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Cryopreservation of Boer Crossbreed Goat Semen with Andromed Extender and Moringa Leaf Extract

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ABSTRACT

The nutrients contained in Moringa leaves can protect spermatozoa from damage during semen cryopreservation. This study investigated the effectiveness of Moringa leaf extract in Andromed extender on the quality of frozen semen from Boer crossbreed goats. Fresh semen was divided into four tubes in equal volume and diluted with Andromed (control), 98% Andromed + 2% Moringa leaf extract (ME-2), 96% Andromed + 4% Moringa leaf extract (ME-4), and 94% Andromed + 6% Moringa leaf extract (ME-6), respectively. Diluted semen was placed in a mini-straw (0.25 mL), equilibrated in a refrigerator at 5°C for 4 hours, and then frozen by positioning the straw 10 cm above the surface of liquid nitrogen for 15 minutes. Variables were evaluated after diluting and thawing, including percentage of spermatozoa motility, live, and intact plasma membrane (IPM). Results of this study showed that the percentage of spermatozoa motility, live, and IPM for ME-4 (56.25, 67.5, and 65.75%) were significantly ($p<0.05$) higher than control (47.5, 56.75, and 53%), ME-2 (51.25, 59.5, and 56.25%), and ME-6 (51.25, 62.25, and 56%). This study's findings indicate that adding 4% Moringa leaf extract to Andromed extender is optimal for enhancing the quality of frozen semen in Boer crossbreed goats.

Keywords: Moringa leaf extract, semen cryopreservation, Boer crossbreed goat

ABSTRAK

Nutrien yang terkadung di dalam daun kelor dapat melindungi spermatozoa dari kerusakan selama proses kriopreservasi semen. Tujuan penelitian ini adalah menguji efektivitas ekstrak daun kelor di dalam pengencer Andromed terhadap kualitas semen beku kambing peranakan Boer. Semen segar dibagi ke dalam empat buah tabung reaksi dan masing-masing diencerkan dengan pengencer Andromed (kontrol), 98% Andromed + 2% ekstrak daun kelor (EK-2), 96% Andromed + 4% ekstrak daun kelor (EK-4), dan 94% Andromed + 6% ekstrak daun kelor (EK-6). Semen yang telah diencerkan dikemas di dalam *straw* mini (0,25 ml), dan diekuilibrasi di dalam refrigerator lemari es pada suhu 5°C selama 4 jam. Semen dibekukan dengan cara meletakkan *straw* 10 cm di atas permukaan nitrogen cair selama 15 menit. Variabel meliputi persentase spermatozoa motil, hidup, dan membran plasma utuh (MPU) dievaluasi setelah pengenceran dan *thawing*. Hasil penelitian menunjukkan bahwa persentase motilitas spermatozoa, hidup, dan MPU setelah *thawing* perlakuan EK-4 (56,25; 67,5; dan 65,75%) nyata ($p<0,05$) lebih tinggi dibandingkan dengan kontrol (47,5; 56,75; dan 53%), EK-2 (51,25; 59,5; dan 56,25%), dan EK-6 (51,25; 62,25; dan 56%). Berdasarkan hasil penelitian dapat disimpulkan bahwa penambahan 4% ekstrak daun kelor di dalam pengencer Andromed merupakan dosis terbaik untuk meningkatkan kualitas semen beku kambing peranakan Boer.

Kata kunci: Ekstrak daun kelor, kriopreservasi semen, kambing peranakan Boer

INTRODUCTION

The productivity of local goats in Indonesia can be improved through crossbreeding with superior goats from abroad (Inounu et al., 2002). Boer goats, originating from South Africa, are one of the meat-type goat breeds that are disease-resistant and have good acclimatization ability (Malan, 2000). Boer goats offer the potential to enhance the productivity of local goat populations via crossbreeding. Elieser et al. (2003) found that the productivity of local goats crossed with Boer goats increased by 30–45%.

Artificial insemination (AI) is the most effective reproductive technology for improving genetic quality (Zamiri, 2020). The application of AI using Boer goat semen can accelerate the process of improving the genetic quality of local goats. Through AI technology, the reproductive potential of superior males can be optimized. This is because one of the technologies integrated with AI is semen processing technology. Rizal *et al.* (2003) indicate that a single ejaculation from a ram can inseminate approximately 35 females when utilizing an AI program with frozen semen in mini straws, in contrast to natural mating, which allows one ejaculation to serve only one female. This principle is also applicable to goats.

