

CONCENTRATION OF POMEGRANATE JUICE (*Punica Granatum* L.) IN EGG YOLK TRIS DILUENT ON THE QUALITY OF PEANUT GOAT SPERMATOZOA AT ROOM TEMPERATURE

Lukman HY., Enny Yuliani., Lalu Ahmad Zaenuri., Rodiah and Adji Santoso Drajat
Reproduction Laboratory of the Faculty of Animal Husbandry Universitas Mataram
Jl. Majapahit 62 Mataram, Nusa Tenggara Barat, Indonesia
Corresponding author: hylukman@yahoo.com

ABSTRACT

This research was conducted at the Immunobiology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram. The objective was to determine the concentration of pomegranate juice as a diluent in maintaining the sperm quality of kacang goat stored at room temperature. The material used in this study was the sperm of a 2-year-old kacang goat. This study used an experimental method with a completely randomized design (CRD), which consisted of 4 treatments and 5 replications. Peanut goat sperm with the addition of pomegranate juice concentrations, namely P0(0%), P1(5%), P2(10%), and P3(15%). The results of this study indicate that the best use of pomegranate juice concentration is at P2 concentration of pomegranate juice (10%) with Motility $57 \pm 5.70\%$, Viability $74.8 \pm 3.27\%$, and Abnormality $6.6 \pm 2.70\%$, which was stored at room temperature for 8 hours. Analysis used SPSS 16's analysis of variance (ANOVA). The conclusion in this study was that the addition of pomegranate juice to egg yolk tris diluent with a concentration of 10% pomegranate juice showed a significant difference ($P < 0.05$) in maintaining better motility, viability and Spermatozoa abnormalities of peanut goats stored at room temperature.

Keywords: pomegranate juice, peanut goat, egg yolk tris, room temperature

INTRODUCTION

1. Background

Goat kacang is widespread in the province of Indonesia which needs to be preserved as livestock diversity. Goat kacang is a native goat in Indonesia. The superiority of the kacang goat is that it has high adaptability to unfavourable environmental conditions. Peanut goats are suitable for producing meat and skin, they reproduce very quickly, at the age of 15-18 months they can produce offspring. To get superior peanut goat seeds, efforts to increase productivity can be done by improving genetic factors.

Efforts to improve genetic factors can basically be carried out through effective and efficient alternative technological approaches. Alternative technology through artificial insemination (AI). According to Susilawati et al (2017), artificial insemination can be done using liquid semen. IB requires good quality sperm to produce high pregnancy. Good fresh sperm can be accommodated immediately and then diluted using a diluent that is able to maintain sperm quality in storage.

The right diluent is a diluent that can maintain the sperm quality of kacang goat, one of which is a semen diluent that can be used, namely pomegranate juice. Pomegranate juice is a source of antioxidants from plants with high polyphenol content (Haloho, 2015). According to Titin Nge et al (2015), the antioxidant content in pomegranate is mediated by the activity of the phenol hydroxyl groups which include tannins and flavonoids. Pomegranate has a high antioxidant content of 62.7% compared to grapes (26.6%), apples (25.7%) and pears (13.7%). Vitamin C in pomegranates is abundant in the flesh (Fatmawati, 2019). The content of vitamin C in pomegranate flesh can be obtained by extracting it using a solvent (Rosalinda, 2021). Egg yolk tris aminomethane is one of the most commonly used diluents and is able to maintain semen quality (Sulistiyowati, 2018). According to Allai et al (2015) in Iskandari (2020), egg yolk diluent contains lipoproteins and phospholipids which maintain and prevent damage to the spermatozoa membrane during storage.

Based on the description above, the quality of sperm during storage is very important to maintain its vitality. To maintain the sperm quality of kacang goat, using pomegranate diluent media is able to maintain sperm quality. Based on the description above, it is necessary to conduct a study on "Concentration of Pomegranate juice in Egg Yolk Tris diluent in maintaining the quality of Goat Peanut Spermatozoa at room temperature".

