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Effect of Trigona Bee Propolis Extract in Duck Egg Yolk Tris Diluent on the Quality of Boer Goat Spermatozoa at 5°C

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A B S T R A C T

This study aimed to determine the quality of Boer goat spermatozoa when stored at 5°C in a duck egg yolk-tris diluent supplemented with propolis extract. The material used was fresh semen collected from ±2-year-old Boer goats using an artificial vagina. This research employed a laboratory experimental method with a Completely Randomized Design (CRD), consisting of four propolis extract treatments: 0% (T0), 0.25% (T1), 0.5% (T2), and 1% (T3), with five replications. Observed variables included motility, viability, and abnormality. Observations were conducted daily at the same time each day. Data were analysed using Analysis of Variance (ANOVA) and subsequently tested with Duncan's multiple range test. The research results show that the best average sperm motility was found in T1, at 54.40±11.14%, followed by T0 at 41.00±12.36%, T2 at 40.40±11.76%, and T3 at 39.60±2.88%. The highest sperm viability was observed in T1, 58.40±6.06%, followed by T3, 49.20±4.65%, T2, 45.60±9.04%, and T0, 44.00±6.85%. The lowest sperm abnormality was found in T1, 4.60±1.87%, followed by T2, 6.00±1.87%, T3, 5.80±4.81%, and T0, 5.00±2.44%. In conclusion, among the four different diluents, the addition of 0.25% propolis extract in Tris-diluted egg yolk diluent showed the highest ability to maintain the quality of Boer goat spermatozoa for three days at a storage temperature of 5°C. Further studies are needed on the effects of different concentrations of propolis extract, various egg yolks, and different temperatures.

Contribution to Sustainable Development Goals (SDGs)
SDG 2 (Zero Hunger)
SDG 3 (Good Health and Well-being)
SDG 12 (Responsible Consumption and Production)

1. INTRODUCTION

The government is developing goats in Indonesia through outreach, providing superior breeding stock, and producing frozen semen in an effort to improve genetic quality through artificial insemination (AI). Artificial insemination is a reproductive technique that allows livestock breeding without the need for males, through a controlled and programmed process. Artificial insemination technology is utilised to enhance livestock populations and optimise the utilisation of semen from males with superior genetic potential, thereby producing large numbers of offspring [1].

The implementation of AI in the field is highly dependent on several factors, one of which is the quality of the semen used. A constraint in using fresh semen is its relatively short shelf life, especially at room temperature [2]. Semen quality can be improved by adding a good diluent as a protective agent and nutrient source for spermatozoa. One such diluent is Tris yellow, which has good osmotic pressure, electrolyte balance, and pH stability [3]. The combination of egg yolk, Tris, fructose, and

citric acid is highly effective in maintaining sperm viability and motility, particularly during refrigerated storage.[4].

Propolis is a bee product consisting of resin collected from plants and then mixed with saliva and enzymes to build hives [5]. Propolis has various pharmacological activities, including antibacterial, anti-inflammatory, antiviral, antifungal, antioxidant, hepatoprotective, immunoregulatory, anticariogenic, local anesthetic, anxiolytic, and antidepressant effects. These pharmacological activities originate from compounds contained in propolis, such as flavonoids, phenolics, ketones, terpenes, esters, sugars, and minerals [5]

Although egg yolk Tris is quite effective as a sperm diluent, it still has several drawbacks, including a limited free radical scavenging ability, inadequate cell membrane protection, and a low antioxidant content. Therefore, the addition of propolis extract, which has antioxidant, antimicrobial, and cell-protective properties, is necessary to increase the effectiveness of spermatozoa protection [4]. This study aimed to determine the optimal concentration of Trigona bee propolis in Tris egg yolk diluent to maintain the quality of Boer goat spermatozoa at 5°C. The benefits of this study are to provide up-to-date information on the effect of Trigona bee propolis extract in Tris egg yolk diluent on Boer goat spermatozoa quality at 5°C for use in artificial insemination.

