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SPERMATOZOA QUALITY OF LIQUID BOER GOAT SEMEN IN TRIS EGG YOLK EXTENDER ENRICHED WITH NON-ENZYMATIC ANTIOXIDANTS

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ABSTRACT

The objective of this study was to determine the effect of standard extender Tris egg yolk supplemented with non-enzymatic antioxidants from guava fruit filtrate (GF) and fig fruit filtrate (FF) on the motility and viability of Boer buck spermatozoa. Semen was collected every four days using an artificial vagina for ten replicates and extended with extenders. The extenders used in this study were Tris + 2.5% egg yolk (T0), T0 + 1.21 mL/100 mL vitamin A from GF (T1), and T0 + 4 mL/100 mL vitamin C from FF (T2). The variables studied were individual motility, progressive motility, and viability of spermatozoa, which were assessed each day for four consecutive days after being stored at 5° C. Data were analyzed using one-way ANOVA. The results showed that 4 days after extended and stored at 5° C, the average individual motility, progressive motility, and viability of spermatozoa in T1 were 45.2±7.94, 33±10.2, and 49±7.69, respectively, while in T2, they were 45.4±6.14, 30.4±8.38, and 50.5±5.88, respectively. The data on the three indicators of spermatozoa quality between T1 and T2 were not significantly different (P<0.05). However, individual motility, progressive motility, and viability of spermatozoa in in T0 were 30.4±5.82; 25.2±7.20 dan 40.2±5.73, respectively and, significantly lower (P<0.05) than that in T2 or T1. It was concluded that semen extended with a Tris egg yolk extender enriched with vitamin A or C that was converted from GF or vitamin C that was converted from FF can be used for artificial insemination until day 4 after storage at 5° C.

Key words: non-enzymatic, spermatozoa, vitamin A, vitamin C

ABSTRAK

Penelitian ini bertujuan mengetahui daya awet pengencer standar Tris kuning telur yang disuplementasi antioksidan nonenzimatik dari filtrat buah jambu biji dan buah tin terhadap motilitas dan viabilitas spermatozoa kambing boer. Semen ditampung menggunakan vagina buatan setiap empat hari sekali sebanyak sepuluh ulangan. Bahan pengencer yang digunakan adalah Tris+2,5% kuning telur (T0), T1 dan T2 berturut-turut T0 + 1,21 ml/100 ml vitamin A (dikonversi dari filtrat buah jambu biji), dan 4 ml/100 ml vitamin C) yang dikonversi dari filtrat buah tin. Variabel yang dinilai adalah motilitas individu, motilitas progesif, dan viabilitas spermatozoa dievaluasi setiap hari selama 4 hari berturut-turut. Hasil penelitian menunjukkan 4 hari setelah pengenceran dan disimpan pada suhu 5° C, rata-rata motilitas individu, motilitas progesif dan viabilitas spermatozoaa adalah 45,8±7,64; 35,0±8,4 dan 52,6±6,69 untuk T1, dan 45,2±6,11; 31,4±8,26 dan 50,5±5,88 untuk T2. Data ketiga indikator kualitas spermatozoaa antara T1 dan T2 tidak menunjukkan perbedaan yang signifikan (P<0,05). Untuk T0 berturut-turut motilitas individu, motilitas progesif dan viabilitas spermatozoa adalah 30,4±5,82; 25,2±7,20 dan 40,2±5,73 dan secara signifikan lebih rendah (P<0,05) dibandingkan dengan T2 atau T1. Penelitian ini menyimpulkan, semen yang diencerkan dengan pengencer Tris kuning telur yang disuplementasi vitamin A yang dikonversi dari filtrate buah jambu biji atau vitamin C yang dikonversi dari filtrate buah tin masih bisa digunakan untuk inseminasi buatan sampai hari ke-4 setelah pengenceran dan penyimpanan pada suhu 5° C.

Kata kunci: antioksidan, non-enzimatis, spermatozoa, vitamin A, vitamin C

INTRODUCTION

To achieve optimal fertility with Artificial Insemination (AI), the quality of liquid semen must also be optimal. Factors that influence the quality of semen are the preservation methods and the type of extender. When preserving liquid semen, an extender should meet all the needs of spermatozoa so that their viability and motility can be maintained longer (Zaenuri *et al.* 2013; Zaenuri *et al.* 2017).

