

Report on viral infections in ART

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ABSTRACT

Human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are widespread infectious agents. In developed countries, effective therapies have turned potentially lethal HCV, HBV and HIV infections into chronic diseases with a good quality of life, also in terms of reproductive chances. Serodiscordant status is the condition in which only one partner is HIV, HBV and/or HCV positive. In fertile serodiscordant couples, natural conception should be encouraged following specific prophylactic strategies, while infertile serodiscordant couples should be referred for treatment performed using assisted reproduction technology (ART). ART centers working with serodiscordant couples must set up an internal management and control system to guarantee the safety of operators, uninfected patients, gametes and embryos, and also of the fetus, especially during the cryopreservation step.

Herein we summarize general features of HIV, HBV and HCV infection, their possible impact on human reproduction, and laboratory recommendations to achieve safe management of serodiscordant couples seeking pregnancy through ART.

KEYWORDS

Viral infections, HBV, HCV, HIV, contamination.

Introduction

Human immunodeficiency virus (HIV), *hepatitis B* virus (HBV) and *hepatitis C* virus (HCV) are widespread infectious agents. In Italy, over one million people are estimated to be infected by HCV, 332,000 by HBV, and almost 4,000 by HIV. Nevertheless, anti-viral therapies have turned potentially lethal HIV, HBV and HCV infections into chronic diseases with a good quality of life, also in terms of reproductive chances. Serodiscordant status is the condition in which only one of the partners in a couple is positive to one or more of these viruses. Serodiscordant status has social and psychological implications; in particular, it limits sexuality and can undermine the couple's welfare, causing feelings of bewilderment, fear, guilt, responsibility, and inadequacy, and leading to breakdowns in communication. Serodiscordant couples therefore need psychological support, especially after the discovery of the infection^[1]. Fortunately, fertile serodiscordant couples can nowadays seek a pregnancy by natural conception thanks to several therapeutic strategies for both the infected partner and the non-infected one^[2,3]. Infertile serodiscordant couples should instead be offered treatment involving assisted reproduction technology (ART). Even though serodiscordant status is not a discriminatory factor for access to infertility treatments, specific procedures are required to protect operators, healthy patients, and the future fetus. A higher risk of cross-contamination and infection may be present during cryopreservation and gamete donation, as suggested by literature data reporting the transmission of viruses and other microorganisms via liquid nitrogen^[4-6]. For this reason, screening and suitable operating procedures are mandatory in order to avoid contamination between cryopreserved

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samples and safeguard the health of patients and the future unborn child.

In this report we summarize recommendations, outcomes, and good laboratory practices allowing safe management of serodiscordant couples seeking pregnancy by ART.

HCV

HCV is a blood-borne RNA virus responsible for hepatitis C. It is transmitted primarily through parenteral exposure (blood products, shared needles, needle stick injuries), although it may also be found in other biological fluids such as saliva, urine, semen, vaginal secretions, and breast milk. Sexual and vertical transmission are considered secondary modes of transmission^[7]. HCV infects over 1% of the world's population, with intravenous drug users, hemophiliacs, homosexual men, sex workers and sexual partners of HCV-infected subjects being the categories at highest risk^[8]. The virus is highly pathogenic: 80% of infected patients develop chronic liver disease, 35% develop cirrhosis, and 5% of the 35% will progress to hepatocellular carcinoma^[9]. There is no vaccine available.

In the ART field, HCV can be transmitted both to operators

and to healthy patients, for example via semen. Good management of HCV serodiscordant couples should begin with counseling aimed at informing the infected partner about the risk of infecting their healthy partner, members of the healthcare team, and the fetus. To prevent HCV infection during ART, sperm washing is strongly recommended in order to reduce the viral load in the semen sample [10]. Moreover, in serodiscordant couples with male infection, employment of elective intracytoplasmic sperm injection (ICSI) is recommended even though conventional IVF has also been demonstrated to reduce the risk of HCV transmission [11].

Data on the impact of HCV on ART outcomes are discordant. Several studies suggest an adverse effect of viral infection on sperm parameters, hormone levels, and pregnancy rates [11-14], while others show no differences in ART results between serodiscordant couples and negative ones [15-17]. Other studies investigating ART outcomes in serodiscordant couples with an HCV-positive female partner suggest that the virus may affect ovarian response to stimulation, fertilization, implantation, and pregnancy rates; however, data are contradictory [18,19].

