Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan
ISSN 2303-2227 eISSN 2615-594X
Accredited by National Journal Accreditation No. 105/E/KPT/2022

Vol. 11 No. 3 October 2023, Page: 133-140
DOI: https://doi.org/10.29244/jipthp.11.3.133-140
Available online at https://journal.ipb.ac.id/index.php/ipthp/index

Effect of Concentration of Antioxidant Quercetin on Semen Quality of Boer Goat during Cold Storage using Andromed Extender

R. N. B. Athalla*, C. D. Nugraha, & Suyadi*
Faculty of Animal Science Universitas Brawijaya Jalan Veteran Ketawanggede, Kec. Lowokwaru, Kota Malang, Jawa Timur, Indonesia 65145
*Corresponding Author: naufal_bagas@student.ub.ac.id, suyadi@ub.ac.id
(Received 16-06-2023; Revised 04-08-2023; Accepted 10-08-2023)

ABSTRACT

Artificial insemination (AI) is a reproductive technology aimed at increasing livestock productivity resulting in animals with superior genetics. Storing semen at cold temperatures can damage sperm quality, one way to improve semen quality is by adding antioxidants such as Quercetin, which has been shown to have a positive effect on sperm quality. The objective of this research is to determine the effect of Quercetin on sperm quality in cold storage using Andromed extender on 5 years old Boer goat using artificial vagina. The method of this research was using Completed Randomized Design (CRD) Factoral two-factors. Variable being tested was motility, viability, abnormality and membrane integrity of spermatozoa. Data was analyze using Analysis of Variance (ANOVA), if there was a significant or significantly differences of treatment then continued by Duncan's Multiple Range Test (DMRT). Result showed that additional of *quercetin* and storage time had a significantly differences (P<0.01) effect on motility, viability and membrane integrity and did not have significant (P<0.05) effect on percentage of abnormality. The best result shown that additional of *quercetin* at 30 µM diluted with Andromed extender for 24-hour cold storage has the best treatment for semen quality.

Keywords: Artificial insemination, quercetin, sperm quality, storage time

ABSTRAK

Pada penelitian ini dilakukan pengamatan terhadap pengaruh penambahan kadar Quercetin dengan konsentrasi 0, 10, 20, dan 30 µM terhadap kualitas semen kambing Boer selama penyimpanan dingin pada suhu 3-5 ^{o}C menggunakan pengencer Andromed. Bahan penelitian ini diperoleh dari kambing Boer berusia 5 tahun menggunakan metode vagina buatan. Metode penelitian yang digunakan adalah Rancangan Acak Lengkap (RAL) Faktorial dua faktor. Variabel yang diuji meliputi motilitas, viabilitas, kelainan, dan integritas membran sperma. Data dianalisis menggunakan Analisis Variansi (ANOVA), dan apabila terdapat perbedaan signifikan antar perlakuan, dilanjutkan dengan Uji Jarak Berganda Duncan (DMRT). Hasil penelitian menunjukkan bahwa penambahan quercetin memberikan perbedaan yang signifikan (P<0.01) pada persentase motilitas dan viabilitas, tetapi tidak signifikan (P<0.05) pada persentase kelainan dan integritas membran. Lama penyimpanan juga memberikan perbedaan yang signifikan (P<0.01) pada persentase motilitas, viabilitas, dan integritas membran, tetapi tidak signifikan (P<0.05) pada persentase kelainan. Hasil terbaik menunjukkan bahwa penambahan quercetin sebanyak 30 µM yang diencerkan dengan pengencer Andromed merupakan perlakuan terbaik untuk kualitas semen selama penyimpanan dingin pada suhu 3-5 ^{o}C, dan waktu penyimpanan selama 24 jam memberikan kerusakan terbaik pada motilitas, viabilitas, kelainan, dan integritas membran.

Kata kunci: Inseminasi buatan, kualitas sperma, lama penyimpanan, quercetin

INTRODUCTION

Artificial insemination (AI) is one of reproduction technology that has objective to increase livestock productivity. AI is expected to assist breeders in improving the genetic quality of livestock by reducing the risk of spreading certain diseases to livestock (Amidia *et al.* 2021). The superiority of AI lies on its potential as a reproductive technology that can increase genetic rates in livestock populations using males that have superior genetics (Moore and Hasler 2017) which have an effect on increasing milk, hair and meat production in livestock. AI has efficiency value because it is able to produce more yields when compared to natural mating. Warmadewi and Bidura (2021) stated that natural mating is capable of producing 50 to 70 cattles in one year, but with AI it is capable of producing 5,000

to 10,000 cattles per year so that AI becomes an efficient reproductive technology.