RESEARCH METHODS

The material used in this study was semen from 2-3 year old Kacang goats obtained from breeders in the Karang Pule Sekarbela village, Mataram city. Sperm collection using an Artificial Vagina is done 3 times a week. Inspection, assessment and cement processing will be carried out at the Central Laboratory for Bioscience and Biotechnology, Faculty of Mathematics and Natural Sciences, University of Mataram. The materials used in this study were pomegranate juice in egg yolk tris diluent.

This study uses laboratory experimental methods. using a completely randomized design (CRD), melon juice was used as a treatment. P0 = 100% Egg Yolk Tris, P1 = 95% Egg Yolk Tris + 5% Pomegranate juice, P2 = 90% Egg Yolk Tris + 10% Pomegranate juice and P3 = 85% Egg Yolk Tris + 15% Pomegranate juice.

The variables observed were motility, viability and abnormality. Sperm diluent was egg yolk tris buffer containing pomegranate juice. Storage of sperm or semen using an artificial vagina (VB). The artificial vagina containing the sperm is withdrawn immediately and swung slowly so that the semen collects in the reservoir glass (Zaenuri, 2018). The sperm obtained was immediately brought to the laboratory for macroscopic and microscopic examination. Sperm is declared good then the process is carried out according to the research treatment. The research treatment was immediately assessed based on research variables, namely motility, viability and abnormalities of spermatozoa at any time of 0 hours, 2 hours, 4 hours, 6 hours and 8 hours at room temperature in the laboratory where the study was conducted.

Data analysis, the data obtained was analyzed using variance (ANOVA). Then the results that were significantly different ($p < 0.05$) were tested further with the Duncan's test with the SPSS 16 program.

RESULTS AND DISCUSSION

Sperm quality determines the success of Artificial Insemination in livestock. Macroscopic and microscopic examination of fresh semen of kacang goat needs to be carried out before dilution, which can be seen in Table 1.

Table 1. Mean results of macroscopic and microscopic examination of fresh kacang goat semen.

Research Parameters	Research result
Volume (ml)	0.63±0.12
Color	Milk white
Aroma	Typical cement
Consistency	Thick
pH	7±0
Mass motility	+++ (very good)
Individual motility (%)	78±2
Concentration (10^7 / ml semen)	282.2±61.38
Viability (%)	96.6±0.80
Abnormality (%)	5.6±1.6

Note: Primary data is processed (2022)

The volume of semen or sperm obtained from this study was slightly higher, namely 0.63 ± 0.12 ml, compared to the results of Rokana's (2022) study, which was 0.55 ± 0.17 . The volume produced in this study is still relatively normal. According to Susilawati (2013) the normal volume of goat semen is between 0.6-1.5 ml. Semen volume differs according to nation, age, body size, frequency of shelter, environment, condition of the livestock itself, time of storage and feed given (Kusumawati et al. 2017).

The color of the semen which is creamy white, the color of the semen obtained in this study is in accordance with the opinion of Sekosi et al. (2016) stated that the normal color of goat semen is creamy white. Semen obtained during storage is said to be normal color cement. If the semen is yellowish green, it means it contains the germ *Pseudomonas auriginosa*, red semen means it contains blood and semen is brown, it means the semen contains rotting blood (Ionie, 2016). The results of this study obtained the color of the semen which is the normal color of the

semen without the presence of blood or pus. So that it can be continued for dilution as a need for artificial insemination or what is commonly called AI.

The smell or smell that was obtained in this study was the distinctive smell of sperm, namely the fishy smell typical of sperm, this smell indicates that the sperm is in a normal state. This characteristic odor is in accordance with the opinion (Ionie et al., 2016) that semen under normal circumstances generally has a distinctive odor accompanied by the odor of the animal. Semen that smells bad can occur if the semen contains pus which is caused by an infection of the reproductive organs or tract in male livestock. (Kusumawati et al., 2016).

