Veterinary Integrative Sciences 2027; 25(1): e2027005-1-12. DOI: 10.12982/VIS.2027.005

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)



Research article

Improving Boer goat semen freezing using poultry egg yolks: A study in the Mekong Delta of Vietnam

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Abstract

Cryopreservation is widely used for long-term sperm preservation and genetic resource conservation. However, the freezing and thawing process can induce oxidative stress, membrane damage, and DNA fragmentation, thereby reducing post-thaw sperm quality. This study evaluated the effects of chicken, duck, and quail egg yolks at concentrations of 0, 5, 10, 15, and 20% on the post-thaw quality of Boer goat sperm. Semen collected from four mature Boer bucks was diluted in a Tris-citrate-glycerol extender supplemented with different egg yolk sources and concentrations, and stored in liquid nitrogen. After 72 h of storage, semen was thawed at 37 °C for 60 s and evaluated for overall and progressive motility, viability, plasma membrane integrity, acrosome integrity, and DNA fragmentation. For all three egg yolk sources, supplementation at 15% produced the highest post-thaw sperm quality ($p<0.05$). Among the three optimal 15% egg yolk treatments, 15% chicken egg yolk resulted in significantly higher overall motility (66.54%), progressive motility (54.67%), viability (71.37%), plasma membrane integrity (51.32%), and acrosome integrity (80.76%), together with significantly lower DNA fragmentation (12.88%), than duck and quail egg yolks ($p<0.05$). Increasing the chicken egg yolk concentration to 20% significantly reduced post-thaw sperm quality compared with 15% supplementation. Under the conditions of the present study, supplementation of a Tris-citrate-glycerol extender with 15% chicken egg yolk provided the best post-thaw sperm quality and may serve as a practical strategy for optimizing Boer goat semen cryopreservation protocols.

Keywords: Boer goat, Cryopreservation, Egg yolk, Freezing, Post-thaw sperm quality

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Funding; *This study was financially supported by the Ministry of Education and Training, Vietnam, Code: B2024-TCT-04.*

Article history; *received manuscript: 4 March 2026,*
revised manuscript: 9 August 2026,
accepted manuscript: 27 August 2026,
published online: 8 October 2026,
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

The success of artificial insemination (AI), genetic improvement, and disease control programs in livestock depends in part on cryopreservation, which enables the long-term storage of semen for genetic resource conservation (Menchaca, 2023). However, the freezing and thawing process can adversely affect sperm quality by inducing oxidative stress, ice crystal formation, and lipid peroxidation of the sperm membrane, thereby reducing sperm motility, viability, and fertility (Hai et al., 2024).

Egg yolk-based extenders are widely used to protect spermatozoa against cryodamage during cryopreservation (Bustani and Baiee, 2021). Egg yolk plays an important role by reducing cold shock, supplying nutrients, and maintaining sperm membrane integrity (Anzar et al., 2019). Various poultry egg yolk sources, including chicken (Swelum et al., 2022), duck (Wulandari et al., 2020), Japanese quail (Maapola et al., 2023), and pigeon (Akhter et al., 2018), have been evaluated for their cryoprotective effects in different animal species, with varying degrees of effectiveness (Chang et al., 2025). However, comparative studies evaluating the effects of chicken, duck, and quail egg yolks on goat sperm cryopreservation remain limited.

Recent studies have further highlighted the importance of optimizing semen cryopreservation protocols through the selection of appropriate cryoprotectants, extenders, and protective additives. Khuong et al. (2024) investigated Boer goat semen cryopreservation in the Mekong Delta and identified 8% glycerol or 8% dimethyl sulfoxide (DMSO) as effective permeating cryoprotectants when used in an extender containing 8% egg yolk. However, that study primarily focused on optimizing the concentration of permeating cryoprotectants and did not evaluate the effects of different poultry egg yolk sources or their concentration-dependent responses. More recently, studies have investigated alternative extender supplements and freezing conditions in canine and boar semen, demonstrating that extender composition can substantially influence post-thaw sperm quality (Santiago et al., 2025; Vui et al., 2025). Nevertheless, comparative evaluations of different poultry egg yolk sources for goat semen cryopreservation remain scarce.

