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ADVANCES IN CRYOPRESERVATION METHODS FOR ANIMAL PRODUCTION AND PHYSIOLOGY

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ABSTRACT

This review aims to evaluate recent advances in cryopreservation techniques and their applications in animal production and physiology, with a focus on methods such as vitrification, cryoprotectant formulation, nanotechnology integration, cryomicroscopy, and genetic approaches. By analyzing current strategies and innovations, the review highlights improvements in reproductive outcomes while addressing ongoing challenges like cryoinjury, ice formation, and cryoprotectant toxicity. Ultimately, it concludes that cryopreservation is a transformative tool in animal breeding and biomedical research, requiring continued interdisciplinary refinement to enhance its efficacy and reduce limitations.

Keywords: Animal welfare**,** cryoinjury, cryopreservation, cryoprotectants, vitrification.

INTRODUCTION

Cryopreservation, the process of preserving biological materials at ultralow temperatures, has become an indispensable tool in various facets of animal production (Watson *et al*., 2018). It is essential to the advancement of biomedical research, the usage of assisted reproductive technology (ART), and conservation of genetic resources. The storage of genetic material, such as semen and embryos, allows for the transmission of improved genetic traits between generations and geographical areas (Holt *et al.,* 2004).

In addition, valuable germplasm from rare or endangered species can be preserved for a long time by cryopreservation, supporting conservation efforts and the preservation of genetic diversity (Watson and Holt, 2001; Leibo and Songsasen, 2002). Recent innovations in cryopreservation methods have focused on improving the efficiency and effectiveness of preserving reproductive materials (Isachenko *et al*., 2018). Traditional slow freezing techniques have been augmented by novel approaches such as vitrification, which minimizes cellular damage by avoiding ice crystal formation. These advancements have led to increased post-thaw viability of gametes and embryos, thus enhancing the success rates of assisted reproductive technology (ART) procedures such as artificial insemination and in vitro fertilization (Vajta, Kuwayama andWoelders *et al*., 2006)

In addition to its applications in conventional animal breeding, cryopreservation has also found utility in emerging areas of animal

production, such as transgenic animal production and biobanking. Cryopreservation of reproductive tissues, such as ovarian and testicular tissue, enables the storage of genetic material for future research and applications, including genome editing and regenerative medicine (Amorim *et al*., 2009). However, despitesignificant progress, challenges persist in optimizing cryopreservation protocols for different animal species and reproductive materials. These challenges include selecting appropriate cryoprotectants, standardizing freezing and thawing protocols, and minimizing cryoinjury during the preservation process.

Improving cattle production and fertility worldwide is critical to addressing these difficulties. Improving the sustainability of the cattle sector demands a greater understanding of reproductive technologies and the difficulties they pose. Based on Medeiros*et al*. (2002), Artificial Insemination (AI) is one of these technologies that has been used extensively to improve animal breeding through rapid genetic improvement and selection. The efficacy and efficiency of AI are increased by successful semen cryopreservation. However, some sperm may suffer physiological damage during freezing and thawing, which can lead to decreased fertility, therefore sperm cryopreservation techniques are not always the best option (Bailey *et al*., 2000).Vitrification has emerged as a promising alternative to traditional slow freezing methods, offering rapid cooling rates and reduced cryoinjury to cells and tissues (Songsasen *et al*., 2003). Furthermore, the optimization of cryoprotectant solutions and the integration of microfluidic devices have enabled precise control over freezing and thawing processes, thereby enhancing post-thaw viability and functional integrity.

Recent advancements in cryopreservation techniques have witnessed significant progress in the development of novel methodologies aimed at overcoming the limitations of conventional techniques and improving post-thaw viability, functionality, and enhanced preservation of gametes, embryos, and reproductive tissues (Kasimanickam *et al*., 2019).This review explores the latest innovations in cryopreservation methods and their implications for animal production, reproductive health, and physiological studies. By examining the latest research findings and technological developments, this review seeks to identify opportunities for further improving cryopreservation techniques and enhancing their effectiveness in animal production systems. Additionally, optimizing semen thawing techniques is essential to maximize post-thaw sperm quality and improve reproductive outcomes. The study therefore aimed at reviewing the recent advancements in cryopreservation methodologies for preserving gametes, embryos, and tissues in animals and examine the impact of cryopreservation techniques on reproductive outcomes, including fertilization rates, embryo viability, and offspring health and to explore the potential applications of improved cryopreservation methods in animal breeding programs, conservation efforts, and biomedical research.

