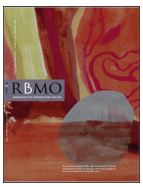
RBMO

ELSEVIER



REVIEW

Cryostorage management of reproductive cells and tissues in ART: status, needs, opportunities and potential new challenges

BIOGRAPHY

Stefano Canosa PhD is a Senior Clinical Embryologist at IVIRMA Global Research Alliance, LIVET, Turin, Italy. He is a member of the clinical embryology scientific board of the Italian Society of Embryology, Reproduction, and Research. His main research areas are fertility preservation, time-lapse microscopy and artificial intelligence.

Stefano Canosa[1], Roberta Maggiulli[2], Danilo Cimadomo[2], Federica Innocenti[2], Gemma Fabozzi[2], Gianluca Gennarelli[1], Alberto Revelli[1], Francesca Bongioanni[1], Alberto Vaiarelli[2], Flippo M. Ubaldi[2], Laura Rienzi[2,3], Giles A. Palmer[4,5,6], Martine Nijs[7,*]

KEY MESSAGE

Cryopreservation and storage of gametes, embryos and tissues is an essential part of the technologies of IVF laboratories. This manuscript summarizes good laboratory practices, but also focuses on the risks and the needs for a cryopreservation and storage programme, besides future perspectives regarding the management of safe cryopreservation in assisted reproductive technology.

ABSTRACT

Among the wide range of procedures performed by clinical embryologists, the cryopreservation of reproductive cells and tissues represents a fundamental task in the daily routine. Indeed, cryopreservation procedures can be considered a subspecialty of medically assisted reproductive technology (ART), having the same relevance as sperm injection or embryo biopsy for preimplantation genetic testing. However, although a great deal of care has been devoted to optimizing cryopreservation protocols, the same energy has only recently been spent on developing and implementing strategies for the safe and reliable storage and transport of reproductive specimens. Herein, we have summarized the content of the available guidelines, the risks, the needs and the future perspectives regarding the management of cryopreservation biorepositories used in ART.

GUIDELINES FOR THE SAFE AND RELIABLE CRYOSTORAGE OF REPRODUCTIVE CELLS AND TISSUES

According to European directives and recommendations, working in compliance with a quality management system is mandatory to ensure safety during the manipulation of human tissues and cells (*European Directorate for the Quality of Medicines & HealthCare of the Council of Europe, 2022*; *Měřička et al., 2012*; *Pirnay et al., 2013*). Revised guidelines for good practice in IVF laboratories have been developed by the most prominent scientific societies to: (i) provide a wide coverage of key aspects regarding andrology and embryology laboratories; (ii) give continuous support to laboratory specialists; and (iii) consequently contribute to improving IVF patient care (*Go, 2019*). Schiewe and coworkers have developed an excellent template for a total quality management programme for the maintenance of a cryogenic inventory (*Schiewe et al., 2019*). As constantly outlined, the frequent revision of such procedures improves

[a] IVIRMA Global Research Alliance, LIVET, Turin, Italy
[b] IVIRMA Global Research Alliance, Clinica Valle Giulia, Rome, Italy
[c] Department of Biomolecular Sciences, University of Urbino 'Carlo Bo', Urbino, Italy
[d] International IVF Initiative Inc, New York, NY, USA
[e] Institute of Life, IASO Hospital, Athens, Greece
[f] IVF 2.0 Ltd, London, UK
[g] CooperSurgical, Ballerup, Denmark.

KEYWORDS

Cryobanking
Cryomanagement
Cryopreservation
Cryostorage
IVF

*Corresponding author. E-mail address: Martine.Nijs@coopersurgical.com (M. Nijs). https://doi.org/10.1016/j.rbmo.2023.06.007 1472-6483/
Declaration: The authors report no financial or commercial conflicts of interest.

appropriate quality and risk management issues, aiming to heighten emergency responses and contingency planning to avoid serious accidents or at least limit their consequences (*Alikani, 2018*; *Alteri et al., 2021a*; *Tomlinson and Morroll, 2008*).

Cryopreservation of reproductive cells and tissues can be considered as a subspecialty of ART, and good laboratory practices during cryopreservation and cryostorage are therefore mandatory. A reliable system of cryogenic storage optimizes patient safety while at the same time minimizing the risk of laboratory liability. Managing cryopreserved reproductive cells and tissues in an embryology laboratory requires skilled personnel and specialized equipment. The American Society for Reproductive Medicine (ASRM) committee's opinion on the cryostorage of reproductive tissues can be used as a valuable guide for developing clinic-specific management policies and procedures (*ASRM/SRBT/SART Practice Committees, 2020a*). Additionally, laboratory guidelines have been developed that outline minimum standards regarding cryostorage management (College of American Pathologists; *Szeptycki and Varghese, 2018*).

Finally, independent IVF centres have demonstrated the benefits of handling human gametes and culturing embryos in a setting with improved heating, ventilation and air-conditioning systems, to optimize clinical outcomes (*Mortimer et al., 2018*). While a cleanroom environment will ensure a low level of contamination from environmental pollutants such as dust particles, airborne microbes, aerosol particles and chemical vapours, few specific protocols for operational standards and maintenance exist in the cryo-room (*Nijs and Verheyen, 2016*).

An emerging new problem is the growing inventory of frozen specimens and the concomitant limitation of cryostorage space. The new standard of care is to cryopreserve a single oocyte or embryo per device, resulting in the cannisters within the dewars exceeding capacity more rapidly. Also, vitrification storage devices take more space in the storage tanks compared with the straws used in slow freezing; more storage tanks are therefore needed to store the same number of samples, diminishing the limited floor space available within most clinics to store the vessels. Depending on local legislation, the cryostorage of samples may become permanent and infinite in duration. These samples will accumulate in the storage tanks, and available cryostorage space will reduce quickly. A redesign and/or rebuild of the cryostorage area, involving extra costs, will be a necessity. Alternatively, external long-term storage options may be required to accommodate new storage.

CRYOPRESERVATION AS A CRITICAL PROCEDURE IN DEFINING HIGH-QUALITY STANDARDS IN IVF LABORATORIES

Safety of short- and long-term cryostorage

The incidence of hazards relating to the handling of cryopreserved samples is difficult to determine. The main risk is represented by premature thawing, which could derive from either an inadequate nitrogen supply during freezing-thawing procedures, equipment failure or improper handling. The latter could occur suddenly and abruptly, as is experienced when searching for specimens in the storage vessels and moving them from the tanks to the bench. Time delays in reading labels may cause devitrification when the specimens are exposed to temperatures of −121°C or higher, making it crucial to reduce either the time length or the number of witness steps. The latter can be accomplished by using automated procedures, as discussed later (*Sansinena et al., 2014*,*2018*). There are further unexpected risks that involve personnel safety. For example, embryologists might experience the explosion of plastic or glass cryo-devices, which emphasizes the need to not underestimate the importance of training (*Schiewe et al., 2016*) and adherence to strict laboratory protocols (*Tomlinson and Morroll, 2008*).

A prolonged storage of human gametes, embryos and ovarian or testicular tissue is currently adopted for both medical and non-medical indications, including fertility preservation in patients facing gonadotoxic treatments, women delaying childbirth for personal reasons and IVF treatments performed using donated gametes (*ASRM/SRBT/SART Practice Committees, 2020a*, *Cobo et al., 2021*; *Jensen et al., 2022*; *Silvestris et al., 2020*). Although long-term cryopreservation is thought to pause cell metabolism and ageing, conflicting results are now available as some authors have raised the question of a putative harmful effect deriving from prolonged cryostorage on oocyte and embryo competence (*Parmegiani and Vajta, 2020*). In addition, the toxic effects of a prolonged exposure to cryoprotectant agents or potential contamination of liquid nitrogen may have a detrimental impact in the long run (*De Santis et al., 2021*; *Gosden, 2011*). One can also speculate that other factors such as temperature fluctuations due to frequent opening of the cryotank lid and lifting canisters to neck level, may have a cumulative impact in the case of short-term storage.

