Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.19.1.11-20
P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 19 Issue 1 January-March 2024

Effect of Thawing on Semen Quality of Boer Goats with Tris-aminomethane Diluent Supplemented with Antioxidant Quercetin

R. P. Sari, C. D. Nugraha, and Suyadi

Faculty of Animal Science, Universitas Brawijaya, Malang
Jl. Veteran Malang 65145 Jawa Timur
Corresponding Author: rossapurnamaa@student.ub.ac.id,
Revised: 2023-07-09, Accepted: 2024-03-26, Publish: 2024-03-30

ABSTRACT

This study aims to determine the effect of the thawing technique on the quality of Boer goat semen with tris aminomethane diluent supplemented with quercetin antioxidants. The materials used in this research are the semen of a Boer goat, which was collected with an artificial vagina. This study used experimental laboratory methods. The design pattern used is a Factorial Complete Randomized Design, which consists of two factors. Each treatment was repeated 4 times. The first factor is the thawing temperature with 3 levels of treatment those were 32°C, 37°C, and 40°C. The second factor is the thawing time with 3 treatment levels: 30 seconds, 60 seconds and 90 seconds. The variables were the motility percentage, viability, abnormality and membrane integrity. The results showed that thawing temperature had a significantly different effect ($P<0.01$) on the percentage of motility, viability, abnormality and membrane integrity. Thawing time had a significantly different effect ($P<0.01$) on the percentage of motility, abnormality and membrane integrity and gave affects significant ($P<0.05$) in the percentage of viability. Meanwhile, the interaction between thawing temperature and time is insignificant ($P>0.05$). The best result shown after thawing is in treatment thawing temperature at 37°C and treatment thawing time at 30 seconds.

Keywords: semen quality, thawing temperature, thawing time

INTRODUCTION

Artificial insemination (AI) is the most effective reproductive technology for genetically improving livestock species. Artificial Insemination (AI) is a technique in which semen collected from male livestock is processed in the laboratory and injected into the female reproductive tract using media at the right time (Singh et al., 2021). The AI program needs to be supported by improving the quality and quantity of semen. Semen's high quality is the basic factor that determines the success of the Artificial Insemination (AI) program because semen influences the dimensions of success for pregnant cows and contributes genetically to the offspring produced (Nugraha et al., 2023). Semen generally used in the AI program is semen processed as frozen semen. Frozen semen is semen that has been diluted and then frozen far below the freezing point of water, which aims to temporarily stop cell activity without damaging cell function. Before being used for AI, frozen semen must go through a thawing process.

Some factors can affect semen quality, namely temperature and length of thawing time. Temperature and thawing time significantly influence the state of spermatozoa, especially the integrity of spermatozoa in semen. The temperature during the thawing process needs to be appropriately considered because temperatures that are too high or too low can hurt the quality of spermatozoa. In addition, the length of thawing also has an important role; thawing time is too short or too long, which can affect spermatozoa's survival. The results of research from Kusumawati et al. (2016) reported that in frozen semen of Simmental cattle, temperature and thawing time affect individual motility, viability and abnormality with temperature treatments of 25°C and 37°C and thawing time of 7 seconds, 15 seconds and 30 seconds, with the optimal thawing technique being 37°C and 30 seconds. The right combination of temperature and thawing time can prevent damage to spermatozoa and maintain the quality of spermatozoa, so they have high fertility ability.

Inappropriate thawing methods can cause spermatozoa to experience increased metabolic processes that require high energy, reducing semen quality in motility, viability, abnormality and membrane integrity. Temperature changes during the thawing process can potentially trigger oxidative stress in sperm. A sudden significant rise in temperature can stimulate the production of free radicals and reduce the activity of antioxidant enzymes, leading to an imbalance

between free radicals and antioxidants in sperm cells. It can then lead to oxidative damage to the sperm cell membrane and sperm DNA. In addition, thawing time that is too long or too short can cause an imbalance between the production of free radicals and the antioxidant system in sperm. It can increase oxidative stress, damaging the sperm cell membrane and other components, such as DNA. Therefore, it is important to keep the thawing temperature and duration stable and avoid drastic temperature changes to reduce the risk of oxidative stress in sperm. Thawing methods in Indonesia are very diverse. To produce good semen quality, the Directorate General of Animal Husbandry standardizes the thawing method, namely the use of water at 37°C for 30 seconds because this temperature is the same as the physiological temperature of livestock and according to the Operational Standard of Work (SOP) of the Balai Besar Inseminasi Buatan (BBIB).

Providing additional antioxidants to the diluent can help protect sperm from the effects of oxidative stress during thawing. Nugraha et al. (2023) state that antioxidants can reduce and suppress free radical reactions. One of the antioxidants that can be used in frozen semen processing is quercetin. Quercetin can prevent lipid peroxidation to neutralize and inhibit the development of free radicals, interact with alpha-tocopherol to delay oxidation and promote gene expression of detoxification enzymes such as NAD(P)H-quinone oxidoreductase, glutathione s-transferase, and UDP-glucuronosyl transferase (Jamadi et al. 2017). Thus, using quercetin as an antioxidant agent in frozen semen processing can be an effective strategy to improve the quality and survival of spermatozoa after freezing. The above description is the basis for researching various thawing methods on the quality of frozen semen of Boer goats with Tris-aminomethane diluent supplemented with quercetin antioxidant. This can provide solutions for farmers and inseminators to improve the quality of frozen semen by IB requirements.

