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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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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.

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.

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Keywords: Andromed, Buck, Liquid semen, Sweet orange essential oil, Viability.

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1. INTRODUCTION

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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].

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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].

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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,

33 by giving milk [7] and raffinose” [8]. In addition, some studies are less effective as a diluent,
34 such as adding coconut water [9].

35 One of the factors that cause the low quality of liquid semen is the growth and
36 contamination of disturbing microorganisms such as bacteria in liquid semen [10]. “Bacterial
37 contamination found in the liquid semen of goats/sheep comes from the male reproductive
38 tract, the environment, handling during the semen dilution process, and diluents” [11].
39 Reducing the population of bacteria in semen can be done with the addition of antibacterial
40 [12].

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

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

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58 **2. MATERIAL AND METHODS**

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

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

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

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$$74 \text{ \% viabilities} = \frac{\text{number of live spermatozoa}}{\text{total spermatozoa counted}} \times 100\%$$

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

81 P₀ = Andromed + Sweet Orange Essential Oil 0%

82 P₁ = Andromed + Sweet Orange Essential Oil 0,25%

- 83 P₂ = Andromed + Sweet Orange Essential Oil 0,5%
 84 P₃ = Andromed + Sweet Orange Essential Oil 0,75%
 85 P₄ = Andromed + Sweet Orange Essential Oil 1%

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88 3. RESULTS AND DISCUSSION

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91 **Table 1.** The results of the study on the percentage of spermatozoa viability in goat semen
 92 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

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

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

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

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

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

120 The decrease in the percentage of surviving spermatozoa was due to the continued
 121 metabolism of spermatozoa even though they were at 3-5 Celcius. The spermatozoa
 122 metabolism, which continues to run, disturbs the electrolyte balance of the spermatozoa,

123 which can cause water or ice crystals to enter the spermatozoa and cause death of the
124 spermatozoa. Spermatozoa metabolism causes an electrolyte imbalance of spermatozoa
125 with solution [20].
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128 **4. CONCLUSION**

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

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144 Authors have declared that no competing interests exist.
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147 **AUTHORS' CONTRIBUTIONS**

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149 This work was carried out in collaboration between all authors. Sukma Aditya Sitepu
150 designed the study, performed the statistical analysis, wrote the protocol and wrote the first
151 draft of the manuscript. Julia Marisa managed the analyses of the study. Aditiya Amanda
152 Rianto managed the literature searches. All authors read and approved the final manuscript
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155 **REFERENCES**

156

- 157 1. Marisa, J., Sitepu, S. A., dan Rianto, A. A. 2022. ANALYSIS OF ACTORS AND
158 ACTIVITIES IN VALUE CHAIN BUSINESS SHEEP FAULTING. In *Proceeding*
159 *International Conference of Science Technology and Social Humanities* (Vol. 1, pp. 69-
160 76).
- 161 2. Sitepu, S. A., Marisa, J., dan Rianto, A. A. 2022. SPERMATOZOA MOTILITY IN BOER
162 BUCK LIQUID SEMEN WITH ADDITION OF SWEET ORANGE ESSENTIAL OIL AND
163 PENICILLIN. In *Proceeding International Conference Keputeraan Prof. H. Kadirun*
164 *Yahya* (Vol. 1, No. 1, pp. 98-103).
- 165 3. Inounu, I.B., Tresnamurti, Subandriyo and H. Martojo, 1999. Lamb production in Prolific
166 Sheep. *Journal of Animal and Veterinary Science* 4(3)
- 167 4. Noor, R.R., A. Djayanegara and L. Schuser, 2001. Selection to Improve Birth and
168 Weaning Weight of Javanese Fat Tailed Sheep. *Arch. Tier Dummerstorf* 44(6)
- 169 5. Wirartha, I. M. 2006. *Socio-economic research methodology*. Yogyakarta: CV Andi
170 Offset.