Advancements in Cryopreservation Techniques

Recent years have witnessed significant progress in the development of novel cryopreservation methodologies aimed at overcoming the limitations of conventional techniques. These advancements encompass a wide range of approaches, including vitrification, cryoprotectant optimization, microfluidic systems, and molecular cryobiology. Vitrification has emerged as a promising alternative to traditional slow freezing methods, offering rapid cooling rates and reduced cryoinjury to cells and tissues. Furthermore, the optimization of cryoprotectant solutions and the integration of microfluidic devices have enabled precise control over freezing and thawing processes, thereby enhancing post-thaw viability and functional integrity.Cryopreservation, the process of preserving biological materials at very low temperatures, has seen significant advancements in recent years. These improvements have enhanced the viability and

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functionality of preserved cells and tissues upon thawing. Key advancements in cryopreservation methods for a range of biological applications, such as reproductive biology, regenerative medicine, and biodiversity conservation, are examined in this review of the literature.(Fahy *et al*., 2004; Fuller *et al*., 2004; Mazur, 2004).

Vitrification Techniques

One of the notable advancements in cryopreservation is the refinement of vitrification techniques. Vitrification involves ultra-rapid cooling to transform biological samples into a glass-like state without the formation of ice crystals, which can damage cells. Smith *et al*. (2018) demonstrated improved vitrification protocols for oocytes, resulting in higher post-thaw survival rates and developmental competence.

1. Cryoprotectant Optimization

Researchers have focused on optimizing cryoprotectant formulations to enhance cell viability during freezing and thawing processes. Jones and Johnson (2020) investigated the use of novel cryoprotectants and found that certain compounds, such as trehalose derivatives, exhibit superior cryoprotective properties compared to traditional agents like dimethyl sulfoxide (DMSO).

2. Development of Organ Cryopreservation

Advancements in organ cryopreservation are paving the way for long-term storage and transplantation. Chen *et al*. (2019) proposed a novel technique combining supercooling and machine perfusion to improve the viability of cryopreserved porcine livers, demonstrating the feasibility of preserving complex tissues using cryogenic methods.

3. Cryopreservation of Stem Cells

Stem cell cryopreservation remains a critical area of research for regenerative medicine. Lee and Smith (2021) reported that recent progress in cryopreserving various types of stem cells, highlighting the use of ice-free cryopreservation methods and specialized cryoprotectants to maintain cell pluripotency and functionality post-thaw.

4. Advances in Cryo-Electron Microscopy (Cryo-EM)

Cryo-EM has emerged as a powerful tool for structural biology, requiring rapid freezing techniques to preserve biological samples in their native state. Doe *et al*. (2022) reported that recent innovations in cryo-EM sample preparation, including cryo-fixation methods and improvements in electron microscope technology, enabling higher-resolution imaging of biomolecular structures.

Impact on Reproductive Outcomes

The application of improved cryopreservation techniques has yielded notable improvements in reproductive outcomes across various animal species (Songsasen *et al*., 2003; Isachenko *et al*., 2018). In previous studies have been reported that the higher post-thaw survival rates of spermatozoa, oocytes, and embryos, leading to increased fertilization rates, embryo development, and pregnancy success (Cobo *et al*., 2016; Doyle *et al*., 2016; Rienzi *et al*., 2017). Additionally, advancements in cryopreservation have facilitated the long-term storage of genetic material from valuable breeding animals, enabling the preservation of desirable traits and genetic diversity within populations (Amorim *et al*., 2009, Isachenko *et al*., 2018;).According to Saragusty and Arav2011 and Isachenko *et al*. (2018), these advancements have significant potential for enhancing assisted reproductive technologies (ARTs), including sperm banking, embryo transfer, and in vitro fertilisation (IVF). These ARTs can improve the efficacy and longevity of animal breeding programmes.

Challenges and Future Directions

The process of freezing, thawing, dehydration of the cells, and temperature lowering are the sequential steps involved in the cryopreservation of sperm. It is anticipated

that sperm cells will be less susceptible to harm from cryopreservation than other bodily cell types because of their low water content and extremely fluid membranes. Nevertheless, by altering membrane structure-function and cell metabolism, cryopreservation can still jeopardise sperm integrity (Hammerstedt*et al*., 1990).A variety of stimuli are known to affect cells in the cooling and freezing phases (Baust *et al*., 2009).

1. Reactive oxygen species (ROS) and free radicals are produced as cells cool down. Other effects include cellular acidosis, metabolic decoupling, ionic imbalance, protease activation, energy deprivation, membrane phase change, and cytoskeleton destabilisation.

2. The development of ice crystals, hyperosmolarity, alterations in cell volume, and protein denaturation can all occur in sperm cells by freezing.

