

Role of Plant Extracts as Natural Additives in Cryopreservation of Bovine and Cattle Germplasm: A Review

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Abstract : Cryopreservation of bovine and cattle germplasm is a key component of artificial insemination programs, enabling long-term genetic preservation and dissemination. However, the freeze–thaw process induces oxidative stress, structural damage, and functional impairment in spermatozoa, thereby reducing fertility outcomes. Recently, plant-derived extracts have gained significant attention as natural additives in extenders due to their antioxidant, antimicrobial, and membrane-protective properties. This review provides a comprehensive overview of the role of plant extracts in bovine and cattle germplasm cryopreservation, emphasizing their bioactive compounds, mechanisms of action, and effects on post-thaw quality. Recent advances, including nano-formulated plant antioxidants, are also discussed. Despite promising findings, further research is needed to standardize extraction methods, optimize dosages, and validate field applications.

Keywords: cryopreservation, plant extracts, antioxidants, phytochemicals, bovine and cattle germplasm, extenders

Highlights

- Natural plant extracts enhance post-thaw sperm quality in bovines
- Antioxidant phytochemicals reduce oxidative stress during cryopreservation
- Improve membrane integrity and fertility potential
- Offer eco-friendly alternatives to synthetic additives
- Natural additives may replace synthetic antioxidants and antibiotics
- Emerging nano-formulations increase antioxidant efficiency
- Standardization and dosage optimization remain key challenges

Graphical Abstract

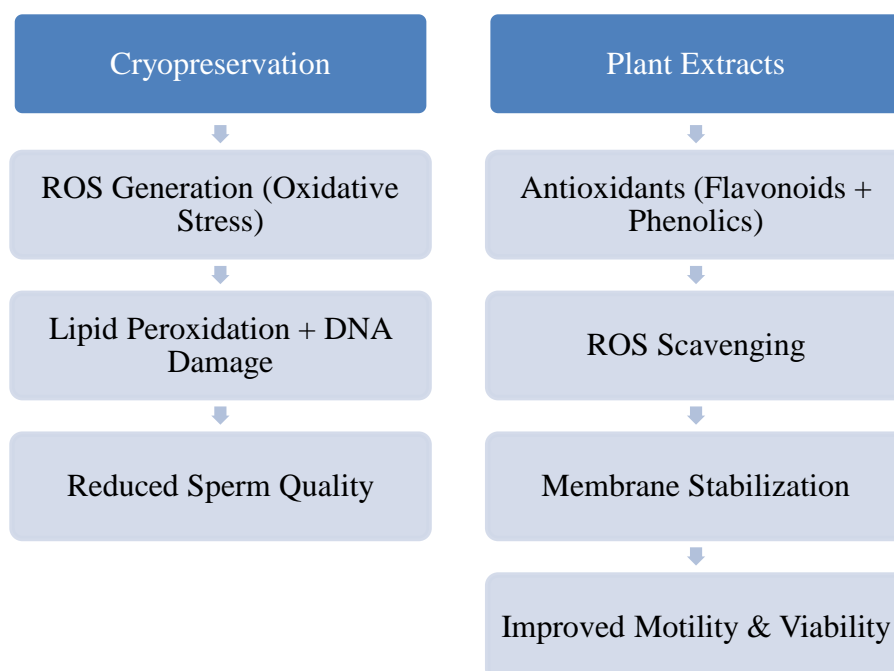


Figure 1: A flow diagram showing the mechanism of antioxidant action of plant extracts in cryopreservation.

1. INTRODUCTION

Cryopreservation has become an indispensable tool in modern bovine and cattle reproduction, allowing the storage and global distribution of superior genetic material. Artificial insemination (AI) has revolutionized bovine and cattle breeding by improving reproductive efficiency and accelerating genetic gain. The success of AI largely depends on the quality of cryopreserved germplasm. However, spermatozoa are highly susceptible to damage during freezing and thawing, leading to decreased motility, viability, and fertilizing capacity (Watson, 2000). Bovine spermatozoa are particularly vulnerable due to their high content of polyunsaturated fatty acids, making them susceptible to lipid peroxidation.

Cells are highly sensitive to temperature fluctuations during freezing and thawing, often leading to compromised function. One of the primary factors contributing to cryo-damage is oxidative stress caused by excessive production of reactive oxygen species (ROS). These ROS attack sperm membranes rich in polyunsaturated fatty acids, resulting in lipid peroxidation and cellular dysfunction (Aitken & Baker, 2006). To mitigate these effects, extenders are supplemented with protective agents, including antioxidants. In response to these challenges, researchers have explored natural alternatives, particularly plant-derived substances, due to their rich antioxidant composition and lower toxicity compared to synthetic compounds.