MATERIALS AND METHODS

The experiment occurred at Sumber Sekar Field Laboratory, Faculty of Animal Husbandry, University of Brawijaya and Biotechnology Laboratory, Faculty of Animal Husbandry, University of Brawijaya. The research was conducted from November 17, 2022, to January 14, 2023.

Goat Semen

The semen used came from a Boer goat at the Sumber Sekar Farm Field Laboratory. This Boer goat was five years old with a body weight of 70 kg. Semen collection was done twice per week, on Monday and Thursday at 08.00 WIB, using the artificial vagina method. It was carried out by experienced Sumber Sekar Animal Husbandry Field Laboratory officers. The requirements for fresh semen used were semen that had mass motility (++), individual motility ≥70%, viability ≥70% and abnormality <10%.

Dilution of Antioxidant Quercetin

Antioxidant quercetin with Sigma Aldrich brand is as much as 100 mg or 0,1 grams and has a molecular weight of 302,24 g/mol. 2. Calculate the amount of volume to be dissolved with the help of the following web: https://www.graphpad.com/quickcalcs/Molarityform.cfm

The total volume obtained was 330,8629 ml, then dissolved with 10% DMSO from the total volume of the solvent, which is 33,08629 ml. 90% distilled water from the solvent volume of 297,77661 ml was added to the solution, then homogenized and transferred to a beaker glass. Repeat until 297 ml of distilled water runs out. A quercetin solution with a concentration of 1000 µM was obtained. Place the quercetin solution on the tube and store it in the freezer.

Tris-aminomethane Egg Yolk Diluent

The diluent used in the study was Tris aminomethane egg yolk diluent. Mixing of Tris aminomethane diluent and egg yolk was done one day before semen collection. The procedure for mixing Tris aminomethane diluent with egg yolk is to separate the yolk and egg white. The egg yolk was put in the ratio of 20% egg yolk and 80% Tris aminomethane diluent into a beaker glass. Add 30 µM quercetin antioxidant into the beaker glass. Homogenize egg yolk, tris aminomethane diluent and quercetin antioxidant using a magnetic stirrer for 15-20 minutes. The solution was put into a centrifuge tube and then centrifuged at 1500 rpm for 30 minutes. After that, the supernatant was put into the tube and stored for 24 hours in a refrigerator. After that, the residue was discarded.

Processing Boer Goat Semen and Thawing Frozen Semen

Calculate the total volume of diluent with the formula:

$$\text{V. Total} = \frac{\text{V. Fresh Semen x Concentration Fresh Semen x } 10^6}{50\text{x}10^6} \text{ X } 0.25$$

Semen and VA1 (1:1) were added to a test tube in a beaker glass containing water at 37°C in a water jacket. Put the test tube containing semen + VA1 in the water jacket into the refrigerator. Calculated the volume of VA2 diluent with the formula:

$$\text{VA2} = \frac{\text{V. Total} - (\text{VA1} + \text{V. Fresh Semen})}{2}$$

After that, put VA2 diluent in a test tube containing semen + VA1 at 12-15°C. Calculate the volume of VB diluent containing 13% glycerol using the formula:

$$\text{VB} = \frac{\text{V. Total}}{2}$$

Then, put the diluent into a test tube containing semen + VA at 4-5°C. Equilibrated at cold temperature for 2 hours at 4-5 ° C. After that, the observation process is done before freezing. Then, filling is done into the straw using a micropipette with a blue tip filled with 0.25 ml. Then, the straw is dealt with in a way; the tip of the straw is closed using tweezers that have been heated over Bunsen. Straw is inserted in the goblet, and the goblet containing the straw is inserted into the canister. Liquid nitrogen vapor was equilibrated at a height of 10 cm for 10 minutes. The canister containing the goblet and straw was immersed in liquid nitrogen at a temperature of -196 ° C. After 24 hours, the straw was frozen and thawed into the water bath according to treatment. After that, the semen that has been thawing is tested for quality.

