DOI: https://doi.org/10.59913/dagr.2026.21976

Comparison of two freezing techniques and two seminal extenders and their effect on post-thaw motility in the cryopreservation of goat sperm

BERČÍK, Jozef[1*] – VOZAF, Jakub[1,2] – BALÁŽI, Andrej[2] – CHRENEK, Peter[1,2]

[1]Institute of Biotechnology, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, Nitra, 949 76, Slovak Republic
[2]NPPC, Research Institute for Animal Production in Nitra, Hlohovecká 2, Lužianky, 951 41, Slovak Republic
*corresponding author: xbercikj@uniag.sk

Abstract

The aim of this study was to compare the effect of two freezing techniques (programmable and manual) and two seminal extenders (Triladyl® and OptiXcell®) on the quality of cryopreserved goat sperm after thawing. The experiment involved ejaculates from goats of the Slovak White Shorthair Goat (n = 3) and Slovak Brown Shorthair Goat (n = 2), collected during the natural reproductive period of goats. After collection, the sperm were diluted, equilibrated for two hours at 4 °C and then frozen using either programmable or manual technique. After thawing, the progressive sperm motility was evaluated. The results showed that the cryopreservation process led to a decrease in sperm motility compared to fresh samples. However, the use of the OptiXcell® extender ensured significantly higher progressive sperm motility after thawing compared to the Triladyl® extender (65.38% vs. 40.06%; $p < 0.05$) for both freezing techniques. On the contrary, no statistically significant differences were found between programmable and manual freezing, indicating their comparable effectiveness (36.85% vs. 40.06% and 57.13% vs. 65.38%, respectively). Based on the results obtained, it can be concluded that the type of extender used has a more significant impact on sperm quality after thawing than the freezing technique itself. Manual freezing appears to be a suitable alternative to programmable freezing, especially under conditions with no access to automated equipment, which may have significant practical benefits for the preservation of goat genetic resources in gene banks.

Keywords: cryopreservation, goat, semen, Triladyl, Optixcell

DOI: https://doi.org/10.59913/dagr.2026.21976

Introduction

Goats are one of the most widespread animal species in the world and offer various uses and benefits (HANIFI et al., 2025). The White Shorthair goat is a dairy breed, developed in the first half of the 20th century by crossbreeding native local goats with imported Saanen breed goats from Switzerland and Germany. The goats are early maturing, hardy, highly fertile with good feed conversion ability. The Brown Shorthair goat breed was developed by crossbreeding native spotted and brown goats with imported Harz breed goats from Germany. Initially bred as hornless, today hornedness is permitted in both sexes. The black triangle behind the ears is a characteristic feature of the breed (CHRENEK et al., 2019).

Sperm cryopreservation plays a key role in the long-term ex-situ in vitro conservation of endangered breeds (RANJAN et al., 2021). It is an important tool for long-term preservation of genetic material, which allows the preservation and transmission of genetic traits through assisted reproduction (ARIYAN et al., 2025). This technique is also important for the breed conservation process and has paved the way for other reproductive biotechnologies. Despite significant progress in this field, frozen and thawed sperm have huge differences in fertility rates (RANJAN et al., 2021). One of the main challenges is the cryosensitivity of caprine sperm, as the lipid-rich membranes of caprine sperm are very susceptible to damage during freezing and thawing. The high lipid content of seminal plasma exacerbates this cryosensitivity, making it difficult to maintain sperm viability after thawing (KUMAR et al., 2025). Cryopreservation significantly impairs sperm quality in goats, resulting in reduced viability, motility, membrane integrity and increased DNA fragmentation. These impairments are primarily due to the excessive production of reactive oxygen species (ROS) during freezing and thawing, which overwhelms the natural antioxidant defences in sperm (TRAN et al., 2025). ROS, such as superoxide anion (O_2-), hydroxyl radical (-OH) and hydrogen peroxide (H_2O_2), directly oxidize polyunsaturated fatty acids in sperm membranes, leading to lipid peroxidation and subsequent cellular dysfunction (SIKKA, 2004; LONE et al., 2018). The sperm membrane of goats is rich in unsaturated free fatty acids, which are susceptible to oxidative stress. Oxidative stress can alter the structure of the sperm head, leading to greater permeability of the cell membrane to fluids, resulting in lower sperm viability (MOHAMMED and OMAR, 2025). In addition, several factors such as breeds, seasons, husbandry practices and cryopreservation protocols are responsible for the poor quality of cryopreserved goat sperm (RANJAN et al., 2021).

