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Effect of *Moringa oleifera* leaves extract on post-thawed semen quality of Senduro Goat

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Abstract. The present study was conducted to evaluate the supplementation of *Moringa* leaves extract in CEP-2 Egg Yolk extender on post-thawed semen quality of Senduro goat semen. Ejaculates were collected once a week for ten weeks from one mature buck using an artificial vagina. CEP-2 egg yolk extender containing a various concentration of *Moringa* leaves extract (1,3,5,7 %) was prepared in mini French straw (0.25 mL). Semen quality was assessed based on post-thawed sperm motility, viability, abnormality and plasma membrane integrity. The result showed that supplementation of different concentration of *Moringa* leaves extract in CEP-2 Egg Yolk extender have a significant effect ($P < 0.05$) on the percentage of sperm motility, viability, and membrane integrity, but there was no significant effect on the percentage of abnormality ($P > 0.05$). In conclusion, the addition of *Moringa oleifera* leaves extract 5 % to semen extender could improve post-thawed semen quality.

1. Introduction

Senduro goat is a wealth of genetic resources of local Indonesian livestock that must be protected and preserved. Senduro goat is a dual-purpose goat, with the potential to be developed and improved in genetic quality so that the productivity of the livestock increases. For the improvement of genetic selection and economic advantages in Senduro goat production, the use of Artificial Insemination (AI) is necessary. The cryopreserved goat semen very important to supply of genetic material for gene banks to encourage valuable individuals [1]. This is especially important in the case of Senduro buck goat. However, the biggest problem to the exploitation of frozen semen is freezing and thawing process of goat sperm generally leads to a decrease sperm quality.

Cryopreservation is associated with oxidative stress. Goat sperms are sensitive to peroxidative damage because of the high content of unsaturated fatty acids in the phospholipids of the plasma membrane and the relative low antioxidant capacity of seminal plasma [2]. The formation of Reactive Oxygen Species (ROS) caused a decrease in the ability of sperm motility and increased the damage that would affect the semen quality. The function of the extender is to supply the sperm cells with a source of energy, protect the cells from temperature-related damage and maintain a suitable environment for the spermatozoa [3,4]. In order to optimize extender for the achievement of best post-thaw semen characteristics which would infer greater fertility, it is important to study the added antioxidant in CEP-2 Egg Yolk extender. *Moringa oleifera* is a plant that has a high antioxidant content, especially in the leaves [5]. Previous research indicated that *Moringa* leaves could improve



the quality of spermatozoa in male rabbits [6] and Wistar rat [7]. *Moringa oleifera* leaves contain high antioxidants and some bioactive compounds flavonoid groups such as quercetin, kaempferol, and proanthocyanidin [8]. Supplementation of *Moringa* leaves extract in CEP-2 Egg Yolk extender is expected to prevent free radicals during processing and storage of frozen semen so that it will maintain the quality of frozen semen. The objectives of this study were to investigate addition of *Moringa* leaves extract in CEP-2 Egg Yolk extender on post-thawed semen quality of Senduro goat.

2. Material and method

Moringa leaves the extraction process as follows: aerated *Moringa* leaves for 16 h then blended until crushed, macerated leaves of *Moringa* that have been destroyed using 70% ethanol solution and left for 24 h, filtered the material that has been macerated with Whatman paper 42 until the filtrate was obtained, filled in the vacuum rotary evaporator at a temperature of 60°C, 35rpm for 1 hour to obtain a paste-shaped extract[9]. Semen was collected from Senduro goats aged from 1.5 to 2.0 years using an artificial vagina once a week. Only samples with $\geq 70\%$ motile sperm and $\leq 10\%$ abnormal sperm were used in this research.

2.1. Extender preparation

Semen was divided to 5 treatments as follows: T0 = 90 % CEP-2 + 10 % Egg Yolk , T1 = T0 + 1 % *Moringa* Leaves Extract (MLE), T2 = T0+3% MLE, T3= T0+5% MLE, T4= T0+7% MLE.

2.2. Freezing and thawing process

Semen loaded in the mini French straw (0.25 mL), cooled for 2 h at 5°C, freezing in the steam of nitrogen liquid for 10 min and then stored for 2 weeks in liquid Nitrogen container. Thawing was done by dipping the straw into the water 37 °C for 30 sec.

2.3 Data analysis

Data analysis using analysis of variance. The Duncan Multiple Range Test was used when there were any differences among the treatments

Table 1. CEP-2 Diluent Composition [10]

Content	The amount of content
NaCl	0,09 g/l
KCl	0,05 g/l
CaCL ₂ (H ₂ O) ₂	0,04 g/l
MgCL ₂ (H ₂ O) ₆	0,08 g/l
NaHCO ₃	0,10 g/l
NaH ₂ PO ₂	0,11 g/l
KH ₂ PO ₄	0,27 g/l
Fructosa	0,27 g/l
Tris Aminomethan	1,61 g/l
Citric acid	0,82 g/l
Aquabidest	90 ml
Penicilin	0,006 g/l
Streptomycin	0,01 g/l
Egg Yolk	10 ml
Bovine Serum Albumin	0,2 g/l

3. Result and Discussion

Percentage of motility, viability, membrane integrity and abnormalities of Senduro goat sperm in different concentration of *Moringa* leaves extract were presented in table 2,3,4 and 5.

