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EDITORIAL

Embryo Cryopreservation: The Significant Other of ART

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In October of 2022, the birth of twin babies to a couple in Oregon made headlines in the USA [1]. The babies were born from adopted embryos that were cryopreserved 30 years ago and donated to the National Embryo Donation Centre, a private faith-based organization. Third-party reproduction has emerged as a treatment option in countries where they are medically and legally available [2], due to the central role of cryopreservation in Assisted Reproductive Technologies (ART).

Embryo cryopreservation was introduced 50 years ago [3,4], and it is now considered a central technique in ART, without which the event of October 2022 may not have taken place. The indication for elective and non-elective oocyte and embryo cryopreservation has grown due to the changing landscape of socio-culture and advancements in medical treatments. Cryopreservation of embryos, which was originally confined to medical indications has since expanded to include elective uses. Elective embryo cryopreservation may be useful for banking, donation, deferred childbearing, preimplantation genetic testing, and storage of surplus embryos. It also maximizes fertility potential per retrieval cycle and has helped pave the way for single embryo transfer, thereby decreasing the risk of multiple gestation pregnancy and the health risks associated with it. Non-elective embryo cryopreservation is indicated for fertility preservation such as in patients undergoing chemotherapy.

The first human pregnancy from a cryopreserved embryo was achieved in 1983 [5], and the first live birth resulting from a cryopreserved embryo was reported in 1985 [6]. The application of embryo transfer with cryopreserved embryos is now widely used as the pregnancy rates are found to be at par with those following non-cryopreserved embryo transfers.

Cryoprotectants in Cryopreservation

Advances in techniques for cryopreservation and development of ideal cryoprotectants have been key developments in ART. Cryopreservation generally requires the use of cryoprotectants (CPAs) or solutions that protect the cells of the embryo from osmotic stress and damage caused by very low-temperature cooling. The application of CPAs is necessary to protect the embryo from freezing injury. Cryoprotectants can be non-permeating or permeating in nature. Non-permeating CPAs are larger molecules that confer cryoprotection but remain outside the cell. These CPAs include sucrose, dextran, trehalose, and Ficoll®.

Permeating CPAs, which provide intracellular protection include dimethyl sulfoxide (DMSO), glycerol, ethylene glycol, and propylene glycol. Apart from being minimally toxic, it is necessary that permeating agents be able to easily cross biological membranes and be water soluble at low temperatures [7]. These CPAs reduce ice crystallization and promote cell dehydration. Although toxic to the cells at high concentrations, CPAs are necessary to protect embryos against cell death during cryopreservation. Over the years, numerous studies have been conducted to search for effective CPAs that are less toxic. This has led to the introduction of protocols for the combined use of several CPAs. Such combinations provide a delicate

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balance between the protective and toxic effects, aimed to maintain the functional roles of the organelles. This effort is challenging as the total molarity of a mixture may not be a reliable indicator of embryotoxicity [8].

Vitrification versus Slow Freezing

Over the years, two methods have been used for embryo cryopreservation, namely slow freezing, and vitrification. Slow freezing was discovered by researchers in 1945, who found that the use of large containers for freezing semen reduced the rate of cooling and gave the best post-thaw motilities [9]. This indicated that slower cooling rates were associated with better cell viability, improved cellular dehydration, and therefore decreased risk of intracellular ice crystal formation. The knowledge was used to further develop cell-specific optimal cooling rates [10] and ice crystal seeding [11].

The concentration of CPAs used in slow freezing is low, thereby exposing embryos to less toxicity. Nonetheless, cooling rates must be slow enough to minimize the risk of cryoinjury because of intracellular ice formation. Slow freezing is normally performed using a programmable freezer. In the early 1970s, two groups used slow freezing with a CPA containing 1 mol/l of DMSO, which resulted in the first survival of murine embryos [3,4]. Later, first pregnancies and birth from frozen thawed human embryos were also recorded [5,6]. There were also reports on human live births with the application of other CPAs such as propanediol and sucrose, which proved to be more reliable and widely adopted [12].

Vitrification, on the other hand, involves the use of high concentrations of cryoprotectants, which exposes embryos to greater toxicity. However, the benefit of this method is that it uses high cooling rates, and intracellular ice formation does not occur as temperature decreases toward sub-zero. Vitrified samples are solidified to a glass-like state. The unique feature of vitrification was discovered by Gay Lussac in the early 19th century with the observation of supercooling. The revival of vitrification took place in 1985 when Rall and Fahy observed that concentrated ethylene glycol (EG) solidified to form an amorphous glass state that prevented ice crystal formation [13]. Since then, vitrification has been reported to produce better embryo survival rates, which in turn improved the effectiveness of embryo transfer and IVF treatment, as well as cumulative pregnancy rates.

