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Effect of Arabica Coffee Supplementation in a Skim Milk–Egg Yolk Extender on Plasma Membrane Integrity, Motility, Viability, and Abnormality of Boer Goat Spermatozoa

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Abstract. *This study evaluated the effect of Arabica coffee extract supplementation in a skim milk–egg yolk extender on sperm motility, viability, abnormality, and plasma membrane integrity in Boer goat semen. Semen was collected once weekly using an artificial vagina. A randomized block design with five treatments and four replicates was used: skim milk–egg yolk extender without coffee extract (P0) and extender supplemented with 0.2 (P1), 0.4 (P2), 0.6 (P3), or 0.8 mL (P4) of Arabica coffee extract. Semen was evaluated macroscopically for pH, volume, color, odor, and consistency and microscopically for motility, viability, abnormality, and plasma membrane integrity. Data were analyzed by analysis of variance using SPSS, followed by Duncan's multiple range test. Fresh semen had a mean pH of 6.45 ± 0.55 and a volume of 1.175 ± 0.204 mL; it was yellowish white, had a characteristic odor, and exhibited a thick consistency. Arabica coffee extract supplementation significantly affected all microscopic parameters ($p < 0.001$). The 0.6 mL treatment (P3) produced the highest motility (74.50 ± 5.26%), viability (87.25 ± 6.34%), and plasma membrane integrity (90.00 ± 3.46%) and the lowest abnormality (17.00 ± 8.28%). These findings indicate that supplementation with 0.6 mL of Arabica coffee extract may improve the quality of Boer goat semen extended with skim milk–egg yolk.*

Keyword: *Arabica coffee, Boer goat, Semen, Semen quality, Spermatozoa*

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INTRODUCTION

The Boer goat is a meat-type breed recognized for its exceptionally large body size. Under favorable management conditions in Australia, Boer bucks can reach 151 kg by two years of age. Further development of the breed requires particular attention to genetic improvement. Boer goats also exhibit favorable reproductive performance, with twin and triplet birth rates of approximately 50% and 7%, respectively. Artificial insemination (AI) is a modern reproductive technology that supports genetic improvement and requires appropriate semen collection, dilution, and storage procedures (Hardyastuti et al., 2023).

A skim milk–egg yolk extender can help maintain sperm quality by providing energy and nutrients and by protecting spermatozoa from cold shock during semen processing (Laos et al., 2021). Egg yolk contains lipoproteins and lecithin that contribute to membrane protection. When semen is stored at very low temperatures for preservation or freezing, spermatozoa may experience cold shock, which can damage or kill the cells (Dzulqarnain et al., 2022).

Coffee was included as a source of biologically active compounds, particularly caffeine, to support sperm survival. Arabica coffee generally contains less caffeine (approximately 0.8–1.4%) than Robusta coffee, which may contain up to 2.5%. Arabica coffee was therefore selected based on its comparatively lower caffeine concentration. Differences in caffeine content influence not only coffee flavor and aroma but also its biological effects (Suaniti et al., 2022). Because sperm mortality can increase before freezing, the present study evaluated different volumes of Arabica coffee extract in a skim milk–egg yolk extender. The skim milk-egg yolk combination serves as both a source of nutrients for spermatozoa and a buffer; meanwhile, the egg yolk contains lecithin and lipoproteins that act as cryoprotectants, thereby protecting sperm membranes from damage caused by cooling (Iskandari et al., 2020). The extender was expected to provide energy and nutrients, while the caffeine-containing coffee extract was evaluated for its ability to support sperm survival and resistance to cold shock before freezing (Aisah et all. 2017).

LITERATURE REVIEW

Boer Goat Semen Preservation

Artificial insemination (AI) has become one of the most widely adopted reproductive biotechnologies for accelerating genetic improvement, increasing

reproductive efficiency, and facilitating the dissemination of superior genetics in goat production systems. The success of AI programs is highly dependent on the quality of semen used during insemination. Semen quality is determined not only by the physiological condition of spermatozoa at ejaculation but also by their ability to maintain structural and functional integrity during dilution, storage, and preservation (Tanga et al., 2021; Leahy & Gadella, 2011)).

Goat spermatozoa are particularly vulnerable to oxidative stress because their plasma membrane contains a high proportion of polyunsaturated fatty acids (PUFAs). During semen processing and storage, excessive production of reactive oxygen species (ROS) induces lipid peroxidation, resulting in decreased membrane fluidity, mitochondrial dysfunction, DNA fragmentation, and impaired fertilizing ability (Adiputra et al., 2023). In addition, temperature fluctuations during cooling may induce cold shock, a phenomenon characterized by irreversible damage to the plasma membrane, acrosome, and mitochondrial membrane, ultimately reducing sperm motility, viability, and fertilization capacity (Grötter et al., 2019).

