Percentage Value of Membrane Integrity and Acrosome Integrity Spermatozoa in Simmental Liquid Semen with Addition Penicillin and Sweet Orange Essential Oil

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Abstract. Growth of bacteria in the Simmental liquid semen is is the cause of the quality of liquid cement to be bad. The addition of antibiotics has been done to inhibit the growth of bacteria in the liquid semen. One of antibiotic that often used is penicillin. The addition of sweet orange essential oil is expected to further suppress the growth of bacteria because it contains antibacterial. The research objective was to determine the value of the percentage Membrane Integrity and Acrosome Integrity on Simmental liquid semen with the addition penicillin and sweet orange essential oil. The method used was experimental using Completely Randomized Design with 5 treatments and 5 replications. The treatment was addition 0%, 0.25%; 0.5%; 0.75% and 1% sweet orange essential oil on tris yolk and penicillin extender. The results showed that addition sweet orange essential oil and tris yolk and penicillin extender resulted the best percentage value of the Membrane Integrity and Acrosome Integrity in Simmental liquid semen was the addition of sweet orange essential oil 1%.

Keywords: Essential Oil, Liquid Cement, Simmental, Sweet Orange, Penicillin.

1. Introduction
The low population and genetic quality of local cattle is one of the causes of the demand for beef in Indonesia is still not sufficient. The characteristics of local cows in Indonesia such as low weight growth and low body weight result in not much meat produced. Demand for meat continues to increase along with population growth, public awareness of the importance of nutritious food for the body and increasing community income. Therefore it is necessary to think about how to improve genetic quality and cattle population.

To improve the genetic quality of local beef cattle can be done by mating with the kind of superior cattle. One of the superior cows is Simmental Cow because it has high body weight, high growth and high carcas. However Simmental Cows are hard to find in Indonesia. Besides that the price of good quality Simmental Cows is very expensive and to maintain it also requires a lot of costs. To optimize the use of Simmental cattle for mating with limited quantities, can be done with Artificial Insemination.
Increased population and productivity of Simmental Cows can be done using Artificial Insemination. Mating with Artificial Insemination can optimize the use of a superior bull to mating many cows. Artificial Insemination has several advantages such as preventing the occurrence of diseases caused by natural mating, optimizing the use of Simmental bull studs for mating programs because in one semen shelter can be used for many cows as well as improving the genetic quality of livestock because it inherits good properties from Simmental Cows.

There are several factors that influence the success percentage of artificial insemination program, including inseminator skills, the timing of Artificial Insemination, and the factors of cattle farmers. In addition, the quality of liquid semen Simmental Cattle used is also very influential to determine the success of Artificial Insemination. The quality of liquid cement is determined by the raw materials used such as fresh cement and the material of semen extender. Liquid cement thinner material must be able to provide protection, especially at the time of cold shock [1]. Liquid cement must be used immediately for Artificial Insemination because the longer the storage period the quality will continue to decline if it is not immediately used [2].

Simmental Cattle liquid semen quality may be lower if contaminated with bacteria in large quantities. Bacterial contamination can occur during the process of fresh semen storage, preparation of liquid semen extender, equipment and the environment. What can be done to inhibit bacterial growth is the addition of antibiotics in liquid semen extender of Simmental Cattle. The antibiotics commonly used in liquid semen extender are penicillin, streptomycin and gentamicin. Penicillin is an antibiotic that can be used as an antibacterial. The addition of the antibiotics less effective because only active against certain types of bacteria. Efforts have been made to combine the use of antibiotics such as GTLS (Gentamisin, Tylosin and Linco-Spectin) [3], penicillin and streptomycin [4] and Andromed® (gentamicin sulfate, spectinomycin, and linkomycin) [5].

