SPERM ONLY PLEASE: Prevention of infections in an assisted reproduction laboratory in a developing country

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Abstract

The aim of the current paper is to discuss prevention of infections in a public sector laboratory-orientated assisted reproduction treatment (ART) setting in Gauteng, South Africa. The reproductive profile with reference to the screening for sexual transmitted infections, affordability of care and resources available, should determine the repertoire of fertility screening and ART scheduling. Alternative viewpoints on resource management of an ART laboratory in a public sector setting, semen decontamination for the human immunodeficiency virus type-1 (HIV-1) seropositive male and the subsequent choice of ART procedures, i.e. intra-uterine insemination (IUI) as a first-line treatment and intracytoplasmic sperm injection (ICSI) are addressed. Assistance for the safe reproduction of HIV-1 serodiscordant couples is globally acceptable, and should be moulded into an accessible ART program for developing countries.

Key words: Affordable, assisted reproduction, developing country, semen decontamination, HIV-1; infections

Introduction

The reproductive health profiles, affordability of and access to health services and resources will influence the reproductive wellbeing of a couple. Assisted reproduction technologies are in general perceived as expensive and childlessness as non-life threatening. Economics therefore dictated that infertility care is at times, not regarded as a public service health priority in developing countries. It is however important that current practices, experiences and facilities of a provider should be reviewed before cost-effective and safe fertility care for the human immunodeficiency virus type-1 (HIV-1) seropositive couple can be established (Frodsham et al., 2006). Therefore, what is considered as “cost-effective” in a society, which carries a high burden of sexual transmitted diseases? The HIV/acquired immune deficiency syndrome (AIDS) epidemic also impacts on the procreation needs of a couple, since childlessness has an universal implication that transverses nationality, income status, physical conditions and gender. The Joint United Nations Program on HIV/AIDS (UNAIDS, 2008) estimated that in 2007, between 30 and 36 million people were living with HIV, with two thirds (67%) of all HIV-positive individuals residing in sub-Saharan Africa. Large variations in HIV prevalence also exist between the different regions within sub-Saharan Africa (Buvé, 2002). South Africa has the unenviable position to have the largest HIV epidemic globally, with an estimated 5.7 million of inhabitants living with HIV in 2007. The epidemic is defined as a hyper-endemic epidemic since 15% of the age group 15 to 49 are living with HIV (UNAIDS, 2008). A South African National Survey on the prevalence and incidence of HIV among more than 13,000 nationals in 2008, estimated that 50.8% of respondents aged 15+ years reported a history of HIV-testing in 2008, with more females (28.7%) participating in testing and knowing their status than their male counterparts (19.9%) (Shisana et al., 2008).

Experiences we have gained at the Reproductive and Endocrine Unit, which is situated in Gauteng, the most populous province of South Africa, indi-
cated that most individuals are knowledgeable of HIV/AIDS and the risks associated therewith, when seeking reproductive assistance. The unit is a tertiary infertility clinic with a diagnostic and therapeutic assisted reproduction laboratory, dealing with the low- and middle-class income population of the northern part of South Africa. It is the only public health care centre active in assisted reproductive technologies, in this part of the country. The aim of this paper is to discuss information relevant to laboratory-orientated services for couples in a public sector South African assisted reproduction treatment (ART) Unit, with reference to conditions where the male is HIV-1 positive.

Infection and contamination control in an ART laboratory

Infections within an ART laboratory can originate through health workers, samples from patients that harbour infectious conditions, the laboratory atmosphere, contaminated equipment and storage vessels, as well as materials and supplies utilized during procedures. The primary objective of any ART Unit should be to exclude all possible pathogens from all potential sources (Elder et al., 2005). By identifying the potential sources, protective measures can be taken to prevent transmission of pathogens. Unhygienic working conditions and waste disposal increases the risk that infections may be transmitted to personnel, patients, cultures, samples and equipment. Elder et al. (2005) recommended that a stringent infection control policy should refer to aspects of hygiene, disposal of clinical waste and protective measures including decontamination procedures. A question consequently arises, if best practice quality control frameworks and directives from developed countries should be used to guide emerging or future assisted reproduction laboratories in the developing world? Mortimer (2005) assessed the impact of the European Union Tissue and Cells Directive (2004) in an ART milieu, and is of the opinion that commonsense can; in some situations indicate an overload of good intentions. The author also recommended that particular circumstances, which could affect reproductive tissues, should be properly considered to ensure that technical regulations are realistic and based on objective evidence.

