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The effect of addition sweet orange essential oil and penicillin in tris yolk extender to Simmental liquid semen against percentage motility, viability and abnormalities of spermatozoa

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Abstract. One of the causes a poor quality of liquid semen is increasing bacterial growth. The addition of sweet orange essential oil and penicillin in Simmental liquid semen is expected to improve the quality of liquid semen because it contains antibacterial. The purpose of this research is to know the value of the percentage of motility, viability and spermatozoa abnormalities in Simmental liquid semen with an addition of combination penicillin with sweet orange essential oil on tris yolk extender. The research hypothesis is the addition of combination penicillin with sweet orange essential oil to tris yolk extender can increase the value of the percentage of motility, viability and spermatozoa abnormalities in Simmental liquid semen. Materials used in this study is the Simmental fresh semen, tris yolk extender, penicillin, and sweet orange essential oil. The experimental design used randomized design with 5 treatments and 5 replications. Treatment on the research is the addition of sweet orange essential oil 0%, 0.25%, 0.5%, 0.75% and 1%. The parameters observed were the evaluation of motility, viability and abnormality spermatozoa before equilibration and after equilibration. The results showed the best result was the addition of 1% sweet orange essential oil and the lowest on the addition of 0% sweet orange essential oil before and after equilibration.

Keywords – sweet orange essential oil, penicillin, tris yolk extender, Simmental, percentage motility, viability, abnormalities, spermatozoa.

1. Introduction

Population and productivity Simmental cattle can be done through reproductive technology, such as Artificial Insemination. Mating with Artificial Insemination can optimize the use of a superior male to mating many females. Artificial Insemination can improve genetic quality and livestock population [12]. The benefits of Artificial Insemination are to minimize the risk of disease transmission through natural mating, not to require many bull for mating programs and the semen used from superior bulls. Superior bull to mating many cows. The benefits of Artificial Insemination are to minimize the risk of disease transmission through natural mating, not to require many bulls for mating programs and the cement used to come from superior bull. Artificial Insemination can increase the profits of farmers [8].

The success of Artificial Insemination is strongly influenced by sperm quality. The quality of sperm after the shelter will experience a decrease if it is not used immediately. Fresh undiluted semen will reduce spermatozoa fertility. Therefore, to maintain the quality of sperm during storage can be



done by adding extenders. The addition of extenders aims to provide a source of energy for sperm so as to ensure the survival of spermatozoa during storage. An important requirement for sperm diluents is to provide protection against cold shock that occurs during freezing and as a buffer to maintain pH stability [11]. Besides being able to provide food as an energy source, maintain the same osmotic pressure as sperm and contain antibiotics to inhibit bacterial growth [15].

One of the factors of the low survival of spermatozoa during liquid semen storage is the development of bacteria. Generally, the addition of antibiotics in liquid semen extenders is done to minimize bacterial growth. Penicillin is one antibiotic that can be used as an antibacterial. The administration of penicillin to the extenders has been carried out by combining several other antibiotics such as GTLS (Gentamicin, Tylosin, and Linco-Spectin) [6] and Andromed® (gentamicin sulfate, spectinomycin, and lincomycin) [10] Essential oils of sweet orange peel containing limonene and linalool are toxic to bacteria [4].

The use of essential oils of sweet orange on Trish egg yolk extender in the liquid semen of Simmental Cattle was carried out and proved to be able to maintain the quality of semen of Simmental Cattle. By combining penicillin and essential oils of sweet orange peel, it is expected that the best antibacterial composition can be obtained that can inhibit bacterial growth but does not reduce the quality of Simmental liquid semen. This study aims to determine the effect of the use of a combination of penicillin and essential oils of sweet orange peel on the quality of Simmental liquid semen.

