Research Article

Potential Use of Bovine Serum Albumin as an Alternative to Egg Yolk in Semen Extenders for Chilled Canine Sperm

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Abstract | This study aimed to evaluate bovine serum albumin (BSA) as a substitute for egg yolk in a diluent medium for chilled canine spermatozoa. Semen was collected from six healthy male French Bulldogs, pooled, and diluted in a Tris–citrate–fructose extender supplemented with either 20% egg yolk (control) or BSA at concentrations of 0.25%, 0.5%, 0.75%, and 1.0%. The samples were cooled to 5°C and stored for 12 days. Sperm quality parameters including progressive motility, viability, membrane integrity, and lipid peroxidation levels were assessed periodically. The results indicated that sperm quality declined gradually over time across all treatment groups ($P<0.05$). However, the supplementation of BSA at concentrations ranging from 0.25% to 0.75% significantly improved motility, viability, and membrane integrity compared to the control group, particularly during the extended storage period. Notably, the 0.5% BSA concentration yielded the best results, maintaining the highest levels of motility (28.10%), viability (37.55%), and membrane integrity (33.08%) on day 12 ($P<0.05$). Conversely, the 1.0% BSA concentration resulted in a significant reduction in sperm quality. Lipid peroxidation levels increased over time in all groups; however, BSA supplementation markedly reduced MDA concentrations, with the 0.5% BSA concentration demonstrating the strongest antioxidant efficacy. In conclusion, BSA serves as an effective alternative to egg yolk for the preservation of canine spermatozoa, with an optimal concentration of 0.5%. The use of BSA improves sperm quality and reduces oxidative damage, offering potential for the development of standardized semen extender containing a novel protein source for canine reproduction.

Keywords | Canine sperm, Bovine serum albumin, Egg yolk, Semen extenders, Chilled storage, Sperm quality

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INTRODUCTION

Canine semen preservation is widely applied in reproductive management due to its convenience, low cost, and suitability for short-distance transport. Compared to freezing, the refrigeration of semen is technically less complex and typically yields higher conception rates when compared under identical conditions regarding the timing of estrus and artificial insemination (AI) techniques (Rijsselaere *et al*., 2002). Consequently, optimizing semen preservation methods remains a key objective in canine reproductive technology.

Semen extenders play an essential role in maintaining sperm viability during storage by stabilizing physicochemical conditions and supporting cellular metabolic activity. Traditional extenders typically comprise buffering systems such as Tris and citric acid to maintain stable pH and osmotic pressure; energy sources such as glucose or fructose to sustain sperm motility, and protective components such

as lipoproteins derived from egg yolk to mitigate cold shock. Additionally, antibiotics such as penicillin, streptomycin, or gentamicin are added to control bacterial proliferation (Dorado *et al*., 2011). However, the use of egg yolk presents several limitations. As a biologically derived product, egg yolk can serve as a source of microbial contamination, including pathogenic and antibiotic-resistant bacteria such as *Escherichia coli*, *Salmonella* spp., and *Pseudomonas* spp. (Mezhoud *et al*., 2016; Hinson *et al*., 2025). The presence of these microorganisms can compromise semen quality, diminish fertility, and increase the risk of endometritis in females following insemination (Althouse *et al*., 2000; Morrell and Wallgren, 2014).

In recent years, there has been increasing interest in developing defined and controlled alternatives to animal-derived protein sources to enhance the biosafety and stability of preservation media. Among these, bovine serum albumin (BSA) is considered a promising candidate due to its well-defined molecular structure, high stability, and multifunctional biological properties. BSA has been demonstrated to stabilize sperm cell membranes by regulating lipid–cholesterol interactions, thereby maintaining membrane fluidity and structural integrity during cooling and storage (Rahimizadeh *et al*., 2021). In addition to its membrane-protective effects, BSA exhibits antioxidant activity by scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation, which are major contributors to sperm damage during storage (Roche *et al*., 2008). Recent studies have also indicated that BSA supplementation can improve sperm motility, viability, and acrosomal integrity across various species, including dogs and livestock (Nang *et al*., 2012; Rahimizadeh *et al*., 2021).

