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Viability of Spermatozoa in Goat's Liquid Semen after Adding Sweet Orange Essential Oil to The Andromed 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. Author JM managed the analyses of the study. Author AAR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To find out the percentage of live spermatozoa after diluting the goat's liquid semen using an andromed extender plus sweet orange essential oil.
Study Design: Randomized Block Design.
Place and Duration of Study: Sample: Galang, Deli Serdang Regency, Indonesia, between December 2022 and January 2023.

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Methodology: The research procedure starts with the preparation of semen extender, collection of fresh semen, dilution of semen and equilibration. This research obtained conducted using a Randomized Block Design consisting of 5 treatment levels and five replications. Semen storage using 3 Bucks, which has done for three days. As a treatment is the addition of sweet orange essential oil as much as (P0) 0%, (P1) 0.25%, (P2) 0.5%, (P3) 0.75% and (P4) 1% on the andromed extender. The observed variable was percentage viability spermatozoa evaluated before equilibration and after equilibration.
Results: The results showed that the addition of sweet orange essential oil had a not significant effect ($P < 0.01$) to viability of spermatozoa. The results of adding sweet orange essential oil to the extender (after equilibration) were 80% (P0), 80% (P1), 80% (P2), 80% (P3) and 81% (P4).
Conclusion: Adding sweet orange essential oil to andromed had no effect on the viability of goat spermatozoa in liquid semen.

Keywords: Andromed; buck; liquid semen; sweet orange essential oil; viability.

1. INTRODUCTION

Beef goat farming can be a solution to increase meat production in Indonesia [1]. Efforts to meet domestic demand for meat still rely on chicken and beef. However, the goat commodity still needs to be developed. One of the obstacles to goat farming is the low population and genetic quality of livestock and light body weight [2]. “To improve the genetic quality of livestock, cross-breeding between local goats and superior goats can be carried out” [3].

To bring in superior-quality male goats is quite tricky and expensive. In addition, maintenance and animal feed are costly. Artificial Insemination in goats can be a solution because farmers do not need to raise superior male goats [4]. “The success rate of Artificial Insemination in goats is highly dependent on the quality of the semen (liquid/frozen) and the implementation of Artificial Insemination. Goat liquid semen requires proper handling and selection of liquid semen diluent to obtain good-quality semen” [5].

There are several diluents for goat semen [6]. “Egg yolk tris diluent plus other ingredients that aim to improve semen quality have been widely carried out, among others, by giving milk [7] and raffinose” [8]. In addition, some studies are less effective as a diluent, such as adding coconut water [9].

One of the factors that cause the low quality of liquid semen is the growth and contamination of disturbing microorganisms such as bacteria in liquid semen [10]. “Bacterial contamination found in the liquid semen of goats/sheep comes from the male reproductive tract, the environment, handling during the semen dilution process, and diluents” [11]. Reducing the population of bacteria in semen can be done with the addition of antibacterial [12].

The cold shock on spermatozoa causes a decrease in the quality of liquid semen because it can disturb and damage spermatozoa [12]. This condition occurs during the dilution process in the manufacture of liquid goat semen. This condition can interfere with the movement of spermatozoa, which causes low motility. Besides that, it can also kill spermatozoa so that the percentage value of viability becomes low [13]. The cryopreservation process can reduce the viability of spermatozoa cells due to cold shock and intracellular changes due to the release of water associated with the formation of ice crystals. In addition, there are additional factors, namely lipid peroxidation and antifreeze factors in semen plasma, such as egg yolk coagulating enzyme, tri glycerol lipase, and anti-motility factors [14].

One of the attempts is to add essential oils to the andromed diluent—the essential oil used as a cement diluent from sweet orange [15]. “Sweet orange essential oil contains limonene and linalool, which are toxic to bacteria” [16]. In this study, a combination treatment of andromed and essential oils was carried out, expected to inhibit bacterial growth and reduce the risk of death of spermatozoa due to cold shock.

2. MATERIALS AND METHODS

This type of research is experimental research, in which the researcher conducts experiments with several treatments on samples obtained from goat semen, andromed diluent, and essential oils. The scope, focus of this research is on observing the viability of semen microscopically by observing it in the laboratory.

