

COMBINED EFFECT OF STREPTOMYCIN AND SWEET ORANGE ESSENTIAL OIL TO MEMBRANE AND ACROSOME INTEGRITY BOER GOAT FROZEN SEMEN

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Abstract

One of factors that cause a bad quality of Boer Goat frozen semen is the growth of bacterial. This can be overcome by adding antibiotics such as streptomycin. To further suppress the growth of bacteria can be added other ingredients that contain antibacterials such as sweet orange essential oil. The purpose of this research is to know the percentage value of Membrane Integrity and Acrosome Integrity on Boer Goat frozen semen with addition sweet orange essential oil and streptomycin. The method used was experimental using Completely Randomized Design with 5 treatments and 5 replications. The treatment in this research is addition 0%, 0,25%; 0.5%; 0.75% and 1% sweet orange essential oil on tris yolk and streptomycin extender. The results showed the best treatment addition combination streptomycin and sweet orange essential oil to percentage Membrane Integrity and Acrosome Integrity is increase 1% sweet orange essential oil.

Keywords: Boer Goat, essential oil, frozen semen, streptomycin, sweet orange.

1. INTRODUCTION

Goat meat demand is high enough to meet the domestic needs and for export. Therefore it is necessary to consider how to improve the population and genetic quality of local goats. Local goats have low body weight so that the meat produced is also small so it is not enough to meet domestic needs and does not meet export requirements abroad. The weight of local goat bodies should be increased so that domestic meat needs can be met and can be exported.

One way to improve the local goat's body weight is to do a cross breeding using superior goat, as Boer Goat. The Boer Goat is one of the superior goats that can be used to improve the genetic quality of local goats. Boer Goats have several advantages of body weight and high litter size. Local goat Indonesia although it has a low body weight but has the advantage as able to adapt to the extreme environment so that the results of the cross is expected to produce good quality goats. The obstacle is very difficult to get a good quality Boer Goat. In addition, maintenance and feed costs to be provided are also very expensive.

Artificial Insemination is the best solution for being able to cross a local goat with a Boer Goat. However, many farmers have not understood about Artificial Insemination in goats (Marisa and Sitepu, 2018). By doing Artificial Insemination breeders do not need to maintain a Boer Goat that is expensive. One Boer Goat can produce sperm that is used for many local doe goat in a one ejaculation. Another advantage of Artificial Insemination is to minimize the danger of disease transmission and good semen quality because it comes from a superior goat.

Goat farm in Indonesia spread to many areas difficult to reach because its distance from downtown and bad road conditions. Therefore, the use of Boer Goat Liquid semen in Artificial Insemination can not be done. Boer liquid cement is not durable, so it can not be used to carry out artificial Insemination in distant places. Liquid cement can be processed into frozen semen in order to be stored for a long time and can be sent to distant areas. Frozen semen that is stored and frozen in containers can still be used for decades.

The success rate of Artificial Insemination in goats is influenced by the quality of frozen semen. One of the causes of the low quality of Boer Goat frozen semen is the development of bacteria. To inhibit bacterial growth in frozen semen can be done by adding antibiotics. The antibiotic that is often used in the manufacture of frozen semen is streptomycin. Provision of streptomycin in diluent semen has been done. But the results obtained are still not good so need to be done another way to suppress the growth of bacteria. The effort that can be done is to combine antibiotics in the diluent. Combination of antibiotics on cement diluents has been done by combining with several other antibiotics such

as GTLS (Gentamicin, Tylosin and Linco-Spectin) Hasan et al., (2000) and Andromed® (gentamicin sulfate, spectinomycin, and linkomycin) (Minitub , 2001).The combination of streptomycin antibiotics has been done by adding penicillin (Rizal and Herdis, 2008).

