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All author's name and affiliations are given below, after references

Semen extenders in caprine reproduction: Composition, application and future prospects

Anchal Sharma, Mukul Anand, Shikha Pareek, Sarvajeet Yadav, Brijesh Yadav, Rahul Dhariya, Amit Kumar and Rashmi

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Abstract
Semen extenders are indispensable in the cryopreservation and Artificial Insemination (AI) of semen, playing a critical role in maintaining sperm viability, motility, and fertilizing ability during storage and transport. Semen extenders are composed of various functional components, including cryoprotectants (e.g., glycerol, DMSO), buffers, energy sources, membrane stabilizers like egg yolk or soy lecithin, and antibiotics, each contributing to the preservation of sperm structure and function during the freeze-thaw process. But goat semen presents unique challenges due to the presence of seminal plasma enzymes like phospholipase A and proteases, which interact negatively with extender components egg yolk and skim milk-based extenders, leading to reduced sperm quality. This enzymatic activity results in the formation of toxic by-products that compromise membrane integrity and motility.

This review presents a comprehensive analysis of the composition, functions, and species-specific applications of semen extenders in goats, highlighting both challenges and recent advances in this domain. It explores species-specific extender formulations tailored to goat sperm physiology and the latest developments, including antioxidant-enriched and vitrification-based approaches to minimize oxidative and cryo-damage.

Keywords: Goat, cryoprotectant, cryopreservation, semen extender

Introduction

The preservation of male gametes through semen cryopreservation has become a cornerstone of modern reproductive biotechnology, particularly in the field of animal breeding and conservation. Among the critical elements ensuring the success of this technique are semen extenders that are specialized solutions used to preserve sperm viability during cryopreservation (freezing and storage at ultra-low temperatures), (Kalobo *et al.*, 2018) [11]. These extenders provide essential nutrients, protect sperm from cold shock, and prevent ice crystal formation, which can damage cell membranes (Bustani *et al.*, 2021) [5]. By minimizing cryo-induced damage and ensuring optimal cellular conditions, these formulations significantly enhance the efficiency of artificial insemination (AI) and long-term genetic preservation.

Each component in a semen extender is meticulously selected to serve a specific physiological function. Cryoprotectants, such as glycerol and ethylene glycol, prevent intracellular ice crystal formation and reduce osmotic stress during freezing (Kumar *et al.*, 2025) [14]. Buffers maintain pH and osmotic balance, while energy sources like glucose and fructose support sperm metabolism. Lipid and protein supplements derived from egg yolk, milk, or soy lecithin stabilize sperm membranes and mitigate cold shock (Rahimi *et al.*, 2024) [27]. Antibiotics are also incorporated to curb microbial contamination.

However, despite the widespread application of these extenders in species like cattle, pigs, and horses, their use in goats presents unique challenges due to species-specific seminal plasma enzymes particularly phospholipase A and proteases that negatively interact with extender components such as egg yolk and milk proteins (Batool *et al.*, 2024) [4].

Corresponding Author:
Anchal Sharma
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

These enzymatic interactions often lead to sperm toxicity, flocculation, or reduced motility in caprine semen, necessitating the development of species-specific, biosecure, and consistent alternatives. Recent advances in extender technology, including soy lecithin-based formulations, synthetic diluters, and antioxidant-enriched media, offer promising solutions to overcome these challenges. For example, supplementation with natural antioxidants such as quercetin (Batool *et al.*, 2024) [4], isoglycyrrhizin (Zhang *et al.*, 2024) [38], and coenzyme Q10 (Oktanella *et al.*, 2024) [24] has shown significant improvements in post-thaw sperm viability, membrane integrity, and mitochondrial function. Similarly, pyridoxine-enriched soy lecithin extenders have been reported to enhance motility and reduce lipid peroxidation (Rahimi *et al.*, 2024) [27].

Innovative cryopreservation strategies such as vitrification, freeze-drying, and nano-based extender systems are also under investigation (Kumar *et al.*, 2025) [14]. These emerging approaches aim to further reduce cryoinjury, ensure consistency across semen batches, and enhance fertility outcomes. Moreover, customized extenders tailored for goat semen are now commercially available or being developed to improve standardization and efficiency in goat artificial insemination protocols.

This review comprehensively explores the functional roles, composition, applications, and species-specific considerations of semen extenders, with a particular emphasis on goat semen. It further highlights the limitations of conventional formulations and discusses recent innovations aimed at improving post-thaw sperm quality and fertility, thereby advancing the field of caprine reproductive biotechnology.

