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RBMO

REVIEW

Fertility preservation during the COVID-19 pandemic: mitigating the viral contamination risk to reproductive cells in cryostorage

BIOGRAPHY

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KEY MESSAGE

This review addresses available literature on the presence of SARS-CoV-2 on tissues, gametes and embryos, with special reference to possible sources of cross-contamination through liquid nitrogen. Strategies for risk-mitigation are extrapolated from reports on other viruses to the current global crisis for safety in fertility treatment services, specifically oncofertility.

ABSTRACT

Reopening fertility care services across the world in the midst of a pandemic brings with it numerous concerns that need immediate addressing, such as the impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on the male and female reproductive cells and the plausible risk of cross-contamination and transmission. Due to the novelty of the disease the literature contains few reports confirming an association of SARS-CoV-2 with reproductive tissues, gametes and embryos. Cryobanking, an essential service in fertility preservation, carries the risk of cross-contamination through cryogenic medium and thus calls for risk-mitigation strategies. This review aims to address the available literature on the presence of SARS-CoV-2 on tissues, gametes and embryos, with special reference to the possible sources of cross-contamination through liquid nitrogen. Strategies for risk mitigation have been extrapolated from reports dealing with other viruses to the current global crisis, for safety in fertility treatment services in general, and specifically for oncofertility.

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https://doi.org/10.1016/j.rbmo.2020.09.013 1472-6483/
Declaration: The authors report no financial or commercial conflicts of interest

KEY WORDS

COVID-19
Cross-contamination
Cryostorage risks
Fertility preservation
SARS-CoV-2

INTRODUCTION

The current pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has focused the attention of healthcare service providers across the globe away from all other non-emergency health problems, including fertility (*ESHRE, 2020a*; *Tesarik, 2020*). The recent guidelines from the American Society for Reproductive Medicine (ASRM) state that the window of opportunity for infertile couples is finite and postponing treatment for too long could reduce the chances of pregnancy (*ASRM, 2020a*). Importantly, individuals needing fertility preservation due to cancer or other conditions that require gonadotoxic treatment need urgent procedures to store their reproductive cells. With fertility services slowly resuming globally (*ESHRE 2020b*), the aim of this review is to formulate a better understanding of the impact of SARS-CoV-2 on male and female gametes and embryos, and determine the risks involved with cross-contamination of viral pathogens such as SARS-CoV-2 during cryostorage in liquid nitrogen (LN2). This review also highlights strategies to mitigate the risks through safety and precautionary measures.

SARS-COV-2 IN REPRODUCTION

Due to the novelty of the COVID-19-causing virus, SARS-CoV-2, few reports are available documenting the detection of this virus on male and female reproductive cells or tissues (*Corona et al., 2020*; *Jing et al., 2020*). SARS-CoV-2 belongs to the Coronavirus family, and as its genome sequence is 82% identical to that of SARS-CoV (*Chan et al., 2020*), the findings from the SARS-CoV family subtype could help in understanding the pathophysiology of SARS-CoV-2 on reproductive cells (*Segars et al., 2020*).

Important structural commonalities between SARS-CoV-2 and SARS-CoV have been recently demonstrated with respect to receptor binding domains (*Lu et al., 2020*) and the mechanism of cell entry (*Hoffmann et al., 2020*). Both viruses use the spike glycoprotein for entry into target cells. Entry into the host cells depends on binding of the virus to angiotensin-converting enzyme 2 (*ACE2*) and occurs after priming of the spike glycoprotein by host cell transmembrane protease, serine 2 (TMPRSS2; *Hoffmann et al., 2020*). As human germ cells and early embryos express high levels of ACE2, there is a potential risk of SARS-CoV-2 being associated with reproductive cells. In male individuals, ACE2 has been shown to be expressed on spermatogonia, Leydig cells and Sertoli cells. Gene set enrichment analysis has shown that gene ontology categories associated with viral replication and transmission are markedly expressed in these cells, lending support to the hypothesis that the human testicle could represent a potential target for SARS-CoV-2 infection (*Wang and Xu, 2020*). Currently, about 27 viruses over a wide range of families have been found in the human semen and, with Zika, Ebola and Marburg viruses, replicating viruses have been demonstrated in the semen and can be sexually transmitted (*Salam and Horby, 2017*). An indication that SARS-CoV, closely related to SARS-CoV-2, may affect the male reproductive system by causing orchitis was demonstrated in six individuals; however, in-situ hybridization did not detect SARS-CoV genomic sequences in the testes, suggesting that the pathology may represent a complication of SARS-CoV infection (*Xu and colleagues 2006*).

