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Male infertility update

The ESHRE Capri Workshop Group*

In September of 1997, the ESHRE Capri Workshop Group considered some of the emerging issues on male fertility (defined as the time to conception). Estimates of male or female factor infertility cannot easily be separated. Some idea of the contribution of the male factor can be obtained from the results of an investigation of a large number of male partners (the ESHRE Capri Workshop 1994). In a large WHO clinical study using a standardized investigation scheme (Rowe *et al.*, 1993), half of the men had normal semen measurements (no demonstrable cause group) and one-quarter had no aetiological factor that could be identified although semen measurements were abnormal (idiopathic abnormal semen group). Varicocele was the most common abnormality on examination, observed in 18% of men, but it was only considered an aetiological factor when associated with abnormal semen measurements. This combination was found in 12% of the men. A consideration of the patterns of diagnoses for both partners of the infertile couple (Farley, 1987) shows that minor degrees of fertility impairment in both partners are seen more frequently than expected. Such fertility impairments are not necessarily associated with infertility when present in only one partner, but may become important when present in both partners. The material discussed during the meeting could be divided into two categories: an evaluation of current information about possible causes of male infertility and recent data on several clinical approaches to alleviate the defect.

Evaluation of current information about possible causes of male infertility

Different areas that must be considered when discussing male reproductive health include: sexuality, virilization, fertilization and the effects of age, body weight and systemic disease on them.

Currently there is a lack of evidence regarding an inverse relationship between male fertility and ageing but age seems to have little effect on seminal fluid volume or sperm concentration (Nieschlag *et al.*, 1982; Handelsman and Staraj, 1985; Johnson, 1986). Body weight has less effect on gamete production than it does in women (Handelsman and Staraj, 1985; Koller *et al.*, 1989). The four following factors and their relation to male fertilizing ability are now considered critically: (i) androgen deficiency; (ii) antisperm antibodies; (iii) testicular histology in men with idiopathic azoospermia; and (iv) genetic abnormalities.

Androgen deficiency

Androgen deficiency can be suspected on the basis of clinical symptoms, disturbed spermatogenesis being one of them.

The clinical diagnosis of androgen deficiency requires biochemical confirmation but optimal biochemical criteria are uncertain (Table I). This uncertainty leads to a substantial underdiagnosis of classical androgen deficiency and to the under-recognition of non-classical partial androgen deficiency secondary to systemic diseases. The implied reference standard for diagnosis of androgen deficiency depends on measurement of the endogenous rate of testosterone production together with markers of tissue androgen sensitivity. Improved diagnostic criteria would require either easier methods to assess the two components of the standard variables or more convenient and valid surrogate variables. Ultimately, the overall or tissue-specific thresholds for androgen deficiency/repletion must be identified. Better understanding is needed of the pathogenesis of non-classical androgen deficiency, including hypothalamic hypogonadism related to catabolic states (cachexia, muscle mass and weight loss) and interaction with cytokines, nutrition, exercize and other hormones. The molecular and cellular basis of androgen effects on muscle and bone – including interaction with nutrition and exercize – are still poorly understood.

More investigation of androgen deficiency and larger controlled clinical trials of androgen therapy are needed. There is also a need for unequivocal definition of the treatment objectives and of the target populations, for long-term safety studies to develop convenient formulations of androgens with selective action.

Antibodies against spermatozoa

Antibodies against spermatozoa can be found in both normal and infertile males and females. Approximately 10% of infertile male partners possess auto-antibodies to spermatozoa in plasma, seminal plasma or attached to the surface of spermatozoa. Approximately 5% of infertile female partners have antibodies to spermatozoa in their circulation or in cervical mucus. It remains unclear whether sperm antibodies are: (i) a

*A meeting was organized by ESHRE (Capri, 7–8 September, 1997) with financial support from Ferring A.G. to discuss the subject. The speakers included J. Collins (Hamilton), P.G. Crosignani (Milano), D. Handelsman (Sydney), T. Hargreave (Edinburgh), I. Liebaers (Brussels), E. Nieschlag (Münster), G. Ragni (Milano), W. Schulze (Hamburg) and A. Van Steirteghem (Brussels). The discussants included J. Cohen (Paris), S.A. Davis (Kiel), E. Diczfalusy (Stockholm), K. Diedrich (Lubeck), R. Edwards (Cambridge), J. Egozcue (Barcelona), J.L.H. Evers (Maastricht), D. Mishell (Los Angeles) and B. Tarlatzis (Thessaloniki). This report was prepared by P.G. Crosignani and B.L. Rubin.

