AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Improving Boer goat sperm cryopreservation efficiency using sucrose and ascorbic acid as cryoprotectants

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Article Info

Article history:
Received 17 May 2025
Revised 19 August 2025
Accepted 28 August 2025
Available online 12 September 2025

Keywords:
Ascorbic acid,
Boer goat,
Cryopreservation,
Sperm,
Sucrose

Abstract

Importance of the work: Cryopreservation of goat sperm is known to cause damage that reduces the sperm's motility, viability, acrosome integrity and antioxidant rate.

Objectives: To optimize Boer goat sperm cryopreservation by adding sucrose and ascorbic acid as cryoprotectants in the medium.

Materials and Methods:
Experiment 1 tested four concentrations of sucrose (0 M, 0.025 M, 0.05 M, 0.1 M). Experiment 2 tested four concentrations of ascorbic acid (0 µM, 40 µM, 60 µM, 80 µM) combined with the optimal sucrose concentration determined from Experiment 1. After 72 hr of storage in liquid nitrogen, sperm quality parameters were assessed.

Results: Based on the results from Experiment 1, 0.05 M sucrose was the optimal concentration for goat sperm preservation with mean ± SD values for overall motility, progressive motility, viability, membrane integrity and acrosome integrity of 75.50 ± 0.68, 59.17 ± 0.64, 79.71 ± 0.42, 63.11 ± 0.55and 83.09 ± 0.75, respectively ($p < 0.05$). Based on the results from Experiment 2, the combination of 0.05 M sucrose and 60 µM ascorbic acid significantly ($p < 0.05$) improved sperm quality, with values for overall motility of 81.88 ± 1.73%, progressive motility of 69.13 ± 0.71%, viability of 81.96 ± 1.44%, membrane integrity of 68.33 ± 1.60%, antioxidant rate of 49.60 ± 1.53% and acrosome integrity of 93.13 ± 1.19%.

Main finding: The cryopreservation medium supplemented with 0.05 M sucrose and 60 µM ascorbic acid protected Boer goat sperm during preservation. The findings highlighted the synergistic effects of sucrose and ascorbic acid, suggesting their potential to greatly enhance goat sperm cryopreservation outcomes.

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https://doi.org/10.34044/j.anres.2025.59.5.10

Introduction

Meat and milk represent growing markets for goat-derived goods and have helped goat farming to spread quickly in many emerging nations, including Vietnam (Nguyen et al., 2023). Among several breeds, the Boer goat (*Capra hircus* L.) has become well-known for its exceptional growth rate, carcass quality and local climatic adaptation (Nguyen et al., 2023). Boer goats are being increasingly included in breeding initiatives designed to increase indigenous cattle output in Vietnam (Tesfaye et al., 2017; Nguyen et al., 2024). However, natural mating has remained the most common approach to reproduction, limiting the effective spread of better genes and raising the issue of susceptibility to spreading diseases (Nguyen et al., 2023).

Accelerating genetic improvement and enhancing reproductive efficiency in goat production systems has been much aided by artificial insemination (AI) (Luo et al., 2019). However, the somewhat low success rate of semen cryopreservation is a main obstacle preventing the general implementation of artificial insemination in goats, as according to the study by Apu et al. (2012), the success rate of AI using frozen-thawed semen in Bengal goats was 43.9%. Cryopreservation of goat sperm can cause severe damage, which is most evident in two main stages: freezing and freezing-thawing. For example, during freezing, the plasma membrane and acrosome of goat sperm can be considerable damaged— including membrane separation, acrosome weakening and mitochondrial structure disruption (Kumar et al., 2024). In addition, hypothermia can cause membrane phospholipids to change from a liquid to a crystalline-gel state, which makes the membrane unstable and substantially reduces sperm viability (Esteve et al., 2025). Furthermore, during freezing and thawing, "cell death" can result from several mechanisms, notably accidental cell death and oxidative stress leading to regulated cell death (Hai et al., 2024). Glycerol and other protectants have been used to reduce the risk of ice crystals; however, 40–50% of spermatozoa still die from oxidative stress or physical damage (Hai et al., 2024). In particular, during the freezing and thawing processes, goat spermatozoa are vulnerable to cryo-induced damage because of their low endogenous antioxidant capacity and high level of polyunsaturated fatty acids, which increase their sensitivity to oxidative stress and membrane disruption (Khalifa et al., 2008; Amid et al., 2016).

