Original Article

Studies of Assisted Reproduction Techniques (ART) for HIV-1-Discordant Couples Using Washed Sperm and the Nested PCR Method: a Comparison of the Pregnancy Rates in HIV-1-Discordant Couples and Control Couples

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SUMMARY: In this study, the efficacy and safety of assisted reproduction techniques with the sperm-washing method and nested PCR assay were evaluated in HIV-1-discordant couples, as many HIV-1-positive people of reproductive age are getting married and wish to have children safely. Twenty-seven HIV-1-discordant couples (husband, positive; wife, negative) were enrolled in this study. The spermatozoa were separated from semen samples by density gradient centrifugation and the swim-up method. HIV-1 RNA and proviral DNA were checked using nested PCR with a detection limit of one copy before fertilization and before embryo transfer. Clinical outcomes were compared with those of matched control couples. Thirty-eight cycles of in vitro fertilization or intracytoplasmic sperm injection were performed in HIV-1-discordant couples, where the pregnancy rates per embryo transfer and per couple were 60.6 and 63.0%, respectively. These rates were significantly higher than those in control couples ($P < 0.05$). Furthermore, all of the females and babies remained HIV-1 negative throughout the study period. Our data strongly suggest that this technique will allow HIV-1-discordant couples to conceive more safely and effectively.

INTRODUCTION

Recently, people infected with human immunodeficiency virus-1 (HIV-1) have been living longer, since the application of highly active antiretroviral therapy (HAART) has greatly improved survival. Many HIV-1-positive people of reproductive age are getting married and wish to have children safely. It would be possible for an HIV-1-infected male to father children without the risk of HIV-1 transmission if HIV-1-free spermatozoa could be obtained from his semen. The clinical value of sperm washing as well as its risks was first reported in 1992 by Semprini et al. (1), and since then, it has been confirmed by many authors examining both methodological issues and clinical data. In this study, we applied the assisted reproduction technique (ART) for HIV-1-discordant couples in which the man was HIV-positive and the woman was negative, using the swim-up method and nested polymerase chain reaction (PCR) assay and tried to elucidate the efficacy and safety of the procedure.

MATERIALS AND METHODS

Patient couples: First, the patients consulted the Department of Hematology of Ogikubo Hospital, and the HIV-1 infection status of the husband was assessed. At this time, couples were also informed of the details of this treatment by one of the doctors in the study group. After the couples confirmed their desire to undergo the treatment, they were referred to Niigata University Hospital and then were again informed of the details of the treatment by another doctor in the study group with a counselor. The explanation included details of the procedure for ovulation induction, oocyte retrieval, and embryo transfer as well as the risks of these procedures, followed by an explanation of the protocol for confirming the elimination of HIV-1 from the husband’s semen. The risk of secondary HIV-1 infection to both mother and baby, if the wife were to conceive, was also thoroughly explained. After the patients confirmed the final decision to participate in this study and gave written informed consent to treatment, the treatment was started. The approval of the ethical committee of Niigata University School of Medicine was obtained.

Semen pretreatments: Semen samples were obtained by masturbation and then tested for sperm concentrations, motility, and deformity. An improved swim-up method was used to collect HIV-1-free spermatozoa from the semen of HIV-1-positive males. Diluted semen was layered over a Percoll solution with a continuous density gradient of 30 - 98% and then centrifuged. We collected the sperm fraction from the end of the tube, and the spermatozoa were collected using the swim-up method as previously described (2). The sperm suspension was divided into two portions, and one half was cryopreserved in a liquid nitrogen container.

Detection of HIV-1 RNA and proviral DNA: The HIV-1 RNA and proviral DNA were measured by the nested PCR
method as previously described (2), with a detection limit of one copy. The oocytes obtained from the HIV-1-negative wives were fertilized after confirming that HIV-1 could not be detected in the washed semen samples by the nested PCR method. Furthermore, the fertilized eggs were cultured for 2 or 3 days and were transferred after a negative result was obtained by the nested PCR procedure in the culture medium of the fertilized eggs.

In vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI): The standard long protocol was adopted for most ovulation stimulation cycles. The short protocol was used for patients who were poor responders. If HIV-1 testing for virion RNA and proviral DNA was negative, the other portion of the sperm sample was thawed for use in conventional IVF or ICSI. IVF or ICSI was offered according to the semen profile of each male. The embryo transfer was conducted only when HIV-1 RNA and proviral DNA were negative by the nested PCR assay.

Seroconversion tests: All female partners who underwent ART, even those who did not conceive successfully, were tested for HIV antibodies, HIV-1 RNA, and proviral DNA in the blood 1, 2, and 3 months after the embryo transfer. Furthermore, babies born to the mothers were tested for HIV-1 RNA and proviral DNA at birth or later.

Control couples: Control couples matched to the woman’s age who underwent conventional IVF or ICSI in Niigata University Hospital between January 2001 and July 2007 were randomly selected to assess clinical efficacy. During this period, 417 patients aged between 24 and 48 years underwent 814 cycles of IVF or ICSI by the long or short protocol. Within these control couples, 465 cycles of IVF from 261 patients (403 embryo transfers) and 209 cycles of ICSI (182 embryo transfers) from 118 patients were used to compare clinical results to those of HIV-1-discordant couples undergoing embryo transfer.

Statistical analyses: The Student’s t test and chi-square test were used to test differences between HIV-1-discordant couples and control couples. Significance was defined as $P < 0.05$.

RESULTS

Twenty-seven discordant couples in which the man was HIV-1 positive and the woman was negative were enrolled in this study at Niigata University Hospital between January 2001 and July 2007. The age of the women ranged from 21 to 41, with a mean of 32.3 years. Of the 27 males, the plasma HIV-1 viral load was <50 copies/ml in 15 patients, and the median plasma HIV-1 viral load of the other 12 was 967 copies/ml (range, 100-100,000). In addition, the median CD4 cell count was 377 cells/ml (range, 96-700) in 27 patients.

Twenty-seven women underwent ovulation induction 38 times. Of these 27 patients, 5 underwent ovulation induction twice, and another 2 underwent ovulation induction 3 and 5 times, respectively. The remaining 20 patients each underwent induction once. Two cycles were cancelled due to poor response. HIV-1 RNA and proviral DNA were not detected by the nested-PCR assay in any of the 36 of spermatozoa samples collected from 27 patients. HIV-1-negative sperm were used for IVF in 12 couples and for ICSI in 18 couples. To date, fertilized eggs were obtained in 26 women and embryo transfer was performed in all 26 women after confirming that HIV-1 RNA and proviral DNA could not be detected in the culture medium of the fertilized eggs. Three cycles were canceled due to the lack of fertilization.

The clinical pregnancy rate per embryo transfer was 60.6% (Table 1). Of the 27 HIV-1-discordant couples, 17 patients (63.0%) conceived and 22 babies were born. Three cases resulted in early abortion. The multiple pregnancy rate was 25.0%, with 4 sets of twins and 1 set of triplets. HIV-1 RNA and proviral DNA were negative in all of the females and infants throughout the study period. The median observation period of born babies was 58 months (range, 10-86). The clinical pregnancy rates per embryo transfer and per couple in the control couples were 30.8% (180 of 585) and 42.5% (161 of 379), respectively. Therefore, the clinical pregnancy rate per embryo transfer as well as that per couple in HIV-discordant couples was significantly higher compared with that in control couples ($P < 0.001$ and $P < 0.05$ by chi-square test, respectively).