In some instances offices, rooms, wards or theatres are modified and adapted to serve as ART laboratories in developing countries, whereby air quality and humidity are the most difficult factors to regulate in our experiences. Contamination with spore-forming bacillus species, fungal spores (e.g. Aspergillus sp, zygomyces or other saprophytic moulds) occur through crevices in the ceiling, and through air conditioning ducts and ventilation systems (Elder et al., 2005). Figure 1A(i) illustrates dust entering through gaps in the suspended ceiling and sticking to a textured wall in the diagnostic spermatology laboratory at Steve Biko Academic Hospital (Reproductive Biology Lab, Pretoria, South Africa) prior to renovations, and Figure 1A(ii) shows the modified sealed ceilings and filters installed in front of ventilation ducts after renovations. The intended clean room for embryo culture before and after constructions is depicted in Figures 1, pictures B(i) and (ii). All basins were removed due to the possibility of the introduction of waterborne pathogens in a clean room environment. Since Pseudomonas spp. were previously identified in tap water from adjacent laboratories and washrooms, we assess the water quality regularly and periodically clean the inside of all laboratory taps using a small bottle brush and a mild sodium hypochlorite solution. Male patients also receive bottled water (intended for irrigation) at our Unit, to wash with, prior to giving a semen sample.

Door closures, digital locks and security gates were installed to improve security and limit access to clean room areas during the upgrade of our laboratory. Monitors were placed strategically adjacent to the clean room areas to (i) observe e.g. follicular fluid screening or embryo selection, (ii) provide visual information in real-time during micro-manipulation training and (iii) decrease the need to enter sensitive areas. Embryo development is also computerized and images can be viewed by students in training, clinical staff and patients, either on the monitors and/or in a printed report format. All ill-fitting wooden pass-throughs, linking the clean rooms to adjacent areas, were replaced with one-way stainless steel and glass double-door pass-throughs (Figure 1B (i) and 1B(ii)). Sturdy washable overlapping blinds replaced flimsy material blinds, and electrical sockets were placed below all work surfaces, to provide a clutter free workspace which is easier to clean. A once off investment to obtain over-pressured separate laboratories for HIV-infected and non-infected patients with separate cryopreservation facilities provide a safe-environment for all our patients and staff. A dust-free laboratory environment is obtained through a high number of fresh air changes per hour, and the placement of in-line HEPA-filters in the ventilation system that functions separate from the hospital’s system. Although the latter additions to the facility were costly and associated with developed countries modus operandi, it was well-worth the efforts. The investment in a basic air sampler (Biotest HYCON®, Dreieich, Germany) and indicator agar strips (Agar Strip TC - Total
count; ref no 941105; Agar Strip YM – Yeast and moulds; ref no 941196; www.biotest.de), proved to be equally empowering. Sampling air using specific indicator agar strips, incubating the strips and learning to interpret and calculate the colony forming units (microbial count) resulted in a quick turn around time between testing and outcome. This interpretation led to lessened costs and provided a practical self-assessment tool to confirm the cleanliness of an area or instrument.

Most of the clinical and laboratory adaptations and control mechanisms to minimize viral transmissions during ART as described by Englert et al. (2004), Gilling-Smith et al. (2005) and Gilling-Smith (2006) have been adopted at our Unit over a period of four years. Entrée level embryologists receive ongoing formal training and are chaperoned for two years, with emphasize on risk-reduction and quality control procedures. An assisted reproduction laboratory is a complex structure geared towards the promotion of optimal cell survival and culture, not only for embryos, but also for viruses and bacteria (Englert et al., 2004; Elder et al., 2005). Intrinsic laboratory factors such as temperature, gas/culture atmosphere, air quality and laboratory hygiene that directly affect gametes and embryos, must be monitored by laboratory staff, while the intake of known and unknown contaminated patient samples are governed mostly by clinical staff. Introduction of infectious conditions into the assisted reproduction laboratory can be prevented if the origin of the contaminant can be identified, contained or removed.

