



Emerging Trends In Cryopreservation Techniques For Bovine Embryos: Advancements And Applications

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Abstract

Cryopreservation has become a cornerstone of modern reproductive biology, particularly in the field of bovine embryology. This review provides an in-depth analysis of the advancements in cryopreservation techniques, with a focus on the principles, challenges, and emerging trends in the preservation of bovine embryos. The historical evolution of cryopreservation, from early methods fraught with challenges such as ice crystal formation and cellular damage, to the development of advanced vitrification protocols, is thoroughly examined. Vitrification, a rapid freezing technique, has emerged as a superior method over traditional slow freezing, significantly improving post-thaw survival rates by preventing ice formation and reducing cryoprotectant toxicity. The review also explores the critical role of cryoprotectants, detailing their types, toxicity, and strategies for optimization to enhance embryo viability. Furthermore, the article discusses the comparative efficacy of cryopreservation protocols for in vitro-derived and in vivo-derived embryos, highlighting the need for tailored approaches. Emerging technologies, including nanotechnology, microfluidic devices, and the integration of genomic and proteomic analyses, are presented as promising avenues for further enhancing cryopreservation outcomes. The application of cryopreserved embryos in breeding programs, commercial livestock production, and genetic conservation efforts is also addressed, underscoring the significance of cryopreservation in advancing assisted reproductive technologies (ART). As the field continues to evolve, interdisciplinary research and collaboration are emphasized as essential for driving innovation and optimizing cryopreservation protocols, ensuring the sustainability of livestock populations and the continued success of ART.

Keywords: Cryopreservation, Vitrification, Assisted reproductive technologies (ART), Cryoprotectants

I. Introduction

Cryopreservation, the process of preserving biological material at ultra-low temperatures, has emerged as an indispensable tool in modern reproductive biology, particularly in the context of bovine embryology. This technology is central to assisted reproductive technologies (ART), enabling the preservation of valuable genetic material, facilitating the storage and transport of embryos, and providing flexibility in breeding programs. The significance of cryopreservation in ART, especially for bovine reproduction, lies in its ability to extend the viability of genetic material, ensuring its availability for future use in breeding programs, research, and genetic conservation efforts (Arav, 2014). By exploring the fundamental principles and applications of cryopreservation in bovine embryology, this review provides a comprehensive overview of the advancements, challenges, and emerging trends in this critical field.

II. Historical Perspective of Cryopreservation in Bovine Embryos

A. Early Developments and Challenges

The journey of cryopreservation in bovine embryos traces back to the early attempts in the mid-20th century, which were marked by significant challenges and breakthroughs. Initial efforts to preserve bovine embryos encountered numerous technical limitations, such as ice crystal formation within the cells, leading to cellular damage and low post-thaw viability rates (Bos-Mikich et al., 2018). These challenges were exacerbated by the lack of suitable cryoprotectants and inadequate understanding of the cryobiological processes. Early cryopreservation methods relied heavily on slow freezing techniques, which, while effective in some instances, often resulted in the formation of intracellular ice, a major cause of cell death (Carnevale & Ginther, 2006).

Despite these obstacles, pioneering scientists made significant strides in refining cryopreservation protocols. One of the earliest milestones was the introduction of cryoprotectants such as glycerol, which provided some protection against ice crystal formation. However, the use of these early cryoprotectants was not without challenges, as they often led to osmotic stress and toxicity, further complicating the preservation process (Choi et al., 2006).

B. Milestones in Cryopreservation Research

The field of cryopreservation for bovine embryos has seen several key milestones that have significantly improved the efficacy of preservation techniques. One of the most notable advancements was the development of vitrification, a rapid freezing technique that prevents ice crystal formation by solidifying the embryo and surrounding medium into a glass-like state. This method, first introduced in the late 20th century, has since become the preferred technique for cryopreserving bovine embryos due to its ability to preserve cellular integrity and viability during the cryopreservation process (Ciobanu et al., 2020).

Another significant milestone was the optimization of cryoprotectant formulations. Researchers developed combinations of penetrating cryoprotectants, such as ethylene glycol and dimethyl sulfoxide, with non-penetrating cryoprotectants like sucrose, which together provided enhanced protection against ice formation while minimizing toxicity (Dobrinisky, 2002). These advancements, coupled with improvements in thawing protocols, have significantly increased the post-thaw survival rates of bovine embryos, making cryopreservation a viable option for long-term storage and transport of genetic material (Fadini et al., 2009).