AI employs liquid or thawed frozen semen from males with superior genetics. This semen is subjected to a preservation process or storage technique at cold temperatures (4-5 °C) to inhibit microorganism activity that might damage spermatozoa membranes. However, prolonged cold storage jeopardizes spermatozoa quality due to nutrient depletion, which reduces energy for metabolism and ultimately leads to spermatozoa death. Nutrient supplementation, such as extenders, is essential for preserving spermatozoa quality (Nurcholis *et al.* 2016) with Andromed being a commonly used extender. Andromed supplementation helps maintain semen quality, utilizing its contained nutrients in spermatozoa metabolism.

Deterioration in spermatozoa quality stems from excessive metabolite levels resulting from heightened metabolic activity during cold storage. This leads to lipid peroxidation reactions between metabolites and spermatozoa membranes. Metabolites generated in metabolic processes are reactive due to the loss of electrons, making them prone to reacting with other molecules (Dewajanti 2017).

Quercetin is a flavonoid compound that can be found naturally in plants and plant food sources such as fruits, whole grains and vegetables such as onions. Quercetin is considered a powerful antioxidant because of its ability to scavenge free radicals and bind transition metal ions, these properties of quercetin allow it to inhibit lipid peroxidation which can damage the spermatozoa membrane. Quercetin also combats inflammation by scavenging free radicals (Baghel *et al.* 2012).

Tvrda *et al.* (2020) explained that quercetin is capable of preserving the quality of Duroc pig semen in terms of motility, membrane stability, and suppressing lipid peroxidation caused by Reactive Oxygen Species (ROS). Furthermore, Kurniawan (2018) explained that the addition of the antioxidant allisin (garlic extract) at a specific concentration using Andromed diluent can maintain the quality of Boer goat semen after 24 hours of cold storage. Therefore, further research is needed to determine the effect of quercetin antioxidant concentration on the quality of Boer goat semen during cold storage using Andromed diluent.

MATERIALS AND METHODS

Materials

The Boer goat semen was collected from the Field Laboratory of Sumber Sekar, Dau Sub-district, Malang Regency, East Java, and then tested at the Biotechnology Laboratory located in Building 4 of the Faculty of Animal Husbandry, Brawijaya University, Malang, East Java. The research was conducted from November 2022 to January 2023 with a semen collection frequency of twice a week at 08:00.

This study used fresh semen from two 5-year-old Boer goats weighing 60 kg and 70 kg, which were located at the Field Laboratory of Sumbersekar, Faculty of Animal Husbandry, Brawijaya University. Collection was done twice a week from November 2022 to January 2023 using artificial vagina method. Tools that used were binocular light microscope Olympus CX 33, test tube, Pyrex brand measuring cup, solution and room thermometer, SelectPette brand micropipette, Assistant haemocytometer brand, bunsen, matches, wire loops, beaker glass, General Care brand object glass and cover brand, Changhong brand refrigerator, Hand Tally Counter (HTC), pH paper brand Macherey Nagel, Axygen brand tips, tweezers, water jacket, test tube, test tube rack, aluminum foil. The diluent used was Andromed diluent brand Sigma Aldrich. The requirement for the fresh semen used was individual motility ≥70% and mass motility 2+. The research was conducted at the Biotechnology Laboratory, Faculty of Animal Husbandry, Brawijaya University, Malang.

Methods

The research method used in this study is a laboratory experiment. The design pattern used is a Completely Randomized Design (CRD) with a factorial two-factor pattern with 4 replications in a 4x4x4 pattern. Factor A includes the treatment of adding quercetin antioxidant levels at 0 (Q0), 10 (Q1), 20 (Q2), and 30 µM (Q3), while factor B includes storage time at 0 (T0), 24 (T1), 48 (T2), and 72 hours (T3).

Dilution of Andromed

Dilution of Andromed diluent begins by preparing the equipment and materials to be used, such as measuring cups, beaker glasses, micropipettes, Andromed, and distilled water. Next, the required volume of distilled water is measured using a measuring cup, then the distilled water and Andromed are homogenized in a ratio of 1:4. The mixture is then transferred to a beaker glass and stored in a safe place.

Dilution of Quercetin

Quercetin is obtained by preparing a stock solution with a concentration of 1000 µM. This is done by dissolving commercially available quercetin. A stock solution with a concentration of 1000 µM, with a mass weight of 100 mg and a molecular weight of 302.24 g/mol, can produce 330.8629 milliliters of stock solution when dissolved in 10% DMSO and distilled water. The use of DMSO as a solvent serves as a cryoprotectant, which is an electrolytic chemical that can reduce the freezing effects caused by ice crystals, thereby maintaining the viability of spermatozoa (Ihsan 2013).