Screening for pathogens and treatment prior to ART

Tremendous improvements in the prevention and screening for sexual transmitted diseases (STDs)
have been made in the South Africa, probably due to HIV/AIDS awareness campaigns in recent times. In the public health sector patients are usually sectioned into groups based on their annual income and access to health insurances, whereby patients with a low income seeking ART, are most often subsidized. Economics clearly influence the choice and scope of sexual transmitted infections (STI) tests, selection of ART procedures and choice of a private or public ART sector in South Africa. Several authors have published data (Table I) on the outcome of intrauterine insemination (IUI), in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) procedures for a large number of HIV-serodiscordant couples (males positive), without any documented case of sero-conversions during the time-periods of the surveys. These reports, from multiple investigators from developed countries, provide directives on the safety and outcome of the ART procedures for HIV-infected individuals in developing countries. ART centres should, however, always consider all risk reduction measures to safeguard all patients and staff when treating patients with known infections. Even if universal precautions are practised, zero risk does not exist (Gilling-Smith et al., 2005).

Microbes in semen

Bacteriospermia does not necessarily imply an infectious condition (Cottell et al., 2000) and can be due to contamination during semen collection. The practice of routine semen cultures in asymptomatic couples is therefore questionable. Four possible approaches to deal with bacterial contaminants in semen are (i) patients with a positive bacterial culture are treated with appropriate antibiotics prior to an assisted reproduction procedure, and re-evaluated to confirm a negative seminal bacterial culture, (ii) provision of guidelines (directives on sample collection) to male-patients to eliminate skin-contaminants, (iii) semen washing and embryo culture medium are supplemented with antibiotics, and the (iv) use of a physical mechanism to reduce microbes in the processed sperm sample, such as density gradient and a mechanical device e.g. the ProInsert™ (Nidacon, Sweden) (Huyser, 2008). Therefore, to pro-actively prevent the unnecessary prescription (costs and use) of antimicrobial medications to our patients, verbal information on laboratory procedures and guidelines on semen collection are provided to male patients in four indigenous languages.

Table I. Summary of published ART outcome for HIV-1 serodiscordant couples (male positive) 2004-2010.

<table>
<thead>
<tr>
<th>ART Procedure</th>
<th>Authors</th>
<th>Cycles</th>
<th>Couples</th>
<th>HIV-1 test of washed fraction</th>
<th>Pregnancy rate</th>
</tr>
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<tbody>
<tr>
<td>IUI</td>
<td>Bujan et al., 2007*</td>
<td>2840</td>
<td>853</td>
<td>Yes**</td>
<td>RNA</td>
</tr>
<tr>
<td></td>
<td>Manigart et al., 2006</td>
<td>68</td>
<td>25</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td></td>
<td>Savasi et al., 2007</td>
<td>2400</td>
<td>581</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Van Leeuwen et al., 2009</td>
<td>174</td>
<td>61</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>5482</td>
<td>1520</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>Bujan et al., 2007*</td>
<td>107</td>
<td>76</td>
<td>Yes**</td>
<td>Yes**</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>107</td>
<td>76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICSI</td>
<td>Bujan et al., 2007*</td>
<td>394</td>
<td>262</td>
<td>Yes**</td>
<td>Yes**</td>
</tr>
<tr>
<td></td>
<td>Garrido et al., 2004</td>
<td>73</td>
<td>73</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td></td>
<td>Manigart et al., 2006</td>
<td>62</td>
<td>20</td>
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<td>Yes</td>
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<tr>
<td></td>
<td>Mencaglia et al., 2005</td>
<td>78</td>
<td>43</td>
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<td></td>
<td>Sauer et al., 2009</td>
<td>420</td>
<td>181</td>
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<td>Yes</td>
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<td></td>
<td>Savasi et al., 2007</td>
<td>278</td>
<td>160</td>
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<td>Yes</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
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<td>739</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF/ICSI</td>
<td>Kashima et al., 2009</td>
<td>38</td>
<td>27</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Prisant et al., 2010</td>
<td>44</td>
<td>28</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>8</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>6976</td>
<td>2390</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*CREAtHE – Centers for Reproductive Assistance Techniques to HIV couples in Europe

