Azoospermic HIV-1 infected patients wishing to have children: proposed strategy to reduce HIV-1 transmission risk during sperm retrieval and intracytoplasmic sperm injection: Case Report

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BACKGROUND: To date, assisted reproductive technology (ART) with sperm washing is offered to serodiscordant couples with an human immunodeficiency virus-1 (HIV-1) infected male partner in order to have a child while reducing the risk of transmission to the woman. However, ART programmes are not possible if the man is azoospermic. We report here the first birth following intracytoplasmic sperm injection (ICSI) using frozen epididymal spermatozoa obtained after surgical sperm retrieval in a HIV-1 infected man with obstructive azoospermia. METHODS: Sperm obtained by micro-epididymal sperm aspiration was frozen after density gradient preparation and tested for HIV-RNA and DNA. ICSI with frozen sperm was performed. RESULTS: A twin pregnancy was obtained following ICSI. Two healthy girls were born. Maternal HIV-1 RNA and HIV-1 serology were negative during pregnancy and at delivery. CONCLUSIONS: This case report demonstrates that ART is possible in azoospermic HIV-1 infected men. On the basis of current knowledge, we propose a strategy to reduce HIV-1 transmission risk during sperm retrieval and ICSI in couples where the man is HIV-1 infected and azoospermic.

Keywords: azoospermic men; HIV; ICSI; virus; serodiscordant couples

Introduction

Human immunodeficiency virus-1 (HIV-1) affects ~40 million persons around the world (UNAIDS/WHO, 2005). Sexual activity is the principal mode of transmission and several studies have reported the presence of HIV-1 in semen (Pasquier et al., 2000; Zagury et al., 1984). In serodiscordant couples where the male partner is HIV-1 infected, consistent condom use is necessary to prevent transmission to the woman. Nevertheless, these couples of reproductive age have a similar desire for children as couples without HIV infection (Chen et al., 2001; Panozzo et al., 2003).

Since 1996, the introduction of highly active antiretroviral therapy (HAART) has radically improved the prognosis of HIV-1 infection and has enabled patients to make plans for the future. Ethics recommendations concerning assisted reproduction have changed (Gilling-Smith et al., 2001; Lyerly and Anderson, 2001). In 1992, Semprini et al. (1992) were the first to report the results of an insemination programme using spermatozoa obtained after specific sperm preparation, allowing serodiscordant couples with an HIV-1 infected male partner to have a child while reducing the risk of transmission to the woman. More recently, and particularly after the introduction of HAART and the changes in ethics recommendations, several groups have reported the results of assisted reproductive technology (ART) programmes for such couples (Bujan et al., 2004b; Gilling-Smith et al., 2006).

These programmes were performed in couples where the man had a sufficient quantity of spermatozoa in the ejaculate to allow sperm washing. In HIV infected men with few spermatozoa, ART was not available because of the need for sufficient cells for adequate virological testing. However, a recent study attempted ART procedures in oligospermic men (Garrido et al., 2006). In azoospermic HIV-infected patients, only one paper has reported an embryo transfer, but without pregnancy, after synchronous sperm retrieval and sperm washing in an intracytoplasmic sperm injection (ICSI) cycle in a man with...
congenital bilateral absence of the vas deferens (Nicopoullos et al., 2004).

In this report, we present the result of an ART programme with frozen epididymal spermatozoa in a couple where the man was azoospermic and HIV-infected, and we propose a guideline for these patients.

Case report
In 2004, a serodiscordant couple, a 45-year-old HIV-1-infected man and a 37-year-old woman, was referred to our centre for fertility counselling. The couple had only condom-protected intercourse in order to avoid transmission to the woman. No condom break was reported and the duration of the relationship was 5 years. HIV tests in the woman were negative.

HIV infection was diagnosed in the man in 1989 and was attributed to sexual transmission. He was co-infected by hepatitis B virus (HBV) with an HBV viral load of 88 000 copies/ml. Medical HIV treatment was first started in 1990 and since 2002 the man had been receiving combination therapy with stavudine, lamivudine and tenofovir. HIV-1 blood viral load was undetectable and CD4 cell count was between 357 and 547 \( \mu l \) for the previous 12 months. In February 2004, HIV-1 blood viral load was still undetectable (<20 HIV-1 RNA copies/ml) and CD4 cell count was 438/\( \mu l \). Medical history included an urinary infection, two episodes of urethritis, one of prostatitis and bilateral epididymitis in 1997. The spermogram showed azoospermia with low semen volume (1.5 and 0.95 ml in two samples) with normal pH. Clinical examination showed normal testis volumes, a left varicocele and bilateral epididymal enlargement with a nodule on the left epididymal tail. Blood levels of FSH (8 mU/ml), inhibin (146 ng/l) and testosterone (893 ng/100 ml) were normal. The karyotype was normal (46XY). In March 2004, semen analysis showed azoospermia with normal pH (8.1) and volume (2.3 ml). The HIV-1 viral load was not detectable in seminal plasma (<100 RNA copies/ml). Biochemical semen markers (carnitine, alpha-glucosidase, glycerophosphocholine, citric acid, zinc and fructose) indicated reduced levels of epididymal markers. Genital ultrasound examination showed a prostatic cyst and bilateral epididymal dystrophy with a cyst on the left side.

