ART outcome in HIV-infected patients

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BACKGROUND: To assess assisted reproductive technique (ART) outcome in couples affected by human immunodeficiency virus (HIV). METHODS: Intrauterine insemination (IUI), IVF and ICSI were performed in 85 couples affected by HIV between January 2000 and June 2005. RESULTS: In 33 of the 85 couples, women were HIV positive—the clinical pregnancy rate (CPR) and cancellation rate (CR) after 34 IUI cycles were, respectively, 25 and 18%. The CPR after 26 IVF and 30 ICSI cycles were, respectively, 37.5 and 18.8% with CRs of 38.5 and 46.7%, respectively. In 38 couples, men were infected—the CPR and CR after 85 IUI cycles were, respectively, 14.7 and 20%; 62 ICSI cycles were performed leading to CPR of 23.4% with a CR of 25%. In 14 couples, the two partners were infected: none of the four IUI cycles carried out was successful (CR, 20%); the CPR and CR after 35 ICSI cycles were, respectively, 12.5% with 31%. All children born had a negative HIV test. CONCLUSION: In couples affected by HIV, an acceptable pregnancy rate was obtained. The worst results were obtained when both partners were infected. The CR was elevated among HIV-infected couples.

Key words: ART/HIV/ICSI/IUI/IVF

Introduction

Since about 5 years ago, certain IVF centres have been treating couples in which one of the partners is affected by human immunodeficiency virus (HIV), using assisted reproductive techniques (ARTs) to reduce the transmission risk to the unaffected partner or to solve existing fertility problems (Delvigne et al., 2001; Ohl et al., 2005; Terriou et al., 2005). These treatments may involve auto-inseminations with or without ovarian stimulation, intrauterine insemination (IUI) (Semprini et al., 1997; Weigel et al., 2001) or IVF associated or not with micro-injection of spermatozoa into the oocytes (ICSI) (Ohl et al., 2005; Terriou et al., 2005). To date, different authors have reported their IUI or IVF data, but these have involved mostly series of seropositive men and seronegative women (Semprini et al., 1997; Gilling-Smith, 2000; Weigel et al., 2001; Sauer and Chang, 2002; Garrido et al., 2004). ART experience in seropositive women is far more limited (Ohl et al., 2005; Terriou et al., 2005; Martinet et al., 2006) due to the fear of vertical HIV transmission (Burns et al., 1997; Garcia et al., 1999; Mofenson et al., 1999; Shaffer et al., 1999). We reported previously the opportunity to use a specific multidisciplinary protocol, assessing HIV couples, including seropositive women. In our 5 years’ experience, in ~39% of the couples seeking help, the woman was infected, whereas either the man alone or both partners were HIV infected in, respectively, 45 and 16% of cases (Y. Manigart et al. unpublished data). The present article reports our results in terms of pregnancy success rates and vertical transmission risk (i.e. the risk of infecting the child to be born), using ART in couples involving at least one infected partner.

Materials and methods

In our centre, we provide help in reducing the transmission risks in HIV-infected fertile couples. After excluding the frequently occurring fertility problems in this group of patients, such as tubal obstruction or cervical pathology (Sobel, 2000; Soncini and Condemi, 2003; Gilles et al., 2005; Ohl et al., 2005), we provide assistance in determining the ovulatory period using either auto-insemination or artificial insemination depending on the couple’s wish. We also provide help for couples with fertility problems to establish a diagnosis and the most appropriate surgical or medical treatment (IUI, IVF, ICSI depending on aetiology).

Between January 2000 and June 2005, 85 couples with at least one HIV-infected partner were accepted in to our ART programme. These couples began 57 cycles of ART (53 IVF or ICSI cycles, 2 transfers of thawed embryos and 2 oocyte donations). Moreover, 124 IUI cycles were also performed.

Couples with man HIV positive

When the man is infected, we always first suggest the possibility of artificial insemination with donor sperm, because we cannot assure the patients of a total absence of risk and because two contaminations have been reported using washed sperm. Only when this procedure is rejected by the couple, even after ensuring that the theoretical and residual risks of contamination associated with washed sperm are
understood, do we consider using washed sperm, as suggested by different groups (Semprini et al., 1992; Frodsham et al., 2006; Sauer, 2006). Depending on the aetiology of infertility, we will proceed to either IUI or IVF, always associated with ICSI, whatever the quality of thawed sperm after appropriate sperm preparation.