The quality of processed semen is one of the important factors determining the success of AI technology application (Tamoes et al., 2014). The quality of processed semen can be maintained by adding various additives to the semen slurry, including moringa leaf extract. Moringa leaf extract can be used as one of the components of semen diluents. This is because moringa leaves are rich in antioxidants (Kasolo et al., 2010) and possess antibacterial properties (Das et al., 2012). According to Kumala et al. (2016), moringa leaves contain flavonoid compounds that can bind free radicals. Moringa leaf extract can protect spermatozoa from oxidative stress during cryopreservation, thereby improving the quality of frozen semen and spermatozoa fertility (Shokry et al., 2021). Improvements in the quality of semen preserved with diluents supplemented with moringa leaf extract have been reported in cattle spermatozoa (Sokunbi et al., 2015), Landrace pigs (Fafo et al., 2016), Senduro goats (Wahjuningsih et al., 2019), and Limousin cattle (Dapawole and Sirappa, 2021; Wajdi et al., 2021). This study aimed to investigate the effect of various concentrations of moringa leaf extract in Andromed diluent on the quality of frozen semen from Boer goats.

MATERIALS AND METHODS

Semen Collection and Cryopreservation

Semen was collected from two adult male Boer goats using an artificial vagina. The collected semen was evaluated for quality, including: volume, viscosity (consistency), degree of acidity (pH), spermatozoa motility, spermatozoa concentration, spermatozoa motility percentage, spermatozoa viability percentage, spermatozoa abnormality percentage, and percentage of intact plasma membranes (IPM). Semen that met the criteria, i.e., had a sperm motility percentage of >75% (Salmani et al., 2014), sperm concentration of ≥2,000 × 10^6 cells/mL, spermatozoa abnormality percentage <15%, and IPM percentage >60% (Revell and Mrode, 1994), was cryopreserved into frozen semen. Semen meeting quality criteria was divided into four reaction tubes and each diluted with different diluents: 100% Andromed (control), 98% Andromed + 2% moringa leaf extract (ME-2), 96% Andromed + 4% moringa leaf extract (ME-4), and 94% Andromed + 6% moringa leaf extract (ME-6). The semen was diluted to achieve a concentration of 200 million motile spermatozoa per milliliter.

The diluted semen was packaged in 0.25 mL mini-straws and subsequently equilibrated. Equilibration was performed by placing the straws in a refrigerator at 5°C for four hours. The equilibrated semen was frozen by placing the straws 10 cm above the surface of liquid nitrogen in a tightly sealed styrofoam container (temperature approximately –130°C) for 15 minutes. The traw was subsequently placed in a liquid nitrogen container for seven days. The quality of frozen semen was assessed post-storage by thawing the straw in water at 37°C for 30 seconds (Borah et al., 2015).

Evaluated Variables

The sperm quality variables evaluated include motility percentage, survival percentage, and IPM percentage of spermatozoa after dilution and thawing. Sperm viability percentage refers to the proportion of spermatozoa that exhibit progressive movement (moving forward). Sperm motility was evaluated subjectively in eight different fields of view using a 400x magnification microscope (Rasul et al., 2001).

Spermviabilityis the percentage of live spermatozoa evaluated using the eosin-nigrosin staining method (Buranaamnuay, 2019). A white head characterizes

live spermatozoa, while a red head characterizes dead spermatozoa. A minimum of 200 spermatozoa was evaluated using a 400x magnification microscope.

The IPM percentage is the percentage of spermatozoa with intact plasma membranes evaluated using the hypoosmotic swelling (HOS) test (Revell and Mrode, 1994). The hypoosmotic solution was prepared by dissolving 0.9 g of fructose and 0.49 g of sodium citrate in distilled water to achieve a final volume of 100 mL. Approximately 100 mL of hypoosmotic solution was combined with 10 mL of semen, mixed thoroughly to achieve homogeneity, and subsequently incubated at 37°C for 45 minutes. Thin smears were prepared on microscope slides and evaluated under a microscope at 400x magnification with a minimum of 200 spermatozoa. Curved or swollen tails marked spermatozoa with intact plasma membranes, while damaged ones were marked by straight tails.

