CASE REPORT

Pregnancy following intracytoplasmic sperm injection from an HIV-1-seropositive man

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The first pregnancy achieved in a seronegative woman following in-vitro fecundation through intracytoplasmic sperm (ICSI) injection from a man with autoimmune deficiency syndrome (AIDS; HIV-1 carrier) is reported. The semen was prepared by PureSperm® and swim-up techniques. Some of the motile spermatozoa obtained were used to detect the presence of HIV-1 using the polymerase chain reaction technique. HIV-1 in DNA or RNA form was not detected using this technique. The remaining spermatozoa were frozen. Ovarian stimulation in the woman was performed with long-protocol analogues and gonadotrophins. Thirteen mature oocytes were recovered, into which the thawed spermatozoa were microinjected. Nine embryos were obtained. Four were frozen, four transferred and one discarded. The woman became pregnant. Analyses for HIV-1 in the woman, performed in the first and third months of pregnancy, gave negative results.

This case provides further experience with washed semen of sufficient quality for performing artificial insemination in HIV-1-serodiscordant couples (101 inseminations, 31 pregnancies, 28 deliveries, 37 babies, all healthy). In women with obstructed Fallopian tubes, or when the semen is not of sufficient quality for artificial insemination techniques to be performed, ICSI can be carried out using frozen, HIV-1-free semen.

Key words: HIV/ICSI/PCR/pregnancy/semen

Introduction

HIV-1-serodiscordant couples (a seropositive man and a seronegative woman) must use a condom during intercourse to prevent infecting the woman with the autoimmune deficiency syndrome (AIDS) virus. They cannot have children even if they are fertile and want to be parents. After the pioneering work by Semprini et al. (1992), washing of HIV-1-seropositive men’s semen was commenced in 1992; their female partners were then inseminated with the fraction of motile spermatozoa obtained after semen washing.

In an article published recently, Semprini (1997) reported that he had carried out >1000 artificial insemination (AI) cycles with washed semen in serodiscordant couples and 250 babies had been born. In no case had the inseminated women, and consequently their babies, been infected.

We have carried out 101 AI cycles with washed semen at the Instituto CEFER, Barcelona, Spain, and 37 babies have been born. No seroconversion of the inseminated women has taken place and all the babies were born seronegative (Marina et al., 1998). Our results corroborate Semprini’s data (1997).

However, there are some cases in which AI is not indicated: women with obstructed Fallopian tubes, cases of low-quality semen and faulty AI methods. In these cases, intracytoplasmic sperm injection (ICSI) or in-vitro fertilization (IVF) must be performed. We report on the first pregnancy achieved at our centre using ICSI in a HIV-1-serodiscordant couple.

Case report

The HIV-1-discordant couple (seropositive man) consulted us for reasons of infertility.

The man, aged 29 years, had known that he was HIV-1-seropositive for 7 years. He had become infected through parenteral drug use. He and his present partner had been in a stable relationship for the previous 8 years. During the first year, they used coitus interruptus as their birth control method. They used a condom after finding out that he was seropositive. When the study was performed, the man was included in Group C 3 of the 1993 Centers for Disease Control (CDC) classification (Castro et al., 1992): he had suffered from a case of cerebral toxoplasmosis and his number of T CD4+ lymphocytes was 75 per mm³. His viral load was <200 copies/ml. He was receiving treatment with stavudine, lamivudine, sulphadiazine, pyrimethamine and folinic acid. His clinical history showed no other significant pathological events. An andrological physical exploration was performed; an andrological physical exploration was performed; a semen analysis was carried out following World Health Organization criteria (WHO, 1992), and semen washing was done using a procedure similar to the method described by Moohan and Lindsay (1995). The procedure was the following: selection by centrifugation (300 g, 20 min) in discontinuous gradients (50%, 70%, 90%) of PureSperm®, followed by the swim-up technique (Martín et al., 1991). The motile spermatozoa obtained after washing were suspended in HTF and divided...
...into two parts. One half was frozen following the technique regularly used in our laboratory (Marina, 1980). The other half was used to detect HIV-1 RNA and DNA using polymerase chain reaction (PCR). The spermatic chromatin was decondensed with dithiothreitol. The details have been published (Von Beroldingen et al., 1989; Dragon et al., 1993; Marina et al., 1998). Briefly, to check for viral RNA, reverse transcription to cDNA (complementary DNA) was done with use of the rTh polymerase enzyme. For the viral DNA test, the Taq polymerase enzyme was used.

At the same time as the study was performed, the HIV-seronegative woman, aged 31 years, was given a fertility test. In addition to her clinical history and a physical exploration, a seronegative woman, aged 31 years, was given a fertility test. Ovarian stimulation was performed following the treatment with gonadotrophin releasing hormone (GnRH) analogue (Pronorm®; Abbott, Madrid, Spain) and recombinant follicle stimulating hormone (FSH; Gonal-F®; Serono, Barcelona, Spain). Ovarian response was monitored through ultrasound of the ovaries via the vagina and serial oestradiol readings. She received 10 000 IU of human chorionic gonadotrophin (HCG; Profasi-HP®; Serono) 36 h before ultrasound-guided follicular puncture via the vagina. The ICSI technique was performed following Palermo et al. (1992).