Since the extent of cryodamage is strongly influenced by the composition of the preservation medium, the selection of an appropriate semen extender represents an important component of an effective cryopreservation protocol. Semen extenders provide an energy source, protect spermatozoa against cold shock and osmotic stress, and help maintain a suitable environment during cooling, freezing and thawing. A

DOI: https://doi.org/10.59913/dagr.2026.21976

number of extenders and additives have therefore been developed and evaluated for both short- and long-term semen preservation (RANJAN et al., 2021).

The use of liquid sperm requires a diluent, because sperm stored at room temperature undergoes metabolism, which causes a decrease in sperm quality (HAFIDHA et al., 2025). A number of extenders and additives have been used and tested for short-term and long-term sperm storage to maintain sperm motility and viability during storage (ARI and YILDIZ, 2018). The most commonly used extenders for goat sperm are either egg yolk-based extenders or non-fat dry skimmed milk-based sperm extenders (RANJAN et al., 2021). Goats are unique among livestock because during sperm donation; they release specific lipase enzymes from their bulbourethral gland that interact with components of the sperm extensor (egg yolk lipids and skim milk triglycerides) to produce a compound that is toxic to sperm. Therefore, the lipase enzyme in the seminal plasma is called egg yolk coagulation enzyme (EYCE). The deleterious interaction between egg yolk and EYCE occurs only in sperm of goat origin but not in bulls, boars or rams (SIAS et al., 2005).

During cryopreservation, equilibration refers to the period after mixing sperm with a cryoprotectant (usually glycerol or other cryoprotectants) to allow the sperm to acclimate to subfreezing temperatures before freezing. Equilibration times of 1 to 2 hours at 4°C are usually recommended to allow the sperm to stabilize and facilitate the gradual diffusion of cryoprotectants into the sperm. If the equilibration time is too short, the sperm may not absorb the necessary amount of cryoprotectant resulting in poor protection during freezing and subsequent damage during thawing. On the other hand, excessive equilibration time of more than 3 hours may lead to overexposure to cryoprotectants, which may cause toxicity or membrane disruption resulting in lower sperm motility and fertility after thawing (KUMAR et al., 2025).

Despite the progress made in the cryopreservation of caprine sperm, further improvements are needed to address issues such as oxidative stress, cryoinjury and environmental factors (KUMAR et al., 2025). The detailed molecular mechanism and extent of structural damage to sperm associated with cryopreservation have not been systematically investigated (RANJAN et al., 2021). Therefore, the aim of the present study was to compare the effects of two freezing techniques (manual and programmable freezing) and two commercial semen extenders (Triladyl® and OptiXcell®) on the progressive motility of cryopreserved goat sperm after thawing. In addition, the study aimed to determine whether manual freezing could provide post-thaw sperm motility comparable to that achieved using a programmable freezing system.

DOI: https://doi.org/10.59913/dagr.2026.21976

Material and methods

Animals

For these experiments, ejaculate samples from goats of the Slovak Brown Short-haired Goat (n = 2) and Slovak White Short-haired Goat (n = 3) breeds were used. During the experiments, the goats were kept separately from the goats in the breeding facility of the NPPC - Research Institute of Animal Production in Lužianky, at an altitude of approximately 154 m. a.s.l. Clinically healthy, sexually mature goats of the Slovak Brown Short-haired Goat and Slovak White Short-haired Goat breeds were involved in the experiments. The individuals were aged from 9 months to 3 years. The goats were fed meadow hay, alfalfa hay, oats (according to body condition score (BCS) and weight) and corn-barley meal (according to BCS and weight). Each goat had a vitamin-mineral supplement and water in its enclosure.