Table I. Diagnosis of androgen deficiency and its interpretations

Diagnosis	Parameters
Excluded	Normal spermatogenesis (testis volume >20 ml, sperm concentration >20 M/ml)
Unlikely	Testosterone 8–20 nM + normal LH presenting with impotence
Confirmed	Testosterone <8 nM (×2) Testosterone 8–20 nM + LH > 1.5× the upper limit of eugonadal normal range

LH = luteinizing hormone.

chance finding; (ii) a primary cause of infertility, or (iii) a sign of underlying defects in sperm membranes or sperm function.

Fifteen clinical studies have assessed the likelihood of pregnancy among infertile couples according to the presence of sperm antibodies in serum (Ansbacher *et al.*, 1973; Ingerslev *et al.*, 1980; Menge *et al.*, 1982; Baker *et al.*, 1983; Blumenfeld *et al.*, 1986; Witkin and David, 1988; Eggert-Kruse *et al.*, 1989; Francavilla *et al.*, 1992; Janssen *et al.*, 1992; Collins *et al.*, 1993; Rajah *et al.*, 1993; Daitoh *et al.*, 1995; Check *et al.*, 1995; Lahteenmaki *et al.*, 1995; Nagy *et al.*, 1995).

Six prospective and nine retrospective cohort comparison studies reported on 224 conceptions among 940 infertile couples in which one or both partners tested positive for sperm antibodies. The likelihood of conception reported in the couples from prospective studies is shown in Table II. Prospective studies show that antibody presence in either partner, when measured by the usual endpoints, is not a useful diagnostic test as a predictor of pregnancy. Antibody positive male partners who have more than 50% of the sperm surface coated with antibodies (about 10% of positive males) have reduced fertility (Abshagen *et al.*, 1998).

Compared with controls, pregnancy rate reductions in the presence of sperm antibodies were: 38% if sperm agglutination or immobilization titres were estimated (seven studies); 18% in the one study that made use of the enzyme-linked immunosorbent assay; 14% if the mixed antiglobulin reaction was tested (three studies) and 6% if immunobead testing was utilized (four studies). In a meta-regression analysis, however, the method used for antibody detection was not a significant variable ($P = 0.79$).

Non-specific treatments, condom use and intrauterine insemination did not avert the lower pregnancy rates observed in the antibody-positive couples.

In six estimates for couples undergoing in-vitro fertilization (IVF), the aggregate pregnancy rate was 28.5% for antibody-positive couples compared with 26.4% for antibody-negative couples (Janssen *et al.*, 1992; Rajah *et al.*, 1993; Check *et al.*, 1995; Daitoh *et al.*, 1995; Lahteenmaki *et al.*, 1995; Nagy *et al.*, 1995).

The better result for antibody-positive couples was due in large part to one study in which 11 (61%) of 18 antibody-positive couples conceived (Daitoh *et al.*, 1995).

In two estimates for couples undergoing intracytoplasmic sperm injection (ICSI), the aggregate pregnancy rate was 26.2% for antibody-positive couples (many of whom had failed fertilization in previous IVF cycles), compared to 28.4% for antibody-negative couples (Lahteenmaki *et al.*, 1995; Nagy *et al.*, 1995).

A separate analysis combined two randomized clinical trials of continuous corticosteroid therapy in men with circulating or attached to sperm antibodies (Haas and Manganiello, 1987; Hendry *et al.*, 1990; Bals-Pratsch *et al.*, 1992; Omu *et al.*, 1996). The typical odds ratio for pregnancy was 3.83 (95% CI 1.33 11.0) for treated couples compared with controls.