HBV

HBV is a double-stranded DNA virus responsible for hepatitis B, a major cause of acute and chronic hepatitis, cirrhosis, and hepatocellular cancer. With about 350 million infected people worldwide, HBV is one of the world's most common infectious diseases. The virus can be parenterally, sexually and vertically transmitted. Moreover, HBV can be transmitted via mucosal exposure (blood, semen, vaginal secretions, and saliva) and during ART [20-22]. Several efficient strategies for prevention of HBV transmission are today available. The HBV vaccine, introduced in 1982, is the most important method of preventing the spread of HBV, both in the general population and among healthcare workers [22]. Good evidence is available supporting the administration, within 12 hours of birth, of HBV immunoglobulins and vaccine in infants born to HBsAg-positive patients, and an appropriate immunoprophylaxis allows breastfeeding of babies born to infected patients [11-23].

In the safe management of serodiscordant couples seeking pregnancy by ART, any treatment should be started only after vaccination of the negative partner, documented by the presence of anti-hepatitis B surface antibodies [1]. Sperm washing is not required if the female partner is immunized against HBV.

HBV infection in the ART setting is an important issue not only for the risk of transmission, but also for the possible effects on gamete competence. Several reports suggest an association between the presence of this virus and a poor reproductive prognosis both in natural and in assisted conception due to increased chromosomal instability in sperm, even without worsening of semen parameters. Sperm progressive motility and fertilization rate both seem to be significantly lower in patients affected by HBV. For these reasons, in serodiscordant couples with an HBV-positive male partner, a mixed (half ICSI, half IVF) approach could be proposed in the first ART cycle, with the aim of evaluating the possible impact of the virus on sperm competence and HBV could be also detected in the oo-

cytes or ovarian tissue. The impact of HBV on ART outcomes such as embryo development, implantation rate, and pregnancy rate are currently under investigation [24-27].

HIV

HIV is a retrovirus that depletes CD4+ T cells and is responsible for *acquired immune deficiency syndrome* (AIDS). HIV is characterized by a single-stranded RNA and by the presence of reverse transcriptase, an enzyme that allows RNA to be copied into DNA. HIV in fact uses a DNA "copy" to infect the host. The first step in the infection is fusion of HIV with the host cell. Then, the viral RNA is converted into DNA and the virus uses the host cell's machinery to replicate itself by reverse transcription. New copies of HIV leave the host cell and go on to infect other cells. There are nearly 37 million people living with HIV/AIDS around the world, the vast majority in developing countries, and more than half are women. Two-thirds of all people newly infected with HIV live in Sub-Saharan African countries, where three-quarters of all AIDS-linked deaths occur [28,29]. HIV is mainly transmitted via unprotected sexual intercourse and facilitated by infections and ulceration of the genital tract, trauma or bleeding during sexual practices, and absence of male circumcision [30]. HIV transmission is directly associated with peripheral blood viral load, with a lower risk in subjects with peripheral viral loads <10,000 copies/mL [31]. Interestingly, the seminal HIV viral load roughly correlates with the plasma one, but is much more variable and isolable. It should also be stressed that infected men with low or undetectable viral loads could have HIV copies in their seminal plasma [32-34].

In the ART field, several women were infected with HIV after donor insemination between 1980 and 1984, before strict donor screening and semen quarantine practices were implemented. Moreover, a significant reduction of the infection has been documented following sperm-wash via density gradient centrifugation and swim-up allowing separation of motile sperm from round cells and seminal plasma [35,36]. In the safe management of HIV serodiscordant couples with a positive male partner, there is good evidence supporting the efficacy of sperm-wash in significantly reducing the risk of infection. In addition, the non-infected partner must be checked periodically for HIV during the treatment and during any resulting pregnancy. Moreover, the storing of HIV-infected samples (i.e., semen, oocytes and embryos) in specially designed tanks is now a widespread practice, making it possible to significantly reduce the risk of viral transmission via liquid nitrogen. Finally, vertical transmission can be reduced thanks to the use of antiretroviral drugs during pregnancy and/or labor, elective cesarean section, and avoidance of breastfeeding. These measures can reduce the risk of HIV transmission to less than 2% [37-39].