Importance of semen extenders in semen dilution and cryopreservation

Semen extenders play a crucial role in both semen dilution and semen cryopreservation, ensuring sperm viability, motility, and fertility potential during storage and transportation Raheja *et al.* (2018) [29]. Semen dilution is necessary to extend the lifespan of sperm, increase the number of insemination doses from a single ejaculate, and preserve sperm quality during short-term storage (Neila-Montero *et al.*, 2024) [23].

Key components of semen extenders and their role in sperm survival

Semen extenders play vital roles in protecting sperm cells during the freeze-thaw process of cryopreservation. Each component contributes uniquely to preserving sperm viability, motility, and fertilizing ability.

- Cryoprotectants are essential for preventing ice crystal formation, which can physically damage sperm cells during freezing (Chang *et al.*, 2021) [6]. They also help in reducing the osmotic stress caused by changes in solute concentration during the freezing and thawing process (Khan *et al.*, 2008) [13]. Based on their mechanism of action, cryoprotectants are broadly classified into penetrating and non-penetrating types:

1. Penetrating Cryoprotectants

These cryoprotectants are small, water-soluble molecules that can permeate the sperm cell membrane and act intracellularly:

- Replace intracellular water.
- Reduce ice crystal formation.
- Stabilize intracellular proteins and membranes.

Cryoprotectant	Species Used	Mechanism of Action	Remarks	References
Glycerol	Cattle, Sheep, Goat, Dog, Horse	Penetrates sperm cells; replaces water; prevents intracellular ice; stabilizes proteins	Gold standard in cattle semen preservation	Andrabi (2007) [2]; Kumar *et al.* (2025) [14]
Ethylene Glycol	Goat, Sheep, Fish	Lower molecular weight; fast penetration; reduces intracellular ice	Suitable for vitrification in goats	Salamon & Maxwell (2000) [31]; Oktanella *et al.* (2024) [24]
Dimethyl Sulfoxide (DMSO)	Poultry, Rabbit, Equine, Fish	Strong permeability; membrane protection; scavenges free radicals	Effective in avian and fish sperm cryopreservation	Watson (2000) [37]; Zhang *et al.* (2024) [38]
Propylene Glycol	Horse, Pig, Rabbit	Intermediate permeability; less toxic than DMSO	Sometimes combined with sugars or proteins	Salamon & Maxwell (2000) [31]; Kumar *et al.* (2025) [14]

2. Non-Penetrating Cryoprotectants

These agents do not enter the sperm cell but act extracellularly to:

- Control dehydration rate.
- Form a protective coat around the sperm.
- Reduce ice formation in the extracellular space.

Cryoprotectant	Species Used	Mechanism of Action	Remarks	References
Sucrose / Trehalose	Bull, Ram, Goat	Osmotic dehydrant; stabilizes membranes; non-toxic	Used in vitrification and combination protocols	Kumar *et al.* (2025) [14]
Polyvinylpyrrolidone (PVP)	Equine, Canine, Avian	Prevents extracellular ice; increases viscosity; physical stabilizer	Common in poultry cryopreservation	Watson (2000) [37]; Andrabi (2007) [2]
Egg Yolk Lipoproteins	Cattle, Buffalo	Provides phospholipids and LDL; protects membranes	Not suitable for goat due to phospholipase interaction	Salamon & Maxwell (2000) [31]; Batool *et al.* (2024) [4]
Milk Proteins (Skim Milk, Casein)	Cattle, Sheep	Provide protein colloids; buffer against cold shock	Alternative to egg yolk in bulls	Andrabi (2007) [2]; Kumar *et al.* (2025) [14]

3. Antioxidant Additives (Functional Cryoprotectants): While not true cryoprotectants, these compounds support cell viability during freezing by neutralizing reactive oxygen species (ROS).

Additive	Species Used	Mechanism of Action	Examples	References
Vitamin C, E	All domestic species	ROS scavenging; lipid membrane protection	Improves sperm motility & DNA integrity	Ansari *et al.* (2023) [3]; Kumar *et al.* (2025) [14]
Glutathione, SOD, Catalase	Goat, Bull, Boar	Enzymatic neutralization of ROS	Enhances post-thaw fertility potential	Oktanella *et al.* (2024) [24]; Zhang *et al.* (2024) [38]
Quercetin, Coenzyme Q10, Resveratrol	Goat, Stallion	Mitochondrial protection; improves ATP and membrane stability	Promising in goats and stallions	Batool *et al.* (2024) [4]; Oktanella *et al.* (2024) [24]

