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Review article

Cryopreservation of animal oocytes and embryos: Current progress and future prospects

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ABSTRACT

Cryopreservation describes techniques that permit freezing and subsequent warming of biological samples without loss of viability. The application of cryopreservation in assisted reproductive technology encompasses the freezing of gametes, embryos, and primordial germ cells. Whilst some protocols still rely on slow-freezing techniques, most now use vitrification, or ultra-rapid freezing, for both oocytes and embryos due to an associated decreased risk of damage caused by the lack of ice crystal formation, unlike in slow-freezing techniques. Vitrification has demonstrated its use in many applications, not only following IVF procedures in human embryology clinics but also following *in vitro* production of embryos in agriculturally important, or endangered animal species, before embryo transfer. Here, we review the various cryopreservation and vitrification technologies that are used in both humans and other animals and discuss the most recent innovations in vitrification with a particular emphasis on their applicability to animal embryology.

1. Introduction

Over the last few decades, cryopreservation techniques have progressed rapidly. This progress has made a significant impact in many fields, with reproductive medicine possibly the most significant. From initial success in cryopreservation of sperm [1], it is now routinely used for the preservation of oocytes, sperm, and embryos within both agricultural systems and in assisted reproductive technology (ART) in humans. Cryopreservation is a process by which biological cells or tissues are preserved at subzero temperatures resulting in a radical decrease in the rate of metabolic processes and the ability to store samples for extended periods [2]. However, as would be expected, freezing cells causes damage and this must be circumvented. The two major causes of cellular damage are the physical damage caused by the formation of ice crystals and the chemical damage that results from changes in intracellular solute concentrations. Both of these damage types can be avoided, or at least ameliorated, by controlling how the temperature is reduced and by modifying the cellular conditions. For instance, the mechanical damage that results from the piercing action of ice crystals can be avoided by making the freezing process very rapid and the significant rise in intracellular solute concentration as the formation of ice crystals increases can be avoided by use of cryoprotectants [3]. Permeating cryoprotectants replace intracellular liquid and decrease ice formation [4]; as such they need to have low toxicity, be capable of penetrating cells, and be able to withstand extremely low temperatures. Examples of commonly used cryoprotectants include glycerol, ethanediol, dimethyl sulfoxide, ethylene glycol, and propanediol [5]. Whilst most cells cannot survive the freezing process without use of a cryoprotectant, it is also important to note that simply using such solutions alone is insufficient for cell survival after freezing (and thawing);

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survival also depends on the cell type and its ability to withstand various stresses caused by physical and physiochemical changes during the process, as well as rates of cooling and warming [5].

2. The promise of vitrification

Vitrification eradicates damage caused due to ice crystal formation during the cooling process. The method involves rapid cooling and liquid solidification due to a substantial rise in viscosity and results in the formation of a solid glass-like form [6]. This solid 'glassy' layer is amorphous; meaning that it can readjust and take the shape of the cell, hence enabling the cell to maintain its structure and remain intact, unlike in slow-freezing, where the formation of ice crystals during cooling prevents the cell from maintaining its structure. See Figure 1 for a schematic comparison of slow-freezing and vitrification.

There have been a number of studies that have compared slow-freezing techniques and vitrification in human embryology [7,8]; whilst it seems that there is a gradual move toward more widespread use of vitrification clinically, the literature to date describes no consensus as to which method is the best. For example, Herrero et al. [9] established that cryopreservation of both human oocytes and blastocysts demonstrate competitive pregnancy rates when compared to those obtained through implantation of fresh samples and that vitrification was preferable in terms of minimized cellular damage and higher post-warming survival rates when compared to traditional freezing processes [9,10]. Whilst some still opt for a traditional slow-cooling method via the use of insemination straws, others now use fast-cooling vitrification techniques, employing an array of different vitrification systems including; thin capillaries or straws, Cryotop, Cryoloop, Cryolock, CryoTip, nylon mesh, plastic blades, Vitri-ingá, electron microscopic grid, Gavi, and the minimum drop size technique [11]. For example, Kuwayama [12] discusses the efficiency of the Cryotop method, wherein he states that cryopreservation of blastocysts using the Cryotop method resulted in more live births when compared to any other vitrification system. Moreover, Mukaida et al. [13] used clinical results from 725 human blastocysts (of which 80.4% survived vitrification and warming) to establish that the Cryoloop system can be used as an effective method for vitrification of human blastocysts. In addition, Sugiyama et al. [14] tested the effect of a plastic blade as a cryopreservation device on survival rates of human embryos and blastocysts following vitrification and warming. Interestingly, this reported that whilst 98.4% of cleavage stage embryos survived vitrification and subsequent warming, all of the blastocysts survived. In a comparative study, Desai et al. [15] comparatively assessed three cryo-devices on the impact of vitrification (nylon mesh, micro-capillary tips, and an electron microscopy grid), of murine preantral follicles and found no significant differences for subsequent *in vitro* development following vitrification. However, a low-survival rate was observed for follicles vitrified using micro-capillary tips, and it was revealed that when a large number of follicles required vitrification, a nylon mesh was most successful. In 2008, Vitri-ingá, was developed and tested on bovine oocytes; the device showed promising results with an 86% survival rate after warming [16]. The method was subsequently adapted for use with human oocytes and in 2010, Almodin et al. [17] evaluated the device's success, by comparing gestational results achieved via use of frozen-warmed human oocytes vitrified using Vitri-ingá and by those that did not undergo vitrification. The technology was tested clinically on 125 human patients, of which 79 patients received embryos that were derived from fresh oocytes, whereas 46 patients were implanted with embryos that were developed using frozen-warmed oocytes vitrified by Vitri-ingá technology; a high-survival rate of 84.9% was reported by oocytes that underwent vitrification. Moreover, no significant differences were reported for fertilization, implantation, or pregnancy rates between the patients of the two groups. Successes of vitrification methods have resulted in IVF clinics around the world progressively shifting away from traditional slow-freezing methods for routine use in ART [18,19]. As is evident here, new vitrification techniques are constantly being developed and these can be broadly classified as open or closed—the distinction depending on the degree to which there is, or is not, direct contact between the media and the liquid nitrogen used during the cooling process.

3. Open and closed vitrification systems: A comparison

In an open system, the oocytes or embryos come into direct contact with liquid nitrogen, whereas in a closed system, they do not. Direct comparisons between these types of systems have been limited; however, the available evidence suggests that the viability of oocytes and embryos after warming can be similar. For instance, Papatheodorou et al. [20] compared open and closed systems by conducting a randomized trial using human sibling oocytes. Whilst survival rates following vitrification using the closed system (82.9%) were slightly lower than that of the open system (91.0%), there was no significant effect on observed, clinical, or ongoing pregnancies between the two groups. Moreover, the closed system group produced higher live birth rates as well as a higher number of healthy babies (27 vs. 18). Researchers in Tokyo demonstrated similar findings, showing no significant difference between blastocyst survival rates using the CryoTip (closed system) and the Cryotop (available as either an open or closed carrier system) [21]. Comparisons were also made between slow-freezing and ultra-rapid vitrification of human embryos, which indicated that vitrification was the most reliable; these results are summarized in Table 1. Similarly, comparisons between Rapid-i and Cryotop [22], and between Vit Kit Freeze/Thaw (Irvine Scientific, CA, USA) Global Fast Freeze/Thaw Kits (LifeGlobal, Canada) [23], indicate that these systems can produce comparable results.

Interestingly, conflicting evidence by Paffoni et al. [24] revealed considerably lower pregnancy rates and a higher ratio of canceled cycles for vitrification of mature human oocytes using a closed system, as opposed to an open