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Medically assisted reproduction in the presence of chronic viral diseases

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Teams practising medically assisted reproduction techniques try to avoid viruses as much as possible. Attitudes towards chronic carriers of viruses are rapidly changing, especially for human immunodeficiency virus (HIV) patients. We focus our attention on the legitimacy of systematic screening before assisted reproductive techniques and the need for specialized approaches including an adapted laboratory for viral hazards as well as the need for a multidisciplinary team. Specificities of HIV, hepatitis C virus (HCV), hepatitis B virus (HBV) carriers and the hypothesis of a reduced fertility potential are discussed. Are male HIV carriers a new indication for assisted reproductive techniques in order to prevent virus transmission? It is largely proven that sperm gradient preparation techniques efficiently decrease viral loads and therefore have a protective effect on contamination risk during assisted reproductive techniques. Although a few thousand assisted reproductive technique cycles were performed in the world for this indication without contamination, it is still too early to demonstrate that this technology is fully safe. Two examples of contaminations during insemination are examined. Many questions remain unresolved, such as the lack of standardized techniques for semen preparation or virus detection or the relative merits of intrauterine insemination or ICSI to prevent HIV contamination during assisted reproductive techniques. The authors plead for well-structured, separate programmes of care linked to research objectives.

Key words: HIV/HCV/HBV/medically assisted reproduction/sexual transmission

Introduction

Sexually transmitted diseases, and, among them, viruses, have always preoccupied teams practising medically assisted reproduction techniques, but mainly as a threat that should be avoided as much as possible. Most of the published reports in the literature concern gamete banking and donation but also cross-contamination between patients during assisted reproductive techniques. Contamination of patients has been described for hepatitis B virus (HBV), hepatitis C (HCV) and human immunodeficiency virus (HIV) in the last 20 years. Hepatitis B contamination has been described during artificial insemination (Berry *et al*., 1987) as well as HCV cross-contamination between patients during assisted conception (Lesourd *et al*., 2000) and demonstrated as possible during artificial insemination (McKee *et al*., 1996). HIV infection in artificial insemination with donor semen (AID) was described in Australia (Stewart *et al*., 1985), but also in Canada and in the USA (Chiasson *et al*., 1990; Araneta *et al*., 1995; Wortley *et al*., 1998) and, quite recently, in Germany (Matz *et al*., 1998). These accidents demonstrated that sperm alone, independently of any sexual contact, can transmit the virus with very similar frequencies to situations of occasional sexual intercourse [4/8 (50%) in Stewart *et al*. (1985) and 7/199 (3.52%) in Araneta *et al*. (1995)].

Cross-contamination in tanks storing biological material (Tedder *et al*., 1995; Clarke, 1999) and infectious disease transmission to graft recipients have been described for hepatitis or HIV as well as for rabies (Kakaiya *et al*., 1991). They are the logical consequence of the excellent viral survival after freezing.

The need for sperm donors to be screened and for sperm to be frozen and placed in quarantine for a period of 6 months, after which the sperm is used in AID only if a new test carried out on the donor is negative, has been stressed in national (American Fertility Society, 1988; Centers for Disease Control and Prevention, 1988b; Human Fertilisation and Embryology Authority, 1991; Barratt *et al*., 1993) and in European (Barratt *et al*., 1998) recommendations. Oocyte capability to carry viral particles is less clear (Baccetti *et al*., 1994, 1999) and there are no reports of transmission of hepatitis or HIV during oocyte donation

procedures. Even if there are fewer trials than for AID, recommendations and attitudes regarding quarantine in oocyte donation are less strict (Human Fertilisation and Embryology Authority, 1991; Barratt *et al*., 1998). In clinical practice, most oocyte donation programmes do not apply quarantine in order to avoid freezing and therefore optimize pregnancy chances (Delbaere *et al*., 2001) except in France (where it is an obligation by law) and in some other programmes (Hamer *et al*., 1995).

Thus patients who are virus carriers are considered a risk and, for the past 20 years, systematic screening for HIV, HBV and HCV was performed in a number of assisted reproduction programmes (Edelstein *et al*., 1990; Abusheikha *et al*., 1999). However, treatment has been denied to most HIV-infected patients and this was more or less openly recommended by various authorities at that time (American Fertility Society Ethics Committee, 1994; Schenker, 1997). Because of the absence of a uniform systematic screening procedure (Balet *et al*., 1998), patients who were chronic carriers of HIV or other viruses have nevertheless undoubtedly been treated at various locations with the centres (and maybe the patients) not being aware of the situation. Only a few rare pioneers such as Semprini in Milan started performing intraconjugal insemination with washed sperm from an infected man as early as the late 1980s (Semprini *et al*., 1992).

In the case of HBV and HCV carriers, policies varied more but in France for example, it was forbidden by law to treat HCV-positive patients with IVF during certain periods of time (see Discussion about systematic screening and legitimacy of screening).

Only in the 1990s did opinions on this delicate matter in assisted reproductive techniques slowly begin to change and the programmes become available to chronic carriers of viruses (Balet *et al*., 1998), bringing forth new questions and new knowledge. Hence the need for this review.

Ethical dilemma

There is no doubt that the debate around HIV patients is now underway in the assisted reproductive techniques field. In a position paper published in *Human Reproduction* in 2001, the authors favoured access to assisted reproductive techniques for both HIV-infected men and women (and this is what was in fact done in the fertility clinic of Erasme Hospital, Brussels) stating that 'what must prevail in the medical decision is a balance between the importance of the message advising against pregnancy and the benefit for patients of being assisted in their plans to have a child. Until recent years, the balance was clearly tilted in favour of the firmness of the message not to become pregnant, not only due to the risks of contamination of the child and the short life expectancy of the parents but also to the few arguments in favour of the efficacy of medical intervention in relation to unprotected sexual intercourse. The review of the literature […] shows that all the parameters have changed and are moving in the direction of intervention by medical teams' (Englert *et al*., 2001a).