However, despite its numerous advantages, the impact of varying BSA concentrations on sperm quality remains inconsistent across studies, with both positive and negative effects observed depending on the dosage and species. This highlights the need for systematic evaluative studies to determine the optimal concentration appropriate for each specific subject. Consequently, this study was conducted to compare the effects of BSA and egg yolk (20%) on canine sperm quality during preservation. Specifically, parameters such as progressive motility, viability, cell membrane integrity, and susceptibility to oxidative stress were assessed to identify a safer and more effective alternative for semen preservation.

MATERIALS AND METHODS

Animals

This study was conducted on six clinically healthy male French Bulldogs, aged 2–3 years. All subjects underwent a comprehensive reproductive health examination prior to inclusion in the study and were maintained under standardized nutritional and management conditions in Tra Vinh Ward, Vinh Long Province, Vietnam. The animals were fed an identical diet, provided with water *ad libitum*, and given daily exercise. All subjects were fully trained for semen collection, and semen was collected on a weekly basis throughout the duration of the study. Experimental procedures were conducted in accordance with ethical guidelines for animal research and were approved by the Animal Ethics Committee of Tra Vinh University.

Canine semen collection and initial quality evaluation

Canine semen was collected periodically once a week, and semen quality was evaluated immediately after sample collection. Sperm motility parameters were analyzed using a computer-assisted sperm analysis (CASA) system to characterize motility characteristics in detail. Sperm morphology and viability were assessed using the eosin–nigrosin staining method Tamuli and Watson (1994). Only semen samples meeting pre-established quality criteria including >70% progressive motility, >90% viability, a sperm concentration of >200 × 10^6 sperm/mL, and <5% abnormal sperm morphology were utilized in the study.

Semen extender preparation and experimental design

In this study, the basic extender used was Tris–citrate–fructose. Five treatments were established, comprising a control treatment supplemented with 20% egg yolk and four treatments supplemented with BSA at varying concentrations (0.25%, 0.5%, 0.75%, and 1%). The detailed composition of the semen extenders is presented in Table 1. All chemicals were supplied by Sigma-Aldrich. Following semen collection, semen samples from six dogs were pooled and aliquoted into five sterile tubes. The seminal plasma was removed via centrifugation at 720×g for 5 minutes, in accordance with the protocol described by Rijsselaere *et al*. (2002). Subsequently, the sperm pellet was diluted using the respective diluent solutions to achieve a final concentration of 200 × 10^6 spermatozoa/mL. The diluted semen samples were gradually cooled at a rate of 0.3°C/minute down to 5°C, following the method of Bouchard *et al*. (1990), and were subsequently stored at 5°C for up to 12 days. The experiment was designed as a completely randomized design with five treatments; parameters were monitored repeatedly throughout the 12-day storage period, with four replicates per treatment.

Assessment of sperm motility

The progressive motility of spermatozoa was assessed by pipetting 10 µL of semen sample onto a glass slide and analyzing it using a computer-assisted sperm analysis (CASA) system (RATO Vision II, China) at

Table 1: Formulation of the extender used for chilled sperm storage.

Components	Control	BSA-0.25	BSA-0.5	BSA-0.75	BSA-1.0
Tris (mg)	3025	3025	3025	3025	3025
Citric acid (mg)	1700	1700	1700	1700	1700
Fructose (mg)	1250	1250	1250	1250	1250
Egg yolk (%)	20	0	0	0	0
BSA (%)	0	0.25	0.5	0.75	1.0
Streptomycin (mg)	100	100	100	100	100
Penicillin (mg)	60	60	60	60	60
Distilled water (mL)	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100

BSA: Bovine serum albumin; Control:20% Egg yolk; BSA-0.25, BSA-0.5, BSA-0.75, and BSA-1.0: 0.25, 0.5, 0.75, and 1.0 % BSA, respectively.

10× magnification (Abah *et al*., 2025). This assessment determines the percentage of progressively motile spermatozoa defined as those capable of moving in a straight direction as well as the percentage of non-progressively motile spermatozoa.