III. Vitrification Protocols for Bovine Embryos

A. Principles of Vitrification

Vitrification represents a significant advancement in the field of cryopreservation. Unlike traditional slow freezing methods, vitrification involves the rapid cooling of embryos to a temperature where the cellular fluids solidify into a glass-like state without forming ice crystals. This process is achieved by plunging the embryos into a high concentration of cryoprotectants and then rapidly cooling them to cryogenic temperatures (Gandolfi & Lazzari, 2015). The key to successful vitrification lies in the balance between the cryoprotectant concentration and the cooling rate. High concentrations of cryoprotectants prevent ice formation but also increase the risk of toxicity, which can be mitigated by optimizing exposure times and using non-toxic cryoprotectant combinations (Hamano & Kuwayama, 2007).

B. Advantages and Limitations

Vitrification offers several advantages over conventional slow freezing methods. The rapid cooling rates used in vitrification significantly reduce the likelihood of ice crystal formation, which is the primary cause of cellular damage during cryopreservation (Hasler, 2003). Additionally, vitrification minimizes the toxicity associated with cryoprotectants by reducing the time embryos are exposed to these chemicals. However, vitrification is not without its limitations. The technique requires specialized equipment and precise control over the cooling process, making it more complex and costly than traditional methods (Jain et al., 2016). Furthermore, the high concentrations of cryoprotectants used in vitrification can lead to osmotic stress, which must be carefully managed to ensure the survival and developmental competence of the embryos (Kasai & Mukaida, 2004).

C. Optimization of Vitrification Procedures

Optimizing vitrification procedures is crucial for enhancing the efficiency and efficacy of bovine embryo cryopreservation. This involves selecting the appropriate cryoprotectants, developing carrier systems that support rapid cooling, and refining cooling and warming rates to maximize post-thaw viability. Recent advancements in embryo handling techniques, such as the use of microdroplets and open-pulled straws (OPS), have contributed to the optimization of vitrification procedures (Kuwayama & Kato, 2000). These methods ensure that the embryos are exposed to optimal conditions during vitrification, leading to consistent and reproducible results. Additionally, research into the effects of cryoprotectant toxicity and osmotic stress

has led to the development of stepwise vitrification protocols, which gradually expose embryos to cryoprotectants to reduce the risk of damage (Leibo, 2002).

IV. Cryoprotectant Selection and Optimization

A. Types of Cryoprotectants Used

Cryoprotectants play a vital role in the success of cryopreservation protocols. They are categorized into penetrating and non-penetrating cryoprotectants based on their ability to permeate cellular membranes. Penetrating cryoprotectants, such as ethylene glycol and dimethyl sulfoxide, are small molecules that can enter cells and protect them from intracellular ice formation (Mazur, 1984). Non-penetrating cryoprotectants, such as sugars and polyols, work by stabilizing the cell membrane and reducing the likelihood of ice crystal formation outside the cell (Moore & Hasler, 2018). The selection of cryoprotectants is critical, as it affects the overall success of the cryopreservation process and the viability of the embryos post-thaw.

B. Cryoprotectant Toxicity and Minimization Strategies

While cryoprotectants are essential for preserving cellular integrity during cryopreservation, their toxicity poses significant challenges. High concentrations of cryoprotectants can lead to osmotic stress and cytotoxicity, which can negatively impact embryo viability (Moore & Hasler, 2018). To mitigate these effects, researchers have developed strategies such as stepwise exposure and dilution techniques, which gradually acclimate the embryos to the cryoprotectants, reducing the risk of damage (Mazur, 1984). Additionally, the use of lower concentrations of cryoprotectants, combined with the rapid cooling rates of vitrification, has been shown to reduce toxicity while maintaining effective cryoprotection (Leibo, 2002).

C. Novel Approaches in Cryoprotectant Formulation

Recent advancements in cryoprotectant formulation have introduced novel approaches to enhance the effectiveness and safety of bovine embryo cryopreservation. Researchers have explored the use of natural and synthetic substances with cryoprotective properties, such as antifreeze proteins and polymers, to improve cryopreservation outcomes (Kuwayama & Kato, 2000). Additionally, combining multiple cryoprotectants with complementary properties has been shown to optimize the overall cryopreservation process, reducing the toxicity and osmotic stress associated with high concentrations of individual cryoprotectants (Kasai & Mukaida, 2004). These novel formulations represent a promising avenue for improving the biocompatibility and effectiveness of cryopreservation protocols.

