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Maha Abd Aljabbar Al-Sammak
Professor of Anatomy and Clinical Embryology, mai@uomosul.edu.iq

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REVIEW

Updates in Cryopreservation and Assisted Reproductive Techniques

Maha Abd Aljabbar Al-Sammak

Professor of Anatomy and Clinical Embryology

ABSTRACT

Cryopreservation is a crucial part of assisted reproductive technologies (ART) that has developed to maintain gametes and embryos viable for long periods of time. Recent technological developments, such as vitrification and innovative cryoprotectant that have significantly raised pregnancy success rates and enhanced embryo viability are highlighted in this review. The integration of state-of-the-art technology like single-cell sequencing is explored, and persistent issues with genetic stability and cryoinjury are addressed. The necessity for strong international regulations is emphasized in the discussion of ethical issues, especially those pertaining to the long-term storage and use of embryos. These developments are influencing the direction of (ART) and improving its efficacy and accessibility globally.

Keywords: Cryopreservation, Embryo, Infertility, In vitro fertilization (IVF)

1. Introduction

Cryopreservation in the context of assisted reproduction (ART). We evaluate technical advancements like vitrification, their impact on pregnancy outcomes, and the consequences for genetic integrity by examining a number of peer-reviewed publications. Future research directions are highlighted by discussing ethical issues and cutting-edge technologies like single-cell sequencing. This review provides a thorough grasp of cryopreservation's role in reproductive medicine by combining data from several ART research. Because it enables the long-term storage of gametes and embryos, cryopreservation is crucial to ART. Recent developments, such as the switch from slow freezing to vitrification, which has improved pregnancy outcomes, are methodically examined in this review. We will also look at how cryopreservation affects genomic integrity, embryo viability, and long-term storage ethics.

As a key factor in the success of in vitro fertilization (IVF) treatments and the preservation of fertility, cryopreservation has transformed the area of assisted reproductive techniques (ART). Gametes and embryos can now be stored for an extended period without losing any of their viability because to major advancements in cryopreservation technology. People with illnesses that affect their ability to procreate, such as males with testicular cancer, cancer patients undergoing gonad toxic therapies, and women who choose to postpone having children for personal or medical reasons, have benefited greatly from this innovation (Cobo et al., 2018).

The development of cryopreservation methods over the last few decades has allowed for many embryo transfers from a single (IVF) cycle, which has increased the number of pregnancies. As a result, fewer ovarian stimulations are required, resulting in less financial, psychological, and physical discomfort (Edgar and Gook, 2012). Additionally, cryopreservation makes it easier to examine embryos for genetic problems before implantation, preventing heritable disorders and identifying genetic abnormalities (Mastenbroek and Repping, 2014).

Even with these developments, there are still issues to be resolved, especially with regard to the possible

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E-mail address: mai@uomosul.edu.iq (M. A. A. Al-Sammak).

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dangers of cryoinjury and the long-term health effects of children derived from cryopreserved embryos. In order to mitigate these hazards, recent studies have concentrated on enhancing cryopreservation methods. One such technique is vitrification, which stops the development of ice crystals and lowers the risk of cryoinjury (Rienzi et al., 2017). However, questions about the relationship between chromosomal abnormalities and cryopreservation remain; therefore, more investigation and the creation of fresh strategies are needed to guarantee the security and effectiveness of these practices (Practice Committees, 2013).

This review aims to give a thorough summary of the situation of cryopreservation in (ART) today, stressing new developments in technology, enduring difficulties, and potential study areas. This study aims to address the ethical issues related to the use of cryopreservation techniques and advance knowledge of how these procedures can improve reproductive results by analyzing the most recent data (Loutradi et al., 2008).

The use of vitrification, a flash-freezing technique, has revolutionized slow freezing. Cryoinjury and decreased embryo survival rates could result from slow freezing, which could cause ice crystals to grow inside cells. However, by preventing the production of ice crystals, vitrification significantly lowers the danger of injury and increases the chances that embryos will survive the thawing process. Numerous (IVF) facilities claim success rates comparable to those of fresh embryo transfers, indicating that vitrification has increased the success rate of frozen-thawed embryo transfers (Rezazadeh Valojerdi et al., 2009).

