2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(2): 202-212.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj25
PII: S232245682400025-14

The Effects of Adding Coconut Water to Egg Yolk Diluent on Motility, Viability, and Abnormality of Etawa Crossbred Goat Sperm

Fachroerrozi Hoesni*, Firmansyah Firmansyah, Sri Arnita Abutani, and Nurhayati Nurhayati

Faculty of Animal Science, Jambi University, Jl. Raya Jambi - Muara Bulian KM. 15, Mendalo Indah, Jambi 36361 Indonesia

*Corresponding author's Email: fachroerrozi_hoesni@unja.ac.id

ORIGINAL ARTICLE
Received: March 20, 2024
Revised: April 19, 2024
Accepted: May 16, 2024
Published: June 25, 2024

ABSTRACT

The Etawah crossbreed goat is a dual-purpose type of goat that can adapt well to tropical regions in Indonesia. The current research aimed to evaluate the effects of adding coconut water to citrate egg yolk diluent on the spermatozoa quality parameters (motility, viability, and abnormality) of the Etawah crossbred goat at the physiology and reproduction laboratory of animal husbandry, Jambi University (Indonesia). The research employed a randomized block design on Etawa crossbreed goats aged around 2-3 years with an average weight of 12 kg per head in six groups. The treatments included 100% citrate diluent of egg yolk without adding coconut water (P0) as a control, 90% citrate diluent of egg yolk + 10% coconut water (P1), 80% citrate diluent of egg yolk + 20% coconut water (P2), 70% citrate diluent of egg yolk + 30% coconut water (P3), 60% citrate diluent of egg yolk + 40% coconut water (P4). The parameters evaluated in this study included viability of spermatozoa, spermatozoa motility, and spermatozoa abnormalities. The five treatment tubes were stored in a refrigerated cabinet at 5°C for 2 days. After this period, semen quality assessment was assessed microscopically. The percentage of live spermatozoa was determined using a staining technique. The spermatozoa motility was assessed based on their ability to move. Abnormal spermatozoa were calculated based on the number of abnormal spermatozoa compared to the total number of spermatozoa. The results of the study showed that the addition of 20% coconut water to the 80% citrate diluent of egg yolk (P2 treatment) reduced the rate of decline in spermatozoa viability and did not increase the number of spermatozoa abnormalities significantly, compared to other groups. There was no decrease in the viability of Etawah crossbreed goat spermatozoa during 2 days of storage at 5°C in all groups. Therefore, it was concluded that coconut water could be added up to 20% into the egg yolk without any significant negative effects on spermatozoa quality parameters evaluated in the current study.

Keywords: Citrate diluent, Coconut water, Egg yolk, Etawah crossbred goat, Spermatozoa resistance

INTRODUCTION

Etawah Crossbred goat is a dual-purpose type of goat that can adapt to tropical areas in Indonesia. This breed results from crossing the Etawah goat from India with local Indonesian goats. The purpose of growing Etawah Crossbred goats is to produce kids rather than for meat production (Pubiandara et al., 2016; Rezki et al., 2016; Barek et al., 2020; Tethool et al., 2022). Many goat mating systems are carried out naturally due to the lack of superior male goats, thereby reducing the productivity of goats (Baldaniya et al., 2020). Therefore, efforts are needed to optimize productivity through artificial insemination technology in Etawah Crossbred goats.

Increased livestock production is a crucial goal to meet the demand for animal protein. Achieving this goal relies on the farmer's capability and access to information about livestock management, especially livestock reproductive technology, which is essential for successful production (Tethool et al., 2022). Artificial insemination is one of the reproduction technology systems. Unfortunately, implementing artificial insemination in goats has not been carried out in-depth in Indonesia, compared to cows. This is due to the technical difficulties of artificial insemination in the field (da Silva Ferreira et al., 2014; Baldaniya et al., 2020; Saputro et al., 2022). Another persistent issue in the artificial insemination program is the semen preservation technique as it is necessary to maintain viability outside the body and minimize sperm mortality rates (Anakkul et al., 2014; Shafiei et al., 2015). Although there are many difficulties with artificial insemination in goats, many countries have successfully implemented this technology in goats (Abdi-Benemar et al, 2020).

Semen is the secretion of the male reproductive glands that are normally ejaculated into the female reproductive tract during copulation and can be collected for artificial insemination purposes (Saputro et al., 2022). According to Oliveira et al. (2014), the benefits of artificial insemination technology include improving the utilization of superior

To cite this paper: Hoesni F, Firmansyah F, Abutani SA, and Nurhayati N (2024). The Effects of Adding Coconut Water to Egg Yolk Diluent on Motility, Viability, and Abnormality of Etawa Crossbred Goat Sperm. *World Vet. J.,* 14(2): 202-212. DOI: https://dx.doi.org/10.54203/scil.2024.wvj25

males, overcoming distance and time constraints, enhancing the genetic quality of livestock, preventing the transmission of diseases, and saving costs. Dilution is one of the methods used to reduce density and extend the survival of spermatozoa. An effective semen diluent must act as a buffer and provide nutrients as a source of energy for spermatozoa (Üstüner et al., 2015; Martínez-Fresneda et al., 2020).

Citrate egg yolk diluent has been widely used as a buffer medium to extend the survival of spermatozoa (Bustani and Baiee, 2021). One advantage of citrate buffer is that it can be mixed directly with egg yolk, serving as an energy source for spermatozoa, protecting them from cold shock, buffering to prevent pH changes due to lactic acid accumulation, and maintaining osmotic pressure and electrolyte balance (Salmani et al., 2013). Egg yolk is commonly used as a diluent since it can protect spermatozoa from cold shock with protective factors, such as lipoproteins and lecithin that act on the sperm cell membrane (Bustani and Baiee, 2021). In addition, egg yolk contains glucose, various proteins, fat-soluble vitamins, and beneficial viscosity for sperm cells (Bogdaniuk et al., 2022). Coconut water is a solution containing carbohydrates and sugars consisting of glucose, proteins, fats, and some minerals that can be used by spermatozoa as an energy source. However, coconut water does not contain cold shock factors like citrate egg yolk diluent that can anticipate a sudden drop in temperature (Baldaniya et al., 2020). The combination of coconut water and citrate egg yolk is expected to maintain the resistance of stored spermatozoa for up to 2 days. The novelty of this research lies in evaluating a combination of adding young coconut water ranging from 10% to 40% and egg yolk citrate ranging from 90% to 60%. The current research aimed to evaluate the effect of adding coconut water to citrate egg yolk diluent on the motility, viability, and abnormality of Etawah crossbred goat spermatozoa.

