

Cryopreservation and Reanimation of Living Cells

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Key Words:

- Cryopreservation
- Oocytes
- Zygoten
- Embryos

Abstract

For decades, cryopreservation has been used for plant cells, unfertilized (oocytes)/fertilized (zygotes) human and animal eggs, and male sperm cells. The cryopreservation process typically involves using liquid nitrogen to quickly deep freeze the cells to -196°C allowing all metabolic processes to stop, preserving them for storage, shipping, or future use. When thawed, the cells resume their normal metabolic processes.

Small individual cells can be frozen quickly with minimal damage to the cell. However, when quickly freezing larger more complex cells, ice crystals form that are large enough to rupture the cell walls. It was found that slow, controlled freezing using temperature ramping between room temperature and -40°C significantly increases survival rates of the cells.¹

Introduction

Preservation and storage of cells provides laboratories the ability to conduct experiments, develop immunizations, perform human and animal stem cell research, and develop plant varieties and hybrids. During early cryopreservation laboratory experiments, it was discovered that quick freezing of ova and zygotes of small lab animals using liquid nitrogen caused damage to the cells. Additional experiments² proved that freezing the cells at a slower rate lowered the occurrence of cell damage.

These results led to significant improvements in the field of reproductive biology of livestock as well as preserving the lineage of precious breeds such as horses and dogs.

Process

The published results from the European Society of Human Reproduction and Embryology as well as studies done by medical and biological institutes³ in cryopreservation of mammalian zygotes reveal that solving the problem of cellular damage caused by rapid freezing needed to be overcome for cryopreservation to become productive in the commercial industry.

Clinical tests by the Department of Experimental Reproductive Biology of Veterinary Medicine, Hannover, Germany⁴ show the best chance of survival of the cells can be achieved by slow, programmed freezing. Mouse sperm and oocytes are prepared for storage by using a controlled freezing to -32°C followed by deep freezing with liquid nitrogen. The cells were then revitalized by thawing in a bath circulator controlled to $+20^{\circ}\text{C}$. The viability of the sperm and oocytes were then examined for cell integrity and showed an improvement of 7.5% over rapid freezing.

Methodology of slow freezing

Oocytes were obtained from mice 14 hours after induced ovulation. To keep the cells alive, they are submersed in a hyaluronidase/ phosphate buffered saline solution and transferred to Petri dishes. To prepare the cells for cryopreservation, DMSO fluid is added incrementally, increasing concentration levels over time. Once complete, 20 to 40 of the oocytes are put into small plastic minitubes and the ends sealed with plastic plugs.

Using the Thermo Scientific Glacier G50 ultra-low refrigerated bath circulator, the minitubes are pre-cooled to $+15^{\circ}\text{C}$ where the temperature is allowed to equilibrate for 5 minutes. From there the temperature ramp begins, controlling the rate of cooling to 1°C per minute until reaching -6°C . Crystallization is initiated at -6°C by touching the minitube with a pre-cooled clamp. The minitubes are held at -6°C for an additional 5 minutes and then further cooled to -32°C at a ramp rate of 0.3°C per minute. Once this process is complete the samples are transferred to a storage system.



Cryopreservation Using Liquid Nitrogen

Thawing

To thaw the samples, the minitubes are taken from the storage container and transferred into the Glacier G50 bath at +20°C where they thaw in about 10 seconds. The anti-freeze solution is then diluted incrementally over time lowering the concentration and then washed twice in Tyrode's medium.

Fertilization

Male sperm cells, which were previously frozen and thawed in the same method as the oocytes, are joined in a Petri dish with an incubation period of 2 hours. After the incubation period, the cells are washed repeatedly in a solution to prepare for cell growth. After 24 hours the cells reach the 2-cell stage and are ready to be transferred to the recipient mice. Once transferred, the gestation of fetal development is 14 days.

Results

The percentage of morphologically intact oocytes after controlled freezing and rapid thawing was 7.5% higher than previous method of rapid freezing and slow thawing. In vitro fertilization and fetal development has the same success rate regardless of whether the sperm and oocytes are freshly collected or frozen and thawed.

Conclusion

The successful research done on the cryopreservation and reanimation of mouse oocytes and spermatozoa has spawned a commercial industry that has adopted the controlled freezing and rapid thawing as the standard

practice for cryopreservation with bovine embryos, plant research, preservation of precious breeds, and human in vitro fertilization.

Keeping your samples safe and getting the best results is important to you. Using the best cryopreservation techniques and procedures is critical. The compact, powerful and portable Glacier G50 bath has the ability to control the temperature ramping needed to safely freeze and thaw your samples. Handling is made simple with racks specifically designed to hold the minitubes. Tracking your experiments is made easy with the digital communication port.



Thermo Scientific Glacier G50
Refrigerated Bath

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APTGLACIER0212

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