RESEARCH ARTICLE

Efficacy of Ovixcell Extender for Cryopreservation of Buck Epididymal Spermatozoa

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Abstract

Cryopreservation of spermatozoa is a cornerstone of reproductive biotechnology, enabling long-term storage of genetic material from livestock and endangered species. However, the freezing-thawing process predisposes spermatozoa to cryoinjuries, including osmotic stress, oxidative damage, and alterations in membrane integrity. This study evaluated the efficacy of Ovixcell, a soy lecithin-based extender, in preserving buck epididymal spermatozoa during cryopreservation. Testes (n=40) were collected from freshly slaughtered bucks, and spermatozoa were retrieved from the cauda epididymides. Samples with >70% motility were cryopreserved using Ovixcell supplemented with 7% glycerol. Sperm quality was assessed pre-freeze and post-thaw using standard procedures, including motility, CFDA and PI dual staining for viability, hypo-osmotic swelling test (HOST) and acrosomal integrity using Giemsa staining. The results showed a significant ($p < 0.0001$) reduction in sperm characteristics from pre-freeze to post-thaw stage. The motility decreased from 82.60% to 63.80%, viability from 80.47% to 61.13%, plasma membrane integrity from 92.16% to 59.40%, and acrosomal integrity from 90.92% to 63.43%. Despite this decline, post-thaw values remained within the acceptable range for further use. The findings confirm that Ovixcell stabilizes sperm membranes effectively and serves as a good sperm extender, supporting artificial insemination and conservation programmes through retrieval and storage of epididymal spermatozoa.

Key words: Buck, Cryopreservation, Ovixcell, Post-thaw, Sperm quality parameters.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.1.28

Introduction

Cryopreservation of spermatozoa is a cornerstone of modern reproductive biotechnology, allowing long term storage of genetic material – spermatozoa from valuable or endangered male animals and supporting artificial insemination programmes across wild animals and livestock species. During the freezing-thawing process, spermatozoa are exposed to fluctuating and extreme temperature ranges that predispose them to multiple forms of cryoinjury, including mechanical stress from ice crystal formation, osmotic shock, and oxidative stress (Januskauskas *et al.,* 2003; Shi *et al.,* 2023). Among the cellular structures, the plasma membrane is particularly susceptible to damage because it is rich in polyunsaturated fatty acids and proteins critical for maintaining cellular integrity and functionality. Cryo-capacitation, a phenomenon observed during sperm cryopreservation, involves biochemical and structural changes in the sperm plasma membrane that mimic the capacitation process normally occurring in the female reproductive tract (Watson, 1995; Srivastava *et al.,* 2013; Castro *et al.,* 2025).

Forward motility of sperm, essential for transport through the female reproductive tract and penetration of the oocyte membranes, is lost or reduced after cryopreservation primarily due to structural damage to flagellar proteins, mitochondrial dysfunction, and oxidative stress, all of which compromise ATP generation (Mortimer *et al.,* 1986). Acrosomal integrity is equally crucial, as the acrosome contains hydrolytic enzymes necessary for zona pellucida penetration. Damage to the acrosome during freezing-thawing may lead to premature or incomplete acrosome reactions, rendering sperm incapable of fertilization. Capacitation like events often lead to premature or spontaneous acrosome reactions, compromising sperm

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How to cite this article: Sakeena, S., Naikoo, M., Khatun, A., Sofi, K. A., Andrabi, M. A., Ahmad, T., & Pampori, Z. A. (2026). Efficacy of Ovixcell Extender for Cryopreservation of Buck Epididymal Spermatozoa. Ind J Vet Sci and Biotech, 22(1), 144-148.

Source of support: Nil

Conflict of interest: None

Submitted 04/09/2025 **Accepted** 30/09/2025 **Published** 10/01/2026

functionality and reducing their lifespan post-thaw (Bailey *et al.,* 2000). Consequently, cryo-capacitation is considered a primary factor responsible for the reduced longevity and survivability of cryopreserved spermatozoa within the female reproductive tract (Talukdar *et al.,* 2015).

