Prevention of infections in an ART laboratory in a developing country

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Introduction

“Any uncertainty about a future event that might threaten an organization’s ability to accomplish its mission” may be labelled as a risk. Proactive prevention as opposed to reactive cure can be seen as prophylactic management to provide quality assisted reproduction technology (ART) services (Mortimer and Mortimer, 2005). Reactive actions such as timeously troubleshooting with corrective adjustments and evidence-based handling of adverse events during ART procedures are also invaluable risk management tools.

Developing countries are constrained by limited access to, or availability of resources, which will impact on the screening, diagnosis and management of infectious diseases. The 2014 West African Ebola outbreak is a current example, with numerous media reports outlining the prevailing stigma and conspiracy theories on the origin and progress of the disease. Undoubtedly disgrace, ignorance, limited finances and resources similarly impede the extent and frequency of screening for sexually transmitted infections (STIs) prior to ART, selection of ART procedures, and choice of a private or public ART provider in low to middle-income countries (Huyser and Fourie, 2010). The aim of the current paper is to discuss the prevention of infections in a laboratory-orientated ART setting in a low to middle-income country, with reference to simplistic risk reduction applications to avoid the introduction and transmission of pathogens. The diagnostic and procedural phases will be briefly discussed, as indicated in Figure 1, i.e. (i) patient evaluation and screening for microbes and (ii-iii) the prevention of procedural and environmental contamination including sperm preparations to reduce infectious agents.

Patient screening for microbes: detection and prevention

Work-up of couples prior to ART treatment and handling of human bodily fluids (blood, follicular fluid and semen) during procedures should be generic for all patients, irrespective of the type of ART procedure (Huyser, 2014). Various viral agents such as cytomegalovirus, hepatitis B (HBV), hepatitis C (HCV), herpes simplex virus type 2, human T-lymphotropic virus, and human immunodeficiency virus (HIV) can be transmitted through semen and vaginal secretions (Elder et al., 2005). A detailed review and guidelines of infections in the female and male partner, and infectious complications of ultrasound-guided oocyte retrieval is provided by Steyaert et al. (2000).

Sexual health screening in most developing countries where ART is offered, is probably less state-regulated with infrequent and fewer tests over time. Most ART practices in developing countries use point-of-care testing that offers overall affordability, and a short time-span to perform, interpret and provide the results. The use of rapid STI-screening tests with high sensitivity, specificity and long shelf-life presently plays a significant role in infectious disease screening and reproductive health management in resource-constrained countries (Huyser and Fourie, 2010). Technological advancement of currently available rapid tests is needed e.g. direct assessment in seminal plasma as...
well as blood plasma, with the ability to grade viral load levels to a certain degree.

Laboratory procedures and staff conduct eliminating and reducing the transmission risk of microbes during ART procedures should be a central theme during training, especially when resources to test or monitor aspiring ART patients are lacking or infrequently provided.

ART procedures: gametes and embryos

Environmental and technical factors

ART laboratory personnel are trained to “treat each sample as potentially infectious” and “to ensure aseptic conditions for gametes, zygotes and embryos” (Magli et al., 2008). Safeguarding through universal precautions and adherence to the safety procedures outlined in the WHO laboratory manual for the examination and processing of human semen (WHO, 2010) is usually provided as guidelines when in doubt. To take extraordinary preventative action implies having knowledge of threats and the degree of risk involved consequently. Awareness and understanding of the vulnerabilities of the ART system wherein laboratory personnel function are invaluable assets when unforeseen equipment failure occurs or instant decisions have to be made to safeguard procedures. Simple laboratory organisational precautions include uncluttered work surfaces, phasing out of sharp-edged glass items, no mouth pipetting, hygienic working conditions (procedures using appropriate equipment and disposables), regular cleaning and decontamination of the laboratory (quality control measures), proper (hazardous) waste disposal and strict access control to different laboratory sections. On a personal level, skin breaks should be protected with waterproof dressings and non-toxic powder-free gloves should be worn at all times, together with appropriate attire (barrier precautions - clothing, masks, gowns, and goggles) (Steyaert et al., 2000; Elder et al., 2005; Magli et al., 2008). Personnel should be immunized against HBV with a baseline serum result available (Elder et al., 2005) as well as against other viral diseases for which vaccines are available (Magli et al., 2008).

