Scientific advances in fertility preservation in recent years; the results have been of great importance in preserving fertility in women, especially in patients exposed to conditions harmful to fertility. Thus, cryopreservation of human gametes, embryos and ovarian tissues has become an essential part of assisted reproduction. This approach limits the number of embryos transferred, while additional eggs and/or embryos can be used for subsequent treatment cycles. There are concerns about this and among them; cryopreservation reduces the potential risk of hyper stimulation syndrome. Cryopreservation is carried out by two techniques; the method of slow freezing, the last of which is a procedure called the vitrification technique. In recent times due to the success and simplicity of glazing, the balance between these two methods of glazing has changed. The use of the slow freezing method has become controversial due to its difficulties, expense, and low success rates in artificial reproduction. Therefore, vitrification always seems to win meager, without provoking a failure of artificial reproduction and in the future cryopreservation will be the most interesting method in the world.

Keywords: Ovarian tissue cryopreservation (OTC), Vitrification, Oocyte cryopreservation, Blastocyst stage cryopreservation.

Abbreviations: OTC: Ovarian Tissue Cryopreservation; FDP: Flexipet-Denuding Pipettes; EM: Electron-Microscopic; ICSI: Intracytoplasmic Sperm Injection; CPAs: Cryoprotectant Agents
INTRODUCTION

The results of fertility preservation have been adhered to that cryopreservation methods for both human eggs and embryos are universally luxurious, and recently, fully ovarian and ovarian tissue, mandatory, in certain circumstances, and useful tools in advanced techniques for artificial reproduction [1-3]. It has been emphasized that the routine use of a good and safe cryopreservation program can provide positive results in artificial reproduction with respect to high cumulative rates of clinical and continuous pregnancy. Additionally, the cost of a live birth can be reduced and the chances of a multiple pregnancy also avoided when a single embryo is transferred Given the results of the effectiveness of anticancer therapies, effective early disease diagnosis of gynecological cancers, increased long-term survival of cancer patients and long-term complications of anti-cancer therapies. From this point of view, fertility preservation is also seen as a viable option where preservation of gametes in females is fundamental. Fertility preservation over the excitement of preserving gametes is not a new idea and has a history of nearly 200 years. It first recorded successful cooling and reheating of sperm in snow [4]. Since then, great progress has been made, and two main methods, slow freezing and vitrification, have been implemented in cryopreservation of human gametes and embryos. Unfortunately, initial applications of cryopreservation had low success with poor cell viability and poor clinical outcomes [5-16]. Potential cell damage that occurs during cryopreservation techniques and toxic damage to antifreeze materials are major limiting factors for clinical success [16,17]. Notably, three potential cellular damages during cryopreservation were previously identified. The first is cryogenic injury that occurs at higher temperatures such as between +15 to -5°C. This injury mainly damages the droplets and the cytoplasmic lipid microtubules including the meiotic spindle [18]. The next and most common damage is the formation of intracellular ice crystals, which are the main source of breakage and damage to the zona pellucida or cytoplasm occurring between -50 to -150°C. The last one was treated below -150°C, which was defined as the least severe. Better and favorable clinical outcomes can be achieved through modification of techniques and the use of both intracellular and extracellular antifreeze materials combined. Then, after the potential damage and toxic cells are overcome through laboratory experience and increased success with each cycle of cryopreservation, the battle for vitrification and slow freezing will begin. Therefore, this study attempts to compare and define the cryopreservation technology that will survive in the future.

