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The Effect Of Variations In Fig Fruit Filtrate Concentration In Tris Albumin Diluent On The Quality Of Boerka Buck Spermatozoa Stored At Room Temperature

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Abstract
Semen quality in form of liquid or frozen is the main factor of the success of Artificial Insemination. Semen quality depend on the quality of diluent. The purpose of this study was to determine the effect of different levels of fig filtrate in Tris-Albumin extender and storage time on the quality of Boerka buck spermatozoa stored at room temperature. This study was conducted at the Animal Reproduction Laboratory, Faculty of Animal Husbandry, University of Mataram, Indonesia. The material used in this study was semen from one Boerka buck, approximately 2 years old, with a body weight of around 80 kg. Semen collection was performed using an artificial vagina. This study used a laboratory experimental method using a completely randomized design (CRD) consisting of 4 treatments and 5 replications. The treatments were divided into 4, namely P0 (standard tris diluent + 2.5% egg yolk), P1 (standard tris diluent + 2.5% egg white), P2 (P1 + 4% fig filtrate), and P3 (P1 + 6% fig filtrate). The variables observed in this study were individual motility, viability and spermatozoa abnormalities. The results of the study after 8 hours of storage at room temperature that the best fig fruit filtrate concentration in Tris-albumin extender was 6%. in conclusion, the concentration of fig fruit filtrate of 6% (v/v) in tris albumin diluent has the highest preservation power on the quality of Boerka buck spermatozoa stored at room temperature.

Key Word*: Albumin, boerka, fig filtrate, quality, spermatozoa.*

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I. Introduction

Public demand for animal protein continues to rise each year in tandem with population growth. Goats play a vital role in Indonesia's agricultural trade system, as evidenced by the country's consistently increasing goat population (Sudrajat et al., 2021). One initiative to enhance the genetic quality of goats in Indonesia is the implementation of artificial insemination (AI) technology. The benefits of AI using semen from superior busck include the elimination of the costs associated with raising bucks, the prevention of disease transmission, and the enhancement of genetic quality (Sriana, 2018; Zaenuri, 2018; Kusumawati, 2017).

Factors influencing the success of artificial insemination (AI) include the quality of liquid or frozen semen, the method of estrus synchronization, and the accuracy of the insemination technique and timing (Zaenuri, 2018). While liquid semen serves as an alternative to frozen semen, it has the disadvantage of a rapid decline in quality, particularly when stored at room temperature. To mitigate significant declines in semen quality, the use of appropriate diluents is essential. A good diluent must provide energy and nutrients to meet the needs of spermatozoa, buffers to stabilize pH, cryoprotectants to prevent damage to spermatozoa, antimicrobial agents, antioxidants, and must be non-toxic and isotonic (Rizal and Riyadhi, 2016; Handoko et al., 2018; Fay et al., 2019; Swari et al., 2019).

Numerous studies have investigated the preservation properties of various diluents on semen quality, applicable to both liquid and frozen storage. Commonly used diluents include egg yolk in sodium citrate buffer or tris (hydroxymethyl)aminomethane (Sumadiasa, 2018). In addition to egg yolk, albumin is also an effective component in semen diluents. Fu et al. (2017) explained that albumin can maintain the abundance of semen proteins by protecting the plasma membrane and acrosome. Perumal et al. (2015) noted that albumin serves as a crucial antioxidant due to its ability to transport Fe2+ and Cu+, which helps minimize hydroxyl radicals (OH-).

Liu et al. (2015) further emphasized that leucine, aspartic acid, serine, glutamic acid, and lysine—amino acids found in egg white albumin—play a vital role in antioxidant activity.

During semen storage, spermatozoa continue to engage in metabolic activities that produce free radicals, leading to a decline in sperm quality. To preserve the quality of spermatozoa in the presence of free radicals, an antioxidant can be added to the semen diluent (Trilaksana et al., 2015). Antioxidants are commonly found in fruits, such as figs. Figs contain approximately 9-10% antioxidants (Siswoyo, 2007). According to Vincen and Morton (2005), figs are rich in several vitamins, including vitamins A and C. The vitamin C content in figs (ascorbic acid) is known to be twice that of dates, three times that of oranges, five times that of grapes, and seven times that of bananas. Vitamin C is one of the essential antioxidants required for semen diluent. The vitamin C content in figs is 2 mg, along with vitamin E (0.11 mg), vitamin K (4.7 mg), and vitamin B6 (0.113 mg) (U.S. Department of Agriculture, 2009). Figs also possess the highest concentration of polyphenols, measuring 1,090-1,100 mg per 100 g of fresh fruit. The phenolic content of dried figs can enhance plasma lipoproteins and provide protection against oxidation (Lukitasari et al., 2014).

