Biotechnology in small ruminant reproduction: an international experience

The 4th CZU Prague hybrid seminar 2025

Extender supplements for the improvement of goat sperm freezability

Abbas Farshad
Veterinary Clinic for Reproductive Medicine and Neonatology,
Justus-Liebig-University of Giessen, Germany

Prague, Czech
06.05.2025

Freezing of Sperm

Advantages

- long-term storage
- Genetic diversity
- Increase in reproduction rate
- Extension of fertility
- International availability
- disease control

Disadvantages

- Increase in the risk of inbreeding
- Defective gene transmission
- Cost-intensive
- Technical challenges
- Requirement for trained professional staff

Damages related to Cryopreservation

- Decreased sperm motility
- Increase in membrane damage
- Increase in acrosome damage
- Increase in DNA damage
- Reduced reproductive capability
- Average 50% loss of sperm

Causes of Damage

- Formation of ice crystals and recrystallization
- Osmotic pressure conditions
- Lipid peroxidation
- Changes in the plasma membrane
- Oxidative **stress**

  (Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), singlet oxygen (O_2), peroxy nitrite ($ONOO^-$).

Ways to Prevent Damage

- Freezing Methods
- Application of Protective Agents

First one: Freezing Methods

(normal, slow, rapid ,

- **Method I: Slow**
  - +4°C to -120°C at a rate of 5°C/min.
- **Method II: Middle**
  - +4°C to 120°C at a rate of 25°C/min.
- **Method III: Fast** +4°C to -12o°C at a rate of 45°C/min.

Second one: Using of Protective Agents

- **Non-permeating Protective Agents:**
  - Non-permeating sugars (Sucrose, Trehalose), L-Carnitine, AFPs, Vitamin E,, Superoxide dismutase (SOD), catalase (CAT), etc.

- **Permeating Protective Agents:**
  - Egg yolk and Lecithin, Glycerin, Dimethyl Sulfoxide (DMSO), MitoQ, L-Cycteine, Vitamin C, etc.

What Protect the used Antioxidants

- Free Radical Scavenging
- DNA Protection
- Membrane Preservation
- Mitochondrial Support

Goat Sperm Cryopreservation: *Challenges and Solutions*

- Cryopreservation protocols for domestic animals are generally similar.

- Goat sperm requires specific modifications based on its specific seminal plasma.

- Phospholipase A and Glycoprotein lipase (SBUIII) present in seminal plasma as two detrimental factors to sperm when egg yolk and milk are used in extenders.

- These enzymes break down lecithin from egg yolks or triglycerides from milk, creating harmful substances (lysolecithin and fatty acids such oleic acid) that trigger early acrosome reactions and lead to cell death.

- This challenge is unique to goats and absent in bulls, boars, or rams.

- ***Therefore:***

UNDERSTANDING THE PHYSIOLOGY OF GOAT SPERM IS ESSENTIAL FOR SUCCESSFUL CRYOPRESERVATION.

Classical Antioxidants in Goat Sperm

Antioxidant	Mechanism	Documented Effects
Vitamin E	Lipid-soluble antioxidant, prevents lipid peroxidation	↑ Motility, ↑ Acrosomal integrity, ↓ MDA
Vitamin C	Restores Vitamin E and scavenges water-soluble ROS.	↑ Viability, ↓ ROS, ↑ Chromatin stability
Glutathione	Intracellular thiol buffer, redox regulation	↑ Membrane integrity, ↓ DNA fragmentation
SOD + Catalase	Enzymatic ROS detoxification	↓ Lipid peroxidation, ↑ Mitochondrial activity
Melatonin	Lipophilic scavenger, anti-apoptotic properties	↑ ATP, ↓ Apoptosis, ↑ Membrane fluidity

Cryoprotective Sugars

Trehalose

Protects membrane phospholipids from freezing damage.

- Enhances post-thaw motility
- Supports membrane recovery
- Improves viability

Sucrose

As non-permeating disaccharide provides osmotic stabilization.

- Reduced osmotic shock
- Maintains acrosomal integrity
- Preserves DNA stability

Pentoxifylline (PTX)

- A methylxanthine derivative and phosphodiesterase inhibitor.
- Increases intracellular cyclic AMP, leading to:
  - Enhanced motility and hyperactivation
  - Reduction in lipid peroxidation
- Improved capacitation potential
- Shown to improve post-thaw fertilizing ability in vitro.

Mitochondria-Targeted Antioxidants

- MitoQ: Mitoquinone mesylateMito
- TEMPO: 2,2,6,6-Tetramethylpiperidin-1-oxyl.
- Designed to focus on mitochondria and directly remove mitochondrial ROS at the source.

- Mechanism:
  - Scavenge superoxide and H_2O_2 within mitochondria
  - Prevent collapse of mitochondrial membrane potential ($\Delta\Psi m$)

- Outcomes:
  - ATP production↑
  - Mitochondrial oxidative damage↓

Antifreeze Proteins (AFPs)

- **Mechanism:**
  - Bind to ice crystals and inhibit growth/recrystallization
  - Reduce mechanical damage to sperm structures

- **Effects:**
  - Post-thaw motility and viability↑
  - Apoptosis and cryoinjury↓
  - Maintain acrosomal and plasma membrane integrity.
  - Motility, viability, and DNA integrity↑

Conclusion and Future Perspectives

- Incorporating antioxidants, cryoprotectants, and functional enhancers significantly improves post-thaw goat sperm quality.
- Understanding the precise mechanisms of freezing-thawing damage is essential for effectively minimizing its negative impact on sperm quality.
- Further research is essential to uncover the exact role of antioxidants in improving the quality of cryopreserved goat sperm.

Thank you very much for your attention.