Buletin Peternakan 42 (2): 97-102, May 2018

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Acredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v42i2.28236

The Quality of Boer Goat Semen Preserved with Sugar Palm Juice

Muhammad Rizal*, Muhammad Riyadhi, and Abrani Sulaiman

Department of Animal Science, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru, 70714, Indonesia

Article history
Submitted: 13 September 2017
Accepted: 19 April 2018

* Corresponding author:
Telp. +62 81348848631
E-mail: icang65@yahoo.com

ABSTRACT

The objective of this study was to examine the effect of seminal plasma on viability of Boer goat spermatozoa and effectiveness of sugar palm juice as an alternative extender during preservation at 5ºC. Semen of two Boer goats were collected using an artificial vagina. Fresh semen were evaluated and divided in equal volume into four tubes. Semen in the first and second tubes diluted with 80% sugar palm juice + 20% egg yolk (P1) and Andromed (P2), respectively. Semen in the third and fourth tubes were centrifuged with 3,000 RPM for 20 minutes, and the supernatant removed. diluted with 80% sugar palm juice + 20% egg yolk (P3) and Andromed (P4), respectively. Diluted-semen were preserved in refrigerator at 5ºC, and quality of the spermatozoa including motile spermatozoa (MS), live spermatozoa (LS), and intact plasma membrane (IPM) were evaluated every day for four days. Results of this study showed that at day-2 preservation, mean percentages of MS, LS, and IPM for P2 (72, 83.4, and 83.4%), P3 (72, 82.6, and 82.2%), and P4 (72, 83, 83.8%) were significantly ($P<0.05$) higher than P1 (3, 24.8, and 25.2%). At day-3 preservation, mean percentages of MS, LS, and IPM for P2 (57, 65.6, and 69.6%) was significantly ($P<0.05$) higher than P3 (21, 34.8, and 31.8%), P4 (22, 33.6, and 31.2%), and P1 (0, 0, and 0%). In conclusion, semen of Boer goat to be preserved with extender containing egg yolk should be removed seminal plasma. Sugar palm juice containing egg yolk could be used as an extender for Boer goat semen, but should be applied in the AI program immediately after the semen is diluted.

Key words: Andromed, Boer goat, Preservation, Sugar palm juice

Introduction

Boer goat is a type of meat goats with a good body conformation, adaptable to changes in ambient temperature, and more resistant to disease (Malan, 2000). The Boer goat is a superior goat imported from South Africa. One of the objectives of importing Boer goats is to improve the performance of Indonesian local goats, such as Kacang goats. According to Inounu *et al*. (2002) one way to increase livestock productivity is to include superior males from foreign breeds to be crossbred with local females.

The application of artificial insemination (AI) technology is a method that can be carried out to accelerate the population growth and to improve the genetic quality of goats. Through the integration of AI into semen processing technology, the reproductive potential of superior males can be optimized for mating with females. This objective can be achieved if the semen is diluted with certain diluents, meeting the quality requirements such as being energy source, buffer capacity, non-toxic, preventing damage to spermatozoa, cheap, and easy to obtain. According to Rizal *et al*. (2003) one ejaculation of male sheep can be used to mated about 35 females when using an AI program by using frozen semen packed in a mini straw; compared to the natural mating where one ejaculation can only be used to mated one female.

One of the main problems in handling goat semen is the presence of enzyme in seminal plasma. The enzyme is synthesized by the bulbouretralis glands (Cowper glands) which, when interacting with egg yolk or milk, will cause coagulation of semen (Ritar and Salamon, 1982; Leboeuf *et al*., 1998; Leboeuf *et al*., 2000). The enzyme is identified as phospholipase A that can hydrolyzed yolk lecithin into fatty acids and lysolecithin. These fatty acids and lysolecithin from the hydrolysis are toxic to goat spermatozoa. Meanwhile, in the process of semen processing, the egg yolk and milk are commonly used as the components of the semen extender. The lecithin contained in the egg yolk is the main reason for its use as the component of semen extender. Lecithin is needed as the protector of spermatozoa plasma membrane from cold shock when semen is stored at low temperatures (Quinn *et al*., 1980; Watson, 1981). This problem can be solved by two alternative methods. The first method: if one of the components of semen extender is egg yolk, so the seminal plasma

should be removed by centrifugation. According to Ustuner *et al*. (2009) and Naing *et al*. (2011) the separation of seminal plasma increases the spermatozoa motility of Saanen and Boer goats after thawing. The second method: if seminal plasma is not removed, it is better to use a semen extender that does not contain egg yolk. Andromed is one of the soybean-based commercial semen extender that does not contain egg yolk, so it can be used for an alternative extender in preservation of goat semen.

