

Male infection: cross-contamination risk management and impact on sperm parameters

Carlotta Zacà¹, Carolina Calesini², Elena Borini¹, Massimo Menegazzo³

¹IVIRMA Global Research Alliance, 9.Baby, Bologna, Italy; ²IVIRMA Global Research Alliance, Extra omnes, Cattolica (RN), Italy; ³Padua University Hospital UOC kidney and pancreas transplant company Regional Diabetes Cell Therapy Center, Padua, Italy

ABSTRACT

Chronic viral infections are considered a risk factor for male fertility given their ability to infect semen. Several studies have shown that semen parameters and gamete DNA integrity can be compromised by human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. These effects on male fertility are also associated with other types of infection, such as human papillomavirus. To date, European directives for viral screening of couples undergoing assisted reproduction treatment requires evaluation only for the three most common infections: HIV, HBV and HCV. In all these cases it is essential that all operators involved in the laboratory are aware of the risk to themselves and to the patients treated, and also of the risk associated with the possibility of cross-contamination with other patients. In IVF centres, specific procedures must be established and respected in the case of serodiscordant couples, in order to guarantee correct management and treatment of biological samples. We can reduce or minimise the risk of cross-contamination by applying specific laboratory protocols for the safe handling of gametes and embryos. This mini review aims to describe the impact of viral infection on sperm and male fertility and the protocols and recommendations for the management of gametes from serodiscordant couples with infectious diseases.

KEYWORDS

Serodiscordant, IVF, cross-contamination, procedures, male infertility, sexual transmission, sperm, viruses.

Introduction

Before starting treatments, assisted reproduction centres should implement effective measures to identify patients infected with blood-borne viruses (BBVs). In the past, systematic screening for human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) infection was performed only by some centres^[1,2]. To date, European Union directives for viral screening of couples undergoing *in vitro* fertilisation (IVF) treatment require that they be evaluated for the presence of the abovementioned infections^[3]. In recent years, the improvement of living conditions linked to the evolution of medical techniques and procedures for the treatment of viral diseases has led to an increased desire for parenthood among people with infections.

Reproductive care aims to minimise the transmission risk both to an un-infected partner and to the future child; this imposes the need for semen processing of men infected with HIV or HCV. Moreover, pre-treatment screening enables the identification of patients representing a risk to staff and other patients. It also minimises the risk of possible transmission in the operating theatre and laboratory environments.

Infected patients seeking treatment can be divided into two groups: i) those who have no fertility disorders and require procedures (i.e., sperm washing) aimed at reducing the risk of viral transmission; ii) infertile couples requiring assisted reproduction treatment (ART) in order to conceive.

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Contact

Carlotta Zacà; carlotta.zaca@9puntobaby.it
Embriologa clinica 9.Baby - Family and Fertility Center
Via Dante, 15 - 40125 Bologna, Italy – Phone: +39 051 2867511

Managing samples derived from virus-positive patients requires significant effort in applying laboratory safety procedures to prevent any form of contamination, especially in relation to the handling of embryos and gametes.

In this respect, team management is crucial; in fact, an employee training programme must be provided and continuously implemented in order to ensure that measures are always in place to safeguard the safety of gametes and embryos and minimise the risk of cross-contamination, even though it is known that the mere presence of measurable viral DNA or RNA is not sufficient to cause infection^[4]. Good communication among clinical staff with reference to procedures, trials, test results, precautions and care offered to the patient is also pivotal. A checklist of good laboratory practice to reduce transmission risk when handling gametes from infected patients was compiled by ESHRE in 2021^[5]. The ESHRE paper lists 78 recommendations on the prevention of viral transmission (horizontal and vertical) before, during and after medically assisted reproduction.

Relevant regulations should describe universal precautions

and personal protection equipment, how to minimise aerosol formation during sample processing and, possibly, how to decontaminate a working area ^[6].

Cross-contamination

Specific laboratory protocols have been developed to safely treat virus-positive patients requiring ART. The measures concern the handling of biological material representing a potential risk of transmission of the infection to staff, to other patients' biological material, or to the partner ^[5].

