Oocyte Cryopreservation in Domestic Animals and Humans

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Oocyte cryopreservation plays important roles in basic research and the application of models for genetic preservation and in clinical situations. This technology provides long-term storage of gametes for genetic banking and subsequent use with other assisted reproductive technologies.

Keywords: animal ; cryopreservation ; oocyte

1. General Aspects of Oocyte Cryopreservation

The success of oocyte cryopreservation was first reported in the 1970s [1][2]. It has become clear that cryopreservation processes inevitably induce cellular and molecular changes that render poor fertilization rate and embryo development [3][4][5]. Controlled-rate slow cryopreservation and vitrification are the two freezing techniques that are clinically applied to oocyte cryopreservation in animals and humans. Slow freezing principally requires a relatively low concentration of cryoprotective agent (CPA), applied with sufficiently slow cooling/freezing rates to ensure a fine control over various factors (i.e., thermal shock) that contribute to cell damage [6]. By gradually decreasing the rate of supra- to sub-zero cooling, the CPA allows adequate cellular dehydration leading to minimal intracellular ice [6][7]. At subzero temperatures, the essential step of slow freezing is so-called “seeding”, a process which induces extracellular ice formation by converting the unfrozen solution to a hyperosmotic state, inducing cell dehydration [8]. With a slow-freezing approach, intracellular water is converted into a glassy phase composed of small intracellular ice crystals [7]. Therefore, super-rapid warming is required for the thawing process to avoid extensive crystallization and cell damage [7]. In contrast, vitrification requires an extremely high concentration of CPA and also an ultrafast freezing rate [9]. During cryopreservation, cells are exposed to several unfamiliar environments, such as chemical toxicity, osmotic changes and low temperature, all of which potentially disrupt cell functions and result in cell death [4][10][11]. Indeed, several factors, including species differences, age and fertility of oocyte donor, stage of oocyte maturation and cryopreservation protocols, have been reported to affect the success of oocyte cryopreservation. Notably, a large variation in oocyte physiology in particular animals cause difficulties in obtaining a consensus on freezing protocols. In some species, such as porcines, high contents of lipids have been claimed to cause poor oocyte freezing ability [12][13]. Thus, the development of freezing techniques and outcomes in terms of fertilization rate, embryo development and pregnancy rate following embryo transfer have been variable among species and laboratories. This aspect is very important for species for which oocytes are not readily available, such as wild species. In this case, anatomically and physiologically related domestic species are logically used to develop suitable cryopreservation techniques. Likewise, the availability of human oocytes for experimental purposes is very limited due to ethical reasons. According to similarities in reproductive physiology between nonhuman primates and humans, such as menstrual cycle length and hormonal profiles in rhesus macaques, nonhuman primates have been important in reproductive biology research during the last two decades [14]. Studies on nonhuman primates as human models in the fields of reproductive biology, reproductive medicine and assisted reproductive technology (ART) have been conducted for decades [14]. For oocyte cryopreservation, research studies using nonhuman primates were established in the late 1980s to early 1990s [15][16] where the principle of osmotic shock being responsible for oocyte quality was agreed [17]. In humans, the first achievement of pregnancy after oocyte cryopreservation was reported in 1986 [18]. However, limited success in oocyte cryopreservation discouraged this technique in routine clinical application for several years [19]. In the 2000s, knowledge of cryobiology by vitrification introduced the possibility of effectively cryopreserving functional oocytes, leading to revolutions in oocyte cryopreservation programs in clinical practice [20]. This was supported by a large randomized clinical trial on oocyte donation that revealed that vitrified oocyte quality was not inferior to fresh oocytes in terms of pregnancy outcomes [21]. Later, oocyte cryopreservation has become a fascinating alternative option for women who attempt in vitro fertilization (IVF) or fertility preservation programs [22]. Benefits of oocyte cryopreservation include increased flexibility to preserve (1) excess oocytes eventually present in each subsequent IVF cycle; (2) fertility in women who are at risk of infertility caused by chemotherapy/radiotherapy/premature ovarian insufficiency (POI) or who prefer to

postpone childbearing and prevent age-related fertility decline (>36 years old) [20][22]. Additionally, oocyte cryopreservation technology could facilitate some advantages in routine IVF programs, such as (1) reducing the number of controlled ovarian stimulation cycles in infertile patients; (2) delaying fresh embryo transfer programs aimed at preventing ovarian hyperstimulation syndrome (OHSS) or to optimize artificial endometrial preparation (AEP) and (3) offering options for infertile couples with religious objections to embryo cryopreservation [5][23]. Consequently, oocyte cryopreservation is now considered a promising tool that could motivate women or infertile patients to preserve their genetic materials for medical or nonmedical reasons.