The utilization of natural components such as sugar palm juice as the component of semen extender can be an alternative to synthetic chemical compounds which are expensive. Sugar palm juice contains 9.16% water, 84.31% sucrose, 0.11% fat, 2.28% protein, 3.66% total mineral, 1.35% calcium, and 1.37% phosphorus (P_2O_5) (BPTP Banten, 2005). Sugar palm juice also contains vitamin A and C and has a pH of 6-7. This fact about the content of chemical compounds is the basis why sugar palm juice can be utilized as one of the alternative semen extender. Sugar palm juice as an alternative extender has been reported to work well in the preservation and application of AI in swamp buffaloes (Rizal and Riyadhi, 2016). The objectives of this study was to examine the effect of seminal plasma on viability of Boer goat spermatozoa and the effectiveness of sugar palm juice as an alternative extender during preservation at 5ºC.

Materials and Methods

Collection, treatment, and preservation of semen

Semen was collected from two male adult Boer goats using artificial vagina. The fresh semen was evaluated for the quality including: volume, mass movement, concentration, degree of acidity (pH), percentage of motility, percentage of abnormality (morphology), percentage of live, and percentage of intact plasma membrane. The fresh semen that qualifies for AI (percentage of motility $\geq$ 70%, concentration ≥ 2,000 x 10^6 cell/ml, and abnormality < 15% (Bearden and Fuquay, 1997), is processed into liquid semen.

The fresh semen was divided into four tubes in equal volume. The semen in the first and second tubes were diluted with 80% sugar palm juice + 20% egg yolk (P1) and Andromed (P2), respectively. The semen in the third and fourth tubes were centrifuged at 3,000 RPM for 20 minutes and seminal plasma was removed. The pellets (spermatozoa) in the third and fourth tubes were diluted with 80% sugar palm juice + 20% egg yolk (P3) and Andromed (P4), respectively. The diluted-semen in the tubes inserted into a glass containing clean water and then preserved in refrigerator at 5ºC. The quality of spermatozoa were evaluated daily until the speramtozoa motility reached 40%.

Sugar palm juice to be used as the extender was first heated to boil without using additives. The heated sugar palm juice was then filtered with filter paper and stored at 5°C for 12 hours before being used as the semen extender. The Andromed extender was consisted of 20% Andromed and 80% aquabidest. All extenders were added of 1,000 µg penicillin and 1,000 IU streptomycin per milliliter extender. Semen was diluted to a concentration of 100 million motile spermatozoa per milliliter.

The variables of spermatozoa quality

The spermatozoa quality variables that were observed including: motility, live spermatozoa (viability), and spermatozoa that had intact plasma membranes. The motility of spermatozoa is the percentage of proportion of progressive moving spermatozoa (moving forward). The motility of spermatozoa was evaluated subjectively using a 400x magnification microscope in eight different fields (Rasul *et al*., 2001).

The viability of spermatozoa is the percentage of live spermatozoa, which is evaluated using eosin-nigrosin staining (Felipe-Perez *et al*., 2008). Spermatozoa with a white or transparent head are live spermatozoa, while the dead are marked by a red head. Spermatozoa were evaluated for a minimum of 200 using a 400x magnification microscope.

Abnormal spermatozoa are the percentage of spermatozoa with primary abnormalities, including abnormalities of the head and tail. Abnormal spermatozoa were evaluated using preparation used for evaluation of spermatozoa viability (Felipe-Perez *et al*., 2008). A minimum of 200 spermatozoa were evaluated with a 400x magnification microscope.