The quality of fresh semen decreases rapidly if the extender is unsuitable (Zaenuri *et al*. 2017). For unextended semen, it is difficult to maintain spermatozoa motility and viability for more than 24 hours, even when the semen is stored at low temperatures. Common semen extenders used include Tris egg yolk, citrate egg yolk, fresh milk egg yolk, skimmed milk egg yolk, AndroMed, and lactose egg yolk (Evan and Maxwell 1989). To maintain semen quality using an extender, some researchers added antioxidants to the extender. Ibrahim *et al*. (2008) explained that there are two types of antioxidants, namely enzymatic antioxidants (SOD, catalase, glutathione, and alpha-lipoic acid) and non-enzymatic antioxidants (vitamins E, C, and A), which are very effective against free radicals.

To improve the preservation effect of semen extenders, several researchers added non-enzymatic organic antioxidants to the extenders, such as local fig fruit filtrate (*Ficus glumerta* Roob) (Zaenuri *et al*. 2013) or *Ficus glumerata* Linn (Zaenuri *et al*. 2014; Zaenuri *et al*. 2017). Sumadiasa *et al*. (2018) also reported that guava fruit filtrate (*Psidium guajava* Linn) is a potential source of non-enzymatic antioxidants. Other research utilized melon juice (Riyadhi *et al*. 2017) and tomato juice (Rosmaidar *et al*. 2013). The addition of fruit juice to the semen extender protects spermatozoa cells from free radicals; hence, spermatozoa viability can be maintained longer. Previous studies (Zaenuri *et al*. 2017) showed that the preservation influence of the semen extender with fig fruit filtrate was significantly better than that of the extender not supplemented with fig fruit filtrate.

Fig Fruit Filtrate (FF) and Guava Fruit Filtrate (GF) is a source of organic non-enzymatic antioxidants that have a high probability of increasing the preservation

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of semen extenders to maintain the quality of buck spermatozoa. Therefore, two types of organic supplements with high non-enzymatic antioxidant content, namely GF and FF, were used in this study. Each 100 g of guava fruit contains 59.5 mg of vitamin A (β-carotene) and 151.4 mg of vitamin C (Sumadiasa 2018). Similarly, fig fruit contains 0.40 mg of vitamin A (β-carotene), 181.50 mg of vitamin C, and 0.81 mg of vitamin E (ά-tocopherol) per 100 g of fresh fig fruit (Zaenuri 2014).

The addition of 4% (v/v) of fresh filtrate of fig fruit in Tris egg yolk extender was able to maintain the percentage of the progressive motility at 48.5±2.24 (Zaenuri *et al*. 2013) of Boer buck spermatozoa 120 hours after being stored at 5° C. The progressive motility of spermatozoa as a result of that study was well above the Indonesian National Standard which is 40% (SNI 2014). Sumadiasa (2018) reported that the addition of 10% guava filtrate to Tris egg yolk extender (v/v) can maintain the percentages of motility and viability of spermatozoa after thawing at 61.03% and 81.44%, respectively.

The role of antioxidants in extenders is to inhibit the chain reaction of free radicals by acting as electron acceptors (Funahashi *et al*. 1993). Kulaksiz and Daskin (2010) added that oxidative molecules play a role in reducing oxidative stress, while compounds in antioxidants protect cells from the dangers of oxygen radicals so that spermatozoa quality can be maintained longer. However, vitamin C acts as an oxidant at low concentrations, while at high concentrations (5 mM), it acts as an antioxidant (Andrabi *et al*. 2008). Therefore, this study was conducted to compare the preservative effect of vitamin C in FF with vitamin A in GF on individual motility and progressive motility of Boer buck spermatozoa during cold storage.

MATERIALS AND METHODS

Vitamin C from Fig Filtrate (FF) and Vitamin A from Guava Filtrate (GF) Preparation

Filtration of the FF was performed using the method of Zaenuri *et al*. (2014). The FF was stored in the refrigerator until it was ready to use. The type of non-enzymatic antioxidant used as the basis for determining the volume of FF was vitamin C. According to Batool *et al.* (2012), the standard concentration of vitamin C in buck semen extender is 0.5 mM/mL or 500 mg/100 mL.

