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Quercetin supplementation in andromed extender on quality of Boer goat semen during room temperature Storage

Suplementasi Quercetin pada Pengencer Andromed terhadap Kualitas Semen Kambing Boer Selama Penyimpanan Suhu Ruang

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ABSTRACT

This research aimed to determine the effect of quercetin antioxidant concentration levels on Boer goat semen quality during room temperature storage using andromed extender. Fresh semen was collected from two of 5-years-old Boer goats with weight of 60 and 70 kg. The method of this research was an experimental laboratory using Completely Randomized Design (CRD) Factorial of two factors, namely the treatment of quercetin concentration levels at 0 µM, 10 µM, 20 µM and 30 µM and the storage time during room temperature at 0 hour, 2 hours, 4 hours, 6 hours and 8 hours. The variables were observed based on individual motility, viability, abnormalities and membrane integrity of spermatozoa. The result showed that the antioxidant quercetin 30 µM have a significant effect to individual motility (P<0.05) but did not have a significant effect to the viability and abnormality of spermatozoa. Antioxidant quercetin gave a highly significant difference on membrane integrity (P<0.01). Storing semen at room temperature for 6 hours resulted in a highly significant difference in individual motility, abnormality and membrane integrity (P<0.01) but did not show a significant difference to viability. The best result showed that the antioxidant quercetin 30 µM was able to maintain the quality of spermatozoa at room temperature storage up to 6 hours.

Kata kunci:
Kambing Boer
Kualitas sperma
Waktu penyimpanan
Quercetin

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh antioksidan quercetin yang ditambahkan dalam pengencer andromed terhadap kualitas semen kambing Boer selama penyimpanan suhu ruang. Materi yang digunakan adalah semen segar yang diperoleh dari 2 kambing Boer berumur 5 tahun dengan bobot badan 60 kg dan 70 kg. Penampungan semen dilakukan setiap 2 kali seminggu menggunakan metode vagina buatan. Pola rancangan yang digunakan adalah Rancangan Acak Lengkap (RAL) Faktorial atas dua faktor, faktor pertama adalah perlakuan konsentrasi quercetin 0 µM, 10 µM, 20 µM, 30 µM dan faktor kedua adalah perlakuan waktu penyimpanan 0 jam, 2 jam, 4 jam, 6 jam dan 8 jam. Variabel yang diamati adalah motilitas individu, viabilitas, abnormalitas dan integritas membran spermatozoa. Hasil penelitian menunjukkan bahwa penambahan antioksidan quercetin 30 µM memberikan perbedaan yang nyata terhadap motilitas individu (P<0,05) namun tidak memberikan perbedaan yang nyata terhadap viabilitas dan abnormalitas spermatozoa. Konsentrasi antioksidan quercetin memberikan perbedaan yang sangat nyata terhadap integritas membran spermatozoa (P<0,01). Waktu penyimpanan semen selama 6 jam pada suhu ruang memberikan perbedaan yang sangat nyata terhadap motilitas individu, abnormalitas dan integritas membran (P<0,01) namun tidak memberikan perbedaan yang nyata terhadap viabilitas spermatozoa. Kesimpulan bahwa penambahan antioksidan quercetin 30 µM mampu mempertahankan kualitas spermatozoa pada penyimpanan suhu ruang selama 6 jam.

INTRODUCTION

Artificial insemination (AI) is an effort to increase productivity and genetic quality in livestock. The success of artificial insemination is influenced by the skills of the inseminator, the time of insemination and the quality of semen produced by the male. AI Programs generally use liquid or frozen semen. The quality of liquid semen at room temperature may deteriorate if the semen is not added with an appropriate diluent. Semen diluents have certain requirements including containing substances that are almost the same as semen, do not contain elements or substances that are toxic to spermatozoa, do not reduce fertility and contain nutrients (Cahyani, et al., 2020). Semen diluent that can be used is andromed. Andromed diluent contains fructose and glycerol, fructose acts as an energy source for spermatozoa while glycerol is an intracellular cryoprotectant that can reduce damage and maintain semen quality during freezing (Sari & Tjandrakirana, 2020).

