Clinical and laboratory management of sero-discordant couples seeking infertility treatments: a mini review

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Summary

An infertility treatment in sero-discordant couples raises many concerns about the risk of viral transmission to the uninfected partner, to the fetus/newborn and to the laboratory personnel. However, fertility treatments should be provided also to individuals with chronic viral infections. The management of these couples should be provided only by centres having the facility to minimize risks of transmissions and cross-contamination. A regular risk assessment analysis should be accomplished in each IVF centre, aiming to provide specific guidelines and standard operating procedures to minimize risks.

KEY WORDS: viral disease, cross-contamination, nucleic acid amplification technique, risk management.

Assisted reproduction technologies (ART) and viral disease: general requirements

An infertility treatment in sero-discordant couples raises many concerns about risk of viral transmission to the uninfected partner, to the fetus/newborn and to the laboratory personnel. The European Tissues and Cells Directive from 2004 (EUTCD; 2004/23/EC), with its supplementary Technical Directives (2006/17/EC, 2006/86/EC), dictates that all anonymous and known directed donors have to be tested serologically for chronic viral infections, including HIV-1/2, HBV, HCV and Syphilis. In certain circumstances, additional testing may be required depending on patient history (e.g. RhD, malaria, CMV, Trypanosoma cruzi). According to this directive, the HTLV-I antibody presence should also be investigated in patients originating from high-incidence areas. Only a certified laboratory, using EC-marked testing kits, should carry out all the biological tests. Before ARTs are started, each IVF center should provide to patients a proper serological screening and a reasonable management of the results in order to perform a risk assessment analysis (in Figure 1, 2 an example of serological screening policy of couples attending for ART is reported). Then, whenever one partner would result positive for any of the investigated disease, specific risk management strategies should be employed. In fact, the detection of a chronic viral disease will not necessarily prevent partner donation. Actually, as suggested by the Ethics Committee of the American Society for Reproductive Medicine (ASRM), fertility treatments should be also provided to individuals with chronic viral infections, including HIV (1). Patients who decide to proceed with fertility treatment, should receive a proper pre-conceptional counseling, in order to clarify the risks of sexual and vertical transmission of their infections. In fact, a chronic viral disease can be
transmitted, even during ART procedures, from an infected partner to an uninfected one (horizontal transmission) or from an infected mother to the fetus or newborn (vertical transmission). Moreover, patients should agree to appropriate interventions, aimed at reducing the transmission risk and then signed an informed consent, emphasizing that risk of transmission cannot be completely eliminated.

On the other hand, the in vitro fertilization (IVF) laboratory providing care to infected couples should have the opportunities to safeguard...
the safety of gametes and embryos and minimize the risk of cross-contamination. To this end, embryologists should be informed about the risks of handling potentially infected biological material and a comprehensive risk management system with written operation procedures and continuous employee training program, should be present and continuously implemented. Moreover, when infected biological samples are involved in the treatment, specific safety measures such as, a separation of the procedures in time or space (using dedicated laboratory space at allocated times and separated storage banks) should be established. Those centers that do not have the resources to provide care to these patients should facilitate referral to a center with specific protocols in place to manage these cases.

Risk management strategies in HIV-Serodiscordant Couples

The HIV presence can be detected in blood, semen and vaginal secretions of infected partner and the infection can be transmitted via sexual contact, via blood and via vertical transmission. Globally, it has been estimated that about 36.7 million people were living with HIV at the end of 2015, with over 80% of these infections transmitted sexually (Global Health Observatory data). An estimated 0.8% of individuals of childbearing age are HIV-infected; the improvements achieved in the medical protocols and the introduction of antiretroviral treatments, have improved life expectancy and life quality of these individuals, increasing then the number of patients seeking for an assisted reproductive treatments, in order to prevent the infection transmission to the partner or to the offspring.

The risk reduction strategies suggested for the treatment of HIV-serodiscordant couples seeking for a pregnancy entails primarily the administration of a highly active antiretroviral therapy, to minimize levels of HIV in the male partner (peripheral blood viral load less than 10,000 copies/mL) (1). A recent Cochrane review including 7 observational studies with a total of 6792 patients has demonstrated that the reduction of HIV transmission in discordant couples was greatest with the use of antiretroviral therapy (2).

During the IVF treatment, sperm-wash procedures, involving density gradient centrifugation followed by a sperm swim-up step, should be employed, aiming to reduce or eliminate HIV-infected white blood cells and the free virus in the fraction utilized for insemination (3). In order to provide an additional measure of safety, the final sperm fraction can be additionally tested by nucleic acid amplification technique (NAT) for the presence of residual detectable HIV, prior to its use for insemination. However, a quantitative assessment of HIV in semen, before and after the washing procedure, indicated that more than 99% of HIV is removed (4).

A recent systematic review and meta-analysis, confirmed the efficacy of semen washing procedure to prevent HIV transmission in 3,994 sero-discordant couples undergoing 11,585 cycles of assisted reproduction. The study reported no cases of HIV transmission following exposure to washed semen and no cases of vertical transmission among 1,026 new-borns, either at birth or at the follow-up evaluations (5). The ASRM Practice Committee also recommends performing HIV-test in the uninfected partner at three-month intervals during treatment and pregnancy (1).

