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## Improved quality of frozen boer goat semen with the addition of sweet orange essential oil on tris yolk and gentamicin extender

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**Abstract**. This research aimed to determine the extent of frozen semen quality Boer Goat by essential oils of sweet orange peel in tris yolk and gentamicin extender. Research has been conducted at the Laboratory Loka Penelitian Kambing Potong Sei Putih, Deli Serdang, North Sumatra in February 2017. This study used a completely randomized design with 4 treatments and 5 replications. Treatments are 0.25; 0.5; 0.75 and 1% essential oils as additional diluent. The parameters were measured percentage Motility, membrane integrity, acrosome integrity and viability Boer Goat frozen semen. The results showed that the addition of essential oils as diluent semen was significant (P <0.01) in the percentage motility, Viability, membrane integrity and acrosome integrity Boer Goat frozen semen. Motility, membrane integrity, acrosome integrity and viability was significantly higher in all treated groups than the control group. The best results of all treatments In the study was the addition of essential oil as much as 1%.

#### 1. Introduction

One effort to meet the needs of meat is to develop a broiler goat farm. The current constraints are the low population and genetic quality of local goats. The growth of local goat meat is still very low, but it has the advantage of easily adapt to the environment and the food was of low quality. It is necessary to consider ways to improve the population and genetic quality of local goats.

Efforts that can be made to increase the population and quality of livestock is to make artificial Insemination by using sperm from superior goats. One of the superior goats that can be used is the Boer Goat. Boer Goats have several advantages such as high body weight, high carcass, litter size 1.7 and rapid growth. It is expected that artificial insemination will increase the population and genetic quality of goats in Indonesia.

Currently the success of the artificial insemination program is still low. This is due to the low quality of frozen semen used, the low inseminator skills and the location of goat farms in rural communities is difficult to reach. The low quality of frozen semen is caused by damage to spermatozoa caused by improper handling of clotting processes. The most critical part of the process of freezing semen is when freezing and thawing. Besides the low quality of frozen semen is also caused by the growth of bacteria that can harm and kill spermatozoa.

The bacteria contamination in Boer goat cement is caused by many factors ranging from the holding process to the freezing. Sperm begins contaminated with bacteria ranging from male

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reproductive tract, cement holding process, dilution process, cooling and freezing. Bacterial contamination can also occur through the air, the materials and equipment used in the manufacture of frozen semen. By adding the antibacterial ingredient in frozen semen diluents boer goat expected to minimize the growth of bacteria.

Generally, to inhibit the growth of bacteria in frozen semen is to add antibiotics, such as gentamicin. Gentamicin can work on gram-negative bacteria. Gentamicin has been used on a commercial diluent is Andromed® [1]. In addition to antibiotics, sweet orange essential oil can also be used as an antibacterial on poer goat frozen semen [2]. Essential oil of sweet orange peel contains the main components include limonene and linalool [3] that are toxic to bacteria [4] Research by the addition of essential oils of sweet orange peel in tris yolk and gentamicin extender on frozen semen boer goat's important to get quality frozen semen Boers better to minimize the growth of bacteria, able to maintain the quality and durability of spermatozoa.

#### 2. Materials and methods

#### 2.1. Place and Time Research

Research has been conducted at the Laboratory Loka Penelitian Kambing Potong Sei Putih, Deli Serdang, North Sumatra in February 2017.

#### 2.2. Materials



Materials Research Used Boer goat bucks (approximately 3–4 years old) were used in the study, sweet orange essential oil, tris yolk and gentamicin as an ingredient diluent, citric acid, fructose, eosin 2% Media as observations of viability, 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 cement and wipes for cleaning equipment.

#### 2.3. Methods

This study used a completely randomized design with 4 treatments and 5 replications. The treatments are:

P<sub>0</sub> = Tris Yolk + Gentamicin + Sweet Orange Oil 0.%

P<sub>1</sub> = Tris Yolk + Gentamicin + Sweet Orange Oil 0.25%

P<sub>2</sub> = Tris Yolk + Gentamicin + Sweet Orange Oil 0.5%

P<sub>3</sub> = Tris Yolk + Gentamicin + Sweet Orange Oil 0.75%

P<sub>4</sub> = Tris Yolk + Gentamicin + Sweet Orange Oil 1%

#### 2.4. Evaluation of microscopic sperm parameters

The observations made are:

Motility: the percentage of spermatozoa moving progressively forward. Evaluation is done by observing spermatozoa in eight different visual field with a light microscope magnification of 400 times [5].

Viability: evaluated using the eosin staining [5]. Live spermatozoa characterized by a head that do not absorb the dye, while the death was marked by a red head. The evaluation was done at a minimum of 200 spermatozoa were observed using a light microscope magnification of 400 times.

Membrane integrity: Evaluation of the integrity of the plasma membrane of the spermatozoa. The evaluation was done using methods hypoosmotic swelling test (HOST). Tests 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 [6].

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 [7]. The evaluation was done at a minimum of 200 spermatozoa by using a light microscope magnification of 400 times.

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#### 3. Results and discussion

Evaluation of the quality of frozen semen Boers done before freezing and after freezing. Observations included the percentage of motility. Viability. Membrane Integrity and Acrosome Integrity. The results of the study can be seen in Table 1.

