SCIENTIFIC PUBLICATIONS CONCERNING THE RISKS OF CONTAMINATION IN IVF LABORATORIES

Anifandis et al, 2021 : SARS-CoV-2 vs. human gametes, embryos and cryopreservation

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, is an unprecedented global situation, and all countries have adopted their own measurements to mitigate the spread of the virus in the first as well as in the subsequent waves of infection. All measures, especially in the first wave of the pandemic, were in combination with recommendations provided by professional and scientific organizations. Similar measures were applied to specific procedures, such as the management of infertility, including in vitro fertilization-embryo transfer (IVF-ET) treatments. Although there is no clear scientific evidence yet that the SARS-CoV-2 may exert negative effects on IVF outcome, especially at the early stages, several clinical reports indicate that the virus may impact male fertility through specific receptors presented at the somatic cells of the testis and used by the virus in order to gain entry to the respective cells. Nevertheless, it is not unreasonable to suspect that the virus may affect sperm function as well as oocyte performance directly through specific receptors or indirectly through other signaling pathways. Despite the good practice of IVF laboratory techniques, culture media may also be contaminated during equilibration when airborne virus's particles can contaminate culture media from an already infected embryology area or staff. Furthermore, although there is no clinical evidence, **liquid nitrogen could be a route of infection for gametes and embryos when it has been contaminated during production or transportation. Therefore, cryopreservation of gametes and embryos must be virus-free. This communication aims to provide some aspects of the possible impact of the virus on gametes and embryos and how it may affect the cryopreservation procedures.**

Molina et al, 2016 : Bacterial and fungal contamination risks in human oocyte and embryo cryopreservation: open versus closed vitrification systems

Objective: To study the contamination risk in open and closed vitrification devices for oocyte/embryo cryopreservation by evaluating the contaminants present (bacteria and fungi) in the thaw medium and in liquid nitrogen (LN) storage containers. Design: Retrospective study. Setting: Human reproduction unit. Patient(s): None. Intervention(s): Retrospective study of vitrification device safety and LN sterility performed from July to October 2014. Main Outcome Measure(s): From each bank container, both open and closed vitrification devices, devitrification media and LN in the containers and as supplied by the company were evaluated for contaminants. An automated system and the corresponding susceptibility to antibiotics were used for bacteria identification. Fungus detection was performed by evaluating the colony morphology and their microscopic characteristics. Result(s): No bacteria or fungi were observed in any of the devitrification media regardless of the type of device used, nor in the LN supplied by the company. No fungi were observed in any of the LN samples tested. **Stenotrophomonas maltophilia and Bacillus spp. were found in all oocyte/embryo bank LN containers.** There was no relationship between the number of samples or the time that each container had been used and the presence of microbiologic contaminants in the LN. At the container's bottom, Acinetobacter lwoffii, Alcaligenes faecalis ssp. faecalis, and Sphingomonas paucimobilis were found. Conclusion(s): Bacteria cross-contamination may not occur in oocyte/embryo banking in either open or closed storage devices. However, **microorganisms can survive in LN.** The bacteria cross-contamination risk is no greater for open than for closed containers. Storage containers should be cleaned periodically owing to the risk of lost straws or small particles of contaminated material.

Bielanski et al, 2009 : Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units

Cryopreservation of sperm, embryos and, more recently, oocytes plays an important and increasing role in assisted reproduction, due to improvements of old, and introduction of new technologies. In parallel, concerns are increasing about the technical and biological safety of these procedures. However, published data regarding the confirmed or theoretical hazards of these procedures are sparse and sometimes contradictory. **The purpose of this review will summarize data and opinions about one of the most disputed risks, the potential hazard of contamination and disease transmission through cryopreservation.** Special attention is concentrated on the weak points of the technology including open vitrification systems, sterilization of liquid nitrogen and safety of commonly used storage tanks including straws and cryovials. Suggestions are also made for practical measures to avoid these dangers while preserving the benefits and perspectives of new cryopreservation technologies.