Many case reports have been published over the years on the safety of long-term cryopreservation, showing successful pregnancies and healthy live births obtained from human oocytes (*Goldman et al., 2015*; *Maxwell et al., 2014*; *Parmegiani et al., 2008*,*2009*; *Quintans et al., 2012*; *Torra-Massana et al., 2023*; *Urquiza et al., 2014*) and spermatozoa (*Huang et al., 2019*; *Pariz et al., 2020*; *Rofeim and Gilbert, 2005*; *Yogev et al., 2010*) stored years before. Furthermore, two independent and complementary systematic reviews and meta-analyses have recently been published supporting the safety of the long-term cryostorage of human embryos at both the cleavage and blastocyst stages (*Canosa et al., 2022*; *Ma et al., 2021*). There is also reassuring evidence concerning frozen-thawed ovarian tissue transplantation after a prolonged storage time (*Andersen et al., 2012*; *Fabbri et al., 2016*).

Although a recent large long-term follow-up study has documented the good health of children conceived from cryopreserved oocytes, one must remain vigilant to regarding stored samples not as inanimate and unchanging but as susceptible to harmful short- and long-term cryostorage (*Gullo et al., 2022*; *Van Reckem et al., 2021*). Finally, this prolonged storage of reproductive tissue specimens has increased the need to implement new procedures, protocols and equipment (*ASRM/SRBT/SART Practice Committees, 2020a*).

Risks associated with contact with liquid nitrogen

Vitrification is currently considered to be the gold standard for both oocyte and embryo cryopreservation since this protocol has proven to be simpler, cheaper, faster and safer than slow-freezing protocols (*Rienzi et al., 2017b*). The efficiency of vitrification is comparable to that of fresh embryo transfers in terms

of pregnancy, miscarriage and live birth rates (*Cobo et al., 2012*; *Feng et al., 2012*; *Roy et al., 2014a*; *Zaat et al., 2021*). Vitrification protocols are characterized by the use of either 'open' or 'closed' carriers. Closed devices are intended to keep the samples physically separated from the liquid nitrogen during the entire cooling, storage and warming procedures, whereas open systems allow direct contact between the sample and the liquid nitrogen (*Vajta et al., 2015*).

Importantly, several microorganisms can survive storage at low temperatures, including that of liquid nitrogen. Samples are in fact stabilized by the presence of several substances currently used in culture media (e.g. serum albumin, sucrose and other sugars), and this inevitably represents a putative source of biological contamination (*Bielanski and Vajta, 2009*). Bacteria possess a relatively high resistance, contrary to fungi, which have been proved to be very sensitive to the freezing procedure and the toxicity of highly concentrated cryoprotectant agents. Indeed, cryopreservation reduces the concentration of fungi in human semen by 90% (*Hubalek, 2003*). Interestingly, bacteria isolated from liquid nitrogen are not ubiquitous environmental micro-organisms but are rare opportunistic pathogens of low significance in producing disease in humans or animals.

It should be acknowledged that some of the isolates may have been derived from laboratory contamination during semen and embryo processing for cryopreservation rather than genuinely being present within the sample (*Bielanski and Vajta, 2009*). In addition, contamination with HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) has been reported in assisted reproductive technology clinics (*Lesourd et al., 2000*) and blood banks (*Tedder et al., 1995*). Although there is no documentation of the cross-contamination of cryopreserved stored human tissue, it is highly recommended that samples from viral carriers be processed in a separate laboratory or designated space within the main laboratory (*ASRM Practice Committee, 2020b*), preferably a biological safety cabinet.

Since HIV, HCV, HBV and possibly other viruses can survive in liquid nitrogen, cross-contamination between samples in liquid nitrogen storage tanks cannot be excluded. So far, the evidence shows that this risk in the liquid phase is probably low. However, to protect cryopreserved specimens from the risk of cross-contamination, it is advised that HIV-, HBV-, and HCV-infected specimens are stored in separate tanks, and liquid nitrogen vapour storage instead of the liquid phase should be considered. Alternatively, an aseptic closed-system vitrification device applying sound sealing can be used to prevent the direct contact of cryopreservation devices with liquid nitrogen (*ASRM Practice Committee, 2020b*, *Mocanu et al., 2021*). Furthermore, specific sperm-washing techniques should be considered to decrease the viral load of potentially infectious ejaculates (*Pomeroy and Schiewe, 2020*).

Of note, European Union directives on tissue manipulation (*European Union Tissues and Cells Directive EUTCD: 2004*/23/EC, *2006a*/17/EC, and *2006b*/ 86/EC) regulate the safety and quality of tissues including reproductive cells processed for human preimplantation through the control of equipment, devices and the environment. These regulations require specific procedures in embryo, oocyte, ovarian and testicular tissue cryopreservation aimed at minimizing the risk of any hypothetical contamination of human cells due to direct contact with accidentally contaminated liquid nitrogen. Nevertheless, these directives report no mandatory statements against direct contact between tissue or cells and liquid nitrogen.

On the other hand, the need to guarantee the absolute sterility of liquid nitrogen for vitrification purposes is still considered a crucial issue, as the contamination of liquid nitrogen and/or its vapour can occur at any step from manufacturing to final use, including transport, storage, distribution and preparation for cryopreservation. The risk of cross-contamination as well as the recommendations to handle reproductive samples to avoid potentially hazardous behaviours have been previously reviewed (*Joaquim et al., 2017*). At present, several approaches have been suggested to obtain sterile liquid nitrogen, such as UV filtration, for a safe vitrification procedure (*Arav et al., 2016*; *Cobo et al., 2010*; *Mcburnie and Bardo, 2002*; *Parmegiani et al., 2012*, *2010*; *Wang et al., 2020*). However, these procedures involve extra costs and extended operator time.

More recently, the unprecedented spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic forced embryologists to critically rethink the appropriateness and safety of all the procedures in IVF laboratories, which resulted in a radical shift of priorities including the management of cryopreservation (*Maggiulli et al., 2020*). During a pandemic involving a respiratory virus, the risk of contamination cannot be overestimated, as even a simple proximity to cryopreservation devices or to the liquid nitrogen surface represents a critical exposure to floating particles (*Lee, 2020*). For this reason, the putative risk of environmental contamination as well as the hypothetical presence of SARS-CoV-2 in gametes and embryos has warranted consideration (*Scarica et al., 2021*).

Closed devices have proved to be as efficient as open ones, but are safer to protect human oocytes from the potential risks of viral contamination during vitrification and storage, especially during the coronavirus 2019 (COVID-19) pandemic (*Porcu et al., 2021*). *Pomeroy and Schiewe (2020)* amended earlier assertions (*Pomeroy et al., 2010*) of the negligible risk of disease transmission based on embryo washing, since embryos being cryopreserved today frequently have a non-intact zona pellucida due to laser drilling and blastocyst biopsy procedures, meaning the trophectoderm layer is exposed to pathogens (e.g. SARS-CoV-2 and others). Closed systems are more important than ever, especially since more randomized controlled studies with oocyte cryopreservation are failing to show a difference compared with the effectiveness of open systems. It should be further emphasized that environmental nitrogen-mediated contamination can also infect reproductive cells during handling or vitrification and warming, irrespective of the use of either open or closed carriers (*De Santis et al., 2021*). Indeed, dilution steps when handling embryos and oocytes before vitrification and after warming represent the most important strategy for reducing the risk of possible contaminants (*Lee, 2020*).

In a constantly changing scenario, where scientific evidence is still limited, the decision on how to preserve and store reproductive tissues and cells is a topic of current debate, which is also driven by the need to avoid greater complexity of management in a time already full of uncertainties. On this basis, a strengths, weaknesses, opportunities and threats risk analysis of the procedure of cryopreservation of reproductive samples during the COVID-19 pandemic has been

recently published, suggesting that new strategies for decision making are warranted during a pandemic (*Alteri et al., 2020*,*2021*b; *Parmegiani and Vajta, 2021*).