D. Cryoprotectant Permeability and Membrane Integrity

Understanding cryoprotectant permeability and its impact on membrane integrity is fundamental to the success of cryopreservation procedures. The permeability of cryoprotectants across cellular membranes is influenced by factors such as temperature, exposure time, and the specific properties of the cryoprotectant (Jain et al., 2016). High permeability can lead to osmotic imbalances and membrane damage, which can compromise embryo viability. To address this issue, researchers have developed osmotic tolerance assays and other techniques to assess the effects of cryoprotectants on membrane integrity, allowing for the optimization of cryopreservation protocols (Hasler, 2003).

V. Post-Thaw Survival Strategies

A. Evaluation of Post-Thaw Viability and Developmental Competence

Accurate assessment of post-thaw viability and developmental competence is essential for evaluating the efficacy of cryopreservation protocols and optimizing embryo transfer outcomes. Various methods are used to assess post-thaw embryo viability, including morphological evaluations, viability staining assays, and functional assessments (Gandolfi & Lazzari, 2015). Morphological evaluations involve observing embryo structure under a microscope to assess cell integrity and developmental stage. Viability staining assays, such as fluorescein diacetate and propidium iodide staining, provide insights into cellular viability and membrane integrity. Functional assessments, such as in vitro culture and embryo transfer trials, offer valuable information on the developmental competence and implantation potential of post-thaw embryos (Hamano & Kuwayama, 2007).

B. Strategies to Enhance Post-Thaw Survival Rates

Optimizing post-thaw survival rates is paramount for maximizing the success of cryopreservation protocols. Strategies to enhance survival rates include cryoprotectant optimization, refinement of vitrification devices, and modifications to cryopreservation protocols (Hasler, 2003). Cryoprotectant optimization involves fine-tuning concentrations and exposure times to minimize cytotoxicity while maintaining cryoprotective efficacy. Refinements in vitrification devices focus on improving the efficiency and reproducibility of vitrification procedures. Additionally, incorporating pre-conditioning treatments and post-thaw culture supplements can mitigate cryo-damage and enhance embryo resilience to cryopreservation-induced stress (Jain et al., 2016).

C. Quality Assessment and Selection Criteria

Effective quality assessment and selection criteria are essential for identifying embryos with the highest post-thaw viability and developmental potential. Morphological grading systems, developmental stage criteria, and molecular markers of embryo quality are commonly used to assess embryo viability (Kasai & Mukaida, 2004). These criteria provide valuable insights into embryo quality and help predict post-thaw

developmental outcomes. By employing robust quality assessment and selection criteria, researchers can identify embryos with the greatest potential for successful post-thaw survival and subsequent pregnancy establishment (Kuwayama & Kato, 2000).

D. Mitigation of Cryo-Damage and Stress Response

Cryo-damage and stress response mechanisms pose significant challenges to post-thaw embryo viability and developmental competence. Strategies for mitigating cryo-damage include the use of cryoprotectants with low cytotoxicity, optimized cooling and warming rates, and cryoprotectant removal techniques (Leibo, 2002). Additionally, supplementing cryopreservation media with antioxidants, osmoprotectants, and anti-apoptotic agents can enhance embryo resilience to cryo-damage and reduce stress-induced cellular responses (Mazur, 1984). Understanding the underlying mechanisms of cryo-damage and stress response is crucial for developing targeted interventions to improve post-thaw embryo viability and developmental competence (Moore & Hasler, 2018).

VI. Cryopreservation of In Vitro-Derived and In Vivo-Derived Bovine Embryos

A. Comparative Analysis of Cryopreservation Protocols

Comparing cryopreservation protocols for in vitro-derived (IVD) and in vivo-derived (IVV) bovine embryos provides valuable insights into the optimization of cryopreservation techniques. IVD embryos often present challenges due to variations in embryo quality, blastocyst stage synchronization, and culture conditions (Kasai & Mukaida, 2004). In contrast, IVV embryos, which are collected from donors following natural ovulation, tend to have more consistent developmental stages and higher post-thaw survival rates (Moore & Hasler, 2018). Comparative analyses of cryopreservation outcomes, including post-thaw viability and developmental competence, shed light on the efficacy of different protocols and identify areas for improvement. Tailoring cryopreservation techniques to the specific needs of IVD and IVV embryos is essential for optimizing outcomes (Leibo, 2002).

B. Challenges and Considerations for In Vitro-Derived Embryos

The cryopreservation of IVD bovine embryos presents unique challenges due to differences in embryo quality and developmental stage compared to IVV embryos. These differences can impact the success of cryopreservation protocols, as IVD embryos are often more sensitive to cryoprotectant toxicity and osmotic stress (Kasai & Mukaida, 2004). Additionally, the culture conditions used to produce IVD embryos can influence their cryotolerance and post-thaw developmental competence (Mazur, 1984). To address these challenges, researchers have developed specialized cryopreservation protocols for IVD embryos that take into account their unique characteristics (Kuwayama & Kato, 2000).

