BACKGROUND: Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) serodiscordant couples with the male infected can be helped to have children minimizing the transmission risk. Our aim was to evaluate the results of our assisted reproduction programme for these couples and to develop adequate strategies for their management.

METHODS: Members of serodiscordant couples: (i) HIV positive males attending our centre for sperm wash and assisted reproduction and (ii) HCV positive males needing assisted reproduction for infertility provided 134 semen samples for sperm wash. Before ICSI treatment, semen was confirmed to be negative for viral presence by reverse transcription and nested PCR after the sperm wash. RESULTS: Sperm washes were effective in 90% of the samples. Regardless of the type of infection, no differences were found in semen quality, embryos obtained and pregnancy rates (40–48% per cycle). To date, 41 pregnancies and 23 newborns were obtained. Fertilization rates were lower in HCV than in HIV serodiscordant couples (59.3±5.3% versus 72.0±8.1%), probably because they were infertile couples for whom we recommended sperm wash and PCR. No seroconversion was detected in the patients’ follow-up. CONCLUSIONS: To date, sperm wash, nested PCR and ICSI is a safe and effective procedure that avoids HIV and HCV transmission with reasonable pregnancy rates, and is cost-effective.

Key words: HCV/HIV/ICSI/nested PCR/semen wash/serodiscordant

Introduction

Antiviral and antiretroviral treatments now efficiently solve the control of life quality and expectancies of HIV-infected males, permitting these men to face the challenge of having their own offspring. Infertile couples with HCV-infected males are nowadays requested in some countries to be included in protocols of controlled assisted reproduction procedures to avoid any risk of nosocomial transmission to the partner, clinic employees, and other patients’ samples sharing laboratories, operation rooms, incubators or nitrogen tanks. Both reasons mean that the number of HIV or HCV serodiscordant couples wishing to conceive and/or needing assisted reproduction is constantly increasing, and to date we still need to clearly determine the clinical protocols to follow in these situations.

Given the low transmission risk, established as one to three infections per 1000 acts of sexual intercourse (or semen exposures) for HIV (De Vincenzi 1994) and even less for HCV (Garrido et al., 2004), all the accumulated data regarding viral transmission through semen with assisted reproduction techniques (mainly intrauterine insemination) do not reflect the real situation. To avoid any accidental infection of the partner, offspring and laboratory staff through assisted reproduction treatments, we must employ the safest techniques available.

HIV belongs to the retroviruses family, having the capacity to synthesize reverse transcriptase, and convert the RNA into DNA and to insert its genome in this manner into the hosts, while HCV is a RNA virus unable to act in this way. This issue is relevant to the detection methods employed in this work (Meseguer et al., 2002).

Commonly, the routine detection methods found in the literature are based on the amplification of defined sequences of the viral nucleic acids, and artificial insemination is the first treatment to be performed, if the gynaecological findings are normal (Semprini et al., 1992; Marina et al., 1998). We have previously demonstrated that the available technologies can be improved in order to detect even a single RNA or DNA viral copy with nested PCR (or reverse transcription and nested PCR for RNA, Meseguer et al., 2002). Nevertheless, an assisted reproduction procedure without using any detection method to determine the virus presence in
the washed sperm has been reported despite the possible risk of transmission (Garrido et al., 2002a).

Also, adequate equipment and trained personnel to work with these samples are mandatory. Strict virology protocols must be implemented in the laboratory (Garrido et al., 2004).

The aim of our study was to determine: (i) the efficiency of the sperm wash, in terms of presence or absence of viral load for HIV and HCV viruses after the wash of the samples, (ii) to determine the results of the assisted reproduction techniques with ICSI in serodiscordant couples for HIV and/or HCV employing washed sperm, (iii) to analyse seroconversion rates by employing these procedures.

Materials and methods

Between August 2001 and October 2003, 91 couples with a seropositive male and a seronegative female partner, divided into couples with HIV positive males (n = 18), couples with HCV/HIV positive males (n = 33), and infertile couples undergoing assisted reproduction treatment where the male was HCV positive (n = 40) were included in this study, providing a total number of 134 ejaculates to be washed. All of them signed an informed consent, and the study was approved by the Institutional Review Board. The mean age of all the men included in the study was 36.6 years old (range 25–47).