It is crucial to remember that the practitioner's first role in this field is one of information and counselling detailing the disease's implications on sex life and reproduction, as well as to promote 'safe sex'. When the desire for a child appears, the first approach consists in examining it openly with patients, remembering that the dynamic of this desire can be different for each member of the couple. Their feelings in relation to expressing their own needs in the face of the child's needs and the risk of his becoming an orphan must also be discussed (Kass, 1994). Discussion about the patient's potential death is unavoidable when handling a desire for a child, but is already well known in fertility clinics with patients with other potentially lethal diseases such as cancer. In fact, it may happen that this discussion leads to a situation in which, even though there is undeniably the desire for a child, the patients decide to give up the idea due to their condition. Nevertheless, the literature shows that, in spite of all the obstacles and difficulties encountered, a significant number of couples in Western Europe (Lindsay *et al*., 1995; Greco *et al*., 1999) and in Africa (Gray *et al*., 1998) do not give up their desire for a child and that seropositivity has relatively little influence (Sunderland *et al*., 1992; Sherr, 1995; Williams *et al*., 1996). For François Delor, 'the diagnosis of seropositivity may be perceived as a sudden and definitive prohibition on having children and this prohibition may be felt, translated or reinterpreted as an unbearable injury or diminishment of identity that may give rise to, or increase, the desire to have a child as a 'compensatory measure', a desire that becomes even more impatient when it arises within a subjective timeframe that is felt to be limited' (Delor, 1997). If the desire persists, then one should examine the possibility of it being overcome either by interfamily circulation of children, as happens in certain African societies, or by adoption or fostering when possible, or finally by the use of artificial insemination with donor semen when it is the male partner who is infected by HIV (Delvigne *et al*., 1990). Up to this point, the health professional is not really confronted with an ethical dilemma. He plays the role of both the patient's private advisor and child protector by doing everything in his power to avoid the risk of the birth of a child infected by the AIDS virus. In the hypothesis of the use of a sperm donor when the man is seropositive, the ethical debate (very active during the 1980s and 1990s) concerning the 'interest of the child' in not rapidly becoming an orphan has died down considerably with the extension of life expectancy for seropositive patients.

The ethical conflict, really, begins when the desire for a 'biological' child persists. It is not a question here of 'opposing the desire to procreate', which would undeniably encroach upon the autonomy of patients (Kass, 1994) who, provided that they are fertile (which will generally but not always be the case; Sharma *et al*., 2003), fortunately do not need the doctor's authorization to attempt unprotected intercourse. It is more a matter of either adopting an attitude of non-collaboration (guaranteeing the soundness of the message of advising against pregnancy) or, on the contrary, providing medical assistance aimed at minimizing the possible risk of contamination (at the risk of weakening the credibility of the recommendation against pregnancy) (Smith *et al*., 1990). This is a difficult choice because one cannot both provide assistance in reproduction and maintain a firm line of advice against pregnancy. However, lack of assistance in the desire for pregnancy leads a number of couples to choose to have unprotected sexual intercourse (Mandelbrot *et al*., 1997), an attitude involving risks in relation to the HIV virus which are known and have also been observed in other particular situations when women have been refused access to medically assisted reproduction (Macaulay *et al*., 1995; Matz *et al*., 1998; Block *et al*., 1999). These couples also tend to distance themselves from the medical structures by which they feel rejected (Nolan, 1990). Moreover, as far as seropositive men are concerned, there are solid arguments

allowing us to consider sperm washing as safer than sexual intercourse both for the partner and for the child to be, even if it is too early to know if this safety could be total. In women, the reduction in the risk of vertical transmission by a factor of $\geqslant 10$ and the progress made in the knowledge of factors influencing the transmission risk encourage more selective counselling. As a result of the growing desire for pregnancy in HIV couples, due to the improvement in their state of health and their new longevity, advice against reproduction automatically becomes weaker: maintaining it indiscriminately could paradoxically have a perverse effect of discrediting all messages of prevention concerning safe sex and reproduction. It is more effective to provide assistance based on the inclusion of couples who are in the most favourable situations from the point of view of the risks of transmission and longevity in properly evaluated protocols: the message of advising against pregnancy for patients not fulfilling these criteria will then be all the more credible.

Other authors stressed the same kinds of reasons to reconsider categorical exclusion of HIV-seropositive individuals from assisted reproductive services (Anderson, 1999; Minkoff and Santoro, 2000; Gilling-Smith *et al*., 2001; Lyerly and Anderson, 2001).

Additional reasons have been put forward by other authors who also consider that the time for a change of attitude has come: the change of recommendations by the American College of Obstetricians and Gynecologists Committee on Ethics (2001) and the American Society for Reproductive Medicine Ethics Committee (2002; Sauer, 2003), the similar (or higher) levels of risks for other medical conditions universally accepted for assisted reproductive techniques (Lyerly and Anderson, 2001; Lyerly and Faden, 2003), the fact that denying individuals assisted reproductive techniques on the basis of their HIV status may be considered as discrimination and subject the physician to liability under the Americans with Disabilities Act (Coleman, 2003).

In contrast to what seems to be now 'politically correct', two recent papers from the Centers for Disease Control and Prevention recommend 'against insemination with semen from HIV-infected men on the basis of lack of best available evidence of efficacy' (Jamieson *et al*., 2001; Duerr and Jamieson, 2003), an important statement that will be analysed below in the section 'Male chronic carriers as new indication for assisted reproductive techniques to prevent virus transmission?'

Assisted reproductive techniques treatment for conventional indications applied to patients who are carriers of chronic viral diseases

Legitimacy of screening

During an oral presentation of in North America on their preliminary experience in treating HIV patients (Englert *et al*., 2002a) we were challenged on the legitimacy of systematic viral screening and on having a separate assisted reproduction laboratory for infected patients, considering that this would be unethical if not discriminatory. Screening for viral disease is a common practice in fertility clinics (Edelstein *et al*., 1990; Balasch *et al*., 1992; Balet *et al*., 1998; Abusheikha *et al*., 1999; Hart *et al*., 2001) even if this screening does not seem to be systematically performed (Balet *et al*., 1998).

There are various reasons justifying screening prospective patients for chronic viral diseases: to be aware of what we are doing; to inform the patient about his treatable (even if not curable) condition for himself and counsel him about the risk of horizontal transmission; to promote either safe sex (HIV) or vaccination of the partner (HBV); to give adequate counselling about the carrying out of their child project despite the newly discovered health condition and the risk of vertical transmission (Englert *et al*., 2001b); to take all the reasonable measures to reduce this risk for the child (semen processing for infected male—see below—early vaccination at birth for HBV, antiretroviral drugs and Caesarean section as well as artificial neonate feeding for HIV: Ministry of Health, French Republic, 2002); to monitor the known nosocomial risk of transmission between patients, already described in assisted reproductive techniques for HBV and HCV (Quint *et al*., 1994; Lesourd *et al*., 2000) and to perform the specialized treatment for those who, like us, think that specialized teams, procedures and structures should be built to treat these patients and who think that universal precautions are not enough to justify including patients known to be infectious (see 'Universal precautions or specialised structures?' below).

On the contrary, the opposite question should be 'What if no screening is applied?' One has to realize that whereas some patients who are chronic carriers of HIV or other viruses are treated for fertility problems without the centres (and perhaps the patients) being aware of it, other patients that are known to be chronic virus carriers are denied treatment, which is totally illogical and can even be considered discriminatory.

