or with a medical disorder. In another Australian study *(29)*, 20 IVF children were assessed using the Griffiths Developmental Scales. The Griffiths scales give a developmental quotient (DQ), again with an average score of 100. After correcting for prematurity, the mean DQ for the IVF infants was found to be 117. Only one infant, born at 33 weeks' gestation, obtained a below-average score. The Griffiths Scales were also administered to 99 IVF children 33–85 months of age in Sweden *(30)*. The mean DQ was above average, and only one child had a DQ of less than 85.

A number of controlled studies have now been reported. The Bayley Scale scores of 65 IVF infants were compared with those of a matched control group of 62 naturally conceived infants at 12 months of age *(31)*. There was no significant difference between the IVF and natural conception children for either mental or physical development as assessed by the MDI and PDI, respectively. Other studies with large samples and matched comparison groups have reported similar findings using the Bayley Scales. No difference in Bayley Scale scores was found between 116 IVF children and 116 non-IVF matched controls between 12 and 45 months of age in Israel *(32)*. In the USA, no difference was found in MDI scores between IVF and matched non-IVF infants between 12 and 30 months of age, and significantly higher PDI scores were found among those conceived by IVF *(33)*.

Using different measures of cognitive development, similar findings have been reported by a number of other researchers. In France, no evidence of psycho-

motor deficit was found in 31 children as assessed by the Brunet-Lezine test at nine months and 18 months in comparison with 31 children conceived by ovulation induction and 31 naturally conceived children *(34)*. Similarly, in a comparison between 26 IVF infants and 29 naturally conceived infants in Israel using the General Cognitive Index, no difference in either the overall score, or in the subscale scores of perception, memory and motor skills, was identified between children from the two family types *(35)*.

With respect to school-age children, the Wechsler Intelligence Scale for Children and tests of visual–motor coordination, visual memory and reading comprehension were administered to IVF children and a comparison group of naturally conceived children with an average age of nine years in Israel *(36)*. All of the children were born at full term. The two groups of children did not differ with respect to any of the measures of cognitive development. Similarly, in an investigation of the scholastic achievement of 370 children 6–13 years of age in France, there was no evidence of low educational attainment among children conceived by IVF in comparison with general population norms *(37)*.

Socioemotional development

In their prospective study of 65 IVF families and 62 families with no history of infertility in Australia, McMahon *et al. (17)* first assessed children's socio-emotional development at the age of four months. All of the babies were singletons. IVF mothers rated their babies as more temperamentally difficult than did the natural conception mothers (although these ratings were within the normal range), and the IVF babies showed more negative behaviours in response to stress. In a follow-up of the families when the babies were one year old, assessments were made of social development, temperament, behaviour problems and test-taking behaviour *(31)*. No differences between the two groups of children were found for social development or test-taking behaviour. Although mothers in both groups reported low levels of behaviour problems, the IVF mothers rated their children as having more behavioural difficulties, and more difficult temperaments, than the control mothers. The authors concluded that these findings may be related to the greater anxiety of IVF mothers about their children's well-being. The security of the infant's attachment to the mother was also assessed at 12 months of age using the Strange Situation procedure *(19)*. IVF children showed predominantly secure attachment relationships, and there was no difference between groups in the proportion classified as insecurely attached.

Thirty-one children between 24 and 30 months of age were compared with 31 natural conception children in Belgium *(22)*. The child's behaviour was rated during an interaction task with the mother on four task-oriented scales (enthusiasm, persistence, reliance on mother and compliance) and three mother-orientated scales (avoidance of the mother, hostility and positive feelings towards her). No differences were found between children from the two family types for any of the scales. In a study of two-year-old to four-year-old children in the Netherlands, 45 IVF children were studied in comparison with 35 natural conception children whose parents had experienced infertility and 35 natural conception children whose parents had no history of infertility *(103)*. The IVF mothers, but not fathers, rated their children as more social and less obstinate than the other mothers, as assessed by a self-report questionnaire. In a large but uncontrolled study of 743 IVF children between 4 and 14 years of age in the USA, a standardized questionnaire of emotional and behavioural problems, the Achenbach Child Behaviour Checklist, showed no indication of raised levels of psychological problems in children conceived by IVF in comparison with general population norms *(38)*. Also using the Achenbach Child Behaviour Checklist, no difference was found in the incidence of emotional or behavioural problems between IVF children 33–85 months of age and a general population control group in Sweden *(30)*.

The European Study of Assisted Reproduction Families *(24)* has obtained data on the socioemotional development of 116 IVF, 111 DI, 115 adopted and 120 natural conception children four to eight years of age in Italy, Spain, the Netherlands and the UK (findings relating to DI children are reported below). Standardized questionnaires of behavioural and emotional problems were completed by mothers and teachers, and the children were administered tests of self-esteem and of their feelings towards their parents. The IVF children did not differ with respect to the presence of psychological disorders or in their perception of their relationship with their parents in any of the four countries studied. In the UK, an assessment was also made of the children's security of attachment to their parents using the Separation Anxiety Test. In addition, interview transcripts

relating to children's psychological functioning were rated by a child psychiatrist who was "blind" to the child's family type. No group differences were found for either security of attachment relationships or for the incidence of psychological disorder as assessed by the child psychiatrist *(23)*. When followed up at the age of 12 years, the IVF children were continuing to function well *(25,26)*.

One study, conducted in Israel, has found a higher incidence of emotional problems among IVF children *(36)*. In a comparison between IVF and naturally conceived children of middle-school age on measures of school adjustment, hyperactivity, trait anxiety, depression, aggression and behavioural problems, the IVF children, particularly the boys, were found to show poorer adjustment to school as rated by teachers and reported themselves to be more aggressive, more anxious and more depressed. The authors were careful to exclude children born prematurely. However, this finding may be explained by the older age of the IVF parents.

Conclusion

Contrary to the concerns that have been raised regarding the potentially negative consequences of IVF for parenting, studies of these families have generally found IVF parents to be well adjusted and to have good relationships with their children. To the extent that differences have been found between IVF and natural conception parents, in the early years these have tended to reflect higher levels of anxiety about parenting by IVF mothers. For example, *(17)* IVF mothers of four-month-old infants were found to report lower self-esteem and lower maternal self-efficacy than natural conception mothers, although these differences had lessened by the child's first birthday *(19)*. In addition, several studies indicated that IVF mothers were more protective of their child *(21)*, allowed their child less autonomy *(22)*, and saw their child as more vulnerable and "special" *(19)*.

These findings must be viewed in the context of a lack of difference between IVF and natural conception families with respect to other measures of maternal feelings, attitudes and behaviour such as separation anxiety and observational measures of maternal behaviour *(17,19)*, maternal interaction with the child *(20)*; acceptance, overindulgence, and rejection of the baby *(21)*; maternal attitudes and emotions and observational measures of maternal interaction *(22)*; and maternal concerns, expectations and burdens *(103)*. There were also more positive results for IVF mothers with respect to feelings toward their baby *(21)* and emotional involvement with their child *(103)*. No differences were identified between IVF and natural conception fathers, with the exception of lower self-esteem and marital satisfaction among IVF fathers of one-year-old children in the Australian study *(19)*.

As IVF children enter the early school years, findings from the European Study of Assisted Reproduction Families *(23,24)* indicate that positive effects prevail. IVF mothers and fathers were found to be more involved with their children than natural conception parents. By the age of 12 years this advantage had disappeared. However, the quality of parenting in IVF families continued to be good, characterized by affection and appropriate control. The few differences in parenting identified between IVF and natural conception mothers and fathers did not reflect dysfunctional relationships between the parents and the child.

With respect to the children themselves, there is no evidence from any of the studies conducted to date to suggest that IVF children are at risk for cognitive impairment. These studies have used a variety of standardized assessments of cognitive ability in children of different ages. The social and emotional development of IVF children also appears to be within the normal range, with only one study reporting a higher incidence of psychological problems among children conceived by IVF. Nevertheless, further research is required to enable conclusions to be drawn about the psychological well-being of IVF children in the middle and later school years.

A number of methodological problems have been associated with studies of IVF families. In particular, mothers of IVF children are generally older than mothers who have given birth without medical intervention, and attempts to match natural conception mothers for maternal age has presented difficulties, as has matching for birth order of the target child and the number of children in the family. These confounding factors may well explain the differences identified between IVF and natural conception parents, although some researchers have attempted to control for these variables statistically. Furthermore, some of the samples studied have been small and the cooperation rates have been less than ideal, and few studies have included an additional comparison group of natural conception parents with a history of infertility. Nevertheless, the available data on parenting and child development in families created by IVF

are generally reassuring. Even if the more positive findings for IVF parents can be explained by factors such as fewer children in the family, there is no evidence to suggest that IVF mothers and fathers experience marked difficulties in parenting compared with their natural conception counterparts. Neither is there evidence for a higher incidence of marital or psychological problems among IVF parents. To the contrary, where differences have emerged, these reflect greater psychological adjustment and marital satisfaction among parents of an IVF child.

DI families

Concerns about DI families

There are concerns additional to those expressed in relation to IVF regarding potentially negative consequences for families where donated sperm have been used in the child's conception. Although DI has been practiced for more than a century to enable couples with an infertile male partner to have children, the majority of adults and children conceived in this way remain unaware that the person they know of as their father is not their genetic parent. In recent years there has been growing unease about the secrecy that surrounds families created by DI. It has been argued that secrecy will have an insidious and damaging effect on family relationships and, consequently, on the child.

Findings suggestive of an association between secrecy and negative outcomes for children have come from two major sources; research on adoption, and the family therapy literature. It is now generally accepted that adopted children benefit from knowledge about their biological parents, and there is a commonly held view that children who are not given such information may become confused about their identity and at risk for emotional problems *(39,40, 41,103)*. In the field of assisted reproduction, parallels have been drawn with the adoptive situation, and it has been suggested that lack of knowledge of, or information about, the donor may be harmful for the child *(42–46)*. Family therapists have argued that secrecy can jeopardize communication between family members, cause tension and result in a distancing of some members of the family from others *(47–49)*. In relation to DI, Clamar *(50)* has argued that keeping the circumstances of conception secret will separate those who know the secret (the parents) from those who do not (the child). A further concern is that parents may feel or behave less positively towards a nongenetic than a genetic child. It has been suggested that the child may not be fully accepted as part of the family which may have an undermining effect on the child's sense of identity and psychological well-being. Fathers, in particular, are expected to be more distant from their nongenetic child (*45)*.

Research on parenting in DI families

Rather fewer studies have been carried out of parenting in families created by DI than of parenting in IVF families where the child is genetically related to both parents. This is perhaps not surprising given the parents' desire to maintain secrecy about the nature of their child's conception. In a review of the 12 studies of parents' disclosure of DI published between 1980 and 1995, Brewaeys *(51)* found that few parents (between 1% and 20%) intended to tell their child about her or his genetic origins and, in eight of the twelve studies, fewer than 10% of parents intended to tell. Although it might be expected that a higher proportion of parents in the more recent studies would be open with their children, this was not the case, a finding replicated by van Berkel *et al. (52)* in a comparison between recipients of DI in 1980 and 1996. The trend towards greater encouragement of disclosure does not appear to have resulted in more parents telling their child. Even in Sweden, where legislation gives individuals the right to obtain information about the donor and his identity, a recent survey found that only 11% of parents had informed their child about the donor insemination *(52)*. It is noteworthy that, in spite of their decision to opt for secrecy, almost half of the parents included in Brewaeys's review *(51)* had told at least one other person that they had conceived as a result of DI, thus creating a risk that the child would find out through someone else. Many parents regretted their earlier openness once the child had been born *(53–55)*.

Brewaeys also examined studies of the characteristics of DI parents *(51)*. She found that in the large majority of cases, DI was felt by parents to be a positive choice and, with few exceptions, fathers reported that DI did not influence their relationship with their child and that they felt themselves to be "real" fathers. However, these investigations were based on questionnaire data of variable quality and no control groups had been employed. With respect to psychological adjustment, there was little indica-

tion of disorder in couples who opted for DI *(55–59)*. In addition, these couples had a low divorce rate and high marital satisfaction *(55–57,60)*.

Parenting in DI families was a major focus of the European Study of Assisted Reproduction Families *(23)*. Details of the samples investigated and the measures used are described in the section on IVF families. In addition to these measures, mothers of children conceived by DI were interviewed about their openness regarding their child's genetic origins. The findings relating to the quality of parenting were the same as for the IVF families, suggesting that genetic ties are less important for family functioning than a strong desire for parenthood. Whether the child was genetically related to two parents (in the case of IVF), genetically related to one parent (in the case of DI), or genetically unrelated to both parents (in the case of adoption), the quality of parenting in families where the mother and father had gone to great lengths to have children was superior to that shown by mothers and fathers who had achieved parenthood in the usual way. It was striking, however, that not one set of DI parents had told their child that she or he had been conceived using the sperm of an anonymous donor. Thus, none of the DI children was aware that their father was not their genetic parent. Nevertheless, half of the DI mothers had told a member of their family, and almost one-third had told one or more friends, so there would always be a risk of disclosure to the child. The main reasons for the parents' decision not to tell the child were concern that the child would love the father less, protection of the father from the stigma associated with infertility, and uncertainty about what and when to tell the child *(61)*.

Similar results were obtained with the inclusion of the additional samples from Italy, Spain and the Netherlands *(24)*. Once again, whether or not donor sperm had been used to conceive the child seemed to make little difference to the quality of parenting in assisted reproduction families. Perhaps surprisingly, there was no evidence that attitudes toward assisted reproduction differed between predominantly Protestant northern Europe and predominantly Catholic southern Europe. Not one of the parents with a child conceived by DI in any of the four countries studied had told their child about the method of their conception.

The families were followed up as the children approached adolescence *(26,62)*. It is at adolescence that issues of identity become salient. Thus it is at adolescence that difficulties for DI families may be expected to arise. To the extent that the experiences of adopted children are relevant to children conceived by DI, early adolescence is the time when adopted children begin to show a greater incidence of behavioural problems in comparison with their nonadopted counterparts *(63,64)*, alongside a greater interest in their biological parents *(39)*. Ninety-four of the DI families were examined when the child was 11–12 years of age, representing a response rate of 89% excluding those who could not be traced, in comparison with 102 of the adoptive and 102 of the natural conception families. The measures are described above in relation to the adolescent follow-up of IVF children.

The findings suggest that DI families with an early adolescent child are characterized by stable and satisfying marriages, psychologically healthy parents, and a high level of warmth between parents and their children accompanied by an appropriate level of discipline and control. No differences were identified between the DI and the IVF families for any of the variables relating to the quality of relationships between parents and the child. Of particular interest is the finding that only 8.6% of the DI children had been told about their genetic origins by the time they had reached 11–12 years.

Research on children in heterosexual DI families

Cognitive development

Five studies, reviewed by Brewaeys *(51)*, have examined the cognitive development of children conceived by DI. The earliest study, conducted in Japan *(65)*, reported higher IQ scores among 54 DI children up to 11 years of age when compared with general population norms. Based on parental reports, two studies in Australia *(66,67)* and a study in Sweden *(68)*, found DI children to be above average in terms of psychomotor development. In the only controlled study, 3-month-old to 36-month-old DI children were compared with naturally conceived children in France *(69)*. The DI children were found to be more advanced with respect to psychomotor and language development according to parental reports. In a study conducted in the USA, 10% of school-age DI children were considered, by their schools, to be gifted *(54)*.

Socioemotional development

The first uncontrolled studies found no evidence of emotional or behavioural problems in children conceived by DI *(66,67)*. Although a higher incidence of psychological problems among DI than naturally conceived children as assessed by an interview with parents has been reported *(69)*, other controlled studies that have used standardized measures have shown no evidence of psychological disorder in children conceived by DI. DI children six to eight years of age were compared with matched groups of adopted and naturally conceived children in Australia *(70)*, and four-year-old to eight-year-old DI children were compared with adopted, IVF and naturally conceived children in the UK *(23)* and Europe *(24)*. When the children were followed up at the age of 12 years, they were found to be functioning well *(26,62)*. In spite of the parents' decision not to tell, the children did not seem to be experiencing negative consequences arising from the absence of a genetic link with their father, or from the secrecy surrounding the circumstances of their birth.

DI families headed by lesbian and single heterosexual mothers

With respect to lesbian mother families, there have been two main concerns; first, that the children would be teased and ostracized by peers, and would develop emotional and behavioural problems as a result, and second, that they would show atypical gender development, i.e. that boys would be less masculine in their identity and behaviour, and girls less feminine, than their counterparts from heterosexual homes. Although there is no evidence for either of these assumptions (see *71,102* for reviews), the early body of research focused on lesbian families where the child had been born into a heterosexual family and then made the transition to a lesbian family after the parents' separation or divorce.

In recent years, controlled studies of lesbian couples with a child conceived by DI have been reported. Unlike lesbian women who had their children while married, these couples planned their family together after "coming out" as lesbian and the children have been raised in lesbian families with no father present right from the start. In the USA, Flaks *et al. (73)* compared 15 lesbian DI families with 15 heterosexual DI families, and Chan *et al. (74)* studied 55 DI families headed by lesbian parents in comparison with 25 DI families headed by heterosexual parents. In the UK, 30 lesbian DI families were compared with 41 heterosexual two-parent DI families and 42 families headed by a single heterosexual mother *(75)*. Similarly, in Belgium, Brewaeys *et al. (76)* studied 30 lesbian mother families with a four-year-old to eight-year-old child in comparison with 38 heterosexual families with a DI child and 30 heterosexual families with a naturally conceived child. The evidence so far suggests that DI children in lesbian mother families do not differ from their peers in heterosexual families in terms of either emotional well-being or gender development. The most striking finding to emerge from these investigations was that co-mothers in two-parent lesbian families were more involved with their children than were fathers in two-parent heterosexual homes. In the Belgian study, information was obtained from parents regarding the decision-making process about whether or not to tell the child about the method of their conception. All of the lesbian mothers intended to tell their children that they had been conceived by DI, and 56% would have opted for an identifiable donor had that been possible *(77)*. The attitude of lesbian mothers toward this issue is in striking contrast to that of heterosexual parents who prefer not to tell.

No studies have been carried out on the quality of parenting of single women who opt for DI as a means of having a child. A small, uncontrolled study of 10 single women requesting DI *(78)* found that an important reason for choosing this procedure was to avoid using a man to produce a child without his knowledge or consent. DI also meant that they did not have to share the rights and responsibilities for the child with a man to whom they were not emotionally committed. Although rare, women who have never experienced a sexual relationship with either sex have also had DI to produce so-called virgin births *(79)*.

Research on single-mother families in general has shown that difficulties in parenting are associated with both economic hardship and lack of social support *(80,81)*. In addition, emotional distress and an associated reduction in parental functioning is common among single mothers following divorce *(83–85)*. In single-mother families with a child conceived by DI, it is important to take social context into account. Single-mother families are not all the same, and anecdotal reports suggest that single women requesting DI tend to be financially secure and to have access to social support from family and friends. Moreover, they have not experienced conflict with, or

separation from, the father of their child. Such circumstances may reduce the potentially negative consequences for parenting or rearing a child alone.

Conclusion

From the information that is currently available, the quality of parenting and the psychological development of children in DI families do not appear to be compromised by the absence of a genetic link between the father and the child. Nevertheless, in spite of the greater encouragement of openness, it seems that parents continue to withhold information from children about their genetic origins. The areas presenting most difficulty for disclosure include the stigma associated with DI, acknowledgement of the father's fertility problem, uncertainty about the best time and method of telling the child, and lack of information to give the child about the genetic father *(42,61,86)*. Unlike adoption, there are no generally accepted stories of what to tell the child about DI, and parents have to explain the facts of life and discuss the father's infertility for the child to understand. Moreover, if an anonymous donor has been used, they have little information to give the child about the genetic father. Due to these factors, most heterosexual DI parents seem to have concluded that nondisclosure is desirable for the protection of both the father and the child.

Although the absence of psychological problems in children and of problems in parent–child interaction in DI families suggests that secrecy does not have an adverse effect on family functioning, this does not necessarily mean that it is better for children not to be told about the nature of their conception. It must be remembered that the children studied so far are young and have not yet developed a sophisticated understanding of their relationship with their parents. Research on adoption has demonstrated that adopted children welcome information about their genetic parents *(87,88)*, and many adoptees do not begin to seek out their genetic parents until they reach adulthood *(89)*. As their children grow older, it becomes more difficult for parents to tell them that they were conceived using donor sperm. Some parents regret not having told their children from the start but feel that it would now present too much of a shock for them and that it is too late *(90)*.

Little is known about children who are aware of their conception by DI, or about the impact of this knowledge on their relationship with their parents, as the only systematic study of such families did not distinguish between parents who had actually told their child about DI and those who simply intended to tell *(86)*. No association was found in this study between attitude towards disclosure and parenting quality. However, fathers who were most concerned about the stigma associated with DI reported less warmth and less fostering of independence in their child, suggesting that perceptions of stigma may have an adverse effect on the relationship between DI fathers and their children. A major problem with investigations of DI families is the low cooperation rate associated with the parents' desire to maintain secrecy about their child's conception. Parents who are most concerned about secrecy are least likely to take part in research. Until families who have told their child have been compared with those who have not, it will not be possible to come to a clear understanding of the consequences of secrecy versus disclosure on parenting in such families. Interestingly, however, in a study of parents who had told their children, the majority (57%) reported feeling good about having done so *(91)*. van Berkel *et al. (52)* also found that parents who had been open with their child did not regret their decision to tell. An exploratory comparison between the few DI children in the European Study of Assisted Reproduction Families who had been told and those who had not, pointed to less conflict between mothers and their children in families where the parents had been open with their child *(26)*. In lesbian DI families, where the child grows up without a father, mothers are more open with their children about the method of their conception. Whether or not single heterosexual mothers with a child conceived by DI opt for secrecy or disclosure remains to be seen. A study of single women requesting DI suggested that they were more likely than married women to intend to tell *(57)*.

Anecdotal reports from adults who are aware of their conception by DI shed some light on the longer-term effects. Whereas some report good relationships with their parents *(43)*, others report more negative feelings including hostility, distance and mistrust *(82,92,93)*. As these adults are not representative of people conceived by DI in general, it is not possible to generalize from their experiences. Most likely, children's reaction to discovering that they were conceived by DI will depend on a number of factors including the quality of their relationship with their parents before the disclosure and the manner in which they find out. Systematic studies of representative

samples are necessary to fully understand the long-term consequences of DI for the individuals concerned.

Egg donation families

Concerns about egg donation families

Although the use of donor sperm to enable couples with an infertile male partner to have children has been practised for many years, it is only since 1983, following advances in IVF, that infertile women have been able to conceive a child using a donated egg *(95,96)*. Egg donation is like DI in that the child is genetically related to only one parent, but in this case it is the mother and not the father who is genetically unrelated to the child. Thus egg donation has made it possible for children to be born to, and reared by, mothers with whom they have no genetic link.

The concerns that have been expressed about egg donation are similar to those raised by DI. It is the absence of a genetic bond between the mother and the child, and the effect of secrecy about the child's conception that have been the topics of greatest debate. But unlike DI where the donor is usually anonymous, egg donors are more often relatives or friends of the parents and may remain in contact with the family as the child grows up. Contact with the genetic mother has been viewed by some as a positive experience for children in that they have the opportunity to develop a clearer understanding of their origins. However, it is not known what the impact of two mothers will be on a child's social, emotional and identity development through childhood and into adult life, or how contact between the genetic mother and the child will affect the social mother's security as a parent and consequently her relationship with the child.

Research on parenting in egg donation families

The first study of parenting in families with a child conceived by egg donation was conducted in France *(20)*. All of the donors were anonymous. The authors reported on 12 families assessed at nine months and 18 months, and nine of these families at 36 months. The quality of the relationship between the mother and her infant was assessed using a procedure based on the mother's body language, vocal dialogue, visual dialogue and attitude toward breastfeeding. It was reported that all of the mother–infant relationships were excellent. However, no details were given of the way in which an "excellent" mother–infant relationship was defined.

A group of 21 families with a three-year-old to eight-year-old child conceived by egg donation was recruited in the UK, and a contrast made between families where the child was genetically related to the father but not the mother (egg donation families) and families where the child was genetically related to the mother but not the father (DI families) *(72)*. The only difference to emerge between egg donation and DI families was that mothers and fathers of children conceived by egg donation reported lower levels of stress associated with parenting than parents of a DI child. Thus it seemed, from the limited information available, that egg donation families, like DI families, were functioning well. However, most of the children in these families had been conceived using the egg of an anonymous donor. What is not known is the effect on parenting of the child being conceived with the egg of a known donor—a relative or family friend—who continues to play a part in family life. Only one set of parents with a child conceived by egg donation had told their child about his genetic origins.

In Finland, 49 families with an egg donation child between six months and four years of age were compared with 92 families with a child born through IVF *(97)*. The large majority of egg donors (84%) were anonymous. However, the eight known donors (sisters or friends) saw the child regularly with no reported difficulties in the relationship between the donor and the mother. Thirty-eight per cent of all parents intended to tell their child that she or he had been conceived by egg donation, a higher proportion than is generally reported for DI parents. Of the eight parents who used a known donor, only two intended to tell the child. As with the DI parents, many (73%) had told someone other than the child. With respect to parenting, significantly fewer egg donation parents than IVF parents expressed concern about their child's behaviour.

Research on children in egg donation families

Cognitive development

Data on 12 egg donation children at nine months and 18 months of age, and on nine of these children at 36 months of age, showed no evidence of psychomotor retardation for any of the children studied *(20)*.

Socioemotional development

Fifty-nine egg donation children and126 IVF children, all between six months and four years of age, were compared with respect to a number of developmental indices as assessed by parental questionnaire *(97)*. There were no group differences in the proportion of children with eating or sleeping difficulties, and the egg donation parents were less likely than the IVF parents to express concern about their child's behaviour. In a study of 21 egg donation children between three and eight years of age in comparison with 41 IVF children, 45 DI children and 55 adopted children, assessments were made of the presence of emotional and behavioural problems by parental questionnaire and the children were administered a standardized assessment of self-esteem *(72)*. There was no evidence of psychological difficulties among the egg donation children.

Conclusion

It appears from the few investigations carried out so far that having a child by egg donation does not have a detrimental effect on parenting. However, most of the parents studied to date had conceived their child using the egg of an anonymous donor. Little is known about the consequences of egg donation when the donor is a relative or friend, a situation that may have more far-reaching effects on family relationships. With respect to the children themselves, the limited data available do not indicate adverse psychological effects arising from the method of their conception. Nevertheless, conclusions cannot be drawn from the small number of existing studies.

Intracytoplasmic sperm injection (ICSI) families

Concerns about ICSI families

The introduction of IVF has paved the way for increasingly "high tech" reproductive procedures such as ICSI wherein a single sperm is injected directly into an egg to create an embryo. Specific concerns have been raised in relation to ICSI, including the use of abnormal sperm, the bypassing of the usual process of natural selection of sperm and the potential for physical damage to the egg or embryo, all of which may produce changes in genetic material *(98)* and may thus have implications for the child's psychological development.

Research on children in ICSI families

Cognitive development

The Bayley Scales were administered to 201 ICSI children at two years of age in Belgium *(100)* and no evidence was found of delayed mental development, although the test was administered to only one-quarter of the original sample. Similar findings were reported in the UK from the administration of the Griffiths Scales to a representative sample of 12–24-month-old singleton ICSI children and a matched comparison group of naturally conceived children *(101)*. The ICSI children scored within the normal range and did not differ from the control group. In contrast, however, significantly lower Bayley Scale MDI scores were found among 89 one-year-old ICSI children when compared with 84 IVF and 80 naturally conceived children, particularly for boys *(98)*. Seventeen per cent of the ICSI children experienced mildly or significantly delayed development (MDI <85) compared with 2% of the IVF and 1% of the natural conception children.

Conclusion

The findings regarding the cognitive development of ICSI children are inconsistent and inconclusive, and only very young children have been studied so far. No investigations have been conducted of the quality of parenting or of the socioemotional development of children in ICSI families.

General conclusions

Creating families by means of assisted reproduction has raised a number of concerns about potentially adverse consequences for parenting and child development. It has been argued, for example, that dysfunctional patterns of parenting may be a feature of these families due to the difficulties experienced by the mothers and fathers in their quest for a child. It seems, however, from the evidence available so far, that such concerns are unfounded. Parents of children conceived by assisted reproduction appear to have good relationships with their children, even in families where one parent lacks a genetic link with the child.

With respect to the children themselves, there is no evidence of cognitive impairment in singleton children born at full term as a result of IVF procedures. The reports of superior cognitive functioning among DI children have not been supported by large-scale controlled studies but could conceivably result from the use of highly educated donors. The findings regarding ICSI children remain unclear. In relation to socioemotional development, assisted reproduction children appear to be functioning well. The greater difficulties of IVF infants are based on maternal reports and probably result from the higher anxiety levels of IVF mothers. Studies of children during the preschool and school-age years generally do not indicate a higher incidence of emotional or behavioural problems among assisted reproduction children.

Nevertheless, few studies have included children at adolescence or beyond, and little is known about the consequences of conception by assisted reproduction from the perspective of the individuals concerned. Moreover, the existing studies are of variable quality. Research in this area is hampered by small, unrepresentative and poorly defined samples, the absence of appropriate control groups, and unreliable and poorly validated measures. In addition, there are some types of assisted reproduction family, such as families created through a surrogacy arrangement or through embryo donation, about whom little is known. The practice of surrogacy, where a woman bears a child for another woman, remains highly controversial. There are two types of surrogacy; partial surrogacy where conception occurs using the commissioning father's sperm and the surrogate mother's egg, and full (IVF) surrogacy where both the egg and the sperm come from the commissioning parents. Although no evidence of speech or motor impairment has been found in singleton children born after IVF surrogacy *(27)*, there are no controlled studies of the consequences of surrogacy for family relationships or children's psychological well-being. It is not known, for example, how the involvement of the surrogate mother with the family as the child grows up will affect the feelings and behaviour of the commissioning mother, particularly when it is the surrogate mother and the commissioning father who are the genetic parents of the child. Embryo donation (sometimes described as prenatal adoption), whereby a donated egg is fertilized by donated sperm in the laboratory and the resulting embryo placed in the womb of the mother-to-be, raises different concerns. Unlike children conceived by egg or sperm donation, who lack a genetic bond with one parent, children born through embryo donation lack a genetic bond with both parents. Furthermore, they lack the information about genetic parents that is usually available to adopted children. Again, no empirical investigations of embryo donation families have yet been carried out.

It is also important to stress that families do not exist within a vacuum; the social environment in which assisted reproduction takes place can have a far-reaching effect on family functioning. In this respect it is noteworthy that assisted reproduction parents who were raising their children under difficult circumstances in Eastern Europe (Bulgaria) reported greater problems in psychological adjustment and in the behaviour of their children than their Western European counterparts *(61)*. Thus the outcomes of assisted reproduction appear to be dependent, to some extent at least, on the cultural context in which these techniques are carried out.

Although existing knowledge about the impact of assisted reproduction for parenting and child development does not give undue cause for concern, there remain many unanswered questions about the consequences of creating families in this way. It is only through systematic, controlled studies of representative samples that the outcomes of assisted reproduction for both parents and children can be fully understood.

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Section 5

Ethical aspects of infertility and ART

Patient-centred ethical issues raised by the procurement and use of gametes and embryos in assisted reproduction

HELGA KUHSE

Introduction

Since 1978, when the world's first *in vitro* fertilization (IVF) baby was born, the world has seen a rapidly increasing array of assisted reproductive technologies (ART). New IVF-based techniques, such as preimplantation genetic diagnosis (PGD), cryopreservation, and micromanipulation of gametes and embryos have provided prospective parents with a range of new reproductive options.

There are various reasons why individuals and couples might want to resort to ART. Estimates and definitions of infertility vary but figures generally suggest that around one in ten couples are affected by infertility at some stage in their lives. Given that it is widely believed that bearing and nurturing a child is a natural and valuable part of life, those unable to have children will often feel a sense of loss and incompleteness and turn to ART for help. In addition, particular options within ART, such as the use of donor gametes and PGD, can help avoid the birth of children suffering from various and sometimes severe chromosomal or genetic abnormalities; and freezing of gametes and embryos can provide protection against disease-related or age-related loss of fertility. In short, assisted modes of reproduction can offer a number of benefits not only to the infertile, but also, increasingly, to various fertile individuals and couples.

ART, from fairly simple artificial insemination to intracytoplasmic sperm injection (ICSI), involves the separation of sexual intercourse and reproduction and will include at least one other party. In addition, in IVF and related techniques, fertilization takes place outside the body, making gametes and embryos available not only for research, but also for testing and manipulation prior to transfer.

The advent of IVF in the late 1970s sparked intense debate about the use of ART, and the social and legal implications they were predicted to have. Many of the central ethical questions raised then are still debated today, and innovations within ART constantly add new dimensions to the debate.

I shall address myself to only a limited set of ethical issues raised for individuals and couples who make use of some forms of ART, such as embryo selection and transfer, PGD, modes of obtaining and manipulating gametes and embryos, cryopreservation, gamete and embryo donation and ovarian stimulation. When focusing on the users of ART, questions of individual harms and benefits, and of respect for autonomy—considerations often captured in the notion of "interests"—are of primary concern. While the interests of those who make use of ART and their possible children, are not, of course, exhaustive of all the ethical issues raised by ART (there are, for example, important questions of resource allocation, of justice and discrimination as well), the interests of those directly affected by ART must be considered the

indispensable backdrop to any adequate overall evaluation of ART.

It is necessary to make explicit two presuppositions on which the provision of ART must necessarily rest: first, that assisted reproduction as such is ethically acceptable; second, that IVF embryos do not have a "right to life". If all assisted reproduction were ethically impermissible, then there would be no point in discussing particular forms of ART and if IVF embryos had a right to life, there would be no point in discussing new technologies, such as embryo preselection and PGD which involve the inevitable destruction of embryos. I believe that there are good grounds for accepting the above assumptions and, in the next two sections, will briefly outline arguments in support of them.

Assisted reproduction is ethically acceptable

Some reject ART as morally unacceptable in itself, that is, as wrong, irrespective of any of the good or bad consequences it might have; others have categorical objections to particular modes of assisted reproduction, such as the use of donor gametes. Such principled objections are typically based on either religious belief or traditional assumptions about the nature of relationships and the role of the family.

These categorical rejections of ART are problematic. First, there are fundamental philosophical problems in seeking to derive ethical principles from religious prescriptions *(1,2)* and, second, it is now widely agreed that religious belief cannot serve as a proper basis for public policies and laws in pluralist and liberal societies. To the extent that no particular vision of how we ought to live can, in the ordinary sense of the term, be demonstrated to be the correct one, individuals must, other things being equal, be free to structure their own lives in accordance with their own self-chosen values and beliefs *(3)*.

While it is true that ART has the capacity to alter traditional ways of forming families and of individuals relating to each other, it is not clear that this is an inherently bad thing. There is no reason to assume that the nuclear family and traditional relationships are the only and best way for human beings to relate to each other and to provide a nurturing environment for children. It may, of course, be the case that certain technologies within ART have the potential to produce more harm than good; but if this were the case, then this would provide us with good reasons to carefully evaluate and perhaps ban those technologies. It would not, however, provide us with any good reasons to reject ART as such.

