J Formos Med Assoc 2011;110(8):495–500

Contents lists available at ScienceDirect

Journal of the Formosan Medical Association

Journal homepage: http://www.jfma-online.com

Original Article

# Outcomes of Assisted Reproductive Techniques for HIV-1-discordant Couples Using Thawed Washed Sperm in Taiwan: Comparison With Control and Testicular Sperm Extraction/Microscopic Epididymal Sperm Aspiration Groups

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**Background/Purpose:** An increasing number of human immunodeficiency virus-1 (HIV-1)-discordant couples in Taiwan have been seeking fertility help. We conducted the first clinical trial in Taiwan of assisted reproductive technology (ART) using sperm washing and viral load measurement.

**Methods:** From 2005 to 2009, we performed 22 ART cycles on 14 HIV-1-discordant couples. The sperm washing involved density gradient centrifugation followed by swim-up method. HIV-1 RNA was checked by real-time reverse transcription-polymerase chain reaction with a sensitivity of 40 copies/mL. In addition, we enrolled two other groups of ART recipients using frozen sperm to compare the clinical outcomes.

**Results:** There were five pregnancies in the fresh cycles (23.8%) of HIV-1-discordant couples and the cumulative pregnancy per couple was 42.9% (6/14). The data were comparable with normal controls and testicular sperm extraction/microscopic epididymal sperm aspiration groups. The nine babies and the 14 women in this study showed no seroconversion.

**Conclusion:** The preliminary data showed good ART results in HIV-1-discordant couples. Fertility services should not be withheld from individuals with HIV-1, although larger series are needed to reach conclusions about safety.

**Key Words:** assisted reproductive technology, human-immunodeficiency-virus-1-discordant, sperm washing

In Taiwan, the first case of acquired immunodeficiency syndrome (a foreigner in transit) was identified in December 1984, and recently Taiwan has shown an increasing incidence of human immunodeficiency virus-1 (HIV-1) infection.[1] Meanwhile, there has been continual improvement in survival among HIV-infected individuals through the use of newer forms of highly active antiretroviral therapy.[2] With the achievement of improved survival rates, married couples now



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**Received:** October 8, 2009
**Revised:** April 6, 2010
**Accepted:** July 21, 2010

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often wish to have children safely. The clinical use and risk of sperm washing and its risks were first reported in 1992 by Semprini et al,[3] and since then it has been confirmed worldwide.[4,5] The guidelines of the American Society for Reproductive Medicine in 2006[6] also recommended Semprini's widely used sperm-washing procedure: the use of gradient centrifugation followed by a sperm swim-up procedure. In the first trial of this procedure in Taiwan, we here present the clinical data of 14 HIV-1-discordant couples and compare the results with two other groups [normal controls and testicular sperm extraction/microscopic epididymal sperm aspiration (TESE/MESA)] who were also using frozen sperm.

## Materials and Methods

### *Subject couples*

The subject couples were seeking reproductive services and were referred to the Department of Obstetrics and Gynecology of National Taiwan University Hospital. HIV-1 infection status of the couples including $CD4^+$ cells and plasma viral load was assessed first. Gynecological examinations, including pelvic ultrasound, and hormone profiles, and semen analysis were subsequently performed to decide which couples would receive intrauterine insemination (IUI, not discussed here) or *in vitro* fertilization (IVF) cycles. From 2005 to 2009, a total of 14 couples (22 stimulation cycles) were enrolled in this study. The institutional review board approved this study (22MD02) in 2003, and signed informed consent was obtained from each couple before they entered the treatment cycle. The indications for IVF in these HIV-1-discordant couples were the same as for other patients.

### *Semen pretreatment*

Semen samples were obtained by masturbation and then examined for sperm concentration, motility and morphology. The samples were processed using a density gradient (three layers at 50%, 70% and 90%, from top to bottom; SpermGrad; Vitrolife, Kungsbacka, Sweden) to separate motile spermatozoa from non-sperm cells, immotile spermatozoa, and seminal plasma. The ejaculate was layered over the gradient and centrifuged at 300*g* for 20 minutes. After centrifugation, the 50% and 70% layers were eliminated, and the sperm pellet recovered and resuspended in 2 mL of fresh human tubal fluid medium (home-made, quality control by mouse embryo culture), divided into four parts, placed in four test tubes and centrifuged at 200*g* for 10 minutes. The supernatant was discarded, 0.5 mL of human tubal fluid medium was subsequently gently layered on the pellet, and the tube was incubated at 37°C for 1 hour. After swim-up, a supernatant volume of 350–400 μL was recovered per test tube (i.e. 1.4–1.6 mL). One of the aliquots was tested for detectable HIV-1 RNA.

### *Detection of HIV-1 RNA in washed semen*

A sample of extract (50 μL) was mixed manually with 50 μL COBAS TaqMan HIV-1 premix reagent and amplified using the real-time polymerase chain reaction (PCR) COBAS TaqMan 48 instrument (COBAS Ampliprep analyzer; Roche Diagnostics, Meylan, France). Target and internal quality standards amplified DNA in each sample were detected and assayed using specific TaqMan probes. Results were visualized on a computer using the Amplilink 3.01 system connecting the COBAS TaqMan 48 and COBAS Ampliprep instruments. The sensitivity ranged from 40 copies/mL to 10,000,000 copies/mL.

### *Assisted reproductive technology (ART)*

Controlled ovarian hyperstimulation was performed mostly by a long gonadotropin-releasing hormone agonist protocol or by short protocols for some poor responders, as previously reported.[7] Recombinant follicle-stimulating hormone (Puregon; Organon, the Netherlands) at 200 IU/day was given starting from Day 3 of the long protocol or Day 5 of the short protocol. Ultrasound-guided transvaginal oocyte retrieval (TVOR) was performed at 34–36 hours after human chorionic gonadotropin (hCG) administration. Subsequently, intracytoplasmic sperm