Collection and dilution of ejaculates

Sperm samples from goats were collected in 2025 during the natural reproductive period of goats (September – November) twice a week in the morning using an artificial vagina (Minitube, Germany) while jumping onto a fixed female. During the collection, the female was fixed in a standing position using simple physical sedation without the use of sedatives.

The sperm were processed immediately after collection at the laboratory of NPPC VÚŽV Nitra. From each goat, we diluted 10 µl of fresh sample with saline solution (0.9% NaCl; Braun, Germany) as required. We diluted the goat sperm samples in the ratio of 1:40 (v/v) or 1:50 (v/v). For diluted samples, we evaluated the volume, concentration and progressive motility of freshly collected sperm. Subsequently, we exposed the collected goat sperm to the cryoprotective medium Triladyl® (Minitube, Germany) or OptiXcell® (IMV Technologies, France) in a ratio of 1:10 (v/v). Equilibration was carried out for 2 h at 4 °C according to BERČÍK et al. (2026).

Freezing techniques

Programmable freezing (PF)

After equilibration, the straws were placed in a pre-cooled automatic freezer (IceCube, Minitube, Germany). After closing the lid, the software started to regulate the flow of nitrogen vapor into the sample compartment according to the set freezing program (+4 °C to −10 °C for 120 s, −80 °C for 450 s, −120 °C for 100 s and −140 °C for 180 s). After completing the freezing cycle, the straws were immersed into liquid nitrogen (−196 °C) in a container (Liquid nitrogen container, KGW-Isotherm, Germany).

The straws were thawed in a water bath at 40 °C for 15 seconds, afterwards the samples were transferred into tubes for 5 min and then analysed.

DOI: https://doi.org/10.59913/dagr.2026.21976

Manual freezing (MF)
We placed the filled straws 4 cm above the liquid nitrogen level on a floating stand in a freezing box (Freezing unit for 90 straws, Minitube, Germany) with liquid nitrogen for 15 minutes (reaching temperatures between -125 and -130 °C) and then slowly immersed them directly into liquid nitrogen (-196 °C).
To thaw, we immersed the straws into a bath for 15 seconds at 40 °C.
The two freezing procedures therefore differed primarily in the control and duration of the cooling phase. Programmable freezing involved a controlled, stepwise decrease in temperature from +4 °C to −140 °C over a total programmed period of 850 s (approximately 14.2 min) before immersion in liquid nitrogen (−196 °C). In comparison, manual freezing involved exposure of the straws to nitrogen vapour for 15 min at 4 cm above the liquid nitrogen surface, where temperatures of approximately −125 to −130 °C were reached, followed by direct immersion in liquid nitrogen. Thus, the overall duration of the freezing phase before immersion in liquid nitrogen was similar between the two techniques (approximately 14.2 vs. 15 min), although temperature reduction was precisely controlled during programmable freezing and passively achieved through nitrogen vapour exposure during manual freezing.

Statistical analyses
Cryopreservation experiments with both techniques were performed in triplicate. Differences between the studied groups were analysed using a one-way ANOVA. Tukey's post-hoc multiple comparison test was used to identify statistically significant differences between individual pairs of groups. The statistical significance level was set at $\alpha = 0.05$. For statistical processing, we used the GraphPad Prism program (version 9.0.0, GraphPad Software, USA).

Ethical approval
The experimental procedures were reviewed by the Ethics Committee of the National Agricultural and Food Centre (NPPC) for the Protection of Animals Used for Scientific and Educational Purposes (approval No. EK NPPC 05/2025). The Committee concluded that semen collection from bucks performed within this study constituted a non-experimental agricultural procedure and therefore did not require additional approval from the State Veterinary and Food Administration of the Slovak Republic.

Results

Volume, concentration and motility of fresh sperm
The average volume of fresh sperm obtained from the studied goats was 0.90 ± 0.23 ml. The average concentration of fresh sperm was $4.04 \pm 2.80 \times 10^9$ ml^{-1}. The overall

DOI: https://doi.org/10.59913/dagr.2026.21976

motility in our study reached an average value of 88.94 % and the average progressive motility was 84.07 %.