Therefore, the present study aimed to compare the cryoprotective effects of chicken, duck, and quail egg yolks at five concentrations (0, 5, 10, 15, and 20%) on the post-thaw quality of Boer goat semen. By simultaneously evaluating egg yolk source and concentration under standardized cryopreservation conditions, this study provides a direct comparison of their effects on post-thaw sperm quality and identifies the most effective extender formulation. The findings are expected to provide practical guidance for selecting the optimal egg yolk source and concentration for Boer goat semen cryopreservation and to support future applications in artificial insemination and genetic resource conservation.

MATERIALS AND METHODS

Animals

Four healthy adult Boer bucks, with a mean age of 18.25 ± 0.5 months and a mean body weight of 48.25 ± 0.65 kg, were obtained from the experimental animal facility of the Stem Cell Laboratory, Can Tho University, Vietnam, and used in this study. The goats were fed a diet formulated to meet the nutritional requirements of adult male bucks according to the nutrient requirements recommended by the National Research Council (NRC, 2007). Animals were fed three times daily according to their assigned rations and had ad libitum access to drinking water. The goats were housed in a clean, well-ventilated facility with a roof and mosquito screens. Routine health examinations and vaccinations were performed throughout the experimental period.

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Animal ethics statement

Ethical approval was obtained for animal care, housing, and semen collection procedures in accordance with the Regulation on Ethics in Animal Experimentation of Can Tho University (CTU Animal Ethics Committee, 2024; approval code: CTU-AEC24013).

Preparation of extenders

Chicken, duck, and quail eggs were obtained from Ba Huân Corporation (Vietnam) and processed aseptically. Egg yolks were separated from the albumen, homogenized, and incorporated into a Tris-citrate-glycerol extender. The extender was prepared by dissolving 250 mM Tris(hydroxymethyl)aminomethane (Biobasic, Canada), 88 mM citric acid (Sigma, USA), and 47 mM D-glucose (Thermo Fisher Scientific, USA) in distilled water, followed by the addition of gentamicin (80 mg/L; Sigma, USA) and glycerol (8%, v/v; Thermo Fisher Scientific) as a cryoprotectant. The prepared extenders were stored at 5 °C and incubated at 37 °C for 15 min before use.

Semen collection

Semen was collected from each Boer buck twice weekly using an artificial vagina (Khuong et al., 2024). Each of the four experiments was conducted using six independent semen collection sessions. During each session, one qualified ejaculate from each of the four Boer bucks was collected and pooled to obtain a homogeneous semen sample. Before pooling, semen quality was assessed based on gross appearance, progressive motility, and sperm viability. Ejaculates showing abnormal coloration, including red discoloration indicative of blood contamination or green discoloration suggestive of pus contamination, were discarded. Only ejaculates with progressive motility >70% and sperm viability >80% were included in the pooled semen samples. All ejaculates used in the study met these quality criteria. The pooled semen sample obtained from each collection session was considered one experimental unit and was equally divided among all treatment groups within that experiment. Consequently, six independent pooled semen batches (n = 6) were generated for each experiment, resulting in a total of 24 pooled semen batches across the four experiments.

Experimental design

Four independent experiments were conducted sequentially. Each experiment used six independent pooled semen batches obtained from six separate semen collection sessions. Thus, a total of 24 independent pooled semen batches were used across the study. The experiments 1, 2, 3 differed only in the source of egg yolk used in the extender.

Experiment 1: The extender was supplemented with chicken egg yolk at concentrations of 0% (control), 5%, 10%, 15%, and 20%.

Experiment 2: The extender was supplemented with duck egg yolk at concentrations of 0% (control), 5%, 10%, 15%, and 20%.

Experiment 3: The extender was supplemented with quail egg yolk at concentrations of 0% (control), 5%, 10%, 15%, and 20%.

Experiment 4: Based on these results, this experiment was conducted to directly compare the optimal treatments under identical experimental conditions.

Cryopreservation protocol

In all four experiments, pooled semen was diluted with the respective extender at 37°C to obtain a final concentration of 2×10^8 sperm/mL. The cryopreservation protocol used in this study followed the standard procedure routinely employed in our laboratory for Boer goat semen cryopreservation and previously described by Khuong et al. (2024). The freezing protocol was identical across all experiments; only the egg yolk source and concentration differed among treatments. Diluted semen was loaded into 0.5-mL French straws at 25°C,

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equilibrated at 15°C for 30 min, cooled to 5°C over 60 min, exposed to liquid nitrogen vapor (5 cm above the liquid nitrogen surface) for 15 min, and finally stored in liquid nitrogen. After at least 72 h of storage, one straw from each treatment within each experimental unit was thawed at 37°C for 60 s. The thawed semen was transferred into an Eppendorf tube, gently mixed thoroughly, and immediately divided into aliquots for the assessment of sperm motility, viability, membrane integrity, acrosome integrity, and DNA fragmentation. Therefore, all post-thaw quality parameters for a given experimental unit were evaluated using aliquots obtained from the same thawed semen sample.