Evaluation of Cement Quality

a. Individual Motility

Boer goat semen is taken using an ose, then placed on an object glass and covered with a cover glass. Observe on a microscope with a magnification of 400x and assess by looking at spermatozoa that move progressively forward for 5 views. Individual motility is given a value of 0% - 100%.

b. Viability

Take one drop of semen using an ose, and place it on the tip of the object glass. Drop one drop of eosin-negrosin dye, then homogenize clockwise. Review using an object glass forming a 45° angle. Wait until dry or heated with a quick movement on the bunsen for 1 - 2 seconds. Observe on a microscope with 400x magnification. Count spermatozoa that absorb colour from at least 200 spermatozoa using HTC (Hand Tally Counter). Then, calculate the percentage of viability with the formula:

$$\text{Viabilitas} = \frac{\text{Viable Spermatozoa}}{\text{Total Spermatozoa}} \text{ X } 100\%$$

c. Abnormality

Take one drop of semen using an ose, and place it on the tip of the object glass. Drop one drop of eosin-negrosin dye, then homogenize clockwise. Review using an object glass forming a 45° angle. Wait until dry or heated with a quick movement on the bunsen for 1 - 2 seconds. Observe on a microscope with 400x magnification. Count normal and abnormal spermatozoa using HTC (Hand Tally Counter). Then, calculate the percentage of abnormality with the formula:

$$\text{Abnormality} = \frac{\text{Abnormal Spermatozoa}}{\text{Total Spermatozoa}} \text{ X } 100\%$$

d. Membrane Integrity

Take 0.1 ml of semen, placed in a test tube, added 150 mOsml / litre HOS solution in a ratio for fresh semen: HOS solution = 1: 10, while semen + diluent: HOS solution = 1: 4. Incubate at 37°C for 30 minutes. After that, take one drop using an ose, place it on an object glass, and then cover it with a cover glass. Observe with a microscope using 400x magnification. Count the number of spermatozoa with circular tails from the total number counted. Then, calculate the percentage of membrane integrity with the formula:

$$\text{Membran Integrity} = \frac{\text{Coiled Tail Spermatozoa}}{\text{Total Spermatozoa}} \text{ X } 100\%$$

Research Design

This study used a laboratory experiment method. The design pattern used was a completely randomized design (CRD) factorial of two factors with a 3x3 pattern. The first factor is thawing temperature of 3 treatment levels,

namely thawing temperature of 32°C, 37°C and 40°C. The second factor is the length of thawing from 3 treatment levels, namely the length of thawing time of 30 seconds, 60 seconds and 90 seconds. Both factors were combined, resulting in a 3x3 = 9 treatment combinations. Each treatment was repeated four times. Each experimental unit consisted of 0,1 ml of Boer goat semen.

Data Analysis

The research data were analyzed statistically using analysis of variance (ANOVA) with a Factorial Complete Randomized Design. Data were analyzed with the help of IBM SPSS Statistics software version 26. If the results obtained showed a real difference in effect ($P < 0.05$) or authenticity ($P < 0.01$), further testing was carried out using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Evaluation of Fresh Semen

Boer goat semen collected is then examined macroscopically, including volume, consistency, pH, odour and colour. In addition, fresh semen is also observed microscopically, including mass motility, individual motility, viability, abnormality and membrane integrity. The results of fresh semen quality evaluation can be seen in Table 1.

Table 1. Quality of Boer goat fresh semen

Variable	Average
Macroscopic	
Volume (ml)	1.1 ± 0.14
Consistency	Concentrated/Thick
Colours	Yellowish-white
Smell / Aroma	Typical
pH	6.5 ± 0.71
Microscopic	
Mass Motility	(+++)
Individual Motility (%)	85 ± 7.07
Concentration (10^6/ml)	6010 ± 947.52
Viability (%)	90.96 ± 2.47
Abnormality (%)	1.92 ± 0.43
Membrane Integrity (%)	69.81 ± 3.94

Evaluation of semen volume is critical in knowing the concentration of spermatozoa per ejaculation. The results of the macroscopic evaluation of fresh semen volume of Boer goats in the study had an average of 1.1 ± 0.14 ml. The volume of fresh semen used in this study is in the normal range, following the explanation of Susilawati (2013), which states that the volume of goat semen per ejaculation has an average of 1 ml with a range of 0.5-1.2 ml. The consistency of fresh semen of Boer goats in this study is thick or concentrated. The consistency of fresh Boer goat semen used in this study is in the normal range, and it follows the opinion of Kostaman and Sutama (2006), who explain that Boer goat semen has a thick consistency.

Semen colour is influenced by the consistency and concentration of spermatozoa contained therein; the thinner and lower the concentration of semen, the fainter the colour of the semen. The fresh semen of Boer goats in this study is yellowish-white, so it can be said to be expected, and there is no contamination. It agrees with Susilawati (2013), explaining that normal semen is yellowish-white or milky white. The smell of semen generally has a distinctive odour from the livestock; if there is an unpleasant odour (rotten), there is the possibility of contaminated semen. The smell of fresh Boer goat semen in this study is that it has a distinctive fishy odour from livestock, so it can be said to be expected, and there is no contamination. It follows the explanation of Agustian et al. (2014), who state that the aroma of Boer goat semen is the fishy smell of livestock.

The concentration of spermatozoa contained therein influences the degree of acidity or pH of semen. The higher the concentration of semen, the lower the pH of the semen will be. The average result of the pH examination of Boer goat semen in this study was 6.5 ± 0.71.

This result follows the opinion of Suyadi et al. (2004) that the degree of acidity (pH) of Boer goat semen is relatively mildly acidic, 6.4-7.6 or an average pH of 6.8. It indicates that the Boer goat semen used in this study is expected.