Intact plasma membrane is the percentage of spermatozoa that have intact plasma membrane. This variable was evaluated using the method of hypo-osmotic swelling (HOS) test (Fonseca *et al*., 2005). The hypo-osmotic solution consisted of: 1.351 g fructose + 0.735 g sodium citrate diluted with aquabidest until 100 ml volume. A total amount of 1.000 µl hypo-osmotic solution were added with 50 µl semen and mixed until homogeneous and then incubated at 37°C for 30 minutes. The thin layer preparation in the object glass was evaluated by a 400x magnification microscope to a minimum of 200 spermatozoa. Spermatozoa with intact plasma membrane are characterized by a circular or bulging tail, while the damaged are marked by a straight tail.

Data analysis

Data on motility, viability, and intact plasma membrane of spermatozoa were collected using a completely randomized design with four treatments and five replications, and the differences of between treatments were tested using the least significant different test. The data were processed using SPSS version 6.0 for windows (SYSTAT, 1996).

Result and Discussion

Characteristics of fresh semen of Boer goat

The results of the study showed that the average volume of fresh semen of Boer goat was 0.64 ml (Table 1). Several researchers have previously reported varying volumes of Boer goat semen, i.e., 0.69 - 1.03 ml (Hastono *et al*., 2002), 1.49 ml (Alawiyah and Hartono, 2006), 0.60 - 0.80 ml (Kostaman and Sutama, 2006), 0.53 ml (Mahmilia *et al*., 2006), 1.02 ml (Hartono, 2010), 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. Mean characteristics of Boer goat fresh semen

Variable	Measurement
Volume (ml)	0,64±0,11
Color	Milky white
Degree of acidity (pH)	6,94±0,09
Consistency	Thick
Mass movement	+++
Spermatozoa concentration (million/ml)	3.016±77,97
Motile spermatozoa (%)	78,00±2,74
Live spermatozoa (%)	89,20±1,30
Abnormal spermatozoa (%)	4,60±1,14
Intact plasma membrane (%)	88,20±0,84

The consistency, pH, and mass movement of Boer goat spermatozoa in this study were thick, 6.94, and +++, respectively (Table 1). The characteristics of goat semen are thick consistency, pH between 6.40 to 7.25 and spermatozoa mass movement of +++ (Alawiyah and Hartono, 2006; Kostaman and Sutama, 2006; Pamungkas *et al*., 2014; Rhochim *et al*., 2017).

Based on the results of observation it was obtained that the average concentration of spermatozoa was 3,016 million cells/ml (Table 1). The average concentration of Boer goat spermatozoa was 2,260 million cells/ml (Hastono *et al*., 2002), 5,154.75 million cells/ml (Alawiyah and Hartono, 2006), 2,700 – 2,730 million cells/ml (Kostaman and Sutama, 2006), 2,290 million cells/ml (Hartono, 2010), 2,981 – 3,568 million cells/ml (Suharyati and Hartono, 2013), 4,125 million cells/ml (Pamungkas *et al*., 2014), and 4,350.4 million cells/ml (Rhochim *et al*., 2017).

The average percentage of motile and live spermatozoa in this study were 78.00% and 89.20% (Table 1). The average percentage of motile and live spermatozoa of Boer goats was 90.00% and 90.77% (Alawiyah and Hartono, 2006), 73.33% and 79.76% (Kostaman and Sutama, 2006), 88.00% and 89.67 (Hartono, 2010), 75% – 93.33% and 96.08% – 98.30% (Suharyati and Hartono, 2013), 79.55% and 85.29% (Pamungkas *et al*., 2014), and 89.00% and 87.00% (Rhochim *et al*., 2017).

The average percentage of abnormal spermatozoa and intact plasma membrane were 4.60% and 88.20%, respectively (Table 1). Various results were reported about the abnormality of goat spermatozoa i.e. 4.33% (Alawiyah and Hartono, 2006), 7.30 – 7.80% (Kostaman and Sutama, 2006), 1.98% (Hartono, 2010), 2.53% (Pamungkas *et al*., 2014) and 0.77% (Rhochim *et al*., 2017), whereas the percentage of intact plasma membrane was 77.25% (Pamungkas *et al*., 2014).

