HIV and Reproductive Care

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Abstract

Three-quarters of individuals infected with HIV are in their reproductive years and can expect an almost normal life expectancy under antiretroviral treatment. Men and women with HIV face the possibility of viral transmission to their partner or offspring if they want to have a child by way of spontaneous conception. When only the man is infected, spermatozoa can be isolated from seminal plasma and leukocytes containing cell-free and cell-associated HIV. After processing, the spermatozoa must be tested for residual contamination and, when negative, can be used for intrauterine transfer or *in vitro* fertilisation (IVF) embryo transfer/intracytoplasmatic sperm injection (ICSI). In women with HIV, self-insemination might be indicated when the couple is fertile or IVF embryo transfer/ICSI when there are infertility problems. Pregnancy should be planned to minimise the risk of drug-induced toxicity for the conceptus while reducing the vertical transmission rate to a minimum. Elective Caesarean birth is the recommended mode of delivery and breastfeeding is contraindicated.

Keywords

HIV, insemination, reproduction, *in vitro* fertilisation (IVF), intracytoplasmatic sperm injection (ICSI), sperm washing, fertility, highly active antiretroviral therapy (HAART)

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At the beginning of the AIDS epidemic, because of the poor prognosis of those infected with the disease, couples with an HIV-infected partner were discouraged from planning a pregnancy. AIDS remains a serious condition worldwide, with continuing mortality. Even in industrial countries where efficient and innovative treatments are available, HIV infection remains a chronic disease with high morbidity.[1] Nevertheless, due to antiretroviral therapies, life expectancy and quality of life of many seropositive patients have dramatically improved over the last 10 years and many couples with an HIV-positive partner can consider pregnancy planning.[2–4]

Assisted reproductive technology (ART) reduces the risk of contaminating the uninfected partner and helps couples conceive.[2,5,6] Furthermore, in recent years the vertical transmission (the risk of infecting the newborn baby) has drastically decreased (to less than 2%) due to the ability to reach undetectable maternal viral loads during pregnancy, the liberal use of Caesarean section and restricted breastfeeding.[7] Finally, certain authors have observed that pregnancy does not worsen the progression of HIV infection or its immunological parameters.[8,9] The American Society for Reproductive Medicine Committee on Ethics[10] and the International Federation of Gynecology and Obstetrics (FIGO)[11] guidelines concerning assisted reproduction in people infected by HIV have been modified to allow assisted reproduction in HIV-discordant couples.

The paper from FIGO[11] reported that access to antiretroviral drugs and ART for populations suffering from HIV or for seropositive patients must be promoted on an equitable basis, and that any restriction on access to assisted reproduction should be clearly justified and not based on discrimination.

Women, including sex workers, have the right to make choices about their sexual behaviour. Public information and access to the means to prevent HIV transmission for women and men at all stages of their reproductive lives are of utmost importance and need to be a concern for all member organisations and individual practitioners. Seropositive healthcare providers have an obligation to ensure that they engage in no behaviour that puts patients at risk. Prevention – by providing information about high-risk behaviour – is essential. The need for patients to behave responsibly to avoid spreading the virus, including the necessity of accepting antiretroviral treatment during pregnancy, must be highlighted.

Finally, FIGO suggested that it is essential to offer appropriate advice to women and men with HIV or whose partners are HIV-positive who wish to reproduce so that their health, the health of their partner and that of any future child is protected. Treatment of seropositive couples by assisted reproductive means that reduce the chance of the woman and her offspring being exposed to HIV are of proven efficiency. It is therefore ethical to offer such techniques in appropriate cases.

Two different medical aspects are analysed in this paper: reproductive assistance in a discordant couple with male positivity and in a discordant couple with female positivity.

Reproductive Assistance in an HIV-discordant Couple with an HIV-positive Male

HIV in Semen

Araneta[12] and Matz[13] reported that semen used for donor artificial inseminations can transmit HIV-1 infection. Studies on the presence of HIV in sperm have also yielded contradictory results. Using different approaches, Baccetti[14] detected HIV-1 particles and HIV-1 DNA in the ejaculated sperm of HIV-seropositive patients. The same group identified a specific HIV receptor, alternative to CD4+, on the sperm membrane: this molecule is a galactosyl–alkyl–acyl glycerol (GalAAG), a glycolipid structurally related to galactosylceramide, the receptor for HIV identified in CD4+ cells.[14,15]

At the same time, other authors emphasised the total absence of HIV particles and nucleic acids in sperm.[16–18] They demonstrated that the separation of seminal fluid and cellular elements from sperm by washing techniques reduces the viral load of semen detected by polymerase chain reaction (PCR) and reverse transcriptase PCR. Semprini et al.[19] were the first to use washed sperm from HIV-1-infected men for intrauterine inseminations (IUI). There are several reports indicating that HIV-1 DNA cannot be found in washed spermatozoa isolated from non-spermatozoa seminal cells and seminal plasma.[4,17,20]

