ICSI for treatment of human immunodeficiency virus and hepatitis C virus-serodiscordant couples with infected male partner

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BACKGROUND: Assisted reproductive technology with semen washing can offer a significant reduction in risk of sexual and vertical transmission of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in serodiscordant couples with infected male partner. METHODS: Among couples coming to our centre for reproductive problems from January 2001 to December 2003, we selected 43 couples with seropositive male and seronegative female: 25 couples with HIV-seropositive males, 10 couples with HIV/hepatitis C virus (HCV)-seropositive males and eight couples with HCV-seropositive males. Sperm samples were washed and used for ICSI. RESULTS: Seventy-eight cycles of ICSI were performed. The mean fertilization rate was 70.34 ± 20.14% (mean ± SD). A mean number of 3.55 ± 1.11 (range: 1–5) embryos of good quality was transferred for each patient. We obtained 22 pregnancies (21 singletons and one twin), with a pregnancy rate per transfer of 28.2% and an implantation rate per transfer of 15.2%. The cumulative pregnancy rate was 51.2%. At follow-up, no seroconversion was detected in any patient. CONCLUSIONS: Our data suggest that sperm wash and ICSI could be useful for reducing the risk of HIV and/or HCV transmission in serodiscordant couples with infected male wishing to have a child, irrespective of their fertility status.

Key words: HCV/HIV/ICSI-seropositive male/sperm wash

Introduction

Three-quarters of individuals infected by human immunodeficiency virus (HIV) or HIV/hepatitis C virus (HCV) are in their reproductive years. Moreover the percentage of HIV heterosexual contamination is increasing, especially in developed countries (Ethics Committee of the American Society for Reproductive Medicine, 2002).

Major advances in pharmaceutical research have greatly improved the prognosis of patients with HIV infection. Correct clinical and therapeutic management of these patients enables the disease to be maintained in a chronic state, in most cases avoiding fatal progression.

The general condition and life expectancy of many patients with HIV infection is very good, and a percentage of young couples can be expected to make plans for the future and to want to have children (Englert et al., 2001). Sexual transmission of HIV is variable and depends on many different factors, such as the number of sexual partners, rather than on the frequency of sexual intercourse (de Vincenzi, 1994). Male-to-female transmission of HIV is estimated to be 1 per 1000 acts of unprotected intercourse (de Vincenzi, 1994; Downs and De Vincenzi, 1996; Mandelbrot et al., 1997; Padian et al., 1997; Pena et al., 2003) and even less in HCV-infected patients (Garrido et al., 2004a). The presence of HCV in semen is controversial: some authors have reported a total absence of HCV RNA in semen (Semprini et al., 1998), while other studies suggest that HCV may be found in the semen with low or high prevalence (Liou et al., 1992; Fiore et al., 1995; Tang et al., 1996). Levy et al. (2000) recently identified HCV RNA in semen of HCV-infected patients.

Araneta et al. (1995) and Matz et al. (1998) reported that semen used for donor artificial insemination can transmit HIV-1 infection. Studies on the presence of HIV in sperm have also yielded contradictory results. Using different approaches, Baccetti et al. (1994) detected HIV particles and HIV DNA in ejaculated sperm of HIV-seropositive patients; the same group identified a specific HIV receptor, alternative to CD4, on sperm membrane: this molecule is a galactosyl-alkyl-acylglycerol (GalAAG), a glycolipid structurally related to galactosylceramide, the receptor for HIV identified in CD4+ cells (Baccetti et al., 1994; Brogi et al., 1998). At the same time, other authors emphasized the total absence of HIV particles and nucleic acids in sperm (Lasheeb et al., 1997; Quayle et al., 1997; Pudney et al., 1998), demonstrating that
separation of seminal fluid and cellular elements from sperm by washing techniques reduces the viral load of semen detected by PCR and RT–PCR.

Semprini et al. (1992) were the first to use washed sperm of HIV-1-infected men for intrauterine insemination (IUI). They recently reported >2000 inseminations and >100 IVF or ICSI cycles with a total of 350 babies born without viral contamination (Semprini et al., 2000).

In this study, we report the results of our protocol for IVF by ICSI in serodiscordant couples in which the male partner was infected by HIV and/or HCV. Taking into account previous reports on this topic, we discuss the possible protective effect of this technique against contamination risk.