- Buffers maintain the pH balance and osmotic stability of the extender solution, creating an environment that supports sperm cell survival (Neila-Montero *et al.*, 2024) [23]. Proper pH is crucial to avoid damage to sperm enzymes and membranes (Mishra *et al.*, 2018). Common buffering agents include Tris (tris hydroxy methyl amino methane), citrate, and phosphate.
- Energy sources provide the necessary nutrients for sperm metabolism, helping maintain motility and cellular functions during storage (Lisboa *et al.*, 2021) [15]. Carbohydrates such as glucose, fructose, and lactose are commonly added to support sperm energy requirements.
- Proteins and lipids serve to protect sperm membranes from cold shock, which occurs due to sudden temperature drops during freezing. These components help stabilize the sperm plasma membrane and prevent structural damage. Egg yolk is widely used in cattle, horse, and dog semen extenders for its high phospholipid content (Manjunath *et al.*, 2021) [19]. Milk proteins also offer membrane protection, while soy lecithin is used as a non-animal alternative, especially in bio secure or standardized semen extender formulations (Aires *et al.*, 2003) [1].
- Antibiotics are included in semen extenders to prevent bacterial contamination, which can damage sperm and reduce fertility. Common antibiotics used include *penicillin*, *streptomycin*, and *gentamicin*, all of which help maintain sterility and extend the shelf life of the diluted semen (Santos *et al.*, 2020) [32].

  Each component in a semen extender has a specific function that provides protection to sperm, ensuring that sperm cells remain viable and functional after the freezing and thawing process.

Challenges associated with goat semen dilution

Dilution of goat semen with egg yolk or skimmed milk-based extenders is a common practice in artificial insemination programs. However, several problems are associated with these extenders, particularly due to the unique composition of goat seminal plasma.

When using egg yolk-based extenders, one major issue is the formation of gel-like clots or flocculates (Sen *et al.*, 2015) [33]. This occurs because goat seminal plasma contains an enzyme called phospholipase A, which reacts with the phospholipids in egg yolk. The enzymatic activity leads to the breakdown of these phospholipids, resulting in the production of harmful substances such as lysolecithin and fatty acids (Rizal *et al.*, 2003) [30]. These substances can damage the sperm membrane and reduce motility and fertility. Additionally, egg yolk composition varies from one batch to another, which affects the consistency of semen preservation. There is also a high risk of microbial contamination since egg yolk is an animal-origin product and cannot be sterilized without losing its protective properties. This makes aseptic preparation difficult and increases the chances of introducing pathogens (Singh *et al.*, 2017) [35]. Similarly, skimmed milk-based extenders also present several challenges. Goat seminal plasma enzymes, particularly proteases, can degrade milk proteins and release toxic by-products that impair sperm quality (Corteel *et al.*, 1981) [8]. Another limitation of skimmed milk extenders is their relatively poor cryoprotective ability during freezing and thawing. As a result, semen preserved with these extenders often shows lower post-thaw motility compared to other extender types. In some cases, the interaction between milk and seminal plasma can lead to the formation of precipitates or clots, which may entrap sperm cells or interfere with microscopic evaluation (Plante *et al.*, 2015) [26]. Like egg yolk, skimmed milk is also subject to batch-to-batch variation, which affects the reproducibility of results. Moreover, milk-based extenders generally have a shorter shelf life and are not very effective for long-term semen storage.

Overall, both egg yolk and skimmed milk-based extenders are less ideal for preserving goat semen due to the presence of specific enzymes in goat seminal plasma that interact negatively with these extender components. For this reason, soy lecithin-based synthetic extenders are increasingly being used as a safer and more consistent alternative. These extenders offer better biosecurity, improved cryoprotection, and standardized composition, making them more suitable for goat semen preservation (Sun *et al.*, 2020) [36].

Routinely use goat semen extender for semen dilution and preservation:

Despite limitation with use of semen extender in goats, different types of semen diluters are used to dilute goat semen depending on the purpose whether for short-term storage, long-term freezing, or biosecurity concerns. These diluters, also called extenders, play a crucial role in preserving the viability and fertility of sperm by protecting them from cold shock, maintaining pH and osmotic balance, providing nutrients, and preventing microbial contamination. The different semen extenders used for goat semen dilution are as follows-

- The most commonly used diluters in goats are egg yolk-based diluters, such as the Tris-egg yolk-citrate-glucose extender (Dhami *et al.*, 2014) [9]. This type of diluter typically contains Tris and citric acid as buffering agents, glucose as an energy source, egg yolk but in a lower concentration to minimize lethal interactive losses and impart protect sperm membranes, and antibiotics to prevent bacterial growth.
- Milk-based diluters, like skim milk-glucose extenders, are often used for short-term preservation (Rahman *et al.*, 2013) [28]. Skim milk proteins help stabilize the sperm cells, while glucose provides energy, and antibiotics are included to inhibit microbial growth (Martínez-Pastor *et al.*, 2011) [20].
- Soy lecithin-based diluters serve as an animal protein-free alternative to egg yolk-based extenders (Fernández-Santos *et al.*, 2016) [10]. Commercial examples include AndroMed® and BioXcell®.These diluters are preferred when avoiding animal-origin biohazards is important, and they offer the advantage of a standardized and sterile composition.