In contrast to this reassuring epidemiological and laboratory background, there are reports using transmission electronic microscopy that indicate the possibility that HIV-1 virions are attached to the sperm cell surface and even within its cytoplasm. Other papers using extraction PCR and *in situ* PCR, respectively, report that proviral HIV-1 DNA can be detected in the spermatozoa of men with AIDS and men infected with HIV-1.[14,21–36]

To investigate these contradictory findings and assess the role of sperm-washing techniques in eliminating both HIV-1 RNA and DNA from semen infected with HIV-1, the authors tested the ejaculate of men infected with HIV-1 before and after processing semen. Testing of the three main seminal fractions – non-spermatozoa cells, cell-free seminal plasma and spermatozoa – was by highly sensitive extractive-nested PCR and *in situ* PCR. All samples of spermatozoa recovered after separation by gradient centrifugation and swim-up (sperm washing) were free of HIV-1 RNA above a threshold of 50 copies/ml and free of proviral DNA. This confirms the findings of previous reports in which nested PCR[4,37,38] was used to assess the validity of sperm washing in HIV-infected semen.

Although other more recent methodologies of sperm washing[39] confirm the validity of the general principle of removing the cellular component of semen, contradictory reports could be due to the inaccuracy of PCR techniques in older studies,[40] too low a threshold (one viral copy) of the PCR assay used to detect viral copies[38,41,42] and improper use of the definition of sperm washing,[43] without the final swim-up of spermatozoa.

In the authors' data of seven seminal plasma samples testing positive for HIV-1 RNA, six were from patients on highly active antiretroviral therapies (HAART). Four men had an elevated blood viral load and three an undetectable viraemia. These results confirm the findings of previous reports demonstrating discrepancies between haematic and seminal HIV-1 concentrations,[4,44] either due to a subtherapeutic concentration of antiretroviral drugs in the male seminal tract or due to production of HIV-1 RNA from localised cells that respond poorly to treatment.

The false-positive detection of HIV-1 DNA by *in situ* PCR in the semen of HIV-1-non-infected men confirms that this technique is inadequate for studying the presence of provirus in semen fractions. The presence of the virus in spermatozoa pellet samples could be due to the presence of non-sperm cells (NSCs) not completely eliminated during semen separation by discontinuous gradient centrifugation before swim-up. Alternatively, these could be real false-positive results due to non-specific hybridisation of *in situ* PCR. Bagasra,[23] Nuovo[34] and Muciaccia[35] showed the presence of provirus by *in situ* PCR in spermatozoa and germ cells at all stages of differentiation, from spermatogonium to round spermatidi. However, none of these studies assessed proper standards for *in situ* PCR specificity in uninfected males. These methodological limitations of *in situ* PCR probably explain why this technique has been abandoned in recent works. Nevertheless, Muciaccia[45] in his study reported the presence of HIV-1 DNA in small amounts of ejaculated abnormal spermatozoa from HIV-1-infected subjects. Interestingly, in these subjects a high percentage (58–80%) of ejaculated spermatozoa had abnormal morphologies and the percentage of spermatozoa with fragmented DNA (9.5–35.4%) greatly exceeded normal values (0.9–4.4%). The authors hold that *in situ* PCR, when correctly performed along with positive and negative controls, is a powerful, highly reliable technique for the detection of viral DNA in human tissue sections and cells.

Semen Processing

In order to eliminate the cell infected by HIV-1 in sperm, we perform sperm washing. The first report of this technique was published in *The Lancet* in 1987.[19] We reported a simple method to process the semen of men infected with HIV to eliminate infected seminal leukocytes from the ejaculate in order to recover uninfected spermatozoa for intrauterine transfer into HIV-uninfected women. Semen analysis was performed and samples were processed using a 40–80% density gradient (PureCeption kit) to separate motile spermatozoa from non-sperm cells, immotile spermatozoa and seminal plasma. The ejaculate was layered over the gradient and centrifuged at 400g for 30 minutes. After centrifugation, the supernatant was removed and the sperm pellet recovered and re-suspended in 3ml of fresh medium (Sage's Sperm Washing Medium). Washing at 400g for 10 minutes was performed and the supernatant was discarded. Subsequently, 1ml of medium was gently layered on the pellet and the tube was incubated at 37°C for one hour. After swim-up, a supernatant volume of about 500µl was recovered and an aliquot of this volume (100µl) was tested for detectable HIV-1 RNA using a realtime PCR assay (Biomerieux) according to the manufacturer's instructions. The remaining washed sperm (400µl) was stored at 4°C for about 22 hours and used for IUI with *in vitro* fertilisation (IVF) embryo transfer/intracytoplasmatic sperm injection (IVFET/ICSI) procedures if the PCR test for HIV-1 was negative.