2. MATERIALS AND METHODS

2.1. Materials

The material used in this study was semen from approximately 2-year-old Boer goats belonging to the Faculty of Animal Husbandry at the University of Mataram, West Nusa Tenggara Province, Indonesia. Semen collection was carried out twice a week using an artificial vagina.

2.2. Research Equipment and Materials

The equipment used for this study included an artificial vagina, collection tubes, Erlenmeyer flasks, test tubes, test tube racks, beakers, measuring cylinders, thermometers, incubators, analytical balances, aluminium foil, and a magnetic stirrer. In addition, this study also utilized a hemocytometer, centrifuge, hot plate, binocular microscope, monitor, counter, pH meter, micropipette, refrigerator, yellow tips, glass slides, cover glass, oven, water bath, filter paper, tissue, scissors, stationery, and a camera. The materials used in this study were propolis, duck egg yolk, 70% ethanol, alcohol, tris (hydroxymethyl) aminomethane, distilled water, fructose, citric acid, streptomycin, eosin-nigrosin, petroleum jelly, sodium citrate, and warm water.

2.3. Research Methods

This study employed a laboratory experimental method with a completely randomised design (CRD), comprising four treatments and 10 replications for each treatment. The treatments consisted of a control (P0) with standard tris egg yolk diluent (TEY), followed by P1 (TKT + 0.25% propolis extract), P2 (TKT + 0.5% propolis extract), and P3 (TKT + 1% propolis extract).

2.4. Propolis Extraction

The first step in producing propolis extract is to clean the propolis of all foreign material and then cut it into small pieces to ensure effective extraction. Second, 10 grams of propolis were placed in an Erlenmeyer flask, and 100 ml of 70% ethanol was added. Third, the mixture was homogenized using a magnetic stirrer to accelerate the dissolution of the active compounds in the propolis. After the extraction process was complete, the solution was filtered using filter paper to remove sediment and impurities such as wax, resulting in a purer propolis extract [6].

2.5. Preparation of Tris Buffer

To prepare the Tris buffer solution, weigh 3.634 g of Tris-aminomethane, 0.50 g of fructose, and 1.99 g of citric acid. Add these ingredients to 100 mL of distilled water in an Erlenmeyer flask and homogenise the mixture thoroughly. Transfer the solution to a beaker filled with water and heat it to 90°C for approximately 10 minutes, then allow it to cool slowly to 32°C. After cooling, add 0.06 g of penicillin and 0.1 g of streptomycin, and homogenize again using a magnetic stirrer for 15 minutes. Store the homogenized buffer solution in a refrigerator at 5°C until use [3].

2.6. Mixing Tris Egg Yolk with Propolis Extract

First, prepare the duck egg yolk. Sterilise the duck eggshells by soaking cotton in 70% alcohol, then dry them. Gently crack the eggshells and remove the egg white. Place the egg yolks on filter paper and tear the yolk membrane to extract the yolk. Mix the buffer solution with the egg yolk by taking 2.5% of the buffer solution (V/V) using a micropipette and replacing it with an equal amount of egg yolk. Next, add the propolis extract by removing a portion of the solution at P0, adjusting the amount of propolis extract according to the treatment. The four treatments are as follows:

PO = 97.5% Tris buffer solution + 2.5% egg yolk
P1 = 97.5% Tris buffer solution + 2.5% egg yolk + 0.25% propolis extract
P2 = 97.5% Tris buffer solution + 2.5% egg yolk + 0.5% propolis extract
P3 = 97.5% Tris buffer solution + 2.5% egg yolk + 1% propolis extract

Stir the mixed solution slowly until it becomes homogeneous.

2.7. Making Eosin Nigrosin Dye

To make Eosin Nigrosin Dye [3] weigh 2.5 grams of nigrosin, 0.7 grams of sodium citrate, and 0.5 grams of eosin using an analytical balance. Transfer all ingredients to a beaker and add 40 mL of distilled water. Mix the ingredients using a magnetic stirrer. Filter the homogenized solution through filter paper and store it until needed.