Some religious and traditional thinkers have appealed to natural law theory in an attempt to provide a more secure grounding for their moral rejection of assisted reproduction. Natural law theory assumes some connection between what is natural and what is good. Particular theories will usually involve either a conflation of "is" and "ought", that is, move illegitimately from claims about particular natural biological functions to what is morally good; or they fail to establish why the affirmation of a range of plausible human goods must lead one to reject certain (often sexual) behaviours, such as masturbation, the use of contraception and the separation of the conjugal act from procreation[i] *(4,5)*.

Gametes and embryos do not have interests or rights

Basic research on IVF embryos is a necessary part of the scientific development of ART and their safe application. Couples often can, and frequently do, donate surplus embryos to research. Even countries, such as Germany, that prohibit all nontherapeutic research, but allow IVF-related treatments, benefit from embryo research conducted elsewhere *(6)*. In addition, various selection procedures, such as PGD of embryos, are aimed at determining which embryos should *not* be transferred—and embryos that are not being transferred to a womb cannot at present develop beyond a certain stage.

This has led to questions—some already well known from the abortion debate—about the moral status of IVF embryos. The fundamental issue is whether it is wrong to experiment on early human embryos or to dispose of them when, for example, PGD shows that they are developmentally inferior or carriers of a chromosomal or genetic condition that would, if implantation and fetal development were to proceed, lead to the birth of an affected child.

i The Roman Catholic Church, for example, rejects any acts that separate the procreative aspect of human intercourse from the unitive, lovemaking aspect of the sexual act. This leads to a rejection of not only masturbation and contraception, but also of all ART.

There are good reasons for rejecting the view that IVF embryos have a right to life. An early IVF human embryo, consisting of no more than a few cells, lacks the capacities that could reasonably ground such a right, or provide the basis for the attribution of any morally significant interests. Lacking a central nervous system, the embryo has no conscious experiences; it cannot feel either pain or suffering, exercise its autonomy, or have any sense of its own existence. In short, unlike more mature human beings or animals, it cannot be harmed or benefited by anything that is done to it. If an IVF embryo is destroyed in the course of experimentation, or simply discarded, no suffering has been inflicted on the embryo; it has not been harmed in a morally significant way and no right to life has been infringed *(7)*.

A human embryo is, of course, derived from the joint genetic material of a mature woman and a man[ii]—and women and men, in distinction from embryos, have various interests or rights. They care, often deeply, about what happens to their genetic material and, in that sense, have an interest in determining what should happen to it[iii] *(8)*.

Given that the progenitors of an embryo have an interest or right to decide what should happen to it, it follows that it would generally be wrong to dispose of an embryo or conduct research on it without the progenitors' consent. But in this case, it would be wrong to do so not because the embryo has a right to life; rather it would be wrong because the action lacked the consent of those who contributed the egg and sperm.

It might be said, of course, that an embryo is morally considerable not because of what it currently is, but because of its potential to become a child and person—that it is this potential which forms the basis of the embryo's interests or rights. The debate over the moral relevance of potential is once again well known from the abortion debate. New complexities are, however, raised if that debate turns from fetuses developing in a woman's womb to IVF embryos *(9)*. Not only do early embryos have the potential to become more than one individual *(7,10)*, but for an IVF embryo to have any potential at all, there must also—in distinction from an *in vivo* fetus—be an additional human intervention to activate the embryo's potential: no natural process is already in place that will, if all goes well, lead to the birth of a child. If an IVF embryo is not transferred to a woman's womb, it has currently no chance of developing into a fetus or child *(9)*.

If one were to accept that an IVF embryo has interests on account of its potential, another complexity presents itself. Would one, then, not also have to say that an egg and sperm, considered jointly, have a rather similar potential? This can perhaps most clearly be seen when we consider a technique, such as ICSI, where a single sperm is injected into an egg. ICSI is very successful in achieving fertilization, and by already having selected a particular egg and sperm we have also already preselected the genetic complement that will be the embryo's. And yet, few of those who believe that embryos have a right to life are worried about the separate disposal of the egg and the sperm that could, if combined, form a unique embryo with a particular genetic code. But if it is not morally wrong to thwart the potential of the egg and sperm, considered jointly, before fertilization has taken place, it is difficult to see why it should be wrong to thwart this potential shortly afterwards.

If human gametes and IVF embryos do not have interests, either on account of what they currently are, or on account of what they have the potential to become, then they cannot be harmed by either destructive embryo experimentation, by practices such as cryopreservation, or by being selected for preimplantation disposal. Many people accept this. Some would, however, want to draw a distinction between experimentation on so-called "surplus" embryos, that is, embryos initially produced with the intention of implanting them, and embryos created specifically for nontherapeutic research[iv] *(11)*. I find it difficult to think of any reasonable grounds that could possibly support this moral distinction *(12)*.

There is a different sense, however, in which the fact that human gametes and embryos have the potential to develop into fetuses, babies and children is morally significant. If gametes or embryos are

ii This would change, of course, if reproductive cloning or parthenogenesis were to become a safe mode of reproduction, or if eggs from female fetuses, for example, were to be used.

iii Considerations such as these may have led the Warnock Committee and subsequent drafters of policies and laws to generally agree that consent to embryo research be required as "a matter of good practice".

iv The Victorian *Infertility Medical Treatment Act 1984*, for example, prohibits the creation of embryos specifically for research, but permits experimentation on "surplus" embryos.

damaged during experimental procedures and are subsequently transferred, this may lead to unsuccessful pregnancies or the births of impaired infants. Once again, this does not show that it is wrong to experiment on *in vitro* gametes and embryos, or establish their interest in, or right to, life; it shows, however, that future children have interests and can be harmed by events that precede their becoming morally considerable beings. It indicates that extreme caution is indicated when performing experimental procedures on embryos or gametes that are destined for reproduction.

Reproductive autonomy and assisted reproduction

Minimally necessary conditions for autonomous choice

I can think of no plausible ethical theory that does not regard personal autonomy or self-determination as an important moral value. Self-determination has instrumental value in so far as it allows people to structure their lives in accordance with their self-chosen values and beliefs, and is also sometimes seen as having intrinsic value. These differences need not, however, concern us here *(13–16)*.

Ideally, health care decisions, including those involving ART, should be autonomous—an idea that is generally captured in the notion of informed consent. To be autonomous, consent requires, at the very least, that it be based on adequate and accurate information and understanding of the potential risks and benefits of alternative courses of action, and that it be voluntary (that is, free from coercion and undue inducement). When it comes to making choices about ART, it has, however, been suggested, that it may be very difficult, if not impossible, for individuals to autonomously choose to enter ART programmes.

One area that has attracted considerable critical attention is the ambiguous and sometimes confusing way in which the success rates of various treatments, and any potential risks associated with them, are presented to would-be parents. But if individuals and couples do not adequately understand the relevant information (or are inadvertently or deliberately misled about potential risks and the likelihood of a successful pregnancy by commercially driven fertility clinics), then they cannot autonomously consent to an ART procedure.

Success rates can be stated in various ways—for example, in terms of a successful pregnancy test, in terms of the birth of a child, or in terms of the birth of a *healthy* child. In an increasing number of countries, national registers collect and publish data on the outcomes of ART. These registers are important, but the mere availability of data is not enough to ensure autonomous decision-making. In light of this, many institutional policies, professional guidelines and laws have recognized the need to provide accurate and understandable information to prospective patients, and for mandatory discussions with independent counsellors. A counsellor can help patients understand the risks and benefits of particular ART, and alert them to alternative reproductive choices.

The above points about the minimally necessary conditions for individuals to be able to autonomously decide for or against making use of ART apply to both women and men. There are, however, significant sexual asymmetries when it comes to burdens and risks associated with assisted reproduction *(17,18)*. On the basis of these asymmetries, it has been argued that women, in particular, cannot autonomously consent to assisted reproduction *(19)*.

Can women autonomously choose to make use of ART?

Invasive modes of collecting sperm present some risks to the men concerned, but in most cases, sperm can be produced in a relatively straightforward way, by masturbation. There is, however, currently no non-invasive and entirely safe way in which eggs can be collected from women. Moreover, prior to egg retrieval, women commonly undergo superovulation regimes to increase the number of eggs available for collection. Superovulation methods pose their own potential hazards, foremost among them the danger of ovarian hyperstimulation syndrome, which has been estimated to range from somewhere between 0.6% to a worrying 14% after IVF. In addition, there is the possibility of adverse long-term effects. While there is no conclusive evidence that drugs used in hyperstimulation contribute to an increase in breast and ovarian cancers in women, or to congenital or embryonic tumours, some disturbing uncertainties remain.

Women, not men, carry pregnancies to term. These pregnancies may fail, and may involve another cycle or cycles of ovarian hyperstimulation, compounding both the physiological and psychological risks. To

increase the success rate of IVF, and to decrease the likelihood of repeated hyperstimulation, it is common practice to transfer multiple embryos, giving rise to increased rates of multiple pregnancies. Multiple pregnancies pose threats to the health of pregnant women as well as that of their fetuses/babies. Various countries, professional bodies, or institutions have established protocols that set limits on the number of embryos that may be transferred, but serious problems remain elsewhere.

Intrauterine insemination (IUI) preceded by hyperstimulation poses particular concerns because the number of embryos that may implant is, in distinction from embryo transfer in IVF procedures, more difficult to control. Women who carry an excessive number of fetuses will often undergo pregnancy reduction—again a procedure that presents considerable physiological and psychological burdens, and which may result in the loss of the pregnancy. Moreover, abortion (of healthy fetuses) is sometimes regarded as morally objectionable, and in some countries it is also illegal.

In light of the considerable burdens imposed by ART on women, and the sometimes slim chances of taking home a baby, the question has been raised whether women are autonomously choosing to resort to ART. The question must also be asked, of course, whether reproductive medicine, in its attempts to produce pregnancies, is failing women. As one writer summed it up in 1987: "Looking for mothers, you only find fetuses." *(20)*.

Pronatalism *(21,22)* is pervasive in many societies. Those unable to have children will frequently be regarded (and regard themselves) as worthless, and go to great lengths in their attempts to produce children.

The role of women has traditionally been defined in terms of bearing and nurturing children. This makes women, much more so than men, subject to pronatalist pressures *(17–19)*. In light of this, the idea that women enter assisted reproduction programmes freely has come under considerable criticisms, particularly from within feminist perspectives. While not all feminists share this view, some feminists reject all ART on the grounds that they bolster the prevailing understanding of women as "fetal containers", and that individual women, enveloped by pervasive pronatalist attitudes, cannot correctly be said to choose autonomously to have children.

But is it correct to say that women cannot autonomously decide to enter assisted reproduction programmes? There are good grounds for a negative answer. While it is correct to say that women are subject to pronatalist pressures, it is not the case, if it ever was, that these pronatalist pressures are so all-pervasive as to preclude autonomous choice. Since the advent of serious education and employment prospects for women, and the availability of safe contraception and abortion, an increasing number of women have chosen to remain childless, to find fulfilment in a wide range of occupations and roles that were closed to their mothers and grandmothers.

Sadly, these options are not open to all women. Some women are constrained by patriarchal social structures, by lack of education and other opportunities, and may find it difficult to resist pronatalist pressures. Nonetheless, it is unlikely that many contemporary women will enter burdensome and often expensive ART programmes unreflectively. The danger that some women may not be acting freely is a reason to provide extensive counselling; it is, however, not a good reason for preventing all women from accessing assisted reproduction *(17)*.

There must be a presumption of patient autonomy. If patient autonomy could be overridden whenever there is a suspicion that a patient does not fully appreciate the balance of risks and benefits, or is assumed to act under some kind of social coercion, then there would be enormous scope for wide-ranging coercive paternalistic interventions by doctors *(18)*. As one writer has summed it up, attempts to protect women from themselves "would treat [them] as legal incompetents, damaging women more than unwise reproductive treatments" *(17)*.

Ethical issues raised by the use of donor gametes and embryos

Ethical parallels between sperm, egg and embryo donations

Artificial insemination by donor sperm (AID) has long been used to achieve successful pregnancies when the male partner is unable to father a child, or when there is a danger of transmitting a hereditary disorder to the offspring. After the development of IVF, the donation of eggs and embryos has also become a possibility.

There are clear ethical parallels between sperm and egg donations. Like sperm, donor eggs can help infertile individuals achieve a pregnancy and have a

child who is genetically related to one parent, and—again like donor sperm—can be used to avoid passing on a hereditary condition to the future child. Donor embryos, enabling a woman to go through pregnancy and childbirth, are usually surplus cryopreserved embryos from couples who have completed their families, but can also be formed from separate egg and sperm donations. Sperm, egg and embryo donations are successful assisted modes of reproduction.

Recourse to donor sperm or eggs might be thought to raise problems in so far as the social father or mother may feel less attached to a child that is not genetically his/hers—a problem which could be compounded by the use of donor embryos. But any problems related to this should largely be able to be overcome by adequate counselling prior to the use of donor gametes or embryos.

One of the central ethical questions relating to egg, sperm and embryo donations is whether a child conceived in this way should or should not be told of her or his origin, and what would happen in each case. Since this issue is discussed in the chapter on "Gamete and embryo donation", I shall comment but briefly. Traditionally (as also in past adoption contexts), it was the almost universal practice to keep the identity of both the sperm donor and the recipient confidential. This practice has been challenged on the grounds that the child has a "right to know the truth", or that it is too difficult to hide the truth. Several countries now allow children when they reach majority to access at least nonidentifying information about their genetic parent(s). Much has been written about this (and about the related issue of whether donors should have access to their genetic offspring), but often in the abstract. It seems clear that ultimately only empirical studies about the impact of different policies on children, their genetic parents and their social families, will be able to guide us towards a sound approach.

Donating eggs

As noted above, women who want to become egg donors must undergo superovulation and egg retrieval procedures. These procedures involve risks and are not in a donor's best medical interests. In light of this, it has sometimes been suggested that women should not be able to donate eggs to strangers. I take a different view. While donations—to the extent that they do not involve substantial payments—are clearly altruistic and beyond the call of duty, as commonly understood, this is hardly a sound reason for paternalistic intervention. Egg donations are not, in principle, different from other widely accepted altruistic donations, involving tissues such as blood and bone marrow. While blood donations involve only minor risks, the same cannot be said about bone marrow donations, where the risks might be comparable to those involved in egg donations.

Things may change, of course, when large amounts of money are offered to potential egg donors. Under such circumstances, it may well be the case that women entering egg donation programmes are motivated not so much by altruism as by monetary considerations. Is this always a bad thing? And could it be argued that poor women in particular are in some sense coerced by the offer of monetary rewards? The answers to these questions are complex. They have long been debated in the organ donations literature, and I shall not attempt to provide an answer here *(23)*.

Egg-sharing is another way in which egg donations can be effected. In this case, the donor is herself undergoing IVF procedures and will share some of her usually limited number of eggs with another woman. In this case, it may, of course, be possible that the donor herself will not succeed in having a baby, whereas the recipient of the donor egg might be successful. If this outcome is known to the donor, it may well be experienced as tragic. Provided, however, that the donor was aware of this possibility, and understood the possible implications, there is no obvious reason why egg-sharing should not be allowed. Once again, it would certainly not be justifiable to seek to prevent women from donating eggs on paternalistic grounds.

Given the scarcity of donor eggs and the growing pool of potential recipients, scientists have begun to look at alternative sources for donor eggs. There are some indications that the maturation of eggs *in vitro* and the transplantation of ovarian tissue may soon be clinical possibilities. This could not only solve the problem of egg shortages, but would also avoid the burdens now resting on egg donors. Ethical objections have, however, been raised to such donations.

Alternative sources of donor eggs

Some scientists in the UK have mooted the idea of using, for infertility treatment, eggs and ovaries retrieved from aborted female fetuses *(24)*. The idea that aborted fetuses might be used in this way has struck some commentators as grotesque *(25)*. But the

mere fact that a potentially beneficial new procedure strikes one as grotesque or in some sense morally repugnant is not by itself a good reason for rejecting it.

Intuitive or "gut-level" responses to what is new and may be experienced as unnatural and in some way threatening will not take us very far. Simple, now widely used and accepted IVF procedures were initially perceived by some as repugnant. While feelings of unease about a procedure may sometimes alert us to subtle ethical considerations and should make us pause, they are ultimately unreliable and any claims to a so-called "wisdom of repugnance" *(26)* or the notion of "moral offence", must be treated with great caution *(27,28)*. After all, it is not so long ago that the "moral offence" experienced by the contemplation of, for example, mixed-race marriages, calls for the emancipation of women, homosexual acts, had given rise to indefensible social practices and laws.

To constitute an ethical judgement, feelings of moral offence and repugnance must be supported by reasons and arguments. Without such reasoned support, expressions of offence and repugnance have no persuasive moral force and must be regarded as expressing ultimately no more than personal sentiment or public prejudice.

Rather than discuss at length the question of whether the use of fetal tissue would be ethically acceptable, let us focus on two less controversial potential sources of donor eggs—donations that have a competent donor's consent—donations of ovaries and eggs after a woman's death, and donations of ovaries removed during unrelated surgery.

It has been argued that the donation of gametes and ovaries is substantially different from the donation or other organs and tissues and should not be allowed—ovaries and eggs, it is said, *give life*; they do not, like other organ donations, *save life*. But, as it stands, the distinction between life-saving and life-giving donations does not provide us with a good reason for thinking that the donation of ovaries or eggs is wrong. After all, why should it be right to save a life, but wrong to give a life? Isn't the giving of life what parents normally do when they decide to have children?

Nor would it be terribly helpful to claim that it would be contrary to the child's best interest to be conceived from an egg retrieved from a donated ovary. It may well be true that children develop their identity and self-understanding, at least in part, through their relationships with their biological parents and might face some psychological and social harm if they learnt that one of their "parents" was a donated ovary, or a "cadaver". But if one wanted to rule out ovary or egg donations on the grounds that the future child will have no relationship with one of her biological parents, then this would, given contemporary donation practices, seem to rule out all egg, sperm and embryo donations from living donors as well *(25)*.

The potential psychological harm to children who have an unusual genetic history should not be minimized. But it should not be overstated either. In many cases, I suspect, children are harmed more by attempts to hide their origins from them, than by honest timely disclosure. In any case, it is unlikely that any potential psychological harm experienced by these children would be so severe as to render their lives so miserable that it would be better if they had never been born. And if one cannot say that a child has been harmed by her or his conception, in what sense, then, can one claim that eggs retrieved from donated ovaries should not be used "for the sake of the child"? (The answer is complex and I will discuss it more fully below under "Should some ART be banned, for the sake of the child?").

Disrespect for the dead

One other point. It has also been claimed that post-mortem use of donated tissues or gametes would amount to disrespect for the dead and the human body *(25)*. Again, this argument is unconvincing. The seemingly most plausible way of showing respect for the dead and their bodies is to respect the wishes of the deceased person as to how she or he would want to be treated when dead. This is recognized in the general acceptance of organ donations. Why should we depart from this principle when the organ is not a liver or a heart, but an ovary or egg? Once again, the answer is not clear.

Postmenopausal mothers

Egg donation opens up the possibility of pregnancy in postmenopausal women in their fifties and sixties. Should older women who want to become mothers be barred from access to ART?

It has been shown that women in their late fifties and early sixties can achieve successful pregnancies with egg donations. There is, of course, an increased possibility that older women do not live long enough to raise children to adulthood. But this argument

against older women becoming mothers will hold only if it is applied equally to older men (seeking assistance in) fathering children, and to younger women and men who have an increased risk of dying before their children reach adulthood.

It is true that there appears to be an age-related increase in obstetrical risks—both for the women concerned and for their fetuses. It is, however, doubtful that an age-related increase in risks is sufficient to justify the categorical exclusion of older women from access to ART. Younger women are not generally barred from accessing ART, just because they have an increased obstetrical risk. And while a line would have to be drawn somewhere as to what magnitude of risk is acceptable (particularly if public funding is involved, and there is a substantial risk that the child will be born damaged), such a line could not plausibly be drawn on the basis of age alone. Some younger women are clearly at greater obstetrical risk than some fit older women. Moreover, we already allow women who make use of ART to take substantial risks in other regards—for example, when it comes to consenting to the transfer of multiple embryos and ovarian stimulation followed by IUI, and to the well-known risks associated with multiple pregnancies.

Would there be other reasonable grounds for not making ART available to older women?

Answers are not easy to come by. As a society we may decide not to provide funds for some ART, but to deny available technologies to groups of individuals on account of their age, their marital status, sexual preferences, and the like, is often based on little more than medical paternalism, or individual or societal prejudice that amounts to unjust discrimination[v] *(29,30)*. While it is frequently assumed that a justification for preventing postmenopausal women, single women, or lesbian couples from accessing ART can be found by appealing to "the best interests of the child", this justifications has—as I have already hinted above and will discuss more fully below—its very own problems *(31)*.

Many other quasi-legal issues raised in the context of using donor gametes and embryos stem from a lack of public policies to regulate the practice, and from broad administrative discretions being in the hands of individual doctors. In many countries, parentage and legitimacy questions are unresolved, and more recent possibilities, such as those raised by cryopreservation and the postmortem use of gametes and embryos, also need regulatory resolution. Such regulatory resolutions must not, of course, rest on either religious or conservative principle alone; rather, to be acceptable from an ethical perspective, any restriction of reproductive freedom must be defensible on universalist grounds.

Preimplantation genetic assessment of gametes and embryos

The primary aim of PGD is to prevent the transmission of genetic and chromosomal disorders to future children. The list of conditions for which PGD of embryos and gametes can be implemented is increasing rapidly. Gametes and embryos found to be wanting will not be transferred, and PGD on embryos might thus be described as the *in vitro* alternative to *in vivo* abortions. Given the link between certain genetic and chromosomal abnormalities and spontaneous abortions, PGD can also increase the success rate of IVF procedures. While PGD itself does not appear to have any detrimental effects, the situation is less clear when it is used in conjunction with ICSI.

Embryos

Those who believe that IVF embryos have a right to life will also generally hold the view that the destruction of an embryo on account of a genetic or chromosal abnormality or reduced developmental potential is wrong, and will reject PGD and other embryo preimplantation selection procedures on those grounds. I have argued above that there are good reasons for rejecting the view that IVF embryos have a right to life and will not repeat these reasons here.

It is, however, important to spell out some of the philosophical and ethically significant implications underlying PGD. In PGD, the cell-cluster which develops after fertilization is biopsied, that is, a cell is removed from the embryo. This cell is, in an important way, like a fertilized egg—inchoate, undifferentiated and totipotent. A totipotent cell has the potential to develop into a separate identical individual, or mono-

v The Victorian *Infertility Act* 1995, for example, restricts access to ART to married couples or de facto heterosexual couples in a stable relationship. This restriction was recently successfully challenged by a doctor in Federal Australian Court, rendering the Victorian legislation inoperative.

zygotic twin, of the biopsied embryo[vi] *(7)*. This means that embryo biopsy might be described as artificial twinning (the German *Embryonenschutzgesetz*, for example, prohibits embryo biopsy and defines the totipotent extracted cell as an embryo *(32)*). The original embryo is preserved, while the extracted cell is tested for abnormalities. Once the cell has been tested, it is destroyed; and, depending on whether an abnormality was found during the test, the original embryo is either also destroyed or transferred in a routine procedure.

Totipotent embryonic cells

As previously noted, PGD has essentially the same aim as prenatal testing, that is, to prevent the birth of an impaired child; but there is also a difference: it involves the destruction of an embryo, even where the test results do not show an abnormality. The embryo on which tests have been performed is destroyed, so that another embryo can be shown to be healthy, but destruction of healthy embryos is not something to which traditional prenatal testing is committed *(33)*. This will not worry those who think that human embryos, despite their potential to develop into persons, lack a right to life; but the implications of the recognition that every totipotent cell might quite properly be described as an embryo, will or should, of course, provide food for thought to those who assert that early IVF embryos (but not each of the embryos' totipotent cells) have such a right to life.

The fact that early embryonic cells are totipotent has other implications as well. It may, for example, soon be possible to cryopreserve individual embryonic cells as clones of the embryo that is being transferred. Parents, concerned with the welfare of their future children, might want to store such embryonic clones, to perhaps one day provide stem cells and other tissues or organs for the child, should this ever be necessary. The potential benefits could be significant. Those who would want to reject this technology need to provide reasons for their rejection; and while such reasons may exist, they cannot, as I argued above, be found in either feelings of repugnance, or merely in the argument that potential human beings have a right to life.

In turning the focus from the moral status of totipotent cells to their potential to grow into fetuses and infants, other possibilities present themselves. Assuming that it would be safe to do so, would it be wrong to artificially induce twinning, or cloning, to allow parents to have monozygotic twins? These twins could be born contemporaneously, but it would of course also be possible to cryopreserve one of the embryonic twins, to allow a later addition to one's family. Monozygotic twinning happens naturally without human intervention, and is seemingly already occurring as an unintended by-product of ICSI. Artificial twinning would give prospective parents the chance to increase the reproductive potential of an embryo, and increase their chances of parenthood. Reasons for the rejection of this technology are again not easy to find. Assuming that the procedure is safe, respect for parental reproductive freedom would seem to support it, as would a focus on the interests of the possible twin[vii] *(28)* (see below "Should some ART be banned, for the sake of the child?").

Other ethical issues

Various questions remain. Most parents choose PGD to avoid the birth of an impaired child, and to avoid a later abortion. PGD is fairly accurate (97%), but the possibility of misdiagnosis means that those who want to achieve a higher degree of certainty will have to undergo prenatal diagnosis as well, which could turn up a positive result. This entails that some women who have made use of PGD will have to decide after all whether they want to abort an affected fetus. While it would, of course, be desirable to have a 100% reliable preimplantation test, such a test is not available, and the fact remains that a 97% reliable test gives at-risk couples much better odds than they would have were PGD not undertaken.

Infertile couples who make use of PGD must of course be adequately informed and counselled about the reliability of PGD, the possibility that other not-tested-for impairments may be present, and the burdensome and risky treatments that will necessarily precede PGD. If *fertile* at-risk couples want to access

vi Strictly speaking, it has the potential to develop into a number of different individuals.

vii It might be said that even if twinning were acceptable if the twins were to be born contemporaneously, consecutive births should not be allowed, because the second twin would lack what has been termed an "open future": there would already be a person who is living a life with the same complement of genes, foreclosing some of the options and opportunities for the latecomer. I am not, however, convinced that this argument is sufficient to justify the prohibition of cloning.

PGD, they must be put into a position where they can adequately weigh up the risks and benefits of choosing between PGD and natural conception, followed, perhaps, by prenatal diagnosis and abortion. If these conditions are met, it seems that here, as in other areas of reproduction, the decision as to what constitutes an acceptable risk–benefit ratio must primarily be the patients'.

PGD makes sex selection possible. While sex selection is widely accepted for the purpose of preventing the transmission of serious sex-linked impairments, many people regard it as ethically unacceptable if done for no reason other than parental preference for a child of a particular sex. I do not think that a restriction of the practice can be ruled out on the basis of either the best interests of the future child, the right to life of the to-be-discarded embryos of the "wrong" sex, or on the grounds that such parental choices are necessarily nonautonomous. The prohibition of sex selection for the purpose of satisfying parental preferences for a child of a particular sex would have to be justified in some other way. A possible candidate for such a justification would be that widely shared societal preferences for a child of a particular sex would create an undesirable imbalance between the sexes.

Special ethical concerns are raised in so far as PGD may detect diseases of which the progenitors of the embryo are not aware. This can be particularly troubling in the case of late-onset diseases, such as Huntington disease[viii]. I shall, however, not discuss these complex questions here. They are not unique to PGD. Essentially the same issues are raised by conventional prenatal and other genetic diagnoses, and there exists an extensive literature on the ethical issues surrounding disclosure questions.

It has also been said that PGD raises eugenic concerns and discriminates against people living with the conditions for which embryos are now being tested, or against parents who cannot afford PGD or other genetic services. These are important issues, but far too numerous and complex to be discussed here. Moreover, they are once again not unique to PGD, but are raised also in various other areas involving genetics and reproductive choices. For any discussion to be adequate, it would need to focus on this wider context, not just on PGD alone *(34,*35*)*.

Should some ART be banned, for the sake of the child?

ART in general are associated with an increase in various obstetrical risks and birth abnormalities. ICSI and the practice of transferring multiple embryos are of particular concern.

Multiple pregnancies

In the case of ART involving IVF, the majority of multiple pregnancies and births are due to the practice of transferring more than one embryo per cycle. In ART involving hormonal stimulation and *in vivo* fertilization, multiple pregnancies are the result of multiple embryos forming in a woman's womb. One important difference between the two practices is that in IVF treatments, the decision to transfer multiple embryos is deliberate, whereas the number of embryos that will form following *in vivo* insemination is not, or less, predictable.

The primary reason for transferring multiple embryos is to increase the chances of a pregnancy resulting from the cycle. Multiple pregnancies are linked to obstetric complications, and may lead to the birth of premature or disabled infants. Evidence suggests that risks—for prematurity, perinatal death, congenital abnormality and neurological problems—appear to increase with each additional fetus carried. On the other hand, and most significantly, there appears to be no scientific evidence that the transfer of more than two embryos enhances the likelihood of pregnancy. If this is correct, then the practice of transferring more than two embryos lacks an empirical justification. It would not only be wasting embryos

viii As an aside: Gianaroli *et al.* (see chapter on "Preimplantation genetic diagnosis" in this volume) assert that a predisposition to late-onset disorders would "certainly not be compatible with the interruption of an ongoing pregnancy", while, at the same time, holding that "the selection for transfer of those embryos that do not carry the mutation under study [and presumably the disposal of those who do] is an additional step towards the prevention of severe diseases." If this is an ethical claim, rather than—say—a report on what the law does/does not permit, it needs to be supported by argument. It is far from clear why it should be ethically sound for doctors to dispose of an IVF embryo that carries the gene for Huntington disease, but not ethically sound for a woman to decide on the abortion of an affected fetus. If this view were to be imposed on women, this might well be regarded as a case of unjustifiable medical paternalism.

which might be cryopreserved for future transfer, but would also impose needless burdens on women, and be causally related to the birth of premature or damaged infants. And yet, Finnström points out (see the chapter on "Outcome of multiple pregnancy following ART: the effect on the child"), few centres or countries have programmes to reduce the incidence of multiple births. Further research is needed to find ways of reducing the incidence of multiple pregnancies following hormone-stimulated IUI, but as far as IVF-preceded pregnancies are concerned, the number of embryos transferred to the womb is up to human decisions; and what that number ought to be, must, to be ethically sound, be based on the best available empirical evidence regarding the likely outcomes of different-number decisions.

ICSI

In ICSI a single sperm is introduced into the egg, to enhance the chances that fertilization will occur, or to make it possible at all, particularly in cases where the sperm is known to be deficient in some way. ICSI is sometimes combined with oocyte activation, to bring about fertilizations that cannot be achieved by ICSI alone.

Both ICSI and oocyte activation raise a number of concerns. One concern arises from reports that the risk for monozygotic twins and for monozygous monochorionic twins (identical twins that share the same chorion) may be higher in the case of ICSI and other zona manipulation techniques. Monochorionic twinning in particular seems to be linked to low birth weight and poorer health and survival of infants. A general concern is that ICSI and related technologies remove natural barriers and allow fertilization by subfertile and genetically dubious or deficient sperm to occur. The latter fact is thought to explain, at least in part, sex chromosome abnormalities in offspring. The outcome of using immature testicular sperm is uncertain.

It is sometimes said that in light of the increased health-related risks that ART children face vis-à-vis their normally conceived counterparts, prospective parents should not make use of ART, because to do so is contrary to the best interests of the child. Similar arguments are frequently put to back up claims to the effect that the use of donor gametes should be banned, or that ART be restricted to married or de facto heterosexual couples "for the sake of the child".

Are these arguments sound? An attempt to answer this question raises some of the most complex questions in philosophical ethics. Space allows me to provide but a sketch of how such an answer might proceed.

Harming possible children

The problem of harm in preconception cases is tricky because, in distinction from individuals who already exist, the child whose interests are at issue is merely a possible child, and when a possible child is brought into existence with the help of ART, it is far from clear that the child has been harmed, even when she or he is worse off relative to a child conceived by normal means.

To illustrate the general point, imagine the following scenario involving unassisted reproduction:

> A woman is advised by her doctor that she should not become pregnant now because she has a condition that is certain to result in a slight mental impairment in her child. There is a safe and effective medication that can cure the condition; if she takes the medication and waits three months before attempting a pregnancy, the risk of the child being mentally impaired will have been eliminated and there is every chance that her child will be normal *(36)*.

Common sense morality holds that the woman ought to postpone her plans to become pregnant. Many people would think this even if the impairment of the child were not so severe as to make the child's life utterly miserable. They would think that if the woman did not wait, she would needlessly be harming her child.

But in distinction from situations where an already existing individual is harmed after conception or birth, discussions of alternative reproductive choices involve what has been called the "nonidentity problem" *(36)*. If the woman were to decide against waiting, she would be conceiving a different child from the one she would conceive if she were to wait. The child would be conceived from a different egg and sperm than the later child. The difficulty is that in a case like this, there seems to be no child that has been harmed—for while the first child would have a mental impairment, she or he would have a subjectively worthwhile life. But if this is so, then it does not make

good sense to suggest that it would have been better for the impaired child if the impairment had been prevented—for that could have been done only by preventing the child's existence. And given that the child has a subjectively worthwhile life, the woman cannot be said to have harmed the impaired child by having brought her or him into existence.

The same kind of reasoning applies to various preconception reproductive choices involving ART where there is an increased risk to the children conceived of physical or mental impairment, or of psychological or emotional problems. To be able to speak about these different undesirable outcomes in a general way, I want to refer to them for now as "making the child worse off".

Next, to allow us to focus on the central issues involved, assume the following: that ICSI is the only way in which men with round-headed sperm can father children. Unfortunately, all children conceived from round-headed sperm will suffer repeated bouts of debilitating depression, but are otherwise normal and regard their lives as worth living.

Men who produce only round-headed sperm and want to embark on parenthood with the help of ART have two main options: father a child with their own sperm, or use donor sperm. While any child produced with the man's own sperm would experience bouts of depression, a child conceived with donor sperm would predictably be healthy. A man in this situation, and his wife, decide to conceive a child with the man's own sperm. Although the couple knowingly bring a child into the world who will be worse off than the alternative child they could have had, the child's life will be worth living. If the couple had chosen to use donor sperm, they would have had a different child.

Someone might say that the couple's decision to use the husband's abnormal sperm was wrong because it failed to take the best interests of their future child into account: it would have been better for their child if it had been conceived with donor sperm. But just as in the case of the woman who refuses to wait for three months before she attempts to become pregnant, the couple's decision to use abnormal round-headed sperm cannot be criticized on the grounds that the child conceived from the abnormal sperm is worse off, or has been harmed, by the parents' decision. It is better for the child conceived with the abnormal sperm that she or he lives rather than not live at all.

