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Optimal Utilization of Ovarian Tissue Cryopreservation: Enhancing Blastocyst Stage Outcomes and Reserving Future Gametes, Embryos, and Ovarian Tissues

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ABSTRACT

Recent years have witnessed significant advancements in preserving fertility, particularly for women facing conditions detrimental to their reproductive health. Cryopreservation of human gametes, embryos, and ovarian tissues has emerged as a pivotal component of assisted reproduction techniques. This approach not only limits the number of embryos transferred but also allows for the storage of additional eggs and embryos for subsequent treatment cycles. One notable benefit is the reduction in the potential risk of hyperstimulation syndrome associated with cryopreservation.

Cryopreservation techniques primarily involve slow freezing and vitrification. Vitrification, known for its simplicity and success rates, has gained preference over slow freezing in recent times. The slow freezing method has become contentious due to its complexities, costs, and lower success rates in artificial reproduction settings. Consequently, vitrification has emerged as the preferred choice, ensuring successful artificial reproduction without compromising outcomes. Looking ahead, cryopreservation, particularly through vitrification, stands as a promising method in the field of fertility preservation.

Keywords: Ovarian tissue cryopreservation; 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

Fertility preservation techniques, particularly cryopreservation of human eggs and embryos, have become indispensable tools in advanced assisted reproduction technologies. These methods are now considered essential, especially in certain circumstances, for preserving ovarian and ovarian tissue. Implementing a reliable and safe cryopreservation program can significantly enhance the success rates of artificial reproduction, leading to higher cumulative rates of clinical and ongoing pregnancies while reducing the cost of live births and minimizing the risk of multiple pregnancies. The increasing effectiveness of anticancer therapies, early diagnosis of gynecological cancers, and improved long-term survival of cancer patients underscore the importance of fertility preservation in modern healthcare [1,2].

The concept of fertility preservation, particularly the preservation of gametes in females, has a long history dating back nearly two centuries. The first successful cooling and reheating of sperm in snow was documented in 1776 by Spallanzani et al., marking the beginning of cryopreservation research. Since then, significant progress has been made, leading to the development of two main cryopreservation methods: slow freezing and vitrification. However, initial applications of cryopreservation faced challenges with low cell viability and poor clinical outcomes, primarily due to potential cell damage during the process and toxic effects of antifreeze materials.

Three main types of cellular damage during cryopreservation have been identified: cryogenic injury, intracellular ice crystal formation, and damage below -150°C. Modifications in techniques and the use of intracellular and extracellular antifreeze materials have helped overcome potential cell damage and toxicity, leading to improved clinical outcomes with each cycle of cryopreservation. As a result, the debate between vitrification and slow freezing has intensified as researchers strive to identify the most effective cryopreservation technology for the future of assisted reproduction.

CRYOPRESERVATION OF OVARIAN TISSUES AND EMBRYOS

Cryopreservation of ovarian tissues and embryos has evolved significantly over the years, with the slow-freezing method being the traditional approach. Slow freezing, also known as equilibrium freezing, involves a gradual cooling process that allows fluid exchange within and outside the cells, minimizing osmotic effects and cellular deformation. This method has been considered safe due to its relatively low concentration of antifreeze solutions, resulting in minimal toxic and osmotic damage.

While slow freezing has been widely used for cryopreservation in the past, vitrification has emerged as a promising alternative in recent years. Vitrification involves rapid cooling of cells to extremely low temperatures (-196°C) after a brief equilibration period, leading to a glass-like solidification. Compared

to slow freezing, vitrification requires higher concentrations of antifreeze solutions and faster cooling rates, which may pose potential toxic and osmotic effects on cells [3-5].

Studies comparing slow freezing and vitrification protocols have shown that both methods can successfully preserve human eggs and embryos. However, slow freezing often yields lower success rates, primarily attributed to the inadequate prevention of ice crystal formation due to the low concentration of antifreeze used in modified slow freezing protocols. In contrast, vitrification offers a novel approach to cryopreservation by completely avoiding ice crystal formation inside cells during cooling and heating processes.