**Table 1**. Summary results of research quality frozen semen Boers.

| Parameters         | Treatments | Observation     |                |
|--------------------|------------|-----------------|----------------|
|                    |            | Before Freezing | After Freezing |
| Motility           | 0%         | 71              | 47             |
|                    | 0.25%      | 73              | 49             |
|                    | 0.5%       | 75              | 51             |
|                    | 0.75%      | 76              | 51             |
|                    | 1%         | 77              | 53             |
| Viability          | 0%         | 79              | 67             |
|                    | 0.25%      | 80              | 71             |
|                    | 0.5%       | 83              | 73             |
|                    | 0.75%      | 85              | 74             |
|                    | 1%         | 86              | 75             |
| Membrane Integrity | 0%         | 80              | 65             |
|                    | 0.25%      | 82              | 67             |
|                    | 0.5%       | 84              | 67             |
|                    | 0.75%      | 86              | 69             |
|                    | 1%         | 87              | 69             |
| Acrosome Integrity | 0%         | 75              | 59             |
|                    | 0.25%      | 78              | 61             |
|                    | 0.5%       | 80              | 63             |
|                    | 0.75%      | 81              | 64             |
|                    | 1%         | 83              | 66             |

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

Based on the results of the research, it is known that it is raising Boer goat frozen semen quality with the addition of sweet orange essential oil in tris diluents gentamicin yolk extender. The result of variance analysis showed that the influence of essential oil as diluent had a very real effect (P <0.01) on sperm motility before both freezing and after freezing. Further BNT test results show that the highest motility, viability, membrane integrity and acrosome integrity are found in the addition of 1% essential oil and the lowest without the addition of essential oil.

Motility is one determinant of the success of the sperm to reach the ovum in the fallopian tube channel and is a measure that is used as the ability of sperm to fertilize an egg. The entire treatment has the requirement to be stored and IB as stated by Evans and Maxwell [8]. a condition worthy of frozen semen motility IB have not less than 40% and have the appropriate national standards of Indonesia [9]. The higher the concentration of essential oil of sweet orange peel, the higher the percentage of sperm motility before and after freezing. Boer Goat Semen is very susceptible to damage during the freezing process for the formation of ice crystals that lead to the death of spermatozoa. During the freezing process ice crystals formed cement will cause the electrolyte concentration increases in the cell that would dissolve the cell wall sheathing lipoprotein spermatozoa

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and the thawing time will change that the sperm plasma membrane permeability die [5]. Besides reduction in sperm motility was also caused by the treatment that causes damage and death of spermatozoa.

Spermatozoa are very susceptible to cell damage due to osmotic pressure changes suddenly due to the rapid melting during the thawing process. Only spermatozoa that have the power of the plasma membrane of the strong can survive. The decrease is also due to the reduced motility of energy supplies spermatozoa used to sustain life and support the movement of spermatozoa [10].

The big difference in sperm motility in each treatment may be due to the high level of use of essential oils of sweet orange in the diluent resulting in lipid peroxidation. According to [11] that hydroxynonenal is one of the lipid peroxidation can inhibit glycolysis and motility. In addition to the damage caused by lipid peroxidation, decreased motility can also occur as a result of several factors such as changes in medium pH. osmotic pressure and the effects of electrolytes and non electrolytes [5].

The decline in the average percentage of each evaluation time is a reasonable decline. According Toelihere [5] on the freezing cement will occur spermatozoa mortality up to 30% of the amount of fresh spermatozoa. Beconi *et al.* [12] states that the addition of cement with good quality antioxidants will maintain the vitality of frozen sperm. but not so in low-quality sperm because peroxidation process can not be treated with antioxidants. In the sweet orange essential oil also contains antioxidants because there are flavonoid compounds therein.

Cold shock is one of the main factors for the cryopreservation of sperm cells which can reduce cell viability. The level of cell sensitivity to cold shock is affected by the cooling rate and temperature interval [13]. Cold shock due to decreased body temperature to below 0 ° C. which would reduce cell viability. The phenomenon of cold shock on cells is not known clearly, but probably related to the phase transition of the membrane lipids that cause phase separation and inheritance - selective permeability of biological membranes of living cells [13].

The results showed a decrease in the quality of spermatozoa from cooling to freezing and thawing. Decrease in sperm motility before freezing decline caused by factors egg-yolk coagulating enzyme in seminal plasma of goats that are toxin and cold shock. The decrease is also due to the reduced motility of energy supplies spermatozoa ged to sustain life and support the movement of spermatozoa. Decrease in sperm motility was also caused by the treatment that causes damage and death of spermatozoa. During the thawing process spermatozoa susceptible to cell damage due to osmotic pressure changes suddenly due to rapid melting. Only spermatozoa that have a strong ability of the plasma membrane that can withstand [10].

The percentage of spermatozoa with high life shows the percentage of intact plasma membranes are also high. Described Tambing [14] that the percentage of live spermatozoa with high indicates that the plasma membrane is still physically intact, so that the sperm cell organelles will be protected, the ions to the metabolic processes and the needs of food substances available. The concentration of lactose must cause a change in osmotic pressure on the diluent in the direction of hypertonic signaling molecules or particles outside cells more than in cells resulting in the expenditure of water from inside the cell to dilute molecules outside the cell that causes the cell will shrink [15].

Intact Membranes Plasma breakdown is usually accompanied by damage to the hood so that acrosome acrosome Whole Hood observation is important because damage can result in loss of the acrosome enzymes proteolytic enzymes and occur mostly during melting back that led to the failure of Artificial Insemination [16].

Another factor that determines the quality of spermatozoa when done thawing at the optimal temperature is glycerol intracellular diffusion occurs more rapidly and prevent the incidence of osmotic shock. According to Evans and Maxwell [8] thawing the frozen semen goat conducted at a temperature of 370C and not through a critical time limit. Thawing frozen semen are done properly will maintain osmotic balance and improve the configuration of the cell membrane lipid protein spermatozoa during the freezing process [17].

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#### 4. Conclusion

The addition of essential oils of sweet orange peel in tris diluents and gentamicin yolk extender on frozen semen boer goat's can improve quality frozen semen Boers better to minimize the growth of bacteria, able to maintain the quality and durability of Boers spermatozoa.

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