Infected sample as source of staff risk

The main risks to IVF staff are through needle puncture or splash injuries. HBV is a well-recognized occupational risk for all health care operators involved in handling blood, body fluids and clinical waste, although exposure risk has been significantly reduced since the introduction of a vaccine. Unfortunately, on the contrary, there are no effective vaccines for HIV or HCV. It is well known that the cross-contamination risk in the IVF laboratory setting can be minimised by using universal precautions when handling infected samples. This involves handling with latex gloves, using eye protection and, where possible, avoiding sharps. The Centres for Disease Control and Prevention ^[7] recommend universal precautions, i.e., handling of all specimens as if they were hazardous, and that these precautions be applied in all laboratories and in all patients ^[8].

Clearly, sanitisation and sterilisation carried out routinely in all assisted reproduction laboratories, irrespective of the viral status of the patient, is a primary measure. However, an IVF laboratory is a complex structure where all components are designed and used to promote adequate conditions for cell survival and culture. Unfortunately, the same conditions also facilitate viral and bacterial growth.

There are specific procedures designed to ensure personnel safety and prevent cross-contamination:

- Vaccination of all personnel against hepatitis B, and other viral diseases for which vaccines are available, is highly recommended.
- Patients must be rigorously screened for infectious diseases.
- Staff must be promptly informed of a scheduled treatment of a viral-positive patient and made aware of the risks of handling infected biological material.
- Procedures should be implemented to manage situations where infection might occur.
- Scheduling full-blown infected patients at the end of a working session of the operating theatre is highly recommended, thus allowing the necessary time for a thorough disinfection of allocated areas and equipment.

Infected samples as source of patient risk

One of the main concerns in managing infected samples in assisted reproduction is the cross-contamination risk to un-

infected samples. Although relative numbers are small, samples from HCV- and HIV-positive women clearly represent a cross-contamination risk in the laboratory setting. Although all follicular fluid samples should be handled as potentially infected and universal precautions should be adopted at all times, the risk to uninfected samples is inevitably higher when samples known to be infected are handled within the same laboratory area. Patients may be exposed to risk if specific protocols are not designed and adopted to prevent cross-contamination of samples. Unsafe conditions and procedures include lack of information, improper labelling of containers, inadequate sanitisation between patients, improper handling of body fluids, and use of blood products contaminated with infectious agents.

Cross-contamination between infected and uninfected patients and samples can potentially occur during oocyte retrieval and embryo transfer as well as during subsequent laboratory procedures such as insemination, injection, incubation and cryopreservation ^[9]. According to the American Society for Reproductive Medicine (ASRM) guidelines on management of the risk of viral transmission during fertility treatments, samples from infected and non-infected patients need to be treated separately ^[10].

The European Society of Human Reproduction and Embryology (ESHRE) Committee of the Special Interest Group on Embryology published guidelines for good practice in IVF laboratories ^[11] and recommended treatments for patients infected with HIV, HBV or HCV ^[5]. The guideline indications include the use of dedicated laboratory space, and processing using disposable instruments within a separate biosafety cabinet for every type of viral infection to prevent cross-contamination of patient specimens and also contamination of healthy samples ^[5].

Recommendations already exist for handling gametes exposed to viral infection in the IVF laboratory.

- Consider scheduling patient treatment at the end of the daily workflow so as to isolate the patient in time.
- Use separate, dedicated instruments and equipment and consider culturing the gametes and embryos in a separate incubator so as to isolate the patient in space.
- Use disposable contact materials and, in particular, use disposable counting chambers for semen.
- At the time of oocyte retrieval, the virus may be present in follicular fluid and may adhere to granulosa cells surrounding the oocyte, hence the need to denude oocytes completely and inseminate via intracytoplasmic sperm injection (ICSI). Remove granulosa and cumulus cells and wash oocytes thoroughly in fresh medium drops to dilute the viral load.
- Semen treatment consists of a modified sperm washing step, separation of sperm from leukocytes and other seminal constituents by centrifugation over a gradient, and separation of motile sperm from the resulting pellet. This final step is achieved by a swim-up step in which washed sperm are moved to a clean tube and allowed to migrate into an overlay of fresh medium.
- Oocytes and embryos are cultured in mini-styled incubators with separate case-specific compartments. This strategy decreases any risk of cross-contamination.
- It is important to wipe down surfaces with 6% hydrogen peroxide followed by distilled water.