MATERIALS AND METHODS

Ethical approval

The Committee of Ethical Clearance of the Faculty of Animal Husbandry, Jambi University (Indonesia), has approved all of the research activities by providing a certificate of ethical clearance ref. 04/UN21.7/ECC/2023

Study design

This research was conducted in the physiology and reproduction laboratory of the faculty of animal husbandry, University of Jambi, Indonesia from June to July 2022. The diluent was made by preparing a citrate solution of 2.9 gr citrate dissolved in 100 cc of distilled water. Then, the citrate thinner and egg yolk were combined (Citric acid = 0.56 gr and egg yolk = 20 ml). After mixing, young coconut water (7-month-old coconut with green skin color) was added to the two diluents according to the specified treatment. The samples used in this study included semen from 2 to 3-year-old Etawah Crossbred male goats collected during the research period. Goat samples were collected from the Mat Beken goat farm in the Jambi City area of Indonesia during the research period with the average weight of goats being 12 kg per head. The samples for each treatment were repeated three times. All treatments appeared once in each replication, and randomization was carried out separately per group. The animals were growing in intensive goat farming. Drinking water was provided *ad libitum*.

The materials used included coconut water from the young coconut, egg yolk, a basic solution of sodium citrate as a diluent, NaCl 3%, eosin, as well as penicillin and streptomycin (1 mg/100ml). Young coconut water and egg yolk were purchased from young coconut and egg traders, and sodium citrate and eosin were obtained from the physiology and reproduction laboratory of the faculty of animal husbandry, University of Jambi. The equipment used in this study included an artificial vagina for semen collection, graduated test tubes, test tubes, a spatula, measuring glass, glass slides, cover slips, a refrigerated cabinet, an erythrocyte pipette, Neubauer counting chamber, an oven, an icebox, thermometer, aluminum foil, counter, syringe, spirit lamp, and microscope (Digital Binocular Microscope, Amscope, United States). This experimental study was designed using a randomized block design with five treatments and six groups. Sperm concentration for each treatment or each group was 3.09 ± 0.89 billion. The treatment in this study included P0 (100% egg yolk citrate + 0% young coconut water), P1 (90% egg yolk citrate + 10% young coconut water), P2 (80% egg yolk citrate + 20% young coconut water), P3 (70% egg yolk citrate + 30% young coconut water), P4 (60% egg yolk citrate + 40% young coconut water).

Research procedure

The process of mixing semen with diluent involved mixing 0.5 ccs of semen with dilution 10 times to get 5 ccs of a mixture of semen and diluent. Following this, each mixture was mixed with the respective treated diluent. Before conducting this research, the necessary equipment, including tools for semen collection (artificial vagina) and laboratory examination, was prepared. Subsequently, these tools were sterilized to ensure cleanliness and prevent contamination caused by microbes. Material sterilization through heating was performed to eliminate germs that could cause damage to

the materials (Toelihere, 1985). Before collecting semen, a diluent was prepared for semen dilution to ensure the quality of semen. Semen quality inspection was carried out by macroscopic and microscopic examination.

The semen from Etawah Crossbred goats was collected using an artificial vagina. After obtaining the semen, the volume obtained for each semen reservoir was 0.5 cc per ml, a macroscopic examination was conducted, including volume, color, odor, consistency, and pH. The semen was then stored in a temporary storage place (icebox) before being transported to the laboratory (physiology and reproduction laboratory of the faculty of animal husbandry, University of Jambi, Jambi Province, Indonesia) for initial microscopic examination (Magnification of 10 and 40 x), including spermatozoa motility and concentration. The semen was diluted according to the predetermined dilution composition, and then the five treatment tubes were stored in a refrigerated cabinet at 5°C for 2 days. After a 2-day storage period, the five tubes were stored in a refrigerator (5°C) for further examination. The observed variables included the percentage of live spermatozoa, spermatozoa motility, and spermatozoa abnormalities after storage for 2 days. The percentage of live spermatozoa was determined using a staining technique. The spermatozoa motility was measured based on the spermatozoa's ability to move. Abnormal spermatozoa were calculated based on the number of abnormal spermatozoa, compared to the total number of spermatozoa.

Statistical analysis

In this study, analysis of variance (ANOVA) was conducted using IBM SPSS Statistics version 26 to examine the significant effects of treatment on the measured parameters. The significant effects of treatment on the measured parameters ($p < 0.05$) were tested by Duncan's Multiple Range Test to compare the means of each treatment result.

RESULTS AND DISCUSSION

Table 1 shows the initial characteristics of the semen from the Etawah crossbred goats used in this study. As indicated, the initial conditions of Etawah Crossbred goat semen used in the study had an average volume of 0.55 ml, spermatozoa concentration of 3.09 billion, spermatozoa motility of 78.72%, percentage of live spermatozoa at 81.67%, and spermatozoa abnormalities at 7.40%. These parameters generally indicate that the semen meets the criteria for being considered studs. However, variations may occur depending on factors, such as the age and maturity level of the male goats, the skills of the semen collectors, and the frequency of semen collection (Wurlina et al., 2020). The declining semen volume tendency after collection may be attributed to the influence of excessively frequent collection.

According to Prastiya et al. (2021), goats suitable for breeding should produce spermatozoa with a volume ranging from 0.5 to 1.5 ml and spermatozoa concentration of 2 to 6 billion/ml. Moreover, spermatozoa motility should fall within the range of 65 to 90%. The findings of this study align with results from several other researchers (Mara et al., 2007; Musaffak et al., 2021), indicating that the parameters are within acceptable ranges. Frequent semen collection can lead to a decrease in semen volume (Bebas et al., 2018). Spermatozoa motility is influenced by the health of the breeding male, age, nutrition, collection frequency, and conditions on the collection day (Ducha et al., 2020). Influential factors on semen quality and quantity include genetics, libido, diseases, nutrition, transportation, and the environment (Susilowati et al., 2022).