Assessment of post-thaw sperm quality

Sperm motility was assessed by placing a 10-µL semen sample on a clean glass slide and covering it with a coverslip. The sample was examined under a light microscope at 400× magnification. Sperm motility was evaluated by the same trained observer following the protocol described by Fumuso et al. (2018). For each sample, motility was assessed in three randomly selected microscopic fields, and the mean value was used for statistical analysis. Sperm were classified as progressively motile (actively moving forward), non-progressively motile (showing movement without forward progression), or immotile (showing no movement).

Sperm viability was assessed using the eosin–nigrosin staining technique, as described by Boccia et al. (2007). Stained smears were examined under a light microscope at 400× magnification. Viable spermatozoa did not take up the eosin dye and appeared white, whereas non-viable spermatozoa absorbed the eosin stain and appeared red. At least 200 spermatozoa were examined, and the percentage of viable spermatozoa was calculated.

Plasma membrane integrity was assessed using the hypo-osmotic swelling test (HOST). Briefly, 20 µL of semen was mixed with 80 µL of hypo-osmotic swelling solution and incubated at 37 °C for 30 min. Following incubation, a smear was prepared and examined under a light microscope at 400× magnification. Spermatozoa with an intact plasma membrane exhibited tail swelling or curling, whereas those with a damaged plasma membrane did not exhibit tail swelling or curling (Tran et al., 2025). At least 200 spermatozoa were examined, and the percentage of spermatozoa with intact plasma membranes was calculated.

Sperm acrosome integrity was assessed using Giemsa staining (Sigma, USA). Stained smears were examined under a light microscope at 400× magnification. Spermatozoa with intact acrosomes exhibited a pink-stained apical region, whereas spermatozoa with damaged acrosomes did not take up the stain (Ministry of Health of Vietnam, 2016).

DNA fragmentation was assessed using the sperm chromatin dispersion (SCD) assay, as previously described by Absalan et al. (2012). The SCD assay is based on the principle that spermatozoa with intact DNA form characteristic halos of dispersed DNA loops around the sperm head following DNA denaturation and protein removal, whereas spermatozoa with fragmented DNA exhibit small or absent halos. The results of these assessments are presented in Figure 1.

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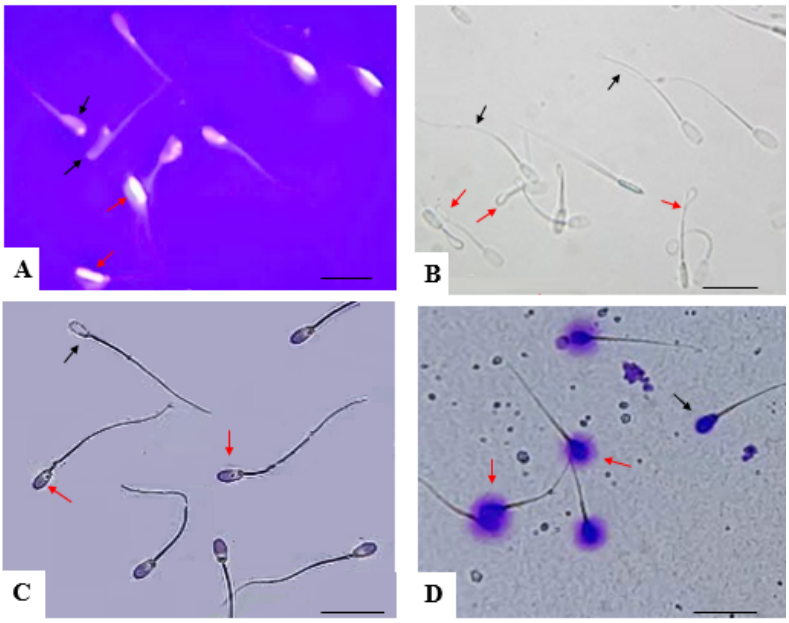