CRYOPRESERVATION OF OVARIAN TISSUES AND EMBRYOS

The method of slow freezing is the former first method, which is also known as equilibrium freezing due to fluid exchange between the excess spaces and within the cell of cells, allowing freezing without serious osmotic effects and deforming cells [19]. Hence, it has been accepted to be a safe procedure due to non-serious toxic and osmotic damage due to the relatively low concentration of antifreeze solutions. In the past decades, the slow cooling procedure has been used for cryopreservation, but over the past few years it has been suggested that the vitrification method may be a valuable alternative to these procedures [20-22]. When comparing the principles, procedures, and results of slow cooling and vitrification protocols, Koleshova and Lobata Kuleshova LL 2002 state that both methods have resulted in successful preservation of human eggs and embryos, but the former gave much lower success rates. To a large extent, it is generally claimed that the low concentration of antifreeze used in modified slow freezing is insufficient in preventing the formation of ice crystals. Ice crystals in and out of the cell are the main sources of fractures and damage to the zona pellucida or cytoplasm resulting in reduced cell viability. Therefore, in early 2005, opinion articles as well as studies began to emphasize that vitrification may be a better alternative to cryopreservation for inoculated animals and human embryos rather than the slow rate method. Vitrification was first introduced clinically in the early 1980s. In 1985, Ral and Fahey reported the effectiveness of vitrification in cryopreservation of embryos. This method is an unbalanced method in which cells rapidly sink into liquid nitrogen at -196°C after a short period of equilibrium and subsequent glass like hardening. However, the method requires a high cooling rate along with higher concentrations of antifreeze, which may lead to toxic and osmotic effects on cells. Thus, an increased possibility of all other cell injury forms with the exception of ice crystal formation has been emphasized. However, contrary to this hypothesis, vitrification offers a new perspective in attempts to develop optimal cryopreservation, by producing glass like cell hardening, completely without ice crystallizing inside cells during the cooling and heating processes. Physically, there is a close correlation with the rate of cooling and concentration of antifreeze materials as the higher cooling rate reduces the required concentration of antifreeze and vice versa. Therefore, establishing specific balances between the highest reliable cooling rate (and warming) and a safe concentration of antifreeze without any toxic effect is critically necessary to prevent cell damage resulting in vitrification [23-26]. Therefore, vitrification bypasses the severe cytotoxicity resulting from the high concentration of antifreeze required by the above techniques, by introducing antifreeze materials with higher membrane permeability and lower toxicity, along with an appropriate concentration of impermeable antifreeze protective Material [27]. Vitrification can be introduced without the use of expensive equipment and can be completed by a single embryologist within a few minutes, providing significant benefits to any busy IVF program. Notably, groups working in the field of vitrification have established their own unique procedures, by making adjustments in antifreeze concentration, cooling rate and/or carriers and have attempted to demonstrate their superiority.

Indications for Ovarian Tissue Cryopreservation (OTC)

Ovarian Tissue Cryopreservation (OTC) has a single objective: maintenance of the ovarian structure and physiology, benefiting multiple target patients in different situations. OTC has been indicated mainly to safeguard fertility in cancer patients at risk of ovarian insufficiency and infertility due to gonadotoxic treatments [28]. Moreover, this is the only fertility preservation alternative for prepubertal patients, since in these cases,
protocols for ovarian stimulation and oocyte collection are not possible [29]. However, patients with benign conditions such as recurrent ovarian cysts, ovarian torsion, endocrine disorders, and autoimmune diseases may also benefit from this promising technique [30]. The OTC technique has enabled the birth of more than 130 healthy babies worldwide [31]. When followed by auto transplantation, success rates are high regarding the reestablishment of ovarian activity (63.9%) and natural live births (57.5%), according to a meta-analysis performed in 2017 [32]. Yet to be mentioned is the growing interest of women in postponing their first pregnancy, owing to education, career planning, or financial instability or even to possible difficulties in finding a partner [33]. The mean age at first pregnancy has increased by 2-4 years in the last 35 years and now is above 30 years [34]. Since both the quality and the amount of follicles decrease considerably with age, cryo storage is an alternative for improving pregnancy outcomes patient, the possibility of transposing the left ovary and removing the right ovary in order to freeze ovarian tissue for oocyte cryo banking. Counseling covered the issue of freezing methods and future technologies as well as the ethical considerations. Laparotomy was carried out and after left ovarian transposition, the right ovary was surgically removed and washed with saline; the outer 2-4 mm layer was dissected into cubes and frozen according to the protocol described by Newton et al. (Figures 1 and 2).