If one wants to criticize the decision as wrong, one needs to abandon what has been called a person-affecting view of benefiting and harming and focus instead on the harms and benefits resulting from alternative reproductive choices that involve the existence of the same number of people. From such a nonperson-affecting view of benefiting and harming, it is, other things being equal, wrong to bring children into the world who are worse off than those who could have lived instead *(37)*.

The nonperson-affecting view of benefiting and harming avoids the nonidentity problem, and can thus explain why it would be wrong for the above couple to use the man's abnormal sperm rather than donor sperm. It might seem difficult, though, to square the nonperson-affecting view with the principle of reproductive autonomy. Reproductive autonomy might be thought to license reproductive decisions that a nonperson-affecting view of ethics would condemn. But there need not be any conflict here. Neither the principle of reproductive autonomy nor the ethical requirement that we avoid nonperson-affecting harm can properly be understood as an absolute. Rather, both considerations are best seen as being constrained by a principle of equality or justice that gives equal consideration to the benefits and harms, or interests of those immediately affected by the decision—that is, to the interests of the prospective parents and any dependants they might already have, as well as the interests of the possible child. A principle of this kind would regard as wrong any reproductive choice that would make a possible child seriously worse off than an alternative possible child, when the prospective parents could have conceived the alternative child without imposing undue burdens on themselves or any dependents they might have *(38)*.

This raises the obvious need to say what constitutes an "undue burden" for the parents. Some people would, no doubt, regard it as an undue burden to forego having genetically related children and think it justifiable to risk making their future children seriously worse off. I am not, however, convinced that we should leave it at that. People sometimes fail to distinguish between having children, and having children that are related to them. One reason for wanting children is to nurture, love and cherish them, to see them grow and develop their own personalities, and to set their own goals in life. Other reasons might be based on the desire for companionship, connectedness to the next generation, or concerns about loneliness, particularly in old age. These and similar reasons for wanting children could undoubtedly be

satisfied by children to whom one is not genetically related. Some people would, however, also think it is important to have genetically related children because they see these children as perpetuating the family, as carrying on the blood line, or as conferring some kind of immortality through the survival of genes. These reasons for having children may well be nonrational *(39)* and counsellors would, I believe, do well if they tried to talk prospective parents out of making such nonrational choices in cases of substantial genetic risk. While genetic counsellors are normally trained to be nondirective, I take the view that it can sometimes be wrong for prospective parents to act in ways that pose a substantial risk of harm for their potential children. If that is the case, nondirectiveness may have to be abolished, for "the sake of the child".

Counsellors will not always succeed. There are currently no public policies that insist that people's unassisted reproductive choices meet the strict moral standards of the nonperson-affecting view. Rather, people are free to have children with whom they like and when they like. They do not need a licence to reproduce and will sometimes make choices that are not for the best—and they will make these choices for seemingly trivial and nonrational reasons. Should attempts not be made to limit such decisions when ART are involved? But there is a problem: what may appear to be a trivial reason for one person may well be a nontrivial reason for another—given that person's values and beliefs. In such cases, reproductive decisions that risk making a child worse off could, at least sometimes, be justified in terms of the parents' own projects, values and attachments. Religious or cultural belief in the wrongness of using donor gametes could, I reluctantly concede, sometimes be such a justifying reason.

This is where the distinction between private morality and public policy can be seen to be important. Ordinarily, each person is best able to determine what constitutes a justifiable or an unjustifiable alternative reproductive choice, given that person's projects, values and beliefs. As the abortion debate has made abundantly clear, what is an unacceptable choice for one person may well be an acceptable choice for another. It is difficult to see, however, how public policies could ever be so finely nuanced as to distinguish adequately between reproductive decisions that would be justified or unjustified in a particular case on the basis of a nonperson-affecting view of benefiting and harming. I fear that the attempt to introduce such coercive restrictive policies in the case of ART would have worse consequences, other things being equal, than granting prospective parents substantial reproductive freedom.

Fortunately, the vast majority of parents will go to great lengths to ensure that any child that will be born to them will have a good chance of being healthy. Some lines must, however, be drawn: strong grounds would exist for denying reproductive assistance to parents who are intent on bringing a child into the world whose life would be so utterly wretched that it would be better if the child had not been born, or where the child would be at substantial risk of serious harm.

Clearly what is meant by it being better that a child not be born, and what constitutes substantial risk of serious harm, must be the subject of further discussion. Suffice it here to note that any acceptable answer would have to be based on both the likelihood of the undesirable outcome and its severity. If the undesirable consequence is very bad, then even a small risk of that outcome eventuating would give us strong grounds for preventing prospective parents from being assisted in implementing their reproductive choice; if the undesirable outcome is not so severe, a higher statistical likelihood of the outcome eventuating would seem acceptable.

These general considerations need to be backed up by empirical data on the relative risks of the various ART, and what they amount to in particular patient-specific contexts and practices. ICSI, for example, may be relatively safe in the case of some sperm abnormalities and unacceptably risky in others.

Reliable empirical data on the risks and severity of the potential psychological or emotional harm experienced by people conceived with the help of ART are also needed. But here the issues are, I believe, somewhat different from those pertaining to serious physical or mental damage. Emotional and psychological harm can, I believe, often be avoided by being truthful to one's children about their origins. But even if some children conceived by, say, donor eggs and sperm were worse off than their naturally conceived counterparts, I find it difficult to believe that this would offer sufficient grounds for preventing parents who cannot, or for good reasons do not choose to, have children by making use of their own gametes. Little can be done about physical and mental damage once it has occurred; but much can be done to prevent emotional and psychological harm. In cases where such harm cannot be prevented, it is unlikely that it will be so all-pervasive as to prevent the person from,

nonetheless, being able to lead a satisfactory life.

Conclusion

My primary focus has been on individuals who may wish to make use of various novel ART, and on the children they hope to conceive. In this context, some of the central unanswered questions relate to the high value placed on genetic parenthood, and on the kinds of risk that it is justifiable for prospective parents to take on behalf of their possible children. Other questions relate to finding reasonable grounds for limiting the novel uses to which individual ART, in their seemingly endless permutations, may be put. It is difficult to find such grounds in the principle of respect for autonomy and the claim that certain applications should be restricted "for the sake of the child". If such grounds exist, then they are likely to be found in a wider consideration of issues, such as the equitable distribution of limited medical resources and questions of justice.

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When reproductive freedom encounters medical responsibility: changing conceptions of reproductive choice

SIMONE BATEMAN

Introduction

Sex is no longer the only means of conceiving human beings. Ever since artificial insemination was first used two centuries ago, a pregnancy can be obtained through the use of techniques generally referred to as reproductive technology[i]. These techniques have created new options for persons who would otherwise not have had children, but they have also progressively altered the practices and relationships that condition and give meaning to reproduction in our society. This is primarily because reproductive technology is most often made available in a medical setting, where relationships are defined in therapeutic terms, where values give precedence to the quality, security and efficiency of a technical act, and where physicians are held responsible for the appropriate management of procedures. Impregnation no longer has to do with the privacy of one's sex life, but with the accomplishment of a medical act. This turning over of conception to professionals deemed competent in reproduction leaves some would-be parents disoriented and even feeling dispossessed of their customary (often felt to be "natural") liberty to make reproductive decisions *(1)*.

Reproduction is today firmly established as an area of legitimate medical intervention; we must remember, however, that the presence of a (usually male) physician at childbirth dates back in Western Europe to only two centuries—a presence whose sole initial justification was the medical monopoly of a new instrument, the forceps, designed to facilitate difficult births. More recent techniques and procedures in the area of obstetrics and gynaecology, such as contraception, abortion, the monitoring of fetal growth or testing for fetal abnormalities during pregnancy, have since helped solidify physicians' claim to professional competence in this domain. Nonetheless, most of these techniques have given rise to much controversy: the idea that it is legitimate to control or interfere with conception and gestation, even under medical auspices, is far from being a consensual issue in many societies.

In most countries, assisted reproduction technology (ART) was originally introduced to treat infertility. Today it is also being offered to fertile heterosexual couples as a means of avoiding the risk of transmitting hereditary disease to their offspring. Both of these circumstances are generally recognized as legitimate indications for treatment. Occasionally

i This term can be applied more generally to include all kinds of techniques, including contraception and abortion, whose purpose is to control or otherwise intervene in the reproductive process. In this paper, we are restricting its use to designate only those techniques whose ultimate objective is to initiate a pregnancy.

fertile heterosexual couples have resorted to ART as a solution to unusual circumstances (such as couples wishing to select a compatible embryo as an organ donor for a living child) *(2)*. Single women and lesbians have also used donor insemination (DI), usually without medical assistance, as a valid alternative to sex for conceiving children. These latter uses of ART remain morally controversial in many countries. But whether or not an infertility problem exists, the question of medical responsibility will invariably be raised whenever a physician is asked to intervene.

Most societies grant physicians considerable authority in deciding what is to be regarded as the best way of implementing a medical procedure; they thus play an important role in defining the values that guide the use of new technology. However, medical reproductive and related genetic practices are procedures whose immediate objective (conceiving a child) and ultimate consequences (demographic, psychological, economic, etc.) are not normally included in the usual scope of a physician's professional competence and responsibility. A physician's involvement in a patient's choices about sexuality and childbearing quite obviously bring into play a broader scope of values than those immediately linked to the professionally responsible practice of medicine—values usually embodied in moral convictions regarding what constitutes a "good" life in terms of sexuality and procreation, family life, health and handicap. The legitimacy of medical authority in this domain may therefore be open to question, creating possibilities for conflict with conceptions of reproductive liberty.

Reproductive liberty *(3)* can be defined as the freedom to make essential choices affecting one's reproductive life. A major decision involves choosing whether or not to have children which, in the case of an affirmative answer, implies decisions concerning with whom, when and in what circumstances. Of course, having children is not necessarily the result of a conscious decision-making process: social pressure, interpersonal conflict and personal ambivalence often interfere with complex biological processes not totally under our control, making the final result of our reproductive lives quite different from our original plans. A couple's decision to have a child by no means guarantees that they will ultimately have one, even when they appeal to medical assistance. Nonetheless, the development of techniques the purpose of which is to increase control over the consequences of our sexual lives and the different aspects of our reproductive decisions—whether or not, with whom, when and in what circumstances—has reinforced the idea of reproduction as a project and a consciously controlled process.

But this increase in control paradoxically requires entry into a new type of reproductive relationship, loosely if not inappropriately defined as therapeutic. Infertility is not strictly speaking a disease: it is certainly a personal and social handicap for the person who is thus affected, but it is not a life-threatening disease. Moreover, the concept of infertility which underlies current use of reproductive technology is a peculiar construct, in which medical and social criteria are closely interrelated: it describes as unable to conceive, not a person with a medically proven infertility problem, but two particular persons—a couple—who are unable to conceive a child together, because one or both are infertile. Treatment, however, invariably concerns the woman, even when she is not infertile, as she is the child bearer. Both DI and intracytoplasmic sperm injection (ICSI) make this point particularly clear: the procedure is carried out on a fertile woman, who could have become pregnant, had she chosen to do so, by having sexual relations with another man. In fact, infertility is a relative concept whose limits are difficult to define: very few patients are diagnosed as completely sterile, and a person diagnosed as infertile might have been able to conceive, had he/she found a more fertile partner.

The fact that infertility is not a disease does not mean that physicians should not try to treat the condition. But assisted conception cannot be considered a cure for either male or female infertility: neither artificial insemination nor *in vitro* fertilization (IVF) enable their infertile beneficiaries to conceive a child on their own. In fact, although these techniques are commonly presented as medical treatment, they constitute an evident deviation from the standard approach to healing. Medical treatment aims, when possible, at identifying and eliminating the cause of a pathological condition: this may be the case in the first phases of an infertility work-up and treatment. When diagnosis has failed to determine a cause, or treatment to provide a cure, a physician can attempt to eliminate or alleviate the symptoms of a disease by some form of palliative treatment. Assisted conception does address infer-tility's primary symptom, the absence of a viable pregnancy, by replicating a defective function it cannot cure. This, however, creates a totally unprecedented therapeutic position in that the immediate medical objective goes beyond the alleviation of suffering: the aim of reproductive technology is to

conceive a human being.

Medical assistance with conception thus consists essentially in providing technical expertise in fertilization and impregnation, a task that transforms physicians, so to speak, into professionally competent reproductive partners. As such, they find themselves called upon to intervene in many crucial procreative decisions. These choices and decisions are all the more difficult to make, in that the facts that inform them are still being accumulated or exist only as probabilities. Moreover, the complexity of the therapeutic relationship, to which must be added the problems raised specifically by the physician's involvement in a reproductive act, concur to create potential for conflict in which it may no longer be evident who, in this novel reproductive framework, should be making fundamental decisions about the coming into existence of a child. Should they be made by those who, as professionals, carry out fertilization and impregnation; by those who, as donors, contribute the reproductive cells; or by those who, as future parents, will ultimately assume responsibility for the child to be born? Deciding who will decide in case of disagreement may involve weighing the physician's responsibility for the safety and the favourable outcome of the procedure against the patient's freedom to make reproductive choices.

Many would-be parents do not consider it necessary to justify their request for assisted conception: the reasons they wish to have children and the conditions in which they intend to raise them concern their private life. But numerous physicians feel that, given their responsibility as professionals, they should refuse or discontinue assisted conception, if they consider that the conditions in which the child is to be born are unacceptable. When would-be parents do not acquiesce to the physician's point of view, a conflict arises which usually leads to attempts at arbitration: in France, this usually means the intervention of a psychologist, referral to an ethics committee, or recourse to a legal procedure *(4)*.

Conflict may arise over questions of access to reproductive technology, in particular with respect to atypical requests for reproductive technology. But even in more conventional situations, there may be conflict over the procedure considered most appropriate, or over the best solution to unforeseen consequences of an otherwise consensually decided procedure. Even though physicians, would-be parents and even donors *(5)* are all apparently endeavouring to achieve the same goal—the birth of a child—there may be significant differences in the way each protagonist approaches this goal. Regardless of the differences among protagonists involved in a conflict, their respective positions often highlight a concurring moral preoccupation: in what physical and social conditions is it acceptable to bring a child into the world? Both physicians and would-be parents, in justifying their viewpoints, frequently refer to a child's best interest. This emotionally charged notion rarely conveys a precise content or meaning, although it does eventually serve to articulate normative concerns about a child's future. Ultimately, the various versions of this argument reveal competing visions of the person, the family, and of life in society; their impact on the future of childbearing will depend on the priorities established among these arguments as conflicts are resolved.

Deciding to have children

One of the main points of conflict between would-be parents and professionals—and usually a major issue on the political agenda—is the type of situation in which reproductive technology may be legitimately requested. Generally speaking, there are two sorts of requests. The first usually comes from heterosexual partners who, unable to achieve a viable pregnancy, seek medical help. Assisted conception is offered in these cases to compensate for a couple's physiological incapacity to reproduce, thus bringing society's support to the conventional (often perceived as the "natural") means of founding a family. The second type of request, more controversial but arising from the very existence of these techniques, comes from persons who do not wish to entertain heterosexual relationships (single, divorced or widowed men and women, gay and lesbian partners), but who nonetheless wish to have children. Providing assisted conception in these circumstances recognizes as legitimate the wish for descendants of those who refuse marriage, partnership or heterosexuality as a way of life and as the only suitable framework for raising children.

In many countries, only the first type of request is considered a socially legitimate reason for access to reproductive technology. Indeed, the whole notion of infertility treatment has been constructed around this indication. And yet, despite apparent differences between these two types of request, they do seem to have three points in common: (i) the wish to avoid sexual relations considered personally, socially or

morally unacceptable and the emotional complications they might entail (fidelity to one's partner appears as a common concern); (ii) the wish to overcome the limitations imposed by one's personal and sexual preferences, in particular that of having chosen an infertile partner or form of relationship; and (iii) the wish for *a child of one's own*, understood as a child emanating from the would-be parents' body and/or gametes. These three points often justify the first type of request, but in many countries, they often invalidate the second. But if they are considered problematic in one case, why is this not true in the other?

The distinction between appropriate and inappropriate requests evidently uses the fecund sexual relation between a man and a woman as the normative model of procreative behaviour—a model that supposedly refers to a "natural" order of procreation. In France, article L. 152-2 of the French 1994 bioethics law draws on this model, as well as on the social value attached to voluntary procreation within a stable relationship, to establish, apparently without ambiguity, the conditions in which assisted conception may legitimately be provided:

> The purpose of assisted conception is to respond to a couple's request to become parents. Its objective is to remedy infertility, the pathological nature of which has been medically diagnosed. It may also be used to avoid transmitting a particularly serious disease to a child. The man and the woman who form the couple should be alive, be of reproductive age, married or able to prove at least two years of cohabitation, and they should give previous consent to the transfer of embryos or to insemination.
>
> [my translation]

The attempt to anchor the legitimacy of assisted conception on the supposedly natural norms of human reproduction (participation of two live persons of different sex and of reproductive age) disregards the manner in which society intervenes to redefine what is natural in humans. To give an example, the age at which it is physiologically possible to have children does not necessarily correspond to the age at which it is considered socially acceptable to have them. A physician would probably not offer assisted conception to an infertile adolescent couple. The biological norms of reproductive capacity are always reassessed in the light of social norms defining the aptitude to become a parent. Even a distinction as biologically evident as morphological sex differences can turn out to be an ambiguous criterion, in the rare cases in which a person has an anomaly of the sexual organs or has undergone hormonal and/or surgical transformation of his/her sex. Sexuality itself is reduced, in this perspective, to its species-oriented function: reproduction.

Legislation thus conceived aims to exclude all normative options that might define assisted conception as anything other than an exceptional venturing away from the "normal" ways of founding a family. The use of reproductive technology as a procreative alternative to sexuality is perceived as too radical an option, with unknown social consequences. And yet the very notion of what constitutes a family has already been profoundly affected by the increase in divorce and remarriage, by the growing number of non-married couples and of same-sex unions. Recent USA census data *(6)* indicate that less than a quarter of the households are made up of married couples with children, whereas the number of families with children but no spouse present are increasing, a trend which is now much advanced and well established in European countries. Procreative plans and behaviour have also undergone considerable change with access to earlier forms of reproductive technology, such as contraception and abortion. And single women, gays and lesbians did not wait for reproductive assistance to have children: many made special sexual arrangements to achieve their purposes and invented new forms of parental relationships with their children *(7)*. Of course, DI has contributed in making these procreative possibilities seem more attractive, particularly to those who wished to dispense with opportunistic sexual relations. And some procreative options were not possible as long as conception took place entirely within the body. Indeed, IVF now allows for heretofore unimaginable dissociations of the reproductive process: the possibility for two separate forms of biological motherhood *(8)*, the separate gestation and birth of embryos conceived at the same time, reproduction with only one or with three sources of gametes *(9,10)*. But many changes in the contemporary family cannot be attributed to these procreative innovations.

The family has never been a natural unit; nor can it be considered the social expression of the biology of reproduction. Family forms have varied considerably through time and space, and, in many societies,

socially and legally sanctioned kinship relationships do not necessarily reflect the biological ties between persons. In many contemporary societies, it is true that the legal ties binding the spouses in a marriage usually carry with them the expectation that the spouses will also be the genitors of the children born to that marriage. This expectation has for centuries rested on the moral value of sexual fidelity. But what institutes the parental tie is a legal act, and not a biological one: even when a society decides to give precedence to biological ties in establishing parenthood, this is a social decision with its corresponding justifications and not an imposition on society of an incontrovertible biological fact *(11)*. Of course, as knowledge accumulates about the facts of reproduction and heredity, our own societies may be attributing an increasing importance to biology in their thinking about the family. The tendency is then to confuse scientific truths about conception and heredity with the social and legal organization of family ties and the emotional and physical experiences underlying the quest for children. As biological metaphors and metonymies for kinship relationships flourish, evolving from the outmoded notion of blood ties to the more scientifically modern notion of genetic ties, we forget that these expressions only signify a more complex whole.

Given that humanity has known so many family forms and been so inventive in finding solutions to the lack of descendants, it is difficult to give credence to the idea that the "traditional" family is in danger. The traditional family may simply be a normative ideal that uses the nuclear family as a landmark to provide social bearings in times of disquieting change. The disadvantage with this approach to innovation is that it defines outright all novelty as unethical or unsafe, while disregarding what may be morally questionable in familiar circumstances. Even if unprecedented options disrupt our usual social and symbolic landmarks in thinking about procreation and the family, they do not, for that reason, threaten our humanity. They do make it vital to rethink the anthropological foundations of procreation in the light of new options.

Whether science and technology represent a threat to family relationships and to social stability may have more to do with our reasons for implementing these new techniques than with the idea that we may, unwittingly, be destabilizing natural human phenomena. The risk of interfering with and destabilizing biological mechanisms is real and should not be minimized, but it is a scientific issue in its own right, related but separate from the issue concerning the way a society deals with limitations imposed to human aspirations by the body and by the environment. The best way to approach the ethical issues concerning the effects of reproductive technology on children and their families may be to examine, not hypothetical options, but those already available and considered acceptable, with the unforeseen problems they raise. We may not have sufficiently explored and understood the novelty of familiar situations and, in particular, the role medicalization plays in that novelty. Some of these issues may have an effect on the family and on procreation that is more profound than is immediately evident: in fact, we may have already gone beyond the controversial issue of whether or not persons in unconventional family situations should use reproductive technology to have offspring. The way decisions are being made about current difficulties may give us clues to underlying trends, and thus to the social choices we are really making.

At first glance, occasions for conflict in situations where the persons being treated are an infertile heterosexual couple appear to be less frequent and less radical. There are nonetheless numerous examples illustrating that, even in the most conventional indications, unforeseen incidents can create difficult ethical dilemmas. I speak of dilemmas purposely, in that many of these situations seem to have no best solution: they either open a choice between two equally unknown possibilities, or offer an option between familiar but inadequate normative references and novel references whose normative pertinence is as yet unknown.

Not all issues underlying decision-making in this area can be handled within the confines of the therapeutic relationship. Some may ultimately call for discussion and decision-making at a political level, the results of which will be diversely affected by the pertinent social, cultural and economic context in which a reproductive procedure is being introduced. In other words, at the heart of the moral issue that involves choosing the best solution for all the protagonists involved, is the social issue of deciding what is the best framework for ensuring the desired outcome.

Avoiding harm and taking risks

No matter how conventional the familial situation of those who request procreative assistance, reproduc-

tive technology involves would-be parents in unprecedented ways of engaging their sexuated bodies in a procreative relationship. Reproductive experience is dismembered and extended in time and space, especially when conception is externalized. This multiplies the number of reproductive events calling for decision-making, and thus the occasions for unforeseen incidents and conflict. The persons directly involved in the conception of a human being are also more numerous: they are not only the would-be parents, but also the professionals technically competent in reproduction and eventually the donors of reproductive cells. If all the protagonists share a moral obligation to act rightly with respect to themselves and to others, those intervening as specialists have an added obligation to provide care meeting the standards of the profession.

Ensuring the quality of medical attention provided to patients requires a translation of this general principle into concrete technical choices. In strictly defined therapeutic situations, these choices usually reflect a consensually defined approach to disease prevention, in harmony with professional standards and guidelines for a particular act; they also ideally reflect the medical staff's basic ethical stance of beneficent action in caring for patients. In the case of reproductive technology, physicians additionally control access to essential technical and biological resources that make fertilization possible in a medical setting; they thus exercise discretionary powers in basic reproductive decisions, in particular in cases where procedures require choosing an appropriate reproductive partner—an egg or a sperm donor. There is still much discussion among physicians concerning the criteria on which these choices should be based and the responsibility associated with the consequences of these choices.

Concretely, questions about medical responsibility usually arise in situations which involve risk-taking, and in which the protagonists hold differing views of what constitutes an acceptable risk in the light of expected benefits (12–14). In reproductive procedures, eventual risks first concern the woman's health, as she is the primary patient. As such, many questions raised by conflict between conceptions of professional responsibility and of patient autonomy do not differ essentially from those raised in a traditional therapeutic situation. But as mentioned earlier, infertility is not a life-threatening disease, nor is assisted conception treatment for infertility. Failure to intervene cannot be said to endanger the woman's health, as might be the case in some routine therapeutic circumstances, whereas there may be important risks in undertaking the procedure. Many forms of assisted conception are physically and emotionally trying experiences for women; they do not always result in a live birth, or may do so only after repeated attempts. Many women may, nonetheless, feel that not having access to the procedure at all could provoke personal distress that is just as serious.

What makes matters even more complicated is the fact that medical concern with risk-taking in reproductive procedures tends to include an, as yet, non-existent third party, the unconceived child. Should professional responsibility for the competent management of a reproductive procedure be guided by the endeavour to do anything more than simply avoid procedures harmful to the woman's health and life? The question is raised acutely in cases where there is a risk that the child will be born with a serious disorder. When such a risk is inherent to the procedure, it is in most cases relatively uncontroversial to stop providing it. But when the risk for harm derives from the medical and the social background of the genitors, the medical stance considered most appropriate is far from consensual.

Establishing a medically appropriate response to a person's request for assistance with conception may be approached either as a question regarding legally defined medical responsibility or as a broader concern with moral responsibility. From a legal perspective, a physician in normal therapeutic circumstances is obliged to propose the necessary means to restore health, but is not held responsible for the outcome of treatment, if the procedure has been performed correctly. In certain situations, physicians occasionally experience these minimal legal requirements as insufficient from a moral perspective. In the case of assisted conception, physicians may even feel that the reverse relationship is true: physicians should not be obliged to propose systematically the means of conceiving, but may be held responsible for the outcome *(12–15)*.

In both perspectives, the fundamental question remains the same: in so far as reproductive technology is not, strictly speaking therapeutic, what criteria define the competent and responsible enactment of a reproductive procedure? An answer to this question requires agreeing on what is the outcome of that procedure. The immediate objective is a viable pregnancy, for as long as this objective is not attained, the procedure will be repeated. There might however

be a limit to the number of times a procedure may be attempted, without harm to the patient. But when the procedure succeeds, does the physician's accountability stop there? Or is the physician ultimately responsible for the state in which the child will be born? If the latter is the case, to what extent is this true? Quite obviously, different definitions of the outcome of a procedure affect working conceptions of appropriate action.

Three recurrent dilemmas are encountered by physicians in routine practice. These dilemmas often oblige them to reconsider the assumptions on which their practice is based. In many cases, the dilemma will first arise as a technical problem requiring a professional response, but as physicians try to solve it, the social and ethical significance of the problem comes to the surface. In some cases, they become a source of conflict with patients. Their immediate solution does not seem to involve finding a right answer, but reaching an agreement among all concerned as to how the priorities in decision-making will be distributed throughout the procedure. This implies identifying the possible consequences on the child and the family of each solution and on whom the responsibility for these particular consequences will rest. The three dilemmas are as follows.

1. In what circumstances is the transition from sexuality to assisted conception justified?

This problem arises in routine practice with both fertile and infertile couples. In the case of infertile couples, the question essentially involves deciding when one should consider that treatment of infertility has failed and that it is legitimate to go on to offer palliative solutions, such as assisted conception. One example of such a dilemma is choosing whether or not to try a curative option such as microsurgery of blocked fallopian tubes, which may not succeed, or go on to the palliative option, IVF. Another is whether or not therapeutic action should be delayed, such as in cases of low fertility where there is a statistically significant chance that a couple might conceive. Medical attitudes vary with respect to this problem: some will persist in delaying treatment or attempting curative solutions if the chances for success seem reasonable, on the basis that restored fertility and thus reproductive autonomy is the best solution for the couple. Others may feel that precious time, in particular with respect to female fertility, may be lost with treatment of uncertain benefit, thus diminishing the chances of arriving at the essential objective, a live birth.

The problem also arises in the treatment of couples who request assisted conception with donor gametes to avoid transmitting a serious disease (such as HIV) or a hereditary disorder to the child. The couple is not infertile and, in the case of recessive hereditary conditions, each partner would have been able to conceive children without that particular risk had they been with another partner who was a non-carrier of the same trait. Moreover, when the male partner is the carrier, he must use some form of contraception during the period of insemination. Abstaining from using one's procreative capacities is often difficult to accept, even when one is perfectly conscious of the risks involved; in fact, on occasion, the child born after DI is afflicted with the disease, because no contraception had been used. In the case of a dominant hereditary condition, the problem is simpler to resolve: a vasectomy or tubal ligation permanently avoids the risk of transmitting a genetically determined condition. Assisted conception is then more easily justified on the grounds of infertility.

In the situations I have observed, contrary to what might be expected, geneticists and physicians are quite reticent to push a couple into a situation in which they must abandon the idea of conceiving on their own. They often prefer recommending genetic counselling and prenatal diagnostic techniques. The use of donor gametes is proposed only when no genetic testing for the condition is available, the genetic condition under consideration is *serious*, and the risk of transmission very *high*. They will also accept requests from couples having had to terminate several pregnancies after amniocentesis.

In all of these situations, it is the high probability of not conceiving a child at all or of conceiving a child stricken by a serious or life-threatening health condition that justifies the move to assisted conception. Physicians perceive the couple's sexual relations, even when the partners are fertile, *as being, for all practical purposes, sterile*[ii]. This approach to the problem validates medical interference in the intimate realm of impregnation. It often also validates the

ii It is my view that a definition of infertility, not as an individual incapacity to conceive (and bear) a child, but as an incapacity to produce a *healthy* child, will play a major role in the way both reproductive technology and genetic diagnosis and therapy will evolve as medical practices in the future.

exclusion of single women, lesbians, and women having reached menopause.

But there are other unusual requests that do not fit so neatly into one of the slots of this dichotomy. For example, most physicians would not refuse to treat any person with a medically diagnosed infertility problem, no matter what their sexual and relational preferences[iii] but many might balk at the idea of extending their services to assisted conception. Another example: although most physicians refuse to offer assisted conception to a menopausal women (who by definition cannot benefit from regular treatment for infertility), most physicians would find it unacceptable not to offer assisted conception to a woman whose premature menopause has been medically induced.

Some cases are highly controversial, even among physicians themselves. One interesting borderline case is the following: what if the persons requesting assisted conception are a couple in which the male partner is a transsexual?[iv] Here, as in the case of premature menopause, the infertility results from medical intervention that amounts to castration: the endocrinological and surgical transformation of a woman into a man. Those who oppose treating such couples feel that, just as in the case of single women or lesbians, the new situation created is not a "natural" situation of heterosexual infertility. But others feel that it is difficult to refuse a request coming from persons whose status as man and woman has been recognized civilly, sometimes even by marriage, and whose infertility is the result of recognized therapy for transsexualism.

Another example: is it a physician's role to propose an alternative in circumstances that create obstacles to heterosexual relations? When these obstacles are related to physical health (for example, the husband is a paraplegic), physicians accept. But when the circumstances are social, such as employment that keeps a couple apart for great lengths of time, physicians tend to refuse. On some rare occasions, the competent administrative authorities in France have asked physicians to inseminate the wife or partner of a man serving a prison term, whose wife will have reached menopause at his release. Here the couple's sterility is due to the social constraints placed on the heterosexual relationship, not only by the prison term, but also by the fact that sexual relations are not allowed in French prisons.

The underlying dilemma in all these unusual situations remains the same as in the conventional situations: when is it justified to abandon sexuality for assisted conception? However, the question is no longer framed in terms of real or effective infertility, either because fertility is not a problem (single women, lesbians, separated couple) or because infertility is an accepted constitutional and socially recognized state (menopause, transsexualism). Most nonmedically motivated requests for assisted conception are founded on the idea of nondiscrimination in access to reproductive procedures. The question remains as to whether there are justifiable limits to free access.

If our societies decide to no longer restrict procreative possibilities to heterosexual relations or to medically justified procedures using marital heterosexuality as a normative reference, two problems are raised. The first requires rethinking our kinship relationships in such a way that they take into account all forms of procreation. The second requires reviewing a physician's responsibility in situations where the demand for professional competence is restricted to implementation of a procedure, with practically no intervention in the area of diagnosis[5]. We must not forget, however, that, in the background, a definition of infertility, not as an individual incapacity to conceive and bear a child but as a conjugated incapacity to produce a healthy child, is probably playing a major role in extending the medical (and therefore apparently noncontroversial) indications for both reproductive technology and genetic diagnosis and therapy.

2. To what extent is a physician's responsibility involved when, in the context of assisted conception, there is a risk of harming either the woman or the future child through the procedure?

As mentioned earlier, many of the responsibility issues in assisted conception are similar to those in any kind of treatment; they require an appropriate control of professional negligence by members of the profession through the setting of standards of practice. There is now relative consensus on how to handle some of the major risk problems of assisted

iii They might refuse to treat persons with a serious disease or a mental health condition.

iv These requests were first made in France in the mid-eighties. They have become more frequent in the past few years.

v This type of medical but nontherapeutic situation already exists with respect to contraception and abortion.

conception, such as ovarian stimulation and the number of embryos to be transferred to the womb after IVF (usually a maximum of three). But there is a tendency to disregard these standards when both physician and patient fear that caution will only be rewarded by the absence of a viable pregnancy.

Consensus is much more difficult to obtain when the risk involves transmitting a serious or life-threatening condition to the future child, because this risk is not immediately linked to the implementation of the procedure but to the medical history of its genitors (parents or donors). As we have just seen, physicians often perceive this risk as a justifiable medical indication for assisted conception even in fertile couples, as well as for screening donors to prevent the inadvertent transmission of disease. This screening in some countries, notably France, includes genetic screening of recipients, to avoid pairing a donor with a recipient who is a carrier of the same recessive trait. Most preventive measures taken in the interests both of the woman and the child are relatively uncontroversial (HIV testing of donors, for example); but can a physician go too far in attempting to prevent disease in children born through assisted conception? The disquieting spectre of eugenics raises its head.

The birth of a healthy child can never be guaranteed, no matter how many preventive measures are taken. Medicine's increased capacity to detect and eventually to prevent genetically determined conditions and malformations does not necessarily mean an increase in control over genetic parameters: they are too numerous, and the relationships between them too complex to be totally under control, even in a single specific situation. Physicians do nonetheless feel that, over and beyond their concern for their immediate patient, the woman, they can and must attempt to maintain the risks incurred by the future child at a limit defined as acceptable. However, the very task of defining such a limit in concrete terms is a complex and controversial process, even among physicians themselves. It is often quite easy for them to agree on the objective data that characterize a particular condition or a procedure, as well as on the objective data that describe the situation of the patient whose case is being considered. But evaluating the seriousness of a condition, the importance of the risk involved (is 5% or 10% a high or low risk?), and consequently deciding whether or not that condition justifies medical intervention (or on the contrary, refraining from intervening), requires subjective appraisal of objective medical data. At this point, the varied clinical experience of physicians with their patients as well as their divergent professional and personal moral views come into play, making consensus as to what constitutes an "acceptable" risk, in some cases, an unattainable ideal.

For example, in the case of donor screening, many physicians feel that it is unacceptable to use the semen of a man certain or suspected of being a carrier of a *serious* genetic condition and, in the case of certain recessive conditions, that can be found *frequently* in the population, and for which there is a *high* risk of transmission. When the genetic origin of a condition has not been clearly established (for example, in the case of a donor who has been cured of cancer), they also feel that, in the absence of conclusive data, it is unacceptable for the physician to take the responsibility of accepting such a donor. Risk-taking here concerns someone else's offspring.