Assisted Reproduction

The ART programme was offered to serodiscordant couples where the man was HIV-positive and seeking medical assistance.[46] Inclusion criteria were adopted to protect not only the couple but the possible child as well: partners were to engage only in protected sexual relations. HIV status had to be monitored and/or treated and long-term compliance had to be assessed by the infectious disease physician. Standard laboratory criteria were adopted:

- $CD4^+$ lymphocytes >200/mm^3 at least twice in the four months prior to treatment;
- stable viral load; and
- infection by an quantifiable, amplifiable strain of HIV-1.

Each couple was interviewed by a psychologist at inclusion and thereafter whenever necessary. Female fertility was assessed by standard procedures.

In clinical practice it is important to screen HIV-discordant couples to determine infertility factors due to the high prevalence of subfertility factors. One of the most important factors is genital tract infection in both males and females. The exact mechanisms involved in male-to-female transmission of HIV-1 are as yet undefined, but circumstantial evidence indicates that genital tract infections may act as facilitating factors. In sub-Saharan and Latin American countries, where heterosexual transfer of the virus is the leading cause of infection, there is also a high prevalence of genital infection carriers. The presence of a sexually transmitted pathogen recruits inflammatory cells in both the male and female genital tract. This may increase the number of HIV-1-infected cells in the semen or vaginal fluid of the seropositive subject, leading to a higher risk of infection for the seronegative partner. Conversely, when genital tract infection is present in the seronegative partner, the uninfected inflammatory cells may become a specific target for the virus.

The ART laboratory used for the procedure was considered to be a 'viral risk' area. It was separated from laboratory facilities used for couples negative for HIV and hepatitis B and C. The ART laboratory complied with standard recommended safety precautions. Specific precautions were implemented against the risk of HIV and hepatitis B and C contamination, as recommended by the French decree of 10 May 2001.[45] The potentially infected gametes and embryos were handled separately. A special biosafety cabinet workstation was used for all tasks that involved handling of sperm, oocytes and embryos.

In the Department of Obstetrics and Gynecology at the Luigi Sacco Biomedical Institute, the IUI pregnancy rate per cycle is 19% and per couple is 78%. Here, the pregnancy rate per couple was higher than the average 57% overall pregnancy rate by IUI in serodiscordant couples summarised by Sauer in 2005.[47] These results could be explained by the routine adoption of ovulation induction with low doses of recombinant fluorescence *in situ* hybridisation (FISH) and timing of ovulation with recombinant luteinising hormone (LH), according to Marina.[40] It could also be due to the standard usage of fresh sperm after realtime PCR or due to a good selection of cases, with an average of four attempts per couple. In addition, other centres used frozen semen.[37] There is a negative impact on the number of available motile sperm after freezing, as already reported,[43] which has a resultant impact on pregnancy rate per IUI.

At the centre at the Luigi Sacco Biomedical Institute more than 3,000 IUIs have been performed. This large number of cases, with safe pregnancy after sperm washing and the consistent biological results that have been published, has led the authors to consider the efficiency of sperm washing to be high.[47]

The efficiency of IUI, its safe outcome after sperm washing with swim-up and its relatively low cost make this first-level procedure the technique of choice in serodiscordant couples with an HIV-positive male partner when no other infertility problems are involved. When the female partner was suffering from infertility factors, the male partner had fewer than $1x10^6$ total motile cells in the final fraction after sperm washing or both partners had a combination of subfertility conditions, IVF/ICSI was performed. The pregnancy rate per embryo transfer was in agreement with similar smaller HIV series[37] and larger non-HIV series.[48] Other markers of outcome were as good in these couples treated after sperm washing as in other infertility series of comparable age: fertilisation rate was 65% by IVF and 88% by ICSI.[38]

The problem with ICSI in serodiscordant couples is the high multiple pregnancy rate and possible obstetric and neonatal complications associated with these pregnancies (14% for Garrido[38] and 57.1% for Pena).[48] The possible additional costs determined by pre-natal and neonatal care in multiple pregnancies should be considered.[49–51] In the authors' experience, the multiple pregnancy rate by IVF/ICSI was 10%, reflecting the special care in superovulation induction and embryo transfer. In 2002, at the Luigi Sacco Biomedical Institute, more than 4,000 IUIs were performed in serodiscordant couples and 1,000 fertilisation *in vitro* and embryo transfer (FIVET)/ICSI cycles without HIV-1 transmission to the female partners with an adequate follow up.