Assessment of sperm viability

Sperm viability was assessed using the Eosin–Nigrosin staining method (Tamuli and Watson, 1994), which distinguishes between live and dead sperm based on the integrity of the plasma membrane. Specifically, 10 µL of semen was mixed with 10 µL of staining solution, smeared onto a glass slide, allowed to air-dry, and observed under a light microscope at 400× magnification. Dead sperm absorbed the eosin stain and appeared pink or red, whereas live sperm remained unstained.

Assessment of sperm plasma membrane integrity

The functional integrity of the sperm plasma membrane was assessed using the Hypoosmotic Swelling Test (HOST), which aims to determine the osmotic responsiveness of the sperm. Specifically, 10 µL of semen was mixed with 100 µL of a hypoosmotic solution (150 mOsm/L), incubated at 38°C for 30 minutes, and subsequently observed under a phase-contrast microscope using a 40× objective lens. A total of 200 sperm were evaluated per sample. Sperm exhibiting curled or swollen tails are considered to possess an intact plasma membrane, whereas sperm with straight tails are considered to have a damaged membrane (Goericke-Pesch *et al*., 2012).

Assessment of lipid peroxidation in sperm

The level of sperm lipid peroxidation was determined by measuring malondialdehyde (MDA) concentration using the TBARS (thiobarbituric acid reactive substances) method, as described by Maia *et al*. (2010), with slight modifications. Specifically, sperm samples were oxidized with iron sulfate, reacted with thiobarbituric acid (TBA), and heated at 95–100°C for 15 minutes to form the MDA–TBA complex. After cooling and centrifugation, the supernatant was collected, and its optical density was measured at a wavelength of 535 nm using a microplate spectrophotometer. MDA concentration was calculated based on a standard curve generated using 1,1,3,3-tetramethoxypropane and was expressed as nmol of MDA per 50 × 10^6 sperm.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics software, version 22.0. Data are presented as mean ± standard deviation. Two-way analysis of variance (mixed ANOVA) was used to assess the effects of storage time and extenders, as well as the interaction between these two factors, on sperm quality parameters. When significant differences were detected, Tukey's post-hoc test was applied for multiple comparisons. A P-value < 0.05 was considered statistically significant. Assumptions of normality and homogeneity of variances were verified prior to conducting the analyses.

RESULTS

Progressive sperm motility

The percentage of progressively motile spermatozoa decreased significantly (P<0.05) throughout the 12-day storage period across all treatments (Table 2). On Day 1, no significant differences were observed among the treatments, with values ranging from 83.68% to 87.55%. From Day 3 onwards, the BSA-1.0 treatment exhibited significantly lower motility compared to the other treatments (P<0.05), while no significant differences were found between the control group and the BSA levels ranging from 0.25% to 0.75%. By Day 12, the BSA-0.5 treatment maintained the highest percentage of progressive motility (28.10%), which was significantly higher than that of BSA-1.0 (17.93%) (P<0.05), while the remaining treatments yielded intermediate values that did not differ significantly.

Sperm viability

Sperm viability was presented in Table 3. Sperm viability decreased progressively over storage time across all treatments (P<0.05). There were no significant differences

Table 2: Progressive sperm motility (%) of canine sperm during 12 days of chilled storage.

Treatments	Day 1	Day 3	Day 6	Day 9	Day 12
Control	86.87±3.56^{A}	81.48±1.32^{aA}	63.21±0.52^{B}	51.45±0.33^{B}	24.76±1.73^{abC}
BSA-0.25	87.55±4.38^{A}	81.20±1.75^{aA}	63.03±1.08^{B}	50.48±1.22^{B}	24.71±1.59^{abC}
BSA-0.5	87.42±4.04^{A}	83.88±1.88^{aA}	65.60±0.40^{B}	51.51±0.97^{B}	28.10±1.95^{aC}
BSA-0.75	86.78±5.45^{A}	82.09±1.73^{aA}	64.87±2.68^{B}	49.68±5.35^{C}	21.20±4.83^{abD}
BSA-1.0	83.68±4.64^{A}	73.70±2.59^{bB}	63.80±6.43^{C}	40.39±12.09^{D}	17.93±5.31^{bE}

Control: 20% Egg yolk; BSA-0.25, BSA-0.5, BSA-0.75, and BSA-1.0: 0.25, 0.5, 0.75, and 1.0 % BSA, respectively. Data are expressed as mean ± standard deviation based on four replicates. Different lowercase letters (a or b) within a column denote significant differences among treatments (P<0.05), while different uppercase letters (A, B, C, D, and E) within a row indicate significant differences among storage times (P<0.05).