But is a physician responsible in the same way for the outcome of a pregnancy, when the risk of transmitting a genetically determined condition emanates from the parents' family history? In some cases, physicians discover that the woman, whose partner is infertile, is herself a carrier of a serious dominant condition, but which she only has in a minor form; this is often the reason why it was first overlooked. In other cases, she might be a carrier of a serious dominant condition (such as polyposis of the colon) which is a late-onset disease of variable penetrance. A more recent problem concerns the appropriateness of proposing ICSI to couples in which male infertility is associated with being a carrier of a statistically frequent and serious recessive condition, cystic fibrosis. Contrary to the situation in which it is decided to exclude a potential donor, because someone else's offspring will be affected, the decision to refuse assisted conception on the basis of the parents' family history, even when this attitude seems medically justified, is experienced by many physicians as a "questionable decision".

Is there a dividing line, and if so where, between the physician's responsibility for controlling the medical factors which intervene during a pregnancy and the couple's autonomy in decision-making regarding their reproductive lives? Does the fact that the birth of a handicapped child implies expensive medical treatment, usually paid for by the state, weigh as a valid counterargument against the fact that ultimately it is the parents who assume responsibility for raising the child? In other words, who assumes responsibility for the consequences of risk-taking in childbearing and how does this affect their right to decide?

These questions are raised most acutely in cases where an at-risk pregnancy is monitored with prenatal diagnostic techniques. The probability of giving birth to a child with a serious disease or malformation can be determined with certainty, but some anomalies of the karyotype may be detected whose consequences for the health of the child are unknown. When in doubt, physicians and parents may have differing attitudes about pursuing the pregnancy. After amniocentesis, a physician may refuse to terminate a pregnancy if this does not seem medically justified, but cannot force a woman to abort, even if from a medical viewpoint this seems acceptable. One could imagine similar scenarios in the case of prenatal genetic diagnosis (PGD), where a physician may refuse to initiate a pregnancy by not transferring affected embryos, but cannot impose a transfer against the woman's will *(4)*.

A patient may legitimately question professional standards, as well as a physician's right to intervene in as personal a decision as whether or not to accept the birth of a child with a mental or physical deficiency. The physician him/herself may question this right to intervene. For in fact, medical and lay definitions of an acceptable risk, which frequently pit patients against physicians, also tend to overlap and eventually to conflict even in the physician's own reasoning: a physician's personal values with respect to the very sensitive problem of reproductive choices, also influence his/her professional attitudes[vi] *(16)*.

What ultimately makes this problem such a troubling one is that, even if professionals are responsible for correctly establishing a probability of risk, once that risk is known, deciding what is an *acceptable* risk becomes a joint moral issue for all the protagonists involved. At length, it is also a social and policy issue because it requires deciding how far physicians may interfere in would-be parents' procreative decisions, to maintain their professional standards of risk-taking. Physicians are, after all, the present gatekeepers of reproductive technology.

3. To what extent is a physician accountable for the social conditions in which a child will be born?

Beyond concern with donor screening, most countries still honour a tradition, stemming from almost a century of practising DI, that allows parents—if they so desire—to conceal from family, friends and even the child, the means by which the child was conceived. This requires a policy of donor anonymity, but also selecting a donor whose physical traits match those of the would-be couple. Minimal matching involves controlling for such traits as skin colour (and in some cases hair and eye colour), as well as blood group. Proceeding in this manner makes it plausible to the ordinary observer that the parents conceived the child.

However, some donors have hereditary traits that do not justify exclusion but that could become markers revealing the parents' recourse to a donor procedure. Several genetic traits fall into this category, but each one poses different problems. A donor who is a known heterozygote for a recessive condition that neither of the parents have, has a 1in 2 possibility of transmitting this trait to the child. However, this trait is not visible, at least in the first generation: at most, the child will himself or herself be a heterozygote for that condition and will therefore be healthy. A cleft palate and a harelip are also hereditary malformations; they are visible, but operable defects. But they do not necessarily function as markers, because sporadic (nongenetically determined) cases are also possible. On the other hand, a polydactyl donor (having more than the usual number of digits on the hands or feet) has a visible hereditary trait, which does not threaten the child's health but which indisputably acts as a marker of DI. Should such a donor be accepted?

Acceptance of such donors raises, as in our preceding dilemma, questions about the extent and the limits of genetic screening. Should physicians avoid the deliberate transmission of any harmful or anomalous genes; or should they attempt only to prevent the most disabling hereditary conditions? In the latter case, it must not be forgotten that evaluating the seriousness of a condition is never a totally objective procedure. Accepting such donors also presupposes that would-be parents are aware of the criteria that guide physician selection of donors in good health, and that they are ready to surpass the need for secrecy. And yet it has been this guarantee of secrecy that has allowed the practice to thrive and

vi An interesting international comparative study, directed by D.Wertz and J. Fletcher of the attitudes of clinical geneticists from 19 different countries confronted with a typical set of difficult cases, shows how values concerning medical decisions related to genetics, which often imply reproductive choices, vary from one society to another, as well as among individual practitioners, in particular according to their age, sex and religious practice.

to become socially acceptable, at least to a certain extent[vii].

In associating, for reproductive purposes, two persons unknown to each other, who will not be recognized socially as the child's parents, the physician quite obviously plays a role that surpasses medical responsibility for correct implementation of a procedure. Paradoxically, this role as mediator in a morally delicate situation has always seemed self-evident. In recent years however, the psychological and moral validity of donor anonymity and of secrecy about the child's origins has been questioned. Semen banks in some countries offer nonidentifying information concerning donors and may even allow would-be parents to choose. Donated eggs are so difficult to obtain, that many physicians are now opting for nonanonymous donations, because women accept more willingly to undergo the risks of the procedure if the beneficiary is a friend or relative. These changes imply reconsidering the physician's role in donor procedures, as well as the criteria and purpose of screening and matching donors with recipients. Many of what may seem self-evident decision-making criteria are implicit suppositions about stable family life, child development, and concepts of health and normality. These must ultimately be submitted to open social discussion and some aspects of donor matching may call for policy decisions.

Questions about the physician's role and responsibility as mediator in a reproductive procedure also arise in nondonor procedures, in particular when an unforeseen incident changes the context in which assisted conception is being offered. One example is the unexpected death of the woman's husband or partner after treatment has been initiated *(4)*. Sometimes frozen semen or embryos are still in storage and, on several occasions, women have requested the treatment be continued, alleging not only that this is their last chance to conceive, but that they wish to have the child of their deceased husband. Physicians' attitudes have varied and, in some cases, they have chosen to pursue treatment; but independently of the final action taken, concern has always been expressed about purposely favouring the birth of a child whose father is deceased. If the initial request was considered acceptable, to what extent, if at all, are the social conditions of a child's birth a physician's responsibility?

Most physicians do not restrict their evaluation of a medical indication for treatment to the physical symptoms. The choice of the most adequate treatment often takes into consideration a patient's finances, family surroundings, mental health, etc. In the case of assisted conception, the social condition of the would-be parents—married and in many countries cohabiting couples of acceptable procreative age—is part of the criteria defining the so-called medical indication. In this sense, the refusal of physicians to pursue treatment when the couple's social condition has changed (death or illness of one of the partners, divorce) appears to be a logical sequel to the initial stance. In this sense, our last dilemma returns to the first, the difference being that what appears as a socially unacceptable context arises from an unforeseen incident in a conventional situation, and not from the characteristics of the initial request.

Conclusion

What are the consequences for a society of having chosen to develop a medically mediated form of reproduction? The fact that would-be parents, whatever their social status, are asking physicians to provide the means of accomplishing what was once an intimate act is hardly an anodyne fact. Whatever the differences in technical variants, reproductive technology appears essentially to be "emancipating" procreation from the usual conditions of heterosexual commerce. Artificial insemination has long since desexualized the act of conception. IVF has now disembodied conception, a trend that could be extended to the rest of pregnancy by creating the conditions for ectogenesis. The prospect of cloning now augurs the emancipation of procreation from what still remains the fundamental requirement of sexual reproduction, the participation of sexually differentiated beings, and introduces the possibility of using reproductive cells (embryonic stem cells) for non-reproductive therapeutic purposes. What seems to be at stake in the development of these practices is a transformation of the anthropological conditions of procreation.

Should these new ways of conceiving become available to all persons who express a desire for

vii The fact that secrecy is the most frequent attitude found among parents of children conceived with donor gametes belies the fact that this particular way of conceiving children and the kinship it establishes is not always perceived and experienced as legitimate.

children, or should they only be dispensed to persons in particular situations? Diverse variables affect both the ways in which the question is raised and the manner in which a society explores the reasons it has for implementing these techniques. Generally speaking, some countries favour a rights approach to issues of choice, giving more space to the political expression of atypical requests; others prefer an approach establishing the limits of what is socially permissible, devolving to physicians as professionals competent in reproduction the task of supervising the respect of these limits. However, the tendency to dissociate issues of reproductive freedom from the medical contexts in which they often arise tends to blind us to the constant interaction of issues of choice with matters of medical responsibility, as well as to the fact that the contours of professional competence and responsibility is a social and ethical issue in its own right.

This paper comes to no major conclusion as to the best way to proceed. How can it, when many of these issues are still emotionally charged objects of moral and political dissent? It does, however, distinguish some strong underlying trends in the development of these practices.

1. There seems to be a trend, even in conservative countries like France, to increasing acceptance of unconventional requests for assisted conception. This is happening, not through open political debate about rights and discrimination, but through the constant redefinition of what constitutes acceptable conditions for access to treatment, in particular through extensive interpretations of infertility and disease prevention.
2. Issues related to changes in the family are basically examined from the point of view of nonmedically motivated requests for assisted conception (single women and lesbians, menopausal women). Less attention is being given to the way medical reasons and, in particular, motives for genetic screening and diagnosis, are progressively shaping aspects of individual procreative choice. Mounting concern with the transmission of genetically determined conditions may have profound effects on the way persons meet and decide to have or not to have children, as well as the type of health care they seek. There may be significant differences between those who decide to take preventive measures and those who do not—differences that most certainly reflect the way persons deal with the disability that afflicts their family.
3. These two trends are intimately linked to evolving conceptions of medical responsibility, an aspect of development in this area of medical practice that is far from getting the attention it needs. In some cases, physicians seem to be offering new options to persons who had no hope of having children; but they are also creating new constraints on procreative liberty. Some constraints imposed by a physician's concern with taking appropriate medical action may be based on valid arguments, pertinent to their field of competence (certain kinds of screening). Other constraints appear more questionable (limiting assisted conception to cases of infertility), but may occasionally derive from a social function attributed to physicians by society as part of the responsible exercise of his/her profession. All of these aspects merit close evaluation and, in the case of the latter, more open social and political debate. For as expectations grow regarding their technical competence and as the scope of their professional activity increases, physicians may, more often than not, be asked to make essential reproductive choices, even by the "patients" themselves.

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Ethical issues arising from the use of assisted reproductive technologies

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Introduction

The purpose of this paper is to address ethical issues arising from four aspects of the employment of assisted reproductive technology (ART), namely:

- the principle of equity;
- the establishment and change of social policies;
- commercialization of human gametes and embryos; and
- conflicts of interest.

Issues will be considered in this sequence, but they are not entirely separate from each other. There is unavoidable overlap among them, and some topics may fit as well under the headings of two or more issues. Similarly, there is some overlap among issues addressed in this and the other background papers on ethical and social concerns in this publication. Accordingly, for the sake of convenient analysis, topics will be presented under headings and subheadings, but they are not to be considered as discrete from each other. Some discussions will relate to others in different sections of the paper, and in other papers. Further, the thrust of some discussions may appear to vary from and even contradict that of others. This is because ethical analysis does not necessarily lead to a self-determined conclusion; rather, it exposes considerations that require or warrant attention, balance and prioritization. Balance and prioritization may be achieved in different ways, depending upon the ethical orientations, principles and levels of analysis that are brought to bear. For instance, deontological or principle-based orientations may produce different outcomes from utilitarian or consequentialist orientations, ethical principles such as beneficence and justice may be ordered in different priorities, and interpersonal or microethics may justify different results from public or macroethics *(1)*.

Different conclusions can be of equal ethical merit, related to the different factors that contribute to undertaking ethical reflection. For instance, much consideration of ART involves gamete and embryo donation, but in the Islamic tradition, where conceiving children and raising them in religious faith are particularly important values, so too is the integrity of a family's genetic lineage *(2)*. Accordingly, in this context, gamete and embryo donation from outside a married couple is ethically unacceptable, but within a marriage artificial techniques may be employed to achieve pregnancy. In contrast, the Roman Catholic branch of Christianity limits acceptable human reproduction to natural intercourse between a married couple *(3)*, but may tolerate transfer of a donated ovum to an infertile woman's reproductive system for natural insemination there by her husband. Artificial conception may therefore be ethically available to a

Muslim but not an observant Roman Catholic couple, and ovum donation may be ethically available to a Roman Catholic but not an observant Muslim couple.

Within some religious faiths, ethical pluralism is rejected, and divergence from authoritative doctrine may be deemed heresy. The modern practice of ethics or bioethics is secular and pluralistic, however *(4)*, recognizing that ethical reasoning on the same issue can justify different conclusions. This is not to say that every option is acceptable, but that adherents of one preferred outcome may well acknowledge that adherents of an alternative preferred outcome are applying approaches that result in different ethical, but not unethical, conclusions.

The principle of equity

Equity and equality

Equity is distinguishable from equality, although the two often coincide. Equality requires the identical treatment of all despite their differences, whereas equity requires equally fair treatment of individuals taking account of ethically significant differences among them. The ethical principle of justice requires that like cases be treated alike (hence the legal preoccupation with precedents) and that different cases be treated in ways that acknowledge the differences, raising ethical concerns of likeness and difference. For instance, the private insurance industry in the USA has long treated men and women as equals in covering contraceptive services for neither. However, women bear the consequences of, particularly unplanned, pregnancy more directly and oppressively than men. The inequity of this equality became clear when insurance companies speedily extended their cover to include the new male potency drug Viagra *(5)*, moving some state legislatures to require coverage of contraception *(6)*.

An initial issue of equity and equality concerning ART is whether people with impaired fertility, including those who turn to ART because their natural reproduction would expose their children to unacceptable risks of harmful genetic inheritance, should be as free to reproduce as people of usual fertility. In many countries and cultures, particularly of the western world, the latter are not subject to legal prohibitions, requirements of marriage or, for instance, medical screening on genetic or other grounds, although they are subject to the regular law on their partners' capable consent and the prohibition of incest. The mature and responsible are not privileged over the immature and irresponsible, nor the wealthy over the poor or the healthy over the infected, but all rank equally as individuals able to exercise choice of reproductive behaviour according to their own preferences and instincts.

In contrast to the capacity of usually fertile individuals to undertake consensual reproductive behaviour in private, is the public attention and regulation to which reproductively impaired individuals are increasingly subject when they propose resort to ART. Particularly in developed countries where ART techniques have been pioneered, such as Australia and the UK, state and national commissions with distinguished memberships have proposed criteria by which ART may become restrictively available to reproductively impaired people. Proposals of many commissions have been enacted into laws or adopted as professional or clinical practices. These may limit access to ART to legally married or cohabiting heterosexual couples in relationships of specific duration, require or facilitate their scrutiny according to medical, genetic and perhaps psychological standards, or screen them by reference to other criteria such as age, personality and criminal or childcare history.

An ethical concern is the extent, if any, to which different approaches towards reproductively impaired and unimpaired people, established in law or practice, can be justified. An important human rights provision is nondiscrimination on grounds of physical and mental disability, according to which reproductively disabled people should be placed at no disadvantage in contrast to people of usual fertility. Another provision is to ensure due protection of children, however, which allows, for instance, lawful removal from their parents' care of children exposed to or at serious risk of abuse or neglect. This provision may afford an ethical justification of laws and practices that bar or scrutinize access to ART of people whose circumstances or histories furnish credible apprehension that, even unintentionally and despite their good will, any children for whose care they became responsible would be at risk of serious disadvantage or neglect. The ethical principle of respect for persons balances rights of autonomy against rights to protection of vulnerable persons, of whom young, dependent children are obvious examples.

The goal of serving the best interests of prospective children is sometimes invoked to justify limiting people's access to ART, even though the consequence

may be that the prospective children whose interests are claimed to be protected are never conceived. The inequality or inequity of controlling the reproduction of infertile people who are dependent on ART, when that of usually fertile people is not and perhaps cannot be controlled, is sometimes explained on pragmatic or utilitarian grounds, and by recognition that, in many countries, fertile people whose parenthood exposes their children to undue risks will be subject to child protective intervention that denies them childrearing opportunities. However, the children of fertile couples are not legally removable from their care on the ground only that public agencies believe that they can better serve the children's "best interests" by placing them elsewhere, and it appears inequitable to invoke a "best interests" criterion legally to deny ART to infertile couples when there is little risk of their future children being abused or neglected.

Disability and pathology

Impairment of fertility may be due to a pathological cause, but it is ethically contentious to describe people seeking access to ART generically as unhealthy or diseased people, or, indeed, apart from their impaired reproductive capacity, as disabled. Infertility itself is not a disease, and alone it does not impair medical health, although among those who want to have their own genetically related children it may impair their health in so far as the World Health Organization recognizes "health" as a state not only of physical well-being but also of mental and social well-being. On this basis, UN conferences have endorsed the definition that: "Reproductive health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, in all matters relating to the reproductive system and to its functions and processes. Reproductive health therefore implies that people are able to have … the capability to reproduce and the freedom to decide if, when and how often to do so. Implicit in this last condition are the right of men and women to be informed and to have … the right of access to appropriate health-care services that will … provide couples with the best chance of having a healthy infant" *(7)*.

Infertility can deny mental or social well-being and be a cause of acute affliction and anguish, evidenced by the extent of physical and financial cost individuals are willing to bear for its relief. However, many countries that provide publicly funded health care for medically necessary services do not fund ART. They usually fund diagnostic services, and may fund drug and surgical treatments, such as of diseased fallopian tubes, that restore fertility, but not ART that does not reverse the medical condition of infertility but overcomes it by artificial means of conception.

The ethical and related human rights principle of nondiscrimination on grounds of disability raises the question of whether states should ethically do more than to permit those with the personal means to avail themselves of accessible ART services to do so; that is, whether ART should be allowed as luxury medicine, like, for instance, cosmetic surgery, available with minimum screening on social or moral grounds to those with the means of purchase, or whether the principle of equity requires some measure of public funding or subsidy of ART services, such as by taxation relief for its cost. States that provide publicly funded health care services to restore natural capacities, including reproductive capacities, may claim that they satisfy their duties of equity in treating all eligible recipients of state medical services alike, and that they have no further ethical responsibilities to those that ordinary care cannot assist. It may be that medical treatment of pathological conditions that cause infertility, such as premature menopause and fallopian tube blockage, discharges the duty of health care equity, and that there is no such duty to relieve remaining disability by provision of costly ART services. Nevertheless, limited access to ART services due to their high cost remains a major equity issue raising questions about reproductive rights of people with limited financial means.

Negative rights and positive rights

Considering impaired fertility as a reproductive disability raises the concern of the appropriate public or macroethical response to the rights of such disabled persons to equitable treatment. Rights are often contrasted by reference to negative and positive rights. Negative rights amount to rights to be left alone, whereas positive rights require that holders be provided, often by state agencies, with means to exercise such rights. Rights to luxury goods and services are usually considered only as negative rights. By analogy to transportation, governments may provide low-cost or subsidized public transit services by road and rail to take people to and from work and between major population centres, but not maintain rural transit networks, provide subsidized airline services, or provide motorized vehicles for private use. Similarly, they may provide routine, low-cost treatment for

pathological causes of infertility and limited higher-cost care for more resistant conditions, but not the more expensive forms of ART. They may explain this in terms of health care economy, and also by reference to cost-effectiveness considerations in the budgeting of public services.

The negative right to ART, meaning individuals' right to acquire access by their own resources, requires that state and other agencies forbear or restrain themselves, or be restrained by judicial or other lawful means, from undue intervention by their creation of barriers or obstacles to equitable access. Many of these barriers have been of a moral nature, prohibiting individuals from unfettered resort to both publicly funded and privately available ART services. Some initial reactions to novel means of conception have exhibited what has been described as "moral panic", meaning an unreasoning fear of subversion of the moral order. It was noted in 1991 that "While the past 40 years has seen the meltdown of the nuclear family and its surrounding myths and ideologies—in less than ten years half of all children born in the United Kingdom will be brought up outside the 'conventional' family—new demons, chimeras and spirits have been summoned to haunt the new families which technological and personal upheavals have introduced" *(8)*.

For instance, unmarried individuals, including single people and partners in same-sex relationships, have been barred from ART by laws or by institutional or professional rules or practices. These have been based on or reinforced by claims that limits are compelled or justified to protect children against births into unstable or otherwise unconventional domestic settings. These speculative claims may be unsupported by empirical data, however, such as is available of the harms suffered by children that live in violent homes. Comparable claims that have denied rights to adoption of children are now yielding in many countries to recognition that children are as well reared in less conventional as in more conventional home environments. It is increasingly recognized that more than conservative orthodoxy and negative speculation based on generic bias are required to deny a right of privately funded access to ART.

Preconceptions about the unsuitability and ineligibility for access to ART of those affected by mental disorders may also require reconsideration on grounds of equity. Mental disorder of a severe nature, although not requiring institutionalization, may justify ineligibility for a childrearing role, whether children result from natural or medically assisted procreation, but many mental disorders are transient, of different levels of severity and amenable to treatment. It has been observed that "The stigma suffered by the mentally ill dates back to antiquity and has its origins in fear, lack of knowledge and ingrained moralistic views. Though erroneous, these associations remain pervasive.... At times, the unusual and even unfounded nature of psychiatric theories and the practitioners who uphold them has compounded the problem" *(9)*. Equity requires that particular applicants for ART be clinically assessed on their individual merits, and not be denied rights of access on grounds of impersonal, collective stigmatization and discrimination.

ART applicants' liability to exclusion on grounds of their physical health should similarly be clinically assessed. Their vulnerability to premature death or disability, leaving young children at risk of orphanage, destitution or neglect, may properly weigh negatively in the balance, but rights of access should not be denied on the basis of negative stereotyping. The *British Medical Journal* has recently observed, for instance, that in view of the prolonged life expectancy of people who are HIV-positive and receiving treatment now available, particularly in developed countries, there is no justification for denying infertility treatment to patients who bear the infection. It reported that "Judicious use of combination antiretroviral therapy during pregnancy and labour, delivery by caesarean section, and avoidance of breastfeeding are proved measures which have reduced the risk of vertical transmission to less than 2%" *(10)*. Exclusion of HIV-positive applicants from ART programmes may be explained not by their incapacities to be suitable parents, but by health care practitioners' inequitable reluctance to treat them as patients *(11)*.

Although potential donors of gametes and surplus embryos may be liable to comparable negative stigmatization, for instance when gay men are rejected as sperm donors, it is doubtful that they have an ethical or equitable right of donation. The question is sometimes posed of, whether human tissue donors, for instance, of blood for transfusion or creation of plasma products, have a general right or only a selective privilege of donation. Egalitarians tend to favour the former in light of the humiliation and loss of self-esteem those whose altruistic offers of donation are rejected may suffer. The right/ privilege distinction may be a false dichotomy, however, since

donation may be neither a right nor a privilege, but only a qualified opportunity; that is, an opportunity to offer to satisfy objectively, scientifically justified criteria of eligibility. For instance, a couple may be admitted to an ART programme as suitable, informed recipients of the service, but not be eligible on genetic or other grounds to donate their gametes or surplus embryos to others. They have no ethical rights of donation, but only the right to offer to donate (see the chapter on "Gamete and embryo donation" for details on the criteria of acceptability).

A related question is whether recipients of ART services can claim a right to choose specific gamete or embryo donors. With the exception, for instance, of the wife of an infertile couple choosing her brother as a sperm donor, couples may claim a right of choice of donors who meet routine criteria, such as being HIV-negative. It has been reported regarding ovum donation, for instance, that "90 percent considered using a sister, 76 percent decided that a sister would be the preferred donor, 70 percent asked a sister to donate, and 60 percent found a sister to be willing" *(12)*. Ethicists and practitioners have raised the concern that family relationships may become blurred or confused by the use of such known donors *(13)*, and issues of blame or regret may arise if donation is followed by an adverse outcome. Allowing ART patients to recruit donors also raises concerns of financial inducements, emotional coercion and exploitation of dependent relationships. The New York State Task Force on Life and the Law recommended that: "When known egg donors are used, informed consent to donation should take place outside the presence of the recipient. Physicians should attempt to determine whether known donors are motivated by undue pressure or coercion; in such cases, the physician should decline to proceed with the donation. When applicable, the informed consent process should include a discussion of the psychological and social ramifications of egg donation within families" *(14)*.

Establishing and changing social policies

Policy evolution

The ethical conduct of a "social policy" suggests pursuit of a principled, deliberative public programme of action designed to serve the interests of a given organized population or society, according to the science of politics or statecraft. However, the conception and birth of children has customarily been regarded as a private or family matter, regulated by the unpredictable chance of nature or as a divine mystery outside decisive human control. The principles of family law within a community reflect its most historical and customary or intuitive values, often embedded in religious beliefs regarding private intimacy, associated with the transition between generations of family traditions, identity and property.

The emergence of ART including gamete donation has confused the genetic cohesion and integrity of traditional family identity *(15)*, and initially triggered conservative responses. First reactions to what reproductive technology shows to have become possible are often more instinctive or visceral than intellectual, and policy responses have tended to focus more on defence against perceived dangers to traditional values than on achieving potentials for human satisfaction and cultural enrichment through new applications of biotechnology. This was observed with the early popularization of artificial insemination, when Kleegman and Kaufman noted in 1966 that:

> Any change in custom or practice in this emotionally charged area has always elicited a response from established custom and law of horrified negation at first; then negation without horror; then slow and gradual curiosity, study, evaluation, and finally a very slow but steady acceptance *(16)*.

Societies progress through this transition at different paces, and establish and change their policies accordingly. Those most influenced by religious concepts are in some ways slowest to progress. For instance, since the Roman Catholic Church adopted the concept of papal infallibility in 1870, its teachings cannot contradict earlier papal pronouncements made *ex cathedra*, and much of its scholarship is devoted to assertion of the authority of conclusions reached in earlier times. Doctrinal reassessment within the church is severely compromised, because it has to be shown consistent with existing authority. Social policies that reflect any variation from church doctrine, such as the doctrine that artificial or "unnatural" means of achieving human conception are illicit, are considered a scandal or heresy, and strongly opposed. Indeed, it has been explained that the modern emergence of secular, pluralistic western bioethics was strongly influenced by the Vatican's intransigence in 1968 on doctrinal

reform regarding artificial contraception *(17)*. In contrast, although Islamic prohibition of gamete and embryo donation is firm, the use of ART to overcome infertility within marriage is accepted, often welcome and even considered necessary *(18)*.

Different popular religious attitudes to relations between human beings and their perceived divine creator can influence policy responses to ART. In many Christian communities, for instance, it is considered offensive and a condemnation that one should assume to "play God" with human conception and birth, as an impertinent human arrogation of divine power and authority. Accordingly, social policy treats the practice of ART conservatively as bordering on impropriety, and detracting from or tampering with the awe and humility with which to face divine authority. In other religious traditions, however, such as Judaism, there is a perceived partnership between humans and their divine creator, so that individuals' "God-given gifts" of skill and initiative are properly employed in scientific advance and in the cure or overcoming of medical impairments, including by ART. In this tradition, the divine creator is described as acting in ways of beneficence, mercy and compassion, and "the human being is required to imitate God in this respect" *(19)*. Social policy in Israel, for instance, is strongly pro-natalist *(20)*, and encourages ART within marriage, provided that ovum donors to Jewish couples are Jewish, in accordance with the first direction given to Adam and Eve in the biblical *Book of Genesis*, chapter 1, verse 28, to "be fruitful and multiply, and fill the earth," reinforced perhaps by demographic and geopolitical incentives.

Problematic and constricting though religiously conditioned social policy may be, it has the ethical advantage over purely secular policy development of invoking profound and enduring principles. In contrast, secular policy-making is more pluralistic but may seem to defy the ethical principle of justice in producing quite different responses to the same circumstance, influenced by idiosyncratic values and priorities and introduced as a consequence of political power rather than of any transcending ethical principle or even conscious tolerance of ethical pluralism. When surrogate motherhood rose to public visibility, for instance, and women were recognized as potentially willing to gestate and surrender children to serve other families, diametrically opposed responses appeared. Some urged and enacted policies that prohibited any woman from undertaking surrogate gestation who had not previously delivered a child, on the principle that truly informed consent to gestation and childbirth could not be given by a woman lacking this experience. Others were fearful of the psychological harm a young child might suffer from recognizing that its mother is willing to give away her child to others, and urged that women with dependent children be prohibited from surrogate gestation *(21)*.

Policy (reform) commissions

Nevertheless, the advent of surrogate motherhood illustrated an ethically defensible process to establish social policy, to evaluate whether existing policy is dysfunctional or inadequate to address new technical possibilities, such as arise from ART, and to change it if necessary. From the late 1970s, many countries and states and provinces such as those of Australia, Canada and the USA, established governmental or other official enquiries into ART, to propose social policy responses to limit, accommodate and/or monitor effects of these new biotechnological capacities on human reproduction and the founding of genetically diverse families *(12,21–29)*. They tended to be composed of members of mixed social, academic, philosophical, religious and other backgrounds who were experienced in development of social policy. They received representations from community groups and individuals, solicited information and opinions they considered necessary or appropriate to fulfil their mandates, and consulted with specialists in technical areas and on social and ethical implications of policy options. They tended not explicitly to invoke the language or categories of ethical discussion, speaking instead of the social values and pragmatic considerations they considered significant, but their discussions and conclusions were amenable to ethical analysis.

The conclusions and array of recommendations that these commissions produced did not always win favour with ethical analysts, and were often greeted with dismay both by libertarians and by many who assessed them from conservative religious perspectives. This was because they tended to recommend acceptance of some practices, such as unpaid gamete and surplus embryo donations, prohibition of others, such as commercial transactions including surrogate motherhood agreements, and, for instance, setting of conditions and time limits by which preserved gametes and embryos had to be let perish.

The commissions contributed to ethical social policy development, in that they opened issues to

public debate, either through their own processes or through generation of public discussion of their conclusions, and sometimes both. They were respectful of those who made oral or written representations to them, although at that time organized religious institutions were better equipped to advance their views than bodies claiming to represent infertile people, they beneficially added to public understanding of the issues and response options raised by ART, and they attempted to justify their balancing of the competing principles and pragmatic considerations that conditioned their conclusions and recommendations. They had different levels of success in having their recommendations enacted in law, but tended to be well respected by medical and related professional associations whose members were practitioners of ART.

The ethical character of these commissions was based more on the transparency and integrity of their processes than on the substance of their conclusions and recommendations, many of which were contentious among ethical analysts and commentators. Many received information and opinions, and formed their own conclusions, before the present emphasis on evidence-based medicine arose. In light of this newer perspective, some of the information they were given and the scientific conclusions they reached might now appear questionable. Further, and perhaps more significantly, they made no approach to advance or consider founding the social policies they explicitly or implicitly adopted on empirical evidence. They almost invariably accepted as true, for instance, that children are better reared in legally married unions than in unmarried unions, and that heterosexual parenthood provides a superior rearing environment to stable same-sex unions. Many uncritically accepted conventional stereotypes of family life and functioning, without seeking or reviewing evidence, for instance, of the incidence and nature of marriage breakdown and family dysfunction within their societies, and the effects on children's well-being. This deficit in these studies raises ethical concerns about the adequacy of this method of establishing, changing or declining to reconsider social policy.

The burden of proof

Commissions of enquiry often include members from the legal profession or judiciary, sometimes as their leaders, and some indeed have been conducted within law reform commissions *(21,26)*. This may provide means to address, though not necessarily to resolve to uniform satisfaction, a key ethical issue of where the burden of proof lies to preserve or change prevailing social policies. The evidence and policy implications arising from individuals' access to ART services and from operation of ART programmes are rarely unequivocably favourable or unfavourable. It is uncertain, for instance, whether treatment that results in an infertile couple having a new family of two or three prematurely born children that suffer respiratory and/or neurological impairments is to be considered successful or unsuccessful, or whether treatment that provides an infertile couple with one or two healthy children following a multiple pregnancy that was "selectively reduced" by ending the lives *in utero* of several embryos or fetuses is to be celebrated or deplored. When a country's social policy is unaccommodating of equivocal new technology, the ethical question is whether potential users can claim an ethical right to policy change to accommodate it, so that opponents have to make the case to preserve the status quo, or whether the burden lies on supporters of the new technology to make the case for policy change. Similarly, when a government proposes a new law to restrict access to a newly developed service, the question is whether the government has to make an ethical case *(30)*, or whether the ethical burden of resistance is on political opponents; the policy is not ethical simply because a government can implement it in law.

When the need for, or desirability of, policy reform is equivocal, and there is as much to be said against policy change as for, and vice versa, the question of whether supporters or opponents of policy change bear the burden of making their case is decisive. Neither case may be made persuasively, and the side bearing the burden will fail to discharge it. Conservative or risk-averse forces will claim that a long-standing and adequate social policy should be changed only when advocates of innovation present a convincing argument in favour, and those of a reformist or socially experimental disposition will claim that prima facie evidence of advantage from innovation should be sufficient to propel policy reform, and that those resistant to reform bear the burden of establishing the case against it. In contrast, however, when a new practice appears to threaten conventional values, such as surrogate motherhood or human cloning, conservative forces want to speed restrictive provisions, and reformists urge caution and time for balanced reflection against precipitate prohibitions *(31)*.

Both conservative and reformist preferences may be based on ethical principles, and often on variants or counterpoints to the same principles. The principle of beneficence may support reform to accommodate the advantages attributable to a new technology, but the duty to do no harm, nonmaleficence, may support its rejection. Supporters of reform may claim that denying a policy that would accommodate the new technology does harm to those it may benefit, and that reform is required by the principle of justice, since the new but excluded practice is like one already accommodated. However, opponents may identify a feature or consequence of the new practice that renders it distinguishable. For instance, advocates of cloning by embryo-splitting may claim that it only simulates natural or spontaneous identical twinning, and so should be allowed, while opponents may claim that it accommodates multiplication by successive twinning of an embryo twinned *in vitro* and, unlike natural twinning, allows identical twins to be gestated and born years apart. A social policy compromise may be to limit induced twinning to a single occasion, and require concurrent implantation of successfully divided embryos. Ethics may provide no self-evident or clear outcome on the merits of a particular case, but provide protagonists of different outcomes with the language and concepts of their advocacy.