Arabica Coffee as a Source of Bioactive Compounds

Arabica coffee (Coffea arabica L.) is one of the world's most economically important agricultural commodities and contains numerous bioactive compounds with potential physiological functions. The principal constituents include caffeine, chlorogenic acids, caffeic acid, diterpenes, trigonelline, flavonoids, and various phenolic compounds that exhibit antioxidant activity (Makiso et al., 2024). These compounds are capable of neutralizing free radicals and reducing oxidative stress, making Arabica coffee a promising natural source of antioxidants for biological applications.

Among these compounds, caffeine has attracted considerable attention because of its physiological effects on sperm function. Caffeine inhibits phosphodiesterase activity, resulting in increased intracellular cyclic adenosine monophosphate (cAMP) concentrations. Elevated cAMP stimulates protein kinase A activation and enhances ATP synthesis within sperm mitochondria, thereby promoting flagellar movement and improving sperm motility (Alonso et al., 2017). Increased ATP availability also supports membrane transport, ion homeostasis, and metabolic activities necessary for sperm survival during storage.

Previous Studies on Natural Antioxidants in Semen Extenders

In recent years, increasing attention has been directed toward the incorporation of natural antioxidants into semen extenders to minimize oxidative damage during storage. Plant-derived antioxidants are considered safer and more environmentally friendly than synthetic compounds because they contain multiple bioactive constituents that function synergistically to neutralize free radicals. Several studies have demonstrated beneficial effects of botanical supplements on goat semen quality. Roselle (Hibiscus sabdariffa) filtrate improved sperm motility, viability, and plasma membrane integrity because of its high anthocyanin and phenolic contents (Kasim et al., 2021). Carrot extract supplementation significantly enhanced sperm quality by providing carotenoids and β-carotene with antioxidant properties capable of protecting sperm membranes from oxidative injury (Tvrdá et al., 2021). Similarly, soybean extract supplementation improved sperm viability during storage owing to its isoflavones and antioxidant. Coconut water has also been successfully incorporated into semen extenders because it supplies sugars, minerals, electrolytes, and natural antioxidants that support sperm metabolism and osmotic balance (Odrada et al., 2023).

RESEARCH METHODS

Preparation of Arabica Coffee Extract

Arabica coffee extract was prepared using a manual brewing method. Thirteen grams of coffee beans were ground to a medium–coarse particle size. The brewing apparatus consisted of a server, dripper, paper filter, and kettle. The coffee was brewed with a total of 250 mL of water for 2 min 30 s to obtain the extract.

Preparation of the Skim Milk–Egg Yolk Extender

Ten grams of skim milk powder were placed in a beaker, and 100 mL of distilled water was added. The mixture was stirred until completely dissolved. A fresh chicken egg was cleaned and cracked, and the yolk was carefully separated from the albumen. Residual albumen was removed, leaving the intact yolk enclosed by the vitelline membrane. After its volume was measured, egg yolk was added to the skim milk solution at 5% (v/v), equivalent to 5 mL per 100 mL of skim milk solution, and mixed thoroughly. The designated amount of Arabica coffee extract was then incorporated, and the extender was stirred until homogeneous.

Semen Collection

Semen was collected in the morning. The artificial vagina was prepared with warm water at approximately 37 °C and lubricated with petroleum jelly. The penis was manually guided into the artificial vagina, which was fitted with a collection tube. The ejaculate was collected directly into the tube and immediately subjected to macroscopic evaluation (Aji et al., 2023).

Fresh Semen Evaluation

Fresh semen collected using the artificial vagina was examined macroscopically and microscopically. Macroscopic evaluation included volume, color, odor, pH, and consistency. Microscopic evaluation included sperm concentration, progressive motility, viability, abnormal morphology, and plasma membrane integrity. Semen volume was measured directly using a graduated collection tube.

Sperm Motility Assessment

Progressive motility was evaluated based on the direction and speed of sperm movement. Only spermatozoa moving actively in a forward direction were classified as progressively motile because progressive movement reflects the ability of spermatozoa to reach the oocyte.

Motility (%) = (Number of progressively motile spermatozoa / Total number of spermatozoa observed) × 100

Sperm Viability Assessment

Sperm viability was determined by comparing live and dead spermatozoa after eosin–nigrosin staining. Spermatozoa with intact plasma membranes excluded the stain and remained colorless, whereas dead spermatozoa with damaged membranes absorbed eosin and appeared reddish to purplish.