Figure 1 Sperm quality test. A - Sperm stained with eosin-nigrosin; Live sperm do not take up the dye (red arrow), dead sperm take up the dye (black arrow). B - Sperm tested for HOS; Sperm with intact cell membranes show tail curling reaction (red arrow), sperm with damaged cell membranes do not have reaction (black arrow). C - Sperm tested for acrosome integrity; Sperm with intact acrosome take up the dye (red arrow), sperm with damaged acrosome do not take up the dye (black arrow). D - Sperm tested for DNA fragmentation; Sperm with halo has intact DNA (red arrow), sperm without halo has fragmented DNA (black arrow). Scale bar = 50µm.

Statistical analysis

Because chicken, duck, and quail egg yolks were evaluated in three independent experiments rather than in a single factorial experiment, data from Experiments 1–3 were analyzed separately using one-way analysis of variance (ANOVA) to assess the effect of egg yolk concentration within each egg yolk source. For Experiment 4, one-way ANOVA was used to compare the three optimal treatments (15% chicken, duck, and quail egg yolks). Mean comparisons were performed using Tukey's post hoc test in R software (version 4.3.1; R Development Core Team). For all analyses, each pooled semen batch obtained from a single collection session was considered one experimental unit (n = 6 per treatment). Results are presented as mean ± standard deviation (SD), and statistical significance was set at $p<0.05$. Accordingly, the reported standard deviations reflect the variation among six independent pooled semen batches rather than the biological variation among individual Boer bucks.

RESULTS

Fresh semen quality was comparable across the four experiments. Overall motility ranged from 81.44% to 82.31%, progressive motility from 72.02% to 72.70%, viability from 86.73% to 87.57%, plasma membrane integrity from 68.79% to 69.81%, acrosome integrity from 92.30% to 93.40%, and DNA fragmentation from 6.82% to 7.25% (Table 1).

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Table 1 Quality characteristics of fresh Boer goat semen before cryopreservation

Sperm quality (%)	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Overall motility	81.47 ± 0.75	81.44 ± 0.68	82.31 ± 0.74	82.17 ± 0.78
Progressive motility	72.64 ± 0.64	72.02 ± 0.83	72.70 ± 0.87	72.25 ± 0.91
Viability	87.06 ± 0.53	86.88 ± 0.78	86.73 ± 0.67	87.57 ± 0.41
Plasma membrane integrity	68.79 ± 0.68	69.08 ± 0.67	69.14 ± 0.63	69.81 ± 0.75
Acrosome integrity	93.40 ± 0.88	92.30 ± 0.74	93.32 ± 0.59	93.11 ± 0.68
DNA fragmentation	6.82 ± 0.64	7.25 ± 0.69	7.20 ± 0.65	7.16 ± 0.53

Experiment 1: The concentration of chicken egg yolk significantly affected all evaluated post-thaw sperm quality parameters ($p<0.05$; Figure 2). Compared with the control (0% egg yolk), supplementation with 5%, 10%, and 15% chicken egg yolk progressively improved post-thaw sperm quality. The 15% chicken egg yolk treatment (C15) produced the highest overall motility, progressive motility, viability, plasma membrane integrity, and acrosome integrity, together with the lowest DNA fragmentation rate ($p<0.05$). Increasing the egg yolk concentration to 20% significantly reduced post-thaw sperm quality compared with the 15% treatment, although all evaluated parameters remained superior to those of the control.