Commercialization of gametes/embryos

Ethical arguments against commercialization

Ethical arguments against commercialization include reference to dangers of exploitation of vulnerable people, such as those who are impoverished, and to the more abstract concept of human dignity *(32)*. A principal argument against allowing human gametes and embryos to be the subject of commercial or profit-earning exchange stems from the ethical principle of respect for persons, which is sometimes considered analogous to the concept of human dignity as applied in Europe. Neither gametes nor embryos are persons, but both may be considered potential persons and what philosophers describe as "the argument from potential" *(33)* requires that they be treated with the respect and dignity due to the persons they have the potential to become. Since abolition of slavery, the concept advanced by the German secular philosopher Immanuel Kant (1724–1804) has prevailed, that people, and by implication potential people, should not be treated as objects, nor only as means to ends. As ends in themselves, individuals have inherent worth and value, not simply the instrumental or utilitarian value ascribed to objects, which are valued only for what can be done with them. Accordingly, it is inconsistent with their inherent worth that human gametes and embryos should become the subject of commercial value, barter and trade.

This ethical reasoning is supported from a variety of extraneous perspectives. A religious view, adopted by the Roman Catholic Church in 1869, displacing earlier concepts of ensoulment that determined when the soul enters the body, is that human life begins at conception or fertilization *(34)*. This view requires that an embryo be afforded the same respect and protection as a born person, although the application of this view to sperm and ova appears more difficult to establish *(35)*. A view from philosophy and political science is that some interests, objects and functions, such as motherhood, should not be amenable to market transactions because of the damage that would result to human values, community and dignity. Margaret Radin, for example, condemns paid surrogate motherhood as devaluing women in general, mothers in particular, and children universally by making them "completely monetizable and fungible objects of exchange", meaning that any one may be replaced by any other and has no individual value in itself, so leading to "an inferior conception of human flourishing" *(36)*.

The ethical argument against commercialization of gametes and embryos is not simply the pragmatic harm this may do to the spirit and practice of altruism. Nor is it the inducement payment affords sellers to conceal and misrepresent reasons why the material they propose to sell may be tainted and harmful to recipients, advanced in a modern classic text opposing paid donation of blood for transfusion *(37)*, and indirectly advocating the moral and practical superiority of (UK) socialized medicine over (USA) market-directed health care. Rather, the argument is that commercialization through commodification damages important ethical values in that it raises functional utility over inherent human worth, invites competitive bidding for superior over inferior products, in the case of gametes and embryos, perhaps because of offensive distinctions in genetic pedigree and racial or ethnic properties, and imposes a monetary tariff on all means by which children are conceived and born. That is, a man's loving act by which his wife conceives their child becomes reduced to his transfer of sperm of a given

market value, and her gestation becomes a service, even when unpaid, that is known to be commercially marketable at an employment rate per month or lesser period.

This impoverishes the quality of human and family life, because it devalues and impersonalizes a profound act of personal commitment and dedication. The social fracture in relationships is comparable to that done by a guest invited to a friend's home for dinner who strips the invitation of its personal character by equating enjoyment of the company and the meal to a restaurant service, and expresses appreciation by placing the assessed money value on the table in cash. A more obvious analogy may be in equating reproduction to prostitution. This description is now often redeemed or mitigated, acknowledging the vulnerability and oppression that direct young persons into this occupation, by being termed "commercial sex work", but its original description implies shameful and immoral debasement, or sacrifice of self-respect for financial gain.

This analogy contributes to another pragmatic reason to oppose commerce in human gametes and embryos; that it would be liable to be exploitive of those vulnerable through poverty who have no other means of earning. Gamete selling is more oppressive of women than is sperm selling of men, since ova recovery, perhaps following superovulation induced by hormonal or other drug treatment, would be considerably more physically invasive and uncomfortable or risk-laden. Similarly, experience shows that infertile couples may be induced to trade a number of their cryopreserved embryos created *in vitro* in exchange for a further treatment cycle, when they cannot afford its financial costs. This payment in kind, in exchange for services rendered, would not be asked or invited of couples that request further treatment on a regular fee-for-service basis.

Ethical arguments allowing commercialization

Few arguments urge commerce or trafficking in human gametes or embryos as positively desirable in itself *(38,39)*, and some who find payments defensible recognize that there is something unsavoury in individuals selling their gametes *(40)*. However, many find that exchange for value may be tolerable, and analogous to practices societies have already accepted. Invoking the ethical principle of justice, that like cases be treated alike, they equate giving and receiving commercial rewards for rendering the service of donation with other payments for products and services that are reputable and tolerated in materialistic and capitalistic or market-based economies. They find contradiction and even hypocrisy in social tolerance and sometimes admiration of some forms of commerce in the overcoming of infertility that accompanies condemnation of giving and receiving commercial rewards for supply of the gametes and embryos that may make treatment possible. For instance, medical practitioners earn professional fees or salaries for their services *(41)*, infertility clinics organize diagnosis and treatment on a for-profit basis, particularly since publicly-funded health services tend not to cover ART services adequately or at all, in some countries sperm banks provide samples for payment, laboratories charge for testing gametes, genetic and other counsellors earn livelihoods by their availability and, for instance, drug companies and equipment manufacturers sell their products for care of infertile patients. The demand or expectation that only those who supply their own sperm, ova or embryos for the same purpose should be altruistic, appears unjust.

Even where the admonition of Richard Titmuss against commercial purchase of blood for transfusion *(37)* is taken seriously, laws often allow payment for whole blood or plasma donation, as an exception from their general prohibition of commerce in human tissues, on pragmatic grounds. The social need for an adequate supply of transfusible blood and blood products overwhelms objections of principle to commercial transactions. The physical dangers to which people are exposed from infertility are less than those posed by loss of blood and by anaemia, but where the claims of infertile patients to have children are respected, commercial incentives to donation, where necessary, may be ethically tolerable. Accordingly, the UK Human Fertilisation and Embryology Authority (HFEA) has suspended its plan to prohibit payment to sperm and ova donors of a modest fee and reasonable expenses *(42)*. Allowance may serve the ethical goal of beneficence, and the burden may fall on those who argue that, on the contrary, commercialization violates the ethic of nonmaleficence, that is the ethic to do no harm, to make their case persuasively. In utilitarian terms, they must show that the harm of society enduring relievable childlessness, and imposing it on those who seek to have children, is less than the harms that would arise from commercial transactions in human gametes and embryos.

The case that would-be sellers might suppress information that would expose their genetic material

and embryos as unsuitable for use is considerably weakened where modern means of genetic diagnosis are available, since they make reliance on the proposed seller's disclosure of personal and family history less necessary. More persuasive may be the claim that payment would induce poor people to undertake what people of means refrain from doing, that is, to make their genetic material and embryos available to strangers. The special emotional burden of donation of extra embryos created in infertility treatment is that the gamete donors may remain childless, while knowing or suspecting that a strange couple have borne and are rearing their child. The risk that impoverished people will become liable to exploitation arises from many sources. These include experience in tissue donation, for instance, when four poor Turkish workers were paid to fly to a London hospital for removal of kidneys for transplantation into wealthy recipients, in documentation of eye and kidney sales in the Republic of Korea under recession *(43)*, and in surrogate motherhood transactions when there are significant wealth differences between commissioning couples and gestational mothers, raising concerns about "how such practices might further oppress poor and disadvantaged women" *(44)*.

Against this, however, it is argued that in order to sustain prohibitions of apparently exploitive practices on ethical grounds, "we need better reasons than our own feelings of disgust" *(45)*. "Protecting" willing, intellectually competent vendors of their gametes and embryos against "exploitation" may disrespectfully deny them their ethical claim to autonomy, and hold them within a paternalistic confine that is itself an oppressive exercise of power over less powerful members of society. They may consider such a sale to be the best option open to them, so that their position is worsened when the option is removed.

The argument that poor people cannot exercise intelligent choice, such as the choice of a healthy, fertile woman to donate ova or of a healthy, athletic man to undertake professional high-risk contact sport such as boxing, is patronizing and insulting. The argument that their choice is not freely made because of the pressure of poverty scarcely provides an ethical justification for further denying their choice. The claim that their choice may not be adequately informed, for instance, because they have not been able to consider or gain access to feasible options, provides a basis for affording them additional, realistic information or opportunities rather than denying them the choice of acting on the information they possess. The objection that ovum sales may involve women in medically unnecessary, invasive and risk-bearing treatments has substance, but the procedures are the same for commercial as for altruistic donors, and although the latter may be willing so to serve only for family members and friends rather than for strangers, the exchange of money does not itself affect the nature of the procedures, and should not affect the care offered by those who counsel or conduct them.

The objection that commercialization of donation unfairly attracts poor people to serve as vendors, and unfairly privileges rich people as purchasers, may be factually correct. However, this does not distinguish gamete and embryo sales from the attraction poor people may feel to sell their labour in low-paying, unpleasant or above-average risk employment, or from the capacity of rich people to purchase superior consumer products and services, including private health care. Where legal prohibitions exclude the capacity of affluent people to purchase the products and services they desire in their jurisdictions of residence, they are allowed to seek them elsewhere, including as "reproductive tourists". In any event, the unjust privileges available to people of means do not provide ethical grounds to deny poor people the opportunity to obtain benefits as they perceive them.

An ethical middle ground—regulation

Even where gametes themselves cannot legally be sold or purchased, donors often receive payments that may not be unlawful. Prohibition of commercial commodification of gametes has not prevented payments from being made to donors, not for their genetic material itself but for the service of making it available. That is, they receive payment not in a commodity transaction but under a service transaction. Men are not paid, for instance, for the genetic properties or volume of their ejaculate, but for the service of offering its availability. In principle, they should receive the scheduled payment even if their sperm are found on analysis to be unsuitable for use in reproduction due, for instance, to a genetic deficiency or viral infection. In the same way that health care professionals are ethically entitled to charge conscionable fees for their services, gamete and embryo donors may claim that it is not unlawful or unethical that they should receive payments that are proportionate to their inconvenience in donation. For instance, in the UK, the Human Organ Transplants Act 1989 provides in section 1 *(1)* that a person commits an offence if (s)he "makes or

receives any payment for the supply of, or for an offer to supply, an organ", but section 1 *(3)* states that "payment" means "payment in money or money's worth but does not include any payment for defraying or reimbursing … (b) any expenses or loss of earnings incurred by a person so far as reasonably and directly attributable to his supplying an organ from his body" *(46)*. Organs cannot be traded, but those supplying them can recover the reasonable costs of that service. Ethical concern that it is inconsistent to allow payment for the service of donation but not for the donated product may be addressed, in part, by recognition that service costs are more measurable in equitable market terms, and less open to the charge of people turning their bodies into "things".

Rates for the supply of gametes and embryos could be independently set or approved under regulations of an appropriate public or publicly accountable agency. This would unlink buying from selling, preclude private barter, and prevent wealthier patients from outbidding less wealthy applicants for infertility treatment. Payments could be made by an independent agency rather than by, for instance, a for-profit clinic, and donations be allocated among clinics according to an equitable formula. This would address an ethical objection to commodification of gametes and embryos, namely, that it unfairly privileges the wealthy through their superior means of purchase.

Both banning commerce in gametes and embryos and permitting their availability according to market principles are ethically problematic. Bans risk exclusion of legitimate benefits, and injustice in light of what else societies permit to be traded, and free operation of market forces risks indignity and indefensible exploitation. In principle, markets may be believed to solve problems of inadequate and surplus supply and, for instance, of quality control, but these concepts seem inappropriate and offensive to common sentiment where human reproduction is concerned. Even in the USA, where supply of health services is widely believed best undertaken through private agencies, there are legal prohibitions of commerce in organs, children and, for instance, surrogate motherhood services *(47)*. The logical virtues of market discipline are subordinated to moral repugnance *(48)*. Nevertheless, the ethics committee of the American Society for Reproductive Medicine has recommended limiting payment to the last few years' "marketplace norm" of US$ 5000 per completed cycle for donated ova *(49)*.

Between the ethical hazards of a prohibited market and an entirely free market is the ethical preference of a regulated market. This is shown in the UK, where the HFEA monitors ART developments, licenses ART centres according to their capacities of equipment and personnel, enforces a Code of Practice, gathers relevant data and informs the public in general and prospective users of services in particular of where they may receive treatment and how successfully particular treatments, and treatment centres, work. The HFEA monitors research initiatives, storage and disposal of embryos, and compliance with legal requirements. The Authority also determines which payments are acceptable and which are not, deciding in 1998, for instance, that it is tolerable for a patient's *in vitro* fertilization (IVF) treatment to be subsidized in return for the donation of some of her ova *(50)*.

The HFEA's observance of the law has also cast illumination on "reproductive tourism". This is often discredited by association with sex tourism, the condemned practice of people, overwhelmingly men, going to usually poor foreign countries to have sexual encounters with local residents that are unlawful in their own countries, such as with legal minors. In 1997, the English Court of Appeal ruled that the HFEA correctly applied legislation of 1990 in denying a widow permission to be inseminated with sperm recovered without his consent from her comatose dying husband *(51)*. The Court noted, however, that the widow was entitled to seek lawful services in countries of the European Community that were unlawful in the UK, and she subsequently was successfully inseminated in Belgium. Accordingly, so-called reproductive tourism need not be regarded only as a devious way to avoid the restrictions of national laws, but may be an ethical means to achieve personal reproductive goals compatibly with the different standards of one's own country and of another where services are lawfully available. Instead of using the pejorative description of "reproductive tourism", with its implications of flawed morality or leisure-time triviality, it may pay ethical respect to those who seek to have children to employ a description such as resort to "transnational services".

Conflicts of interest

Conflict in reality or in appearance

In ethics as in law, conflicts of interest clearly arise when those who induce others to depend on their

integrity and good faith place their own interests above those of such dependants. Accountability for conflict of interest goes far beyond this, however, because it also arises when those in whom others are encouraged to trust are in a position to favour their own interests, whether or not they actually succumb to the temptation of self-interest. Practitioners in health care professions, on whose specialized knowledge and training lay people must necessarily depend for the services they feel they need, are almost invariably enmeshed in multiple functions and commitments that require an exercise of choice among options, some of which might appear more favourable to themselves than others, and some of which might appear less favourable to the interests of patients to whom they have conscientious duties. Conflict of interest arises not just from the actual prioritization of self-interest, but also from an appearance that self-interest might be indulged at the cost of a reliant patient. For many reproductive health care practitioners, in publicly funded facilities as well as in private, for-profit centres, conflict of interest created by the appearance of conflict of interest, is inescapable.

Conflict is more obvious in some cases, of course, than in others. Professional fee-splitting is considered conflictual because it risks dissipation of the practitioner's allegiance to the patient *(52)*. Practitioners who are also owners or financial shareholders in for-profit clinics, who advise clinic patients to take more costly or prolonged treatments than appear indicated, are vulnerable to the suspicion of conflict. So equally, however, are practitioners on fixed salaries in publicly funded services, who advise patients whose care would be costly of material resources and/or caregivers' time that their prospects of successful treatment are poor, and that they should reconcile themselves to clinical failure and perhaps pursue an alternative such as adoption. When practitioners serving fee-paying patients with the same medical characteristics advise them that further treatment is worthwhile because it may succeed, it may appear that the former practitioners are unethically serving goals of institutional economy, contrary to their patients' interests, that the latter practitioners are unethically profiteering or serving futile extravagance, at their patients' cost, or both.

Practitioners therefore need not be employed in for-profit clinics to fall under suspicion of being in a conflict of interest. Private clinics that genuinely can present themselves as non-profit institutions, for instance, may pay staff members, who may also be proprietors, inflated salaries, and function to cover their costs, which are boosted by paying such salaries. Although these clinics may accordingly be non-profit, they may be sources of considerable personal enrichment to their practitioners.

Conflicts may appear in the options and advice that practitioners offer patients on preservation and disposal of their gametes and embryos. If clinics make profits from storage, or storage fees contribute to pay the costs of storage facilities, clinic personnel may have an apparent interest to recommend or offer preservation, reinforced by the incentive this may give donors to remain in treatment programmes. It has been reported, for instance, that a facility in New York charges $ 500 for three months' storage of embryos *(53)*. As against this, however, patients' compliance with requests or recommendations that patients should make surplus ova and/or embryos available for donation to other patients, may provide clinics with access to scarce materials through which treatments can be offered to additional patients, and with incentives to super-ovulate women patients in ways that may be contrary to their health interests and reproductive options. The HFEA in the UK accepted transfer of ova for fees or as part-payment in kind for infertility treatment late in 1998 *(50)*, and has now allowed similar donation of embryos *(42)*. Practitioners' interests in preserving and employing patients' gametes and embryos in these ways are not necessarily contrary to patients' interests, but opportunities for clinics' and practitioners' own advantage exist from which conflict may appear.

The definition of "infertility" and "genetic risk"

Particular difficulty arises from different, legitimate definitions of what constitutes infertility, and from what outcomes of natural reproduction present prospective children with genetic risks. In Canada, for instance, the Royal Commission on New Reproductive Technologies, following the practice of the World Health Organization, conservatively defined infertility as a failure to conceive following 24 months of normally frequent unprotected sexual intercourse *(29)*, whereas clinics often admit applicants on the basis of 12 months' failure. Clearly, more couples are infertile by a 12-month test than by a 24-month test. This raises the concern of whether clinics are being aggressively entrepreneurial and self-serving in admitting applicants of normal fertility or slight subfertility, claiming

credit for pregnancies during the following 12 months that occurred or would have occurred naturally, or even applying procedures that obstruct pregnancies that would have happened without their interventions.

Clinics may justify a 12-month test, however, on rational and compassionate grounds. Their clients, or patients, tend not to be young, newly married couples, but couples in which the female partners are approaching, at or a little beyond so-described advanced maternal age, meaning about 35 years of age or above. They may be in second or later marriages, perhaps having had children in earlier relationships but wanting to have families in their new marriages. When women's capacity to achieve pregnancy is in natural decline, clinics are reluctant to require that they wait a further year or more to become eligible for treatment. Further, with a rising risk of abnormality in a later-conceived child, particularly Down syndrome, delay in access to ART may be clinically contraindicated. Accordingly, clinics' apparent haste in admitting applicants to treatment on an assessment of their infertility may not be clinically suspect or unethical.

Assessments of genetic or dysgenic risks to future children that may induce couples to forgo natural reproduction and turn to gamete or embryo donation, or to IVF with their own gametes and preimplantation genetic diagnosis (PGD), may become more refined with advances in genetic understanding. However, questions are likely to remain of calculations of genetic risk, how prospective children's predispositions or susceptibilities to illness or injury due to genetic inheritance are explained to prospective parents, and what inherited conditions or abnormalities render a child's nonexistence preferable to its existence, in its own interests, those of its prospective parents or those of others such as existing children of the family. A background concern is the qualification a practitioner or counsellor has to undertake genetic counselling of ill-informed and perhaps apprehensive applicants for ART. Considerable room exists, by choice of language, emphasis, nuance, contrast or analogy, which may be deliberate or unconscious, to control or influence patients' decisions. Eugenic and aesthetic themes may infiltrate discussions, on practitioners' or counsellors' initiatives. Their preferences for children of particular stature, appearance and propensity can distort prospective parents' exercise of the choices that, ethically, they should be informed and empowered to make. Practitioners and genetic counsellors must show that they can be relied upon to be self-conscious of their own values and biases, and to exercise the self-restraint to suppress any tendencies to impose their own preferences that may be in conflict with those of their patients.

Resolution of conflicts of interest

In an idealized clinical setting for ART, conflicts of interest would be avoided. Although real settings are frequently far from ideal, the ethical principles of beneficence, nonmaleficence and perhaps justice compel practitioners' efforts to minimize the incidence and extent of conflicts. For instance, clinicians should not ask their patients to volunteer to be subjects of research studies of which they are the principal investigators, lest they unethically abuse their patients' dependency on them for their own interests *(54)*. Similarly, clinicians should not accept or be required to be gatekeepers of departmental or other collective resources on which treatment of their individual patients must draw, lest they may favour their patients to the disadvantage of colleagues' patients, or violate their ethical duty of allegiance by sacrificing their patients' interests to a perception of departmental, institutional or other extraneous priorities. As departmental or institutional gatekeepers, they are unethically compromised in discharge of duties owed to individual patients who rely on their disinterested judgment, clinical integrity and capacity for supportive advocacy of their interests. In many legal systems, these ethical responsibilities to patients are reinforced by the law.

Because conflicts of interest consist in appearance as well as reality, they are frequently inescapable. They may then be ethically resolved by due disclosure. Disclosures should be to those at risk of suffering disadvantage from a conflicted exercise of choice, or at least to a superior officer whose duty is to ensure ethical management of conflicts, and that those that consist in appearance do not evolve to consist in reality too; that is, that an apparent conflict is confined to the superficial level of mere appearance. In the doctor–patient setting, the doctor's conflict should in principle be disclosed to the patient. For instance, doctors with financial interests in the profits of drug companies whose products they are inclined to prescribe, or for instance, in clinical laboratories to which they propose to refer their patients for the testing of their biological samples, should so inform the patients, and provide them with alternative drug or laboratory options in which they are disinterested.

Physicians' interests in these regards are not necessarily unethical. They may be based on a genuine conviction that these companies or laboratories provide superior products and services or, for instance, on the conviction that, as interest-holders, the physicians can ensure maintenance or improvement of their products or standards. If these convictions are sincerely held, indeed, it may be unethical for a physician to seek to avoid the appearance of conflict of interest by prescribing inferior products or referring patients to inferior services; disclosure may be the ethical ideal for patients' informed choice.

It has been seen that a conflict arises when a person who wants therapeutic care from a clinician is asked by that clinician, or by a colleague on his or her behalf, to consider entering a study that the clinician is proposing to conduct. The proposal requires that the person be clearly informed that treatment under the study is not intended primarily as therapy, and that, if the study design includes randomization between an unproven intervention and a placebo, it may include no proven medical treatment at all. Disclosure to the person seeking care is ethically necessary, but not sufficient, because those asking physicians for care often accept the so-called "therapeutic fallacy" that the medical treatment they are offered in research studies is intended for their personal well-being. Accordingly, proposed investigators must also submit their study designs, including details of how subjects are to be recruited and informed, to independent ethics review committees. These committees will address how adequately prospective subjects are informed that the studies are primarily intended to advance scientific knowledge rather than their personal therapy, and how capable such subjects are to decline involvement in studies and instead to obtain the therapy they seek.

A modern classic of unethically resolved conflict of interest arose in the much-discussed legal case of *Moore versus Regents of the University of California (55)*. A patient whose cells were found to have unusually valuable genetic properties was asked to provide additional tissues so that investigators, presenting themselves only as his therapists, could patent and trade in a cell line they biotechnologically developed from them. The Supreme Court of California dismissed his claims based on his property interest in his cells or the cell-line, but allowed it to proceed for his lack of informed consent and the investigators' breach of the fiduciary duty they owed him. This is the way courts may reinforce the ethical duty of more powerful parties not to benefit themselves at the cost of those they induce to depend on their superior knowledge *(56)*.

A particular conflict that may affect ART clinics is how they report and advertise their treatment outcome data. Independent monitoring systems, such as in Sweden, may provide the public with reliable data. Similarly, governmental agencies in, for instance, the UK and USA, require clinics to submit annual reports of their practices, including numbers of patients and conditions treated, procedures undertaken and results. The Centers for Disease Control and Prevention (CDC) in the USA *(57)*, and the HFEA in the UK *(58,59)* publish quite detailed aggregated annual data reports, and include warning that the data do not allow reliable comparisons among clinics, for instance, because they will have treated different types of patients with different severities of reproductive disorders. Nevertheless, the news media have at times publicized the data in the form of a table that ranks clinics in order of their performance, or, as the CDC report is entitled, their "success rates".

The conflict of interest, arising at both micro-ethical and macroethical levels, is that clinics can influence their success rates by the choice of patients they accept and how they treat them. They can achieve higher success rates by accepting only patients below certain age levels, who are more subfertile than infertile, and whose conditions afford greatest prospects of successful treatment. Clinics that, as a matter of social justice and commitment, or of research interest to advance care, accept patients who have less promise of success and who are more difficult to treat, are liable to appear lower in rankings of success. Clinics operated for profit, that promote their services by commercial advertisement, have an incentive to boost their competitive status by screening out applicants with poorer prospects of reproductive success, and admitting those of borderline infertility. Clinic success rates may be achieved at a loss of social equity in access to services.

A more immediate ethical concern is whether clinics recommend more traditional infertility treatments before recourse to ART, even when their use might compromise later ART, or whether ART will be first recommended when more traditional, less expensive procedures might succeed. Recommended care should be based on practitioners' clinical judgement directed to each patient's conscientiously assessed best interests. An incentive to achieve a clinic's financial success or an impressive publishable

success rate may present a practitioner with an unethical conflict of interest. Disclosure of the profit-seeking status and preferred practice of clinics to regulatory authorities, and indirectly or directly to prospective patients, may afford such patients desperate for reproductive success only limited means to exercise independent choice. Professional ethics and self-regulation have a significant role in monitoring the integrity of clinical practice and guarding the public and prospective patients against unethical practice.

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Section 6

National and international surveillance of assisted reproductive technologies and their outcomes

The Swedish experience of assisted reproductive technologies surveillance

KARL NYGREN

Introduction

In addition to the standard peer review mechanisms that exist in most countries, of scientific articles, seminars and national professional meetings, Sweden has established two permanent and independent systems to monitor assisted reproductive technology (ART) outcomes. Furthermore, several national ad hoc research projects have been conducted using information from these two national databases.

Annual clinical summary reports on cohort data covering treatment outcomes

The first child born after *in vitro* fertilization (IVF) treatment in Sweden was delivered in 1982. Since 1987, Swedish law has required all IVF clinics to submit annual summary reports on the results of treatment. These summaries report on cohort data covering treatments starting during a specific year and including the outcome of the resulting pregnancies. The data are usually available one year after the end of the treatment period. The reports are sent to the Centre for Epidemiology at the National Board of Health and Welfare. This Centre has expert statisticians and demographers who are responsible for the preparation of an annual national report, in consultation with one of the practising IVF doctors in Sweden who acts as a senior consultant to the Centre.

In 2001, for the first time, the report has been expanded to include trends in the data over the time period 1991–1997 *(1)*.

These annual reports have been widely used by the medical profession, by couples seeking information, and also by the general public, usually through the media. The reports have not explicitly included clinic-specific data, although by Swedish law such information must be available. The reason for avoiding the publication of individual clinical data in the reports is the awareness that the public would have great difficulties in interpreting these data and that the public could easily be misled. This position has been strengthened by negative experience in the UK and the USA where the publication of results from individual centres has resulted in some confusion.

The summaries from each clinic contain the following data:

- The number of IVF procedures, pregnancies and deliveries by ART procedure including standard IVF, intracytoplasmic sperm injection (ICSI), frozen and thawed cycles and unstimulated cycles;
- The number of cycles started, number of ovum aspirations and number of embryo transfers;
- Abortions and ectopic pregnancies, reported separately;

- Deliveries, reported as simplex, duplex, triplets or higher order;
- The number of caesarean sections;
- The number of liveborn children;
- The proportion of deliveries per cycle, per ovum aspiration and per embryo transfer;
- The number of IVF treatments, reported by ART procedure, the indications for treatment and the women's age, in five-year intervals;
- The number of embryos replaced per procedure, related to pregnancy and pregnancy outcome;
- The proportion of children who are born with a low birth weight, i.e. below 2500 g.

Before embarking on this relatively simple and inexpensive system of surveillance, the Swedish authorities discussed whether a system of direct reporting of individual cycle data would be preferable. It was decided, however, that such a system would be very expensive and would probably necessitate a separate authority. It was also felt that a validation system for the clinical annual summaries could be set up by cross-linkage to other reporting systems and registers already in operation in Sweden.

Therefore, this relatively simple approach works very reliably in Sweden and it is planned to continue with this system. Each year, the forms for reporting are reviewed and often revised. This depends on the dynamics of developments in ART. Changes have included data on the different types of ICSI that have been incorporated into clinical practice and the most recent change is the inclusion of information on elective one-embryo transfers.

Recent descriptions of time trends have shown an increasing number of ART procedures in Sweden as well as an increasing effectiveness reported as delivery rates per procedure. In addition, there is a declining multiple pregnancy rate. Ten years ago, triplets or higher-order pregnancies accounted for 5% of deliveries, whereas today it is close to zero; similarly, the twinning rate has gone down from approximately 28% to 22% over the same period (Figures 1–3).

Over recent years, the forms for reporting data have also been adjusted to the norms that have been adopted in Europe through the European Society for Human Reproduction and Embryology (ESHRE). Therefore, today, each clinic reports its data only once a year to the Centre of Epidemiology. Further, international reporting comes from the Centre itself. The burden on the clinics to report data is, therefore, minimal.

Surveillance of children born after IVF

A separate surveillance system has been established to collect data concerning the health of children born since ART procedures were introduced in Sweden *(2)*.

This system operates through separate reporting from all clinics on all women who have delivered one or more children after ART treatment. Each Swedish citizen has a unique ten-digit identification number (PIN) and the clinic report includes each woman's

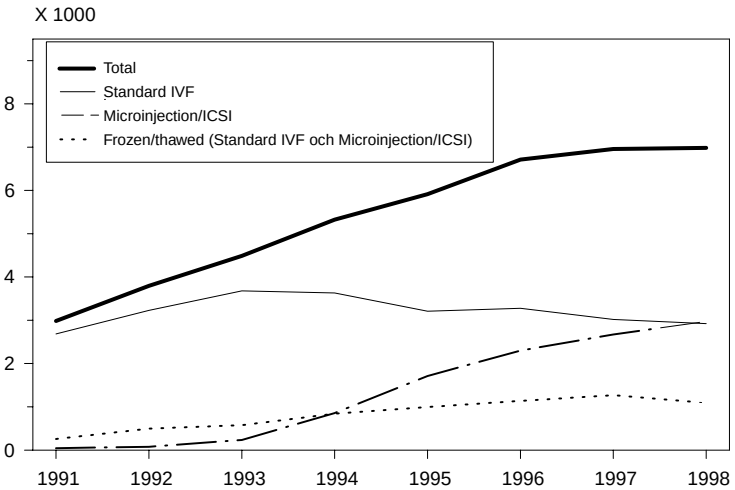

Figure 1. Number of embryo transfers, by ART procedure, 1991–1998

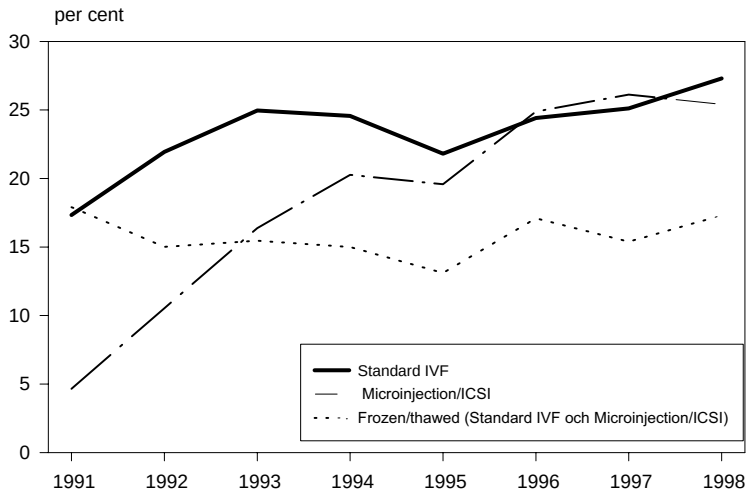

Figure 2. Number of deliveries per embryo transfer (per cent), by ART procedure, 1991–1998

PIN. The Centre for Epidemiology has developed a specific register covering all ART deliveries in Sweden and reports have been produced covering two time-periods, the first covering all ART children born between 1982 and 1995, and the second covering the years 1996–1997.

The ART delivery register is then cross-linked with the already existing Medical Birth Registry, the Cancer Registry and the Registry for Malformations. These also use the same PINs and, therefore, each woman can be identified in all registers. The PIN for the ART children is identified through the Medical Birth Registry.

The integrity of the individual's data in each registry is guaranteed and protected by law. Results from these registries may never be published showing an individual's data.

All deliveries in Sweden are reported to the Swedish Medical Birth Registry, which has been in existence since 1973. The registry contains information collected during pregnancy, delivery and the immediate postpartum period and is based on standard medical documents used in all Swedish antenatal care centres, delivery and neonatal units. The registry also

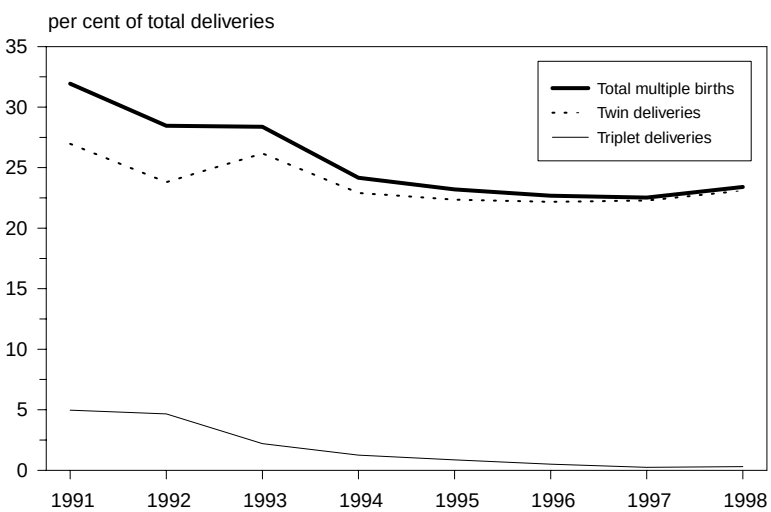

Figure 3. Multiple births, rate of all deliveries, 1991–1998

includes information about health and various social conditions before and during pregnancy. Medical data on the outcome of delivery and on neonates, such as birth weight, duration of pregnancy and diagnosis during the neonatal period, including malformations, are included. The quality of the registry has been assessed and shows that the drop-out frequency is very low.

The Swedish registers of congenital malformation and of cancer are also well established and the information from the first time period (1982–1995) includes approximately 6000 children born following an ART procedure. They were compared to all other children born during the same time-period, about 1.4 million children. These results have been published recently and constitute the largest follow-up of ART children published worldwide *(2)*. The main conclusion of the report was that the increased rate of multiple pregnancies, in this case mostly twins, carries with it an increased risk of prematurity and consequent increases in perinatal mortality and morbidity. The report did not find that the ART technique itself caused problems. The report concluded that the high rate of multiple pregnancies (including twins) must be reduced or eliminated.

The Centre of Epidemiology at the Swedish Board of Health and Welfare, together with the ART professionals, have decided to continue this follow-up of the entire ART population, at timely intervals, to accumulate even more powerful data, with the possibility of describing time trends at a later stage.

Additional ad hoc projects

In addition to the two permanent surveillance systems described above, a number of ad hoc projects have been undertaken.

One of these has been the collection of data on neurological disturbances in children born after ART treatment. Again, this report has included all ART children born compared to selected controls. The results show that the increased rate of prematurity creates a subsequent increase in the risk of cerebral palsy *(3)*. Again, the conclusion is that the ART technique itself does not cause these problems but that the increased rate of multiple pregnancies is to blame.

Another ad hoc national project covers the emotional and psychosocial development of ART children at the age of approximately 8–10 years. The results are yet to be published.

Conclusions

The two independent surveillance systems, one covering direct clinical results, the other covering follow-up of the health of IVF children, together give a valid and true picture of the situation in Sweden. The two systems will continue to operate but will be modified according to the dynamic situation in the area of ART.