Spermatozoa retrieved from the cauda epididymides of deceased animals and its cryopreservation represent a "hidden reservoir" of functional gametes with immense value for *ex-situ* conservation of genetic resources (Luvoni and Morselli, 2017: Martínez-Fresneda *et al.*, 2019). To mitigate the cryoinjuries, osmoticially, nutritionally and cryoprotectant (egg yolk or soy lecithin) optimized semen extenders are employed during the freezing process (Purdy, 2006). Ovixcell is a commercially available extender that uses soy lecithin as a membrane-stabilizing agent, offering an alternative to traditional egg yolk-based extenders. Its use has been extensively documented in ram semen cryopreservation, and recent studies have explored its applicability for buck semen. Saratsi *et al*. (2023) demonstrated that supplementation of Ovixcell with 2.15 mM fumaric acid enhanced post-thaw sperm viability, membrane integrity, acrosomal status, and mitochondrial function, highlighting the potential for further optimization of soy lecithin-based extenders. Further, Ovixcell was found to maintain higher sperm viability, membrane functionality, and acrosome integrity than AndroMed, though egg yolk-based extender still provided slightly better overall preservation of buck semen at 5°C for 48 h (Saratsi *et al.,* 2024). Thus, the present study focused on evaluating the efficacy of Ovixcell extender in preserving buck epididymal spermatozoa during cryopreservation, using functional sperm parameters and to contribute in refining epididymal semen cryopreservation protocols for bucks' future breeding and conservation programmes.

Materials and Methods

Harvesting and Processing of Testes

The study was conducted at the Division of Animal Reproduction, Gynaecology and Obstetrics, Shuhama-Alusteng, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir (J&K, India). A total of 40 buck testicles were collected and studied in 10 replicates to ensure consistency and reliability of the results. Fresh testes were obtained from a local abattoir immediately after slaughter. Each pair of testes was carefully excised, trimmed of excess tissue, and individually packed in sterile polythene bags to prevent contamination and fluid leakage. The samples were then placed on ice packs and transported in an insulated ice chest to the laboratory to maintain a temperature close to 4 °C, minimizing metabolic activity and preventing sperm deterioration during transit. Upon arrival at the laboratory, the testicles (weighing more than 80 g) were processed immediately for retrieval of cauda epididymal spermatozoa under aseptic conditions. The tunica vaginalis and surrounding connective tissue were carefully excised, after which an incision was made in the tunica albuginea to expose the testicular parenchyma. The epididymis was then carefully dissected from the testis. This meticulous handling facilitated the recovery of high-quality spermatozoa suitable for cryopreservation and sperm evaluation.

Collection and Extension of Spermatozoa

The cauda epididymides separated from the testes were further incised and placed in 3 mL of Ovixcell extender (Commercial soybean-lecithin based extender, IMV Technology, France) and incubated for 20 min at room temperature to allow sperm to swim out. Sperm motility was then initially evaluated on a pre-warmed slide by adding one drop of sperm sample with a drop of extender. Samples exhibiting more than 70% motility were considered viable. Further, the cauda epididymides tissues were thoroughly rinsed with an additional 1 mL of Ovixcell, and the collected sperm samples were pooled and then diluted with the extender for subsequent use.

Sperm Cryopreservation

The harvested semen was diluted with Ovixcell, a soy lecithin-based extender, supplemented with 7% glycerol as a cryoprotectant to a final concentration of 250 to 300 million sperm/mL. The diluted semen was carefully loaded into 0.25 mL French mini straws and sealed to prevent contamination or leakage. The straws were then gradually cooled to 4-5 °C and allowed to equilibrate for 4 h. For freezing, the straws were placed in a Styrofoam box positioned above liquid nitrogen, exposing the semen to nitrogen vapour at approximately -70 °C to -120 °C for 10 min, and then plunged in liquid nitrogen at -196 °C temperature and stored for long-term preservation. Thawing was performed after two weeks by immersing the straws in a 37 °C water bath for 30 to 60 seconds. Semen samples were evaluated pre-freeze and immediately following thawing for motility, viability, acrosome integrity and HOST to assess the success of cryopreservation.