2. Key information

1. Effects of Cryopreservation on Pregnancy Rates and Embryo Viability:

Cryopreservation is a successful method for preserving embryo viability and improving pregnancy outcomes, as evidenced by recent studies. For example, Behr et al. (2002) found a strong relationship between increased pregnancy rates and the successful transfer of cryopreserved blastocysts. They also out that a notable rise in successful post-thawing pregnancies can be attributed to advancements in cryopreservation methods, such as the use of tailored cryoprotectant and controlled-rate freezing. Additionally, the invention of vitrification has decreased cryoinjury, protecting embryos' integrity and developmental potential. Improvements in cryopreservation methods are directly associated with higher pregnancy rates. Recent studies show that vitrification has raised the post-thaw survival rate of embryos to over 90%, up from 50–70% using previous slow-freezing techniques. Vitrification allows for pregnancies that are more successful from frozen-thawed embryo transfers by maintaining the embryo is developmental potential in addition to its structural integrity. For example, tailored cryoprotectant solutions and controlled-rate freezing have decreased thawing hazards and increased the likelihood of post-thaw embryo survival.

2. The genetic stability of embryos that have been cryopreserved:

In (ART) research, the genetic integrity of cryopreserved embryos continues to be a key concern. While cryopreservation normally protects the genetic integrity of embryos, the stress of freezing and thawing might result in chromosomal abnormalities that are not negligible, according to Michelmann and Nayudu (2006). This is in line with research conducted by Son, Lee and Lim (2003), which showed that even if vitrification is very successful in preventing the production of ice crystals, the risk of aneuploidy still exists, especially in older embryos. The significance of genetic screening prior to embryo transfer in reducing the likelihood of genetic disease transmission is highlighted by these findings. Because freezing and thawing embryos may result in chromosomal defects, genetic stability is essential to the effectiveness of cryopreservation. Although recent research has found that the freezing procedure may cause minor epigenetic modifications, vitrification has been demonstrated to reduce such concerns. Although these alterations are not always detrimental, more research is necessary, especially in light of their potential long-term effects on progeny. A promising method for identifying these alterations at the molecular level is single-cell RNA sequencing, which enables scientists to comprehend how freezing affects gene expression and embryonic development. Changes in gene function that do not result from modifications to the DNA sequence are referred to as epigenetic modifications. Offspring development and health may be impacted by these alterations. Preimplantation genetic testing (PGT) and other sophisticated genetic screening techniques have made it feasible to check embryos for chromosomal abnormalities before to implantation, greatly lowering the possibility of transferring embryos with genetic defects.

3. Prognosis for Offspring Resulting from Cryopreserved Embryos:

Wennerholm (2000) carried out a thorough analysis of the obstetric results in children born from cryopreserved embryos and discovered no discernible rise in congenital defects when compared to infants conceived spontaneously. In order to properly evaluate the health effects of cryopreservation, longer-term follow-ups are necessary, according to more recent research, such the one conducted by Li et al. (2014). While the preliminary results are encouraging, more research is necessary since cryopreservation methods may cause minute epigenetic modifications. The development of cryoprotectant solutions is one of the most exciting new developments in cryopreservation. Cryoprotectants are compounds that prevent freezing damage to biological tissue. However, at excessive amounts, they may be harmful. Optimized quantities of cryoprotectants are currently used in modern cryopreservation techniques, which lessen their toxicity while preserving them effectively. Another important development is closed-system vitrification, which removes the possibility of contamination. Additionally, automation has started to play a significant part; AI-powered cryopreservation devices offer more uniformity in freezing and thawing procedures. The procedure of sealing an embryo or gamete in a sterile, closed container and then freezing it is known as closed-system vitrification. This lowers the chance of infection and avoids contamination, which were major issues with previous open vitrification techniques that exposed embryos directly to liquid nitrogen.

4. Enhancement of Cryopreservation Procedures:

An important area of research is still the continuous improvement of cryopreservation methods. Results have been markedly enhanced by developments in cryoprotectant compositions and the use of automation in cryopreservation procedures. For instance, new advancements in closed-system vitrification have increased results uniformity throughout (IVF) laboratories and decreased the possibility of contamination. These advancements raise the effectiveness and accessibility of (ART) techniques while also improving the safety of cryopreservation (Takahashi et al., 2017). Cutting-edge technologies are set to further revolutionize cryopreservation. Single-cell RNA sequencing, for example, allows for the analysis of gene expression in individual cells, providing unparalleled insight into how cryopreservation affects embryos at a molecular level. This technique can help identify which embryos are most likely to survive and develop successfully after thawing, enabling more informed decisions about embryo selection. Additionally, advancements in artificial intelligence (AI) are being used to automate the cryopreservation process, ensuring consistent results and reducing human error.

