THE EFFECT OF ADDING SWEET ORANGE ESSENTIAL OIL IN ANDROMED EXTENDER ON GOAT SPERM MOTILITY

Sukma Aditya Sitepu[1]*, Julia Marisa[2], Aditiya Amanda Rianto[3]
[1] Animal Husbandry Department, Universitas Pembangunan Panca Budi – Indonesia
[2,3] Agrotechnology Department, Universitas Pembangunan Panca Budi – Indonesia

*Corresponding author: sukmaaditya@dosen.pancabudi.ac.id

ARTICLE INFO

Date received : 1 Nov 2022
Revision date : 14 Nov 2022
Date received : 23 Nov 2022

Keywords:
Essential Oils, Liquid Semen, Motility, Spermatozoa

ABSTRACT

This study aimed to determine the effect of andromed diluent added with various levels of sweet orange essential oil on buck spermatozoa. Observations were made microscopically, namely by observing the motility of buck spermatozoa. This study hypothesized that adding sweet orange essential oil to andromed diluent could increase and maintain the percentage value of spermatozoa motility in buck semen. The materials used in this study were buck fresh semen, andromed and sweet orange essential oil. The experimental design used in the study was a non-factorial, Completely Randomized Design (CRD) with five treatments and five replications. The treatment added 0%, 0.25%, 0.5%, 0.75%, and 1% sweet orange essential oil. The parameters observed were the motility evaluation of buck spermatozoa. The results showed the motility of spermatozoa with the addition of sweet orange essential oil P0 = 70%, P1 = 70%, P2 = 75%, P3 = 75%, and P4 = 75%. It concluded that the best result and recommended use in Buck Artificial Insemination was the addition of 1% essential oil (P4).

INTRODUCTION

Good quality buck is quite challenging to obtain and expensive. Besides, care and animal feed is expensive (Marisa et al., 2022). Artificial insemination in goats can be a solution because breeders do not need to raise a buck. The success rate of Artificial Insemination in goats is highly dependent on the quality of the semen and the implementation of Artificial Insemination (Sitepu & Marisa, 2020). Goat liquid semen requires proper handling and selection of liquid semen diluent in order to obtain good quality semen.

There are several extender for buck cement. Widya's research (2011) using egg yolk tris diluent plus other ingredients aimed at improving semen quality has been widely carried out, among others, by giving milk (Widya, 2011), raffinose (Gunawan & Kain, 2008). In addition, some studies are less effective as a diluent, such as adding coconut water (Anggreani, 2005). Over time, the diluent material grows. Currently, an andromed extender is more practical in its use.

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. Bacterial contamination found in goat's liquid semen comes from the male reproductive tract, the environment, handling during the semen dilution process, and diluents (Toelihere, 1993). Reduce the population of bacteria in the semen can be done with the addition of antibacterial.

The cold shock on spermatozoa causes a decrease in the quality of liquid semen because it can disturb and damage spermatozoa. This condition occurs during the dilution and freezing process in manufacturing frozen semen. This condition can interfere with the movement of spermatozoa which causes low motility. Bacteria contained in liquid semen can reduce motility because it inhibits movement, kills, and makes spermatozoa abnormal. Motility is the movement of spermatozoa to reach the egg, so this parameter is the most important in microscopic observation to determine the quality of liquid semen.

One of the attempts is to add essential oils to the andromed diluent. Essential oils that have been used as semen extenders are those derived from sweet oranges (Sitepu & Putra, 2017). The sweet orange essential oil contains limonene and linalool, which are toxic to bacteria (Fisher & Phillips, 2006). In this study, a combination treatment of andromed and essential oils was carried out, which was expected to inhibit bacterial growth and reduce the risk of damage due to cold shock by observing the percentage of motility, viability, and abnormalities of spermatozoa.

LITERATURE REVIEW

Characteristics of Buck Spermatozoa

Spermatozoa are the male gametes produced by the seminiferous tubules of the testis. The number of spermatozoa contained in a certain amount of semen will affect its appearance. Semen that is watery and clear contains a small number of spermatozoa. Whereas semen that is cloudy and thick under normal circumstances has a high concentration of spermatozoa (Salisbury & VanDemark, 1985).

