Human Reproduction Update 2000, Vol. 6, No. 5 pp. 432–441

Infections in IVF: review and guidelines

S.R.Steyaert[1,*], G.G.Leroux-Roels[1] and M.Dhont[2]

Departments of [1]Clinical Chemistry, Microbiology and Immunology, and [2]Obstetrics and Gynecology, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

Since the inception of in-vitro fertilization (IVF), questions about contamination and the transmission of infection have been raised. In this review, screening for *Chlamydia trachomatis*, as well as medical and ethical considerations on IVF in couples infected with hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV), are discussed. IVF is not contraindicated in case of HBV or HCV infection, but the decision is far more delicate in case of HIV infection. If donor gametes or embryos are used, prevention of infectious disease transmission resides in accurate donor selection, including screening for *C. trachomatis*, HIV, HBV, cytomegalovirus and *Treponema pallidum*. In the embryology laboratory, microbial contamination of the IVF system deserves attention, and can be prevented by using sterile technique and supplementing culture media with screened sera or serum substitutes and antibiotics. Persons whose biological material is to be cryopreserved should be screened for HBV, HCV and HIV, and separate containers should be used for infected and non-infected material. Finally, transmission of infectious diseases to laboratory personnel can be prevented by adherence to strict safety guidelines, wearing of protective clothing, HBV vaccination, prohibition of mouth pipetting, and developing a plan for the disposal of bio-hazardous material.

Key words: biosafety/contamination/ethics/IVF/screening

TABLE OF CONTENTS

Introduction
The fertility clinic
The embryology laboratory
Conclusions
References

Introduction

Assisted reproductive techniques such as IVF are increasingly being used. Besides the well-established prognostic factors for success, such as the age of the woman, the hyperstimulation protocol used, the number and the quality of transferred embryos (Lens and Rijnders, 1996), other factors such as contamination and transmission of infection can also impinge on the success rates of an IVF programme.

The purpose of this review is to highlight issues relating to infections during IVF practice, and to propose guidelines which may contribute to infection control and laboratory safety. In the first part, the discussion will focus on issues of infections in the fertility clinic, from the couple seeking infertility treatment, to infectious complications of ultrasound-guided oocyte retrieval, and finally a screening policy for donors of gametes and embryos. The second part will discuss issues of infections encountered in the embryology laboratory. First, microbial contamination of the IVF culture system, then, a safe cryopreservation protocol for gametes and embryos and, finally, the occupational risk of infectious diseases for laboratory technicians will be addressed.

The fertility clinic

IVF in couples with diseases that can be transmitted

Similar to the screening policies applied for women who do not need assisted fertilization techniques, testing for rubella and syphilis is recommended (Ron-El *et al*., 1992). Recently, the Chief Medical Officer's Expert Advisory Group on *Chlamydia* (Expert Advisory Group, 1998) has called for action to reduce the prevalence and morbidity of *Chlamydia trachomatis* infection. The Group recommends that consideration be given to screening couples attending for infertility investigations and treatment. With respect to the offspring that may result from the treatment of infertility, the European Society of Human Reproduction and Embryology (ESHRE) recommends the screening of both partners for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) before assisted reproductive technology procedures are started (Van den Eede, 1995). When one or both partners are found positive for one of these viral infections, the responsible physician may be confronted with a medical and ethical dilemma.

Infections in the female partner

C. trachomatis

This is the most common sexually transmitted disease in Europe and the United States, but awareness among sexually active women about chlamydial genito-urinary infection is poor (Centers

*To whom correspondence should be addressed at: Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium. Tel: +32 9 240 34 22; Fax: +32 9 240 4985; E-mail: sophia.steyaert@rug.ac.be

for Disease Control, 1997; Macmillan *et al*., 1999). Antibodies to *C. trachomatis* are more common in women with tubal factor infertility than in age-matched pregnant women (Persson *et al*., 1999). Unlike the association between cervical antibodies to *C. trachomatis* or human 60 kDa heat-shock protein and poor IVF outcome, reduced pregnancy rates in tubal factor patients are unrelated to these antibodies in the sera of patients (Spandorfer *et al*., 1999).

Screening is recommended before IVF (Expert Advisory Group, 1998). This guideline is not surprising, as lower genital tract infection with *C. trachomatis* is often asymptomatic, and as women undergoing infertility investigations and treatment may be at risk for upper-tract dissemination through hysterosalpingography, laparoscopy and dye hydrotubation, hysteroscopy, intrauterine insemination and/or embryo transfer (Macmillan and Templeton, 1999). Another reason for screening is the prevention of perinatal transmission of *C. trachomatis*, which can cause neonatal conjunctivitis and pneumonia (Centers for Disease Control, 1997). Newly available technologies such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) may become the test of choice for screening and detection of *C. trachomatis* (Cohen *et al*., 1998; Paavonen *et al*., 1998; Macmillan and Templeton, 1999). *Chlamydia* serology has little value, because previous chlamydial infections elicit long-lasting antibodies that cannot be easily distinguished from the antibodies produced in a current infection (Centers for Disease Control, 1993).

When *C. trachomatis* is detected, both partners should be treated. Indeed, if the male partner is not treated, reinfection may occur (Centers for Disease Control, 1993).

Chronic hepatitis B

Pregnancy does not seem to influence the course of a hepatitis B infection, and such an infection has no adverse effects on pregnancy (Mishra and Seef, 1992; Fédération des BLEFCO, 1997).

It is recommended that the hepatitis B surface antigen (HBsAg; Centers for Disease Control, 1991b) and hepatitis Be antigen (HBeAg) or hepatitis B virus (HBV) DNA (Fédération des BLEFCO, 1997) be determined in order to estimate the perinatal transmission risk (Table I). Indeed, the chance for a woman with a chronic HBV infection to transmit the infection to her child is 2–15% when she is only HBsAg-positive, and 80–90% if she is HBsAg-positive and HBeAg- or HBV DNA-positive (Botta-Fridlund, 1994; Fédération des BLEFCO, 1997; Pawlotsky, 1997; Michielsen and Van Damme, 1999). A chronically infected patient who is HBsAg-positive and HBeAg-negative can still be infectious, even when there are antibodies against HBeAg. These patients very often harbour a mutant HBV that is unable to secrete HBeAg but is otherwise fully competent to infect a host (Miyakawa *et al*., 1997; Scaglioni *et al*., 1997). In order to assess the infectivity in such a patient, it is recommended that HBV DNA be determined.

When the future mother is HBsAg-positive, the couple should be informed about the evolution of the disease in the mother and about infectivity and pregnancy, and they should sign a document containing this information. When the woman is also HBeAg- or HBV DNA-positive, a hepatologist should be consulted for treatment. HBV DNA levels should be monitored, and the assisted fertilization should preferentially be performed at a moment when the viral load is low (Fédération des BLEFCO, 1997). The transmission of perinatal HBV infection can be effectively prevented if the infant of a HBsAg-positive woman receives appropriate immunoprophylaxis. Hepatitis B vaccination and one dose of hepatitis B immunoglobulins, administered within 24 h after birth, are 85–95% effective in preventing both HBV infection and the chronic carrier state. Vaccination should be completed at 1 and 6 months. Testing for HBsAg and anti-HBsAg at 9–15 months of age will determine the success of the therapy and, in the case of failure, will identify HBV carriers or infants who require revaccination (Centers for Disease Control, 1991b). Breastfeeding does not seem to play an important role in the transmission of hepatitis B (Michielsen and van Damme, 1999). Finally, if not yet done, the partner and household contacts of HBsAg-positive women should be vaccinated (Centers for Disease Control, 1991b).