Evaluation of Fresh Semen Quality

The collection of fresh semen is done by preparing a female teaser to be tied in a teaser cage, and then preparing a male with libido characteristics. Before the male is introduced to the female, an artificial vagina is prepared, and then false mountings are performed 3-5 times to increase the male's libido. After the false mountings, allow the male to mount the female, and attach the artificial vagina at a 45-degree angle to the erect penis of the male. The semen is collected in a collection tube and stored in a safe place.

Semen Dilution

Before proceeding with the dilution process, it is necessary to evaluate the quality of the semen

macroscopically and microscopically, based on the criteria of individual motility ≥70% and mass motility 2+. Once the semen meets the criteria, it can proceed to the next stage. Semen dilution is done by mixing the semen with Andromed diluent that has been mixed with the calculated amount of quercetin. The semen dilution is adjusted according to the four treatments to be tested subsequently.

Research Variables

Fresh semen that has been collected is then tested macroscopically and microscopically at the Biotechnology Laboratory, Faculty of Animal Husbandry, Brawijaya University. Semen that meets the criteria is diluted using Andromed diluent and supplemented with the antioxidant quercetin. The variables observed in this study are macroscopic (color, pH, volume, odor, and consistency) and microscopic (motility, viability, abnormalities, and membrane integrity).

RESULT AND DISCUSSION

Evaluation of Fresh Boer Goat Semen Quality

Fresh semen plays an important role in artificial insemination (AI) as good fresh semen is characterized by mass motility of 2+ and individual motility of ≥70%. If fresh semen does not meet these criteria, the quality of semen will decrease over time, especially during cold storage. The average quality of fresh Boer goat semen observed during the study is presented in Table 1.

Volume of Semen

The results of the study on Boer goat semen, as observed macroscopically in Table 1, indicate an average semen volume of 1.4 ± 0.1 ml/ejaculation, this measurement was carried out utilizing a measuring scale applied to the collection tube after semen collection. The average semen volume obtained in this study is higher compared to Lestari *et al.* (2014), who reported Boer goat semen volume ranging from 0.69-1.03 ml/ejaculate. The findings of this study fall within the broader spectrum of goat semen volumes, which commonly range between 0.5-1.5 ml/ejaculate (Rahayu *et al.* 2014). The variation in semen volume can be attributed to factors such as breed, age, body size, season, feed level, and collection frequency (Husin *et al.* 2007). Semen volume itself has a positive correlation with body weight, where the volume of semen produced tends to increase with higher body weight, although the correlation is low. According to Saputra *et al.* (2019) body weight influences semen volume by approximately 9%, while the remaining 91% is determined by factors other than body weight.

Table 1. Fresh semen quality of boer goats

Parameter		Average ± SD
Macroscopic Test	Volume (ml)	1.4 ± 0.1
	Color	Yellowish White
	Odor	Typical
	Consistency	Concentrated
	pH	6.33 ± 0.58
Microscopic Test	Mass Motility	(+++)
	Individual Motility (%)	90 ± 0
	concentration (106/ml)	5.096,7 ± 1.435,5
	Viability (%)	91.64 ± 4.53
	Abnormality (%)	0.69 ± 0.1
	Membrane Integrity (%)	66.53 ± 7.64

Color

The average color of Boer goat semen in this study is off-white to yellowish, which is consistent with Rosmaidar *et al.* (2013) who described fresh Boer goat semen as creamy white or milky white, indicating normal semen quality. Wattimena (2006) explained that the creamy color is due to the secretion of the pigment riboflavin by the vesicular glands, with a thick consistency and distinct odor. On the other hand, according to Mugiyanti *et al.* (2017), clear semen indicates poor sperm quality, while brown or reddish semen indicates contamination with blood due to injury in the reproductive tract, and yellow semen indicates contamination with urine. Semen color also correlates with sperm concentration, where creamy-colored fresh semen with a thick consistency indicates a high sperm concentration, while watery semen with a thin consistency indicates a low sperm concentration (Saputra *et al.* 2019).

Odor

The fresh semen examined in this study possesses a characteristic animal odor. A distinct and non-foul scent typically signifies normal semen devoid of microbial contamination (Thasmi *et al.* 2020). Conversely, a foul odor within the semen usually points to contamination arising from infections in the animal's reproductive organs.

Consistency

The texture of fresh semen examined in this study is notably thick. Consistency is intrinsically connected to color and concentration, whereby a lighter color of semen typically corresponds to a lower concentration and a thinner consistency. Setyowati (2019) noted that medium or thick consistency, combined with a concentration of 1,500 x 10^6, characterizes thick goat semen.

pH

The pH value of Boer goat semen is 6.33 ± 0.58, which is within the normal range for goat or sheep semen, which typically falls between 5.9-7.3 (Thasmi *et al.* 2020).

Mass Motility

The mass motility of fresh semen is categorized as very good or scored as (+++), as indicated by the formation of rapidly moving black cloud waves.

