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Effects of Adding *Aquilaria malaccensis* Leaf Extract on Motility and Post-Thaw Semen Quality Using Computer-Assisted Sperm Analysis in Saanen Goats

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ABSTRACT

The quality of frozen semen from Saanen goats plays a vital role in the success of artificial insemination. The present study aimed to evaluate the effects of different semen diluents on post-thaw semen quality and sperm kinematic parameters using computer-assisted sperm analysis (CASA). Fresh semen samples were obtained from two 3-4-year-old Saanen goats, weighing 64 kilograms and 92 kilograms, with an average fresh motility of 80%. Semen quality was assessed under a microscope, focusing on motility, viability, abnormalities, concentration, and total motile spermatozoa. In the current laboratory experiment, five treatment groups were used with 10 replicates. The first group served as the negative control and consisted of tris-egg yolk without *Aquilaria malaccensis* leaf extract, the second group contained tris-egg yolk with 0.10 mg of *Aquilaria malaccensis* leaf extract, the third group consisted of tris-egg yolk with 0.15 mg of *Aquilaria malaccensis* leaf extract, the fourth group contained tris-egg yolk with 0.20 mg of *Aquilaria malaccensis* leaf extract, and the fifth group received the AndroMed® (positive control). The present results indicated that the use of different diluents had a significant effect on post-thawing semen quality, including motility, progressive motility, average path distance, curved line distance, straight line distance, average path velocity, straight line velocity, curvilinear velocity, amplitude of lateral head, beatcross frequency, and wobble. However, there was no significant difference in post-thawing semen quality in terms of individual motility, viability, abnormalities, concentration, total motile spermatozoa, straightness, and linearity. The use of tris-egg yolk diluents supplemented with *Aquilaria malaccensis* leaf extract at a concentration of 0.10 mg and AndroMed® indicated that the highest results among all treatments were demonstrated by the highest post-thaw semen quality.

Keywords: Antioxidant, *Aquilaria malaccensis*, Sperm kinematic, Saanen goat

INTRODUCTION

The agricultural and livestock sectors in Indonesia have exhibited significant development and are considered promising contributors to future economic growth. Livestock husbandry, particularly the production of small ruminants such as goats, constitutes a vital component of the agricultural sector. Recent statistics from the Central Statistics Agency of Indonesia reported that the goat population in Indonesia was approximately 15.7 million head (Directorate General of Livestock and Animal Health Services, 2024). Saanen goats are among the most important dairy goat breeds due to their high milk production capacity and adaptability to diverse environmental conditions (Zeljić Stojiljković et al., 2025). Previous studies reported that Saanen goats produced an average of 2.55 kg of milk per day, a yield that was higher than that of most dairy goat breeds (Ferro et al., 2017). Therefore, Saanen goats were extensively used as a genetic foundation for dairy goat improvement programs worldwide (Upadhyay et al., 2024). Genetic quality improvement in livestock can be achieved by increasing livestock productivity through the application of artificial insemination (AI) technology (Mansour, 2025).

Artificial insemination improves reproductive performance and pregnancy outcomes while reducing the need to maintain breeding males, thereby increasing production efficiency (Ayantoye et al., 2025). The success of AI is largely dependent on the quality of frozen-thawed semen. Luo et al. (2019) reported that outcomes are influenced by post-thaw semen quality, the timing of insemination, and the effectiveness of estrus synchronization protocols. Sharafi et al. (2022) reported that the cryopreservation of goat semen is associated with several physiological challenges due to the high sensitivity of spermatozoa to cold shock. During cooling, freezing, and thawing, sperm cells may experience membrane

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damage, reduced motility, osmotic stress, and elevated reactive oxygen species (ROS) levels, which can ultimately impair semen quality. The ROS-induced oxidative stress is a major factor contributing to sperm damage during cryopreservation. High ROS levels can lead to DNA fragmentation, lipid peroxidation, acrosomal damage, and mitochondrial dysfunction, all of which reduce sperm viability and fertilization capacity (Wang et al., 2025). Therefore, the quality of semen after thawing is often lower than that of fresh semen, underscoring the need to develop appropriate diluents that protect spermatozoa from oxidative stress. Zhang et al. (2022) further indicated that excessive ROS disrupts plasma membrane integrity, acrosome integrity, sperm motility, and fertilization function.

The effectiveness of a semen diluent depends on its ability to provide metabolic substrates, maintain physicochemical stability, protect sperm membrane integrity, and minimize cryoinjury during freezing and thawing (Bustani and Baiee et al., 2021). Liang et al. (2023) reported that goat semen cryopreserved with Optidyl® (egg yolk-based) and Andromed® (soybean lecithin-based) diluents exhibited significantly higher post-thaw sperm motility than semen preserved in skim milk-based diluents. Moreover, semen frozen with skim milk diluent demonstrated lower membrane integrity, acrosome integrity, and mitochondrial activity after thawing. Different types of diluents frequently used in semen cryopreservation include tris-egg yolk and skim milk. In addition to conventional diluents, commercial formulations such as AndroMed® and Ovixcell® have been developed and are widely used for the cryopreservation of Saanen goat semen due to their standardized composition and protective effects on sperm quality (Nisfimawardah et al., 2023). Adding 2% of the natural antioxidant black cincau leaf extract to skim milk yolk diluent resulted in a 50.40% increase in motility compared to 43.42% without the addition (Wahjuningsih et al., 2021). The integration of natural antioxidants offers a viable alternative, as it is more environmentally sustainable compared to antioxidants derived from egg yolk. Tris-egg yolk diluent contains natural antioxidant compounds derived from egg yolk, including low-density lipoproteins (LDL), phospholipids, and α-tocopherol, which contribute to membrane protection and reduce oxidative damage during cryopreservation (Mahiddine and Kim, 2021).

Aquilaria malaccensis leaf extract (ALE) contains bioactive compounds such as flavonoids, phenolics, and terpenoids that act as antioxidants and anti-inflammatory agents (Batubara et al., 2020). The antioxidant potential of ALE can improve membrane integrity, prevent lipid peroxidation, and maintain sperm motility during freezing (Ismail et al., 2019). The application of ALE in goat semen cryopreservation remains limited. Furthermore, comprehensive studies have compared the effects of different diluents on frozen-thawed semen quality, evaluated using both conventional microscopic assessment and computer-assisted sperm analysis (CASA; Anand and Yadav, 2016). The CASA is widely recognized as a reliable tool for the objective evaluation of sperm motility and kinematic characteristics, providing more precise and reproducible measurements than conventional microscopic assessments (Shah et al., 2023). The present study aimed to assess the potential of ALE as a natural antioxidant and to identify the optimal diluent formulation for improving the cryopreservation and post-thaw quality of Saanen goat semen.

MATERIALS AND METHODS

Ethical approval

All study procedures were carried out in accordance with Standard Operating Procedures (SOPs) at the Artificial Insemination Center (AIC) in Lembang, Bandung, West Java, Indonesia, in accordance with ISO/IEC 17025-2008 and were supervised by a veterinarian. Ethical guidelines and research procedures were provided by AIC and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia, with number No. 77-KEP-FKHUB-2026.

Study design

This study utilized two male Saanen goats aged 3 to 4 years, located at the AIC in Lembang, Bandung, West Java, Indonesia. The goats were allowed to graze in the morning on forage primarily composed of Indigofera zollingeriana and *Calliandra calothyrsus*. Each goat received a daily diet comprising 2 kg of forage, 2.5 kg of leguminous feed, and 1 kg of concentrate. The concentrate used at AIC Lembang was reported to have contained 16–18% crude protein (CP) and 60–75% total digestible nutrients (TDN). The goats weighed 64 kg and 92 kg, respectively. Semen collection was performed twice a week for five weeks using an artificial vagina (IMV, France). Initially, semen was collected using false mounting to enhance libido and facilitate collection within the goat enclosure.