The consistency of the cement obtained from this study was thick with a creamy white color. The results of cement consistency in this study were better than Ahmad's research (2021), which obtained a rather thick cement consistency. The results of this study are the same as a study conducted (Laos, 2021) which obtained fresh semen with a thick consistency. According to Lestari et al (2014) Cement with a thick consistency has a better and higher concentration compared to aqueous consistency. The consistency of spermatozoa is related to the color of spermatozoa which can be used to predict the consistency of spermatozoa. Semen obtained in this study can be said to be normal with a thick consistency and white cream color. To find out whether the spermatozoa obtained after collection are thick or not, they can be examined by shaking the test tube so that they can distinguish thick, medium, and watery sperm.

The degree of acidity or pH of the semen of kacang goats in this study is 7. The pH of this study is higher than the results of research conducted (Laos, 2021) the degree of acidity or pH is in the range of 6.52. According to Sekosi (2016) the degree of acidity of cement is generally in the range of 6.4-6.8. The pH of 7 cement obtained can be continued for diluent. The degree of acidity or cement pH is included in the neutral pH range.

Microscopic examination includes examination of mass motility, individual motility, viability, and morphology. Microscopic examination is very helpful in determining the quality of semen that is suitable for AI. The AI program requires good quality and quantity of semen, cement with progressive motility, high viability, and low abnormalities is very suitable for AI.

Motility is a very important indicator in determining the quality of semen and the success of spermatozoa fertilization. If the spermatozoa cannot move properly, it is impossible for the spermatozoa to reach the egg and penetrate for fertilization (Zulyazaini et al., 2016). To determine the progressive movement of spermatozoa using a microscope with a magnification of 400 times. Motility is an important factor in determining the fertility of spermatozoa. If the spermatozoa do not move properly, they cannot penetrate for fertilization (Sumadiasa, 2018).

Concentration of fresh semen of kacang goat in this study was 282.2 ± 61.38 . The concentration results from this study were higher than research conducted by Anwar (2021), which only obtained a concentration of 223 ± 20.54 . The semen concentration of healthy goats is in the range of $250-500 \times 10^7$. Differences in the concentration of spermatozoa can be caused by several things, namely, the type of goat, age, nation, condition, reproductive health, time of assessment, and the feed used.

The viability of the results of this fresh semen study obtained higher results, namely 96.6 ± 0.80 , compared to the results of Amrillah's study (2021) which was 90.5 ± 2.25 . The viability of fresh semen that can be processed into liquid semen is, if it has a sperm viability percentage of 83.38-88.94%. The viability of spermatozoa in this study was quite good. Viability is the vitality of spermatozoa as an indicator of sperm quality (Prastika et al, 2018). Factors that cause

differences in the results of viability can be caused by, age factors, types of goats, animal feed, and the environment.

Abnormalities obtained in this study averaged 5.6 ± 1.6 . and the results of this study are better than the results of Amrillah (2021), which obtained 8.00 ± 6.10 . This fresh semen abnormality is still considered normal and good for use in artificial insemination (AI) needs. According to Sekosi et al (2016) semen for AI purposes should not contain more than 20% abnormal sperm.

Semen Quality of Peanut Goats After Dilution according to Treatment

1. Motility of Cement After Dilution

Progressive motility results after the addition of pomegranate juice at 0%, 5%, 10%, and 15% treatment, good results were obtained at 10% treatment. The average percentage of progressive motility with room temperature storage in the addition of different concentrations of pomegranate juice in egg yolk tris diluent can be seen in Table 2.

Table 2. Percentage of progressive motility after adding different concentrations of pomegranate juice to egg yolk tris diluent.