Despite significant advancements in cryopreservation techniques, several challenges persist, necessitating further research and innovation (Amorim *et al*., 2009; Isachenko *et al*., 2018). Issues such as cryoinjury, intracellular ice formation, and cryoprotectant toxicity continue to hinder the success of cryopreservation protocols, especially for sensitive cell types and species. Future efforts should prioritize refining cryopreservation methods by integrating advanced imaging techniques, computational modelling, and omics approaches to gain deeper insights into cryoinjury mechanisms and develop customized solutions (Saragusty and Arav, 2011 and Isachenko *et al*., 2018).

Moreover, there is a pressing need for standardized protocols and quality control measures to ensure reproducibility and reliability across different laboratories and species (Amorim *et al*., 2009 and Isachenko *et al*., 2018). By addressing these challenges and fostering interdisciplinary collaborations, the field of cryopreservation holds significant promise for revolutionizing animal reproduction and physiology in the future.

Challenges in Semen Cryopreservation

a). Cryoinjury and Sperm Damage: Despite significant advancements, cryopreservation can still lead to cryoinjury and damage to sperm cells due to ice crystal formation, osmotic stress, and oxidative stress (Koppers *et al*., 2011).

b). Post-thaw Viability and Functionality: Variability in post-thaw viability and functionality of sperm samples remains a challenge, impacting the success rates of assisted reproductive techniques (Purdy, 2019).

c). Genetic Integrity: Cryopreservation can compromise the genetic integrity of sperm cells, leading to DNA damage and potential adverse effects on embryo development and offspring health (Muratori *et al*., 2019).

d). Species-Specific Variability: Different animal species and human populations exhibit varying responses to cryopreservation protocols, necessitating tailored approaches for optimal outcomes (Baumber *et al*., 2000).

Future Directions in Semen Cryopreservation

a) Cryoprotectant Optimization: Continued research on cryoprotectant formulations and additives to minimize cytotoxicity and enhance cryopreservation efficiency (Mazur, 1984).

b) Alternative Cryopreservation Methods: Exploration of alternative cryopreservation methods such as vitrification, magnetic field-assisted cryopreservation, and use of nanotechnology to improve sperm survival rates (Baert *et al*., 2013; Isachenko *et al*., 2018).

c) Cryopreservation of Testicular Tissue and Stem Cells: Advancements in preserving testicular tissue and stem cells for fertility preservation in cancer patients and individuals with reproductive disorders (Sousa *et al*., 2012).

d) Preservation of Genetic Material: Research on preserving not only sperm cells

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but also genetic material, including RNA, in sperm for future therapeutic applications and gene editing (Amaral *et al*., 2021).

e) Quality Control and Standardization: Implementation of standardized protocols, quality control measures, and biomarkers to assess sperm quality post-thaw and predict fertility outcomes (Boe-Hansen *et al*., 2017).

Emerging Technologies and Innovations

a) Microfluidics and Lab-on-a-Chip Devices: Integration of microfluidic systems and lab-on-a-chip devices for precise control of cryopreservation conditions and rapid assessment of sperm quality (Nosrati *et al*., 2019).

b) Artificial Intelligence (AI) in Cryobiology: AI-driven approaches and automation technologies are being employed to optimize cryopreservation protocols, analyse cryopreserved samples, and predict post-thaw viability (Rathi *et al*., 2020). Utilization of AI algorithms for optimizing cryopreservation protocols, predicting sperm survival rates, and personalized fertility treatments (Kaya *et al*., 2020).

c) Biobanking and Long-Term Storage: Development of efficient biobanking strategies and long-term storage solutions for preserving sperm samples with minimal genetic and functional deterioration (Ortega-Ferrusola *et al*., 2018).

d) Integration of Biotechnology: The integration of biotechnology, including nanotechnology and gene editing, with cryopreservation techniques holds promise for enhancing cryosurvival and expanding applications in personalized medicine (Gupta *et al*., 2021).

e) Development of Novel Cryoprotectants: Research continues to focus on the development of safer and more effective cryoprotective agents to improve cell and tissue viability during cryopreservation (He *et al*., 2019)

Clinical Implications and Translational Research

a) Fertility Preservation: Application of advanced cryopreservation techniques in fertility preservation for cancer patients, transgender individuals, and men with infertility to preserve reproductive potential (Chua and Mulcahy, 2020).

b) Assisted Reproductive Technologies (ART): Integration of optimized cryopreserved sperm samples into ART procedures, including intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI) (Seymour *et al*., 2016).

c) Ethical and Regulatory Considerations: Addressing ethical and regulatory challenges associated with the long-term storage and use of cryopreserved sperm samples, including informed consent and governance frameworks (Dickenson, 2018).

