THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212

Popular Article

August, 2023; 3(08), 2037-2042

Freeze-dried sperm spotted on paper recovered later to produce offspring

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https://doi.org/10.5281/zenodo.8270428

Introduction

Across the world livestock is an asset and safety net to enhance the living standard of billions of households. Similarly, farm animals play a contributing role in rural livelihoods and the economy of developing countries (Sansoucy *et al*., 1995). Without high-yielding productive animals, the livestock farm may not run economically for a sustainable period. To maintain productivity at an optimum level there must be an incessant supply of superior male germplasm in the form of quality semen/sperm. The genomic element of sperm should be able to transmit enough qualitative traits with high heritability to the offspring (Kumaresan *et al*., 2020). The collection and long-term preservation of sperm for artificial insemination is a complex process. After post-collection, sperm should be refrigerated or frozen adapting a set procedure without delay. A slight deviation in the storage system for any reason can affect sperm motility and a reduction in viable sperm count, resulting in low fertility. Unlike many plants and animals, human cells cannot withstand freezing temperatures as witnessed in frostbite during environmental freezing. Once viable cells are chilled below freezing temperature there is double trouble: formation of ice crystals and increased salt concentration which is deleterious to eukaryotic cells.

Innovation in cryopreservation

Progress in live cell preservation at low temperatures (cryopreservation) is being driven empirically and often by trial and error. The word '**cryo**' is used in the formation of compound words, to describe "icy cold" or "frost". The criticality of cryopreservation of cells lies in how to prevent intracellular ice crystal formation (Gosden 2011). Characteristically liquid nitrogen is used for the long-term storage of sperm collected from animals and humans (Macpherson 1960). Normally semen collected from donors is processed to store spermatozoa in liquid nitrogen for future use in artificial

insemination or in-vitro fertilization to produce healthy offspring (Benson *et al*., 2012). The temperature of liquid nitrogen (LN2) is 196°C below the freezing point of pure water (-196°C). Such low temperature is ideal for long-term storage of sperm. Scientifically all the biochemical and photodynamic reactions of living cells virtually halt at -135°C therefore it is the acceptable norm to store sperm in LN2 for an indefinite period. During long-distance shipment liquid nitrogen provides an ideal temperature to retain sperm viability. Alternatively, carbon dioxide gas is compressed at high pressure resulting conversion of gaseous carbon dioxide to form dry solid ice that attains a very low temperature near about -80°C which is equally good for sperm preservation and transportation. Till 1963, human semen was preserved at -75°C on "dry ice" (Sherman 1964). Subsequently, once liquid nitrogen was developed, it replaced the dry ice. Invariably glass ampoules filled with liquid sperm are subjected to freezing followed by packing in dry ice or immersed in LN2 for shipment. This cryopreservation technique is widely used as the most convenient and successful method for the long-term preservation of sperm of various animal species. Glass is vulnerable to breakage and has its own weight is the major drawback of adding transport cost and material loss during transit. In place of glass vials, now high-quality plastic cryovials are used to avoid breakage.

Problems remain with liquid nitrogen.

It is worth noting here that around the world liquid nitrogen and dry ice may not be readily available in nook and corner of the country. Additionally, frozen sperm are highly sensitive to temperature variation, so there is an absolute requirement of cold chain maintenance from the production unit to the user's end during logistics. Uncontrolled temperature variation during storage or during transportation of sperm causes deterioration in sperm quality. Low-quality sperm are unable to produce healthy offspring thereby conception rate declines. Once the conception rate declines it escalates the maintenance cost of unproductive animals. It is not always possible to ensure an absolute freezing temperature due to delays in transportation caused by road disruption or power outages, or any mechanical breakdown that may arise during transportation. All these can cause the melting of the frozen semen rendering it virtually unusable for artificial insemination. On the other hand, there are many species in the animal world whose sperm are very sensitive to low temperatures and cannot withstand conventional cryopreservation protocols, so the preservation of sperm collected from such animals is more intricate.