2. Research method

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

- P₀ = Penicillin + sweet orange Essential oil 0%
- P₁ = Penicillin + sweet orange Essential oil 0.25%
- P₂ = Penicillin + sweet orange Essential 0.5%
- P₃ = Penicillin + sweet orange Essential 0.75%
- P₄ = Penicillin + sweet orange Essential 1%

2.1. Evaluation of microscopic sperm parameters

2.1.1. Motility

The percentage of spermatozoa moving progressively forward. The evaluation was done by observing spermatozoa in eight different visual field with a light microscope magnification 400 times.

2.1.2. Viability

Percentage of dead spermatozoa after thawing was evaluated with eosin staining (eosin-Y 1.67g and sodium citrate 2.9 g dissolved in 100 ml distilled water) as described by [2]. The evaluation was done at a minimum of 200 spermatozoa was observed using a light microscope magnification of 400 times. Live spermatozoa characterized by a head that do not absorb the dye, while the die is characterized by a red head.

2.2. Abnormalitas Spermatozoa

Deviations in the form of sperm morphology that can reduce the fertility of spermatozoa. Calculated abnormalities are too large head abnormalities, head too small, double head (duplicate head), circular tail and double tail. Abnormalities are calculated by the formula:

$$\frac{\text{Number of spermatozoa abnormalities}}{\text{Total sperm counted}} \times 100\%$$

3. Results and discussion

The results of motility, viability, and abnormality of spermatozoa in liquid semen of Simmental Cattle before and after equilibration can be seen in the recapitulation table below.

Table 1. Recapitulation of motility, viability, and abnormalities of Simmental Cattle semen before equilibration and after equilibration.

Parameter	Treatment	Observation	
		Before Equilibration	After Equilibration
Motility	P ₀	70±0.00	69±2.74
	P ₁	72±0.00	70±2.24
	P ₂	73±2.24	72±2.24
	P ₃	75±2.74	74±2.74
	P ₄	77±2.24	75±0.00
Viability	P ₀	77±2.50	75±1.68
	P ₁	78±2.50	77±2.50
	P ₂	80±1.68	79±0.87
	P ₃	82±2.72	80±2.72
	P ₄	84±1.68	82±2.50
Abnormality	P ₀	7±1.37	8±1.37
	P ₁	6±1.24	7±1.45
	P ₂	4±1.15	6±0.72
	P ₃	3±0.75	4±1.24
	P ₄	3±1.24	3±1.42

Description: Different superscripts in columns show very real differences ($P < 0.01$).

3.1. Motility of Simmental spermatozoa

The results of research on observations of semen motility of Simmental Cattle before dilution using trish yolk extender and sweet orange essential oil and observations after equilibration showed different results for each treatment. According to [5], sperm motility or sperm motility is one of the determinants of the success of spermatozoa to reach the ovum in the fallopian tubes and the simplest way to assess sperm for Artificial Insemination. The results of variance analysis showed that the effect of essential oils as extenders had a very significant effect ($P < 0.01$) on the motility of spermatozoa both after dilution and after equilibration. This result is also the same as the treatment of Boer Goats using gentamicin and sweet orange essential oil [13]. Judging from the average observation of each treatment that the motility of all treatments meets the standards to be used as cement for Artificial Insemination due to motility above 69% and that number has met the requirements for Artificial Insemination. Where the conditions for cement that are suitable for Artificial Insemination have motility of not less than 40% [3].

[7] states that hydroxynonenal is a lipid peroxidation that can inhibit glycolysis and sperm motility. In addition to damage caused by lipid peroxidation, decreased motility can also occur due to several factors. According to [15] factors that can reduce motility are changes in medium pH, osmotic pressure and electrolyte and non-electrolyte effects. The best sperm motility that can be used and meet the standards in this study is the addition of 1% sweet orange essential oil. The results showed that the sperm motility of liquid semen increased with the addition of sweet orange essential oil both before dilution and after dilution. Addition of sweet orange essential oil to liquid semen Simmental cattle is appropriate because it increases the percentage of spermatozoa motility Simmental Cattle. This is consistent with the statement of [11] that the quality of semen will decrease if the liquid cement extender is not appropriate.

