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Percentage Viability Spermatozoa in Post-Thawing 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: To ddetermined the effect of the addition of sweet orange essential oil for tris yolk extender to percentage viability spermatozoa in Boer Buck frozen semen.
Study Design: Randomized Block Design.
Place and Duration of Study: Sample: 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. This research obtained conducted using a Randomized Block Design consisting of 5 treatment levels and five replications. Semen storage using 3 Boer bucks, which has done for three days. As a treatment is the addition of sweet orange essential oil as much as (P_0) 0%, (P_1) 0.25%, (P_2) 0.5%, (P_3) 0.75% and (P_4) 1% on the tris yolk extender. The observed variable was percentage viability 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 44.8% (P_0), 49% (P_1), 51.8% (P_2), 55.2% (P_3) and 59% (P_4).
Conclusion: It can be concluded that addition of sweet orange oil to tris yolk extender at 1% gave best results.

Keywords: Boer buck; post-thawing; sweet orange essential oil; viability spematozoa.

1. INTRODUCTION

Good quality breeds will determine the livestock business's success and goat breeders [1]. To get good breeds can be done by mating using a Boer buck. Boer buck has several advantages, namely high body weight, fast growth, and high carcass. However, to maintain good quality Boer buck is quite expensive and difficult to obtain. Artificial Insemination is a solution to do doe mating with Boer buck, which has good quality.

The success of Artificial Insemination in goats depends on the quality of frozen semen. The bacteria present in frozen semen can damage and kill spermatozoa. Besides, the process of diluting and freezing goat semen causes cold shock so that the quality of the semen decreases [2]. It can do by adding antibacterial and antioxidant ingredients to the frozen goat semen extender to prevent damage caused by bacteria and cold shock [3].

The use of antibiotics has done made on frozen semen extenders. Antibiotics act as growth inhibitors and kill bacteria in frozen semen [4]. In the livestock business, the use of antibiotics has begun to abandon. The use of natural ingredients is widely studied to replace antibiotics. Sweet orange essential oil is one ingredient that can use as a source of antibacterial and antioxidant properties. The sweet orange essential oil contains limonene and linalool, which have antibacterial properties [5]. Besides, sweet orange essential oil contains flavonoids which function as antioxidants [6]. Antioxidants can reduce damage to spermatozoa during the freezing process of semen (cold shock) [7]. This study aims to determine the effect of adding sweet orange essential oil to spermatozoa's viability in the frozen semen of Boer Goats.

2. MATERIALS AND METHODS

The study conducts experimentally with a quantitative or objective approach. Experimental research was carried out using various levels of sweet orange essential oil and then comparing it to without giving sweet orange essential oil. Activities in the empirical study aimed to assess the effect of sweet orange essential oil and determine the impact on giving it compared to providing sweet orange essential oil.

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

Materials used were fresh semen of Boer bucks, sweet orange essential oil, nutrient agar, nutrient broth, Tris (hydroxymethyl) aminomethane (3.32 g), Citric Acid (1.86 g), Fructose (1.37 g), Glycerol (6 ml), egg yolk (20 ml), aqua dest (100 ml), 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= Tris Yolk Extender + Sweet Orange Essential Oil 0%
- 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%

The parameters observed were the evaluation of semen before freezing and after freezing, namely:

2.1 Viability Evaluation

Evaluated using eosin staining. 10 µl of cement plus 50 µl placed in a glass slide, then stirred until homogeneous. Attach the tip of the glass object to the solution, then push it along the glass object. The Glass object heat at 37^0C. Peparat observed using a 400 magnification microscope. Living spermatozoa are marked by a head that does not absorb dyes, while a redhead marks dead ones. The evaluation carried out on a minimum of 200 spermatozoa observed using a 400 times magnification light microscope. The percentage of live spermatozoa calculated according to the formula:

$$\text{Viability} = \frac{\text{sperm with do not absorb color}}{\text{total sperm counted}} x\ 100\%$$

3. RESULTS AND DISCUSSION

The average results of the viability study of Boer buck frozen semen before freezing and after freezing can see in Table 1. The highest research results obtained in the treatment of 1% (P_4) with a value of 59% and the lowest in the treatment of 0% (P_0) with a value of 44.8%. All treatments in this study meet the standards as an extender, meaning that the use of sweet orange essential oil as an extender can maintain the sperm viability of Boer buck. Frozen semen that will use for Artificial Insemination must have a minimum value of 40% [8]. The results of the analysis of variance showed that the effect of the addition of sweet orange essential oil was significantly different ($P<0.01$) on the viability of spermatozoa in the frozen semen Boer buck.