As a necessary condition to be included in this programme, the men were requested for a full report of their disease by the physician providing care for their infection, although many HCV patients were not followed at all.

For HIV positive males, CD4+ T-cell counts in blood, blood viral load, treatment received, co-infections, and a general description of the male patient status were detailed.

The results are presented in a global manner, without considering the infection. In the following analysis, different subgroups were constituted depending on the presence of each virus. The HIV-infected patients displayed all the stages of the infection (A, B or C) according to the Centers for Disease Control classification (CDC) (Table I), the A2 group being the most represented with 18 patients. Six of the HIV or HIV/HCV (10.3%) were not receiving antiretroviral treatment where the male was HCV positive (n = 40) were included in this study, providing a total number of 134 ejaculates to be washed. All of them signed an informed consent, and the study was approved by the Institutional Review Board. The mean age of all the men included in the study was 36.6 years old (range 25–47).

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We found no scientific or ethical reason to reject any patient due to their blood viral load or CDC classification status. The only reason to avoid an assisted reproduction treatment was a positive result after the sperm wash, and this obliged us to repeat the procedure later.

All the samples were processed in a laboratory separate from the laboratories where non-infectious samples were prepared, and all the safety guidelines followed have been published elsewhere (Bellver et al., 2002). Briefly, the viral risk area was only dedicated to these biological samples, including biosafety cabinet workstation, exclusive centrifuge, and nitrogen tank.

Semen parameters were analysed according to World Health Organization (WHO, 1999) criteria. Total count and motility before and after the wash were recorded. Sperm morphology was not analysed for safety reasons: it is not recommended to work with glass or blades when treating HCV/HIV positive samples. Sperm wash was only performed in those samples with >2 X 10^6 of total progressive motile sperm in the whole semen sample.

The HIV infection was acquired by parenteral drug addiction in 25 of patients (48.8%), plasma donation in one (0.2%), sexual transmission in 11 (21.6%), blood transfusion in six (11.7%), and eight infection acquisitions (15.5%) were unknown. The infection source for HCV was never known.

Regarding the female population, different gynaecological findings were observed: 59 were considered as normal (64.8%), 15 were aged >36 years (16.4%), five were low responders (5.5%), eight had endometriosis (8.6%). Depending on the patient’s characteristics we treated these women with two assisted reproduction procedures: ICSI with their own oocyte or with oocytes obtained from young healthy donors. In HIV serodiscordant couples, 11 procedures were performed with donated oocytes, while seven procedures were performed with donated oocytes in HCV serodiscordant couples.

Only females with demonstrated absence of HIV and HCV antibodies were accepted in the study. They were also requested to practice sex with condoms.

HCV seropositive males presented a median viral load of 125 000 copies (range 31 750–2 500 000); of these, 35 had detectable viral load for HCV (47.9%), and only two had liver biopsy revealing liver damage. In all, only five males have been treated with different interferon protocols.

**Sperm wash**

Ejaculates obtained after a sexual abstinence of 3–5 days were allowed to liquefy, and then were diluted 1:1 (vol:vol) with Sperm Medium (MediCult, Denmark). They were then pelleted at 400 g for 10 min, and the supernatants were discarded. A volume of Sperm Medium equal to the initial volume of semen was added, and then layered onto a triple concentration gradient (90, 70 and 45%, Pure-Sperm; Nidacon, Sweden) of 1–1.2 ml of each layer, and centrifuged 20 min at 300 g.

Each pellet was obtained and washed with 5 ml of Sperm Medium, and re-pelleted again. Supernatants were discarded and a swim-up of 0.5–0.7 ml was done. After 45 min, the upper 0.35 ml of each tube supernatant was aspirated, pooled and split into two samples. One half was immediately submerged in liquid nitrogen for PCR determinations, and the other half was frozen with Sperm Freezing Medium (MediCult), according to the manufacturer’s instructions, and stored until their use after a negative result for viral presence (Meseguer et al., 2002).

