

# The Effect of Addition of Moringa Leaf Extract in Lactose Extender on Frozen Semen Quality of Boer Crossbreed Goat

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## RESEARCH PAPER

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## The Effect of Addition of Moringa Leaf Extract in Lactose Extender on Frozen Semen Quality of Boer Crossbreed Goat

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**Key words:** Moringa leaf extract, Frozen semen, Boer crossbreed goat

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### Abstract

Moringa leaves contain various nutrients that can protect spermatozoa during semen cryopreservation. This study aimed to examine the effect of moringa leaf extract in tris extender on the quality of frozen semen of Boer crossbreed goat. Semen was collected with an artificial vagina. Fresh semen was divided into four tubes and diluted with 73% tris extender + 20% egg yolk + 7% glycerol (control), 71% tris extender + 20% egg yolk + 7% glycerol + 2% moringa leaf extract (MLE-2), 69% tris extender + 20% egg yolk + 7% glycerol + 4% moringa leaf extract (MLE-4), and 67% tris extender + 20% egg yolk + 7% glycerol + 6% moringa leaf extract (MLE-6), respectively. Diluted-semen was loaded in a mini straw and then stored in a liquid nitrogen container for seven days. The quality of spermatozoa including motility, viability, and intact plasma membrane (IPM) were observed after diluting and thawing. The result showed that the addition of moringa leaf extract into the tris extender could improve the spermatozoa quality of frozen semen of Boer crossbreed goat. At the stage after thawing, the percentage of motility, percentage of viability, and percentage of IPM of MLE-4 (72.2, 65.2, and 65.0%) were significantly higher ( $p < 0.05$ ) compared to the control (43.7, 53.5, and 53.7%). It can be concluded that the addition of 4% moringa leaf extract is the appropriate concentration in producing frozen semen of Boer crossbreed.

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### Introduction

One method to improve the performance of Indonesian local goats is to cross them with Boer goats through the application of artificial insemination (AI) technology. An important factor in the success AI is the quality of semen processing (Tamoës *et al.*, 2014). The quality of semen processing can be maintained by adding various additive compounds to the semen extender, including moringa leaf extract. This is because moringa leaves contain high antioxidants (Kasolo *et al.*, 2010) and antibacterial (Das *et al.*, 2012).

Compounds that are antioxidants can inhibit the work of free radicals in damaging the plasma membrane of spermatozoa during semen preservation (Sitepu *et al.*, 2018), to maintain the quality of spermatozoa. Moringa leaves contain flavonoid compounds that can bind free radicals (Kumala *et al.*, 2016).

Improved semen quality preserved with an extender supplemented with moringa leaf extract was reported in bovine spermatozoa (Sokunbi *et al.*, 2015) and Landrace pig spermatozoa (Fafo *et al.*, 2016).

This study aims to examine the effect of various concentrations of moringa leaf extract in tris extender on the quality of frozen semen of Boer crossbreed goat. It is expected that the compounds contained in moringa leaf extract can protect against spermatozoa cell damage during the cryopreservation process so that the quality can be maintained and meet the requirements for use in the AI program.

### Materials and methods

#### Semen collection and cryopreservation

Semen was collected from two male Boer crossbreed goats using an artificial vagina (Fig. 1). Fresh semen was evaluated for quantity and quality, including macroscopic (volume, viscosity, degree of acidity (pH)), and microscopic (mass movement, concentration, motility, viability, and abnormality of spermatozoa, as well as the percentage of spermatozoa with intact plasma membrane). Fresh semen was divided into four tubes and diluted with

73% tris extender + 20% egg yolk + 7% glycerol (control), 71% tris extender + 20% egg yolk + 7% glycerol + 2% moringa leaf extract (MLE-2), 69% tris extender + 20% egg yolk + 7% glycerol + 4% moringa leaf extract (MLE-4), and 67% tris extender + 20% egg yolk + 7% glycerol + 6% moringa leaf extract (MLE-6), respectively. The semen was diluted to a concentration of 200 million motile spermatozoa per milliliter.