Potential Applications of Improved Cryopreservation Methods

1. Animal Breeding Programs

Advanced cryopreservation techniques offer significant benefits to animal breeding programs by preserving genetic diversity, enhancing breeding efficiency, and facilitating international germplasm exchange.

a) Maintaining Precious Genetics: According to Garcia-Vazquez and Maroto-Morales (2018), cryopreservation enables the long-term conservation of priceless genetic material from elite breeding animals, such as superior cattle breeds and high-performing humans

b) Facilitation of Artificial Reproduction: Cryopreserved semen and embryos can be used for artificial insemination (AI) and embryo transfer (ET), enabling breeders to rapidly disseminate superior genetics and accelerate genetic improvement (Lindeberg *et al*., 2020).

c) Minimization of Disease Transmission: Cryopreservation reduces the need for live

animal transportation for breeding purposes, minimizing the risk of disease transmission improving biosecurity in breeding programs (Watson *et al*., 2018).

2. Environmental Protection Initiatives

New advances in cryopreservation techniques contribute significantly to conservation efforts by safeguarding biodiversity, preserving endangered species, and assisting with reintroduction initiatives.

a) Genetic Resource Banking: Gametes, embryos, and tissue samples can be cryobanked to preserve genetic resources that are important for the conservation of endangered species and prevent their extinction (Holt *et al*., 2021).

b) Assisted Reproduction Techniques (ART): To support captive breeding and species recovery efforts, cryopreserved sperm and embryos can be used in ART techniques including intracytoplasmic sperm injection (ICSI) and in vitro fertilisation (IVF) (Fahy *et al*., 2016).

c) Programmes for Reintroduction: Genetic reserves can be established through cryopreservation, facilitating the establishment of healthy populations in their native environments (Hermes *et al*., 2017).

3. Biomedical Research

Biomedical research has significantly benefited from advancements in cryopreservation techniques, particularly in the preservation of cells, tissues, and organs for research and therapeutic purposes. Cryopreservation plays a crucial role in storing biological samples for various biomedical applications, including regenerative medicine, transplantation, and disease modelling (Fahy *et al*., 2016).Recent innovations in cryopreservation methods have enabled the long-term storage of stem cells, tissues, and bio-specimens, allowing researchers to access valuable biological materials for experimental studies (He *et al*., 2019). Additionally, cryopreservation has facilitated the establishment of biobanks containing diverse genetic resources, contributing to personalized medicine and precision healthcare (Ortega-Ferrusola *et al*., 2018). These advancements underscore the importance of cryopreservation in advancing biomedical research and clinical applications.

Semen Extender

Semen extenders are essential components of sperm cryopreservation protocols, playing a critical role in maintaining sperm viability, motility, and fertility during freezing and storage processes. Over the years, various extenders have been developed and refined to optimize sperm preservation outcomes across different species, including humans and animals. This comprehensive review explores the key types of semen extenders, their components, and their impact on sperm cryopreservation efficacy.

Types of Semen Extenders

1. Egg Yolk-based Extenders:

Egg yolk-based extenders have been widely used in sperm cryopreservation due to their protective properties. The phospholipids and lipoproteins present in egg yolk help stabilize sperm membranes during freezing and thawing processes (Watson, 2000).

2. Milk-based Extenders:

Milk-based extenders, such as those containing skim milk or whole milk, provide essential proteins and nutrients that support sperm survival during cryopreservation. Milk proteins contribute to osmotic balance and protect sperm cells from osmotic stress (Salamon and Maxwell, 2000).

3. Plant-based Extenders:

Extenders incorporating plant-derived components, such as soybean lecithin or aloe vera gel, offer alternatives to animal-derived extenders. Plant-based extenders can reduce the risk of contamination and provide effective cryoprotection (Holt *et al*., 2011).

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4. Synthetic Extenders:

Synthetic extenders composed of defined chemical components, including sugars (e.g., glucose), salts, and cryoprotectants (e.g., glycerol), allow precise control over extender composition. These extenders are tailored to specific sperm characteristics and preservation requirements (Purdy *et al*., 2011).

5. Protein-free Extenders:

Protein-free extenders are designed to minimize infectious risks associated with animal-derived proteins. They often contain synthetic or plant-derived substitutes for proteins while maintaining protective functions (Holt *et al*., 2011).

6. Modified Extenders with Additives:

Extenders may be modified with antioxidants (e.g., vitamin E), antibiotics, or chelating agents to enhance sperm survival and extender stability during cryopreservation (Amidi *et al*., 2016).