From an ethical point of view, there is no sound reason to advise against IVF treatment in a woman chronically infected with HBV, since in similar circumstances a spontaneous pregnancy is thought to be acceptable.

Table I. Vertical transmission rate for HBV, HCV and HIV related to the serostatus of the mother

Infection	Serostatus of the mother		Vertical transmission rate (%)
HBV	HBsAg+	HBeAg– and HBV DNA–	2–15
	HBsAg+	HBeAg+ and HBV DNA+	80–90
HCV	HCV Ab+	HCV RNA–	<1
	HCV Ab+	HCV RNA+	11
	HCV Ab+	HCV RNA+ and HIV Ab+	16
HIV	HIV Ab+		15–20

HBsAg = hepatitis B surface antigen; HBV DNA = hepatitis B virus DNA; HBeAg = hepatitis B e antigen; HCV Ab = hepatitis C virus antibodies; HCV RNA = hepatitis C virus RNA; HIV Ab = human immunodeficiency virus antibodies.
–, negative; + positive.

Chronic hepatitis C

Hepatitis C does not seem to manifest itself differently in pregnant women than in non-pregnant women. Otherwise, hepatitis C does not seem adversely to affect pregnancy (Mishra and Seef, 1992; Fédération des BLEFCO, 1997).

The serostatus of the future mother should be assessed, since this allows appreciation of the transmission risk to her child (Table I). For a woman who has antibodies (Ab) against HCV and who is HCV RNA-negative, the peripartal transmission rate is <1% (Michielsen and Van Damme, 1999). For a woman who has HCV-Ab and who is HCV RNA-positive, the transmission rate reaches 11% (Michielsen and Van Damme, 1999). For a mother who is concomitantly infected with HCV and HIV, the transmission rate ranges from 0% to 36%, with an average of 16% (Michielsen and Van Damme, 1999). These higher

transmission rates might be related to the increased HCV viraemia observed in HIV co-infected mothers (Novati *et al*., 1992; Zanetti *et al*., 1995).

When the future mother has HCV-Ab, the couple should be informed about the evolution of the disease in the mother, and about infectivity and pregnancy. The couple should also sign a document containing this information. When HCV RNA is positive, a hepatologist should be consulted for management of the hepatitis. Treatment with interferon-α is possible before starting the IVF procedure, but contraindicated during pregnancy (Fédération des BLEFCO, 1997). Interferon-α reduces the viral load, but sustained responses are seen in only 15–20% of patients treated (McHutchison *et al*., 1998). The novel combination of interferon-α and ribavirin seems to be more effective than treatment with interferon-α alone (Davis *et al*., 1998; McHutchison *et al*., 1998). The viral load should be followed, and IVF should be started at a moment of low viral load, preferentially 6 months after cessation of therapy. This drug-free period is recommended by the manufacturer. Treatment of HCV RNA-positive patients before IVF could also contribute towards minimizing the risks of nosocomial HCV transmission. Indeed, a recent report of HCV transmission during the ancillary procedures for assisted conception warrants prudence when including HCV-infected patients (Lesourd *et al*., 2000). In contrast to HBV, it is not possible to protect the neonate of a HCV-infected mother at birth. The development of an HCV vaccine is hampered by the presence of multiple viral genotypes (Choo *et al*., 1994), and post-exposure administration of immunoglobulins appears to offer little protection (Mishra and Seef, 1992). Children of HCV-infected mothers should be followed with HCV RNA testing soon after birth and few months thereafter or, alternatively, by HCV antibody screening at 18 months (Palomba *et al*., 1996; Centers for Disease Control, 1998c). There is no need to discourage breastfeeding (Michielsen and Van Damme, 1999).

Since women with a chronic HCV infection are allowed to become pregnant spontaneously, the ethical considerations are similar to those cited for HBV.

HIV

Most HIV-infected women are in their reproductive years (Coggins and Segal, 1998), and it is quite likely that they continue to want and to have children (European Collaborative Study, 1996). This tendency may rise as medical treatment is becoming increasingly effective (Minkoff, 1998). Although there is good evidence that pregnancy does not affect early progression of HIV disease (Berrebi *et al*., 1990; Alger *et al*., 1993), its effect on maternal health in women with advanced disease is less certain (Newell and Thorne, 1997). Studies of asymptomatic women have not shown an increased risk of obstetric complications and adverse perinatal outcome (Alger *et al*., 1993). Little information is available on the use of anti-retroviral drugs other than zidovudine during pregnancy (Mofenson, 1996). However, guidelines for optimal anti-retroviral therapy and for initiation of therapy in pregnant HIV-infected women are the same as those delineated for non-pregnant adults. The decision to use anti-retroviral drugs during pregnancy should be made by the woman following discussion regarding benefits and risks, and will also depend on the likelihood of adherence to the prescribed treatment regimen (Centers for Disease Control, 1998b).

The mother-to-child transmission rate of HIV in Europe is estimated between 15% and 20% (Table I), and is thought to increase with disease progression (European Collaborative Study, 1996). The prognosis for an HIV-infected child is not good: 17% of infected babies become ill and die before one year (European Collaborative Study, 1991). In infants whose HIV infection is maternally acquired, the rate of disease progression varies directly with the severity of the disease in the mother at the time of delivery (Blanche *et al*., 1994). Even if the child is not infected, there is still a chance that the HIV-infected parent(s) may die in the child's early infancy (Newell and Thorne, 1997).

Every couple in which the future mother is HIV-positive should be counselled by a multidisciplinary team (Stratton *et al*., 1992) to enable them to make an informed decision. Counselling should include information regarding what is known about infectivity, pregnancy, risk factors for perinatal transmission, prophylaxis, parental illness and death, and psychosocial factors (Smith *et al*., 1991). If assisted fertilization techniques are started, prophylactic anti-retroviral treatment with zidovudine should be given to the mother during pregnancy and labour, and to the newborn for the first 6 weeks of life (Connor *et al*., 1994). No adverse effects were observed in HIV-uninfected children with in-utero and neonatal exposure to zidovudine (Culnane *et al.*, 1999). Elective Caesarean section is another intervention that may reduce the vertical transmission risk (The International Perinatal HIV Group, 1999). The additional risk of transmission through breastfeeding is estimated to be approximately 14% among children born to mothers who were already HIV-positive (Dunn *et al*., 1992). Therefore, HIV-infected mothers in industrialized countries are advised to refrain from breastfeeding (Global Programme on AIDS, 1992). The neonate should be tested for HIV-DNA within 48 h after birth, at age 1–2 months, and at age 3–6 months (Centers for Disease Control, 1998a). HIV infection can be definitively excluded if HIV antibody is negative at age 18 months and if the child has no clinical symptoms of HIV infection, and virological assays for HIV are negative (Centers for Disease Control, 1998b).

Do medical staff have the right to deny the pregnancy wish of a well-informed couple in which one or both partners are HIV-positive? While the dilemma is most likely minimal for women with advanced HIV disease, the issue is less clear-cut in those who are asymptomatic and have CD4 counts within the normal range. The only guideline issued by the Human Fertilisation and Embryology Authority relevant to this issue is that doctors should put the welfare of the child first (Olaitan *et al*., 1996). A legal analysis of infertility treatment and HIV has been discussed (Smith *et al*., 1991), but whatever decision is taken regarding the treatment, it is important for the medical team to explain this decision and its rationale clearly and honestly to the couple (Olaitan *et al*., 1996).

