A Review on Applications & Advantages of Cryopreservation in Different Fields of Science

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Abstract

Cryopreservation is a practical application of cryobiology which is the study of biological material or system at temperature below normal. It is a process where cells, whole tissues or any other substances susceptible to damage caused by chemical reactivity or time are preserved by cooling to sub-zero temperature. It's goal is to replace some of the water with other compound like DMSO & glycerol. It is applied in different fields of science like fishery science, medical science, micro biology & many other fields. Most importantly now-a-days it is used in fertility treatment and storage of biological specimens either for future or simply as a record of biodiversity. It is the only technique that ensures the safe and cost-efficient long-term conservation of various types of plants.

Keywords: Cryopreservation, cryobiology, DMSO, sub-zero, fertility, biodiversity.

1. Introduction

Cryopreservation is a long term storage technique with very low temperature to preserve cells, whole tissues or any other substances susceptible to damage for extended period of time at a relatively low cost.

This techniques are available for the preservation of micro-organisms, tissues, primary cells, established cell lines, small multicellular organisms, complex cellular structures like embryos, nucleic acid, protein. Traditional cryopreservation has relied on coating the material to be frozen with a class of molecules termed cryoprotectants. New methods are constantly being investigated due to the inherent toxicity of many cryoprotectants.

The 1st mammalian cells to be cryopreserved successfully were the spermatozoa[1]. Since then many methods have been developed for various type of cells, tissues & organs. Research into fundamental cryobiology has provided the basis for new cryopreservation methods such as vitrification.

In present review it has been tried to show the application of modern methods of cryopreservation in different fields of science with their utility.

2. Objective of cryopreservation

In general when a tissue is subjected to low temperature, ice crystals will eventually form. These crystals may disrupt the cell membrane leading to the death of the cell.

The goal of cryopreservation is to replace some of the water with other compound like DMSO (dimethyl sulfoxide) and glycerol and these are mixed into a solution with media in which cells are placed in a liquid nitrogen freezer usually at -196^0C. When the media begins to freeze, salt concentration outside the cells will become greater than inside the cell and the water will leave the cells to be replaced by the cryopreservation. The main objective is to minimize damage to biological material during low temperature freezing and storage[2].

3. Types of cryoprotective agents

There are two broad categories of cryoprotective agents exist- i) Permeating cryoprotectants and ii) Non-permeating cryoprotectants.

Cryoprotectants that are permeable to cell membrane like DMSO, methanol, propanediol, ethylene glycol and glycerol. Non-permeating cryoprotectants are not permeable to cell membrane.e.g- sucrose, glucose, dextran, egg, yolk serum, skim milk. The fuctions of a cryoprotective agents are to increase the extra cellular osmolality to promote dehydration during cooling and to bind residual water thereby preventing the deleterious effects of ice formation(IIF)[3].Many compounds have been tried as cryoprotective agents, either alone or in combination.although there are no absolute rules in cryopreservation, glycerol and DMSO have been widely used and traditionally have been demonstrated to be the most effective agents for preserving living cells and organism. The choice of cryopreservative agent is dependent upon the type of cell to be preserved. For most cell glycerol is more preferable than DMSO due to its less toxicity. However DMSO is more penetrating and is usually the agent of choice for larger, more complex cells such as protists[4].

4. Method of cryopreservation

There are three main methods of freeze samples at ultra low temperature (i.e. with liquid nitrogen) – i) slow freezing ii) Vitrification & iii) ultra-rapid freezing.

i) **Slow freezing :-** It involves step-wise programmed decrease in temperature. The procedure is lengthy and requires the use of expensive instrumentation. The process does not exclude ice crystal formation.

ii)**Vitrification :-**It refers to any process resulting in "glass formation", the transformation from a liquid to a solid in the absence of crystallization. It involves the use of a medium that has a very high solute concentration to begin with. Thus ice cannot form. The vitrified state & the associated physico-chemical condition obtained using vitrification methods are to some extent similar to those obtained by slow cooling, but the way of reaching those point is quite different[5]. It is rapid cooling of a sample in the presence of a cryopreservation that increases viscocity and depresses the freezing temperature inside of the cell. It is a simple, inexpensive and rapid process of more newly developed technology. It increase the embryos and oocyte survival rate. Unfortunately common cryoprotectants are toxic and the immersion of solution directly in liquid nitrogen can be cause of contamination of embryos and oocytes with bacterium, mushroom and virus.

iii)**Ultra-rapid freezing :-**It is a midway technique between slow freezing and vitrification. It is quicker than slow-freezing technique, does not involve the use of programmable machines and requires lower concentrations of cryoprotectant agents (CPA) than those used invitrification

Experimental results demonstrate that this technique has lower performances than slow freezing's and vitrification's one[6].

5. Application of cryopreservation technique

a) In Animal Husbandry:-The introduction of cryopreservation technology leads a major breakthrough in animal husbandry[7] .Since the 1st successful cryopreservation of bull semen has been used to propagate the rare and endangered species using assisted reproduction techniques. Every year, more than 25 millions cows are artificially inseminated with frozen-thawed bull semen[8] and many bovine calves have been produced using the transfer of cryopreseved embryos into cow[9].

b) In fishery science:-The 1st report on fish sperm cryopreservation was published by Blaxter (1953). To date milt (semen) of over 200 species of fresh water and marine fish have been cryopreserved and have been adequated for the purpose of cryobanking[(10,11,12)]. In fish aquaculture the successful cryopreservation of gametes and embryos could offer new commercial possibilities, allowing the unlimited production of fry and potentially healthier and better conditioned fish as required[13]. Cryopreservation of aquatic sperm is relatively common in the breeding and management of fish species, including salmonid, cyprinids, silurids and Acipenseridae (familia) is well documented[(14)]. However, cryopreservation of embryos and oocytes of aquatic species have not been successful, except for eastern oyster eggs (*Crassotrea virginica)*[15].