Table 1. The initial characteristics of fresh semen in the Etawah crossbred goat

Description	Mean ± Standard Deviation
Volume (ml)	0.55 ± 0.07
Spermatozoa concentration (billion)	3.09 ± 0.89
Spermatozoa motility (%)	78.72 ± 0.78
Spermatozoa live percentage (%)	81.67 ± 2.87
Spermatozoa abnormality (%)	7.40 ± 2.08

The results indicated that adding young coconut water with egg yolk citrate diluent had a significant effect on the percentage of live spermatozoa ($p < 0.05$, Table 2). In this regard, treatment P2 yielded the best results (58.40%), followed by P3 (56.20%), P1 (52.80%), and P4 (54.00%). Treatment P2 achieved an optimal balance between egg yolk citrate diluent and young coconut water, effectively neutralizing metabolic waste, such as lactic acid and thereby prolonging the survival of spermatozoa. Conversely, treatments P1, P3, and P4 indicated an imbalance in neutralizing metabolic waste, potentially leading to inadequate neutralization of the remaining metabolic by-products and an elevated number of dead spermatozoa due to increased spermatozoa activity. In treatment P0, the percentage of live spermatozoa was 50.20%, which was lower than that in other treatments (P1, P2, P3, and P4). This condition indicated a lack of nutritional availability for the survival of spermatozoa in this treatment. Additionally, coconut water as a diluent contains

various sugars, such as glucose, sucrose, and fructose to meet the energy needs of spermatozoa during storage (Salmani et al., 2014; Huang et al., 2022; Zaenuri et al., 2023). Wondim et al. (2022) stated that the high activity of spermatozoa can lead to their death. The availability of sufficient nutrition for spermatozoa is very important to slow down the decline in the percentage of spermatozoa death and prevent the acceleration of metabolism. This prevention is crucial as it hinders the accumulation of lactic acid, which in turn reduces the pH of semen (Pahlevy et al., 2022). The accelerated accumulation of lactic acid can poison spermatozoa as it reduces semen pH. If a buffering solution within the medium does not neutralize the remaining metabolic by-products, it can result in poisoning and death of spermatozoa (Saputro et al., 2022; Huang et al., 2022). The analysis of variance revealed a significant effect of adding young coconut water to egg yolk citrate diluent on spermatozoa motility ($p < 0.05$, Table 3). Treatment P2 (52.60%) provided the highest spermatozoa motility, compared to other groups. This could be attributed to the optimal balance achieved in treatment P2, where the addition of 20% coconut water provided an ideal condition for maintaining spermatozoa viability during the 2-day storage period (Figure 1).

The average results for each treatment on spermatozoa motility revealed a decreasing trend in P4 and P0 (Figure 2). In treatment P4, it is likely that spermatozoa still had energy reserves to survive and undergo metabolism by utilizing glucose from egg yolk citrate and young coconut water. This observation is consistent with the findings of Michael et al. (2019), Souza et al. (2021), and Thiangthientham et al. (2023), who highlighted that spermatozoa store glucose in the form of glycogen, which is converted into ATP when energy is depleted. Then, in P0, there was also a decrease in spermatozoa motility probably due to the lack of nutrients for the survival of spermatozoa. This is consistent with studies conducted by Ranjan et al. (2020) and Nurcholis et al. (2021), indicating that sufficient nutrients for spermatozoa are crucial to maintaining the rate of decrease in spermatozoa motility.

Table 2. The effect of adding coconut water to citrate egg yolk diluent on the percentage of live spermatozoa in Etawa crossbreed goat

Coconut water addition (%)	Replication					Mean ± Standard Deviation
	1	2	3	4	5	
P0 (0)	50.00	50.00	50.00	53.00	48.00	50.20 ± 3.65^{C}
P1 (10)	52.00	50.00	55.00	57.00	50.00	52.80 ± 3.58^{BC}
P2 (20)	55.00	60.00	57.00	65.00	55.00	58.40 ± 6.05^{A}
P3 (30)	50.00	58.00	60.00	60.00	53.00	56.20 ± 4.62^{AB}
P4 (40)	60.00	55.00	50.00	55.00	50.00	54.00 ± 3.77^{B}

^{ABC}: The different capital superscript letters in the s column are significantly different at the 5% level; P0: 100% egg yolk citrate without young coconut water, P1: Combination of 90% egg yolk citrate and 10% young coconut water, P2: Combination of 80% egg yolk citrate and 20% young coconut water, P3: Combination of 70% egg yolk citrate and 30% young coconut water, and P4: Combination of 60% egg yolk citrate and 40% young coconut water.

Table 3. The effect of adding coconut water to citrate egg yolk diluent on spermatozoa motility male Etawa crossbreed

Coconut water addition (%)	Replication					Mean ± Standard Deviation
	1	2	3	4	5	
P0 (0)	36.40	37.00	42.10	44.10	45.04	40.93 ± 4.98^{B}
P1 (10)	39.70	46.00	52.60	53.40	46.19	47.58 ± 5.28^{A}
P2 (20)	48.80	53.70	52.80	55.60	48.51	52.60 ± 2.93^{A}
P3 (30)	44.40	46.70	53.60	54.50	46.91	49.14 ± 4.28^{A}
P4 (40)	53.20	41.00	45.20	46.36	44.18	45.99 ± 4.48^{AB}

^{AB}: Different capital superscript letters in the same column are significantly different at the 5% level; P0: 100% egg yolk citrate without young coconut water, P1: Combination of 90% egg yolk citrate and 10% young coconut water, P2: Combination of 80% egg yolk citrate and 20% young coconut water, P3: Combination of 70% egg yolk citrate and 30% young coconut water, and P4: Combination of 60% egg yolk citrate and 40% young coconut water.

