

EPIDIDYMAL SPERMATOZOA QUALITY OF ETAWA CROSSBREED GOAT IN TRIS EXTENDER SUPPLEMENTED WITH VARIOUS LACTOSE CONCENTRATIONS

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ABSTRACT

This research was conducted to investigate the effect of various concentrations of lactose supplementation in Tris extender for maintaining the quality of Etawa crossbreed goat epididymal spermatozoa stored at 3-5° C. Semen in the control group was diluted with a tris extender containing 20% egg yolk without lactose. Semen in the test groups was diluted with a tris extender containing 20% egg yolk and added with 0.3% (0.3 g per 100 mL extender) and 0.6% lactose for group TL1 and TL2, respectively. Parameters evaluated of the fresh epididymal spermatozoa were motility, concentration, percentage of live, and abnormality of spermatozoa, while for diluted-spermatozoa were motility and percentage of live spermatozoa. Spermatozoa observation was conducted until it reaches 40% motility. The results showed that the mean percentage of motility, live sperm, concentration, and abnormality of epididymal spermatozoa were 70%; 81%; $3,220 \times 10^6$ cells/mL; and 4.30%, respectively in all group. After dilution, the percentage of motility and live spermatozoa were also 70% and $81.00 \pm 1.58\%$, respectively in all groups. The decreasing of spermatozoa motility was observed on day 4 of storage, in which percentage of spermatozoa motility in control group ($40.00 \pm 0.00\%$) was significantly lower ($P < 0.05$) than those in TL1 ($44.00 \pm 2.24\%$) and TL2 ($45.00 \pm 0.00\%$) groups. Percentage of live spermatozoa in control ($63.20 \pm 2.68\%$) was not significantly different ($P > 0.05$) than TL1 ($65.40 \pm 1.95\%$) and TL2 ($65.60 \pm 1.95\%$). In conclusion, the supplementation of lactose into Tris extender could maintain the epididymal spermatozoa of Etawa crossbreed for 3 days of storage at 3-5° C.

Key words: epididymal spermatozoa, Etawa crossbreed, lactose, preservation

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh penambahan laktosa pada berbagai konsentrasi dalam pengencer Tris kuning telur terhadap kualitas spermatozoa kauda epididimis kambing peranakan etawa (PE) yang disimpan pada suhu 3-5° C. Pengencer Tris kuning telur digunakan sebagai kontrol (K), perlakuan pengencer Tris kuning telur + 0,3% laktosa (TL1), dan pengencer Tris kuning telur + 0,6% laktosa (TL2). Pengamatan spermatozoa asal epididimis meliputi persentase motilitas, konsentrasi, persentase hidup spermatozoa, dan abnormalitas. Pengamatan spermatozoa setelah pengenceran meliputi pengamatan persentase motilitas dan hidup/mati. Pengamatan dilakukan sampai motilitas spermatozoa mencapai batas minimum 40%. Hasil penelitian menunjukkan motilitas, persentase hidup, abnormalitas, dan konsentrasi spermatozoa masing-masing adalah 70%; 81%; 4,30%; dan $3,220 \times 10^6$ sel/ml. Setelah pengenceran, persentase motilitas dan hidup spermatozoa pada K, TL1, dan TL2 adalah sama yakni 70% dan $81 \pm 1,58\%$. Penurunan motilitas terjadi pada hari ke-4, pada K1; TL1; dan TL2 masing-masing adalah $40,00 \pm 0,00$; $44,00 \pm 2,24$; dan $45,00 \pm 0,00\%$ ($P < 0,05$). Persentase hidup spermatozoa pada K; TL1; dan TL2 masing-masing adalah $63,20 \pm 2,68$; $65,40 \pm 1,95$; dan $65,60 \pm 1,95\%$ ($P > 0,05$). Disimpulkan bahwa laktosa mampu mempertahankan kualitas spermatozoa epididimis kambing PE yang disimpan selama tiga hari pada suhu 3-5° C.

Kata kunci: spermatozoa epididimis, kambing peranakan Etawa, laktosa, preservasi

INTRODUCTION

The application of reproductive technologies for the population improvement of domestic animals has been developed through the application of artificial insemination (AI). Semen used for AI was usually collected using an artificial vagina (AV), but there is an alternative to obtain the spermatozoa from the cauda epididymis (Rizal *et al.*, 2007). Axner *et al.* (1998) states that the spermatozoa obtained from the cauda epididymis have similar ability to the spermatozoa of ejaculated semen especially in motility and oocytes fertilization. Therefore, the use of cauda epididymis from abattoir waste can be used as an alternative of spermatozoa sources. Previous studies of spermatozoa collected from cauda epididymis have been reported in several animals such as in cows (Graham, 1994), deer (Soler *et al.*, 2003), african buffalo (Herrick *et al.*, 2004; Harshan *et al.*, 2005; Herold *et al.*, 2006), and Garut sheep (Rizal *et al.*, 2004).