During the storage period spermatozoa will undergo metabolism and will produce free radicals in the form of reactive oxygen species (ROS) which will cause lipid peroxidase and oxidative stress. Increased levels of ROS are cytotoxic which results in loss of motility and viability of spermatozoa. Oxidative stress resulting from excessive production of ROS can have a negative effect on the integrity of the spermatozoa membrane. The metabolic process in spermatozoa will produce free radicals in the form of ROS and there will be a decrease in antioxidant capacity and lipid peroxidase which can cause cell damage, so antioxidants need to be added. Antioxidants are compounds that can prevent or inhibit oxidation (Awaliah, et al., 2020). One of the antioxidants that can be added is quercetin.

Quercetin is one of the antioxidants that can counteract and inhibiting free radicals, quercetin will interact with alpha-tocopherol to delay oxidation and increase gene expression of detoxification enzymes. El-Khawagah et al. (2020) stated that flavonoids have antioxidant activity where quercetin compounds, which are one class of flavonoid compounds are able to counteract ROS and hydroxyl radicals. Quercetin with five OH groups is known to have strong membrane interactions. The results of research by Seifi et al. (2017) revealed that quercetin antioxidant was able to maintain motility, viability, and membrane integrity in Mahabadi goat semen by inhibiting the development of free radicals to prevent the process of the lipid peroxidation.

Based on the description, quercetin was one of the effective antioxidants to counteract ROS and there was a need for further research regarding the addition of the antioxidant quercetin to the quality of Boer goat semen during storage at room temperature. So that, this research aimed to determine the effect of quercetin antioxidant concentration levels on Boer goat semen quality during room temperature storage using andromed extender.

MATERIALS AND METHODS

Research Materials

The research was conducted on 17 November 2022 to 14 January 2023. Semen evaluation was conducted at the Biotechnology Laboratory, Faculty of Animal Science, Brawijaya University, Malang. The material used fresh semen from two 5 years old Boer goats with body weights of 60 kg and 70 kg collected in the Field Laboratory of Sumber Sekar. Semen collection was carried out twice a week on Monday and Thursday at 08.00 WIB using an artificial vagina conducted by experienced officers from the Field Laboratory of Sumber Sekar. The semen that has been collected was stored in a thermos and immediately taken to the laboratory to for macroscopic and microscopic evaluation. Semen used had a minimum mass motility ++ and individual motility of at least 70%. The diluent used was andromed available at the Biotechnology laboratory, Faculty of Animal Science, Brawijaya University and quercetin antioxidant brand Sigma Aldrich obtained from Nitra Kimia.

Research Methods

This study used a laboratory experimental method and the design method used a Completely Randomized Design (CRD) factorial of two factors consisting of 4 replicates with 4×5 pattern. The first factor was quercetin concentration from 4 treatments namely 0 µM, 10 µM, 20 µM and 30 µM and the second factor was storage time at room temperature storage from 5 treatments namely 0 hour, 2 hours, 4 hours, 6 hours and 8 hours. The research data were analysis using ANOVA, if results obtained showed a real difference in effect

(P<0.05) or authenticity (P<0.01) then proceed with Duncan test.

Dilution of Antioxidant Quercetin

The preparation of quercetin solution was carried out by dissolving 100 mg of quercetin which have a molecular weight of 302.24 g/mol with 10% DMSO in 33 ml increments. Quercetin and DMSO that have been dissolved then added 297 ml of distilled water to the quercetin solution gradually, then homogenized and a quercetin solution with a concentration of 1000 µM will be produced. Pour the quercetin solution into a collection tube and store in the freezer.