In conclusion, the overall likelihood of pregnancy is reduced by 12–18% in the presence of antibodies to spermatozoa in the female and 18% in the male partner respectively; and prospective studies making use of recent methods for detecting sperm antibodies show less impact on fertility than retrospective studies based on sperm agglutination and sperm immobilization methods. Nevertheless, where more than 50% of spermatozoa are antibody-bound, significant reductions in pregnancy rates were observed.

Although corticosteroid therapy seems to be effective, the side effects, including aseptic necrosis of the hip and osteoporosis, would deter many couples from considering this therapy.

The results of IVF and ICSI, the current standard treatments for couples with sperm antibodies, are similar to the respective results among couples without evidence of immunological infertility. With severe male immune infertility (>80% of spermatozoa antibody bound), ICSI may be the preferable treatment (Rajah *et al.*, 1993; Lahteenmaki *et al.*, 1995; Nagy *et al.*, 1995).

The main reason for testing for antibodies is to identify couples who may warrant earlier referral for assisted reproductive technology (ART).

Testicular histology in men with idiopathic non-obstructive azoospermia

To investigate whether the histological analysis predicts the chance of success for testicular sperm extraction (TESE), Schulze *et al.* (1984) carried out an accurate qualitative and quantitative evaluation of testicular biopsy samples.

Whenever possible, bilateral testicular biopsies were taken. Each biopsy sample was divided into four portions. One part was embedded in plastic and semi-thin sections were made (Holstein *et al.*, 1988, 1994). A second portion of the biopsy, which spatially overlapped with the first, was immediately subjected to a mild enzymatic sperm extraction procedure (test-TESE; Salzbrunn *et al.*, 1996). The remaining two biopsy fragments were cryoconserved, so that they could be used for the therapeutic TESE/ICSI treatment in the event that spermatozoa were detected. The characterization of normal spermatogenesis followed the criteria established by Holstein and Roosen-Runge (1981), Silber and Rodriguez-Rigau (1981) and Schulze and Rehder (1984). Pathological changes were classified according to a scoring system developed by De Kretser and Holstein (1976) (Table III).

The results of this analysis of a large number (N-576) of biopsies are reported in Table IV.

The patients were divided according to their follicle-stimulating hormone (FSH) concentrations. Among the patients with normal FSH (<8 mIU/ml), 188 of 233 biopsies (80.7%) gave scores >7 and contained mature spermatids. Of the biopsies

Table II. Relative likelihood of conception in couples with sperm antibodies: prospective studies, excluding ART treatments

Antibodies	Number of estimates	Pregnancy rate (PR)		
		Aggregate PR (%)		OR (95% CL) in affected couples
		affected	control	
In the male partner	3	23.5	26.4	0.84 (0.37, 1.91)
In the female partner	5	27.0	28.6	0.88 (0.49, 1.57)

ART = assisted reproductive technology.

Table III. Scoring system for testicular biopsies (De Kretzer and Holstein, 1976)

Score 1:	No seminiferous epithelial cells, tubular sclerosis
Score 2:	No germ cells, Sertoli cells only (SCO)
Score 3:	Spermatogonia only
Score 4:	No spermatids, few spermatocytes, arrest at primary spermatocyte stage
Score 5:	No spermatids, many spermatocytes
Score 6:	Few early spermatids, disturbance of spermatid differentiation
Score 7:	No late spermatids, many early spermatids
Score 8:	Few late spermatids
Score 9:	Many late spermatids, disorganized tubular epithelium
Score 10:	Full spermatogenesis

In the event that regional differences in the spermatogenetic status are noted within a histological section (e.g. focal occurrence of seminiferous tubules with SCO), then the scoring is differentiated accordingly [e.g. if there are both tubules with spermatogenesis (score 9) and Sertoli cell only area (score 2) the combined score will be 9,2].

with scores >7, 23 (12.2%) showed regional differences in the spermatogenetic status.