Infections in the male partner

Chronic hepatitis B

To prevent transmission of HBV to the female partner, and thus to prevent perinatal transmission to the child, the woman should be vaccinated (Centers for Disease Control, 1991b). Once the anti-HBsAg titre has risen above 10 mIU/ml (Centers for Disease

Control, 1991b), she is protected and IVF can be carried out (Botta-Fridlund, 1994; Fédération des BLEFCO, 1997; Pawlotsky, 1997).

Some years ago, it was shown (Hadchouel *et al*., 1985) that HBV DNA may be present in integrated form in the spermatozoa, at least during the acute phase of HBV infection. Integration of HBV DNA might be explained by the reverse transcriptase activity of the viral DNA polymerase (Pawlotsky, 1997). The hypothesis of a new type of vertical transmission via the germline raises new questions to be answered in the future.

Chronic hepatitis C

A vaccine for HCV is not available (Choo *et al*., 1994), but as the sexual transmission rate of HCV is small (Rooney and Gilson, 1998), IVF is not contraindicated (Duffaut and Valla, 1997; Fédération des BLEFCO, 1997; Pawlotsky, 1997). The couple should, however, be informed and should sign a document with the information given. When a chronic active hepatitis is concerned, it is recommended that the male partner be treated before starting an IVF procedure (Duffaut and Valla, 1997).

Since HCV is a RNA virus lacking reverse transcriptase activity, it is impossible that the viral RNA can integrate into the genome of the host (Pawlotsky, 1997).

HIV

As discussed earlier, every couple in which the future parent is HIV-positive should be carefully counselled. If assisted fertilization is started, it will be of great importance to reduce the transmission risk of HIV to the female partner and the future child. It has been shown that anti-retroviral treatment results in a significant drop of the viral load in semen (Gupta *et al*., 1997; Vernazza *et al*., 1997). However, replication-competent provirus DNA has been demonstrated in the seminal cells of HIV-1 infected men after highly active anti-retroviral therapy (Zhang *et al*., 1998). It is strongly recommended that the spermatozoa be isolated and that contamination with white blood cells be avoided as seminal white blood cells are host cells for HIV in the semen of HIV-infected men (Mermin *et al*., 1991; Quayle *et al*., 1997). Some data have been published on the removal of cell-associated HIV from the semen of HIV-seropositive men by gradient centrifugation and repeated washing, followed by a swim-up procedure (Semprini *et al*., 1992; Chrystie *et al*., 1998).

Controversy surrounds the question of whether spermatozoa and sperm cells can be infected with HIV. A number of groups have presented data suggesting that HIV attaches to and infects spermatozoa (Bacetti *et al*., 1994; Bagasra *et al*., 1994). The first group (Baccetti *et al*., 1994) described the transfer of HIV-1 by the spermatozoon into the oocyte. These findings raised new questions concerning not only the problem of HIV transmission to children, but also transmission to women (Douglas *et al*., 1998).

Infectious complications of ultrasound-guided oocyte retrieval

Ultrasound-guided oocyte retrieval is now being used as the method of choice for oocyte collection (Lenz *et al*., 1987). Complications are only rarely reported, even though the treatment is not without potential risks. The occasional finding of bacteria in a follicular aspirate (Artley *et al*., 1993) suggested a possible mechanism for postoperative infection. Others (Curtis *et al*., 1991) assessed the infectious risk of oocyte retrieval by culturing peritoneal fluid of 25 women with unexplained infertility, and found peritoneal cultures negative in all patients but one. In other reports, postoperative infections occurred with a rate varying between 0.3% and 1.5% (Bergh and Lundkvist, 1992; Bennett *et al*., 1993; Tureck *et al*., 1993).

Postoperative pelvic infections are obviously the result of direct inoculation of vaginal organisms into the peritoneal cavity by the collecting needle (Bennett *et al*., 1993). Disinfection of the vagina is, however, not advisable as shown by comparative vaginal disinfection with a 1% solution of povidone-iodine and normal saline (van Os *et al*., 1992). Disinfection of the vagina with povidone-iodine was related to a lower pregnancy rate per puncture, whereas rinsing with saline did not seem to increase the risk of infection. The potential embryotoxic effect of disinfectants (especially iodine compounds) has been described by others (Gembruch *et al*., 1988). Regarding the low incidence of postoperative infections, the value of using prophylactic antibiotics is questionable (Bennett *et al*., 1993).

Screening of donors

In addition to adequate history-taking and physical examination, The American Fertility Society, the European Society of Human Reproduction and Embryology (ESHRE) and the British Andrology Society recommend the screening of semen donors for *C. trachomatis*, HIV, HBV, *Treponema pallidum* and cytomegalovirus (CMV) (The American Fertility Society, 1990; Van den Eede, 1995; British Andrology Society, 1999). Whenever possible, oocyte donors should be screened for infectious diseases, including HIV. Since a quarantine period for HIV is not practicable for fresh oocyte donation, recipients should be counselled and informed that there is a risk of HIV infection (Van den Eede, 1995). Donors of oocytes to be frozen should be screened in a similar manner as semen donors, for whom the screening guidelines will be discussed here.

Urethral swab for C. trachomatis *and* Neisseria gonorrhoeae

As conventional microbiological techniques can fail to detect *C. trachomatis* in semen and urethral specimens, it is recommended that DNA amplification techniques such as PCR be used for the detection of *C. trachomatis* (van den Brule *et al*., 1993). A urethral swab for *Neisseria gonorrhoeae* is recommended by The American Fertility Society and the British Andrology Society, not by ESHRE. Urethral swabs should be obtained initially and should be repeated at 6 month intervals or more frequently if clinically indicated (The American Fertility Society, 1990).

Serum screening for HIV, HBV, syphilis and CMV

Screening for HIV is based on the detection of HIV-antibodies (The American Fertility Society, 1990). Recently, a woman became infected with HIV following artificial insemination with fresh semen from a seronegative donor (Matz *et al*., 1998). Therefore, considering the seroconversion time of an HIV infection, it is no longer warranted to use fresh semen (Hamer *et al*., 1995). The initial serum screening for HIV should be repeated after 6 months, and the frozen semen specimen should be released for use only after this quarantine and if the test results are negative again. As PCR techniques that allow for the detection of

virus particles in semen samples become more available and practicable, the 6-month quarantine principle might be changed in the future (The American Fertility Society, 1990).

For HBV screening, serum HBsAg is the marker of choice (The American Fertility Society, 1999; Centers for Disease Control, 1991a). It is the earliest indicator of the presence of an acute infection, as well as being a marker of chronicity (Hoofnagle and Di Bisceglie, 1991). Serum HBsAg should be obtained initially and at 6-month intervals (The American Fertility Society, 1990).

Serological screening for *T. pallidum* is still recommended considering the extremely high morbidity associated with congenital infection (Hollier and Cox, 1998). Serological tests should be obtained initially and need not be repeated unless clinically indicated (The American Fertility Society, 1990).

To screen for CMV, serum antibody titres IgG and IgM should be obtained (The American Fertility Society, 1990, The British Andrology Society, 1999). If the CMV titres of a donor are negative, the donor should be re-tested at 6-month intervals, and the quarantined samples should not be released if there is a seroconversion suggesting recent CMV infection (The American Fertility Society, 1990, The British Andrology Society, 1999). If the donor is sero-positive (IgG positive and IgM negative), opinions differ. Some authorities believe that the donor semen may be used with CMV-positive women; others feel that CMV-positive women may still be vulnerable because there are multiple strains of CMV and because CMV recurrence in a previously infected woman is more likely if she is multiply exposed to CMV by sexual activity prior to pregnancy (The American Fertility Society, 1990, The British Andrology Society, 1999).