Extract preparation

Aquilaria malaccensis leaves were collected from an agarwood plantation located in Lamandau Regency, Central Kalimantan, Indonesia. The preparation of ALE was initiated by drying the leaves in an oven at 55°C for 72 hours. The dried leaves were subsequently macerated in 70% ethanol at a 1:10 (w/v) ratio (100 g leaf powder in 1000 mL solvent)

for 24 hours. The mixture was then filtered to separate the filtrate from the residue, and the maceration extract was collected and stored. The remaining residue was further extracted using microwave-assisted extraction (MAE). The resulting extract was centrifuged at 3500 rpm for four minutes, and the supernatant was collected. Subsequently, the supernatant was filtered through Whatman No. 1 filter paper, and the ethanol was removed on a rotary vacuum evaporator at 60°C for 45 minutes, yielding a concentrated extract. Finally, the crude extract was stored in dark glass bottles and kept under refrigerated conditions until further use.

Diluent preparation

The present study used two types of diluents, namely tris-egg yolk (AIC, Lembang, Indonesia) with the addition of ALE at 0 mg (T0), 0.10 mg (T1), 0.15 mg (T2), 0.20 mg (T3), and the patented AndroMed® diluent (minitube, Germany; T4). The tris-based diluent consisted of a buffer solution containing 2.42 g of tris hydroxymethyl aminomethane (Merck, Germany) supplemented with antibiotics, 1.28 g of citric acid (Merck, Germany), 2.16 g of fructose (Merck, Germany), 0.1 g of penicillin (Meiji, Japan), 0.1 g of streptomycin (Meiji, Japan), and 100 ml of water for injection. The buffer was stirred with a magnetic stirrer for 4 hours, then 74 mL of the homogenized buffer was removed. This formed the basis of the Andromed thinner buffer, which was then mixed with 20 mL of egg yolk and 6 mL of glycerol (Merck, Germany) to create the tris egg yolk diluent. Homogenization was performed for 30 minutes, followed by centrifugation at 4000 rpm for 30 minutes to separate the solution from the precipitate. The resulting solution was then stored in a refrigerator at 4-5℃ (AIC, 2026). The AndroMed® diluent contained buffer, citric acid, glycerol, fructose, aquabidest, phospholipids, spectinomycin, 15 mg of lincomycin, 5 mg of tylocin, and 25 g of gentamicin.

The process of preparing semen diluent was carried out by placing tris-egg yolk diluents, AndroMed®, and ALE in a water bath at 37℃. There were five treatment groups. The first group received a tris-egg yolk diluent without ALE (T0), the second group was treated with a tris-egg yolk diluent containing 0.10 mg/100 mL of ALE (T1), the third group comprised a tris-egg yolk diluent with 0.15 mg/100 mL of ALE (T2), the fourth group was administered a tris-egg yolk diluent with 0.20 mg/100 mL of ALE (T3), and the fifth group solely received AndroMed® (T4). Following supplementation, each diluent was vortex-mixed for three minutes and maintained in a water bath to preserve temperature stability. Treatment T4 was prepared by diluting the commercial diluent with distilled water at a 1:4 ratio (20 mL of AndroMed® to 80 mL of distilled water), yielding a final volume of 100 mL without ALE supplementation (Nisfimawardah et al., 2023). Semen dilution was performed in a single step by adding fresh semen to the five dilution treatments, using Formula 1.

$$\text{Volume total} = \frac{\text{fresh semen (mL)} \times \text{concentration} \times 10^6}{50 \times 10^6} \times 0.25 \text{ (mL)} \qquad \text{(Formula 1)}$$

Semen dilution at the AIC in Lembang, Indonesia, was performed in a one-step procedure, in which the calculated volume of semen was mixed directly with the total volume of diluent at one time. To ensure the quality of semen during cold storage, semen mixed with a diluent at a temperature of 37°C was placed within a water jacket filled with water maintained at 37°C and subsequently stored in a refrigerator set to a temperature of 4-5°C for a duration of 30 minutes. The semen mixture is then removed from the water jacket and kept at 4-5°C for an additional three hours. The pre-freezing motility analysis should meet a minimum threshold of 55%, in accordance with the Indonesian National Standard (INS, 2014). When motility fell at or below 55%, the freezing procedure was not initiated.

Semen collection

Semen was collected twice weekly in the morning using a goat artificial vagina (IMV, France) containing 500 mL of warm water maintained at 50°C. The artificial vagina was pumped and coated with lubricating gel to prevent injury when the semen was collected. The volume of Saanen goat semen obtained was 1-2 mL. Once the semen had been collected, the samples were sent to the laboratory at AIC in Lembang, Indonesia, which specialized in semen quality testing. Then, the semen samples were placed in a container designed to protect them from environmental exposure, such as light.

Semen freezing procedures

The cryopreservation procedure consisted of three evaluation stages, including fresh semen, chilled semen prior to freezing, and frozen-thawed semen. During the fresh semen stage, semen quality was assessed based on motility (≥ 70%), viability, and sperm abnormalities using a microscope (Olympus BX-53, Japan) at 400x magnification. Then, equilibration was carried out in a cool top for three hours, and a pre-freezing analysis was performed to assess semen quality, including motility ≥ 55%, viability, and abnormalities, using a light microscope (Olympus BX-53, Japan) at 400x magnification. Semen samples meeting the quality requirements were subsequently packaged and sealed into 0.25-

mL straws in accordance with the designated treatment groups. The filled straws were subsequently subjected to a 7-minute pre-freezing step at approximately −136°C, followed by immersion in liquid nitrogen (LN_2) at −196°C and storage. Each stage of evaluating fresh semen, liquid semen (before freezing), and frozen semen (after freezing) was compared using CASA (AndroVision®, Germany).

Evaluation of semen quality

Spermatozoa motility

Sperm motility was assessed by preparing a 20 µL of semen sample on an object glass and covering it with a coverslip. A light microscope at 400x magnification was used to observe the semen sample. Sperm motility was assessed at three stages following the INS (2014) guidelines, immediately after collection (fresh semen, ≥70%), prior to cryopreservation (liquid semen, ≥ 55%), and after thawing (frozen semen, ≥ 40%). Motility assessment was performed using five fields of view, according to Formula 2 (Susilawati, 2011).

$$\text{Motility (\%)} = \frac{\text{Motility 1 + Motility 2 + Motility 3 + Motility 4 + Motility 5}}{5} \times 100\% \quad \text{(Formula 2)}$$

Spermatozoa viability

Viability or sperm survival was determined by preparing a semen smear from 10 µL, adding 20 µl of eosin-nigrosin dye, mixing thoroughly, and spreading the mixture thinly and slowly for evaluation. Sperm viability was assessed using a light microscope at 400× magnification. Spermatozoa that absorbed the stain were classified as non-viable, whereas unstained spermatozoa were considered viable, which indicated live cells (Zou et al., 2021). The evaluation was performed by counting at least 200 live and dead spermatozoa, as described by Formula 3 (Susilawati, 2011).

$$\text{Viability (\%)} = \frac{\text{Viability}}{\text{Viability + nonviability}} \times 100\% \quad \text{(Formula 3)}$$

Spermatozoa abnormality

Sperm abnormalities were assessed by preparing a semen smear of 10 µl, adding 20 µl of eosin-nigrosin stain, mixing thoroughly, and then spreading the mixture thinly and slowly for evaluation. The evaluation of abnormalities was performed using a microscope (Olympus BX-53, Japan) set at 400x magnification. The abnormalities assessed include the shape of the head (too large, small, or flat) and the middle or tail (broken, curved, or abnormal). The evaluation was performed by counting at least 200 live and dead spermatozoa using the Formula 4 (Susilawati, 2011).

$$\text{Abnormality (\%)} = \frac{\text{Sperm Abnormal}}{\text{Sperm abnormal + sperm normality}} \times 100\% \quad \text{(Formula 4)}$$

Spermatozoa concentration

Sperm concentration was measured as the number of sperm per unit volume to determine the dilution requirements for semen. The concentration was determined using an SDM 6 spectrophotometer (Minitube, Germany) in accordance with the semen freezing protocol established by the AIC, Lembang, Indonesia, which incorporated the addition of fresh semen, 0.9% NaCl, and aquabidest. The findings were subsequently presented on the device in units of millions per milliliter.

Total motile spermatozoa

Total motile spermatozoa (TMS) were the total number of motile spermatozoa calculated by multiplying motility by concentration. At the time of post-thawing, TSM was calculated as the product of motility and concentration (Zou et al., 2021).