Figure 1: (Left) Computed tomography: Rectal adenocarcinoma (arrows). (Right) Transposed ovary: Evidence of follicular development (F) After pelvic radiotherapy (arrows).

Figure 2: (Left) Right ovary: cystic formation © with intracystic vegetations (arrows) (vaginal ultrasound) 9resistance index: 0.40. (Right) Left ovary: Borderline tumour (arrows) which occurred 3 months after removal of the right ovary. T: Tumour; O: Ovary.

This case, as well as case report 3, highlighted the possibility of the rapid occurrence of a borderline tumour of the contralateral ovary even if a biopsy had previously demonstrated the absence of any suspected lesions. In this case, the rapid occurrence and growth of the ovarian tumour led to an overdue removal of ovarian cortex with almost no oocytes. In these four case studies, the indications and patients’ desire for cryopreservation of ovarian tissue is clear. Case reports 3 and 4 strongly suggest removing a large ovarian cortex biopsy (1 cm long, 4 mm wide) at the time of first-look surgery that is at the time of the initial surgery for a unilateral ovarian borderline tumour (Figure 3).

Vitrification

More recently, human ovarian tissue has also been cryopreserved by means of vitrification. So far, only 2 live births have been reported after cryopreserving human ovarian tissue using this procedure [35,36]. Vitrification is a process of converting a super cooled liquid into a glass-like amorphous solid, preventing ice crystal formation. Vitrification processes are based on an ultrafast cooling rate combined with a high concentration of CPAs Theriogenology [37]. However, high concentrations of CPAs have toxic effects on the cells. Because of this, vitrification methods usually use a combination of two or more CPAs [37,38], so that the sum of their concentrations supports vitrification, while the low concentration of each CPA reduces their toxic effects [39]. In theory, any permeating CPA may be used for vitrification (Figure 4). However, ethylene glycol is being established as the best choice [40], because of its low toxicity and rapid diffusion into cells. Another factor that influences vitrification is the volume of the sample. The smaller the sample, the less liquid is required to be cooled, and the lower the probability of ice crystal formation [41]. To achieve low volumes of liquid, different approaches are used, such as medium droplets [41, 42], a solid surface, a silver closed vitrification system, and plastic straws. Cryopreservation by vitrification is attractive because it is a quick and easy procedure and does not require special and expensive equipment. Although it seems simple to perform, if cooling rates are not fast enough, crystallization may occur. In a successful vitrification, the tissue and surrounding solution become transparent, whereas failed vitrification is characterized by an opaque white sample, meaning ice crystals have formed. Despite the growing popularity of this type of preservation, it is still rarely used for ovarian tissue preservation. Unlike the slow freezing procedure, there is no standard vitrification protocol for ovarian tissue. Apart from the 2 babies reported by the Japanese group [42,43], promising results using vitrified ovarian tissue were also reported by Kiseleva et al. [44]. In their case study, vitrified ovarian tissue showed recovery of its reproductive potential after auto transplantation [45].

Figure 3: (Left) Vaginal echography: Large ovarian cystic tumour with numerous thick septa. (Right) Computed tomography: Cystic structure appeared within 3 months, indicating rapid growth of the left ovarian borderline tumour. K: Cyst; U: Uterus.

Figure 4: Method of freezing.
Oocyte cryopreservation

Cryopreservation of human gametes and embryos has been known to result in different success rates based on the developmental stages of cells [46]. Mainly, the immature cells seem to be more sensitive than the latter stages, concerning clinical and laboratory applications or procedures. The methods of cryopreservation, especially vitrification, surely affect cells and cause damages due to harmful non-physiological conditions. The human oocyte ultra-structure is quite sensitive to changes of temperature and extracellular osmotic pressure. Thus, during freezing and thawing human oocyte can undergo several types of cellular damage such as cytoskeletal disorganization, chromosomal or DNA abnormalities, spindle disintegration, premature cortical granule exocytosis, related hardening of the zona pellucida and plasma membrane disintegration [47,48].