Semen culture

According to the ESHRE committee, a semen culture for sexually transmitted diseases should always be obtained, while The American Fertility Society recommends an optional semen culture in case of leukocytospermia which is defined as >10 white blood cells per high-powered field The donor should be reexamined at 6-month intervals when follow-up tests are obtained (The American Fertility Society, 1990; Van den Eede, 1995).

Serum screening for HCV

Although not recommended by ESHRE or The American Fertility Society, screening of gamete donors for biological markers of HCV is mandatory in France (Gromb *et al*., 1995) and the United Kingdom (McKee *et al*., 1996), and recommended by the British Andrology Society Centers for Disease Control (Centers for Disease Control, 1991a) and the National Institutes of Health Consensus Development (National Institutes of Health Consensus Development Conference Panel Statement, 1997).

The embryology laboratory

Microbial contamination in an IVF culture system

An IVF culture system should be a sterile system (Cottell *et al*., 1996). Therefore, correct procedures are needed for preparing and storing culture media. In order to avoid viral contamination from the serum component, the use of safe supplements is mandatory. Since neither seminal fluid nor the vagina are sterile environments (Toth and Lesser, 1981; Masfari *et al*., 1986), care must be taken when carrying out associated clinical and laboratory procedures in order to minimize microbial transfer.

Preparation of culture medium

Endotoxin concentration in IVF media have been cited as a cause of reduced clinical successes (Snyman and Van der Merwe, 1986; Fishel *et al*., 1988). In both reports, the source of endotoxin was culture medium contaminated upon receipt or during incorrect storage.

It is apparently extremely important that highly purified water be used that is not only free of organics, but also of pyrogens (Marrs, 1986). If water is produced on site, a comprehensive programme of quality control for the water system must be in place, including endotoxin tests and bacterial contamination (colony) testing (The American Fertility Society, 1992). Endotoxin testing of purchased water is recommended if it is not certified to be endotoxin-free (The American Fertility Society, 1992). All media preparation should be performed using sterile techniques, including location and appropriate environment (The American Fertility Society, 1992). As far as possible, disposable materials should be used (The American Fertility Society, 1992). When glassware (Erlenmeyer flasks, pipettes, etc.) is used, it should be properly cleaned with cleansing solutions, rinsed with ultra-pure water and, if necessary, sonicated to remove substances that adhere to the surface (Hammitt *et al*., 1990). Whenever possible, glassware should be heat-sterilized (The American Fertility Society, 1992). Appropriate refrigerated facilities should be available for the storage of media (The American Fertility Society, 1992).

Microbial contamination of the culture medium from the serum component

The hazard associated with the use of serum and serum substitutes in culture media is illustrated by a hepatitis B epidemic that occurred among women treated at an IVF centre in the Netherlands. The epidemic was caused by a human serum pool contaminated with hepatitis B virus (van Os *et al*., 1991).

Contamination can be prevented by using only the patients' own blood for preparing the culture medium (van Os *et al*., 1991). When donor serum is necessary, one should use the serum of a thoroughly screened (HBV, HCV, HIV, *T. pallidum*) donor or fetal cord blood (van Os *et al*., 1991; The American Fertility Society, 1992; Van den Eede, 1995; Peeters, 1996). A safe alternative is the use of serum substitutes such as pasteurized plasma protein solution or human serum albumin (Ménézo *et al*., 1984; Peeters, 1996; Laverge *et al*., 1997). These products should be tested as a serum source, or should be certified to be free from HBV, HCV and HIV (The American Fertility Society, 1992). Their use is becoming more and more general and, as has been shown, without loss of quality (Ashwood-Smith *et al*., 1989; Laverge *et al*., 1997). Much research is being done on the production of recombinant plasma proteins such as human serum albumin, but these products are not yet available (Goodey, 1993). The use of animal products such as bovine serum albumin is contraindicated because the absence of bovine pathogens can never be ascertained, and because patients can become sensitized to such foreign proteins (Ashwood-Smith *et al*., 1989).

Microbial contamination of the culture medium from follicular fluid

Commensals of the female tract can contaminate follicular fluid and lead to contamination of an IVF culture system (Cottell *et al*., 1996; Liversedge *et al*., 1996). Contamination of the follicular fluid originates from the vagina during the transvaginal oocyte collection process (Cottell *et al*., 1996).

Disinfection of the vagina is however not advisable because of the potential embryotoxic effect of disinfectants, especially iodine compounds (van Os *et al*., 1992). Gentle cleaning of the oocyte after retrieval in a small dish containing culture medium is likely to dilute any contaminating microbes. This physical processing, in combination with the bacteriostatic activity of penicillin and streptomycin present in the medium, appears to be effective in managing commensal contamination (Cottell *et al*., 1996).

Microbial contamination of the culture medium from seminal fluid

Bacteriospermia is a common phenomenon that does not necessarily point towards infection (Stovall *et al*., 1993). Contamination of the culture system by seminal microorganisms may however lead to oocyte degeneration (Huyser *et al*., 1991), suboptimal fertilization rates (Hewitt *et al*., 1985) or impaired embryonic development (Guillet-Rosso *et al*., 1987).

There are several approaches to reduce microbial contamination of the culture medium from the seminal fluid. The first involves counselling of the partner or sperm donor about a sterile technique for semen collection. Detailed verbal and written instructions (Boucher *et al*., 1995) should include, besides a 2- to 3-days abstinence period, the washing of hands and genital areas with soap and water before towel drying (World Health Organization, 1992). Routine semen cultures are not beneficial in asymptomatic couples undergoing IVF (Stovall *et al*., 1993; Liversedge *et al*., 1996). The benefits of antibiotic treatment in asymptomatic couples is also limited (Eggert-Kruse *et al*., 1988; De Geyter *et al*., 1994; Liversedge *et al*., 1996). Physical processing of spermatozoa by sperm washing and a swim-up processing technique is effective in reducing the commensals present in semen (Wong *et al*., 1986; Cottell *et al*., 1997). The addition of culture medium may provide further reduction of commensals by significant dilution (Stovall *et al*., 1993). Another effective measure in managing commensal contamination is the enrichment of culture media with antibiotics such as penicillin and streptomycin (Karlström *et al*., 1991; Cottell *et al*., 1997). If bacteria persist after processing, this may be due to insensitivity of the bacteria to the antibiotics used and lead to bacterial overgrowth of the culture medium (Cottell *et al*., 1997).

An overview of the guidelines proposed to prevent microbial contamination of the culture medium is given in Table II.

Viral contamination from cryopreservation

At present, spermatozoa and human embryos are commonly frozen in liquid nitrogen using straws (Brotherton, 1990; Naaktgeboren, 1996). Since a straw may leak or shatter during freezing, or its plug may be blown during thawing (Russell *et al*., 1997), the potential for contamination of liquid nitrogen represents a real danger. This danger is illustrated by the recent report of hepatitis B transmission from a contaminated cryopreservation tank (Tedder *et al*., 1995). In this particular case, the contaminated specimens were bone marrow and peripheral blood stem cells, but it is quite likely that this can also happen during the preservation of semen, oocytes and embryos.