Cauda epididymis is a temporary spermatozoa reservoir prior to ejaculation. Therefore, spermatozoa

obtained from the cauda epididymis need to be proceed appropriately in order to survive for a certain duration and have an optimum fertility (Solihati *et al.*, 2008). The processing needed to maintain the viability and fertility rates are through the addition of diluent. Various diluent could be used to dilute the spermatozoa, but it must contain substances required by sperm cells. According to Toelihere (1993), a diluent should be able to provide nutrients for spermatozoa, protecting the spermatozoa from cold shock, providing a buffer to prevent the pH changes, maintaining the osmotic pressure and electrolyte balance, preventing the growth of bacteria, and increasing the volume of insemination.

One of the substances that can maintain the quality of semen is carbohydrate particularly sugars group (saccharides), either monosaccharide, disaccharide or oligosaccharide. This is due to the ability of carbohydrates as a resource of nutrients, as well as an extracellular cryoprotectant that able to protect the cell plasma membrane from damages (Supriatna and Pasaribu, 1992). The use of sugar for maintaining the

quality of spermatozoa during the preservation and the cryopreservation process in livestock has been widely reported by several researchers, such as the use of glucose in the sheep frozen semen (Molinia *et al.*, 1993), trehalose in pampinta sheep frozen semen (Aisen *et al.*, 2000; Aisen *et al.*, 2002), sucrose and trehalose in cattle frozen semen (Woelders *et al.*, 1997), lactose in goat frozen semen (Singh *et al.*, 1995), and Garut sheep liquid semen (Rizal, 2006), and dextrose, trehalose, raffinose, and sucrose in Garut sheep frozen semen (Rizal *et al.*, 2006).

In this research, lactose derived from the disaccharide group which function as an energy source, a water removal from the cells to reduce the ability of water to form ice crystals, and as an osmotic buffer to prevent the cell from swelling and to stabilize the cell membrane (Tambing *et al.*, 2003) was used. Based on the previous studies, the addition of lactose in tris-egg yolk as extender for spermatozoa obtained from cauda epididymis of etawa crossbreed (PE) goat was expected can be an energy source for the spermatozoa and reduce the destruction to the plasma membrane of spermatozoa due to lipid peroxidation. Therefore, it will be able to produce a qualified liquid semen of PE goats.

MATERIALS AND METHODS

Cauda epididymis was collected from three PE goats (6 pairs of testes) aged 2-4 years old. One of testicle disqualified due to not fulfill the criteria required. A collected testicular goat came from a traditional abattoir in Martapura, Banjar Regency. The collection, evaluation, and dilution of cauda epididymis spermatozoa were conducted at the Laboratory of Reproductive and Animal Breeding, Department of Animal Science, Faculty of Agriculture, University of Lambung Mangkurat, Banjarbaru.

Collection and Dilution of Cauda Epididymis Spermatozoa

Testis and epididymis from PE goats were put in a flask containing ice packs, thereafter, it was brought to the laboratory for collection and dilution process of spermatozoa. First, the epididymis was separated from the testes, then the cauda epididymis was separated from the caput and the corpus of epididymis. Furthermore, spermatozoa were collected with a combination of slicing and pressure methods into the epididymal tissues following a rinsing using 2.5 mL of sodium chloride (NaCl 0.9%) (Rizal, 2004). Finally, collected spermatozoa were divided into four tubes with the same volume.

The evaluated parameters were sperm concentration, the percentage of abnormalities, motility, and live spermatozoa. According to Hafez and Hafez (2000), a good quality standard of cauda epididymis spermatozoa and had prerequisites to be further processed as sample were those have motility $\geq 70\%$, concentration $> 2.000 \times 10^6$ cells/mL and sperm abnormalities $< 15\%$. The cauda epididymis

spermatozoa that fulfill these requirements were then diluted with 80% tris base diluent + 20% egg yolk (for control/K), 80% tris base diluent + 20% egg yolk + 0.3 g lactose/100 mL of diluent (TL1), and 80% tris base diluent + 20% egg yolk + 0.6 g lactose/100 mL of diluent (TL2).