Dilution of Andromed

The andromed solution made by preparing of 20 ml andromed and 80 ml distilled water. Andromed and distilled water were poured into a measuring cup in a ratio of 1:4 and gently homogenize, then poured the andromed solution into a collection tube and store in the refrigerator at 4-5°C.

Fresh Semen Evaluation

Evaluation of fresh semen was carried out microscopic and macroscopic. Macroscopic observations were included evaluation of color, odor, semen volume, consistency and pH. Microscopic observations were included evaluation of mass motility, individual motility, concentration, viability, abnormality and HOS Test observations.

Semen Dilution

The fresh semen have been evaluated, then diluted and divided according to the amount of andromed diluent that have been added with quercetin antioxidant. The volume of diluent used was obtained from the calculation of the amount of fresh semen concentration. Semen that has been diluted was stored at room temperature and then evaluated for semen quality at 0 hour, 2 hours, 4 hours, 6 hours and 8 hours. Evaluation of semen quality included individual motility, viability, abnormality and membrane integrity.

Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) and processed with IBM SPSS version 26 software. If the data obtained significant differences, it was continued with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Evaluation of Fresh Semen Quality

Fresh semen were collected directly from the male must be evaluated before use, to determine macroscopic and microscopic characteristics. Only fresh semen that have more than the quality standard would be used for the research process. The results of the fresh semen quality evaluation can be seen in Table 1.

Table 1. Fresh semen quality of Boer goats

Variables	Average
Macroscopic Test	
Volume (ml)	1.48±0.41
Color	Yellowish White
Smell	Typical
consistency	Concentrated
pH	6.25±0.5
Microscopic Test	
Mass motility	(+++)
Individual motility (%)	86.25±4.79
Concentration (106 /ml)	4482.5±1701.7
Viability (%)	89.51±5.57
Abnormalities (%)	0.52±0.03
Membrane integrity (%)	67.25±5.89

The examination of fresh semen volume is one of the requirements to determine the quantity of fresh semen produced after the collection process. The results of fresh semen volume on Boer goats in this study showed mixed results in each collection which ranged from 1-2 ml/ejaculate with the average obtained 1.48±0.41 ml. The volume of semen produced was in the normal range in accordance with the results research of Afiati (2015) with the average semen volume of sheep and goats was 0.5-2 ml. The amount of semen volume of Boer goats when collected can vary because it is influenced by individual differences in livestock, age, nutrition and frequency of ejaculation (Lestari et al., 2014).

The results of the semen color examination showed that the semen of Boer goats in this study was normal, namely yellowish white. Rahayu & WM (2014) stated that the appearance of normal Boer goat semen was milky white to yellowish white. The color of the semen from the collection could be used as a parameter to determine the quality of

the semen, where the color of the deviated semen can mean that the semen has been contaminated by foreign objects. Red semen indicated the presence of blood from the genital tract while yellowish green semen indicated the presence of germs from the *Pseudomonas aeruginosa* species and semen contained suppuration if there were clots in the semen (Kholis, 2013).

Based on the observations, the odor of the semen showed a distinctive smell. This indicated that the semen collected meets the criteria of normal semen standards. Research Results from Suyadi & Iswanto (2012) showed that the characteristic odor in goat semen indicated normal conditions and was an indication of the absence of contamination in the semen. Generally, normal semen have a distinctive odor accompanied by the smell of the livestock. If there was a foul odor or other unnatural odor then semen have been contaminated with bacteria so it cannot be used for further research.

Observations showed that the consistency of semen obtained at the time of collection was included in the thick category. Semen that have a thick consistency indicated that the semen contained higher spermatozoa. The level of semen consistency has a positive correlation with the concentration of spermatozoa contained in it. Research results of Nahriyanti et al., (2017) showed that a slightly thick to thick consistency indicated that the semen was normal and have a high spermatozoa content.