- It has been experimentally demonstrated that cross-contamination between liquid nitrogen (LN₂) and embryos may occur when infectious agents are present in LN₂ and oocytes/embryos are not protected by a hermetically sealed device [112]. Since HIV, HCV, HBV and possibly other viruses can survive in LN₂, treat and cryopreserve the samples in heat-sealed cryopreservation straws to prevent direct contact of cryo-containers with LN₂. Sanitisation of instruments to restore standard operating conditions and separation of frozen storage according to virus (HIV, HBV, HCV) is mandatory.

Cross-infection between partners

IVF procedures must aim to reduce infection of patients. Modified sperm washing protocols are available for the treatment of infected patients. After treatment and before cryopreservation, if possible, viral screening is recommended [110].

Viral infection: impact on male fertility, treatment protocols and recommendation

It is known that chronic viral infections can infect sperm and are considered a risk factor in male infertility [113]. Recent studies have demonstrated that the presence of BBVs in semen impairs DNA integrity and other sperm parameters. In fact, infections can result in temporary or permanent infertility, impairing hormone levels, testicular function and spermatogenesis [114].

HIV

Impact on male fertility

HIV RNA and leukocyte antigens are detected in the testis, epididymis, prostate and seminal vesicles [115]. Patients infected with HIV-1 can develop chronic orchitis and, consequently, progressive hypergonadotropic hypogonadism, which suggests that testicular steroidogenesis is impaired [116,117].

Sexual transmission plays a major role in the spread of HIV-1. The semen of infected men may contain high levels of HIV-1, and virus can be recovered from seminal cells or seminal fluid. Seminal cells are mixtures of spermatozoa, precursors of germ cells, T lymphocytes, macrophages and epithelial cells. HIV-1 proviral DNA has been detected in several of these cell types, mainly lymphocytes, monocytes and macrophages [118].

In vitro studies have demonstrated that HIV attaches to spermatozoa through heparin sulphate proteoglycans [118] but there is no evidence of the virus's transmission by sperm to the foetus (vertical transmission).

HIV treatment aims to suppress viral replication and maintain plasma HIV-1 below detection levels with antiretroviral therapy. Antiretroviral therapy, however, has gonadotoxic effects that are difficult to distinguish from the direct impact that HIV-1 has on male fertility. The latter can affect sperm parameter values and lead to decreased total sperm count, decreased progressive motility, and an increase in sperm counts with abnormal morphology [119].

Protocols and recommendation

If the male partner is HIV positive, and patients are good candidates for intrauterine insemination (IUI), HIV viremia should be minimised (peripheral blood viral load less than 10,000 copies/mL) through the use of highly active antiretroviral therapy, to reduce levels of HIV in semen, followed by a special washing of sperm, which is a safe, cost-effective and widely used treatment method [120].

An efficient sperm washing procedure was proposed by Semprini in 1992 [121]. It appears to be a safe and effective method for achieving pregnancy in HIV-discordant couples in which the man is HIV infected. The procedure consists of:

1. initial dilution of the seminal fluid 1:2
2. 30 minutes centrifugation (1600 RPM) with silica-based discontinuous density gradient, which allows infected leukocytes to be eliminated
3. double washing followed by a second centrifugation
4. 1h sperm swim-up at 37°C with 5% CO₂

A systematic review and meta-analysis published in 2016 [122] demonstrated that this sperm washing protocol appears to significantly reduce the risk of transmission in HIV-discordant couples requiring ART, regardless of viral suppression in the male partner. In fact, no HIV transmission occurred in 11,585 cycles of assisted reproduction using washed semen among 3,994 women.