In order to improve the safety of cryopreservation, all patients and donors whose spermatozoa, oocytes or embryos will be cryopreserved, should be screened for HBV, HCV, HIV and *T. pallidum* (Tedder *et al*., 1995; Peeters, 1996). If a patient or donor is HIV-positive, one can argue whether samples which may have potential risks for a future child should be stored in the first place (Hargreave and Ghosh, 1998). If storage is considered, it is highly recommended that the infected materials be stored in separate containers for each infection (Tedder *et al*., 1995; Massey *et al*., 1996). When gametes or embryos are taken up from medium, the external surface of the straw can become contaminated. Therefore, the serum used in media should be screened (see above) (Peeters, 1996). The use of 0.5% chloramine for external decontamination has been suggested, although the efficacy is certainly not 100% reliable (Naaktgeboren, 1996; Peeters, 1996). It has been shown (Russell *et al*., 1997) that straws filled using a traditional 'dip and wipe' method and sealed with polyvinyl alcohol (PVA) powder demonstrated a significant degree of leakage, while straws filled using an aseptic technique did not leak, irrespective of the sealing method used (PVA powder, plastic spheres and plasticine modelling clay). Since screw-cap tubes may also leak during freezing (Naaktgeboren, 1996), they do not represent a superior alternative to straws. Glass ampoules should certainly not be used as they may shatter during the

Table II. Proposed guidelines to avoid microbial contamination of the culture medium

During preparation of the culture medium
1. Use highly purified water
2. Perform endotoxin tests and bacterial contamination testing
3. Use sterile technique
4. Use disposable equipment whenever possible
5. Clean glassware properly, and heat-sterilize
6. Store media at 4°C
From the serum component
7. Use the patients' own blood; or
8. Use thoroughly screened sera or fetal cord blood; or
9. Use serum substitutes
From follicular fluid
10. Rinse the vagina with normal saline
11. Reduce number of microorganisms by physical processing
12. Enrich culture medium with penicillin and streptomycin
From semen
13. Give detailed verbal and written instructions for semen collection
14. Reduce number of microorganisms by physical processing
15. Enrich culture medium with penicillin and streptomycin

freezing–thawing procedure (Brotherton, 1990), and as sharp rims represent an infectious threat to laboratory technicians. Finally, the storage container should be periodically emptied and cleaned. This guideline is based on the risk of lost straws or small particles of contaminated material falling to the bottom of a large container (Hargreave and Ghosh, 1998).

Laboratory technicians at risk of blood-borne pathogens

Employees working in a reproductive laboratory are exposed to semen, blood and follicular fluid which are potentially infected with HBV, HCV and HIV. Infection can be transmitted through contact with the skin or the mucous membranes, or by ingestion. In general, HBV infection is the most common laboratory-acquired infection. The infectious risk for laboratory workers is approximately 10-fold that of the general public, and almost 3-fold that of other hospital employees (Sewell, 1995). This risk is so high because the virus is frequently present at very high concentrations (10^7–10^8 infectious particles/ml) in the blood of a patient. HCV is less contagious because it circulates in the blood at lower concentrations (10^4–10^6 infectious particles/ml). HIV is generally present in the blood at still lower concentrations than HCV. The risk of becoming infected following a needle stick injury is from 2% to 40% for HBV, 3% to 10% for HCV and 0.2% to 0.5% for HIV (Gerberding, 1995). The transmission risk for HIV is believed to be greater for injuries with a hollow-core needle than for injuries with a solid needle (Gerberding, 1995; Sewell, 1995).

In 1992, The American Fertility Society formulated guidelines for human embryology laboratories including safety guidelines and infection control measures which are outlined in the following paragraph (The American Fertility Society, 1992).

Laboratory technicians should handle every body fluid sample as if it were contaminated. Although the intact skin is an excellent barrier, small cuts and abrasions are often present (Sewell, 1995). Therefore, protective, non-toxic (non-powdered) gloves should be worn when handling fresh or frozen body fluids or any containers that have come into contact with body fluids. Extraordinary precautions should be taken to avoid accidental wounds from sharp instruments contaminated with body fluids. A laboratory coat or disposable gown should be worn in the laboratory, and removed upon leaving the laboratory. Safety glasses or goggles are suggested where appropriate. The use of a mouth mask, or in some cases full face protection, should be considered when procedures are conducted which have a high potential for creating aerosols or droplets. To avoid the formation of aerosols, tubes should always be capped when centrifuged, and centrifugation should be carried out in a closed centrifuge. Bench tops and instruments should be cleaned and decontaminated regularly using, for example, diluted sodium hypochlorite. The laboratory should contain a hand-washing sink and an eyewash station. Hands should be washed after removing gowns and gloves, and immediately if they become contaminated with body fluids. All handwashing should be done with disinfectant soap and hot water. Mouth pipetting is still being used in many reproductive laboratories to strip eggs and to verify fertilization. In the USA however, mouth pipetting is prohibited by The American Fertility Society and by the regulations of the OSHA (National Institute for Occupational Safety and Health) (Gerrity, 1993). Mechanical pipetting devices should be used for the manipulation of liquids in the laboratory. Eating, drinking, smoking, application of make-up, or manipulation of contact lenses are not allowed in the laboratory. Food and beverages should not be stored in refrigerators designated for the storage of clinical specimens and other laboratory materials. Reproductive laboratories must have a plan to manage the disposal of bio-hazardous materials. Finally, since an effective and safe hepatitis B vaccine has been available for several years, all employees working in the laboratory should be offered vaccination for HBV.

An overview of these guidelines is given in Table III.

Safety programme

In order to reduce transmission of infectious diseases in the embryology laboratory, a comprehensive safety programme should be designed and implemented. Not only are written policies mandatory in a safety programme, but also continuous employee training. In order to obtain tight adherence to safety practices, it is imperative that laboratory technicians are provided with an understanding of the programme's safety principles (The American Fertility Society, 1992).

Conclusions

Assisted procreation is a novel domain of medicine where numerous acquisitions of modern science are rapidly applied. IVF has not only been fuelled by progress made in areas of gynaecology, endocrinology, cell biology and biochemistry, but fundamental work in microbiology has also had a major impact on the safety and decision-making in the fertility clinic.

Within the laboratory, as well as in the clinic, a series of high-

Table III. Proposed guidelines to protect laboratory workers from the hazard of occupational infections

1.	Handle every body fluid as if it were contaminated
2.	Wear protective, non-toxic (non-powdered) gloves
3.	Wear a laboratory coat or disposable gown
4.	Wear safety glasses or goggles where appropriate
5.	Wear mouth masks or full face protection if formation of aerosols or droplets is possible
6.	Cap tubes while centrifuged
7.	Use a closed centrifuge
8.	Clean and decontaminate bench tops and instruments regularly
9.	Provide a hand-washing sink and an eyewash station
10.	Wash hands after removing gowns and gloves, and immediately if they become contaminated
11.	Use mechanical devices for pipetting
12.	Prohibit eating, drinking, smoking, application of make-up, or manipulation of contact lenses in the laboratory
13.	Do not store food and beverages in refrigerators designated for the storage of clinical specimens and other laboratory material
14.	Implement a plan for disposal of bio-hazardous materials
15.	Vaccinate employees for HBV

risk events are undertaken, during which infectious agents can be transmitted. It is not only laboratory technicians and healthcare workers who are at risk, but also the future mothers and their desired offspring. It should be the challenge of each fertility clinic to reduce this risk, and this requires both guidelines and standard operating procedures to be established by each centre. The maintenance of these rules requires the permanent attention and awareness of all participants in the process—a situation which can only be achieved by the installation of a safety programme of quality assurance, together with the continuous motivation and education of the staff, and counselling of the couple who require assistance.