Individual Motility

The average percentage of individual motility is 90 ± 0%. This value is higher than the average value reported by Ihsan (2017), which was 70 ± 0% for individual motility. It is also higher than the average motility reported in general by Ihsan (2017) reported an average individual motility of fresh Boer goat semen to be 71.67 ± 3.72%.

Concentration

The concentration of fresh semen in this study was measured at 5,096.7 ± 1,435.5 x 106/ml, which surpasses the findings of Agustian *et al.* (2014), who reported a concentration of 3,810.2 ± 1,590.31 x 106/ml. Nevertheless, this concentration remains within the acceptable range. As highlighted by Rosmaidar *et al.* (2013), the variability in concentration can be ascribed to individual dissimilarities, age, and management practices.

Viability

The viability of the semen in this study was 91.64 ± 4.53%, which is higher compared to the findings of Mugiyanti *et al.* (2019) who reported a viability percentage of 79.81±8.63% for fresh Boer goat semen. This result indicates good semen quality, as Susilawati (2013) stated that the percentage of live spermatozoa should be between 70-90% motile.

Abnormalities

The observed percentage of abnormalities in this study was recorded at 0.69 ± 0.1, a figure that contrasts with the findings of Thasmi *et al.* (2020) who documented a percentage of 7.8 ± 1.48% for fresh semen abnormalities. The discernible divergence in average values is likely attributed to inherent variations in the spermatogenesis process during sperm formation.

Membrane Integrity

The membrane integrity in this study was 66.53 ± 7.64, which is higher compared to Kurniawan (2018) value of 65.54±0.84. However, the obtained result is still considered good and can be further processed.

Evaluation of Individual Motility of Stored Boer Goat Semen at Cold Temperature (3-5 ^{o}C)

Individual motility is a parameter indicating the percentage of live and active spermatozoa in semen. The tested semen must have a value of ≥70% to proceed to the next stage. The analysis of variance results for the motility of Boer goat sperm with the addition of quercetin antioxidants Q0, Q1, Q2, and Q3 at different storage durations T0, T1, T2, and T3 can be seen in Table 2. The analysis of variance results in Table 2 shows that the addition of the antioxidant quercetin to Boer goat semen significantly influences individual motility (P<0.01). Further post hoc analysis using Duncan's test indicates significant differences between Q0 and Q1, Q2, and Q3. The mean and standard deviation of the highest concentration of quercetin supplementation are found in Q3 with a value of 44.69±16.91. This occurs because the addition of quercetin to the Andromed extender can improve the detrimental effects of cryopreservation on spermatozoa (Jamadi *et al.* 2017) because Andromed contains essential ingredients for supporting spermatozoa viability, such as fructose, citric acid, buffer, antibiotics, and glycerol. Glycerol, in particular, acts as an intracellular cryoprotectant that can diffuse into sperm cells and be metabolized to produce energy and form fructose (Ihsan 2013). The decrease in spermatozoa motility in this treatment is likely due to the gradual depletion of essential nutrients, such as sodium and plasma proteins, which are required for the high metabolic demands of sperm transport through the female genital tract (Udeh and Oghenesode 2011). However, the findings of this study differ from those previous studies, as higher concentrations of quercetin did not decrease the motility of Boer goat semen. Additionally, Tvrda *et al.* (2020) mentioned that the addition of 50 μM quercetin to semen actually had a toxic effect on mitochondria, leading to a significant decrease in sperm motility. In conclusion, the Q3 treatment, with the addition of 30 μM quercetin, is considered the best because it can maintain individual spermatozoa motility due to the availability of nutrients in Andromed and the inhibition of oxidative processes caused by ROS through the addition of quercetin as an antioxidant.

The analysis of variance results indicate that the storage duration significantly affects individual motility of Boer goat semen (P<0.01). Further post hoc analysis using Duncan's test shows highly significant differences between T0 and T1, T2, and T3. Mean and standard deviation of the highest storage duration are found in T2 with a value of 41.56±12.47, while the mean and standard deviation of the lowest storage duration are found in T3 with a value of 21.25±5.6. The decrease in motility over time is likely due to oxidative damage associated with prolonged semen storage, which can disrupt essential structures necessary for the survival of spermatozoa. It can be concluded that the addition of the antioxidant quercetin.