Statistical analysis

The present study used a randomized block design (RBD) with five treatment groups and 10 replicates. The data obtained in this study were analyzed using RStudio version 4.4.3 and one-way ANOVA with Duncan's Multiple Range Test. The significance level was established at a p-value less than 5% ($p < 0.05$) for all statistical analyses.

RESULTS AND DISCUSSION

Fresh Saanen goat semen

Fresh semen was an indicator of semen quality before freezing. Fresh semen was obtained immediately after semen collection and placed in a sample test tube in the laboratory. In accordance with INS (2014), fresh semen exhibiting motility ≥70% was deemed eligible for the dilution stage. The current results on the quality of fresh Saanen goat semen are presented in Table 1.

The fresh semen exhibited a volume of 2.5 ± 0.53 mL, a milky-white color, a pH of 6.5, a precise odor, and a thick consistency. These macroscopic characteristics indicated normal semen quality and were consistent with previous findings reported by Cardenas-Padilla et al. (2024), who observed fresh semen motility of more than 70% accompanied by high semen consistency. Microscopic evaluation revealed a mass motility score of +++ (vigorous wave motion), along with individual motility of 80.00 ± 10.54%, viability of 75.50 ± 0.74%, abnormalities of 4.46 ± 0.31%, sperm concentration of 3578 ± 223.32 × 10^6 spermatozoa/mL, and TMS of 2850 ± 302.59 × 10^6 spermatozoa/mL. These findings indicated that the ejaculates met the minimum quality requirements for further processing, as individual motility exceeded the recommended threshold of 70%. The current findings were consistent with those of Dapawole and Sirappa (2021), who indicated that fresh semen used in dilution should be at least 70% in goats.

Table 1. Fresh semen quality of Saanen goats

Parameter	**Macroscopic finding** (Mean + SD)
Volume (ml)	2.5 ± 0.53
Color	Milky white
pH	6.5
odor	Typical
Consistency	Thick
Parameter	**Microscopic finding** (Mean + SD)
Mass motility	+++
Individual motility (%)	80 ± 10.54
Viability (%)	75.50 ± 0.74
Abnormality (%)	4.46 ± 0.31
Concentration (million/ml)	3578 ± 223.32
Total motile spermatozoa (million/ml)	2850 ± 302.59

Mass motility was assessed based on the intensity of wave motion. +: Weak wave motion samples, ++: Moderate wave motion samples, and +++: Vigorous wave motion samples

Evaluation of Saanen goats' frozen semen

The supplementation of ALE in the tris-egg yolk diluent was intended to enhance the antioxidant capacity of the medium and provide additional protection to spermatozoa during the cryopreservation process. This finding was consistent with previous studies showing improved sperm preservation during cryopreservation. The present study focused on the effect of AML supplementation on post-thaw semen quality parameters. The beneficial effects of ALE might be attributed to its high content of bioactive compounds, including flavonoids, phenolic compounds, tannins, and terpenoids, which exhibit strong antioxidant activity. These compounds act as free radical scavengers, thereby reducing ROS accumulation and minimizing oxidative stress during the cryopreservation process (Eissa et al., 2022). AndroMed® was used as the commercial diluent due to its soy lecithin content and being free of animal-derived ingredients such as egg yolk; therefore, it protected the spermatozoa during cold storage (Bustani and Baiee, 2021). The current results on Saanen goats' semen quality were presented in Table 2.

There was a significant difference in pre-freezing sperm motility across the groups ($p < 0.05$; Table 2). Motility was assessed using a microscope across five fields of view as a percentage. Treatment T1 demonstrated higher motility after freezing than T0 (42.00 ± 10.33%), T3 (39.00 ± 13.08%), and T4 (43.50 ± 11.80%). The current results demonstrated that Treatment T1, supplemented with 0.10 mg of ALE, exhibited superior post-thaw motility compared with T0 (42.00 ± 10.33%), T3 (39.00 ± 13.08%), and T4 (43.50 ± 11.80%). These findings indicated that adding 0.10 mg of ALE provided optimal protection during cryopreservation, thereby preserving plasma membrane integrity and maintaining the functional activity of the sperm flagellar apparatus. Studies evaluating the supplementation of *Aquilaria malaccensis* extract in a tris-egg yolk extender were limited; therefore, the present findings were discussed in comparison with previous studies that employed extracts from other plant sources. Similar results were reported by Wahjuningsih et al. (2019), who reported post-thawing motility of 63% by adding 3% moringa leaves' extract. The differences in motility among treatments were due to the bioactive content of ALE, including flavonoids and phenolics, which can protect the sperms against oxidative stress induced by ROS (Ismail et al., 2019). Kaltsas (2023) indicated that mitochondrial integrity and ATP production decreased due to ROS, thereby impairing motility. This finding was consistent with the current results, which demonstrated that 0.20 mg of ALE was mildly toxic when the antioxidant concentration exceeded normal limits. The current result was consistent with those of Isnaini et al. (2019), who reported that the addition of 6% mangosteen peel extract antioxidants resulted in only 24.5% motility. This suggested that excessive antioxidant addition may induce toxic effects on spermatozoa.

Table 2. Evaluation of Saanen goats' frozen semen using a light microscope (400× magnification)

Parameter	**Fresh semen** (Mean + SD)	**Before freezing** (Mean + SD)					**After freezing** (Mean + SD)				
		T0	T1	T2	T3	T4	T0	T1	T2	T3	T4
Motility (%)	80 ± 10.54	60.00 ± 6.67^{ab}	63.00 ± 7.89^{a}	58.00 ± 5.37^{bc}	56.50 ± 5.80^{c}	63.50 ± 4.74^{a}	42.00 ± 10.33	49.00 ± 9.66	40.00 ± 16.83	39.00 ± 13.08	43.50 ± 11.80
Viability (%)	75.50 ± 0.74	62.60 ± 5.53^{b}	67.45 ± 6.43^{a}	64.20 ± 5.41^{ab}	66.19 ± 4.62^{ab}	68.30 ± 6.09^{a}	54.31 ± 9.49	57.74 ± 9.24	55.51 ± 10.91	56.94 ± 9.99	51.65 ± 8.79
Abnormality (%)	4.46 ± 0.31	6.35 ± 1.02	5.55 ± 0.73	5.85 ± 0.95	6.41 ± 0.96	5.87 ± 1.14	6.96 ± 1.29	6.42 ± 0.83	6.82 ± 0.94	7.56 ± 0.59	6.47 ± 0.95
Concentration (million/straw)	3578 ± 223.32	61.14 ± 1.95	63.68 ± 3.12	63.24 ± 3.82	64.14 ± 2.54	64.65 ± 4.11	60.63 ± 2.47	63.12 ± 3.34	62.64 ± 3.86	63.57 ± 2.56	64.08 ± 4.16
TMS (million/straw)	2850 ± 302.59	36.75 ± 4.8	40.13 ± 5.6	36.69 ± 4.2	36.30 ± 4.5	41.09 ± 4.4	25.46 ± 6.48	31.04 ± 6.90	25.17 ± 11.20	24.78 ± 8.40	27.73 ± 7.39

[ab]Different supper script letters in the same row indicated a significant difference ($p < 0.05$). TSM: Total motile spermatozoa, ALE: *Aquilaria malaccensis* leaf extract, T0: Tris-egg yolk treatment group, T1: Tris-egg yolk + ALE at 0.10 mg, T2: Tris-egg yolk + ALE at 0.15 mg, T3: Tris-egg yolk + ALE at 0.20 mg, T4: AndroMed®

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Viability is the survival rate of spermatozoa assessed using semen preparation with eosin-nigrosin staining (Abedin et al., 2023). The use of different diluent formulations resulted in significant differences in sperm viability prior to freezing ($p < 0.05$); however, post-thaw viability did not differ significantly among treatments ($p > 0.05$). Despite the lack of statistical significance after thawing, treatments supplemented with ALE (T1, T2, and T3) consistently exhibited higher viability than the control treatment (T0). The current results indicated that the antioxidant compounds present in ALE may have provided protective effects against cryodamage by maintaining sperm plasma membrane stability throughout the freezing and thawing procedures. The current results indicated that spermatozoa cell membranes were susceptible to lipid peroxidation because ruminants have a high content of unsaturated fatty acids in their cell membranes. Saratsi et al. (2024) indicated that spermatozoa viability decreased with storage time, while the addition of cryoprotectants to the diluent increased the viability of spermatozoa stored in liquid nitrogen.