Calculation to find out FF concentration is as follows: The average content of vitamin C in figs is 18.15 mg per 1000 mg, or 1.815 mg per 100 g of fresh fruit, and the average water content is 35.91 mL per 100 g (Zaenuri 2014). After being juiced, then extended with distilled water in a ratio of 1:1, the concentration of vitamin C becomes: = $\frac{1}{2}$ x 1,815 mg/100 g= 0,9075 mg/71.815 mL= 0.1264 mg/100 mL (noted, 71.815 comes from water content of fresh fruit × 2). The needs of vitamin C in extender were 500 mg/100 mL extender. The content of vitamin C in FF is 0.1264 mg/100 mL, then the volume of FF added: (500/0.1264 mg)/100 mL extender= 3.955 mL/100 mL converted to 4 mL/100 mL extender.

Guava fruit filtration was performed as described by Sumadiasa *et al*. (2015). The concentration of GF for this study was based on the requirement for vitamin A (β-carotene) in the extender, which is 0.002 g/100 mL according to Siahaan *et al*. (2012).

The average content of carotene in guavas was 59.5 mg/100 g of fresh fruit with an average liquid content of 75% (75 mL). The liquid guava juice was diluted with 100 mL of aquabidest (1:1), so that the concentration of carotene becomes: 59.5/100 g= 29.73 mg/71.815= 0.414 mg/100 mL (noted, 71.815 come from the water content of fresh fruit × 2). The need for Vitamin A in the extender was 0.002 g/100 ml (Siahaan *et al*. 2012). The content of vitamin A in GF is 0.414 mg/100 mL, then the volume of GF is added: (500/0.414 mg)/100 mL extender= 1.207/100 mL, which is converted to 1.21 mg/100 mL.

Extender Preparation for Treatment

The control extender (T0) was Tris egg yolk as described by Evans and Maxwell (1989). Treatment extenders were T1 (T0 + 4 mL FF/100 mL) and T2 (T0 + 1.21 mL GF/100 mL (v/v)). The control and treatment extenders were homogenized with a magnetic stirrer before being used to dilute the semen.

Semen collection and Extension

The semen used in this study was collected from a 2.5-year-old, 75-kg Boer Cross buck (F3). Semen was collected by an artificial vagina at every four consecutive days. Initial evaluation for fresh semen was immediately performed in the laboratory. Spermatozoa concentration was the same for each treatment, which was 50×10^6/0.25 mL. The dilution rate is calculated in the following way: Frequency of extended= (% motility of sperm × concentration/mL x volume of semen)/(50×10^6)= A. If the volume of fresh semen is 1 mL, then 1/A= b mL (fresh semen). b mL of fresh semen are then extended in 1 ml of extender so that the concentration of spermatozoa per 1 mL of the extender is 200 10^6 (Zaenuri *et al*. 2013). The semen was extended by gradually adding the treatment extender to the tube containing fresh semen. The tube was gently shaken to make the semen and extender homogeneous. Each treatment was divided into four tubes based on the observation schedule. In addition, the tube was incubated in a water bath at 37° C for 30 minutes to allow the antibiotics in the extender to act optimally. To avoid cold stress, the incubated semen was transferred to a glass beaker containing 50 mL of distilled water at a temperature of 18-21° C and placed in a refrigerator so that the temperature of the treated semen would gradually decrease to 5° C after one hour (Menchaca *et al*. 2005).

Liquid Semen Evaluation

Fresh semen was evaluated directly in the laboratory. The minimum requirements for processable

semen are that it has a minimum 0.8 mL in volume, 70% spermatozoa motility, and a spermatozoa concentration of 2.5 109/mL (Zaenuri *et al*. 2017). Meanwhile, the examination of treatment semen included spermatozoa concentration/mL, percentage of individual motility, and progressive motility. Treatment semen evaluation was performed immediately after dilution and every 24 hours for 4 consecutive days. A progressive motility evaluation was assessed by placing a drop of semens on a glass slide and covering it with a coverslip. Observations were made using a light microscope (Nikon, Japan) with 400× magnification on two hundred spermatozoa in several fields of view. The percentage of progressively motile spermatozoa is the number of progressively motile spermatozoa divided by the number of observed spermatozoa (Menchaca *et al*. 2005). The percentage of individual spermatozoa motility is calculated subjectively (Zaenuri *et al*. 2017).