Marin et al, 2020 : Experimental Evidence Reveals Both Cross-Infection and Cross-Contamination Risk of Embryo Storage in Liquid Nitrogen Biobanks

This study was conducted to demonstrate the potential hazards of cross-infection and cross-contamination of embryos during storage in liquid nitrogen biobanks. **For the harmless and successful cryopreservation of embryos, the vitrification method must be chosen meticulously to guarantee not only a high post-thaw survival of embryos, but also to reduce the risk of disease transmission when those embryos are in storage for long periods.**

Pomeroy et al, 2020 : Cryopreservation and IVF in the time of Covid-19: what is the best good tissue practice (GTP)?

Examine good tissue practices as relates to in vitro fertilization, biopsying, and vitrification to compare current knowledge of ova, sperm, and embryos as vectors for disease transmission as it relates to our current knowledge regarding the SARS-CoV-2 virus. Unknown risks relating to the SARS-CoV-2 virus and sperm, ova, and embryos necessitate a re-examining of how human IVF is performed. Over the last decade, improvements in cryosurvival and live birth outcomes have been associated with zona pellucida breaching procedures (e.g., blastocyst collapsing and biopsying). In turn, today embryos are generally no longer protected by an intact zona pellucida when vitrified and in cryostorage. **Additionally, high security storage containers have proven to be resilient to potential cross-contamination and reliable for routine human sperm freezing and embryo vitrification**. Several options to current IVF practices are presented that can effectively mitigate the risks of cross-contamination and infection due to the current Covid-19 pandemic or other viral exposures. The question remains; is heightened security and change warranted where the risks of disease transmission likely remain negligible?

Joaquim et al, 2017 : Risk of Contamination of Gametes and Embryos during Cryopreservation and Measures to Prevent Cross-Contamination

The introduction and widespread application of vitrification are one of the most important achievements in human assisted reproduction techniques (ART) of the past decade despite controversy and unclarified issues, mostly related to concerns about disease transmission. Guidance documents published by US Food and Drug Administration, which focused on the safety of tissue/organ donations during Zika virus spread in 2016, as well as some reports of virus, bacteria, and fungi survival to cryogenic temperatures, highlighted the need for a review of the way how potentially infectious material is handled and stored in ART related procedures. It was 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. Thus, **this review summarizes pertinent data and opinions regarding the potential hazard of infectious transmission through cryopreserved and banked reproductive cells and tissues in LN2. Special attention is given to the survival of pathogens in LN2, the risk of cross-contamination, vitrification methods, sterility of LN2, and the risks associated with the use of straws, cryovials, and storage dewars.**

Bielanski et al, 2014 : Biosafety in embryos and semen cryopreservation, storage, management and transport

This chapter summarizes pertinent procedures, data and opinions on the potential hazards of disease transmission through liquid nitrogen (LN)-cryopreserved and banked germplasm and tissues for somatic cell nuclear transfer (SCNT) The importance of applying internationally adopted sanitary washing procedures to germplasm as a crucial step towards their successful microbial-free cryopreservation and storage is emphasised. Special attention is given to the survival of pathogens in LN, variety of vitrification methods, sterility of LN, risks associated with the use of straws and cryovials, and LN Dewars including dry shippers. It was experimentally demonstrated that cross-contamination between LN and embryos may occur, when infectious agents are present in LN and if embryos are not protected by use of a sealed container. **It is important, therefore, to prevent direct contact of germplasm and reproductive tissues with LN during cryopreservation and their storage as a mandatory measure for reducing the risk of contamination. This includes the usage of hermetically sealed high quality shatter proof freezing containers and/or the application of a secondary enclosure such as "double bagging or straw in straw"**. A periodic disinfection of cryo-Dewars should be considered as an additional precaution to diminish the potential for inadvertent cross-contamination. It would be advisable to use separate LN Dewars to quarantine embryos derived from infected donors of valuable genotypes or from unknown health status, extinction-threatened species.