Based on the value of fresh semen quality, it can be concluded that the semen produced by Boer goats in this study qualifies to be processed into liquid semen or frozen semen. Good fresh semen should have a percentage of motile spermatozoa ≥ 70% and mass movement of ++ or +++ (Evans and Maxwell, 1987), a percentage of abnormal spermatozoa 6 – 10% (Delgadillo, 1992; Ax *et al*., 2000), and a percentage of intact plasma membrane ≥ 60% (Revell and Mrode, 1994).

Quality of spermatozoa during semen preservation

The results showed that on the second day of preservation, the semen containing seminal plasma and diluted with egg yolk-containing extender had a very drastically reduced quality of spermatozoa. On the second day of preservation, the mean percentage of motile spermatozoa for P1 (3.8%) was significantly ($P < 0.05$) lower than P2 (72%), P3 (72%), and P4 (72%) (Table 2). The same was true of the percentage of live spermatozoa and intact plasma membrane (Table 3 and Table 4).

A very drastic decrease the quality of spermatozoa in the semen diluted with egg yolk-containing extender (treatment P1) is presumably Boer goat seminal plasma contains an enzyme which, when reacted with egg yolk, can kill spermatozoa. According to Ritar and Salamon (1982) goat seminal plasma is containing phospholipase A enzyme that synthesized by the bulbouretralis gland (Cowper gland). The phospholipase A enzyme, when interacting with egg yolk or milk, will cause coagulation of semen (Leboeuf *et al*., 1998; Leboeuf *et al*., 2000). Phospholipase A enzyme can hydrolyze yolk lecithin into a fatty acids and lysolecithin. These fatty acids and lysolecithin are toxic to goat spermatozoa.

Table 2. Mean percentage of motile spermatozoa during preservation at 5°C

Treatments	Day of preservation				
	1	2	3	4	5
P1	78,00±2,74	3,80±1,30^{a}	0,00±0,00^{a}	0,00±0,00^{a}	0,00±0,00^{a}
P2	78,00±2,74	72,00±2,73^{b}	57,00±2,74^{c}	40,00±3,53^{b}	19,00±2,24^{b}
P3	78,00±2,74	72,00±2,73^{b}	21,00±2,24^{b}	0,00±0,00^{a}	0,00±0,00^{a}
P4	78,00±2,74	72,00±2,73^{b}	22,00±5,70^{b}	0,00±0,00^{a}	0,00±0,00^{a}

^{a,b,c} Superscript in the same column showed significantly differences ($P<0.05$).

Table 3. Mean percentage of live spermatozoa during preservation at 5°C

Treatments	Day of preservation				
	1	2	3	4	5
P1	89,20±1,30	24,80±1,90[a]	0,00±0,00[a]	0,00±0,00[a]	0,00±0,00[a]
P2	89,20±1,30	83,40±2,70[b]	65,60±4,28[c]	53,00±2,91[c]	35,60±2,70[b]
P3	89,20±1,30	82,60±2,07[b]	34,80±2,77[b]	16,00±4,18[b]	0,00±0,00[a]
P4	89,20±1,30	83,00±2,34[b]	33,60±3,85[b]	18,00±2,74[b]	0,00±0,00[a]

[a,b,c] Superscript in the same column showed significantly differences (P<0.05).

Table 4. Mean percentage of intact plasma membrane during preservation at 5°C

Treatments	Day of preservation				
	1	2	3	4	5
P1	88,20±0,84	25,20±1,64[a]	0,00±0,00[a]	0,00±0,00[a]	0,00±0,00[a]
P2	88,20±0,84	83,40±1,14[b]	69,60±1,14[c]	50,80±1,92[c]	36,20±192[b]
P3	88,20±0,84	82,20±1,64[b]	31,80±4,97[b]	19,00±1,58[b]	0,00±0,00[a]
P4	88,20±0,84	83,80±1,92[b]	31,20±1,64[b]	19,80±1,30[b]	0,00±0,00[a]

[a,b,c] Superscript in the same column showed significantly differences (P<0.05).