Table 3: Sperm viability (%) of canine sperm during 12 days of chilled storage.

Treatments	Day 1	Day 3	Day 6	Day 9	Day 12
Control	96.23±1.09^{A}	89.84±0.62^{B}	71.90±1.26^{dC}	58.87±1.42^{D}	34.35±1.18^{abE}
BSA-0.25	97.32±0.25^{A}	88.68±0.67^{B}	74.06±0.35^{cC}	58.55±1.26^{D}	33.27±2.33^{bcE}
BSA-0.5	96.69±0.62^{A}	89.63±1.50^{B}	76.62±0.37^{bC}	59.91±2.43^{D}	37.55±1.89^{aE}
BSA-0.75	96.90±0.55^{A}	89.92±1.15^{B}	77.31±0.37^{abC}	58.21±0.58^{D}	32.41±1.37^{bcE}
BSA-1.0	96.39±1.37^{A}	88.71±4.22^{B}	78.52±0.73^{aC}	60.93±0.95^{D}	30.09±1.78^{cE}

Control: 20% Egg yolk; BSA-0.25, BSA-0.5, BSA-0.75, and BSA-1.0: 0.25, 0.5, 0.75, and 1.0 % BSA, respectively. Data are expressed as mean ± standard deviation based on four replicates. Different lowercase letters (a, b, c, or d) within a column denote significant differences among treatments (P<0.05), while different uppercase letters (A, B, C, D, and E) within a row indicate significant differences among storage times (P<0.05).

Table 4: Sperm plasma membrane integrity (%) of canine sperm during 12 days of chilled storage.

Treatments	Day 1	Day 3	Day 6	Day 9	Day 12
Control	91.97±1.09^{A}	85.47±1.07^{B}	67.74±2.72^{cC}	53.79±1.90^{aD}	28.60±2.80^{abE}
BSA-0.25	92.58±1.59^{A}	84.75±1.12^{B}	69.46±2.51^{bcC}	54.36±0.21^{aD}	27.98±1.77^{abE}
BSA-0.5	92.98±0.37^{A}	85.81±2.20^{B}	71.74±0.00^{abC}	54.85±0.07^{aD}	33.08±2.33^{aE}
BSA-0.75	92.96±1.38^{A}	84.52±2.47^{B}	73.37±0.55^{abC}	54.12±3.36^{aD}	28.24±0.44^{abE}
BSA-1.0	91.70±2.43^{A}	87.60±1.20^{B}	75.62±0.58^{aC}	47.33±1.60^{bD}	24.38±3.74^{bE}

Control: 20% Egg yolk; BSA-0.25, BSA-0.5, BSA-0.75, and BSA-1.0: 0.25, 0.5, 0.75, and 1.0 % BSA, respectively. Data are expressed as mean ± standard deviation based on four replicates. Different lowercase letters (a, b, or c) within a column denote significant differences among treatments (P<0.05), while different uppercase letters (A, B, C, D, and E) within a row indicate significant differences among storage times (P<0.05).

among the treatments on days 1 and 3. However, from day 6 onwards, distinct differences emerged. On day 6, the BSA-1.0 treatment exhibited the highest viability rate, followed by BSA-0.75 and BSA-0.5, while the control treatment recorded the lowest value (P<0.05). By day 12, the BSA-0.5 treatment maintained the highest viability rate (37.55%) significantly higher than the other treatments, while BSA-1.0 recorded the lowest value (30.09%) (P<0.05).