References

Alger, L.S., Farley, J.J., Robinson, B.A. *et al*. (1993) Interactions of human immunodeficiency virus infection and pregnancy. *Obstet. Gynecol*., **82**, 787–796.

Artley, J.K., Braude, P.R. and Cooper, P. (1993) Vaginal squamous cells in follicular aspirates following transvaginal puncture. *Hum. Reprod*., **8**, 1272–1273.

Ashwood-Smith, M.J., Hollands, P. and Edwards, R.G. (1989) The use of Albuminar 5^{TM} as a medium supplement in clinical IVF. *Hum. Reprod*., **4**, 702–705.

Bacetti, B., Benedetto, A., Burrini, A.G. *et al*. (1994) HIV-particles in spermatozoa of patients with AIDS and their transfer into the oocyte. *J. Cell Biol*., **127**, 903–914.

Bagasra, O., Farzadegan, H., Seshamma, T. *et al*. (1994) Detection of HIV-1 proviral DNA in sperm from HIV-1 infected men. *AIDS*, **8**, 1669–1674.

Bennett, S.J., Waterstone, J.J., Cheng, W.C. *et al*. (1993) Complications of transvaginal ultrasound-directed follicle aspiration: a review of 2670 consecutive procedures. *J. Assist. Reprod. Genet*., **10**, 72–77.

Bergh, T. and Lundkvist, Ö. (1992) Clinical complications during in-vitro fertilization treatment. *Hum. Reprod*., **7**, 625–626.

Berrebi, A., Kobuch, W.E. and Puel, J. (1990) Influence of pregnancy on human immunodeficiency virus disease. *Eur. J. Obstet. Gynecol. Reprod. Biol*., **37**, 211–217.

Blanche, S., Mayaux, M.J., Rouzioux, C. *et al.* (1994) Relation of the course of HIV infection in children to the severity of the disease in their mothers at delivery. *N. Engl. J. Med*., **330**, 308–312.

Botta-Fridlund, D. (1994) Risque de transmission des virus B et C en procréation médicalement assistée (PMA). *Contracept. Fertil. Sex*., **22**, 298–301.

Boucher, P., Jejeune, H., Pinatel, M.C. *et al*. (1995) Spermoculture: improvement of the bacteriological quality of samples by direct counselling before semen collection. *Fertil. Steril*., **64**, 657–660.

British Andrology Society (1999) British Andrology Society guidelines for the screening of semen donors for donor insemination. *Hum. Reprod*., **14**, 1823–1826.

Brotherton, J. (1990) Cryopreservation of human semen. *Arch. Androl*., **25**, 181–195.

Centers for Disease Control (1991a) Public health service inter-agency guidelines for screening donors of blood, plasma, organs, tissues, and semen for evidence of hepatitis B and hepatitis C. *Morbidity and Mortality Weekly Report*, **40** (RR-4), 1–17.

Centers for Disease Control (1991b) Hepatitis B Virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination: recommendations of the immunization practices advisory committee (ACIP). *Morbidity and Mortality Weekly Report*, **40** (RR-13), 1–19.

Centers for Disease Control (1993) Recommendations for the prevention and management of *Chlamydia trachomatis* infections, 1993. *Morbidity and Mortality Weekly Report*, **42** (RR-12), 1–39.

Centers for Disease Control (1997) *Chlamydia trachomatis* genital infections – United States, 1995. *Morbidity and Mortality Weekly Report*, **46**, 193–198.

Centers for Disease Control (1998a) Guidelines for the use of antiretroviral agents in pediatric HIV infection. *Morbidity and Mortality Weekly Report*, **47** (RR-4), 1–31.

Centers for Disease Control (1998b) Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. *Morbidity and Mortality Weekly Report*, **47** (RR-5), 43–82.

Centers for Disease Control (1998c) Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease. *Morbidity and Mortality Weekly Report*, **47** (RR-19), 1–39.

Choo, Q.L., Kuo, G., Ralston, R. *et al*. (1994) Vaccination of chimpanzees against infection by the hepatitis C virus. *Proc. Natl Acad. Sci. USA*, **91**, 1294–1298.

Chrystie, I.L., Mullen, J.E., Braude, P.R. *et al*. (1998) Assisted conception in HIV discordant couples: evaluation of semen processing techniques in reducing HIV viral load. *J. Reprod. Immunol*., **41**, 301–306.

Coggins, C. and Segal, S. (1998) AIDS and reproductive health. *J. Reprod. Immunol*., **41**, 3–15.

Cohen, D.A., Nsuami, M., Etame, R.B. *et al*. (1998) A school-based *Chlamydia* control program using DNA amplification technology. *Pediatrics*, **101**, E1.

Connor, E.M., Sperling, R.S., Gelber, R. *et al*. (1994) Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. *N. Engl. J. Med*., **331**, 1173–1180.

Cottell, E., McMorrow, J., Lennon, B. *et al*. (1996) Microbial contamination in an in vitro fertilization-embryo transfer system. *Fertil. Steril*., **66**, 776–780.

Cottell, E., Lennon, B., McMorrow, J. *et al*. (1997) Processing of semen in an antibiotic-rich culture medium to minimize microbial presence during in vitro fertilization. *Fertil. Steril*., **67**, 98–103.

Culnane, M., Fowler, M.G., Lee, S.S. *et al*. (1999) Lack of long-term effects of in utero exposure to zidovudine among uninfected children born to HIV-infected women. *JAMA*, **281**, 151–157.

Curtis, P., Amso, N., Keith, E. *et al*. (1991) Evaluation of the risk of pelvic infection following transvaginal oocyte recovery. *Hum. Reprod*., **6**, 1294–1297.

Davis, G.L., Esteban-Mur, R., Rustgi, V. *et al*. (1998) Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. *N. Engl. J. Med*., **339**, 1493–1499.

De Geyter, Ch., De Geyter, M., Behre, H.M. *et al*. (1994) Peroxidase-positive round cells and microorganisms in human semen together with antibiotic treatment adversely influence the outcome of in-vitro fertilization and embryo transfer. *Int. J. Androl*., **17**, 127–134.

Douglas, G.C., Fazely, F. and Hu, J. (1998) Transmission of HIV to the placenta, fetus and mother and implications of gametic infection. *J. Reprod. Immunol*., **41**, 321–329.

Duffaut, M. and Valla, D. (1997) Procréation médicalement assistée et infection par le virus de l' hépatite C. *Contracept. Fertil. Sex*., **25**, 534–537.

Dunn, D.T., Newell, M.L., Ades, A.E. *et al*. (1992) Risk of human immunodeficiency virus type 1 transmission through breastfeeding. *Lancet*, **340**, 585–587.

Eggert-Kruse, W., Hofmann, H., Gerhard, I. *et al*. (1988) Effects of antimicrobial therapy on sperm-mucus interaction. *Hum. Reprod*., **3**, 861–869.

European Collaborative Study (1991) Children born to women with HIV-1 infection: natural history and risk of transmission. *Lancet*, **337**, 253–263.

European Collaborative Study (1996) Characteristics of pregnant HIV-1 infected women in Europe. European Collaborative Study. *AIDS-care*, **8**, 33–42.

Expert Advisory Group (1998) *Chlamydia trachomatis – Summary and Conclusions of CMO's Expert Advisory Group*. Department of Health, London, UK, pp. 1–22.