2. Principles of Oocyte Cryopreservation

Oocyte cryopreservation is an important tool for preserving germ cells for subsequent uses such as fertilization, as cytoplasts for somatic cell nuclear transfer, and also for genome banking for patients and valuable animal species. However, oocytes are very susceptible to damage during cooling and cryopreservation. Furthermore, oocytes also have a relatively low membrane permeability to water and cryoprotectants [24]. Although the optimization of freezing procedures has resulted in improvements in oocyte quality, oocyte structures such as the plasma membrane [25] and cytoskeleton [26] [27] have been shown to be very sensitive to cryoinjury, frequently resulting in cellular disruption and cell death. Several factors have been shown to influence the outcome of oocyte cryopreservation, such as the stage of oocyte maturation during freezing, types of cryoprotectants used and freezing techniques. Immature oocytes are arrested at prophase I (germinal vesicle stage) where the condensed chromatins are protected within the nuclear membrane. Following maturation, the oocytes complete nuclear and cytoplasmic maturation promptly for fertilization and further embryonic development. Results obtained from the cryopreservation of immature and mature oocytes have been contradictory and variable among species and laboratories. In principle, the cryopreservation of immature oocytes is beneficial over mature oocytes, as they do not have a cold-sensitive meiotic spindle. However, cryopreservation processes per se disrupt oocyte structure and the signals responsible for oocyte maturation. Therefore, maturation and fertilization rates of frozen–thawed oocytes are generally poor when compared to noncryopreserved oocytes. These poor results of oocyte cryopreservation have been reported to involve cryoinjury at several levels, such as excessive formation of lethal intracellular ice [28], chromosome abnormality [29], disturbance of hyperosmotic stress [30][31], disruption of actins and microtubules [32] and zona pellucida hardening [33]. More recently, studies have also indicated that cryopreservation induces changes in gene and protein expressions [34][35][36][37].

3. Cryopreservation Techniques

Techniques for the cryopreservation of oocytes, as well as of sperm and embryos, are generally classified as controlled-rate slow freezing and “ice-free” vitrification. Conventional slow freezing requires a programable freezer that can substantially control the optimal freezing rate. During cooling, the temperature is gradually decreased to below the freezing point where ice is formed. However, ice formation occurs in the extracellular and intracellular regions. Excessive ice formation within cells, especially intracellular ice formation, disrupts cell structure and function, which results in apoptosis or cell death. The initiation of the outgrowth of extracellular ice formation via seeding ice crystals is generally performed to mitigate the excessive formation of ice during supercooling. At this stage, extracellular ice is formed and the osmolarity of the extracellular fluid is also gradually increased. The oocytes will be in the dehydrated stage during freezing due to the unfrozen intracellular water flowing out to balance the osmolarity. As the oocytes are the largest cells and have low membrane permeability to cryoprotectants, most cryopreservation requires a freezing rate that is slow enough for sufficient CPA permeability. However, oocyte membrane permeabilities to CPA and cryotolerant have been demonstrated to differ among species. Although theoretical models can be used to predict the optimal freezing rate, empirical study is frequently required to test the freezing protocols prior to use. If the temperature is reduced too rapidly, excessive intracellular ice will be formed. In contrast, oocytes will undergo severe dehydration if the freezing rate is too slow. Therefore, the optimal freezing rate is the slow process that achieves a balance between adequate cellular dehydration and minimal intracellular ice formation. By using this technique, low concentrations of CPA are generally required, thus minimizing osmotic shock and CPA toxicity.

In contrast to slow freezing, vitrification allows the rapid transition from a liquid phase to a glasslike stage or water solidification. Vitrification is another promising technique for living cell cryopreservation [19]. Principally, the definition of vitrification is a “process of glass solidification of a liquid or water-based solution without ice crystal formation” [38]. To achieve this result, high concentrations of CPAs (both permeable and nonpermeable CPAs) are loaded onto living cells before deep freezing in liquid nitrogen [19][39]. However, this procedure causes extreme osmotic stresses and chemical

toxicity [19][39]. Different devices can be modified for efficient vitrification such as open-pulled straw [40], solid surface [3], cryoloop [41], electron microgrids [42] and cryotop [43].

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