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Original article

Potential of Basil (*Ocimum basilicum*) leaf extract in Tris-egg yolk extender on sperm motility and plasma membrane integrity of Sapudi ram at room temperature

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ABSTRACT

This study aimed to determine the effect of basil (*Ocimum basilicum*) leaf extract in a Tris-egg yolk extender on the motility, and plasma membrane integrity of Sapudi ram spermatozoa at room temperature. Fresh semen samples were collected from Sapudi ram using an artificial vagina. A completely randomized design was used, consisting of four treatment groups with five replicates each. Data were analyzed using SPPS with factorial analysis of variance (ANOVA) and Duncan's test. The control group (T0) received Tris-egg yolk extender without basil leaf extract. Treatment groups T1, T2, and T3 received 1%, 2%, and 3% basil leaf extract, respectively, in the extender. Results showed that sperm motility and plasma membrane integrity (Table 4) tended to decrease with increased storage time. Among the treatment groups, the addition of 1% basil leaf extract in the Tris-egg yolk extender (T1) resulted in significantly higher ($p < 0.05$) both sperm motility and plasma membrane integrity compared to the control (T0). However, higher concentrations of basil leaf extract (T2 and T3) did not result in significant differences ($p > 0.05$) in sperm motility after three hours of storage compared to the 1% treatment (T1). In conclusion, the addition of 1% basil leaf extract to a Tris-egg yolk extender was effective in maintaining sperm motility and plasma membrane integrity of Sapudi ram spermatozoa stored at room temperature for up to three hours.

Keywords: basil leaf extract, plasma membrane integrity, Sapudi ram, sperm motility, Tris-egg yolk extender

INTRODUCTION

Sapudi sheep are a native Indonesian breed classified under the Fat-Tailed Sheep category, primarily found in East Java, particularly on Sapudi Island (Ibrahim *et al*., 2023). This breed has considerable potential for meat production due to its high adaptability and relatively strong resistance to diseases (Sodiq and Tawfik, 2004). Data from local farmers and recent studies indicate that Sapudi sheep exhibit a growth rate of approximately 0.2-0.3 kg/day, which is comparable to or higher than that of other indigenous breeds. Moreover, the reproductive efficiency of Sapudi sheep is noteworthy, with a natural kidding rate of 1.8-2.0 lambs per year, an

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age at first mating between 8-12 months, and a kidding interval of around 8-10 months (Muarifah *et al*., 2019). Given these attributes, Sapudi sheep hold substantial potential to contribute to local food security and rural livelihoods. However, without immediate conservation and structured breeding programs, this valuable genetic resource is at risk of irreversible loss, which would undermine efforts toward sustainable livestock development and food self-sufficiency.

Reproductive management in sheep can be achieved through natural mating or artificial insemination (AI). Artificial insemination is a key reproductive technology that plays a crucial role in enhancing genetic quality and productivity (Halaweh *et al*., 2025). In Indonesia, sheep husbandry remains largely traditional, and the application of AI is still limited and mostly experimental. Nonetheless, AI is expected to become an integral part of future national sheep breeding strategies (Sujarwanta *et al*., 2024). The main advantage of AI lies in its ability to improve production efficiency, allowing a single high-quality sire to produce thousands of offspring annually (Gibbons *et al*., 2019).

Semen dilution is essential for maintaining semen quality for multiple inseminations and increasing the volume of usable semen. Commonly used extenders include Tris-egg yolk and skimmed milk-egg yolk, as both provide essential nutrients, protect sperm membranes from cold shock, and act as buffers (Madrigali *et al*., 2021). Egg yolk contains lipoproteins and phospholipids that help stabilize sperm membranes and prevent damage during cryopreservation (Chang *et al*., 2025). Among extenders, Tris-egg yolk has proven most effective in preserving sperm motility, viability, and membrane integrity at 5°C (Maia *et al*., 2009).

Numerous studies have sought to improve both the quality and quantity of liquid semen during storage, including through the supplementation of extenders with antioxidants (Madrigali *et al*., 2021). Basil (*Ocimum basilicum*) leaves are known to contain various bioactive metabolites such as flavonoids, terpenoids, essential oils, phytosterols, and lignin (Tateishi *et al*., 2024). Flavonoids like scutellarin and apigenin can scavenge free radicals and act as potent antioxidants (Zahra *et al*., 2024). Antioxidants help improve ram sperm quality by preventing cell membrane damage (Rather *et al*., 2016). Sperm motility is a critical indicator of semen quality and fertility potential (Van de Hoek *et al*., 2022). The sperm plasma membrane functions as a selective barrier regulating the exchange between the intracellular and extracellular environments. Its integrity is vital for sperm viability and fertilizing ability (Tapia *et al*., 2012).