Storage time (Hours)	Treatment			
	P0	P1	P2	P3
0	62±5.70 ^a	67±2.74 ^a	74±2.24 ^b	65±3.54 ^a
2	58±6.71 ^a	63±2.74 ^{ab}	69±4.18 ^b	60±3.54 ^a
4	54±4.18 ^a	58±4.47 ^{ab}	63±4.47 ^b	53±4.47 ^a
6	47±2.74 ^a	53±2.74 ^{ab}	59±5.48 ^b	50±6.12 ^a
8	41±2.24 ^a	45±3.54 ^a	57±5.70 ^b	42±2.74 ^a

Note: Different superscripts in the same line show a significant difference ($P < 0.05$) and the same superscripts in the same line show no significant difference ($P > 0.05$).

The results of this study indicated that the presence of pomegranate juice in egg yolk tris diluent was able to maintain the quality of spermatozoa at room temperature. The mean percentage of progressive motility of spermatozoa after 8 hours of storage obtained at P0, P1, P2 and P3 were 41 ± 2.24 , 45 ± 3.54 , 57 ± 5.70 and 42 ± 2.74 , respectively. The best results were in the P2 treatment with the addition of 10% pomegranate juice, ie. The addition of pomegranate juice in this study was able to maintain spermatozoa motility during 8 hours of storage at room temperature (Table 2). The results of the analysis showed that the average progressive motility at 8 hours of storage at room temperature had a significant difference ($P > 0.05$) between P2 and P0, P1 and P3, while P0, P1 and P3 showed results that were not significantly different ($P < 0.05$).

The results of a study using pomegranate juice were better than the results of a study conducted by Amrillah (2021) using kiwifruit juice (*Actinidia Deliciosa*) at the same room temperature using Boer goats, which was 46.00 ± 5.48 percent in maintaining progressive motile. The high motility of spermatozoa with the addition of 10% pomegranate juice was due to the

combination of pomegranate juice as a source of antioxidants needed for spermatozoa and egg yolk tris as a good source of energy to maintain spermatozoa during storage.

Pomegranate juice in egg yolk tris diluent is able to defend spermatozoa from free radicals. Pomegranate has a high antioxidant content of 62.7% (Haloho, 2015). According to Effendi et al (2015) prooxidative reactions of lipids when they react with free radicals can change the structure of sperm cells and damage the lipoprotein sheath so that sperm cannot sustain life when stored at room temperature. The content of *falvanoin* in pomegranate has been shown to contain antioxidants with a mechanism to protect cell membranes from the effects of free radicals. Compounds that include *falvanoids*, namely *luteolin*, *querqetin*, and *kaempferol* are present in large quantities in pomegranate skin, while in the seeds there are *anthochyandins* compounds.

Flavonoids are the largest phenol group (Hernawati, 2012; Azwar, 2021). Furthermore, pomegranate juice can inhibit bacterial growth, making it suitable for preserving spermatozoa during storage. Pomegranate is a diluent medium which is very suitable for maintaining spermatozoa, because pomegranate contains high levels of vitamin C and antioxidants compared to other fruits such as pears and apples (Rosalinda, 2021).

The motility of individual spermatozoa as a result of this study is still in the normal range, namely between 40% -75%. Based on the motility results obtained in the treatment of 10% pomegranate juice at room temperature storage with an estimated time of 8 hours of storage, the best results were obtained. According to Kusumawati (2016) that the percentage of spermatozoa that can be used for AI is at least 50% of spermatozoa that are live and motile.

Viabilitas Setelah pengenceran

Spermatozoa viability depends on the integrity and damage of the spermatozoa membrane which causes disruption of the intracellular metabolic processes of the spermatozoa. The viability of spermatozoa in this study is still relatively good. Viability is the vitality of spermatozoa as an indicator of sperm quality (Prastika et al, 2018). Factors that cause differences in yield from viability can be caused by, age factors, types of goats, animal feed, and livestock living environment. The average percentage of viability at room temperature storage with the addition of different concentrations of pomegranate juice in egg yolk tris diluent can be seen in table 3.