Based on the description provided, a study titled "The Effect of Adding Fig Filtrate to Tris Albumin Diluent on the Quality of Boerka Buck Sperm Stored at Room Temperature The results are anticipated to serve as a reference for future research in this area.

II. Material And Methods

Research Materials

The material used in this study was semen collected from a Boerka buck, which is a cross between a Boer buck and a Kacang buck. The goat was approximately 2.5 years old and weighed around 75 kg. Semen collection was performed every five days, totaling five collections, using an artificial vagina.

Fig Filtrate Filtration

The figs used were ripe and uniform in size. They were cleaned with tap water, rinsed with distilled water, and then dried with paper towels. The figs were cut into several pieces, weighed, and placed in a blender. Next, distilled water equal to the weight of the figs was added, and the mixture was blended until smooth. The pulp was then centrifuged at 3,500 rpm for 20 minutes. The supernatant was collected using a micropipette and centrifuged again at the same speed and duration as the initial centrifugation. The resulting clear liquid, referred to as fig filtrate (abbreviated as FT), was transferred into a prepared sterile tube using a micropipette and stored in the refrigerator until needed (Zaenuri et al., 2013). *Preparing the Tris buffer Basic Diluent*

The buffer, or basic diluent, consisted of 3.786 g of Tris and 2.172 g of citric acid. These two components were combined in an Erlenmeyer flask with 100 mL of distilled water, heated on a hot plate until boiling, and then allowed to cool. Once the temperature in the Erlenmeyer flask dropped to 37°C, 0.06 g of penicillin, 0.1 g of streptomycin, and 0.625 g of fructose were added and homogenized using a magnetic stirrer. The Tris buffer solution was stored at 5°C until needed (Evans and Maxwell, 1987).

Preparing Albumin, Egg Yolks, and Treatment Diluents

Chicken eggs disinfected with 70% alcohol were dried using tissue paper. The shells were cracked, and the yolks and egg whites were separated and stored in sterile containers. The composition of the treatment diluents was as follows: P0 = control (standard Tris diluent + 2.5% egg yolk), P1 = standard Tris diluent + 2.5% albumin, P2 = P1 + 4% FT, and P3 = P1 + 6% FT (v/v).

Evaluation of Fresh Semen and Treated Semen

Semen was collected using an artificial vagina every five days. The macroscopic examination includes the following parameters: Semen color, volume, consistency, and acidity. Good-quality semen is typically milky white or yellowish-white. Clear white semen indicates poor sperm quality, while brown or reddish semen suggests the presence of blood or pus, which may result from a wound or infection in the genital tract (Susilawati, 2013). Cream-colored semen is indicative of a high sperm concentration (Ariantie et al., 2014). Semen volume can be directly measured using the scale on the collection cup. Consistency is assessed by gently shaking the tube containing the semen; the speed at which the semen returns to its original position reflects its consistency. Thicker semen generally correlates with a higher sperm concentration (Sumadiasa, 2018). The degree of acidity (pH) is measured using pH paper. Acidity is a crucial factor, as it significantly affects sperm viability (Zaenuri et al., 2023b).

Microscopic examination involves assessing mass motility by placing a drop of buck semen on a glass slide and observing it under a microscope with a magnification of 10×. Mass motility is classified into four categories: Very Good (+++), characterized by large, numerous, dark, thick, and active waves that move quickly from place to place; Good (++), indicated by small, thin, sparse, unclear, and slow-moving waves; Fair (+), where no waves are visible but only active progressive individual movements; and Poor (N, necrospermia,

or 0), which is defined by few or no individual movements (Zaenuri et al., 2023b; Sumadiasa, 2018). Additionally, individual motility can be evaluated by placing fresh or treated semen on a glass slide and covering it with a cover slip. Observations are made under a light microscope with a magnification of 40×, and motility is assessed subjectively across several fields of view. The percentage of motility from these fields is summed and averaged (Zaenuri et al., 2023; Sumadiasa, 2018).

Sperm viability is assessed by placing a drop of semen on the edge of a glass slide. An eosin dye drop is added to the semen, which is then homogenized. A smear is prepared and allowed to dry for several minutes at room temperature. Sperm viability is evaluated using a light microscope at 400× magnification. Dead spermatozoa absorb the dye, while live spermatozoa remain transparent. To determine the percentage of spermatozoa viability, 200 spermatozoa (both dead and live) are counted across several fields of view. The percentage of viable spermatozoa is calculated by dividing the number of live spermatozoa by 200 and multiplying by 100 (Zaenuri, 2013). Sperm morphology is assessed using the same preparation method as for viability. Abnormal spermatozoa morphology is characterized by large heads, broken or absent heads, conjoined spermatozoa, and double tails (Zaenuri et al., 2023; Sumadiasa, 2018).