Evaluation of Viability of Stored Boer Goat Semen at Cold Temperature (3-5 ^{o}C)

Viability is a test conducted to determine the percentage of live spermatozoa using staining techniques with eosin-nigrosin, by examining the condition of the spermatozoa head. In good-quality sperm, the intact

Table 2. Semen motility percentage of boer goats after treatment

Treatment	Cold Storage				Average ± SD
	T0	T1	T2	T3	
Q0	48.75±8.54	26.25±04.79	21.25±02.50	13.75±02.50	27.50a±15.07
Q1	55.00±5.77	37.50±06.45	32.50±06.45	20.00±04.08	36.25b±14.51
Q2	57.50±9.57	47.50±09.57	32.50±05.00	25.00±04.08	40.63bc±14.63
Q3	62.50±9.57	55.00±10.00	35.00±07.07	26.25±04.79	44.69c±16.91
Average ± SD	55.94d±05.72	41.56c±12.47	30.31b±06.16	21.25a±05.68	

Means in the same column or row with different superscript differ significantly (P<0.01)

SD = Standart Deviation

plasma membrane prevents the eosin-nigrosin stain from penetrating the cell membrane, resulting in a white or clear head color for live sperm and a dark or bluish color for dead sperm. The analysis of variance (ANOVA) results for the viability of Boer goat spermatozoa with the addition of quercetin antioxidant concentrations of 0, 10, 20, and 30 µM at storage durations of 0, 24, 48, and 72 hours can be seen in Table 3. The analysis of variance in Table 3 shows that Boer goat semen with the addition of quercetin antioxidant concentrations significantly affects the individual viability of Boer goat semen during cold storage at 3-5 °C (P<0.01). Further post-hoc tests using Duncan›s test indicate a significant difference between Q0 and Q2 as well as Q3, but no significant difference from Q1. Q3 exhibits the highest mean value with 72.14±8.2, which is still above 70%, indicating good viability that can lead to fertility. Bintara (2011) stated that goat sperm viability is expected to be 90%. However, if viability is not less than 50%, it can still result in good fertility. Q3 demonstrates that quercetin can optimally preserve the integrity of spermatozoa by attaching to the middle part of the spermatozoa, where mitochondria are located, responsible for energy production in spermatozoa, thus maintaining energy production for the maintenance of spermatozoa›s body (Naseer *et al.* 2018).

The analysis of variance in Table 3 shows that the storage duration of Boer goat semen significantly affects viability (P<0.01). Duncan's test also indicates a significant difference between T0 and T2 as well as T3, but no significant difference from T1. T0 has the highest mean value with 78.17±03.70. This is because lower storage temperature and longer storage duration lead to decreased viability and motility of spermatozoa due to biochemical activity affected by osmotic disturbances and cell membrane damage caused by oxidative stress (Pahlevy *et al*. 2022) resulting from ROS accumulation formed during cooling and long-term storage, where endogenous antioxidants in seminal plasma are insufficient to counteract the accumulated ROS during storage. Although antioxidants do not completely prevent detrimental changes caused by storage, they significantly reduce their effects (Łukaszewicz and Kowalczyk 2020), as evidenced in Table 3 T0 has the highest percentage of viability, while T3 has the lowest percentage. The analysis of variance also shows that the interaction between Boer goat semen with the addition of quercetin antioxidant concentrations and storage duration does not significantly affect individual viability of Boer goat semen during cold storage at 3-5 °C (P<0.05).

Evaluation of Abnormality of Stored Boer Goat Semen at Cold Temperature (3-5 °C)

Abnormality refers to the condition of abnormalities or damage that occur in the head, tail, and acrosome of spermatozoa, which can be observed with specific staining techniques. Spermatozoa abnormalities are caused by the age of the animal, individual variations, and the physical condition of the animal itself. In the present study, the acceptable percentage of spermatozoa abnormalities is less than 20%. Spermatozoa abnormalities are directly proportional to spermatozoa viability, where abnormalities play an important role because a higher number of normal spermatozoa also have longer viability compared to abnormal spermatozoa (Putranti *et al*. 2010). The analysis of variance results for the abnormalities of Boer goat spermatozoa with the addition of quercetin antioxidant concentrations of 0, 10, 20, and 30 µM during storage for 0, 24, 48, and 72 hours can be seen in Table 4. The analysis of variance results in Table 4 indicates that the addition of quercetin antioxidant concentrations in Boer goat semen showed significant differences, while storage time did not have a significant

Table 3. Semen viability percentage of boer goats after treatment

Treatment	Cold Storage				Average ± SD
	T0	T1	T2	T3	
Q0	73.47±04.34	67.46±03.72	59.16±03.01	53.81±03.12	63.48a±08.72
Q1	77.43±04.77	69.88±02.53	61.50±05.50	58.87±04.26	66.92ab±08.43
Q2	79.55±04.27	69.52±05.28	62.62±04.30	59.59±03.89	67.82bc±08.86
Q3	82.23±04.71	75.09±02.65	67.36±04.02	63.88±04.51	72.14c±08.20
Average ± SD	78.17d±03.70	70.49c±03.25	62.66ab±3.45	59.04a±04.13	

Means in the same column or row with different superscript differ significantly (P<0.01)