Materials and methods

Patients

Among couples coming to the Centre for reproductive problems from January 2001 to December 2003, we selected 43 out of 59 (72.9%) serodiscordant couples with seropositive male and seronegative female, who wanted to conceive children with the minimum risk. We treated 25 couples with HIV-seropositive males, 10 couples with HIV/HCV-seropositive males and eight couples with HCV-seropositive males. All couples underwent common selection tests for ICSI including blood test, karyotype and sperm evaluation. In order to reduce the possibility of viral contamination in selected semen samples, our selected couples were only accepted for ICSI if the male partner had a plasma viral load of <50 copies of HIV and/or HCV RNA/ml, as detected by PCR. Inclusion criteria and treatment protocol satisfied the clinical programme described by Sauer and Chang (2002). At enrolment, a full report of disease course and current antiretroviral treatment was obtained for all HIV-seropositive men from their specialists. HIV-seropositive males had to be in good general health and, in cases of HIV infection, with a stable CD4+ T-cell count for the past 6 months.

Three out of eight HCV-seropositive males had not been followed at all, and only two patients had received interferon therapy.

Female partners (mean age 34.95 ± 2.9 years) were accepted for ICSI only if HIV- and HCV-seronegative by standard antibody screening tests. Couples were also required to use condoms during sexual intercourse.

General reproductive screening was performed in all couples before ICSI, and counselling by specialists in maternal fetal medicine and psychiatry was suggested and made easily accessible. Informed consent and a statement acknowledging the possibility of infection were obtained from couples before each ICSI cycle.

Semen analysis

Semen parameters were evaluated according to World Health Organization (1999) criteria. Sperm count and progressive motility were analysed before and after washing. Sperm morphology was not evaluated in order to minimize the contamination risk for laboratory staff when working with HIV/HCV-positive samples.

Sperm treatment

Ejaculates obtained after a sexual abstinence of 3–5 days were allowed to liquefy, and were then diluted 1:1 with Sperm Medium (Cook Italia, Italy). They were pelleted at 400 g for 10 min and supernatants were discarded. A volume of Sperm Medium equal to the initial ejaculate volume was added. The suspension was layered onto a triple density gradient apparatus (90, 70 and 45%, PureSperm; Nidacon, Sweden) at 1–1.2 ml per layer, and centrifuged at 300 g for 20 min.

Pellets were washed with 5 ml of Sperm Medium for 20 min at 2000 g, and pelleted again. Supernatants were discarded and sperm wash-up performed with 0.5–0.7 ml. After 45 min, the upper 0.35 ml of each tube was harvested and pooled. The purified sperm were counted and the concentration was adjusted to 4–5 × 10^6 motile sperm/ml (Bellver et al., 2002; Meseguer et al., 2002). The final sperm samples were stored in tightly capped tubes and incubated at 37°C until ICSI.

Ovarian stimulation

Ovarian stimulation was performed by the GnRH agonist long protocol as reported by Diaz et al. (2000). Patients received 0.1 mg/day of triptorelin (Decapeptyl; Ipsen Pharma Barcelona, Spain) from mid-luteal phase of the preceding cycle, until vaginal ultrasound demonstrated ovarian quiescence. The GnRH analogue was decreased to 0.05 mg/day until the day of hCG administration. Recombinant FSH (Puregon; Organon, Italy; or Gonal-F; Serono S.A., Italy) was used for ovarian stimulation and initial doses were calculated according to age, basal serum FSH and estradiol (E2) levels. Serum E2 levels were determined on day 3 to adjust gonadotrophin doses. hCG (10 000 IU, Gonalis; AMSA, Italy) was administered when three or more follicles reached 18 mm in diameter and oocyte retrieval was scheduled 34 h later.

ICSI procedure

ICSI was performed on mature oocytes as described by Palermo et al. (1995), using a Narishige micromanipulator. Eighteen hours after ICSI, the presence of two pronuclei was checked in order to determine normal fertilization. Culture of embryos was carried out in a separate incubator until embryo transfer, 72 h after oocyte retrieval. The embryos were evaluated morphologically and graded according a 5-point scale (1: poor quality embryo; 5: good quality embryo). Clinical pregnancy was determined by detecting a gestational sac with fetal heartbeat at 7 weeks.

Seroconversion tests

During pregnancy, HIV/HCV antibodies were tested every 3 months by standard enzyme-linked immunosorbent assay (ELISA) antibody screening tests (ELISA kits supplied by Abbott Diagnostic Division, Rome, Italy). At birth and 3 months later, infants and mothers were tested for HIV DNA and HCV RNA by PCR, using a procedure allowing detection of <10 copies of viral genome per ml of whole blood.