Sperm Quality Evaluation

Sperm motility: A drop of diluted sperm sample was placed on a grease-free slide maintained at 37°C on a biotherm stage, covered with a cover slip and was examined under the phase contrast microscope (40X). The numbers of spermatozoa that move in forward direction were estimated (Mortimer *et al.,* 1986) and the percentage was determined.

Sperm viability: Sperm viability was evaluated using fluorescent microscopy with Carboxyfluorescein Diacetate (CFDA) - a non-fluorescent, cell-permeable dye and Propidium Iodide (PI) - a membrane-impermeable nucleic acid dye, following the method described by Harrison and Vickers (1990). A staining medium was prepared by combining 20 µL of a CFDA stock solution (25 mg/mL in DMSO, stored in the dark at –20 °C) with 60 µL of a PI stock solution (5 mg/mL in isotonic saline, stored in the dark at

–20 °C). Thin smears prepared from the processed semen were examined under a fluorescence microscope (Magnus, India) equipped with a Mag Cam MU2A camera and Mag Vision software (2.3 MP, 1/1.19" CMOS sensor). The viable spermatozoa exhibited bright green fluorescence with blue filter (480 nm), whereas non-viable spermatozoa appeared orange-red to fully red with the green filter (535 nm). For each slide, 200 spermatozoa were counted across randomly selected fields, and the percentage of viable spermatozoa (Fig.1) was calculated.

Acrosomal integrity: The acrosomal status was evaluated by Giemsa staining technique (Watson, 1995) using thin, fixed, overnight stained smears under the microscope (100X). More than 200 spermatozoa were examined, and the percentage of sperm with intact acrosome (Fig. 2) was determined using standard formula.

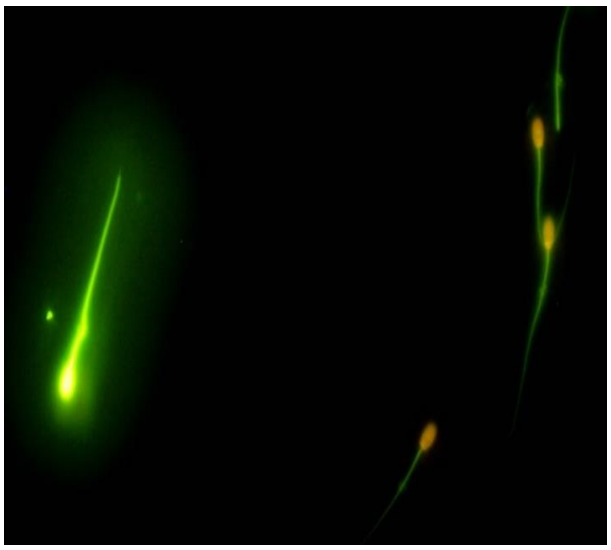

Fig. 1: CFDA and PI staining (live spermatozoa with green fluorescence and dead spermatozoa with red to orange-red fluorescence)