**Multicentre study between 8 centers; all centers except one, performed HIV-1 DNA and RNA validation of sperm samples
If needed, HIV-negative patients are issued with a non-spermicidal polyurethane condom (Male-FactorPak®, Apex Medical Technologies, California). A large retrospective study by Fourie et al. (2009) indicated that microbes were present in 53% of all semen samples (n=929) tested at the Pretoria Unit during 2007-2009. Micro-organisms commonly cultured in samples during diagnostic semen analyses were: coagulase negative staphylococci (27%), Ureaplasma spp. (11%), Mycoplasma spp. (5%), α-haemolytic streptococci (5%), Escherichia coli (5%), Enterococcus faecalis (4%), and Enterobacter spp. (3%). The positive cultures and the reported sensitivities, will then determine the antimicrobial-options available to the patient. Questionable is the infrequent practice at some ART Units, to only request microbial testing of semen and viral screening (HIV-1) of male patients judged to be at risk of infections. To assume on appearances and judgement of the females’ blood pathology results that the male is negative, is a risky endeavour for all involved.

Sexual transmissible pathogens

Only a limited number of papers have been published on the prevalence of sexual transmitted diseases in South Africa. Pham-Kanter et al. (1996) provided an informative historical perspective on the disease burden of classical transmitted infections, and statistics on ulcerative infections, gonorrhoea, chlamydia and other organisms. A review by Johnson et al. (2005) updated the study by Pham-Kanter and colleagues (1996) to provide information on STI prevalence rates between 1985 and 2003 in South Africa. Bamba (1999) indicated that the most common STIs reported in Africa, besides HIV include chancroid, human papilloma virus, herpes simplex, trichomoniasis and candidiasis, while gonorrhoea, syphilis and chlamydia also contribute to damages of Fallopian tubes. Infectious conditions of the male reproductive tract may also impact on HIV-1 shedding and thus the HIV load in semen (Dejuqc-Rainsford and Jégou, 2004; Kalichman et al., 2008). The origin, clinical presentation and transmission through semen of other sexual transmitted viruses, such as cytomegalovirus (CMV), hepatitis B, C, D (HBV, HCV, HDV) and human T-lymphotrophic virus (HTLV-I) have been reviewed elsewhere (Dejuqc-Rainsford and Jégou, 2004; Elder et al., 2005). Sub-Saharan Africa is reported to have the highest HCV prevalence rate in the world, compared to European and North American populations. African countries with the highest HIV infections tend to have the lowest estimated HCV incidences (Madhava et al., 2002). The mode of hepatitis transmission and implications for infertile patients, as well as health workers have been described in detail by the Practice Committee of the American Society for Reproductive Medicine (ASRM, 2006).