Non-pregnant women were tested for HIV/HCV antibodies 3 and 6 months after embryo transfer.

Results

Table I shows the results of analysis of semen parameters according to World Health Organization (1999) criteria. The mean number of sperm was 51.7 ± 7.1 suggesting that sperm production was not affected by the infection in this group of patients. In our patients, the mean percentage of sperm with progressive motility was 31.7 ± 9.1; HIV/HCV-infected men showed a mean progressive motility of 27.9 ± 7.5. Sperm selection increased the percentage of sperm with progressive motility to a mean percentage of 65.5 ± 9.1. This confirmed that the standardized washing method for infected semen selected sperm samples with improved motility in
Values are expressed as percentages, or mean ± SD.

Table I. Semen parameters of 43 males with human immunodeficiency virus (HIV) and/or hepatitis C virus (HCV) infection before (B) and after (A) sperm selection

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 43)</th>
<th>HIV (n = 25)</th>
<th>HIV/HCV (n = 10)</th>
<th>HCV (n = 8)</th>
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<tr>
<td></td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
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<tr>
<td>Ejaculate volume (ml)</td>
<td>3.6 ± 0.6</td>
<td>1 ± 0.1</td>
<td>3.8 ± 0.4</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>Sperm number/ml (× 10^6)</td>
<td>51.7 ± 7.1</td>
<td>6.5 ± 1.3</td>
<td>49.3 ± 8.1</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>31.7 ± 9.1</td>
<td>65.5 ± 9.1</td>
<td>29.5 ± 6.9</td>
<td>63 ± 7.7</td>
</tr>
</tbody>
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Values are expressed as mean ± SD.

a similar way to selection procedures for uninfected sperm samples (Garrido et al., 2004b).

The 43 serodiscordant couples with HIV- and/or HCV-infected male partner and seronegative female partner underwent 78 cycles of ICSI (Table II): 13 couples underwent one cycle, 25 couples two cycles and five couples three cycles. None of the cycles was cancelled before oocyte retrieval. The mean number of oocytes retrieved was 11.8 ± 6.10. The mean fertilization rate was 70.34 ± 20.14. Two days after oocyte retrieval, a mean number of 3.55 ± 1.11 embryos of good quality was transferred. Four or five embryos were transferred only if the women were aged >36 years and had failed the first ICSI cycle. A total of 22 pregnancies (21 singleton and one twin) resulted, with a pregnancy rate per transfer of 28.2% and an implantation rate per transfer of 15.2%. The cumulative pregnancy rate was 51.2%.

With regard to the seroconversion tests performed in all women after embryo transfer, irrespective of pregnancy, all were negative for HIV and HCV at 3, 6 and 9 months after embryo transfer. All babies were negative for viral infection at birth and 3 months later.

Discussion

Current antiretroviral treatments in individuals with HIV/HCV infection in their reproductive years are effective enough to lead many of these patients to contemplate raising families. Recent advances in the treatment of HIV infection have sharply reduced patient mortality and improved their quality of life. Many couples with infected male and seronegative female want to have children, and reproductive counselling and care can offer a considerable reduction in the sexual and vertical transmission rate in couples. Assisted reproduction techniques play a primary role in making this possible. Intrauterine insemination (IUI) of ‘washed sperm’ has been suggested as a means of removing seminal plasma and non-motile cells from the gamete fraction (Semprini et al., 1992). ICSI with processed semen has been proposed for serodiscordant couples with Fallopian tube or male infertility (Marina et al., 1998; Loutradis et al., 2001).

Although Dussaix et al. (1993) and Baccetti et al. (1994) demonstrated HIV viral particles and nucleic acids in ejaculated sperm, and viral genome has been found in spermatozoa, and less often (<1%) in spermatozoa, of seropositive males (Muciaccia et al., 1998a,b), Quayle et al. (1997) did not detect any viral DNA in motile sperm selected according to Semprini’s (1992) procedure: recently Garrido et al. (2004b) and Pena et al. (2003) used this washing technique to safely perform assisted reproduction in >2000 serodiscordant couples. In any case, ICSI after sperm washing minimizes the risk of exposure of oocytes and women to potentially HIV/HCV-infected sperm. More sensitive methods of separating non-infected sperm were recently proposed by Politch et al. (2004) who demonstrated the effectiveness of a new sperm-washing device that uses a double tube and a gradient in excluding HIV particles from the motile sperm fraction.