Informed consent should of course be obtained before screening and post-test counselling and care should be available for patients detected as chronic carriers. Patients should of course be free to refuse the screening, but the centre should then handle their gametes as those of patients who are chronic viral carriers. Our view is that screening is unethical or discriminatory if a patient known to be positive treated unethically or discriminatorily. But if these patients are respected and appropriate counselling and treatment is offered, then the above reasons in favour of screening and the interest of the child to be born uninfected should result in considering screening as the rule and absence of screening as the exception.

Assisted reproduction procedures: universal precautions or specialized approaches?

Whilst the risk of contamination of staff exists but is extremely low (Weiss *et al*., 1988), nosocomial contamination between patients has been described both for the HIV virus (Blank *et al*., 1994) and in assisted reproductive techniques for the HCV and HBV (Quint *et al*., 1994; Lesourd *et al*., 2000). Cross-contamination in tanks storing biological material has also been clearly demonstrated (Tedder *et al*., 1995; Clarke, 1999) as well as occasional nosocomial contamination of the workers for HIV (Weiss, 1988). Preventive measures especially regarding the most dangerous of these viruses, HBV, should be taken (Bonanni and Bonaccorsi, 2001). It therefore seems essential to evaluate each stage in these complex technologies very attentively to ensure their safety as much as possible. In the clinical part of the programme, special attention should be paid to vaginal echography, to the oocyte retrieval step as well as to anaesthesia. These steps were the ones incriminated in the HCV contamination described by Lesourd

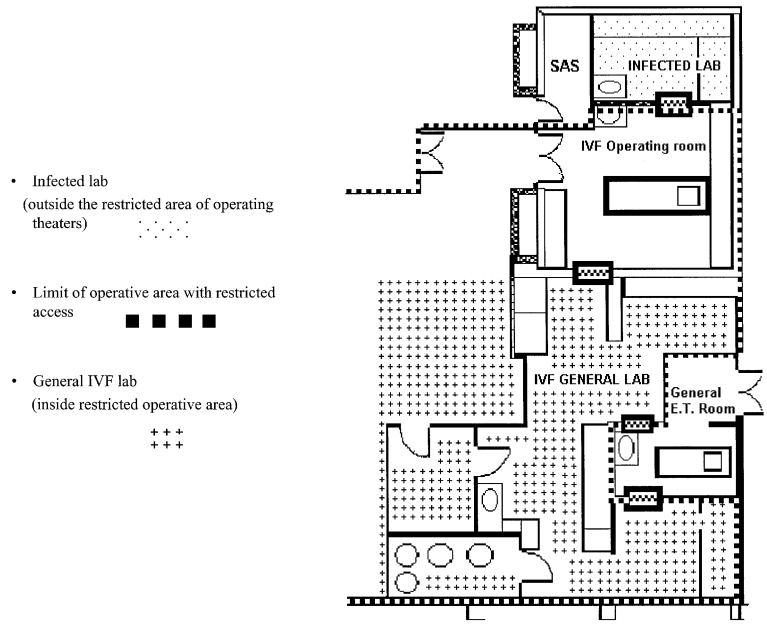

Figure 1. Separate adapted L2 laboratory.

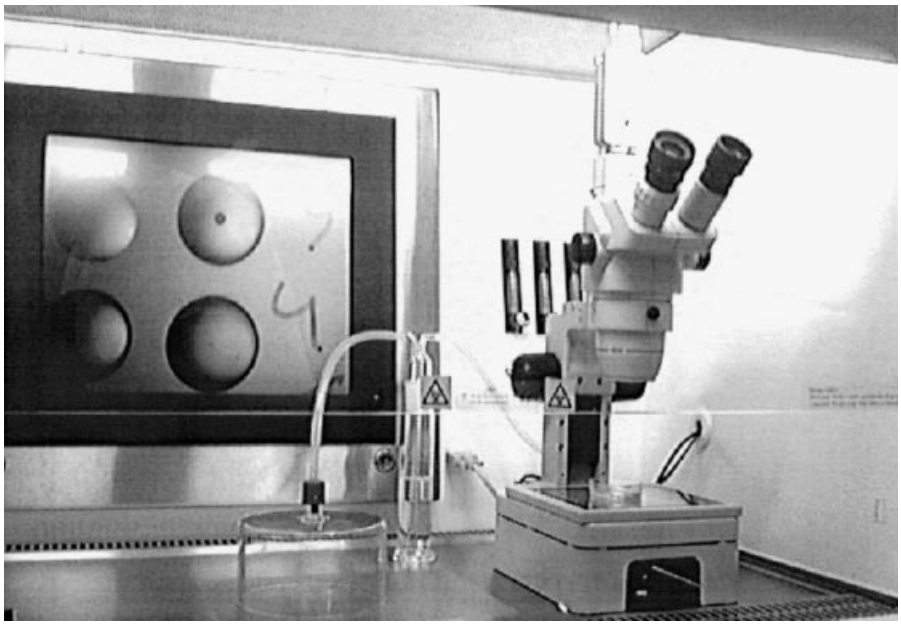

Figure 2. Vertical flow cabinet with videovision.

et al. (2000). An IVF laboratory is a complex structure where everything is planned to promote adequate conditions for cell survival and culture, a condition also favourable for viruses and bacteria. Infection is thus a normal concern and the assisted reproduction laboratory for virally infected patients developed in Brussels was adapted to meet most of the recommendations for HIV laboratories: a separate adapted L2 laboratory (devoted to infected patients, with an airtight chamber and safety access procedures) was set apart for treating the biological liquids of patients (semen, oocytes and embryos) when HIV, HCV or HBV carriers were concerned (Figure 1). A vertical laminar flow cabinet for viral culture with 100% recirculation of filtered air was adapted with a microscope and video vision to offer a safe workplace to the laboratory workers (Figure 2). The 'infected laboratory' is equipped with the whole range of facilities for IVF, ICSI and intrauterine insemination (IUI) procedures (Englert *et al*., 2002b). The cost of a laboratory by itself is a small investment in the whole assisted reproduction budget where recurrent costs of wages of highly specialized workers represent a much more important financial investment than a single investment of around $250 000. The development of this specialized laboratory has also improved our standard procedures for the general assisted reproduction laboratory, i.e. full suppression of mouth pipetting, progressive replacement of old horizontal flows by vertical ones. Tank storage