The composition of tris base diluents consisted of 2.42 g tris (hydroxymethyl) aminomethane, 1.28 g of citric acid, and 2.16 g fructose dissolved in aquabidestilata up to 100 mL, then added with 1,000 IU penicillin and streptomycin per milliliter diluents. Each tube contains 5 mL of diluted spermatozoa. Furthermore, the tubes were sealed and preserved in a refrigerator at 3-5° C (Solihati *et al.*, 2007).

Evaluation of the Cauda Epididymis Spermatozoa The percentage of the spermatozoa motility

The motility rate assessment was performed by observing the number of spermatozoa moving forward (progressive). It was determined subjectively on eight different fields of view with a light microscope at 400x magnification. The numbers given was in the range of 0-100% with a scale of 5% (Toelihere, 1993).

The spermatozoa concentration

The calculation of the spermatozoa concentration was done using haemocytometer. Concentration of undiluted spermatozoa was counted at five Neubauer counting chambers (upper right, upper left, lower right, lower left and middle counting chambers) under a microscope at 400x magnification. Concentration of spermatozoa was then calculated using a formula $n \times 10^6/\text{mL}$ spermatozoa, where n is the number of spermatozoa counted at Neubauer counting chambers (Toelihere, 1993).

The percentage of live spermatozoa

The percentage of live spermatozoa is the number of a live and dead spermatozoa observed by using an eosin 2%. The live spermatozoa were marked by a white head or do not absorb the color, while the dead spermatozoa were marked by a red head or absorbs the color. Observations were performed at 10 different fields of view using a microscope at 400x magnification (Toelihere, 1993).

Percentage of the sperm abnormality

The abnormal percentage of spermatozoa was evaluated using an eosin 2%. Spermatozoa abnormalities were observed by viewing malformed of 200 sperm cells using a microscope at 400x magnification (Toelihere, 1993).

Evaluation of the quality of spermatozoa after dilution

Evaluation of the spermatozoa quality after diluting with lactose diluent and preserved in a refrigerator at 3-5° C include a microscopic evaluation, that is the percentages of motility and live spermatozoa. Observations were done every day until the percentage of sperm motility less than 40%.

Data Analysis

Data were analyzed using analysis of variance (one way ANOVA) with three treatments and five replications. The difference among treatments were tested using Least Significant Difference (LSD) test (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

Characteristic of the Cauda Epididymis Spermatozoa

Characteristic of cauda epididymis spermatozoa of PE goats were presented in Table 1. According to Hafez and Hafez (2000), the goat spermatozoa criteria that could be diluted for AI must have a value of $\geq 70\%$ for motility, $>2000 \times 10^6$ cells/mL for sperm concentration, and $<15\%$ for abnormalities. The results of the present study showed that cauda epididymis spermatozoa of PE goat meet the requirement to be diluted because the spermatozoa motility, concentration, and abnormalities were 70%, $3,220 \pm 727.50 \times 10^6$ cells/mL, and $4.3 \pm 1.15\%$, respectively.

Table 1. Characteristic of spermatozoa obtained from cauda epididymis

Variabels	Average \pm SD
Volume (mL)*	2.5 \pm 0.00
Motility (%)	70.0 \pm 0.00
Concentration ($\times 10^6$ cell/mL)	3,220 \pm 727.50
Life sperm (%)	81.0 \pm 1.58
Abnormality (%)	4.30 \pm 1.15

* = Volume after added NaCl 0.9% until volume 2.5 mL

The spermatozoa motility of PE goat in this study was 70% which was similar to the motility of the ejaculated sperm reported by Souhoka *et al.* (2009). However, this data was lower compared to the cauda epididymis sperm motility reported by Hamdan *et al.* (2010) in local Aceh goats (82.77%).

The concentration of cauda epididymis spermatozoa of PE goats in this study was $3,220 \times 10^6$ cells/mL. This concentration was higher compared to those reported by Hamdan *et al.* (2010) in local Aceh goats (3.180×10^6 cells/mL). The lower spermatozoa concentration was also reported by Bintara (2011), in kacang and PE goats after fresh ejaculate ($2,865 \times 10^6$ cells/mL). The differences in the concentration of spermatozoa goats reported by various researchers can be influenced by

several factors, such as feed, age, and different animal (Devendra and Burns, 1983). Devendra and Burns (1983) reported the range values of goat spermatozoa concentration were between $1,800-4,000 \times 10^6$ cells/mL.

The percentage of live and abnormalities of cauda epididymis spermatozoa of PE goats were 81.0%, and 4.3%, respectively. The percentage variation of the life spermatozoa values of goats has been reported by several researchers, such as 82.29% in local Aceh goats (Hamdan *et al.*, 2010), 83.89% in PE goats (Souhoka *et al.*, 2009), and 80% in kacang and PE goats (Bintara, 2011). For spermatozoa abnormalities, variation of several values has been reported such as 7.12% in PE goats (Souhoka *et al.*, 2009), 8.2% in kacang and PE goats (Bintara, 2011), and 7.23% in local Aceh goats (Hamdan *et al.*, 2010).

