

Assisted reproductive technologies to establish pregnancies in couples with an HIV-1-infected man

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ABSTRACT

For HIV-1-infected men and women the introduction of highly active antiretroviral therapy (HAART) in 1996 led to a spectacular increase in life expectancy and quality of life. In Western society where HAART is readily available, HIV-1 is now considered to be a chronic disease and as a consequence quality of life is an important aspect for men and women with HIV-1. Many of them express the desire to father or mother a child. Assisted reproductive technologies, including intrauterine insemination (IUI), *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) in combination with semen washing have been used to decrease the risk of HIV-1 transmission in HIV-1-infected discordant couples with an HIV-1-infected man. This article aims to summarise the current state of the art of assisted reproductive technologies for couples with an HIV-1-infected man and to discuss current trends and dilemmas in the treatment of these couples.

KEYWORDS

Assisted reproductive techniques, HIV-1, reproduction

INTRODUCTION

From its initial presentation in the early 1980s until 1996, HIV-1 infection almost inevitably led to AIDS, which was a death sentence. Life expectancy after the diagnosis was on average only ten months in 1987, and only 20 months after the introduction of zidovudine in 1990.¹ Because of such a short life expectancy, patients were advised

not to get pregnant.² Couples with one HIV-1-infected partner, i.e. HIV-1-discordant couples, had a high risk of horizontal transmission of the virus to their uninfected partner, and HIV-1-infected women had a high risk of vertically transmitting the virus to their child.^{2,3} As a consequence, these couples were advised to always use condoms, irrespective of other contraceptives. If women nonetheless did become pregnant, they were advised to undergo first-trimester abortion.⁴

In 1990, in an era where data on HIV-1 in semen or spermatozoa were not yet available, the Italian gynaecologist Semprini started carrying out intrauterine inseminations (IUI) of HIV-negative women with processed semen from their HIV-1-infected partners, in order to reduce the risk of horizontal HIV-1 transmission.⁵ In the hope of selecting HIV-1-free motile spermatozoa, Semprini processed semen from HIV-1-infected men by combining density-gradient centrifugation with swim-up of spermatozoa. After negative testing for HIV using immunofluorescence with monoclonal antibodies against HIV p17, the final sperm fraction was used for IUI, in which processed semen is inserted directly into the uterine cavity with a syringe. For more than ten years, Semprini remained the only clinician providing fertility care for HIV couples, but he received a lot of criticism from his colleagues.⁶ Arguments against IUI with processed sperm during that time were 1) the short life expectancy of the future father, 2) the very low sensitivity and therefore a high chance of a false-negative result of the test that was used to detect any residual HIV in processed semen before insemination, and 3) the report by the Centre for Disease Control (CDC) of one case of HIV-1 transmission after IUI with processed

semen in 1990, although in this specific case the semen did not undergo the combination of density-gradient centrifugation and swim-up of spermatozoa that was used by Semprini and was not tested for the presence of HIV before insemination.⁷

In 1996, the introduction of highly active antiretroviral therapy (HAART) led to a spectacular increase in life expectancy, AIDS-free survival, and quality of life of HIV-1-infected men and women with access to this therapy.⁸ These radically changed clinical circumstances led to the publication of numerous debates in authoritative journals with a plea to reconsider the ban on reproduction for HIV-1-infected couples.⁹⁻¹¹ The first argument to offer HIV-1-infected couples artificial reproductive technologies was 'harm minimisation'. IUI with processed semen seemed to be safe, as seroconversions of the treated women or their offspring after IUI with HIV-negative sperm had never been described.¹² Withholding these techniques could lead patients to practice unprotected intercourse with an unknown but presumably higher risk of HIV-1 infection. Second, in 1998 the US Supreme Court declared that an asymptomatic HIV-1 infection should be considered a handicap falling under the protection of the Americans with Disabilities Act (ADA).¹³ As discrimination of people with any handicap under the ADA is unlawful, it was felt that the categorical exclusion of people with an HIV infection from assisted reproductive technology programmes was also unlawful.¹⁴ The third –moral– argument was that medical interventions should not be discriminatory. Couples with HIV infection were not essentially different from couples with other chronic diseases or couples with an increased chance of having offspring with anomalies, for whom there was no ban on assisted reproductive technologies, for instance couples with diabetes, and women in their forties who have an increased chance of having a child with Down's syndrome. The final argument was that a doctor has to respect a patient's autonomy when the risks seemed acceptable, even if the patient's values, preferences and decisions conflicted with the values of the doctor.