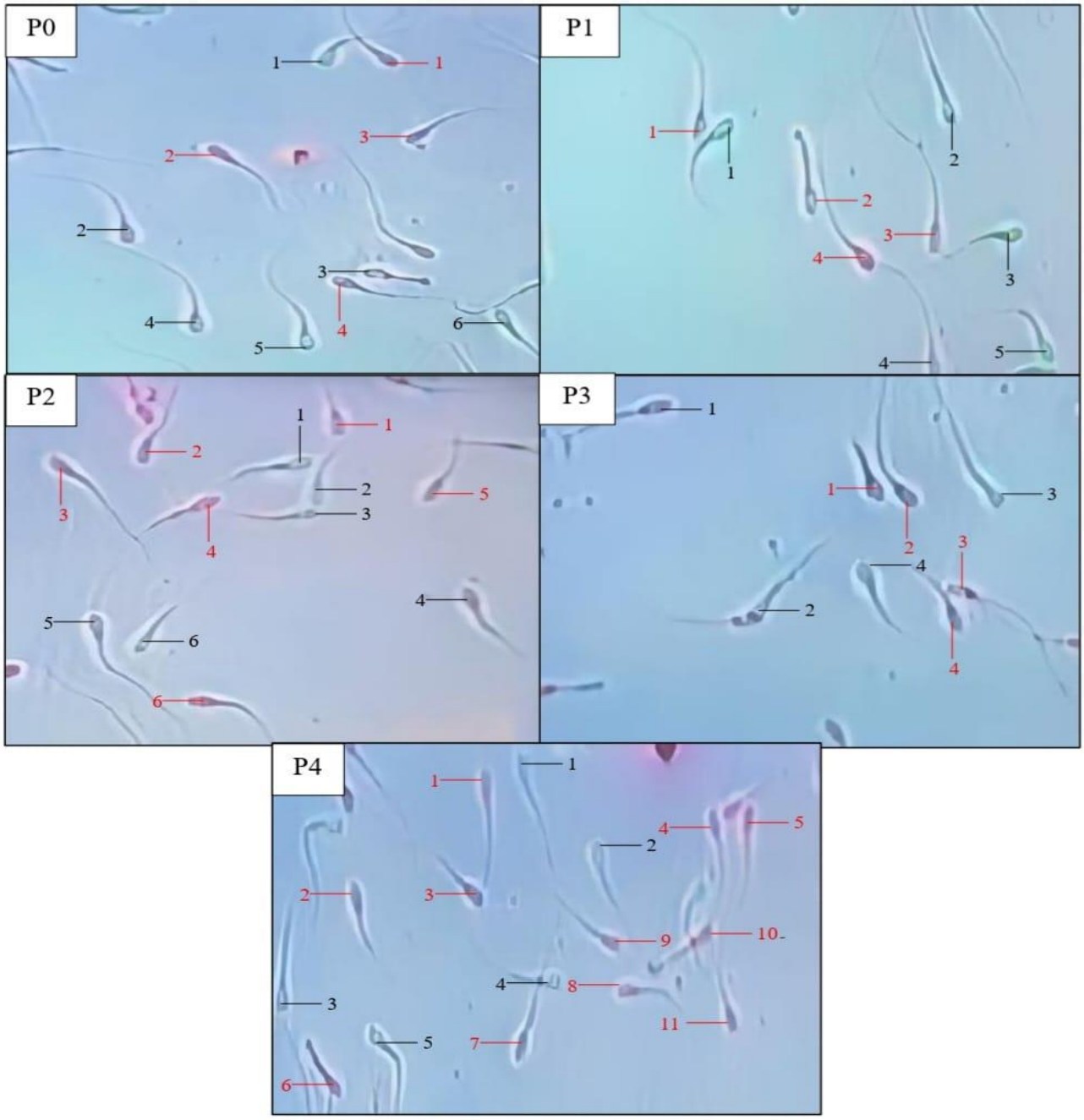

Figure 1. Viability of Etawah crossbreed goat sperm at 400x magnification. Black lines with numbers indicate live spermatozoa. Red lines with numbers indicate dead spermatozoa. P0: 100% egg yolk citrate without young coconut water, P1: Combination of 90% egg yolk citrate and 10% young coconut water, P2: Combination of 80% egg yolk citrate and 20% young coconut water, P3: Combination of 70% egg yolk citrate and 30% young coconut water, and P4: Combination of 60% egg yolk citrate and 40% young coconut water.