2.8. Evaluation of Fresh and Treated Semen

Macroscopic evaluations are performed after each semen collection [7]. and include assessments of volume, color, consistency, and pH. Measure the semen volume directly using the scale marked on the collection tube. Examine the semen colour in a well-lit area to ensure clarity. Check consistency by gently tilting the collection tube or by taking a small amount and feeling it with your thumb and index finger; classify the semen viscosity as thin, medium, or thick. Measure the pH using litmus paper by dipping the paper into fresh semen, observing the color change, and comparing it to a pH standard.

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2.9. Microscopic evaluation of spermatozoa

2.9.1. Mass Motility

A drop of semen is placed on a glass slide, examined under a microscope at 10x magnification, and the semen movement is observed. Mass motility will appear as a black cloud of waves moving. The criteria for assessing sperm mass motility are: excellent (+++) if the waves are fast and thick; good (++) if the waves are quite agile; poor (+) if the movements are slow and have rather thin waves; and very poor (-) if the sperm have very little or no movement. . Individual motility is observed by placing a drop of semen on a glass slide, covering it with a coverslip, and examining it under a microscope at 10x or 40x magnification. Motility is assessed subjectively and expressed as a percentage [8].

2.9.2. Viability

Viability is observed by placing a drop of semen on one-third of the edge of a glass slide, adding a drop of dye, and homogenizing it. A smear is then prepared with the other side of the glass slide at a 30-degree angle, pushing it forward to form a thin smear. The live and dead semen are then viewed under a microscope at 400x magnification, and the number of live (colorless) and dead (stain-absorbing) spermatozoa is recorded, with a total of 200 cells [9].

Formula for calculating viability:

$$X = \frac{a}{b} x\ 100\%$$

Where:
x = spermatozoa viability.
a = live spermatozoa.
b = total spermatozoa observed.
Abnormalities

Assessing spermatozoa abnormalities is performed by preparing a smear on a glass slide in the same manner as for viability testing. Observed spermatozoa abnormalities included heads detached from the body, broken tails, broken/tangled tails, large/small heads, flat heads, double heads/tails, and other abnormal shapes. Evaluation of spermatozoa abnormalities involved 200 cells observed using a light microscope at 400x magnification .[5]. Abnormal spermatozoa were counted from the 200 observed spermatozoa. The percentage of abnormality was calculated using the formula [9].

$$Y = \frac{c}{d} x\ 100\%$$

Where:
Y = spermatozoa abnormality.
c = number of abnormal spermatozoa.
d = total semen observed.

2.9.3. Semen Dilution and Storage

The total semen diluent was calculated by subtracting the total diluent from the volume of semen used or to be diluted [10].

Formula for calculating total diluent:

$$x = \frac{a\ x\ b\ x\ c}{\text{dosis IB } 125\ x\ 10^6} - a$$

Where:
x = total diluent
a = semen volume
b = progressive motility
c = concentration

The semen, evaluated for known sperm concentration and motility, was diluted and placed into test tubes as sample containers. The test tubes were labeled with paper labels according to the treatment. The samples were then placed in a refrigerator at 5°C and evaluated every 24 hours.

2.10. Observed Variables

The variables observed in this study were the percentage values of motility, viability, and abnormalities of Boer goat spermatozoa.

2.11. Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA) [11]. Results with significant differences ($P < 0.05$) were further tested using the Duncan test in SPSS 27.

3. RESULT AND DISCUSSION

3.1. Examination of Fresh Boer Goat Semen

The fresh semen data from this study are listed in Table 1. The Boer goat semen volume obtained was 0.6 ± 0.17 ml. This result is lower than the study by Ref.][5], which found that Boer goat semen volume was in the range of 0.70 ± 1.50 ml per ejaculation. However, the semen volume from this study was considered normal according to Ref. [8].