Therefore, this study aimed to explore the potential of basil leaf extract as an additive in Tris-egg yolk extender to improve motility and plasma membrane integrity of Sapudi ram semen stored at room temperature (27-28°C).

MATERIALS AND METHODS

This study used semen from 2-year-old Sapudi ram owned by the Faculty of Veterinary Medicine, Universitas Airlangga. The research was conducted at the Artificial Insemination Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, in October 2024. The research protocol was approved by the Research Ethics Commission of Universitas Airlangga (Certificate No. 0536/HRECC.FODM/V/2024).

Basil leaf extraction

One hundred grams of dried basil leaf powder was extracted through a maceration process using 600 mL of 96% ethanol. After soaking, the solution was filtered using filter paper to obtain the filtrate, which was then evaporated using a rotary evaporator to remove the solvent and produce a concentrated extract (A'yuni *et al*., 2023).

Semen collection techniques

Prior to semen collection, the ram was bathed and its prepuce cleaned. Semen was collected using an artificial vagina and a teaser

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ewe. False mountings were performed 2–3 times to increase libido (Bahadi *et al*., 2023). The artificial vagina was prepared to body temperature, with the tip lubricated with vaseline and used at a 45° angle. A test tube wrapped in dark material was attached to prevent semen exposure to direct light. The collected semen was evaluated both macroscopically and microscopically. Ejaculates with a sperm motility of ≥70% were processed for semen dilution (Triyaningrum *et al*., 2024). Semen collection was performed twice a week, with five ejaculates used as replicates.

Fresh semen evaluation

The collected semen was evaluated macroscopically and microscopically. Macroscopic evaluation included assessment of colour, volume (measured directly from the tube scale), pH (using pH indicator paper), and consistency (categorized as thin, medium, or thick). Microscopic evaluation included assessment of sperm concentration, motility, viability, abnormality, and mass movement (Quraini *et al*., 2022).

Semen extender preparation

All chemicals used in the extender formulation were sourced from Merck Millipore (Merck KGaA, Darmstadt, Germany). The stock solution was prepared by mixing 1.363 g of tris amino methane, 0.762 g of citric acid, 1.5 g of lactose, 0.5 g of fructose, 2.7 g of raffinose, and 80 mL of distilled water.

This mixture was homogenized using a magnetic stirrer for 10-15 minutes, heated in a water bath at 100°C, then cooled to 37°C. Streptomycin (0.1 g) and penicillin (0.1 g) were added, followed by further homogenization for 10-15 minutes. The solution was refrigerated, and after three days, the sediment was discarded, leaving the supernatant as the stock solution. The Tris-egg yolk extender was prepared by mixing 80 mL of the stock solution with 20 mL of egg yolk (Sari *et al*., 2024).

In this study, the T0 group used the Tris-egg yolk extender without any basil leaf extract. Treatment groups T1, T2, and T3 used the same extender supplemented with 1%, 2%, and 3% (w/v) basil leaf extract, respectively. Five qualified ejaculates were used as replicates. Each fresh semen sample was divided into four equal parts and diluted with the corresponding extender at a ratio of 1 : 10 (semen : extender).

Motility evaluation

Sperm motility was assessed by placing one drop of diluted semen on a glass slide, adding one drop of physiological NaCl solution, and covering it with a cover slip. Observations were made under a microscope at 400× magnification for individual motility and 100× magnification for mass motility (Miranda *et al*., 2023).

Plasma membrane integrity evaluation

Plasma membrane integrity was evaluated using the hypoosmotic swelling test (HOS test) with a 150 mOsm/L fructose-sodium citrate solution. Semen was mixed with the hypotonic solution and left to stand for 30 minutes. The mixture was then combined with eosin-nigrosin stain, homogenized on a glass slide, and fixed. Observations were performed under a microscope at 400× magnification to assess plasma membrane integrity (Miranda *et al*., 2023).

Data Analysis

Data were presented in tabular form and analyzed using factorial analysis of variance (ANOVA). When significant differences were observed, Duncan's multiple range test was used for post hoc analysis at a 5% significance level. All analyses were performed using SPSS version 26 for Windows.

RESULTS

Semen used in this study was required to meet acceptable quality standards. The quality of fresh Sapudi ram semen was evaluated both macroscopically (Table 1) and microscopically (Table 2). Sperm motility (Table 3) and plasma membrane integrity (Table 4) tended to decrease with increasing storage time at room temperature. Based on treatment groups, the

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addition of 1% basil leaf extract in the Tris-egg yolk extender (T1) resulted in significantly higher ($p < 0.05$) motility and plasma membrane integrity compared to the control group (T0), which contained no basil extract. The use of higher concentrations of basil leaf extract (2% and 3%) in groups T2 and T3 did not show significant differences ($p > 0.05$) compared to the 1% group (T1) in terms of sperm motility after three hours of storage at room temperature. However, the integrity of the sperm plasma membrane showed variable percentages among the groups.