remains a problem that is not yet fully resolved. A minimal organization implies the need to avoid mixing infected specimens with the general specimens and to replace breakable straws by high security ones (Clarke, 1999; Benifla *et al*., 2000; Letur-Konïrsch *et al*., 2003). It is nevertheless unclear if a specific tank should be used for each of the three viral types, but in that case co-infected specimens necessitate a fourth tank at least for co-infected HIV-HCV specimens, a common feature in HIV patients infected by i.v. drug use. Special attention should be given to motivation and training of the fertility clinic staff who are not accustomed to handle infected patients (especially HIV positive patients) and will transitionally express anxiety. Some can even react aggressively as an expression of fear for their own safety. Some special safety procedures were also developed both for staff and in relation to the risk of inter-patient contamination, by taking infected patients at the end of the programme for vaginal echography, for oocyte retrieval, as well as for transfer and insemination. The segregation of these patients in space and/or time has been chosen by others (C.Gilling-Smith *et al*., unpublished study) and is imposed by law in France to assisted reproduction clinics that want to treat HIV- or HCV-infected patients (Decree Concerning Assisted Reproductive Treatment of Patients with Viral Risks: *Journal Officiel de la République française*, May 15, 2001, cited by Ohl *et al*., 2003). Specific precautions edited for laboratory staff working with HIV positive samples are usually required (Weiss *et al*., 1988) and should be adapted to the special situation of assisted reproduction laboratories. Regular training of the staff is also essential.

In complete opposition, the CDC recommends universal precautions, i.e. handling all specimens as if they were hazardous, which is certainly the philosophy to apply in a general laboratory (Centers for Disease Control and Prevention, 1988a; Duerr and Jamiesson, 2003). But, as recalled earlier in this chapter, a zero risk does not exist and patients in a fertility clinic (like in any hospital unit) are subject to a small risk of nosocomial infection. Even though risk is part of life, it seems safer and more ethically acceptable to handle patients with the same levels of risk together, i.e. detected viral carriers in one laboratory, negatively screened patients in another, rather than to mix patients with clearly different risk levels. This strategy also reassured our usual patients who were aware of the existence of our special programme for infected patients through our desire for full transparency and also from time to time interest from the media. Various groups performing assisted reproduction cycles indeed fear to frighten and loose patients from their general programme, which has not been the case in our clinic since the installation of the specialized laboratory.

In addition to these architectural and procedural adaptations, multidisciplinary teams are essential in order to give a comprehensive approach to patients planning a child while chronically ill with a transmissible and potentially lethal disease. The team in Brussels includes an assisted reproduction clinician, a biologist, a specialist in internal medicine, an obstetrician and a paediatrician all specialized in HIV patients as well as a psychologist and the head of the Virology Laboratory and the AIDS Reference Laboratory at the Free University Brussels. These professionals are all working within the same academic hospital (Erasme Hospital, Brussels). All requests and patients' files are reviewed collectively (Englert *et al*., 2001b).

Do these patients have specificities?

As long as the patients are referred for the usual infertility indications to assisted reproduction clinics, one should imagine that they do not express any special characteristics: this is clearly not the case. The ethical question linked to the unusual situation of these patients planning a child while chronically ill with a transmissible and potentially lethal disease, was discussed earlier (Englert *et al*., 2001a). Sauer (2003) reported that these patients are 'typically married, well-educated and middle class or above social status', thus a politically correct situation to assess for having children but this is probably largely due to recruitment bias in a programme where a single trial costs $12 000 (Sauer, 2003). HCV carriers are usually contaminated by i.v. drug use [it has been shown that within 12 months of use, 80% of i.v. drug users are HCV carriers (Di Bisceglie, 1998) and blood transfusion contamination is becoming a rare event since screening for HCV was introduced, using continuously improving techniques since the 1990s (Donaghue *et al*., 1992; Schreiber *et al*., 1996)]. In our preliminary experience of nearly 70 patient carriers of HIV, most of them were either African migrants or previous drug users. Both these situations are more often encountered in disadvantaged social classes and they imply that other difficulties may be present. These difficulties need to be assessed and eventually taken into account in patient counselling and management. Another situation sometimes encountered is that of heterosexual couples in which contamination occurred in a male homosexual relationship, implying a couple's dynamic that has not been studied extensively but that can certainly be qualified as unusual.

Very few papers compare the fertility (potential to reproduce) of patient carriers of viral diseases with that of a control population, this being a logical consequence of the reluctant position of the medical profession towards the child project of infected patients.

Semen analyses in patients at different stages of AIDS have been performed and consistently give a correlation between semen analyses and clinical status, AIDS stage and viral (or CD4+) count in the direction of an alteration of semen analysis with illness progression (Krieger *et al*., 1991; Crittenden *et al*., 1992; Politch *et al*., 1994; Lasheeb *et al*., 1997; Muller *et al*., 1998). Decreased androgen levels were demonstrated by several authors usually but not systematically in association with progressive immunosuppression in HIV-affected men (Dobs *et al*., 1988; Croxson *et al*., 1989; Villette *et al*., 1990; Christeff *et al*., 1992; Grinspoon and Bilezikian, 1992; Laudat *et al*., 1995; Chatterton *et al*., 1996; Christeff *et al*., 1996; Schurmeyer *et al*., 1997; Christeff *et al*., 1999). In clinically healthy patients under retroviral therapy, Politch *et al*. (1994) did observe normal semen parameters but significant alterations were described by Dulioust *et al*. (2002) on a large series of healthy HIV carriers, most of them under antiretroviral (ARV) therapy. ARV has been reported to influence sexual hormones in men (Collazos *et al*., 2002a,b) and is present in the genital tract: concentrations of various ARV in semen have been established in a view of treatment efficacy (Taylor *et al*., 2000) but no information is available on the safety of these drugs regarding a hypothetical teratogenicity on the future conceptus in case of pregnancy obtained during ARV therapy in men. It should also be stressed that significant frequency of sexual dysfunction has been reported in men using antiretroviral drugs, particularly

protease inhibitors in large patient cohorts and was not related to hormonal causes (Schrooten *et al*., 2001; Collazos *et al*., 2002a,b).

In women, menstruation disturbances were described in advanced stages of AIDS or low CD4+ counts (Hinz *et al*., 2002) while normal menstrual patterns were described in UK and US studies independently of their CD4+ levels (Shah *et al*., 1994; Ellerbroeck *et al*., 1996). Chirgwin *et al*. (1996) described increased amenorrhoea frequency and delayed menstruation in HIV seropositive women regardless of their CD4+ count or stage of disease but they were positively correlated with past drug abuse, an observation corresponding with data regarding ovarian function of HCV carriers (see below).