The size of the spermatozoa head in goats varies between species but usually is 8 to 10 µm long, 4 µm wide, and 1 µm thick (Evan & Maxwell, 1987). Spermatozoa heads are generally oval and slightly flattened, and a nucleus contains chromosomes (Morel, 1999). The acrosome covers the front end of the head. Thin sac with a double membrane containing acrosin, hyaluronidase, and other hydrolytic enzymes that play a role in the penetration of the corona radiata and the zona pellucida in the fertilization process (Bearden & Fuquay, 1984). The equatorial part acts as a place that initiates the process of attaching and fusing the spermatozoa membrane with the oocyte membrane during the fertilization process (Morel, 1999). The tail of spermatozoa contains radially arranged axial filaments. This axial filament starts from the top centrioles and runs up to the tip of the tail (Bearden & Fuquay, 1984).

Essential oil

Sweet orange peel has a distinctive aromatic odor and a bitter taste, which contains: essential oil containing Limone, and the glucosides hesperidin, isohesperinda, aurantiamarina, and resin. The macroscopic description of the sweet orange peel generally includes spiral-shaped chips, and some are long in shape. The outer surface is slightly yellowish brown to orange-brown, + 3 mm thick, hard and brittle, the inner surface is flat, orange-brown, and there is a small quantity of spongy tissue. If this skin is damaged, the oil cavities with a diameter of about 1 mm will be visible (Kartasapoetra, 2001).

Fresh fruit peel contains about 0.8% essential oil with main components such as α-pinene (1.59%), β-pinene (7.29%), β-mirsene (4.59%), octanal (0.70 %), Limonene (82.06%), Osimene (0.14%), 7. 4-Thujanol (0.06%), 1-Octanol (0.13%), β-Linalool (1.61%) , α-limonene dipoxide (0.04%), 1,3,5-Tris (Methylene), cycloheptane (0.04), trans-p-2,8-Mentadien-1-ol (0.05), Citronellal (0.13), 4-Methyl-1-(1-Methylethyl)-3-cyclohexene-1-ol (0.17), α-Terpineol (0.30), trans-Piperitol (0.04), n -Decanal (o,18), B-Sitronello l(0,13), Karvona (0,05), Perillal (0,06), Nonanal (0,03), Elementa (0,14), α-Kariofilena (0.06) (Agusta, 2010). Many essential oil components contain various compounds of various types, coupled with their volatile nature at room temperature. The obstacle encountered when analyzing the components that make up essential oils is the loss of some components during the preparative process. Since the discovery of gas chromatography, obstacles in analyzing essential oil components have come in the ongoing analysis process. The evaporation effect can be avoided or even eliminated (Agusta, 2010).

Antibacterial Ingredients in Semen

Continuous use of antibiotics can cause resistance to certain types of bacteria. The emergence of bacteria resistant to one (anti micro bacterial resistance) or certain types of antibiotics (multiple drug resistance) dramatically complicates the treatment process. Bacterial resistance to antibiotics has several consequences. For example, infectious diseases caused by bacteria that fail to respond to treatment can result in prolonged illness (Utami, 2012).

The level of sensitivity of bacteria to a type of antibiotic determines through an antibiotic sensitivity test. In addition to knowing the sensitivity of bacteria to certain antibiotics, this test also aims to determine the potential antibiotics against microorganisms. Inhibition of the growth of microorganisms by antibiotics as a clear zone (zone of inhibition) around the growth of microorganisms. The extent of the inhibition zone indicates the level of microorganisms' resistance to antibiotics (Vandepitte et al., 2003).

Kohanski et al. (2010) stated that antibiotics could inhibit the growth or multiplication of bacteria (bacteriostatic) or kill bacteria in the infection process. Bacteria will respond sensitively or be resistant to the properties of these antibiotics. Mutschler (1991) explained the mechanism of action of antibiotics against bacteria through one of the following five pathways. They were disrupting bacterial cell metabolism, inhibiting

bacterial cell wall synthesis, damaging the integrity of the bacterial cell membrane, inhibiting bacterial cell protein synthesis, and inhibiting synthesis or damaging cell nucleic acids bacteria.