Based on the observation it was found that the Andromed extender (treatment P2) was the best treatment because it was able to maintain the quality of spermatozoa for three days of preservation. Andromed contains various substances such as Tris hydroxy aminomethane, citric acid, fructose, lecithin, glycerol, and antibiotics (Minitüb, 2001) that are needed to protect spermatozoa during preservation at 5 – 10°C. Lecithin (phosphatidyl choline) contained in the Andromed extender is the product of extracts from soybeans. This soy lecithin has the same function as lecithin contained in egg yolks, which protects the spermatozoa from the damage caused by cold shock. According to Kayser *et al*. (1992) and White (1993), lecithin in egg yolks protects spermatozoa from bad effects of cold shock.

On the third day of preservation, the quality of semen diluted in the absence of seminal plasma (treatment P3 and P4) were lower than that of semen containing seminal plasma and diluted with Andromed (treatment P2) (Table 2, 3, and 4). This phenomenon shows that seminal plasma has an important role in maintaining the life of spermatozoa. The seminal plasma contains various nutrients, which may play a role in protecting spermatozoa during preservation, cannot be completely replaced by the nutrients contained in the extenders. The seminal plasma contains various nutrients such as carbohydrates, proteins, vitamins, minerals, hormones, etc. that serve to support the life of spermatozoa (Hafez and Hafez, 2000). Rizal *et al*. (2008) reported that the semen of Etawa Crossbreed (EC) goat, which was diluted with egg yolk-containing extender without the removal of seminal plasma, was coagulated. This caused the spermatozoa to die. Afterwards, it was reported that the quality of EC goat spermatozoa preserved at low temperatures without seminal plasma was lower than that of spermatozoa preserved with ram seminal plasma.

The results obtained in this present study differ from those reported by some previous researchers. Alawiyah and Hartono (2006), Kostaman and Sutama (2006), Pamungkas and Anwar (2013), Rosmaidar *et al*. (2013), Suharyati and Hartono (2013), Pamungkas *et al*. (2014), and Rhochim *et al*. (2017) reported that Boer goat semen diluted with egg yolk-containing extenders did not cause spermatozoa death when preserved at 5°C. This may be due to concentration differences of the phospholipase A enzyme in seminal plasma.

The results showed that the Boer goat semen diluted with Andromed and without removed seminal plasma (treatment P2) could be preserved at 5°C for three days and still feasible to be used in the AI program, because the percentage of motile spermatozoa was about 40%. Based on the Indonesian National Standard (SNI, 2014), the eligible goat semen used in the AI program must have a minimum percentage of motile spermatozoa of 40%. The semen diluted with treatment P3 and treatment P4 could be stored for one day, while the semen diluted with treatment P1 should be used in the AI program immediately after it was diluted.

Conclusion

It can be concluded that the Boer goat semen to be stored with extender containing egg yolk should be separated from the seminal plasma. Andromed is the best diluent for Boer goat semen, and can maintain quality of spermatozoa for three days of preservation at 5°C. Sugar palm juice containing egg yolk can be used as the Boer goat semen extender, but it should be applied in the AI program immediately after the semen is diluted.

Acknowledgment

This research is part of an Applied Product Research scheme funded by the Ministry of Research, Technology and Higher Education for the fiscal year of 2017 with the contract number 070/SP2H/LT/DRPM/IV/2017. The author would like to thanks the goat farmers of Maju Bersama Sejahtera Farmers Group, Desa Cindai Alus, Kecamatan Martapura, Kabupaten Banjar, and the Laboratory of Reproduction and Animal Breeding, Department of Animal Science, Faculty of

Agriculture, Lambung Mangkurat University for the support and assistance so that this research can be finished.

Refferences

Alawiyah, D. dan M. Hartono. 2006. Pengaruh penambahan vitamin E dalam bahan pengencer sitrat kuning telur terhadap kualitas semen beku kambing Boer. Jurnal Pengembangan Peternakan Tropis 31: 8-14.