Discussion

In developed countries, effective therapies have allowed lethal infections like HCV, HBV and HIV to be turned into chronic diseases associated with good life expectations, also

in terms of reproductive chances. For fertile serodiscordant couples, natural conception following specific prophylactic strategies should be encouraged, while infertile serodiscordant couples should be offered treatment with ART. In recent years, serodiscordant couples have increasingly turned to ART centers. Their desire for parenthood is identical to that of any other couple, even though their management must involve specific counseling and recommendations. Counseling is crucial to inform patients about the risk of sexual and vertical transmission of the infection; it should include information on safe sexual practices, such as the use of condoms during the infertility treatment and the pre- and post-pregnancy period, as well as guidance on appropriate lifestyle choices. Moreover, in serodiscordant couples, serial diagnostic testing of the uninfected partner is important, also during the first year following the child's birth. A clear and explicit informed consent process is essential, in which it must be made clear that the risk of transmission cannot be entirely eliminated, even when all the necessary precautions are taken and the best risk reduction strategies are implemented. Appropriate psychological, medical and obstetric assistance should ideally be provided by a multidisciplinary medical team, for the protection of patients, and also of the future unborn child.

In the management of serodiscordant couples seeking pregnancy by ART, is important to remember that a risk of viral contamination has been documented in ART clinics and blood banks. Even though this risk can be reduced, it cannot be entirely eliminated. For this reason, ART centers working with serodiscordant couples must set up an internal management and control system designed to guarantee the safety of operators and uninfected patients, and also of the fetus. Correct and safe management of serodiscordant couples should follow recommendations, such as the treatment of infected samples in a separate laboratory or in a designated space within the main laboratory to minimize the risk of cross-contamination. The risk of cross-contamination during ART is probably greatest during the cryopreservation step because viruses and other microorganisms can survive in liquid nitrogen. In order to protect the cryopreserved samples against the risk of cross-contamination, the use of separate tanks and sterile nitrogen is strongly recommended. Other possible strategies to avoid cross-contamination via liquid nitrogen are the use of "closed" freezing devices or the use of "double enveloping". Another possibility is to store cryopreserved samples in nitrogen vapor, although the literature is conflicting with regard to this approach as it has been demonstrated that contaminants persist in the vapor phase^[40-45].

An important aspect of ART in serodiscordant couples, recently discussed by Borges *et al.*^[46], is the cost of microbial contamination during infertility treatment. Although data on the economic impact of microbial contamination in the global ART industry are scarce, this impact is found to be considerable when estimated on the basis of the prevalence of contamination (about 0.7%, according to literature), the number of IVF cycles per year, and the cost of the procedure. For instance, of the approximately 285,000 IVF cycles performed in the USA in 2017, nearly 2000 cycles presented microbial contamination and a decline in cell quality, without successful embryo transplantation in most cases. This translates into a mean cost per

patient of US\$ 10,000 (according to current US market prices) and an overall cost of about US\$ 20 million per year, excluding the ancillary costs linked to couples who do not achieve pregnancy due to potentially undetectable contamination of embryo cultures. Similarly, in 2017, over 75,000 cycles of IVF were carried out in 119 licensed ART clinics across the UK where the cost of IVF is around £5000 (US\$ 6500) per cycle of treatment. Applying the same parameters used to estimate the cost of microbial contamination in the USA, the overall cost in the UK corresponded to about US\$ 3.4 million. In Brazil, each IVF cycle costs, on average, R\$ 15,000 (US\$ 4000). On the basis of the 36,370 IVF cycles reported in 2017 and the estimated 4.8% rate of microbial contamination reported in national registers, the total annual cost of microbiological contamination in ART laboratories and clinics reaches nearly R\$ 26.1 million (US\$ 7 million), a non-negligible cost for IVF clinics and patients.

Of greater concern, IVF failure has the potential to be an emotionally and financially exhausting experience for patients. This has enormous implications for couples of increasingly higher age, when compared to the mean age of the women undergoing ART procedures. Furthermore, longitudinal studies should be carried out to identify possible adverse effects of the presence of microorganisms in contact with embryos conceived *in vitro*, and the possible implications for adult life. Meanwhile, there is also a need to discuss new ways to improve or modify the preparation and cryopreservation of gametes. These discussions should be developed with a view to improving the safety of ART by reducing the impacts, both subtle and overt, of contamination in the embryo culture and cryopreservation steps.

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