Oocyte cryopreservation is technically challenging due to the biological characteristics of the metaphase II (MII) oocyte (Figure 5). These include large cell size, high water content and a delicate, active meiotic spindle structure. Oocyte cryopreservation by vitrification is the standard practice to maximise the probability of oocyte survival (Figure 6).

Figure 5: Oocyte cryopreservation is a mainstream treatment.

Figure 6: Oocyte survival.

Blastocyst stage cryopreservation

Blastocyst freezing has three major rationales: 1. The superiority of blastocyst-stage over earlier stage freezing in terms of implantation per thawed embryos which improves the overall expectations for cryopreservation programmes; 2. Maximizing the cumulative pregnancy rates per oocyte retrieval; 3. Extended in-vitro culture of human embryos is becoming more common, encouraging the routine use of blastocyst transfer in IVF programs which is of reduced chances for multiple pregnancies [49]. Blastocysts and further stages of human embryos have different physiological requirements than early stage embryos. These requirements affect the survival rate of the organism exposed to harmful conditions like ultra-rapid freezing [50]. A major factor that affects the survival rate of blastocyst is the fluid-filled cavity called blastocoel. As expected, the formation of intracellular ice crystals is directly proportional to the volume of blastocoel. Vanderzwalmen et al. initially encountered low survival rates after vitrification of blastocysts [51]. However, they were able to overcome the problem by reducing the size of the blastocoelic cavity through puncturing it with a special pipette before the procedure [52], by kewise, Mukaida et al. reported artificial shrink-age in blastocyst vitrification with increased success of the technique [53,54]. Nonetheless, nowadays, without artificial shrinkage or puncturing, blastocyst vitrification has tended to be extremely successful with increased clinical outcomes.

CONCLUSION

Cryopreservation of both ovarian tissue and the whole organ has been proved a feasible technique in fertility preservation. Nevertheless, transplantation of ovarian tissue or cortex is precisely working whereas some advances in improving follicular survival in the whole ovary transplantation is necessary. However, in the near future the cryopreservation of ovarian tissue and the whole ovary seems to replace oocyte banking for fertility preservation. On the other hand, oocyte banking should be the selected method in fertility preservation and artificial reproductive techniques where absolute follicular depletion or loss is not expected, especially in the ethical point of view. Nonetheless, great opportunities have been witnessed in cryopreservation and all developmental stages of gametes or generated embryos, as well as tissue, and the whole ovary could be cryopreserved with amazing success. OTC has been increasingly applied to preserve fertility of cancer patients and women with benign conditions and, more recently, has been discussed as a strategy for postponing pregnancy and menopause in healthy women. While more robust results have been reported for slow freezing procedures, various centers worldwide have started to test vitrification protocols. Nevertheless, optimization of both cryopreservation strategies and thawing/warming protocols is necessary to improve the survival of follicles in cryopreserved ovarian tissue. Vitrification seems to have replaced the former slow rate freezing protocols by improved survival and clinical outcomes. Although different stages of human gametes and embryos show different physiological needs and features that can affect the survival rate, especially, upon laboratory procedures. Undoubtedly, outcomes of vitrification of human embryos at different developmental stages are quite encouraging. Therefore, vitrification should be accepted as a real, viable and a more efficient alternative for cryopreservation of human embryos.

References

4. Spallanzani L. Opuscoli di Fisica Anamale e Vegitabile Opuscola II. Observationi e sperienze intorno ai vermi celli spermatica dell homo e degli animali; Modena: 1776.


42. Wusteman M, Robinson M, Pegg D. Vitrification of large tissues with dielectric warming: biological problems and some approaches to their solution. Cryobiology. 2004;48(2):179–89.