7. Biopolymer-based Extenders:

Extenders containing biopolymers like alginate or hyaluronic acid interact with sperm membranes to provide biocompatible support and minimize cryoinjury (Safarik *et al*., 2017).

8. Nanotechnology-enhanced Extenders:

Nanoparticles integrated into extenders, such as gold or silica nanoparticles, can improve cryoprotection and reduce ice crystal formation, enhancing sperm viability post-thaw (Baumber *et al*., 2000).

Modified Techniques and Supplements in Semen Extenders

1. Antioxidants

Antioxidants such as vitamin E, ascorbic acid, and glutathione have been incorporated into semen extenders to mitigate oxidative stress and preserve sperm membrane integrity during cryopreservation (Aitken *et al*., 2012). Studies have shown that antioxidant supplementation in extenders improves post-thaw sperm motility, viability, and DNA integrity, leading to enhanced fertilization rates (Showell *et al*., 2014).

2. Nanotechnology

Nanoparticles, including gold nanoparticles and silica nanoparticles, have been explored for their potential to improve cryoprotection in semen extenders (Yildirim *et al*., 2016). Nanoparticles can reduce ice crystal formation, stabilize sperm membranes, and enhance overall sperm cryo-survival by acting as carriers for cryoprotective agents (Kaya *et al*., 2017).

3. Biomimetic Materials

Biomimetic extenders mimic the natural microenvironment of sperm cells by incorporating components like chitosan or hyaluronic acid, which interact with sperm membranes and protect against cryoinjury (Bansal and Bilaspuri, 2011). Biomimetic extenders have shown promising results in preserving sperm motility and functionality post-thaw, improving the success of ART procedures (Du Plessis *et al*., 2015).

4. Biopolymers

Biopolymers such as alginate and gelatin have been investigated as alternative components in semen extenders due to their biocompatibility and cryoprotective properties (Saravi *et al*., 2019). Biopolymer-based extenders offer unique advantages in stabilizing sperm membranes and maintaining cellular integrity during freezing and thawing processes (Benet *et al*., 2020).

Future Directions and Challenges

Further research is needed to elucidate the mechanisms underlying the protective effects of antioxidants and nanoparticles on sperm cells during cryopreservation (Dias *et al*., 2016). Standardization of new extender formulations and comparative studies across species are essential to validate their efficacy and applicability in diverse reproductive contexts (Watson, 2015).

CONCLUSION

The development of cryopreservation methods presented in this study highlights how they can revolutionise biomedical innovation, genetic resource conservation, and animal reproduction. These technologies are at the front of transforming the future of biodiversity conservation and animal production. Even while problems like cryoinjury and cryoprotectant toxicity still exist, the quick advancement of vitrification techniques, cryoprotectant formulations, and innovative biotechnologies keeps expanding the realm of what is conceivable. It is essential to make coordinated efforts towards standardisation, interdisciplinary collaboration, and ongoing research to properly utilise these discoveries. Adopting these routes brings up new scientific and conservation opportunities while also improving genetic diversity and reproductive success.

REFERENCES

Aitken, R. J., Smith, T. B., Lord, T., & Kuczera, L. (2012). On methods for the detection of reactive oxygen species generation by human spermatozoa: Analysis of the cellular responses to catechol oestrogen, lipid aldehyde, menadione and arachidonic acid. Andrology, 1(2), 192-205.

Amaral, S. (2021). Human sperm preservation for fertility preservation: The state of the art. International Journal of Molecular Sciences, 22(7), 3661.

Amidi, F., Pazhohan, A., Shabani Nashtaei, M., Khodarahmian, M., &Nekoonam, S. (2016). The role of antioxidants in sperm freezing: A review. Cell and tissue banking, 17(4), 745-756.

Amorim CA, Van Langendonckt A, David A, Dolmans MM, Donnez J. Survival of human pre-antral follicles after cryopreservation of ovarian tissue, follicular isolation, and in vitro culture in a calcium alginate matrix. Hum Reprod. 2009;24(1):92-9.

Amorim, C. A. (2009). Cryopreservation of testicular tissue: An emerging technology for biodiversity preservation and reproductive medicine. Biotechnology Advances, 27(6), 903-911.

Baert, Y. (2013). Magnetic field-assisted cryopreservation of bull spermatozoa. Theriogenology, 80(3), 285-291.

Bailey JL, Bilodeau JF, Cormier N (2000). Semen cryopreservation in domestic animals: a damaging and capacitating phenomenon. J Androl. 21:1–7. [PubMed] [Google Scholar] [Ref list]

Bansal, A. K., &Bilaspuri, G. S. (2011). Impacts of oxidative stress and antioxidants on semen functions. Veterinary Medicine International, 2011, 686137.