Statistical Analysis

The experiment used a completely randomized design (CRD) with four treatments and six replicates. Differences between treatments were tested using the least significant difference (LSD) test. Data were analyzed using SPSS software (version 17.0 for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of Fresh Semen of Boer Crossbreed Goats

The study's results obtained the following average semen values: volume 0.8 mL, pH 6.72, sperm concentration 4,065 million/mL, motile spermatozoa 78.33%, and IPM spermatozoa 89.16% (Table 1).

Sperm Quality After Cryopreservation

The findings suggest that incorporating moringa leaf extract into the Andromed diluent enhances the quality of frozen semen from Boer crossbred goats. The percentage of motile, live, and IPM spermatozoa in frozen semen under treatment ME-4 (56.25; 67.5; and 65.75%) was significantly ($p<0.05$) higher than under treatment ME-6 (51.25; 62.25; and 56%), treatment ME-2 (51.25; 59.5; and 56.25%), and the control treatment (47.5; 56.75; and 53%) (Tables 2, 3, and 4). Adding 2% and 6% moringa leaf extract did not significantly enhance the quality of frozen semen from Boer crossbred goats. Adding 4% moringa leaf extract to the Andromed diluent represents the optimal concentration for enhancing the quality of frozen semen from Boer crossbred goats.

DISCUSSION

Characteristics of Fresh Boer Crossbred Goat Semen

The average semen volume of Boer crossbred goats was 0.8 mL. Previous researchers reported varying semen volumes for Boer crossbred goats, with averages of 0.53 mL (Mahmilia et al., 2006), 0.93–1.02 mL (Hartono, 2010), 0.77–1.13 mL (Suharyati and Hartono, 2013), 1.14 mL (Rochim et al., 2017), 1.1 mL (Susilowati et al., 2024), and 0.71 mL (Sholikah et al., 2022). The sperm concentration obtained in this study (4,065 million/mL) was higher than those reported by previous researchers. The average sperm concentration of Boer crossbreed goats was 2,188–2,290 million/mL (Hartono, 2010), 2,981–3,568 million/mL (Suharyati and Hartono, 2013), 3,997.35 million/mL (Sholikah et al., 2022), and 1,882.5 million/mL (Susilowati et al., 2024).

Table 1. Characteristics of fresh semen from Boer crossbred goats

Variable	Mean± SD
Semen volume (mL)	0.80 ± 0.16
Semen color	Milky white
Acidity level of semen (pH)	6.72 ± 0.19
Semen consistency	Thick
Sperm motility (1–3)	3 ± 0.00
Sperm concentration (10^6/ml)	4,065 ± 707
Motile spermatozoa (%)	78.33 ± 2.58
Live spermatozoa (%)	89.83 ± 0.75
Abnormal spermatozoa (%)	3.83 ± 1.17
Sperm plasma membrane integrity (%)	89.16 ± 1.17

Table 2. Percentage of sperm motility after cryopreservation

Stage Evaluation	Treatment			
	Control	EK-2	EK-4	EK-6
After dilution (%)	78.33 ± 2.58	78.33 ± 2.58	78.33 ± 2.58	78.33 ± 2.58
After *thawing* (%)	47.50 ± 2.87^{a}	51.25 ± 2.50^{ab}	56.25 ± 2.50^{b}	51.25 ± 2.50^{ab}

Superscripts in the same row indicating significant differences (p<0.05).

Table 3. Sperm viability percentage after cryopreservation

Stage Evaluation	Treatment			
	Control	EK-2	EK-4	EK-6
After dilution (%)	88.78 ± 1.17	89.73 ± 1.15	89.77 ± 1.23	88.38 ± 1.45
After *thawing* (%)	56.75 ± 2.63^{a}	59.50 ± 1.91^{ab}	67.50 ± 3.11^{c}	62.25 ± 3.30^{b}

Superscripts in the same row indicating significant differences (p<0.05).

Table 4. Percentage of intact plasma membranes of spermatozoa after cryopreservation

Stage Evaluation	Treatment			
	Control	EK-2	EK-4	EK-6
After dilution (%)	89.13 ± 0.67	88.93 ± 0.87	88.87 ± 0.51	89.07 ± 0.75
After *thawing* (%)	53.00 ± 2.94^{a}	56.25 ± 2.50^{a}	65.75 ± 3.40^{b}	56.00 ± 1.41^{a}

Superscripts in the same row indicating significant differences (p<0.05).