Vitrification has gradually replaced slow freezing as the preferred method of cryopreservation in the field of reproductive medicine due to its unique capability of eliminating the formation of ice crystals. In comparison with slow freezing techniques, vitrification of human oocytes and embryos has been reported to result in higher survival rates and better clinical outcomes [14]. It requires less time to perform and does not require highly specialized and expensive equipment like programmable freezers.

Freeze-all strategy in ART

The phrase "freeze-all" refers to the cryopreservation of all mature oocytes or viable embryos following ovarian stimulation. In recent years, there has been a move towards the freeze-all strategy where women above 35 years, and those diagnosed with ovarian hyperstimulation syndrome (OHSS) were highly encouraged to have their embryos cryopreserved for future attempts at fertilization [15]. The need to cryopreserve arose as ovarian hyperstimulation in ART led to the production of supernumerary embryos. As most clinics advocate the transfer of single embryos to reduce the risk of complications related to multiple embryo transfers, cryopreservation has become a valuable procedure to preserve surplus oocytes and embryos in ART cycles. The ability to cryopreserve supernumerary embryos following the initial transfer reduces the need for and the risk of repeated ovarian hyperstimulation. It expands the cumulative success of single IVF cycles for female patients [16].

Issues and Challenges in the Cryopreservation of Embryos

Although the technology and use of embryo cryopreservation are necessary, the verification of safety to the mother and transferred frozen embryo remains lagging. There have been numerous reports of risks and issues over the years [17]. Some studies have shown increased risks of placental issues, pregnancy-induced hypertension, and pre-eclampsia after frozen embryo transfer (FET) [18,19,20].

A cumulative meta-analysis has also reported increased risks for large babies and hypertensive disorders of pregnancy with FET [21]. At subcellular levels, cryopreservation has been reported to alter chromatin architecture, which subsequently affects embryonic cleavage and progression [22]. At the molecular level, cryopreservation of embryos in which embryos are exposed to osmotic shock and CPA toxicity may trigger epigenomic changes. The use of DMSO as a CPA has also been shown to induce drastic alterations in cells and their epigenetic landscape [23]. Cryopreservation parameters could also lead to oxidative stress, which could alter DNA methylation [24]. Clearly, there is still much to learn about the long-term consequences of cryopreservation of embryos on the mother and the newborn.

Worldwide, the number of children born after ART using FET currently exceeds the number of children born after fresh embryo transfer. Given that many embryos are usually produced during an ART cycle, many couples face the dilemma of what to do with unused cryopreserved embryos after the ART procedures are completed. For couples who plan to continue to expand their family, this is less critical because they can choose to save unused cryopreserved embryos for the next pregnancy. However, for couples who no longer have a need, the decision on what to do with the spare embryos may not be simple or straight forward. Personal, socio-cultural, and religious factors will, no doubt, come into play when the time comes to decide on the fate of the spare frozen embryos [25]. Although there are options for embryo adoption or embryo donation for scientific research or embryo disposal, all of these, however, may not be ideal choices for all the couples.

Embryo adoption can be an open adoption, in which the recipients are known, or closed adoption, in which the recipients are unknown [26]. For some couples, the decision to donate may seem natural but to others it might pose a dilemma, thinking of their genetic offspring being raised by another couple. Embryo donation for research might seem an easy route in the belief that it will help advance science and develop better subfertility treatments for the future [27]. But when one begins to explore this issue further, this option may not be as clear or straight forward as it seems at first. Should there be a limit to the type of research that can be carried out on these donated embryos? Can we control the type of research that can be carried out on these embryos? Do we have clear cut guidelines on these? How can we ensure that these donated embryos do not get into the hands of some rogue scientists? Disposing of the extra embryos – how can it done? Disposal of embryos after thawing is generally done either in the cryogenic facility or ART clinic. Is this acceptable to all couples? Could the disposal procedure called compassionate transfer be offered more often? In this procedure, supernumerary frozen-thawed embryos are transferred into the patient during her infertile period, where they are left to degenerate [28]. This might be a more acceptable way of disposable to some couples and provides them with a better peace of mind. Studies are needed on the views and feelings of couples who have undergone ART using cryopreserved embryos on how they feel about the way the spare embryos were handled. This will certainly help the experts in advising the couples on the handling of spare embryos. It is important that the issue of spare embryos is clearly explained to all couples before they undergo ART. It might help them make a better decision on whether to go with ART or not, and what to do with the extra embryos afterwards should they decide to undergo ART.

In view of some evidence pointing to the possible detrimental effects of embryo cryopreservation, ART practitioners should continue to tread with caution [29]. Although the freeze-all strategy presents opportunities to improve clinical outcomes, indications for freeze-all should be considered thoroughly. At this point, new insights from long-term studies are warranted to improve the safety of FET for maternal and offspring health. There is also a need for each society or community to develop policies and practices for the handling of the spare or extra embryos, particularly as the ART technology is spreading rapidly and more and more embryo banks are being established throughout the world.

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