Cryoprotectants are important

The preservation of sperm at extremely low temperatures necessitates the presence of several essential chemicals, to protect the sperm from the heat shock effect. These critical elements have been proven to be vital and essential for sperm life, so their inclusion in sperm diluent is mandatory as a cryoprotectant. Without cryoprotectants, cells rupture due to "Solution effect" injury. In the absence of these chemical agents, sperm cannot tolerate sub-zero temperatures, resulting in higher sperm mortality. Once the scientific group observed that glycerol solution can protect chicken sperm from

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Published: **20.08.2023**

freezing (Polge *et al*.,1949), the cryoprotective property of glycerol revolutionized cattle production through sperm banking (Gosden 2011). Cryoprotectants effectively depress the melting point of water and reduce ice formation. The presence of 5-15% concentration of cryoprotectants in semen diluent is optimum to protect the sperms to withstand heat shock during freezing and post-freeze-thaw operation before insemination. Ethylene glycol, dimethyl sulfoxide, and glycerol are some commonly used agents used as cryoprotectants (Kar *et al*., 2019). Although several organic and inorganic ingredients have been known to protect sperm during cryopreservation (preservation at extremely low temperatures), the most ideal cell-protecting substance without any ill effect on sperm has not yet been traced. For this reason, experts are swiftly searching for better options to store sperm using the freeze-drying technique.

Freeze-drying is an act of daring

Freeze-drying (FD) is a physical process by which the aqueous substance is frozen and then subjected to vacuum evaporation by which frozen material is dried by the sublimation of ice. It is intended to remove the water content from the biological substance so that leftover too little moister in the samples will be inadequate to support microbial growth or chemical reactions. In this way, biological materials can be preserved for a longer duration without deterioration. In late 1930 the freeze-drying technique was applied to preserve disease-causing microbes (Flosdorf and Kimball 1939). A decade time later attempt to preserve prokaryotic cells was initiated using sperm collected from avian species (Polge *et al*., 1949). Earlier efforts of freeze-dried fowl sperm at ambient temperature ended with highly unsatisfactory results. On rehydration, after 2 hours of post-storing at ambient temperature, the revival of sperm motility could not be re-claimed beyond 50% therefore considered unfit for artificial insemination. Later freeze-dried sperm from humans (Sherman, 1954) and bulls (Bialy & Smith, 1957) were found to be of low viability on rehydration with disappointing outcomes. Since then, several workers have worked on their bench to achieve victory but, the successful birth of full-grown pups obtained from freeze-dried mouse sperm has been authenticated in the late nineties (Wakayama and Yanagimachi 1998). This has proved the possibility of storing male germplasm for future use. Recently, the freeze-dried technique is one of the innovative areas of research that got an entry into the laboratory of a peer group of the scientific community to find its efficacy for the long-term preservation of live spermatozoa.

Storing sperm on paper easy to recover

One of the meddling research groups, from Japan, through various trial and error approaches finally got success to develop a groundbreaking method of collecting mice sperm on postcard-sized sheets of paper instead of glass vials for freeze drying. In a subsequent series, the paper with freeze-dried sperm was wrapped between two layers of plastic sheets to avoid contamination during storage and handling. After screening several types of papers used in their laboratory, unexpectedly, the best results were recorded accidentally with one of the papers used for weighing chemicals in their