The key to successful vitrification lies in establishing a balance between cooling rates and antifreeze concentrations. Higher cooling rates allow for lower concentrations of antifreeze, reducing the risk of cytotoxicity. Vitrification also benefits from the use of antifreeze materials with higher membrane permeability and lower toxicity, along with appropriate concentrations of impermeable antifreeze protectants.

Furthermore, vitrification offers practical advantages over slow freezing, as it can be performed quickly and efficiently by a single embryologist without the need for expensive equipment. Different groups have developed unique vitrification protocols by adjusting antifreeze concentrations, cooling rates, and carriers to optimize outcomes.

INDICATIONS FOR OVARIAN TISSUE CRYOPRESERVATION (OTC)

Ovarian tissue cryopreservation (OTC) serves a singular purpose: preserving the structure and function of ovarian tissue, benefiting diverse patient populations in various scenarios. Primarily, OTC is recommended to safeguard fertility in cancer patients vulnerable to ovarian insufficiency and infertility resulting from gonadotoxic treatments. Notably, OTC stands as the sole fertility preservation option for prepubertal patients, as ovarian stimulation and oocyte collection protocols are not viable in these cases. Additionally, individuals with benign conditions such as recurrent ovarian cysts, ovarian torsion, endocrine disorders, and autoimmune diseases may also find OTC beneficial [Figure 1-3].

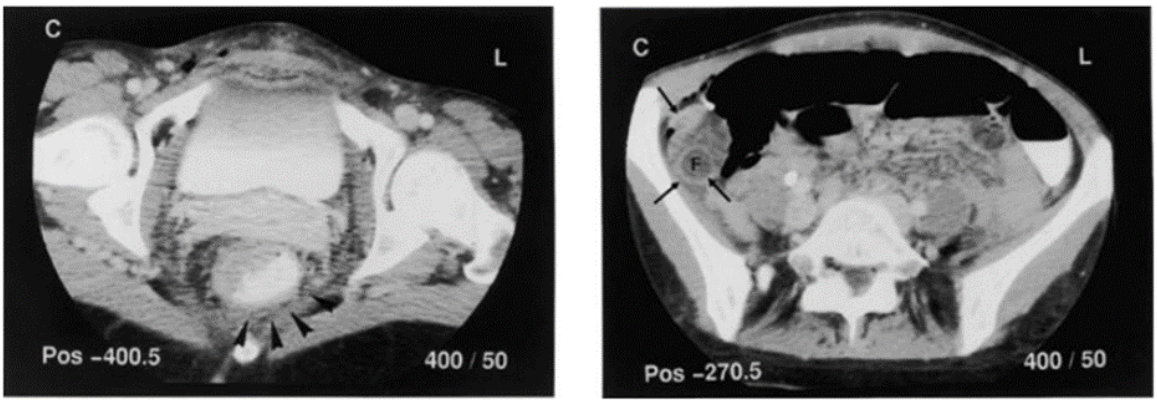

Figure 1: (Left) Computed tomography: Rectal Adenocarcinoma (arrows). (Right) Transposed ovary: Evidence of Follicular development (F) after pelvic radiotherapy (arrows).