3.2. *Viability of Simmental spermatozoa*

[14] states that immovable spermatozoa do not necessarily die so they do not absorb color, while in interpretation with a moving and immovable basis are considered immobile. In spermatozoa that are alive and moving but there is a defect in the cell wall, it can absorb colors considered dead, while other interpretations are considered not dead. The results showed that the average value of all treatments for the percentage of live spermatozoa before equilibration was 84% and after equilibration 82%. The results of the analysis of various percentages of dead life spermatozoa are very significantly different ($P < 0.01$) both on the observation before equilibration and after equilibration.

This difference in average viability can be due to physical influence at the time of treatment so that it can cause death. Friction between spermatozoa can cause abnormalities as well as death. The occurrence of a decrease in spermatozoa viability after the cooling and freezing process can be caused due to physical influence during the treatment that caused death. The physical effect is caused by friction between spermatozoa, between spermatozoa and tube walls, or between fat globules of egg yolks so that there is a tendency to decrease viability along with different levels of dilution. The National Standardization Agency determines the quality of a cow's liquid cement after the freezing process must show a minimum viability percentage value of 40% [1]. Quality degradation after the cooling and freezing process is caused by spermatozoa experiencing cold shock. Another factor that can cause a decrease in sperm quality is because during the freezing process the formation of ice crystals occurs, so the concentration of electrolytes in the cell increases and dissolves the lipoprotein sheath of the spermatozoa wall [15]. [9] stated that during freezing and storage of cement there is a membrane imbalance, which can reduce sperm resistance so that after thawing the quality of cement becomes low.

3.3. *Abnormalities of Simmental spermatozoa*

Spermatozoa abnormalities are a condition in which spermatozoa experience disability in one or all parts of the body of the spermatozoa. Spermatozoa abnormalities consist of primary, secondary and tertiary abnormalities. Primary abnormalities occur during the process of spermatogenesis and the presence of testicular disorders. Secondary abnormalities occur after spermatozoa leave the seminal tube to the male reproductive tract, while tertiary abnormalities occur after ejaculation to the handling process. The results of variance analysis showed that the effect of the addition of streptomycin combination with sweet orange essential oil as a diluent had a very significant effect ($P < 0.01$) on abnormalities of spermatozoa both after freezing and after freezing. The results of further BNT tests show that the best abnormalities are in P4 treatment, which is 3% before equilibration and 3% after equilibration.

Increased abnormalities of spermatozoa after cooling and freezing are caused by the physical influence of spermatozoa which can cause abnormal spermatozoa. temperature changes can cause changes in the permeability of the sperm cell wall membrane and result in disharmonism, membrane breakdown, and enzyme secretion. This condition can cause an increase in sperm abnormalities. The number of abnormal spermatozoa increases will cause low semen fertility of the animal. Spermatozoa cells are deformed, although they can fertilize an egg but usually end up with the death of a child before birth. Another factor that influences the increase in abnormalities is the inadvertent action at the time of treatment, liquefying the cement with the same isotonic fluid, cold shock, heat, and nutritional disorders. Spermatozoa who experience abnormality are caused by physical influence at the time of treatment, where spermatozoa rub against each other causing abnormalities as well as death. Factors that affect abnormal percentages are the act of not carefully diluting semen with fluid that is not the same as isotonic, cold shock, heat, nutritional disorders or endocrine disorders that affect normal spermatogenesis [16].

4. Conclusion

Addition of essential oils of sweet orange and penicillin in liquid semen extender Simmental cattle can increase the percentage value of motility, viability and spermatozoa abnormality and are suitable

for use in Artificial Insemination. The best results obtained are the addition of essential oils as much as 1% and the lowest is without the addition of essential oils. Fertility testing is needed to see the success rate of Artificial Insemination using Simmental Cattle liquid semen.

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