Fédération des BLEFCO (1997) Propositions d'attitudes en matière de dépistage des hépatites B et C préalables à la tentative d' AMP intra-couple. *Contracept. Fertil. Sex*., **25**, 313–324.

Fishel, S., Jackson, P., Webster, J. *et al*. (1988) Endotoxins in culture medium for human in vitro fertilization. *Fertil. Steril*., **49**, 108–111.

Gembruch, U., Diedrich, K., Al-Hasani, S. *et al*. (1988) Die transvaginale, sonographisch gesteuerte follikelpunktion. *Geburtshilfe Frauenheilkd*., **48**, 617–624.

Gerberding, J.L. (1995) Management of occupational exposures to bloodborne viruses. *N. Engl. J. Med*., **332**, 444–451.

Gerrity, M. (1993) Legislative efforts affecting the reproductive biology laboratory. *Curr. Opin. Obstet. Gynecol*., **5**, 623–629.

Global Programme on AIDS (1992) Consensus statement from the WHO/UNICEF constitution on HIV transmission and breastfeeding. *Weekly Epidemiol. Rec*., **67**, 177–184.

Goodey, A.R. (1993) The production of heterologous plasma proteins. *Trends Biotechnol*., **11**, 430–433.

Gromb, S., Beylot, J. and Lazarini, H.P. (1995) Sperm conservation and HIV infection. *Med. Sci. Law*, **35**, 197–200.

Guillet-Rosso, F., Fari, A., Taylor, S. *et al*. (1987) Systematic semen culture and its influence on IVF management. *Br. J. Obstet. Gynaecol*., **94**, 543–547.

Gupta, P., Mellors, J., Kingsley, L. *et al*. (1997) High viral load in semen of human immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. *J. Virol*., **71**, 6271–6275.

Hadchouel, M., Scotto, J., Huret, J.L. *et al*. (1985) Presence of HBV DNA in spermatozoa: a possible vertical transmission of HBV via the germ line. *J. Med. Virol*., **16**, 61–66.

Hamer, F.C., Horne, G., Pease, E.H.E. *et al*. (1995) The quarantine of fertilized donated oocytes. *Hum. Reprod*., **10**, 1194–1196.

Hammitt, D.G., Walker, D.L. and Syrop, C.H. (1990) Improved methods for preparation of culture media for in-vitro fertilization and gamete intra-fallopian transfer. *Hum. Reprod*., **5**, 457–463.

Hargreave, T.B. and Ghosh, C. (1998) The impact of HIV on a fertility problems clinic. *J. Reprod. Immunol*., **41**, 261–270.

Hewitt, J., Cohen, J., Fehilly, C.B. *et al*. (1985) Seminal bacterial pathogens and *in vitro* fertilization. *J. In Vitro Fertil. Embryo Transfer*, **2**, 105–107.

Hollier, L.M. and Cox, S.M. (1998) Syphilis. *Semin. Perinatol*., **22**, 323–331.

Hoofnagle, J.H. and Di Bisceglie, A.M. (1991) Serologic diagnosis of acute and chronic viral hepatitis. *Semin. Liver Dis*., **11**, 73–83.

Huyser, C., Fourie, F., Le, R., Oosthuizen, M. *et al*. (1991) Microbial flora in semen during *in vitro* fertilization. *J. In Vitro Fertil. Embryo Transfer*, **8**, 260–264.

Karlström, P., Hjelm, E. and Lundkvist, Ö. (1991) Comparison of the ability of two sperm preparation techniques to remove microbes. *Hum. Reprod*., **6**, 386–389.

Laverge, H., De Sutter, P., Desmet, R. *et al*. (1997) Prospective randomized study comparing human serum albumin with fetal cord serum as protein supplement in culture medium for in-vitro fertilization. *Hum. Reprod*., **12**, 2263–2266.

Lens, J.W. and Rijnders, P.M. (1996) The embryo. In Bras, M., Lens, J.W., Piederiet, M.H., Rijnders, P.M., Verveld, M. and Zeilmaker, G.H. (eds), *Laboratory Aspects of In-Vitro Fertilization*. Organon, The Netherlands, pp. 177–203.

Lenz, S., Leeton, J. and Renou, P. (1987) Transvaginal recovery of oocytes for *in vitro* fertilization using vaginal ultrasound. *J. In Vitro Fertil. Embryo Transfer*, **4**, 51–55.

Lesourd, F., Izopet, J., Merran, C. *et al*. (2000) Transmissions of hepatitis C virus during the ancillary procedures for assisted conception. *Hum. Reprod*., **15**, 1083–1085.

Liversedge, N.H., Jenkins, J.M., Keay, S.D. *et al*. (1996) Antibiotic treatment based on seminal cultures from asymptomatic male partners in in-vitro fertilization is unnecessary and may be detrimental. *Hum. Reprod*., **11**, 1227–1231.

Macmillan, S. and Templeton, A. (1999) Screening for *Chlamydia trachomatis* in subfertile women. *Hum. Reprod*., **14**, 3009–3012.

Macmillan, S., Walker, R., Oloto, E. *et al*. (1999) Ignorance about *Chlamydia* among sexually active women – a two centre study. *Hum. Reprod*., **14**, 1131–1135.

Marrs, R.P. (1986) Laboratory conditions for human in-vitro fertilization procedures. *Clin. Obstet. Gynecol*., **29**, 180–189.

Masfari, A.N., Duerden, B.I. and Kinghorn, D.R. (1986) Quantitative studies of vaginal bacteria. *Genitourin. Med*., **62**, 256–263.

Massey, E.J., Dasani, H., Jones, P. *et al*. (1996) Storage facility exists for sperm from patients positive for antibody to hepatitis C virus. *Br. Med. J*., **313**, 1078.

Matz, B., Kupfer, B., Ko, Y. *et al*. (1998) HIV-1 infection by artificial insemination. *Lancet*, **351**, 728.

McHutchison, J.G., Gordon, S.C., Schiff, E.R. *et al*. (1998) Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N. Engl. J. Med*., **339**, 1485–1492.

McKee, T.A., Avery, S., Majid, A. *et al*. (1996) Risks for transmission of hepatitis C virus during artificial insemination. *Fertil. Steril*., **66**, 161–163.

Ménézo, Y., Testart, J. and Perrone, D. (1984) Serum is not necessary in human in vitro fertilization, early embryo culture, and transfer. *Fertil. Steril*., **42**, 750–755.

Mermin, J.H., Holodniy, M., Katzenstein, D.A. and Merigan, T.C. (1991) Detection of immunodeficiency virus DNA and RNA in semen by the polymerase chain reaction. *J. Infect. Dis*., **164**, 769–772.

Michielsen, P.P. and Van Damme, P. (1999) Viral hepatitis and pregnancy. *Acta Gastroenterol. Belg*., **62**, 21–29.

Minkoff, H.L. (1998) Human immunodeficiency virus infection in pregnancy. *Semin. Perinatol*., **22**, 293–308.

Mishra, L. and Seef, L.B. (1992) Viral hepatitis, A through E, complicating pregnancy. *Gastroenterol. Clin. North Am*., **21**, 873–887.

Miyakawa, Y., Okamoto, H. and Mayumi, M. (1997) The molecular basis of hepatitis B e antigen (HbeAg)-negative infections. *J. Virol. Hepat*., **4**, 1–8.

Mofenson, L.M. (1996) The role of antiretroviral therapy in the management of HIV infection in women. *Clin. Obstet. Gynecol*., **39**, 361–385.