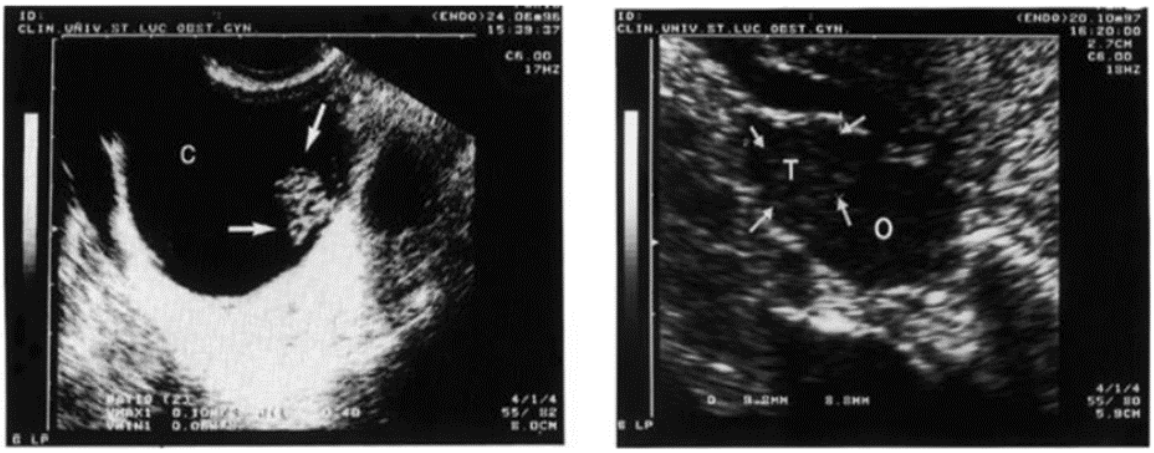

Figure 2: (Left) Right ovary: cystic formation (C) with intracystic vegetations (arrows) (vaginal ultrasound (resistance index: 0.40). (Right) Left ovary: borderline tumor (arrows) which occurred 3 months after removal of the right ovary. T=tumour; O=ovary.

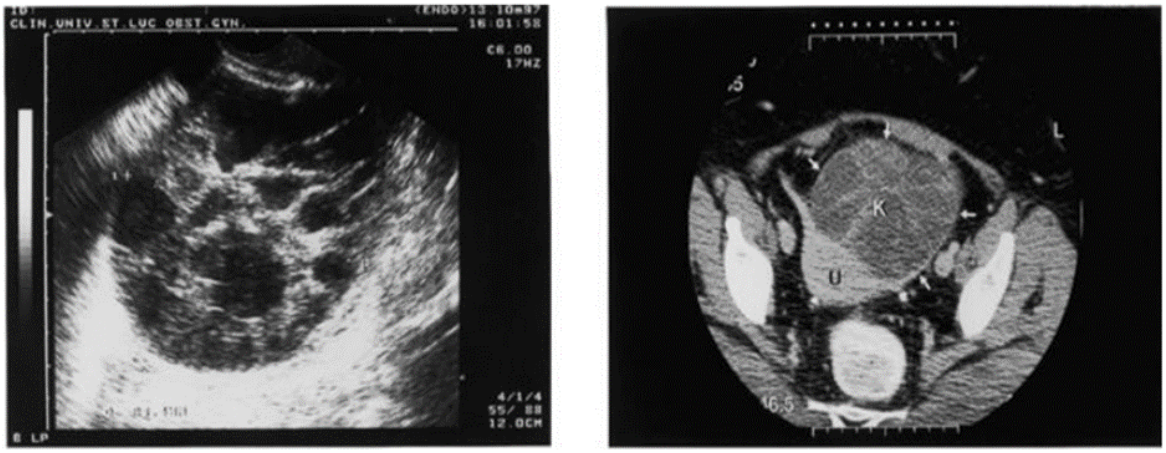

Figure 3: (Left) Vaginal Echography: large ovarian cystic tumor with numerous thick septa. (Right) Computed tomography: cystic structure appeared within 3 months, indicating rapid growth of the left ovarian borderline tumor. K=cyst; U=uterus.

The efficacy of OTC is underscored by the birth of over 130 healthy babies worldwide. Particularly when followed by auto-transplantation, OTC exhibits high success rates in restoring ovarian function (63.9%) and achieving natural live births (57.5%), as indicated by a 2017 meta-analysis.

Furthermore, there is a growing trend among women to delay their first pregnancy due to educational pursuits, career planning, financial instability, or challenges in finding a partner. Over the past 35 years, the average age at first pregnancy has increased by 2–4 years and now exceeds 30 years. Given the substantial decline in both follicle quality and quantity with age, cry storage emerges as a viable option for enhancing pregnancy outcomes [6].