The semen color obtained in this study was cream. The consistency and concentration of sperm can influence the colour of semen; the thicker the consistency and the higher the concentration, the darker the colour of the semen.

The aroma of goat semen is also distinctive, depending on the animal being collected, indicating that the semen is in normal condition. This is consistent with the opinion of [12], who stated that normal semen generally has a distinctive odor, accompanied by the odor of the animal.

Consistency or viscosity is a semen property related to sperm concentration [13]. The consistency or viscosity of Boer goat semen, according to this study, was moderate, indicating normal semen consistency. Ref. [14] found that consistency is positively correlated with sperm concentration, with ratings ranging from "thin" to "medium".

The average acidity or pH of fresh Boer goat semen obtained from this study was 6.6 ± 0.55, which is within the normal range. This result is consistent with the study by Ref. [15], which found a pH of 6.5 ± 0.1. Semen pH can be influenced by factors such as goat breed, feed, environment, and the physiological condition of the bull during semen collection. The results showed that semen pH was normal, slightly acidic, and supported sperm viability and motility.

Tabel 1. Macroscopic and Microscopic Examination Results of Fresh Boer Goat Semen

Assessment parameters	Assessment results
Volume (ml)	0.6±0.17
Color	Cream
Aroma	Typical cement
pH	6.6±0.55
Consistency	Medium
Mass motility	+++ (good)
Individualmotility (%)	82±2.74
Concentration (10^9/ml)	1.780±0.11
Viability (%)	87±2.74
Abnormality	5.6±2.70

Source. Research results data (2025)

Table 1 also presents the mass motility obtained from this study, which was very good (+++), characterised by a large number of thick, dark, and small to large waves visible. Mass motility reflects the quality of individual motility, at 82 ± 2.74%. This result is higher than the results reported by Ref. [16], which ranged from 75% to 80% in Boer, PE, and Sanen goats. Furthermore, the individual sperm motility in this study exceeded the Indonesian National Standard (SNI 4869-3, 2023), which requires a minimum of 70%. Therefore, the semen from this study is suitable for further processing.

The average sperm viability in fresh semen in this study was 87±2.74%, which is considered very good. According to [17], fresh semen for processing must contain at least 70% viable spermatozoa. The average spermatozoa abnormality in fresh semen obtained from this study was 5.6±2.70%, higher than the results conducted by Ref. [12], which was 3.81±1.73%, and [9], which was 2.9%. Spermatozoa for artificial insemination purposes should not contain more than 15% abnormal spermatozoa [9].. The average abnormality of Boer goat spermatozoa in this study was still within the minimum standard range for the IB program, no more than 20%.

3.2. Semen Quality after Treatment

3.2.1. Spermatozoa Motility

Table 2 shows that the average sperm motility of Boer goats with the addition of propolis extract in each treatment showed no significant difference between days 0 and 3 of storage. Ref. [1] explained that the addition of propolis to the dilution medium did not significantly affect sperm motility in PE goats after storage at 5°C until day 3 (P > 0.05). This is likely because the antioxidant activity of propolis was not sufficient to withstand oxidative damage during initial storage.

Table 2. Average Individual Motility of Boer Goat Sperm in DuckEgg Yolk Tris Diluent plus Propolis Extract Stored at a Temperature of 5℃.

Storage (days)	Motility			
	P0	P1	P2	P3
0	82.00±2.73	83.60±4.15	83.20±3.42	83.80±3.96
1	74.60±4.56	78.60±4.15	73.60±5.77	76.60±6.34
2	56.20±13.42	65.20±10.13	59.00±9.59	60.40±9.37
3	41.00±12.36	54.40±11.14	40.40±11.76	39.60±2.88

Source. Research results data (2025)

The average individual motility percentage in P1 tended to be higher, although not significantly different (P > 0.05) compared to P0, P2, and P3, up to day 3 (Table 2). This is likely due to the optimal concentration of 0.25% propolis extract, which provides protection for spermatozoa during cold storage. The lowest motility was in P3, at 39.60 ± 2.88%. This is suspected because excessively high propolis concentrations can have a toxic effect on spermatozoa [6].