Sperm abnormalities were evaluated by examining morphological defects in spermatozoa, including abnormalities of the head, midpiece, and tail regions. These defects might be attributable to inherent developmental disorders or to physical damage sustained during cryopreservation (Humann-Ziehank, 2026). Sperm abnormality rates did not differ among the diluent treatments either before freezing or after thawing ($p > 0.05$). Nevertheless, numerical variations were observed, with Treatment T3 exhibiting the highest percentage of abnormalities before freezing (6.41 ± 0.96%). Following thawing, the highest rate of abnormalities was observed in T0 (6.96 ± 1.29%), whereas T1 demonstrated the lowest rate of abnormalities (6.42 ± 0.83%). The current findings indicated that ALE supplementation did not adversely affect sperm morphology and may have helped maintain structural integrity during cryopreservation. The increase in sperm abnormalities observed after cryopreservation may have been attributed to structural damage induced during the freezing and thawing processes (Zang et al., 2025). Cryoinjury was reported to affect sperm membranes, acrosomes, mitochondria, and tail structures, leading to morphological defects and reduced sperm quality. The addition of 1% and 2% soybean lecithin to tris egg yolk diluents was reported to enhance membrane protection during cryopreservation, thereby minimizing sperm morphological damage and improving post-thaw semen quality (Suwor et al., 2025). The abnormality values observed in the present study remained below 5%, indicating that the tris-egg yolk diluent supplemented with ALE, as well as the AndroMed® diluent, did not induce substantial structural damage to spermatozoa and remained within the acceptable range for semen cryopreservation. Representative images of sperm viability and morphological abnormalities are illustrated in Figure 1.

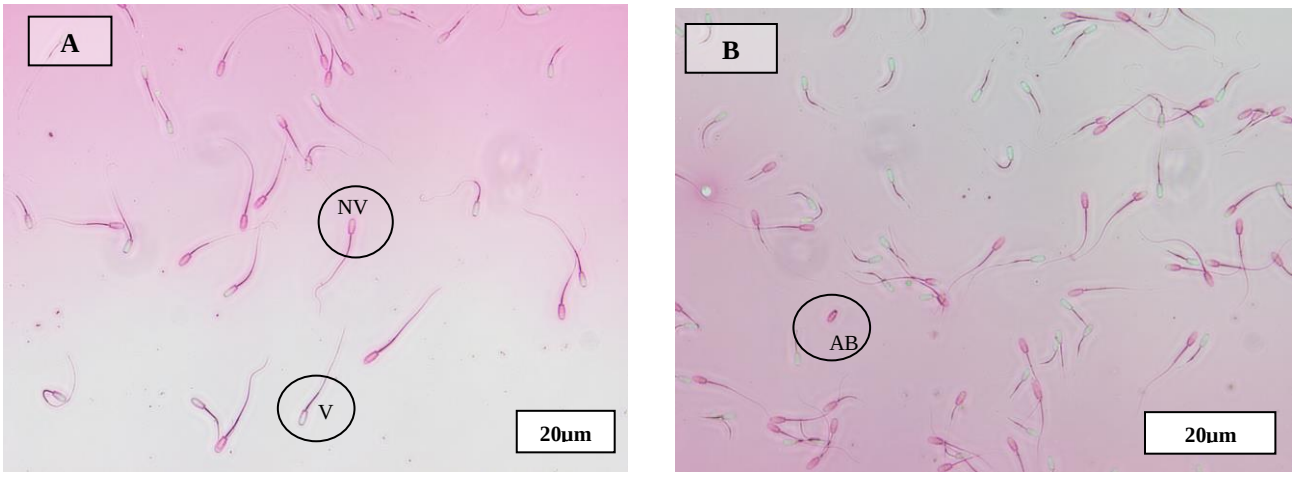

Figure 1. Spermatozoa viability and abnormalities post-thawing using a light microscope. **A**: Viability preparation, **NV**: Non-viable, **V**: Viable (head does not absorb color), **B:** Abnormality preparation, **AB**: Abnormal spermatozoa (the sperm head was detached from the tail). Eosin–nigrosin staining, 400x magnification.

Sperm concentration was measured before freezing and after thawing. No significant differences in sperm concentration were observed among the diluent treatments at either evaluation stage ($p > 0.05$). The lowest post-thawing concentration was observed in T0, which was 60.63 ± 2.47 million/straw, and the highest post-thawing concentration was observed in Treatment T4, which was 64.08 ± 4.16 million/straw. The concentration across treatment groups was stable across diluents, ranging from 60 to 64 million/straw. This finding indicated that supplementation with ALE did not affect the number of spermatozoa but helped maintain their functional quality during cryopreservation. Similar results were reported by Hassan et al. (2021), who found that adding natural antioxidants to semen diluents improved post-thaw sperm motility and viability without affecting sperm concentration. Likewise, El-Seadawy et al. (2022) reported that antioxidant supplementation primarily protected sperm membranes against oxidative damage and enhanced sperm function, whereas sperm concentration remained unchanged. Therefore, the beneficial effects of ALE were likely associated with its ability to preserve sperm physiological integrity rather than alter sperm cell numbers. Sperm concentration was influenced by individual male factors, such as age, genetic background, reproductive status, and

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testicular size, as well as by semen collection frequency. Similarly, Kumar et al. (2025) found that genetic factors, breed, boar age, and physiological conditions affect fresh semen concentration and sperm survival during freezing.

The TMS was a combined parameter that integrated sperm concentration and motility (Novita et al., 2021). The TMS was considered essential for evaluating semen quality prior to AI, as it provided a comprehensive estimate of the number of motile spermatozoa available for fertilization. The current results indicated that the use of a diluent did not significantly affect TMS before freezing and post-thawing ($p > 0.05$). The highest post-thaw TMS was observed in Treatment T1 (31.04 ± 6.90 million/straw), whereas Treatment T3 demonstrated the lowest value (24.78 ± 8.40 million/straw). These findings indicated that adding 0.10 mg of ALE to the tris-egg yolk diluent improved sperm survival during cryopreservation. The phospholipid content of egg yolk and the antioxidant properties of ALE suppressed oxidative damage. These findings are consistent with those of Isnaini et al. (2019), who found that freezing semen using 2% mangosteen peel extract resulted in a motility value of 56.5%. Additionally, Tethool et al. (2022) indicated that egg yolk protected spermatozoa motility.

Evaluation of motility

The evaluation of frozen semen, before and after freezing, can be conducted either visually with a microscope or systematically using CASA to assess different motility parameters of spermatozoa (Fernandez-Novo et al., 2021). The inclusion of CASA reduced potential bias in semen evaluation by objectively quantifying sperm motility and motion characteristics through computerized analysis. This approach minimized the influence of observer subjectivity, fatigue, and differences in technical expertise, resulting in more accurate and reproducible assessments compared with conventional visual examination. Additionally, sperm motility parameters are crucial indicators of fertility, helping to identify males with lower reproductive potential and to select genetically superior sires for breeding programs (Hackerova et al., 2025). A total of 14 parameters were used in CASA, including motile, progressive, distance average path (DAP), distance curved line (DCL), distance straight line (DSL), velocity average path (VAP), velocity straight line (VSL), velocity curved line (VCL), straightness (STR), linearity (LIN), amplitude of lateral head (ALH), beat cross frequency (BCF), wobble (WOB), and head activity (HAC; Blagojević et al., 2024). The results of sperm motility using CASA before and after freezing stages were indicated in tables 3 and 4.