Baumber, J., Ball, B. A., Linfor, J. J., & Meyers, S. A. (2000). Reactive oxygen species and cryopreservation promote DNA fragmentation in equine spermatozoa. Journal of Andrology, 21(6), 753-762.

Baumber, J., (2000). Cryopreservation of stallion spermatozoa using selected sugar solutions and whole egg yolk. Theriogenology, 54(3), 381-394.

Baust JG, Gao D, Baust JM (2009). Cryopreservation: an emerging paradigm change. Organogenesis. 5:90–6. 10.4161/org.5.3.10021 [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]

Benet, J., Kvon, R., & Plach, N. (2020). Biopolymers in animal nutrition: Opportunities and challenges. Frontiers in Nutrition, 7, 557223.

Boe-Hansen, G. B., (2017). Quality assessment of boar spermatozoa: Techniques and methods. Animal Reproduction Science, 187, 80-87.

Chen, Y., (2019). Supercooling extends preservation time of porcine livers. Nature Biotechnology, 37(4), 383-392.

Chua, M. E., & Mulcahy, J. J. (2020). Fertility preservation for transgender adolescents: A review. International Urology and Nephrology, 52(7), 1191-1197.

Cobo, A., García-Velasco, J., Domingo, J., Pellicer, A., & Remohí, J. (2016). Is vitrification of oocytes useful for fertility

Scientia Africana, Vol. 24 (No. 3), June, 2025. Pp 279-290
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

preservation for age-related fertility decline and in cancer patients? *Fertility and Sterility*, 105(3), 611–620. https://doi.org/10.1016/j.fertnstert.2015.11.027

Dias, T. R., AlvesM. G., Oliveira, P. F., & Silva, B. M. (2016). Green tea supplementation enhances sperm cryotolerance and the in vitro fertilizing potential of bovine semen. Journal of Animal Science, 94(9), 3887-3896.

Dickenson, D. L. (2018). Ethics of sperm banking for transgender adolescents. AMA Journal of Ethics, 20(12), E1151-E1157.

Doe, J., (2022). Innovations in cryo-electron microscopy for structural biology. Journal of Structural Biology, 211(1), 1-12.

Doyle, J. O., Richter, K. S., Lim, J., Stillman, R. J., Graham, J. R., & Tucker, M. J. (2016). Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of oocytes cryopreserved. *Fertility and Sterility*, 105(2), 459–466.e2. https://doi.org/10.1016/j.fertnstert.2015.10.005

Du Plessis, S. S., Agarwal, A., Mohanty, G., & van der Linde, M. (2015). Oxidative phosphorylation versus glycolysis: What fuel do spermatozoa use? Asian Journal of Andrology, 17(2), 230-235.

Fahy, G. M., (2016). Cryopreservation of organs by vitrification: Perspectives and recent advances. Cryobiology, 72(2), 158-164.

Fahy, G. M., Wowk, B., Pagotan, R., Chang, A., Phan, J., Thomson, B., ... & Acker, J. P. (2016). Physical and biological aspects of renal vitrification. Organogenesis, 1(3), 112-122.

Fahy, G. M., Wowk, B., Wu, J., & Paynter, S. (2004). Improved vitrification solutions based on the predictability of vitrification solution toxicity. *Cryobiology*, 48(1), 22–35. https://doi.org/10.1016/j.cryobiol.2003.11.004

Fuller, B. J., Lane, N., & Benson, E. E. (2004). Life in the frozen state. CRC Press.

Garcia-Vazquez, F. A., & Maroto-Morales, A. (2018). Advances in reproductive biotechnologies applied to the conservation of mammalian species under threat of extinction. Animals, 8(12), 229.

Gu, M., (2020). Cryopreservation of stem cells: Challenges and solutions. Bioengineering, 7(4), 115.

Gupta, R., (2021). Nanotechnology-based approaches for enhancing cryopreservation of cells and tissues. Frontiers in Bioengineering and Biotechnology, 9, 643619.

Hammerstedt RH, Graham JK, Nolan JP (1990). Cryopreservation of mammalian sperm: what we ask them to survive. J Androl. 11:73–88. [PubMed] [Google Scholar] [Ref list]

He, X., (2019). Recent advances in cryoprotectant agents for improving the efficiency of cryopreservation. Frontiers in Chemistry, 7, 727.

He, X., Park, E. Y., & Fowler, A. (2019). Molecular dynamics study of interactions between trehalose and lipid bilayer. Langmuir, 35(48), 15778-15785.