Evaluation of spermatozoa viability was performed using a smear preparation. A drop of semen was mixed with a drop of eosin nigrosin, homogenized by the tip of other object glass then slide to the end of object glass, and left on a glass object for 30 seconds. Then, a smear preparation was made. The smears were viewed under a light microscope (Nikon, Japan) with 400× magnification. Spermatozoa that absorbed color were dead, while those that did not absorb color or were transparent were live spermatozoa. The percentage of live spermatozoa is the number of live spermatozoa divided by the number of observed spermatozoa multiplied by 100 (Zaenuri *et al*. 2017).

Data Analysis

The statistical significance of the result was assessed by a one-way analysis of variance (ANOVA) using the SPSS 16.0 program. The data were presented as Mean±SE. Probability P<0.05 was considered significantly different.

RESULTS AND DISCUSSION

Fresh Semen Evaluation

The results of the macroscopic and microscopic examination of the fresh semen of the Boer-cross buck are shown in Table 1. The semen volume was 1-1.8 mL with an average of 1.54±0.32 mL/ejaculate. Zaenuri *et al*. (2017) observed that semen volume correlates with spermatozoa concentration. The lower semen volume, the less spermatozoa concentration was observed, however, it did not affect fertility. Semen volume in this study was lower than that reported by two previous researchers, which were 2.64 mL (Pratiwi *et al.* 2007) and 2.44 mL (Armansyah *et al*. 2018). However, the value was within the range reported by other researchers, namely 0.960 mL/ejaculate (Putra *et al*. 2012), 0.70-1.50 mL (Suyadi *et al*. 2004), 0.640 (Rizal *et al*. 2018), 1.03 mL (Prestiya *et al*. 2020), 0.77 to 1.13 mL (Suharyati and Hartono 2013), 0.80 mL (Pamungkas *et al*. 2014), and 1.14 mL (Rhochim *et al.* 2017).

Table 1. Quality of Boer buck fresh semen (n= 10)

Parameter	Average
Volume (mL)	1.56±0.22
Spermatozoa concentration (10^9)	2,350±156,5
Viscosity	Moderate
Acidity (pH)	7.07±0.04
Mass motility (%)	+++
Individual motility (%)	92.2±2.78

The average spermatozoa concentration in this study was 2.32±168.5 × 10^9 (Table 1). Similarly, several studies also reported that the average spermatozoa concentration was 3,016±77.97 × 10^9 (Rizal *et al*. 2018), 2,981-3,568 × 10^9 (Suharyati and Hartono 2013), 4,125 × 10^9 (Pamungkas *et al*. 2014), and 4,350 × 10^9 cells/mL (Rhochim *et al*. 2017).

The viscosity, pH, and mass motility of spermatozoa as a result of this study were moderate, 7.06±0.05 and +++, respectively (Table 1). The semen viscosity in this study was comparable with data reported by Rizal *et al*. (2018), Pamungkas *et al*. (2014), and Rhochim *et al*. (2017). The pH of semen from this study was also normal, as reported by several previous researchers, such as 6.2–6.8 (Putra *et al*. 2012), 6.5-6.9 (Sumadiasa, 2018), 6.40-7.25 (Pamungkas *et al*. 2014), and Rhochim *et al*. (2017). The pH decreases with increasing storage time due to the anaerobic metabolic activity of spermatozoa, which produces lactic acid (Salisbury and VanDermak, 1985).

Spermatozoa mass motility was found to be +++ in this study (Table 1). These results were in agreement with the findings of previous studies (Pamungkas *et al*. 2014; Kusumawati *et al*. 2017; Rhochim *et al*. 2017; Rizal *et al*. 2018). Progressive motility of spermatozoa was observed immediately after storage. The current results (90.2±3.89%) were higher than those of several previous studies. Masyitoh *et al*. (2018) reported that the progressive motility was 76.00±0.04% and 77±0.1% (Zaenuri 2014) immediately after storage. Ax *et al*. (2008) stated that the motility of fresh spermatozoa that could be further processed was above 70%. Thus, the fresh semen from this study was eligible to be processed.