Motility is one of the indicators of semen quality related to spermatozoa fertility. The higher the mass motility scale, the better the semen quality. The observation of mass motility is (+++), has a fast movement, looks like big waves, very dark and thick. This indicates that the semen is good quality and suitable for further processing. Susilawati (2011) explains that the criteria for assessing the motion of spermatozoa mass are excellent (+++) visible large, dark, thick, and active waves that move quickly and move around; good (++) visible small waves are thin, rare, less transparent and move slowly; less sound (+) if no waves are seen but progressive active individual movements; bad (0) seen only a few individual movements.

Individual motility is one of the criteria determining the quality of semen, seen from the number of progressively motile spermatozoa compared to all spermatozoa in one microscope view. The motility of individual spermatozoa in the observation results is 85 ± 7.07%. The motility is still within the normal range. Yulnawati and Setiadi (2005) stated that the use of fresh semen below the standard of 70% will result in a rapid decline in quality.

Spermatozoa concentration is the number of spermatozoa cells contained in one millilitre of semen. The calculation method uses a red blood cell counting device or haemocytometer. The average concentration of Boer goat semen observed was 6010 ± 947.52 (10^6/ml). Susilawati (2013) stated that the concentration of goat semen ranged from 2.5-5.0 x 10^9. Assessment of spermatozoa concentration is critical because it is used to determine the quality of semen and the addition of diluent to be used.

Viability is one of the indicators determining the quality of semen related to the life and death of spermatozoa. Spermatozoa can be assessed by calculating the number of live spermatozoa after staining using eosin-negrosin. Live spermatozoa are characterized by not absorbing colour, and dead spermatozoa will absorb the colour. The average viability of fresh semen of Boer goats from the observation results is 90.96 ± 2.47%, indicating that the semen is still classified as good. Susilawati (2013) states that the percentage of live spermatozoa is 70-90% motile.

Several abnormalities, such as small head, large head, conical head shape, oblique head, double head, round head, double tail, misaligned acrosoma, and large neck, characterize primary abnormality. Secondary abnormalities are characterized by detached head, broken neck, broken tail and curled tail (Rizal and Herdis, 2008). In this study, abnormal sperm that are often found are secondary abnormalities. The average abnormality of fresh Boer goat semen obtained was 1.92 ± 0.43%. Susilawati (2013) explained that if the abnormality reaches 15%, the semen can no longer be used for IB.

Spermatozoa membrane integrity is the integrity of the spermatozoa membrane or a state that shows the mechanism of physiological function of the membrane. The average percentage of fresh semen membrane integrity of Boer goats during the study was 69.81 ± 3.94%. The result of fresh semen membrane integrity is lower than Pamungkas et al. (2014), which amounted to 77.52 ± 7.35%. The intact plasma membrane of spermatozoa has a positive correlation with spermatozoa motility. In contrast, the better the plasma membrane of spermatozoa will cause the movement of spermatozoa to be more active.

Post-thawing Spermatozoa Motility

Individual motility is one of the most important parameters to evaluate spermatozoa fertility. Individual motility can be seen from the number of spermatozoa moving progressively compared to all in five microscope views. Samsudewa (2008) states that post-thawing motility (PTM) of frozen semen that is not suitable for IB is that which has a spermatozoa motility value <40%. Data on the average percentage of individual motility in frozen semen of Boer goats after thawing can be seen in Table 2.

Based on the data in Table 2. the results of the analysis of variance showed that thawing temperature had a very significant effect (P <0.01) on the motility of individual Boer goat spermatozoa with Tris aminomethane diluent that had been supplemented with quercetin antioxidant as much as 30 µM. The results showed that the highest mean value in the thawing temperature treatment was at 37°C with an average of 45.00 ± 9.77% and the lowest mean of 29.58 ± 7.22% at 40°C. A thawing temperature of 37°C is a temperature that can maintain the motility of individual post-thawing Boer goat spermatozoa.

Table 2. Post-thawing Boer Goat Frozen Semen Motility

Post-thawing Spermatozoa Motility (%)				
	Thawing Time			Average
Thawing Temperature	30 second	60 second	90 second	
32°C	31.25±2.50^{bc}	36.25±2.5^{bc}	32.50±2.89^{bc}	33.33±3.26^{a}
37°C	51.25±8.54^{d}	48.75±6.29^{d}	35.00±5.77^{bc}	45.00±9.77^{b}
40°C	37.50±2.89^{c}	28.75±2.5^{ab}	22.50±5.00^{a}	29.58±7.22^{a}
Average	40.00±10.00^{b}	37.92±9.41^{b}	30.00±7.07^{a}	

Notes: Different notations on the same line indicate very significant differences (P<0.01)

It is estimated that high temperatures can make spermatozoa experience death. It is supported by the opinion of Zelpina et al. (2012), who state that high temperatures in the thawing media will cause the metabolic process of spermatozoa to increase, requiring high energy as well. Such conditions cause spermatozoa to lose energy, resulting in the death of spermatozoa quickly.