These debates gradually changed the initial unwillingness to accept HIV-1-infected couples into assisted reproductive technologies programmes. In addition, the improved

prognosis of patients with HIV-1 infection following the introduction of combination antiretroviral therapy had led to more HIV-1-discordant and HIV-1-concordant couples wishing to mother or father a child.¹⁵ Furthermore, far more sensitive polymerase chain reaction-based methods to detect the presence of HIV-1 not only in blood but also in other body fluids including semen had become available.¹⁶ Following this mind shift, Semprini's method has been copied and refined by many others, and assisted reproductive technologies are now increasingly being offered to these couples around the globe.^{17,18} The Academic Medical Centre in Amsterdam is currently the only hospital in the Netherlands providing fertility care for HIV-1-discordant and HIV-1-concordant couples. The current state of the art of assisted reproductive technologies for couples with an HIV-1-infection is summarised below and current trends and dilemmas in the treatment of these couples are discussed.

ASSISTED REPRODUCTIVE TECHNOLOGIES

At present, the rationale of assisted reproductive technologies in HIV-1-infected couples can be threefold: to overcome subfertility for the same indications as in non-HIV-1-infected couples, to minimise the risk of HIV-1 transmission in case of an HIV-1-serodiscordant couple with an HIV-1-infected man or to prevent HIV-1 superinfection with a different HIV-1 strain in seroconcordant couples (*table 1*). A treatment algorithm has been published to guide in the careful evaluation of these couples.¹⁹

The basic principle underlying assisted reproductive technologies in HIV-1-discordant couples with an HIV-1-infected man is the processing of semen, during which HIV-1-free, motile spermatozoa with a normal morphology are separated from seminal plasma and other non-seminal cells. This is achieved by combining density gradient centrifugation with swim-up, and testing of the spermatozoal fraction for HIV-1, using PCR-based methods.²⁰ After a negative test, the remaining spermatozoa

Table 1. Artificial reproductive techniques in human immunodeficiency virus type-1 (HIV-1) discordant and concordant couples

Man	Woman	Risk for (super)infection partner	Primary goal of artificial reproductive techniques	HIV semen processing
HIV+	HIV-	Yes	Prevent HIV-1 transmission	Yes
HIV-	HIV+	No ^a	Overcome subfertility	No
HIV+	HIV+	No ^b	Overcome subfertility	No
HIV+	HIV+	Yes ^b	Prevent HIV-1 transmission	Yes

^aWhen self-insemination is used; ^b risk of superinfection depends on whether or not partners are infected with the same HIV strain, and whether or not one or both partners are being treated with HAART.

can be used for assisted reproductive technologies such as intrauterine insemination (IUI), *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI). Since the lower limit of detection of the PCR tests used is never nil, the risk of HIV-1 transmission by assisted reproductive technologies can never be completely eliminated. These assisted reproductive technologies in HIV-1-discordant couples should therefore be considered risk-reduction and not risk-elimination strategies.

INTRAUTERINE INSEMINATIONS IN THE ACADEMIC MEDICAL CENTRE

In 2003 the AMC started a programme offering assisted reproductive technologies for HIV-1-discordant couples with an HIV-1-infected man. Both therapy-naïve men and men receiving HAART are eligible for the programme.

A standardised fertility work-up is performed to assess possible fertility problems. In addition, HIV-1-semen processing is done to ascertain whether two million spermatozoa remain after processing, for both the PCR test and the actual insemination, since this number is a prerequisite for treatment.