Ax, R. L., M. Dally, B. A. Didion, R. W. Lenz, C. C. Love, D. D. Varner, B. Hafez, and M. E. Bellin. 2000. Semen evaluation. In: Reproduction in Farm Animals 7th edn. E. S. E. Hafez and B. Hafez (eds). Lippincott Williams & Wilkins, Baltimore. pp 365-375.

Bearden, H. J. and J. W. Fuquay. 1997. Applied Animal Reproduction 4th edn. Prentice Hall, Upper Saddle, New Jersey.

BPTP Banten. 2005. Kajian Sosial Ekonomi Gula Aren di Banten. Balai Pengkajian Teknologi Pertanian Banten, Serang.

Delgadillo, J. J., B. Leboeuf, and P. Chemineau. 1992. Abolition of seasonal variations in semen uality and maintenance of sperm fertilizing ability by photoperiodic cycles in goat bucks. Small Rum. Res. 9: 47-59.

Evans, G. and W. M. C. Maxwell. 1987. Salamon's Artificial Insemination of Sheep and Goats. Butterworths, London.

Felipe-Perez, Y. E., M. L. Juarez-Mosqueda, E. O. Hernandez-Gonzalez, and J. J. Valencia. 2008. Viability of fresh and frozen bull sperm compared by two staining techniques. Acta Vet. Bras. 2: 123-130.

Fonseca, J. F., C. A. A. Torres, V. V. Maffili, A. M. Borges, A. D. F. Santos, M. T. Rodrigues, R. F. M. Oliveira. 2005. The hypoosmotic swelling test in fresh goat spermatozoa. Anim. Reprod. Sci. 2: 139-144.

Hafez, E. S. E. and B. Hafez. 2000. Reproduction in Farm Animals 7th edn. Lippincott Williams & Wilkins, Baltimore.

Hartono, M. 2010. Kualitas semen kambing peranakan Boer. Jurnal Penelitian Pertanian Terapan 10: 52-58.

Hastono, I. K. Sutama, P. Situmorang, I. G. M. Budiarsana, T. Kostaman, U. Adiati, M. S. Hidayat, dan Mulyawan. 2002. Pengaruh intensitas ejakulasi terhadap kualitas semen kambing Peranakan Etawah dan Boer. Kumpulan Hasil-hasil Penelitian APBN Tahun Anggaran 2001. Balai Penelitian Ternak Ciawi, Bogor. Hlm. 181 – 190.

Inounu, I., N. Hidayati, A. Priyanti, dan B. Tiesnamurti. 2002. Peningkatan produktivitas domba melalui pembentukan rumpun komposit. Laporan Hasil Penelitian Tahun Anggaran 2001. Buku I. Ternak Ruminansia. Balai Penelitian Ternak, Bogor.

Kayser, J. P., R. P. Amann, R. K. Shidefer, E. L. Squires, D. J. Jasko, and B. W. Pickett. 1992. Effects of linear cooling rate on motion characteristics of stallion spermatozoa. Theriogenol. 30: 601-614.

Kostaman, T. dan I. K. Sutama. 2006. Studi motilitas dan daya hidup spermatozoa kambing Boer pada pengencer Tris-sitrat-fruktosa. Jurnal Sain Veteriner 24: 58-64.

Leboeuf, B., E. Manfredi, P. Boue, A. Piacere, G. Brice, G. Baril, C. Broqua, P. Humbolt, and M. Terqui. 1998. Artificial insemination of dairy goats in France. Livestock Prod. Sci. 55: 193-203.

Leboeuf, B., B. Restall, and S. Salamon. 2000. Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci. 62: 113-141.

Mahmilia, F., M. Doloksaribu, dan F. A. Pamungkas. 2006. Karakteristik semen kambing Boer. Prosiding Seminar Nasional Peternakan dan Veteriner. Bogor, 5 – 6 September 2006. Puslitbang Peternakan, Bogor. Hlm. 533-536.

Malan, S. W. 2000. The improved Boer goat. Small Rum. Res. 3: 165-170.

Minitüb. 2001. Certificate of AndroMed. MinitübAbfull und Labortechnik GmbH & Co KG, Germany.