The analysis of the variance of the data in Table 2. shows that the length of thawing has a genuine influence (P <0.01) on the motility of individual spermatozoa. The results showed that the treatment of thawing time with 30 seconds gave the highest average of 40.00 ± 10.00% and the lowest average of 30.00 ± 7.07% at 90 seconds. A thawing time of 30 seconds is a time that can maintain the motility of individual post-thawing Boer goat spermatozoa. The possibility of too long thawing time can cause an increase in free radicals. It is supported by the opinion of Datta et al. (2009), which states that spermatozoa that are too long exposed to oxygen cause an increase in the production of free radicals that produce lipid peroxides, as a factor causing damage to the spermatozoa membrane.

The data in Table 2. analyzed, showed that the interaction of temperature and thawing time had a very significant effect (P < 0.01). A good combination of temperature and thawing time causes minor damage to sperm cells, so they can still fertilize eggs (Aprilina et al., 2014). According to the observations, the interaction of temperature and thawing time with a combination of 37°C thawing temperature and 30 seconds thawing time gave the highest average of 51.25 ± 8.54% and the lowest average in the combination of 40°C thawing temperature and 90 seconds thawing time with an average of 22.5 ± 5%. According to Ansary et al. (2010), good motility is achieved through the thawing technique using water at 37°C and 30 seconds because lower thawing temperatures will produce lower motility numbers. Thawing temperatures that are too high do not give good results. If the temperature change from liquid nitrogen to thawing temperature is too high, the spermatozoa metabolic process will occur faster. The metabolic process of spermatozoa requires energy; if spermatozoa runs out of energy, it results in weak progressive movement of spermatozoa and can even cause death.

Post-thawing Spermatozoa Viability

Viability is one of the parameters to evaluate semen quality by looking at the percentage of live and dead spermatozoa. Assessment of spermatozoa can be seen based on the calculation of the number of live spermatozoa after staining using eosin-negrosin and then observation with a 400x magnification microscope. The number of live and dead spermatozoa was observed in several fields of view so that a minimum cell count of 200 spermatozoa cells was obtained. Live spermatozoa look bright because they do not absorb eosin-negrosin dye, while dead spermatozoa look dark because they absorb eosin-negrosin dye. Data on the average percentage of viability in frozen semen of Boer goats after thawing can be seen in Table 3.

Based on the data in Table 3. the results of the analysis of variance showed that thawing temperature had a significant effect (P<0.05) on the viability of Boer goat spermatozoa with Tris aminomethane diluent that had been supplemented with quercetin antioxidant as much as 30 µM. The results of the observations in Table 3. indicate that the highest mean value in the treatment of 37°C thawing temperature with an average of 64.89 ± 5.31%, and the lowest mean of 60.21 ± 5.95% at 40°C temperature. The thawing temperature of 37°C is the temperature that can maintain the viability of post-thawing Boer goat spermatozoa. It is suspected that high temperatures can make spermatozoa experience death. Oyeyemi et al. (2000) stated that when there is an inappropriate temperature change extracellularly, the permeability of hydrophilic

Table 3. Post-thawing Boer Goat Frozen Semen Viability

Post-thawing Spermatozoa Viability (%)				
	Thawing Time			Average
Thawing Temperature	30 second	60 second	90 second	
32°C	60.30±3.95	61.72±3.73	60.97±3.91	60.99±3.55[a]
37°C	68.24±4.83	65.83±4.50	60.60±4.37	64.89±5.31[b]
40°C	64.44±4.01	60.17±6.35	56.02±5.11	60.21±5.95[a]
Average	64.32±5.15[b]	62.57±5.15[ab]	59.20±4.69[a]	

Notes: Different notations on the same line indicate significant differences (P<0.05)

phospholipids is damaged and causes membrane fluidity to be disrupted, resulting in spermatozoa death.

The analysis of variance from the data in Table 3. showed that the length of thawing had a significant effect (P <0.05) on the viability of spermatozoa. The results showed that the treatment of thawing time with 30 seconds gave the highest average of 64.32 ± 5.15% and the lowest average of 59.20 ± 4.69% at 90 seconds. Thawing 30 seconds is the ideal time for thawing Boer goat spermatozoa. It is estimated that if thawing is too long, spermatozoa will be exposed to oxygen, which can cause death. The factor that causes the low percentage of live spermatozoa after thawing is the amount of lactic acid from the metabolic products of spermatozoa that cannot be oxidized. Thawing time that is too long will cause metabolic activity to increase and take place en masse, resulting in increased lactic acid production (Aprilina et al., 2014).

Data analysis in Table 3. shows that the interaction of temperature and thawing time does not have a significant effect (P>0.05) on the viability of spermatozoa. It shows that thawing temperature and thawing time influence the viability of spermatozoa. Still, the thawing temperature and thawing time do not affect each other on the viability of frozen semen of Boer goats.

Post-thawing Spermatozoa Abnormality

Abnormality is one of the parameters to evaluate semen quality by looking at the percentage of normal and abnormal spermatozoa. Assessment of spermatozoa can be seen based on the calculation of the number of normal spermatozoa and abnormal spermatozoa after staining using eosin-negrosin then observed with a magnification microscope. Data on the average percentage of abnormalities in the frozen semen of Boer goats after thawing can be seen in Table 4.

