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Acrosome Integrity Spermatozoa Test with Addition of Gentamicin and Sweet Orange Essential Oil in Simmental Bull Liquid Semen

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Authors' contributions

This work was carried out in collaboration between both authors. Author SAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JM managed the analyses of the study. Both authors read and approved the final manuscript

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ABSTRACT

Aims: The purpose of this study was to determine the percentage value of spermatozoa acrosome integrity with the addition of gentamicin and sweet orange essential oil on the tris yolk extender on Simmental bull liquid semen.
Methodology: The material that was used in this research included Simmental bull fresh semen, tris yolk extender, gentamicin, and sweet orange essential oil. Tris yolk extender prepares with Tris (hydroxymethyl) aminomethane (3.32 g), citric acid (1.86 g), fructose (1.37 g), glycerol (6 ml), egg yolk (20 ml), aqua dest (100 ml). The experimental design used in the study was a nonfactorial Complete Randomized Design with five treatments and five replications. The treatments comprised the addition of sweet orange essential oil at 0%, 0.25%, 0.5%, 0.75% and 1% inclusion levels. The parameter observed was the evaluation of the percentage value of acrosome integrity before and after equilibration equilibration.
Results: The results showed that the percentage value of acrosome integrity in spermatozoa linearly increased along with the addition of sweet orange essential oil. The addition of sweet

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orange essential oil had a very significant effect (P <0.01) on the percentage of spermatozoa acrosome integrity in Simmental bull liquid semen. The percentage of acrosome integrity on the addition of 0% (P_0) sweet orange essential oil is 60%, the addition of 0.25% (P_1) is 64%,the addition of 0.5% (P_2) is 67%, the addition of 0.75% (P_3) is 69%, and the addition of 1% (P_4) is 73%.
Conclusion: The addition of 1% (P_4) sweet orange essential oil can increase the value of acrosome integrity on spermatozoa, which subsequently improve the quality of Simmental bull liquid semen.

Keywords: Acrosome integrity; essential oil; gentamicin; liquid semen; sweet orange; simmental.

1. INTRODUCTION

Beef demand in Indonesia have not been met and still rely on exports. Therefore It is imperative to recognize the various operational policies that have been set, such as improving the quality of beef cattle, helping capital for farmers, controlling reproductive diseases and animal health, developing feed, and developing the quality of beef cattle breeds [1]. Artificial Insemination (A.I) is an advanced livestock breeding program ranging from organization, counseling, semen production, detection of estrus, and Artificial Insemination (deposition of semen into livestock reproduction channels) to evaluating the success of Artificial Insemination.

One of the important factors of Artificial Insemination is the quality of Simmental bull liquid semen used. The best quality of liquid semen is the optimum requirement for fertility of spermatozoa [2]. Spermatozoa must be preserve for some time after storage. Therefore semen needs to be mixed with a diluent solution that guarantees its physical and chemical needs or rather requirements [3]. Semen dilution aims to increase the volume of semen and not reduce the quality of the semen [4]. The use of semen diluents must be able to maintain the viability of spermatozoa before use. The normal condition of the diluent is that it must be able to provide nutrients, allow the spermatozoa to move progressively, be non-toxic, and be able to protect the spermatozoa from cold shock [5].

One of the consequences of low liquid semen quality is perhaps the bacteria contained in Simmental bull liquid semen. Bacteria can cause damage to acrosomes in spermatozoa [6]. Spermatozoa with damaged acrosomes cannot fertilize the ovum, hencefertility is low. The higher percentage of acrosome integrity improves fertility in Artificial Insemination of cattle.The addition of antibiotics to Simmental bull liquid semen diluents can inhibit and kill bacteria [7]. One antibiotic that can be use for bull semen dilution is gentamicin. Gentamicin is an active agent against gram-negative and positive bacteria [8]. The addition of one type of antibiotic is still not enough to kill bacteria in the liquid semen, so that much work done by combining antibiotics with other ingredients that contain antibacterial.

Sweet orange essential oil containing limonene and linalool are toxic to bacteria [9]. The results of previous studies by adding a combination of penicillin and Sweet orange essential oil have been shown to increase the percentage of intact plasma membranes in Simmental spermatozoa [10]. In this research, a combination of gentamicin and sweet orange essential oil is expected to increase the percentage of spermatozoa acrosome integrity in Simmental bull liquid semen.

2. MATERIALS AND METHODS

The study was conducted experimentally with a quantitative or objective approach. Experimental research was carried out using treatments at various levels of sweet orange essential oil and gentamicin and then comparing them with no administration of sweet orange essential oil (control). Activities in experimental research aimed to assess the effect of giving sweet orange essential oil the effect on giving it when compared to without giving sweet orange essential oil.