When mature spermatids were detectable in the histological section (score >7), then a positive result was obtained in the test-TESE. In some individual cases, even with scores <8, some spermatozoa could be extracted. Altogether, it was possible to isolate mature spermatids or spermatozoa suitable for ICSI for 114/125 patients (91.2%) with idiopathic azoospermia and normal gonadotrophin levels.

In contrast, in azoospermic males with elevated FSH (>8 mIU/ml), mature spermatids were detected in 99/343 (28.9%) biopsy samples.

Of the 99 biopsies with mature spermatids, 51 (51.5%) displayed regional variation in spermatogenetic status.

The post-operative TESE-test gave a more optimistic prognosis for this patient group than anticipated from the histological examination.

It was possible to extract mature spermatids or spermatozoa from the tissue of 105/191 (55%) patients with azoospermia and elevated gonadotrophin levels.

Nevertheless, the predictive value of the histological examination for the TESE result decreases for those cases with low scores.

In order to improve the success of ICSI using testicular spermatozoa (and thus also to reduce the risk of a frustrated treatment), a 'diagnostic' sperm extraction (TESE-test) carried out along with the histological evaluation (Salzbrunn *et al.*, 1996) is recommended.

Genetics of male infertility

The causes of male infertility still remain largely unknown. However, an increasing list of genetic defects leads to reproductive failure. Surveys showed in somatic karyotypes a higher incidence of numerical and structural chromosomal aberrations in infertile and subfertile men than in the neonatal population: 50 times more 47,XXY karyotypes, four times more 47,XYY karyotypes, 60 times more 46,XX karyotypes, 20 times more 46,X, derY karyotypes were found; Robertsonian translocations, reciprocal translocations, inversions, and additional marker chromosomes were respectively 8.5, five, eight and three times more frequent in subfertile men than in newborns. Most of the sex chromosomal aberrations were found in men without spermatozoa in their ejaculate (Van Assche *et al.*, 1996). More recently, the *SRY* gene on Xp was shown to be necessary to trigger male differentiation in embryos but its presence alone was insufficient to impose normal sperm production (Bogan and Page, 1994). Microdeletions of the q11 region of the Y-chromosome related to the dysfunction of deleted in azoospermia (*DAZ*) and RNA-binding motifs genes (*RBM* genes) have been reported in azoospermic as well as in oligoasthenoteratozoospermic men. Several Y-linked and at least one autosomal gene on human chromosome 3, classified as *YRRM*, *AZF* or *DAZ* in various situations are implicated not only in testicular but also in ovarian development (Ma *et al.*, 1992; Vogt *et al.*, 1996, 1998; Zhong *et al.*, 1996; Cooke *et al.*, 1996; Haberman *et al.*, 1998). Other mutations on the X chromosome such as these present in the Kallmann gene, the androgen-receptor gene and mutations in autosomal genes such as those causing myotonic dystrophy and the *CFTR* gene are known to interfere with normal spermatogenesis (Mak and Jarvi, 1996). Certainly more genes are involved, some acting on their own, some acting in collaboration with other genes and also interacting with external factors. Finally mitochondrial DNA may play a role in male infertility (St John *et al.*, 1997).

Approaches to deal with male infertility

In this broad area the following items have been discussed: (i) the role of human gonadotrophin preparations in male infertility treatment; (ii) surgical treatments; (iii) ICSI: are there any limits? and (iv) cryopreservation of semen before chemotherapy.

Table IV. Distribution (%) of histological scores in testicular biopsies carried out in 316 men with idiopathic non obstructive azoospermia

FSH	No. of patients	No. of biopsies	Score									
			1	2	3	4	5	6	7	8	9	10
Normal	125	233	0	10.3	1.3	2.1	0.4	5.2	0	18.5	44.6	17.6
Elevated	191	343	2	45.8	4.4	10.2	1.7	6.7	0.3	17.8	9.3	1.8

FSH = follicle-stimulating hormone.