Viability (%) = (Number of unstained spermatozoa / Total number of spermatozoa observed) × 100

Assessment of Sperm Abnormalities

Sperm abnormalities were assessed based on morphological defects in the head, midpiece, or tail. Primary abnormalities originate during spermatogenesis, whereas secondary abnormalities may arise after ejaculation because of semen handling, environmental changes, or osmotic stress. Abnormal structures may impair movement or

reduce the ability of spermatozoa to penetrate the oocyte, thereby lowering fertilization potential.

Abnormality (%) = (Number of abnormal spermatozoa / Total number of spermatozoa counted) × 100

Plasma Membrane Integrity Assessment

Plasma membrane integrity was assessed using the hypo-osmotic swelling (HOS) test. Ten microliters of semen were mixed with 1 mL of HOS solution, homogenized, and incubated at 37 °C for 30–45 min. A drop of the incubated mixture was placed on a glass slide, covered with a coverslip, and evaluated under a light microscope at 400× magnification. Spermatozoa with intact plasma membranes were identified by curled or swollen tails, whereas cells with damaged membranes had straight tails. The percentage of spermatozoa reacting to the HOS solution was calculated as follows:

Plasma membrane integrity (%) = (Number of HOS-reactive spermatozoa / Total number of reactive and nonreactive spermatozoa) × 100

Treatments

The study consisted of five treatments with four replicates:

P0 Semen + skim milk–egg yolk extender

P1 Semen + skim milk–egg yolk extender + 0.2 mL Arabica coffee extract

P2 Semen + skim milk–egg yolk extender + 0.4 mL Arabica coffee extract

P3 Semen + skim milk–egg yolk extender + 0.6 mL Arabica coffee extract

P4 Semen + skim milk–egg yolk extender + 0.8 mL Arabica coffee extract

Time and Location

The study was conducted from October to November 2025. Boer goat semen was collected at the Maju Berkah Mapan Farmers Group farm, Jl. Usaha Tani, Jl. Bungur No. 3, RT 1, Lempake, North Samarinda District, Samarinda, East Kalimantan 75118. Semen quality was evaluated at the Laboratory of Animal Reproduction and Breeding, Faculty of Agriculture, Mulawarman University.

Materials and Equipment

The materials used were 13 g of Arabica coffee, chicken eggs, skim milk powder, HOS solution for plasma membrane integrity assessment, eosin–nigrosin stain for viability assessment, distilled water, and petroleum jelly as a lubricant for the artificial

vagina. The equipment included an artificial vagina, a light microscope, glass slides, coverslips, micropipettes, test tubes, an ice box, and tissue paper.

Research Procedure

The study began with direct observation to select a sexually mature buck based on age and dentition. Semen was then collected using an artificial vagina and transferred to a cooled container to minimize sperm mortality. Arabica coffee extract was prepared and incorporated into the skim milk–egg yolk extender according to the treatment. After all materials had been prepared, semen samples were mixed with the respective extenders and evaluated for motility, viability, abnormalities, and plasma membrane integrity.

Statistical Analysis

The experiment was arranged in a randomized block design with five treatments and four replicates. Data were analyzed by analysis of variance (ANOVA) using SPSS (Statistical Product and Service Solutions). When treatment effects were significant, Duncan's multiple range test was used for mean separation.

RESULTS AND DISCUSSION

Macroscopic Evaluation

Macroscopic evaluation of fresh Boer goat semen included pH, volume, color, odor, and consistency. The results are presented in Table 1.

Table 1. Macroscopic characteristics of fresh Boer goat semen

Parameter	Mean ± SD / Description
pH	6.45 ± 0.55
Volume (mL)	1.175 ± 0.204
Color	Yellowish white
Odor	Characteristic
Consistency	Thick

Semen pH

The mean pH of fresh Boer goat semen was 6.45 ± 0.55. This value was slightly lower than those reported by Saanen Di Balai et al. (2021), who recorded mean pH values of 6.63 ± 0.075, 6.65 ± 0.059, and 6.70 ± 0.07 in September, October, and November, respectively, for fresh semen from Etawah Crossbred, Boer, and Saanen goats. Although a slight increase occurred in November, all values remained within the normal range. The present value was also within the generally accepted pH range of 5.9–7.3 for goat and sheep semen (Sirat et al., 2025).

Macroscopic evaluations of fresh semen from Etawah Crossbred, Boer, and Saanen bucks at the Lembang Artificial Insemination Center have similarly shown relatively stable and normal pH values. These values are regarded as normal because they remain within the general pH range of 5.9–7.3 for goat and sheep semen (Fahlevi et al., 2025).