(i) Screening for HIV in ART

Participation in an ART program implies agreement to disclose the HIV status to the participating partner (ESHRE Taskforce on Ethics and Law, 2004). Cost implications, as well as restricted access to pathology laboratories, could be reasons for the periodical screening of patients for selected blood borne viruses in some South African ART Units (Huyser, 2008). Although access to reliable HIV-1 testing for diagnostic and monitoring purposes is limited in Sub-Saharan Africa, most resource-constrained countries do have reference laboratories, especially in capital cities (Rouet and Rouzioux, 2007). A trade-off therefore exists between the cost for screening of sexual transmitted infections, indirectly assessing health risk indicators and the accommodation of patients in ART practices. Most South African ART centres screen patients who enter an ART program, using an automated enzyme-linked immuno sorbent assay (ELISA) – i.e. HIV Combo testing for HIV-1 & 2 antibodies plus p24 (Abbott 4th Generation assay). Positive HIV ELISA results are then confirmed with a second HIV Combo ELISA (e.g. Roche Diagnostics) using the same blood sample. A positive result is then reported with the request for second blood sample to confirm the initial test (using ELISA), and to verify the origin of the sample (see testing strategy II, WHO, 2005). Branched-chain DNA test (bDNA) or polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) analyses, are usually requested to evaluate blood plasma viral load (BPVL). These commercial assays are also used to determine seminal plasma viral load (SPVL), as well as the efficacy of the decontamination procedure to obtain purified sperm samples after processing. For a review on current commercial methods to determine SPVL, with reference to performance advantages and limitations; see Chan and McNally (2008). These molecular validation techniques are labour-intensive as well as time consuming and especially expensive to patients with minimal or no health insurance. A retrospective survey in 2009 (N = 200 patients), indicated that less than 40% of patients that requested ART, have the benefit of health insurance. Extrapolation of costs to present day PCR analyses (confirmation of viral genome) at a private pathology laboratory in South Africa, indicated that HIV-1 analyses and CD4/8 amounts to a cost of approximately 90 €. Newer real-time technology (real-time
PCR) seems to offer excellent cost-benefits, have an expanded dynamic range, reduces the amount of manual handling and risk of contamination through automation (Chan and McNally, 2008) to benefit higher throughputs in resource-poor environments (Scott et al., 2009). Comparing three real-time assays for HIV-1 subtype C VL quantification in South Africa, the latter-mentioned authors, are of opinion that the Abbott m2000 RealTime HIV-1 assay, will benefit a high throughput in the South African National Health Laboratory Service (to be implemented in mid 2010).

Blood and semen/purified sperm samples are usually couriered to pathology laboratories in major cities in South Africa for viral validations, using the previous-mentioned commercial assays. Patients are required to return for a follow-up appointment to obtain their results and to initiate treatment, since the results are usually available within 24-72 hours. A delay before treatment can result in further transmission, while a number of patients may prefer not to return and/or are unreachable for a follow-up appointment. Some patients may be apprehensive to return, or perhaps fear further cost escalations and therefore remain untreated. A test that provides immediate results will avert a delay between performing a test and obtaining the results (Ward and Weber, 2010). The WHO guidelines on HIV rapid testing and counselling services in resource-constrained settings indicated that most rapid tests are single tests, easy to perform, with a shelf-life of approximately 12 months, can be stored at 20-30°C, and cost between 0.40 – 1.20 US$ for a single test (WHO, 2004). Although the ELISA screening test is less expensive than the previously mentioned molecular assays for viral validation, it is still costly for most patients and requires an infrastructure and trained staff to perform and interpret results. The development of applicable rapid tests could play a significant role in future reproductive health screening and management of patients in low-resource settings.