The research materials used were fresh goat semen, essential oil, andromed diluent, citric acid, fructose, 2% eosin, straw, liquid N2, aquabidestillata, warm water (45-55ºC), and tissue. The research tools used were a 400 ml beaker glass, deck glass, tissue, stir bar, a set of an artificial vagina, test tube, water bath, electric microscope, thermometer, pipette, object glass, cover glass, pH meter, Bunsen burner, denominator, and holding cage.

The observed parameter was viability using eosin staining. Live spermatozoa by a head that does not absorb the dye, while a redhead marks dead spermatozoa. The evaluation of a minimum of 200 spermatozoa using a light microscope with a magnification of 400 times. The formula to know the percentage of live spermatozoa:

> % viabilities = number of live spermatozoa / total spermatozoa counted X 100%

The research method describes the observed values using the non-factorial, Completely Randomized Design with five treatments and five replications. The population and samples in this study were goat semen, andromed extender, and various levels of essential oils with the following treatments:

> P_0 = Andromed + Sweet Orange Essential Oil 0%
> P_1 = Andromed + Sweet Orange Essential Oil 0,25%
> P_2 = Andromed + Sweet Orange Essential Oil 0,5%
> P_3 = Andromed + Sweet Orange Essential Oil 0,75%
> P_4 = Andromed + Sweet Orange Essential Oil 1%

3. RESULTS AND DISCUSSION

The results of the viability test of goat spermatozoa after semen equilibration showed that the lowest percentage values were without treatment, 0, 0.25, 0.5, and 0.75%, namely 80% viability, while the highest was with the addition of 1% sweet orange essential oil (P4), namely 81%. The data obtained shows that the addition of sweet orange essential oil increased the viability percentage of goat spermatozoa after equilibration. The higher the level of administration of sweet orange essential oil will further increase the percentage value of viability of spermatozoa. The analysis of variance showed that adding the combination of andromed with sweet orange peel essential oil as a diluent had no significant effect ($P<0.01$) on the viability of spermatozoa after equilibration.

The percentage of viability of spermatozoa in frozen semen of goats/sheep continued to decrease with the length of storage at room temperature. The longer the storage, the percentage value of spermatozoa viability decreases. The best results were by adding 1% sweet orange essential oil because it had the highest viability percentage value during storage at room temperature. However, even though the percentage of viability continued to increase, the analysis of variance showed no significant results, so, in terms of the percentage of viability, adding sweet orange essential oil to andromed goat semen diluent was not recommended.

“All treatments meet the requirements for Artificial Insemination. Standardization bodies determine that the quality of semen after the freezing process must show a minimum viability percentage of 40%” [17]. “Decreased quality of spermatozoa after being caused by spermatozoa experiencing cold shock” [18].

“Spermatozoa that do not move are not necessarily dead, so they do not absorb color, whereas, in the interpretation based on moving and not moving, they are considered immotile. Spermatozoa that are alive and moving, but have defects in their cell walls, able to absorb color are dead, while other interpretations are considered not dead” [19].

Table 1. The results of the study on the percentage of spermatozoa viability in goat semen after equilibration

Parameter	Treatment	After Equilibration (%)
Viabilities	0%	80±2.31
	0.25%	80±2.36
	0.5%	80±2.08
	0.75%	80±2.44
	1%	81±2.22

Note: Different superscripts in the column show no significant difference (P<0.01)

The decrease in the percentage of surviving spermatozoa was due to the continued metabolism of spermatozoa even though they were at 3-5 Celcius. The spermatozoa metabolism, which continues to run, disturbs the electrolyte balance of the spermatozoa, which can cause water or ice crystals to enter the spermatozoa and cause death of the spermatozoa. Spermatozoa metabolism causes an electrolyte imbalance of spermatozoa with solution [20].

4. CONCLUSION

The results showed that adding sweet orange essential oil to the andromed extender did not significantly increase the viability of goats spermatozoa. The andromed extender is suitable enough to be used as a diluent, so there is no need to add other ingredients to increase the goat's liquid semen.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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