Risks associated with improper handling of samples

In general, a major problem in cell and tissue cryopreservation is the lack of standardization. There are many different cryo-carriers, vitrification solutions, warming solutions and vitrification protocols for each model. Handling differences within the centre (*Tannus et al., 2018*), between centres and even among cryobanks (*Gianaroli et al., 2022*) may create issues that ultimately affect IVF outcomes after the use of cryopreserved samples.

Working with liquid nitrogen and its associated cryogenic equipment has raised health and safety concerns due to the possibility of accidents resulting in staff injury and loss/damage of patient material due to either equipment failure or human error. Organized staff training and periodic revision through staff meetings are of critical importance to properly sustain high standards in cryopreservation and cryostorage. As an example, repeated withdrawals of samples without safe liquid nitrogen submersion in liquid nitrogen baths or dewar flasks during handling or identification can result in episodes of varying temperatures that should be minimized to avoid a possible accumulative decline of subsequent viability. This concern is greatest in liquid nitrogen vapour storage since the goblet or visotube holding devices are air filled, not liquid nitrogen filled.

In addition, it is also important to take into consideration the incidence of hazards related to the cryopreservation protocol, namely slow freezing or vitrification. During slow freezing oocytes and embryos are exposed to a gradual decrease in temperature using a controlled-rate slow-cooling machine, which involves specimen handling during the cryopreservation such as a transfer from laminar flow and the freezer, or manual seeding performed at −7°C. On the other hand, vitrification has apparently proved to be much simpler and faster than slow freezing but is characterized by the need for skilled operators. While both methods endure the same environmental insult of very low temperatures, vitrified samples with a minimal volume encasing or surrounding them, positioned on a cryo-device, are more sensitive to temperature changes and the transition shift to devitrification, and thus to subsequent damage (*McDonald et al., 2011*). Although serious events are rare, careful risk management practice should be viewed as a high priority (*Tomlinson, 2018*).

Failure of the liquid nitrogen supply to cryogenic dewars may lead to involuntary thawing-warming, potentially resulting in the loss of samples. Events like these mostly derive from storage system failure and not from human errors. This strongly underlines how important appropriate emergency procedures are. Indeed, fertility clinics are less likely to be proven 'negligent' when care is provided in line with the common principles of best practice and with the latest technological advances. Conversely, the use of inadequate emergency procedures would pave the way for allegations of negligence (*Tomlinson and Pacey, 2003*).

In this regard, one of the earliest events was reported in 2003. At the time, many frozen sperm samples were lost, including samples from patients who had undergone sperm cryopreservation before chemotherapy (*Nesbit et al., 2022*). A failure of the cooling apparatus caused the accident. Unfortunately, all the samples were lost as they were all stored in a single tank. Most recently, three high-profile cases occurred in the USA, all resulting in the loss of thousands of embryos and gametes. In one event the alarm was turned off during a manual tank refill while the automatic filling apparatus was undergoing maintenance; the second event is thought to have been due to insufficient tank levels of liquid nitrogen; and the third event occurred because of a failure of the tank's vacuum pump (*Nesbit et al., 2022*). All these events resulted in class action lawsuits against the clinics and/or staff.

It is thought that most errors in cryostorage occur as a result of equipment failure or a lapse in the supply of liquid nitrogen. To minimize such risks it is recommended that tanks undergo regular maintenance and be monitored daily or even continuously for evidence of leakage. Facilities should retain an excess supply of liquid nitrogen in case supplies are diminished before a replacement can be delivered by supply companies. Furthermore, an extra cryostorage tank should always be available in the event of a malfunction. The spare tank should have similar characteristics to the largest tank in the facility. Lastly, it is advised that samples from individuals or couples should be distributed across multiple tanks, if possible, to ensure that patient samples are not lost if a container malfunctions (*Nesbit et al., 2022*). As a result, US Food and Drug Administration and European Union regulations are now more likely to be adopted to set standards in cryobanking. The UK Human Tissue Authority regulators of the cell and tissue banking community specify that 'wherever possible, CE-marked medical devices must be used' (*Tomlinson, 2018*).

Moreover, it is advised that a periodic storage inventory survey be conducted whereby stored samples have their position within the vessel and identity checked with either a written registry or digital records (*Frith and Blyth, 2014*). For large storage facilities, this involves the physical removal of a large quantity of samples and checking their identity, which itself poses a degree of risk of the accidental premature warming of storage devices (*Mortimer, 2004*; *Tomlinson and Morroll, 2008*). Multiple inventorying of long-term samples may have an accumulative impact on cell viability, particularly in reference to open vitrification devices in liquid nitrogen vapour storage.

To reduce the risk of failure, IVF clinics should re-examine their practices to meet strict standards, particularly in the provision of: (i) continuous electronic monitoring systems for relevant quality control parameters such as storage tank temperature and liquid nitrogen level; (ii) tank alarm systems with on-site and remote notification possibility; and (iii) detailed disaster plans including the availability of spare tanks to be used in the event of forced specimen removal from a failing tank or for other unforeseen events (*Alikani and Parmegiani, 2018*). Furthermore, daily quality control practices and weekly operational qualification monitoring of evaporation rates are critical preventative measures (*Schiewe et al., 2019*).

MAINTENANCE OF DEWARS AND STORAGE TANKS

Small-capacity dry shippers are commonly used for moving samples within a clinic or shipping samples to other centres. In general, these cryopreservation vessels are

double-walled, vacuum-insulated vessels made of aluminium with a fibreglass composite neck. The vessel absorbs liquid nitrogen into its walls and when it is fully charged sustains a low temperature; it should be used only for liquid nitrogen and not for other liquids. If the vessel is orientated in any other way than vertically upright this may accelerate the evaporation rates and possibly damage the vessel. Alternatively, mishandling (a fall, hit or jarring blow) can cause an immediate or premature vacuum failure that will result in a temperature increase.

A new vessel and its packaging should be examined upon receipt for any evidence of damage (e.g. dents, or damage to the cork or neck) and then cleaned using the standard cleaning procedure. The empty vessel should be weighed, before being filled slowly with liquid nitrogen and closed. Excessive frosting or sweating on the outside of the vessel after the first few hours indicates a compromised vacuum. The vessel is weighed again after 2 h. If the weight is different, the vessel could be leaking or the vacuum wall damaged. These situations may warrant the rejection of non-qualifying containers (i.e. retirement). Weight checks should be carried out as a regular maintenance procedure during the year.

An annual, or preferably monthly, maintenance check should include a summary of evaporation rates. In this test, the weight of a full vessel is measured over multiple days to define the expected evaporation rate and is compared with the normal evaporation rate provided by the supplier. After each use of a dry shipper a performance qualification form should be completed to supplement a periodic operational qualification review.

CLEANING OF SHORT-TERM USE DEWARS AND LONG-TERM USE STORAGE TANKS

The life expectancy of most liquid nitrogen dewars is 10 years (*Rienzi et al., 2017a*). They are designed according to standard requirements for safety, durability and performance. The maintenance and cleaning instructions provided by the supplier must be strictly followed over time. Mishandling contrary to these basic instructions may lead to indirect damage to the cryopreserved samples.

In addition, the long-term maintenance of storage containers may mandate their periodic cleaning with solutions that do not react with aluminium or stainless steel. A generally accepted practice of using 10% chlorine bleach with 90% water solution is the best method for decontamination. Other cleaners and disinfectants that can be used safely include hydrogen peroxide, a chlorine–water mixture and denatured alcohol. It is important that all surfaces being sanitized are thoroughly rinsed and that all cleaning solution residues are removed after cleaning. It is also suggested that the unit is inverted to drain and dry completely before being refilled. Routinely, any household detergent or mild soap solution is suitable.

Before cleaning the tanks, any cryopreserved samples need to be transferred in a safe and temperature-controlled way to a similar cryostorage vessel. All transfers need to be witnessed, to guarantee the chain of custody. The vessel is emptied in a well-aerated environment and moved outside the cryostorage area to eliminate the risk of ambient volatile organic compounds during the cleaning procedure. After safely removing the vessels with the specimens, the liquid nitrogen should be allowed to equilibrate and the bottom of the tank is then inspected for miscellaneous devices or debris. Once it has been confirmed that the vessel is empty of specimens, it can then be moved.