The pH of fresh semen ranged from 6-7 with an average of 6.25±0.5. The degree of acidity or pH was evaluated by placing the semen on litmus paper and then matched with the indicator listed on the litmus paper box. Aisah et al., (2017) explained that the pH of semen could be said to be normal and still suitable for further processing if the semen have a degree of acidity between 6.00-7.00.

The result of the mass motility examination in this study was 3+ or very good, indicating that the semen was eligible for further processing. Muhammad et al., (2017) explained that the fresh semen used must meet the requirements, namely mass motility of at least 2+ and individual motility of at least 70%. The quality of mass motility could be said to be very good (+++) if the movement of spermatozoa was seen to form large waves, many, thick, dark and active and move quickly moving around the place.

The result of individual motility evaluation was 86.25±4.79%. The resulting average showed that spermatozoa were normal and have good mobility. Observation of individual motility was done by assessing spermatozoa that move progressively forward while spermatozoa that move backward or circularly were not included.

The average concentration obtained during the study was 4482.5±1701.7 million/ml. It was not much different from the results of the study Pamungkas & Batubara (2014) which obtained the concentration of Boer goat semen 4.125 million/ml. Fresh semen concentration needed to be known for determining semen quality, male reproductive power and to determine the amount of semen dilution. Masyitoh et al., (2018) explained that the value of spermatozoa concentration in the range of 2000-6000 million/ml was still normal.

Based on the observation of fresh semen of Boer goats, the average viability obtained was 89.51±5.57%. The results of the viability observations obtained were not much different from the results of Agustian et al. (2014) which obtained an average viability of 89.26±2.63%. It indicated that the semen was still classified as normal and this result was higher than the research of Salim et al. (2019) which obtained the viability of Boer goat semen 74±11.9% which indicated that the semen produced have good quality. Calculation of live and dead spermatozoa was done using certain dyes and must be checked selectively (Munazaroh et al., 2013).

The observation of fresh semen abnormality of Boer goats was 0.52±0.03%, the average abnormality was considered normal. Abnormality was one indicator in determining the quality of spermatozoa because abnormal cell structures can cause interference and obstacles during fertilization (Afiati et al., 2015). Abnormality was characterized by abnormalities in the head and tail such as curved, coiled and broken tails (Susilawati, 2013). Abnormality that reaches >20% then the quality of the semen was doubtful (Susilawati, 2011).

The observation of the integrity of the fresh semen membrane of Boer goats was 67.25±5.89%, the average was still considered normal even though this result was smaller than the research of Akbar et al., (2013) with the results of membrane integrity 83.67±3.58%. Spermatozoa that showed bubbling and coiled tails as a result of the HOST

test, indicating that the spermatozoa were in good condition because the plasma membrane was still intact (Rodiah et al., 2015).

Individual Motility of spermatozoa at Room Temperature Storage

Spermatozoa motility was used as the main requirement for determining semen quality because it was closely related to the ability of spermatozoa to fertilize. Calculation of motility percentage was done subjectively by observing the semen under a microscope. Progressively moving spermatozoa were compared with all observed spermatozoa and scored with a percentage of 0-100% (Nubatonis et al., 2022). Analysis of variance on motility of Boer goat spermatozoa with the addition of antioxidant concentrations of quercetin 0 µM, 10 µM, 20 µM and 30 µM at storage times of 0 hour, 2 hours, 4 hours, 6 hours and 8 hours can be seen in Table 2.

The results of analysis of variance in Table 2. showed that the addition of antioxidant concentration of quercetin 0 µM, 10 µM, 20 µM and 30 µM gave a significant difference to the motility of individual spermatozoa (P<0.05). The results of Duncan's further test obtained the highest addition of quercetin antioxidants in the 30 µM treatment, the average in the 30 µM treatment was 63.00±17.35%. The average of the addition of quercetin antioxidant concentration was lowest in the 0 µM treatment with a value of 54.50±17.24%. The 30 µM treatment with the addition of 30 µM quercetin showed that the antioxidant concentration of 30 µM quercetin had an effect in maintaining the quality of motility of individual spermatozoa. According to Najafi et al., (2020) Quercetin antioxidants can provide protection against oxidative damage to spermatozoa. Decreased individual motility can occur because the nutrient content in the dilution decreased and the length of storage time causes a rapid increase in metabolism so that spermatozoa motility decreased. Dilution of spermatozoa with certain materials would obtain different motility depending on the pH content, diluent material, handling method, storage method and storage temperature (Fitriani et al., 2022).