Another way is to do a combination of antibiotics with an antibacterial-containing materials, such as sweet orange essential oil (Sitepu, 2018). Sweet orange essential oil containing limonene and linalool are toxic to bacteria (Fisher and Phillips, 2006).The use of sweet orange essential oil as antibacterial in Boer Goat frozen semen has been done and proved able to maintain the quality of Boer Goat semen (Sitepu, 2018).The combination of streptomycin and essential oils of sweet orange peel is expected to inhibit bacterial growth and improve the quality of frozen semen of Boer Goat.

Microscopic analysis is important to test the quality of the Boer goat's frozen semen. One of the tests carried out was the Membrane Integrity and Acrosome Integrity Membrane Integrity testing is essential and fundamental in the fertilization process (Lodhi et al., 2008). Some physiological processes during fertilization (cotitation, acrosome reactions, spermatozoa pooling and ovum) require active membranes and are unlikely to be fertilized with inactive membrane conditions. The integrity function of the spermatozoa plasma membrane is an important factor in the metabolism of spermatozoa, capacitance, acrosome reactions, and the binding of spermatozoa to the surface of the egg cell (Baqir et al., 2009).

Acrosome Integrity are the most important part of spermatozoa and play a role in the fertilization process. There are enzymes in the Acrosome Integrity (hyaluronidase, acrosine, CPE (Corona Penetrating Enzyme) which are needed to penetrate the culumus oophorus and the pellucida zone. Therefore this part of the Acrosome Integrity is an important part to be considered in semen evaluation (Tambing, 1999).

2. MATERIALS AND METHOD

2.1 Materials

Materials Research Used Boer goat fresh semen, sweet orange essential oil, tris yolk extender, streptomycin, formolsaline for observations acrosome integrity, akuabidestilata, Hypo osmotic Swelling Test (HOST) for observation membrane integrity, liquid N2 to freeze semen review, straw packing for a review of the semen and wipes for cleaning equipment.

2.2 Method

This study used a completely randomized design with 5 treatments and 5 replications. The treatments are:

P₀ = Streptomycin + essential oil 0%

P₁ = Streptomycin + essential oil 0.25%

P₂ = Streptomycin + essential oil 0.5%

P₃ = Streptomycin + essential oil 0.75%

P₄ = Streptomycin + essential oil 1%

Evaluation of microscopic sperm parameters

Membrane integrity: Evaluation of the integrity of the plasma membrane of the spermatozoa. The evaluation was done using methods hypoosmotic swelling test (HOST). Test done by mixing 0.1 ml to 9.9 ml cement hypoosmotic medium. Once mixed, the preparations are incubated in a water bath temperature of 37 ° C for 30 minutes (Rodriquezgil et al., 1994).

Acrosome integrity: Evaluation of the integrity of the sperm acrosome hood characterized by head spermatozoa black thick cement when exposed in physiological saline solution containing 1% formalin (Saacke and White, 1972). The evaluation was done at a minimum of 200 spermatozoa by using a light microscope magnification of 400 times.

3. RESULTS AND DISCUSSION

Percentage Membrane integrity

Membrane integrity is an absolute requirement for successful fertilization. If the plasma membrane is damaged, the metabolic process will be disrupted, ATP synthesis does not run normally and can be fatal to sperm, resulting in decreased motility and survival of the Boer Goat spermatozoa.



Table 1. Percentage Membrane integrity of Boer Goat semen before and after freezing

Parameters	Treatments	Observation	
		Before Freezing	After Freezing
Membrane Integrity	P ₀	78 _± 2.24	64 _± 2.74
	P ₁	80 _± 2.24	66 _± 2.24
	P ₂	82 _± 0.00	67 _± 2.74
	P ₃	84 _± 2.74	68 _± 2.24
	P ₄	86 _± 2.74	70 _± 2.24

Explanation: *Different superscripts in the columns show very significant differences (P < 0.01).*