Effect of diluent type on sperm motility after thawing

The cryopreservation process significantly affected the progressive sperm motility, as mostly the progressive motility of fresh sperm was higher than the measured progressive motility values, which were evaluated after thawing in both extenders. However, the effects of cryopreservation were not the same for both extenders. The percentages of progressive sperm motility (Table 1) after thawing were significantly ($p < 0.05$) higher when using OptiXcell® (65.38%) than when using Triladyl® (40.06%) extenders.

Table 1: The sperm motility after thawing using different extenders

Extender	Triladyl		OptiXcell	
Freezing type	MF (%)	PF (%)	MF (%)	PF (%)
PM	40.06 ± 6.66^{b}	36.85 ± 8.32^{b}	65.38 ± 8.90^{a}	57.13 ± 18.17^{a}

[a,b] Values within rows with different superscripts are different; $p < 0.05$. Abbreviations: PM, progressive motility; MF, manual freezing; PF, programmable freezing.

Effect of freezing technique on sperm motility after thawing

The results show no statistically significant difference in the use of programmable and manual freezing when using Triladyl, indicating their mutual homogeneity. There was also no significant difference in the use of programmable and manual freezing techniques, when using the Optixcell sperm extender, indicating similar values within these two groups.

Discussion

The average fresh sperm volume values in our study were lower and concentrations - higher compared to the study on Malya and Boer goats (KASHOMA et al., 2025). The results of the study on male Korean Native Black Goats and Crossbred Goats (EUN-DO et al., 2025), where the collection was performed by electroejaculation, also show lower sperm volumes in males and higher concentrations compared to our results. The volumes and concentrations in our study were similar to those observed on Saanen goats (NISFIMAWARDAH et al., 2025). The sperm concentrations in our study were also similar to those concentrations in Gulabi goats (MUHAMMAD et al., 2025).

Total motility of fresh sperm in our study was similar to that of Gulabi goats (MUHAMMAD et al., 2025) and higher than that of Saanen goats (NISFIMAWARDAH et al., 2025). The progressive motility of fresh sperm in our

DOI: https://doi.org/10.59913/dagr.2026.21976

study was higher than that of Boer, Saanen and Anglo-Nubian goats in the study of HANIFI et al. (2025) but lower compared to Malya and Boer goats in the study of KASHOMA et al. (2025).

The progressive motility after thawing in our results was higher compared to the study on Saanen goats using an Optixcell extender (NISFIMAWARDAH et al., 2025). The motility in our study was also significantly higher compared to the study on rams (SETIADI et al., 2025). Total motility after thawing was approximately the same as in Gulabi goats using the extender Triladyl (MUHAMMAD et al., 2025). VOZAF et al. (2021) achieved progressive motility values of 74.8% with Triladyl at 6-hour equilibration, which are higher values than in our study.

Although cryopreservation is the most important procedure for success in assisted reproductive biotechnology, this process causes significant structural and molecular changes in mammalian sperm (YANEZ-ORTIZ et al., 2021). Cryopreservation strongly alters acrosome integrity, destroys sperm function and alters membrane fluidity, phospholipid and protein patterns, all of which are associated with a decrease in enzymatic activity, motility, viability and the ability of sperm to fertilize an egg (SHADAN et al., 2004). The decrease in sperm viability after freezing is associated with intracellular changes after cryopreservation, leading to the formation of intracellular ice crystals that cause sperm membrane destruction, increase permeability and, ultimately, sperm death (KHAN et al., 2021).

OptiXcell® resulted in higher post-thaw progressive sperm motility than Triladyl®. No significant differences were observed between manual and programmable freezing. However, the small sample size (n = 5) and evaluation of motility alone limit the generalizability of the findings. Manual freezing may therefore be a useful alternative when programmable equipment is unavailable, but further studies with more animals and additional sperm quality parameters are needed. The main limitation of the present study is the relatively small number of bucks included (n = 5), which may limit the generalisability of the findings and the ability to fully account for inter-individual variability in semen characteristics and response to cryopreservation.