Evaluation of Semen after Treatment

Individual motility and progressive motility

The data on the average percentage of individual motility and progressive motility of buck spermatozoa in extenders with and without vitamin A or vitamin C are shown in Table 2. The percentage of individual and progressive motility in this study showed that T1 and T2 had a relatively similar protective effect against individual and progressive motility compared to T0. The percentage of individual motility in T1 and T2 was not significantly different (P<0.05). However, the percentage of individual spermatozoa motility was significantly (P<0.05) higher in both T1 and T2 than that of the T0.

The percentage of individual spermatozoa motility in T0, T1, and T2 found in this study was higher than that in extender containing 80% palm sugar + 20% egg yolk or in Andromed, which was assessed 4 days after

extension and stored at 5° C, 0% and 40.00±3.53%, respectively (Rizal *et al*. 2018). Also, the addition of 37.5 g and 75 g of PGF2α into physiological NaCl was only able to maintain the percentage of motility of spermatozoa 62.0±3.46 and 361.8±10.12 at 4 hours after extension and storage at 5° C (Prestiya *et al.* 2020). The percentage of spermatozoa individual motility in the extender of 80% Tris + 20% egg yolk and 80% Tris (without raffinose) + 20% egg yolk 4 days from extension and storage at 5° C were 51.5±9.73 and 55.5±8.32, respectively (Rhochim *et al*. 2017).

The percentage of progressively motile spermatozoa assessed 4 days after extension and storage at 5° C is documented in Table 2. Extender T1 was relatively better at maintaining the spermatozoa progressive motility (33.0±10.2) than T2 (30.4±8.38) although both T1 and T2 were not significantly different (P<0.05). However, T1 and T2 showed a significantly better ability (P<0.05) to maintain the percentage of spermatozoa progressive motility than P0 (24.6±7.3).

The percentage of progressively motile spermatozoa found in this study was relatively lower than that of previous studies. Zaenuri *et al*. (2013) reported that the addition of 6-7% (v/v) fig fruit filtrate (*Ficus glumerata* Robb) in Tris egg yolk extender could maintain the progressively motile spermatozoa between 40.0±2.11 and 54.5±2.93 at 4 days after dilution and storage at 5° C. Apriliana (2019) also found that progressive spermatozoa motility in Tris egg yolk + 7% freeze-dried fig fruit filtrate was 48.4±1.4%. Similarly, Saaban (2018) and Rosmaidar *et al*. (2013) reported the higher progessive spermatozoa motility in 2% soy lecithin extender (52.73±27.51%) and in the egg yolk citrate extender + 20% tomato juice (48.20±14.68%), respectively. However, the progessive spermatozoa motility in the present study was relatively higher as compared to the data presented by Mugiyati *et al*. (2017) who observed that the use of Tris extender + 20% egg yolk + pink coconut water could only maintain the progressive motility of 14±23.07%. In addition, Lubis *et al.* (2013) reported progressive motility of spermatozoa in skim milk + 0.3 g of vitamin C was only 36.20±6.30 after 3 days of storage at 5° C.

The decrease in individual and progressive spermatozoa motility in cold storage is not only due to the type of extender but also due to the adaptation process that spermatozoa undergo in a new environment and atmosphere (Agustian *et al.* 2014; Zaenuri *et al.* 2017). In this study, individual and progressive motility decreased significantly (P<0.05) in P0 than in T1 and T2. This might be due to the role of organic antioxidants such as vitamin A and vitamin C from GF and FF filtrate added to T1 and T2. Extenders containing antioxidants can inhibit chain reactions that generate free radicals by acting as electron acceptors (Funahashi *et al.* 1995). Kulaksiz and Daskin (2010) also found that oxidative molecules would reduce the effect of oxidative stress, while compounds in antioxidants would protect cells from the dangers of oxygen radicals, thus preserving spermatozoa quality longer.