In IUI, women receive millions of sperm, and in traditional IVF, oocytes are exposed to thousands of sperm. Englert et al. (2004) reported two cases of viral contamination during insemination. ICSI enables fertilization of the oocyte with a single sperm, considerably reducing the risk of viral transmission with respect to other assisted reproduction techniques (Loutradis et al., 2001). Since each oocyte is exposed to a single sperm, the risk of viral transmission must decrease sharply, if it indeed exists after sperm processing (Pena et al., 2003). We treated couples with HCV-seropositive males by the same procedure, since HCV can be detected in semen, albeit with low prevalence (Levy et al., 2000).

Several authors recommend virological testing of selected sperm before any insemination or IVF cycle. However, Pena

Table II. Outcome of 73 ICSI cycles in 43 serodiscordant couples with an human immunodeficiency virus (HIV) and/or hepatitis C virus (HCV)-infected male

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 43)</th>
<th>HIV (n = 25)</th>
<th>HIV/HCV (n = 10)</th>
<th>HCV (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean no. of oocytes retrieved</td>
<td>11.8 ± 6.10</td>
<td>9.6 ± 1.90</td>
<td>10.1 ± 2.0</td>
<td>9.8 ± 3.10</td>
</tr>
<tr>
<td>Mean fertilization rate</td>
<td>70.34 ± 20.14</td>
<td>68.34 ± 15.2</td>
<td>66.34 ± 18.3</td>
<td>71.4 ± 12.2</td>
</tr>
<tr>
<td>Mean no. of embryos transferred</td>
<td>3.55 ± 1.11</td>
<td>2.5 ± 0.5</td>
<td>3.2 ± 1.0</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>Clinical pregnancy rate per embryo transfer (%)</td>
<td>28.2</td>
<td>27.6</td>
<td>27.8</td>
<td>29.2</td>
</tr>
<tr>
<td>Implantation rate per transfer (%)</td>
<td>15.2</td>
<td>14.8</td>
<td>14.6</td>
<td>16.3</td>
</tr>
<tr>
<td>Cumulative pregnancy rate (%)</td>
<td>51.2</td>
<td>50.2</td>
<td>50.8</td>
<td>52.6</td>
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</table>

Values are expressed as percentages, or mean ± SD.
et al. (2003) advised against HIV testing of semen and no test is currently available to assess the infectious status of the single sperm, as would be needed for ICSI. The sensitivity of laboratory assays for detecting virus is usually between 200 and 800 copies/ml. Even if HIV RNA RT–PCR is used to test sperm before IUI or IVF, the procedure is therefore not risk-free, because small numbers of virus particles may go undetected. Semen contains polymerase inhibitors which interfere with the reactions, yielding false negatives (Levy et al., 2000; Garrido et al., 2004a). Moreover, Semprini et al. (2001) suggested that gamete micromanipulation may bypass mechanisms protecting against viral infections when fertilization occurs in vivo or in vitro. Our patients were informed about the efficacy of the sperm-washing method in reducing the viral load of sperm, and they agreed to undergo ICSI with selected sperm, without any attempt to detect HIV in semen after preparation. All couples considered the low residual risk to be acceptable, and stated this in a written informed consent.

Advantages of ICSI over IUI also include the considerably better success rate. The fewer attempts necessary to achieve pregnancy should also reduce potential viral exposure from repeated cycles (Pena et al., 2003), irrespective of the fertility potential of the treated couples. Levy et al. (2000) suggest that there is risk of contamination not only through sexual transmission, but also through assisted reproduction, and Garrido et al. (2004b) suggested that HIV- and HCV-serodiscordant couples may be safely treated by sperm wash and ICSI to avoid viral transmission. In the present series, horizontal or vertical transmission of infection did not occur in any of the 43 couples who underwent 78 cycles of ICSI. This result is in line with those of other authors regarding the feasibility and efficacy of the procedure for minimizing risk of viral transmission in serodiscordant couples, while maximizing pregnancy rates. Nevertheless, only a few thousand cycles have been performed worldwide and it is still too early to demonstrate that these techniques are completely risk-free. Many aspects remain unsolved, such as standardization of sperm preparation procedure, methods and limits of viral detection in semen, and the benefits of different assisted reproduction techniques (IUI or ICSI). More research is needed to define guidelines for current treatment of serodiscordant couples and more cases need to be reported before any confirmation can be given. Such criteria would increase access to assisted reproduction techniques for these couples, reduce the spread of viral infection and improve quality of life.

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References