Based on the data in Table 4. the results of the analysis of variance showed that thawing temperature gave a very significant effect (P <0.01) on the abnormities of Boer goat spermatozoa with Tris aminomethane diluent that had been supplemented with quercetin antioxidant as much as 30 µM after thawing. The observation results from the data table 4. show that the highest mean value in the treatment of thawing temperature is in the treatment of 40 ° C with an average of 6.90 ± 1.38% and the lowest mean of 5.55 ± 1.07% at a temperature of 37 ° C. The highest mean value in the treatment of thawing temperature is 40 ° C with an average of 6.90 ± 1.38%. The length of thawing also has a very significant effect (P <0.01) on spermatozoa abnormality. The results showed that the treatment of thawing time with 90 seconds gave the highest average of 7.28 ± 1.24% and the lowest average of 5.72 ± 1.06% at 30 seconds. Thawing in 30 seconds is ideal to provide resistance to Boer goat spermatozoa.

Table 4. Post-thawing Boer Goat Frozen Semen Abnormality

Post-thawing Spermatozoa Abnormality (%)				
	Thawing Time			Average
Thawing Temperature	30 second	60 second	90 second	
32°C	6.83±1.02[d]	6.20±0.88[bcd]	6.89±0.95[d]	6.64±0.92[a]
37°C	4.84±0.23[a]	5.24±0.50[ab]	6.58±1.29[cd]	5.55±1.07[b]
40°C	5.50±0.54[abc]	6.83±0.73[d]	8.37±0.79[e]	6.90±1.38[b]
Average	5.72±1.06[a]	6.09±094[a]	7.28±1.24[b]	

Notes: Different notations on the same line indicate very significant differences (P<0.01)

The analysis of variance from the data in Table 4 shows that the interaction of temperature and thawing time has a significant effect (P <0.05). Data in Table 4. shows that the interaction of temperature and thawing time, the combination of 37°C thawing temperature and 30 seconds thawing time, gives the lowest average of 4.84 ± 0.23% and the highest average in the combination of 40°C thawing temperature and 90 seconds thawing time with an average of 7.28 ± 1.24%. The increase in abnormality may be due to kneading, temperature shock and oxygen contamination. Other factors that affect the increase in abnormality are less careful actions during treatment, thawing semen with fluids that are not equally isotonic, cold shock, heat shock, and nutritional disorders (Susilawati, 2011).

Post-thawing Spermatozoa Membrane Integrity

Membrane integrity is one of the parameters to evaluate the quality of semen by looking at the condition of the plasma membrane of spermatozoa. Assessment of spermatozoa can be seen based on the calculation of the number of spermatozoa whose membranes are still intact and spermatozoa whose membranes have been damaged after being added to HOS (Hypo Osmotic Swelling) solution and incubated for 30 minutes at 37°C, then observed with a 400x magnification microscope and observed in several fields of view so that a minimum cell count of 100 spermatozoa cells is obtained. Spermatozoa whose membrane conditions are intact are seen at the end of the tail in a circle, while spermatozoa whose membranes are damaged are seen with straight spermatozoa tails. Data on the average percentage of viability in frozen semen of Boer goats after thawing can be seen in Table 5.

Based on the data in Table 5. the results of the analysis of variance showed that thawing temperature had a very significant effect (P <0.01) on the integrity of the Boer goat spermatozoa membrane with Tris aminomethane diluent, which had been supplemented with quercetin antioxidant as much as 30 µM. The observation results from Table 5. show that the highest mean value in the thawing temperature treatment is at 37°C with an average of 23.63 ± 3.38% and the lowest mean of 18.91 ± 4.56% at 40°C. A thawing temperature of 37°C is a temperature that can maintain the integrity of the Boer goat spermatozoa membrane. It is suspected that high temperatures can cause spermatozoa to experience heat shock. Following Aprilina et al. (2014), the thawing temperature of 40°C will take longer to adjust to the ambient temperature. As a result, there will be a heat shock effect on spermatozoa, thus affecting the membrane's stability, which impacts the quality of frozen semen.

The results of the variance analysis from the data are in Table 5. showed that the length of thawing had a very significant effect (P <0.01) on the integrity of the membrane post-thawing. The results showed that the treatment of thawing time with 30 seconds gave the highest average of 22.93 ± 3.66% and the lowest average of 18.06 ± 3.42% at 90 seconds. A thawing time of 30 seconds is the time that can maintain the integrity of the Boer goat spermatozoa membrane. It is suspected that too long thawing can cause an increase in free radicals. It agrees with Datta et al. (2009), which states that spermatozoa that are too long exposed to oxygen cause an increase in the production of free radicals that produce lipid peroxides as a factor causing damage to the spermatozoa membrane. The results of the variance analysis of the data are in Table 5. showed that the interaction of temperature and thawing time had a very significant effect (P <0.01) on the integrity of the Boer goat semen membrane with Tris aminomethane diluent that had been supplemented with quercetin antioxidant as much as 30 µM.