**Method of sperm preparation**

Semen samples were collected by masturbation into a sterile container after 3 days of sexual abstinence. After 30 min liquefaction, standard analysis of semen samples was carried out according to the World Health Organization (WHO) guidelines (WHO, 1999). Afterwards, samples were diluted with an equal volume of Earles Balanced Salt Solution (EBSS) and then processed in a first step on density gradient layers (Pure Sperm) of 90 and 45% (20 min at 400 × g), followed by washing with EBSS medium. In a second step, the retrieved 90% layer pellet underwent a swim-up technique. The swim-up supernatant, after sperm count, was divided in two: half was cryopreserved and used for insemination and the other tested for HIV RNA and DNA. Nucleic acids were extracted from the washed sperm. The RT–PCR test was described by Vandamme et al. (1995). The proof of the absence of viral DNA and RNA in the sperm pellet is of course mandatory.

**Couples with woman HIV positive**

Procreation assistance is offered to reduce transmission risks in normally fertile couple. We help the woman determining the time of ovulation and using artificial insemination or auto-insemination depending on the couple’s wish. The results concerning couples who used auto-insemination are not presented because no ART was applied. Couples in whom fertility problems were diagnosed were offered surgery or ART (IUI, IVF, ICSI) depending on the aetiology of infertility.

**IUI protocol**

When no major problem of female infertility was encountered, and the HIV-infected sperm found to be of good quality, this sperm was used after washing or when moderate sperm anomalies (more than 1 × 10^6 motile sperm in the final preparation) were encountered. IUI has been performed also when HIV-infected women fail successful auto-insemination for several months or in the case of insufficient cervical mucus or when idiopathic infertility is encountered. Whenever IUI was scheduled, we stimulated our patients with 100 mg of Clomiphene citrate (from day 3 till day 7) and supplemented them with ethinyl estradiol 0.05 mg (from day 8 till day 12) for the first two cycles of treatment. After two cycles, we used gonadotrophins starting on day 6 or 8 of the cycle, using 75 or 150 U of gonadotrophins (Menopur, Ferring, Sweden) according to patient’s age (threshold at 35 years of age) for two further cycles. Ovulation stimulation was monitored by measuring serum estradiol, LH and progesterone and by echographic measurement of follicle and endometrium growth, from day 11 of the cycle till ovulation. The patient was inseminated only once, 24 or 36 h after spontaneous or induced ovulation (using 10 000 IU HCG; Pregnyl, Organon, Netherlands), respectively. The cycle was cancelled if a risk of multiple pregnancies was encountered in order not to hamper obstetrical outcome and also not to increase the risk of vertical transmission in the case of an HIV-infected woman.

**IVF protocol**

IVF was carried out when justified by the infertility aetiology or after four consecutive IUI failures. IVF cycles were conducted using similar stimulation schemes as in non-infected patients (Delvigne et al., 2003). Oocyte retrieval and culture procedures have been previously described (Bertrand et al., 2004).

**Laboratory procedures**

For sperm preparation, we used the classical gradient procedure in case of non-infected sperm. We proceeded systematically to ICSI, independently of sperm quality when the man was HIV infected in order to maximally reduce oocyte–sperm contact. ICSI was also carried out when sperm quality was defective. Until July 2003, we used to transfer one or two embryos depending on their quality, in patients younger than 35 years and maximally three embryos in older women. Since July 2003, Belgian legislation subordinates social security reimbursement to limitation of transferred embryos to one in women younger than 35 years for a first IVF attempt, to two embryos for a second trial or beyond and to two embryos for the first two IVF trials in women aged between 36 and 39 years and three thereafter. No limitation exists for women aged ≥40 years: over 40 years, we transfer a maximum of three embryos for the first trial and of four to six embryos for their second trial. The embryos of good quality that are not transferred are frozen.

All these protocols were approved by our local ethics committee. Written informed consent is endorsed by the couple before the beginning of ART treatment.

Statistical analysis, i.e. descriptive analyses and comparison between groups by one-way analyses and non-paired Mann–Whitney U-test, was performed using the SPSS program. The level of significance was set at $P < 0.05$.