As SARS-CoV-2 has been found in the blood of 1% of symptomatic patients (*Wang et al., 2020*), the possibility of male reproductive tract infections, especially in patients with systemic or local inflammation compromising the blood–testes/deferens/epididymis barriers, remains a concern. Until now the presence of SARS-CoV-2 in human semen has been controversial as the few studies performed have reported conflicting data. Li and colleagues (*Li et al., 2020*) reported the presence of the virus in the semen of 26.7% patients in the acute phase of infection and 8.7% of recovering patients. However, the study included only sparse detailed about procedures adopted to avoid contamination of semen samples, and proof of the real infectivity of the virus detected in the semen is lacking. Conversely, Pan and colleagues (*Pan et al., 2020*) failed to detect the virus through reverse transcription (RT-)PCR in the semen of 34 patients diagnosed with COVID-19 based on clinical symptoms and confirmed with quantitative RT-PCR when patients generally demonstrated milder symptoms. It is noteworthy that semen samples were collected about 1 month after diagnosis of COVID-19 positivity, i.e. possibly after clearance of the virus from the patients' bodies. Similarly, Paoli and co-workers (*Paoli et al., 2020*) failed to detect the presence of the virus in urine and semen of one patient 8 days after SARS-CoV-2 PCR detection but 1 day before a further negative pharyngeal swab.

Other recent studies (*Guo et al., 2020*; *Song et al., 2020*) have also not detected SARS-CoV-2 RNA in semen samples in patients with recent infection or recovering, or in a testicular sample of one patient who died of COVID-19. However, Holtmann and colleagues (*Holtmann et al.(2020)* have reported impairment of semen parameters in patients with moderate SARS-CoV-2 infection even though the virus was not detected in the semen, but whether this impairment was due to the infection itself or the associated treatment was not determined. Overall, due to the limited number of participants analysed, the stage of infection at the time of semen sampling and conflicting evidence, the possibility that SARS-CoV-2 is present in the semen of infected patients cannot be completely ruled out, especially in asymptomatic cases (*Kashi, 2020*).

From the available literature it appears that the female reproductive tract is less impacted by SARS-CoV-2 than the male (*Segars et al., 2020*) as SARS-CoV has not been demonstrated in the ovaries and uterine tissues by immunohistochemistry and in-situ hybridization studies (*Ding et al., 2004*), eve though ACE2 is widely expressed in the ovary (*Jing et al., 2020*), uterus and vagina (*Vaz-Silva et al., 2009*). The presence of ACE2 indicates the female reproductive organs as potential targets for SARS-CoV-2 infection; however, no evidence of infection or sexual transmission has been reported to date (*Cui et al., 2020*).

Gametes obtained from patients with other viral illnesses, such as human immunodeficiency virus (HIV) infection and hepatitis, must be treated with special precautions to reduce exposure of the non-infected partner and cross-contamination of reproductive tissue within the laboratory (*ASRM 2013*). It is yet to be seen whether these precautions should currently be recommended for SARS-CoV-2, given the insufficient

evidence for transmission through blood or sexual contact (*Chang et al., 2020*; *Cui et al., 2020*). Although there is no universal recommendation for screening oocyte or semen donors for SARS-CoV-2, ASRM recommends screening questions for asymptomatic donors, to avoid potential infections (*ASRM, 2020b*). These are areas in which further investigation is necessary to assure the safety of stored gametes and the safety of patients undergoing assisted reproduction.

FERTILITY PRESERVATION DURING COVID-19

With the emerging global and regional success of oncofertility (*Ataman et al., 2016*; *Rashedi et al., 2020*), and the American Society of Clinical Oncology recommending guidelines to offer fertility preservation services to oncological patients (*Oktay et al., 2018*), many young and adult cancer patients preserve their spermatozoa, testicular tissue, ovarian tissue, oocytes or embryos for a fertile future. Multiple cryopreservation protocols using various cryoprotectants have been developed for each of the reproductive materials to ensure long-term safe storage and effective recovery while also sustaining fertility potential (*Rodriguez-Walberg et al., 2019*). The reproductive materials are cryopreserved in either open or closed cryodevices. Cryoprotected cells are then plunged into LN2, which has the ability to maintain an ultra-low temperature of –196°C (*Joaqium et al., 2017*), enabling prolonged conservation of the reproductive material.