Counseling sessions often address various aspects, including freezing techniques, emerging technologies, and ethical considerations. Surgical procedures typically involve transposing the left ovary and extracting the right ovary to freeze ovarian tissue for oocyte cryo-banking. The surgical process entails laparotomy,

followed by the removal and saline washing of the right ovary. The outer layer of the ovary is dissected into cubes and frozen according to established protocols, ensuring preservation efficacy.

VITRIFICATION

In recent times, human ovarian tissue preservation has also adopted the technique of vitrification. Although only two live births have been reported following the cryopreservation of human ovarian tissue using this method, vitrification offers distinct advantages. Vitrification involves the transformation of a supercooled liquid into a glass-like amorphous solid, effectively preventing ice crystal formation. This process relies on an ultrafast cooling rate combined with a high concentration of cryoprotective agents (CPAs). However, the high concentrations of CPAs can exert toxic effects on the cells. To mitigate this, vitrification methods typically utilize a combination of two or more CPAs, where the cumulative concentration supports vitrification while reducing individual CPA toxicity.

Among the CPAs, ethylene glycol emerges as a preferred choice due to its low toxicity and rapid diffusion into cells [7]. Additionally, the volume of the sample plays a critical role in vitrification success. Smaller samples require less liquid to be cooled, minimizing the likelihood of ice crystal formation [7]. Various techniques are employed to achieve low liquid volumes, including medium droplets, solid surfaces, closed vitrification systems, and plastic straws. Vitrification is appealing for its simplicity and speed, requiring no specialized or expensive equipment. However, if cooling rates are not sufficiently rapid, crystallization may occur. Successful vitrification results in transparent tissue and surrounding solution, while failed attempts manifest as opaque white samples indicating ice crystal formation.

Despite its advantages, vitrification for ovarian tissue preservation remains underutilized compared to slow freezing methods. Unlike slow freezing, there is no standardized vitrification protocol for ovarian tissue. In addition to the two reported babies by a Japanese group, promising outcomes with vitrified ovarian tissue have been observed by Kiseleva et al. In their study, vitrified ovarian tissue demonstrated the restoration of reproductive potential following auto transplantation [8].

OOCYTE CRYOPRESERVATION

Cryopreservation of human gametes and embryos yields varying success rates depending on the developmental stage of the cells. Immature cells appear to be more sensitive to cryopreservation methods, especially vitrification, which can induce cellular damage due to non-physiological conditions. The ultrastructure of human oocytes is particularly susceptible to temperature changes and extracellular osmotic pressure alterations. During freezing and thawing processes, human oocytes may experience several types of cellular damage, including cytoskeletal disorganization, chromosomal or DNA abnormalities, spindle disintegration, premature cortical granule exocytosis, and hardening of the zona pellucida and plasma membrane disintegration. These cellular changes highlight the complexity and challenges associated with oocyte cryopreservation.

Oocyte cryopreservation presents technical challenges due to the biological characteristics of the metaphase II (MII) oocyte, including its large size, high water content, and delicate, active meiotic spindle structure. Vitrification has become the standard practice for oocyte cryopreservation to maximize oocyte survival [9].

BLASTOCYST STAGE CRYOPRESERVATION

Blastocyst freezing is justified by three primary reasons: First, blastocyst-stage freezing yields superior implantation rates per thawed embryos, enhancing overall expectations for cryopreservation programs. Second, it maximizes cumulative pregnancy rates per oocyte retrieval. Third, the increased use of extended in-vitro culture of human embryos encourages the routine adoption of blastocyst transfer in IVF programs, thereby reducing the likelihood of multiple pregnancies [10-13].