Table 3. Evaluation of sperm motility before freezing using computer-assisted sperm analysis in Saanen goat semen

Before freezing / Kinematic parameters	T0	T1	T2	T3	T4
Motile (%)	75.42 ± 8.72	79.50 ± 8.99	79.36 ± 8.81	77.39 ± 11.06	75.03 ± 3.81
Progressive (%)	71.27 ± 10.74	76.34 ± 10.72	76.04 ± 9.67	73.08 ± 13.47	69.27 ± 6.03
DAP (µm)	17.05 ± 5.93	15.56 ± 3.46	16.11 ± 4.60	17.74 ± 7.08	14.54 ± 6.03
DCL (µm)	35.50 ± 12.50^{a}	33.90 ± 8.27^{ab}	34.90 ± 10.47^{ab}	37.51 ± 15.06^{a}	30.72 ± 11.40^{b}
DSL (µm)	12.95 ± 4.85	11.07 ± 2.63	11.76 ± 3.60	13.35 ± 5.92	11.58 ± 5.80
VAP (µm/sec)	52.76 ± 15.27	57.58 ± 12.72	56.06 ± 11.42	55.49 ± 15.08	55.22 ± 9.59
VSL (µm/sec)	41.70 ± 12.43	44.04 ± 9.31	43.38 ± 8.58	43.55 ± 12.50	45.84 ± 9.90
VCL (µm/sec)	105.85 ± 31.90	119.18 ± 29.62	115.86 ± 26.38	107.49 ± 39.55	112.75 ± 21.06
STR (%)	0.79 ± 0.02^{ab}	0.77 ± 0.01^{b}	0.77 ± 0.02^{b}	0.78 ± 0.02^{b}	0.82 ± 0.04^{a}
LIN (%)	0.40 ± 0.03^{b}	0.37 ± 0.02^{b}	0.38 ± 0.02^{b}	0.39 ± 0.01^{ab}	0.41 ± 0.04^{a}
ALH (µm)	0.90 ± 0.37^{b}	1.38 ± 0.30^{a}	1.37 ± 0.31^{a}	1.37 ± 0.40^{a}	1.21 ± 0.22^{b}
BCF (Hz)	9.01 ± 2.00	11.47 ± 1.07	11.69 ± 1.46	11.96 ± 2.28	11.60 ± 2.88
WOB (%)	0.50 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.50 ± 0.02	0.49 ± 0.04
HAC (rad)	0.29 ± 0.08	0.32 ± 0.07	0.32 ± 0.07	0.31 ± 0.09	0.30 ± 0.05

^{a,b} Mean different superscript letters that vary in each row indicate significant differences at $p < 0.05$. T0: Tris-egg yolk, T1: Tris-egg yolk + 0.10 mg of ALE, T2: Tris-egg yolk + 0.15 mg of ALE, T3: Tris-egg yolk + 0.20 mg of ALE, T4: AndroMed®. DAP: Distance average path, DCL: Distance curved line, DSL: Distance straight line, VAP: Velocity average path, VSL: Velocity straight line, VCL: Velocity curved line, STR: Straightness, LIN: Linearity, ALH: Amplitude of lateral head, BCF: Beat cross frequency, WOB: Wobble, HAC: Head activity.

Table 4. Evaluation of sperm motility after freezing using computer-assisted sperm analysis in Saanen goat semen

After freezing / Kinematic parameters	T0	T1	T2	T3	T4
Motile (%)	66.11 ± 18.34[a]	68.01 ± 17.63[a]	63.81 ± 19.89[a]	57.65 ± 22.48[a]	45.15 ± 15.72[b]
Progresif (%)	61.74 ± 20.71[a]	62.79 ± 20.76[a]	58.41 ± 22.56[a]	53.25 ± 24.54[a]	40.56 ± 16.19[b]
DAP (µm)	15.44 ± 4.94[a]	16.06 ± 6.21[a]	14.39 ± 6.56[a]	15.10 ± 7.05[a]	11.10 ± 4.87[b]
DCL (µm)	30.66 ± 9.76[a]	30.86 ±10.99[a]	29.32 ± 11.81[a]	30.13 ± 13.66[a]	23.77 ± 10.31[b]
DSL (µm)	12.50 ± 4.52[a]	13.21 ± 5.91[a]	11.56 ± 6.08[ab]	12.47 ± 6.53[a]	9.25 ± 4.50[b]
VAP (µm/sec)	44.45 ± 16.23[a]	46.41 ± 17.90[a]	42.45 ± 18.18[ab]	40.46 ± 18.84[ab]	34.08 ± 13.94[b]
VSL (µm/sec)	36.89 ± 13.95[a]	39.03 ± 16.33[a]	35.06 ± 16.28[ab]	34.02 ± 16.69[ab]	29.20 ± 12.36[b]
VCL (µm/sec)	87.42 ± 35.17[a]	88.38 ± 35.02[a]	84.67 ± 35.32[a]	79.50 ± 37.88[ab]	70.73 ± 30.49[b]
STR (%)	0.83 ± 0.04	0.83 ± 0.05	0.82 ± 0.04	0.83 ± 0.05	0.85 ± 0.03
LIN (%)	0.43 ± 0.05	0.44 ± 0.07	0.41 ± 0.05	0.42 ± 0.05	0.41 ± 0.02
ALH (µm)	1.03 ± 0.34[a]	1.02 ± 0.34[a]	1.01 ± 0.37[a]	0.95 ± 0.39[ab]	0.78 ± 0.29[b]
BCF (Hz)	12.42 ± 3.24[a]	12.76 ± 3.66[a]	11.75 ± 3.27[a]	11.54 ± 4.20[a]	9.26 ± 3.47[b]
WOB (%)	0.52 ± 0.05[a]	0.53 ± 0.05[a]	0.50 ± 0.03[ab]	0.51 ± 0.03[ab]	0.48 ± 0.02[b]
HAC (rad)	0.23 ± 0.09[a]	0.23 ± 0.09[a]	0.22 ± 0.09[a]	0.21 ± 0.10[ab]	0.18 ± 0.07[b]

[a,b] Mean different superscript letters that vary in each row indicate significant differences at $p < 0.05$. T0: Tris-egg yolk, T1: Tris-egg yolk + 0.10 mg of ALE, T2: Tris-egg yolk + 0.15 mg of ALE, T3: Tris-egg yolk + 0.20 mg of ALE, T4: AndroMed®. DAP: Distance average path, DCL: Distance curved line, DSL: Distance straight line, VAP: Velocity average path, VSL: Velocity straight line, VCL: Velocity curved line, STR: Straightness, LIN: Linearity, ALH: Amplitude of lateral head, BCF: Beat cross frequency, WOB: Wobble, HAC: Head activity.

According to the present results, diluent type did not significantly influence TSM prior to cryopreservation ($p > 0.05$). However, significant differences were observed among treatments after thawing ($p < 0.05$), indicating that the protective effects of the diluents became more pronounced following freeze-thaw stress. Treatment T1 exhibited the highest total motility values both before freezing (79.50 ± 8.99%) and after thawing (68.01 ± 17.63%). A similar pattern was observed for progressive motility, which did not differ among the treatment groups prior to the freezing process ($p > 0.05$) but was significantly influenced by the treatments following thawing ($p < 0.05$). Treatment T1 exhibited the highest progressive motility values both before freezing (76.34 ± 10.72%) and after thawing (62.79 ± 20.76%). Although progressive motility decreased across all treatments following cryopreservation, the extent of the decline differed among different diluent formulations. The lowest post-thaw progressive motility was observed in Treatment T4 (40.56 ± 16.19%), whereas Treatment T1 maintained the highest value. The current findings indicated that supplementation with ALE enhanced the diluent's ability to preserve sperm motility during cryopreservation, thereby reducing the detrimental effects of freeze-thaw stress on sperm function. Treatment T1 consistently exhibited higher total and progressive motility values, suggesting that supplementation with 0.10 mg of ALE enhanced sperm resistance to cryoinjury during freezing and thawing. The improved post-thaw motility observed in the present study indicated a protective effect on the integrity of the sperm plasma membrane and flagellar function. Damage to the flagellar apparatus during cryopreservation may impair flagellar motility and propulsion, thereby decreasing sperm motility after thawing. However, motility and progression remained above 40% in Treatment T1, likely due to flavonoids and phenolics diluent acting as potent antioxidants. The present results were in agreement with the findings of Hassan et al. (2021), who reported that *Turraea fischeri* leaf extract enhanced post-thaw sperm quality due to its high flavonoid and phenolic content. These antioxidant compounds have been demonstrated to neutralize excessive ROS produced during equilibration and cryopreservation, thereby safeguarding spermatozoa from oxidative damage. Consequently, the preservation of membrane integrity and mitochondrial function contributed to improved sperm motility following thawing. Meanwhile, the reduction in motility and progression observed in Treatment T4 suggested that patented or practical diluents containing lesitin were not fully effective in mitigating oxidative stress in goat semen, which was highly sensitive to thermal and osmotic pressures (Nisfimawardah et al., 2023). These findings were likely associated with the inherent sensitivity of goat spermatozoa to cryopreservation-induced oxidative stress, as their plasma membranes were rich in polyunsaturated fatty acids, which are particularly susceptible to lipid peroxidation induced by ROS. The results of the CASA motility assessment were presented in Figure 2.