Critically, minimizing cellular damage and sustaining sperm functioning during cryopreservation depend on the use of antioxidants in semen extenders and cryoprotectants (CPAs) (Yi et al., 2024). Sucrose, as a non-permeating extracellular CPA, encourages osmotic dehydration, hence lowering intracellular ice generation and preserving membrane integrity (Hossain and Osuamkpe, 2007; Moura et al., 2022.). Sucrose has been shown to enhance post-thaw sperm quality by offering osmotic buffering and retaining cellular structure (Farshad and Akhondzadeh, 2008; Khalili et al., 2009).

Antioxidants are commonly added to reduce oxidative stress, protect sperm membranes and improve survival and motility after freezing or thawing. For example, some typical antioxidants that have been widely studied include: glutathione (GSH)—improving motility and the membrane/acrosome integrity index in post-thawed goat sperm (Rawash et al., 2018); vitamin E (α-tocopherol)—protecting membrane lipids from peroxidation and increasing the ratio of live/motile sperm (Penitente-Filho et al., 2014); melatonin—both a free radical scavenger and a stabilizer of mitochondrial function, increasing motility after thawing (Cardenas-Padilla et al., 2024); and quercetin with its good ability to limit reactive oxygen species (ROS) damage (Kumar et al., 2024). Another useful addition to cryopreservation media is ascorbic acid, sometimes known as vitamin C, a strong water-soluble antioxidant as it scavenges ROS, therefore shielding sperm cells from oxidative damage and maintaining motility, viability and acrosomal integrity (Amidi et al., 2016; Beygi et al., 2021). The antioxidant qualities of ascorbic acid have been reported to improve sperm quality in several mammalian species (Gangwar et al., 2015; Singh et al., 2020; Rakha et al., 2023).

Although both sucrose and ascorbic acid have been researched separately, few studies have looked at their combined usage in enhancing goat sperm cryopreservation techniques. Thus, the aim of the current work with Boer goats was to assess the combined effects of sucrose and ascorbic acid on post-thawed sperm quality. The hypothesis of the study was that the addition of sucrose and ascorbic acid to Boer goat semen dilutions would significantly improve sperm quality parameters after the freeze-thaw process. The project sought to develop a scientifically based and practically useful technique to enhance genetic conservation and reproductive biotechnology in systems of tropical goat farming.

Materials and Methods

Chemicals

Citric acid, sucrose and glucose were purchased from Sigma (USA), glycerol from Fisher Scientific (USA), L(+)-ascorbic acid from Acros (USA) and sodium citrate and Tris-hydroxylmethyl aminomethane from Biotech (Vietnam).

Animals

Four mature male Boer goats, with a mean ± SD age and weight of 18.25 ± 0.5 month and 48.25 ± 0.65 kg, respectively, were obtained from the experimental animal facility of the Stem Cell Laboratory at Can Tho University, Vietnam. Their diet was designed to fulfill the nutritional requirements of adult male goats, as advised by the NRC (2007). The goats were fed three times daily on the specified rations and drinking water was available *ad libitum*. The roofed barn area where the goats were housed throughout the experiments were constructed to be spacious and cool and were equipped with mosquito nets and maintained in a clean condition.

Preparation of extenders

A Tris extender was prepared consisting of Tris-hydroxymethylaminomethane (250 mM), citric acid (88 mM), D-glucose (47 mM) and gentamicin antibiotic (80 mg/L), glycerol (8% volume per volume) and egg yolk (15%). The extender was stored at 5ºC and incubated at 35ºC for 2 hr before use.

Experimental design

Semen samples were collected using an artificial vagina twice weekly in the early morning (06:00–08:00 hours) based on 3 ejaculates/male goat.

Experiment 1: Following collection, the semen was diluted with Tris extender supplemented with four concentrations of sucrose (0 M, 0.025 M, 0.05 M, 0.1 M) at an appropriate ratio so that the sperm concentration was adjusted to 2×10^8 sperm/mL.

Experiment 2: After collection, the semen was diluted with Tris extender supplemented to the optimal sucrose concentration determined in Experiment 1, in addition to four concentrations of ascorbic acid (0 µM, 40 µM, 60 µM, 80 µM) at an appropriate ratio so that the sperm concentration was adjusted to 2×10^8 sperm/mL.

Each samples was transferred to a separate French straw. Each straws was stabilized at 15°C for 30 min, followed by 5°C for 60 min and then exposed to liquid nitrogen vapor for 15 min before being directly plunged into liquid nitrogen for storage. After 72 hr of storage, the samples were thawed at 37°C for 60 s and evaluated for quality based on the following parameters: overall motility, progressive motility, sperm viability, membrane integrity, antioxidant rate and acrosome integrity. Each treatment group consisted of samples taken from four male goats, resulting in a total sample size of 12 for each treatment group (Fig. 1).