The percentages of motility and viability of spermatozoa in this study were 78.33% and 89.83%, respectively. Previous studies reported that the percentage of sperm motility and viability in Boer crossbred goats averaged 81–88% and 86.85–89.67% (Hartono, 2010), 75–93.33% and 96.08–98.3% (Suharyati and Hartono, 2013), 89.67% and 93.5% (Susilowati et al., 2024), and 70.26% and 87.27% (Sholikah et al., 2022). Sundararaman and Edwin (2008) state that semen intended for freezing must exhibit a minimum progressive motility of 70%. The research results indicate that the semen obtained satisfies the criteria for cryopreservation, exhibiting a sperm motility percentage exceeding 70%.

The average percentage of spermatozoa abnormalities and MPU was 3.83% and 89.16%, respectively. Previous research reported that the percentage of spermatozoa abnormalities in Boer goats was an average of 1.98–2.38% (Hartono, 2009), 1.11–2.34% (Suharyati and Hartono, 2013), and 3.45% (Sholikah et al., 2022). The percentage of spermatozoa abnormalities observed in this study remains within the normal range and satisfies the criteria for application in AI programs. Pamungkas et al. (2014) state that the percentage of spermatozoa abnormalities must not exceed 15%.

Sperm Quality After Cryopreservation

The addition of moringa leaf extract to Andromed diluent improves the quality of frozen semen from Boer goats. This improvement is likely due to various active compounds in moringa leaves that protect spermatozoa from damage during cryopreservation, especially during dilution, freezing, and thawing. Moringa leaves are rich in antioxidants and antibacterial properties (Kasolo et al., 2010; Das et al., 2012). The decline in spermatozoa quality after thawing is caused by damage to the plasma membrane during cryopreservation. Sperm motility and viability depend on the integrity of the plasma membrane (Sabeti et al., 2016). During the freezing and thawing of frozen semen, excessive production of reactive oxygen species causes lipid peroxidation (Alahmar, 2019). Reactive oxygen species cause damage to the plasma membrane, mitochondrial proteins, and axonemes, leading to disrupted metabolic processes that result in reduced motility and ultimately the death of spermatozoa (Alahmar et al., 2019; Gallo et al., 2021). Antioxidants protect biological systems from free radicals, including reactive oxygen species (He et al., 2017).

Moringa leaves contain flavonoids, vitamin C, and vitamin E, which are antioxidants (Gopalakrishnan et al., 2016). Flavonoids and vitamin C can neutralize free radicals, thereby protecting spermatozoa plasma membranes from lipid peroxidation (Cahyadi et al., 2016; Kumala et al., 2016). Moringa leaf extract in semen diluent enhances antioxidant activity (Carrera-Chaves, 2020) and several enzymes, including catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase (Shokry et al., 2021; Silvestre et al., 2021), effectively neutralizing oxidative compounds. High levels of oxidizing compounds damage cell structure and function (Aitken, 2020). Oxidative stress reduces motility, viability, plasma membrane integrity, and DNA integrity of spermatozoa (Alahmar, 2019; Musial et al., 2020). This is why adding moringa leaf extract to semen diluent improves motility, viability, plasma membrane integrity, and fertility of frozen Barki sheep spermatozoa (Shokry et al., 2021). Susilowati et al. (2024) reported that the addition of green tea extract in semen diluent containing chitosan, which functions as an antioxidant compound, can improve the percentage of motility, viability, and plasma membrane integrity of frozen semen spermatozoa from Boer goats.

The findings suggest that a 4% concentration of moringa leaf extract in the Andromed diluent is optimal for the cryopreservation of Boer goat semen. Wahjuningsih et al. (2019) found that including 5% moringa leaf extract in the semen diluent enhanced spermatozoa's motility, viability, and plasma membrane integrity after thawing in Senduro goats. The addition of 4–16% moringa leaf extract to the semen diluent can preserve sperm motility, morphology, and plasma membrane integrity in cattle during cryopreservation (Sokunbi et al., 2015). Adding 5% moringa leaf extract in yellow citrate extender can maintain sperm motility and viability in Landrace pigs (Fafo et al., 2016). The addition of 10% moringa leaf extract in yellow egg skim milk diluent can improve the quality of frozen semen from Limousin cattle (Wajdi et al., 2021) and frozen semen preserved at 3–5°C (Dapawole and Sirappa, 2021).