Medical history and investigation results suggested obstructive azoospermia with bilateral epididymal obstruction, probably due to the genital infection.

The woman had negative HIV and HBV screening tests. She had regular cycles. Her body mass index was 20.2. Hysterosalpingography revealed no tubal abnormalities, ultrasound showed normal ovaries, and FSH as well as estradiol levels on day 4 were in the normal range.

The couple had several meetings with an andrologist, reproductive biologist and psychologist for comprehensive counselling, as is the practice in our centre. They were advised that (i) although there is a high probability of spermatozoa retrieval during surgical exploration, there is no guarantee that these spermatozoa can be used for ICSI, particularly if HIV genomes are detected in epididymal sperm; (ii) that the sperm washing protocol is a risk-reduction method and does not completely eliminate the risk of HIV transmission; (iii) that, under French law, if the seminal plasma collected on the day of surgery contains more than 10 000 copies of the HIV genome, ART will not be performed. Moreover, the couple was informed of the need for HIV infection to be well controlled before and during all tests and ART procedures, of the importance of condom protection during intercourse and the necessity for repeated visits to our centre for clinical tests and procedures. Finally, so that counselling should be complete, the sperm donor option was explained to the couple.

After the couple had been fully informed, HBV vaccination was carried out in the woman and was effective (HBs antibodies 21 IU/l). After the patient had given his consent, surgical exploration was performed under general anaesthesia in November 2004. On the day of surgery, HIV-RNA was undetectable in seminal and blood plasma. Surgical exploration found a hypotrophic left testis and a right testis of normal volume. The vasa deferentia were normal. The right epididymis was enlarged but the left was normal.

Micro-epididymal sperm aspiration (MESA) was unsuccessful in the left caput epididymis, whereas spermatozoa were retrieved in the caput and corpus of the right epididymis. The spermatozoa obtained underwent sperm washing using differential density gradient centrifugation over 50, 70 and 90% layers of PureSperm (JCD S.A., Lyon, France). Subsequently, the 50% fraction (12.9 \( \times 10^6 \) cells) and an aliquot (2.3 \( \times 10^6 \) cells) of the 90% fraction were tested for HIV-1 DNA and RNA according to a previously published method (Pasquier et al., 2000). The remainder of the 90% fraction was frozen in liquid nitrogen according to the standard freezing procedures used for sperm banking in our centre. Four straws each containing 1.17 \( \times 10^6 \) spermatozoa were obtained.

According to the French law, both the 50% and 90% fractions were tested, and both had undetectable HIV genomes (<20 copies/ml). In September 2005, ICSI was performed by a previously reported method (Lesourd et al., 2006). Briefly, after ovulation stimulation using a combination of Gn-RH agonist and recombinant FSH in a long protocol followed by ultrasonically guided ovarian puncture, ICSI was performed on 8 oocytes. Two embryos were obtained and transferred on day 2 after oocyte collection. A twin pregnancy occurred. Because of maternal age, the patient opted for amniocentesis showing normal karyotypes on both fetuses (46XX). A caesarean section was performed at 35 weeks of gestation and two healthy girls (1910 g and 2230 g) were delivered.

Blood HIV-RNA detection and antibody screening were negative in the woman 1, 3 and 6 months after ICSI and at delivery.

Discussion
This is the first report of live birth following ICSI in a serodiscordant couple using frozen epididymal spermatozoa from an HIV-1-infected man, who had obstructive azoospermia. To date, only one study has reported embryo transfer in a serodiscordant couple using fresh epididymal spermatozoa from the HIV-1-infected man, but no pregnancy was achieved (Nicopoullos et al., 2004). Moreover, in this case, we demonstrate that the use of frozen sperm was successful.
In serodiscordant couples with an HIV-infected male partner, sperm washing was introduced more than 15 years ago allowing these couples to have a child with a greatly reduced risk of HIV transmission to the woman. More than 3000 cycles have been reported and none of the women have seroconverted (Bujan et al., 2007). Various ART methods have been used, but in most cases, spermatozoa fractions were tested for HIV-1 genomes before their use in ART. Some teams have proposed ICSI as the method of choice to reduce the transmission risk, and in a few studies no viral test was done before ART. However, such protocols are controversial (Bujan et al., 2006). As numerous spermatozoa are needed for classic sperm washing methods, a modified method with only two sperm washes was recently proposed for HIV-1 patients with severe oligoasthenozoospermia (Garrido et al., 2006).

Usually, MESA or testicular sperm aspiration (TESA) is proposed in non-infected men with obstructive or non-obstructive azoospermia in order to collect spermatozoa for ICSI. For azoospermic HIV-1 infected men, such methods can be offered if there is a high probability of obtaining virus-free spermatozoa for ICSI.