Table 1 Macroscopic examination of fresh Sapudi ram semen

volume (mL)	0.92 ± 0.13
consistency	thick
color	creamy white
pH	6-7

Table 2 Microscopic examination of fresh Sapudi ram semen

concentration (million/mL)	1968 ± 50.20
motility (%)	84 ± 2.24
viability (%)	93.86 ± 0.61
abnormality (%)	6.002 ± 1.59
sperm mass movement	+++

+++ = very good, characterized by strong wave motion, thick grey clouds, and active movement

Table 3 Sperm motility of Sapudi rams extended with basil leaf extract during room temperature storage

	0 hour	1st hour	2nd hour	3rd hour
T0	72.0 ± 2.73 ^{fg}	59.0 ± 4.18 ^{cd}	49.0 ± 4.18 ^{b}	39.0 ± 5.47 ^{a}
T1	77.0 ± 2.73 ^{g}	68.0 ± 6.70 ^{ef}	59.0 ± 6.51 ^{cd}	49.0 ± 6.51 ^{b}
T2	76.0 ± 2.23 ^{g}	65.0 ± 3.53 ^{de}	58.0 ± 5.70 ^{c}	48.0 ± 5.70 ^{b}
T3	76.0 ± 2.23 ^{g}	68.0 ± 4.47 ^{ef}	58.0 ± 5.70 ^{c}	46.0 ± 6.51 ^{b}

Different superscripts in the same column indicate significant differences ($p < 0.05$); T0: semen extended with Tris-egg yolk extender; T1, T2, and T3: semen extended with Tris-egg yolk extender + 1%, 2%, and 3% (w/v) basil leaf extract, respectively.

Table 4 Intact plasma membrane of Sapudi ram spermatozoa extended with basil leaf extract during room temperature storage

	0 hour	1st hour	2nd hour	3rd hour
T0	55.88 ± 1.29 ^{gh}	51.86 ± 1.18 ^{ef}	48.84 ± 1.49 ^{cde}	45.64 ± 1.53 ^{ab}
T1	60.06 ± 1.90 ^{i}	55.22 ± 1.34 ^{fg}	52.14 ± 1.82 ^{ef}	47.18 ± 1.55 ^{bcd}
T2	54.08 ± 3.13 ^{fg}	49.72 ± 3.10 ^{cde}	46.06 ± 3.85 ^{ab}	42.92 ± 4.12 ^{a}
T3	58.60 ± 2.98 ^{hi}	53.40 ± 3.24 ^{fg}	49.82 ± 2.48 ^{de}	46.24 ± 3.15 ^{abc}

Different superscripts in the same column indicate significant differences ($p < 0.05$); T0: semen extended with Tris-egg yolk extender; T1, T2, and T3: semen extended with Tris-egg yolk extender + 1%, 2%, and 3% (w/v) basil leaf extract, respectively.

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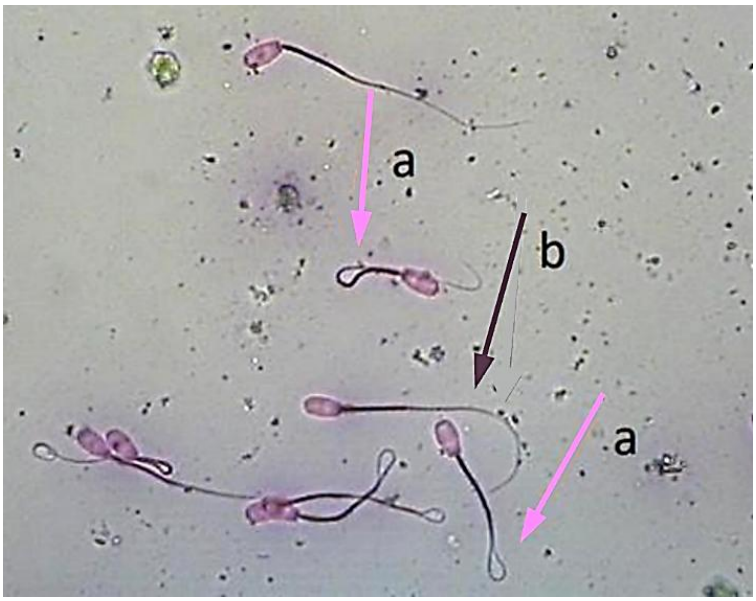

Figure 1 Hypoosmotic swelling test of Sapudi ram spermatozoa. (a) spermatozoa with intact plasma membranes (tails curled); (b) spermatozoon with a damaged plasma membrane (tail straightened).