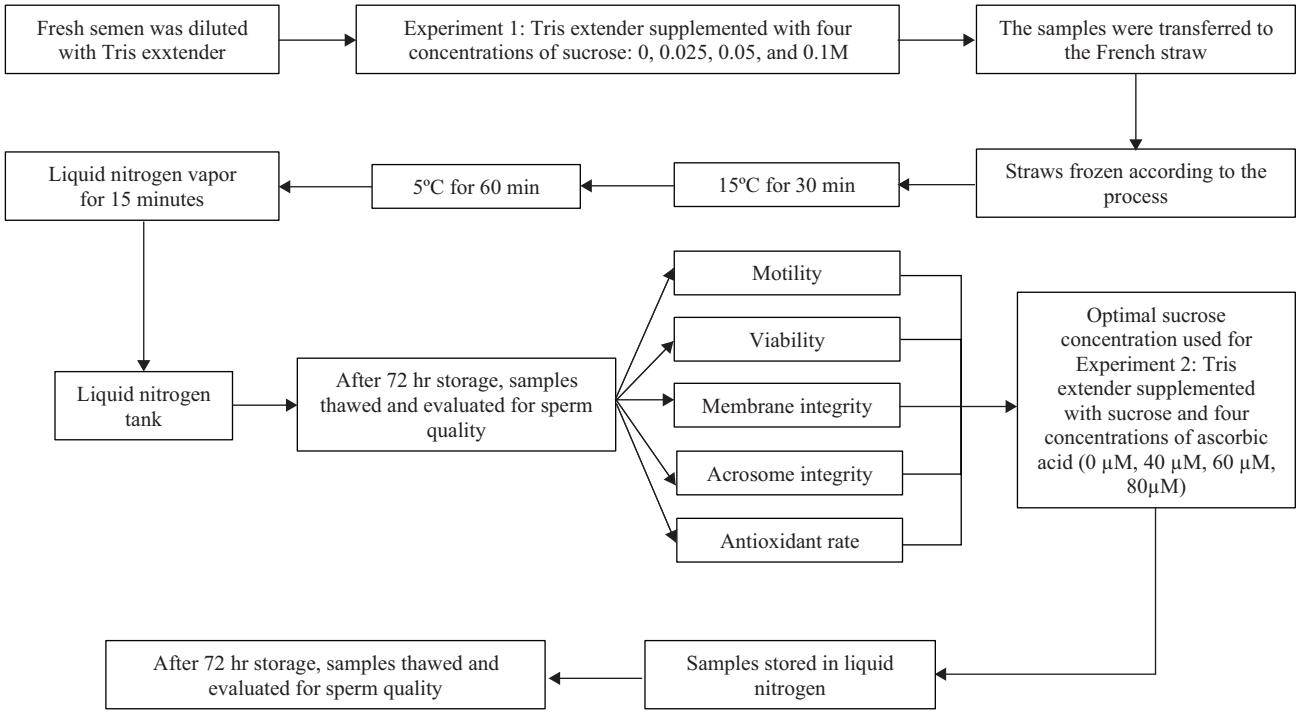

Fig. 1 Experimental design for semen cryopreservation with Tris extender. Experiment 1 tested sucrose (0–0.1 M), and Experiment 2 combined the optimal sucrose level with ascorbic acid (0–80 µM); post-thaw sperm quality was evaluated.

Assessment of sperm motility

Each sample (10 µL) of sperm was placed on a clean glass slide and viewed under an Eclipse Si microscope (Nikon;Japan) at 40× magnification (Fumuso et al., 2018). Sperm motility was assessed and classified into overall and progressive motility. The percentage of each type of sperm motility was calculated by dividing the number of motile sperm by the total number of sperm counted.

Assessment of sperm viability

Sperm viability was examined by eosin-nigrosin staining (Agha-Rahimi et al., 2014). Dead spermatozoa were stained with the dye, while live spermatozoa were not stained. The sperm samples were viewed under the Eclipse Si microscope at 40× magnification and the sperm viability rate was calculated (Fig. 2). The sperm viability rate (%) was calculated by dividing the number of live sperm by the total number of sperm counted.