SD = Standart Deviation

Table 4. Semen abnormality percentage of boer goats after treatment

Treatment	Cold Storage				Average ± SD
	T0	T1	T2	T3	
Q0	04.05±00.50	04.25±00.67	04.17±01.59	04.65±00.66	04.28±00.26
Q1	03.39±00.28	03.87±00.64	03.85±01.22	04.03±01.23	03.78±00.28
Q2	03.22±00.17	03.50±01.06	03.62±01.15	04.11±01.37	03.61±00.37
Q3	03.39±00.39	03.18±00.74	03.46±00.76	03.59±01.19	03.40±00.17
Average ± SD	03.51±00.37	03.70 ±00.47	03.77±00.31	04.10±00.44	

SD = Standart Deviation

Table 5. Semen membrane intgegrity percentage of boer goats after treatment

Treatment	Cold Storage				Average ± SD
	T0	T1	T2	T3	
Q0	39.09±05.33	37.54±06.38	30.51±02.22	26.31±03.11	33.36±06.00
Q1	40.40±06.68	37.18±03.30	31.92±02.42	28.97±03.51	34.62±05.14
Q2	41.05±03.34	37.17±04.97	32.35±02.63	28.90±02.73	34.87±05.34
Q3	43.15±04.79	40.37±02.81	34.92±03.16	30.80±02.15	37.31±05.52
Average ± SD	40.92b±01.69	38.07b±01.55	32.42a±01.84	28.75a±01.85	

Means in the same column or row with different superscript differ significantly (P<0.01)

SD = Standart Deviation

effect (P<0.05) on the abnormality of Boer goat semen. Table 4 shows that the goats are still categorized as healthy since they have abnormality levels of less than 6%. Bintara (2011) stated that a healthy goat may have around 6% to 10% abnormal spermatozoa. The abnormalities observed in spermatozoa are caused by disturbances in the goat's reproductive tract, as well as during the semen collection, handling, and mixing processes with extenders (Kurniawan 2018). Abnormalities in semen can be categorized into several types, such as no tail, abnormal head, abnormal tail shape, abnormal tail shape with proximal cytoplasmic droplet, and abnormalities in the distal droplet of the tail (Rahayu *et al.* 2014). In this study, the abnormalities found include the absence of a tail, abnormal head with double heads, and abnormal tail shapes. Table 4 indicates that Q3, with a concentration of quercetin at 30 µM, showed the lowest average decrease with a value of 03.40±00.17, while Q0 showed the highest average decrease with a value of 04.28±00.26. Q3, with the addition of quercetin at 30 µM, yielded the best results in maintaining spermatozoa abnormality. This is because quercetin can suppress intracellular enzyme leakage in spermatozoa and inhibit the production of transaminase enzymes in the extracellular fluid (Khawagah *et al*. 2020).

Evaluation of Membrane Integrity of Stored Boer Goat Semen at Cold Temperature (3-5 °C)

Membrane integrity is one of the indicators of successful fertilization in livestock. The morphology of the sperm head, particularly in the acrosome region, plays a role in binding to the zona pellucida (Sun *et al*. 2021), so any damage can affect sperm fertility. The analysis of variance results for the viability of Boer goat spermatozoa with the addition of quercetin antioxidant concentrations at 0, 10, 20, and 30 µM during storage periods of 0, 24, 48, and 72 hours can be seen in Table 5.

The analysis of variance results in Table 5 shows that the addition of quercetin antioxidant concentrations to Boer goat semen does not significantly affect membrane integrity (P<0.05). The research results indicate that the addition of quercetin in Q1, Q2, and Q3 can suppress the average decrease in membrane integrity until T1, with the highest average value observed in Q3 with a 30 µM addition, with a value of 37.31±05.52. These results differ from Siari *et al*. (2021), where the best treatment was found in the supplementation of quercetin (15 mM) in Red Fowl extender, which improved sperm motility, plasma membrane integrity, viability, acrosome integrity, chromatin condensation, and mitochondrial activity. The differences could be attributed to variations in extender types, livestock species, environmental conditions, livestock age, laboratory techniques, and semen cooling procedures. Additionally, spermatozoa cannot survive during preservation due to plasma membrane damage caused by oxidative stress, which can reduce semen reproductive potential and mitochondrial activity. Moreover, during cryopreservation, the formation of lipid membranes alters fluidity levels, making the plasma membrane more vulnerable to disturbances and further cell damage, ultimately leading to membrane death (Hassan *et al.* 2021).

The analysis of variance results shows that Boer goat semen with different storage times significantly influences membrane integrity (P<0.01). The highest average value is found in T1, with a 24-hour storage period, compared to the control (T0) with a value of 38.07±01.55, and it significantly differs from T2 and T3. This difference in results is due to cold shock during cold storage, which increases spermatozoa metabolism and produces lactic acid, leading to a decrease in pH levels and a toxic environment. Additionally, heat shock also affects the incubation process at 37 °C for 30 minutes.