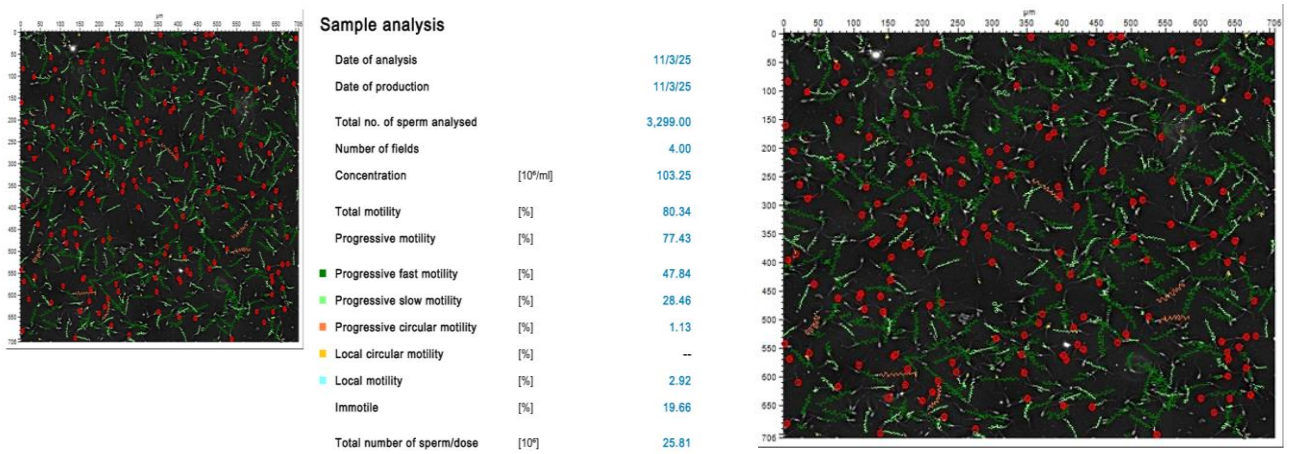

Figure 2. Kinematic parameters using computer-assisted sperm analysis AndroVision® (Minitube, Germany)

The CASA-derived parameters (DAP, DCL, and DSL) and velocity parameters (VAP, VSL, and VCL) exhibited patterns that closely aligned with the motility results. Higher values of these kinematic parameters were generally associated with improved sperm motility, suggesting enhanced preservation of sperm movement efficiency and fertilizing potential following cryopreservation. Treatments T1 and T2 demonstrated higher values for DAP, DCL, and DSL than Treatment T4, indicating that spermatozoa movement patterns were more directed and stable. The spermatozoa exhibited an enhanced capacity to maintain longer, more consistent movement paths, as evidenced by increased DAP, DCL, and DSL values. These parameters indicated that the spermatozoa move greater distances while maintaining forward progression more efficiently. Additionally, treatments T1 and T2 exhibited higher VAP and VCL values, indicating enhanced preservation of sperm kinematic performance following thawing. These improvements may have reflected improved mitochondrial function and energy metabolism, as sperm motility largely depends on ATP generated by mitochondria in the midpiece. Preservation of mitochondrial structural integrity during cryopreservation likely contributed to the maintenance of sperm velocity and overall motility (Zhang et al., 2025). However, the highest ALE supplementation level (0.20 mg) in Treatment T3 resulted in lower VAP and VSL values than in treatments T1 and T2. Although VAP and VSL values remained higher than those observed in Treatment T4 supplemented with AndroMed®. The current findings indicated that increasing the extract concentration beyond the optimal level did not further enhance sperm kinematic performance and may have reduced the beneficial effects of antioxidant supplementation. A similar trend was reported by Hassan et al. (2021), who observed that moderate concentrations of *Turraea fischeri* leaf extract improved post-thaw sperm quality, whereas excessive supplementation did not provide additional benefits and adversely affected sperm function. These findings indicated that the addition of high doses of ALE can negatively affect enzymatic activity and flagellar motility. The present results were consistent with those of Wahjuningsih et al. (2019), who reported that 6% *Moringa oleifera* leaf extract reduced sperm motility, indicating that high concentrations of antioxidants may exert detrimental effects on sperm physiological function. Adding 0.10 mg of ALE in the present study maintained a greater movement and velocity after thawing, indicating that this concentration provided optimal protection against cryopreservation-induced damage while preserving sperm kinematic performance.

The STR, LIN, and WOB parameters, along with head-oscillation metrics such as ALH displacement, BCF, and HAC, were used to describe sperm movement patterns in greater detail. These kinematic indicators provided valuable information regarding movement efficiency, trajectory control, and flagellar function, thereby enabling a more comprehensive assessment of sperm quality following cryopreservation. The highest post-thaw STR and LIN values were recorded in treatments T4 and T1, respectively, indicating that spermatozoa exhibited straighter swimming and greater forward progression after cryopreservation. This finding indicated that 0.10 mg of ALE contributed to the preservation of sperm kinematic function by reducing unnecessary lateral movement and improving swimming efficiency. The present findings were consistent with those of İnanç et al. (2018), who reported that increased sperm LIN was positively correlated with penetration distance and reproductive performance. In the current study, treatments T1 and T2 exhibited the highest post-thaw ALH and BCF values, indicating higher preservation of sperm flagellar function following cryopreservation. Higher ALH and BCF values reflect stronger flagellar movement and optimal tail-beat activity. These are crucial for sustaining sperm hyperactivation, aiding capacitation, and enhancing the spermatozoa's capacity to penetrate the zona pellucida during fertilization. This finding was consistent with the study conducted by Xu et al. (2023), who demonstrated that the capacitation process was influenced by sperm motility and metabolic alterations following thawing. Similar observations were reported by Giaccagli et al. (2021), who found that mitochondrial activity was essential for sperm hyperactivation and fertilizing ability by regulating sperm motility. Furthermore, Tourmente et

al. (2022) found that capacitation involves metabolic changes that boost sperm motility and energy use, highlighting a strong link between energy metabolism, sperm movement, and fertilization ability. In contrast, Treatment T4, which used the AndroMed® diluent, exhibited lower ALH, BCF, WOB, and HAC values after thawing, suggesting impaired head oscillation and flagellar activity resulting from cryopreservation-induced structural damage. The reduction in these kinematic parameters indicated a decline in sperm movement efficiency and functional performance following the freeze-thaw process. Conversely, treatments T1 and T2 maintained relatively stable values for these parameters, indicating that the combination of tris-egg yolk diluent and ALE provided superior protection against cryoinjury. The present findings indicate that the ALE-supplemented diluent more effectively preserved sperm kinematic function and structural integrity than treatments T0 and T4. This observation was consistent with that of Rizal et al. (2025), who reported that the motility of frozen goat semen was 47.5% with AndroMed® diluent alone and increased to 56.25% when 4% moringa leaf extract was added to the diluent. The improved post-thaw motility and kinematic parameters observed in treatments T1 and T2 indicated that the antioxidant capacity of ALE was most effective at appropriate concentrations, thereby enhancing sperm protection during freezing and thawing.