During IUI treatment mild ovarian hyperstimulation takes place with recombinant follicle stimulating hormone (FSH). On the day of insemination the semen is produced in the morning, processed, and half of the spermatozoa fraction with at least one million spermatozoa is tested for the presence of HIV-1 RNA. The test, which includes both positive and negative internal controls, was validated in our own hospital and has a lower limit of detection of 10 HIV-1-RNA copies per portion of one million spermatozoa. IUI is only performed in the afternoon with the remaining part of the spermatozoa fraction, which contains at least one million spermatozoa, when HIV-1-RNA tests in the spermatozoa fraction are negative. The woman undergoes standard HIV testing every three IUI cycles or at 4, 12 and 24 weeks gestation. The child undergoes an HIV test at the age of six months.

Since the start of the programme, 61 HIV-1-discordant couples have been accepted (*table 2*). These 61 couples underwent 266 IUI cycles. In 174 cycles (65%) IUI was performed, and in 92 cycles (35%) the insemination was cancelled. In 46 cycles the insemination was cancelled before ovulation, because of a risk of multiple pregnancy (more than two dominant follicles on trans-vaginal ultrasound), ovulation during the weekend (no possibility to perform the PCR test) or for personal reasons. In 46 cycles the insemination was cancelled after ovulation, because the number of spermatozoa was lower than two million spermatozoa after semen processing on the day of insemination, the HIV-1 RNA test after processing was positive or not reliable, or because of other reasons.

Table 2. Results of intrauterine inseminations in human immunodeficiency virus type-1 (HIV-1) discordant couples with an HIV-1-infected male partner in the Academic Medical Centre from 2003-2008

Results	N (%)
Couples	61
Cycles	266
Cancel insemination	92 (35)
Inseminations:	174 (65)
• Clinical pregnancies	32 (52)
• Miscarriage	6
• Ectopic pregnancies	1
• Ongoing pregnancies	25 (41)
• Twins	5
• Babies born	30
• Seroconversions	0

Thirty-two women became pregnant (52%), 25 of these women had an ongoing pregnancy (41%), i.e. a viable pregnancy on ultrasound at 12 weeks gestation, of whom 20 were singletons and five were twin pregnancies. The percentage of clinical pregnancies, i.e. a pregnancy visible on ultrasound, and the percentage of ongoing pregnancies, was 12 and 9%, respectively, per IUI cycle, and 18 and 14%, respectively, per insemination. As of June 2009, 30 children have been born; none of the mothers or children have seroconverted for HIV-1.

Ten couples returned for second children, thus far nine of these women have an ongoing pregnancy.

Our clinical pregnancy rate for first children (18%) is comparable with the 15.1% pregnancies per intrauterine insemination (IUI) which were reported in the largest reported series of IUI in discordant couples with an HIV-1-infected man. This series described pooled data of 2840 IUI cycles carried out in Europe.¹⁷ Seroconversion for HIV-1 did not occur in either of the HIV-1 negative women or babies.

INFLUENCE OF HIV INFECTION AND HAART ON SEMEN QUALITY

IUI is noninvasive and less costly than IVF or ICSI.²¹ However, many HIV-1-infected men are excluded from IUI, because their semen qualities are poor, already prior to the semen processing, or after the intensive semen processing due to its low efficiency (5-10% recovery rate, unpublished data). As a result, only men with good semen quality can opt for IUI. In the AMC, out of 177 men who underwent a semen processing test, 56 (32%) had less than two million spermatozoa after processing.