Moringa leaves were extracted using a rotary vacuum evaporator method (Sokunbi *et al.*, 2015; Kumala *et al.*, 2016). Moringa leaf extract was put into an effendorf tube and stored in the freezer before use (Fig. 2).

The diluted semen was loaded in mini straws (0.25 ml) and equilibrated in the refrigerator at 5°C for four hours. Freezing of semen was carried out by placing straws 10 cm above the surface of liquid nitrogen in closed styrofoam (temperature about -130°C) for 15 minutes. Next step, straws were put into liquid nitrogen and stored in the container. After seven days, straw samples for each treatment were thawed (in water at 37°C for 30 seconds) to evaluate (Fig. 3).

#### Observed variables

The observed spermatozoa quality variables were motility, viability, and intact plasma membrane (IPM) after diluting and thawing.

The motility of spermatozoa was evaluated according to Rasul *et al.* (2001). The viability of spermatozoa was evaluated by eosin-nigrosine staining (Feline-Perez *et al.*, 2008) and spermatozoa with IPM was evaluated by the method of hypoosmotic swelling (HOS) test (Revel and Mrode, 1994).

#### Statistical analysis

Data were analyzed by analysis of variance in form of a completely randomized design (CRD) with four treatments and six replications. Different treatments were tested with the least significant difference (LSD) (Steel and Torrie, 1993). Data is processed using SAS statistical software program (SAS 9.1, 2001).

## Results and discussion

### Characteristics of Boer crossbreed goat semen

The quality of fresh semen of Boer crossbreeds was generally good, where the main parameters such as motility, abnormality and IPM were 77.5%, 4.33%, and 88.3%, respectively (Table 1).

Fresh semen that meets the requirements to be processed for storage as frozen semen has a

spermatozoa motility >75% (Salmani *et al.*, 2014), abnormality <15% (Bearden and Fuquay, 2000) and IPM >60% (Revell and Mrode, 1994).

### Spermatozoa quality after cryopreservation

The results showed that the addition of moringa leaf extract in tris extender could improve the spermatozoa quality of Boer crossbreed goat frozen semen.

**Table 1.** Characteristics of fresh semen of Boer crossbreed goat.

Semen attributes	Mean ± SD
Semen volume (ml)	0.75 ± 0.13
Semen color	Milky white
Semen pH	6.92 ± 0.09
Semen consistency	Thick
Spermatozoa mass movement	+++
Spermatozoa concentration (million/ml)	4,483 ± 883
Spermatozoa motility (%)	77.5 ± 2.7
Spermatozoa viability (%)	88.3 ± 0.7
Spermatozoa abnormality (%)	4.33 ± 1.03
Spermatozoa with the intact plasma membrane (%)	88.3 ± 1.03

At the stage after thawing, the percentage of motility, percentage of viability, and percentage of the intact plasma membrane of MLE-4 treatment (52.2, 65.2, and 65.0%) were significantly higher ( $p < 0.05$ ) compared to control (43.7, 53.5, and 53.7%) (Table 2, 3, 4). The addition of moringa leaf extract of as much as 2% did not significantly improve the quality of frozen semen of Boer crossbreed goat, furthermore, the addition of 6% moringa leaf extract was

significant ( $p < 0.05$ ) from the control treatment in improving the quality of Boer crossbreed goat frozen semen. This indicates that the addition of 4 and 6% moringa leaf extract in tris extender is a suitable concentration for improving the quality of frozen semen of Boer crossbreed goat. Observations also showed that the movement quality of individual spermatozoa in the MLE-4 treatment was better than the other treatments.

**Table 2.** Motility of Boer crossbreed goat spermatozoa.

Stage of evaluation	Treatments			
	Control	MLE-2	MLE-4	MLE-6
After diluting (%)	77.5 ± 2.74	77.5 ± 2.74	77.5 ± 2.74	77.5 ± 2.74
After thawing (%)	43.7 ± 2.50 <sup>a</sup>	46.2 ± 4.79 <sup>ab</sup>	52.2 ± 2.89 <sup>c</sup>	51.2 ± 2.50 <sup>bc</sup>

Description: <sup>abc</sup>Superscript in the same line shows significantly different ( $p < 0.05$ )

MLE = Moringa leaf extract.