The addition of other materials that contain antibacterial can be used to minimize the growth of bacteria in the liquid semen Simmental Cattle. Besides giving antibiotics such as penicillin, can also add sweet orange essential oil. Addition of sweet orange essential oil has been carried out by [6] on Boer Goat semen, which shows increased quality with additions of up to 1%. Additionally sweet orange essential oil can also be added to the Simmental Cattle semen extender to improve the quality [7].

The content of linalool and limonane is very high in sweet orange essential oil so that it can be used as an antibacterial [8]. The results of the study by [7] show that the addition of sweet orange essential oil to the tris yolk extender can improve the quality of Simmental Cows semen. The addition of sweet orange essential oil and penicillin is expected to further improve the quality of Simmental Cows liquid semen. To see the quality of liquid semen was carried out by microscopic analysis, namely observations made by observing spermatozoa using a microscope. The aim of the study was to determine the effect of addition of sweet orange peel essential oil on tris yolk and penicillin extender to the percentage value Membrane Integrity and Acrosome Integrity in Simmental liquid semen.

2. Methods

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

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

2.2. 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). Tests were done by mixing 0.1 ml to 9.9 ml cement hyposmotic medium. Once mixed, the preparations are incubated in a water bath temperature of 37 °C for 30 minutes.

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

3. Results and Discussion

The results of membrane integrity and acrosome integrity of spermatozoa in liquid semen Simmental cattle before equilibration and after equilibration can be seen in Table 1.

| Parameters       | Treatment | Before equilibration | After equilibration |
|------------------|-----------|----------------------|---------------------|
| Membrane Integrity | P₀       | 70                   | 68                  |
|                   | P₁       | 75                   | 72                  |
|                   | P₂       | 77                   | 73                  |
|                   | P₃       | 78                   | 75                  |
|                   | P₄       | 81                   | 78                  |
| Acrosome Integrity | P₀       | 66                   | 61                  |
|                   | P₁       | 70                   | 65                  |
|                   | P₂       | 72                   | 67                  |
|                   | P₃       | 73                   | 70                  |
|                   | P₄       | 75                   | 72                  |

Note: Different superscripts on the column show very significant differences (P <0.01)

3.1. Membrane Integrity

Observation of membrane integrity spermatozoa is very important to test the quality of liquid cement in Simmental Cattle. The lowest percentage value without the addition of sweet orange essential oil and its value increases with increasing sweet orange essential oil given. The results of various membrane integrity analysis showed the effect of sweet orange essential oil on the extender material had a very significant effect (P <0.01) both on observation before and after equality. The highest average value of membrane integrity of Simmental cattle before equality is shown in treatment P₄, which is 81% and after equality of 78%. The presence of toxic substances originating from dead spermatozoa or from substances contained in the extender that have undergone oxidation due to storage causes high levels of free radicals which can damage the integrity of the plasma membrane of the spermatozoa. If spermatozoa membrane integrity has been damaged, then the sperm metabolism will be disturbed and begin to lose motility, resulting in the death of spermatozoa [10].

The results of the study showed a decrease in the quality of liquid semen before the equilibration until after equilibration. The decrease in the value of membrane integrity percentage is related to the motility of spermatozoa. The motility of spermatozoa after equilibration has decreased due to the egg-yolk coagulating enzyme factor in toxic plasma semen and cold shock. The decrease in motility is caused by the reduced energy supply of spermatozoa needed to maintain the life and movement of the spermatozoa. Decreased spermatozoa motility Simmental cattle are also caused by treatments that cause damage and death of spermatozoa. During the spermatozoa equilibration process Simmental cattle are
very susceptible to cell damage due to changes in osmotic pressure. Only spermatozoa have a strong plasma membrane that can survive to fertilize the egg cell [11].

The difference in percentage of membrane integrity in each treatment was caused by differences in the concentration of sweet orange essential oil in each treatment. The membrane integrity is very necessary for spermatozoa because damage to the plasma membrane will affect the metabolic process and is related to the viability of spermatozoa. If the membrane integrity is damaged then the cell metabolism process will be disrupted and result in the death of spermatooza. Acrosomes contain many enzymes that are needed during fertilization, therefore the success of acrosome fertilization process must be intact, because the enzymes in it function to lyse the oocyte protective layer [12].