METHOD

The research parameter observed was the motility of spermatozoa before and after equilibration. Motility knows as the percentage of spermatozoa that move progressively forward. The evaluation knows by observing spermatozoa in eight fields of view with a light microscope with 400 times magnification. The population and sample in this study were Buck semen which had added andromed and various levels of sweet orange essential oil with the following treatments:

P0 = Andromed + Sweet Orange Essential Oil 0%
P1 = Andromed + Sweet Orange Essential Oil 0.25%
P2 = Andromed + Sweet Orange Essential Oil 0.5%
P3 = Andromed + Sweet Orange Essential Oil 0.75%
P4 = Andromed + Sweet Orange Essential Oil 1%

RESULTS AND DISCUSSION

Results of Buck's liquid semen before and after equilibration using the andromed extender and the addition of orange essential oil can be seen in Table 1.

Table 1. Spermatozoa motility before equilibration

Parameter	Treatment	Before Equilibration (%)
Motility	0%	70±0.00
	0,25%	70±0.00
	0,5%	75±0.00
	0,75%	75±0.00
	1%	75±0.00

Note: Different superscripts in the column show a significant difference (P<0.01)

The Buck spermatozoa motility test results before equilibration showed that the lowest percentage value was without treatment (P0), namely 70%. In contrast, the highest was adding 1% sweet orange essential oil (P4), namely 75%. The data obtained show that adding sweet orange essential oil increased the percentage value of Buck spermatozoa motility before freezing. The higher the administration level of sweet orange essential oil, the higher the percentage value of spermatozoa motility.

The variance analysis showed that adding sweet orange peel essential oil as an andromed diluent had a significant (P<0.01) effect on spermatozoa motility before equilibration. The results of the BNT follow-up test showed that the highest motility was in the P4 treatment, which was 75% before equilibration.

Table 2. Spermatozoa motility after equilibration

Parameter	Treatment	After Equilibration (%)
Motility	0%	70±0.00
	0,25%	70±0.00
	0,5%	75±0.00
	0,75%	75±0.00
	1%	75±0.00

Note: Different superscripts in the column show a significant difference (P<0.01)

The buck motility after semen equilibration showed that the lowest percentage value was without treatment (P0), namely 70%. In contrast, the highest was adding 1% sweet orange essential oil (P4), namely 75%. The data obtained show that adding sweet orange essential oil increased the percentage of motility after equilibration. The higher the administration level of sweet orange essential oil, the higher the percentage value of spermatozoa motility.

The analysis of variance showed that adding sweet orange peel essential oil to andromed diluent had a significant (P<0.01) effect on motility after equilibration. The results of the analysis follow-up test showed that the highest motility is P4 treatment, which was 75% after equilibration.

The percentage of spermatozoa motility in buck semen continued to decrease with the length of storage at room temperature. The longer the storage, the percentage value of spermatozoa motility

decreased. The best result was adding 1% sweet orange essential oil because it had the highest motility percentage value during storage.

CONCLUSION

The results showed that with the addition of 1% sweet orange essential oil to Buck's liquid semen, it was suitable for use in the artificial insemination of goats.

REFERENCES

Agusta, A. 2010. Minyak Atsiri Tumbuhan Tropika Indonesia. ITB, Bandung.

Anggraeny, Y.N., L. Afandhy dan Rasyid. 2005. Efektifitas Substitusi Pengencer Tris Sitrat dan Kolesterol Menggunakan Air Kelapa dan Kuning Telur Terhadap Kualitas Semen Beku Sapi Potong. Seminar Nasional Teknologi Peternakan dan Veteriner 2005.

Arifiantini, I., T. Wresdiyati., dan E. F. Retnani. 2006. Pengujian morfologi spermatozoa sapi Bali *(Bos sondaicus)* menggunaan "Williams". Jurnal Pengembangan Peternakan Tropis 31 : 105 – 110.

Bearden, H. J., and J. Fuquay. 1984. Applied Animal Reproduction, 2nd Edition, A Reston Book Prentice Hall Inc. Eagle Wood, New Jersey.