Observations of the motility of individual spermatozoa showed that quercetin supplementation at storage time room temperature significantly improved sperm motility at higher concentrations (P <0.01). Table 2. showed a very significant difference between storage times of 0 hour, 2 hours, 4 hours, 6 hours and 8 hours. The storage time of 0 hour was a very significant difference to 2 hours, 4 hours, 6 hours and 8 hours. Storage of individual motility of spermatozoa at room temperature with a storage time of 4 hours was a very significant difference to the storage time of 6 hours and 8 hours. The highest storage time was at 0 hours with an average and standard deviation of 74.69±8.26% while the lowest storage time was at 8 hours with an average and standard deviation of 32.50±6.58%. Semen quality will continue to decline naturally due to changes in the semen environment starting from the dilution process to storage where the semen needs to adapt to its environment. Exposure to temperature during the storage process would cause oxidation by ROS and the danger of ROS could be inhibited by the presence of antioxidants (Aplugi et al., 2020).

Viability of Spermatozoa at Room Temperature Storage

Viability was one of the indicator determining the quality of semen because it was related to the survival of spermatozoa. Viability of Boer goat spermatozoa can be tested with eosin-nigrosin staining which was characterized by a

Table 2. Individual motility of spermatozoa at room temperature storage

Quercetin Concentration	Room Temperature Storage					Average (%)
	0 Hour	2 Hours	4 Hours	6 Hours	8 Hours	
0 µM	72.50±5.00	65.00±5.77	57.50±5.00	51.25±10.3	26.25±4.79	54.50a ±17.24
10 µM	73.75±4.79	67.50±5.00	63.75±7.50	51.25±8.54	30.00±4.08	57.25ab ±16.82
20 µM	75.00±10.0	68.75±13.1	62.50±9.57	52.50±9.57	35.00±5.77	58.75ab ±16.85
30 µM	77.50±13.2	72.50±15.0	67.50±11.9	58.75±10.3	38.75±4.79	63.00b ±17.35
Average (%)	74.69a ±8.26	68.44ab ±9.95	62.81b ±8.75	53.44c ±9.26	32.50d ±6.58	

Different notations on the same line indicates a significant difference (P<0.05).

Table 3. Viability of spermatozoa at room temperature storage

Quercetin Concentration	Room Temperature Storage					Average (%)
	0 Hour	2 Hours	4 Hours	6 Hours	8 Hours	
0 µM	83.25±6.16	78.04±4.23	76.10±9.99	75.94±8.14	74.45±12.7	77.55±8.40
10 µM	83.69±3.19	78.63±4.82	78.33±5.21	75.83±5.16	72.10±9.32	77.71±6.53
20 µM	78.74±6.31	84.90±5.49	76.55±10.3	76.28±7.28	76.56±6.45	78.61±7.35
30 µM	81.46±5.74	83.20±3.48	78.46±7.80	79.08±6.20	81.99±7.59	80.84±5.94
Average (%)	81.78±5.31	81.19±5.08	77.36±7.75	76.78±6.23	76.27±9.16	