Cell metabolism will take place well if the plasma membrane of the cell is in an intact state so that it is able to properly regulate traffic in and out of the cells of all the substrates and electrolytes needed in the metabolic process [13]. The metabolic process will be disrupted if the plasma membrane is damaged. ATP synthesis does not run normally so that the sperm will become damaged so that the value of the percentage of viability and motility of the sperm in the liquid cement of the Simmental Cow decreases.

Spermatozoa with a high percentage value of viability indicate that the plasma membrane is still intact, so that spermatozoa cell organelles will be protected and ions for metabolic processes and food requirements are available [14]. The high lactose concentration causes changes in osmotic pressure on the extender in the hypertonic direction which indicates more particles outside the cell than in the cell so that water is released from the cell so that the cell will shrink [15].

During the maturation process of spermatozoa in the epididymis, there is also a change in the composition of the components of the constituent compounds of spermatozoa plasma membranes. The plasma membrane of spermatozoa cells will lose some cholesterol, so there is an increase in the ratio between unsaturated fatty acids and cholesterol. This causes the plasma membrane to become more fragile. Such a condition of plasma membrane is physiologically needed to facilitate spermatozoa undergoing the capacitation process in the uterus and at the time of fusion with the oocyte plasma membrane during fertilization [16].

3.2. Acrosome Integrity
Acrosome Integrity percentage value is very important because it plays a role in the process fertility. The results of the variance analysis showed that the addition of sweet orange essential oil to extender had a very significant effect ($P <0.01$) on the percentage value of Acrosome Integrity before equilibration and after equilibration. The highest percentage value of Acrosome Integrity is in treatment P4 before equilibration which is 75% and 72% after equilibration.

The results showed that the percentage of the Acrosome Integrity would be higher if there was an increase in the level of sweet orange essential oil in the Simmental semen extender. Damage to the Acrosome Integrity is usually accompanied by damage to the acrosome hood so that the observation of the Whole Acrosome Hood is important because damage to the acrosome can result in the loss of proteolytic enzyme enzymes and most occur during dilution of cement which causes failure of Artificial Insemination [17].

The Acrosome Integrity is the layer that covers the nucleus, inside it is a collection of enzymes that function to help the nucleus enter the cytoplasm of the egg at fertilization, by damaging the layer of wrapping the egg through an acrosome reaction. The percentage of Acrosome Integrity shows a significant correlation with fertility. The most important aspect in evaluating semen quality is the investigation of the ability to experience an acrosome reaction. This process is related to activation and release of proteolytic enzymes from acrosomes which are very crucial during the penetration of eggs [12]. In the Acrosome Integrity there are hyaluronidase enzymes, acrosine and Corona Penetrating Enzyme which are needed to penetrate the cumulus oophorus and pellucida zone. Therefore this part of the Acrosome Integrity is an important part to consider in evaluating liquid semen [14].

Viability of spermatozoa is also influenced by the pH of semen. Changes in pH to a more acidic direction occur because of the accumulation of lactic acid which is the result of cell metabolism, namely the breakdown of fructose. Lactic acid is toxic to spermatozoa. With the percentage of Acrosome Integrity decreasing, this results in decreased spermatozoa fertilization [12]. Physiologically there is a relationship between the motility and integrity of the plasma membrane and the survival of spermatozoa. Damage to the plasma membrane will cause the loss of necessary enzymes [18].

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4. Conclusion
Addition of essential oils of sweet orange and penicillin in Simmental liquid semen diluent can increase the percentage value of membrane integrity and acrosome integrity. The best results obtained is the addition of essential oils as much as 1% and the lowest is without the addition of essential oils.

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