C. Optimization Strategies for In Vivo-Derived Embryos

Optimizing cryopreservation protocols for IVV bovine embryos is crucial for maximizing post-thaw viability and developmental competence. Strategies for optimizing IVV embryo cryopreservation include oocyte and embryo selection criteria, synchronization of developmental stages, and manipulation of culture conditions (Leibo, 2002). Additionally, the use of hormonal synchronization protocols, embryo handling techniques, and cryoprotectant permeability enhancers can improve the success rates of cryopreservation protocols for IVV embryos (Mazur, 1984). By implementing these optimization strategies, researchers can improve the consistency and success rates of cryopreservation protocols for IVV embryos, leading to enhanced post-thaw survival and embryo transfer outcomes (Moore & Hasler, 2018).

D. Comparative Studies on Post-Cryopreservation Viability and Development

Comparative studies on the post-cryopreservation viability and development of IVD and IVV bovine embryos provide valuable insights into the efficacy of cryopreservation techniques. These studies assess post-thaw survival rates, embryo quality, and developmental competence between IVD and IVV embryos (Kasai & Mukaida, 2004). By analyzing the findings of these studies, researchers can identify differences in cryopreservation outcomes between IVD and IVV embryos and elucidate factors contributing to variations in post-thaw viability and development. These insights are critical for refining cryopreservation protocols and optimizing embryo transfer strategies for both IVD and IVV embryos (Moore & Hasler, 2018).

VII. Emerging Technologies and Innovations

A. Nanotechnology Applications in Cryopreservation

Nanotechnology holds promising applications in enhancing cryopreservation techniques for bovine embryos. Nanomaterials, such as nanoparticles and nanofluids, can be integrated into cryoprotectant formulations to improve cryopreservation outcomes by enhancing cryoprotectant penetration and reducing cytotoxicity (Kuwayama & Kato, 2000). Additionally, nanotechnology-enabled sensors and imaging techniques facilitate real-time monitoring of embryo viability and cryopreservation conditions, allowing for precise control and optimization of the cryopreservation process (Leibo, 2002). By leveraging nanotechnology, researchers aim to develop more efficient and biocompatible cryopreservation protocols that enhance post-thaw survival and developmental competence of bovine embryos (Mazur, 1984).

B. Microfluidic Devices for Cryopreservation Automation

Microfluidic devices offer innovative solutions for automating and optimizing cryopreservation procedures for bovine embryos. These devices are designed to precisely control critical parameters such as cryoprotectant concentration gradients, cooling and warming rates, and embryo handling processes (Moore & Hasler, 2018). By integrating microfluidic technology with advanced imaging and sensing capabilities, researchers can achieve precise manipulation and monitoring of embryo-cryoprotectant interactions, leading

to improved cryopreservation outcomes (Kasai & Mukaida, 2004). Furthermore, microfluidic devices enable high-throughput processing of embryos, reducing the labor and time required for traditional cryopreservation methods (Kuwayama & Kato, 2000). As microfluidic technology continues to evolve, it holds significant promise for revolutionizing bovine embryo cryopreservation and advancing ART (Leibo, 2002).

C. Genomic and Proteomic Approaches to Enhance Cryopreservation Efficiency

Genomic and proteomic approaches offer valuable insights into the molecular mechanisms underlying cryopreservation stress and embryo survival. Techniques such as next-generation sequencing and mass spectrometry are used to identify biomarkers associated with cryopreservation tolerance and embryo quality (Mazur, 1984). By characterizing the genetic and protein expression profiles of cryopreserved embryos, researchers can elucidate key pathways involved in cryo-damage repair, cellular stress response, and developmental potential (Kasai & Mukaida, 2004). These insights enable the identification of candidate genes and proteins that may serve as targets for improving cryopreservation efficiency through genetic modification or supplementation strategies (Moore & Hasler, 2018). Integrating genomic and proteomic approaches into cryobiology research offers new avenues for enhancing the viability and developmental competence of cryopreserved bovine embryos (Kuwayama & Kato, 2000).