Table 4. Spermatozoa abnormality at room temperature storage

Quercetin Concentration	Room Temperature Storage					Average (%)
	0 Hour	2 Hours	4 Hours	6 Hours	8 Hours	
0 µM	3.91±0.76	4.15±0.52	4.78±0.41	4.45±0.54	4.49±0.71	4.35±0.62
10 µM	3.59±0.66	3.75±0.83	4.52±0.99	4.24±0.57	5.55±1.17	4.33±1.05
20 µM	3.45±0.50	3.48±0.41	3.99±0.78	4.24±0.96	4.26±1.02	3.89±0.78
30 µM	3.22±0.63	3.30±0.76	3.87±0.79	4.52±0.91	4.40±0.81	3.86±0.89
Average (%)	3.54a±0.63	3.67a±0.67	4.29b±0.79	4.36b±0.70	4.67b±1.00	

head that did not absorb color or was transparent, dead spermatozoa have increased membrane permeability and absorb color (Munazaroh et al., 2013). Viability of Boer goat spermatozoa at room temperature storage can be seen in Table 3.

The observation of Boer goat sperm viability in Table 3 showed that the concentration of antioxidant quercetin did not give a significant difference to the viability of Boer goat spermatozoa. The treatment of 0 µM and 10 µM did not give a significant difference as well as 20 µM and 30 µM. The content of andromed extender and the addition of the antioxidant quercetin were sufficient to protect the spermatozoa plasma membrane so that it was not easily damaged during the process storing. Viability of spermatozoa have the highest average in the 30 µM treatment while the lowest average was in the 0 µM treatment. Table 3. showed the average viability of Boer goat spermatozoa was above 70%. According to Muhammad et al. (2017), the percentage of spermatozoa viability above 70% indicated that the semen met the standards for artificial insemination. The percentage of spermatozoa viability depended on the integrity of the spermatozoa membrane. Damage to the spermatozoa membrane could cause disruption of metabolic processes so that the spermatozoa would be weakened (Muhammad et al., 2017).

The results of the analysis in Table 3. showed that the storage time of spermatozoa viability did not give a significant difference to the viability of Boer goat spermatozoa. Storage time of 0 hour without the addition of antioxidant quercetin with an average of 81.78±5.31% haev the highest average result on sperm viability. Storage time of 8 hours at room temperature gave the lowest average with an average of 76.27±9.16%. The availability of nutrients during the storage period could affect the viability of spermatozoa. Nutrients were required for the production of energy which could be used as mechanical energy or as chemical energy.

Abnormalities of Spermatozoa at Room Temperature Storage

The quality of spermatozoa was determined by the structure of the spermatozoa, if there was damage to the structure of the spermatozoa (abnormality). It would inhibit and interfere with the course of fertilization which have an impact on low pregnancy rates. Abnormal spermatozoa could not fertilize oocytes. The level of abnormality could be influenced by temperature, length of storage, age and also genetic factors. Abnormality of Boer goat spermatozoa at room temperature storage can be seen in Table 4.

The result of spermatozoa abnormality showed that the concentration of quercetin antioxidants did not give a significant difference

Table 5. Spermatozoa membrane integrity at room temperature storage

Quercetin Concentration	Room Temperature Storage					Average (%)
	0 Hour	2 Hours	4 Hours	6 Hours	8 Hours	
0 µM	34.03±3.41	33.94±3.66	28.64±6.15	24.02±4.57	19.88±4.62	28.10a±7.00
10 µM	38.79±4.72	34.97±6.85	32.74±6.64	29.17±2.62	23.49±2.94	31.83b±6.99
20 µM	40.33±2.87	40.20±6.27	38.74±2.57	27.34±6.26	22.45±5.13	33.81b±8.80
30 µM	42.14±5.43	42.40±2.71	40.84±1.34	34.58±4.71	25.65±4.15	37.12c±7.45
Average (%)	38.82a±4.90	37.88ab±5.89	35.24b±6.55	28.78c±5.78	22.87d±4.40	

Different notations on the same line indicates a very significant difference (P<0.01)

to the abnormality of Boer goat spermatozoa. The average without quercetin antioxidant concentration had shown 4.35±0.62% which was higher than the average of 10 µM, 20 µM and 30 µM. The high average abnormality of spermatozoa was thought to be due to a kneading error at the time of semen preparation, improper kneading could increase the abnormality of Boer goat spermatozoa. Abnormality that occured in spermatozoa included primary abnormal, secondary abnormal and tertiary abnormal. Abnormality of spermatozoa classified as secondary abnormalities were abnormaly in the tail such as broken or broken tails that occured due to adaptation to environmental changed and less careful semen preparation (Ardhani et al., 2019).