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The Latin American Registry of Assisted Reproduction

FERNANDO ZEGERS-HOCHSCHILD

Introduction

Over the past few decades, remarkable progress has been made in modern reproductive technology. Latin America has not been untouched by these developments, and a serious and systematic effort has been made in the transfer of technology and its application in the developing countries of Latin America. In contrast to the introduction of new scientific developments, these countries have been less effective in generating laws to regulate reproductive health and, to date, no country in Latin America has adopted laws regulating the practice of modern reproductive technologies. In addition, access to fertility treatment has not been part of the agenda of national health authorities and, furthermore, private health insurance companies do not cover expenses related to fertility treatment. For many legislators, procedures such as *in vitro* fertilization and embryo transfer (IVF-ET), are considered morally unacceptable and others consider them to be a luxury that should not be supported with state funds. An extreme example of this is Costa Rica where the high court has recently banned IVF-ET, by considering it morally unacceptable *(1)*. These are some of the conditions contributing to restricted and inequitable access to modern reproductive technology in Latin America. Today, more than 90% of the centres in the area of assisted reproductive technology (ART) are private institutions which do not receive university or government support, and restrict access almost exclusively to couples who can pay the high costs involved. Assuming a prevalence of infertility of 10% in women aged between 20 and 40 years of age, 20% of whom will at some time require ART, and considering the number of ART cycles performed per country, the number of women with access to these therapies does not exceed 0.7%–2.8% of those who would theoretically benefit from these treatments *(2)*.

Since 1984, when the birth of an IVF-ET baby was first reported in Latin America *(3)*, ART has spread throughout the entire region. Given the lack of national regulatory bodies and the reluctance to deal with controversial issues, very little is known about the number and type of procedures performed by each country up to 1990, when a multinational registry of assisted reproduction was initiated. This activity, known as the *Registro Latinoamericano de Reproducción Asistida (RLA)* has been published annually since 1992 (reporting cycles initiated during 1990 and births taking place up to 1991). Today, it covers more than 90% of initiated cycles in centres located from Mexico in the north to Chile in the south *(4)*.

From the very start, centres voluntarily agreed to adhere to a registration protocol and to provide their data to the registry, with the understanding that the results of individual clinics or countries would not be disclosed. Results were to be reported and published

as summaries for Latin America as a whole, with pregnancy rates according to diagnostic category, age of female partner, etc. The outcome of clinical pregnancies was also recorded together with gestational age at delivery and perinatal mortality rates.

The Latin American Registry was initiated with three objectives in mind: first, to create an educational tool that, together with health professionals, would allow couples to evaluate the costs and benefits of ART procedures; second, to develop a comprehensive, regional database to serve as an external reference for each centre's self-evaluation; and finally, to have a robust database, allowing for epidemiological research to be conducted.

When the Registry started, very few centres had protocols for data collection, only one-third had computerized registration and there was little experience in participating in multicentre trials. Over the years, the Registry has served as an educational instrument, contributing in the development of ART in centres that otherwise would have never been involved in data registration and research.

After four years of continuous publication of the results from the Registry, it was decided that the time had come to undertake further tasks. It was then that the "Red Latinoamericana de Reproducción Asistida" (Latin American Network of Assisted Reproduction) was born. Latin America was divided in five subregions and a board of regional directors was elected to represent and coordinate subregional activities. Apart from continuing the Registry, the Latin American Network has focused most of its efforts on education and multinational research. The major effort has been in the area of quality control of IVF laboratories, training health professionals and the transfer of new technology. A continuous accreditation process has also been established, in which standard conditions are required for centres to participate in the Network and to have their results published in the Registry. The accreditation process is carried out by teams, consisting of a biologist and a clinician, belonging to centres located in a different subregion. The centre that is being evaluated voluntarily agrees to open its records to the accreditation team, which checks for consistencies between the data reported in the Registry and the actual files and laboratory data. The referees also check for the existence of: quality control protocols for laboratory facilities, air purity, culture media, incubators, etc.; clinical and laboratory procedures such as ovulation induction, ovum pick-up, fertilization and preimplantation development and manipulation, etc.; the follow-up of pregnancies; signed consent forms, and consistencies in the results reported to the Registry. The centre needs to report a minimum of 20 cycles per year and the results must be at least one standard deviation below the mean for Latin America.

Methodology

Selection of centres

During the first five years, the policy was to allow as many centres as possible to be part of the Registry. It was assumed that, because individual results were not disclosed, centres would see no benefit in manipulating their results. However, after some time, it was obvious that inconsistencies found in the data provided by a few centres were not only the result of involuntary errors; some of these centres were in fact manipulating their results to fit the computer program developed to check for inconsistencies. Some centres were consistently under the minimum efficiency required and were not participating in the educational activities developed by the Network. These were the two major reasons why eight out of 101 centres were excluded from the Registry in 1999. Presently, once a new centre expresses its willingness to be part of the Network, it receives the computer software and submits its results which are checked for consistencies. It is then visited by an accreditation team and, if accepted, the centre is included as part of the Registry and the Network. When the Registry started in 1990, 19 centres in eight countries reported 2460 cycles, in 1999, 93 centres in eleven countries initiated more than 14 763 cycles.

Type of software and distribution

The computer program is sent to the participating centres in the form of three 3.5" floppy disks, or via email. Each centre is given a security code according to the country of origin and name of the centre. The data produced by each centre are entered in tables which have an inbuilt validation system and once all the information has been completed, the centre generates a file which is sent to the central office. The Registry central office double-checks for inconsistencies before the data are included in the report.

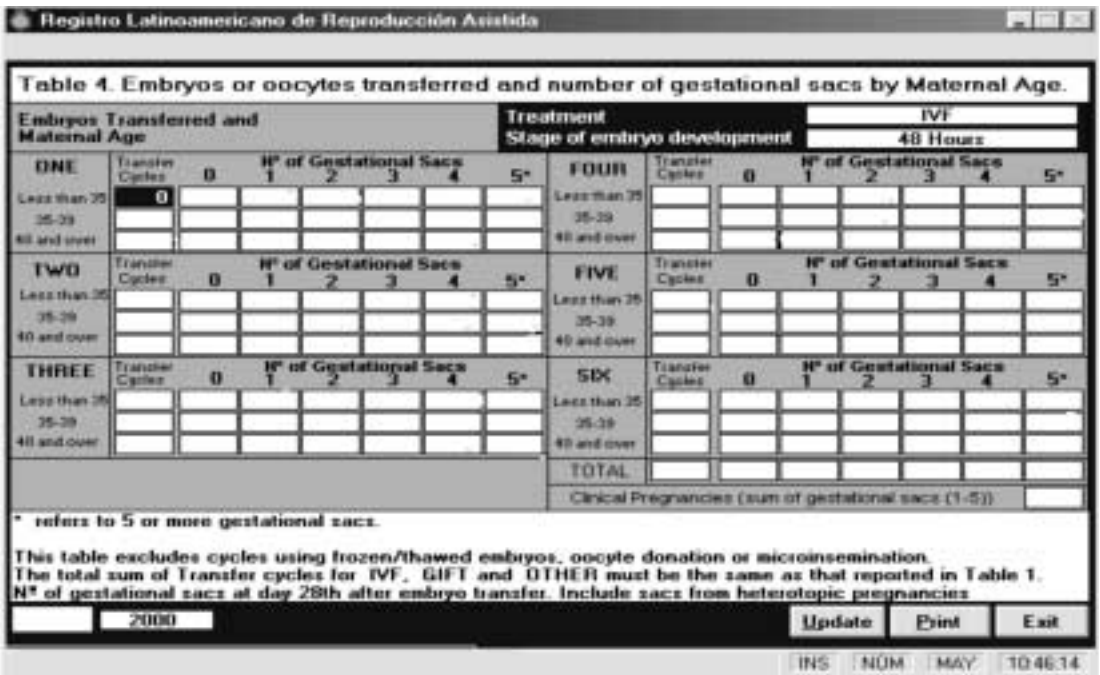

Figure 1

Type of information produced

The following information is obtained for each ART procedure:

- Number of initiated cycles, follicular aspirations and transfer cycles;
- Pregnancy, delivery and live-birth rates by follicular aspiration and by transfer cycles;
- Pregnancy, implantation and multiple gestation rates according to the age of the female partner, and/or number of transferred embryos, and/or stage of development at the time of ET;
- Outcome of clinical pregnancies, including spontaneous and induced abortion, ectopic pregnancies, stillbirths, live births and early neonatal deaths;
- Gestational age at delivery, birth weight, major malformations and cytogenetic analysis of abortion material (where available).

Figure 1 provides an example of the forms used for data recording by the clinics for pregnancy, implantation and multiple gestation rates, and Figure 2 is an example of the forms used for data recording on outcome of clinical pregnancies, birth weights, etc.

Surveillance system

There are two levels of surveillance. One is inbuilt in the computer software and includes a program which checks for inconsistencies. It does not allow the operator to continue with the program if there is inconsistency between the numerical information entered in a certain table and the numerical information available in previous tables. The program advises the operator on the source of the inconsistency. If the operator wishes to continue running the program, he needs to correct the conflicting figures. The question arises as to whether this "correction" of numbers does in fact reflect the truth. The second level of surveillance checks the consistency in the centre, between the reported numbers and actual numbers that should be present in the patients' files and/or in the specific protocols for clinical and laboratory procedures performed by each centre. These data are checked by the accreditation teams. This process was conducted in all clinics between December 1999 and March 2000 and it is expected that every participating clinic will be reassessed every three years.

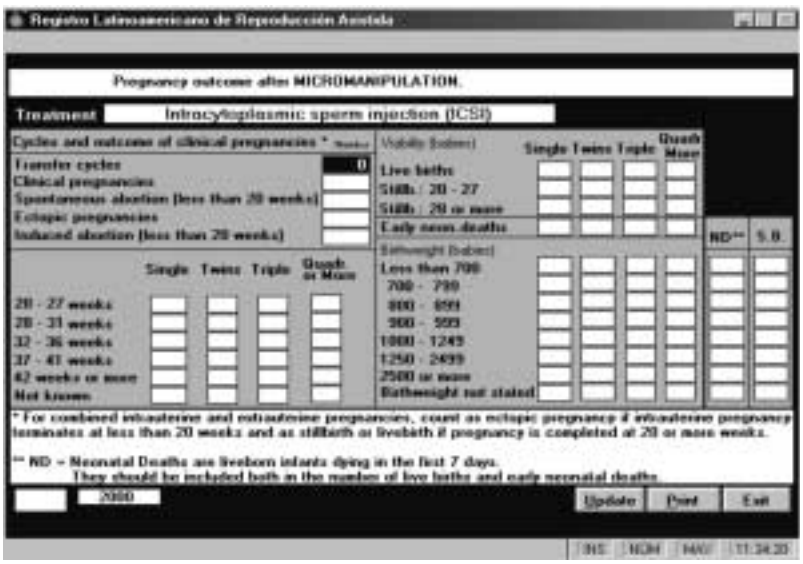

Figure 2

Results

The Registry is an educational tool for both couples and centres. To inform infertile couples, each centre should use their own data together with an external registry. In this way, couples or individuals can examine their chances of success and, at the same time, evaluate the relative performance of that centre in relation with other centres or with a region as an external reference.

The way the data are processed in the RLA makes it possible to address some of the questions that arise routinely when examining costs and benefits of ART procedures. Much of this information should be part of the documents used to prepare couples for a particular therapy. The following are examples of frequently asked questions from infertile couples:

> *I am 40 years old. What are the chances of becoming pregnant transferring only two or three embryos?* The answer can be found in Figure 3.

Indeed, this question can also be addressed as the chances of delivering a normal child. It is also possible to stratify the answer according to the day of ET.

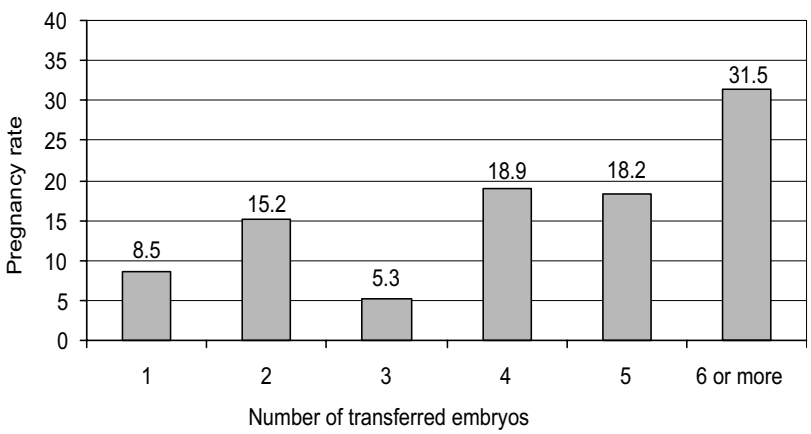

Figure 3. Clinical pregnancy rate according to the number of transferred embryos in women ≥40 years of age (IVF). (Source: 1998 Latin American Registry)

Table 1. Multiple gestation according to the age of the female partner and the number of transferred embryos (IVF)

Number of embryos transferred	Age (years)	Number of pregnancies	Overall multiple gestation (%)	High-order multiple gestation (%)
1	<35	27	7.4	0.0
	35–39	22	4.5	0.0
	>39	9	11.1	0.0
	Sub-total	**58**	**6.9**	**0.0**
2	<35	55	10.9	0.0
	35–39	41	24.4	0.0
	>39	15	6.7	0.0
	Sub-total	**111**	**15.3**	**0.0**
3	<35	143	31.5	4.9
	35–39	75	16.0	2.7
	>39	4	0.0	0.0
	Sub-total	**222**	**25.7**	**4.1**
4	<35	216	38.0	14.8
	35–39	114	26.3	7.0
	>39	17	23.5	7.9
	Sub-total	**347**	**33.4**	**11.8**
5	<35	73	53.4	20.5
	35–39	75	30.7	8.0
	>39	10	40.0	0.0
	Sub-total	**158**	**41.8**	**13.3**
6	<35	36	27.8	11.1
	35–39	30	26.7	10.0
	>39	11	36.4	27.3
	Sub-total	**77**	**28.6**	**13.0**
	Total	**973**	**29.0**	**8.3**

Source: 1998 Latin American Registry

I am 32 years old. What are my chances of having a multiple pregnancy if you transfer three embryos? The answer can be found in Table 1.

This question can also address the chances of having a triplet or a twin pregnancy.

If I get pregnant, what are my chances of delivering a normal child? The answer can be found in the outcome of pregnancy (Table 2) and the risk of major malformations (Table 3).

The Registry can be used as a database for epidemiological studies. Although the forms used in the Latin American Registry have changed in the past ten years, the main body of information has remained unchanged, allowing for longitudinal analyses.

Table 2. Outcome of clinical pregnancies

	IVF	ICSI	OD (fresh)
Clinical pregnancy	973	1124	333
Spontaneous abortion	18.5%	20.9%	18.6%
Ectopic pregnancy	2.6%	1.2%	2.1%
Stillbirth	1.0%	1.3%	0.9%
Deliveries with ≥1 live birth	77.9%	76.6%	78.4%

Source: 1998 Latin American Registry

Figure 4 shows how ART in Latin America has grown over the years. Between 1990 and 1998, 27 859 transfer cycles produced 6952 clinical pregnancies, 5239 deliveries with live births and 6480 live births. In 1990, 21 centres produced almost 2500 cycles. In 1998, 84 centres produced 12 274 cycles. In terms of the number of initiated cycles, the major contributors are Brazil (42.9%), Argentina (22.7%), Mexico (11.1%), Colombia (5.9%) and Chile (5.7%).

Figure 5 shows how the age of the female partner has changed. In 1990, 65.6% of the population was under 35 and only 8.7% were 40 years or more in age. In 1998, only 50% were under 35 and the proportion of women aged 40 years of age or more increased to 14%. The age median increased from 33 in 1990 to 35 in 1998. These factors need to be considered when performing longitudinal analyses on pregnancy and delivery rates in the region.

Figure 6 shows that, in spite of the increase in the women's age, between 1990 and 1998, the clinical pregnancy rates and delivery rates increased significantly. The years in which the changes were more dramatic coincided with the initiation of continuous education programmes by the Network (1995–1996).

Figure 7 shows how technology transfer has changed over the years. It took five years from the birth of Louise Brown in Great Britain to the birth of the first IVF baby in Latin America, but it took only

Table 3. Malformation rate in ART

Year	Number of live births observed	Malformation %
1996	1711	0.7
1997	1343	1.2
1998	807	2.0

Source: 1998 Latin American Registry

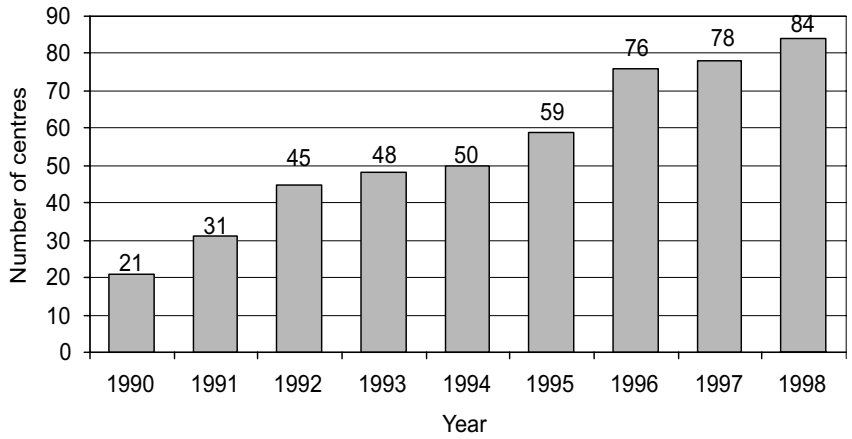

Figure 4. Centres reporting data

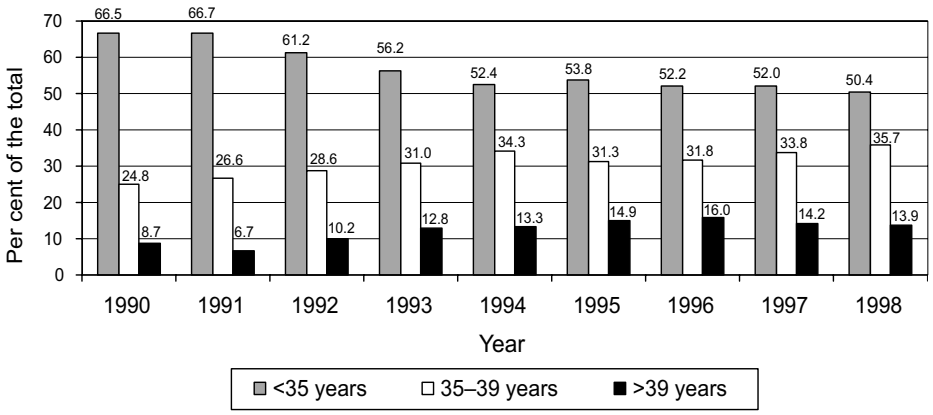

Figure 5. Age of women receiving IVF

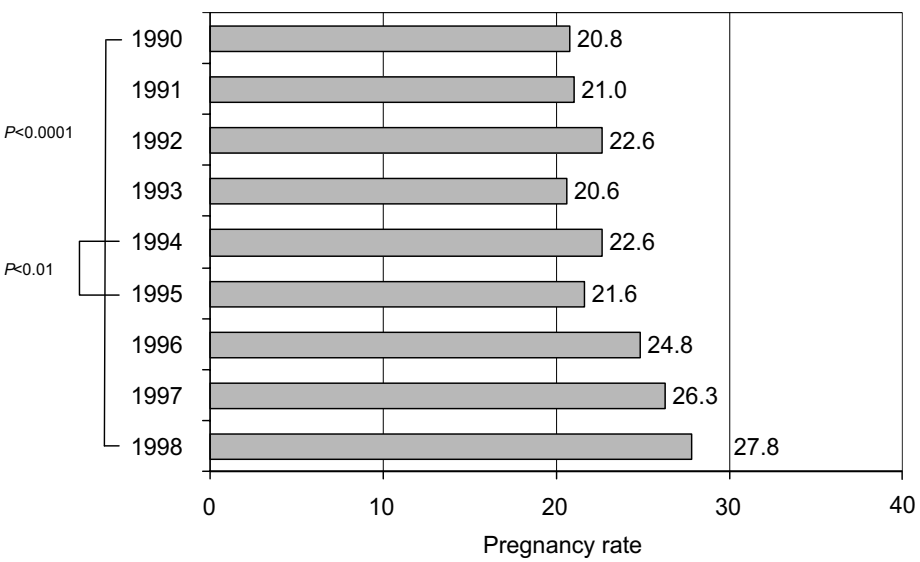

Figure 6. (A) Pregnancy rate per transfer in IVF

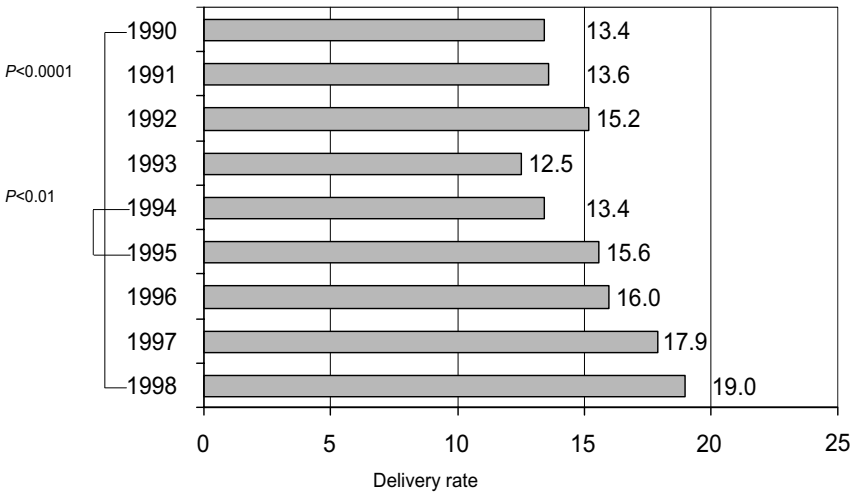

Figure 6. (B) Delivery rate per transfer in IVF

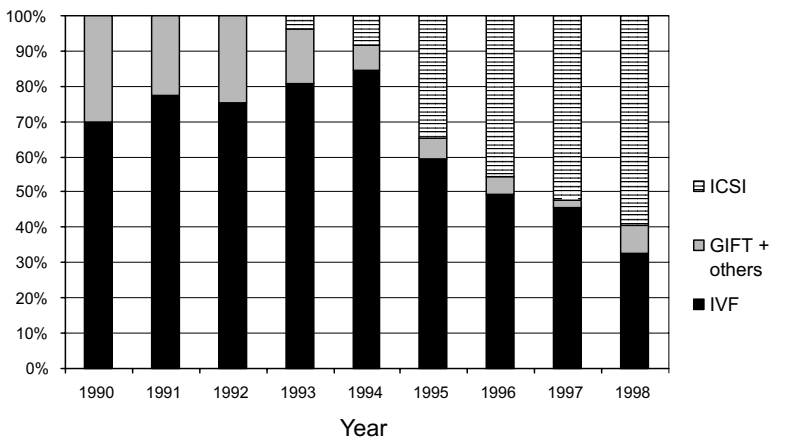

Figure 7. Percentage of the total number of attempted oocyte retrievals according to procedure

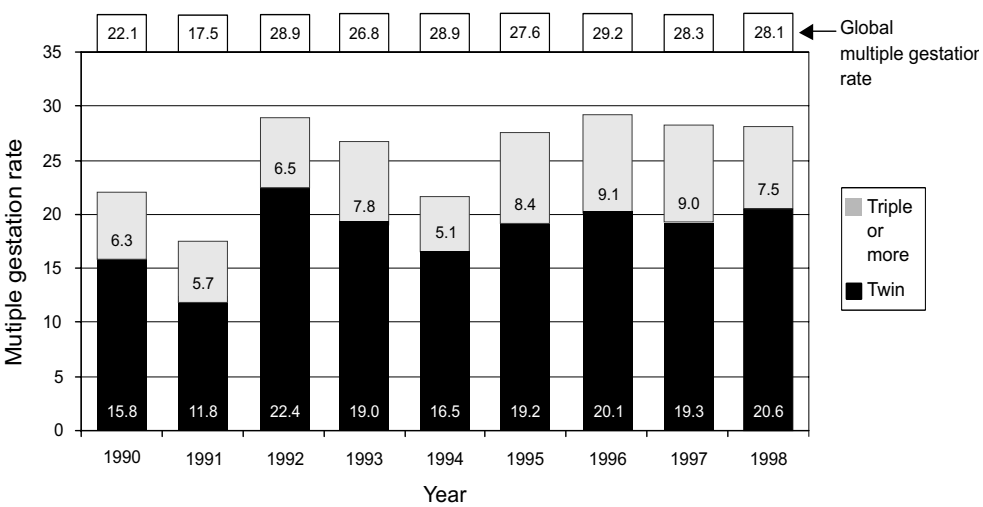

Figure 8. Multiple gestation rate in ART

Table 4. Perinatal outcome according to the order of gestation (IVF + ICSI)

	Single		Double		Triple		≥QUAD	
	n	%	*n*	%	*n*	%	*n*	%
Total births	1172	100	707	100	277	100	52	100
Stillbirths	13	1.1	17	2.4	27	9.7	4	7.7
Live births	1159	98.9	690	97.6	250	90.3	48	92.3
Neonatal deaths	8	0.7	11	1.6	6	2.2	5	9.6

Source: 1998 Latin American Registry

one year to transfer the technology of ICSI from Europe to Latin America. Today, ICSI is performed by 88.2% of centres and represents almost 67% of the ART procedures undertaken in Latin America. Although this technique has opened the possibility of becoming a parent to otherwise azoospermic or severely oligozoospermic men, it is surprising how much ICSI has spread, considering that male factors do not appear to be increasing in the region.

Multiple gestation deserves special attention, considering that selective fetal reduction and therapeutic termination of pregnancy are illegal procedures in Latin American countries. Over the past four years, the average number of embryos transferred has been 3.2 and the rate of multiple gestation has remained above 28%, which is unacceptably high. However, what gives most cause for concern is the proportion of higher-order multiple gestations (triplets and more) (Figure 8).

Table 1 shows the overall and higher-order multiple gestation rates according to the number of embryos transferred and the woman's age. The rate of multiple gestation in women under 35 years of age is similar when three or when four embryos are transferred (31.5% and 38%, respectively). However, the rate for triplets and higher-order pregnancies increases from 4.9% to 14.8% for three and four transferred embryos, respectively, and reached 20.5% when five embryos are transferred. The high rates of stillbirth and perinatal mortality are strongly related to low birth weight associated with multiple gestation (Table 4).

Conclusions

One of the great achievements of the Latin American Registry and the Latin American Network of Assisted Reproduction has been to provide scientists and clinicians responsible for ART centres with a common objective. This started with the Registry and later evolved into continuous education and multinational research. The Registry has provided centres with a recording system which, using a common language to register results and compare success rates, allows for better and more fluent interaction between professionals in the region. Furthermore, it has provided infertile couples with an educational instrument that, when used properly, helps in their decision-making process.

Different from the recently formed European registry, which is mostly a scientific compilation of national registries, the Registry consists of individual centres located in twelve countries. In fact, the few national registries in the region (Argentina, Brazil, Chile and Mexico), have been created from information provided by the Registry. In Latin America, there are no national bodies responsible for regulating ART. Therefore, without these regulatory bodies, all the surveillance programmes, the transfer of technology and voluntary regulations have been generated by the scientific community associated with the Latin American Network of Assisted Reproduction. The activities of the ART community in Latin America can be found on the web site www.redlara.com.

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Assessment of outcomes for assisted reproductive technology: overview of issues and the US experience in establishing a surveillance system

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Introduction

Every year, infertility affects millions of couples who desire children. In the USA, the latest population-based estimates of infertility are derived from the 1995 National Survey of Family Growth *(1)*. The estimates vary, depending on the question asked, but each suggest a substantial disease burden. Among women of reproductive age, 10% of those surveyed (representing 6.1 million women) reported they had difficulty conceiving or carrying a pregnancy to term, 2% (representing 1.2 million women) reported they had an infertility health care visit in the past year and 15% (representing 9.3 million women) reported they had an infertility visit at some point in the past. Infertility visits were defined to include medical advice, diagnostic tests, drugs, surgery or other treatments. Among married couples in which the woman was of reproductive age, 7% (representing 2.1 million couples) reported they had not conceived after twelve or more months of unprotected intercourse.

In the USA, as elsewhere, couples have increasingly turned to technological advances such as assisted reproductive technology (ART) procedures to overcome their infertility. The first infant conceived with ART in the USA was born in 1981. In 1998, more than 80 000 ART procedures were performed in the USA, and more than 28 000 infants were born as a result of these procedures *(2)*.

As the use of ART increased throughout the 1980s, there was a growing concern over the information infertility patients were receiving. Many clinics offering ART began tabulating and reporting their success rates, both directly to patients and sometimes through paid advertisements. These rates used various definitions for numerators and denominators, resulting in confusing and often exaggerated depictions of clinics' successes. In response to this concern, Congress enacted the Fertility Clinic Success Rate and Certification Act (FCSRCA) in 1992, mandating that all ART clinics report success rate data to the government in a standardized fashion *(3)*. ART was defined to include all fertility treatments in which both ovum and sperm are handled. Thus assisted insemination (also known as intrauterine insemination [IUI] or artificial insemination) is not considered ART in the USA surveillance system; nor are treatments in which ovarian stimulation medications are given but there is no intention of retrieving ova. The Centers for Disease Control and Prevention (CDC) was charged with implementing FCSRCA and it published the first report under the law in 1997 *(4)*. That report was based on ART procedures initiated in 1995. The medical professional organizations, the American Society for Reproductive Medicine (ASRM) and the Society for Assisted Reproductive Technology (SART) supported this legislation and along with the patient advocacy association, RESOLVE, they have support-

ed CDC's activities in implementing the law.

In this report, methodological issues in defining ART success rates and collecting success rate data are discussed, the USA programme for reporting ART success rates is described, lessons learned are reviewed and future directions are discussed.

Defining ART success rates

How do you count a success?

In defining and reporting success rates, the first issue to consider is the numerator. Although seemingly straightforward, many options exist for defining success. These can be considered sequentially from positive pregnancy test to healthy, thriving child. Each option must be considered in light of both the relevance to the intended audience (consumer, scientist, physician, policy-maker) and the feasibility of accurately collecting the data.

At a fundamental level, the goal of ART is to overcome a couple's infertility and achieve pregnancy. To the ART physician, then, a reasonable measurement of success is pregnancy. To the couple undergoing the ART procedure, the goal is not just pregnancy, but the birth of a healthy child. This is not to suggest that physicians do not also desire that their patients have a live birth of a healthy child. Rather, from a scientific and clinical standpoint, the ART physician intervenes to bring about the fertilization of oocytes and subsequent implantation of embryos. Thus, when assessing medical practice and advances in ART technology, it is appropriate and even preferable at times to define success in terms of the most proximate outcome—pregnancy. Indeed for many physicians and laboratory personnel, when developing and testing new *in vitro* culture techniques and treatment protocols, an even more explicit evaluation is often favoured, such as assessing the percentage of ova that were successfully fertilized (i.e. fertilization rate) or assessing the number of embryos that successfully implanted among all those that were transferred into the uterus (i.e. implantation rate). Such measures provide useful information that cannot be gleaned by merely calculating the live-birth rate, a composite measure that encompasses factors not only related to successful fertilization and implantation, but also to factors associated with the maintenance of pregnancy.

The numerator of any success rate, then, must be consistent with the objective the success rate indicator is intended to serve. If the objective is to compare differing treatment approaches, the more proximate outcome, pregnancy, gives a better indication of the direct treatment effect and is thus preferable. Concurrent evaluation of the more distal outcome, live birth, might also be useful in such assessments, as this measure may provide insight into additional indirect effects of the treatment. If the objective in defining a success rate is to present scientific data to prospective patients such that they can make an informed choice about whether to undergo ART treatment, the best single measure is the more distal outcome, live birth. This measure is closer to the patient's goal of becoming a parent, rather than just achieving pregnancy.

The complexity of defining the numerator extends beyond the decision as to whether the outcome of choice for a given objective or audience is pregnancy or live birth. Within each of these broad categories, further definition is desirable to ensure comparability across rates. For the purposes of ART, pregnancy is most reasonably defined as the presence of a gestational sac, visible sonographically, in the uterus. This reflects evidence of implantation and coincides with the time a pregnancy becomes recognizable clinically. This definition would exclude apparent "chemical" pregnancies (i.e. transient increases in human chorionic gonadotrophin [hCG] in the woman's serum without evidence of a gestational sac). Live birth is typically defined as the birth of one or more infants that show signs of life. This definition is acceptable, but does not disentangle the infant's health status at birth. Rather, both singleton and multiple-birth deliveries are included in the definition and normal-birth-weight, healthy infants born at term with a high probability of surviving the infant period are not distinguished from infants born with morbidities that put them at risk for infant death. The ability to subdivide live-birth deliveries into more meaningful subgroups is hampered by the difficulties in collecting data on ART outcomes.

As the numerator moves along the continuum from pregnancy to healthy child, it becomes increasingly difficult to collect complete and accurate data at the population level. The paramount barrier is that in many countries, including the USA, medical care is often not coordinated among infertility, obstetric and pediatric providers. In the USA, the responsibility for reporting ART success-rate data rests with the infertility clinic that provided the ART treatment. These clinics do not typically provide care for patients

beyond the first trimester of pregnancy. Thus clinic personnel must work to track patients once they are released from the clinic's care to ascertain the outcome of each pregnancy. The scope of this follow-up is limited by the feasibility of collecting various medical data. In the USA, data collection extends only to the point of birth and is limited to data that can be reliably collected from either parents or obstetric providers, such as birth occurrence, number of infants liveborn, date of birth and birth weight(s). Information on birth defects and other anomalies present at or shortly after birth, other medical complications and health conditions diagnosed during infancy, and infant deaths cannot be reliably ascertained with the current system. Thus the USA live-birth rate can be further subdivided into singleton and multiple-birth rates and a general assessment of low birth weight is feasible, but it is not possible to address specific infant health outcomes.

Who should count in the denominator?

In addition to accurately defining, collecting and reporting numerator data, it is important to carefully consider which patients and procedures should be included in the denominator of each success rate and to adhere to standard criteria when collecting denominator data. Much of the confusion in defining the denominator stems from the fact that an ART procedure is not really a procedure at a single point in time, but rather ART is more appropriately considered as a cycle of treatment. A typical ART procedure or cycle begins with ovarian stimulation and monitoring, progresses to ovum retrieval, fertilization (generally *in vitro*), embryo transfer (ET), and hopefully implantation, pregnancy and live-birth delivery. A cycle may be discontinued at any of these steps, for clinical reasons such as unsuccessful ovum retrieval or ET, because of medical complications (e.g. ovarian hyperstimulation syndrome [OHSS]) that arise during the cycle, or because the couple chooses to withdraw from treatment. Thus, the success rate that is calculated may be highly variable depending on which procedures are included in the denominators. Figure 1 depicts how fresh, nondonor ART cycles (i.e. excluding those cycles using embryos that were previously frozen or ova or embryos donated from other women) progressed in 1998. As can be seen, the denominators (and thus the rates) will be quite different depending on the stage of treatment chosen.