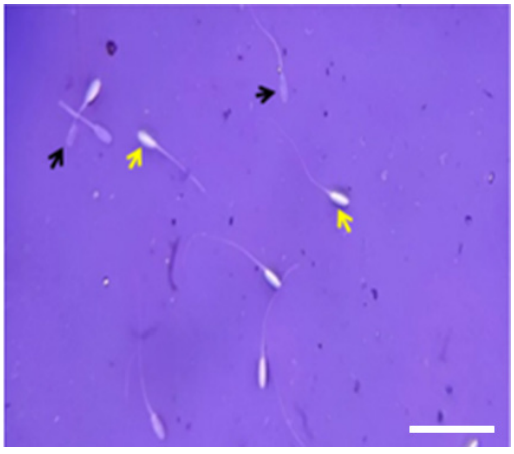

Fig. 2 Goat sperm stained with eosin-nigrosin, showing live sperm with no uptake of dye (yellow arrows) and dead sperm uptake of dye (black arrows). Scale bar = 50 µm.

Assessment of sperm membrane integrity

Sperm membrane integrity was determined following incubation of 20 µL of semen and 80 µL of the hypo-osmotic swelling solution (sodium citrate 0.012 M and fructose 0.035 M) at 37°C for 30 min for the hypo-osmotic swelling test to be conducted (Tran et al., 2025). Sperm with an intact cell membrane showed a tail-curling reaction, while sperm with a damaged cell membrane did not show any reaction. Sperm samples were viewed under the Eclipse Si microscope at 40× magnification and the sperm membrane integrity rate was calculated (Fig. 3). The percentage sperm membrane integrity rate was calculated by dividing the number of sperm with curved tails by the total number of sperm counted.

Assessment of sperm antioxidant capacity

Free radical scavenging activity using the ABTS+ decolorization method was determined according to the method of Nenadis et al. (2004) based on the ABTS+ solution gradually losing its blue color, corresponding to the presence of antioxidants. The test was determined by pipetting 10 µL of sperm sample into 990 µL of ABTS+ free radical solution and was incubated for 6 min at room temperature (28–30°C). The absorbance was measured at 734 nm. The antioxidant capacity of sperm was expressed as the percentage of ABTS+ free radical reduction (inhibition; I), calculated using Eq. (1):

$$I = (A - a) / A \times 100 \quad (1)$$

where A is the spectral absorption value of the control sample (at 734nm) and a is the spectral absorption value of the experimental sample (at 734nm).

Assessment of acrosome integrity

Sperm acrosome integrity was analyzed using the Giemsa staining method and observed using the Eclipse Si microscope at 40× magnification. Sperm with intact acrosomes revealed a pink-stained pole region while sperm with aberrant acrosomes did not absorb the dye (Ministry of Health of Vietnam, 2016).

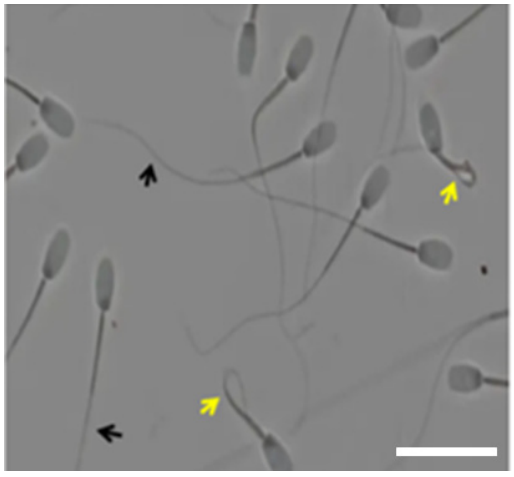

Fig. 3 Sperm tested using hypo-osmotic swelling, with sperm having intact plasma membranes showing tail coiling (yellow arrows), while sperm with damaged plasma membranes show no response (black arrows). Scale bar = 50 µm.

The percentage of sperm with intact acrosomes was determined by counting the number of sperm stained with the dye divided by the total number of sperm counted (Fig. 4). The percentage sperm acrosome integrity rate was calculated by dividing the number of sperm with intact acrosomes by the total number of sperm counted.

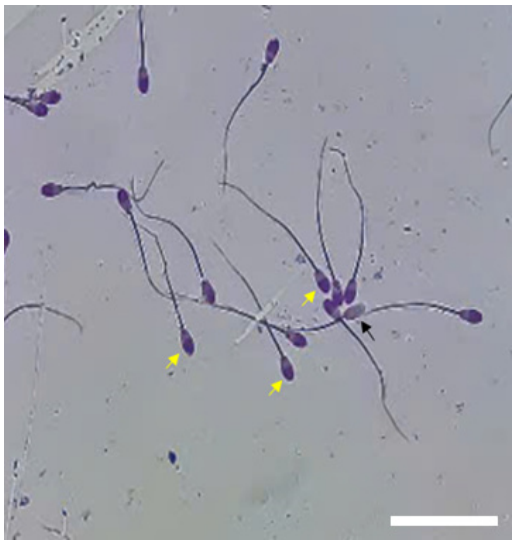

Fig. 4 Sperm stained for acrosome evaluation, with sperm with normal acrosomes showing staining at head apex (yellow arrows), while sperm with abnormal acrosomes show no staining at head apex (black arrow). Scale bar = 50 µm.