**Results**

In 33 couples (39%), the woman was HIV infected, in 38 couples (45%) the man was infected and in 14 couples (16%) both partners were infected (Tables I and II). Among the 33 couples with infected women, 56 IVF (26 IVF and 30 ICSI), 1 transfer of thawed embryos, 2 oocyte donations and 34 IUIs were performed. The aetiology of infertility for couples treated with IVF/ICSI was tubal defects (75%) and sperm insufficiency (∼25%) and rarely idiopathic infertility. Twenty-four IVF/ICSI cycles (43%) and six IUI cycles (18%) had to be cancelled.

Thirty-four IUIs were initiated and 28 performed in eight couples resulting in seven pregnancies (pregnancy rate of 25% per insemination cycle); one pregnancy aborted spontaneously and one was a twin pregnancy. Finally, seven seronegative babies were born. Seventy-five per cent of the couples involving an HIV-positive woman, treated using IUI, obtained an ongoing pregnancy.

Twenty-six IVF cycles were carried out in 16 couples. Ten IVF cycles were cancelled (38.5%), mostly for poor ovarian response ($n = 7$). Seven clinical pregnancies were obtained (including one after transfer of thawed embryos from oocyte donor cycle, one miscarriage, four singleton and one twin pregnancy). Finally, 37.5% of the couples with an HIV-positive woman, treated using IVF, obtained an ongoing pregnancy.

Thirty ICSI cycles were performed in 14 couples (Table I). Fourteen ICSI cycles were cancelled (46.7%), generally for poor ovarian response ($n = 9$), and in two cases, fertilization failure was observed. Three clinical pregnancies were obtained: one ectopic pregnancy and two singletons, which means that 14% of couples with an HIV-positive woman, treated with ICSI, obtained an ongoing pregnancy.

No complications (ovarian hyperstimulation syndrome, infection or blood loss after oocyte retrieval) were observed during IVF and ICSI procedures. However, two retrieval failures

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Table I. Principal assisted reproductive technique (ART) results in couples in whom the woman, the man or both partners were HIV infected

<table>
<thead>
<tr>
<th></th>
<th>Women HIV + IVF + ICSI</th>
<th>Women HIV + IVF</th>
<th>Women HIV + ICSI</th>
<th>Men HIV + ICSI</th>
<th>Couple HIV + ICSI</th>
<th>Total</th>
<th>P (B)</th>
<th>P (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>32</td>
<td>16</td>
<td>16</td>
<td>47</td>
<td>24</td>
<td>37.0</td>
<td>0.015</td>
<td>0.005</td>
</tr>
<tr>
<td>Age of women (SD)</td>
<td>35 (3.4)</td>
<td>35.7 (3.4)</td>
<td>35.7 (3.9)</td>
<td>38.3 (3.5)</td>
<td>36.6 (3.8)</td>
<td>37.0</td>
<td>0.015</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean E2 max pg/ml (range)</td>
<td>2321 (1467–3816)</td>
<td>1677 (1314–2786)</td>
<td>3379 (2379–4206)</td>
<td>2983 (2005–3722)</td>
<td>2255 (1791–3063)</td>
<td>2636 (1706–3527)</td>
<td>0.013</td>
<td>Not significant</td>
</tr>
<tr>
<td>Number of oocytes (range)</td>
<td>7.0 (3.0–105)</td>
<td>4.0 (3.0–7.5)</td>
<td>8.5 (4.8–13.2)</td>
<td>8.0 (6.0–10.3)</td>
<td>6 (4–9)</td>
<td>7.0 (4.0–10.0)</td>
<td>0.015</td>
<td>Not significant</td>
</tr>
<tr>
<td>Fertilization rate % (range)</td>
<td>75 (57–100)</td>
<td>85 (68–100)</td>
<td>65 (51–84)</td>
<td>50 (41–68)</td>
<td>56 (33–69)</td>
<td>60 (42–80)</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>Number of embryos (range)</td>
<td>4 (2–7)</td>
<td>4.0 (1.5–6.0)</td>
<td>5.0 (3.0–7.8)</td>
<td>4 (2–5)</td>
<td>3 (2–4)</td>
<td>4.1 (2.0–5.0)</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
<tr>
<td>Top rate embryos% (range)</td>
<td>0 (0–25)</td>
<td>0 (0–22)</td>
<td>11 (0–25)</td>
<td>0 (0–28)</td>
<td>0 (0–19)</td>
<td>0 (0–25)</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
<tr>
<td>Pregnancy rate/transfer (%)</td>
<td>28.1</td>
<td>37.5</td>
<td>18.8</td>
<td>23.4</td>
<td>12.5</td>
<td>22.3</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
</tbody>
</table>
occurred. Finally, 51 and 42% of all couples with HIV-positive women who were treated using ART obtained a clinical or an ongoing pregnancy, respectively.