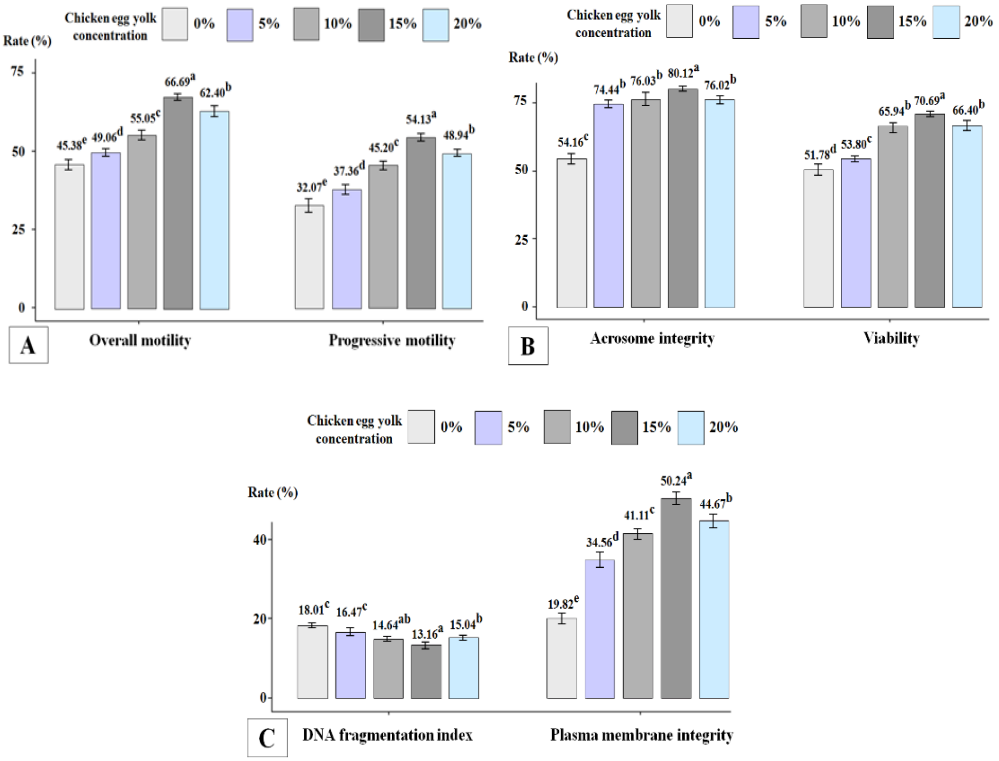


Figure 2 Goat sperm quality after cryopreservation with Tris-citrate-glycerol extender supplemented with different concentrations of chicken egg yolk.
A - Sperm motility, B - Sperm acrosome integrity and viability, C - Sperm DNA fragmentation index and plasma membrane integrity. Values for each parameter with different superscripts are significantly different ($p<0.05$).

Experiment 2: A similar concentration-dependent response was observed with duck egg yolk (Figure 3). Supplementation with 15% duck egg yolk (D15) resulted in significantly better post-thaw sperm quality than all other duck egg yolk

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concentrations (p<0.05), including the highest overall motility, progressive motility, viability, plasma membrane integrity, and acrosome integrity, together with the lowest DNA fragmentation rate. The direct comparison among the optimal 15% egg yolk treatments was subsequently performed in Experiment 4.