Although elective procedures using ART are being preferably cancelled or postponed during this pandemic, fertility preservation is an emergency requirement even in limited resource settings, as cancer treatment Should not ideally be delayed (*Salama et al., 2020a*). However, there are concerns about risks of cross-contamination during cryostorage and reintroduction of virus when patients need fertility restoration.

VIRAL CROSS-CONTAMINATION IN CRYOTANKS

Cryostorage, while being beneficial, also carries multiple concerns pertaining to contamination of LN2, leading to transmission of infectious diseases between samples (*Tomlinson, 2005*) or transmission of infectious disease to the patient themself years later during fertility restoration. Although the presence of SARS-CoV-2 in semen and female reproductive fluids is still under debate, a relevant risk in cryopreservation is the potential transmission of pathogens during preparation procedures and cryostorage. Noticeably, the transmission of pathogens in samples stored in fertility cryobanks has never been reported (*Yakass and Woodward, 2020*), but concerns of cross-contamination among cryopreserved samples arose after a report of human hepatitis virus transmission from bone marrow transplants stored in the same LN2 tank (*Tedder et al., 1995*).

One of the reasons for the cryo-resistance of viruses could be the use of specific cryoprotectants to protect the germplasm during the process of freeze-thawing; this might also confer protection on the enveloped viruses, leading to transmission of the pathogen to the other stored biomaterials (*Alikani, 2018*; *Hubalek, 2003*; *Bielanski et al. 2000*; *Bielanski et al. 2009*). Reports of viruses such as HIV, hepatitis, influenza virus and papillomavirus retaining their infectivity after cryopreservation has brought a realization that exposure of LN2 to the virus can be a biohazard leading to cross-contamination (*Bielanski, 2012*; *Byers, 1999*; *Merrill et al., 2018*; *Schaefer et al., 1976*; *Tedder et al., 1995*).

Results of studies on viral cross-contamination in ART cryobanks have been controversial. Bielanski and colleagues (*Bielanski et al., 2000*) reported an absence of transmission of bovine viral diarrhoea virus and bovine herpesvirus 1 from infected semen and embryo straws to non-infected samples stored in the same LN2 tanks, but cross-contamination to frozen embryos was seen during experimental contamination of LN2 with the same viruses. On the other hand, Cobo and colleagues (*Cobo et al., 2012*), failed to detect the presence of viral RNA or DNA sequences in LN2 used for oocyte or embryo vitrification in patients seropositive for HIV, hepatitis C virus, and hepatitis B virus undergoing ART cycles.

While repeated washing could effectively eliminate the viral pathogens (ASRM, 2020), the possibility that viruses could cross the zona pellucida is a major concern. The zona pellucida is a glycoprotein envelope that acts as a physical barrier against microorganisms and might protect oocytes and embryos from viral infection when it is intact (*Van Soom et al., 2010*). As there is no evidence of SARS-CoV-2 infection of oocytes and embryos, studies reporting the ability of other viruses to permeate the zona pellucida should be taken into consideration. Silva-Frade and colleagues (*Silva-Frade et al., 2010*) localized bovine herpesvirus type 5, and Queiroz-Castro and colleagues (*Queiroz-Castro et al., 2019*) identified bovine herpesvirus 1, within bovine oocytes, demonstrating that the zona pellucida was an ineffective barrier against such viral infections. During certain ART procedures, the continuity of the zona pellucida could be accidentally or intentionally breached (intracytoplasmic sperm injection, embryo biopsy, assisted hatching), representing an additional concern considering that human preimplantation embryos highly express ACE2, which is required by SARS-CoV-2 for cell entry (*Yan et al., 2013*).

From a clinical perspective, patients can be reassured that their gametes are not infected by SARS-CoV-2 and can therefore be cryopreserved, but SARS-CoV-2 testing should be made mandatory for all individuals undergoing fertility preservation (*Dellino et al., 2020*). Apart from contaminated clinical samples, there is a substantial risk of iatrogenic infection of the samples in the laboratory from the operators. It should be noted that the SARS-CoV-2 particles could be present during cryopreservation and might survive after thawing as viruses are usually resistant to the freeze-thaw process (*ASRM 2020a*). This warrants the need for guidelines in safe handling and cryopreservation of biomaterials during fertility preservation.

