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CRYOPRESERVATION OF GAMETES AND EMBRYOS: A REVIEW

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Abstract

Cryopreservation is the process of freezing cells or tissues and putting them in liquid nitrogen at -196 °C. Water ice crystals are the primary cause of the issue since they cause cell death, especially in large cells like oocytes, which have a meiotic spindle that degenerates during this process. Sub-zero temperatures are not a normal environment for cells.

Importantly, cryopreservation is a viable alternative for individuals who wish to save their germ cells for future use or who experience gonadal failure due to any kind of illness in order to preserve their fertility.

The ability to freeze sperm, oocytes, and embryos has been accessible for a long time, with the first birth using thawed oocytes occurring in 1983. From the mid-2000s onward, egg verification via intracytoplasmic sperm injection has increased pregnancy rates. There are certain complications and occurrences associated with births utilizing assisted reproductive technologies (ART). These dangers may be related to infertility or ART procedures.

Since ART takes place when the epigenome is most fragile, cryopreservation alters the epigenome of gametes and embryos. Cryoprotective substances also cause changes to the integrity of embryos and germ cells. Notably, cryopreservation significantly affects sperm motility, modifies the proteome profile, impairs vital cellular activities, and impacts cell survival.

This approach has been frequently used since the 1980s; however, there is a dearth of understanding of molecular alterations. According to the growing view, cryopreservation causes molecular alterations that impair metabolism, cytoarchitecture, calcium homeostasis, epigenetic status, and cell survival, compromising fertilization in ART.

Key words: *Ovarian Stimulation, Follicle Monitoring, Egg Retrieval, Oocyte Maturation, Sperm Preparation, Insemination Techniques*

Introduction Mohd – Ovaish, Rajwinder Kaur, Dipneet Kaur

Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues for a long period of time [1]. Depending on the cell types or given cells among different mammalian species, there is great diversity in cryobiological response and cryosurvival during the freezing and thawing cycle [2]. Cryopreservation processes can generally be grouped into the following types [3]. slow freezing, The major steps in cryopreservation are,the mixing of cryoprotective agent CPAs with cells or tissues before cooling of the cells or tissues to a low temperature at 0.3 to -2ºC and its storage,warming of the cells or tissues removal of cryoprotective agent CPAs from the cells or tissues after thawing. Cryoprotective agent like glycerol, dimethyl sulfoxide DMSO, Polymers, Proteins are use to protect cells from ice induce damage.

Cryoinjury mechanisms involving osmotic rupture caused by extra- or intracellular concentrated solutes and intracellular ice formation have been highly suggested [4]. both processes of which are dependent on the cooling rate [5].

Objective

To investigate the molecular alterations induced by cryopreservation in germ cells and embryos, focusing on how these changes impact metabolism, cytoarchitecture, calcium homeostasis, epigenetic status, and cell survival, with the aim of improving the success rates and safety of assisted reproductive technologies (ART).

Statement of problem

Cryopreservation, involving the freezing of cells or tissues at -196 °C with liquid nitrogen, is a vital method for safeguarding germ cells and ensuring fertility, especially for individuals experiencing gonadal failure or opting to delay reproduction. Nevertheless, the formation of ice crystals during freezing inflicts substantial damage, particularly on larger cells like oocytes. Although cryopreservation has been integral to assisted reproductive technologies (ART) since the 1980s, it is still plagued by various complications and risks. These issues encompass altered epigenetic landscapes, compromised cellular integrity, diminished sperm motility, and reduced cell viability. The precise molecular changes triggered by cryopreservation—such as disturbances in metabolic processes, cytoarchitecture, calcium homeostasis, and proteome composition—are not fully understood. This lack of comprehensive knowledge impedes the refinement of ART outcomes, highlighting the need for further investigation into the molecular mechanisms through which cryopreservation impacts cellular and reproductive health.

Hypothesis: Cryopreservation induces specific molecular alterations in germ cells and embryos, including disruptions in metabolism, cytoarchitecture, calcium homeostasis, and epigenetic status, which significantly compromise cell survival and fertilization outcomes in assisted reproductive technologies (ART).

Null Hypothesis: Cryopreservation does not induce any significant molecular alterations in germ cells and embryos, and thus does not affect metabolism, cytoarchitecture, calcium homeostasis, epigenetic status, cell survival, or fertilization outcomes in assisted reproductive technologies (ART).