Traditional extenders include synthetic antioxidants; however, concerns regarding toxicity and long-term effects have prompted interest in natural alternatives. Recently, plant extracts have emerged as promising natural alternatives due to their rich composition of bioactive compounds and minimal side effects (El-Sheshtawy et al., 2016). Their incorporation into extenders has shown encouraging results in improving sperm quality during cryopreservation. Plant-derived extracts, rich in bioactive compounds, have emerged as promising candidates due to their strong antioxidant capacity and biological compatibility (Ros-Santaella & Pintus, 2021; Mphaphathi et al., 2024).

2. Cryopreservation-Induced Damage

Bovine sperm is particularly vulnerable due to its high lipid content, which increases susceptibility to oxidative damage. These factors collectively contribute to reduced fertility rates following artificial insemination.

The cryopreservation process imposes both physical and biochemical stress on spermatozoa. Ice crystal formation, membrane damage, mitochondrial dysfunction, protein denaturation, osmotic imbalance, and oxidative stress collectively contribute to structural and functional deterioration. Excess ROS production leads to lipid peroxidation, enzyme inactivation, and DNA fragmentation (Bailey et al., 2000).

Recent studies further confirm that oxidative stress is a major limiting factor affecting post-thaw sperm quality and fertility potential (Bastan et al., 2025). Mitochondrial dysfunction during cryopreservation also reduces ATP production, thereby impairing sperm motility.

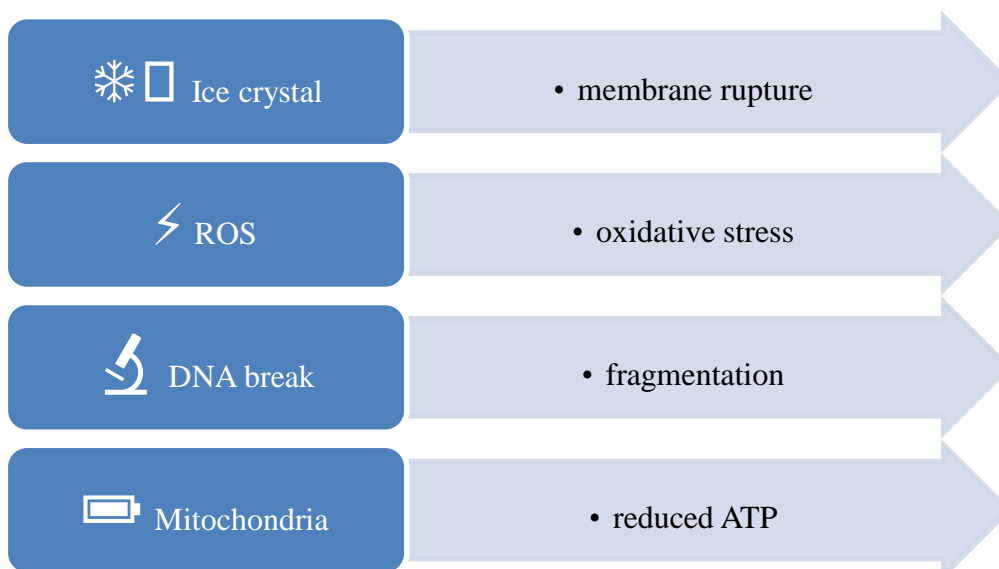


Figure 2: Structural and biochemical damage in spermatozoa during cryopreservation.

3. Need for Natural Additives

Extenders are specialized media designed to maintain sperm viability during storage. They typically contain buffers, energy sources, cryoprotectants (e.g., glycerol), antibiotics, and antioxidants (Salamon & Maxwell, 2000).

Although synthetic antioxidants are effective, concerns regarding toxicity, cost, and resistance have prompted the search for natural alternatives. Plant extracts provide a sustainable solution due to their antioxidant and antimicrobial properties (Ghareeb et al., 2020).

4. Bioactive Compounds in Plant Extracts

Plant extracts contain a diverse array of phytochemicals that contribute to their protective effects.

4.1 Phenolic Compounds and Flavonoids

Phenolics and flavonoids act as potent antioxidants by scavenging free radicals and preventing lipid peroxidation (Rice-Evans et al., 1997). These compounds are abundant in plants such as green tea and pomegranate.

