



## **Spermatozoa Motility of Boer Buck Semen with Addition of Sweet Orange Essential Oil to Tris Yolk Extender**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author SAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZU, Jaswandi and Hendri managed the analyses of the study and literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** The sweet orange essential oil contains antioxidants and antibacterials that can inhibit bacterial growth and prevent damage due to cold shock so that spermatozoa motility increases. Determine the effect of the addition sweet orange essential oil to tris yolk extender to spermatozoa motility Boer Buck frozen semen.

**Study Design:** Randomized Block Design.

**Place and Duration of Study:** Laboratory Reproduction of Loka Penelitian Kambing Potong Sei Putih Indonesia, between January and March 2019.

**Methodology:** The research procedure starts with the preparation of semen extender, collection of fresh semen, dilution of semen, equilibration, freezing of semen, and thawing. Semen collected from 3 boer bucks were analyzed for the study using randomized block design as experimental design. Sweet orange essential oils as treatment used at 4 treatment level at (P<sub>0</sub>) control group (P<sub>1</sub>) 0,25%, (P<sub>2</sub>) 0,5%, (P<sub>3</sub>) 0,75% and (P<sub>4</sub>) 1% on the tris yolk extender. The observed variables was motility spermatozoa evaluated before freezing and after freezing (Post-Thawing).

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**Results:** The results showed that the addition of sweet orange essential oil had a very significant effect ( $P < 0.01$ ). The results of adding sweet orange essential oil to the extender (Post-Thawing) were 36% ( $P_0$ ), 40% ( $P_1$ ), 40% ( $P_2$ ), 44% ( $P_3$ ) and 50% ( $P_4$ ).

**Conclusion:** The best values the motility 50% ( $P_4$ ). It can conclude that the best result of adding sweet orange essential oil to tris yolk extender is ( $P_4$ ) 1%.

*Keywords: Boer buck; motility; post-thawing; sweet orange essential oil.*

## 1. INTRODUCTION

Breeding is one of the success factors of goat farm [1]. The genetic quality of local goats in many countries is still low, especially body weight. The impact is that many countries currently cannot meet the needs of goat meat. Improving the genetic quality of goats is essential to increase meat production so that meat demand fulfilled. Utilization of livestock reproduction technology, such as Artificial Insemination by utilizing semen from superior goats such as Boer buck can improve the genetic quality of local goats. Boer goats are the superior type of meat goat in the world because of their high body weight. Boer Goat Weight is 35 - 45 kg at the age of five to six months, with an average weight gain of between 0.02 - 0.04 kg per day [2].

The quality of goat's frozen semen determines the success of artificial insemination. One of the causes of low-quality frozen semen is bacteria. To inhibit bacterial growth in frozen goat semen is to add antibacterial ingredients. Sweet orange essential oil is a natural ingredient that can be used as an antibacterial because it can inhibit and be active against bacteria [3]. The sweet orange essential oil contains the main components, including limonene, linalool, pinene, and oktanal [4]. Limonene and linalool are toxic to bacteria [3]. The sweet orange essential oil also contains antioxidants [5]. Antioxidants can reduce damage caused by cold shock in spermatozoa [6].

To determine the quality of sperm can do by observing of spermatozoa motility. Spermatozoa motility is one of the criteria for determining spermatozoa's quality by looking at the progressively moving spermatozoa. Assessment is carried out by observing progressive movements and compared with spermatozoa that have reverse or only rotating movements [7]. The addition of sweet orange essential oil to the tris yolk extender can increase the percentage of spermatozoa motility in Boer buck frozen semen.

## 2. MATERIALS AND METHODS

The research carried out in the laboratory by adding sweet orange essential oil as an antibacterial increase the percentage of motility spermatozoa of the post-thawing Boer buck semen. The research procedure starts from a collection of fresh semen, dilution semen, equilibration, semen freezing, and thawing.

Materials used were fresh semen Boer buck, sweet orange essential oil, nutrient agar, nutrient broth, Tris yolk extender, eosin 2%, liquid nitrogen, incubator, autoclave, oven, petri dish, beaker glass, cover glass, and denominator.