(ii) HIV-1 validation in seminal plasma and purified sperm samples

It is debatable if an HIV/ART policy should be adopted based on the BPVL and/or SPVL of patients requesting ART in a resource-limited setting. An undetectable or low BPVL together with a repeatable, easy to perform, and cost-effective semen decontamination procedure is desirable in this environment. We initiated an experimental semen decontamination program at our Unit, to test the ability of a decontamination procedure (using a novel tube insert [ProInsert™] combined with density gradient centrifugation) to remove HIV-1, HCV, CMV and white blood cells from in vitro spiked semen samples (Loskutoff et al., 2005; Huyser et al., 2006). Subsequently we evaluated the effectiveness of the decontamination procedure to remove a variety of bacteria and yeast from in vitro spiked semen samples obtained from HIV-seronegative donors; and in vivo derived HIV-1 RNA as well as proviral DNA from semen of HIV-seropositive men (Huyser 2008; Fourie et al., 2009). Commercial validation assays were used to determine HIV-1 RNA VL (Cobas AmpliPrep-Cobas Taqman HIV-1 version 2, Roche Diagnostics, Indianapolis, USA; linear reportable range 20-10,000,000 copies/ml) and to evaluate proviral DNA qualitatively (AmpliCor HIV-1 DNA, version 1.5 Roche diagnostics) in all neat semen samples from HIV-1 seropositive males and corresponding post-processed sperm aliquots. These validations were performed on all samples, by an independent private pathology laboratory. Evaluation of fifty semen samples indicated that 64% of the neat semen samples tested positive for HIV-1 RNA, DNA or both (26%, 20% and 18%, respectively). The decontamination method was effective in the removal of all HIV-1 proviral DNA and RNA (range 138 - 801,440 RNA copies/ml) in neat semen. Validation results were, however, usually received after 48 hours, which necessitates the cryopreservation of the purified sperm. Cryopreservation of the final sperm sample has major consequences for ART management of the HIV-serodiscordant couple (Leruez-Ville et al., 2002; Nakhuda and Sauer, 2007), especially in the developing world. Cryopreservation and the resultant sperm quality after thawing may negate a planned IUI procedure, and may necessitate the implementation of IVF procedures (Leruez-Ville et al., 2002). This implies that laboratory staff should be able to cryopreserve and store sperm samples in hermetically sealed ionomeric resin staws (CryoBioSystems, CBS™) (Tomlinson, 2005) within dedicated liquid nitrogen canisters. The volume of neat semen/sperm needed to perform the molecular viral validation analyses is also a limiting factor. Leruez-Ville et al. (2002) mentioned that some ART groups proposed the use of selected spermatozoa without viral validation, where HIV detection in (previous) processed semen was negative. The authors cautioned that HIV RNA detection was most likely to be positive in sperm samples that originate from seminal plasma with a high VL (> 20,000 copies/ml). Seminal viral load will most definitely impact on the efficiency of sperm processing methods to obtain purified sperm samples. An upper limit of 1.5 × 10^5 RNA copies/ml in seminal plasma was indicated by Fiore et al. (2005), for discontinuous density gradient centrifu-
The researchers quantified HIV-1 RNA using a NASBA assay with a lower detection limit of 80 copies/ml. Although South African health insurances often do not cover infertility treatment and do not subsidize HIV testing in neat semen or purified sperm samples, we are of opinion that SPV quantification should be a starting point for any semen decontamination attempt. We therefore verify HIV-1 RNA and DNA in seminal plasma (see previous molecular assay specifications), and test the final sperm aliquot only if the validation test was positive in the neat semen sample.

Sperm processing techniques for the HIV-infected male

Assisted reproduction procedures for patients with blood borne viruses are generically geared towards risk-reduction to avoid horizontal transmission to both the male and female, and perinatal transmission to the infant. Minimal invasive procedures such as self insemination or intra-uterine insemination can be used when the female is HIV-positive (Gilling-Smith, 2006). The main objective when the male partner is HIV-positive is the removal of cell-free and cell-associated pathogens from sperm fractions, while optimal sperm quality is preserved (Semprini et al., 2004; Nakhuda and Sauer, 2007). This is obtained through sperm washing and decontamination techniques, using density gradients, swim-up steps and/or decontamination devices (Politch et al., 2004; Loskutoff et al., 2005). More than 60% of all neat semen samples from HIV-positive males processed at our Unit tested positive for HIV-1 RNA quantitative (range 138 - 801,440 RNA copies/ml), DNA qualitative or both RNA and DNA positive. Using the ProInsert™ without a final swim-up step, HIV-1 proviral DNA and RNA were not detected in any of the processed sperm samples within the detection range of the commercial PCR tests. The ProInsert™ facilitates precise density gradient layering, prevention of recontamination of the sperm pellet after centrifugation, and provides a bio-secure method for the operator. The term “sperm decontamination” is used (Huyser et al., 2006; Huyser, 2008; Fourie et al., 2009) since different interpretations of the term “sperm washing” exists. Semprini and Hollander (2007) refer to sperm washing as a three-step seminal processing method, i.e. density gradient centrifugation, washing of the sperm pellet and spontaneous migration of the spermatozoa; as was designed nearly two decades ago (Semprini et al., 1992). Persico et al. (2006) is of the opinion that it is improper.

![Flow chart for ART management of HIV positive males](image)

*Fig. 2. — Flow chart for ART management of HIV positive males (adapted from Ombelet et al., 2003; Semprini and Hollander, 2007)

1. RNA – Quantitative (Cobas AmpliPrep - Cobas Taqman HIV-1 version 2, Roche Diagnostics, linear reportable range 20
-10,000,000 copies/ml);
2. DNA qualitative (Amplicor HIV-1 DNA, version 1.5 Roche diagnostics).*
to refer to sperm washing (Leruez-Ville et al., 2002), if the final swim-up step of spermatozoa is not included during processing. Procedures such as centrifugation, washing as well as separation solutions, may vary in different laboratories. A balance must always be reached between removal of non-sperm cells from semen and maintaining sperm quantity and quality (Gilling-Smith, 2003), which have a direct impact on the choice and success of ART procedure for a couple. Repetitive washes can cause sperm damage and loss of sperm quality and quantity during repeated centrifugations (Gilling-Smith, 2003).