Statistical Analysis

The statistical significance of the results was assessed using a one-way analysis of variance (ANOVA). Results that were significantly different were further analyzed using Duncan's test with SPSS version 16.0 software. The data are presented as Mean ± SD. A probability value of $p < 0.05$ was considered statistically significant (Sawyer, 2009).

III. Results And Discussion

Fresh Semen Evaluation Results

The assessment of fresh semen in Boerka bucks included parameters such as volume, color, aroma, consistency, pH, mass motility, individual motility, viability, and abnormalities. The results of the examination and evaluation of Boerka buck semen over five collections, along with their average values, are presented in Table 1.

Table 1. Macroscopic and microscopic characteristics of fresh Boerka buck semen from the study.

Parameters	Averages
Volume (mL)	1,58 ± 0,50
colour	Cream
Aroma	Typical goat aroma
consistency	Moderata
acidity (pH)	6,8 ± 0,04
Mass motility	++ to +++
Individual motility (%)	91 ± 2,24
Viability (%)	97 ± 0
Abnormality (%)	4 ± 7,26

Note. Semen Microscopic and macroscopic characteristics from the study

The average semen volume recorded in this study was 1.58 ± 0.50 mL, which aligns with findings from other studies on Boer goats: 1.54 ± 0.32 mL (Ramadanthi, 2020) and 1.33 ± 0.2 mL (Putri et al., 2019), as well as 0.96 ± 0.06 mL (Putra et al., 2012). Variations in semen volume can be influenced by several factors, including the type of livestock, breed, age, body size, frequency of collection, environmental conditions, diet, and the overall health of the livestock (Kusumawati et al., 2017).

The color of Boer buck semen collected over five sessions was cream with a medium consistency, indicating a relatively high concentration of spermatozoa per milliliter. High-quality semen is typically whitish or creamy (Rusdin, 2006; Sekosi et al., 2016), milky white (Sugatha, 2018), or yellowish white (Putri et al., 2019). Variations in semen color are influenced by the consistency and concentration of spermatozoa; the thicker the consistency and the higher the concentration, the darker the semen color (Putri et al., 2019). The semen analyzed in this study can be considered normal, as there were no brown hues indicating contamination with blood, nor any greenish tint suggesting the presence of putrefactive bacteria (Kusumawati et al., 2017).

The aroma of semen observed in this study is characteristic of goat semen. A foul-smelling semen indicates an abnormality, as it may contain pus resulting from an infection in the male animal's reproductive organs or tract (Ama et al., 2017). The consistency of Boerka buck semen in this study is classified as moderate, aligning with the definitions provided by Sumadiasa (2018) and Putra et al. (2012), which state that good semen should neither be too runny nor too thick. In contrast, abnormal semen resembles the color and consistency of coconut water (Anwar et al., 2019). The pH of the semen in this study was measured at 6.8 ± 0.04 (Table 1). Generally, the pH of semen ranges from 6.4 to 6.8 (Sekosi et al., 2016) and can reach up to 7 (Kusumawati et al., 2017). Deviations from these normal pH values can lead to a more rapid decline in sperm viability (Sujoko

et al., 2009). Variations in semen pH are influenced by several factors, including species, temperature, sampling frequency, age, and season (Susilawati, 2011).

The motility of the sperm mass in this study was classified as good, with values ranging from ++ to +++. These results are consistent with those of Kusumawati et al. (2017) and Manehat et al. (2021), which indicated that the semen quality was very good (+++). The individual spermatozoa motility observed in this study was 80%, which is higher than the motility of fresh spermatozoa from Kacang bucks at 75.2% (Kusumawati et al., 2017) and 78.3% in Boer bucks (Putri et al., 2019). Additionally, previous studies reported motility rates of more than 50% (Rahayu et al., 2014) and 70% (Putri et al., 2019). The average spermatozoa viability obtained in this study was 97±0%, surpassing the findings of Anwar et al. (2019), which reported 78% viability in Boer bucks and 86.70% in Bligon bucks (Maria et al., 2020). The percentage of abnormalities in this study was 4±7.26%. According to artificial insemination (AI) standards, the percentage of abnormal spermatozoa should not exceed 15% (Kusumawati et al., 2017) or 20% (Sekosi et al., 2016). Fresh semen suitable for further evaluation must meet minimum volume requirements of 0.5 mL, 70% individual motility, and a concentration of 2.5 x 10^9 per mL (Zaenuri et al., 2025). Therefore, the fresh semen produced in this study can be further processed.