Reproductive Assistance in an HIV-discordant Couple with an HIV-positive Female

Some preliminary studies suggest that HIV-infected women may have a decreased fertility rate[52] and a higher frequency of menstruation disturbances associated with low CD4 cell counts[53] and upper genital tract infections.[54] In addition, severe ovarian dysfunction, such as premature ovarian failure or ovarian resistance to stimulation, has also been described.[55,56] Ovarian resistance to hyperstimulation may add to this effect because a greater number of units of gonadotrophins were needed to adequately stimulate these patients. This resistance may reflect an underlying subclinical (normal menses) and subanalytical (comparable basal FISH values) hypogonadism. Superovulation may be considered a functional stress test on the ovary.

Very few data are available on the presence of viral material in the cumulus oophorus complex of infected women. Baccetti[26] exposed unfertilised human oocytes partly surrounded by follicular cells to low doses of HIV-1 and found that they remained negative for the presence of HIV-1 DNA. This suggests the resistance of oocytes to HIV-1 penetration, possibly as a result of the absence of specific receptors for the virus, as assessed by immunocytochemistry. Bertrand[57] was unable to detect the presence of HIV-1 genetic material in the follicular fluid or flush fluids of patients with undetectable plasma viral loads. Nevertheless, in one of his patients with a low but detectable load, HIV-1 RNA was detected in one follicular fluid and one flush.

A paper from Martinet[58] evaluates the ovarian response to IVF stimulation of HIV-positive patients compared with control patients. No significant difference was observed between HIV-positive patients and matched negative controls in terms of ovarian response to stimulation. The pregnancy rate calculated per transfer was 14%, which is lower than that obtained by Ohl (23.9%)[59] but similar to the results of Terriou and colleagues (16.1%).[60] The latter authors

performed ICSI and IVF in a series of 29 seropositive women (66 cycles). They compared their results with an age-matched group of uninfected women and with their overall uninfected population. Higher cancellation rates and lower pregnancy rates were observed when the overall population was considered, but these differences disappeared when using an age-matched group.

Coll et al.[61] also found a clinical pregnancy rate of 16.2% among infected patients (n=50), which was half that of a group of age-matched uninfected patients (37.5%). When they restricted their analysis to cycles with oocyte donation, these differences disappeared (36 versus 44% of patients). These authors therefore concluded that poor IVF results in HIV-positive women may be due to reduced ovarian response. Ovarian resistance to hyperstimulation may be involved in this effect because a greater number of units of gonadotrophins were needed to adequately stimulate these patients. As stated before, this resistance may reflect underlying subclinical and subanalytical hypogonadism and superovulation may be a useful functional stress test on the ovary.

Finally, Guibert et al.[62] observed increased FSH levels on the third day of the cycle in a population of 80 HIV-positive patients compared with a control group of similar age (n=70), and concluded that HIV infection accelerates ovarian reserve depletion. On the other hand, when selecting women below 42 years of age with normal basal FISH and inhibin levels, no difference in ovarian response was observed between HIV-positive and HIV-negative patients (n=14 for each group). The authors at the Luigi Sacco Biomedical Institute concluded from these results that IVF is not influenced by HIV infection in patients with a normal ovarian reserve. The discrepancies in the results between studies may be explained by heterogeneities in the populations studied. Differences in the matching processes and lack of power of the various studies are other possible explanations.

Pelvic inflammatory disease has been shown to reduce ovarian stimulation due to direct damage to the ovaries, follicle loss or mechanical alterations in follicle development. This may result from adherences or a deficiency in ovarian vascularisation.[63,64] For instance, a significantly higher prevalence of serum immunoglobulin G (IgG) antibodies to *Chlamydia trachomatis* was observed in poor responders, suggesting a possible detrimental effect of *C. trachomatis* on subsequent ovarian function.[64] This may explain a tendency towards reduced ovarian response among HIV-positive patients.

Conclusion

Reproductive counselling for individuals with HIV might motivate them to ask for reproductive care in order to limit the risk of infecting uninfected partners, or of superinfection if the partner is also infected. By offering reproductive care to men infected with HIV, it is possible to strengthen the message that, by protecting their partners from becoming infected through unprotected sex, they could in the future become the healthy mother of an uninfected child. For uninfected women with an infected partner, the optimal solution remains protecting themselves from becoming infected to avoid perinatal transmission of HIV. For women who are HIV-1-positive, the problem remains the risk of vertical transmission. Significant progress has been made in this area, but additional research into the mechanisms of vertical transmission of HIV is still needed. ■

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