Advantage of fish semen is not only useful management tool, it offers several benefits such as stock protection from being totally eliminated due to sudden outbreak of disease, natural disaster, over exploitation etc. Fish germplasm also plays a significant role in human genomic studies because its relatively small size of the genome makes it easier for sequencing and ideal models for studying the human disease[16].

c) In medical science:- Low temperature have been used in medicine and to prevent food spoilage since ancient time. Now- a- days it is used in fertility treatment the transport of human organs and the long- term storage of biological specimens, either for future or simply as a record of biodiversity.

i) Cryopreservation of sperm :-Today human sperm cryopreservation is widely used to store donor and partner spermatozoa before assisted reproduction treatments to preserve spermatozoa before therapy for malignant diseases, vasectomy or surgical in fertility treatments and to ensure the recovery of a small number of spermatozoa in several male factor infertility[17] .

It is commonly called sperm-banking is a procedure to preserve sperm cells. For human sperm the longest successful storage is 21 years.

ii) Cryopreservation of oocyte:- Human oocyte cryopreservation is a new technology in which a woman's eggs are extracted, frozen or stored. Egg freezing benefits two groups of women. One those who are diagnosed with a medical condition whereby the nessary treatments for cure may render them sterile or unable to produce viable eggs.The second who are delaying their childbearing for personal reasons. Eggs frozen at the age of 35 are more usable than fresh oocytes produced at age 43 years of age.

iii) Cryopreservation of testicular tissue:-Cryopreservation of immature testicular tissue is a developing method to avail reproduction to young boys who need to have gonado toxic therapy.

iv) Embryo cryopreservation:-Embryo cryopreservation is used most often to store good-quality excess embryos resulting from an IVF treatment cycle. Embryos can be stored for a patient who elects to have her eggs fertilized with donar sperms. Pregnancies have been reported from embryos stored for 16 years[18].

v) Cryopreservation of ovarian tissue :- Ovarian tissue cryopreservation is considered to be an experimental technique for fertility preservation. This procedure is an option for patients who require immediate gonadotoxic treatment of aggressive malignancies when there is insufficient time to allow the woman to undergo ovulation induction, oocyte retrieval and crypreservation oocytes and/or embryos. Ovarian tissue crypreservation is the only option available for fertility preservation in young girls who are prepubertal or in woman who have hormone-sensitive malignancies or whose reproductive potential is threatened by future of cryopreservation[(19,20)].

vi) Cryopreservation of stem cell:-An important application of cryopreservation is in the freezing and storage of hematopoietic stem cell, which are found in the bone marrow rescue, hematopoietic stem cells are collected from a patient's bone marrow prior to treatment with high-dose chemotherapy. Following treatment, the patient's cryopreserved cells are thawed and infuse back into the body. This procedure is necessary, since high dose chemotherapy is extremely toxic to the bone marrow[21].

d) Preservation of micro-biology cultures :- Bacteria and fungi can be kept short term refrigerated however, cell division and metabolism is not completely arrested and thus is not an optimal option for long term storage or to preserve cultures genetically or phenotypically as cell divisions can led to mutations.

e) To conserve plant biodiversity-The conservation of plant biodiversity is animportant issue concerning the human population worldwide. Conservation of plant biodiversity can be performed in situ and ex situ. These two methods are complementary and are not exclusive. They offer different alternatives for conservation,but selection of the appropriate strategy should be based on a number of criteria, including the biological nature of the species and the feasibility of applying the chosen methods[22]. At present biotechnological methods have been used to conserve endangered, rare crop ornamental, medicinal and forest species for short-,medium-, and long- term.

For long-term conservation cryopreservation is the most effective tool, as itmaintain the living cells, tissues, organs at ultralow temperature(usually that of liquid nitrogen,-196ºc).At liquid nitrogen temperature, all metabolic activity and cell divisions are stopped and cells will not undergo genetic changes during storage. Crypreservation is the ony technique that ensures the safe an cost-efficient long term conservation of various categories of plants, including non-orthodox seed species, vegetatively propagated plants, rare and endangered species and biotechnology products[23].

6. Future of cryopreservation

Vitrification method of cryopreservation may bring new opportunities to research protocols. It is still an experimental procedure. There are two major concern about vitrification - toxicity of high concentration of cryoprotectants used and microbial contamination of liquid nitrogen.

Several IVF programs have adopted the vitrification method as the sole procedure for day-3 human embryos and for human blastocysts, with excellent survival and pregnancy rates. The challenge now is to find a protocol to successfully vitrify human oocytes for which the slow freezing method has yet to produce acceptable.

7. Conclusion

Cryopreservation of gametes and embryos are already routinely applied in mammalian. Cryopreserved oocytes, sperms and embryos are used for artificial insemination and embryo transfer in the livestock industry.

The practical application of cryopreservation in the aquatic species needs more vigorous research efforts in this area and the efforts may be prioritized on endangered, economical value and representative species from various aquatic habitats. The establishment of cryobanks to utilize the cryopreservation world-wide would be a significant and promising task in future.

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