Statistical analysis

Statistical significance was set at $p < 0.05$ and all analysis and graphical presentation was undertaken using the R software, version 4.3.1 (R Core Team, 2023). Statistical analyses were performed to assess the effects of the sucrose (Experiment 1) and ascorbic acid (Experiment 2) concentrations on post-thaw sperm quality parameters. A linear mixed model analysis of variance was applied, followed by Tukey's *post hoc* test for multiple comparisons. In both experiments, the treatment concentrations were considered fixed effects, while individual goats and ejaculations were treated as random effects. All results were reported as mean ± SD values.

Ethical review

Ethical approval for the study, including animal care, housing and semen collection, was obtained according to the guidelines of the Regulation on Ethics in Animal Experimentation of Can Tho University, Vietnam (CTU-AEC24004).

Results

Effect of sucrose concentration on goat semen quality after cryopreservation

Based on the results of Experiment 1, the sucrose concentration significantly affected the post-thaw sperm quality, as shown in Fig. 5. The 0.05 M sucrose treatment resulted in the highest overall motility (75.50 ± 0.68%) and progressive motility (59.17 ± 0.64%), indicating improved sperm motion characteristics. In addition, viability peaked at 79.71 ± 0.42%, suggesting enhanced membrane integrity and resistance to cryoinjury. Membrane integrity (63.11 ± 0.55%) and acrosome integrity (83.09 ± 0.75%) were significantly higher in this group than the other treatments. However, the antioxidant activities were not significantly different ($p \geq 0.05$) across treatments. Notably, both lower (0.025 M) and higher (0.1 M) sucrose concentrations yielded reduced sperm quality parameters, indicating that deviations from the optimal osmotic environment either failed to protect or introduced osmotic stress.

Effect of ascorbic acid concentration on goat semen quality after cryopreservation

Based on the results of Experiment 2 (Fig. 6), which evaluated the inclusion of ascorbic acid at different concentrations (0 µM, 40 µM, 60 µM, 80 µM) in the sucrose-optimized extender, 60 µM ascorbic acid significantly enhanced all assessed sperm parameters. Overall motility reached 81.88 ± 0.83% and progressive motility was 69.13 ± 0.85%, while the highest viability was 87.08 ± 0.47%. In addition, membrane integrity (68.33 ± 0.76%) and acrosome integrity (93.16 ± 0.55%) were improved markedly compared to the other groups. The antioxidant capacity, as measured by ABTS+ decolorization, was significantly greater at 60 µM (31.96 ± 0.69%), suggesting a robust reduction in oxidative stress post-thaw. Concentrations above or below this level resulted in comparatively lower sperm performance, likely due to suboptimal free radical scavenging or potential pro-oxidant effects at higher doses.

Generally, these findings showed that the combination of 0.05 M sucrose and 60 µM ascorbic acid in the extender synergistically protected the structural integrity and increased the oxidative defense mechanisms, resulting in improved cryopreserved sperm quality.