Table 1. Clinical outcomes of IVF/ICSI cycles in 27 couples

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Range</th>
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<tbody>
<tr>
<td>Couples (n)</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>32.3 ± 5.0</td>
<td>21-41</td>
</tr>
<tr>
<td>Cycles (n)</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Total gonadotropin dose (IU)</td>
<td>2,022.4 ± 777.4</td>
<td>1,050-4,200</td>
</tr>
<tr>
<td>Retrieved oocytes (n)</td>
<td>9.8 ± 6.2</td>
<td>1-22</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>50.6 (179/354)</td>
<td></td>
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<tr>
<td>Transferred embryos (n)</td>
<td>2.2 ± 0.6</td>
<td>1-3</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>34.8 (24/69)</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy rate per embryo transfer (%)</td>
<td>60.6 (20/33)</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy rate per couple (%)</td>
<td>63.0 (17/27)</td>
<td></td>
</tr>
<tr>
<td>Delivered pregnancy rate (%)</td>
<td>85.0 (17/20)</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancy rate (%)</td>
<td>25.0 (5/20)</td>
<td></td>
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<tr>
<td>Maternal seroconversion (n)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Delivered offspring seroconversion (n)</td>
<td>0</td>
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IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.
embryo transfer of IVF and ICSI were 30.3 and 31.8%, respectively (Tables 2 and 3). The implantation rate and the clinical pregnancy rate per embryo transfer were significantly higher in the HIV-1-discordant couples, especially for IVF treatment (The \( P \)-values appear in Tables 2 and 3; statistical analyses were performed using the chi-square test).

### DISCUSSION

The heterosexual transmission rate of the HIV-1 virus is not very high, but a risk does exist. The rate of male-to-female transmission of HIV-1 in stable heterosexual relationships is estimated to be approximately 1 per 1,000 acts of unprotected intercourse (3). The transmission rates are perhaps greater with advanced stages of the disease, the presence of ulcerative genital infection, a history of previous sexually transmitted disease in the female partner, and the presence of postcoital bleeding. Araneta et al. have reported that the risk of transmission with intrauterine insemination (IUI) using non-washed semen from an infected man is 3.52% (4). For HIV-1-discordant couples with male infection, techniques such as sperm washing would further reduce the risk of transmission. The clinical value of sperm washing and the absence of seroconversions were first reported in 1992 (1). Since then, the method has been confirmed with regard to clinical issues by many authors (5-10). They have reported pregnancy rates per IUI cycle ranging from 15 to 31% (Table 4). However, their method may be suboptimal because it has not been proven to remove HIV-1 RNA completely, and they have not checked proviral DNA in infected cells in the semen. Zhang et al. have reported that HIV-1 may be present as proviral DNA in seminal cells in HIV-1-infected men who have achieved undetectable levels of viral RNA in plasma with HAART (11), and this HIV-1 could be transmitted sexually.

Although IUI therapy may be simpler and less expensive than IVF or ICSI therapy, IVF or ICSI involves a lower exposure of sperm cells compared to that in IUI, which requires millions of sperm to be placed in the uterine cavity. Another advantage of IVF or ICSI over IUI relates to the increase in pregnancy rates per treatment cycle. IVF or ICSI should decrease the number of attempts needed to establish a success-
ful pregnancy, thus further reducing potential viral exposure from repetitive treatment cycles. Since 1998, several groups have reported the results of IVF or ICSI in HIV-1-discordant couples with an HIV-1-infected male partner (3,7,10,12-16). The pregnancy rates reported in these studies were higher than those obtained using the IUI technique (Table 4). In the present study, the pregnancy rates per embryo transfer of IVF and ICSI were 72.7 and 54.5%, respectively. These data were significantly better than those of the control group for both IVF and ICSI treatment (Tables 2 and 3). Although the reason for the higher success rate in HIV-discordant couples than in control couples remains unclear, it is assumed that the females in the HIV-discordant couples, unlike many of those in the control couples, were endocrinologically normal. Furthermore, all of the females and babies remained HIV-1 negative throughout the study period.

Although the number of patient couples treated in this study was smaller that in previously reported studies, we nonetheless were able to establish the safety of the modality. The HIV-1 RNA and proviral DNA were measured twice, just after adjustment of the semen and just before embryo transfer, by the nested PCR method with a detection limit of one copy, for each patient.

In conclusion, the technology employed in this study is considered to offer promising results for HIV-1-discordant couples, allowing those who wish to conceive to do so more effectively and safely. In future, however, it will be necessary to increase the number of patients examined in order to more fully elucidate the safety and efficacy of this technique.

ACKNOWLEDGMENTS

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REFERENCES