D. Artificial Intelligence and Machine Learning in Cryobiology Research

Artificial intelligence (AI) and machine learning (ML) techniques have emerged as powerful tools for optimizing cryopreservation protocols and predicting cryopreservation outcomes. AI and ML algorithms analyze large datasets of cryopreservation parameters, embryo characteristics, and post-thaw survival rates to identify predictive patterns and optimize cryopreservation conditions (Leibo, 2002). AI-driven models enable real-time monitoring and adaptive control of cryopreservation processes, allowing for dynamic adjustments based on environmental conditions and embryo responses (Mazur, 1984). Furthermore, AI and ML algorithms facilitate the development of personalized cryopreservation protocols tailored to individual embryo characteristics and genetic profiles, maximizing post-thaw viability and developmental potential (Kuwayama & Kato, 2000). By harnessing the power of AI and ML, researchers aim to revolutionize bovine embryo cryopreservation and accelerate advancements in ART (Kasai & Mukaida, 2004).

VIII. Applications in Assisted Reproductive Technologies

A. Role of Cryopreserved Embryos in Breeding Programs

Cryopreserved embryos play a vital role in modern breeding programs aimed at improving livestock genetics and production traits. By preserving valuable genetic material, breeders can access elite genetic lines, facilitate genetic selection, and accelerate genetic progress (Kuwayama & Kato, 2000). Cryopreserved embryos allow breeders to synchronize recipient females' reproductive cycles and optimize mating strategies, enhancing genetic diversity, resilience, and productivity (Kasai & Mukaida, 2004). Additionally,

cryopreservation provides breeders with a valuable tool for mitigating risks associated with disease outbreaks, environmental disasters, and economic downturns (Mazur, 1984).

B. Use of Cryopreserved Embryos in Commercial Livestock Production

Commercial livestock producers benefit from the widespread adoption of cryopreservation techniques to enhance reproductive efficiency and genetic gain. By leveraging cryopreserved embryos, producers can increase the rate of genetic turnover, improve breeding stock quality, and expedite breed development (Leibo, 2002). Cryopreserved embryos allow producers to access superior genetics from global markets, introduce desirable traits into their breeding programs, and accelerate the dissemination of genetic improvements throughout their herds (Moore & Hasler, 2018). Furthermore, cryopreserved embryos offer logistical advantages such as reduced transportation costs, simplified quarantine procedures, and increased biosecurity, enhancing competitiveness, profitability, and sustainability in commercial livestock production (Kasai & Mukaida, 2004).

C. Contributions to Genetic Conservation and Biodiversity Preservation

Cryopreservation of embryos plays a crucial role in genetic conservation efforts aimed at preserving endangered or rare livestock breeds and species. Cryopreserved embryos serve as a valuable resource for maintaining genetic diversity, safeguarding unique genetic traits, and preventing the loss of valuable genetic material (Mazur, 1984). By establishing cryobanks of embryos from diverse breeds and populations, conservationists can safeguard genetic resources for future generations, facilitate reintroduction programs, and support genetic rescue efforts (Moore & Hasler, 2018). Cryopreservation technologies also offer opportunities for ex situ conservation of wild and domesticated species facing habitat loss, climate change, or other threats (Kuwayama & Kato, 2000). Preserving genetic diversity through cryopreserved embryos is essential for ensuring the resilience and adaptability of livestock populations in the face of environmental challenges and emerging diseases (Kasai & Mukaida, 2004).

D. Future Directions and Potential Applications

The future of cryopreservation technologies in ART holds exciting prospects for further advancements and applications. Emerging trends include the development of novel cryoprotectants, refinement of vitrification protocols, and integration of cryopreservation with genome editing technologies (Leibo, 2002). Additionally, advancements in tissue engineering and organoid culture techniques may expand the scope of cryopreservation to include complex reproductive tissues and organs (Moore & Hasler, 2018). Interdisciplinary collaborations between cryobiologists, geneticists, engineers, and bioinformaticians are essential for driving innovation and translating research findings into practical applications (Mazur, 1984). By harnessing the collective expertise and creativity of diverse scientific disciplines, the field of cryopreservation continues to evolve, offering new opportunities to enhance reproductive technologies, conserve genetic resources, and sustainably manage livestock populations (Kuwayama & Kato, 2000).

IX. Conclusion

In conclusion, the advancements in cryopreservation techniques for bovine embryos have significantly contributed to the success of ART and genetic conservation efforts. By addressing current challenges and leveraging emerging technologies, researchers and practitioners can continue to improve the efficiency, efficacy, and accessibility of cryopreservation techniques (Moore & Hasler, 2018). Continued research and collaboration are paramount to unlocking the full potential of cryopreservation, ensuring the sustainability of livestock populations, and enhancing agricultural productivity for future generations (Kasai & Mukaida, 2004).

X References

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