The presence of HIV-1 in semen is now well documented and several factors associated with semen shedding of HIV have been studied, such as genital infection, blood viral load, HIV-1 infection status and semen polykaryon cells (Cohen et al., 1997; Dejucq and Jegou, 2001; Bujan et al., 2004a). In contrast, the presence of HIV-1 in epididymis is poorly documented. One study found HIV-1 positive cells in the epididymis epithelium and connective tissue of epididymis and prostate (Pudney and Anderson, 1991). In a second study, HIV-1 proviral DNA was detected in the epididymis (Purohit et al., 1992). Both studies were performed on patients who had died because of AIDS. On the other hand, only two studies to date have sought the virus in the testes of asymptomatic HIV-1 infected men (Muciaccia et al., 1998a; Paranjpe et al., 2002). Using in situ PCR hybridization, two studies detected HIV DNA within testicular germ cells in asymptomatic (Muciaccia et al., 1998a,b) or AIDS-infected men (Nuovo et al., 1994; Shevchuk et al., 1998). However, these results remain highly controversial, as a number of other studies did not demonstrate germ cell infection (Quayle et al., 1997; Pudney et al., 1998; Quayle et al., 1998; Roulet et al., 2006), even using similar detection techniques (Pudney et al., 1998).

Lymphocytes and macrophages can both support HIV-1 replication and have been observed in the human epididymis, rete testis, vas deferens, seminal vesicles and prostate (el-Demiry et al., 1985) and testis (el-Demiry et al., 1987), and increased numbers of macrophages have been found in testicular biopsies from infertile men (Frunieri et al., 2002). In the human testis, resident macrophages appear to be the only cell type expressing sufficient amounts of CD4, CXCR4 or CCR5 (Habasque et al., 2002; Roulet et al., 2006) and DC-SIGN (Roulet et al., 2006) to be detected by immunohistochemistry, while no expression of these receptors was evidenced in germ cells or in seminiferous tubules. More recently, in organotypic culture of the human testis, Roulet et al. (2006) demonstrated that the testis was susceptible to be productively infected by HIV-1, but infected cells were detected exclusively in the interstitial tissue and were found to be resident macrophages bearing the above-mentioned HIV receptors.

The effects of HAART on blood viral load are well known. This treatment very significantly reduces viral load in blood and seminal plasma. Under treatment, an undetectable HIV-1 seminal viral load argues in favour of good dissemination and effectiveness of HAART in the genital tract, although this is not systematically the case because of possible virus-specific resistance due to virus compartmentalization. Although the relationship between HIV-1 presence in the testis and in the genital compartment is not known, we believe that the effectiveness of the treatment on semen argues in favour of efficient antiretroviral molecule distribution and probably reduces the possibility of HIV-1 RNA in the testis. Although limitation of the penetration of some antiretroviral molecules by P-glycoprotein expressed at the blood-testis barrier (Fromm, 2004) has been described, its consequences in vivo have still to be evaluated. Detection of HIV-1 in cells in or seminal HIV-1 viral load could be of great interest but, particularly in the context of azoospermia, its sensitivity is highly dependent on the quantity of cells tested and is extremely variable. The detection of HIV-1 RNA in cells and the reliability of the tests performed are a matter for discussion with the virologists.

In order to reduce the risk of HIV transmission, we suggest the following strategy (Fig. 1): (i) treatment optimization to obtain undetectable blood and seminal HIV-1 viral load, (ii) careful surgery to reduce as much as possible blood contamination of epididymal sperm or testis fragments, (iii) if the blood and seminal HIV-1 viral load are both undetectable, density gradient centrifugation to isolate spermatozoa for ICSI if the quantity of cells allows this, or two washes if cells are too few for density gradient centrifugation and (iv) if the blood or/and seminal HIV-1 viral load is detectable, HIV-1 RNA detection in an aliquot of 90% fraction obtained after density gradient centrifugation or in the flushes from first washes. If HIV-1 RNA is present, the spermatozoa cannot be used, whereas if it is absent, the spermatozoa may be used for ICSI.

The method we propose does not guarantee that the risk of HIV-1 transmission is totally excluded. However, to date, it reduces the risk to the minimum. The couple must be aware that this method is a risk-reduction option and the consent of both partners is required after they have been fully informed. Moreover, it should be noted that clinical and laboratory procedures must be performed not only according to universally accepted precautions, but also according to the recommendations for ARTs in virus carriers (Englert et al., 2004). In our case, we performed an extra step in that sperm testing for HIV RNA was done even though the patient had an undetectable blood and semen viral load. This is because it is required by French law.

As azoospermic patients with HIV-1 infection are not numerous, we suggest that all ICSI attempts in this population should be recorded in a database to evaluate safety and efficiency.
Acknowledgements
The authors particularly thank Ms Nathalie Dejucq-Rainsford, Ph.D., for her attentive reading of the paper and pertinent comments and suggestions, and Ms Nina Crowte for language editing.

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Figure 1: Proposed strategy for HIV-1 infected azoospermic men in order to reduce the risk of HIV transmission to the female during ART procedures and NOA, non-obstructive azoospermia; OA, obstructive azoospermia.


Submitted on January 18, 2007; resubmitted on May 9, 2007; accepted on May 17, 2007