Among the 38 couples in whom only the man was HIV positive, 62 ICSI cycles, 1 transfer of thawed embryos and 85 IUI cycles were initiated. The most frequent aetiologies of infertility for couples treated with ICSI were tubal defect (39%), sperm insufficiency (22%) and anovulation (22%).

In most patients for whom IUI was used, the indication consisted in avoiding transmission using washed sperm (65%), whereas 26% of the couples suffered from male infertility and 9% of ovulation disorders. All sperm used for IUI and ICSI were found to be negative for HIV DNA and RNA. Eighty-five IUI cycles were initiated and 68 performed in 25 couples, resulting in 10 pregnancies [clinical pregnancy rate (CPR) per insemination of 14.7%]. There was, one miscarriage, one twin pregnancy and eight singleton pregnancies. Finally, 40 and 36% of couples with HIV-positive men, treated using IUI, obtained a clinical and ongoing pregnancy, respectively.

Sixty-two ICSI cycles were performed in 20 couples. Fifteen ICSI cycles were cancelled (24%), mostly for poor ovarian response to stimulation (n = 11). Eleven pregnancies were obtained, among which six were singletons, three were twin pregnancies and two miscarriages. Fifty-five and 45% of all couples with HIV-positive men, treated using IUI, obtained a clinical and ongoing pregnancy, respectively.

Finally, 55 and 47% of all couples with HIV-positive men, treated using ART, obtained a clinical or an ongoing pregnancy, respectively, after resorting to ICSI or IUI using washed sperm. All women and newborns were found to be negative for HIV.

In 14 couples in whom both partners were HIV infected, 35 ICSI cycles and five IUI cycles were initiated. The aetiology of infertility of couples treated using ICSI was mostly tubal defect (73%) and sperm insufficiency (27%). Treatment was cancelled in seven ICSI cycles (31%) and in one IUI cycle all due to poor ovarian response. None of the IUI trials resulted in a pregnancy. ICSI treatments resulted in only three pregnancies in two couples, and none was ongoing.

When comparing couples with infected women, infected men or both infected partners, differences in age distributions, fertilization rates and number of transferred embryos were observed (Tables I and II). Fewer embryos were transferred in HIV-infected women than in uninfected women. The pregnancy rates per cycle vary from 8.6–23.1% and per transfer from 12.5–37.5% between these three groups. The worst results were observed in couples in whom both partners are infected, but this difference did not reach statistical significance. High cancellation rates (CRs) (mainly due to poor ovarian response) were observed, ranging between 20.0 and 46.7%.

**Discussion**

Currently, a consensus has been reached in many European countries to include HIV-infected patients in ART programmes (Shenfield et al., 2004) to treat their infertility pathologies and to avoid HIV contamination to the uninfected partner. Because HIV infection has been associated with a reduced fertility (Sobel, 2000; Clark et al., 2001; Dulioust et al., 2002; Nicopoulos et al., 2004), it has also become necessary to evaluate the ART results in HIV-infected patients (Coll et al., 2005; Ohi et al., 2005; Terriou et al., 2005). Our study analyses the ART outcome in a cohort of 85 couples with at least one HIV-infected partner and with the man, the woman or both partners infected. Because *primum non nocere* remains one of the major medical principles, we evaluated first the HIV transmission risk. Pregnancy in a seropositive woman implies the possibility of a vertical transmission risk of HIV to the baby. In this series of patients, all babies born after ART were seronegative. Besides this risk, a risk of teratogenicity may also coexist. To minimalize the latter, retroviral treatment may be stopped during the first trimester or modified by avoiding known teratogenic treatments such as efavirenz. This was the case for two patients who had to modify their treatment before ART. We did not change the treatment of women who were using highly active antiretroviral therapy (HAART) and had an undetectable viral load. Most untreated patients began their treatment around 24 weeks of pregnancy. Women who reach an undetectable viral load delivered vaginally, whereas otherwise a Caesarean section is proposed. At delivery and during post-partum, we also applied classical recommendations (The European Mode of Delivery Collaboration, 1999; Ioannidis et al., 2001; Dorenbos et al., 2002).