CONCLUSION

Adding 0.10 mg of *Aquilaria malaccensis* leaf extract to a tris-egg yolk diluent enhanced protection against cryopreservation-induced damage, as evidenced by higher post-thaw motility, progressive motility, and improvements in movements, velocity, and head oscillation measures. Therefore, *Aquilaria malaccensis* leaf extract exhibited potential as a natural antioxidant additive to enhance the functional quality of frozen Saanen goat semen and supported artificial insemination programs. The utilization of *Aquilaria malaccensis* has the potential to enhance the efficacy of future artificial insemination programs by enhancing the availability of high-quality frozen semen and promoting the dissemination of superior genetic resources.

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Authors' contributions

Lailatun Nisfimawardah, Firdaus Husein, Monasdir, Ika Fitriana Dyah Ratnasari, Eros Sukmawati, and Sri Wahjuningsih contributed to manuscript writing, data analysis, and study design. Lailatun Nisfimawardah collected data from the field and laboratory. All authors contributed to reviewing the data of the study, performed statistical analysis, and approved the final draft of the manuscript. All authors have read and approved the final edition of the manuscript before publication.

Availability of data and materials

All data generated and analyzed during this study were included in this published article. Additional data supporting the findings of the present study were available from the corresponding author upon reasonable request.

Competing interests

The authors declared no personal or financial interests in this study.

Ethical considerations

The authors declared that this manuscript has been checked by all authors and was originally submitted to the journal for publication. No AI tools were used to conduct or write this study. The authors accept full responsibility for the originality of the study before submission and before publication.

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REFERENCES

Abedin SN, Baruah A, Baruah KK, Kadirvel G, Katiyar R, Khargharia G, Bora A, Dutta JY, Sinha S, Tamuly S et al. (2023). *In vitro* and *in vivo* studies on the efficacy of zinc-oxide and selenium nanoparticle in cryopreserved goat (*Capra hircus*) spermatozoa. Biological Trace Element Research, 201(10): 4726-4745. DOI: https://www.doi.org/10.1007/s12011-022-03551-6

Anand M and Yadav S (2016). Assessment of motion and kinematic characteristics of frozen-thawed Sirohi goat semen using computer-assisted semen analysis. Veterinary world, 9(2): 203. DOI: https://www.doi.org/10.14202/vetworld.2015.203-206

Ayantoye JO, Kolachi HA, Zhang X, Shahzad M, Kandil OMT, Wan P, and Zhao X (2025). Advances in timed artificial insemination: Integrating omics technologies for enhanced reproductive efficiency in dairy cattle. Animals, 15(6): 816. DOI: https://www.doi.org/10.3390/ani15060816

Batubara R, Hanum TI, Handika A, and Affandi O (2020). The screening of phytochemical and antioxidant activity of *Aquilaria malaccensis*leaves (*Aquilaria malaccensis*) from two sites in North Sumatra, Indonesia. Biodiversitas Journal of Biological Diversity, 21(4): 1588-1596. DOI: https://www.doi.org/10.13057/biodiv/d210440

Blagojević J, Stanimirović Z, Glavinić U, Vakanjac S, Radukić Ž, Milošević M, and Maletić M (2024). Impact of supplemented nutrition on semen quality, epigenetic-related gene expression, and oxidative status in boars. Animals, 14(22): 3297. DOI: https://www.doi.org/10.3390/ani14223297

Bustani GS and Baiee FH (2021). Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. Veterinary World, 14(5): 1220-1233. DOI: https://www.doi.org/10.14202/vetworld.2021.1220-1233

Cardenas-Padilla AJ, Jimenez-Trejo F, Cerbon M, Chavez-Garcia A, Cruz-Cano NB, Martinez-Torres M, Alcantar-Rodriguez A, and Medrano A (2024). Sperm melatonin receptors, seminal plasma melatonin and semen freezability in goats. Theriogenology, 225: 98-106. DOI: https://www.doi.org/10.1016/j.theriogenology.2024.05.034

Dapawole RR and Sirappa IP (2021). Effect concentration of moringa (*Moringa oleifera* Lam) leaf extract in citrate-egg yolk in maintaining motility and viability of spermatozoa of Kacang goat. Jurnal Sain Peternakan Indonesia, 16(4): 340-346. DOI: https://www.doi.org/10.31186/jspi.id.16.4.340-346

Directorate general of livestock and animal health services (2024). Livestock and animal health statistics 2024. Ministry of Agriculture of the Republic of Indonesia. Available at: https://ditjenpkh.pertanian.go.id

Eissa MA, Hashim YZH, Abdul Azziz SS, Salleh HM, Isa MLM, Abd Warif NM, Abdullah F, Ramadan E, and El-Kersh DM (2022). Phytochemical constituents of *Aquilaria malaccensis* leaf extract and their anti-inflammatory activity against LPS/IFN-γ-stimulated RAW 264.7 cell line. ACS Omega, 7(18): 15637-15646. DOI: https://www.doi.org/10.1021/acsomega.2c00439

El-Seadawy IE, Kotp MS, El-Maaty AMA, Fadl AM, El-Sherbiny HR, and Abdelnaby EA (2022). The impact of varying doses of moringa leaf methanolic extract supplementation in the cryopreservation media on sperm quality, oxidants, and antioxidant capacity of frozen-thawed ram sperm. Tropical Animal Health and Production, 54(6): 344. DOI: https://www.doi.org/10.1007/s11250-022-03344-y

Fernandez-Novo A, Santos-Lopez S, Barrajon-Masa C, Mozas P, de Mercado E, Caceres E, Garrafa A, Gonzalez-Martin JV, Perez-Villalobos N, Oliet A et al. (2021). Effect of extender, storage time and temperature on kinetic parameters (CASA) on bull semen samples. Biology, 10(8): 806. DOI: https://www.doi.org/10.3390/biology10080806

Ferro MM, Tedeschi LO, and Atzori AS (2017). The comparison of the lactation and milk yield and composition of selected breeds of sheep and goats. Translational Animal Science, 1(4): 498-506. DOI: https://www.doi.org/10.2527/tas2017.0056

Giaccagli MM, Gómez-Elías MD, Herzfeld JD, Marín-Briggiler CI, Cuasnicú PS, Cohen DJ, and Da Ros VG (2021). Capacitation-induced mitochondrial activity is required for sperm fertilizing ability in mice by modulating hyperactivation. Frontiers in Cell and Developmental Biology, 9: 767161. DOI: https://www.doi.org/10.3389/fcell.2021.767161

Hackerova L, Pilsova A, Pilsova Z, Zelenkova N, Tymich Hegrova P, Klusackova B, Chmelikova E, Sedmikova M, Simonik O, and Postlerova P (2025). Boar sperm motility assessment using computer-assisted sperm analysis: Current practices, limitations, and methodological challenges. Animals, 15(3): 305. DOI: https://www.doi.org/10.3390/ani15030305

Hassan SA, Khalil WA, Hassan MAE, Yousif AI, Sabry OM, Wink M, and Sobeh M (2021). Antioxidant and antiapoptotic effects of a *Turraea fischeri* leaf extract on cryopreserved goat sperm. Animals, 11(10): 2840. https://www.doi.org/10.3390/ani11102840

Humann-Ziehank E (2026). Selenium deficiency in sheep and goats. Encyclopedia of livestock medicine for large animal and poultry production. Springer Nature., Cham, Switzerland, pp. 1180-1182. DOI: https://www.doi.org/10.1007/978-3-031-61549-8_231

İnanç ME, Cil B, Tekin K, Alemdar H, and Daşkin A (2018). The combination of CASA kinetic parameters and fluorescein staining as a fertility tool in cryopreserved bull semen. Turkish Journal of Veterinary & Animal Sciences, 42(5): 452-458. DOI: https://www.doi.org/10.3906/vet-1801-83

Indonesian national standard (INS) (2014). INS No. 4869.3-2014 Freezing semen, part 3. Sheep and goat. Indonesian National Standard Agency, Jakarta. Available at: https://www.pesta.bsn.go.id/produk/detail/9884-sni486932014

Ismail F, Wahab AYA, Isa MLM, Muhammad H, Ismail RASR, and Razak RNHA (2019). The effects of *Aquilaria malaccensis* leaves aqueous extract on sperm of Sprague Dawley Rats towards early embryogenesis. IIUM Medical Journal Malaysia, 18(2): 59-67. DOI: https://www.doi.org/10.31436/imjm.v18i2.96