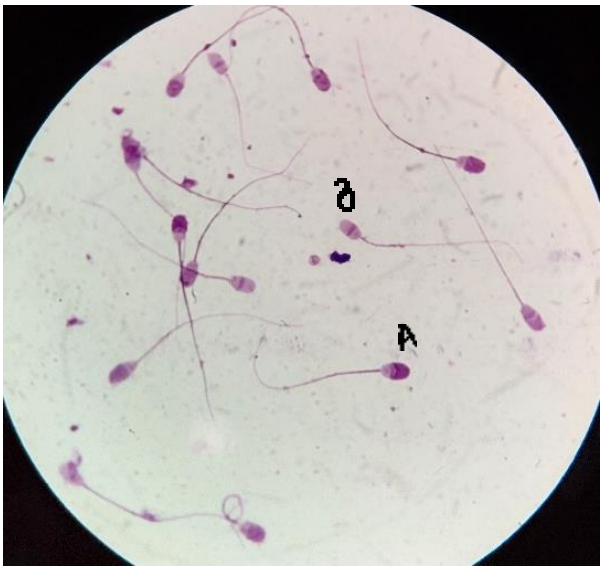

Fig. 2: Giemsa staining for acrosomal integrity (A: Intact acrosome, B: completely lost acrosome).

Hypo-osmotic swelling test (HOST): The HOST was performed to evaluate the plasma membrane integrity of the sperm present in the extended sample as per the method described by Vasquez *et al.* (2012). Spermatozoa with visible coiling of tail were considered as HOST reactive. A total of 200 spermatozoa were counted to determine HOST (Fig. 3) using standard formula.

Fig. 3: Hypo-osmotic swelling test (coiled means HOS reactive and non-coiled means non-reactive

Statistical Analysis

The data recorded in the experiment were analysed by students paired t-test to detect the level of significance, if any between pre-freeze and post-thaw sperm quality parameters.

Results and Discussion

In the present study, the use of Ovixcell extender in cryopreservation of buck epididymal spermatozoa showed a highly significant ($p < 0.0001$) decline in sperm functional attributes after thawing over pre-freeze values (Table 1). The progressive motility decreased from 82.60 ± 0.74% in the pre-freeze stage to 63.80 ± 0.92% at post-thaw stage. This decline was in accordance with earlier reports of bucks and rams' semen, where cryopreservation negatively affected motility due to structural and functional alterations in flagellar proteins and mitochondrial activity (Fernandez-Santos *et al.,* 2006; Sharafi *et al.,* 2022). The impaired mitochondrial membrane potential caused by oxidative stress and lipid peroxidation during freezing-thawing has been identified as a primary factor limiting post-thaw motility (Agarwal *et al.,* 2021).

Similarly, sperm viability declined from 80.47 ± 1.02% to 61.13 ± 1.37%. Reduced viability from pre-freeze to post-thaw stage reflects loss of plasma membrane integrity, osmotic imbalance, and increased production of reactive oxygen species (ROS). This agreed with the findings of Saratsi *et al.* (2023), who demonstrated that although Ovixcell maintains

relatively higher viability compared with other lecithin-based extenders, the post-thaw survival is still lower than fresh semen values. The relatively high pre-freeze and post-thaw motility and membrane integrity demonstrated that Ovixcell effectively stabilizes sperm membranes, likely due to the presence of plant-based phospholipids that substitute for egg yolk in maintaining membrane fluidity during cryopreservation (Bodu *et al.,* 2025). The post-thaw motility (64%) and viability (61%) obtained in this study were comparable to previous findings in bucks using Ovixcell extender, emphasising its suitability as a non-egg yolk alternative for epididymal sperm preservation (Marco-Jiménez *et al.,* 2004; Saratsi *et al.,* 2024).

Table 1: Efficacy of Ovixcell for cryopreservation of buck epididymal spermatozoa

S.No	Sperm quality parameters	Mean (± SE) Values	
		Pre-freeze	Post-thaw
1	Motility	82.60 ± 0.74a	63.80 ± 0.92b
2	Viability	80.47 ± 1.02a	61.13 ± 1.37b
3	HOS test	92.16 ± 0.20a	59.40 ± 2.78b
4	Acrosomal integrity	90.92 ± 0.86a	63.43 ± 2.63b

Means with different superscripts differ significantly ($p<0.0001$).