FCSRCA specifies that live-birth success rates for USA clinics must be based on "...the number of ovarian stimulation procedures attempted" *(3)*. This is the rate that is perhaps most informative to consumers because it represents the average chance of having a liveborn infant among couples that begin ART treatment. At the time a couple is considering ART, they, of course, do not know whether and when they will withdraw from treatment; thus using the number of women who undergo ovarian stimulation with the intent of ART (i.e. cycle starts) as the denominator provides the most realistic picture of the success rate. Presentation of supplemental rates based on other denominators (e.g. cycles that progress to ovum retrieval, cycles that progress to ET) provides additional information on the likelihood of progressing from one stage to the next, once the cycle is initiated. Such rates may also be more informative when presenting data related to specific ART treatment effects. For example, we have consistently found that among couples diagnosed with male factor infertility, the live-birth rate, considering cycles in which one or

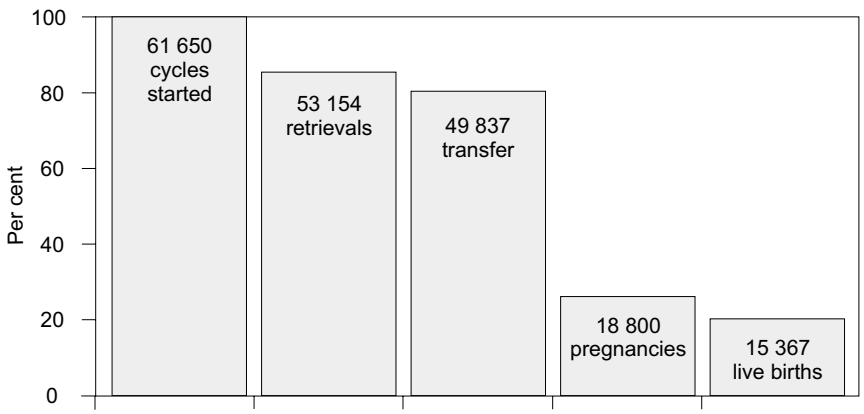

Figure 1. Outcome of ART cycles using fresh, nondonor eggs or embryos, by stage, 1998

more ova were retrieved (live birth per retrieval rate), was slightly higher when intracytoplasmic sperm injection (ICSI) was used, indicating that ICSI may improve the chances of fertilization among such couples *(2)*. However, when we limited analysis of the live-birth rate to those cycles that progressed to ET (live birth per transfer rate), we found no difference between ICSI and non-ICSI cycles. This finding suggests that ICSI does not have an additional impact on the success rate once fertilization is achieved.

A separate issue in defining denominators is whether rates should be calculated based on ART procedures or based on women who undergo ART procedures. Because it is possible and in fact not uncommon for a woman to undergo more than one ART cycle in a given year, the number of ART treatment cycles is not equal to the number of women undergoing ART. Currently, success-rate data in the USA and elsewhere are collected according to cycles, and it is not yet possible to reliably link cycles for the same woman. This, of course, presents a number of methodological problems. Whether or not a woman undergoes multiple cycles is likely to be related to the probability that a given cycle will be successful. First it is possible to undergo multiple cycles in the same year if pregnancy is not achieved, presenting a bias toward couples who are less successful. On the other hand, the decision of whether or not to undergo a second, third or more, cycles of treatment may in part be driven by assessment of various intermediate points in the previous cycle, such as the response to ovarian stimulation. Thus it is possible that among patients with one unsuccessful cycle, those with a more optimistic prognosis for success disproportionately choose to undergo further cycles. Additionally, for some patients the chance of success may actually improve with each successive cycle of treatment because the provider may adjust the treatment protocol on the basis of an assessment of the previous cycle. Thus cycles are not independent events and statistical comparisons of rates must be made cautiously.

Further issues in defining and interpreting success rates

In addition to developing standard definitions for numerators and denominators, success rates must be considered in light of the populations from which they were drawn and a host of patient and treatment factors associated with success. As previously discussed, both national and within-clinic success rates are based on a population of couples who *chose* to undergo ART within a given year (and in some cases chose to undergo multiple cycles of ART). Thus, even when collecting and reporting population-based data, the success rate does not necessarily represent the theoretical target population of interest—all potential ART users. It is important to collect as much information as is feasible to describe the population and to adjust or stratify success rates on key variables known to influence success.

First, because ART encompasses a variety of treatment approaches, success rates should be presented separately by ART procedure. At a minimum, success rates should be subdivided according to (i) whether embryos were fertilized as part of the current procedure (fresh) or had been fertilized during a prior procedure and frozen until the current procedure (frozen); and (ii) whether the source of ova was the patient (nondonor) or a woman serving as an ovum or embryo donor (donor). If the sample size allows, it is desirable to also examine success rates separately according to the method of embryo transfer, including *in vitro* fertilization (IVF) and transcervical ET (IVF-ET), gamete intrafallopian transfer (GIFT) or zygote intrafallopian transfer (ZIFT). Other treatment factors that should be examined include the number of embryos (or ova, in the case of GIFT) transferred, and specific procedures used, such as ICSI or assisted hatching.

Consideration of patient factors should, at a minimum, include the age of the woman undergoing ART. Age has been shown in numerous studies to be one of the strongest predictors of ART success among women who use their own ova *(2,5,6)*. Other patient factors that should be considered for evaluation include infertility diagnosis, patient history including previous pregnancies and births, infertility treatment history, and clinical factors such as follicle stimulating hormone (FSH) levels.

Evaluation of both treatment and patient factors is limited by the feasibility of collecting detailed clinical data on a population level and by the small sample sizes within clinics and even within certain subgroups on a national level. At a minimum, presentation of clinic-specific success rates should address differences across clinic patient populations in distributions of patient age and the general categories of ART treatment including fresh-nondonor, frozen-nondonor, fresh-donor, frozen-donor. A comprehensive evaluation of other factors is also recommended, but is generally limited by sample size to national-level data only.

In addition to stratification or adjustment for potential confounding factors, clinic-level statistics should incorporate a measure of the variance or uncertainty of key statistics attributable to small sample sizes. Even though the data within each clinic are, in one sense, population-based, presentation of confidence limits in addition to rates is preferred because it is important to convey the limitations inherent in comparing rates based on too few observations.

A final consideration in presenting ART success rates is the delay associated with data collection and reporting. To collect live-birth outcome data, a minimum lag time of nine months from cycle start is required. Additional time must be allowed for data compilation and review, analysis, report preparation and publication. The USA process is described in the following section. At best, there is an approximate two-year delay between when ART cycles were performed and when success rates are reported. This is not unusual for a public health surveillance system. However, because ART is still a relatively new technology that continues to advance rapidly, reported success rates in this field do not always represent current clinical practices.

Collecting and reporting ART success rate data in the USA

Overview

As previously discussed, medical centres in the USA that perform ART are required to report annually to CDC data on every ART procedure initiated. FCSRCA was enacted in 1992; however, since this mandate was not funded, implementation was delayed until 1997. To date, CDC has published reports for ART cycles initiated in 1995, 1996, 1997 and 1998 and the preparation of the 1999 report is currently under way.

CDC's primary objective in developing the ART reporting process was to produce an annual report that provided accurate information about ART and was accessible to the public. To fulfil this objective, CDC entered into partnership with ASRM, ASRM's affiliate, SART, and RESOLVE, the National Infertility Association. ASRM and SART are medical professional societies representing reproductive health professionals and clinics that perform ART in the USA. RESOLVE is a national consumer organization representing persons experiencing infertility. CDC meets regularly with these organizations to review and revise the reporting process, and the format and content of the published reports.

The ART reporting process includes data collection, validation, analysis, writing, editing and review, and publication. Table 1 presents the steps in this process with a time-line for the data flow. Altogether, there is an approximate two-year lag from when the last ART cycle of a given reporting year is performed to the publication of the annual ART report. A detailed description of the reporting process is provided below.

Data collection

SART had been collecting data from its member clinics and publishing annual reports on pregnancy success rates in its peer-reviewed journal, *Fertility and Sterility*, since 1989. Rather than duplicate SART's reporting system, and thereby burden ART clinics, CDC contracted with SART to obtain annually a copy of their clinic-specific database. All USA clinics that perform ART are now asked to submit data to SART for inclusion in its database. Both SART and non-SART member clinics are now eligible to participate in SART's data system. All clinics that submit their data to this CDC-supported SART system are considered to be in compliance with FCSRCA.

SART maintains an index of ART clinics known to be in operation in each year and tracks clinic reorganizations and closings. It is the responsibility of the practice director of each clinic to notify SART of the clinic's existence and any changes in address, location or key staff. This requirement, along with all the ART reporting requirements, was published in the Federal Register of the United States *(7)*. SART also follows up reports of ART physicians or clinics not on its list. These reports generally originate from consumers looking for a particular clinic in the annual ART report. Most often follow-up reveals that the clinic has only recently opened (and thus was not eligible for inclusion in the previous ART report).

Each year, SART distributes a database-management software system and instructions to all ART clinics. Clinics abstract data from clinic records for all ART cycles they initiated in a given reporting year, 1 January to 31 December, and enter their data using the SART software. An ART cycle is considered to begin when a woman begins taking ovarian stimulatory drugs or starts ovarian monitoring with the intent of having embryos transferred. The data file is organized with one record per cycle. Multiple cycles from a single

Table 1. Activities in ART reporting process and usual time line

Activity	Usual time line
ART cycles are performed	January–December, Year 0*
Data collection	
SART distributes data collection materials to clinics	January, Year 0
SART distributes any updates to the data system to clinics	by September, Year 1
Clinics submit data to SART	December, Year 1
SART compiles clinic data and submits to CDC	February, Year 2
SART and CDC review data and ask clinics to reconcile errors	February–March, Year 2
SART submits final national dataset to CDC (cycle-level data)	April, Year 2
SART submits final clinic tables dataset to CDC (aggregate data)	June, Year 2
Data validation	
CDC selects sample of reporting clinics for validation	March, Year 2
SART teams conduct site visits for all selected clinics	April–June, Year 2
SART and CDC review validation data	June, Year 2
Data analysis and publication	
CDC conducts analysis, develops graphics and text	April–July, Year 2
CDC, Division of Reproductive Health conducts initial proof of numbers in each clinic table	June–July, Year 2
CDC, ASRM, SART, RESOLVE participants review drafts of the report	July–August, Year 2
CDC editorial staff review and edit national report, format clinic tables and appendices and conduct editorial proof for entire report	July–September, Year 2
Desktop publisher lays out report for CDC	September–October, Year 2
Desktop version of report proofed by CDC editorial staff (corrections made by desktop publisher if necessary)	October–November, Year 2
Report cleared for publication by CDC science director	November, Year 2
Files forwarded to printer and CDC web site team	November, Year 2
Report published, printed version and on CDC web site	December, Year 2
Final proof of web site against printed publication	December, Year 2
Report release and dissemination	January, Year 3

*Year 0 refers to reporting year, Years 1–3 refer to the 1–3 years subsequent to the reporting year.

patient are not linked. Data collected include patient demographics, medical history and infertility diagnoses, clinical information pertaining to the ART procedure, and information on resultant pregnancies and births.

The medical director of each clinic is required to submit the clinic's data file to SART by an established deadline and to verify, by signature, that the data reported are accurate. The current deadline for data submission is in December, one year subsequent to the reporting year in question. (For example, the deadline to report data on cycles initiated in 1999 was 15 December 2000.) This allows sufficient time for all pregnancies conceived in 1999 to have reached completion and for clinic personnel to compile these data. All clinics known to be in operation throughout a given reporting year that fail to submit the required materials to SART by the required deadline are considered to *not* be in compliance with the federal reporting requirements of FCSRCA. These clinics are notified by both SART and CDC that they will be listed as nonreporting clinics in the annual ART report.

Beginning with the reporting year 2000 data-collection cycle, SART asked clinics to submit a portion of their data for each ART cycle within three days of the cycle start (i.e. before the outcome of the cycle was known). SART developed an internet-based system to process these submissions. These prospectively reported data will be linked with the full dataset submitted at the end of the data-collection period. The feasibility of this system is still being evaluated but, in time, prospective reporting may become a requirement.

After the reporting deadline, SART compiles the individual clinic data files and submits the national data file to CDC. CDC and SART then work together to review the data and identify inconsistencies and logic errors. Clinics with errors for key data elements are asked to reconcile the discrepancies and submit updates to SART. SART then compiles and submits

the final national dataset to CDC.

In addition to data entry for each ART cycle, SART's database software system includes programming that uses the cycle-level data to calculate key ART statistics for each clinic. Each year, SART refines this program in conjunction with CDC to ensure that these clinic-level statistics meet the needs of the fertility clinic tables section of the annual CDC ART report (see below—Data analysis and publication). Once the national dataset of individual ART cycles is finalized, SART additionally compiles an *aggregate*-level dataset of clinic statistics (clinic tables dataset) and submits this file to CDC.

Data validation

Once all the data have been reported, reviewed and corrected, a sample of reporting clinics (generally 8%–10%) are chosen for data-validation site visits. SART and CDC jointly decide upon the criteria for clinic selection each year in advance of data collection (thus ensuring that the criteria are not influenced by review of any clinic's data). In general, most of the clinics are chosen using a simple random sampling scheme with weighting to reduce the likelihood that a clinic previously validated will be chosen again. Additionally, clinics with a statistical value above a predesignated target level for a key indicator, such as live-birth rate, may be automatically selected. For each clinic selected, a proportion of ART cycles (usually 50) are sampled using a stratified random sampling scheme such that cycles in which a live birth occurred and cycles in which no live birth occurred are selected proportionate to the total live birth per cycle rate. Two-person teams from the SART Validation Committee conduct the data validation visits. (A representative from at least one non-SART member clinic sits on this committee.) A CDC representative attends a portion of the visits to observe the process. During the visits, the validation teams compare data that were reported to SART with clinic records. They note discrepancies on paper data validation forms and these forms are then forwarded to CDC for data entry and analysis. CDC calculates error rates within and across clinics and for each data item validated. SART and CDC review these findings.

The data-validation process is meant to be primarily educational and to identify particular problem areas in the data-collection process such that they may be corrected in subsequent data-collection cycles. Within this context, SART may institute global changes to their data-collection software or process on the basis of the validation findings and may contact individual clinics after validation to review specific problems identified. In nearly all instances, validation results are not expected to affect a clinic's status in the annual ART report. However, in rare instances, validation may reveal an unacceptable error rate. The prevailing consideration in deciding if a clinic's data are unacceptable is whether or not the validation findings suggest that publication of the clinic's data, as reported, present a misleading account of that clinic's "true" success rate. If such a situation arises, SART and CDC may work with the clinic and allow for corrections in time for the publication of the annual report. If timely corrections are not possible, the clinic may opt to remove its data from the annual report and be listed as a nonreporter. To date, error rates for all clinics validated have been within acceptable limits. Moreover, the majority of errors identified have been minor, e.g. date misrecorded by one or two days, and the impact of errors on the success rates reported has been estimated to be minimal.

Data analysis and publication

CDC holds the primary responsibility for data analysis and for writing and publishing the annual ART success rates report. The report is co-authored by ASRM, SART and RESOLVE. Both printed and web site versions of the report are published. Over the four years that CDC has been involved in this process, requests for printed reports have declined while hits to the ART Report web site have increased dramatically.

The report is laid out in three main sections. The national report presents the overall US success rates and provides some analyses of how patient and treatment factors affect the rates.

The fertility clinic tables section provides one page of key statistics for each clinic. The format and content of the most current clinic table is shown in Figure 2. As can be seen, the table is actually divided into three subsections: (i) an ART cycle profile that provides information on the various types of ART performed at the clinic and the distribution of patient infertility diagnoses being treated; (ii) a pregnancy success rates section that provides several key pregnancy and live-birth success rates, confidence limits around the live birth per cycle success rate, data on the number of embryos transferred, and statistics related to multiple births; and (iii) a current clinic services section that provides various data on patient

Clinic X
City, State

1998 ART CYCLE PROFILE

Type of ART[a]		Patient diagnosis	
IVF	**Procedural factors:**	Tubal factor	Uterine factor
GIFT	With ICSI	Male factor	Other factors
ZIFT	Unstimulated	Ovulatory dysfunction	Unexplained
Combination		Endometriosis	

1998 PREGNANCY SUCCESS RATES Data verified by Dr XY Zee

Type of cycle	Age of woman <35	35–37	38–40	>40[d]
Fresh embryos from nondonor eggs				
Number of cycles				
Percentage of cycles resulting in pregnancies[b]				
Percentage of cycles resulting in live births[b,c]				
(reliability range)				
Percentage of retrievals resulting in live births[b,c]				
Percentage of transfers resulting in live births[b,c]				
Percentage of cancellations[b]				
Average number of embryos transferred				
Percentage of pregnancies with twins[b]				
Percentage of pregnancies with triplets or more[b]				
Percentage of live births having multiple infants[b,c]				
Forzen embryos from nondonor eggs				
Number of transfers				
Percentage of transfers resulting in live births[b,c]				
Average number of embryos transferred				
Donor eggs				
Number of fresh embryo transfers				
Percentage of fresh transfers resulting in live births[b,c]				
Average number of fresh embryos transferred				
Number of frozen embryo transfers				
Percentage of forzen transfers resulting in live births[b,c]				
Average number of frozen embryos transferred				

CURRENT CLINIC SERVICES AND PROFILE (as of 1/15/2000)

Current name: Clinic X

Services offered:	***Clinic profile:***
Donor egg?	SART member?
Donor embryo?	Verified laboratory accreditation?
Single women?	
Gestational carriers?	*Additional information on laboratory accreditation is*
Cryopreservation?	*available in Appendix C*

[a] Reflects patient and treatment characteristics of ART cycles performed in 1998 using fresh, nondonor eggs or embryos.
[b] When fewer than 20 cycles are reported in an age category, rates are shown as a fraction. Calculating percentages from fractions may be misleading and is not encouraged.
[c] A multiple-infant birth is counted as one live birth.
[d] Among women >40, rates change with every year of age. Refer to Figure 10 for average chances of success by year of age.

Figure 2. Clinic table format, *1998 Assisted Reproductive Technology Success Rates Report*

services, such as whether donor ova and embryos are available, and clinic information, such as whether the laboratory used is accredited. Of note is that the statistics presented in this table are stratified according to the patient's age and the general cycle type (i.e. fresh versus frozen embryos and nondonor versus donor ova or embryos). Finally, several appendices are provided in the report. These include a glossary of key terms, an explanation of confidence limits and how to interpret the data using confidence limits, contact information for all clinics included in the report, and a listing of all clinics that were required to report under FCSRCA, but did not submit data. (Being listed as a nonreporter is the only sanction for not reporting data as required under FCSRCA; however, this appears to be a fairly strong incentive for reporting as clinics have voiced concern about the business implications of being included on such a nonreporter list.)

Once CDC receives the final dataset from SART, data analyses for the national report begins concurrent with data validation. The CDC scientific staff in the Division of Reproductive Health conduct the analyses and develop the graphics and text to present key findings. Because the primary audience for this report is current and prospective ART patients, the data must be presented in a simple and straightforward manner. Thus, in general, only univariable or bivariable analyses are presented. However, for each finding presented, additional descriptive analyses and statistical adjustments or stratifications are often also conducted and reviewed. If these additional analyses have an impact on the final result, the presentation of the data or the text are adapted to provide a more accurate interpretation than the unadjusted data suggest. ASRM, SART and RESOLVE co-authors also review the draft versions of the report and comment on the content and presentation of the data.

During this stage of the process, CDC staff also conduct an initial check of the numbers in each clinic table: (i) to ensure consistency between the two datasets compiled from the clinic submissions (the national dataset and the clinic tables dataset); and (ii) to ensure consistency between the final electronic version of each clinic table and the corresponding printed versions of the tables that each clinic medical director was required to sign as verification of data accuracy.

All data and text files for the report are next forwarded to the CDC editorial staff. They review and edit the national report, develop the final format for the clinic tables and appendices, and proofread the entire report. They then forward the report to a desktop publishing contractor for layout. Once the desktop version is proofread and approved by CDC, the desktop publisher prepares files that are forwarded concurrently to a printer for publication of hard-copy reports and to the Division of Reproductive Health web site team. As a final check, all text and data are proofread once more to ensure that the Web site and printed versions agree. The report is then released and disseminated.

Table 2. Use of assisted reproductive technology in the United States, 1995–1998

	1995	1996	1997	1998
ART clinics in the United States	–	315	359	390
ART clinics that submitted data	281	300	335	360
ART cycles reported	59 142	64 036	71 826	80 634
Live-birth deliveries resulting from ART cycles	11 609	14 388	17 054	19 891
Liveborn infants resulting from ART cycles	16 520	20 659	24 582	28 500

Notes:Total number of ART clinics was not tracked for 1995
Because more than one infant may be born during a live-birth delivery, the number of live-birth deliveries and liveborn infants are not equivalent.

Key findings

Since CDC began tracking ART success rates, there has been a marked increase in both the number of clinics performing ART and the number of ART cycles reported to CDC (Table 2). This, coupled with a modest rise in success rates each year, has led to a corresponding increase in the number of live-birth deliveries and infants conceived through the use of ART. Over 28 500 infants were born as a result of ART cycles performed in 1998. Some clinics (5%–7% of those in operation) did not report data, despite the federal mandate. Most of these are believed to be fairly small practices and it is thus estimated that more than 95% of all ART cycles have been reported each year.

Each year, the majority of cycles were classified as fresh-nondonor and in 1998, 76.5% of the cycles carried out were of this type. Cycles are further subdivided as IVF with transcervical ET, GIFT or ZIFT. As seen in Figure 3, the vast majority of cycles were fresh-nondonor IVF.

In each report, a series of success rates are

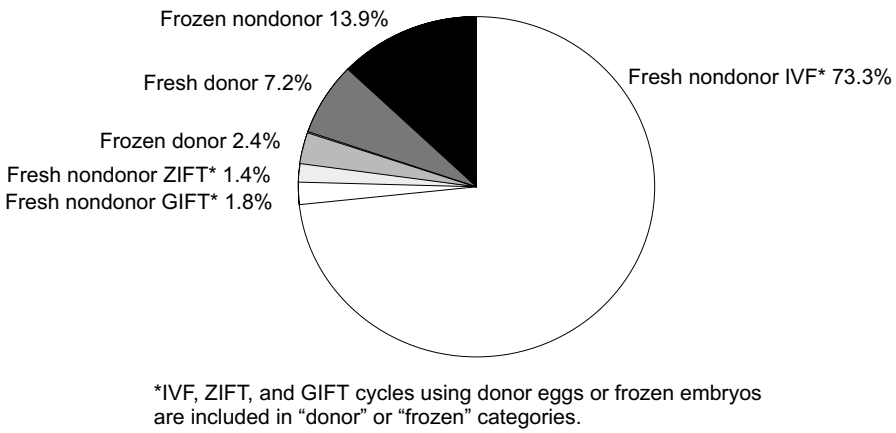

Figure 3. Types of ART procedures—United States, 1998

presented to provide a complete picture of a woman's chances of pregnancy and live-birth delivery at various stages of the ART cycle. Figure 4 shows success-rate data for fresh-nondonor cycles in 1998. While over 30% of cycles started resulted in a pregnancy, only 24.9% resulted in a live-birth delivery. As expected, the live-birth rate varied according to the denominator used. Among cycles that progressed to ET, 30.8% resulted in a live-birth delivery. Success rates were lower for cycles that used frozen embryos (19.3% live births per transfer for frozen-nondonor cycles in 1998) and higher for cycles that used donor embryos (41.0% and 23.2% live births per transfer for fresh and frozen-donor cycles, respectively).

We have consistently found that the age of the female patient is one of the strongest predictors for success among nondonor cycles; success rates decline steadily from the mid-thirties onward. In contrast, age has had little impact on the success of cycles that used donor ova, suggesting that the age of the woman who was the source of the ova is of primary importance (Figure 5).

In each report, we also have examined the effects of several other patient and treatment factors on ART success. These include infertility diagnosis, previous births, number of embryos transferred, ART type (IVF, GIFT, ZIFT), use of ICSI and clinic size. The findings have been fairly consistent over the years. Having previously given birth has been associated with increased success rates in all age groups. Transfer of three or more embryos has also been generally associated with increased success (however, this finding was variable by age and was limited in that embryo availability and patient choice drive decisions on ET).

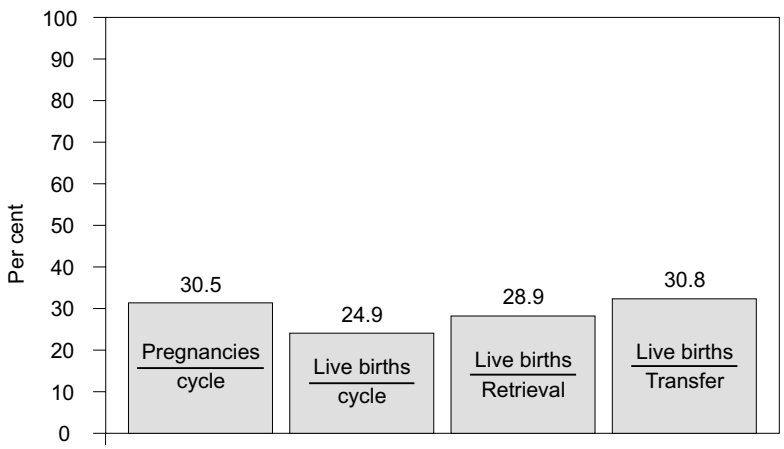

Figure 4. Success rates for ART cycles using fresh, nondonor eggs or embryos, by different measures, 1998

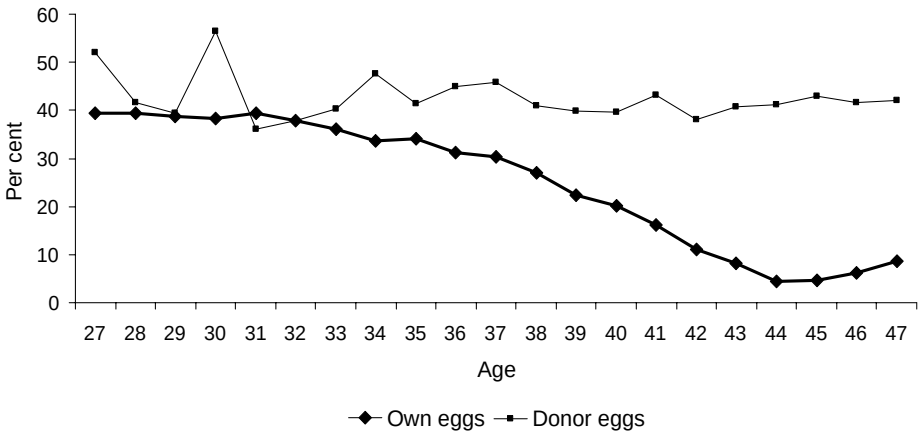

Figure 5. Live births per transfer for fresh embryos from own and donor eggs, by age of patient, 1998

ICSI was associated with slightly higher success rates among couples diagnosed with male-factor infertility. Finally, larger clinics tended to have higher success rates. There has been little variation in success rates across infertility diagnosis or ART type categories. However, to date, diagnostic criteria have been poorly defined. Beginning with the collection of the 1999 ART data, infertility diagnostic criteria have been more explicitly defined to standardize this data element and to allow for analyses of couples diagnosed with multiple causes of infertility.

In each report, limited data on adverse pregnancy outcomes such as spontaneous abortion and ectopic pregnancy, and data on infant outcomes such as multiple birth have also been presented. Treatment factors on ART success are also examined. The proportion of multiple-gestation pregnancies and multiple-infant births associated with ART has remained fairly constant over the past four years. In 1998, 39% of pregnancies had more than one fetus (defined by the number of fetal hearts observed on ultrasound) and 38% of live-birth deliveries included more than one infant (Figure 6). This compares with a multiple-birth rate of less than 3% in the general population *(8)*.

Lessons learned

Like all large-scale surveillance projects, implementation of FCSRCA has presented a series of challenges. During the past four years, a number of issues have arisen that have prompted both SART and CDC to review and refine various phases of the reporting

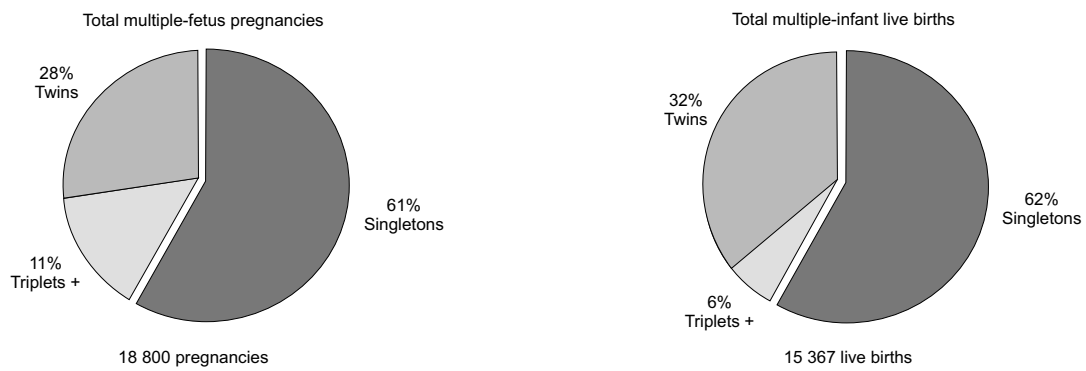

Figure 6. Risk of having multiple-fetus pregnancy and multiple-infant live birth from ART cycles using fresh, nondonor eggs or embryos, 1998

process. We believe such refinements have led to more efficient data collection and processing and improvements in data quality and data presentation. Like all surveillance systems, the ART data system is not static; indeed it is a particular challenge to keep ART data collection current with the ever-evolving innovations in the field.

The challenges can be divided into two general categories: data-collection and data-reporting issues. The challenges with data collection generally stem from the large number of individual ART clinics in operation. This makes coordination of the data flow quite cumbersome. To proceed in both a timely, fair and impartial manner, both SART and CDC recognized early on that explicit deadlines and guidelines for data submission were needed. Additionally, with such a large number of clinics now performing ART, clinical practice is increasingly variable across clinics; thus, relatively soon it became apparent that some data elements had been ambiguously defined. For example, clinics were asked to select the primary infertility diagnosis (tubal factor, male factor, ovulatory dysfunction, endometriosis, uterine factor, other factors and unexplained infertility) for each cycle reported. During a joint SART–CDC review, two issues emerged. Diagnostic protocols were fairly variable between clinics, and forcing selection of a primary diagnosis among couples with multiple diagnoses often resulted in an arbitrary decision by clinic personnel. In response, more explicit criteria were developed on how to classify each diagnosis for the purposes of the ART surveillance system and a data entry scheme was developed that allows for entry of multiple diagnoses.

The other major challenge that has been faced is the presentation of these complex data and ideas in a simple and clear manner that is informative to the general public. To help us assess whether the reports were fulfilling their objectives, two series of focus groups with current and prospective ART patients were undertaken. The latest of these was conducted in 1999 and was based on an evaluation of the 1996 *ART Report*. The groups were generally satisfied with both the content and the format of the report. Some areas of confusion were noted, however. Many of the focus-group participants had trouble understanding some of the more clinical and statistical terms and concepts used in the report. Some of the most difficult included age-adjusted rate, 95% confidence intervals and multiple-gestation pregnancy. To a lesser extent, even the basic indicators presented caused confusion. For example, some participants could not understand the differences between the live birth per cycle, live birth per retrieval and live birth per transfer rates. In response to these problem areas, adjusted rates are no longer given and instead the focus in the clinic tables is on rates stratified by patient age and cycle type. Confidence intervals are still included because we can find no simpler way to provide a sense of the often large variance measures inherent in data from small clinic samples, but we have expanded our explanation of how to understand and interpret a confidence interval. We altered some of the report terminology to provide more familiar descriptions for certain indicators; for example, we now refer to multiple-fetus pregnancies rather than multiple gestations. We expanded the national report to provide a clearer picture of the overall concepts of rates, why rates with different denominators are presented, and how to interpret the various rates presented. Finally, we added a section to the report that include answers to many commonly asked questions about the report process and content.

In addition to the focus groups, feedback is received each year from various individuals in the public domain as well as ART providers. We review their comments with SART and RESOLVE and consider revisions and additions to data presentation. A particularly challenging issue that has arisen is how to present, fairly, data for clinics that were in operation during a given reporting year but have undergone reorganization by the time of report publication. Reorganization can include a change of key staff (medical, practice and/or laboratory directors) or a change in clinic ownership or affiliation. Although only a handful of such reorganizations occur each year, they have required a great deal of attention, as each party involved is understandably concerned that the ART report represent their situation accurately. In response to these concerns, a policy has been developed to present each clinic according to the clinic name at the time of data collection (e.g. all clinics in the 1998 report are presented according to their 1998 clinic names). Changes in clinic names and any clinic reorganizations that have occurred after the reporting year are noted.

Future directions

As the field of ART continues to evolve, the ART surveillance system will also evolve. SART, with

support from CDC and RESOLVE, continues to review and revise its reporting system. As previously noted, SART has already made several important changes. It has clarified many case definitions and addressed several ambiguities in classifying ART cycle type and outcome. This includes developing more explicit criteria for documenting live-birth deliveries in clinic records and adding a provision for reporting the source of information for each live birth. Additionally, SART has added several data elements to the system to capture better patient and treatment differences that might be affecting success rates. These include data on clinical indices such as the patient's hormone levels, and improved data on pregnancy history, infertility history and the history of previous infertility treatment. Several data items have been streamlined or discarded because they were not deemed useful. The revised reporting system was instituted in the1999 ART data-reporting cycle and CDC plans to assess the accuracy and completeness of the new data items and incorporate analyses of these factors in future reports.

CDC and SART are also investigating the feasibility of various mechanisms that will allow the linkage of data from ART cycles performed on the same patient. Such a system will permit the evaluation of ART usage patterns within and between clinics and over time, and the assessment of cumulative patient success rates. As previously mentioned, SART is also working on the development of systems to improve data quality, such as adapting its data system to allow for prospective reporting.

Finally CDC, ASRM, SART and RESOLVE continue to struggle with various "nonmethodological" issues inherent in this surveillance system. ART, like other medical specialties, is increasingly conducted in independent clinics that must consider cost and profit issues to remain viable. Since this surveillance system is clinic-based, there is often concern about the impact that the ART report may have on clinic business practices. Thus, it is especially important that success-rate data are collected in a fair and impartial matter, that erroneous data are corrected to the fullest extent possible and that all success rates are presented in the appropriate context with full disclosure of the limitations in interpreting and comparing success rates. Various concerns related to this issue have been addressed already. The challenge is to meet the requirements specified in the national legislative mandate, to provide a report that is fair to clinics, but also to present these data in a meaningful way at a level that is appropriate for the general public.

