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ARTICLE

Semen decontamination for the elimination of seminal HIV-1

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Abstract The risk of human immunodeficiency virus (HIV) transmission to the female partner, or potential offspring of an HIV-1 infected man can be reduced using semen decontamination procedures before assisted reproductive treatment (ART). The objective of this study was to determine the efficiency of decontaminating semen samples ($n = 186$) from 95 HIV-1 sero-positive patients. Aliquots of neat semen were submitted for viral validation by qualitative and quantitative polymerase chain reaction. Semen samples were processed by density gradient centrifugation in combination with a ProInsert™ tube after which aliquots of the processed sperm samples were analysed for the presence of HIV-1. Fifty-four percent of all tested neat semen samples tested positive for HIV-1 DNA, RNA or both (13.4%, 11.3% and 29.0%, respectively). From a total of 103 processed sperm samples that were submitted for viral validation, two samples tested positive for HIV-1 DNA and none for RNA. In conclusion, semen processing with the ProInsert™ followed by viral validation of processed sperm samples should be carried out when providing ART to couples where the male partner is HIV-1 sero-positive.

KEYWORDS: density gradient centrifugation, HIV-1, semen decontamination, semen processing

Introduction

The general transformation towards a more accommodating approach to the provision of assisted reproduction treatment (ART) for human immunodeficiency virus type-1 (HIV-1) sero-positive patients is in part due to the improved clinical care of infected patients, as well as by improvements made in risk-reduction procedures during ART (Zutlevics, 2006). Concerns about the treatment of patients with potential seminal pathogens during ART include the following: the probability of vertical and horizontal transmission of the specific pathogen; the welfare of children

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with infected parents that may pass away due to the infection (Lyerly and Anderson, 2001); and the possibility of nosocomial transfer of pathogens in an ART laboratory (Englert et al., 2004).

Infection with the human immunodeficiency virus (HIV) is no longer considered as life threatening to patients. Improvements made in multi-drug highly active antiretroviral therapy (HAART) has resulted in infection with HIV becoming a manageable, chronic disease (Lambert-Niclot et al., 2012). Most infected individuals, however, are in their reproductive years with the desire to have their own genetically related offspring (Kanniappan et al., 2008; Paiva et al., 2007; Sauer, 2005), and it would be unethical to deny access to ART (Englert et al., 2001; Gilling-Smith et al., 2001). A recent South African HIV prevalence survey in 2012 by Shisana et al. (2014) indicated that 12.2% of the national population (6.4 million persons) were HIV positive. Men aged between 35 and 39 years presented with an HIV prevalence of 28.8% (31.6% of women tested positive for HIV), followed by 25.6% and 17.3% for men aged between 30 and 34 years and 25 and 29 years, respectively. In women, HIV prevalence was 36% and 28.4% for the latter age groups. A 2009 survey investigating the sero-prevalence of HIV-1 among patients ($n = 303$) seeking ART at the Reproductive Biology Laboratory (RBL) at Steve Biko Academic Hospital (SBAH), indicated that 14.4% of couples were sero-discordant (7.1% male positive and 7.3% female positive), and 9.3% of couples were sero-concordant (unpublished data). A total of 16.4% (9.3% + 7.1%) of male patients seeking ART at SBAH are therefore HIV-1 sero-positive.

Shedding of HIV into the semen of patients taking HAART may occur (Halfon et al., 2010; Zhang et al., 1998), possibly owing to inflammation and co-infections, poor adherence to their HAART regimens, or both. Halfon et al. (2010) described a 3% HIV-1 RNA for blood plasma viral load (BPVL) negative and seminal viral load (SVL) positive, with an overall rate of 7% detectable HIV-1 in semen. Similarly, 6.6% of all semen samples of patients seeking ART between 2002 and 2011 tested positive for HIV-1 RNA, despite undetectable BPVL (Lambert-Niclot et al., 2012). Conversely, Sheth et al. (2009) noted a relatively high cell-free virus (HIV-RNA) load in seminal plasma (48%), and are of the opinion that cell-associated HIV (HIV-DNA) may also persist in semen despite HAART. A small test-sample by Zhang et al. (1998) indicated that four out of seven HIV-1 positive men with undetectable BPVL, presented with positive proviral DNA in semen samples. The infectious potential of semen, even from men receiving HAART, should therefore not be underestimated. Assisted reproductive procedures combined with semen decontamination should be carried out to reduce the risk of vertical and horizontal transmission of the virus (Huyser and Fourie, 2010). Semen processing to harvest an optimal quality sperm sample is a well-established and effective procedure in ART laboratories. Technique failure, however, does occur, probably as a result of re-contamination of the processed sperm samples after density gradient centrifugation (Loskutoff et al., 2005), resulting in the detection of HIV in processed sperm samples (Fiore et al., 2005; Leruez-Ville et al., 2002). Risk-reduction procedures carried out during semen decontamination should be evaluated to ensure the effective and safe ART for HIV-1 sero-positive male patients.

In this study, the effectiveness of semen processing by discontinuous density gradient centrifugation in combination with the ProInsert™ for the removal of HIV-1 from semen samples of HIV-1 sero-positive patients was evaluated.

Materials and methods

Institutional approval for the study was received from SBAH and the University of Pretoria's Ethics Committee, on 8 October 2012 (protocol number 37/08).

Participants

HIV-1 sero-positive patients that participated in the semen decontamination programme at RBL were required to provide the Unit with recent (<3 months old) blood viral validation results (HIV-1 RNA and CD4 count). Leukocytes expressing the CD4 receptor are the major target cells for HIV, and decreased CD4 counts are correlated with a supressed immune response (Lawn et al., 2006). Patients with CD4+ lymphocyte counts below 300 cells/μL were therefore excluded from the programme, and were referred to a virologist for antiviral therapy. Patients could only enter into the ART programme once the CD4+ lymphocyte count increased to above 300 cells/μL, and semen decontamination was successfully performed. The semen decontamination procedure and potential risks involved in using purified sperm from HIV-1 positive men were discussed with patients. Participants were then required to sign an informed consent form providing the Unit permission to perform the semen decontamination procedure, submit a neat diagnostic semen sample and purified sperm sample for HIV-1 validation, cryopreserve and store purified sperm samples, and use sperm samples for potential ART use, should these samples present with HIV-1 loads below the lowest limit of detection (LLD). A unique identification code was assigned to each participant in the programme to ensure anonymity.

Collection of semen samples

Between 2008 and 2012, 95 HIV-1 infected men that enrolled in the semen decontamination programme were requested to provide two semen samples by masturbation per week (Monday and Wednesday, or Tuesday and Thursday), for 1–2 consecutive weeks, depending on the quality of the processed sperm samples. Most patients therefore were required to provide the Unit with four semen samples. In cases in which sperm yield was too low for a specific ART procedure, however, additional semen samples were required from the patient. The first semen samples delivered each week served as diagnostic samples, and those delivered on the second visit of each week were processed for therapeutic use during ART. Patients were instructed to seal the semen-containing sample cup using a re-sealable zipper storage bag, placed in a polystyrene cup and covered with a lid. Upon receiving semen samples from the patients, the samples were