Semen Volume

The mean semen volume in this study was 1.175 ± 0.204 mL. In comparison, previously reported monthly mean volumes were 2.30 ± 0.54 mL in September, 2.64 ± 0.43 mL in October, and 2.60 ± 0.53 mL in November. Differences in semen volume and pH may be influenced by breed, body weight, nutrient intake—particularly energy and protein—and semen collection management. Despite the variation, the macroscopic characteristics indicated that the fresh goat semen was of acceptable quality for artificial insemination (Sutriana et al., 2020).

Semen Color

The semen collected in this study was yellowish white. Ahmad Zaenuri et al. (2021) similarly reported yellowish-white semen in Boer, Etawah Crossbred, and Kacang goats. This color is considered normal for goat semen and generally reflects normal physiological conditions, including appropriate consistency and sperm concentration. The similarity among breeds indicates that color alone may not differentiate initial semen quality when all samples are within the normal range (Stefanus et al., 2021).

Semen Odor

Fresh Boer goat semen had a characteristic odor. Odor is an important macroscopic parameter in semen quality evaluation. Semen from Etawah Crossbred, Boer, and Saanen goats at the Lembang Artificial Insemination Center was reported to have the characteristic slightly fishy odor typical of goat semen (Balai et al., 2021). The odor observed in the present study therefore supported the assessment that the semen was normal and suitable for further processing.

Semen Consistency

The semen had a thick consistency. Semen consistency is an important macroscopic indicator of fresh semen quality because it is positively associated with sperm concentration. Normal Boer goat semen is generally thick and yellowish white. A thick consistency indicates a high sperm concentration and is desirable for breeding or artificial insemination programs. However, consistency may become moderate or watery when

bucks ejaculate too frequently, thereby reducing the recovery period required for sperm production (Balai et al., 2021).

Microscopic Evaluation

Microscopic evaluation included sperm motility, viability, abnormalities, and plasma membrane integrity. The results are presented in Table 2.

Table 2. Microscopic characteristics of Boer goat semen following supplementation with Arabica coffee extract

Treatment	Motility (%)	Viability (%)	Abnormality (%)	Plasma membrane integrity (%)
P0	59.75 ± 4.57	67.00 ± 12.62	29.25 ± 7.58	74.75 ± 5.50
P1	64.25 ± 6.18	69.25 ± 12.20	26.75 ± 8.88	77.50 ± 6.45
P2	65.00 ± 6.18	72.00 ± 15.25	25.00 ± 8.52	80.00 ± 6.27
P3	74.50 ± 5.26	87.25 ± 6.34	17.00 ± 8.28	90.00 ± 3.46
P4	69.25 ± 7.36	78.00 ± 12.19	23.50 ± 13.37	83.75 ± 6.23
p-value	<0.001	<0.001	<0.001	<0.001

Values are presented as mean ± standard deviation (n = 4).

Sperm Motility

Mean sperm motility ranged from 59.75% to 74.50%. The highest value was observed in P3 (74.50 ± 5.26%), whereas the lowest was recorded in P0 (59.75 ± 4.57%). The treatment effect was statistically significant ($p < 0.001$). The higher motility in P3 indicates that supplementation with 0.6 mL of Arabica coffee extract in the skim milk–egg yolk extender was more effective than the control treatment in maintaining progressive sperm movement. This value was lower than the mean motility of 78.0 ± 2.5% reported by Anwar et al. (2019). The difference may have been associated with the distance between the semen collection site and the laboratory, which could have allowed semen quality to decline during transportation. Progressive motility is a critical indicator of semen quality because it reflects the ability of spermatozoa to move forward and reach the oocyte for fertilization (Zaenuri et al., n.d.).

Sperm Viability

Mean sperm viability ranged from 67.00% to 87.25%. The highest value was observed in P3 (87.25 ± 6.34%), whereas the lowest was recorded in P0 (67.00 ± 12.62%). The treatment effect was statistically significant ($p < 0.001$), indicating that supplementation with 0.6 mL of Arabica coffee extract in the skim milk–egg yolk extender improved viability relative to the control (Kudratullah, & Sudrajat, A. 2021). Sperm viability is an important parameter for semen evaluation because it indicates the proportion of living spermatozoa with functional cellular structures. Anwar (2019) stated

that viability is commonly assessed using eosin–nigrosin staining. This method is based on differences in membrane permeability between live and dead cells. Dead spermatozoa have damaged plasma membranes that allow eosin to enter and stain the head red, whereas live spermatozoa have intact plasma membranes that exclude the dye and therefore remain transparent or unstained (Masyitoh et al., 2018).