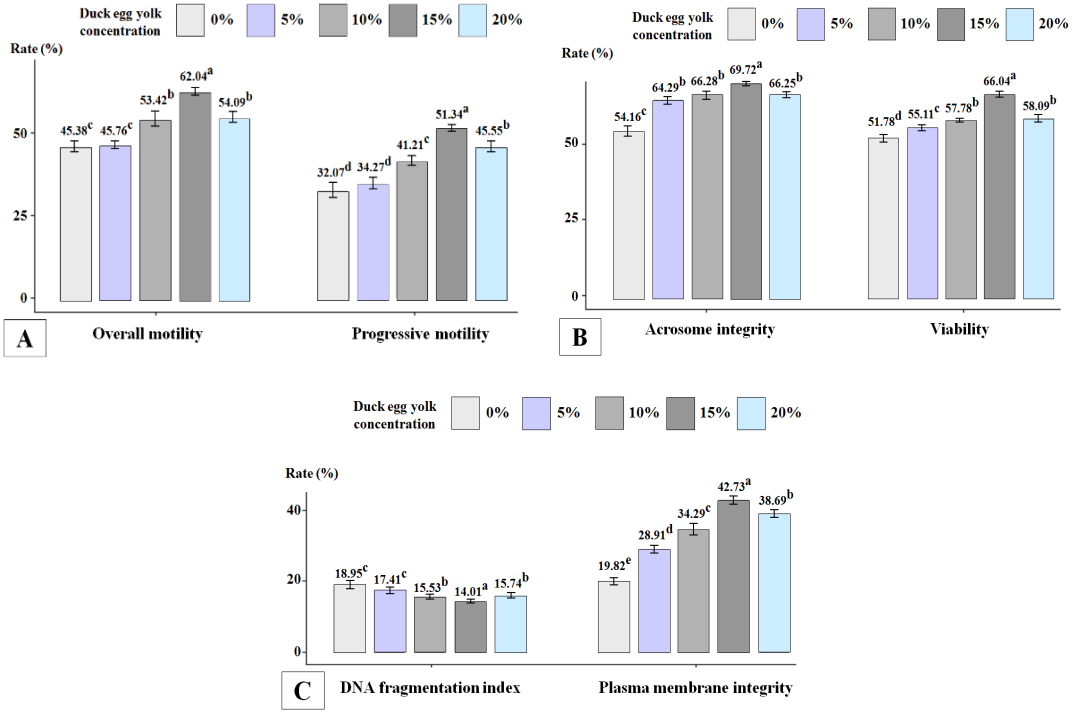


Figure 3 Goat sperm quality after cryopreservation with Tris-citrate-glycerol extender supplemented with different concentrations of duck egg yolk.
A - Sperm motility, B - Sperm acrosome integrity and viability, C - Sperm DNA fragmentation index and plasma membrane integrity. Values for each parameter with different superscripts are significantly different (p<0.05).

Experiment 3: Quail egg yolk also improved post-thaw sperm quality compared with the control (Figure 4). Consistent with the findings of the previous experiments, supplementation with 15% quail egg yolk (Q15) produced significantly better post-thaw sperm quality than all other concentrations tested (p<0.05). Nevertheless, the overall post-thaw sperm quality achieved with quail egg yolk was generally lower than that obtained with either chicken or duck egg yolk.

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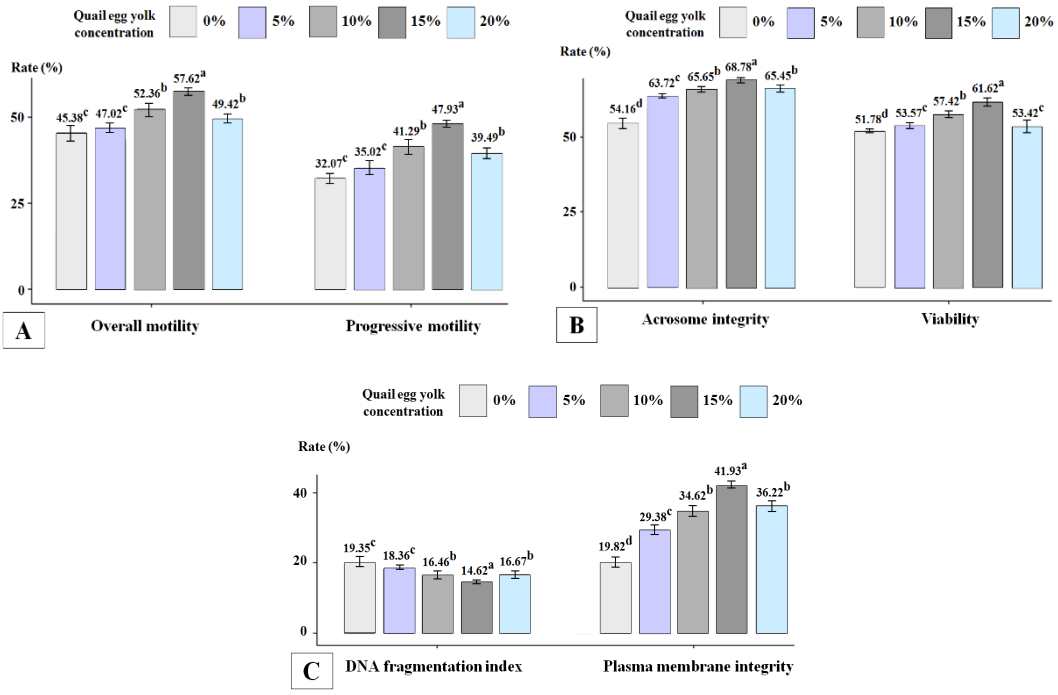


Figure 4 Goat sperm quality after cryopreservation with Tris-citrate-glycerol extender supplemented with different concentrations of quail egg yolk.
A - Sperm motility, B - Sperm acrosome integrity and viability, C - Sperm DNA fragmentation index and plasma membrane integrity. Values for each parameter with different superscripts are significantly different ($p<0.05$).

Experiment 4: Based on the results of Experiments 1–3, the optimal concentration for each egg yolk source was identified as 15%. Therefore, these optimal treatments were further evaluated. The results demonstrated that supplementation with 15% chicken egg yolk produced significantly higher overall and progressive motility, viability, plasma membrane integrity, and acrosome integrity, together with lower DNA fragmentation, than 15% duck and quail egg yolks ($p<0.05$) (Table 2).