Plasma membrane integrity

Table 4 presents the plasma membrane integrity of canine spermatozoa over 12 days of storage. Plasma membrane integrity declined significantly over time across all treatments (P<0.05). No significant differences were observed among the treatments on days 1 and 3. From day 6 onwards, the supplementation of BSA demonstrated a marked improvement compared to the control. On day 6, the BSA-1.0 treatment achieved the highest value (75.62%), which was significantly higher than that of the control (P<0.05). However, by day 9, this treatment had declined significantly relative to the other treatments. By day 12, the BSA-0.5 treatment maintained the highest membrane integrity (33.08%) significantly higher than that of BSA-1.0 (24.38%) (P<0.05) while the remaining treatments yielded intermediate values.

Sperm lipid peroxidation

Malondialdehyde (MDA) content an indicator of lipid peroxidation increased significantly (P<0.05) throughout the 12-day storage period across all treatments (Table 5). On Day 1, no significant differences were observed among the treatments, with MDA values ranging from 9.71 to 12.31 nmol/50 × 10^{6} spermatozoa. However, from Day 3 onwards, distinct differences began to emerge. The control treatment consistently exhibited the highest MDA content, indicating a greater degree of oxidative damage, whereas

Table 5: Malondialdehyde (MDA) levels (nmol/50×10^6 sperm) in canine sperm over 12 days of chilled storage.

Treatments	Day 1	Day 3	Day 6	Day 9	Day 12
Control	10.16±0.4^{E}	18.69±2.65^{aD}	32.23±2.04^{aC}	39.03±1.31^{aB}	56.38±1.64^{aA}
BSA-0.25	10.35±0.66^{D}	11.45±2.02^{bD}	16.79±1.49^{bcC}	29.65±2.47^{bB}	36.02±2.03^{cA}
BSA-0.5	9.71±1.02^{D}	9.96±1.91^{bD}	13.83±2.43^{cC}	25.01±0.93^{bB}	31.19±1.78^{cA}
BSA-0.75	10.85±1.21^{D}	12.25±1.93^{bD}	21.29±1.94^{bC}	26.99±0.55^{bB}	35.44±1.71^{cA}
BSA-1.0	12.31±1.49^{D}	14.45±2.19^{abD}	31.22±1.2^{aC}	35.82±2.66^{aC}	51.06±0.62^{bA}

Control: 20% Egg yolk; BSA-0.25, BSA-0.5, BSA-0.75, and BSA-1.0: 0.25, 0.5, 0.75, and 1.0 % BSA, respectively. Data are expressed as mean ± standard deviation based on four replicates. Different lowercase letters (a, b, or c) within a column denote significant differences among treatments (P<0.05), while different uppercase letters (A, B, C, D, and E) within a row indicate significant differences among storage times (P<0.05).

the BSA-supplemented treatments showed significantly lower values (P<0.05). Among the treatments, BSA-0.5 demonstrated the lowest MDA content throughout the storage period particularly on Day 6 (13.83 nmol/50 × 10^6 spermatozoa) and Day 12 (31.19 nmol/50 × 10^6 spermatozoa) values that were significantly lower than those of the control and BSA-1.0 treatments (P<0.05). The BSA-0.25 and BSA-0.75 treatments also reduced lipid peroxidation compared to the control, though their efficacy was inferior to that of BSA-0.5. Conversely, the BSA-1.0 treatment exhibited relatively high MDA levels particularly during the later stages of storage and did not differ significantly from the control on Days 6 and 9. By Day 12, although BSA-1.0 showed lower MDA levels than the control, they remained significantly higher than those of the lower-concentration BSA treatments (P<0.05). Overall, the level of lipid peroxidation increased gradually over the storage period. The addition of BSA helped reduce oxidative damage, with a concentration of 0.5% providing the most effective protection, whereas a higher concentration (1.0%) offered less effective protection.