**PCR techniques for HIV and HCV applied to sperm**

Nucleic acids were extracted from the washed sperm using the Nuclisense® method (Organon Teknika, Spain) following the manufacturer’s instructions. Two extractions were run in parallel, one from the sperm sample and the other with the sperm samples after the addition of HIV RNA obtained from HIV-infected plasma, to detect the presence of transcription or amplification inhibitors after the nucleic acid extraction procedure. Both extracted samples were used for two HIV RNA transcriptions to detect genes from the gag and pol region, followed by a nested DNA amplification. The same samples were used to amplify HIV proviral DNA by a nested amplification to detect both genes (gag and pol). The other extraction, run

---

**Table I.** Centers for Disease Control (CDC) classification of our male population

<table>
<thead>
<tr>
<th>CDC classification</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
in parallel with added HIV RNA before nucleic acid extraction, was used as a positive control to detect the presence of inhibitors of the transcription or amplification. Negative controls to detect the presence of amplicon contamination were also performed.

For HIV RNA transcription we used the antisense external primers to anneal with nucleotides 1696 to 1676 and 3286 to 3265 for gag and pol genes respectively. Standardized conditions for transcription were followed using 100 mmol/l dithiothreitol, 1 mmol/l each dNTP, 0.2 mmol/l antisense primer, 20 IU RNAasin (Promega, Spain) and 5 IU Avian Myeloblastosis Virus reverse transcriptase (AMV) transcriptase (Promega) in a final volume of 20 ml. Nested DNA amplification used the external primers to anneal with nucleotides (from ARV2/SF2 sequence) 1224–1243 and 1696–1676 and internal primers to anneal with nucleotides 1316–1335 and 1524–1504 for the gag region. External primers annealing with nucleotides 2623–2642 and 3286–3265 and internal primers annealing with nucleotides 2716–2741 and 3250–3227 for the pol region were used. PCR standardized conditions were followed including 6 μl of RT or previous PCR product, 2.5 and 2 mmol/l MgCl2 for gag and pol region respectively, 0.2 mmol/l each dNTP, 0.2 mmol/l each primer and 2 IU Taq polymerase (Promega, Spain) in a 20 ml final volume. β-Actin gene amplification was performed to confirm the presence of DNA in the extraction from sperm suspension. Results were read from a 2% agarose gel electrophoresis after ethidium bromide staining. In all the samples a consistent result (either positive or negative) was obtained. The assay failure rate was zero.

HCV nested PCR protocols were described in a previous work, in order to amplify viral Non-coding Region (NCR) in a protocol comparable to reverse transcription and nested PCR for HIV but with the adequate specific primers (Meseguer et al., 2003).

Ovarian stimulation and ovum donation

Protocols for ovarian stimulation

For ovarian stimulation, both GnRH agonist and antagonist protocols were used. For GnRH agonist, long protocol was employed as previously described (Díaz et al., 2000). Briefly, patients started administration of 0.1 mg of leuprolide acetate (Procrin; Abbott S.A., Spain) or triptorelin (Decapeptyl; Ipsen Pharma; Spain) in the mid-luteal phase of the previous cycle, until negative vaginal ultrasound defined ovarian quiescence. The dose of GnRH analogue was then decreased to 0.05 mg until the day of hCG administration. GnRH antagonists were used following the low dose daily protocol recently described (Bosch et al., 2003): starting on stimulation day 6, 0.25 mg of the GnRH antagonist Cetrotide (Cetorelix; Serono S.A., Spain) was administered on a daily basis until the day of hCG administration.

Recombinant FSH (Gonal-F; Serono S.A., Spain; or Puregon; Organon Española, Spain) and hMG (Lepor; Farma Laboratories, Spain; or Menopur; Ferring, Spain) were used for ovarian stimulation. Initial doses were determined according to patients’ age and basal serum FSH and estradiol (E2) levels. On stimulation day 3, serum E2 level was assessed and gonadotrophin doses adjusted according to a step-up or step-down protocol. hCG (Profasi 10 000 UI; Serono S.A., Spain) was administered when three or more follicles reached 18 mm in diameter and oocyte retrieval was scheduled 36 h later.