A typical cleaning procedure for a small emptied stainless steel vessel includes rinsing the vessel inside and outside with sterilized water followed by spraying or fully filling the vessel with the cleaning solution. The cleaning solution is agitated in the vessel to ensure the coverage of all surfaces. The vessel is then decanted, placed upside down and left to dry completely before being put into service again. Dry shipper tanks can be cleaned in a similar manner, using the cleaning product as advised by the supplier. A study on the use of biocides for cleaning/disinfecting dry shippers identified sodium hypochlorite and peracetic acid as effective (*Bielanski and Vajta, 2009*). Drying of the adsorption material takes a considerably longer time compared with a stainless steel dewar. Replacing the adsorption material regularly can also be an option, and the use of one-time or disposable dry shippers is becoming increasingly popular.

The materials and equipment used during the cryopreservation procedures and storage also need to be cleaned regularly; these include the forceps, tongs, canisters, canes, heat-sealing devices and slow-freezing devices as well as the gloves and aprons used for personal protection. As previously mentioned, one strategy is to use UV radiation to successfully sterilize the liquid nitrogen for further vitrification procedures (*Parmegiani et al., 2010, 20102*). The temporary storage tanks, dewar flasks and Styrofoam baths used for transporting straws or devices between the long-term storage tanks and the IVF laboratory bench should be cleaned after use by rinsing or spraying with a cleaning solution (e.g. a commercially available solution of 6% H_2O_2), followed by rinsing with sterile water, and should be left to dry upside down.

Specialized companies offer cleaning/decontamination services for large vapour-phase or liquid nitrogen tanks. Cleaning protocols should be clearly described in the standard operating procedures and periodically reviewed or revised with new information, and all cleaning and maintenance interventions should be logged. Although specialized companies offer cleaning/decontamination services for large vapour-phase or liquid nitrogen tanks, it is strongly suggested that training courses should be established for all staff members on liquid nitrogen handling and cryostorage compliance. Widely used aluminium dewar tanks are only supported by the weld at their adjoining necks and are therefore susceptible to weakening at that point, which will alter the integrity of the vacuum and ultimately the evaporation rates.

Clear instructions should be in place to always handle the storage vessels with care; excessive handling and repositioning of empty tanks in everyday use and especially during the cleaning, rinsing and drying process can compromise their integrity. Furthermore, it is recommended that staff competency in emergency preparedness should be periodically tested by simulating a critical catastrophic event in the cryostorage room. And finally, moving cryopreserved samples to temporary tanks is a critical procedure, and care should be taken in avoiding clerical errors that could result in positional errors in the vessels.

TRANSPORT OF CRYOPRESERVED SAMPLES

In clinical practice, patients often transfer cryopreserved cells and tissues between clinics and donor banks. Such transfers may be local, within the country or across borders, or even across continents, and all these transfers demand particular attention given the intrinsic value of the biological material. It is strongly advised that patients are counselled on the potential risks of shipping their cryopreserved biological materials, including the signing of an informed consent/agreement regarding liability.

The shipment of cryopreserved samples is complex, involving several critical steps such as sample transfer between storage containers. As previously mentioned, cryopreserved samples are highly sensitive to temperature fluctuations, which may occur during handling and storage. This is particularly true when open vitrification systems are used. Temperature and humidity can change in uncontrolled ways, and this fluctuation may be harmful to reproductive samples. Despite the predominant use of liquid nitrogen for storage, the shipment of cells/tissue is generally performed with tanks or dry shippers that use nitrogen vapour (−150° C). Disposable vapour-phase tanks can also be used for transport. However, the associated risk of sample devitrification, defined as the process of crystallization of a formerly crystal-free, amorphous glass state that can lead to damage during the warming of cells, should not be underestimated (*Alikani and Parmegiani, 2018*; *Tomlinson and Morroll, 2008*). Recovery failure and a lower survival rate for shipped oocytes compared with their non-shipped counterparts suggest that some abnormal conditions could occur during shipping, including exposure to elevated temperatures and air pressure, vibration and physical shock (*McDonald et al., 2011*).

Most experts agree that professional courier services are the safest means of transportation, reducing risk liabilities to the shipper, since they provide electronic data logging of temperature, location and container positioning to ensure that proper conditions are maintained the entire duration of a 24–48 h journey (*Parmegiani et al., 2017*). Companies involved in cold chain logistics should undergo scrutiny by IVF clinics in the form of audits and undergo an investigation of their adherence to international standards (*ISO 21973:2020*).

As in other fields of medicine, there is a need to develop common guidelines aimed at providing a unified process applying standard operating procedures, forms and communication networks to reduce, or preferably eliminate, any possibility of error (*Simione and Sharp, 2017*). Standards have been drafted providing general requirements to ensure cell quality, safety and efficacy during the transportation process, which have important repercussions in the IVF industry (ISO 21973:2020, White Paper, Cryoport). Locally approved decrees can also be taken into consideration to control and implement procedures for transporting cryopreserved biological material.

Among these policies, it is worth mentioning a document drafted by the Italian Society of Embryology, Reproduction, and Research (*Paoli et al., 2021*). The Society identified critical communication procedural steps between Italian centres and created new forms for patient authorization, requests from the recipient centre, critical checks carried out by both the sending and recipient centres, the start of sample transfer, collection, transport of and taking responsibility for the biological material, and acknowledgement of the arrival of samples and also of any adverse event that may have occurred. It is strongly advised that patients are counselled on the potential risks associated with shipping their cryopreserved biological materials, and this should also include signing an informed consent form or agreement around liability.

REPRODUCTIVE OUTCOMES WHEN CRYOPRESERVED SAMPLES ARE USED

The improvement of cryopreservation practices for both oocytes and embryos through the adoption of vitrification protocols has been a game changer (*Rienzi et al., 2012*). Reliable post-thaw viability and higher adoption of single embryo transfer have led to the acceptance of the cumulative live birth rate per cycle becoming the most appropriate measure of success in IVF (*Maheshwari et al., 2015*). Furthermore, freeze-all cycles represent a feasible strategy to decrease the risk of severe ovarian hyperstimulation syndrome (*Zhu et al., 2018*) and reduce the maternal, gestational and perinatal risks associated with multiple gestations by performing single euploid embryo transfers (*Conforti et al., 2021*). Although most scientists agree that fresh oocytes produce more stable live birth rates per started cycle compared with cryopreserved oocytes (*Kushnir et al., 2018*), oocyte vitrification has proven to be a successful strategy for fertility preservation for women, especially those with a medical indication (*Cornet-Bartolome et al., 2020*).

Despite this, limited data are available on the health of the children born from cryopreserved oocytes, especially in cancer patients, emphasizing the need for well-designed follow-up studies of pregnancies and a long-term follow-up of children born (*Fraison et al., 2023*; *Porcu et al., 2022*).

THE AUTOMATED FUTURE OF CRYOMANAGEMENT

Even with technological advancements on the horizon, IVF is primarily a manual process that is time-consuming and largely operator dependent. Only a fraction of the available procedures have thus far been semi-automated and integrated into routine use, specifically slow freezing, automatic witnessing and the time-lapse monitoring of embryo development.

To address the need to better control all the important variables during the vitrification process, including temperature, embryo position, media volume, media concentration and exposure times, and to minimize the variability across users and clinics, automated vitrification has been proposed for the optimization and repeatability of the results (*Dal Canto et al., 2019*; *Roy et al., 2014b*). Although promising in terms of clinical performance and enhanced standardization, automated vitrification may lead to longer processing times and less convenience for staff while not necessarily increasing efficiency or eliminating potential clinical errors compared with conventional manual processes (*Hajek et al., 2021*). Robotic systems and microfluidic platforms are currently being developed and marketed but at present remain clinically untested (*Arav and Patrizio, 2022*; *Arav et al., 2018*; *Cianchetti et al., 2018*; *Meseguer et al., 2012*; *Miao et al., 2022*; *Roy et al., 2014b*; *Tirgar et al., 2021*).