Nevertheless, the experimental design allowed repeated evaluation of semen samples under standardised laboratory conditions and provided preliminary information on the effects of the investigated cryopreservation manipulations. Future studies involving a larger and more diverse population of bucks are warranted to confirm the present findings and to better characterise individual variability in response to the cryopreservation protocol.

Acknowledgement

This work was supported by the Slovak Research and Development Agency (grant no. APVV-23-0089).

DOI: https://doi.org/10.59913/dagr.2026.21976

References

ARI, U.C. – YILDIZ, S. (2018): Storage of Goat Semen. Türkiye Klinikleri.

ARIYAN, F. – FARZINPOUR, A. – FARSHAD, A. – SHARIFI, A. (2025): Protective Effects of Lactobacillus plantarum Secretions on Goat Sperm Quality During Cryopreservation. Biopreservation and Biobanking. https://doi.org/10.1177/19475535251395868

BERČÍK, J. – VOZAF, J. – BALÁŽI, A. – CHRENEK, P. (2026): Cryopreservation of goat sperm: Effect of manual versus programmable freezing and different equilibration lengths on progressive motility. In Winter School – Animal Biotechnology 2026. SUA in Nitra. 78 p. https://doi.org/10.15414/2026.9788055229416

EUN-DO, L. – MAYANGE, T.B. – DONG-KYO, K. – GA-EUN, K. – SANG-RAE, C. – KWAN-WOO, K. (2025): A Study on the Frozen Semen and Artificial Insemination Efficiency of Korean Native Black Goats and Crossbred Goats. Journal of the Korea Academia-Industrial cooperation Society, 26(1), 47–52. https://doi.org/10.5762/KAIS.2025.26.1.47

HAFIDHA, F.G. – UTOMO, B. – SOEHARSONO – LESTARI, T.D. – HIDAJATI, N. – HERMADI, H.A. (2025): The Effect of Kemangi Leaf (Ocimum Basilicum) Extract in The Egg Yolk Skim Milk Diluent on The Plasma Membrane and Motility of Boerawa Goats' Sperm. Media Kedokteran Hewan, 36(3), 273–281. https://doi.org/10.20473/mkh.v36i3.2025.273-281

HANIFI, H. – UMAR, S. – HANDARINI, R. – MAULANA, T. (2025): Kinematic Characteristics of Spermatozoa in Different Goat Breeds Using Computer Assisted Semen Analysis (CASA). Jurnal Agripet, 25(2), 209–213. https://doi.org/10.17969/agripet.v25i2.47621

CHRENEK, P. – MAKAREVICH, A. – KUBOVIČOVÁ, E. – BULLA, J. – SUPUKA, P. (2025): Slovenské národné plemená zvierat. Slovenská poľnohospodárska univerzita. 99 p.

KASHOMA, I.P. – KELLER, W.S. – KICHUKI, M.M. (2025): Comparative Efficacy of Soybean Milk and OptiXcell Extenders for Cryopreservation of Goat Semen. Tanzania Veterinary Journal, 40(1). https://doi.org/10.4314/tvj.v40i1.6

KHAN, I.M. – CAO, Z. – LIU, H. – KHAN, A. – RAHMAN, S.U. – KHAN, M.Z. – SATHANAWONGS, A. – ZHANG, Y. (2021): Impact of Cryopreservation on Spermatozoa Freeze-Thawed Traits and Relevance OMICS to Assess Sperm Cryo-Tolerance in Farm Animals. Frontiers in Veterinary Science, 8. https://doi.org/10.3389/fvets.2021.609180

KUMAR, A. – KANAKA, K.K. – MEHTA, H. – SINGH, G. – THAKUR, N.S. (2025): A Review of Current Challenges, Influential Factors, and Advancements in Goat Semen Cryopreservation. Archives of Current Research International, 25(3), 398–411. https://doi.org/10.9734/acri/2025/v25i31131