According to Hafez and Hafez (2000), total of goat sperm abnormality for AI should be less than 15%. This statement is supported by Bearden and Fuquay as cited by Prihatiny (2008) that 8-10% spermatozoa abnormalities did not have a significant influence on fertility rate, but if spermatozoa abnormalities was $>25\%$, it will decrease the fertility rate.

The Percentage of Motility and Life Spermatozoa after Dilution

The percentage of motility after diluting with lactose diluents was observed every day until exhibited the minimum value of 40% (National Standardization Agency, 2014). The results of sperm motility after diluting with lactose diluents were presented in Table 2. The decrease of sperm motility up to 40% was occurred on day-4 for control (K), but the sperm motility in TL1 and TL2 was still 44% and 45%, respectively ($P < 0.05$).

The percentage of life spermatozoa after diluting with lactose diluents and stored in a refrigerator at 3-5°C for five days were presented in Table 3. Percentage of live spermatozoa showed a decline every day and on day 4 were 63.2%, 65.4%, and 65.6% in group K, TL1, and TL2, respectively ($P > 0.05$). The declining of life spermatozoa was in line with the decrease of sperm motility.

The percentage of sperm motility in TL1 and TL2 were higher than K ($P < 0.05$). It showed that addition of lactose provided a protection as well as a energy sources for spermatozoa during the preservation process. According to Rizal (2009), lactose can be metabolized

Table 2. Percentage of spermatozoa motility during preservation

Treatments	Percentage of spermatozoa motility (day)			
	1	2	3	4
Control	70.00 \pm 0.00 ^a	51.00 \pm 41.8 ^b	45.00 \pm 3.54 ^b	40.00 \pm 0.00 ^b
TL1	70.00 \pm 0.00 ^a	59.00 \pm 2.24 ^a	52.00 \pm 2.74 ^a	44.00 \pm 2.24 ^a
TL2	70.00 \pm 0.00 ^a	59.00 \pm 2.24 ^a	54.00 \pm 2.24 ^a	45.00 \pm 0.00 ^a

^{a,b} A different superscript in the same column show a significant difference ($P < 0.05$)

Table 3. Percentage of live spermatozoa during preservation

Treatments	Percentage of live spermatozoa (day)			
	1	2	3	4
Control	81.00 \pm 1.58 ^a	75.40 \pm 2.88 ^a	68.40 \pm 3.78 ^b	63.20 \pm 2.68 ^a
TL1	81.00 \pm 1.58 ^a	76.60 \pm 2.30 ^a	71.60 \pm 1.82 ^{ab}	65.40 \pm 1.95 ^a
TL2	81.00 \pm 1.58 ^a	78.40 \pm 2.51 ^a	72.20 \pm 1.64 ^a	65.60 \pm 1.95 ^a

^{a,b} A different superscript in the same column show a significant difference ($P < 0.05$)

by the spermatozoa through glycolysis or the Krebs cycle to produce energy, adenosine triphosphate (ATP). The ATP will be then used by spermatozoa as a source of energy in the moving process, thus, the spermatozoa can keep their motility and at the same time maintaining their viability. In addition, the ability of carbohydrates as a source of nutrients as well as a compound that function as an extracellular cryoprotectants, enable to protect the plasma membrane cell from damages (Supriatna and Pasaribu, 1992).

The percentage of live spermatozoa on day-4 of TL1 and TL2 were not significantly different ($P>0.05$) compared to K. The value of live spermatozoa percentage should be higher than the percentage of motility. This is because a live spermatozoa will still show a movement, although the movement on the site, rotating or backwards. However sperm motility assessment conducted only on spermatozoa that moving progressively. According to Toelihere (1993), the standard percentage of live spermatozoa in liquid semen after preserved for AI was $\geq 50\%$. Therefore, preservation at 3-5° C of spermatozoa obtained from the cauda epididymis until day-4 in this study was still allowed to be used for artificial insemination (AI).

CONCLUSION

In conclusion, the addition of lactose into Tris diluents could maintain the quality (motility and viability) of epididymis spermatozoa of PE goats preserved in a refrigerator at 3-5° C and this liquid semen is still suitable to be used for the AI program.

ACKNOWLEDGEMENT

We would like to gratefully thank the animal breeding and reproduction laboratory and the traditional slaughterhouse in Martapura, Banjar for their support during this research.

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