Oocyte donation

Oocyte donor recruitment and management has been previously described, they were screened for sexually transmitted diseases (HIV and HCV included) (Garrido et al., 2002b). The protocol for steroid replacement has been also described. It included pituitary desensitization with one dose of 3.75 mg i.m. triptorelin (Decapeptyl; Ipsen Pharma, Spain) beginning in the secretory phase of the previous cycle. Patients started administration of E2 valerate (Progynova; Schering, Spain) on cycle day 1; 2 mg were administered from day 1 to day 8, 4 mg from day 9 to day 11, and 6 mg from day 12 onwards. On day 14, vaginal ultrasound and serum E2 determination were performed to evaluate endometrial receptivity, and E2 valerate doses were continued until donation became available. On the day of the donation, 800 mg/day of micronized intravaginal progesterone (Progell; Laboratorios Effik, Spain) were added.

IVF with ICSI

The microinjection was performed as previously described (Meseguer et al., 2003). Briefly, morphologically normal sperm were sought in the sperm droplet, and then immobilized and aspirated, tail first, into the tip of a micromanipulation pipette. A metaphase II oocyte was held on the holding pipette, and the injection pipette was pushed through the zona pellucida injecting a single spermatozoon.

Injected oocytes were incubated in 20 ml drops and fertilization was assessed after 18 h, and embryo cleavage 24 h thereafter. Embryos were transferred into the uterine cavity 48–72 h after ICSI. The remaining embryos were frozen for eventual future transfers. Clinical pregnancy was determined by observing a gestational sac with fetal heartbeat at 7 weeks of pregnancy. Embryo freezing–thawing protocols can be found elsewhere (Cobo et al., 2002).

Statistical analysis

r-Tests were employed for comparisons between groups when the data followed a normal distribution. Non-parametric tests were used to compare study parameters in fertile and infertile males when the data did not follow a normal distribution. Subsequently, in these cases, Mann–Whitney U-tests were carried out. Significance was defined as P < 0.05. The statistical analysis was performed using MedCalc Software (Belgium).

Results

Sperm washes

The results of the analysis for the detection of the presence of viral molecules after the sperm washes can be found in Table II. Positive results of the nested PCR after wash in samples from infected males is always (independently of the viral infection subgroups) ~10%, most commonly HCV RNA in those males co-infected with both viruses HIV DNA in men infected with only HIV was never found in our samples.

In those cases where a positive result was obtained, the sample was destroyed, and another sperm wash was programmed after 2–3 weeks. We never obtained a positive result after a second wash. Failure of the assay to yield a diagnosis was zero.

Table II. Sperm wash results for HIV and/or HCV detection

<table>
<thead>
<tr>
<th>Blood</th>
<th>n</th>
<th>HIV positives (%)</th>
<th>HCV positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RNA</td>
<td>DNA</td>
</tr>
<tr>
<td>HIV</td>
<td>26</td>
<td>2 (7.7)</td>
<td>0</td>
</tr>
<tr>
<td>HIV/HCV</td>
<td>52</td>
<td>4 (7.7)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td>HCV</td>
<td>56</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. Among all the HIV washed samples, either with or without HCV, nine (11.5%) resulted positive with any test.
Table III. Seminal characteristics depending on the infection