Viability

Sperm viability is the ability of spermatozoa to survive dilution and is an important factor in determining the quality of a bull's spermatozoa. The average viability data from this study are shown in Table 3.

Table 3. Average viability of Boer goat spermatozoa in egg yolk tris diluent plus propolis extract stored at 5°C.

Storage (days)	Viability			
	P0	P1	P2	P3
0	79.00±6.40	86.80±4.02	79.20±9.88	81.20±7.59
1	73.40±5.59^{a}	82.60±4.15^{b}	71.00±7.77^{a}	75.40±6.80^{ab}
2	61.80±8.92	69.20±7.22	61.60±8.29	62.40±8.08
3	44.00±6.85^{a}	58.40±6.06^{b}	45.60±9.04^{a}	49.20±4.65^{a}

Source. Research results data (2025)

The average viability percentage at P1 on day 3 was the highest (58.40±6.06%) and significantly higher (P<0.05) than the viability percentages at P0, P2, and P3, which were 44.00±6.85%, 45.60±9.04%, and 49.20±4.65%, respectively. The high viability at P1 may be attributed to the optimal antioxidant activity of the bioactive compounds present in propolis, including flavonoids, phenolic acids, and terpenoids. Ref. [10][18] stated that the higher the viability of spermatozoa, the greater the chance of fertilization during copulation, whether natural or artificial.

3.2.2. Spermatozoa Abnormalities

Table 4 shows that the average abnormality of Boer goat spermatozoa with propolis extract added during storage from day 0 to day 3 did not show a significant difference (P>0.05). However, there was a tendency for treatment P1 to show a lower percentage of abnormalities compared to the other treatments. This indicates that the addition of propolis extract at a concentration of 0.25% (v/v) was able to suppress morphological damage to spermatozoa during storage at 5°C.

Table 4. Average abnormality of Boer goat spermatozoa in duck egg yolk tris diluent plus propolis extract stored at 5°C.

Storage (days)	Abnormality			
	P0	P1	P2	P3
0	3.40±1.81	3.00±1.22	3.00±1.22	4.00±1.87
1	5.20±3.42	5.00±4.52	7.00±4.63	4.00±1.87
2	5.40±3.71	4.00±2.34	3.20±1.30	4.40±3.20
3	5.00±2.44	4.60±1.67	6.00±1.87	5.80±4.81

Source. Research results data (2025)

Abnormality is a condition where spermatozoa experience damage or abnormalities in specific parts and is one parameter in determining the level of sperm fertility. Morphological abnormalities in spermatozoa can be divided into two categories: primary and secondary (or tertiary). Primary abnormalities occur

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during the formation process in the testes, while secondary abnormalities occur after they leave the animal's body and result from semen processing. Secondary abnormalities, such as broken tails and curling, are often observed, which can occur due to friction during preparation or homogenization. This is consistent with [19] and [5], who stated that the midsection of spermatozoa is more sensitive to damage.

This study supports the findings of [1] that the addition of propolis does not always have a significant effect; however, it still helps maintain sperm quality by preventing an increase in the percentage of abnormalities. This is thought to be due to the flavonoids and phenolics in propolis, which function as natural antioxidants. Abnormalities can also occur due to excessive pressure, high heating, rapid cooling, or contamination with water, urine, germs, or antiseptics. Furthermore, according to Ref. [14] and [20], the mixing time with the treatment ingredients also influences sperm damage.

4. CONCLUSION

The optimal concentration of propolis extract in Tris-diluent duck egg yolk to maintain Boer goat sperm quality for 3 days at 5°C is 0.25% (v/v). Further research is needed on various livestock species using Tris-diluent chicken egg yolk and varying concentrations of propolis extract.

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