Prolonged storage of spermatozoa results in a reaction between spermatozoa and oxygen, which causes the formation of free radicals. The free radicals formed trigger the peroxidation of membrane lipids which reduces spermatozoa viability and motility (Siahaan *et al*. 2012). In this unfavorable condition, β-carotene present in GF filtrate acts to reduce free radicals. According to Surjohudojo (2000), β-carotene is a fat-soluble compound that plays an important role in preventing the occurrence of excessive lipid peroxides in the cell plasma membrane. According to Oshima *et al*. (1993), β-carotene can protect liposomes (a vesicle with a single phosphorus layer) from damage caused by a brief oxygen attack.

Vitamin C in FFF plays a very important role as an antioxidant compound in reducing free radicals and as a free radicals scavenger against free radicals formed by spermatozoa metabolism; hence, cell function disorders can be avoided (Zaenuri *et al*. 2017). In addition, Vitamin C has polarity properties; thus, it can act as a substance against free radicals, is able to neutralize and react with superoxide anions, hydroxyl radicals, singlet oxygen, and lipid peroxides, and prevents spermatozoa clumping (Suhartono *et al*. 2007). Vitamin C in the form of L-ascorbic acid, as an antioxidant ascorbic acid, is classified as a reducing agent because it has a low redox potential, but it is also effective against oxidizing agents. Ascorbic acid can break the radical reaction generated by peroxidation, where ascorbic acid reacts directly in the liquid phase with free radicals and transforms into a slightly reactive ascorbyl (Padayatti *et al*. 2003).

Spermatozoa viability

Spermatozoa viability is the percentage of live and dead spermatozoa observed using the differentiation technique of smear preparations stained with eosin-negrosin (Evans and Maxwell 1989; Kusumawati *et al*. 2017). The data on the average viability of Boer buck spermatozoa resulting from this study are presented in Table 3. As noted in Table 3, it can be seen that the addition of vitamin A and vitamin C to an extender based on Tris yolk preserved the quality of buck spermatozoa according to SNI for 4 days (40%), for frozen semen as a comparison. However, the percentage of spermatozoa viability as a result of this study was lower than the addition of 7% FF filtrate into standard extender Tris egg yolk (66.0±7.9) on day 4 after extension and storage at 5° C (Zaenuri *et al*. 2013). However, the percentage of spermatozoa viability from this study was relatively the same as those of previous studies using FF species *Carica* Linn (Zaenuri *et al*. 2014; Zaenuri *et al*. 2017) and GF (Sumadiasa 2015).

The results of statistical analysis showed that the percentage of spermatozoa viability could survive until the 4th day of storage (49.6±7.69) in P1 or slightly lower but not significantly different (P≤0.05) compared to P2 (50.5±5.88). However, both values in P1 and P2 were above the Indonesian National Standard, which is 40% (SNI 2014). Both P1 and P2 showed a significant

preservation activity (P≤0.05) to maintain spermatozoa viability of Boer bucks compared to P0 which was 40.7±5.77. Spermatozoa viability (Figure 1 and Table 3) was much higher than the viability of Boer bucks spermatozoa extended in Tris aminomethane + 20% egg yolk + pink coconut water after being stored at cold temperatures for 4 days (Mugiyati *et al*. 2017). However, three researchers reported higher percentages of spermatozoa viability in which semen was prolonged in Tris + soy lecithin and stored cold for 4 days (Saaban *et al*. 2018), in Tris aminomethane + 20% egg yolk (Rhochim *et al*. 2017), and in the addition of 0.002% β-carotene (Siahaan *et al*. (2012).

Liquid semen stored at 5° C undergoes changes in membrane structure which eventually reduce their viability due to oxidative stress, triggering the formation of reactive oxygen (Zaenuri *et al*. 2014). Immotile spermatozoa are not necessarily dead spermatozoa. Inappropriate environmental factors affect the ability of spermatozoa to move under the microscope (Zaenuri *et al*. 2014). Eosin nigrosin staining was carried out to determine live and dead spermatozoa. Absorption of eosin color on spermatozoa indicates that the spermatozoa had damaged their plasma membrane. Rosmaidar *et al*. (2013) stated that the absorption of the color of dead spermatozoa was due to the absence of a potential difference between sodium and potassium ions between cells inside and outside so that eosin bound to sodium would easily diffuse and show color. Meanwhile, the live spermatozoa remained colorless because eosin was bound to sodium (Figure 1).