In a first group of HIV seropositive women seen in Brussels, severe ovarian dysfunction under the form of premature ovarian failure or ovarian resistance to stimulation was astonishingly frequently observed. Even if the size of the sample was too small to come to any conclusion, it could be a field for further investigation, especially since Clark *et al*. (2001) suggested the same observation in a retrospective analysis of a serum bank of 52 patients and an African study demonstrated reduced fecundity (significantly fewer pregnancies and fewer live births than controls) in a group of healthy women screened during pregnancy and unaware of their seropositivity status, strongly suggesting a reduced fertility independently of the AIDS stage and of the 'state of' malnutrition (Yaro *et al*., 2001). Moreover, little attention was given to the possible impact on the reproductive function of various endocrine perturbations linked to AIDS but also to ARV therapy (especially protease inhibitors) known to strongly influence lipid metabolism and insulin resistance and thus could have consequences on folliculogenesis and ovulation regulatory processes (Ng *et al*., 1994; Barbaro, 2002; Bhasin *et al*., 2001). Concerning prevention of vertical transmission from the infected pregnant woman to her child, major improvements have been achieved in the nineties thanks to ARV therapy during pregnancy and especially during labour and delivery and to the demonstration of the efficacy of iterative Caesarean section and artificial feeding of the child (for review see Englert *et al*., 2001a; Ministry of Health, French Republic, 2002). Research should now focus on new forms of treatment such as combined therapies (McGowan *et al*., 1999), which challenge (due to their efficacy) the need for a systematic Caesarean section (Beckerman *et al*., 1999; Brocklehurst, 1999). More detailed attention should also be given to the question of the innocuity of antiviral molecules for the child, a subject which is still highly controversial and of crucial importance. Whereas a French group has described a very rare pathology of the mitochondria in several children exposed to Zidovudine (Blanche *et al*., 1999; Barret *et al*., 2003), a far-reaching American study has revealed no particular pathology in these children (Culnane *et al*., 1999). Furthermore, it is known that mitochondria are a favourite target for antiviral drugs in the category of nucleoside analogues (Brinkman *et al*., 1998). Protease inhibitors have not been associated with congenital risks in three recent studies (Cooper *et al*., 2000; Morris *et al*., 2000; Dorenbaum *et al*., 2002), but the protease inhibitors efavirenz and ddC should be avoided during pregnancy for potential teratogenicity (Ministry of Health, French Republic, 2002) and the association of protease inhibitors d4T and ddI for their potential toxicity to the mother (Marcus *et al*., 2002). It should be stressed that none of the antiviral molecules have been categorized by the US Food and Drug Administration as having been proven to be safe during pregnancy through controlled studies (category A) and that the extended use of antiretroviral drugs has completely changed the situation regarding reproduction: while most of the studies on drug toxicity have been performed on women who initiated treatment during pregnancy to prevent vertical transmission, most pregnant women and couples seeking infertility treatment are already under ARV treatment using poly-therapy. This raises a new concern regarding teratogenicity during male and/or female gametogenesis and in the first trimester of pregnancy.

A lot less information is available for patient carriers of HCV, despite the fact that, in Europe, seroprevalence for HCV is 0.5–2%, and 3% of the world's population are chronic carriers of the virus (Roudot-Thoraval *et al*., 1993). The sexual transmission rate is low, due to the general low viral load in semen (Debono *et al*., 2000; Dore and Kaldor, 2000). The virus can be undetectable in semen but is often detected in cervico-vaginal secretions and menstrual blood (Bresters *et al*., 1993; Koda *et al*., 1996; Semprini *et al*., 1998; Debono *et al*., 2000; Leruez-Ville *et al*., 2000; Levy *et al*., 2000; Pasquier *et al*., 2000; Bourlet *et al*., 2002a,b). Half of the couples, where both partners are seropositive, are carrying different genotypes of the virus, demonstrating independent parenteral contamination (Roudot-Thoraval, 1993). The sexual transmission risk is increased in cases of genital lesion due to traumatic intercourse or to associated sexually transmitted diseases (Roudot-Thoraval *et al*., 1997). Vertical mother-to-child transmission is ~5%, and occurs mostly during an initial viraemia during pregnancy even if chronic carriers are also at risk. This risk is highly correlated with the viral load, being ~11% in case of mothers with HCV-RNA positive and 0.8% in case of HCV-RNA negative mothers in a meta-analysis collecting 758 children born from HCV mothers (Michielsen and Van Damme, 1999) and 4.7 and 0% in a study collecting 403 children born from HCV mothers (Resti *et al*., 1998). Interferon in combination with ribavirin, now used to lower HVC viral loads in blood, has been shown to decrease HCV viral loads in the seminal plasma (Levy *et al*., 2002) but a severe toxicity of ribavirin on murine spermatogenesis has been shown (Narayana *et al*., 2002). These treatments are contra-indicated during pregnancy and vaginal delivery. Breastfeeding has not been proven to increase the vertical transmission risk (Resti *et al*., 1998, 2002). In addition to the ethical dilemma of offering infertility treatment to these patients, the risk of nosocomial and professional transmission within the highly complex IVF procedure should not be underestimated. Even if HCV occupational transmission is not increased in medical settings (Pradat *et al*., 2000), patient-to-patient transmission through AID and IVF has been described (McKee *et al*., 1996; Lesourd *et al*., 2000).

In a recent case–control study, we observed a statistically significant association between HCV infection in women and altered ovarian function: cancelled cycles are increased, while lower estradiol levels are achieved and fewer oocytes are obtained for increased stimulation in non-cancelled cycles (Table I). Furthermore, low implantation rates are present for HCV seropositive men (Y.Englert, 2003). There are no data in the literature to which these results may be compared, but they look similar to some of the observations made earlier in this section for HIV seropositive women. The frequently low socio-economic

Table I. Ovarian stimulation parameters during IVF for hepatitis C virus (HCV)-infected men and women compared to matched controls

	Partners of HCV positive men (n = 48)	Controls for HCV men (n = 96)	HCV seropositive women (n = 33)	Controls for HCV women (n = 66)
Cancelled cycles (n)	2	7	8	4[a]
Amount of FSH (IU)	3356 ± 1902 (43)	3383 ± 1704 (82)	3522 ± 1385 (23)	2936 ± 1488[a] (61)
Days of stimulation	11.5 ± 2.7 (43)	11.4 ± 2.7 (82)	11.2 ± 2.5 (23)	11.5 ± 2.5 (61)
Estradiol maximum (pg/ml)	3352 ± 1994 (43)	2733 ± 1205 (82)	2184 ± 903 (23)	2975 ± 1328[b] (61)
Oocytes collected	11.6 ± 5.6 (43)	11.9 ± 5.9 (82)	9.8 ± 4.5 (23)	12.2 ± 7.0 (61)

Results are means ± SEM (n) (Y.Englert, unpublished study).
[a]Significantly higher compared to controls for HCV seropositive women (P = 0.018).
[b]Significantly lower compared to controls for HCV seropositive women (P = 0.014).

levels of these patients may be associated with other characteristics that could be responsible for the results observed.