The role of human gonadotrophin preparations in male infertility treatment

Because of the high success rate of gonadotrophin-releasing hormone (GnRH) or human chorionic gonadotrophin (HCG)/human menopausal gonadotrophin (HMG) in inducing pregnancies in the partners of hypogonadotrophic men (Kliesch *et al.*, 1994), these therapies were also applied to patients with normo- or hypergonadotrophic infertility. Numerous non-controlled open studies, over a period of more than two decades, attempted to demonstrate their effectiveness. However, a placebo-controlled, prospective, double-blind, randomized study of HMG/HCG treatment in normogonadotrophic men with oligoasthenoteratozoospermia (OAT) did not demonstrate any beneficial effect on a complex of sperm parameters and pregnancy rates, when compared with placebo (Knuth *et al.*, 1987).

With the arrival of purified HMG and later of recombinant human FSH preparations investigators again felt compelled to use these preparations empirically for treatment of idiopathic male infertility.

In 1992, a non-controlled study (Acosta *et al.*, 1992) reported an increase in in-vitro fertilization pregnancy rates in wives of men treated with highly purified HMG who had failed to fertilize oocytes on previous occasions. Recent studies also appear to be either non-controlled (Glander and Kratzsch, 1997) or to suffer from an inadequate number of subjects (Merino *et al.*, 1996; $n = 14$) or from an inadequate control group (Bartoov *et al.*, 1994). The only study employing randomization of the 148 couples allocated for IUI failed to provide placebo treatment in parallel to purified HMG, but left the control group untreated (Matorras *et al.*, 1997). Even so, these authors did not find a significantly higher pregnancy rate in the HMG-treated group.

A double-blind, randomized, placebo-controlled study with recombinant human FSH (Kamischke *et al.*, 1998) showed no improvement in classical semen parameters, even though there were a significant increase in testicular volume and an increased chromatin condensation in the sperm heads. Therefore, currently there is no basis for FSH or HMG treatment in idiopathic male infertility. Moreover, knock-out studies in mice revealed how a total deficiency of endogenous FSH had little or no effect on male fertility, but led to sterility in females (Rajendra Kumar *et al.*, 1997). Perhaps there are other regulatory systems that operate when FSH is depleted, although this evidence questions the role of FSH in male fertility.

Surgical treatments

Surgery to prevent later infertility

Repair of infantile hernia and hydrocele

There is some anecdotal evidence suggesting that persistent congenital hydrocele may keep the testis too warm and impair later fertility.

Surgery for testicular maldescent

This treatment probably makes little difference to later fertility but there is benefit in placing the testes in a position where they can be easily examined and testicular malignancy can be detected. Weak evidence exists that an operation before testicular development may spare later fertility (Whitaker, 1975).

Varicocele treatment in adolescents

Two prospective randomized trials indicate that both ipsi- and contralateral testis growth is promoted in those adolescents who were treated as compared with testicular size of those who were not (Laven *et al.*, 1992; Niedzielski *et al.*, 1997). The inference is that early treatment may prevent fertility problems in adult life but this has not been proven.

Surgery to help fertility in adult men

Varicocele treatment

There is controversy about the efficacy of this treatment, with conflicting results from four prospective studies (Nilsson *et al.*, 1979; Nieschlag *et al.*, 1995; Madgar *et al.*, 1995). All the studies had biases but it is notable that the two with positive results include slightly younger couples than the two with negative results. Further studies are needed and some are in progress (Holland and Germany). In future studies objective diagnostic tests to detect varicocele should be used: colour Doppler has been shown to be 97% sensitive and 94% specific (Trum *et al.*, 1996). The Tauber technique of antegrade sclerosis is a simple, low cost, day case, local anaesthetic procedure which is worthy of further evaluation (Tauber and Jojnsen, 1994).

Operations for testicular maldescent

These do not promote fertility when performed in adults.

Reversal of vasectomy

The results after first-time microsurgical vasectomy reversal exceed those of other treatments (Hargreave, 1994). The partner should be assessed and Fallopian tube patency tests should be performed if she has any history of disorder that may have caused tubal damage. If occlusion is detected then the best management will be MESA or TESA and ICSI.