DOI: https://doi.org/10.59913/dagr.2026.21976

LONE, S.A. – PRASAD, J.K. – GHOSH, S.K. – DAS, G.K. – BALAMURUGAN, B. – VERMA, M.R. (2018): Study on correlation of sperm quality parameters with antioxidant and oxidant status of buffalo bull semen during various stages of cryopreservation. Andrologia, 50(4). https://doi.org/10.1111/and.12970

MOHAMMED, Z.A. – OMAR, C. (2025): Effect of Various Levels of Sage (Salvia officinalis) Essential Oil Drenching on Some Semen Parameters in Different Periods of Saanen Goat Bucks. Basrah Journal of Agricultural Sciences, 38(2), 455–466. https://doi.org/10.37077/25200860.2025.38.2.34

MUHAMMAD, T. – KALWAR, Q. – RAHIMOON, M.M. – SOOMRO, H. – HUSSAIN, F. – LAGHARI, S.A. – SABOOR, A. – KOREJO, R.A. (2025): Effects of Triladyl® Extender and Vitamin E on Fresh and Frozen Thawed Semen of Gulabi Bucks. Planta Animalia, 4(5), 445–450. https://doi.org/10.71454/PA.004.05.0250

NISFIMAWARDAH, L. – HUSEIN, F. – ASTUTI, E. – MONASDIR, M. – RISKAYANTI, R. – NUGROHO, A.A. (2025): Utilization of Various Diluents on the Quality of Frozen Semen from Saanen Goats. Jurnal Ilmiah Peternakan Terpadu, 13(1), 179–193. https://doi.org/10.23960/jipt.v13i1.p179-193

RANJAN, R. – KUMAR, M. – GANGAWAR, C. – KHARCHE, S.D. (2021): Developments in Goat Semen Cryopreservation. Animal Reproduction Update, 1(1), 41–45. https://doi.org/10.48165/aru.2021.1205

SETIADI, D.R. – MAGFIRA – RIWU, R.M.J. – ARIFIANTINI, R.I. (2025): Effectiveness of various commercial extenders in cryopreserving semen of local indonesian rams. Jurnal Bioteknologi & Biosains Indonesia, 12(1), 68–77. https://doi.org/10.55981/jbbi.2025.9208

SHADAN, S. – JAMES, P.S. – HOWES, E.A. – JONES, R. (2004): Cholesterol Efflux Alters Lipid Raft Stability and Distribution During Capacitation of Boar Spermatozoa. Biology of Reproduction, 71(1), 253–265. https://doi.org/10.1095/biolreprod.103.026435

SIAS, B. – FERRATO, F. – PELLICER-RUBIO, M.T. – FORGERIT, Y. – GUILLOUET, P. – LEBOEUF, B. – CARRIERE, F. (2005): Cloning and seasonal secretion of the pancreatic lipase-related protein 2 present in goat seminal plasma. Biochimica et Biophysica Acta, 1686(3), 169–180. https://doi.org/10.1016/j.bbalip.2004.09.008

SIKKA, S.C. (2004): Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. Journal of Andrology, 25(1), 5–18. https://doi.org/10.1002/j.1939-4640.2004.tb02751.x

TRAN, K.T.T. – LU, S.N.C. – NGUYEN, D.L.K. (2025): Curcumin as a protective agent for goat sperm viability and DNA integrity against bisphenol A toxicity and cryopreservation stress. Veterinary Research Communications, 49(6). https://doi.org/10.1007/s11259-025-10878-7

VOZAF, J. – MAKAREVICH, A. – BALÁŽI, A. – VASICEK, J. – SVORADOVÁ, A. – OLEXÍKOVÁ, L. – CHRENEK, P. (2021): Cryopreservation of ram semen:

DOI: https://doi.org/10.59913/dagr.2026.21976

Manual versus programmable freezing and different lengths of equilibration. Animal Science Journal, 92(1). https://doi.org/10.1111/asj.13670

YANEZ-ORTIZ, I. – CATALAN, J. – RODRIGUEZ-GIL, J.E. – MIRO, J. – YESTE, M. (2021): Advances in sperm cryopreservation in farm animals: Cattle, horse, pig and sheep. Animal Reproduction Science, 246. https://doi.org/10.1016/j.anireprosci.2021.106904