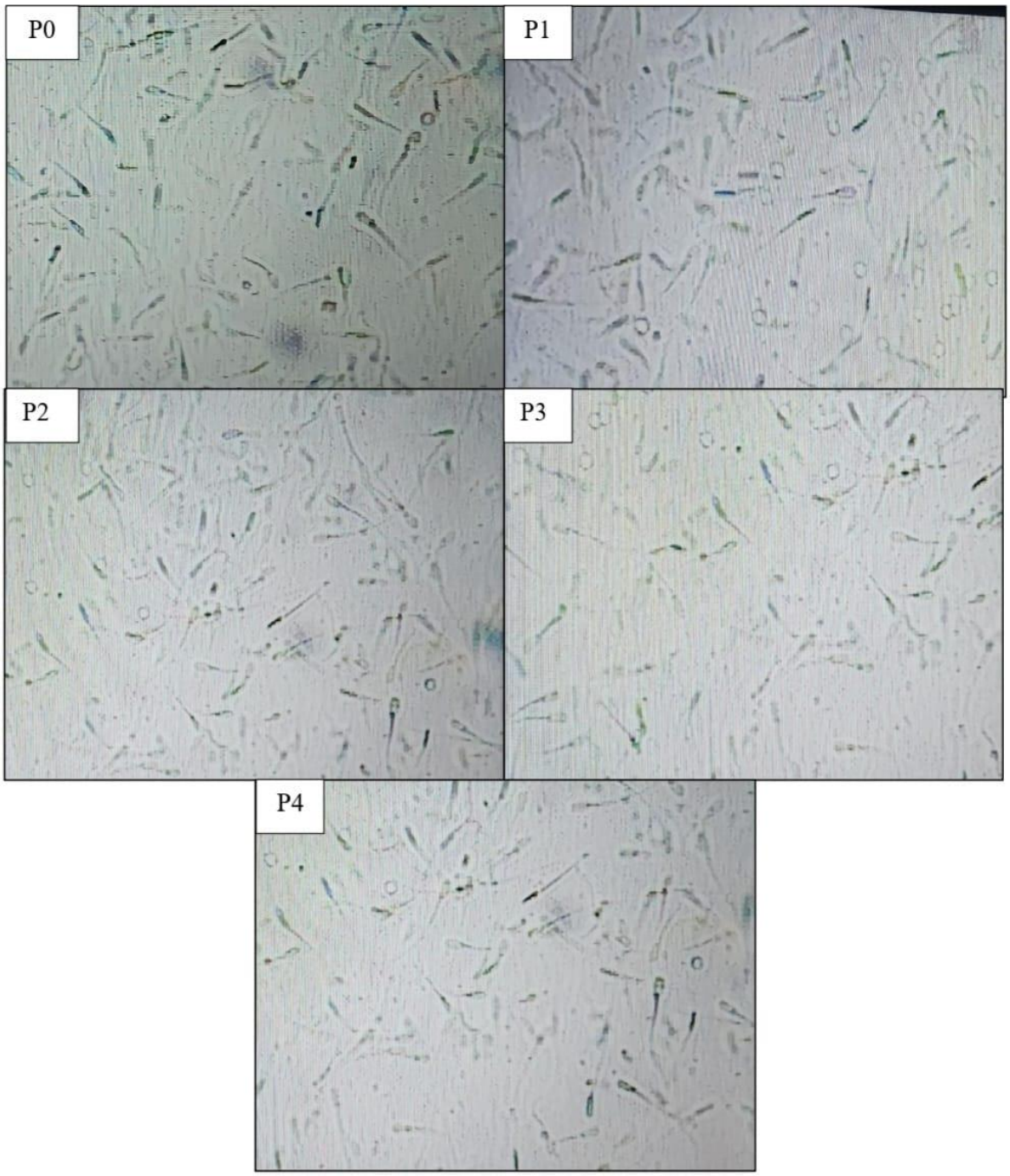

Figure 2. The sperm motility of Etawah crossbreed goats in different treatments (P0 to P4). P0: 100% egg yolk citrate without young coconut water, P1: Combination of 90% egg yolk citrate and 10% young coconut water, P2: Combination of 80% egg yolk citrate and 20% young coconut water, P3: Combination of 70% egg yolk citrate and 30% young coconut water, and P4: Combination of 60% egg yolk citrate and 40% young coconut water.

The results showed that storing semen from Etawah crossbreed goats did not significantly affect spermatozoa abnormalities between treatments ($p > 0.05$, P0, P1, P2, P3, and P4, Table 4). Moreover, the average percentage of spermatozoa abnormalities remained below 10% (Figure 3). This finding suggests that the 2-day storage period in a refrigerator can maintain the morphological condition of spermatozoa because the spermatogenesis process could affect spermatozoa abnormalities in the seminiferous tubules and during the movement of spermatozoa in the epididymis. In this study, the average percentage of spermatozoa abnormalities in Etawah Crossbred goats only involved primary abnormalities and did not vary significantly between treatments. Studies by Lukusa and Kabuba (2020) and Diansyah (2022) indicated that abnormalities or abnormal shapes of spermatozoa from failed spermatogenesis processes include coiled tails, broken necks, severed heads and necks, double heads, and double tails. This was also emphasized by Zamiri (2020), Nurcholis et al. (2021), and Macêdo et al. (2022) indicating that abnormal shapes resulting from failed spermatogenesis processes include large heads (macrocephalus) or small heads (microcephalus), short heads, broad heads, and double tails.

The preparation of smears and the storage process can lead to abnormalities in spermatozoa. The preparation of smears can result in abnormalities, such as coiled tails, broken necks, and severed heads and necks (Üstüner et al., 2015; Sutama, 2021). If the percentage of spermatozoa abnormalities exceeds 15%, it means that goats are experiencing infertility and cannot fertilize eggs (Menéndez-Blanco et al., 2019). Anand et al. (2017) and Wurlina et al. (2020) reported that spermatozoa abnormalities can be caused by the influence of semen pH, osmotic pressure, and cold-shock stress during storage.

Table 4. The effect of adding coconut water to citrate egg yolk diluent on spermatozoa abnormality in male Etawa crossbreed goats

Coconut water addition (%)	Replication					Mean ± Standard Deviation
	1	2	3	4	5	
P0 (0)	2.65	4.14	2.00	6.30	4.00	3.81 ± 1.66
P1 (10)	5.15	2.50	5.20	2.50	7.40	4.55 ± 2.08
P2 (20)	3.30	4.70	2.45	2.00	7.10	3.91 ± 2.06
P3 (30)	4.55	4.30	5.50	2.88	7.40	4.92 ± 1.67
P4 (40)	5.60	4.30	2.38	2.51	2.00	3.35 ± 1.54

P0: 100% egg yolk citrate without young coconut water, P1: Combination of 90% egg yolk citrate and 10% young coconut water, P2: Combination of 80% egg yolk citrate and 20% young coconut water, P3: Combination of 70% egg yolk citrate and 30% young coconut water, and P4: Combination of 60% egg yolk citrate and 40% young coconut water