Surgery to promote sperm delivery

Circumcision for phimosis

If there is a cellular ejaculate, the penis should be examined to exclude any phimosis as the cause for chronic infection.

Surgery for penile congenital abnormality

These are rare problems but any man who indicates that the penis is too deformed to allow sexual intercourse should have an examination of the erect penis after a pharmacological induced erection, e.g. after injection of prostaglandin into the corpora cavernosa (Fabbri *et al*., 1997; Bogaert, 1997). More often than not a significant anatomical problem will be revealed.

Surgery for urethral stricture

Stricture is best prevented by prompt treatment of sexually transmitted (STD) urethritis. Urethral stricture may be suspected if the man has a poor urine stream, poor sperm motility and has seepage of semen sometime after ejaculation.

Surgery to obtain spermatozoa for assisted conception

Testicular biopsy

As already reported in this paper, there is a change in practice: whereas testicular biopsy used to be performed for diagnostic purposes it is now increasingly performed in order to obtain spermatozoa for IVF or ICSI. Currently there are considerable differences in technique between centres and several questions remain to be answered. Is there any role for the diagnostic biopsy as a separate procedure to assess patients prior to MESA? When biopsy is performed to search for spermatozoa during an assisted conception attempt, how radical should the biopsy be before deciding that spermatozoa cannot be found? What are the complications of very radical attempts to find spermatozoa and what should men be told about hormone problems afterwards? Some men, having attempted sperm recovery, will have carcinoma *in situ* in their testicles: will this be recognized when biopsies are taken in the IVF–ICSI unit? Are second look testicular biopsies worthwhile if the first IVF–ICSI attempt fails? Is it better to freeze spermatozoa extracted from testis tissue or to freeze the tissue intact?

ICSI: are there any limits?

Since the birth of the first ICSI child in January 1992 (Palermo *et al*., 1992) this assisted fertilization procedure is now widely applied world-wide to alleviate severe male factor infertility (Tarlatzis, 1996; Van Steirteghem *et al.*, 1996). It can be used (i) for patients with quantitative sperm anomalies since only a single live (motile) spermatozoon is needed to micro-inject each fertilizable metaphase-II oocyte and (ii) for patients with qualitative semen anomalies, since ICSI bypasses most steps in the oocyte–sperm interaction required for fertilization in natural conception or conventional IVF. ICSI can also be used (i) with epididymal spermatozoa obtained by microsurgical or percutaneous epididymal sperm aspiration for most patients with obstructive azoospermia and (ii) with testicular spermatozoa retrieved from a testicular biopsy by open excisional surgical procedure or fine needle aspiration in the patients with obstructive azoospermia and some patients with non-obstructive azoospermia (Tournaye *et al.*, 1995; Verheyen *et al.*, 1995; Devroey *et al.*, 1996; Silber *et al.*, 1996; Crabbé *et al.*, 1997; Nagy *et al.*, 1997).

The practice of 6 years of ICSI (1991–1996) at the Brussels Free University Centre for Reproductive Medicine involved 6353 planned treatment cycles. ICSI could not be carried out in 3% of these cycles because no mature oocytes or no spermatozoa were available; the latter was the case for about 50% of patients with non-obstructive azoospermia due to germ-cell aplasia (Sertoli cell only syndrome) or maturation arrest (Tournaye *et al.*, 1997). There are no good clinical predictors for sperm retrieval in testicular biopsies from patients with non-obstructive azoospermia. ICSI was carried out with ejaculated spermatozoa in 87% of the cycles. About 10% of the injected metaphase II oocytes were damaged by the injection procedure. Normal fertilization (2-PN oocytes) occurred in 72% of the injected oocytes and 79% of these normally fertilized oocytes developed into cleaved embryos, which were of sufficient morphological quality to be transferred. The transfer of at least one embryo was possible in 93% of the cycles. The comparison of ICSI results was similar for the four types of spermatozoa used. The pregnancy (serum HCG positive) and delivery rate per transfer were 34% and 27% for ICSI with ejaculated spermatozoa, 43% and 31% for ICSI with freshly collected epididymal spermatozoa, 30% and 29% for ICSI with frozen–thawed epididymal spermatozoa and 29% and 22% for ICSI with testicular spermatozoa.