Sperm plasma membrane integrity as assessed by HOST, decreased drastically from 92.16 ± 0.20% before freezing to 59.40 ± 2.78% after thawing. The sperm plasma membrane is highly enriched with polyunsaturated fatty acids, making it vulnerable to lipid peroxidation during temperature fluctuations (Januskauskas *et al.,* 2003). The resulting disruption of membrane fluidity and permeability leads to leakage of intracellular enzymes, impaired ion exchange, and reduced fertilizing potential. Similar declines in HOST response after cryopreservation have been documented in bucks and rams (Bucak *et al.,* 2010; Lone *et al.,* 2021).

Acrosomal integrity was also significantly reduced from 90.92 ± 0.86% pre-freeze to 63.43 ± 2.63% in post-thaw semen. Damage to the acrosome is often associated with cryo-capacitation and destabilization of acrosomal membranes, which can trigger premature acrosome reactions (Bailey *et al.,* 2000; Srivastava *et al.,* 2013). Such alterations compromise the sperm ability to penetrate the oocyte. The extent of acrosomal damage observed was comparable to previous reports in goat and ram spermatozoa cryopreserved with both egg yolk and soy lecithin-based extenders (Saratsi *et al.,* 2024).

Overall, the typical decrease in sperm quality parameters from pre-freeze to post-thaw stage was suggestive of sperm cell osmotic imbalance, ice crystal formation, lipid peroxidation, and loss of membrane fluidity (Lone *et al.,* 2021; Castro *et al.,* 2025). However, despite the significant decline, the post-thaw values obtained with Ovixcell were within the acceptable range for artificial insemination and were in accordance with earlier reports using the same extender in ruminants (Marco-Jimenez *et al.,* 2004; Saratsi *et al.,* 2024). Further, the findings highlight the positive potential of Ovixcell in retrieving and conserving genetic material from valuable or endangered animals at post-mortem**,** thereby supporting *ex-situ* genetic resource conservation strategies (Martínez-Fresneda *et al.,* 2019).

Conclusions

The present findings demonstrated that cryopreservation of buck epididymal spermatozoa using Ovixcell extender resulted in sperm quality parameters within the highly acceptable range for use in assisted reproductive technologies. The relatively better preservation of sperm functional parameters highlights the efficacy of Ovixcell, which, due to its plant-based phospholipid composition, serves as a reliable and bio-secure extender. These findings emphasize the potential application of Ovixcell in long-term storage of epididymal spermatozoa from genetically valuable or endangered species including bucks, thereby contributing to genetic conservation and assisted breeding programmes.

Acknowledgement

The authors are thankful to abattoir owners of Jammu and Kashmir for providing the fresh testicular samples immediately after slaughtering the bucks.

References

Agarwal, A., Hamada, A., & Esteves, S.C. (2021). Oxidative stress in male infertility: Causes, effects, and potential therapeutics. *Andrology, 53*(1), 1-10.

Bailey, J.L., Bilodeau, J.F., & Cormier, N. (2000). Semen cryopreservation in domestic animals: A damaging and capacitating phenomenon. *Journal of Andrology, 21,* 1-8.

Bodu, M., Hitit, M., Greenwood, O.C., Murray, R.D., & Memili, E. (2025). Extender development for optimal cryopreservation of buck sperm to increase reproductive efficiency of goats. Frontiers in Veterinary Science*, 12,* 1-18.

Bucak, M.N., Sariozkan, S., Tuncer, P.B., Sakin, F., Atessahin, A., Kulaksiz, R., & Cevik, M. (2010). The effect of antioxidants on post-thawed Angora goat (*Capra hircus ancryrensis*) sperm parameters, lipid peroxidation and antioxidant activities. *Small Ruminant Research, 89*(1), 24-30.

Castro, M., Leal, K., Pezo, F., &Contreras, M.J. (2025). Sperm membrane: Molecular implications and strategies for cryopreservation in productive species. *Animals, 15*(12), 1-18.

Fernandez-Santos, M.R., Esteso, M.C., Montoro, V., Soler, A.J., & Garde, J.J. (2006). Cryopreservation of Iberian red deer (*Cervus elaphus hispanicus*) epididymal spermatozoa: Effects of egg yolk, glycerol, and cooling rate. *Theriogenology, 66*(8), 1931-1942.