With respect to traceability and quality control, electronic witness systems are more commonly employed from egg retrieval to embryo cryopreservation. These systems use either barcode readers or radiofrequency identification (RFID) tags to identify gamete and embryo sources with dishes and devices in relation to the patient source(s). Modified RFID labels are capable of working in liquid nitrogen to locate, identify and quantify cryo-devices while they are being stored or relocated (*Palmer et al., 2020*). These labelling and coding systems have the advantage of providing witness traceability of the entire IVF process to the eventual location in the storage tank by a series of 'matching' steps; each critical step is electronically documented, thus providing an improvement in timing and efficiency compared with manual witnessing (*Holmes et al., 2021*).

An automated system may be seen as an additional expense and added set-up process for a well-staffed, centralized laboratory where manual witnessing is being effectively implemented, but it has the potential to drastically reduce the accidental warming of cryo-devices during sample verification. Such a process provides a more optimal cryostorage chain of custody. Finally, the use of RFID logging of cryo-devices is under investigation to further improve the safe handling of reproductive tissues and improve their traceability in cryobanks. Current systems require excessive specimen manipulation and are prone to misplacement by human error.

Automated platforms are being developed to provide high levels of safety and security and an improved chain of custody for the management, identification and storage of frozen human gametes and embryos. It is proposed that automated racking and storage processes using integrated software and hardware would prevent the types of catastrophes that might result from manual processes. Automation may reduce incidents such as the misidentification, misplacement or loss of reproductive material (*Abdullah et al., 2022*; *Tomlinson and Morroll, 2008*). In an automated sample inventory system, samples would be safely maintained at controlled temperatures and constantly controlled for their location; these proposed higher levels of safety and security need to be proven (*Logsdon et al., 2021*).

When transitioning from purely manual procedures to automated processes involving robotic mechanics, electronics and sensor locations, concern must focus on the reliability of stored gametes and embryos if malfunctions occur. Additionally, if a new storage system requires a complete change of the type of vessel (e.g. canes, visotubes or beacons) used in the storage process, one must weigh its compatibility with traditional systems and potential excess sample manipulations under various conditions previously described (i.e. in storage, shipping and emergency handling). Such potential improvements to the traditional way of storage should be thoroughly investigated and validated for improved safety and against integrity failure, malfunctions and heightened maintenance.

Regardless of new technologies every single IVF centre should have a clear and reliable protocol for the identification of patients and traceability of cells during manipulation. Findings by a 'failure mode and effects analysis', applied to sample traceability would support IVF groups in better recognizing critical steps in their protocols, understanding identification and witnessing the process and, in turn, enhancing safety by introducing validated corrective measures (*Intra et al., 2016*; *Rienzi et al., 2017a*). Established quality control practices must continue to be balanced when validating the potential pros and cons of new technologies from a risk–liability–cost perspective.

CONCLUSIONS

There is an undeniable growth in the cryo-inventory within IVF laboratories due to improvements in blastocyst culture and embryo and oocyte cryopreservation protocols, the practice of single-embryo transfer and the more widespread use of medically assisted reproduction in general. Likewise, the efficacy of pregnancy success after IVF and the increasing opportunity for fertility preservation contributes to samples remaining unused and possibly unclaimed, presenting a challenge of cryomanagement and ultimately storage space within a clinic. Hence, the management of cryopreserved reproductive tissues in an IVF laboratory is becoming an increasing burden for clinic staff, requiring skilled personnel, specialized equipment and strict adherence to quality control measures.

The mounting administrative demand for monitoring and managing cryo-inventories calls for advances in this area to help embryologists manage these samples and reduce risk liability and cost perspective.

The implementation of improved cryotank designs, more sensitive and reliable early alert monitoring systems and automated platforms may reduce the risks associated with both the storing and inappropriate handling of reproductive samples. Clinics should investigate retention time and dormant samples to manage storage policies from within the clinic and even drive the discussion to the development of off-site solutions. Once these innovative inventory management solutions are properly validated and clinically trialled, they have great potential to improve current diligence. Whatever the technology used, the objective should always be to implement a complementary quality management system for the cryostorage of cells and tissues to ensure the safety and proper identification of samples, thereby preventing mishandling and mitigating the risk of potentially catastrophic events.

DATA AVAILABILITY

No data was used for the research described in the article.

ACKNOWLEDGEMENTS

The authors sincerely thank Dr Rhodri J. Walters, a British publisher and professional proof reader (AIProofreading.com), for the expert proofreading of their manuscript.

REFERENCES

Abdullah, K.A.L., Atazhanova, T., Chavez-Badiola, A., Shivhare, S.B., 2022. Automation in ART: paving the way for the future of infertility treatment. Reprod. Sci. 1–11.

Alikani, M., 2018. Cryostorage of human gametes and embryos: a reckoning. Reprod. Biomed. Online. 37, 1–3.

Alikani, M., Parmegiani, L., 2018. Human reproductive cell cryopreservation, storage, handling, and transport: risks and risk management. Semin. Reprod. Med. 36, 265–272.

Alteri, A., Petrisli, E., Nolan, P., Pisaturo, V., Fehily, D., Navarro, A., Strong, D.M., Cardillo, M., Costa, M., 2021a. Learning from incidents in medically assisted reproduction: the notify library as a learning tool. Reprod. Biomed. Online. 43, 581–585.

Alteri, A., Pisaturo, V., Somigliana, E., Vigano, P., 2020. Cryopreservation in reproductive medicine during the COVID-19 pandemic: rethinking policies and European safety regulations. Hum. Reprod. 35, 2650–2657.

Alteri, A., Pisaturo, V., Somigliana, E., Vigano, P., 2021b. Reply: COVID-19 in liquid nitrogen: a potential danger still disregarded. Hum. Reprod. 36, 260–261.

Andersen, C.Y., Silber, S.J., Bergholdt, S.H., Jorgensen, J.S., Ernst, E., 2012. Long-term duration of function of ovarian tissue transplants: case reports. Reprod. Biomed. Online. 25, 128–132.

Arav, A., Natan, Y., Kalo, D., Komsky-Elbaz, A., Roth, Z., Levi-Setti, P.E., Leong, M., Patrizio, P., 2018. A new, simple, automatic vitrification device: preliminary results with murine and bovine oocytes and embryos. J. Assist. Reprod. Genet. 35, 1161–1168.

Arav, A., Natan, Y., Levi-Setti, P.E., Menduni, F., Patrizio, P., 2016. New methods for cooling and storing oocytes and embryos in a clean environment of -196 degrees C. Reprod. Biomed. Online. 33, 71–78.

Arav, A., Patrizio, P., 2022. Automation in Oocyte and Ovarian Tissue Vitrification. In: Grynberg, M., Patrizio, P. (Eds.), Female and Male Fertility Preservation. Springer, Cham. https://doi.org/10.1007/978-3-030-47767-7_31.

ASRM/SRBT/SART Practice Committees, 2020a. Cryostorage of reproductive tissues in the in vitro fertilization laboratory: A committee opinion. Fertil. Steril. 114, 486–491.

ASRM Practice Committee, 2020b. Recommendations for reducing the risk of viral transmission during fertility treatment with the use of autologous gametes: A committee opinion. Fertil. Steril. 114, 1158–1164.

Bielanski, A., Vajta, G., 2009. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Hum. Reprod. 24, 2457–2467.

Canosa, S., Cimadomo, D., Conforti, A., Maggiulli, R., Giancani, A., Tallarita, A., Golia, F., Fabozzi, G., Vaiarelli, A., Gennarelli, G., Revelli, A., Bongioanni, F., Alviggi, C., Ubaldi, F.M., Rienzi, L., SIERR, 2022. The effect of extended cryo-storage following vitrification on embryo competence: a systematic review and meta-analysis. J. Assist. Reprod. Genet. 39 (4), 873–882.

Cianchetti, M., Laschi, C., Menciassi, A., Dario, P., 2018. Biomedical applications of soft robotics. Nat. Rev. Mater. 3, 143–153.