DISCUSSION

The present study clearly demonstrates that the supplementation of BSA at appropriate concentrations significantly improved the quality of canine spermatozoa preserved for 12 days, as evidenced by increased progressive motility, viability, and cell membrane integrity, as well as reduced lipid peroxidation. These results suggest that BSA can serve as an effective substitute for egg yolk in semen extenders intended for short-term preservation. Throughout the storage period, sperm quality gradually declined over time a finding consistent with the detrimental effects of cold shock, oxidative stress, and the depletion of metabolic energy during preservation. However, the rate of this decline was markedly slower in the groups supplemented with BSA, particularly at concentrations ranging from 0.25% to 0.75%. Notably, the group supplemented with 0.5% BSA consistently exhibited the highest quality in terms of progressive motility, viability, and cell membrane integrity compared to the other treatments. These results align with previous studies. Specifically, Rachmawati *et al.* (2020) reported that the addition of 0.2% BSA significantly improved sperm quality during storage. Similarly, Nang *et al.* (2012) demonstrated that supplementing a preservation medium with 8 mg/mL of BSA at 4°C enhanced sperm viability and protected sperm DNA integrity. These studies collectively affirm the critical protective role of BSA in maintaining sperm function during preservation.

The superior efficacy of BSA groups ranging from 0.25% to 0.75% particularly the 0.5% concentration can be attributed to several physiological mechanisms. First, BSA helps maintain colloid osmotic pressure, thereby preventing excessive water movement across the sperm cell membrane and averting cellular shrinkage or swelling. This equilibrium is crucial for safeguarding membrane integrity under low-temperature conditions. Second, BSA is an amphipathic protein capable of interacting with the sperm cell membrane. It regulates the lipid-to-cholesterol ratio, thereby stabilizing the phospholipid bilayer, minimizing phospholipid loss, and maintaining membrane fluidity; this, in turn, prevents structural damage during the cooling process (Rahimizadeh *et al.*, 2021). Furthermore, BSA contains numerous amino acids such as glutamic acid, aspartic acid, lysine, and arginine that can serve as metabolic substrates to support sperm energy metabolism (Wolfe *et al.*, 2001). Structurally, BSA possesses 35 cysteine residues that form 17 disulfide bonds, enhancing the protein's stability and resistance to denaturation. Consequently, BSA retains its protective function even under harsh storage conditions (Majorek *et al.*, 2012).

Conversely, the addition of 1% BSA significantly compromised sperm quality particularly progressive motility and membrane integrity during the later stages of storage. This suggests that excessively high concentrations of BSA may be detrimental. The decline in quality observed at the 1% concentration may be attributed to elevated extracellular osmotic pressure, which induces rapid water efflux from the sperm cells, resulting in shrinkage and structural damage (Khan and Ijaz, 2008). Concurrently, high BSA concentrations increase the viscosity of the

medium, thereby impeding sperm motility and accelerating energy expenditure. Consequently, the sperm rapidly deplete their energy reserves, leading to reduced motility and a shortened lifespan (Ibrahim *et al*., 2013).

Lipid peroxidation assessed via MDA content increased significantly over time across all treatment groups, reflecting progressive oxidative damage during the preservation process. However, the supplementation of BSA markedly reduced MDA accumulation compared to the control group utilizing egg yolk. Notably, the 0.5% BSA group exhibited the lowest MDA levels throughout the preservation period, whereas the control group recorded the highest values. This demonstrates that BSA possesses potent antioxidant capabilities and effectively protects spermatozoa against oxidative stress. The higher MDA content observed in the egg yolk group may be attributed to its inherent biological nature. Egg yolk contains lipoproteins that are susceptible to degradation and oxidation over time, thereby diminishing protective efficacy and promoting lipid peroxidation. Conversely, as a purified protein with minimal enzymatic impurities, BSA functions as a "protective scaffold" or a stable carrier that resists rapid degradation (Monteiro *et al*., 2009). Furthermore, BSA possesses the capacity to bind to and neutralize ROS and toxic metabolites, thereby contributing to enhanced antioxidant efficacy. This synergistic combination of membrane stabilization and antioxidant activity accounts for the superior preservation performance observed in the BSA-supplemented treatment groups. Taken together, the research results demonstrate that BSA serves not only as an effective substitute for egg yolk but also offers superior protective effects during long-term preservation, owing to its high stability and antioxidant capacity. Among the concentrations investigated, 0.5% BSA was identified as the optimal level for preserving canine spermatozoa under refrigerated conditions.