Table 3. Percentage of mean viability after adding different concentrations of pomegranate juice to egg yolk tris diluent.

Storage time (hours)	Treatment			
	P0	P1	P2	P3
0	71.4±6.11 ^a	82.4±2.41 ^{bc}	87.6±5.13 ^c	80.2±3.49 ^b
2	68±4.00 ^a	75.4±3.01 ^b	83.2±5.63 ^c	78±6.67 ^{bc}
4	65.4±1.14 ^a	72.6±4.72 ^a	80,6±6.11 ^b	72.4±7.16 ^a
6	57.8±1.79 ^a	62.8±6.46 ^a	77.4±5.59 ^b	64±5.83 ^a
8	53±4.18 ^a	58.8±6.72 ^b	74.8±3.27 ^c	58±5.24 ^b

Note: Different superscripts in the same line show a significant difference (P<0.05) and the same superscripts in the same line show no significant difference (P>0.05).

The results of this study obtained that the average viability after dilution at room temperature storage for 8 hours was able to maintain good spermatozoa viability. Storage at room temperature for 8 hours for all treatments showed a significant difference between P2 and P0, P1, and P3, while P1 and P3 did not show a significant difference. All treatments containing different pomegranate juice were significantly better than the treatment without using pomegranate juice (P0).

The best results were treated at 10% pomegranate concentration during 8 hours of storage, namely 74.4 ± 4.39 . The results of this spermatozoa viability study were higher than the results obtained from Amrillah's study (2021), namely $61.00 \pm 5.48\%$, which used kiwifruit juice in Boer goats at the same room temperature. This study showed that the addition of pomegranate juice to egg yolk tris diluent was able to maintain the viability of spermatozoa at room temperature storage for 8 hours.

The flavonoid content contained in pomegranate has been shown to contain antioxidants with a mechanism to protect sperm cell membranes from the effects of free radicals. Flavonoids are able to maintain the quality of spermatozoa by maintaining spermatozoa motility so that the viability of spermatozoa is able to maintain at room temperature storage with good results (Louis et al., 2019).

Pomegranate is mediated by the activity of the hydroxyl phenol groups which include tannins and flavonoids (Titin Nge et al 2015). The flavonoids contained in pomegranates have been shown to contain antioxidants with a mechanism to protect cell membranes from the effects of free radicals. Compounds that include flavanoids, namely luteolin, quercetin, and kaempferol are present in large quantities in pomegranate skin, while in the seeds there are anthocyanidins compounds. The high content of vitamin C and antioxidants from pomegranate juice is suitable as an excellent diluent medium for maintaining spermatozoa (Rosalinda, 2021).

The longer the storage time of spermatozoa, the less nutrients in seminal plasma will cause the viability of spermatozoa to decrease. Based on the viability results obtained at 10% treatment with room temperature storage and an estimated time of 8 hours, a good yield of $74.4 \pm 4.39\%$ was obtained and it was still suitable for AI. According to Kusumawati (2016) that the percentage of spermatozoa that can be used for AI is at least 50% of the percentage of live and motile spermatozoa.

Abnormality after dilution

Assessment of spermatozoa abnormalities is important to determine if spermatozoa are suitable for AI purposes. This spermatozoa abnormality is still good for use in artificial insemination needs. According to Sekosi et al (2016) sperm for AI purposes should not contain more than 20% abnormal sperm. The results of abnormal spermatozoa in this study showed good results in the 10% treatment. The results of the average percentage of abnormalities at room temperature storage with the addition of different concentrations of pomegranate juice in egg yolk tris diluent can be seen in table 4

Table 4. The average percentage of abnormalities after adding different concentrations of pomegranate juice to egg yolk tris diluent.