Cobo, A., De Los Santos, M.J., Castello, D., Gamiz, P., Campos, P., Remohi, J., 2012. Outcomes of vitrified early cleavage-stage and blastocyst-stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. Fertil. Steril. 98, 1138–1146 e1.

Cobo, A., Garcia-Velasco, J.A., Remohi, J., Pellicer, A., 2021. Oocyte vitrification for fertility preservation for both medical and nonmedical reasons. Fertil. Steril. 115, 1091–1101.

Cobo, A., Romero, J.L., Perez, S., De Los Santos, M.J., Meseguer, M., Remohi, J., 2010. Storage of human oocytes in the vapor phase of nitrogen. Fertil. Steril. 94, 1903–1907.

Conforti, A., Picarelli, S., Carbone, L., La Marca, A., Venturella, R., Vaiarelli, A., Cimadomo, D., Zullo, F., Rienzi, L., Ubaldi, F.M., Alviggi, C., 2021. Perinatal and obstetric outcomes in singleton pregnancies following fresh versus cryopreserved blastocyst transfer: a meta-analysis. Reprod. Biomed. Online. 42, 401–412.

Cornet-Bartolome, D., Rodriguez, A., Garcia, D., Barragan, M., Vassena, R., 2020. Efficiency and efficacy of vitrification in 35 654 sibling oocytes from donation cycles. Hum. Reprod. 35, 2262–2271.

Dal Canto, M., Moutier, C., Brambillasca, F., Guglielmo, M.C., Bartolacci, A., Fadini, R., Renzini, M.M., Buratini, J., 2019. The first report of pregnancies following blastocyst automated vitrification in Europe. J. Gynecol. Obstet. Hum. Reprod. 48, 537–540.

De Santis, L., Parmegiani, L., Scarica, C., Sierr, Italian Society of Embryology Reproduction and Research, 2021. Changing perspectives on liquid nitrogen use and storage. J. Assist. Reprod. Genet. 38, 783–784.

European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM), 2022. Guide to the quality and safety of tissues and cells for human application, fifth ed Council of Europe, Strasbourg.

European Union Tissues and Cells Directive EUTCD:2004/7/23/EC, 2004: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2004/l_102/l_10220040407en00480058.pdf, 07/2023.

European Union Tissues and Cells Directive EUTCD: 2006/17/EC, 2006a: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_038/l_03820060209en00400052.pdf, 07/2023.

European Union Tissues and Cells Directive EUTCD: 2006/86/E, 2006b: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_294/l_29420061025en00320050.pdf, 07/2023.

Fabbri, R., Macciocca, M., Vicenti, R., Pasquinelli, G., Caprara, G., Valente, S., Seracchioli, R., Paradisi, R., 2016. Long-term storage does not impact the quality of cryopreserved human ovarian tissue. J. Ovarian. Res. 9, 50.

Feng, G., Zhang, B., Zhou, H., Shu, J., Gan, X., Wu, F., Deng, X., 2012. Comparable clinical outcomes and live births after single vitrified-warmed and fresh blastocyst transfer. Reprod. Biomed. Online. 25, 466–473.

Fraison, E, Huberlant, S, Labrune, E, Cavalieri, M, Montagut, M, Brugnon, F, Courbiere, B., 2023 Mar 1. Live birth rate after female fertility preservation for cancer or haematopoietic stem cell transplantation: a systematic review and meta-analysis of the three main techniques; embryo, oocyte and ovarian tissue cryopreservation. Hum. Reprod. 38 (3), 489–502.

Frith, L., Blyth, E., 2014. Assisted reproductive technology in the USA: is more regulation needed? Reprod. Biomed. Online. 29, 516–523.

Gianaroli, L., Ferraretti, A.P., Perruzza, D., Terzuoli, G., Azzena, S., Crippa, A., Dworakowska, A., Tabanelli, C., Magli, M.C., 2022. Oocyte donation: not all oocyte cryobanks are the same. Reprod. BioMed. Online. 44, 271–279.

Go, K.J., 2019. A subspecialty of the assisted reproductive technologies: cryogenic inventory maintenance. J. Assist. Reprod. Genet. 36, 15–17.

Goldman, K.N., Kramer, Y., Hodes-Wertz, B., Noyes, N., Mccaffrey, C., Grifo, J.A., 2015. Long-term cryopreservation of human oocytes does not increase embryonic aneuploidy. Fertil. Steril. 103, 662–668.

Gosden, R., 2011. Cryopreservation: a cold look at technology for fertility preservation. Fertil. Steril. 96, 264–268.

Gullo, G., Scaglione, M., Cucinella, G., Chiantera, V., Perino, A., Greco, M.E., Lagana, A.S., Marinelli, E., Basile, G., Zaami, S., 2022. Neonatal outcomes and long-term follow-up of children born from frozen embryo, a narrative review of latest research findings. Medicina (Kaunas) 58.

Hajek, J., Baron, R., Sandi-Monroy, N., Schansker, S., Schoepper, B., Depenbusch, M., Schultze-Mosgau, A., Neumann, K., Gagsteiger, F., Von Otte, S., Griesinger, G., 2021. A randomised, multi-center, open trial comparing a semi-automated closed vitrification system with a manual open system in women undergoing IVF. Hum. Reprod. 36, 2101–2110.

Holmes, R., Wirka, K.A., Catherino, A.B., Hayward, B., Swain, J.E., 2021. Comparison of electronic versus manual witnessing of procedures within the in vitro fertilization laboratory: impact on timing and efficiency. F&S Reports. 2, 181–188.

Huang, C., Lei, L., Wu, H.L., Gan, R.X., Yuan, X.B., Fan, L.Q., Zhu, W.B., 2019. Long-term cryostorage of semen in a human sperm bank does not affect clinical outcomes. Fertil. Steril. 112, 663–669 e1.

Hubalek, Z., 2003. Protectants used in the cryopreservation of microorganisms. Cryobiology 46, 205–229.

Intra, G., Alteri, A., Corti, L., Rabellotti, E., Papaleo, E., Restelli, L., Biondo, S., Garancini, M.P., Candiani, M., Vigano, P., 2016. Application of failure mode and effect analysis in an assisted reproduction technology laboratory. Reprod. Biomed. Online. 33, 132–139.

ISO 21973:2020: https://www.iso.org/standard/72326.html, 07/2023.

Jensen, C.F.S., Dong, L., Gul, M., Fode, M., Hildorf, S., Thorup, J., Hoffmann, E., Cortes, D., Fedder, J., Andersen, C.Y., Sonksen, J., 2022. Fertility preservation in boys facing gonadotoxic cancer therapy. Nat. Rev. Urol. 19, 71–83.

Joaquim, D.C., Borges, E.D., Viana, I.G.R., Navarro, P.A., Vireque, A.A., 2017. Risk of contamination of gametes and embryos during cryopreservation and measures to prevent cross-contamination. Biomed. Res. Int., 1840417.

Kushnir, V.A., Darmon, S.K., Barad, D.H., Gleicher, N., 2018. New national outcome data on fresh versus cryopreserved donor oocytes. J. Ovarian. Res. 11, 2.

Lee, B.U., 2020. Cryogenic aerosol generation: airborne mist particles surrounding liquid nitrogen. Int J Environ Res Public Health 17.