Table 1 shows a decrease in the percentage value of spermatozoa viability in the frozen semen of Boer Goats. It is normal to have a reduction in the average percentage at each evaluation time. In freezing semen, spermatozoa die up to 30% of the total spermatozoa [9]. Cold shock occurs due to a decrease in body temperature to below 0° C, which will reduce cell viability. The phenomenon of cold shock in cells is unknown, but it is probably related to the lipid membrane transition stage, which causes a separation stage and a selective decrease in permeability properties of the biological membrane of living cells [10]. The addition of antioxidants can reduce mortality in spermatozoa [11].

The sweet orange essential oil can increase viability because it contains antibacterial and antioxidant properties. The content of limonene and linalool in sweet orange essential oil acts as an antibacterial that can reduce and kill bacteria [12]. Sweet orange essential oil is active against gram-positive and gram-negative bacteria [5]. Antibacterial can prevent the death of spermatozoa caused by bacteria in frozen semen. Sweet orange essential oil contains flavonoids which can function as antioxidants [13]. Antioxidants can prevent the death of spermatozoa caused by cold shock during the semen freezing process [14].

The addition of antioxidants will maintain the viability of frozen semen if the semen used is of good quality [7]. However, not with low semen quality because peroxidation cannot overcome by adding antioxidants. One of the requirements for semen extender is the addition of an antibacterial-containing material [15]. Bacteria contained in frozen semen can reduce the quality of semen, including the percentage of spermatozoa viability [8].

Lipid peroxidation will cause structural damage and disruption of spermatozoa metabolism resulting in dead spermatozoa. Antioxidants are nucleophilic compounds that can suppress free reactions and able to end the reaction cycle [16]. Antioxidants have the effect of proper resistance to peroxidation. For storage should be carried out at low oxygen concentrations and contain antioxidants in the extender [11]. It can do by using a closed container so that it can control the environment around the spermatozoa [17]. The implementation of artificial insemination can do if the female has estrus so that the percentage of fertility is high [18]. Goat farmer's income will increase if the breedings produced good quality [19].

The activity of sperm and bacteria in frozen semen will cause a metabolic process to form lactic acid due to metabolism [20]. The metabolic products of sperm and bacteria can have toxic effects on sperm [21]. Excess lactic acid levels can cause poisoning and high mortality for sperm [22]. Sweet orange essential oil plays a role in reducing the number of bacteria in the frozen semen of Boer Goats. The amount of lactic acid formed as a result of bacterial metabolism can also suppress. The inhibitory activity of metabolic processes, which aim at the reaction of the active ingredients in sweet orange essential oil, works more widely in maintaining spermatozoa's survival.

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

Parameter	Treatment	Observation	
		Before freezing	After freezing
Viability (%)	P_0	$(65.2\pm3,27)^d$	$(44.8\pm2,05)^e$
	P_1	$(68.2\pm2,05)^c$	$(49\pm2,64)^d$
	P_2	$(70.2\pm1,79)^c$	$(51.8\pm2,38)^c$
	P_3	$(71.8\pm1,64)^b$	$(55.2\pm4,60)^b$
	P_4	$(74.2\pm1,09)^a$	$(59\pm2,34)^a$

Note: Different superscripts in each variable column shows very significant differences (P <0.01)

The results of other studies indicated that apart from the viability of spermatozoa, antioxidants and antibacterials also showed good results on other semen quality parameters. Supplementation extra virgin olive oil (EVOO), betaine (BET) and ginger (GIN), as a natural antioxidant improved motility, intact acrosome, and membrane integrity in rabbit bucks semen [23]. The addition of butylated hydroxytoluene, which contains antibacterial and antioxidant properties, can increase motility, membrane integrity, acrosome integrity, and viability of cooled and frozen Boer goat spermatozoa at different concentrations [24]. The antibacterials in natural honey can increase sperm motility, live sperm count, intact acrosome, and hypo-osmotic swelling test (HOST) reacted spermatozoa [25].

4. CONCLUSION

The best result for the percentage of spermatozoa viability in Boer buck frozen semen is the addition of 1% sweet orange essential oil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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