This study was authorized by the Centre’s Ethics and Research Committee, and the couple gave their informed consent in writing.

The man’s clinical history did not contribute any other significant pathological data. Physical exploration gave normal results. Study of the ejaculate showed the volume to be 1.8 ml; sperm count 84×10⁶/ml; sperm motility (grade 2 plus grade 3) 60%; normal sperm morphology 60%. After washing, a total of 1.24×10⁶ normal motile spermatozoa was obtained.

The spermatozoa which had been frozen (0.62×10⁶) were thawed on the day ICSI was to be performed. The PCR technique for detecting HIV-1 RNA and DNA gave a negative result on the remaining spermatozoa.

Seventeen oocytes were obtained through follicular puncture, 13 of which were in metaphase II. ICSI was performed on these 13 oocytes. Nine embryos were obtained. Four were frozen and four were transferred on day +3 after the follicular puncture. One embryo did not divide.

On day 28 of the cycle, 15 days after follicular puncture, the βHCG concentration was 203.3 mUI/ml. Pregnancy was confirmed by ultrasound scanning on day 40 (one sac).

PCR analysis for HIV-1 RNA and DNA in the woman’s serum, performed 1 month after embryo transfer, gave a negative result. Testing for HIV-1 antibodies in the woman 3 months after transfer also gave negative results.

**Discussion**

Does a doctor have the right to refuse infertility treatment to a well-informed couple where one of the partners is HIV-1 seropositive? The right to be treated is questioned in cases of seropositive women where the viral process is in an advanced stage. The necessary anti-retrovirus medication would have to be suspended during the first 3 months of pregnancy owing to its possible teratogenic effects (Olaitan et al., 1996). However, a seropositive man in the C3 stage according to CDC classification (Castro et al., 1992), as in the case under study, does not need to suspend the anti-retrovirus medication. This factor is not taken as a basis for deciding whether he should be treated for infertility. Despite his advanced stage of AIDS, C3, his semen was normal according to WHO (1992) criteria. However, Dondero et al. (1996) observed in HIV-1-seropositive men a reduction of sperm count, motility and morphology despite the fact that their patients were not in such an advanced stage; none of them were in stage C3. Although the semen was normal, recovery of motile spermatozoa after the washing processes was poor: 1.6×10⁶. This number is insufficient for detecting the presence of HIV-1 using the PCR technique and to perform AI with any certainty of achieving pregnancy. ICSI and IVF was therefore indicated.

Of note is the fact that spermatozoa survived without seminal plasma when using the same freezing technique as for whole semen, i.e. spermatozoa with seminal plasma. This allows for washing and analysing for HIV-1 using PCR in semen samples obtained before follicular puncture is performed.

A third point is the possible existence of HIV-1 within the spermatozoon. The presence of intraspermatic HIV-1 in seropositive men has been described by Baccetti et al. (1990, 1991, 1994, 1996), Bagasra et al. (1994) and Scofield et al. (1994). Other authors have detected HIV-1 only in seminal plasma and/or in the HIV-1 target cells present in the ejaculate (lymphocytes and macrophages), but not in gametes (Quayle et al., 1997). The presence of intraspermatic HIV-1 has also been detected in the semen of HIV-1-seronegative men after it has been infected in vitro with HIV-1 (Dussaix et al., 1993; Bagasra et al., 1994; Baccetti et al., 1994). The paper of Kuji et al. (1998) does not corroborate the presence of intraspermatic HIV-1 after in-vitro culture of spermatozoa with HIV-1. The presence of HIV-1 in spermatogonia, spermatocytes and, to a lesser degree, in spermatids has been demonstrated (Nuovo et al., 1994). However, the study was carried out on the testicles, mainly atrophied, of men who had died from AIDS, a situation which cannot entirely be extrapolated to the case under study of a man with a low viral load and normal sperm count. Semprini’s (1997) extensive clinical experience and our own (to date, >1500 AI cycles have been performed by the two groups) suggests that motile spermatozoa are not HIV-1 vectors. If they are, they are not capable of infecting inseminated women since — and this is merely a hypothesis — the inoculant is very small and below the sensitivity limit of the PCR technique, i.e. <200 copies/ml of HIV-1 in virion form and <10 infected cells.

The ICSI technique involves using only one spermatozoon per oocyte (four spermatozoa in the case under study, since four embryos were transferred) and it would therefore seem logical that risk of infection is lower than in AI, where women receive millions of spermatozoa.

In view of the case discussed here, we consider that ICSI can be performed on serodiscordant couples when AI is not feasible or is unsuccessful.
References


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