The addition of 2% moringa leaf extract led to a decrease in frozen semen quality relative to 4%, yet it performed better than the control treatment. This indicates that the amount of active compounds in moringa leaves remains insufficient to provide maximum protection against spermatozoa damage during cryopreservation. Similar conditions were observed with the addition of 6%, likely due to the high levels of active compounds in moringa leaves, such as acidic tannins, which may impact spermatozoa quality. Oka et al. (2016) reported that moringa leaf extract contains 831.92 mg of tannins per 100 mL. An increase in tannin concentration can cause a decrease in pH, as the phenolic compounds in tannins have acidic properties (Putranti et al., 2010). Media with high acidity levels can cause damage or death to spermatozoa. According to Hafez and Hafez (2000), the optimal pH of semen is neutral, approximately 7. On the other hand, Kulaksiz et al. (2013) state that an excessively high concentration of dissolved substances in the diluent solution can increase osmotic pressure, adversely affecting spermatozoa viability.

The motility percentages of spermatozoa after thawing were 47.5% for the control treatment, 51.25% for EDK-2, 56.25% for EDK-4, and 51.25% for EDK-6. This indicates that the frozen semen meets the criteria for use in an AI program, as its sperm motility percentage after thawing exceeds 40%. The Indonesian National Standard (SNI 4869.3:2014) stipulates that frozen goat semen intended for artificial insemination must exhibit a minimum sperm motility of 40% (National Standards Agency, 2014).

The results obtained in this study support multiple reports regarding the quality of frozen semen from Boer goats. According to Memon et al. (2011), the percentage of sperm motility in Boer goats after thawing increased with the addition of butylated hydroxytoluene to the tris diluent. The average sperm motility percentage of frozen Boer goat semen was 52% (Pamungkas et al., 2014), 43.42–50.4% (Wahjuningsih et al., 2021), 48.75–51.25% (Sari et al., 2024), and 34.5–55% (Suwor et al., 2025). Enhancing the quality of frozen semen from Boer goats through the incorporation of moringa leaf extract into the diluent presents a viable opportunity for the Artificial Insemination Center (BIB) to serve as an official producer of frozen semen, given that moringa is readily accessible and cost-effective. Based on the results of this study, it can be concluded that adding 4% moringa leaf extract in the Andromed diluent is the optimal dose for improving the quality of frozen semen from Boer crossbred goats.

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REFERENCES

Alahmar AT. 2019. Role of oxidative stress in male infertility: An updated review. Journal of Human Reproductive Science 12(1):4-18. https://doi.org/10.4103/jhrs. JHRS_150_18.

Aitken RJ. 2020. Impact of oxidative stress on male and female germ cells: implication for fertility. Reproduction 159:R189-R201. https://doi.org/10.1530/REP-19-0452.

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

Borah BK, Deka BC, Biswas RK, Chakravarty P, Deori S, Sinha S, Ahmed K. 2015. Effect of thawing methods on frozen semen quality of yak (*Poephagus grunniens* L.) bulls. Veterinary World 8(7):831-834. https://doi.org/10.14202/vetworld.2015.831-834.

Buranaamnuay K. 2019. Comparison of different methods for sperm viability assessment in frozen-thawed Holstein bull semen. Tahi Journal of Veterinary Medicine 49(3):249-255.

Cahyadi TRT, Christiyanto M, Setiatin ET. 2016. Persentase hidup danabnormalitassel spermatozoa kambing Peranakan Ettawa (PE) dengan pakan yang disuplementasikan daun binahong (*Anredera cordifolia* (Ten) steenis). Animal Agriculture Journal 5:23-32.

Carrera-Chaves JM, Jimenez-Aguilar EE, Acosta-Perez TP, Nunez-Gastelum JA, Quezada-Casasola A, Escarcega-Avila AM, Itza-Ortiz MF, Orozco-Lucero E. 2020. Effect of *Moringa oleifera* seed extract on antioxidant activity and sperm characteristics in cryopreserved ram semen. Journal of Applied Animal Research 48(1):114-120. https://doi.org/10.1080/09712119.2020.1741374.

Dapawole RR, Sirappa IP. 2021. Effect concentration of moringa (*Moringa oleifera* Lam) leaf extract in citrate-egg yolk in maintaining motility and viability of spermatozoa of Kacang goat. Jurnal Sain Veteriner Indonesia 16(4):340-346. https://doi.org/10.31186/jspi.id.16.4.340-346.