<table>
<thead>
<tr>
<th>Seminal parameters</th>
<th>HIV (total) (n = 78)</th>
<th>HIV only (n = 26)</th>
<th>HIV/HCV (n = 52)</th>
<th>HCV (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.7 ± 0.5</td>
<td>3.4 ± 0.4</td>
<td>3.7 ± 0.3</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Concentration (sperm/ml)</td>
<td>77.6 ± 18.4</td>
<td>88.4 ± 21.3</td>
<td>74.2 ± 15.3</td>
<td>90.6 ± 22.3</td>
</tr>
<tr>
<td>A</td>
<td>0.3 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>B</td>
<td>45.0 ± 16.3</td>
<td>8.0 ± 12.3</td>
<td>47.0 ± 10.3</td>
<td>45.0 ± 10.3</td>
</tr>
<tr>
<td>A + B</td>
<td>45.0 ± 16.6</td>
<td>39.2 ± 10.9</td>
<td>47.0 ± 11.3</td>
<td>45.3 ± 9.4</td>
</tr>
<tr>
<td>C</td>
<td>13.0 ± 2.5</td>
<td>14.3 ± 3.8</td>
<td>13.2 ± 2.3</td>
<td>17.3 ± 6.3</td>
</tr>
<tr>
<td>Total motile progressive (×10⁹/ejaculate)</td>
<td>133.8 ± 14.9</td>
<td>114.7 ± 18.6</td>
<td>137.9 ± 13.4</td>
<td>97.3 ± 20.3</td>
</tr>
<tr>
<td>Volume resuspended (ml)</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Concentration post wash (sperm/ml)</td>
<td>8.0 ± 2.0</td>
<td>6.5 ± 0.9</td>
<td>8.3 ± 0.6</td>
<td>7.3 ± 1.2</td>
</tr>
<tr>
<td>A post wash</td>
<td>4.8 ± 2.0</td>
<td>5.7 ± 2.1</td>
<td>4.5 ± 1.6</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>B post wash</td>
<td>70.0 ± 14.8</td>
<td>64.0 ± 13.3</td>
<td>72.4 ± 12.9</td>
<td>72 ± 15.3</td>
</tr>
<tr>
<td>A + B post wash</td>
<td>74.8 ± 13.8</td>
<td>70.1 ± 12.8</td>
<td>77.0 ± 13.0</td>
<td>73 ± 15.0</td>
</tr>
<tr>
<td>C post wash</td>
<td>16.1 ± 5.0</td>
<td>16.3 ± 3.7</td>
<td>6.9 ± 3.0</td>
<td>6.0 ± 2.0</td>
</tr>
<tr>
<td>Total motile progressive post wash</td>
<td>8.6 ± 2.0*</td>
<td>7.1 ± 0.6</td>
<td>9.0 ± 1.1</td>
<td>6.5 ± 1.0</td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>4.2 ± 2.0*</td>
<td>5.7 ± 1.8</td>
<td>3.3 ± 1.0*</td>
<td>10.7 ± 2.0*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. *P < 0.05.

Table IV. Global cycle results depending on the infection

<table>
<thead>
<tr>
<th></th>
<th>HIV (total)</th>
<th>HIV only</th>
<th>HIV/HCV</th>
<th>HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular aspirations (FA)</td>
<td>73</td>
<td>27</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>Oocytes obtained</td>
<td>920</td>
<td>352</td>
<td>568</td>
<td>621</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>742</td>
<td>293</td>
<td>449</td>
<td>514</td>
</tr>
<tr>
<td>Zygotes</td>
<td>511</td>
<td>211</td>
<td>300</td>
<td>305</td>
</tr>
<tr>
<td>Frozen embryos</td>
<td>169</td>
<td>78</td>
<td>91</td>
<td>68</td>
</tr>
<tr>
<td>Transferred embryos</td>
<td>154</td>
<td>59</td>
<td>95</td>
<td>116</td>
</tr>
<tr>
<td>Metaphase II/FA</td>
<td>9.9 ± 2.3</td>
<td>10.9 ± 2.0</td>
<td>10.2 ± 2.0</td>
<td>10.5 ± 3.0</td>
</tr>
<tr>
<td>Fertilization rate/metaphase II (%)</td>
<td>68.9 ± 6.3</td>
<td>72.0* ± 8.1</td>
<td>66.8 ± 6.0</td>
<td>59.3* ± 5.3</td>
</tr>
<tr>
<td>Embryos/FA</td>
<td>4.3 ± 2.0</td>
<td>5.1 ± 1.8</td>
<td>4.0 ± 2.0</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>Cleavage rate (embryos/metaphase II) (%)</td>
<td>57.3 ± 5.3</td>
<td>62.4 ± 5.6</td>
<td>53.4 ± 8.3</td>
<td>44.9 ± 10.2</td>
</tr>
<tr>
<td>Embryo transfers</td>
<td>64</td>
<td>23</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Embryos/transfer</td>
<td>2.4</td>
<td>2.6</td>
<td>2.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Positive pregnancy test</td>
<td>29</td>
<td>9</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Negative pregnancy test</td>
<td>29</td>
<td>12</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Newborns</td>
<td>19</td>
<td>4</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Pregnancy rate/cycle (%)</td>
<td>46.0</td>
<td>40.1</td>
<td>48.7</td>
<td>40.1</td>
</tr>
<tr>
<td>Frozen embryo (FE) transfer</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>FE positive pregnancy tests</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>FE negative pregnancy tests</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>FE pregnancy rates (%)</td>
<td>20</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are expressed as total numbers, or mean ± SEM. *P < 0.05.

Semen parameters

The results of the WHO analysis of the semen parameters are shown in Table III. Similar results were obtained in all groups.