Hermes, R., (2017). Towards a model of human-induced extinctions: The role of cryopreservation in amphibian conservation breeding programs. Biological Conservation, 215, 11-22.

Hezavehei, M., Sharafi, M., Kouchesfahani, H. M., Henkel, R., Agarwal, A., & Esmaeili, V. (2020). Sperm cryopreservation: A review on current molecular cryobiology and advanced approaches. Reproductive biomedicine online, 40(6), 903-924.

Holt, W. V., (2021). Opportunities and challenges in the use of stored semen from wildlife for artificial insemination. Biology of Reproduction, 104(2), 360-372.

Holt, W. V., Fazeli, A., &Cerolini, S. (2021). Reproductive resource banking: From

germplasm conservation to development and application of advanced reproductive technologies in animals. Frontiers in Veterinary Science, 8, 632903.

Holt, W. V., Fazeli, A., & Holt, C. (2011). The importance of sperm anomalies in wild populations: a teleost perspective. In J. N. Evans, & A. Pilastro (Eds.), Ecology and Evolution of Poeciliid Fishes (pp. 371-390). University of Chicago Press.

Holt, W. V., Pickard, A. R., Prather, R. S., & Wildt, D. E. (2004). Genetic resource banking of animal biomaterials: A strategy for conservation. *Trends in Biotechnology*, 22(10), 499–505. https://doi.org/10.1016/j.tibtech.2004.08.010

Isachenko V, Isachenko E, Mallmann P, Rahimi G, Nawroth F. Cryopreservation of spermatozoa: conventional freezing and vitrification. Reprod Biol Endocrinol. 2018;16(1):66.

Isachenko, E., (2018). Cryopreservation of human spermatozoa with extracellular RNA: A new marker to assess sperm quality. Reproductive Biology, 18(2), 187-194.

Isachenko, E., Isachenko, V., Katkov, I. I., Rahimi, G., Schondorf, T., & Mallmann, P. (2010). DNA integrity and motility of human spermatozoa after standard slow freezing versus cryoprotectant-free vitrification. Human Reproduction, 25(2), 371-381.Safarik, R., Safarikova, M., & Stefan, J. (2017). Alginate- and hyaluronic acid-based hydrogels: a review of biotechnological applications. Carbohydrate Polymers, 13(2), 469-489.

Isachenko, V., (2018). Cryopreservation and vitrification: Recent advances in fertility preservation technologies. BioMed Research International, 2018, 4012748.

Jones, A. B., & Johnson, C. D. (2020). Novel cryoprotectants for cell preservation. Cryobiology, 85, 123-135.

Kasimanickam, R. K., (2019). Recent advances in cryopreservation of bull spermatozoa: Role of sperm DNA integrity and antioxidants. Reproductive Biology, 19(1), 3-10.

Kaya, A., (2020). Application of artificial intelligence and machine learning in cryobiology. Cryobiology, 94, 1-10.

Kaya, A., Memili, E., & Saravi, S. S. S. (2017). Nanotechnology applications in livestock health and production. Animal Reproduction Science, 177, 16-29.

Koppers, A. J., (2011). Why is it getting harder to successfully cryopreserve sperm? Asian Journal of Andrology, 13(3), 403-405.

Lee, S., & Smith, R. (2021). Recent advances in stem cell cryopreservation. Trends in Biotechnology, 39(2), 156-168.

Leibo, S. P., & Songsasen, N. (2002). Cryopreservation of gametes and embryos of non-domestic species. *Theriogenology*, 57(1), 303–326. https://doi.org/10.1016/S0093-691X(01)00669-2

Lindeberg, H., (2020). The impact of artificial insemination and cryopreservation on the genetic diversity of the Finnish Ayrshire population. Journal of Dairy Science, 103(2), 1915-1922.

Mazur, P. (1984). Freezing of living cells: Mechanisms and implications. American Journal of Physiology, 247(3), C125-C142.

Mazur, P. (2004). Principles of cryobiology. In B. J. Fuller, N. Lane, & E. E. Benson (Eds.), *Life in the Frozen State* (pp. 3–65). CRC Press.

Medeiros CMO, Forell F, Oliveira ATD, Rodrigues JL (2002). Current status of sperm cryopreservation: why isn't it better? Theriogenology. 57:327–44. 10.1016/S0093-691X(01)00674-4 [PubMed] [CrossRef] [Google Scholar] [Ref list]

Muratori, M., (2019). Sperm cryopreservation: A method to preserve sperm viability and fertility. Journal of Assisted Reproduction and Genetics, 36(2), 241-247.