Harrison, R.A.P., & Vickers, S.E. (1990). Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. *Journal of Reproduction and Fertility, 88,* 343-52.

Januskauskas, A., Johannisson, A., &Rodriguez-Martinez, H. (2003). Subtle membrane changes in cryopreserved bull semen in relation with sperm viability, chromatin structure and field fertility. *Theriogenology, 60,* 743-758.

Lone, F.A., Naikoo, M., Shah, S.M., Darzi, S.A., & Farooq, J. (2021). Effect of idebenone, resveratrol and taurine on the sperm quality and lipid peroxidation of cryopreserved crossbred ram semen. *Cryoletters, 42*(3), 146-153.

Luvoni, G.C., & Morselli, M.G. (2017). Conservation of feline semen. *Animal Reproduction Science, 181,* 56-63.

Marco-Jimenez, F., Puchades, S., Mocé, E., Viudes-de-Cartro, M.P., Vicente, J.S., & Rodriguez, M. (2004). Use of powdered egg yolk vs fresh egg yolk for the cryopreservation of ovine semen. *Reproduction in Domestic Animals, 39*(6), 438-441.

Martínez-Fresneda, L., Castaño, C., Bóveda, P., Tesfaye, D., Schellander, K., & García-Vázquez, F.A. (2019). Epididymal and ejaculated sperm differ on their response to the cryopreservation and capacitation processes in mouflon (*Ovis musimon*). *Scientific Reports*, 9(1), 1-13.

Mortimer, D., Pandey, I.J., & Sawers, R.S. (1986). Relationship between human sperm motility characters and sperm penetration into human cervical mucus *in vitro*. *Journal of Reproduction and Fertility, 78,* 93-102.

Purdy, P.H. (2006). A review on goat sperm cryopreservation. *Small Ruminant Research, 63,* 215-225.

Saratsi, A., Samartzi, F., Panagiotidis, I., Basioura, A., Tsiokos, D., Ligda, C., & Rekkas, CA. (2023). Post-thaw parameters of buck semen quality after soy lecithin extender supplementation with fumaric acid. *Veterinary Sciences, 10*(9), 1-13.

Saratsi, A., Samartzi, F., Tsiokos, D., Theodosiadou, E.K., Panagiotidis, I., Ligda, C., &Rekkas, C.A. (2024). Effect of three commercially available extenders containing phospholipids of different sources on Skopelos buck liquid-stored sperm quality. *Veterinary Sciences, 11*(10), 1-17.

Sharafi, M., Borghei-Rad, S.M., Hezavehei, M., Shahverdi, A., & Benson, J.D. (2022). Cryopreservation of semen in domestic animals: A review of current challenges, applications, and prospective strategies. *Animals, 12*(23), 1-24.

Shi, H., Li, Q.Y., Li, H., Wang, H.Y., Fan, C.X., Dong, Q.Y., Pan, B.C., Ji, Z.L., &Li, J.Y. (2023). ROS-induced oxidative stress is a major contributor to sperm cryoinjury. *Human Reproduction, 39*(1), 250-262.

Srivastava, N., Jerome, A., Srivastava, S.K., Ghosh, S.K., &Amit, K. (2013). Bovine seminal PDC–109 protein: A review of biochemical and functional properties. *Animal Reproduction Science, 138,* 1-13.

Talukdar, D.J., Ahmed, K., & Talukdar, P. (2015). Cryocapacitation and fertility of cryopreserved semen. *International Journal of Livestock Research, 5,* 1-18.

Vasquez, J., Florentini, E.A., & Valdivia, M. (2012). Hypo-osmotic swelling test (HOS) in semen evaluation. *Reproduction in Domestic Animals, 47*(6), 83-87.

Watson, P.F. (1995). Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. *Reproduction Fertility and Development, 7,* 871-891.