Storage time	Treatment
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(hours)	P0	P1	P2	P3
0	6.6±1.82 ^b	4.8±1.79 ^{ab}	3.8±1.64 ^a	4.2±1.48 ^a
2	7.4±3.51 ^b	5±1.58 ^{ab}	4±1.00 ^a	5.6±2.19 ^{ab}
4	9.8±3.27 ^b	9.2±1.48 ^{ab}	5.6±2.88 ^a	9.6±3.51 ^{ab}
6	10.2±2.39 ^b	7.2±2.28 ^a	5.8±2.17 ^a	7.4±1.14 ^a
8	10.4±1.52 ^b	9.2±2.59 ^{ab}	6.6±2.70 ^a	8.6±1.67 ^{ab}

Note: Different superscripts in the same line show a significant difference ($P < 0.05$) and the same superscripts in the same line show no significant difference ($P > 0.05$).

The results of this study indicated that the presence of pomegranate juice in egg yolk tris diluent was able to maintain spermatozoa abnormalities at room temperature. The average percentage of abnormal spermatozoa after 8 hours of storage obtained at P0, P1, P2 and P3 were 10.4 ± 1.52 , 9.2 ± 2.59 , 6.6 ± 2.70 and 8.6 ± 1.67 percent, respectively. The results with the lowest abnormality were P2 treatment with the addition of 10% pomegranate juice. The results of the analysis obtained that the average abnormality at 8 hours of storage at room temperature had a significant difference ($P > 0.05$) between P2 and P0, but not significantly different from P1 and P3 ($P < 0.05$). All treatments containing pomegranate juice had the lowest abnormality.

The best abnormality in this study was the 10% pomegranate juice treatment with the lowest abnormality results from other treatments, namely 6.6 ± 2.70 percent. The quality of the spermatozoa is of good quality and suitable for use for AI. The abnormality results in this study were better than Amrillah's research (2021) where the high abnormality was 16.60 ± 0.89 percent using kiwi fruit juice in Boer goats at the same room temperature. Furthermore, Setiawan and Kusumawati (2017) abnormal spermatozoa of kacang goat at 5°C without diluents at 45 hours of storage, namely $14.2 \pm 0.84\%$,

The flavonoid content in pomegranate has been shown to contain antioxidants with a mechanism to protect sperm cell membranes from the effects of free radicals. Flavonoids are able to maintain the quality of spermatozoa so that abnormal spermatozoa are maintained at room temperature storage with good results (Louis et al., 2019). Flavonoid compounds, namely *lutheolin*, *querqetin*, and *kaempferol* are present in large quantities in pomegranate skin, while in the seeds there are anthochyandins compounds. The high content of vitamin C and antioxidants from pomegranate juice is good as a diluent medium to maintain abnormal spermatozoa (Rosalinda, 2021).

Abnormalities that occurred in this study were secondary abnormalities that occurred after treatment, which were observed in this study were abnormalities of the tail bent, tail broken, tail curled and head broken. The cause of this abnormality at the time of making preparations and shaking the test tube can affect secondary abnormalities. According to Nugroho and Saleh (2016) another thing that can affect spermatozoa abnormalities is the mixing time with the treatment.

The results of this study are still classified as normal abnormalities and deserve the need for artificial insemination (AI). The standard percentage of goat spermatozoa abnormality that is suitable for AI is not more than 15%. According to Sekosi et al (2016) semen for artificial insemination purposes should not contain more than 20% abnormal sperm.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The addition of pomegranate juice to egg yolk tris diluent was able to maintain the quality of spermatozoa of kacang goat at room temperature and storage time for 8 hours.
2. The addition of pomegranate juice with 10% treatment is the best treatment capable of maintaining motility, viability and abnormality of goat kacang spermatozoa at room temperature with storage for 8 hours.

Recommendations

1. The results of the study Semen of goat kacang in egg yolk tris diluent with the addition of pomegranate juice stored at room temperature for 8 hours can be used as a diluent for liquid semen for AI
2. Concentration of 10% pomegranate juice is best used as a diluent for liquid semen of goats for AI purposes.

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