Cumulative evidence suggests that ART is safe and effective for avoiding horizontal and vertical transmission in HIV serodiscordant couples [123].

HBV

Impact on male fertility

During HBV infection, the virus can be found in many secretions, including semen, and in other tissues beyond the liver and blood [124,125]. HBV is able to not only pass through the blood-testis barrier and enter the male germ cells but also integrate into their genomes [126].

Current data show an increased incidence and risk of infertility in men infected with HBV. Moreover, they showed that exposure to hepatitis B virus S protein induced oxidative stress in sperm cells up to the stage of apoptosis, as revealed by phosphatidylserine externalisation, caspase activation, and DNA fragmentation. In view of these results, it is possible to conclude that HBV-infected males have significantly impaired sperm quality compared with that of control men [127].

HBV can be vertically transmitted, and spermatozoa transfected with HBV are susceptible to apoptosis and have reduced fertilisation capacity [128].

The prevalence of virus shedding in semen was found to be around 68% in a population of chronically HBV-infected men [129].

Protocols and recommendation

In couples with a male partner infected by HBV, if the female partner is vaccinated against HBV, sperm washing is considered unnecessary for preventing the risk of sexual transmission. Therefore, ART should be postponed until the couple has re-

ceived the necessary prevention measures, i.e. vaccination ^[10]. If the female partner is HBV-positive, the couple must be informed of the need for specific vaccination of their newborn ^[30].

HCV

Impact on male fertility

HCV infection is associated with alterations in semen quality, including decreased sperm count, reduced motility, and abnormal sperm morphology ^[24,31].

It is noteworthy that treatment with ribavirin and interferon further worsened semen quality in HCV-infected patients ^[32]. In chronically HCV-infected men, viral replication is associated with worsening of all sperm functional tests; this worsening includes high levels of seminal ROS and high sperm DNA fragmentation.

Protocols and recommendation

Unlike HBV, there is no vaccine against HCV. Therefore, it is essential to employ risk-reduction measures during assisted reproduction involving affected patients.

For HCV-positive males with detectable viral load in serum, the density gradient with swim-up protocol ^[33,34] can be used before IUI or ICSI to reduce the viral concentration in the final sperm sample.

The infected partner can be treated with peginterferon alfa and ribavirin to reduce viral load before assisted reproduction treatment ^[35]. It is recommended that this treatment be administered for 48 weeks.

During this period, two forms of contraception should be used followed by a six-month washout period to reduce the teratogenic and embryotoxic risks of ribavirin, a pregnancy category X drug.

There is a small, but measurable, risk of HCV transmission via semen. When the male partner is HCV infected, sperm washing can reduce the viral load in semen. It is also recommended to reduce the risk of transmission to his partner. Using IVF with ICSI has also been demonstrated to reduce the risk of transmission of HCV when the male is seropositive ^[34].

As described elsewhere ^[36] ejaculates obtained after a sexual abstinence of 3-5 days are allowed to liquefy, and then diluted 1:1 (vol:vol) with sperm medium.

They are then pelleted at 400 g for 10 min, and the supernatants are discarded. A volume of sperm medium equal to the initial volume of semen is added, and then layered onto a triple concentration gradient (90, 70 and 45%, PureSperm; Nidacon, Sweden) of 1-1.2ml of each layer, and centrifuged 20 min at 300 g. Each pellet thereby obtained is then washed with sperm medium and re-pelleted again. Supernatants are discarded and another swim-up of 0.5-0.7ml is executed. After 45 min, the upper 0.35 ml of each tube supernatant is collected, pooled and split into two samples.

One half is immediately plunged into liquid nitrogen for polymerase chain reaction (PCR) determinations, and the other half is frozen with sperm freezing medium, according to the manufacturer's instructions, and finally stored until use, following a negative result for viral presence ^[36].

HPV

Impact on male fertility

HPV is a non-enveloped, double-stranded circular DNA virus, a member of the *Papillomaviridae* family ^[37].

It is the most common sexually transmitted virus in humans, with over 80% of the sexually active adults being infected by one HPV type at least once in their lifetime.