No data on the reproductive function of HBV carriers are available. The large contamination within an IVF clinic in 1991 did not impair the chances of these 79 contaminated women conceiving since 18 of them were pregnant at the time of contamination and five out of 18 during a second trial a few months later (Quint, 1994). Nevertheless, no comparative evaluation of their fecundity with a control group is available. HBV is known to be present and infectious not only in blood but also in semen, saliva and vaginal secretion (Alter *et al*., 1986; Hadler and Margolis, 1993) underlining the importance of vaccination of relatives of infected persons.

Co-infected semen for HIV and HCV have been described (Pasquier *et al*., 2000) and preliminary evidence of co-infected semen for HIV, HCV and hepatitis G virus or GB virus were recently published (Semprini *et al*., 1998; Bourlet *et al*., 2002b). There is thus still a lot to study about these viruses and the reproduction processes of their chronic carriers.

Male patients who are chronic carriers: a new indication for assisted reproductive techniques to prevent virus transmission ?

Sexual transmission of HIV

Transmission of HIV during sexual intercourse through vaginal penetration is extremely variable (Royce *et al*., 1997; Peterman *et al*., 1998). Whereas transmission is relatively low in stable couples (non-transmission over extremely long periods is reported), very effective transmission during casual sexual relations has been described (Clumeck *et al*., 1989). Numerous factors are known to explain these variations, such as the infectiousness of the viral strain, the degree of advancement of the disease, the viral load, the sex of the infected partner, the existence of associated sexually transmitted diseases and the nature of the sexual practices (Vernazza *et al*., 1999). The risk of contamination by sexual contact in a stable couple is between 0.1 and 0.5% (De Vincenzi, 1994; Gray *et al*., 2001), much lower than that connected with occasional intercourse, as in the case of prostitution, for example (Cameron *et al*., 1989; Mastro *et al*., 1994). Several studies show a correlation between the level of infectiousness and the size of the viral load; this is observed whatever the means of transmission: blood transfusion (Busch *et al*., 1996), sexual relations (Ragni *et al*., 1998; Gray *et al*., 2001), and vertical mother-to-child transmission (St Louis *et al*., 1993). One should not draw the hasty conclusion from these observations that in the case of a low or undetectable viral load the risk of contamination disappears. This type of message is inexact and would lead to the abandonment of safe sex by infected people undergoing antiviral treatment. This fear would seem to be justified according to the results of certain recent surveys (Kravcik *et al*., 1998) but not constantly (Lavoie *et al*., 1998). We know today that the correlation between the circulating viral load and the viral load in the semen, whilst undeniable, is relatively low. In fact, compared to measurements taken from the blood, the spermatic compartment has a certain peripheral autonomy: there is local replication (presence of viral DNA and RNA in the semen), with the viral concentration being sometimes lower and sometimes higher than in the plasma, and the viral strains sometimes separate, with different resistance characteristics (Byrn *et al*., 1997; Coombs *et al*., 1998; Eron *et al*., 1998; Tachet *et al*., 1999). Furthermore, there are contradictory data in the literature today on the extent of the variability of the viral load in the semen of patients otherwise considered to be stable with respect to the evolution of their illness (Gilliam *et al*., 1997; Coombs *et al*., 1998). All these notions are extremely important in understanding and preventing sexual transmission through medically assisted procreation.

Use of medically assisted reproduction in seropositive cases

Unfortunately, it has been amply demonstrated that the sperm used in artificial insemination can transmit HIV-1: one has to remember the first case of infection in artificial insemination with a donor (AID) in Australia (Stewart *et al*., 1985), but also other cases in Canada, the USA (Chiasson *et al*., 1990; Araneta *et al*., 1995; Wortley *et al*., 1998) and, quite recently, Germany (Matz *et al*., 1998). These accidents also demonstrated that sperm alone, independently of any sexual contact, could transmit the virus with a variation in frequency very similar to situations of sexual intercourse [4/8 (50%) in Australia and 7/199 (3.52%) in the USA]. The presence of viral particles has been demonstrated in the seminal plasma in the free form and in the cellular part in the intracellular form both through co-culture and through PCR (Mermin *et al*., 1991). Its presence has been confirmed through an autopsy on the white corpuscles of the tissues of the entire male genital tract (Pudney and Anderson, 1991) and in the semen of men who have had a vasectomy (Anderson *et al*., 1991).

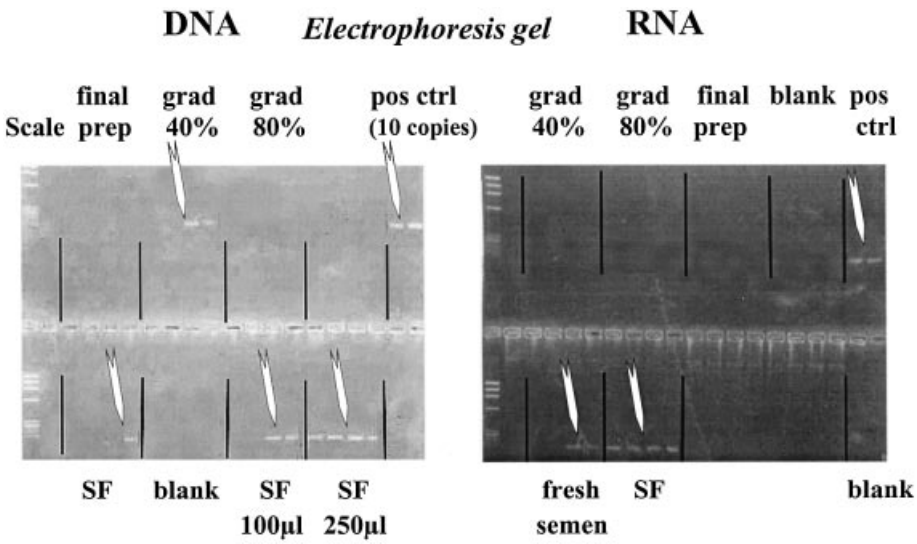

Figure 3. HIV PCR detection (RNA and DNA) before, during and after sperm washing in fresh semen, in seminal fluid (SF), 40 and 80% separation gradient (grad40%, grad80%) and in final sperm preparation (final prep) (B.Lesage *et al*., unpublished study).