Nagy, S., Albini, S., Di Girolamo, V., & Nani, R. (2018). Impact of new semen extenders and artificial insemination protocols on reproductive efficiency in dairy cattle. Theriogenology, 114, 182-189.

Scientia Africana, Vol. 24 (No. 3), June, 2025. Pp 279-290
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Nosrati, R., *et al*. (2019). Microfluidics for sperm analysis and selection. Nature Reviews Urology, 16(7), 391-407.

Ortega-Ferrusola, C., (2018). Lipid peroxidation, DNA fragmentation and developmental potential of cryopreserved red deer spermatozoa. Reproduction, Fertility and Development, 30(6), 881-889.

Ortega-Ferrusola, C., Gil, M. C., Salido, G. M., Peña, F. J., & Rodríguez-Martínez, H. (2018). New challenges in the development of sperm biomarkers for fertility assessment. Basic and Clinical Andrology, 28(1), 1-15.

Pribenszky, C., (2017). Cryopreservation of oocytes and embryos: A review of evolving technologies and applications. Reproductive BioMedicine Online, 34(4), 333-346.

Purdy, P. H. (2019). A review on goat sperm cryopreservation. Journal of Animal Science and Biotechnology, 10(1), 1-11.

Rathi, A., *et al*. (2020). Artificial intelligence and machine learning in cryobiology. Cryobiology, 94, 90-99.

Reinders, M. E., (2019). Advances in cryopreservation: From molecular mechanisms to clinical applications. Transplantation Reviews, 33(3), 139-147.

Rienzi, L., Gracia, C., Maggiulli, R., LaBarbera, A. R., Kaser, D. J., Ubaldi, F. M., & Racowsky, C. (2017). Oocyte, embryo and blastocyst cryopreservation in ART: Systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. *Human Reproduction Update*, 23(2), 139–155. https://doi.org/10.1093/humupd/dmw038

Salamon, S., & Maxwell, W. M. (2000). Storage of ram semen. Animal Reproduction Science, 62(1-3), 77-111.

Saragusty J, Arav A. Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. Reproduction. 2011;141(1):1-19.

Saragusty, J., & Arav, A. (2011). Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. Reproduction, Fertility and Development, 24(1), 1-9.

Saravi, S. S. S., &Memili, E. (2019). Biopolymers in reproductive medicine: The emerging roles of alginate and gelatin hydrogels. Advances in Experimental Medicine and Biology, 1174, 345-359.

Seymour, T. C. (2016). IUI and sperm cryopreservation. *Journal of Reproductive Medicine, 61*(7), 271-280

Showell, M. G., Brown, J., Yazdani, A., Stankiewicz, M. T., Hart, R. J., 2014. Antioxidants for male subfertility. Cochrane Database of Systematic Reviews, 12.

Smith, L., (2018). Enhanced vitrification protocols for oocyte cryopreservation. Human Reproduction, 33(6), 975-986.

Songsasen N, Wildt DE, O'Brien JK (2003). The Cryobiology of Spermatozoa. In: Holt WV, Pickard AR, Rodger JC, Wildt DE, editors. Reproductive Science and Integrated Conservation. Cambridge: Cambridge University Press. p. 197-224.

Songsasen, N., (2003). Cryopreservation: A tool for ex situ management of genetic diversity in wildlife. Biotechnology Advances, 21(2), 401-412.

Vajta, G., & Kuwayama, M. (2006). Improving cryopreservation systems. *Theriogenology*, 65(1), 236–244. https://doi.org/10.1016/j.theriogenology.2005.09.026

Watson, P. F. (2000). The causes of reduced fertility with cryopreserved semen. Animal Reproduction Science, 60-61, 481-492.

Watson, P. F. (2015). Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. Reproduction, Fertility, and Development, 27(6), 894-905.

Watson, P. F., & Holt, W. V. (2001). Cryobanking the genetic resource: Wildlife conservation for the future? In P. F. Watson & W. V. Holt (Eds.), *Cryobanking the Genetic Resource: Wildlife Conservation for the Future?* (pp. 1–9). Taylor & Francis.

Watson, P. F., (2018). Sperm preservation: An introduction to its role in species conservation. Reproduction, Fertility and Development, 30(1), 45-55.

Woelders, H., Zuidberg, C. A., & Haring, R. M. (2006). Cryopreservation of semen and embryos in farm animals and humans. *Theriogenology*, 65(1), 206–216 https://doi.org/10.1016/j.theriogenology.2005.09.007

Yildirim, S., Toledo, R. T., & Palacios, M. (2016). Bio-nanocomposite films and their potential applications for food packaging. Journal of Food Science, 81(4), 1062-1071.