Semen Evaluation After Treatment

Sperm Motility

Individual motility was assessed across several fields of view under a microscope at 400x magnification and averaged (Kusumawati et al., 2017). Generally, the highest individual motility is characterized by progressive movement or active forward motion (Annur, 2018). The average percentage of individual sperm motility is presented in Table 2.

Table 2. Average individual spermatozoa motility of Boerka buck in various treatments of adding fig fruit filtrate
in albumin Tris-based diluent.

Stprage time (Hours)	Treatments			
	P0	P1	P2	P3
0	80,02±0,00a	80,03±0,00a	80,05±0,00a	80,03±1,12a
2	76,12±2,24b	77,21±2,74ab	75,14±0,01b	79,11±2,21a
4	70,06±3,53a	72.02±2,74ab	69,05±3,53b	70,12±3,53a
6	65,20±6,12ab	64,21±2,24b	62,13±2,74b	70,07±3,53a
8	57,12±5,70ab	58,12±2,74ab	54,14±4,18b	62.11±2,74a

Note: Different superscripts in the same row indicate significant differences ($p<0.05$).

The results of this study indicate that the addition of 6% (v/v) fig filtrate, equivalent to 0.5 g/100 mL of vitamin C, to a Tris-albumin-based diluent is optimal for maintaining the individual motility of Boerka buck spermatozoa stored at room temperature. The motility recorded at the eighth hour after dilution and storage was 62 ± 2.74 (P3). In comparison, individual motility at P0, P1, and P2 was recorded at 57.12 ± 5.70, 58.12 ± 2.74, and 54.14 ± 4.18, respectively. This improvement may be attributed to the antioxidant properties of the 6% fig filtrate, which can inhibit the chain reaction of oxygen during the formation of free radicals by acting as an electron acceptor (Funahashi and Sano, 2005). Additionally, the inclusion of 0.2 g of vitamin C has been shown to maintain Boer buck sperm motility at 40.20 ± 3.35% (Lubis et al., 2013) and 52.44 ± 4.17% (Pahlevy et al., 2022) when stored at 5°C for three days.

According to Kulaksiz and Daskin (2010), oxidative molecules can mitigate the effects of oxidative stress, while antioxidant compounds safeguard cells from the harmful effects of oxygen radicals, thereby preventing a rapid decline in sperm quality. During semen dilution and storage, a reaction occurs between sperm and oxygen, resulting in the formation of free radicals. These free radicals initiate membrane lipid peroxidation, which diminishes sperm viability and motility (Siahaan et al., 2012). The addition of fig filtrate, which is rich in vitamin C, enhances this protective effect. Vitamin C, in the form of L-ascorbic acid, acts as an antioxidant and is classified as a reducing agent due to its low redox potential, making it effective against oxidizing agents (Suhartono et al., 2007). Ascorbic acid can interrupt the radical reactions produced by peroxidation, as it reacts directly in the liquid phase with free radicals, converting into a slightly reactive active form known as ascorbyl (Padayatti et al., 2003).

Several studies have investigated the use of natural antioxidants derived from fruit juice as additives to diluents, aiming to protect spermatozoa from cell damage caused by free radicals and to maintain their quality. The results of these studies include: 52.73 ± 27.51 (2% soy lecithin) (Saaban, 2018), 48.20 ± 14.68 (80%

tomato juice in egg yolk citrate diluent) (Rosmaidar et al., 2013), 67.9 ± 1.8 (10% guava fruit filtrate in CEP-2 diluent) (Sumadiasa, 2015), and 61.1 ± 6.6 (4% fig fruit filtrate) (Zaenuri et al., 2013).

Sperm Viability

Viability refers to the percentage of live and dead spermatozoa assessed using the differentiation technique of eosin-nigrosin stained smears (Kusumawati et al., 2017). Sperm that absorb the dye are considered dead, while those that do not are classified as live (Sriana, 2018).

Table 3. Average viability of Boerka buck spermatozoa stored at room temperature.

Storage time (Hours)	Treatments			
	P0	P1	P2	P3
0	91.28±1.14a	89.25±1.41c	85.14±0.02b	86.61±1.14a
2	85.63±4.16a	83.65±1.95b	80.37±2.4c	84.02±0.3a
4	79.11±1.13a	79.10±2.39a	77.26±2.28b	78.15±1.03a
6	74.00±1.04a	71.81±3.27b	70.03±3.53b	73.34±1.17a
8	69.09±5.15a	62.52±4.50b	64.12±3.83b	68.88±5.85a

Note: Different superscripts in the same row indicate significant differences ($p<0.05$).