Risk management strategies in HBV-Serodiscordant Couples

HBV infection is the most common infection disease worldwide. Although a vaccine against hepatitis B has been available since 1982, it has been estimated that roughly 350 million people have came into contact to the virus, showing serological evidence of recent or past infection (6). HBV can be transmitted by perinatal or horizontal transmission, but it is most commonly spread by percutaneous or mucosal exposure to infected blood and body fluids, including seminal fluids. HBV DNA has been detected as a free virus in seminal plasma, but it also may be present as an integrated genome in spermatozoa and it can be transmitted through artificial insemination. A report of acute viral hepatitis B in a patient following artificial insemination with donor sperm, (that was subsequently found to be positive for hepatitis B surface antigen) has been published in 1987 (7), suggesting the need.
to perform a routine screening of semen donors. Moreover, in 1991, some human embryos were exposed to HBV-contaminated human serum utilized to prepare culture dishes (8). After embryo-transfers were preformed, the procedure had a positive outcome for 18 women who experienced serologically detectable HBV infections during the first trimester of pregnancy. Two patients were tested positive for HBSAg and HBV DNA at the time of delivery. Fortunately, no HBV DNA was detected in serum or lymphocytes from the exposed 22 infants (8).

In the management of HBV-serodiscordant couples, the first step should entail the vaccination of the virus-free partner against HBV (1). Fertility treatments may be initiated once the presence of anti-hepatitis B surface antibody can be clearly detected in the vaccinated partner. If the female partner is immunized against HBV, advanced sperm washing procedures, such as density gradient are not strictly required. However, the employment of these safety measures may help to minimize the risk of HBV-contamination of other gametes or embryos that are from uninfected patients in the same laboratory and reduce the risk of transmission of the infection to the new-born as well as to the IVF operators.

In case of HBV positive female partner with a serological evidence of the infection (HBsAg-positive), immunoprophylaxis with HBV vaccine and immunoglobulin, should be provided to new-borns within 12 hours after birth (1).

**Risk management strategies in HCV-Serodiscordant Couples**

HCV is a globally prevalent pathogen, infecting over 1% of the world’s population (9). HCV is spread primarily through contact with the blood of an infected person. However, less common modes of transmission involves the sexual exposure and the vertical transmission, when the mother has high levels of circulating HCV RNA.

The primary aim of providing care to HCV-discordant couples through ARTs is to prevent the uninfected partner from horizontal transmission and minimize the risk of vertical transmission if the female partner is HCV-negative. In fact, unlike HBV, there is no vaccine for HCV and the only available treatment (with peginterferon alfa and ribavirin) should be considered prior to fertility treatment in order to reduce the viral load. ARTs treatments should be started when the viral load has a low value and at least 6 months after the therapy is completed (10).

The presence of HCV RNA has been reported in follicular fluid of HCV-positive women regardless the degree of blood contamination (11). HCV was also present in the seminal plasma of chronically HCV-infected males at varying prevalence with no correlation with HCV virus load (12). However, HCV is a single-stranded RNA virus lacking of reverse transcriptase; therefore, it cannot succeed in DNA integration within infected cells, gametes or embryos (10).

Although the risk of HCV transmission during ARTs is not documented and still debated, the employment of risk reduction measures during assisted reproduction is essential to reduce the potential risk of nosocomial contamination. In case of HCV-positive male, recommendations to reduce the risk of transmission to the partner involves a sperm washing procedure by density gradient centrifugation followed by a sperm swim-up step in order to remove the seminal plasma and reduces the HCV viral load to undetectable levels (13). ICSI should be also preferred as insemination method when the male partner is seropositive (14).

**Safety laboratory measures during assisted reproduction procedures**

Recently, different guidelines have been published in order to prevent the risk of nosocomial contamination during the treatment of patients with a blood borne virus infection (10, 15). The European Society of Human Reproduction and Embryology Committee of the Special Interest Group on Embryology (15) recommends the use of dedicated laboratory space at allocated times. The IVF centre should be provided with separate laboratory or laboratory area with dedicated equipment (e.g. incubators, flow hoods) to handle potentially infected samples; in alternative, the treatment of infected patients should be scheduled last in work list. The IVF personnel should strictly adhere to safety precautions (for instance, the use of single-use disposable materials and personal protective equipment) and be accurately informed and trained on suitable decontamination process. Special concerns
have been raised as regards to the cryopreservation procedures. These viruses have the ability to survive and retain their virulence, even after the exposure to the liquid nitrogen temperature (16). Cross-contamination with HBV of five bone marrow samples cryopreserved in liquid nitrogen and the subsequent cross-infection of patients has been reported (17). To avoid a potential cross-infection, different separate frozen storage banks for sperm, oocytes and embryos should be employed according to the detected infection. The use of vapour storage of both gametes and embryos may be a sustainable storage alternative to reduce the risk of cross-contamination (18). Others safety precautions include the use of heat-sealed high security straws for semen cryopreservation or, more generally, the employment of sealing techniques to prevent the direct contact with liquid nitrogen.

**Conclusion**

Fertility treatments of sero-discordant couples should be exclusively performed in centres having the facility to provide the required resources to minimize risks. An accurate counselling of these couples is fundamental to prevent the transmission of the infections to the partner or to the offspring.

A quality management system with detailed and regular risk assessment should be established in each IVF center, aiming to provide specific guidelines and standard operating procedures and increase the awareness of the operators handling patients with blood borne virus infections.

**References**