Naing, S. W., A. W. Haron, M. A. K. Goriman, R. Yusoff, M. Z. A. Bakar, K. Sarsaifi, M. M. Bukar, M. Thein, T. Kyaw, and M. M. San. 2011. Effect of seminal plasma removal, washing solutions, and centrifugation regimes on Boer goat semen cryopreservation. J. Trop. Agric. Sci. 34: 271.

Pamungkas, F. A. dan Anwar. 2013. Daya tahan hidup spermatozoa kambing Boer dalam pengencer tris kuning telur yang disimpan pada temperature berbeda. Prosiding Seminar Nasional Peternakan dan Veteriner. Bogor, 4-5 Agustus 2013. Pusat Penelitan dan Pengembangan Peternakan, Bogor. Hlm. 331-338.

Pamungkas, F. A., A. Batubara, dan Anwar. 2014. Kriopreservasi spermatozoa kambing Boer: perbandingan dua bahan pengencer terhadap kualitas post-thawing dan kemampuan fertilisasinya. Jurnal Ilmu Ternak dan Veteriner 19: 130-137.

Quinn, P. J., P. Y. W. Chow, and I. G. White. 1980. Evidence that phospholipid protects ram spermatozoa from cold shock at plasma membrane site. J. Reprod. Fertil. 60: 403-407.

Rasul, Z., N. Ahmad, and M. Anzar. 2001. Changes in motion characteristics, plasma membrane integrity and acrosome morphology during cryopreservation of buffalo spermatozoa. J. Androl. 22: 278-283.

Revell, S. G. and R. A. Mrode. 1994. An osmotic resistance test for bovine semen. Anim. Reprod. Sci. 36: 77-86.

Ritar, A. J. and S. Salamon. 1982. Effects of seminal plasma and of its removal and of egg yolk in the diluent on the survival of fresh and frozen-thawed spermatozoa of the Angora goat. Aust. J. Biol. Sci. 35: 305-312.

Rizal, M., M. R. Toelihere, T. L. Yusuf, B. Purwantara, dan P. Situmorang. 2003. Karakteristik penampilan reproduksi pejantan domba garut. Jurnal Ilmu Ternak dan Veteriner 8: 134-140.

Rizal, M., Herdis, M. Surachman, dan W. M. M. Nalley. 2008. Pengaruh plasma semen domba priangan terhadap daya hidup spermatozoa kambing Peranakan Etawah yang disimpan pada suhu 3-5ºC. Jurnal Ilmu Ternak dan Veteriner 13: 23-29.

Rizal, M. dan M. Riyadhi. 2016. Fertilitas semen kerbau rawa (Bubalus bubalis carabanensis) yang diencerkan dengan pengencer nira aren. Jurnal Veteriner 17: 457-467.

Rhochim, A., M. A. Salim, N. Isnaini, dan T. Susilawati 2017. Pengaruh penghilangan rafinosa dalam pengencer Tris aminometan kuning telur terhadap kualitas semen kambing Boer selama simpan dingin. Jurnal Ternak Tropika 18:27-35.

Rosmaidar, Dasrul, dan 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: 7-17.

Standar Nasional Indonesia (SNI). 2014. Semen Beku Bagian 3: Kambing dan Domba (SNI 4869.3-2014). Badan Standardisasi Nasional, Jakarta.

Suharyati, S. dan M. Hartono. 2013. Peningkatan kualitas semen kambing Boer dengan pemberian vitamin E dan mineral Zn. Jurnal Kedokteran Hewan 7: 91-93.

SYSTAT. 1996. Statistics (Version 6.0 for Windows), SPSS. Chicago, IL, USA.

Ustuner, B., U. Gunay, and A. Nur. 2009. Effect of seminal plasma, egg yolk, and season on the freezability of Saanen buck semen. Bull. Vet. Inst. Pulawy 53: 369-374.

Watson, P. F. 1981. The roles of lipid and protein in the protection of ram spermatozoa at 5ºC by egg yolk lipoprotein. J. Reprod. Fertil. 62: 483-492.

White, I. G. 1993. Lipid and Ca uptake of sperm in relation to cold shock and preservation: A review. Reprod. Fertil. Dev. 5: 639-658.