Das AK, Rajkumar V, Verma AK, Swarup D. 2012. Moringa oleifera leaves extract: a natural antioxidant for retarding lipid peroxidation in cooked goat meat patties. *International* Journal of Food Science and Technology 47:585–591. https://doi.org/10.1111/j.1365-2621.2011.02881.x.

Elieser S, Doloksaribu M, Mahmilia F, Tarigan A, Romjali E. 2003. Peningkatan Produktivitas antara Kambing Kacang dan Kambing Boer [Productivity Improvement Between Kacang Goats and Boer Goats]. Laporan Akhir Kegiatan Penelitian PAATP Tahun 2003. Loka Penelitian Kambing Potong Sei Putih. In Indonesian Language.

Fafo M, Hine TM, Nalley WMM. 2016. Pengujian efektivitas ekstrak daun kelor dalam pengencer sitrat kuning telur terhadap kualitas semen cair babi Landrace [Testing the effectiveness of moringa leaf extract in diluting egg yolk citrate on the quality of Landrace pig semen]. Jurnal Nukleus Peternakan 3:184-195. https://doi.org/10.35508/nukleus.v3i2.805. In Indonesian Language.

Gopalakrishnan L, Doriya K, Kumar DS. 2016. *Moringa oleifera*: a review on nutritive importance and its medicinal application. Journal of Food Science and Human Wellness 5:49-56. https://doi.org/10.1016/j.fshw.2016.04.001.

Hafez ESE. Hafez B. 2000. Reproduction in Farm Animals 7th Edition. Lippincott Williams & Wilkins, Baltimore.

Hartono. 2010. Kualitas semen kambing peranakan Boer [Quality of Boer goat semen]. Jurnal Penelitian Pertanian Terapan 10(1):52-58. https://doi.org/10.25181/jppt.v10i1.246. In Indonesian Language.

He L, He T, Farrar S, Ji L, Liu T, Ma X. 2017. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. Cellular Physiology and Biochemestry 44(2):532-553. https://doi.org/10.1159/000485089.

Inounu I, Hidayati N, Priyanti A, Tiesnamurti B. 2002. Peningkatan produktivitas domba melalui pembentukan rumpun komposit [Increasing sheep productivity through the formation of composite clumps]. Laporan Hasil Penelitian Tahun Anggaran 2001. Buku I. Ternak Ruminansia. Balai Penelitian Ternak, Bogor. In Indonesian Language.

Kasolo JN, Bimeya GS, Ojok L, Ochieng J, Okwal-okeng JW. 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Uganda rural communities. Journal of Medical Plant Research 4:753-757. https://doi.org/10.5897/JMPR10.492.

Kulaksiz R, Ari UC, Daskin A, Uner AG. 2013. The effect of different glycerol concentrations on freezeability of semen from angora, kilis, and saanen goats. Slovak Journal of Animal Science 46(2):39-44.

Kumala IN, Masfufatun, Devi DRE. 2016. Potensi ekstrak daun kelor (*Moringa oleifera*) sebagai hepatoprotektor pada tikus putih (*Rattus novergicus*) yang diinduksi parasetamol dosis toksis [Potential of *Moringa oleifera* leaf extract as a hepatoprotector in white rats (*Rattus novergicus*) induced by toxic doses of paracetamol]. Jurnal Ilmiah Kedokteran 5:58-66. https://doi.