4.2 Tannins and Saponins

They stabilize cell membranes and exhibit antimicrobial activity, reducing bacterial contamination (Cowan, 1999). Tannins exhibit antimicrobial and membrane-stabilizing properties, while saponins contribute to anti-inflammatory effects (Liman et al., 2022).

4.3 Vitamins and Carotenoids

Plant-derived vitamins such as vitamin C and E enhance antioxidant defense mechanisms and improve sperm viability and function. (Surai, 2002).

Table 1. Major Phytochemicals in plant extracts and their functions in cryopreservation

Phytochemical	Source	Function in cryopreservation
Flavonoids	Green tea, Moringa	Antioxidant, ROS scavenging
Phenolics	Pomegranate	Prevent lipid peroxidation
Tannins	Fennel, herbs	Membrane stabilization
Saponins	Various plants	Anti-inflammatory
Vitamins (C, E)	Fruits, leaves	Enhance antioxidant defense

5. Mechanisms of Action of Plant Extracts

Plant extracts exert protective effects through multiple mechanisms:

5.1 Antioxidant Activity

Plant extracts scavenge ROS and enhance endogenous antioxidant systems such as superoxide dismutase (SOD) and catalase (CAT), thereby reducing oxidative damage (Aitken et al., 2014).

5.2 Membrane Stabilization

Phytochemicals interact with sperm membranes, preserving their lipid bilayer structural integrity during freezing and thawing (Bansal & Bilaspuri, 2011).

5.3 Antimicrobial Effects

Plant extracts inhibit microbial growth, reducing the need for synthetic antibiotics in extenders (Ghasemi et al., 2016).

5.4 DNA protection

Prevention of fragmentation through reduced oxidative damage.

5.5 Mitochondrial support

Maintenance of ATP production and motility

Recent advancements indicate that nano-encapsulation of plant compounds enhances their bioavailability and effectiveness in extenders (Khalil et al., 2024).

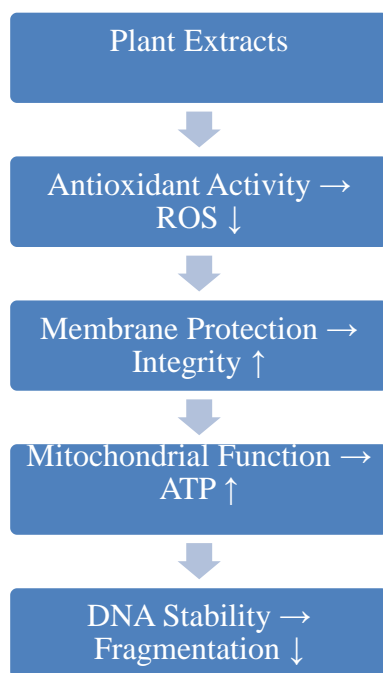


Figure 3. Protective mechanisms of plant extracts on sperm cells

6. Common Plant Extracts Used in Cryopreservation

Several plant extracts have been evaluated for their effectiveness:

6.1 Moringa oleifera: Supplementation improves sperm motility, viability, and antioxidant status due to its high flavonoid content (Siddique et al., 2021).

6.2 Camellia sinensis (Green Tea): Rich in catechins, green tea extract enhances sperm motility and reduces oxidative stress via catechins (El-Sheshtawy et al., 2016).

6.3 Punica granatum (Pomegranate): Contains strong antioxidants that enhance sperm quality and viability. It also reduces bacterial contamination (Türk et al., 2008).

6.4 Zingiber officinale (Ginger): Improves sperm motility and reduces lipid peroxidation in cryopreserved (Khalifa et al., 2014).

6.5 Cinnamomum zeylanicum (Cinnamon): Enhances membrane integrity, sperm motility, and overall post-thaw quality (Alahmar, 2019). It enhances membrane integrity and reduces enzyme leakage (Alam et al., 2024).

6.6 Phoenix dactylifera (Date Palm): Improves acrosomal integrity and sperm viability due to its antioxidant properties (Ali et al., 2017).

6.7 Curcumin: Improves sperm quality and oocyte fertilization potential (Lin et al., 2025)

6.8 Kaempferia parviflora: Reduces lipid peroxidation and enhances viability (Saiyamanon et al., 2025)

However, not all plant extracts produce positive effects, as their efficacy depends on concentration and composition (de Souza Silva et al., 2020).