As mentioned above (see sexual transmission), the relationship between the viral concentration in the plasma and the semen is not constant and there is no agreement in the literature today on the extent of the variation of the concentration in viral particles measured on samples taken successively from the same patient considered to be clinically stable. There is still no unanimous answer to the crucial question of whether the spermatozoon can itself act as a vector for the virus (Zagury, 1984; Krieger *et al*., 1991; Pudney and Anderson, 1991; Van Voorhis *et al*., 1991; Schofield, 1992; Dussaix *et al*., 1993; Baccetti *et al*., 1994; Bagasra *et al*., 1994; Nuovo *et al*., 1994; Quayle *et al*., 1997; Pudney *et al*., 1998; Quayle *et al*., 1998). On the other hand, it is clearly established that methods of preparing the sperm in which the seminal plasma is removed by washing and sperm are separated from the other cellular elements of the sperm may reduce the viral load up to a level undetectable by the most sensitive techniques as illustrated in Figure 3 (Heimerl *et al*., 1993; Baccetti *et al*., 1994; Lasheeb *et al*., 1997; Pudney *et al*., 1998; Quayle *et al*., 1998; Kim *et al*., 1999; B.Lesage et al., unpublished study). The capacity of falling below an undetectable level is nevertheless related to the viral load, as illustrated in the small series of cases presented in Table II (B.Lesage *et al*., unpublished study). It has been demonstrated that antiviral treatments that are very active at the plasma level reduce the viral load in the sperm (Anderson *et al*., 1992; Gilliam *et al*., 1997; Gupta *et al*., 1997; Vernazza *et al*., 1997a,b; Zhang *et al*., 1998; Bourlet *et al*., 2001). All these data have led some teams to use medically assisted reproduction techniques allowing sero-different couples in which the man is carrying the virus to have children using the man's own sperm. In a recent review, Sauer (2003) summarized 3221 cycles performed worldwide, most of them in Europe but also recently in the USA. Dr Semprini, the pioneer of the use of intraconjugal insemination with washed sperm, announced >2000 inseminations, 100 IVF and a few ICSI cycles in 800 women, allowing the birth of 350 children without the slightest contamination (Semprini *et al*., 2000). Spanish teams have reported 101 intraconjugal inseminations with washed sperm in 63 women, having led to the birth of 37 children without any contamination (Marina *et al*., 1998), a series that has been extended to 458 cycles as reported recently (Marina, 2001, cited by Sauer 2003) and 155 cycles using IUI (Tur et al., 1999, cited by Sauer, 2003). French teams presented a collaborative study that is systematically using ICSI to keep any contact between the infected biological material and the recipient to a minimum, and report 97 ICSI cycles (Jouannet, 2001), IUI for 62 cycles (Bujan *et al*., 2001) and five IUI and 49 IVF or ICSI cycles (Ohl *et al*., 2003). A German publication reported 143 cycles (Weigel, 2001), a UK team 66 IUI cycles and 12 IVF cycles (Gilling-Smith, 2000). Recently a publication from the USA reported 55 cycles also using systematic recourse to ICSI (Sauer and Chang, 2002), a series now extended to 103 cycles (Sauer, 2003). These data always underestimate the reality (the teams who have published have certainly increased the numbers of cases since their last publication) and one can estimate that ~4000 cycles have been performed in assisted reproduction clinics to date, taking into account that various groups performed assisted reproduction cycles and are reluctant to publish, due to the anxiety

Table II. HIV detection in semen before and after preparation according to blood viral load

Blood viral load	Primary testing	%	Final testing
$x < 50$	7/41	17	0/41
$50 < x < 1000$	7/20	35	0/20
$1000 < x < 10\,000$	4/8	50	0/8
$x > 10\,000$	15/16	94	6/16 (37)
Total	33/85	39	6/85 (7)

Values in parentheses are percentages.
The blood viral load is given in number of HIV-1 RNA copies/ml. The results give the number of positive tested samples for HIV-1 RNA presence within each range. Primary testing was made on fresh semen, seminal plasma and separation gradient supernatant. Final test reveals presence of HIV-1 RNA in selected motile sperm obtained after a two gradient separation protocol and double washing (detection limit in semen: 20 copies/ml) (B.Lesage *et al*., unpublished study).

of frightening and losing the patients of their general programme (personal communication). Many additional questions regarding these treatments remain to be evaluated and standardized, such as sperm preparation methods, laboratory techniques for HIV detection, types of assisted reproductive techniques used, number of cycles to be performed, etc.

Is the efficacy of assisted reproductive technology to prevent male-to-female HIV transmission evidence-based?

As noted previously, one can estimate that ~4000 cycles have performed worldwide in assisted reproduction clinics using the combination of gradient separation followed by HIV undetectable level within the final semen preparation before the use of assisted reproductive techniques, without any reported contamination. One has to consider that the two known cases of contamination through IUI should not be regarded as method failures: the contamination that occurred in the USA and reported by the CDC at the beginning of the 1990s (Anonymous, 1990) used a method of sperm centrifugation followed by insemination of the cell pellet, i.e. sperm and round cells. It thus demonstrated that leukocytes and macrophages known to be present in any normal semen are able to transmit the disease, which after all is not really a surprise. The second but unpublished case that recently occurred in Japan (anonymous personal communication) illustrated two procedure risks: the treated sperm came from a patient with a high viral load (a situation known to be linked to a lower chance of reaching undetected levels after sperm preparation) unfortunately linked to an absence of HIV detection of the final preparation used for insemination. The sperm preparation method used is unknown but involved a gradient method. In both cases, other sources of contamination such as unprotected intercourse were considered very unlikely to have occurred, even if absolute proof is impossible to produce.

Nevertheless, the results summarized previously should be examined carefully: Dr Semprini's large series has never been the subject of a meticulous publication covering the methodology and it is considered that loss at follow-up is an unfortunately frequent event. Very wide variations in methodology for case selection, sperm preparation methods, assisted reproductive techniques used as well as for methods and sensitivity of HIV detection in final preparation make a meta-analysis difficult. But the main reason for caution should be the comparison to the natural risk rate. Considering a male-to-female sexual transmission risk per coital act in stable couples on an average of 0.001 (Gray *et al*., 2001); considering that patients with low viral loads have lower transmission rates (0.0001 in Gray *et al*., 2001); considering that patients treated by sperm processing methods were usually carefully selected for carrying low-to-undetectable viral loads, either spontaneously or thanks to antiretroviral drugs: it is easy to understand that the number of treatments needed (NNTT) to prove efficacy is far from being reached. Taking into account the hypothesis of a 0.001 contamination risk and that according to Hanley and Lippman-Hand (1983) NNTT without contamination would be 3000 cases to demonstrate that the risk of contamination is less than the natural risk: with the hypothesis of a 0.0001 contamination risk, NNTT without contamination would reach 30 000 cases! These calculations could be criticized: they do not take into account that male-to-female transmission is more efficient than the opposite and implicitly consider that all these couples have lived together without using safe sex methods before discovering the man's seropositivity. But couples having met after seropositivity was discovered and having always used safe sex methods may be exposed to considerably higher transmission rates if they are considered to be in a situation close to the one of occasional sexual contact (see sexual transmission above). This situation will be more and more frequent in the future and will paradoxically increase the risk of transmission within stable relationships during natural reproduction as well as after sperm preparation. In our view, sperm preparation and assisted reproduction is a useful method: proven efficacy of gradient technologies for reducing viral load in sperm below a detectable range and the very well demonstrated correlations between viral load and contamination probability are strong indications in favour of these methods. Since it has not been experimentally demonstrated—and will not be so for a considerable length of time—that these methods significantly decrease the transmission risk, are efficient or even carry no residual risk of transmission, it is thus very important to instruct the patients to stay within evaluated and carefully structured experimental programmes for a prolonged period. The CDC statement of 'lack of best available evidence of efficacy' (Jamieson *et al*., 2001; Duerr and Jamieson, 2003) mentioned earlier in this paper, should be, in the view of the authors, understood with regard to these considerations.