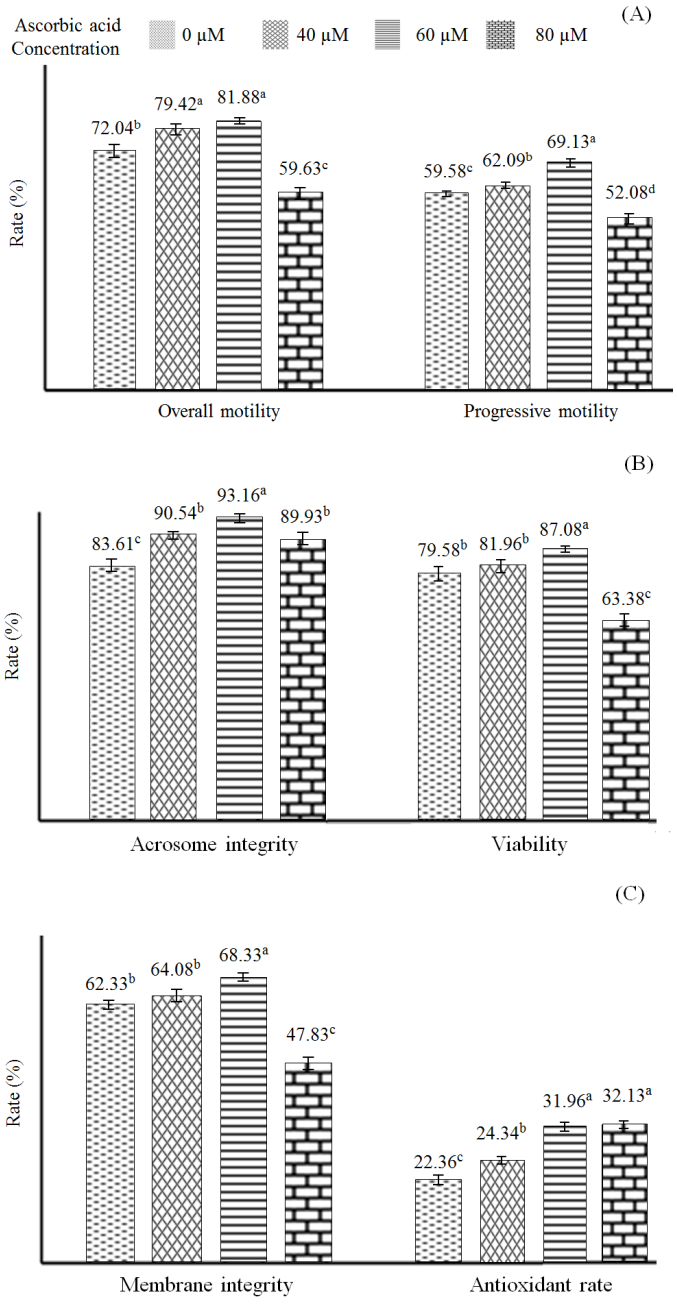

Fig. 5 Effects of Ascorbic acid concentration on post-thaw sperm quality. Different lowercase superscripts denote significant differences ($p < 0.05$) within each trait. Bars and error bars represent mean values and ± SD, respectively.