CONCLUSION

The addition of quercetin antioxidant can effectively suppress the average decrease in semen quality in terms of motility, viability, abnormality, and membrane integrity. The best results were obtained with the addition of a quercetin concentration of 30 µM (Q3) in combination with Andromed extender, which helps maintain Boer goat sperm quality against oxidative reactions caused by free radicals. Quercetin and Andromed extender provide optimal results after a 24-hour storage period (T1) in terms of motility, viability, abnormality, and membrane integrity, but they have not yet achieved optimal results beyond 72-hours (T3) of storage, as longer storage periods can affect the structure of sperm membranes. The addition of quercetin concentration and storage time did not interact with each other, indicating no relationship between quercetin concentration and storage time regarding sperm quality.

REFERENCES

Agustian, M. F., M. N. Ihsan, & N. Isnaini. 2014. Pengaruh lama simpan semen dengan pengencer tris aminomethan kuning telur pada suhu ruang terhadap kualitas spermatozoa kambing boer. Ternak Tropika Journal of Tropical Animal Production. 15(2):1-6.

Amidia, L., F. Hoesni, & B. Rosadi. 2021. Analisis keberhasilan inseminasi buatan (ib) ternak sapi berdasarkan karakteristik inseminator di Kabupaten Kerinci. Jurnal Ilmiah Universitas Batanghari Jambi. 21(2): 467-476.

Baghel, S. S., N. Shrivastava, R. S. Baghel, P. Agrawal, & S. Rajput. 2012. A review of *quercetin*: antioxidant and anticancer properties. World J Pharm Pharmaceutical Sci. 1(1):146-160.

Bintara, S. 2011. Rasio spermatozoa x: y dan kualitas sperma pada kambing Kacang dan Peranakan Ettawa. Sains Peternakan: Jurnal Penelitian Ilmu Peternakan. 9(2):65-71.

Dewajanti, A. M. 2017. Peranan Enzim Glukosa 6 Fosfat Dehidrogenase dalam Mempertahankan Integritas Membran Sel Darah Merah terhadap Beban Oksidatif. Jurnal Kedokteran Meditek. 21(56):1-8.

El-Khawagah, A. R., M. M. Kandiel, & H. Samir. 2020. Effect of quercetin supplementation in extender on sperm kinematics, extracellular enzymes release, and oxidative stress of egyptian buffalo bulls frozen–thawed semen. Frontiers in Veterinary Science. 7(1):1-10.

Husin, N., T. Suteky, & K. Kususiyah. 2007. Uji kualitas semen kambing nubian dan peranakannya (Kambing Nubian X PE) serta kambing Boer berdasarkan lama penyimpanan. Jurnal Sain Peternakan Indonesia. 2(2):57-64.

Ihsan, M. N. 2012. Penggunaan telur itik sebagai pengencer semen kambing. Ternak Tropika Journal of Tropical Animal Production. 12(1):10-14.

Ihsan, M. N. 2013. Pembekuan vitrifikasi semen kambing boer dengan tingkat gliserol berbeda. Journal of Tropical Animal Production. 14(2):38-45.

Kurniawan, N. F. 2018. Pengaruh Konsentrasi Sari Bawang Putih Dalam Pengencer Andromed Terhadap Kualitas Semen Kambing Boer Setelah 24 Jam Pada Suhu Dingin. *Skripsi*. Fakultas Peternakan. Universitas Brawijaya: Malang.

Lestari, T. P. S., M. N. Ihsan, & N. Isnaini. 2014. Pengaruh waktu simpan semen segar dengan pengencer andromed pada suhu ruang terhadap kualitas semen kambing boer. Ternak Tropika Journal of Tropical Animal Production. 15(1):43-50.

Łukaszewicz, E., A. Jerysz**, & A. Kowalczyk.** 2020. Effect of semen extenders on viability of ISA Brown and Hubbard Flex roosters' sperm stored for 24 h. Poultry Science. 99(5):2766-2774.

Moore, S. G., & J. F. Hasler. 2017. A 100-Year Review: Reproductive technologies in dairy science. Journal of dairy science. 100(12):10314-10331.

Mugiyati, M., N. Isnaini, M. A. Salim, & T. Susilawati. 2017. Pengaruh air kelapa merah yang muda dan tua sebagai pengencer terhadap kualitas semen kambing boer selama penyimpanan dingin. Ternak Tropika Journal of Tropical Animal Production. 18(1):20-26.

Naseer, Z., E. Ahmad, H. S. Şahiner, E. T. Epikmen, M. Fiaz, M. R. Yousuf, S. A. Khan, I. Serin, A. Ceylan, & M. Aksoy. 2018. Dietary quercetin maintains the semen quality in rabbits under summer heat stress. Theriogenology. 122(1):88-93.