DISCUSSION

The macroscopic and microscopic parameters of fresh Sapudi ram semen in this study were suitable for artificial insemination. All measured values fell within the ranges reported by Miranda *et al*. (2023) and Sigit *et al* (2024). Fresh semen used for artificial insemination should have a motility greater than 70% (Purdy *et al*., 2010). In this study, ejaculates were subjected to treatment groups and evaluated for quality based on sperm motility and plasma membrane integrity after 0, 1, 2, and 3 hours of storage at room temperature (27–28°C).

The decline in sperm motility during storage is likely due to a reduction in seminal plasma concentration. Spermatozoa require a period of adaptation to their new environment following dilution (Rajabi-Toustani *et al*., 2021). Decreased motility is caused by disturbances in membrane permeability, reduced metabolic activity, and membrane damage, which collectively impair sperm movement. This decline is primarily due to diminished energy reserves within the spermatozoa, which are critical for maintaining viability and motility (Van de Hoek *et al*., 2022).

This study indicates that sperm motility can be maintained for up to three hours with the addition of basil leaf extract to a Tris-egg yolk extender. Basil leaf extract functions as an antioxidant (Nadeem *et al*., 2022), offering protection against lipid peroxidation by neutralizing reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH) (Gulcin, 2025). These ROS may increase in association with higher motile sperm counts or spermatozoa with compromised membranes (Zhang *et al*., 2024). The antioxidant properties of basil leaf extract helped preserve sperm motility and morphology (Zaini *et al*., 2020).

Antioxidants neutralize ROS by interacting with lipids in spermatozoa, thereby preventing oxidative damage. Lipids, which are highly susceptible to oxidative attack, are protected from peroxidation and apoptosis by antioxidant binding (Zhang *et al*., 2024), thereby sustaining sperm motility and slowing its decline. Gündoğan *et al*. (2003) reported that semen can still be considered of acceptable quality if motility remains above 50%. Extended ram semen used for artificial insemination should have motility above 30% to be deemed viable for successful fertilization (Madrigalia *et al*., 2010).

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In this study, ram semen extended with Tris-egg yolk containing basil leaf extract maintained motility above 40%.

Plasma membrane integrity

Exposure of ram semen to open air post-collection and during storage can lead to oxidative stress due to ROS generation (Wang *et al*., 2025). ROS can progressively damage the sperm plasma membrane, eventually penetrate the cell and attack cytoplasmic components, leading to cell death (Juárez-Rojas *et al*., 2022). The flavonoid content in basil leaf extract functions as an antioxidant, capable of neutralizing free radicals by donating protons (Romano *et al*., 2022), thereby reducing plasma membrane damage in spermatozoa (Mishra *et al*., 2024).

The mechanism by which flavonoids act as antioxidants involves their ability to neutralize free radicals and prevent cellular damage caused by oxidative stress. Free radicals are unstable molecules due to their electron deficiency, which drives them to scavenge electrons from stable molecules—resulting in damage to DNA, proteins, and lipids (Zahra *et al*., 2024). The chemical structure of flavonoids allows them to donate electrons to free radicals, thereby halting the oxidative chain reactions (Hassanpour *et al*., 2023).

Administering antioxidants at appropriate doses can provide optimal protection against free radicals, particularly in the plasma membranes of spermatozoa. These membranes are rich in unsaturated fatty acids, making them especially vulnerable to lipid peroxidation (Kowalczyk *et al*., 2023). Antioxidants help inhibit such peroxidative reactions during semen handling, thereby preserving membrane integrity (Paul *et al*., 2017). Maintaining the integrity of the plasma membrane also protects the acrosomal vesicles, which located just beneath the sperm head membrane, from mechanical damage, ensuring the preservation of both vesicle structure and sperm motility (Liang *et al*., 2022).

CONCLUSIONS

The addition of 1% (w/v) basil (*Ocimum basilicum*) leaf extract to Tris-egg yolk extender effectively maintained the sperm motility and plasma membrane integrity in Sapudi ram spermatozoa stored at room temperature.

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AUTHOR'S CONTRIBUTIONS

Pratama Putra Banusiandana (PPB), Suherni Susilowati (SS), Emy Koestanti Sabdoningrum (EKS), Nove Hidajati (NH), Sri Mulyati (SM), Tri Wahyu Suprayogi (TWS).

PPB, SS, EKS: conceived the idea and designed the overall framework of this manuscript. PPB: conducted data acquisition, analysis and interpretation of data, and manuscript drafting. NH, SM, and TWS: critically read and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

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