CONCLUSIONS

This study demonstrates that bovine serum albumin (BSA) serves as an effective and reliable alternative to egg yolk for the cold storage of canine spermatozoa. The supplementation of BSA at an optimal concentration (0.25%–0.75%) specifically 0.5% significantly improved motility, viability, and membrane integrity, while simultaneously reducing lipid peroxidation, thereby extending sperm longevity throughout a 12-day storage period. Conversely, supplementation at a high concentration (1.0%) exerted detrimental effects, underscoring the critical importance of optimizing the concentration used.

From a practical perspective, replacing egg yolk with BSA offers numerous benefits, including enhanced stability of the diluent medium, reduced biological variability, and improved antioxidant capacity. These findings provide a solid foundation for developing standardized semen extender incorporating a novel protein source for use in canine reproduction. Future studies should focus on evaluating fertilization potential and practical applications to facilitate the adoption of BSA-containing media into routine breeding programs.

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NOVELTY STATEMENT

This study demonstrates, for the first time, the optimal use of bovine serum albumin (BSA) as a standardized alternative to egg yolk in chilled canine semen extenders. It identifies 0.5% BSA as the most effective concentration for improving sperm quality and reducing oxidative damage during extended storage (12 days). Additionally, the study highlights the negative effects of excessive BSA, emphasizing the importance of concentration optimization for developing reliable, egg yolk–free semen preservation systems.

AUTHOR'S CONSTRIBUTION

Nguyen Van Vui and Huynh Thanh Tan designed and conceptualized the study; Huynh Thanh Tan conducted the experiments and analyzed the data; Nguyen Van Vui and Huynh Thanh Tan drafted the manuscript; Nguyen Van Vui critically reviewed and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors confirm that ChatGPT was used solely to improve the English grammar of the manuscript. All content was carefully reviewed, revised, and validated by the authors to ensure accuracy and originality.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abah KO, Ligocka-Kowalczyk Z, Partyka A, Prochowska S, Fontbonne A, Niżański W (2025). Exploring the characters of sperm lipid peroxidation, enzymatic antioxidant activity, and spermatozoa zona binding capacity with aging in dogs. Sci. Rep., 15: 1–11. https://doi.org/10.1038/s41598-025-16410-w

Althouse GC, Kuster CE, Clark SG, Weisiger RM (2000). Field

investigations of bacterial contaminants and their effects on extended porcine semen. Theriogenology, 53: 1167–1176. https://doi.org/10.1016/S0093-691X(00)00261-2

Bouchard GF, Morris JK, Sikes JD, Youngquist RS (1990). Effect of storage temperature, cooling rates and two different semen extender on canine spermatozoal motility. Theriogenology, 34: 147–157. https://doi.org/10.1016/0093-691X(90)90586-I

Dorado J, Gálvez MJ, Murabito MR, Muñoz-Serrano A, Hidalgo M (2011). Identification of sperm subpopulations in canine ejaculates: effects of cold storage and egg yolk concentration. Anim. Reprod. Sci., 127: 106–113. https://doi.org/10.1016/j.anireprosci.2011.07.005

Goericke-Pesch S, Klaus D, Failing K, Wehrend A (2012). Longevity of chilled canine semen comparing different extenders. Anim. Reprod. Sci., 135: 97–105. https://doi.org/10.1016/j.anireprosci.2012.08.032

Hinson C, Tonouhewa A, Azokpota P, Daube G, Korsak N, Sessou P (2025). Global prevalence and antibiotic resistance profiles of bacterial pathogens in table eggs: A systematic review and meta-analysis. Vet. World, 18: 939–954. https://doi.org/10.14202/vetworld.2025.939-954

Ibrahim SF, Ariff Johor A, JOthman F, Yee LP, Firdaus Yahaya M, Hanan Fatihah Jaafar F, Mohd Bakri N, Osman K (2013). Albumin-V: Role in bovine spermatozoa viability, motility and membrane elasticity. J. Agric. Sci., 5: 83–89. https://doi.org/10.5539/jas.v5n3p83