Blakely, J., dan D. H. Bade., 2008. Ilmu Peternakan. Gadjah Mada University Press, Yogyakarta.

Fisher, K., and C. A. Phillips. 2006. The effect of lemon, orange and bergamot essential oils and their components on the survival of Campylobacter jejuni, Escherichia coli O157, Listeria monocytogenes, Bacillus cereus and Staphylococcus aureus in vitro and in food systems. J Appl Microbiol. 2006 Dec;101(6):1232-40.

Gunawan. M dan E. M. Kaiin. 2008. Kualitas Sperma Sapi Beku Dalam Media Tris Kuning Telur Dengan Konsentrasi Raffinosa yang Berbeda. Seminar Nasional Teknologi Peternakan dan Veteriner 2008.

Hafez, E. S. E., dan B. Hafez. 2005. Reproduction in Farm Animal 7 th ed. Lippincott Williams and Walkins, South Carolina.

Kartasapoetra. 2001. Budidaya Tanaman Berkhasiat Obat. Rineka Cipta, Jakarta.

Leboeuf, B., E. Manfredi., P. Boue, A. Piacere., G. Brice., G. Baril., C. Broqua., P. Humblot., and M. Terqui.1998. Artificial insemination of dairy goats in France. Livestock Prod. Sci. 55: 193-203.

Marisa, J., Sitepu, S. A., and Kurniawan, R. 2022. ANALYSIS OF SHEEP FARMER SUPPLY CHAIN INTEGRATION LANGKAT REGENCY. In *Proceeding International Conference Keputeraan Prof. H. Kadirun Yahya* (Vol. 1, No. 1, pp. 104-112).

Marisa, J., Sitepu, S. A., dan Kurniawan, R. 2022. *Budaya Organisasi dan Integrasi Rantai Pasok Ternak Domba*. Tahta Media Group.

Pane, P., 2008. Pemuliaan Ternak Sapi. PT. Gramedia Pustaka, Jakarta.

Paulenz, H., L. So¨derquist., T. A Dnøy., K. Soltun., P. A. Sæther., K. R. Fjellsøy, and K. A. Berg. 2005. Effect of cervical and vaginal insemination with liquid semen stored at room temperature on fertility of goats. Anim. Rep. Sci. 86 (2005) 109–117.

Purdy, P. H. 2010. A Review on Goat Sperm Cryopreservation. Small Ruminat Research.

Rizal, M., dan Herdis. 2008. Inseminasi Buatan Pada Domba. Jakarta. Rineka Cipta, Jakarta.

Salamon, S., dan W. M. C. Maxwell. 2000. Storage of Ram Semen. Anim Reprod Sci 62:77-111.

Salmah. N. 2014. Motilitas, Persentase Hidup dan Abnormalitas Spermatozoa Semen Beku Sapi Bali Pada Pengencer Andromed dan Tris Kuning Telur. Fakultas Peternakan Universitas Hasanudin. Makassar.

Sitepu, S. A., dan Putra, A. 2017. Pengaruh penambahan minyak atsiri kulit Jeruk Manis pada pengencer tris kuning telur terhadap kualitas semen post-thawing Sapi Simmental. *Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science)*, *19*(3), 149-155.

Sitepu, S. A., dan Julia Marisa, S. P. 2020. *Manajemen Usaha Ternak Perah Kambing Peranakan Etawa*. Mitra Cendekia Media.

Toelihere, M. R. 1993. Fisiologi Reproduksi pada Ternak. IPB Press, Bogor.

Williamson G. dan W. J. A. Payne. 1993. Pengantar Peternakan di Daerah Tropis. Universitas Gadjah Mada Press, Yogyakarta.

Widjaya. N. 2011. Pengaruh Pemberian Susu Skim dengan Pengencer Tris Kuning Telur Terhadap Daya Tahan Hidup Spermatozoa Sapi pada Suhu Penyimpanan 5^0C. Sains Peternakan Vol. 9 (2), September 2011: 72-76.

Yuwanta, T. 2010. Telur dan Kualitas Telur. Gadjah Mada University Press. Yogyakarta.