Table 5. Post-thawing Boer Goat Frozen Semen Membrane Integrity

Post-thawing Spermatozoa Membrane Integrity (%)				
	Thawing Time			Average
Thawing Temperature	30 second	60 second	90 second	
32°C	19.15±0.80^{b}	22.33±3.03^{bc}	20.15±0.79^{b}	20.54±2.19^{a}
37°C	26.31±2.04^{d}	24.08±1.08^{cd}	20.05±1.76^{b}	23.63±3.38^{b}
40°C	23.34±3.11^{cd}	19.41±1.31^{b}	13.98±2.41^{a}	18.91±4.56^{a}
Average	22.93±3.66^{b}	21.94±2.71^{b}	18.06±3.42^{a}	

Notes: Different notations on the same line indicate very significant differences (P<0.01)

The observation results are in the data in Table 5. shows that the combination of 37°C thawing temperature and 30 seconds thawing time gives the highest average of 26.31 ± 2.04% and the lowest average in the combination of 40°C thawing temperature and 90 seconds thawing time with an average of 13.98 ± 2.41%. It is estimated that the cause of membrane damage is due to shock and lipid peroxides. Salim et al. (2012) believe thawing temperature and duration are inappropriate, resulting in spermatozoa membrane damage due to heat stress and contact with oxygen. The spermatozoa membrane composed of phospholipids is reduced due to the generation of fatty acids from the cell peroxidation process.

CONCLUSION

Based on the results of the discussion, it can be concluded that thawing temperature treatment affects the percentage of individual motility, viability, abnormality and membrane integrity. The highest average of individual motility, viability, abnormality and membrane integrity is found in the treatment with 37°C thawing temperature. In treating thawing length, the percentage of individual motility, viability, abnormality and membrane integrity is influenced. The highest average of individual motility, viability, abnormality and membrane integrity was found in the 30-second thawing treatment. In addition, the interaction between thawing temperature and thawing time influences the percentage of individual motility, abnormality and membrane integrity. The average individual motility, disorder and membrane integrity were highest at 37°C and 30 seconds of thawing time. Based on the results of this study, it can be concluded that the thawing temperature of 37°C and thawing time of 30 seconds show the best motility, viability, abnormality and membrane integrity so that it can provide the best quality in frozen semen of Boer goats.

REFERENCES

Agustian, M.F., M.N. Ihsan dan N. Isnaini. 2014. Pengaruh Lama Simpan Semen dengan Pengencer Tris Aminomethan Kuning Telur pada Suhu Ruang terhadap Kualitas Spermatozoa Kambing Boer. J. Ternak Tropika. 15 (2): 1-6.

Ansary, M.S., A. Bushra, Rakha and S. Akher. 2010. Effect of Straw Size and Thawing Time on Quality of Cryopreserved Buffalo (*Bubalus bubalis*) Semen. J Reproductive Biology. 11(1): 49-54.

Aprilina, N., S. Suharyati dan P.E. Santosa. 2014. Pengaruh Suhu dan Lama Thawing di Dataran Rendah terhadap Kualitas Semen Beku Sapi Simmental. Jurnal Ilmiah Peternakan Terpadu 2(3): 96-102.

Arifiantini, R. I., Wresdiyati, T dan Retnani, E. F. 2006. Pengujian MorfologiSpermatozoa Sapi Bali *(Bos sondaicus*) Menggunakan Pewarnaan "Williams". J. Indontrop. Anim. Agric. 31 (2):105 -110

Datta, U., M.C. Sekar, M.L. Hembram, R. Dasgupta. 2009. Development of a New Methode to Preserve Caprine Cauda Epididymal Spermatozoa in situ at 10°C. Procedings. Departement of Veterinary Gynaecology & Obstetrics Faculty of Veterinary and Animal Sciences West Bengal University of Animal and Fishery Sciences. Kolkata West Bengal. India.

Hafez, B. and E.S.E. Hafez. 2008. Reproduction in Farm Animals. 7th ed. Blackwell Publishing. Australia.

Herdis. 2015. Daya Motil dan Keutuhan Membran Plasma Spermatozoa Domba Garut (Ovis aries) pada Penambahan Kolestrol dalam Pengencer Semen Tris Kuning Telur. JSTI. 17(1): 16-23.

Isnaini, N. 2011. Viabilitas Spermatozoa Kambing Boer Pasca Pendinginan Dan Pembekuan Menggunakan Pengencer Dasar Tris Dengan Level Trehalosa Yang Berbeda. J. Ternak Tropika 12 (1): 27-37.

Jamadi, A.S., E. Ahmad, M. Ansari and H. Kohram. 2017. Antioxidant effect of quercetin in an extender containing DMA or glycerol on freezing capacity of goat semen. Cryobiology. 75: 15-20.

Kostaman, T. dan I.K. Sutama. 2006. Studi Motilitas Dan Daya Hidup Spermatozoa Kambing Boer Pada Pengencer Tris-Sitrat-Fruktosa. J. Sain Veteriner 24 (1): 58-63.