Human papillomavirus is frequently detected in semen and urethral swabs from asymptomatic men. In the past, HPV semen infection was always considered transient and without clinical consequences. In a recent study, nested PCR showed the presence of HPV DNA sequences in 10% of semen samples from asymptomatic young adult males who had had unprotected intercourse ^[38].

HPV can be localised at different levels: in sperm, in exfoliated cells or in both sites. Curiously, infertile patients had both a prevalent infection in sperm and a higher percentage of infected sperm, while exfoliated cells were significantly more affected in patients with other risk factors. In fertile control subjects HPV infection was never found in sperm cells ^[39].

The proposed mechanisms underlying male infertility associated with HPV infection include direct modification of semen quality, damage to sperm DNA integrity, and production of anti-sperm antibodies that can interfere with sperm motility and sperm-oocyte binding ^[40].

The hypothesis of a direct viral effect on sperm is substantiated by the findings of high sperm DNA fragmentation rates and decreased progressive sperm motility in infertile men with asymptomatic chronic high-risk HPV infection ^[41].

Overall, some questions remain unanswered and many studies are required to clarify the mechanisms underlying the effects of HPV in both the female and the male reproductive system, and to determine the role of HPV persistence in fertility alterations. It is also known that oncogenic or high-risk HPV plays a significant role in the development of vulvar, vaginal, penile, anal and oropharyngeal cancers.

Protocols and recommendation

Human papillomavirus infection has been detected in the sperm of a large percentage of sexually active males. Standard sperm selection techniques are ineffective in removing HPV. The application of an efficient swim-up procedure is therefore recommended. Garolla *et al.* ^[42] suggested a "direct" swim-up method, which reduced the number of HPV-infected sperm by ~24% ($p < 0.01$), and a "modified" swim-up procedure, which is able to remove HPV DNA completely from both naturally and artificially infected sperm. In this modified protocol, enzymatic treatment with heparinase-III coincided with a decrease in sperm motility, sperm viability and DNA integrity, but these effects were not statistically significant. Unfortunately, heparinase-III is not licensed for assisted reproduction in humans. In a study published by the same group in 2021 ^[43], the authors tested the possibility of using hyaluronidase as an innovative method for removing HPV virions bound to sperm cells before proceeding with ART ^[44]. The experimental treatment is associated with complete loss of HPV-VLP in association with a reduction of sperm viability and progressive motility.

The sperm sample is first incubated for 1 h at 37°C in fresh SWM with HPV at the ratio of 1 mg HPV-VLP per 1×10^6 spermatozoa per mL. After the incubation the sample is incubated with hyaluronidase at the concentration of 80 IU/mL at 37°C for 30 min.

After this second incubation sperm is washed in order to eliminate excess of reagents and processed with the standard swim-up procedure.

HPV virions bound to sperm cells are detected by immunofluorescence. Using the 80 IU/mL concentration, total removal of HPV virions was observed without any alterations of cell morphology, DNA integrity, capacitation and acrosome reaction according to 2010 WHO criteria^[43,44].

Conclusion

Semen as a vector for sexual and vertical viral transmission must be studied further; nevertheless, the mere presence of viral nucleic acids in semen does not indicate infection. Further research should be aimed at detecting accurate viable viral loads and particles in semen capable of causing disease.

It is essential that all operators are aware of the risk of cross-contamination, not only between serodiscordant partners but also between patients and operators. To ensure adequate safety measures, infected patients should be treated only in IVF laboratories with dedicated areas and equipment. Alternatively, the treatment of infected patients must be carried out in defined working time frames, provided that, following the procedures, the areas and equipment are fully disinfected. In IVF centres specific procedures must be established and followed for serodiscordant couples, to ensure correct management and treatment of biological samples. Samples should, in any case, always be considered and treated as potentially contaminated. Patients and their gametes/embryos properly treated for HIV and HCV pose no infection risk to their partners or other patients undergoing assisted reproduction. Laboratories can simply and effectively mitigate risk from individuals with active blood-borne viral infection by individual preparation and treatment of gametes.

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