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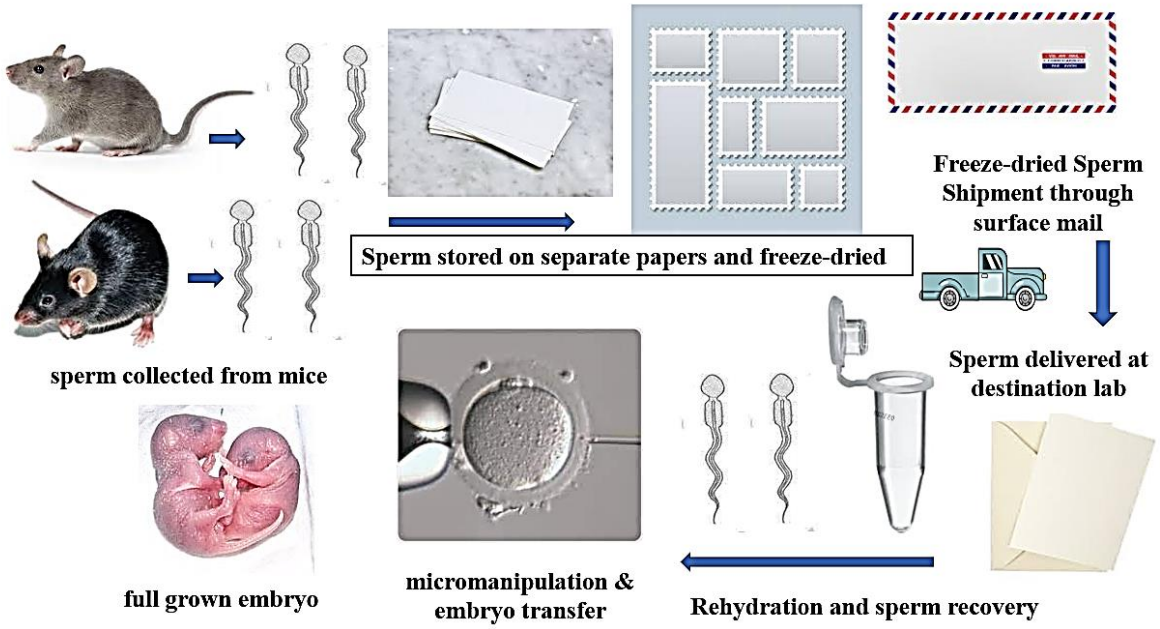

Fig-1. Schematic diagram showing stages from sperm collection up to successful embryo transfer in mice

laboratory (Ito *et al*., 2021). Qualitatively this paper is superior and devoid of any toxicity toward live cells therefore considered to be ideal for the freeze-drying process. It was easy to absorb sperm on those papers and convenient to recover the sperm with rehydration. Since this paper does not release any chemical components, scientifically it is judged to be a completely sterile container for sperm. Paper sheets loaded with sperm collected from different species of mice were serially stacked one by one, and sequentially page numbering was done for species identification. Finally, all the pages were stitched together in the form of a book for surface transportation under ambient temperature to a destination lab located 200 km from the sperm collection unit. In the next step, the sperm stored in the paper was rehydrated for retrieval and managed to reproduce its reproductive potential. The results of the research prove that the morphology and functionality of the sperm preserved in this latest method were unchanged (Fig-1). Although not all spermatozoa were viable, the spermatozoa's inherent nucleic acid-enriched genetic constituents remained intact, in this new protocol (Ito *et al*., 2021). Using in-vitro fertilization, multiple healthy embryos were generated out of these sperm in their laboratory. Afterward adapting the embryo transfer protocol healthy embryos were implanted in the recipient to grow further. Pups born from sperm preserved in this new practice showed no malformations in adulthood and without any reproductive anomaly. No birth defects were observed in the offspring born to them in subsequent generations. Previously this group had successfully sent frozen sperm collected from mice in a dry state to the space station (Wakayama *et al*., 2017). According to scientific evidence, sperm cells conventionally do not need to be alive for reproduction. This groundbreaking discovery heralds a new chapter in cryopreservation techniques. Sperm collected from mice, foxes, hamsters, horses, and sheep have been successfully preserved using this technique. This is an extraordinary achievement, drying the samples in this way, does away with the need for cold storage

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(Ito *et al*., 2021). Freeze drying technique has long been used for the preservation of bacteria and viruses, but it is only the beginning of its successful application to the preservation of sperm.