Table 2. Individual motility of sperm (%) in different concentration of *Moringa* leaves extract

Stages	Treatments				
	T0(0%)	T1(1%)	T2	T3	T4
Before Freezing	36.00±7.38 ^a	37.00±4.22 ^a	40.50±8.96 ^{ab}	45.50±7.62 ^b	40.50±7.62 ^{ab}
Post Thawing	32.00±6.32 ^a	31.50±4.74 ^a	37.50±8.58 ^{ab}	40.50±7.62 ^b	35.50±7.62 ^{ab}

Means±SEM within rows having unlike letters are significantly different at $P < 0.05$ **Table 3.** Sperm viability (%) in different concentration of *Moringa* leaves extract

Stages	Treatments				
	T0	T1	T2	T3	T4
Before Freezing	50.47±3.01 ^a	53.33±2.27 ^{ab}	55.26±1.37 ^{ab}	60.57±3.25 ^b	56.13±2.13 ^{ab}
Post Thawing	37.96±1.73 ^a	45.08±1.99 ^{ab}	44.05±2.53 ^{ab}	45.53±2.68 ^b	38.41±3.72 ^{ab}

Means±SEM within rows having unlike letters are significantly different at $P < 0.05$ **Table 4.** Plasma membrane integrity (%) in different concentration of *Moringa* leaves extract

Stages	Treatments				
	T0	T1	T2	T3	T4
Before Freezing	45.53±7.16 ^a	46.86±6.56 ^{ab}	42.80±7.97 ^a	52.26±3.13 ^b	46.81±7.35 ^{ab}
Post Thawing	38.51±5.92 ^a	39.67±8.56 ^a	39.84±4.59 ^a	47.24±6.42 ^{ab}	41.63±6.65 ^b

Means±SEM within rows having unlike letters are significantly different at $P < 0.05$ **Table 5.** Sperm Abnormality (%) in different concentration of *Moringa* leaves extract

Stages	Treatments				
	T0	T1	T2	T3	T4
Before Freezing	3.00±1.8	4.28±1.88	3.05±1.17	3.93±1.99	3.05±2.29
Post Thawing	3.14±1.19	3.19±1.08	3.11±1.12	3.44±1.12	3.46±1.13

Based on the table showed that the addition of *Moringa* leaves extract to CEP-2 Egg Yolk extender had a significantly different effect ($P < 0.05$) on motility, viability, membrane integrity, but non significantly different ($P > 0.05$). Addition of *Moringa* leaves to extract as much as 5% provides optimal results compared to other treatments. The antioxidant content of *Moringa* leaves was able to maintain the spermatozoa membrane and protect the sperm damage caused by free radicals. Membrane integrity is not only important for metabolism but also specific changes in membrane components, especially during fertilization. Damage of plasma membrane would cause the loss of sperm motility and ability to conceive because of loss of cellular components and inactivation of proteins essential enzyme in the acrosome [12,13]. *Moringa* leaves as a source of antioxidants which contain flavonoids, saponins, alkaloids, tannins, carotenoids (especially lutein and carotene), quercetin and phenol [14,15]. The negative effect of supplementation 7% *Moringa* leaves extract on sperm quality may be due to too high antioxidant concentrations. Excessive concentration of antioxidants will lose its effectiveness as an antioxidant and even form a prooxidant. Changes in antioxidant function become prooxidant, or free radicals cause more unsaturated fatty acids that are subjected to free radicals. The decrease in sperm quality is due to the presence of antinutrients in *Moringa* leaves, one of which is tannins. Increasing tannin levels can inhibit the movement of spermatozoa, because tannins can bind complex proteins or proteins bound to Ca, Mg, Na, and K ions, carbohydrates and fats.

It can be seen in table 2,3,4 that there was different sperm quality between before freezing and post-thawing at the same concentration of *Moringa* supplementation. Decreasing sperm quality during the process is due to cell membrane phospholipids of sperm that are permanently damaged and a

decrease in cell membrane function [11]. The decrease in motility value of motility, viability and plasma membrane integrity after freezing process can be caused by osmotic shock when sperm was frozen, so that ice crystals form in cells that damage the structure of the plasma membrane. Moreover, freezing and thawing of sperm increase the reactive oxygen species (ROS), producing DNA damage cytoskeleton alterations, inhibition of the sperm-oocyte fusion and affecting the sperm axoneme that is associated with the loss of motility. Cryopreservation as a technique for storage of goat semen has advantages, but freezing and thawing induces detrimental effects regarding sperm ultrastructural, biochemical and functional damage, resulting in a reduction of motility, membrane integrity and fertilizing ability. This research demonstrated that cryopreservation leads to decrease sperm quality.

4. Conclusion

The addition of 5% Moringa leaves extract in CEP-2 egg yolk extender was able to maintain the quality of Senduro goat semen during cryopreservation.

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