Research methodology

We used the CENTRAL databases to conduct an electronic literature search of PubMed, google scholar review. The following key word combinations were employed: There were no language limitations. Verifications is a newer and highly promising method. It involves rapid cooling to avoid ice crystal formation. Similar to slow freezing, the cells are exposed to a cryoprotectant solution. The samples are plunged directly into liquid nitrogen or another cryogenic medium. Vitrified samples are stored at ultra-low temperatures. Verification is more effective for oocytes and early-stage embryos. a small amount of liquid nitrogen is needed. Verification is widely used for oocytes, embryos, and spermatozoa.

Inclusion Criteria: Those paper which is related to my topic, cryopreservation of gamete, cryopreservation of embryo, cryopreservation of sperm are reviewed.

Exclusion Criteria: those paper which don't related like oligozoospermia patients freezing, asthenozoospermia patient freezing ,oocyte donor vitrification, embryo donor vitrification.

Sperm cryopreservation

The most frequent cryoprotectant for sperm is glycerol (10% in culture media). Sucrose or other di- and trisaccharides are frequently added to glycerol solutions. Cryoprotectant media can be supplemented with egg yolk or soy lecithin, and the two have no statistically significant changes in motility, morphology, ability to bind to hyaluronate in vitro, or DNA integrity after thawing [6].

Additional cryoprotectants can be employed to improve sperm viability and fertility after freezing. Treatment of sperm with heparin binding proteins prior to cryopreservation reduced cryoinjury and ROS production [7].
The use of nerve growth factor as a cryoprotectant reduces sperm cell death rates while increasing motility following thawing. Incorporating cholesterol into sperm cell membranes with cyclodextrins prior to freezing boosts sperm vitality [8]
Semen is frozen using either a controlled-rate, slow-cooling method (slow programmed freezing, or SPF) or a newer flash-freezing procedure called vitrification. Vitrification improves post-thaw motility and cryosurvival over slow programmed freezing [9].

Oocytes cryopreservation

is a method for preserving a woman's eggs (Oocytes). This procedure has been used to delay pregnancy. When pregnancy is desired, the eggs can be thawed, fertilized, and transferred into the uterus as embryos. According to several research, the majority of infertility issues are caused by aging-related germ cell degeneration [10].
The procedure's success rate varies depending on the age of the woman, with the odds being higher in younger, adult women.

Embryos cryopreservation

Cryopreservation of embryos is the method of preserving an embryo at sub-zero temperatures, usually at an embryonic stage equivalent to pre-implantation, that is, from fertilisation to the blastocyst stage.
Embryo cryopreservation is typically conducted as part of in vitro fertilization (which usually involves ovarian hyperstimulation, egg harvesting, and embryo transfer). The ovarian hyperstimulation is preferably performed with a GnRH agonist rather than human chorionic gonadotrophin (hCG) for final oocyte maturation, as it reduces the risk of ovarian hyperstimulation syndrome with no evidence of a difference in live birth rate [11
Cryopreservation complements these technologies by allowing the storage of gametes and embryos, increasing the chances of desired future pregnancies.
Cost Efficiency: Cryopreservation reduces the need for repeated retrieval cycles, making fertility treatment more accessible.
Risk Reduction: It enables single embryo transfer, minimizing the risk of multiple gestation pregnancies
Convenience: Cryopreserved gametes and embryos can be transported and used for various purposes, including gestational carriers, family planning, and donor options
Cryopreservation can induce changes in the epigenome of gametes and embryos. The use of cryoprotective agents affects germ cell and embryo integrity, researchers continue to explore ways to optimize cryopreservation methods for ovarian and testicular tissue
Cryopreservation of gametes and embryos plays a vital role in expanding reproductive options, ensuring fertility preservation, and advancing assisted reproductive technologies. It's a fascinating field that combines science, medicine, and hope for the future

Conclusion

The field of molecular alterations caused by cryopreservation is not well studied. Further data is needed on the activation of cell death-related signalling pathways, long-term impacts on progeny produced from cryopreserved embryos, sperm, or oocytes, and changes to the transcriptome, epigenome, and proteome. In order to better understand these modifications, more advanced techniques must be applied to reveal the molecular changes occurring in cryopreserved embryos and germ cells. This will help identify potential molecular targets that could aid in improving the cryopreservation processes. New technologies, such as single-cell RNA sequencing, may offer useful information to understand these modifications.

Recommendation

Further studies are required to confirm the present findings in large number of studies, along with the determination of underlying The success rates of using vitrification compared to slow freezing for cryopreservation of gametes and embryos are significantly different. The post thaw survival rate offered by vitrification is phenomenally good, almost 90%, making IVF results comparable with cycles done with fresh eggs/embryo.

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