- 171 6. Hastono and Masbulan E. 2001. Performance of people's sheep reproduction in Garut
172 Regency, In: Proceedings. National Seminar on Animal Husbandry and Veterinary
173 Technology. Bogor, Indonesia, 17-18 September 2001. pp 100-105.
- 174 7. Toro-Mujica, P., García, A., Gómez-Castro, A. G., Acero, R., Perea, J., Rodríguez-
175 Estévez, V., and Vera, R. 2011. Technical efficiency and viability of organic dairy sheep
176 farming systems in a traditional area for sheep production in Spain. *Small Ruminant*
177 *Research*, 100(2-3)
- 178 8. Sitepu, S. A., and Marisa, J. 2019. Percentage value of membrane integrity and
179 acrosome integrity of spermatozoa in simmental liquid semen with addition of penicillin
180 and sweet orange essential oil. In IOP Conference Series: Earth and Environmental
181 Science (Vol. 327, No. 1, p. 012027). IOP Publishing.
- 182 9. Affandy, L., U. Umiasih, D. Pamungkas and D.E. Wahyono, 1997. Reproductive
183 Response of Fat Ekr Sheep To Differences in Feed Energy Levels. GratiPasuruan
184 Agricultural Technology Research and Assessment Installation. National Seminar on
185 Animal Husbandry and Veterinary
- 186 10. Sitepu, S. A., and Zaituni, U. 2018. Improved quality of frozen boer goat semen with the
187 addition of sweet orange essential oil on tris yolk and gentamicin extender. In IOP
188 Conference Series: Earth and Environmental Science (Vol. 122, No. 1, p. 012125). IOP
189 Publishing.
- 190 11. Pamungkas, D., LAffandy, D.B. Wijono and K Ma'sum, 1996. Characteristics of Fat-
191 tailed Sheep Farms in Rural Seed Centers in East Java. Pros. Scientific Meeting on
192 Animal Husbandry Research Results, Ciawi, Bogor.
- 193 12. Latief, A., Rahardja, D.P., Yusuf, M., 2004. Improving the Reproductive Efficiency of
194 Beef Cattle Through Accelerating the Emergence of Postpartum Festive. Livestock
195 Production Department. Hasanuddin University.
- 196 13. Marisa, J., and Sitepu, S.A. 2020. Relationship analysis between production factors and
197 business production of beef cattle livestock in West Binjai District, Indonesia. *Asian*
198 *Journal of Advanced Research and Reports*, 1-7.
- 199 14. Ashari, M., Suhardiani, R. A., and Andriati, R. 2018. Analysis of Reproductive Efficiency
200 of Fat-tailed Sheep in East Lombok District. *Indonesian Journal of Animal Husbandry*
201 *Science and Technology* Volume, 4(1), 207-213
- 202 15. Dixon, A. B., Knights, M., Winkler, J. L., Marsh, D. J., Pate, J. L., Wilson, M. E., and
203 Inskeep, E. K. 2007. Patterns of late embryonic and fetal mortality and association with
204 several factors in sheep. *Journal of Animal Science*, 85(5)
- 205 16. Slingsby, L. S., and Watterman-Pearson, A. E. 2002. Comparison between meloxicam
206 and carprofen for postoperative analgesia after feline ovariohysterectomy. *Journal of*
207 *Small Animal Practice*, 43(7), 286-289.
- 208 17. Hasan, F., Sitepu, S. A. P., and Alwiyah, A. 2017. The Influence of Parity Against a
209 Percentage of Estrus of Ekor Tipis Sheep that are Synchronized Estrus Using
210 Prostaglandin F2 α (PGF2 α). *Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan*, 5(1),
211 46-48.
- 212 18. Ritonga, M. Z., Putra, A., Sitepu, S. A., Suriadi, S., Bakti, Z. A., Kurniawan, A., and
213 Pinem, R. S. (2020). Detection And Prevalence Of Gastrointestinal Helminthes In
214 Samosir Goats On Samosir Island, North Sumatra, Indonesia. *The International Journal*
215 *of Tropical Veterinary and Biomedical Research*, 5(1).

- 216 19. Sitepu, S. A., and Udin, Z. 2020. Total Bacteria of Post-Thawing Boer Buck Semen with
217 Addition of Sweet Orange Essential Oil to Tris Yolk Extender. *Asian Journal of*
218 *Biochemistry, Genetics and Molecular Biology*, 5(3), 1-5.
- 219 20. Hafez, E. S. E., dan B. Hafez. 2005. *Reproduction in Farm Animal* 7 th ed. Lippincott
220 Williams and Walkins, South Carolina.