Nurcholis, A. R., & M. Yamin. 2016. Kriopreservasi semen domba garut menggunakan tris kuning telur yang disuplementasi omega-3 minyak ikan salmon. Jurnal Veteriner. 17(2):309-315.

Lestari, T. P. S., M. N. Ihsan, & N. Isnaini. 2014. Pengaruh waktu simpan semen segar dengan pengencer andromed pada suhu ruang terhadap kualitas semen kambing boer. Ternak Tropika Journal of Tropical Animal Production. 15(1):43-50.

Łukaszewicz, E., **A. Jerysz, & A. Kowalczyk.** 2020. Effect of semen extenders on viability of ISA Brown and Hubbard Flex roosters' sperm stored for 24 h. Poultry Science. 99(5):2766-2774.

Moore, S. G., & J. F. Hasler. 2017. A 100-Year Review: Reproductive technologies in dairy science. Journal of Dairy Science. 100(12):10314-10331.

Mugiyati, M., N. Isnaini, M. A. Salim, & T. Susilawati. 2017. Pengaruh air kelapa merah yang muda dan tua sebagai pengencer terhadap kualitas semen kambing boer selama penyimpanan dingin. Ternak Tropika Journal of Tropical Animal Production, 18(1):20-26.

Naseer, Z., E. Ahmad, H. S. Şahiner, **E. T. Epikmen, M. Fiaz, M. R. Yousuf, S. A. Khan, I. Serin, A. Ceylan, & M. Aksoy.** 2018. Dietary quercetin maintains the semen quality in rabbits under summer heat stress. Theriogenology. 122(1):88-93.

Nurcholis, A. R., & M. Yamin. 2016. Kriopreservasi semen domba garut menggunakan tris kuning telur yang disuplementasi omega-3 minyak ikan salmon. Jurnal Veteriner. 17(2):309-315.

Pahlevy, J. R., H. Ratnani, F. Fikri, T. I. Restiadi, A. L. Saputro, & B. Agustono. 2022. The addition of vitamin C in tris–egg yolk extender maintained Sapera goat semen quality in 5 °C storage. Ovozoa: J Anim Reprod. 11(1):1-8.

Rosmaidar, R. Dasrul, & T. M. Lubis. 2013. Pengaruh penambahan sari buah tomat dalam media pengencer terhadap motilitas dan viabilitas spermatozoa kambing Boer yang disimpan pada suhu 3–5 °C. Jurnal Ilmiah Peternakan. 1(1):7-17.

Saputra, A. K., M. D. I. Hamdani, S. Suharyati, & M. Hartono. 2019. Korelasi antara bobot badan, lingkar skrotum, dan volume semen kambing saburai di wilayah sumber bibit Kabupaten Tanggamus. Jurnal Riset dan Inovasi Peternakan (Journal of Research and Innovation of Animals). 3(1):7-11.

Setyowati, S. 2019. Kualitas dan Profil Kalsium Intraseluler Spermatozoa Post Thawing Kambing Senduro dengan Pengencer Andromed® pada Suhu Pembekuan -80 °C dan -196 °C.

Susilawati, I. D. A. 2021. Kajian Pustaka: Sumber Reactive

Oxygen Species (ROS) Vaskular. Stomatognatic-Jurnal Kedokteran Gigi. 18(1):1-10.

Susilawati, T., N. Isnaini, A. P. A. Yekti, I. Nurjannah, & E. Errico. 2016. Keberhasilan inseminasi buatan menggunakan semen beku dan semen cair pada sapi Peranakan Ongole. Jurnal Ilmu-Ilmu Peternakan (Indonesian Journal of Animal Science. 26(3):14-19.

Thasmi, C. N., M. Ikhsanuddin, H. Hamdan, D. Dasrul, M. N. Salim, & A. Azhar. 2020. Effect of Noni Fruit Extract (Morinda citri-folia L.) in CitrateYolk Diluent on The Boer Goat Spermatozoa Motility Stored at Temperature 5 ℃. In E3S Web of Conferences. 151(1):1-5.

Tvrdá, E., M. Debacker, M. Ďuračka, J. Kováč, & O. Bučko. 2020. Quercetin and naringenin provide functional and antioxidant protection to stored boar semen. Animals. 10(10):1-16.

Udeh, I., & B. Oghenesode. 2011. Effects of type of extender and storage conditions on the motility of goat spermatozoa. International Journal of Animal and Veterinary Advances. 3(5):282-286.

Warmadewi, D. A., & I. G. N. G. Bidura. 2021. Comparative study of artificial insemination versus natural mating methods on the quality of Bali cattle calves. International Journal of Fauna and Biological Studies. 8(2):82-88.