Biosafety for ART procedures are classified as level 2, with supplementary precautions when processing HIV and HBV/HCV-positive samples. It is prudent to ensure separate facilities and equipment for semen handling and cryopreservation within an ART laboratory (Elder et al., 2005). Additionally specimens from patients who tested positive for blood borne viruses should be processed, cryopreserved and stored in dedicated areas using

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**Fig. 1. — Elements that influence the prevention of infections during an ART program: (i) Diagnostic phase, i.e. patient assessment and screening, and (ii-iii) the procedural phase including sperm processing, environmental and technical factors (Huyser, 2014).**
Semen samples should be handled with extreme care as a biohazard by laboratory personnel (WHO, 2010). In-house statistics have indicated the positive identification of microbes in approximately 50% of all semen samples obtained for ART procedures, with gram-negative species present in only a fraction of samples (Huyser and Fourie, 2010; Fourie et al., 2012). In addition, over 50% of all neat semen samples from HIV-1 infected males tested consistently positive for HIV-1 RNA (quantitative result) or DNA (qualitative result) over a 5-year period (Huyser and Fourie, 2010; Fourie et al., 2014) (Table I – revised data).

Antimicrobial treatments are prophylactic or empirically prescribed to intended ART patients when pathology services are not readily available to the couple. Sperm purification through discontinuous density gradient centrifugation is used, in preparation for intra-cytoplasmic sperm injection (ICSI). In this scenario seroconcordant or discordant couples are restricted to the ICSI procedure with confounding costs; in cases where ICSI is not offered at the ART Unit the couples have to add travel and accommodation costs.

An array of sperm processing methods are commercially available, from simple office-based semen preparation kits for intra-uterine insemination to density gradient centrifugation with an additional mechanical device to reduce microbe recontamination in the processed sample (ProInsert™ system, Nidacon, Sweden) (Huyser and Fourie, 2010; Fourie et al., 2014). Once the enriched sperm fraction is removed, the device can be capped and discarded as biohazardous waste. In our program, semen decontamination eliminated HIV-1 RNA (100% non-detection) and proviral DNA from 98.75% of semen samples of HIV-1 positive patients, as shown in Table I. A robust and affordable semen decontamination method is a valuable risk reduction tool to eliminate bacteria (Fourie et al., 2012) and viruses (Huyser and Fourie, 2010; Fourie et al., 2014) within a low-resource setting.

Cryopreservation is often an expensive necessity during ART in developing countries. An array of protocols, devices and publications on biosafety trials outlining the potential for disease transmission and pathogen survival during cryopreservation are currently available. If a cryopreservation protocol could be designed that can be universally applied, the worldwide use thereof could translate in an economical and safe system (Scholz, 2012). According to Scholz (2012) commercial liquid nitrogen does not pose a sufficient high risk for contamination, whereby prevention of cross-contamination in containers using closed or semi-closed vitrification devices that permits sealing as well as a high cooling rate should be sufficient to warrant sterility, good survival and development of the specimen cryopreserved.

### Table I. — HIV-1 RNA and DNA detection in neat semen and purified sperm samples from HIV-1 positive males (N = 104) in preparation for an ART cycle.

<table>
<thead>
<tr>
<th>Neat Semen Samples (N = 236)</th>
<th>Processed Sperm Samples (N = 160)</th>
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<tbody>
<tr>
<td><strong>DNA &amp; RNA</strong></td>
<td><strong>DNA &amp; RNA</strong></td>
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<tr>
<td>Negative</td>
<td>Positive</td>
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<tr>
<td>DNA</td>
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<td><strong>DNA &amp; RNA</strong></td>
<td><strong>DNA &amp; RNA</strong></td>
</tr>
<tr>
<td>Negative</td>
<td>DNA</td>
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<tr>
<td>N = 109</td>
<td>N = 37</td>
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<td>46.19%</td>
<td>19.91%</td>
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<td>N = 47</td>
<td>N = 43</td>
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<td>15.68%</td>
<td>18.22%</td>
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<td>98.75%</td>
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<td>N = 158</td>
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Conclusion

The prevention of infections within any ART laboratory is a team effort which starts with the couple’s reproductive health work-up to identify and avoid vertical and horizontal transmission and nosocomial transfer of pathogens during an ART cycle. Infection management forms part of quality control and risk reduction. Minimizing or averting risks implies having the tools and experience to recognize the consequences and likelihood of threats within a laboratory. Semen decontamination will reduce contamination risks, but will not eliminate all infectious agents from semen. Cost-benefit risk prevention and reduction tools created for ART laboratories in developing countries can equally benefit ART laboratories in developed countries.

References


Fourie JM, Loskutoff N, Huyser C. Semen decontamination for the elimination of seminal HIV-1. RBM Online. 2014 (accepted for publication).