Lesourd, F., Izopet, J., Mervan, C., Payen, J.L., Sandres, K., Monrozies, X., Parinaud, J., 2000. Transmissions of hepatitis C virus during the

ancillary procedures for assisted conception. Hum. Reprod. 15, 1083–1085.
Logsdon, D.M., Grimm, C.K., Schoolcraft, W.B., Mccormick, S., Schlenker, T., Swain, J.E., Krisher, R.L., Yuan, Y., Collins, M.G., 2021. Evaluation of the TMRW vapor phase cryostorage platform using reproductive specimens and in vitro extended human embryo culture. F. S. Sci. 2, 268–277.
Ma, Y., Liu, X., Shi, G., Liu, Y., Zhou, S., Hou, W., Xu, Y., 2021. Storage time of cryopreserved embryos and pregnancy outcomes: a dose-response meta-analysis. Geburtshilfe. Frauenheilkd. 81, 311–320.
Maggiulli, R., Giancani, A., Fabozzi, G., Dovere, L., Tacconi, L., Amendola, M.G., Cimadomo, D., Ubaldi, F.M., Rienzi, L., 2020. Assessment and management of the risk of SARS-CoV-2 infection in an IVF laboratory. Reprod. Biomed. Online. 41, 385–394.
Maheshwari, A., Mclernon, D., Bhattacharya, S., 2015. Cumulative live birth rate: time for a consensus? Hum. Reprod. 30, 2703–2707.
Maxwell, S.M., Goldman, K.N., Labella, P.A., Mccaffrey, C., Noyes, N.L., Grifo, J., 2014. Live birth in a 46 year old using autologous oocytes cryopreserved for a duration of 3 years: a case report documenting fertility preservation at an advanced reproductive age. J. Assist. Reprod. Genet. 31, 651–655.
McBurnie, L.D., Bardo, B., 2002. Validation of Sterile Filtration of Liquid Nitrogen. Pharmac. Tech. North America 26, 74–82.
Mcdonald, C.A., Valluzo, L., Chuang, L., Poleshchuk, F., Copperman, A.B., Barritt, J., 2011. Nitrogen vapor shipment of vitrified oocytes: time for caution. Fertil. Steril. 95, 2628–2630.
Měřička, P., Straková, H., Štěrba, L., 2012. Safety and quality assurance in donation, harvest and preservation of cells and tissues of human origin-a review of own experience. Conference Paper, http://www.researchgate.net/publication/270888540.
Meseguer, M., Kruhne, U., Laursen, S., 2012. Full in vitro fertilization laboratory mechanization: toward robotic assisted reproduction? Fertil. Steril. 97, 1277–1286.
Miao, S., Guo, C., Jiang, Z., Wei, H.X., Jiang, X., Gu, J., Hai, Z., Wang, T., Liu, Y.H., 2022. Development of an open microfluidic platform for oocyte one-stop vitrification with cryotop method. Biosensors (Basel) 12.
Mocanu, E., Drakeley, A., Kupka, M.S., Lara-Molina, E.E., Le Clef, N., Ombelet, W., Patrat, C., Pennings, G., Semprini, A.E., 2021. ESHRE guideline: medically assisted reproduction in patients with a viral infection/disease. Hum. Reprod. Open. hoab037.
Mortimer, D., 2004. Current and future concepts and practices in human sperm cryobanking. Reprod. Biomed. Online. 9, 134–151.
Mortimer, D., Cohen, J., Mortimer, S.T., Fawzy, M., Mcculloh, D.H., Morbeck, D.E., Pollet-Villard, X., Mansour, R.T., Brison, D.R., Doshi, A., Harper, J.C., Swain, J.E., Gilligan, A.V., 2018. Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting. Reprod. Biomed. Online. 36, 658–674.
Nesbit, C., Porter, M.B., Esfandiari, N., 2022. Catastrophic human error in assisted reproductive technologies: a systematic review. J. Patient Saf. 18, e267–e274.
Nijs, M., Verheyen, G., 2016. Personnel practices in an IVF clean room facility,. In: Esteves, S., Varghese, A.C., Worrilow, K.C. (Eds.), Clean Room Technology in ART Clinics: A Practical Guide. CRC Press, Boca Raton, pp. 193–202.
Palmer, G., Parker, P., Dawson-Smith, K., Igbokwe, N., 2020. Improving sample identification and reducing risks during cryostorage of vitrified gametes and embryos using radio frequency identification (RFID) tags that operate while samples are immersed in liquid nitrogen. Cryoletters. C/O Royal Veterinary College, Royal College St, London Nw1 0tu, England. Cryo Letters 177–178.
Paoli, D., Dal Canto, M., Baldi, E., Cervi, M., Ciotti, P.M., Ciriminna, R., Dabizzi, S., Farace, D., Garello, C., Garolla, A., Giacchetta, D., Gualtieri, R., Menegazzo, M., Minasi, M.G., Oneta, M., Pisaturo, V., Rienzi, L., Scarica, C., Taliani, G., De Santis, L., 2021. Cryopreserved gamete and embryo transport: proposed protocol and form templates-SIERR (Italian Society of Embryology, Reproduction, and Research). Biopreserv Biobank 19, 27–32.
Pariz, J.R., Monteiro, R.A.C., Hallak, J., 2020. Long-term sperm cryopreservation does not affect post-thaw survival rates. JBRA Assist. Reprod. 24, 3–8.
Parmegiani, L., Accorsi, A., Bernardi, S., Arnone, A., Cognigni, G.E., Filicori, M., 2012. A reliable procedure for decontamination before thawing of human specimens cryostored in liquid nitrogen: three washes with sterile liquid nitrogen (SLN2). Fertil. Steril. 98, 870–875.
Parmegiani, L., Accorsi, A., Cognigni, G.E., Bernardi, S., Troilo, E., Filicori, M., 2010. Sterilization of liquid nitrogen with ultraviolet irradiation for safe vitrification of human oocytes or embryos. Fertil. Steril. 94, 1525–1528.
Parmegiani, L., Fabbri, R., Cognigni, G.E., Bernardi, S., Pocognoli, P., Filicori, M., 2008. Blastocyst formation, pregnancy, and birth derived from human oocytes cryopreserved for 5 years. Fertil. Steril. 90, e7–10 2014.
Parmegiani, L., Garello, C., Granella, F., Guidetti, D., Bernardi, S., Cognigni, G.E., Revelli, A., Filicori, M., 2009. Long-term cryostorage does not adversely affect the outcome of oocyte thawing cycles. Reprod. Biomed. Online. 19, 374–379.
Parmegiani, L., Maccarini, A.M., Rastellini, A., Bernardi, S., Troilo, E., Arnone, A., Lanzilotti, S., Filicori, M., 2017. Oocyte vitrification/storage/handling/transportation/warming, effect on survival and clinical results in donation programmes. Curr. Trends. Clin. Embryol. 4, 34–40.
Parmegiani, L., Vajta, G., 2020. The effect of cryostorage duration on vitrified embryos: has vitrification suddenly become unsafe? Hum. Reprod. 35, 2632–2633.
Parmegiani, L., Vajta, G., 2021. COVID-19 in liquid nitrogen: a potential danger still disregarded. Hum. Reprod. 36, 260.
Pirnay, J-P., Vanderkelen, A., De Vos, D., Draye, J-P., Rose, T., Ceulemans, C., Ectors, N., Huys, I., Jennes, S., Verbeken, G., 2013. Business oriented EU human cell and tissue product legislation will adversely impact Member States' health care systems. Cell. Tissue. Bank. 14, 525–560.
Pomeroy, K.O., Harris, S., Conaghan, J., Papadakis, M., Centola, G., Basuray, R., Battaglia, D., 2010. Storage of cryopreserved reproductive tissues: evidence that cross-contamination of infectious agents is a negligible risk. Fertil. Steril. 94, 1181–1188.
Pomeroy, K.O., Schiewe, M.C., 2020. Cryopreservation and IVF in the time of Covid-19: what is the best good tissue practice (GTP)? J. Assist. Reprod. Genet. 37, 2393–2398.
Porcu, E., Tranquillo, M.L., Notarangelo, L., Ciotti, P.M., Calza, N., Zuffa, S., Mori, L., Nardi, E., Dirodi, M., Cipriani, L., Labriola, F.S., Damiano, G., 2021. High-security closed devices are efficient and safe to protect human oocytes from potential risk of viral contamination during vitrification and storage especially in the COVID-19 pandemic. J. Assist. Reprod. Genet. 38, 681–688.
Porcu, E, Cipriani, L, Dirodi, M, De Iaco, P, Perrone, AM, Zinzani, PL, Taffurelli, M, Zamagni, C, Ciotti, PM, Notarangelo, L, Calza, N, Damiano, G., 2022 Mar 10. Successful Pregnancies, Births, and Children Development Following Oocyte Cryostorage in Female Cancer Patients During 25 Years of Fertility Preservation. Cancers (Basel) 14 (6), 1429.
Quintans, C.J., Donaldson, M.J., Urquiza, M.F., Carretero, I., Pasqualini, R.A., Horton, M., Pasqualini, R-S., 2012. Live birth of twins after IVF of oocytes that were cryopreserved almost 12 years before. Reprod. Biomed. Online. 25, 600–602.
Rienzi, L., Bariani, F., Dalla Zorza, M., Albani, E., Benini, F., Chamayou, S., Minasi, M.G., Parmegiani, L., Restelli, L., Vizziello, G., Costa, A.N., Italian Society Of Embryology Reproduction and Research, 2017a. Comprehensive protocol of traceability during IVF: the result of a multicentre failure mode and effect analysis. Hum. Reprod. 32, 1612–1620.
Rienzi, L, Cobo, A, Paffoni, A, Scarduelli, C, Capalbo, A, Vajta, G, Remohi, J, Ragni, G, Ubaldi, FM., 2012. Consistent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study. Hum. Reprod. 27, 1606–1612.
Rienzi, L., Gracia, C., Maggiulli, R., Labarbera, A.R., Kaser, D.J., Ubaldi, F.M., Vanderpoel, S., Racowsky, C., 2017b. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum. Reprod. Update. 23, 139–155.
Rofeim, O., Gilbert, B.R., 2005. Long-term effects of cryopreservation on human spermatozoa. Fertil. Steril. 84, 536–537.
Roy, T.K., Bradley, C.K., Bowman, M.C., Mcarthur, S.J., 2014a. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil. Steril. 101, 1294–1301.
Roy, T.K., Brandi, S., Tappe, N.M., Bradley, C.K., Vom, E., Henderson, C., Lewis, C., Battista, K., Hobbs, B., Hobbs, S., Syer, J., Lanyon, S.R., Dopheide, S.M., Peura, T.T., Mcarthur, S.J., Bowman, M.C., Stojanov, T., 2014b. Embryo vitrification using a novel semi-automated closed system yields in vitro outcomes equivalent to the manual Cryotop method. Hum. Reprod. 29, 2431–2438.
Sansinena, M., Santos, M.V., Chirife, J., Zaritzky, N., 2018. In-vitro development of vitrified-warmed bovine oocytes after activation may be predicted based on mathematical modelling of cooling and warming rates during vitrification, storage and sample removal. Reprod. Biomed. Online. 36, 500–507.
Sansinena, M., Santos, M.V., Taminelli, G., Zaritky, N., 2014. Implications of storage and handling conditions on glass transition and