The type of assisted reproductive technique used is still a matter of controversy: in the largest experience, Semprini used a simple insemination technology, i.e. injecting usually ~10^6 motile sperm and used variable detection methods for >10 years with relatively low sensitivity, without contamination. His initial report (Semprini *et al*., 1992) used detection of HIV antigen by indirect immunofluorescence with monoclonal antibodies against HIV p17, and various papers published the use of commercial kits with sensitivity between 200 and 800 HIV-1 RNA copies/ml, suggesting that these methodologies were already sufficiently sensitive to avoid contamination. Recently, home-made RT–PCR protocols have been developed to allow an increase in sensitivity up to 50 copies/ml (Y.Englert, unpublished data). On the other hand, some groups suggested the use of ICSI based on the theoretical idea that using only one single spermatozoon per inseminated oocyte would be safer (Kunstmann *et al*., 2000; Sauer and Chang, 2002). Whereas this is certainly an unproven approach, which has the disadvantage of being invasive and costly, it also raises the question of an unnecessary exposure (except in the case of associated male infertility) to unknown risks linked to the rupture of the cellular oocyte membrane and the unusual full entry of the sperm membrane and acrosome within the oocyte (Piomboni and Baccetti, 2000; Gordon, 2002; Anderson and Politch, 2003). Unlike Sauer (2003), the authors recommend screening the final sperm preparation using sensitive PCR test for HIV, an approach already recommended by others as necessary even for the semen of treated patients (Garrido *et al*., 2002; Leruez-Ville *et al*., 2002; Anderson and Politch, 2003).

As stressed in the section 'Do these patients have specificities?', there are no valid data for women as well as for men on the risk associated with ARV therapy used during conception, and careful follow-up of pregnancies and children is needed to demonstrate the safety of these approaches.

Assisted reproductive techniques for HCV chronic male carriers

Even fewer data are available concerning HCV. Despite some controversy, it is acknowledged that HCV can sometimes be detected in the semen of chronically infected patients, but at a low concentration, and sexual contamination is considered to be exceptional (see above). Recently, efforts were made to standardize laboratory techniques to detect HCV in semen (Bourlet *et al*., 2003) and sperm gradient techniques have been proven to be able to reduce viral load in sperm preparation before use in assisted reproductive techniques (Pasquier *et al*., 2000; Bourlet *et al*., 2002a,b; Cassuto *et al*., 2002; Levy *et al*., 2002). The use of these techniques to prevent male-to-female transmission of HCV during reproduction is not assessed at all today, since it was demonstrated that sexual transmission is a rare event (Brettler *et al*., 1992; Dore and Kaldor, 2000).

Discussion and conclusions

In recent years, interest in the reproductive desire of patients who are carriers of chronic viral diseases, primarily HIV but also HCV, has increased. This interest has opened a new fields in assisted reproduction: new indications (in the case of fertile sero-different couples with the objective of semen decontamination), new adaptations of the technology for sperm preparation, new viral detection methods adapted to semen, adapted laboratories specially designed for viral hazards and new risks management. There is no doubt that the ethical debate around HIV patients is the one which is actually moving the field. A huge contrast indeed exists between the passionate climate surrounding the HIV patients' wish for a child and the lack of interest in HCV chronic carriers treated for years without much reflection or attention, although the questions surrounding the contamination risks and the ethical dilemma are very similar. This is a reminder that rationality is not the only fuel feeding the ethical debate. Many publications focused on viral contamination of semen, mainly to understand contamination routes and risks. But it is only recently, thanks to progress made in prevention of transmission and in therapy, that the reproductive wishes of patients who are carriers of chronic viral diseases have been taken into account as an entity with its own logic that is distinct (and complementary) from sexual desires and needs.

Knowledge is now accumulating on the fertility characteristics of these patients, on sperm preparation, on the use of assisted reproductive techniques for infected patients, and on results obtained. The efficacy of gradient separations to reduce viral loads in sperm preparations is well established, and adapted PCR technology for detection in semen is nowadays available. The need to treat these patients, within adapted procedures and with the help of a multidisciplinary team, has been progressively established as an useful tool to reduce the transmission risk. Although a few thousand cycles have been performed in the world without contamination, it is still too early to be able to demonstrate that these technologies are fully safe. Two known cases of contamination during insemination remind everybody that there is certainly a risk when some steps of the procedures are skipped. This review underlines the fact that many questions still remain unresolved: there are no standardized techniques for semen preparation nor for virus detection methods and limits. There is no consensus about the relative merits of IUI or ICSI to prevent partner contamination of HIV seropositive males during reproduction attempts. Nor is there agreement about the level of prevention efficacy of these assisted reproductive techniques on horizontal contamination rates of partners of infected males and the possible residual risks linked to couples. Indirect observation suggests that women carriers of HIV or HCV viruses may have reduced fertility potential, but additional work is necessary to explore this field and understand the influence of chronic viral infection on the reproductive function. Safety issues must also be addressed: concerning laboratory procedures, patient-to-patient contamination risks, but also potential teratogenic risks linked to ARV treatments during the periconceptional period—a very different field than the one, partially explored today, of drug toxicity during the second half of pregnancy. According to our actual knowledge, we take positions on various issues of conflict: yes to systematic screening before assisted reproductive techniques, yes to treating chronically infected patients, yes to a separate 'infected laboratory' and to adapted procedures; no to the systematic use of ICSI for contamination prevention, and no to skipping the semen HIV detection post preparation. The authors stress the number of unresolved questions and plead for well-structured separate programmes of care linked to research objectives.

Moreover, patients who are HIV chronic carriers give a new dimension to assisted reproductive techniques: the teams have to adapt to the patient's stimulating views on risk and risk management in contrast with a society that is clinging to the 'no risk' myth. These patients, chronically confronted with life and death issues, having various cultural backgrounds and life experiences, give us all the opportunity for different, enriching experiences and develop less conventional thinking processes.

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