Isnaini N, Ihsan MN, and Wahjuningsih S (2019). Mangosteen peel extract in Tris-egg yolk extender improves fertility of cryopreserved goat sperm. Livestock Research for Rural Development, 31: 53. Available at: http://www.lrrd.org/lrrd31/4/nuruli31053.html

Kaltsas A (2023). Oxidative stress and male infertility: The protective role of antioxidants. Medicina, 59(10): 1769. https://www.doi.org/10.3390/medicina59101769

Kumar A, Kanaka KK, Mehta H, Thakur NS, and Singh G (2025). A review of current challenges, influential factors, and advancements in goat semen cryopreservation. Archives of Current Research International, 25(3): 398-411. DOI: https://www.doi.org/10.9734/acri/2025/v25i31131

Liang J, Larbi A, Lv C, Ali S, Wu G, and Quan G (2023). Fertility results after exocervical insemination using goat semen cryopreserved with extenders based on egg yolk, skim milk, or soybean lecithin. Reproduction in Domestic Animals, 58(3): 431-442. DOI: https://www.doi.org/10.1111/rda.14304

Luo J, Wang W, and Sun S (2019). Research advances in reproduction for dairy goats. Asian-Australasian Journal of Animal Sciences, 32(8): 1284-1295. DOI: https://www.doi.org/10.5713/ajas.19.0486

Mahiddine FY and Kim MJ (2021). Overview on the antioxidants, egg yolk alternatives, and mesenchymal stem cells and derivatives used in canine sperm cryopreservation. Animals, 11(7): 1930. DOI: https://www.doi.org/10.3390/ani11071930

Mansour HAEH (2025). Integration of assisted reproductive technologies and artificial intelligence for optimizing fertility and genetic selection in livestock production. Discover Applied Sciences, 7(10): 1143. DOI: https://www.doi.org/10.1007/s42452-025-07380-9

Nisfimawardah L, Firmawati A, Ihsan MN, Susilawati T, and Wahjuningsih S (2023). Semen cryopreservation quality and sperm kinematics of Saanen goats using different diluents. World's Veterinary Journal, 13(2): 300-309. DOI: https://www.doi.org/10.54203/scil.2023.wvj32

Novita A, Ciptadi G, Wahjuningsih S, Amaliya A, Sawitri W, and Susilawati T (2021). The influence of individual factors on the characteristic and production of frozen semen of Bali Cattle. Journal of Advanced Veterinary Research, 11(3): 162-166. DOI: Retrieved from https://www.advetresearch.com/index.php/AVR/article/view/732

Rizal M, Syarifuddin NA, Sumantri I, Wahdi A, Riyadhi M, Rizqiana S, Bahtiyar S, and Rifani MA (2025). Cryopreservation of Boer crossbreed goat semen with AndroMed® extender and moringa leaf extract. Jurnal Acta Veterinaria Indonesiana, 13(2): 175-182, 2337-3202. DOI: https://www.doi.org/10.29244/avi.13.2.%25p

Saratsi A, Samartzi F, Tsiokos D, Theodosiadou EK, Panagiotidis I, Ligda C, and Rekkas CA (2024). Effect of three commercially available extenders containing phospholipids of different sources on skopelos buck liquid-stored sperm quality. Veterinary Sciences, 11(10): 494. https://www.doi.org/10.3390/vetsci11100494

Shah SAH, Haider MS, Ahmed H, Fayyaz MH, and Andrabi SMH (2023). Cryopreservation protocol resolving the temperature challenges of long-distance transportation of Beetal buck (*Capra hircus*) sperm. Small Ruminant Research, 226: 107030. DOI: https://www.doi.org/10.1016/j.smallrumres.2023.107030

Sharafi M, Borghei-Rad SM, Hezavehei M, Shahverdi A, and Benson JD (2022). Cryopreservation of semen in domestic animals: A review of current challenges, applications, and prospective strategies. Animals, 12(23): 3271. DOI: https://www.doi.org/10.3390/ani12233271

Susilawati T (2011). Spermatology. Universitas Brawijaya Press., Malang, pp. 1-176. Available at: https://b2n.ir/u32230

Suwor F, Kubota S, Nawong S, Thuangsanthia A, Toyra M, Paengkoum P, and Ponchunchoovong S (2025). Effects of cryoprotectant combinations on post-thawed sperm quality, biomolecular changes, DNA methylation, and pregnancy rates in Boer goat semen. Veterinary Sciences, 12(2): 178. DOI: https://www.doi.org/10.3390/vetsci12020178

Tethool AN, Ciptadi G, Wahjuningsih S, and Susilawati T (2022). Deterioration of frozen semen of Bali cattle after cooling at 5°C. World's Veterinary Journal, 12(4): 395-404. DOI: https://www.doi.org/10.54203/scil.2022.wvj50

Tourmente M, Sansegundo E, Rial E, and Roldan ER (2022). Capacitation promotes a shift in energy metabolism in murine sperm. Frontiers in Cell and Developmental Biology, 10: 950979. DOI: https://www.doi.org/10.3389/fcell.2022.950979

Upadhyay A, Alex R, Dige MS, Sahoo S, Khan KD, Das P, Vohra V, and Gowane GR (2024). Optimizing the genetic evaluation criteria for the small herd of Saanen x Beetal crossbred dairy goats of Indian sub-tropic. Small Ruminant Research, 241: 107402. DOI: https://www.doi.org/10.1016/j.smallrumres.2024.107402

Wahjuningsih S, Ciptadi G, Ihsan MN, Isnaini N, and Rahayu S (2019). Supplementation of *Moringa oleifera* leaves' extract in Tris-egg yolk extender on the quality and fertility of cryopreserved Senduro goat sperm. Livestock Research for Rural Development, 31: 185. Available at: http://www.lrrd.org/lrrd31/12/yunin31185.html

Wahjuningsih S, Ihsan M, Siswoyo DA, Fatmila DT, and Firmawati A (2021). Extract of cincau (*Mesona palustris* B.) supplementation in semen extender improves boer goat sperm cryopreservation. Journal of Advanced Veterinary Research, 11(4): 247-253. Available at: https://advetresearch.com/index.php/AVR/article/view/762

Wang Y, Fu X, and Li H (2025). Mechanisms of oxidative stress-induced sperm dysfunction. Frontiers in Endocrinology, 16: 1520835. DOI: https://www.doi.org/10.3389/fendo.2025.1520835

Xu B, Bai X, Zhang J, Li B, Zhang Y, Su R, Wang R, Wang Z, Lv Q, Zhang J et al. (2023). Metabolomic analysis of seminal plasma to identify goat semen freezability markers. Frontiers in Veterinary Science, 10: 1132373. DOI: https://www.doi.org/10.3389/fvets.2023.1132373

Zang S, Zou S, Chen X, Pan B, Ning A, Qin J, Wei Y, Du K, Ye J, Liang Q et al. (2025). Abnormalities in mitochondrial energy metabolism induced by cryopreservation negatively affect goat sperm motility. Frontiers in Veterinary Science, 11: 1514362. DOI: https://www.doi.org/10.3389/fvets.2024.1514362

Zeljić Stojiljković K, Mićić N, Bogdanović V, Đedović R, Pihler I, Stojiljković N, and Stanojević D (2025). Genetic evaluation of milk production traits in the Serbian Saanen goat population. Animals, 15(20): 3008. DOI: https://www.doi.org/10.3390/ani15203008

Zhang X, Hu ZT, Li Y, Li YX, Xian M, Guo SM, and Hu JH (2022). Effect of astragalus polysaccharides on the cryopreservation of goat semen. Theriogenology, 193: 47-57. DOI: https://www.doi.org/10.1016/j.theriogenology.2022.08.007

Zou J, Wei L, Li D, Zhang Y, Wang G, Zhang L, Cao P, Yang S, and Li G (2021). Effect of glutathione on sperm quality in Guanzhong dairy goat sperm during cryopreservation. Frontiers in Veterinary Science, 8: 771440. DOI: https://www.doi.org/10.3389/fvets.2021.771440