There are exceptional circumstances in which no injected oocytes fertilize and these normally can be associated with oocyte factors (very few metaphase-II oocytes or gross abnormalities in the oocytes) or with sperm factors (only totally immotile ejaculated spermatozoa being available for the injection or round-headed spermatozoa being injected) (Liu *et al.*, 1995). In ICSI, most of the laboratory steps are similar to conventional IVF. Therefore all the limitations of IVF, such as effect of maternal age or oocyte quality, apply also when the sperm insemination is done by ICSI. There have been recent reports on ICSI with round or elongated spermatidis. The use of these immature haploid male germ cells in the clinic requires further studies in terms of (i) definition of the target group, (ii) non-invasive reliable identification of these spermatids and (iii) assessment of its efficiency and innocuousness in preclinical experimentation (Angelopoulos *et al.*, 1997; Vanderzwalnen, 1997).

Because of the novelty of the ICSI procedure and of possible as yet unknown aspects of the outcome, couples were counselled and agreed to participate in prospective follow-up studies of the pregnancies and of the children born after ICSI. The first ICSI child was born in January 1992, and as of March 1997, 1672 children have been born in the Brussels Free University programme. Examination of 977 fetal prenatal karyotypes revealed the presence of 15 de-novo abnormal karyotypes, of which eight were sex chromosome abnormalities. The proportion of children with major congenital malformations causing functional impairment or requiring surgical correction was 2.3%.

With ICSI several thousand children have been born and the great majority of them are healthy. The incidence of major

malformations is not increased over that for IVF or naturally conceived children (Bonduelle *et al.*, 1996; Wisanto *et al.*, 1996). The possible slight increase in de-novo, mainly numerical sex chromosomal, aberrations of a paternal origin causes concern. It could be related to the existence of a greater number of 'chromosomally abnormal spermatozoa' (Liebaers *et al.*, 1995; Van Assche *et al.*, 1996). Transmission of balanced structural chromosomal aberrations, Y-deletions, X-linked or autosomal gene mutations causing in- or subfertility now occurs more frequently but can be prevented in a number of situations by preimplantation diagnosis (Liebaers *et al.*, submitted). The origin and frequency of de-novo deletions, especially on the Y chromosome, requires detailed studies.

In summary, the work-up is necessary for cases of male infertility requiring ICSI with ejaculated, epididymal or testicular spermatozoa. It should include at least a personal history and physical examination, a family history, karyotyping (conventional with and without FISH) which becomes mandatory for cases with azoospermia or of severe oligozoospermia with $<10\times10^6$ spermatozoa/ml.

A Yq11 analysis is essential in cases of non-obstructive azoospermia or extreme oligozoospermia with $<10^6$ spermatozoa/ml.

A CFTR analysis (also of the female partner) is needed for cases with congenital absence of the vas deferens (CAVD). Other tests have to be performed according to the history and physical examination and/or within the frame of research. This includes potential cases carrying the Kallmann gene, the myotonic dystrophy gene, or the androgen receptor gene, and also for mDNA, sperm chromosome analysis, and meiotic studies in the testes. Finally, further follow-up must be conducted on children born after ICSI and especially after ICSI with epididymal or testicular spermatozoa and eventually with spermatids. Karyotypes and more specific tests such as Y deletions should be performed at birth if not done prenatally.

Cryopreservation of semen before chemotherapy

Combined chemotherapy for various types of cancer, such as Hodgkin's disease and testicular tumours and for bone-marrow transplantation has progressed greatly in recent years. Many of the patients are young or even adolescent. Since there are no human data showing preventive measures helpful to preserve spermatogenesis, the cryopreservation of spermatozoa obtained before the start of treatment is perhaps the only known way to ensure the fertility of these young men.