Naaktgeboren, N. (1996) Cryopreservation. In Bras, M., Lens, J.W., Piederiet, M.H., Rijnders, P.M., Verveld, M. and Zeilmaker, G.H. (eds), *Laboratory Aspects of In-Vitro Fertilization*. Organon, The Netherlands, pp. 247–270.

National Institutes of Health Consensus Development Conference Panel statement (1997) Management of hepatitis C. *Hepatology*, **26**, 2S–10S.

Newell, M.-L. and Thorne, C. (1997) Pregnancy and HIV infection in Europe. *Acta Paediatr. Suppl*., **421**, 10–14.

Novati, R., Thiers, V., d'Arminio Monforte, A. *et al*. (1992) Mother-to-child transmission of hepatitis C virus detected by nested polymerase chain reaction. *J. Infect. Dis*., **165**, 720–723.

Olaitan, A., Reid, W., Mocroft, A. *et al*. (1996) Infertility among human immunodeficiency virus positive women: incidence and treatment dilemmas. *Hum. Reprod*., **11**, 2793–2796.

Paavonen, J., Puolakkainen, M., Paukku, M. *et al*. (1998) Cost-benefit analysis of first-void urine *Chlamydia trachomatis* screening program. *Obstet. Gynecol*., **92**, 292–298.

Palomba, E., Manzini, P., Fiammengo, P. *et al*. (1996) Natural history of perinatal hepatitis C virus infection. *Clin. Infect. Dis*., **23**, 47–50.

Pawlotsky, J.M. (1997) Assistance médicale à la procréation et hépatites virales: la position du virologue. *Contracept. Fertil. Sex*., **25**, 530–533.

Peeters, M.F. (1996) Safety aspects. In Bras, M., Lens, J.W., Piederiet, M.H., Rijnders, P.M., Verveld, M. and Zeilmaker, G.H. (eds), *Laboratory Aspects of In-Vitro Fertilization*. Organon, The Netherlands, pp. 271–283.

Persson, K., Osser, S., Birkelund, S. *et al*. (1999) Antibodies to *Chlamydia trachomatis* heat shock proteins in women with tubal factor infertility are associated with prior infection by *C. trachomatis* but not by *C. pneumoniae*. *Hum. Reprod*., **14**, 1969–1973.

Quayle, A.J., Xu, C., Mayer, K.H. *et al*. (1997) T lymphocytes and macrophages, but not motile spermatozoa, are a significant source of human immunodeficiency virus in semen. *J. Infect. Dis*., **176**, 960–968.

Ron-El, R., Bracha, Y., Herman, A. *et al*. (1992) Prerequisite work-up of the couple before in-vitro fertilization. *Hum. Reprod*., **7**, 483–486.

Rooney, G. and Gilson, R.J. (1998) Sexual transmission of hepatitis C virus infection. *Sex. Transm. Infect*., **74**, 399–404.

Russell, P.H., Lyaruu, V.H., Millar, J.D. *et al*. (1997) The potential transmission of infectious agents by semen packaging during storage for artificial insemination. *Anim. Reprod. Sci*., **47**, 337–342.

Scaglioni, P.P., Melegari, M. and Wands, J.R. (1997) Biologic properties of hepatitis B viral genomes with mutations in the precore promotor and precore open reading frame. *Virology*, **233**, 374–381.

Semprini, A.E., Levi-Setti, P., Bozzo, M. *et al*. (1992) Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet*, **340**, 1317–1319.

Sewell, D.L. (1995) Laboratory-associated infections and biosafety. *Clin. Microbiol. Rev*., **8**, 389–405.

Smith, J.R., Forster, G.E., Kitchen, V.S. *et al*. (1991) Infertility management in HIV positive couples: a dilemma. *Br. Med. J*., **302**, 1447–1450.

Snyman, E. and Van der Merwe, J.V. (1986) Endotoxin-polluted medium in a human in vitro fertilization program. *Fertil. Steril*., **46**, 273–276.

Spandorfer, S.D., Neuer, A., LaVerda, D. *et al*. (1999) Previously undetected *Chlamydia trachomatis* infection, immunity to heat shock proteins and tubal occlusion in women undergoing in-vitro fertilization. *Hum. Reprod*., **14**, 60–64.

Stovall, D.W., Bailey, L.E. and Talbert, L.M. (1993) The role of aerobic and anaerobic semen cultures in asymptomatic couples undergoing in vitro fertilization: effects on fertilization and pregnancy rates. *Fertil. Steril*., **59**, 197–201.

Stratton, P., Mofenson, L.M. and Willoughby, A.D. (1992) Human immunodeficiency virus infection in pregnant women under care at AIDS clinical trials centers in the United States. *Obstet. Gynecol*., **79**, 365–368.

Tedder, R.S., Zuckerman, M.A., Goldstone, A.H. *et al*. (1995) Hepatitis B transmission from contaminated cryopreservation tank. *Lancet*, **346**, 137–140.

The American Fertility Society (1990) New guidelines for the use of semen donor insemination: 1990. *Fertil. Steril*., **53**, Supplement 1.

The American Fertility Society (1992) Guidelines for human embryology and andrology laboratories. *Fertil. Steril*., **58**, Supplement 1.

The International Perinatal HIV Group (1999) The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1. *N. Engl. J. Med*., **340**, 977–987.

Toth, A. and Lesser, M.L. (1981) Asymptomatic bacteriospermia in fertile and infertile men. *Fertil. Steril*., **36**, 88–91.

Tureck, R.W., Garcia, C-R., Blasco, L. *et al*. (1993) Perioperative complications arising after transvaginal oocyte retrieval. *Obstet. Gynecol*., **81**, 590–593.

van den Brule, A.J.C., Hemrika, D.J., Walboomers, J.M.M. *et al*. (1993) Detection of *Chlamydia trachomatis* in semen of artificial insemination donors by the polymerase chain reaction. *Fertil. Steril*., **59**, 1098–1104.

Van den Eede B. (1995) Investigation and treatment of infertile couples: ESHRE guidelines for good clinical and laboratory practice. *Hum. Reprod*., **10**, 1246–1271.

van Os, H.C., Drogendijk, A.C., Fetter, W.P.F. *et al*. (1991) The influence of contamination of culture medium with hepatitis B virus on the outcome of in vitro fertilization pregnancies. *Am. J. Obstet. Gynecol*., **165**, 152–159.

van Os, H.C., Roozenburg, B.J., Janssen-Caspers, H.A.B. *et al*. (1992) Vaginal disinfection with povidone iodine and the outcome of in-vitro fertilization. *Hum. Reprod*., **7**, 349–350.

Vernazza, P.L., Gilliam, B.L., Flepp, M. *et al*. (1997) Effect of antiviral treatment on the shedding of HIV-1 in semen. *AIDS*, **11**, 1249–1254.

Wong, P.C., Balmaceda, K., Blanco, J. *et al*. (1986) Sperm washing and swim-up technique using antibiotics removes microbes from human semen. *Fertil. Steril*., **45**, 97.

World Health Organization (1992) *Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*. Cambridge University Press, Cambridge, UK.

Zanetti, A.R., Tanzi, E., Paccagnini, S. *et al*. (1995) Mother-to-infant transmission of hepatitis C virus. Lombardy Study Group on Vertical HCV Transmission. *Lancet*, **345**, 289–291.

Zhang, H., Dornadula, G., Beumont, M. *et al*. (1998) Human immunodeficiency virus type 1 in the semen of men receiving highly active antiretroviral therapy. *N. Engl. J. Med*., **339**, 1803–1809.

Received on February 21, 2000; accepted on June 21, 2000