Table 2 Experiment 4: Comparison of the optimal extender formulations identified from three independent experiments

	Overall motility (%)	Progressive motility (%)	Viability (%)	Plasma membrane integrity (%)	Acrosome integrity (%)	DNA fragmentation (%)
C15	66.54 ± 0.57^{a}	54.67 ± 0.71^{a}	71.37 ± 0.55^{a}	51.32 ± 0.72^{a}	80.76 ± 0.85^{a}	12.88 ± 0.54^{a}
D15	61.85 ± 0.69^{b}	51.42 ± 0.67^{b}	66.25 ± 0.63^{b}	43.06 ± 0.66^{b}	68.78 ± 0.86^{b}	14.23 ± 0.48^{b}
Q15	58.42 ± 0.71^{c}	47.81 ± 0.75^{c}	61.57 ± 0.61^{c}	42.35 ± 0.76^{b}	69.11 ± 0.72^{b}	14.77 ± 0.61^{b}

Values with different superscripts within a column are significantly different ($p<0.05$). C15: Chicken egg yolk 15%, D15: Duck egg yolk 15%, Q15: Quail egg yolk 15%.

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DISCUSSION

The present study demonstrated that egg yolk concentration affected post-thaw sperm quality within each egg yolk source, while comparison of the optimal 15% treatments showed differences in post-thaw sperm quality among the three egg yolk sources. Our results indicate that supplementation of Tris-citrate-glycerol extender with 15% chicken egg yolk provided significantly superior post-thaw sperm quality, as evidenced by higher sperm motility, viability, plasma membrane integrity and acrosome integrity, as well as lower levels of DNA fragmentation when compared with equivalent concentrations of duck or quail egg yolk. These results are supported by the findings of some previous studies including Benchaib et al. (2003) and Akhter et al. (2018). On the other hand, extenders without egg yolk constantly produced poor sperm quality, which emphasizes the indispensable function of egg yolk components in protecting spermatozoa during cryopreservation (Donnelly et al., 2001). Across all three egg yolk sources, supplementation with 15% egg yolk consistently produced the highest post-thaw sperm quality, suggesting that an intermediate egg yolk concentration provides the most favorable balance between cryoprotection and extender properties.

There was a clear dose-dependent effect of egg yolk on post-thaw quality of goat sperm; supplementation of Tris-citrate-glycerol extender with 15% egg yolk was ideal, even though 5% and 10% egg yolk supplementation also showed some protective effect. Thus, a 15% egg yolk concentration appears to provide an appropriate level of cryoprotective components, including low-density lipoproteins (LDL), cholesterol, and intrinsic antioxidants, which have been reported to help preserve the structural and functional integrity of the sperm membrane (Marri and Richner, 2014; Sen et al., 2015). On the other hand, it appeared that raising the egg yolk concentration to 20% significantly reduced post-thaw sperm quality compared to those obtained by 15% egg yolk supplementation. One possible explanation is that increasing egg yolk concentration to 20% may promote the formation of larger yolk aggregates, thereby increasing extender viscosity and impairing sperm movement during cryopreservation. Similar mechanisms have been proposed in previous studies (Blanch et al., 2014; Tarig et al., 2017).

Egg yolk is widely recognized as a non-permeating cryoprotectant that forms a protective barrier around sperm cells. Its low-density lipoprotein (LDL) fraction is considered the principal cryoprotective component because it interacts with the sperm plasma membrane to add necessary phospholipids and cholesterol, minimizing cold shock effects and lowering the risk of membrane rupture during freezing and thawing (Akhter et al., 2018). Moreover, egg yolk contains antioxidants including vitamin E, selenium, and carotenoids that scavenge reactive oxygen species produced during cryopreservation, thereby helping preserve plasma membrane integrity and DNA integrity (Zhong and Zhou, 2013).