RISK MITIGATION DURING CRYOSTORAGE

Several reproductive societies and clinical groups have provided recommendations and guidelines on the management of ART patients during the current SARS-CoV-2 global pandemic (*Alteri et al., 2020*; *Dellino et al., 2020*; *ESHRE, 2020b*; *Huyser, 2014*; *SART, 2020*; *Vaiarelli et al., 2020*). The European Society for Human Reproduction and Embryology (ESHRE) and ASRM recommend testing both the partners for SARS-Cov-2 before initiating ART

treatment (*ASRM, 2020a*; *ESHRE, 2020b*). Esteves and colleagues (*Esteves et al., 2020*) have proposed remedies that include identifying oncology patients who can neither delay the treatment nor experience infertility after gonadotoxic therapy and giving them priority for fertility preservation after testing for SARS-CoV-2 (*Esteves et al., 2020*).

Cross-contamination during cryostorage possibly arises from the type of cryopreservation device used, for example an open or closed cryosystem. In a closed device, such as sealed straws, the reproductive material is not directly exposed to LN2, thus reducing the hazard of cross-contamination (*Shapiro et al., 2020*). However, the use of screw-capped plastic vials can lead to contact with surrounding LN2 by creation of a vacuum in the vial due to condensation of the atmosphere at such a low temperature, which draws in the LN2 (*Woods and Thirumala, 2011*).

Several closed carrier systems have been employed for cryopreservation; these include the microvolume air cooling device (*Punyawai et al., 2015*), high-security vitrification straw or CryoTip, Fujifil Irvine Scientific, USA, some of which have been used for human embryo cryopreservation, hermetically isolating the reproductive cells (*Abdel-Hafez et al., 2011*; *Arav, 2020*; *Kuwayama et al., 2005*). Hence, ESHRE in its latest guidelines recommends the use of high-security straws and vapour-phase storage for cryopreserving reproductive samples from COVID-19-positive patients (*ESHRE, 2020b*). To avoid contamination while using open carrier systems during vitrification, Arav and colleagues (*Arav et al., 2016*) have devised a bench-top device to produce cooled clean liquid air that has a similar temperature to that of LN2. It may also be safe to use a secondary enclosure for cryodevices or to store samples from patients who are infected, or suspected to be, in separate LN2 cryotanks to avoid disease transmission via contaminated LN2 (*Bielanski et al., 2009*). Sharing of LN2 between patient samples during cryopreservation is not advisable (*Pomeroy and Schiewe, 2020*). LN2 has been used as an efficient cryogenic medium but, considering the risk of cross-contamination, vapour-phase nitrogen has been shown to be a safer alternative (*Abdel-Hafez et al., 2011*; *ASRM 2020c*), although the risk cannot be negated (*Grout and Morris, 2009*). Vapour-phase nitrogen storage appears to be practical, especially for unwashed semen samples or those awaiting viral test results (*Schiewe et al., 2019*).

While taking these precautions it is also important to ensure the sterility of LN2 (ASRM 2020; *Bielanski et al., 2009*; *Larman et al., 2014*), using certain devices (http://www.freepatentsonline.com/5737926.html), especially with regard to viral pathogens whose route of transmission is different from those of blood-borne viral pathogens. Small volumes of LN2 required for vitrification can be filtered using 0.2 µm filters (*McBurnie and Bardo, 2002*), but its efficacy for eliminating virus such as SARS-COV-2 remains to be proved. Periodic disinfection of the cryotanks and tools used during cryopreservation, by ultraviolet exposure or chemical disinfectants, is recommended to reduce cross-contamination (*Bielanski et al., 2009*; *Larman et al., 2014*). Sterilization of LN2 has been reported by Vajta and colleagues (*Vajta et al., 1998*), who performed cooling in LN2 that had been filtered through 0.2 µm pore size disposable filters to eliminate contaminants, and by Parmegiani and co-workers (*Parmegiani et al., 2009*), via emission of a minimum ultraviolet radiation dose in a temperature-controlled manner over a short interval for effective sterilization. Care should be taken, however, during cleaning that stored reproductive samples are not put at risk during removal from the cryotank (*Tao et al., 2020*). Although no studies have yet been reported on the presence of SARS-Cov-2 in commercially produced medical-purpose LN2, contamination risks should be considered.