- In some tropical regions, coconut water-based diluters are used. Coconut water naturally contains sugars and electrolytes beneficial for sperm, and it is often combined with egg yolk or glycerol for better efficacy (Pandey *et al.*, 2018) [25].
- Glycerolated diluters are specifically used for the cryopreservation of goat semen. Glycerol serves as a cryoprotectant that helps prevent damage from ice crystal formation during the freezing process (Chaudhary *et al.*, 2017) [7]. These diluters are usually based on Tris, egg yolk, or soy lecithin.
- There are also commercial ready-to-use extenders available in the market that are specifically formulated for goat semen. Products like Triladyl®, Androhep®, and Optixcell® are commonly used due to their consistency, sterility, and ease of preparation.

The selection of a suitable semen diluter depends on several factors such as the desired storage duration (short-term at 4°C or long-term in liquid nitrogen), the sensitivity of goat sperm to cold shock, biosecurity considerations, and the availability of extender components.

Advances and future strategies

Recent advancements in goat semen extender technology have been driven by the limitations of traditional extenders such as egg yolk- and skim milk-based formulations. To overcome these challenges, research is now focused on the development of synthetic and species-specific extenders, particularly animal origin free diluter example soy lecithin-based extenders. These extenders eliminate animal-derived proteins, thus reducing the risk of microbial contamination and avoiding enzymatic reactions that are detrimental to spermatozoa. Additionally, the incorporation of novel cryoprotectants such as ethylene glycol, dimethyl sulfoxide (DMSO), and propylene glycol has been explored to enhance cryoprotection. These agents have demonstrated improved ability to reduce intracellular ice crystal formation and osmotic damage during freezing and thawing, resulting in better post-thaw viability compared to glycerol alone (Sharafi *et al.*, 2022; Lv *et al.*, 2019) [34, 17]. Another promising area of advancement is the use of antioxidants including glutathione, ascorbic acid, and vitamin E within extender formulations to combat oxidative stress, which is a key contributor to sperm DNA damage during cryopreservation. The addition of such antioxidants has been associated with improved mitochondrial function, membrane integrity, and DNA stability in post-thaw goat sperm (Lukusa *et al.*, 2019; Khan *et al.*, 2021) [17, 12]. Emerging technologies, such as nanoparticle-based delivery systems, have further enhanced the efficacy of antioxidants by providing targeted protection and sustained release during the freeze-thaw process (Malekpour Afshar *et al.*, 2021) [18].

Despite these advancements, future research must focus on optimizing the biochemical composition of extenders, particularly with respect to osmolarity, pH, and ion concentrations that align with the physiological requirements of goat sperm. Moreover, vitrification, an ultra-rapid freezing method that prevents ice crystal formation altogether, is gaining attention as an alternative to conventional cryopreservation, although its practical application in goats requires further refinement (Lv *et al.*, 2019) [17]. Integrating-omics technologies such as proteomics and metabolomics can provide deeper insights into the molecular responses of sperm cells to cryopreservation stress, aiding in the development of precision-designed extenders (Mekonnen *et al.*, 2023) [21].

Conclusion

In conclusion, the field of goat semen extender technology is advancing rapidly, with plant-based extenders, novel cryoprotectants, antioxidants, and nanotechnology paving the way for improved cryopreservation outcomes. Future research should focus on refining extender formulations to align with the unique biological characteristics of goat sperm, integrating molecular tools to elucidate cryo-induced damage mechanisms, and exploring innovative freezing techniques such as vitrification for enhanced fertility and genetic conservation

Conflict of Interest

Not available

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Reference

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Author's Name and Details

Anchal Sharma
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

Mukul Anand
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

Shikha Pareek
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

Sarvajeet Yadav
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

Brijesh Yadav
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

Rahul Dhariya
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

Amit Kumar
Department of Veterinary, Physiology, College of Veterinary

Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

Rashmi
Department of Veterinary, Physiology, College of Veterinary Sciences and Animal Husbandry, Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

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