The longer the storage time, the less nutrient was available which lead to spermatozoa death. Nutrients are used by spermatozoa as a source of energy; consequently, less availability of nutrients can lead to a decrease in viability. The addition of organic additives to the semen extender aims to overcome the effects of free radicals produced by spermatozoa metabolism during storage; thus, spermatozoa viability can be maintained for longer. The added organic additives are rich in antioxidants, which inhibit the chain reactions involved in the formation of free radicals by acting as

Table 2. The effect of additional vitamin A from guava or C from fig fruit filtrate in a Tris egg yolk-based extender stored at 5° C on the average percentages of individual and progressively spermatozoa motility

Parameter (%)	Storage time (days)	Treatments		
		T0	T1	T2
Individual motility	0	82.4±1.81[a]	81.4±1.46[a]	82.2±2.67[a]
	1	64.3±4.72[a]	75.4±5.64[b]	73.6±3.44[b]
	2	55.3±4.06[a]	64.6±6.19[b]	63.4±4.61[ab]
	3	44.1±2.34[a]	57.2±7.61[b]	56.1±4.12[b]
	4	30.4±5.82[a]	45.8±7.64[b]	45.2±6.11[b]
Progressive motility	0	79.3±0.82[a]	79.8±3.24[a]	77.0±2.62[a]
	1	54.6±4.66[a]	62.4±8.71[b]	58.4±8.39[ab]
	2	45.9±4.98[a]	53.2±7.11[b]	50.6±9.22[b]
	3	36.4±6.62[a]	45.0±10.2[b]	43.0±11.4[b]
	4	25.2±7.2[a]	35.0±8.4[b]	31.4±8.26[b]

[a, ab, b] Different superscripts in the same rows indicate significant differences (P<0.05)

Table 3. Spermatozoa viability of buck spermatozoa in Tris egg yolk-based extender supplemented with and without vitamin A from GF filtrate and vitamin C from FF filtrate

Storage times (days)	Extender		
	T0	T1	T2
0	84.4±1.15[a]	83.4±1.34[a]	83.7±2.53[a]
1	68.2±4.12[a]	75.9±4.74[b]	75.3±2.77[b]
2	62.2±6.04[a]	68.2±7.48[a]	66.4±4.56[a]
3	53.8±5.64[a]	61.8±7.74[b]	59.4±4.54[b]
4	40.2±5.73[a]	52.6±6.69[b]	50.5±5.88[b]

[a, ab, b] Different superscripts in the same rows indicate significant differences (P<0.05)

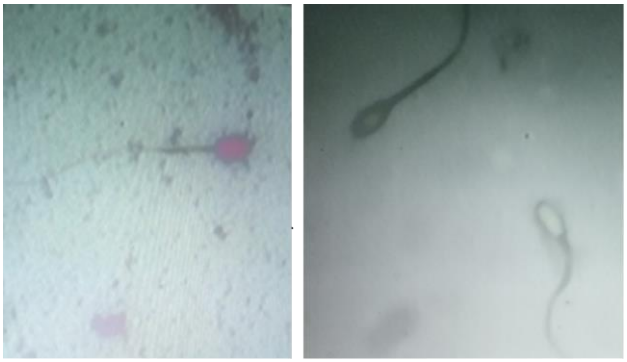

Figure 1. Sperm viability of Boer goats using a light microscope with 400x magnification. Live sperm (right) dead sperm (left)

electron acceptors (Funahashi *et al*. 1993; Sano 2005). As a result, oxidative molecules reduce the effect of oxidative stress, while compounds in antioxidants function to protect cells from the dangers of oxygen radicals so that spermatozoa quality lasts longer (Kulaksiz and Daskin 2010).

CONCLUSION

The addition of 4 mL/100 mL vitamin C from fig fruit filtrate and 1.21 mL/100 mL vitamin A from guava fruit filtrate in Tris egg yolk-based extender is able to provide a better preservation rule for Boer buck spermatozoa than the standard extender without the addition of vitamin A or vitamin C. Liquid semen in both extenders can still be used for artificial insemination up to day 4 after dilution and storage at 5° C.

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