**ART procedures**

It is noted by Semprini and Hollander (2007) that until 1995 a large number of IUls were performed for serodiscordant couples (males HIV-positive). During this period antiretroviral medication was not available and no PCR assays existed for viral validation of the processed sperm sample. Some authors advocate the use of a less expensive and labor-intensive IUl-procedure, compared to IVF-ICSI, in the absence of tubal pathology and a sperm sample of sufficient quality (motility and concentration) (Semprini and Hollander, 2007; Van Leeuwen et al., 2009). Others prefer to navigate or apply the IUl procedure as a special detour and rather perform IVF and/or employ the ICSI procedure (Mencaglia et al., 2005; Prisant et al., 2010; Sauer et al., 2009; see Table I). Another possible avenue is the singular use of anti-retroviral pre-exposure prophylaxis (PrEP) prior to unprotected intercourse in carefully selected patients with suppressed VL (Van Leeuwen et al., 2009; see also http://www.creathe.org: Timed intercourse and PrEP). An overwhelming safety record of centers that offer ART to serodiscordant couples where the male is positive (Table I), aided our Unit to design a flow chart (Figure 2) to perform IUl as an inexpensive and safe first-line treatment (Ombelet et al., 2003). Semprini and Hollander (2007) described the indications for ART procedures similarly, with the addition of IUl in spontaneous ovulation cycle or hormonal stimulated cycle, in combination with female age. The discourse by Bujan et al., (2009) on an article by Sauer et al., (2009) regarding the use of ICSI, and the reply of the latter-mentioned author, provide insight into the choice of a specific ART procedure for a serodiscordant couple where the male is HIV-positive.

**Conclusion**

An HIV-positive adult may not necessarily be infertile, but the practice of “safer sex” may precipitate in involuntary childlessness. Therefore, should ART treatment options for serodiscordant and concordant couples be based on viral status and/or presence of male and female factors in a resource-constraint setting? Also, is there any difference in the choice of ART procedure for HIV-infected individuals in a developed versus a developing country? In this regard best practice quality control frameworks and directives from developed countries can be used to guide emerging or future assisted reproduction laboratories in the developing world. Financial constraints and logistics might preclude the continuing monitoring of the plasma VL over time and secondly, the submission of seminal and purified semen samples for viral validations. As viral containment (e.g. use of antiretroviral medications or sperm processing techniques) become standardized globally, the requirement to perform a post-processing viral validation can not be compulsory. A robust and efficient simple semen decontamination procedure will certainly provide additional protection to overcome some of these hurdles within a low-resource setting. This does not imply that viral screening is to be omitted, but perhaps unattainable in certain scenarios in a developing country. We have monitored and evaluated the introduction of contaminants into the ART laboratory from all possible sources to develop and deploy a prevention-based strategy within the laboratory. On the other hand, an ART laboratory is part of a Unit and can not be viewed as sovereign in its actions and policies. If a Unit’s patient population carries a high burden of STIs, and screening of these patients is not regularly performed or checked in a developing country, then the unprepared assisted reproduction laboratory can be a disaster waiting to happen.

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**References**


Prismatic N, Tubiana R, Lefebvre G et al. HIV-1 or hepatitis C chronic infection in serodiscordant infertile couples has no impact on infertility treatment outcome. Fertil Steril. 2010; 93:1020-3


Semprini AE, Hollander L. Treatment of HIV-discordant couples: the Italian experience. In Oehinger SC and Kruger TF (eds) Male Infertility: Diagnosis and Treatment,
World Health Organization, Guidelines for HIV diagnosis and monitoring of antiretroviral therapy. WHO, Regional Office for South-East Asia, New Delhi 2005.