All treatment regimens are toxic to spermatogenesis with the effects wearing off after some years in some instances (e.g. platinum-based regimens for testis cancer) whereas in others recovery is minimal even after a decade [e.g. mechlorethamine-oncovin-procarbazine-prednisone (MOPP) for Hodgkin's disease].

Ideally three sperm samples should be collected before chemotherapy starts. On balance, collection of samples after the start of treatment is preferable to not offering storage at all. This advice is despite the theoretical arguments, based on animal experimentation, that such spermatozoa might be adversely affected by the drugs or irradiation since these concerns are not known to be clinically significant in humans.

Pre-treatment semen analysis

Spermatogenesis is often impaired when men come to collect semen samples. This may be due to fever, weight loss or iatrogenic factors such as investigations, as well as other unknown factors (Berthelsen *et al.*, 1984; Ragni *et al.*, 1985; Viviani *et al.*, 1991; Botchan *et al.*, 1997). Due to the advent of ICSI, if the treatment is likely to lead to irreversible azoospermia (e.g., MOPP for Hodgkin's disease, bone marrow transplantation) then any spermatozoa should be frozen regardless of sperm concentration or quality in semen samples.

Post-treatment semen analysis

Following standard cisplatinum treatment, 80–100% of patients with testicular tumours had azoospermia or severe oligoasthenoteratozoospermia (Fossa *et al.*, 1985). Nevertheless, cisplatinum much more frequently allows eventual recovery, but it may take years and most men actually recover despite having only one testis, some with greater sperm production than their pre-treatment levels (when they had two).

The incidence of abnormal semen in patients with Hodgkin's disease varies, as this depends on the type of combination chemotherapy administered (Viviani *et al.*, 1991). The percentage of normal semen post-treatment may vary from 44–100%, depending on the drugs used. Recovery of spermatogenesis is very rare after MOPP but is the rule after adriamycin-bleomycin-vinblastine-dacarbazine (ABVD) (Ragni *et al.*, 1996).

In essence, recovery takes time and the longer one waits (e.g. childhood cancers) the greater the chance of recovery after less toxic regimens.

Currently, there are no pre-treatment parameters (seminal or hormonal) which are able to predict what the reproductive condition of the patient will be after treatment.

Advantage of pre-treatment cryopreservation of semen

Since chemotherapy is associated with high gonadal toxicity, the only way to preserve the reproductive capability of these patients is the pre-treatment cryopreservation of their semen (Sanger *et al.*, 1992). Ideally, three ejaculates should be frozen so that sufficient attempts at ART may be made. Ideally spermatozoa should be obtained and frozen prior to the start of treatment but in unusual circumstances where this is not possible sperm cryostorage may be undertaken even after the start of treatment. Studies conducted in animal models suggest that chemotherapy may have a mutagenic effect on late stage germinal cells (Meistrich, 1993). The clinical significance to the patient and his partner of such experimental findings remain unclear. These facts should be explained to the patient but should not preclude cryostorage. It is known that freezing leads to a loss in the quantity of spermatozoa and this is even more evident when an abnormal semen sample is cryopreserved, as occurs for many of these patients. For this reason, an attempt was made to optimize the freezing process. According to some authors, motility and cell survival are significantly better with slow freezing utilizing computer controlled biological freezers, especially for abnormal semen, than with rapid freezing methods using nitrogen vapours (Ragni *et al.*, 1990).

Pregnancy rates following the use of cryobanked semen are related to the type of assisted reproduction technique used. A considerable number of pregnancies has been reported following the use of different techniques.

In conclusion, until an effective and less toxic chemotherapy is found, we recommend that cryopreserving spermatozoa before therapy is beneficial for two reasons. Firstly, cryopreservation is a form of medical insurance in which spermatozoa are set aside and may be used if needed. These patients should be given general advice about the need for contraception when recovery is unpredictable, and advised to seek medical help early if fertility is required.

Secondly, various assisted reproduction techniques, from simple insemination to ICSI, may be used, depending on the quality of semen post-thawing. ICSI enables pregnancy to be achieved even with a reduced number of spermatozoa. Cryopreservation may also be recommended today for patients with severe semen disorders.

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