5. Future Directions and Ethical Issues:

The ethical issues surrounding the long-term storage and potential usage of embryos have gained prominence as cryopreservation becomes more often employed. The scientific community debates topics such as what to do with leftover embryos and the effects of long storage times. In order to overcome these issues, Wennerholm (2000) and Michelmann and Nayudu (2006) both stressed the importance of having precise rules and moral frameworks. In order to guarantee the ongoing safety and effectiveness of cryopreservation methods, future studies should also investigate the application of cutting-edge technology, such as single-cell sequencing. This will help to better understand the molecular changes that take place throughout this process. The usage and storage of embryos have raised more urgent ethical concerns as cryopreservation has grown in popularity. What should happen, for instance, to embryos that are left undeveloped for years? Should they be thrown away, utilized for study, or given to other couples? Furthermore, research on the long-term health of offspring derived from cryopreserved embryos is expanding. Even while there hasn't been a noticeable rise in congenital abnormalities, further research is required to completely comprehend the possible long-term repercussions. These ethical concerns have prompted calls for clearer global guidelines and policies regarding embryo storage and disposal. There is also a growing recognition of the need for transparent informed consent processes to ensure that patients fully understand the potential long-term implications of storing embryos, including legal rights and responsibilities concerning their future use.

3. Conclusion

With vitrification marking a significant advancement in the preservation of gametes and embryos, cryopreservation has developed into an essential part of (ART). This analysis shows that vitrification is the recommended technique in contemporary (ART) clinics since it not only increases embryo survival rates but also improves overall pregnancy outcomes. By eliminating the need for recurrent ovarian stimulations, vitrification has been incorporated into (ART) regimens, which has helped patients experience less physical and psychological stress and save related expenses.

To more clarification, we can say:

1. Clinical Effectiveness:

Compared to conventional slow-freezing methods, vitrification is clearly a better method for preserving embryos, according to a large body of research. Vitrification lowers cryoinjury and preserves greater embryo viability after thawing by limiting the production of ice crystals; multiple studies have shown post-thaw survival rates of over 90%. As a result, cryopreservation results are comparable to those of fresh embryo transfers, increasing both pregnancy and live birth rates.

2. Genetic and Epigenetic Stability:

The findings examined suggest that there is little risk of genetic and epigenetic changes during cryopreservation, despite worries about this possibility. According to current studies, there is no discernible rise in congenital defects or chromosomal abnormalities in infants born from cryopreserved embryos as opposed to those developed spontaneously. To keep an eye on these results and guarantee the safety of vitrified embryos for upcoming generations, more long-term research is necessary.

3. Long-term Storage and Ethical Issues:

Cryopreservation presents significant ethical issues, especially in relation to the extended storage of embryos. As improvements in vitrification make it possible to keep embryos indefinitely without sacrificing their viability, this problem is become increasingly widespread. Managing the long-term use of these embryos requires the creation of international standards and ethical frameworks, particularly when parental consent may evolve over time. The fate of extra embryos must be clearly defined, including whether they are to be discarded, donated to other couples, or used in research.

4. Future Research and Technological Integration:

Looking forward, the incorporation of advanced molecular techniques, such as single-cell RNA sequencing, promises to deepen our understanding of the effects of cryopreservation at the cellular level. This technology will help identify subtle molecular changes that may occur during the freezing and thawing process, leading to improvements in cryopreservation protocols. Furthermore, AI-driven automation systems are poised to enhance the precision and consistency of cryopreservation, reducing human error and improving outcomes on a global scale.

By combining state-of-the-art technology with moral considerations, cryopreservation has the potential to revolutionize ART in addition to preserving fertility. To fully exploit the promise of cryopreservation, researchers, ethicists, and reproductive medicine professionals must continue their interdisciplinary partnership. This will guarantee that the method continues to be a safe, efficient, and morally sound choice for preserving fertility, helping patients all around the world.

Conflict of interest

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