Sperm Abnormalities

Mean sperm abnormality ranged from 17.00% to 29.25%. The lowest value was observed in P3 (17.00 ± 8.28%), whereas the highest was recorded in P0 (29.25 ± 7.58%). The treatment effect was statistically significant ($p < 0.001$). Sperm abnormality is defined as the percentage of sperm cells with abnormal morphology, particularly in the head, midpiece, or tail, relative to the total number of spermatozoa evaluated. Abnormality is an important criterion for determining whether semen is suitable for artificial insemination, for which the proportion of abnormal spermatozoa is generally expected not to exceed 20%. The abnormality value in P3 was 17.00 ± 8.28%, which was below this threshold.

Environmental conditions may contribute to sperm abnormalities. Relevant factors include ambient temperature, scrotal temperature, and the temperature inside the artificial vagina during collection. Extreme temperature changes can affect ejaculate quality, while high temperature and low humidity may induce heat stress and increase sperm abnormalities (Zakaria et al., 2020). Barek et al. (2020) reported a low initial abnormality value of 3.45 ± 0.40% in fresh goat semen.

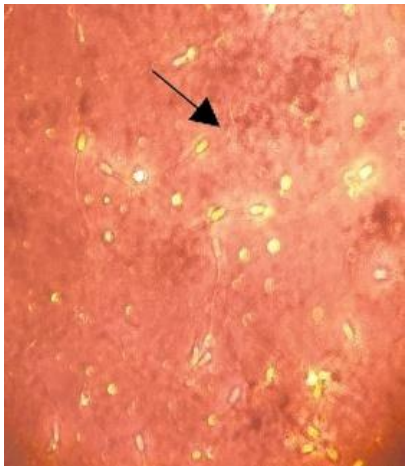
Plasma Membrane Integrity

Mean plasma membrane integrity ranged from 74.75% to 90.00%. The highest value was observed in P3 (90.00 ± 3.46%), whereas the lowest was recorded in P0 (74.75 ± 5.50%). The treatment effect was statistically significant ($p < 0.001$), demonstrating a difference between the control extender and the extender supplemented with 0.6 mL of Arabica coffee extract. The P3 treatment produced the highest plasma membrane integrity and therefore appears to be a potentially effective alternative extender formulation.

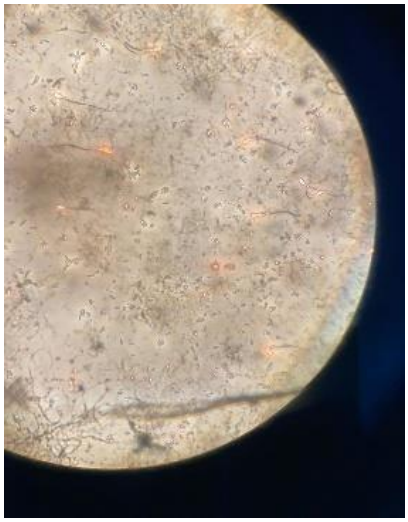
Plasma membrane integrity is a crucial indicator because it is closely related to sperm viability and resistance to damage during chilled storage. Plasma membrane damage, which is commonly caused by cold shock during cooling, can markedly reduce sperm viability (Sholikah et al., 2022). Therefore, supplementation with 0.6 mL of

Arabica coffee extract produced the strongest effect in maintaining plasma membrane integrity in this study.

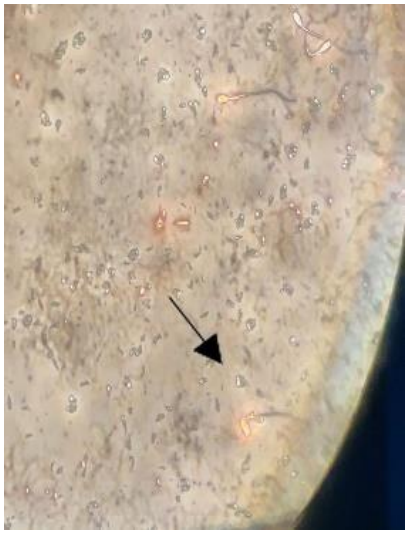
Sperm motility



Sperm viability



Plasma membrane integrity



Sperm abnormalities

Figure 1. Microscopic evaluation of Boer goat spermatozoa

CONCLUSION

Arabica coffee extract supplementation significantly influenced sperm motility, viability, abnormalities, and plasma membrane integrity. Among the treatments, supplementation with 0.6 mL of Arabica coffee extract in the skim milk–egg yolk extender produced the highest motility, viability, and plasma membrane integrity and the lowest abnormality. Therefore, this formulation may serve as an alternative extender for Boer goat semen and has potential for application in artificial insemination programs

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