PERCENTAGE VALUE OF MOTILITY AND VIABILITY SPERMATOZOA IN BOER GOAT FROZEN SEMEN WERE ADDED STREPTOMICIN AND SWEET ORANGE ESSENTIAL OIL

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Abstract

The purpose of this study was to determine the percentage value of motility and viability of spermatozoa in frozen semen Boer Goat by adding a combination of streptomycin with sweet orange essential oil to tris yolk extender. The material that will be used in this study are Boer Goat fresh semen, tris yolk extender, streptomycin and sweet orange essential oil. Tris yolk extender was prepared with Tris (hydroxymethylaminomethan) (3.32g), citric acid (1.86g), fructose (1.37g), glycerol (6ml), egg yolk (20ml), aquabides (100ml). The experimental design used in the study was a non factorial complete randomized design with 5 treatments and 5 replications. The treatment given is the addition of sweet orange essential oil 0%, 0.25%, 0.5%, 0.75% and 1%. The results showed that the addition of a combination of streptomycin and sweet orange essential oil to the extender had a very significant effect (P<0.01) on the motility and viability of Boer Goat spermatozoa before and after freezing. The best average value of motility and viability spermatozoa in the addition of essential oils is 1% (P4). Before freezing by 75% motility and viability 82% and after freezing by 50% motility and viability 67%.

Introduction:

Boer Goat is one of the superior goats whose sperm can be used for the Artificial Insemination program. The success of Artificial Insemination program in goats depends on the quality of frozen semen. One of the causes of the low survival of spermatozoa during frozen semen storage is caused by the development of bacteria. Generally, the addition of antibiotics in material extender semen frozen done to minimize the growth of bacteria. Provision of streptomycin in a semen extender is commonly done. But its use is still considered unfavorable so many attempts to suppress bacterial growth. One effort is to combination streptomycin with sweet orange essential oil which contains limonene and linalool are toxic to the bacteria. If bacterial growth can be suppressed, the quality of frozen semen automatically such as the percentage value of motility, viability and abnormality will increase.

Material and Methods:

The evaluation was done at a minimum of 200 spermatozoa by using a light microscope magnification of 400 times.
The research material is Boer Goat semen which has been added tris yolk extender, streptomycin and various levels of sweet orange essential oil with the treatment given are:

- \( P_0 \) = Streptomycin + Sweet Orange Essential Oil 0%
- \( P_1 \) = Streptomycin + Sweet Orange Essential Oil 0.25%
- \( P_2 \) = Streptomycin + Sweet Orange Essential Oil 0.5%
- \( P_3 \) = Streptomycin + Sweet Orange Essential Oil 0.75%
- \( P_4 \) = Streptomycin + Sweet Orange Essential Oil 1%

The research method is carried out experimentally with a quantitative or objective approach. Experimental research carried out by making some treatments use a variety of sweet orange essential oil levels and compared them without giving sweet orange essential oil (control). Activities in experimental research aim to assess the effect of administration of sweet orange essential oil or test whether there is an influence on the administration when compared to without administration of sweet orange essential oil. The experimental design used in the study was a non factorial complete randomized design with 5 treatments and 5 replications. The parameters observed were the evaluation of semen before freezing and after freezing, namely:

### Spermatozoa Motility:
Percentage of spermatozoa that move progressively forward. Evaluation is done by observing spermatozoa at eight different fields of view with a 400 times magnification light microscope.

### Viability:
Evaluated using eosin staining. Living spermatozoa are marked by a head that does not absorb dyes, while dead ones are marked by a red head. Evaluation is carried out on a minimum of 200 spermatozoa observed using a 400 times magnification light microscope. The percentage of live spermatozoa is calculated according to the formula:

\[
\% \text{ viability} = \frac{\text{the number of spermatozoa that do not absorb color}}{\text{total spermatozoa calculated}} \times 100\%
\]

### Results and Discussion:
The results of motility, viability and spermatozoa abnormalities test in frozen semen of Boer Goat after equilibration and freezing can be seen in Table 1.