Sperm viability data presented in Table 3 indicate no significant difference between P0 and P3, as well as between P2 and P3, eight hours after dilution and storage at room temperature. However, the viability in P1 and P2 was significantly lower ($p > 0.05$) than that in P0 and P3. This difference is suspected to be attributed to the role of egg yolk in maintaining spermatozoa viability. As an extracellular cryoprotectant, egg yolk serves as a nutrient source, energy source, and provides extracellular protection for spermatozoa against cold shock, owing to its lipoprotein and lecithin content (Kulaksiz and Daskin, 2010).

According to Fu (2017), albumin helps maintain the abundance of semen proteins by protecting the plasma membrane and acrosome. Perumal et al. (2015) state that albumin is a vital antioxidant due to its role in transporting Fe^{2+} and Cu^{+}, which can reduce hydroxyl radicals (OH^{-}). Liu et al. (2015) also noted that the amino acids leucine (Leu), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), and lysine (Lys) found in egg white albumin play a significant role in antioxidant activity. The total amino acid content in chicken egg white albumin is 43.23% (Sun et al., 2019). Egg white protein primarily consists of ovalbumin (54%), ovotransferrin (12%), lysozyme (3.4%), and ovomucin (3.5%) (Liu et al., 2015). In contrast, in P3, the concentration of FT (6%) is higher than in P1 and P2. Therefore, even though it does not contain egg yolk, FT supplementation enhances its ability to maintain spermatozoa viability (Zaenuri et al., 2023). However, the sperm viability observed in this study was lower than the results of previous studies, which reported 76.84% (Isnaini, 2011) using mung bean sprout filtrate with skim milk diluent and 87.50% (Astuti, 2022) using carrot filtrate in egg yolk tris diluent. Nevertheless, the results of this study were higher than those of Efitri (2020), which reported an average spermatozoa viability percentage of 55.9% using guava fruit filtrate and fig filtrate with egg yolk tris diluent.

Sperm Abnormalities

Abnormalities significantly impact fertility, as irregular cell structures can create complications during fertilization. Factors affecting sperm quality include coiled tails, broken tails, and malformed heads. Data on sperm abnormalities from this study are presented in Table 4.

Table 4. Average abnormality of Boerka buck spermatozoa stored at room temperature

Storage time (Hours)	Treatments			
	P0	P1	P2	P3
0	9.0±6,78a	10.1±2.00b	8.1±2.6c	9.2±2.61b
2	10.1±5.54a	10.3±5.86a	9.2±4.97a	9.5±2.49a
4	11.2±2.92a	11.1±1.14a	11.0±2.07a	10.0±2.74a
6	11.3±2.28b	12.1±2.41c	11.4±3.91b	10.1±2.35a
8	13.2±3.36b	12.4±2.86c	11.6±1.41a	10.4±1.92a

Note: Different superscripts in the same row indicate significant differences ($p<0.05$).

The data presented in Table 4 indicate that the percentage of spermatozoa abnormalities increases with storage time at room temperature. The highest rate of decline was observed at P0 (4.2%), which was significantly higher than the rates at P1, P2, and P3, which were 2.3%, 3.5%, and 1.2%, respectively. However, the results of this study reveal a higher abnormality rate compared to Aeni's (2018) study, which also utilized fig filtrate but involved goat sperm, reporting an average abnormality of 4.2±2.5% on day 3 at 5ºC. Similarly,

when compared to Nuraini's (2021) study, which employed yellow watermelon filtrate in a quail egg yolk Tris diluent, buck sperm exhibited an abnormality rate of 4.86±2.34% after 9 hours at room temperature. The acceptable percentage of spermatozoa abnormalities for artificial insemination (AI) is defined as 5-20% (Garner and Hafez, 2000) and 8-10% (Bearden and Fuquary, 2004); however, if the abnormality rate exceeds 25%, fertility may be compromised. This threshold aligns with the AI standards set by Sekosi et al. (2016), which state that semen intended for AI should not contain more than 20% abnormal sperm. Therefore, the abnormality percentage observed in this study is considered to reflect good quality. The low percentage of spermatozoa abnormalities at P3 may be attributed to the nutritional content of figs, including carbohydrates, protein, vitamin A, and vitamin C, which can help maintain spermatozoa quality (Kubby, 1997).

IV. Conclusion

A 6% (v/v) concentration of fig filtrate in Tris-albumin diluent exhibited the most significant preservation effect on the quality of Boerka buck spermatozoa stored at room temperature.

Conflict of Interest

The authors state that no conflict of interest.

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