The addition of moringa leaf extract in tris extender was able to improve the quality of frozen semen of Boer crossbreed goat, presumably because moringa leaves contain several active substances that protect

spermatozoa from damage during the cryopreservation process, especially during dilution, freezing and thawing. Moringa leaf contains high antioxidants and antibacterial (Das *et al.*, 2012).

**Table 3.** Viability of Boer crossbreed goat spermatozoa.

Stage of evaluation	Treatments			
	Control	MLE-2	MLE-4	MLE-6
After diluting (%)	87.8 ± 1.25	88.0 ± 1.09	87.9 ± 1.31	87.9 ± 1.54
After thawing (%)	53.5 ± 1.29 <sup>a</sup>	58.0 ± 2.16 <sup>ab</sup>	65.2 ± 3.09 <sup>c</sup>	62.0 ± 2.58 <sup>bc</sup>

Description: <sup>abc</sup>Superscript in the same line shows significantly different ( $p < 0.05$ ).

MLE = Moringa leaf extract.

Antioxidant compounds can inhibit the work of free radicals that can damage spermatozoa so that the integrity of the plasma membrane of spermatozoa cells can be maintained during the cryopreservation process (Sitepu *et al.*, 2018).

The intact cell plasma membrane will have a positive effect on the percentages of motility and survival of

spermatozoa. Moringa leaf also contains compounds that function as antioxidants in the form of flavonoids, vitamin C and vitamin E (Gopalakrishnan *et al.*, 2016). Vitamin C can ward off free radicals and protect the plasma membrane of spermatozoa cells (Cahyadi *et al.*, 2016). Kumala *et al.* (2016) stated that moringa leaves contain flavonoid compounds that can bind free radicals.

**Table 4.** The intact plasma membrane of Boer crossbreed goat spermatozoa.

Stage of evaluation	Treatments			
	Control	MLE-2	MLE-4	MLE-6
After diluting (%)	87.3 ± 1.15	87.9 ± 1.45	88.1 ± 1.67	88.1 ± 1.27
After thawing (%)	53.7 ± 0.96 <sup>a</sup>	56.0 ± 1.26 <sup>ab</sup>	65.0 ± 1.15 <sup>c</sup>	59.0 ± 0.82 <sup>b</sup>

Description: <sup>abc</sup>Superscript in the same line shows significantly different ( $p < 0.05$ ).

MLE = Moringa leaf extract.

A semen extender added with 4-16% moringa leaf extract was able to maintain the motility, morphology, and integrity of the plasma membrane of bovine spermatozoa cells (Sokunbi *et al.*, 2015).

**Fig. 1.** Collection of semen using an artificial vagina.

It was also in line with Fafu *et al.* (2016) who reported that 5% moringa leaf extract added to egg yolk citrate extender was effective in maintaining the motility and

viability of Landrace pig spermatozoa.

**Fig. 2.** Moringa leaf extract is packaged in an effendorf tube.

The lower quality of frozen semen with the addition of 2% moringa leaf extract compared to 4% indicates that the active substances contained in moringa leaves are still insufficient to optimally protect spermatozoa from damage during the cryopreservation process.



**Fig. 3.** Evaluation and processing of Boer crossbreed goat semen with a simple method.

In this study, the percentage of spermatozoa motility from frozen semen in all treatments showed more than 40%, so it could be used in the AI program based on SNI 4869.3:2014.

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#### Conclusion

Based on the results of the study it can be concluded that the addition of moringa leaf extract in tris extender could improve the quality of frozen semen of Boer crossbreed goat. The addition of 4% moringa leaf extract is the appropriate concentration in producing frozen semen of Boer crossbreed.

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