#### Table 1: Recapitulation of motility, viability and abnormality of Boer Goat semen after equilibration and freezing.

| Parameter | Treatment | Observation  |  |
|-----------|-----------|--------------|---|
|           |           | After Equilibration | After Freezing |
| Motility  | \( P_0 \) | 68±2.74       | 40±2.24       |
|           | \( P_1 \) | 70±0.00       | 43±2.24       |
|           | \( P_2 \) | 71±2.24       | 45±2.74       |
|           | \( P_3 \) | 73±2.74       | 48±2.24       |
|           | \( P_4 \) | 75±0.00       | 50±2.74       |
| Viability | \( P_0 \) | 75±1.68       | 58±0.76       |
|           | \( P_1 \) | 77±2.50       | 60±2.56       |
|           | \( P_2 \) | 79±0.87       | 63±2.42       |
|           | \( P_3 \) | 80±2.72       | 65±2.56       |
|           | \( P_4 \) | 82±2.50       | 67±2.78       |

Ket: Different superscripts in the column show very significant differences (\( P <0.01 \))

### Spermatozoa Motility:
The results of the analysis of variance showed that the effect of adding a combination of streptomycin with sweet orange peel essential oil as a diluent had a very significant effect (\( P <0.01 \)) on the motility of spermatozoa both after equilibration and freezing. Further test results showed that the highest motility was found in the \( P_4 \) treatment, namely 75% after equilibration and 50% after freezing. These results indicate an increase in the level of individual motility after equilibration and after freezing. This value is included in good motility standards because according to Evans and Maxwell (1987) the proper semen requirements for IB programs have motility above 40%. During the cement freezing process Ice crystals that are formed will cause electrolyte concentrations to increase in cells which will dissolve the lipoprotein envelope of spermatozoa cell walls and at thawing time will change the permeability of the plasma membrane so that spermatozoa will die (Toelihere, 1993).
The results showed a decrease in the quality of spermatozoa during freezing and thawing (thawing). Spermatozoa motility after the cooling process has decreased, this decrease is caused by egg-yolk coagulating enzyme factors in goat semen plasma which are toxic, as well as due to cold shock (Widjaya, 2011). Decreased spermatozoa motility is also caused by treatments that cause damage and death of spermatozoa. During the thawing process spermatozoa are susceptible to cell damage due to sudden changes in osmotic pressure caused by rapid thawing. Only spermatozoa have the ability of a strong plasma membrane to survive (Maxwell and Watson, 1996).

**Spermatozoa Viability**

Observations viability needs to be done because the sperm that do not move are not necessarily dead. Partodihardjo (1982) states that immovable spermatozoa are not necessarily dead so they do not absorb color, whereas in interpretation on the basis of moving and not moving are considered immotile. In spermatozoa that live and move but there are defects in the cell wall, can absorb colors considered dead, while other interpretations are considered not dead.

The results of the various analyzes showed that the effect of adding a combination of gentamicin with sweet orange peel essential oil as a extender had a very significant effect ($P <0.01$) on the viability of spermatozoa both after equilibration and freezing. Further test results showed that the best viability was found in the P4 treatment, namely 82% after equilibration and 67% after freezing.

This difference in average viability can be caused by physical influences at the time of treatment so that it can cause death. Friction between spermatozoa can cause abnormalities as well as death. The decrease in spermatozoa viability after the cooling and freezing process can be caused by physical influences during the treatment which causes death. The physical effect is caused by friction between spermatozoa, between spermatozoa and tube walls, or between fat globules from egg yolks, causing a tendency to decrease viability along with different dilution rates.

The quality of goat semen after the freezing process must show a live spermatozoa (viability) of at least 40% (Sitepu et al, 2018). Decreased quality of spermatozoa after the cooling and freezing process is caused by spermatozoa experiencing cold shock (Toelihere, 1993). During freezing and storage of membrane imbalance occurs, which can reduce the resistance of spermatozoa so that after thawing the quality of the semen becomes low (Maxwell and Watson, 1996).

**Conclusion:**

The best of use a combination sweet orange essential oil and streptomycin in the semen extender is the addition 1% essential oil, which can increase the percentage value of motility and viability spermatozoa in Boer Goat frozen semen.

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