In couples involving a seropositive man not using condoms, the transmission risk to the uninfected woman has been estimated to range between 0.15 and 0.2% per sexual intercourse but may be much higher when other sexually transmitted diseases coexist or when the viral load is high (Laga et al., 1993; Grosskurth et al., 1995; Zhang et al., 1998). This risk is much reduced by using washed sperm containing an undetectable viral load (Semprini et al., 1992). Although it is hopeful that the international database (Creathe, 2003) will soon confirm the effectiveness of the latter procedure, a doubt still exists to date about the eventuality of a residual transmission risk. In this study, we tested the HIV-negative women every 3 weeks for 3 months after the ART procedure and then every 3 months till the end of the pregnancy; none of these patients were infected during the ART programme.

Contrary to some other groups, we choose an aggressive sperm preparation (with three centrifugations), which may reduce its final quality but often results in viral-free sperm preparations. In this way, we avoid treating men with HAART for reproductive reasons only in order not to diminish future therapeutic possibilities.

The results of IUI and IVF/ICSI were analysed in couples with one or two affected partners. Although not significantly different, the worst pregnancy rates were observed when the two partners were infected. This may be due to the small sample size and/or a higher mean age of the patients. Coll et al. (2005) have been the only authors to observe worse prognosis for HIV-infected women (n = 50) as compared with couples in whom the man was infected (n = 50). These differences disappeared when considering infected women who were treated using oocyte donation (n = 25). Although our sample size is in the same range, we have not found such results. Further studies using a larger cohort will be necessary to clarify this point.
Other authors compared couples in whom only the woman was infected and compared them to seronegative controls and found similar outcomes after IVF/ICSI (Terriou et al., 2005). Ohl et al. (2005) observed poor results after IUI in 10 HIV-infected patients. It may be hypothesized that if HIV infection impairs the ART results, the cumulative negative effect of an HIV infection present in both partners may be responsible of poor results. Larger studies are needed to confirm this hypothesis. Nevertheless, when only one partner was infected and IUI was used, pregnancy rates per cycle were comparable with that in the general IUI population (~20%).

On the contrary, high CRs were found and seemed to be generally associated with insufficient ovarian response, which may be related to high rates of tubal sterility, as observed also by others (Sobel, 2000; Ohl et al., 2005; Terriou et al., 2005; Frodsham et al., 2006). Coll et al. (2005) found poor CPRs in HIV-infected women, as compared with age-matched uninfected patients. But, when they restricted their analysis only to cycles with oocyte donation in HIV-infected women, these differences disappeared, suggesting that poor IVF results may be due to reduced ovarian response and oocyte quality in HIV-positive women. Terriou et al. (2005) performed ICSI and IVF in seropositive women and compared their results with age-matched uninfected women and with their overall uninfected population. They observed higher CRs in HIV-infected patients and lower pregnancy rates only when the overall population was the control group. These differences disappeared when using an age-matched group. Similarly, we did not find a difference in ovarian response between HIV-infected women who were matched to a control group of uninfected women (Martinet et al., 2006).

The ovarian response to stimulation was similar whether the women, the men or both partners were HIV positive. Higher fertilization rates were obtained for HIV-positive women treated by IVF than in the other groups of patients. This may be due to better sperm quality. Terriou et al. (2005) observed similar results in HIV-infected women treated with systematic ICSI for sanitary precautions. Other authors found that satisfactory ovarian stimulation was more difficult to achieve, needing higher total doses of recFSH and longer duration of stimulation, when comparing with a non-matched general ART group (Ohl et al., 2005) or with an age-matched group (Terriou et al., 2005) or in a logistic regression model (Coll et al., 2005).

Some authors reported higher rates of miscarriage (Brocklehurst and French, 1998; Ohl et al., 2005), but as Terriou et al. (2005) found, we observed miscarriage rates in the same range as in our general population. Globally, acceptable results have been obtained, when considering that ART–HIV programmes started in Europe had to include a cohort of aged patients, who had to wait for long time before being treated by ART.

To our knowledge, our study is the first to report ART results in a small series of couples in whom both partners were HIV infected, in whom we observed discouraging results in terms of ongoing pregnancies. This may have been due, at least partially, to a very high rate of age-related cancellations. However, it remains to be firmly assessed whether these low results can be related to viral prognostic factors.

References
Create (2003) European network for centres providing assisted reproduction to couples afflicted by HIV. Yahoo! Groups (http://health.groups.yahoo.com/group/create/).


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