Based on Table 4. of spermatozoa abnormality storage time at room temperature gave a very difference to the abnormality of spermatozoa (P <0.01). Storage time 0 hours at room temperature storage gave a significant difference to the treatment of storage time 2 hours, 4 hours, 6 hours and 8 hours. This result obtained the highest average of 4.67±1.00% at 8 hours storage time while the lowest average was 3.54±0.63% at 0 hours storage time. This was due to plasma membrane damage that occured which caused changes in spermatozoa morphology. Spermatozoa would experience an increase of abnormality every hour due to the influence of semen treatment at the time of handling fresh semen, mixing semen with diluents and at the time of making reviews (Rosary et al., 2018).

Membrane integrity of Spermatozoa at Room Temperature Storage

Membrane integrity in Boer goat spermatozoa could be tested through the HOS Test (Hypoosmotic Swelling Test). The plasma membrane of spermatozoa consisted of carbohydrates associated with lipid and protein (Musaffak et al., 2021). The plasma membrane functions to protect and maintained electrolyte balance both intracellular and extracellular (Azzahra et al., 2016). The integrity of spermatozoa correlated with the mobility of spermatozoa, if the plasma membrane of spermatozoa was damage, the metabolism of spermatozoa would be disrupted. Spermatozoa whose metabolism was disturbed could cause loss of mobility and result in cell death. The integrity of the spermatozoa membrane at room temperature storage could be seen in Table 5.

The result in Table 5 showed that the concentration of antioxidant quercetin gave a very significant difference to membrane integrity between treatments 0 µM, 10 µM, 20 µM and 30 µM (P < 0.01). Duncan's further test results obtained 0 µM gave a very significant difference to 10 µM, 20 µM and 30 µM, as well as 10 µM which gave a very significant difference to 20 µM and 30 µM. Based on observations, the highest mean and standard deviation were found in the 30 µM treatment with the provision of 30 µM quercetin antioxidant concentration and an average of 37.12±7.45%. The lowest mean and standard deviation were in the 0 µM treatment without quercetin antioxidant with a mean value of 28.10±7.00%. The concentration of quercetin antioxidant in the 30 µM treatment was able to reduce free radicals that occured in spermatozoa. Antioxidants were able to reduce free radicals or reactive oxygen species (ROS). Antioxidants were able to stop the process of cell destruction by giving electrons to free radicals.

The result in Table 5. showed that storage time at room temperature gave a very significant difference to the integrity of the spermatozoa membrane (P<0.01). Based on the observations,

it was found that the 0-hour room temperature storage time had the highest average with an average value of 38.82±4.90% and the 8 hours storage time had the lowest average with an average value of 22.87±4.40%. Membrane integrity decreased during room temperature storage which was thought to be due to rupture or leakage of the spermatozoa plasma membrane. Spermatozoa that have intact and live plasma membranes were characterized by swollen heads and circular tails while damaged and dead plasma membranes were characterized by heads that do not swell and tails that were straight. Susilawati (2013) explained that spermatozoa with intact membranes if placed in hypoosmotic media would try to increase the volume of water in their bodies so that the fluid inside and outside the spermatozoa remains balanced.

CONCLUSIONS

The addition of quercetin antioxidant was able to maintain the quality of spermatozoa at room temperature storage. Supplementation of the antioxidant quercetin 30 µM in Boer goat semen gave the best results in maintaining the motility of individual spermatozoa up to 6 hours of storage.

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