Table 2. Plant extracts and their effects on quality

Plant Extract	Key Compounds	Observed Effects	Reference
<i>Moringa oleifera</i>	Flavonoids	↑ motility, viability	Siddique et al., 2021
<i>Camellia sinensis</i>	Catechins	↓ oxidative stress	El-Sheshtawy et al., 2016
<i>Punica granatum</i>	Polyphenols	↑ sperm quality	Türk et al., 2008
<i>Zingiber officinale</i>	Gingerol	↑ motility	Khalifa et al., 2014
<i>Cinnamomum zeylanicum</i>	Cinnamaldehyde	↑ membrane integrity	Alahmar, 2019
<i>Phoenix dactylifera</i>	Antioxidants	↑ acrosome integrity	Ali et al., 2017

7. Effects on Post-Thaw Quality

7.1 Motility

Plant extracts significantly improve progressive motility by protecting mitochondrial function and reducing oxidative damage (Bansal & Bilaspuri, 2011).

7.2 Viability and Membrane Integrity

Enhanced membrane stability results in higher percentages of live sperm cells post-thaw (Bailey et al., 2000). Improved membrane stability leads to higher survival rates post-thaw (Mphaphathi et al., 2024).

7.3 DNA Integrity

Reduction in oxidative stress helps maintain DNA integrity, stability, and minimize fragmentation (Aitken & Baker, 2006).

7.4 Fertility Outcomes

Some studies report improved fertilization rates; however, large-scale field validation is still required (Salamon & Maxwell, 2000).

Table 3. Impact of plant extracts on different parameters

Parameter	Effect of Plant Extracts	Mechanism
Motility	Increased	Mitochondrial protection
Viability	Increased	Membrane stabilization
Acrosome integrity	Improved	Reduced lipid peroxidation
DNA integrity	Maintained	ROS reduction
Fertility rate	Moderately improved	Overall sperm protection

8. Advantages of Plant-Based Additives

Plant-Based Additives have many advantages, such as: Natural and eco-friendly, Cost-effective and widely available, Reduced toxicity compared to synthetic additives, Potential alternative to antibiotics, Multifunctional (antioxidant + antimicrobial), and Reduced reliance on synthetic additives.

9. Limitations and Challenges

Despite their benefits, plant extracts face several challenges, such as a lack of standardization in extraction methods, variability in effective dosages, limited large-scale field validation studies, and potential toxicity at high concentrations. These limitations highlight the need for further research to optimize their use in cryopreservation.

Table 4. Advantages vs limitations of plant-based additives

Advantages	Limitations
Natural and eco-friendly	Lack of standardization
Cost-effective	Dose variability
Antioxidant + antimicrobial	Possible toxicity at high doses
Reduces antibiotic use	Limited field trials

10. Future Perspectives

Future studies should focus on:

- Isolation and characterization of active compounds
- Standardization of extraction and dosage protocols
- Dose optimization and toxicity evaluation
- Development of nano-formulations for improved delivery
- Integration with advanced cryopreservation techniques
- Large-scale fertility trials under field conditions

Recent developments in nanotechnology and plant-based antioxidants provide promising avenues for improving preservation efficiency (Khalil et al., 2024).

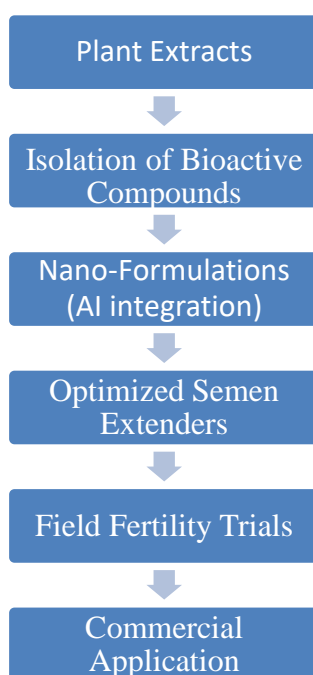


Figure 4. Future research directions

11. Conclusion

Plant extracts offer a promising natural alternative for improving bovine and cattle germplasm cryopreservation. Their antioxidant, antimicrobial, and membrane-stabilizing properties significantly enhance post-thaw sperm quality. However, further research is necessary to standardize their application and validate their effectiveness under practical conditions. The incorporation of plant-derived additives into extenders could play a transformative role in livestock reproductive biotechnology.

Declarations:

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical Approval

Not applicable.

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