Sperm concentration means are ~75–90 × 10⁹/ml, thus confirming previous studies, demonstrating that sperm concentration is not apparently affected in HIV positive males. Also, HCV-infected males display good count results, being ≥4-fold what WHO criteria consider as normal.

Sperm motility seems to be slightly impaired in comparison to what WHO states as normal, given that the mean does not reach in any case 50% of A + B types, although the lowest values were displayed by those males with only HIV infection.

After the sperm wash procedure, results were again similar among groups, and means of >70% of A + B forms is always found, suggesting that these semen samples display an adequate preparation, and behave in a similar way to those from non-infected males.

Comparing between groups, only the recovery rate, expressed as the percentage of recovered sperm after preparation in comparison to the initial amount, was different between patients infected with HIV and HCV versus patients with only HCV.

If this finding represents that co-infection is diminishing sperm ability to capacitate is still unknown and needs further investigation.

Cycle results

The results of treatment according to the viral infection are shown in Table IV. The total number (all patients grouped) of follicular aspirations was 124, with a total number of 1541 oocytes obtained, of which 1256 were metaphase II. Cancellation rate was 10.1% (14 cancellations). To decide
cancellation, less than three follicles $>18$ mm or $<450$ pg/ml
$E_2$ on the day of hCG administration had to be found.

The mean number of cycles per couple was $1.4 \pm 1.3$. The
number of zygotes obtained was 816, and 237 of these were
elected to be frozen. Finally 270 were transferred back to the
mother. Total cycles with transfer of embryos obtained within
the cycle were 113 (91.1%). A total of 41 pregnancies was
obtained (33.0% per cycle, and 41% per couple), divided into
one abortion, six biochemical pregnancies and 23 newborns
to date. Six pregnancies were twins, (14%), while the remain-
ing were singletons. Thirty-three pregnancies were obtained
in the first cycle (80.4%), and 11 (19.6%) in the second.

Regarding frozen embryos, four pregnancies were
achieved out of 16 attempts (25.0%).

The viral infection results demonstrate similar success
rates with respect to the different parameters analysed, with
fertilization and pregnancy rates comparable among groups,
except for the results of frozen–thawed embryos, where the
low numbers available made the sample unrepresentative.

Comparing the results in each group, the parameters ana-
lysed were similar except for fertilization rates. The success
of fertilization per metaphase II oocyte was significantly
higher in patients with only HIV than in couples where the
male partner was infected with HCV.

**Seroconversion tests**

Seroconversion tests for the partner were programmed 3 and
6 months after finishing every treatment (each embryo trans-
fer). To date, none of them has been seroconverted, neither
for HIV nor for HCV.

Women were tested in our clinic ($n = 41$) for the same
infection as their respective male partner, whereas the others
were controlled in their respective hospitals every 6 months,
as a routine screening for women at risk of infection. None rep-
ported a positive result, the transmission rate thus being zero.

**Discussion**

Nowadays, given that long-term survival issues in HIV-
infected patients have ceased to be a major problem, these
males are now seeking the possibilities that science offers to
obtain their own children, avoiding the employment of donor
amates, as well as any risk of transmission to their partner
and children. In parallel, HCV-infected males undergoing
assisted reproduction procedures are becoming more con-
scious that, although low, there is a possibility of trans-
mision to the partner in IVF cycles, and also to the medical
and laboratory staff and other patients. This makes adequate
treatment obligatory in some countries, and strongly rec-
ommended in others.

These viruses must also be considered from a different
point of view, regarding the methods employed to detect
them. While HIV is a retrovirus, that can be present as an
RNA molecule or as a part of the sequence of the host cell,
after the reverse transcription to DNA. HCV is only present as
RNA

Subsequently, for RNA tests of viral presence, different
positive and negative controls to both RNA extraction and
reverse transcription must be included (Meseguer et al.,
2002) in order to avoid false positive/negative results.

The analysis of HIV DNA, free or integrated inside the
human cells, is also necessary for HIV. With our analysis,
we only are able to determine the viral presence in a repre-
sentative population of sperm cells. The results obtained will
be extended to the half sample that will be employed for
assisted reproduction if virus sequences are not found.