In a longitudinal cohort study involving 55 men not yet receiving antiretroviral therapy, we found that once these men were chronically infected with HIV-1, semen parameters were not affected by ongoing HIV-1 infection during the

observation period of 77 weeks on average.²² We observed in this study that delaying treatment in HIV-1-infected patients until CD4 cell counts reached around 200 cells/mm³ had no adverse effect on semen quality. Ongoing HIV-1 infection, therefore, probably does not appear to affect the chance to qualify for IUI. In contrast, the percentage of progressively motile spermatozoa decreased significantly from 28 to 17% during 48 weeks of follow-up in another longitudinal cohort study involving 34 men who started first-line HAART.²³ The negative effect on the percentage of progressively motile spermatozoa by HAART may thus have a negative effect on the chance to qualify for IUI. The impact of HAART on semen quality becomes more relevant in view of guidelines increasingly recommending earlier initiation of HAART.^{24,25} As a result, more men will use HAART for a longer period during their HIV-1 infection. The impact of this policy on semen quality has not yet been studied, but according to the outcome of our study on HAART and semen quality, it may be associated with a negative impact on the percentage of progressively motile spermatozoa.

The consequences of the observed reduction in the percentage of progressively motile spermatozoa during HAART on IUI outcome remain unknown. Although data acquired from a non-HIV-1-infected population show that progressively motile spermatozoa determine the chance to conceive successfully by IUI,^{26,27} the only study that described predictors of success in HIV-IUI was flawed by the *a priori* inclusion of men with good semen qualities only.^{28,29}

ASSISTED REPRODUCTIVE TECHNOLOGY OF CHOICE: IUI, IVF OR ICSI

There is no uniformity in assisted reproductive technologies that are offered by various centres around the world to HIV-1-discordant couples.¹⁷ Most centres perform IUI, as HIV-1-infected couples are not infertile unless proven otherwise, so ICSI should not be used routinely. In men with *a priori* lower semen qualities or a sperm yield lower than two million spermatozoa after semen processing, ICSI is the only realistic treatment option.^{18,30-34} In ICSI a single spermatozoon is injected directly into an oocyte, during which the pellucid zone of the oocyte is penetrated artificially. So far, the results of over 1300 cycles of ICSI have been published and not a single case of HIV-1 transmission to the woman or the child has been reported.^{17,18,30-36} The largest numbers of ICSI cycles have been published in Europe and the USA. In Europe, clinical pregnancy rates, and live birth rates per ICSI cycle of 31 and 16% respectively have been reported.¹⁷ In the USA, clinical pregnancy rates and live birth rates per ICSI cycle of 36 and 29%, respectively, have been published.³⁶

Despite the lack of scientific evidence, some authors even advocate the sole use of ICSI to prevent HIV-1 transmission irrespective of semen quality.²¹ Arguments used in favour of ICSI are that pregnancy rates are generally higher with ICSI than with IUI, and thus less cycles of ICSI are needed to achieve pregnancy, with less exposure to possibly HIV-1-contaminated spermatozoa, but randomised studies that compare pregnancy rates in ICSI and IUI are lacking.³⁶ A second argument in favour of ICSI is that, in contrast to IUI, only a single spermatozoon in minute amounts of medium is used, thus decreasing the likelihood of contamination with HIV-1.^{31,33} As no HIV-1 infections have ever been observed after IUI this argument lacks validity. At present, the joint perspective of the Dutch Society of Obstetrics and Gynaecology, the Dutch Society of Clinical Embryologists and the Dutch Working Group of Clinical Virologists is not to perform ICSI in HIV-1-infected men and women, reasoning that the injection of a single spermatozoon, potentially carrying an HIV-1 particle, directly into an oocyte may lead to incorporation of the viral genome into the future embryo, with unknown but possible catastrophic consequences, for instance iatrogenic HIV-1-infected children.