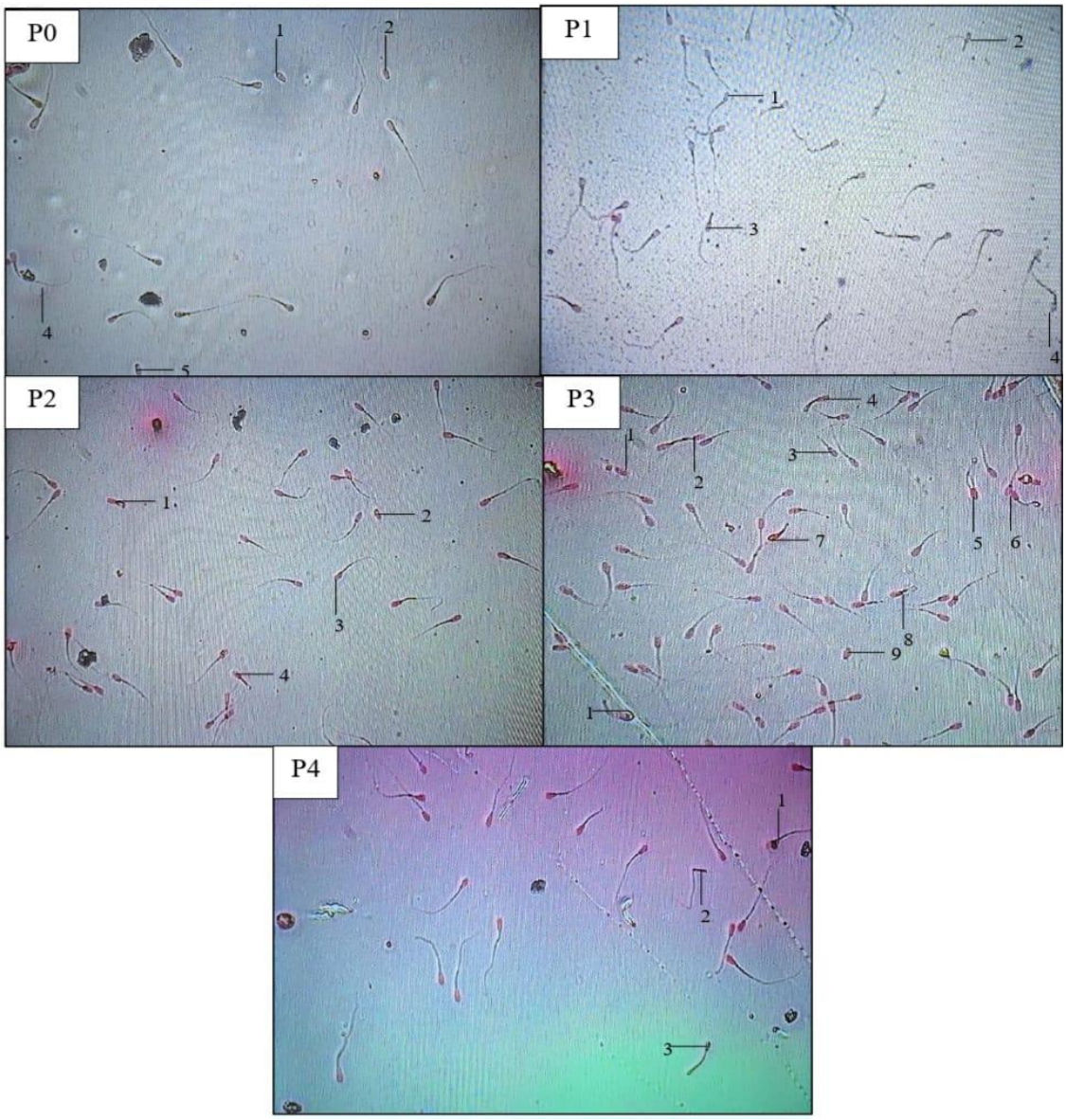

Figure 3. Etawah crossbreed goat sperm abnormalities, 400x magnification. Black lines with numbers indicate abnormalities. Red-colored spermatozoa indicate that they are classified as abnormal P0: 100% Egg Yolk Citrate without Young Coconut Water, P1: Combination of 90% Egg Yolk Citrate and 10% Young Coconut Water, P2: Combination of 80% Egg Yolk Citrate and 20% Young Coconut Water, P3: Combination of 70% Egg Yolk Citrate and 30% Young Coconut Water, and P4: Combination of 60% Egg Yolk Citrate and 40% Young Coconut Water.

CONCLUSION

It was concluded that the addition of up to 20% young coconut water to the egg yolk citrate diluent could reduce the rate of decline in spermatozoa viability in Etawah crossbreed goats and did not increase the number of spermatozoa abnormalities significantly. Further research is needed to evaluate other diluents in different temperatures.

DECLARATIONS

Funding
This research was funded by the faculty of animal husbandry at the University of Jambi, Indonesia.

Availability of data and materials
The data of the current study are available by a reasonable request.

Ethical considerations
The authors confirmed that the manuscript has been reviewed and submitted to this journal for the first time. The text of the manuscript was checked for plagiarism by authors before submission and all sentences were written by authors originally.

Acknowledgments
The author would like to thank the Dean of the faculty of animal husbandry at Jambi University, who approved and supported this research

Authors' contributions
Fachroerrozi Hoesni conceptualized, managed, and supervised the study. Fachroerrozi Hoesni and Firmansyah drafted the manuscript and performed all the experimental procedures. Fachroerrozi Hoesni, Firmansyah, Sri Arnita Abutani, and Nurhayati conducted data analysis and interpretation. All authors read and approved the final manuscript.

Conflict of interests
There is no conflict of interest to declare.

REFERENCES

Abdi-Benemar H, Khalili B, Zamiri MJ, Ezazi H, Ardabili GS, Moghadam SH, and Simanoor N (2020). Effects of astaxanthin supplementation on the freezability, lipid peroxidation, antioxidant enzyme activities and post-thawing fertility of ram semen. Small Ruminant Research, 192: 106213. DOI: https://www.doi.org/10.1016/j.smallrumres.2020.106213

Anakkul N, Suwimonteerabutr J, Tharasanit T, Khunmanee S, Diloksumpan P, Berg DK, and Techakumphu M (2014). Sperm distribution and fertilization after unilateral and bilateral laparoscopic artificial insemination with frozen-thawed goat semen. Theriogenology, 82(8): 1137-1144. DOI: https://www.doi.org/10.1016/j.theriogenology.2014.07.032

Anand M, Baghel G, and Yadav S (2017). Effect of egg yolk concentration and washing on sperm quality following cryopreservation in Barbari buck semen. Journal of Applied Animal Research, 45(1): 560-565. DOI: https://www.doi.org/10.1080/09712119.2016.1232265

Baldaniya RV, Chaudhari NF, Modi LC, Patel CM, Puri G, and Patel JM (2020). Effect of coconut water in tris egg yolk citrate extender on cauda epididymal buck spermatozoa motility preserved at refrigeration temperature. Indian Journal of Animal Health, 59(1): 55-61. Available at: https://ijah.in/upload/snippet/255_63.pdf

Barek ME, Uly K, Hine TM, Nalley M, and Belli HLL (2020). Pengaruh Penambahan Sari Wortel Dalam Pengencer Sitrat Kuning Telur Terhadap Kualitas Spermatozoa Kambing Bligon [The effect of carrot juice supplementation in citrate - egg yolk extender on spermatozoa quality of bligon goat]. Jurnal Nukleus Peternakan, 7(2): 109-117. DOI: https://www.doi.org/10.35508/nukleus.v7i2.3152