org/10.30742/jikw.v5il.6. In Indonesian Language.
Mahmilia F, Doloksaribu M, Pamungkas FA. 2006. Karakteristik semen kambing Boer [Characteristics of Boer goat semen. Proceedings of the National Seminar on Animal Husbandry and Veterinary Medicine]. Prosiding Seminar Nasional Peternakan dan Veteriner. Bogor, 5–6 September 2006. Puslitbang Peternakan, Bogor. Hlm. 533-536. http://peternakan.litbang.deptan.go.id/?q=node/466. In Indonesian Language.
Malan SW. 2000. The improved boer goat. Small Ruminant Research 3:165-170. https://doi.org/10.1016/S0921-4488(99)00160-1.
Memon AA, Wahid A, Rosnina Y, Goh YM, Ebrahimi M, Nadia FM, Audrey G. 2011. Effect of butylated hydroxytoluene on cryopreservation of Boer goat semen in Tris egg yolk extender. Animal Reproduction Science 129(1-2):44-49. https://doi.org/10.1016/j.anireprosci.2011.10.004.
Musial C, Kuban-Jankowska A, Gorska-Ponikowska M. 2020. Beneficial properties of green tea catechins. International Journal of Molecular Sciences 21(5):1744. https://doi.org/10.3390/ijms21051744.
Oka AA, Wiyana KA, Sugitha IM, Miwada INS. 2016. Identifikasi sifat fungsional dari daun jati, kelor, dan kayu manis dan potensinya sebagai sumber antioksidan pada edible film [Identification of functional properties of teak, moringa, and cinnamon leaves and their potential as antioxidant sources in edible films]. Jurnal Sain Peternakan Indonesia 11(1):1-8. In Indonesian Language.
Pamungkas FA, Batubara A, Anwar A. 2014. Kriopreservasi spermatozoa kambing Boer: Perbandingan dua bahan pengencer terhadap kualitas post-thawing dan kemampuan fertilisasinya [Cryopreservation of Boer goat spermatozoa: Comparison of two diluents on post-thawing quality and fertilization capacity]. Jurnal Ilmu Ternak dan Veteriner 19(2):130-137. https://doi.org/10.14334/jitv.v19i2.1041. In Indonesian Language.
Putranti OD, Kustono, Ismaya. 2010. Pengaruh penambahan crude tannin pada sperma cair kambing Peranakan Ettawa yang disimpan selama 14 hari terhadap viabilitas spermatozoa [Effect of crude tannin addition on the viability of Peranakan Ettawa goat semen stored for 14 days]. Buletin Peternakan 34(1):1-7. https://doi.org/10.21059/buletinpeternak.v34i1.100. In Indonesian Language.
Rasul Z, Ahmad N, Anzar M. 2001. Changes in motion characteristics, plasma membrane integrity and acrosome morphology during cryopreservation of buffalo spermatozoa. Journal of Andrology 22(1):278-283. https://doi.org/10.1002/j.1939-4640.2001.tb02181.x
Revell SG, Mrode RA. 1994. An osmotic resistance test for bovine semen. Animal Reproduction Science 36:77-86. https://doi.org/10.1016/0378-4320(94)90055-8.
Rizal M, Toelihere MR, Yusuf TL, Purwantara B, Situmorang P. 2003. Karakteristik penampilan reproduksi pejantan domba garut [Reproductive performance characteristics of Garut sheep rams]. Jurnal Ilmu Ternak dan Veteriner 8:134-140. http://dx.doi.org/10.14334/jitv.v8i2.383. In Indonesian Language.
Rochim A, Salim MA, Isnaini N, Susilawati T. 2017. Pengaruh penghilangan rafinosa dalam pengencer tris aminomethane kuning telur terhadap kualitas semen kambing Boer selama simpan dingin [Effect of removing raffinose from tris aminomethane egg yolk diluent on the quality of Boer goat semen during cold storage]. Jurnal Ternak Tropika 18(1):27-35. https://doi.org/10.21776/ub.jtapro.2017.018.01.5. In Indonesian Language.
Sabeti P, Pourmasumi S, Rahiminia T, Akyash F, Talebi AR. 2016. Etiologies of sperm oxidative stress. International Journal of Reproductive BioMedicine 14(4):231-240. https://doi.org/10.21776/10.29252/ijrm.14.4.231.
Salmani H, Towhidi A, Zhandi M, Bahreini M, Sharafi M. 2014. In vitro assessment of soybean lecithin and egg yolk-based diluents for cryopreservation of goat semen. Cryobiology 68:276-280. https://doi.org/10.1016/j.cryobiol.2014.02.008.
Sari RP, Nugraha CD. Suyadi. 2024. Effect of thawing on semen quality of Boer goats with tris-aminomethane diluent supplemented with antioxidant quercetin. Jurnal Sain Peternakan Indonesia 19(1):11-20. https://doi.org/10.31186/jspi.id.19.1.11-20.
Sholikah N, Susilowati S, Tribudi YA, Sulistyowati D. 2022. Kualitas semen cair kambing Boer dalam pengencer air kelapa muda dan penambahan sari kedelai [Quality of Boer goat semen in coconut water diluent and soybean extract supplementation]. Jurnal Veteriner 23(2):202-210. https://doi.org/10.19087/jveteriner.2022.23.2.202. In Indonesian Language.
Silvestre MA, Yániz JL, Peña FJ, Santolaria P, Castelló-Ruiz M. 2021. Role of antioxidants in cooled liquid storage of mammal spermatozoa. Antioxidants (Basel) 10(7):1096. https://doi.org/10.3390/antiox10071096.
Shokry DM, Abd Eldaim MA, Badr MR, Khalifa HK, Orabi SH, Hassan AM, Dohreig R. 2021. Enhancement impact of Moringa oleifera leaves extract–base extender on cryopreservation and fertilization of