This research conducted using a Randomized Block Design consisting of 5 treatment levels and five replications. The group is a holding of fresh semen using 3 Boer buck once every three days. As treatment is the addition of sweet orange essential oil to the tris yolk extender. Duncan's test tested differences between treatments. The treatments observed:

$P_0$ = Control Group

$P_1$ = Tris Yolk Extender + Sweet Orange Essential Oil 0.25%

$P_2$ = Tris Yolk Extender + Sweet Orange Essential Oil 0.5%

$P_3$ = Tris Yolk Extender + Sweet Orange Essential Oil 0.75%

$P_4$ = Tris Yolk Extender + Sweet Orange Essential Oil 1%

Motility of spermatozoa during pre and post freezing:

### 2.1 Spermatozoa Motility

Percentage of spermatozoa that move progressively forward. Evaluation is done by observing spermatozoa at eight different fields of view with a 400 times magnification light microscope [8].

### 3. RESULTS AND DISCUSSION

#### 3.1 Spermatozoa Motility

The results of observations of motility observation of Boer buck semen before and after freezing using sweet orange essential oil can see in Table 1. Motility observation data shows that the use of sweet orange essential oil as an extender that meets the standards is all treatment after equilibration. After equilibration (before freezing), the percentage of spermatozoa motility of all treatments is above 60%. The quality of semen that meets the standards in the process of making frozen semen, one of which is the motility of more than 60% [9]. The higher motility will increase the percentage of fertility success. The availability of good quality breeds will increase the profitability of livestock farming [10].

Table 1 shows the average results of the highest motility of Boer buck frozen semen after freezing is 1% (P<sub>4</sub>) with a value of 50%, and the lowest is at the control group (P<sub>0</sub>) with a value of 36%. The results showed that the frozen semen of Boer buck control group P<sub>0</sub> did not meet the requirements for use in Artificial Insemination. The mean percentage of motility in the control group was only 36%. To qualify, the minimum value of the percentage of motility in Artificial Insemination is 40% [9]. The percentage of motility is low because the extender used does not have antibacterial properties. One of the requirements for semen extender is the addition of materials that contain antibacterial properties. Bacteria contained in frozen semen can reduce the percentage of spermatozoa motility [11].

Treatments P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub>, and P<sub>4</sub> meet the standards as diluents, meaning that the use of sweet orange essential oil can maintain the sperm motility of Boer buck. The results of the analysis of variance showed very significantly different (P < 0.01) on the motility of spermatozoa in the frozen semen of Boer buck. Semen that is suitable for use in Artificial Insemination has motility of not less than 40% [12].

Spermatozoa motility is one of the factors spermatozoa can reach the ovum in the fallopian tubes and the most straightforward way in the assessment of sperm for Artificial Insemination [13]. The high motility will increase the ability of spermatozoa for fertilization [14]. The use of essential oils as an extender maintains motility after equilibration and after freezing. It is due to

the essential oil functions as an excellent antioxidant for sperm. The semen of good quality, the addition of antioxidants can maintain the survival of frozen sperm [15]. However, low-quality sperm due to peroxidation can not overcome by the addition of antioxidants.

Spermatozoa motility provides an overview of spermatozoa's ability to travel within the female reproductive tract and fertilize the ovum. The factors that influence the speed and movement of buck semen spermatozoa are the method of collecting semen, environmental conditions, handling and care for shelter, holding intervals, variations in individual buck, and seasons [11]. The other factors that influence are the number of ejaculates, the buck's age, changes in temperature, and livestock breeds [16].

**Table 1. The effect of supplementation of sweet orange essential oil on tris yolk extender on the motility percentage of boer buck semen before freezing and after freezing**

Parameter	Treatment	Observation	
		Before Freezing	After Freezing
	P <sub>0</sub>	(62±4.47) <sup>b</sup>	(36±5.47) <sup>c</sup>
	P <sub>1</sub>	(68±4.47) <sup>a</sup>	(40±0.00) <sup>b</sup>
Motility (%)	P <sub>2</sub>	(70±0.00) <sup>a</sup>	(40±0.00) <sup>b</sup>
	P <sub>3</sub>	(70±0.00) <sup>a</sup>	(44±5.47) <sup>b</sup>
	P <sub>4</sub>	(70±0.00) <sup>a</sup>	(50±0.00) <sup>a</sup>

*Note: Figures having the same superscript across the rows / between treatment groups do not differ significantly*

#### 4. CONCLUSION

The best result is the addition of 1% sweet orange essential oil, and it is suitable for use in artificial insemination.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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