potential devitrification of oocytes and embryos. Theriogenology. 82, 373–378.
Scarica, C., Parmegiani, L., Rienzi, L., Anastasi, A., Cimadomo, D., Klinger, F.G., Licata, E., Sosa Fernandez, L., De Santis, L., 2021. SARS-CoV-2 persistence at subzero temperatures. J. Assist. Reprod. Genet. 38, 779–781.
Schiewe, M., Schiewe, E., Vu, V., Zozula, S., Anderson, R., 2016. Liquid nitrogen vapor sealing of straw containers can be unsafe and detrimental to embryo survival. Austin. J. Reprod. Med. Infertil. 3, 1038–1041.
Schiewe, M.C., Freeman, M., Whitney, J.B., Vermilyea, M.D., Jones, A., Aguirre, M., Leisinger, C., Adaniya, G., Synder, N., Chilton, R., Behnk, E.J., 2019. Comprehensive assessment of cryogenic storage risk and quality management concerns: best practice guidelines for ART labs. J. Assist. Reprod. Genet. 36, 5–14.
Silvestris, E., De Palma, G., Canosa, S., Palini, S., Dellino, M., Revelli, A., Paradiso, A.V., 2020. Human ovarian cortex biobanking: a fascinating resource for fertility preservation in cancer. Int. J. Mol. Sci. 21 (9), 3245.
Simione, F., Sharp, T., 2017. Best practices for storing and shipping cryopreserved cells. In Vitro Cell. Dev. Biol. Anim. 53, 888–895.
Szeptycki, J., Varghese, A., 2018. Recent Advancement in the Maintenance of Laboratory Quality Control in Practical Guide in Andrology and Embryology. Jaypee Medical, India. doi.org/10.5005/jp/books/18073_19.
Tannus, S., Dahan, M.H., Tan, J., Tan, S.L., 2018. Issues related to human oocyte vitrification: a consideration of the facts. J. Assist. Reprod. Genet. 35, 1157–1158.
Tedder, R.S., Zuckerman, M.A., Goldstone, A.H., Hawkins, A.E., Fielding, A., Briggs, E.M., Irwin, D., Blair, S., Gorman, A.M., Patterson, K.G., et al., 1995. Hepatitis B transmission from contaminated cryopreservation tank. Lancet. 346, 137–140.
Tirgar, P., Sarmadi, F., Najafi, M., Kazemi, P., Azizmohseni, S., Fayazi, S., Zandi, G., Ziaie, N., Shoushtari Zadeh Naseri, A., Ehrlicher, A., Dashtizad, M., 2021. Toward embryo cryopreservation-on-a-chip: a standalone microfluidic platform for gradual loading of cryoprotectants to minimize cryoinjuries. Biomicrofluidics. 15, 034104.
Tomlinson, M., Morroll, D., 2008. Risks associated with cryopreservation: a survey of assisted conception units in the UK and Ireland. Hum. Fertil. (Camb) 11, 33–42.
Tomlinson, M.J., 2018. Safe storage of gametes and embryos: no time for complacency. Semin. Reprod. Med. 36, 289–298.
Tomlinson, M.J., Pacey, A.A., 2003. Practical aspects of sperm banking for cancer patients. Hum. Fertil. (Camb) 6, 100–105.
Torra-Massana, M, Miguel-Escalada, I, Vassena, R, Rodríguez, A., 2023 May 3. Long-term storage of vitrified oocytes does not affect pregnancy and live birth rates: analysis of 5362 oocyte donation cycles. Reprod. Biomed. Online. S1472-6483(23)00274-2.
Urquiza, M.F., Carretero, I., Cano Carabajal, P.R., Pasqualini, R.A., Felici, M.M., Pasqualini, R.S., Quintans, C.J., 2014. Successful live birth from oocytes after more than 14 years of cryopreservation. J. Assist. Reprod. Genet. 31, 1553–1555.
Vajta, G., Rienzi, L., Ubaldi, F.M., 2015. Open versus closed systems for vitrification of human oocytes and embryos. Reprod. Biomed. Online. 30, 325–333.
Van Reckem, M., Blockeel, C., Bonduelle, M., Buysse, A., Roelants, M., Verheyen, G., Tournaye, H., Hes, F., Belva, F., 2021. Health of 2-year-old children born after vitrified oocyte donation in comparison with peers born after fresh oocyte donation. Hum. Reprod. Open. hoab002.
Wang, M., Isachenko, E., Todorov, P., Rahimi, G., Mallmann, P., Katkov, Ii., Isachenko, V., 2020. Aseptic technology for cryoprotectant-free vitrification of human spermatozoa by direct dropping into clean liquid air: apoptosis, necrosis, motility, and viability. Biomed. Res. Int., 2934315.
Yogev, L., Kleiman, S.E., Shabtai, E., Botchan, A., Paz, G., Hauser, R., Lehavi, O., Yavetz, H., Gamzu, R., 2010. Long-term cryostorage of sperm in a human sperm bank does not damage progressive motility concentration. Hum. Reprod. 25, 1097–1103.
Zaat, T., Zagers, M., Mol, F., Goddijn, M., Van Wely, M., Mastenbroek, S., 2021. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane. Database. Syst. Rev. 2, CD011184.
Zhu, Q., Chen, Q., Wang, L., Lu, X., Lyu, Q., Wang, Y., Kuang, Y., 2018. Live birth rates in the first complete IVF cycle among 20 687 women using a freeze-all strategy. Hum. Reprod. 33, 924–929.

Received 9 August 2022; received in revised form 12 June 2023; accepted 14 June 2023.