Conclusion

Recent studies have shown that spermatozoa preserved in the new process can be stored for more than 90 days at -30 degrees centigrade without impairing spermatogenesis and embryo development. Compared to surface-stored sperm, offspring from freeze-dried stored sperm did not develop any genetic defects. Full-term pregnancy has not been compromised with this approach. Mouse sperm can be transported through airmail or surface mail exposed to ambient temperature on the other hand, since these spermatozoa can remain active for only 3 days at ambient temperature, the scientific groups are aiming how to preserve the spermatozoa at normal temperature for at least 30 days. Through this experiment, the scientists want to show that the genetic constituents of sperm are a valuable biological resource for the future and its conservation is essential. More detailed information disseminated in this manuscript is available in the following publications enlisted here as references:

References

Benson J D, Woods E J, Walters E M, Critser J K (2012). The cryobiology of spermatozoa. *Theriogenology*. 78(8):1682–1699 doi: 10.1016/j.theriogenology.2012.06.007.

Bialy G, and Smith V R (1957). Freeze-drying of bovine spermatozoa. *Jour. Dairy Sci*. 40(7): 739–745. doi: 10.3168/jds.s0022-0302(57)94548-4

Flosdorf E W, and Kimball A C (1939). Studies with H. Pertussis: Maintenance of Cultures in Phase I. *J. Bacteriol*. 39(3):255-61. doi: 10.1128/jb.39.3.255-261.1940. 35, 696–704.

Gosden R (2011). "Cryopreservation: a cold look at technology for fertility preservation." *Fertil. and Steril.* 96(2): 264-268. doi: 10.1016/j.fertnstert.2011.06.029.

Ito D, Wakayama S, Emura R, Ooga M, Wakayama T (2021). "Mailing viable mouse freeze-dried spermatozoa on postcards." *iScience* 24(8) 102815. doi:10.1016/j.isci.2021.102815

Kar M, Chourasiya Y, Maheshwari R, Rakesh K. Tekade R K(2019).Current Developments in Excipient Science: Implication of Quantitative Selection of Each Excipient in Product Development, Editor(s): Rakesh K. Tekade, In Advances in Pharmaceutical Product Development and Research, Basic Fundamentals of Drug Delivery, Academic Press,2019, 29-83

Kumaresan A, Das Gupta M, Datta T K and Morrell J M (2020). Sperm DNA Integrity and Male Fertility in Farm Animals: A Review. Front. Vet. Sci. 7:321. doi: 10.3389/fvets.2020.0032

Macpherson J W (1960). Semen storage in liquid nitrogen. *Can. Vet. J.* 1 (7)292-294

Polge C, Smith A U, and Parkes A S (1949). Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature*. 164(4172):666. doi: 10.1038/164666a0.

Sansoucy R, Jabbar M A, Simeon Ehui S, Fitzhugh (1995). The contribution of livestock to food security and sustainable development. In book: Livestock Development Strategies for Low-Income Countries: Proceedings of the joint FAO/ILRI roundtable, ILRI, Addis Ababa, Ethiopia Publisher: FAO, Rome and ILRI, Addis Ababa Editors: T R Wilson, S Ehui, S Mac

Sherman J K. (1954). Freezing and freeze-drying of human spermatozoa. *Fertil. Steril*. 5(4):357-71. doi: 10.1016/s0015-0282(16)31685-5.

Sherman, J K (1964) Research on Frozen Human Semen. Past, Present, and Future. *Fertil. Steril.* 15(5) 485-499. doi: 10.1016/s0015-0282(16)35343-2.

Wakayama S, Kamada Y, Yamanaka K, Kohda T, Suzuki H, Shimazu T *et al.*, (2017). Healthy

offspring from freeze-dried mouse spermatozoa held on the International Space Station for 9 months. *Proc. Natl. Acad. Sci*. U.S.A. 114,5988–5993 (2017).

Wakayama T and Yanagimach R (1998). Development of normal mice from oocytes injected with freeze-dried spermatozoa. Nat. Biotechnol. 16(7): 639–641. doi: 10.1038/nbt0798-639.