Khan MIR, Ijaz A (2008). Effects of osmotic pressure on motility, plasma membrane integrity and viability in fresh and frozen-thawed buffalo spermatozoa. Animal, 2: 548–553. https://doi.org/10.1017/S1751731108001596

Maia S, Dimas S, Cecilia C, Rodello L, Cristina I, Gallego S (2010). Lipid peroxidation and generation of hydrogen peroxide in frozen-thawed ram semen cryopreserved in extenders with antioxidants. Anim. Reprod. Sci., 122: 118–123. https://doi.org/10.1016/j.anireprosci.2010.08.004

Majorek KA, Porebski PJ, Dayal A, Zimmerman MD, Jablonska K, Stewart AJ, Chruszcz M, Minor W (2012). Structural and immunologic characterization of bovine, horse, and rabbit serum albumins. Mol. Immunol., 52: 74–82. https://doi.org/10.1016/j.molimm.2012.05.011

Mezhoud H, Chantziaras I, Iguer-Ouada M, Moula N, Garmyn A, Martel A, Touati A, Smet A, Haesebrouck F, Boyen F (2016). Presence of antimicrobial resistance in coliform bacteria from hatching broiler eggs with emphasis on ESBL/AmpC-producing bacteria. Avian Pathol., 45: 493–500. https://doi.org/10.1080/03079457.2016.1167837

Monteiro JC, Gonçalves JSA, Rodrigues JA, Lúcio CF, Silva LCG, Assumpção MEOA, Vannucchi CI (2009). Influence of ascorbic acid and glutathione antioxidants on frozen-thawed canine semen. Reprod. Domest. Anim., 44: 359–362. https://doi.org/10.1111/j.1439-0531.2009.01434.x

Morrell JM, Wallgren M (2014). Alternatives to antibiotics in semen extenders: A review. Pathogens, 3: 934–946. https://doi.org/10.3390/pathogens3040934

Nang CF, Osman K, Budin SB, Ismail MI, Jaffar FHF, Mohamad SFS, Ibrahim SF (2012). Bovine serum albumin: Survival and osmolarity effect in bovine spermatozoa stored above freezing point. Andrologia, 44: 447–453. https://doi.org/10.1111/j.1439-0272.2011.01203.x

Rachmawati A, Ismaya, Widyobroto BP, Bintara S, Susilawati T (2020). Effect of different bovine serum albumin (BSA) levels on the sperm viability of ongole cross bred bull during 5°C storage. IOP Conf. Ser. Earth Environ. Sci., 478: 012068. https://doi.org/10.1088/1755-1315/478/1/012068

Rahimizadeh P, Topraggaleh TR, Bucak MN, Ziarati N, Hasirbaf A, Taher-Mofrad SMJ, Maroufizadeh S, Shahverdi A (2021). Effect of bovine serum albumin supplementation in tris-soybean lecithin-based extender on quality of chilled ram epididymal spermatozoa. Biopreserv. Biobank., 19: 33–40. https://doi.org/10.1089/bio.2020.0041

Rijsselaere T, Van Soom A, Maes D, De Kruif A (2002). Effect of centrifugation on *in vitro* survival of fresh diluted canine spermatozoa. Theriogenology, 57: 1669–1681. https://doi.org/10.1016/S0093-691X(02)00663-5

Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E (2008). The antioxidant properties of serum albumin. FEBS Lett., 582: 1783–1787. https://doi.org/10.1016/j.febslet.2008.04.057

Tamuli MK, Watson PF (1994). Use of a simple stating technique to distinguish acrosomal changes in the live sperm subpopulation. Anim. Reprod. Sci., 35: 247–254. https://doi.org/10.1016/0378-4320(94)90040-X

Wolfe CA, James PS, Gunning AP, Ladha S, Y Christova RJ (2001). Lipid dynamics in the plasma membrane of ram and bull spermatozoa after washing and exposure to macromolecules BSA and PVP. Mol. Reprod. Dev., 59: 306–313. https://doi.org/10.1002/mrd.1035