Blastocysts and subsequent stages of human embryos have distinct physiological requirements compared to early-stage embryos. These requirements influence the survival rate of embryos exposed to harsh conditions such as ultra-rapid freezing. The blastocoel, a fluid-filled cavity within the blastocyst, plays a significant role in this context. The formation of intracellular ice crystals is directly correlated with the volume of the blastocoel. Initially, low survival rates were observed after vitrification of blastocysts, but this challenge was addressed by techniques such as reducing the size of the blastocoelic cavity by puncturing it with a special pipette or inducing artificial shrinkage. Nowadays, blastocyst vitrification has become highly successful, yielding increased clinical outcomes even without artificial shrinkage or puncturing.

CONCLUSION

Cryopreservation of ovarian tissue and the entire ovary has emerged as a feasible technique in fertility preservation. While transplantation of ovarian tissue or cortex has proven effective, improvements in enhancing follicular survival in whole ovary transplantation are necessary. Soon, cryopreservation of ovarian tissue and the whole ovary may replace oocyte banking for fertility preservation. However, oocyte banking remains a preferred method in fertility preservation and artificial reproductive techniques, particularly where absolute follicular depletion or loss is not expected, from an ethical standpoint. Remarkable progress has been made in cryopreservation across all developmental stages of gametes, embryos, tissues, and the whole ovary, with promising success rates. Ovarian tissue cryopreservation is increasingly applied to preserve fertility in cancer patients, women with benign conditions, and healthy women considering pregnancy postponement and menopause delay strategies. While slow freezing procedures have shown robust results, centers worldwide are exploring vitrification protocols. Optimization of cryopreservation strategies and thawing/warming protocols is essential to enhance follicular survival in cryopreserved ovarian tissue. Vitrification has surpassed slow rate freezing protocols due to improved survival and clinical outcomes. Despite the physiological differences among human gametes and embryos at different developmental stages, encouraging outcomes have been observed with vitrification across these stages. Consequently, vitrification should be recognized as a viable and efficient alternative for cryopreservation of human embryos.

REFERENCES

1. Al-Hasani S, Diedrich K, van der Ven H, Reinecke A, Hartje M, Krebs D. Cryopreservation of human oocytes. Hum Reprod. 1987; 2: 695-700.
2. 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.
3. van Uem JF, Siebzehnrübl ER, Schuh B, Koch R, Trotnow S, Lang N. Birth after cryopreservation of unfertilized oocytes. Lancet. 1987; 1: 752-753.

4. Kuleshova LL, Lopata A. Vitrification can be more favorable than slow cooling. Fertil Steril. 2002; 78: 449-454.
5. Katayama KP, Stehlik J, Kuwayama M, Kato O, Stehlik E. High survival rate of vitrified human oocytes results in clinical pregnancy. Fertil Steril. 2003; 80: 223-224.
6. Testart J, Lassalle B, Belaisch-Allart J, Forman R, Frydman R. Cryopreservation does not affect future of human fertilised eggs. Lancet. 1986; 2: 569.
7. Ménézo Y, Nicollet B, Herbaut N, André D. Freezing cocultured human blastocysts. Fertil Steril. 1992; 58: 977-980.
8. Fabbri R, Porcu E, Marsella T, Rocchetta G, Venturoli S, Flamigni C. Human oocyte cryopreservation: New perspectives regarding oocyte survival. Hum Reprod. 2001; 16: 411-416.
9. Fosas N, Marina F, Torres PJ, Jové I, Martín P, Pérez N, et al. The births of five Spanish babies from cryopreserved donated oocytes. Hum Reprod. 2003; 18: 1417-1421.
10. Veeck LL. Does the developmental stage at freeze impact on clinical results post-thaw? Reprod Biomed Online. 2003; 6: 367-374.
11. Rall WF, Fahy GM. Ice-free cryopreservation of mouse embryos at -196 degrees C by vitrification. Nature. 1985; 313: 573-575.
12. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online. 2005; 11: 300-308.
13. Vajta G, Nagy ZP. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. Reprod Biomed Online. 2006; 12: 779-796.