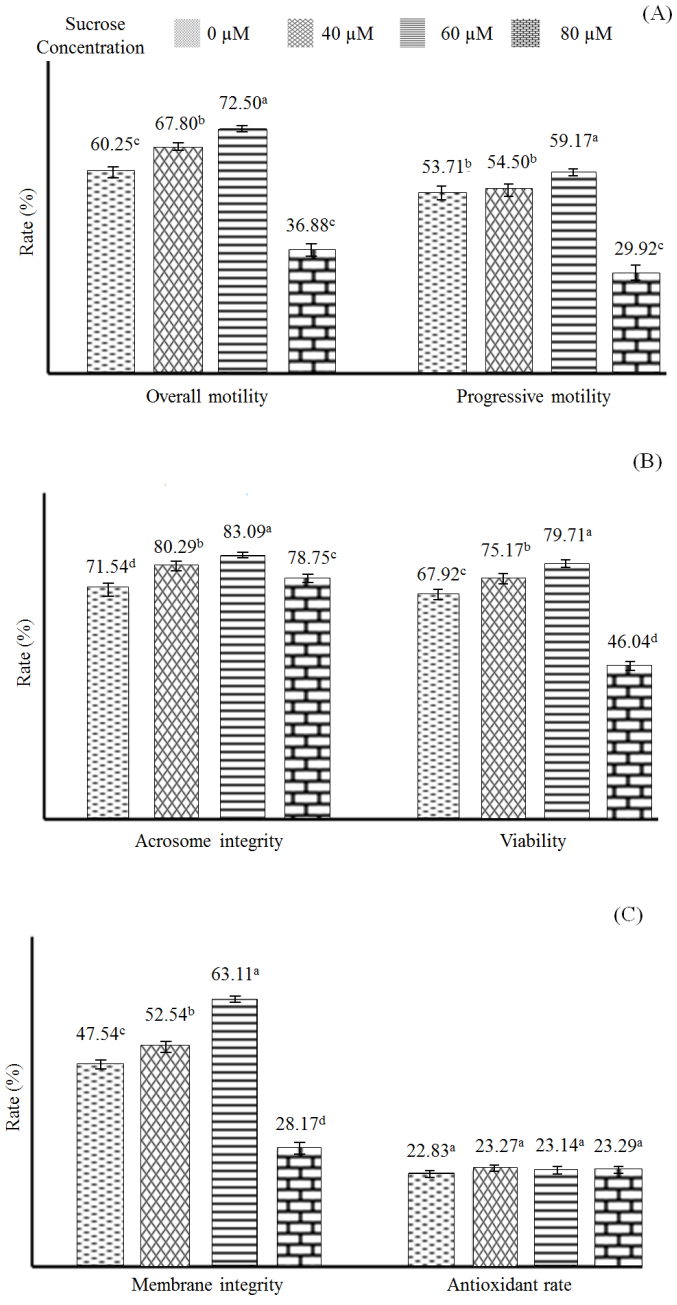

Fig. 6 Effects of Sucrose concentration on post-thaw sperm quality, where Different lowercase superscripts denote significant differences ($p < 0.05$) within each trait. Bars and error bars represent meand values and ± SD, respectively.

Discussion

This work assessed goat sperm quality following cryopreservation in liquid nitrogen under the influence of sucrose and ascorbic acid. Particularly in terms of motility, viability, membrane integrity and acrosome integrity following freezing, the findings showed that adding sugar and ascorbic acid produced considerable increases in sperm quality parameters. In the framework of cryopreservation, especially for artificial insemination and reproductive uses in cattle, these criteria are critical markers of sperm quality.

In Experiment 1, sucrose supplementation at the lowest concentration (0.025 M) improved the sperm quality indices compared to the control group without sucrose supplementation, indicating the protective effect of sucrose, even at low concentrations. Similarly, in Experiment 2, the combination of ascorbic acid at concentrations of 40 μM and 0.05 M sucrose resulted in a significant improvement in sperm viability after cryopreservation. These findings suggested that even at minimal concentrations, sucrose and ascorbic acid could still exert beneficial physiological effects, playing an important role in minimizing the damage caused by cryopreservation. Although the improvement was not significant, it showed that sucrose and ascorbic acid have a positive effect on sperm quality even at very small doses. The highest sperm quality in Experiment 1 resulted from the 0.05 M sugar concentration. These findings validated the important part sucrose plays as an extracellular cryoprotectant during sperm cryopreservation. As a key to preserving sperm viability during the freezing and thawing cycles, sucrose helps stop the production of intracellular ice crystals. Particularly affecting the sperm membrane and intracellular components, ice crystal development can induce cellular damage that reduces sperm motility and fertilizing ability (Hossain and Osuamkpe, 2007). Sucrose reduces the freezing point of the cryoprotective medium by interacting with water molecules, thereby reducing the production of ice crystals inside sperm cells (Herdis et al., 2019).

Recent research has revealed that sucrose is a strong cryoprotectant, therefore mitigating cold stress-induced damage and enhancing post-thaw sperm quality. For example, sucrose affected the osmotic pressure of cryopreservation solutions, which reduced cell rupture upon thawing (Moura et al., 2022). These results were consistent with other research (Khalili et al., 2009), which reported that sucrose was useful in retaining sperm quality and sustaining motility and survival during cryopreservation in many cattle species.

Furthermore, sperm quality after thawing was improved by the addition of ascorbic acid to the cryopreservation mixture in Experiment 2 based on the strong antioxidant action of ascorbic acid, as ROS species that are known to harm sperm cells are neutralized by ascorbic acid. ROS can cause lipid peroxidation, therefore affecting sperm motility, viability and membrane integrity (Amid et al., 2016). In the cryopreservation medium, ascorbic acid serves as a non-enzymatic antioxidant that lowers oxidative stress, resulting in increased sperm survival following thawing. This outcome was consistent with other research such Al Aslam et al. (2014) and Gangwar et al. (2015). In addition, Al Aslam et al. (2014) reported that adding ascorbic acid supplements enhanced goat post-thaw sperm motility and viability.

As a non-enzymatic antioxidant, ascorbic acid guards sperm cells against oxidative damage brought on by ROS. Ascorbic acid supplementation in cryopreservation conditions can lower oxidative stress, thereby improving sperm quality and raising fertilizing capacity after thawing (Singh et al., 2020; Beygi et al., 2021). The findings of the current study support the work of Almeida-Monteiro et al. (2017), which reported that by lowering oxidative stress during freezing, ascorbic acid enhanced sperm quality and acrosome integrity.

By separate processes, both sucrose and ascorbic acid protect sperm quality. Mostly acting as an external cryoprotectant, sucrose helps sperm cells avoid the development of internal ice crystal. For example, Hossain and Osuamkpe (2007) reported that reduced motility and fertilizing capacity resulted from the significant damage to the sperm cell membrane and other intracellular structures caused by ice crystal formation during freezing. In addition, sucrose affected the osmotic features of the cryopreservation media, lowering osmotic stress during thawing and so preserving sperm viability (Moura et al., 2022).