Bebas W, Gorda W, Trilaksana IGNB, Laksmi DND I, and Pemayun TGO (2018). Lactose-astaxanthin increased the frozen semen quality of Gembrong goat in conservation efforts. Jurnal Veteriner, 19(3): 390-396. Available at: https://ojs.unud.ac.id/index.php/jvet/article/view/31308/26828

Bogdaniuk AO, Yurchuk TO, and Petrushko MP (2022). Seasonal differences in sperm characteristics and the level of DNA fragmentation in fresh and cryopreserved sperm of Saanen goats. Cytology and Genetics, 56(5): 410-416. DOI: https://www.doi.org/10.3103/S0095452722050036

Bustani GH 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

Diansyah AM, Yusuf M, Toleng AL, and Dagong MIA (2022). Characteristik and kinematics of Bali-Polled bull sperms. Advances in Animal and Veterinary Sciences, 10(8): 1787-1796. DOI: http://www.doi.org/10.17582/journal.aavs/2022/10.8.1787.1796

Ducha N, Budijastuti W, and Rahayu DA (2020). Effect of addition of different egg yolks in basic tris-soya diluent on quality, membrane integrity of Senduro Goat sperm, and free radicals during storage at temperature of 4-5°C. Journal of Physics: Conference Series, 1569(4): 04208. DOI: https://www.doi.org/10.1088/1742-6596/1569/4/042081

da Silva Ferreira V, Mello MRBD, Fonseca CEMD, Dias ÁCF, Cardoso JM, Silva RB, and Martins Júnior WP (2014). Effect of seminal plasma and egg yolk concentration on freezability of goat semen. Revista Brasileira de Zootecnia, 43(10): 513-518. DOI: http://www.doi.org/10.1590/S1516-35982014001000001

Huang M, Cao XY, He QF, Yang HW, Chen YZ, Zhao JL, and Quang FS (2022). Alkaline semen diluent combined with R848 for separation and enrichment of dairy goat X-sperm. Journal of Dairy Science, 105(12): 10020-10032. DOI: https://www.doi.org/10.3168/jds.2022-22115

Lukusa K and Kabuba J (2020). Semen collection methods and cooling rates affect post-thaw sperm motility and kinematic parameters of semen goat. Asian Pacific Journal of Reproduction, 9(5): 239-246. DOI: https://www.doi.org/10.4103/2305-0500.294666

Macêdo LFBD, Texeira LSDA, Porfírio KDP, Costa SCDS, Silva LHM, Nascimento WM, and Paula NRDO (2022). Assessment of coconut water added to Numida meleagris egg yolk as cryoprotectant for goat semen. Animal Reproduction, 19(3): e20210114. DOI: https://www.doi.org/10.1590/1984-3143-AR2021-0114

Mara L, Dattena M, Pilichi S, Sanna D, Branca A, and Cappai P (2007). Effect of different diluents on goat semen fertility. Animal Reproduction Science, 102(1-2): 152-157. DOI: https://www.doi.org/10.1016/j.anireprosci.2007.02.007

Martínez-Fresneda L, O'Brien E, Sebastián AL, Velázquez R, Toledano-Díaz A, Tesfaye D, Schellander K, García-Vázquez FA, and Santiago-Moreno J (2020). *In vitro* supplementation of testosterone or prolactin affects spermatozoa freezability in small ruminants. Domestic Animal Endocrinology, 72: 106372. DOI: https://www.doi.org/10.1016/j.domaniend.2019.06.004

Menéndez-Blanco I, Catala MG, Roura M, Soto-Heras S, Piras AR, Izquierdo D, and Paramio MT (2019). Intracytoplasmic sperm injection (ICSI) of prepubertal goat oocytes using fresh and frozen-thawed semen. Small Ruminant Research, 170: 137-142. DOI: https://www.doi.org/10.1016/j.smallrumres.2018.11.026

Michael AJ, Alexopoulos C, and Pontiki EA (2009). Effect of antioxidant supplementation in semen extenders on semen quality and reactive oxygen species of chilled canine spermatozoa. Ukraina Animal Reproduction Science, 112(1-2): 119-135. DOI: https://www.doi.org/10.1016/j.anireprosci.2008.04.007

Musaffak TR, Sumartono, and Humaidah N (2021). Differences in semen quality of fresh liquid and frozen goats of etawah and saanen goas. International Journal of Animal Science, 4(3): 75-84. DOI: https://www.doi.org/10.2430/04.03.2021.75-84

Nurcholis N, Furqon A, Arifiantini RI, and Salamony SM (2021). Supplementation of pandanus conoideus oil in cryopreservation diluents for maintaining the semen quality of Ongole Grade Bull. Tropical Animal Science Journal, 44(2): 146-151. DOI: https://www.doi.org/10.5398/tasj.2021.44.2.146

Oliveira T, Thomas M, and Espadanal M (2014). Assessing the determinants of cloud computing adoption: An analysis of the manufacturing and services sectors. Information & Management, 51(5): 497-510. DOI: https://www.doi.org/10.1016/j.im.2014.03.006

Pahlevy JR, Ratnani H, Fikri F, Restiadi TI, Saputro AL, and Agustono B (2022). The addition of vitamin C in tris–egg yolk extender maintained Sapera goat semen quality in 5 C storage. Ovozoa: Journal of Animal Reproduction, 11(1): 1-8. DOI: https://www.doi.org/10.20473/ovz.v11i1.2022.1-8

Prastiya RA, Munir MM, and Nugroho AP (2021). The protective impacts of α-tocopherol supplementation on the semen quality of Sapera goat preserved at 4^0C. Tropical Animal Science Journal, 44(3): 261-266. DOI: https://www.doi.org/10.5398/tasj.2021.44.3.261

Pubiandara S, Suharyati S, and Hartono M (2016). Pengaruh penambahan dosis rafinosa dalam pengencer sitrat kuning telur terhadap motilitas, persentase hidup dan abnormalitas spermatozoa sapi ongole [Effect of additional dose of raffinose in egg yolk citrate diluent on motility, survival percentage and abnormalities of spermatozoa in Ongole cattle]. Jurnal Ilmiah Peternakan Terpadu, 4(4): 292-299. Available at: https://jurnal.fp.unila.ac.id/index.php/JIPT/article/view/1396/1282