Our results demonstrated that supplementation with chicken egg yolk resulted in superior post-thaw sperm quality compared with duck and quail egg yolks under the conditions of the present study. The superior post-thaw sperm quality observed with 15% chicken egg yolk may be partially explained by differences in the biochemical composition of chicken egg yolk compared with duck and quail egg yolks. Previous studies have reported that chicken egg yolk contains higher levels of polyunsaturated fatty acids and antioxidant vitamins, which may contribute to improved oxidative protection and membrane stabilization during cryopreservation (Akhter et al., 2018; Wulandari et al., 2020). However, the biochemical composition of the egg yolks was not analyzed in the present study; therefore, this explanation should therefore be regarded as a plausible explanation rather than a direct finding of our study. Similarly, the intermediate performance of duck egg yolk and the comparatively lower performance of quail egg yolk may also reflect differences in their biochemical composition, as suggested by previous studies (Swelum et al., 2023).

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The findings of the present study have practical relevance for Boer goat production in the Mekong Delta of Vietnam. The high incidence of disease and reproductive disorders that hinder herd development and economic sustainability (Khuong et al., 2022) present serious problems for the goat farmers of this area. The use of a Tris-citrate-glycerol extender supplemented with 15% chicken egg yolk may improve the post-thaw quality of Boer goat semen and thereby facilitate semen preservation for artificial insemination. This optimized protocol may contribute to more effective semen preservation for artificial insemination and support future genetic improvement and conservation programs. However, its practical reproductive benefits should be confirmed through in vivo fertility studies. Crucially for the preservation of valuable species and for reducing losses during disease outbreaks or other adverse conditions, this optimized protocol also supports the building of genetic resource banks (Khuong et al., 2022).

Moreover, our work provides a basis for future studies aimed at isolating the specific protective fractions within chicken egg yolk, such as purified LDL and evaluating the potential benefits of incorporating additional antioxidants. These improvements may further enhance post-thaw sperm quality and should be evaluated for their effects on in vivo fertility in future studies (Blanch et al., 2014; Tarig et al., 2017).

Overall, our results demonstrate that supplementation of a Tris-citrate-glycerol extender with 15% chicken egg yolk provides superior post-thaw sperm quality in Boer goats by effectively balancing membrane stabilization and antioxidant protection. These findings contribute to our understanding of goat sperm cryobiology and provide a practical basis for optimizing semen cryopreservation protocols. Nevertheless, further studies evaluating fertility and artificial insemination outcomes are required before the reproductive benefits of this protocol can be confirmed.

A limitation of the present study is that semen was collected from only four Boer bucks and pooled before assignment to treatment groups. Although this approach minimized individual variation and facilitated the evaluation of treatment effects, it may not fully capture the biological variability among individual Boer bucks. In addition, the present study evaluated only post-thaw sperm quality under laboratory conditions and did not assess fertility or conception outcomes following artificial insemination. Therefore, further studies using a larger number of animals and individual-based experimental designs are recommended.

CONCLUSIONS

The results of the present study demonstrated that supplementation of a Tris-citrate-glycerol extender with 15% chicken egg yolk significantly improved the post-thaw quality of Boer goat semen compared with duck and quail egg yolks. Specifically, semen cryopreserved with this formulation exhibited superior post-thaw sperm motility, viability, membrane integrity, acrosome integrity, and lower DNA fragmentation than the other treatments. These findings suggest that chicken egg yolk is an effective cryoprotective supplement for Boer goat semen cryopreservation. Further studies evaluating in vivo fertility and artificial insemination outcomes are needed to confirm the practical reproductive benefits of this protocol.

ACKNOWLEDGEMENTS

We thank the staff at the Stem Cell Laboratory, Can Tho University for their support and assistance during the research process.

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CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

Khuong Thi Thanh Tran: Funding Acquisition (lead), Methodology (lead), Investigation (equal), Writing – Original Draft (lead), Review & Editing (equal).
Duy Lam Khanh Nguyen: Conceptualization (lead), Methodology (equal), Investigation (equal), Formal Analysis (lead), Writing – Original Draft (equal).
Tran Nam Van Be: Methodology (equal), Formal Analysis (equal), Software (lead), Writing – Original Draft (equal), Review & Editing (equal).
Giang Thi Tran: Conceptualization (equal), Investigation (lead), Formal Analysis (equal), Review & Editing (lead).

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How to cite this article;
Khuong Thi Thanh Tran, Duy Lam Khanh Nguyen, Nam Van Be Tran and Giang Thi Tran. Improving Boer goat semen freezing using poultry egg yolks: A study in the Mekong Delta of Vietnam. Veterinary Integrative Sciences. 2027; 25(1): e2027005-1-12.

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