When a positive result is yielded, we are unable to deter-
mine whether this is a consequence of the presence of sem-
nal plasma residues, immune cells that escaped from the
purification protocols, or even whether single sperm cells are
carrying the viruses inside them.

Nevertheless, using single sperm to inseminate oocytes,
instead of several thousands in classic IVF, or even millions
with intrauterine insemination (IUI) techniques, is theoreti-
cally safer, since the woman’s exposure to potentially
dangerous sperm is minimized. This is against other authors’
theories which claim that we could be introducing viral par-
ticles into the oocyte. We believe that sperm cells carrying
viruses (if any) and fertilizing an oocyte by IVF/IUI also act
in this way, introducing the viral genome inside.

Both situations (HCV/HIV) converge to nearly the same
protocols in the andrology laboratory: the sperm wash. With
this treatment of the samples, we can eliminate round cells, seminal plasma and the majority of immotile
sperm efficiently. Moreover, we will overcome PCR tech-
nical difficulties linked to seminal plasma polymerase
inhibitors.

Once this semen wash is performed, several procedures
to detect HIV and HCV presence have been historically
employed with different sensitivity. Nested PCR has been
demonstrated to be able to detect one single viral copy
(Meseguer et al., 2002). Moreover, this methodology is prob-
ably responsible for the positive rates after wash of $\sim 10%$
This is higher than any value we can find in the literature
(Semprini et al., 1992; Marina et al., 1998); obviously these
studies used tests with lower sensitivity.

The next step is to decide which kind of assisted reproduc-
tion treatment will be applied. Several considerations must
be taken into account.

Given the low risks, artificial insemination has been the
method of choice for some time (Semprini et al., 1992;
Marina et al., 1998). This technique has the advantages of
low economic cost and less inconvenience for the woman,
but nevertheless presents some inconveniences, such as the
need for a sperm wash result on the same day (and, if a posi-
tive result is found, the cycle will be cancelled), the low
numbers of sperm inseminated, and the high risk of viral
transmission (due to the exposure to thousands of ‘potentially
infecting’ sperm). Also the whole sperm preparation must be
employed within the same cycle.

We support a different approach with these couples,
including ICSI. Although more expensive, safety reasons, as
well as cost-effectiveness issues, encouraged us to follow this
protocol.

First, with regard to safety, we are exposing the woman to
an infinitesimal risk: we employ just one single sperm for
each oocyte, from a previously negative sample with nested PCR. Semen samples are frozen, and nPCR can be confirmed as many times as needed, given that the sample is not necessarily employed the same day. Also, sperm wash can be performed before the cycle, thus not cancelling any cycle because of a positive result.

Second, regarding the costs, pregnancy rates are two to three times higher with ICSI than IUI, and a sperm wash that resulted negative can be employed in as many cycles as required, thus avoiding the need for new washes. Many IUI patients unable to achieve pregnancy will subsequently proceed to IVF cycles. Also, many males will provide semen samples that will not be adequate for IUI after the extensive procedure of sperm wash, since the recovery of sperm is very low in order to avoid possible positives (see Table III).

Pregnancy rates are acceptable in all the groups, and are as expected for patients attending our centre for assisted reproduction undergoing ICSI cycles. Also we must consider that, although it was presumed that the women were not an infertile population, gynaecological study showed that some clearly infertile women were included in these protocols which may have had a negative influence on the results. More than 60% had a gynaecological finding to classify them as subfertile.

Another consideration is the source of patients. HIV serodiscordant couples form a cohort of patients with infertility features equal to the general population. Conversely, HCV-infected males are not counselled to avoid procreation due to the transmission risk: these are already infertile couples infected with HCV. This may account for the low pregnancy rates displayed.

Finally, different studies have excluded patients regarding several disease features such as variable viral blood load in the past 6 months, elevated viral loads, not receiving antiretroviral treatment, low CD4 levels, etc. We strongly support the idea that every patient requesting this kind of help must proceed to IVF cycles. Also, many males will provide semen samples that will not be adequate for IUI after the extensive procedure of sperm wash, since the recovery of sperm is very low in order to avoid possible positives (see Table III).

In summary, we provided information supporting sperm wash, nested PCR and ICSI as the safest and most reasonable method to treat HIV and HCV serodiscordant couples seeking pregnancy.

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References


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