WASHING PROCEDURES TO ELIMINATE PATHOGENS

Permegiani and colleagues (*Permegiani et al., 2012*) have reported a safe three-step washing procedure with sterile LN2 to eliminate pathogens from human cryopreserved specimens to minimize the risk of contamination; however, as this was only studied for bacteria and fungi, its effectiveness on viral pathogens is yet to be seen. Nevertheless, it may be advisable to follow this procedure in the current scenario to eliminate viral pathogens (*ASRM, 2020a*). In addition, repeated and efficient washing of gametes and embryos before cryopreservation and after thawing can reduce infectivity by high dilution of the viral particles (*ASRM 2020a*; *Bielanski, 2009*). Such washing procedures dilute the infective agents to far below the threshold concentration required for causing a clinical infection, to an infection probability of <0.0002% even with open vitrification systems (*Vajta et al., 2015*); however, measures should be taken to prevent even such a low probability. Sperm-washing procedures such as double-density gradient followed by swim-up has been shown to separate motile spermatozoa free of viral particles in men infected with HIV or hepatitis C virus, and such sperm-wash procedures could be used for other viral infections (*ASRM, 2013*).

Based on the above discussion, implementing the following cross-contamination risk-mitigation strategies may ensure safety for patients, personnel and laboratories offering cryo-facilities:

- Periodic disinfection of cryocontainers or ensuring the sterility of factory-derived LN2 (*Bielanski and Vajta, 2009*).
- Storage of infected samples and samples suspected to be infected in separate cryo-containers away from samples from other healthy patients (*Bielanski and Vajta, 2009*).
- Use of closed carrier devices during cryopreservation to prevent direct contact with LN2, or 'double-bagging' of cryodevices (*Bielanski and Vajta, 2009*).
- Storage of samples in vapour-phase nitrogen instead of LN2 to minimize risk for unwashed semen samples or those awaiting viral test results (*Schiewe et al., 2019*)
- Repeated washing of gametes/embryos before cryopreservation and after thawing to dilute any virus present and therefore lessen infectivity (*ASRM, 2020a*).

SAFETY ISSUES

While the above stated precautions can minimize the risk of cross-contamination, thereby ensuring patient safety, the safety of the personnel working in the laboratory is equally important (*Vajta et al., 2013*). In many embryology laboratories across the world, standard precautions are not regularly followed while handling LN2 (*Vajta et al., 2013*), proving to be a health hazard

for the laboratory personnel. It has been demonstrated that aerosol mist particles 1–5 µm in size are generated near the LN2 surface, up to a distance of 10–20 cm, which could be due to evaporation and floating of the particles (*Lee, 2020*). This could pose a risk to personnel who are handling contaminated LN2 containers. Hence, it is important for personnel in cryo-units to use cryo-accessories such as safety goggles or face visors and protective clothing (*Vajta et al., 2013*) while handling cryo-samples and containers, to ensure safety and efficiency.

CONCLUSION

With fertility services resuming across the world, it is important for healthcare providers to be aware of the impact of SARS-CoV-2 on male and female reproductive cells and tissues, and of the risk of cross-contamination and transmission through cryobanking services. Although there is controversial evidence on the presence of SARS-CoV-2 in seminal plasma from COVID-19-infected or recovering patients, there are so far no reports demonstrating its presence in female reproductive cells and tissues. The current COVID-19 scenario is expected to last for at least several months to a year, with rebounds expected to occur (*ASRM, 2020a*); therefore, new strategies have to be adopted when offering fertility services, especially pertaining to cryopreservation, to combat COVID-19.

Cryobanking using LN2 carries the risk of cross-contamination by viral pathogens leading to disease transmission, so it is important to understand and mitigate such risks through safety and precautionary measures. Some of the measures include testing both partners for SARS-CoV-2 before initiating treatment, use of closed-carrier cryodevices, sanitary cryostorage protocols and efficient washing of gametes or embryos during cryopreservation, which will help in reducing the risk of disease transmission. However, the feasibility of adopting these strategies, especially in developing countries, with limited resources and already existing challenges to support oncofertility programmes, would require a strong global network that enables the sharing of resources, methodologies and experiences to build competency (*Salama et al., 2020b*).

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Received 6 July 2020; received in revised form 9 September 2020; accepted 9 September 2020.