Ranjan R, Goel AK, Kharche SD, Priyadharsini R, Ramachandran N, Singh MK, Kumar R, Dige MS, Bhushan S, Choudhary UB et al. (2020). Effect of cervical insemination with frozen semen on fertility of Indian goat breed. The Indian Journal of Animal Sciences, 90(4): 557-559. DOI: https://www.doi.org/10.56093/ijans.v90i4.104195

Rezki ZM, Sansudewa D, and Ondo YS (2016). Pengaruh Pengencer Kombinasi Sari Kedelai dan Tris terhadap Kualitas Mikroskopis Spermatozoa Pejantan Sapi PO Kebumen. [Effect of combination diluent of soybean extract and tris on the microscopic quality of spermatozoa of PO Kebumen cattle bulls]. Jurnal Sain Peternakan Indonesia, 11(2): 67-74. DOI: https://www.doi.org/10.31186/jspi.id.11.2.67-74

Salmani H, Nabi MM, Vaseghi-Dodaran H, Rahman MB, Mohammadi-Sangcheshmeh A, Shakeri M, Towhidi A, Zare Shahnehand A, and Zhandi M (2013). Effect of glutathione in soybean lecithin-based semen extender on goat semen quality after freeze-thawing. Small Ruminant Research, 112(1-3): 123-127. DOI: http://www.doi.org/10.1016/j.smallrumres.2012.12.015

Salmani H, Towhidi A, Zhandi M, Bahreini M, and Sharafi M (2014). *In vitro* assessment of soybean lecithin and egg yolk based diluents for cryopreservation of goat semen. Cryobiology, 68(2): 276-280. DOI: https://www.doi.org/10.1016/j.cryobiol.2014.02.008

Saputro AL, Syadid UBS, Susilowati S, Prastiya RA, Agustono B, Mumtazi F, and Farhan MR (2022). Effect of vitamin C and E supplementation and combination of both in egg yolk Tris Diluter on the quality of Sapera Goat spermatozoa in the 5° C cooling process. Pharmacognosy Journal, 14(5): 661-665. DOI: https://www.doi.org/10.5530/pj.2022.14.150

Shafiei M, Forouzanfar M, Hosseini SM, and Esfahani MHN (2015). The effect of superoxide dismutase mimetic and catalase on the quality of postthwed goat semen. Theriogenology, 83(8): 1321-1327. DOI: http://www.doi.org/10.1016/j.theriogenology.2015.01.018

Souza RS, Machado WM, França CSD, Mugabe LC, Pinheiro EEG, Carneiro IDMB, and Barbosa LP (2021). Docosahexaenoic acid in diluent for goat semen cryopreservation. Animal Reproduction, 18(3): e20210027. DOI: https://www.doi.org/10.1590/1984-3143-AR2021-0027

Susilowati S, Mustofa I, Wurlina W, Hernawati T, Oktanella Y, Soeharsono S, and Purwanto, DA (2022). Green tea extract in the extender improved the post-thawed semen quality and decreased amino acid mutation of Kacang buck sperm. Veterinary Sciences, 9(8): 403. DOI: https://www.doi.org/10.3390/vetsci9080403

Sutama IK (2002). The effect of equilibration time on the quality of frozen semen of Ettawa crossbred and Boer goat. International Seminar on Tropical Animal Production (ISTAP), pp. 141-147. Available at: https://journal.ugm.ac.id/istapproceeding/article/view/32900/19885

Tambing SN, Toelihere MR, Yusuf TL, and Sutama I (2000). Pengaruh gliserol dalam pengencer tris terhadap kualitas semen beku kambing Peranakan Etawah. Jurnal Ilmu Ternak dan Veteriner, 5(2): 1-8. Available at: https://repository.pertanian.go.id/server/api/core/bitstreams/2dd9cbab-d2c6-4852-abe1-76640a4d37de/content

Tethool AN, Ciptadi G, Wahjuningsih S, and Susilawati T (2022). Karakteristik dan Jenis Pengencer Semen Sapi Bali: Suatu Review [Characteristics and Types of Bali Cow Semen Diluents: A Review]. Journal of Tropical Animal and Veterinary Science, 12(1): 45-47. DOI: https://www.doi.org/10.46549/jipvet.v12i1.214

Thiangthientham P, Kallayanathum W, Anakkul N, Suwimonteerabutr J, Santiviparat S, Techakumphu M, Loi P, and Tharasanit T (2023). Effects of freeze-drying on the quality and fertilising ability of goat sperm recovered from different parts of the epididymis. Theriogenology, 195: 31-39. DOI: https://www.doi.org/10.1016/j.theriogenology.2022.10.013

Toelihere MR (1985). Fisiologi reproduksi pada ternak [Reproductive physiology in livestock]. Penerbit Angkasa. Bandung. Available at: https://books.google.co.id/books?id=-v1wNAAACAAJ

Üstüner B, Nur Z, Alcay S, Toker MB, Sağirkaya H, and Soylu MK (2015). Effect of freezing rate on goat sperm morphology and DNA integrity. Turkish Journal of Veterinary and Animal Sciences, 39(1): 110-114. DOI: https://www.doi.org/10.3906/vet1407-70

Wondim B, Taye M, Alemayehu K, Rouatbi M, Getachewe T, Haile A, and Rekik M (2022). he efficiency of estrus synchronization protocols and articifial insemination in the abergelle goat on-station and on-farm conditions of northern Ethiopia. African Animal Resesearch, 50(1): 518-525. DOI: https://www.doi.org/10.1080/09712119.2022.2108815

Wurlina W, Safitri E, Susilowati S, and Meles DK (2020). The effect of crude guava leaf tannins on motility, viability, and intact plasma membrane of stored spermatozoa of Etawa crossbred goats. Veterinary world, 13(3): 530-537. DOI: https://www.doi.org/10.14202/vetworld.2020.530-537

Zaenuri LA, Rodiah ASD, Lukman HY, and Yuliani E (2023). Spermatozoa quality of liquid boer goat semen in Tris egg yolk extender enriched with non-enzymatic antioxidants. Jurnal Kedokteran Hewan June, 17(2): 55-61. DOI: https://www.doi.org/10.21157/j.ked.hewan.v17i2.26913

Zamiri JM (2020) Update on semen cryopreservation in sheep and goats: A review. Iran small, 8(1): 1-15. DOI: http://www.doi.org/10.22103/jlst.2020.15927.1321