NATURAL CONCEPTION IN DISCORDANT COUPLES WITH AN HIV-1-INFECTED MAN

Some infectious disease specialists, ethicists, and fertility specialists feel that HIV-1-discordant couples should not only be informed about assisted reproductive technologies, but also about the possibility of natural conception when they request reproductive advice.^{37,38}

The argument in favour of natural conception is that the estimated risk of HIV-1 transmission in HIV-1-serodiscordant couples is lower than 1/1000 unprotected intercourse at blood plasma HIV-1-RNA concentrations lower than 1700 copies/ml,³⁹ and is estimated to be even lower during successful HAART,⁴⁰ when the blood plasma HIV-1-RNA concentration, the most important predictor of sexual HIV-1 transmission, decreases to below the limit of detection.⁴¹

In these couples, natural conception should only take place after optimisation of factors that limit the chance of HIV-1 transmission and improve the chance to conceive. This would include: 1) a fertility screen, with couples diagnosed as infertile being offered assisted reproductive technologies, 2) the initiation of HAART, 3) the exclusion or treatment of genital tract infections, and 4) the avoidance of unprotected intercourse other than around the established time of ovulation –timed intercourse– and immediate abstinence from unprotected sex as soon as pregnancy is achieved.^{37,38,42,43} In the Kantonsspital St.

Gallen, Switzerland, HIV-1-discordant couples with an HIV-1-infected man on HAART are currently offered three cycles of timed, unprotected intercourse with pre-exposure prophylaxis with tenofovir 245 mg provided to the woman, 36 and 12 hours before intercourse (<http://www.creathe.org>). The results of this strategy in terms of pregnancy rates and seroconversions have not yet been published. The concept of unprotected intercourse is heavily debated for several reasons. First, the safety of natural conception will be difficult to prove due to the low seroconversion rate during unprotected intercourse.³⁹ A 3.8% HIV-1-transmission rate in pregnancy was observed in 1997 in previously HIV-negative women who conceived naturally from their HIV-1-infected male partners.⁴⁴ However, in this study only 20% of men were using antiretroviral therapy and none of them were using HAART. A recent retrospective study did not report HIV-1 transmission in HIV-1-discordant couples achieving pregnancy when the HIV-1-infected man or woman had an undetectable blood plasma HIV-1-RNA level under HAART.⁴² Unfortunately, both studies are flawed by the inclusion of successful pregnancies only, couples who were unsuccessfully trying to conceive were not included and both studies do not mention the number of unprotected coital acts needed to achieve pregnancy.^{42,44} The actual seroconversion rate during natural conception may thus have been higher. Second, the exact chance of HIV-1 transmission in an individual couple is difficult to predict, as HIV-1 may be intermittently present in the male and female genital tract at variable concentrations, sometimes irrespective of HAART or genital tract infections and is detectable in seminal plasma in 5% of men who are using HAART for at least six months.^{45,46} Third, we have recently reported that although the risk of HIV-1 transmission is reduced to almost nil by assisted reproductive technologies, the fear for HIV-1 transmission among these couples is not proportionally reduced, and anxiety for HIV-1 transmission is still present among these couples.⁴⁷ To our knowledge, there are no data on the psychological impact of natural conception in HIV-1-discordant couples with an HIV-1-infected man.

RECOMMENDATIONS

In our view four issues in the use of assisted reproductive technologies for HIV-1-infected men deserve priority to be clarified further. First, it is unclear at present whether natural conception is a safe strategy. With the knowledge we have today, a randomised controlled trial comparing natural conception with pre-exposure prophylaxis to IUI with semen washing could demonstrate feasibility, safety and effectiveness. Second, HIV-1-discordant couples' attitudes towards natural conception must be explored. Third, the consequences on IUI outcome of the observed reduction in the percentage of progressively motile

spermatozoa during HAART are unknown. It is therefore important to identify prognostic factors for IUI outcome, and to adjust assisted reproductive technology protocols accordingly. Fourth, despite the wide application of ICSI in HIV-1-discordant couples, its safety has not yet been proven. The safety of ICSI is currently being studied in *in vitro* studies in the Academic Medical Centre in Amsterdam, the Netherlands. The objectives of these studies are to investigate whether human oocytes can become infected via intracytoplasmic injection with HIV-1.

As fertility care for HIV-1-infected couples is complex, it is crucial that HIV physicians, reproductive gynaecologists, reproductive biologists and virologists work together on the fertility treatment of HIV-1-infected patients and extend their knowledge on reproductive issues, in order to offer these couples up-to-date assisted reproductive support.

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