On the other hand, ascorbic acid is a strong antioxidant that counteracts ROS and stops lipid peroxidation (Memon et al., 2013). Furthermore, ROS can damage sperm membranes through oxidation, decreasing their viability and motility (Wang et al., 2025). By neutralizing ROS, ascorbic acid helps to shield sperm cells from such harm, improving acrosome integrity and sperm survival after thawing (Amidi et al., 2016). Supplementing cryopreservation media with ascorbic acid has been shown to reduce oxidative stress and enhance sperm quality, which increases the likelihood of successful fertilization following thawing (Almeida-Monteiro et al., 2017; Singh et al., 2020).

Notably, based on the results of the current study, the protective effects of sucrose and ascorbic acid were dose-dependent; thus, excessive concentrations may compromise sperm quality. Although no definitive threshold has been established for goats, based on the current data, sucrose at 0.10 M impairs post-thaw sperm quality—likely exceeding the optimal concentration. This trend was consistent with findings in other species. For example, Arando et al. (2017) reported that increasing sucrose concentrations during ram sperm vitrification reduced motility, membrane integrity and morphological normalcy, potentially due to osmotic stress. Likewise, Díaz-Jiménez et al. (2019) found that 0.1 M sucrose improved donkey sperm motility post-thaw, while higher concentrations (0.25–0.3 M) resulted in poorer outcomes, suggesting a critical concentration threshold beyond which sucrose becomes detrimental. A similar biphasic effect was observed with ascorbic acid. While moderate levels (40 µM) improved post-thaw viability, high-dose supplementation (80 µM) reduced motility, viability and membrane integrity. This paradoxical response may reflect a pro-oxidant shift at supra-physiological levels, particularly in the presence of metal ions, as noted by Sharafi et al. (2022) and Gangwar et al. (2015). Excessive vitamin C can generate ROS, triggering lipid peroxidation and mitochondrial dysfunction. Supporting this, Al-Dean et al. (2024) showed that low-to-moderate doses enhanced ram sperm quality, whereas higher doses had no benefit or even adverse effects. Together, these findings support a U-shaped dose-response relationship for both cryoprotectants. Therefore, optimizing concentrations is crucial, as overuse may lead to osmotic shock (sucrose) or redox imbalance (ascorbic acid), counteracting their intended protective roles during cryopreservation.

The current study's findings suggest that adding ascorbic acid and sucrose to the cryopreservation medium could greatly enhance the quality of sperm after freezing and thawing. Ascorbic acid lowers oxidative stress and shields sperm membranes from damage caused by ROS, while sucrose aids in preventing ice crystals from forming on sperm. Both compounds have a beneficial effect on acrosome integrity, viability and overall motility, which increases the likelihood of fertilization following thawing. More research is necessary to understand better the mechanisms of action of these cryoprotectants and assess other aspects, such as DNA fragmentation and *in vivo* fertility outcomes. Crucially, the current study is highly relevant to Vietnam, where goat farming—particularly in the Mekong Delta and mountainous areas—is an essential part of rural livelihoods and agricultural development (Nguyen et al., 2023). Effective cryopreservation methods that utilize locally available cryoprotectants, such as sucrose and ascorbic acid, can help to expand the use of AI, enhancing reproductive efficiency, protecting valuable goat breeds and lowering dependency on natural mating. Ultimately, this helps Vietnam's food security, sustainable livestock production and genetic advancement. A more sophisticated and efficient method for semen preservation in reproductive biotechnology could be provided by the incorporation of sucrose and ascorbic acid into cryopreservation media, to improve the structural and functional preservation of goat spermatozoa.

Conclusion

Both sucrose and ascorbic acid had positive effects on goat sperm quality after cryopreservation in liquid nitrogen, improving membrane integrity, progressive motility, viability, head integrity and antioxidant capacity. Concentrations of 0.05 M sucrose and 60 µM ascorbic acid in the cryopreservation medium of 8% glycerol and 15% egg yolk produced the best sperm quality after 72 hr of cryopreservation. These findings open up new research directions on the use of sucrose and ascorbic acid in the cryopreservation of goat sperm in particular, and of cattle sperm in general.

Conflict of Interest

The authors declare that there are no conflicts of interest

Acknowledgements

This study was funded in part by the Ministry of Education and Training, Vietnam (Code: B2024-TCT-04). This study would not have been possible without the contributions of staff time and the facilities of Stem Cell Laboratory, Can Tho University, Vietnam.

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