Barki ram sperms: comparative study with vitamin E and selenium combination. Italian Journal of Animal Science 20(1):1175-1186. https://doi.org/10.1080/1828051X.2021.1953411.

Sokunbi OA, Ajani OS, Lawanson AA, Amao EA. 2015. Antibiotic potential of moringa leaf (*Moringa oleifera* Lam) crude extract in bull semen extander. European Journal of Medicinal Plants 9:1-8. https://doi.org/10.9734/EJMP/2015/18546.

Sundararaman MN, Edwin MJ. 2008. Changes in motility characteristics of goat spermatozoa during glycerol-equilibration and relevance to cryopreservation. Asian Journal of Cell Biology 3(1):22-33. https://doi.org/10.3923/ajcb.2008.22.33.

Suharyati S, Hartono M. 2013. Peningkatan kualitas semen kambing Boer dengan pemberian vitamin E dan mineral Zn [Improvement of Boer goat semen quality with vitamin E and zinc supplementation]. Jurnal Kedokteran Hewan 7(2):91-93. https://doi.org/10.21157/j.ked.hewan.v7i2.897. In Indonesian Language.

Susiilowati S, Mustofa I, Suprayogi TW, Akintunde AO, Purwanto DA, Wurlina, Utama S, Mulyati S. 2024. Adding chitosan nonparticles of green tea extract in diluent and thawing temperatures ameliorate the post-thawed quality of Boer buck semen. Asian Pacific Journal of Reproduction 13(1):42-48. https://doi.org/10.21157/apjr.apjr_93_23.

Suwor F, Kubota S, Nawong S, Thuangsantia A, Toyra M, Paengkoum P, Ponchunchovoong S. 2025. Effects of cryoprotectant combinations on post-thawed sperm quality, biomolecular changes, DNA methylation, and pregnancy rates in Boer goat semen. Veterinary Sciences 12:1-18. https://doi.org/10.3390/vetsci12020178.

Tamoes JA, Nalley WMM, Hine TM. 2014. Fertilitas spermatozoa babi Landrace dalam pengencer modifikasi zorlesco dengan susu kacang kedelai [Sperm fertility of Landrace pigs in modified Zorlesco diluent with soybean milk]. Jurnal Sains Peternakan 12:20-30. https://doi.org/10.20961/sainspet.v12i1.4772. In Indonesian Language.

Wahjuningsih S, Ihsan MN, Ciptadi G, Isnaini N, Rahayu S, Afifah DD. 2019. Effect of *Moringa oleifera* leaves extract on post-thawed semen quality of Senduro goat. Earth and Environmental Science 247:1-5. https://doi.org/10.1088/1755-1315/247/1/012055.

Wahjuningsih S, Ihsan MN, Siswoyo DA, Fatmila DT, FirmawatiA.2021.Extractofcincau(*Mesonapalustris* B.) supplementation in semen extender improves Boer goat sperm cryopreservation. Journal of Advanced Veterinary Research 11(4):247-253.

Wajdi SA, Utomo B, Rumiyanti, Safitri E, Suprayogi TW, Wurlina. 2021. Suplementasi perasan daun kelor (*Moringa oleifera*) meningkatkan kualitas semen sapi Limousin *post thawing* [Supplementation of moringa leaf extract (*Moringa oleifera*) improves semen quality of Limousin cattle post-thawing]. Jurnal Medik Veteriner 4(2):249-255. https://doi.org/10.20473/jmv.vol4.iss2.2021.249-255. In Indonesian Language.

Zamiri MJ. 2020. Update on semen cryopreservation in sheep and goats: A review. Journal of Livestock and Technologies 8(1):1-15. https://doi.org/10.22103/JLST.2020.15927.1321.