The results of the analysis variety Membrane Integrity showed the effect of essential oils on tris yolk and streptomycin extender had a very real effect ($P < 0.01$) on observations before and after freezing. The highest percentage value of Boer Goat Membrane Integrity before freezing at P₄ treatment was 86% and after freezing at P₄ treatment was 70%. The results showed a decrease in the quality of spermatozoa from the process of cooling to freezing and thawing. The decrease in spermatozoa Membrane Integrity before freezing has decreased due to egg-yolk coagulating enzyme factor in toxin semen plasma and cold shock. This decrease in Membrane Integrity is also due to a decrease in the energy supply of spermatozoa used to sustain life and support sperm motion. Decreased Membrane Integrity of spermatozoa is also due to the treatment that causes damage and death of spermatozoa. During the thawing process spermatozoa are very susceptible to cell damage due to sudden changes in osmotic pressure caused by rapid melting. Only spermatozoa have strong plasma membrane power capabilities that can survive (Maxwell and Watson, 1996).

Spermatozoa with a high percentage of live shows a high percentage of intact plasma membranes as well. Tambing et al (1999) pointed out that spermatozoa with a high percentage of life indicate that the plasma membrane is still intact physically, so that spermatozoa cell organelles will be protected, ions for metabolic processes and nutritional needs are available. High concentrations of lactose lead to changes in osmotic pressure in the diluent to the hypertonic direction indicating molecules or particles outside the cell more than in the cell so that discharge of water from within the cell to dilute molecules outside the cells that cause cells will shrink (Supriatna and Pasaribu, 1992).

Percentage Acrosome Integrity

Acrosome Integrity is an important part of spermatozoa and a role in the fertilization process. There are enzymes in the acrosome integrity (hyaluronidase, acrosine, CPE (Corona Penetrating Enzyme) which are needed to penetrate the culumus oophorus and the pellucida zone. Therefore this part of the acrosome integrity is an important part to be considered in semen evaluation (Tambing, 1999).

Table 2. Percentage Acrosome Integrity Boer Goat before and after freezing.

Parameters	Treatments	Observation	
		Before Freezing	After Freezing
Acrosome Integrity	P ₀	74 _± 2.74	58 _± 2.24
	P ₁	76 _± 0.00	60 _± 2.74
	P ₂	78 _± 2.24	62 _± 2.74
	P ₃	79 _± 2.74	63 _± 2.74
	P ₄	82 _± 0.00	65 _± 2.24

Explanation: *Different superscripts in the columns show very significant differences (P < 0.01)*

The result of variance analysis showed that the effect of essential oil on the dilution material was very significant ($P < 0.01$) to the percentage of Acrosome Integrity after dilution and equilibration. The highest percentage of Acrosome Integrity on P₄ treatment before freezing was 82% and 65% after freezing. The results showed that the percentage of the Acrosome Integrity will be higher if there is increase of sweet orange essential oil level in the Boer Goat semen diluent. The Damage of Acrosome Integrity usually accompanied by damage to Acrosome Integrity so that the observation is very important because destruction of acrosomes can result in the loss of proteolytic enzyme enzymes and most occur at the time of re-melting which causes the failure of Artificial Insemination (Valcarcel et al., 1997).

Physiologically there is a relationship between motility, Membrane Integrity, Acrosome Integrity and spermatozoa survival. Membrane Integrity damage will cause the loss of necessary enzymes (Salisbury and Van DeMark, 1985). Another factor that determines the quality of spermatozoa when thawing at optimum temperature is intracellular glycerol diffusion occurs faster and prevention of osmotic shock events. According to Evans and Maxwell (1987)

thawing on frozen goat semen was done at 37°C and not over the critical time limit. Thawing of frozen semen are done properly will maintain osmotic balance and improve the configuration of the cell membrane lipid protein spermatozoa during the freezing process (Farstad, 1996).

4. CONCLUSIONS

The best use of a combination sweet orange essential oils and streptomycin is the addition of 1% sweet orange essential oil which can increase the percentage of the Membrane Integrity and Acrosome Integrity on Boer Goat frozen semen. Further research must be done using a combination of other antibiotics as well as fertility test through artificial insemination..

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