ART use continues to grow in the USA and throughout the world. The USA ART surveillance system and the annual reports published from this system provide standardized information on ART success rates for patients as well as for the medical and public health communities. These data are being used by patients and providers to make informed choices about ART. CDC, ASRM, SART and RESOLVE have made a great deal of progress in developing the current surveillance system and will continue to collaborate on this increasingly important public health issue.

Appendix

To obtain a printed version of the most recent *Assisted reproductive technology success rates report*, call CDC's Division of Reproductive Health, info line: 1-770-488-5372. For immediate electronic access to any of the published reports, connect to the CDC web site: www.cdc.gov/nccdphp/drh/art.htm.

Acknowledgements

We are grateful to the many individuals at the Society for Assisted Reproductive Technology (SART), the American Society for Reproductive Medicine (ASRM), and RESOLVE, The National Infertility Association who have contributed to the development and continued review of the assisted reproductive technology reporting system in the USA and the annual *Assisted reproductive technology success rates reports.*

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International registries of assisted reproductive technologies

KARL NYGREN

Introduction

International data collection and reporting of assisted reproductive technology (ART) results are still very much in their infancy. The same is true for national reporting systems; however, there are large differences between countries. Many countries have no reporting systems at all while others do, although with different levels of completeness, working definitions and validation.

The degree of complexity of ART data reporting escalates from case reports via clinic reports through national reporting systems and regional reporting to world reports. As the reporting widens and includes more and more treatment cycles, the data tend to become more incomplete and originate from different validation systems and definitions. However, this situation is a very dynamic one. More and more countries are now establishing national reporting systems, on which regional and world reporting rely.

A commonly adopted strategy, at least among those responsible for national and international registration of data, is that each clinic should report their results only once. National bodies, be they governmental or nongovernmental, either on an obligatory or a voluntary basis, should collect, audit and publish national data, preferably on an annual basis, in their own country. They should also report their national data to regional bodies, where such bodies exist. At present, four regional bodies report data annually: Australia and New Zealand *(1)*, Latin America *(2)*, USA and Canada *(3)* and Europe *(4)*. Regional bodies are also under preparation in South-East Asia.

The International Working Group for Registers on Assisted Reproduction, which is now a Task Force under the International Federation of Fertility Society (IFFS), has so far produced five world reports. The most recent report was published in 2001*(5)*.

Why register?

ART is a very dynamic field and will possibly remain dynamic for a long time. The dynamics includes medical improvements, new treatment modalities, ethical issues, cost–benefit analyses for allocation of resources and national legislation. Infertile couples, ART professionals, the industry, media, politicians, legislators and the public at large all need relevant information on results and outcomes of ART. They need data from an individual clinic or an individual country and also international data, so that they can make relevant comparisons to elucidate the characteristics of ART in their own setting. Therefore, the different levels of reporting complement each other.

How to register?

When a national registration on ART is established, there are two basic options to choose from. First, each clinic in the country is asked to prepare an annual report covering items decided by the registrating body of that country. The data should ideally be cohort data covering treatments starting over a period of one year and the outcome of those treatments, which means that data usually cannot be collected until the end of the second year.

The second option is to collect data from each cycle individually, preferably in a direct system where details are reported before the outcome of each treatment is known.

The annual reporting system has, for example, been chosen by the Scandinavian countries. The system is simple and cheap but it has a potential problem with data validation.

The individual cycle reporting system has been chosen, for example, in Germany and the UK *(4)*. It is much more expensive and complicated to follow but has advantages as regards validation of the data.

The collection of national data must then be followed by a process of auditing and publishing. To publish only crude data is not acceptable to the consumers of the reports. The data have to be audited, important time-trends have to be highlighted and—a very controversial issue—the reporting of "success rate" per clinic has to be handled with care. (This last issue is difficult indeed and is highlighted by the problems created in the UK, where the authority tried to construct one simple measurement of success in ART treatments. Despite the good intention, this resulted in a distortion of available data so that couples were misled and clinics started to adopt procedures that were not necessarily medically indicated.) (Personal communication with Professor Allan Templeton)

The publication of national data should ideally be once a year. The experience from several countries is that such publication gets good coverage in the media so that the public can share the results. Internationally, the four regional reports in existence collect data from their region and publish annual reports along the same lines as the national reports.

It is hoped that in the future the world reports will be published on a biannual basis.

What should be reported?

Ultimately, each country should report the results of direct treatment (pregnancy rates, etc.), pregnancy outcome (deliveries and children born), child development and primary or secondary side-effects for the women.

Each of these reporting areas need different strategies for data collection. Most registries today only deal with the first of these categories, reporting the immediate results of ART treatments including the proportion of pregnancies, abortions, ectopic pregnancies and deliveries (single or multiples) in relation to started cycles, ovum pick-up rates and the number of embryo transfers. Different modalities of treatment are reported separately (standard *in vitro* fertilization [IVF], intracytoplasmic sperm injection [ICSI], frozen and thawed embryo transfers, etc.). This category of reporting sometimes, but not always, also includes information on the type of delivery (normal, forceps, vacuum extraction, caesarean section) and possibly the birth weight and the gestational week of delivery.

The immediate outcome of the deliveries regarding the health of the babies born (detailed information on birth weight, week of delivery, malformations, perinatal death rates, etc.) usually requires a quite different strategy of data collection and is much more complex to organize. In many countries specific reporting systems have to be set up and they are difficult to manage nationwide. Therefore, monitoring of the outcome for the children from individual clinics is usually easier to perform. One exception is the Nordic countries where national medical birth registries have been in existence for some time and data are collected on all children born in the country. By cross-linkage to specific ART registers it is much easier to have a complete and correct information of the outcomes for the ART children and compare these and national data *(6)*.

Later child development follow-up focuses not only on psychosocial but also on late medical problems such as neurological sequelae. In this case, data collection is usually even more cumbersome and necessitates much smaller samples of individuals. Reports in this category are still scarce.

Which ART categories?

The reporting systems described so far usually cover different types of IVF but not other medical treat-

ments, such as induction of ovulation. One of the main medical problems with ART treatment is the persistent occurrence of high levels of multiple pregnancies. However, it may well be that this problem is of the same or even higher magnitude after ovarian stimulation without ART. In this field, so far, only clinic reports have appeared in the literature and there have been no systematic national efforts at data collection. It is clear that this gap has to be filled and in Sweden efforts are under way to do so.

Where to go from here?

For future refinement of ART reporting, three areas appear to be the most important.

Definitions

Each country has its own working definitions covering important determinants such as "When does a treatment cycle actually start?", "What is a biochemical pregnancy?", "Where is the dividing line between a late abortion and a birth?", "What is a major malformation?" This situation has not come about by chance but is the result of different cultures, different ethical views on ART, legislation on legal abortion, etc. Nevertheless, for the sake of comparison, a harmonization of definitions would be highly desirable and the International Working Group is preparing a list of definitions used in ART.

Validation of register data

The possibility of validating data nationally and ensuring that they are complete is rapidly improving in many countries. One mechanism is through the process of accreditation, based on inspections of clinics. Another possibility is to cross-link data from different registries. Today, some countries have highly sophisticated validation systems although the data are not complete. Examples of this include Germany and the USA where reporting is not obligatory.

Again, the situation is dynamic and improving in many countries. The driving force behind the change is most probably that potential consumers of this information in each country realize the importance of the data being both valid and complete.

Guidelines

The International Working Group for Registers on Assisted Reproduction has taken, as one of its tasks, the formulation of guidelines for the establishment of national registries on ART. The Group realizes that such guidelines must recognize different ways in different countries of reaching the same goal, namely complete and valid data.

Summary of available data

Pregnancy rates, usually expressed as the proportion of pregnancies per embryo transfer, differ immensely between countries. There are three reasons for these very large differences. One may be differences in skills in different countries. The most powerful reason, however, is the very different policies concerning the number of embryos transferred at each procedure. These numbers vary amongst countries from four or more (examples are Eastern and Southern Europe, Latin America and also partly in the USA) down to one or two embryos per transfer as in the Nordic countries. A third reason is the very varied practice of fetal reduction (the technique to reduce the number of fetuses to transform a high-order multiple pregnancy to a low-order multiple pregnancy). This procedure is very controversial in some countries but it is practised frequently in France and the USA but very rarely in the Nordic countries *(6)*.

Spontaneous abortions are heavily age dependent (increasing with the age of the woman) but the average after ART is not increased from the normal rate and is usually recorded as around 18% of pregnancies.

Ectopic pregnancies are fewer today compared to the early days of ART treatment. This shift depends on the fact that women with tubal damage dominated the early years of ART treatment, whereas today they form only a relatively smaller part of the indications for treatment. Whereas ectopic pregnancy rates formerly were reported as 3%–5% per embryo transfer, most countries today report an incidence of about 1%–2%, which is not significantly higher than the national rates in the population as a whole *(1–5)*.

Multiple pregnancy rates are very different in different countries. Characteristically, they are low in northern Europe and Australasia and high in Latin America, the USA and Southern and Eastern Europe. The USA reports a multiple pregnancy rate of 37% while Europe reports a corresponding rate of 29% *(3,4)*.

Epidemiology

Today about one million children have been born worldwide after IVF treatments *per se* and the number of children born after other forms of ART is very uncertain but is possibly about the same. Therefore, around 2 million children have been born after assisted reproduction.

Availability

The availability of ART services differs widely among countries. One extreme example is the Nordic countries, where there are 1000 to 1500 treatments per million inhabitants. In contrast, the availability in the USA is about 200 treatments per million inhabitants. In several developing countries, including China and India, the availability is certainly extremely low *(5)*.

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Recommendations

The meeting participants agreed upon the following recommendations which are grouped under the headings of the various topics that were discussed. While in most cases it is self-evident as to whom the recommendation is directed, some recommendations specify the group or entity that would be expected to take the appropriate action.

Section 1: Infertility and assisted reproductive technologies in the developing world

Papers

Infertility and social suffering: the case of ART in developing countries (Abdallah S. Daar, Zara Merali)
ART in developing countries with particular reference to sub-Saharan Africa (Osato F. Giwa-Osagie)

Despite infertility being a universal health problem, infertile couples—especially in developing countries—have limited access to infertility services. Infertility has been argued to be relatively unimportant in low-resource settings where fatal and infectious diseases remain uncontrolled. While inadequate access to assisted reproductive technology (ART) is a worldwide issue, it is particularly pronounced in resource-poor settings. Improved access depends on a number of factors, including increased recognition of infertility as a public health problem by policy-makers, the development of simplified management schemes for infertility and reduction in the cost of ART to the consumer.

Recommendations

- Infertility should be recognized as a public health issue worldwide, including developing countries.
- Research is needed on innovative, low-cost ART procedures that provide safe, effective, acceptable and affordable treatment for infertility.

Section 2: Infertility and assisted reproductive technologies from a regional perspective

Papers

Assisted reproductive technology in Latin America: some ethical and sociocultural issues (Florencia Luna)
Attitudes and cultural perspective on infertility and its alleviation in the Middle East area (Gamal I. Serour)
Social and ethical aspects of assisted conception in anglophone sub-Saharan Africa (Osato F. Giwa-Osagie)
ART and African sociocultural practices: worldview, belief and value systems with particular reference to francophone Africa (Godfrey B. Tangwa)
Sociocultural attitudes towards infertility and assisted reproduction in India (Anjali Widge)
Sociocultural dimensions of infertility and assisted reproduction in the Far East (Ren-Zong Qiu)

Cultural perspectives regarding infertility and ART are as diverse as the societies in which they exist. However, one common theme is that infertility is perceived universally by the infertile as a stigma. In many cultures infertility is usually blamed on the female partner. As a result, the burden of infertility is heavier for women, especially in societies that define womanhood through motherhood. The feeling of stigmatization exacerbated by family, peer and media pressure leads to psychological, marital and social problems. The infertile couple, but more often the female partner, seeks assistance and advice from members of the family, traditional healers, primary health care providers or directly from infertility specialists. Often the infertile couple is especially vulnerable to chicanery by both traditional and conventional health care providers.

ART is practised in many countries and a wide range of perceptions exists, depending on the particular culture and society. Such perceptions are often influenced by religious views and traditional cultural values. Although in some societies ART may not be perceived with a positive attitude, the number of clinics that offer ART services is on the rise. The media are often responsible for creating a very promising image of ART and of what it can offer to the infertile couple. However, it is well known that in many countries the public lacks basic knowledge about infertility, its causes and its prevention, as well as accurate information about realistic possibilities for infertility treatment.

Recommendations

- Policy-makers and health staff should give attention to infertility and the needs of infertile patients.
- Governments should improve education in infertility and reproductive health for the general public and health care professionals.
- A gender perspective needs to be applied by health care providers to infertility management and treatment.
- Infertility management should be integrated into national reproductive health education programmes and services.
- Physicians should provide adequate investigation facilities and treatment for the infertile couple in a culturally sensitive and ethically acceptable manner.
- Where appropriate, traditional healers should be included in the dialogue between patients and health care providers concerning the treatment of infertility.
- Where public funding is insufficient, alternative sources of funding for public sector ART programmes should be sought.

- Cost-effective options, including the establishment of national networks of satellite clinics to screen and refer appropriate couples to specialist centres, should be examined as a means of improving access to ART.
- ART should be complementary to other ethically acceptable, social and cultural solutions to infertility.
- Public awareness of infertility and its causes should be increased to improve preventative behaviour and to diminish the stigmatization and social exclusion of infertile men and women.
- The dissemination of public information on the options for treatment of infertility, including adoption and the ethical and legal issues involved, should be improved.

Section 3: Recent medical developments and unresolved issues in ART

a. Gamete source, manipulation and disposition

Papers

Gamete source and manipulation (Herman Tournaye)
Ovarian stimulation for assisted reproductive technologies (Jean-Noel Hugues)
ICSI: technical aspects (Henry E. Malter, Jacques Cohen)
ICSI: micromanipulation in assisted fertilization (André Van Steirteghem)
Cryopreservation of oocytes and ovarian tissue (Helen M. Picton, Roger G. Gosden, Stanley P. Leibo)
Cryopreservation of human spermatozoa (Stanley P. Leibo, Helen M. Picton, Roger G. Gosden)
Gamete and embryo donation (Claudia Borrero)

Intracytoplasmic sperm injection (ICSI) has now become a widely used procedure and is indicated for the treatment of male infertility; however, it should not be considered as an alternative to routine *in vitro* fertilization (IVF). The current methods of sperm selection for ICSI are unable to prevent the injection of aneuploidic spermatozoa. As a result, the health of ICSI offspring has been of considerable concern. To date, available evidence on the short-term health of ICSI children appears to be reassuring.

A possible factor in the failure of implantation is nonrupture of the zona pellucida. Recently, techniques have been developed to assist in the hatching of the embryo through the zona pellucida. These include drilling with an acidified medium, zona slitting, zona thinning and, most recently, laser assisted hatching. Few randomized studies have been conducted; however, retrospective analysis has yielded equivocal results comparing implantation and pregnancy rates from assisted hatching and control embryos.

The purpose of ovarian stimulation is the accelerated development of oocytes suitable for fertilization either in conjunction with *in vivo* insemination (donor insemination/artificial insemination from husband [DI/AIH]) or IVF–ICSI. In the past decade, there have been considerable advances in the understanding of ovarian follicular physiology together with the development and introduction of more specific drugs for ovarian stimulation.

One of the major issues in ART is the high frequency of multiple pregnancy, and the associated premature births and neonatal morbidity and mortality. Whereas the incidence of multiple births following IVF/embryo transfer (ET) or ICSI is determined by the number of embryos transferred, there is far less control over the number of oocytes fertilized following ovarian stimulation and DI/AIH. In clinics where both procedures are carried out and whether two to three embryos are transferred after IVF/ET or ICSI, high-order multiple pregnancies are almost exclusively the result of multiple *in vivo* fertilizations following ovarian stimulation.

Common to all methods of ovarian stimulation is the potential for ovarian hyperstimulation syndrome (OHSS). In the past decade, prevention of OHSS has been improved by the identification of high-risk patients and closer monitoring of induced folliculogenesis, including serial serum estradiol estimations and ultrasound assessment of the cohort of growing follicles. The prognosis of ART in older women is consistently poor. In that respect, couples should be informed about the high risk of stimulation cycle cancellation and counselling should be provided on alternative solutions.

Concerns remain over possible associations between induced folliculogenesis and uterine and ovarian cancer. However, concerns about breast cancer would seem to have been allayed.

Cryopreservation of male and female gametes has been a critical issue in the development of ART. Cryopreserved/thawed sperm and oocytes increase the flexibility of ART services, while they also allow patients who might become infertile after medical interventions to restore fertility. Despite the clear evidence of individual patient variation in the success of sperm cryopreservation, the methodology for sperm freezing has changed little over the past 20 years.

In recent years, the prospect of ovarian tissue cryopreservation has attracted the attention of the medical community as well as the media. Despite sensational reports on the implications of such options for the conservation of future fertility, limited information is available on the optimal cryopreservation techniques for immature oocytes that would be recovered from unstimulated ovaries or ovarian cortical tissue. Although cryopreserved ovarian cortical tissue may be used as the source of immature female gametes and ovarian cortex autografting has the potential to restore reproductive function, long-term viability and safety data are not yet available.

One of the reasons advocated for retrieval of immature oocytes from unstimulated ovaries has been the limited availability of oocyte donors. Egg-sharing has been considered as a way of overcoming this problem. While the first presents significant technical difficulties, the latter poses complex psychological and ethical questions.

Recommendations

- Existing data on the efficacy, safety and outcome of ICSI should be analysed as a matter of high priority.
- The effect of ICSI treatment on the risk of congenital malformation should continue to be monitored.
- Further animal studies are needed to evaluate the safety of the following procedures currently being investigated or proposed for the treatment of infertility:

  —ICSI using round spermatids

  —*in vitro* spermatogenesis

  —spermatogonial stem-cell maturation

  —*in vitro* growth and maturation of oocytes

  —cytoplasmic transfer

  —haploidization of diploid somatic cell nuclei.

  All of these procedures should be considered experimental.
- Nonobstructive azoospermia should be defined by clinical, biochemical and histological features prior to selection for ICSI.
- Methods need to be developed to detect aneuploidic spermatozoa so that these can be excluded from sperm preparations to be used for ICSI.
- Because of the risk of aneuploidy in testicular spermatozoa, it is important to combine ICSI with preimplantation genetic diagnosis (PGD) to prevent aneuploidy in offspring.
- Further research is needed on the outcome of pregnancies resulting from the use of nonejaculated sperm.
- Research is needed on the role of prenatal testing during ICSI pregnancy.
- Sensitive test systems (e.g. micro-array probes [gene chips]) need to be developed for screening ICSI candidates for known genetic traits that can be transmitted to subsequent generations.

- Low-dose ovarian stimulation protocols should be used so that fewer follicles develop.
- To reduce high-order pregnancies following ovarian hyperstimulation for non-IVF/ICSI-ET, if the number of preovulatory follicles is more than two, the cycle should be converted to IVF/ICSI-ET, or if access to IVF/ICSI is not available, the cycle should be cancelled.
- Further research is needed on the efficacy of ovarian stimulation regimens in which exogenous recombinant-follicle stimulating hormone (rFSH) or r-luteinizing hormone (rLH) are administered.
- Research is needed on the optimal regimen for oral contraceptives in programming gonadotrophin-releasing hormone (GnRH) antagonist cycles.
- Further research is needed on noninvasive measurement of ovarian blood flow for monitoring the induction of ovulation.
- Research is needed on uterine receptivity using ultrasonographic measurement of peristaltic uterine contractions.
- Research is needed on the efficacy of the vaginal route of administration of progesterone administration (particularly sustained-release formulations) compared with intramuscular injection for luteal support.
- Studies need to be conducted on the use of progesterone and/or estradiol for luteal phase support with or without human chorionic gonadotrophin (hCG) administration as regards implantation and miscarriage rates.
- Further research is needed on the application of freeze-drying for human gamete cryopreservation.
- Protocols for the cryopreservation of human gametes, embryos and gonadal tissues need to be optimized.
- Protocols for the cryopreservation of sperm need to be improved to reduce the observed variation in sperm survival.
- Sperm donors need to be screened for hereditary as well as infectious diseases.
- Procedures for the separation of X- and Y-bearing spermatozoa should be used for preventing the transmission of sex-linked diseases.
- Further scientific and ethical research is needed on the application and use of spermatozoa retrieved postmortem or from patients in a persistent vegetative state.
- Clear guidelines need to be established for the optimal stage of oocyte maturation for cryopreservation that maximizes gamete survival and developmental potential but which minimizes the risk of aneuploidy.
- More research is needed on aneuploidy screening of oocytes after cryopreservation and of embryos generated following ovum cryopreservation.
- More research is needed on autotransplantation of frozen-thawed human ovarian tissue.
- There is a need for long-term viability and safety data on the restoration of reproductive function following ovarian autografting or the *in vitro* growth of oocytes.
- Data are needed on the short-term and long-term medical and psychological issues related to oocyte "sharing", with a particular focus on the donor.
- Long-term psychological follow-up is needed of gamete donors and recipients and of the resulting offspring.

b. Embryo selection methods and criteria

Papers

Embryo culture, assessment, selection and transfer (Gayle M. Jones *et al.*)
Preimplantation genetic diagnosis (Luca Gianaroli *et al.*)

Following fertilization, the zygote needs to mature to a stage that is suitable for uterine implantation. This process (which involves the transition from the zygote to the morula and then to the blastocyst) normally occurs in the fallopian tube and uterus, but can be induced in the laboratory. This *in vitro* process of early embryo development—termed embryo culture—has become a highly sophisticated set of laboratory procedures involving culture media and culture conditions, selection of embryos for transfer, techniques and timing of ET, assessment of endometrial receptivity and luteal phase support in the postimplantation period. None of these procedures has been fully optimized and they are under constant refinement and improvement.

The transmission of genetic disorders remains a major problem in reproductive medicine. Until recently, couples have been screened mainly by using maternal age or family history. Invasive procedures such as amniocentesis and chorionic villus sampling have been extensively used in high-risk populations. However, the detection of a genetic abnormality implies a decision to be made by the woman as to whether or not the pregnancy should be terminated. In many countries this option is not available. PGD provides the possibility for screening for chromosomal and genetic disorders prior to implantation; and if such abnormalities are detected, the ET need not proceed. PGD is now an established technique for the detection of genetic and chromosomal abnormalities in preimplantation embryos and may be used for detection of chromosomal or monogenic diseases in high-risk couples.

Recommendations

- The optimal *in vitro* culture environment for human embryos needs to be established and refined and tested by randomized controlled trials of sufficient power.
- Research is needed to determine the cleavage stage of the zygote or blastocyst that is optimum for transfer in terms of pregnancy outcome.
- The criteria for uterine receptivity in relation to implantation and continuing pregnancy need to be better defined.
- More specific criteria for predicting the developmental potential of the embryo need to be developed.
- Research is needed on embryo morphology and growth rate including:
  - —the regulation of cell cycles in the preimplantation embryo
  - —the aetiology of cell fragmentation in the preimplantation embryo
  - —determination of the role of cytoplasmic and nuclear polarity in the viability of preimplantation embryos
  - —relationship of embryo viability with follicular and oocyte parameters both within an ovulatory cycle cohort and between cycles and patients.
- Further research is needed on the sensitivity, specificity and predictive value of PGD alone for the detection of aneuploidy. At present, amniocentesis or chorionic villus sampling are recommended after PGD.

- Techniques need to be developed for the simultaneous screening for fetal single-gene disorders and chromosome aneuploidies using multiple markers.

c. Multiple pregnancies and multiple births

Papers

Multiple pregnancy in assisted reproduction techniques (Ozkan Ozturk, Allan Templeton)
Outcome of multiple pregnancy following ART: the effect on the child (Orvar Finnstroem)
Multiple birth children and their families following ART (Jane Denton, Elizabeth Bryan)

The number of embryos transferred is the major factor determining multiple pregnancy rates in ART. Elective single embryo transfer reduces the risk of multiple pregnancy and may result in a comparable pregnancy rate to that obtained with two embryo transfers. It has been shown that successive single frozen-thawed embryo transfers can achieve good cumulative conception rates while minimizing the risk of multiple pregnancy. In turn, this will reduce the excess maternal morbidity and mortality associated with multiple pregnancy.

The high percentage of multiple births following ART is mainly responsible for the high rate of preterm births, neonatal complications and congenital anomalies. The increasing numbers of preterm babies, especially from high-order pregnancies, have resulted in an exponential increase in the cost of neonatal care. As a result of the increased health risks and costs, fetal reduction may be offered as an option in order that some of the adverse effects of a triplet or higher-order pregnancy may be reduced. However, there are considerable ethical and legal implications of this technique. What strategies should be implemented to reduce multiple pregnancies associated with ART remains an unresolved issue.

Recommendations

- Prior to treatment, health personnel should provide patients with full information about ovarian stimulation and the risks and implications of multiple pregnancy.
- In view of the maternal and fetal risks of multiple pregnancy, no more than two embryos should be transferred per cycle.
- To encourage single embryo transfer, any additional spare embryos should be cryopreserved for subsequent treatment cycles—if needed, and where it is not illegal to do so.
- Prospective studies are needed to compare the outcomes of elective single embryo transfers with two embryo transfers.
- Methods need to be developed and tested for the selection of embryos for elective one- or two-embryo transfers.
- Ovarian stimulation protocols need to be optimized to reduce the likelihood of multiple pregnancy as a result of treatment with gonadotrophins followed by intrauterine insemination (IUI).
- The efficacy, side-effects, patient acceptability and costs of IVF, versus ovarian stimulation and IUI, need to be compared in patients deemed suitable for either treatment (unexplained infertility, mild endometriosis, and mild male factor infertility).
- More data are required on malformation rates following ART, especially in twin pregnancies.
- As monozygous multiple pregnancies have a much higher perinatal mortality rate than dizygous

pregnancies, ART specialists and obstetricians should determine zygosity by assessing whether there are one or two chorionic membranes (chorionicity). This can be defined by ultrasound scan prior to 14 weeks' gestation as the chorion is optimally seen in early pregnancy. It is particularly important in ART multiple pregnancies in which the risk of monozygosity may be increased.

- The incidence and possible causes of monozygotic twinning in pregnancies resulting from ART need to be determined.

Section 4: Social and psychological issues in infertility and ART

Papers

Consumer perspectives (Sandra Dill)
Gender, infertility and ART (Ellen Hardy, Maria Yolanda Macuch)
Family networks and support to infertile couples (Pimpawun Boonmongkon)
Parenting and the psychological development of the child in ART families (Susan Golombok)

Consumers have a key role in improving the availability and quality of infertility services especially through local and national support groups. A crucial issue in the management of infertility, including ART, is that infertile couples need to have sufficient information so that they may make informed choices as to where and how they will be diagnosed and treated.

Since the early days of ART more than two decades ago, there have been concerns about the health and psychological well-being of ART offspring. As it is now possible to have up to five parents (an oocyte donor, a sperm donor, a surrogate mother and two social parents), many questions have been raised about the consequences of such possibilities for psychological development through childhood and into adult life. Additional concerns have been raised regarding the functioning of the ART family as a whole. How the relationships and dynamics in an ART family are affected by the partial or complete absence of genetic links between the child and the parents in cases of gamete donation, or by the secrecy or disclosure of the circumstances of conception to the child, are questions of critical importance especially with the ever-growing number of people resorting to ART for solving their infertility problem.

Existing studies have shown that singleton ART children born at term do not appear to differ from spontaneously conceived children with respect to psychological well-being or the quality of their relationship with their parents. Although data are limited, and research in this area lags behind the fast developments and advances in ART, there is no evidence of developmental delay among singleton ART children.

In many societies infertility problems are blamed on women irrespective of the diagnosis. As a result, traditionally, the burden of infertility has been heavier for women. ART has improved the chances of infertile couples to have children but, at the same time, it has further increased the imbalance with the share of the burden of treatment relying more heavily on women. Several forms of ART have been criticized for causing that imbalance and for reinforcing the conventional gender roles. The increased expectations regarding ART resulting from media publicity, the greater economic and emotional costs and the greater physical demands made on women, as well as failed ART, result in significant psychological distress and have significant social consequences for the couple.

Infertile men and women suffer the effects of their infertility throughout their lifespans and develop coping mechanisms in the context of their family and sociocultural environment. In many circumstances different forms of family and other social supports exist to help both partners. Research has focused on how women cope with infertility, but less information is available on the coping mechanisms of men with childlessness.

Recommendations

- Consumers of ART services should work closely with professionals and with governments to document

the health implications of infertility.

- Local and national patient support groups play an important role in advocating improved treatment of infertility and their establishment should be encouraged.
- Consumer groups should continue to play a critical role in advocating further research on ART, improving public health education, and striving to increase equity of access to ART.
- The terminology used to describe gamete and embryo donors and recipients, surrogate mothers, and their relationship to the offspring, needs to be defined and agreed upon.
- Prior to treatment, patients undergoing ART procedures involving gamete or embryo donation should be counselled about the implications of disclosure and nondisclosure to the child of its genetic origins. Subsequently, parents should have access to support when they are considering being open with the child about his/her genetic origins.
- Further studies should be conducted on the effects on the child of secrecy and disclosure about his/her genetic origins resulting from gamete or embryo donation.
- Further studies are needed of the consequences for parenting and child development of surrogacy, gamete and embryo donation and the use of donor insemination by lesbian and single heterosexual women.
- Studies are needed on the quality of parenting and the psychological well-being of children in families which have had multiple births by ART.
- Long-term epidemiological studies are needed on the psychomotor and cognitive development of ART children.
- In addition to the counselling provided in infertility clinics as routine support to infertile patients and their families, support groups, media discussions and internet exchange can also provide valuable inputs and these should be encouraged.
- The nature of gender-specific, psychosocial reactions to ART procedures and outcomes should be investigated.
- More research is needed on how men and women perceive and respond to infertility at different stages in their lives.
- Research is needed on how to reduce the consequences of infertility and especially its impact on women.

Section 5: Ethical aspects of infertility and ART

Papers

Patient-centred ethical issues raised by the procurement and use of gametes and embryos in assisted reproduction (Helga Kuhse)
When reproductive freedom encounters medical responsibility: changing conceptions of reproductive choice (Simone Bateman)
Ethical issues arising from the use of assisted reproductive technologies (Bernard M. Dickens)

ART has continuously challenged social norms, moral and ethical standards and legal systems. Although few areas in medicine have generated as much controversy as ART, in the past two decades ART has nevertheless been offered to many thousands of infertile couples and more than one million children have been born worldwide as a result of ART interventions. The impact of the controversies and the challenge can be seen at the individual, societal and political/legal levels.

The many possibilities that ART offers to infertile couples are often outnumbered by moral and ethical dilemmas regarding, among others, gamete donation, surrogacy, PGD, fetal reduction, embryo research or destruction of stored embryos. Many of the ethical concerns are present at the decision-making process of the infertile couple regarding treatment. At this stage of the process, the involvement of the health care provider is critical and has been the subject of debate. Health care professionals must respect patients' autonomy and have an obligation to ensure that patients have accurate and adequate information on the risks and benefits of particular treatments and of possible alternative treatments.

The controversies and, at the same time, the wide application of ART have impacted on the relationships and practices that condition and give meaning to reproduction in society. Issues of choice, reproductive freedom and medical responsibility should be seen within the broad scope of changing values in modern society.

The state often attempts to capture the controversies surrounding ART through regulation. In some cases, rapid recourse to legal regulation of ART may be counterproductive because it may restrict the opportunity for informed debate about existing options. Where laws and regulations restrict certain ART procedures, the ethics and legality of physicians' referral to other ART services require clarification.

Whether at the individual, societal or legal level, there are many unresolved issues in ART, including whether gamete and/or embryo donors and/or surrogate mothers should receive financial payment and, if so, whether or not it should be regulated; whether or not the right to equitable access to infertility treatment including ART should be provided to individuals and partners other than heterosexual married couples; the ethical acceptability of sperm and embryo management for purposes of sex selection; the fate of stored embryos if no contact can be made with the donors after a specified time interval.

Recommendations

- Procedures need to be established to ensure continuing multidisciplinary debate to shape the ethical framework of ART. This debate must include public participation.
- Procedures should be developed to allow stored embryos, for which there will be no clinical use, to be used for research or discarded. This research could be used to improve ART or the understanding of

embryo development, but must be legally permissible and carried out only with the prior informed consent of the donors.

- Informed consent should be provided by the donors of gametes, prior to their donation. This consent should address all possible immediate and future uses of the gametes:

  —for gamete or embryo research

  —to create embryos that will be used for treatment of the donors

  —to create embryos that will be used to treat others

  —the final disposition of the gametes if not used for treatment or research.

  The gamete providers should state what can, and cannot, be done with the gametes they provide or the embryos derived therefrom.

- Newly introduced procedures, as well as those currently used in ART, should be followed by surveillance of all treated individuals and offspring and include child development.

- It is proposed that the following terminology relating to ART should be used in the context of the above recommendations:

  —Animal studies *in vitro* and *in vivo*, and studies on human cells, gametes or embryos for which appropriate informed consent has been obtained, should be described as "preclinical research";

  —Initial clinical studies designed to test a scientific hypothesis derived from "preclinical research" should be described as "clinical research".

  —This is followed by well-designed, "randomized controlled trials" to provide statistical validation of the hypothesis developed from the "clinical research".

Section 6: National and international surveillance of ART and their outcomes

Papers

The Swedish experience in assisted reproductive technologies surveillance (Karl Nygren)
The Latin American Registry of Assisted Reproduction (Fernando Zegers-Hochschild)
Assessment of outcomes for assisted reproductive technology: overview of issues and the US experience in establishing a surveillance system (Laura A. Schieve *et al*.)
International registries of assisted reproductive technologies (Karl Nygren)

ART has been developed over the past two decades, during which time it has received considerable media and public attention. Concerns about safety and efficacy of the various ART procedures have led to governmental inquiries, legislation in some countries and, in others, action by professional bodies to develop monitoring schemes. A number of countries have set up ART surveillance providing information for policy-makers, health care professionals and consumers. There is significant variation among countries in the quality and quantity of data available, as well as in the definitions used to describe the procedures and their outcomes.

Both national and international data collection of reported ART results are still in the early stages of development, mainly as a result of difficulties in reaching consensus on definitions and the lack of standardized methodologies for data collection and analysis.

Recommendations

- The definitions of terms commonly used in ART should be agreed upon between ART providers and national and international registries.
- Guidelines need to be developed for the establishment of national and international ART registries.
- National ART surveillance programmes should be developed. These programmes should include non-ART methods of treatment and IUI with ovarian hyperstimulation.
- ART registries should be linked, where possible, with national health registries.
- Regional and global systems for managing data on ART outcomes should be further developed to allow analysis of time trends, of geographical differences and to provide information on new ART procedures.
- The principal outcome statistic of ART results should be singleton and multiple live-birth rates per treatment cycle initiated.
- National and international data on twin and higher-order multiple pregnancy rates resulting from ART and other forms of infertility treatment should be published.
- ART statistics need to emphasize the birth rates of healthy infants as well as rates of malformations, neonatal morbidity and mortality, and abnormalities of pregnancy.
- Malformation rates should include those associated with abortion, stillbirth and live birth.
- Data on fetal reductions should be included in ART registries.