Kusumawati, E.D. 2017. Inseminasi Buatan. Malang: Media Nusa Creative

Kusumawati, E.D. 2015. Sexing Spermatozoa Kambing. Media Nusa Creative: Malang

Kusumawati, E.D., A.T.N. Krisnaningsih, dan R.R. Romadlon. 2016. Kualitas

spermatozoa Semen Beku Sapi Simental dengan Suhu dan Lama thawing yang Berbeda. Jurnal Ilmu-Ilmu Peternakan 26 (3): 38 – 41.

Kusumawati, E.D., K.N. Utomo, A.T.N. Krisnaningsih dan S. Rahadi. 2017. Kualitas Semen Kambing Kacang Dengan Lama Simpan Yang Berbeda Pada Suhu Ruang Menggunakan Pengencer Tris Aminomethan Kuning Telur. JITRO. 4 (3): 42 – 51.

Nugraha, C.D., D.D. Pangestu, dan Suyadi. 2023. Pengaruh Konsentrasi Antioksidan Genisteine Terhadap Kualitas Semen Sapi Peranakan Ongole Selama Proses Penyimpanan Suhu Dingin. Prosiding Seminar Nasional Teknologi dan Agribisnis Peternakan X: "Peningkatan Kapasitas Sumberdaya Peternakan dan Kearifan Lokal untuk Menghadapi Era Society 5.0", Fakultas Peternakan Universitas Jenderal Soedirman: 20 – 21 Juni 2023. Hal. 393 – 399.

Nugraha, C.D., R.F. Putri, A. Ardiantoro, W.A. Septian, A. Furqon, Kuswati, N. Widodo and Suyadi. 2023. Effect of Genistein Supplementation in Tris-Aminomethane Base Extender on Characteristics of Pre-Freezing and Post-Thawed Sperm Ongole Grade Bull. Advances in Animal and Veterinary Sciences 11 (7): 1135 – 1143.

Oyeyemi, M., M.O. Akusu and O.E. OlaDavis. 2000. Effect of Successive Ejaculations on the Spermiogram of West African Dwarf Goats (*Capra hircus* L.). Jurnal Veterinarski Arhiv. 70(4): 215-221.

Pamungkas, F. A., A. Batubara, dan Anwar. 2014. Kriopreservasi Spermatozoa Kambing Boer: Perbandingan Dua Bahan Pengencer terhadap Kualitas Post-thawing dan Kemampuan Fertilisasinya. Jurnal Ilmu Ternak dan Veteriner. 19: 130-137

Rizal, M. dan Herdis. 2008. Inseminasi Buatan Pada Domba. Jakarta: Rineka Cipta.

Salim, M. A., T. Susilawati dan S. Wahyuningsih. 2012. Pengaruh Metode Thawing terhadap Kualitas Semen Beku Sapi Bali, Sapi Madura dan Sapi PO. Agripet. 12 (2): 14 -20.

Samsudewa, D. 2008. Pengaruh Berbagai Metode Thawing terhadap Kualitas Semen Beku Sapi. Seminar Nasional Teknologi Peternakan dan Veteriner. Fakultas Peternakan, Universitas Diponegoro, Semarang.

Sayoko, Y., M. Hartono, dan P. E. Silotonga 2007. Faktor-faktor yang Mempengaruhi Persentase Spermatozoa Hidup semen Beku Sapi Pada Berbagai Inseminator di Lampung Tengah. Kumpulan Abstrak Skripsi Jurusan Produksi Ternak Fakultas Pertanian. Universitas Lampung, Lampung.

Singh, M., R. T. Mollier, N. Pongener and P. R. Sharma. 2021. Artificial insemination in pig for enhancing the pig productivity in North Eastern Hill region. Indian Farming 71 (2): 20 – 22.

Susilawati, T. 2013. Pedoman Inseminasi Buatan pada Ternak. Malang: UB Press.

Susilawati, T.. 2011. Spermatology. UB Press: Malang.

Suyadi, T. Susilawati dan N. Isnaini. 2004. Uji Coba Produksi Semen Beku Kambing Boer. Laporan Penelitian Kerjasama Ditjen Peternakan – Fakultas Peternakan Universitas Brawijaya Malang.

Toelihere, M. R. 1985. Fisiologi Reproduksi pada Ternak. Angkasa, Bandung: Penerbit Angkasa

Yulnawati dan M. A. Setiadi. 2005. Motilitas dan Keutuhan Membran Plasma Spermatozoa Epididimis Kucing selama Penyimpanan pada Suhu 4°C. J Med Vet. 21(3): 100-104.

Yulnawati., Herdis, H. Maheswari, A. Boediono dan M. Rizal. 2009. Potensi Reproduksi dan Upaya Pengembangbiakan Kerbau Belang Tana Toraja. Seminar dan Lokakarya Nasional Kerbau.

Zelpina, E., B. Rosadi dan T. Sumarsono. 2012. Kualitas Spermatozoa Post thawing Dari Semen Beku Sapi Perah. Jurnal Ilmu-Ilmu Peternakan 15 (2): 98-100.