The population and sample in this study were Simmental bull fresh semen which had added with tris yolk extender, Gentamicin, and various levels of sweet orange essential oil with the treatment given as follows:

P_0 = Gentamicin + Sweet orange essential oil 0%
P_1=Gentamicin +Sweet orange essential oil 0,25%
P_2 =Gentamicin +Sweet orange essential oil0,5%
P_3=Gentamicin+Sweet orange essential oil0,75%
P_4= Gentamicin +Sweet orange essential oil1%

Data collection techniques used during the study were to observe the parameters, namely the evaluation of Simmental bull liquid semen before equilibration (After dilution of liquid semen) and after equilibration. Research methods describe the value of observations according to a nonfactorial Complete Randomized Design with five treatments and five replications. The research data were analyzed using the SAS 9.0 program and compare the differences in means test using the Duncan Multiple Range Test.

An evaluation of the acrosome integrity of spermatozoa marked by the tip of the head of the thick black spermatozoa.If semen exposed to a physiological NaCl solution containing 1% formalin [11]. Evaluations performed on a minimum of 200 spermatozoa observed using a 400 times magnification light microscope. The percentage of acrosome integrity is calculated based on the formula:

$$\text{Acrosome Integrity} = \frac{\text{sperm with a colored acrosom}}{\text{total sperm counted}} x\,100\%$$

3. RESULTS AND DISCUSSION

The results of Simmental bull liquid semen before equilibration and after equilibration using a combination of antibiotic gentamicin and sweet orange essential oil on tris yolk extender with acrosome integrity observations is shown in Table 1.

Acrosome integrity test of spermatozoa in Simmental bull results before equilibration showed that the lowest percentage value is without sweet orange essential oil (P_0), which is 65% while the highest is with the addition of 1% sweet orange essential oil (P_4) which is 76%. From the data obtained, it has been shown that the addition of sweet orange essential oil increases the value of acrosome integrity spermatozoa Simmental bull percentage before semen equilibration. The higher level of administration of sweet orange essential oil will further increase the value of acrosome integrity spermatozoa.

The results of the analysis of variance showed that the effect of the addition of a combination of gentamicin with sweet orange essential oil as a diluent had a very significant effect (P <0.01) on the acrosome integrity of spermatozoa on both before and after equilibration.

The results of the acrosome integrity spermatozoa of Simmental bull tested after semen equilibration showed that the lowest percentage value was without treatment (P_0), which recorded 60% while the highest was the treatment with the addition of 1% sweet orange essential oil (P_4) which recorded 73%. From the data obtained, it has been shown that the addition of sweet orange essential oil increases the value of acrosome integrity spermatozoa Simmental bull percentage before semen equilibration. The higher level of administration of sweet orange essential oil will further increase the value of acrosome integrity spermatozoa [10]. Since it perhaps enhances membrane integrity and thus prevents cold shock.

The value of the percentage of acrosome integrity in spermatozoa of Simmental bull after semen equilibration is higher compared to before semen equilibration. It reveals a decrease in the quality of semen during the equilibration process. The highest increase in the percentage of acrosome integrity of spermatozoa shown in the treatment without the addition of sweet orange essential oil (P_0) by 5%. In comparison, the increase in the percentage of the lowest spermatozoa acrosome integrity value shown in the addition of 1% (P_4) in sweet orange essential oil.

The results of the analysis of variance showed that the effect of the addition of a combination of

Table 1. Summary of results of research simmental bull semen acrosome integrity before and after equilibration

Parameter	Treatment	Observation	
		Before equilibration	After equilibration
Acrosome Integrity	0%	65±1.77	60±0.57
	0,25%	67±0.98	64±1.54
	0,5%	71±1.12	67±1.22
	0,75%	74±1.64	69±1.75
	1%	76±2.25	73±1.88

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

gentamicin with sweet orange essential oil as a diluent had a very significant effect (P <0.01) on the acrosome integrity of spermatozoa on both before and after equilibration. Giving the fact that semen extenders are known to stabilize sperm cell membrane, besides antibiotics apparently prevents agglutination of sperm cells or rather clumped semen occurrence in infected ejaculate.

The quality of Simmental bull liquid semen is one of the most important factor for successful fertilization after Artificial Insemination [12]. Microscopic determinants of spermatozoa quality that are feasible to conduct Artificial Insemination include membrane integrity and acrosome integrity of spermatozoa [13].

The results showed that the highest percentage of acrosome integrity in P4 treatment after dilution was 76% and 73% on both before and after equilibration respectively. The results showed that an increase in the concentration of sweet orange essential oil in the diluent apparently leads to decrease of the average percentage of acrosome integrity of Simmental bull sperm as a consequence. However, acrosome integrity plays an important role in the process of fertilization for the successful conduct of Artificial Insemination. Changes that do occur in the acrosome areoften related to fertility than sperm motility [14]. Since acrosome is vital in the process of capacitation, sperm reaction and sperm-oocyte encounter.

The low percentage of acrosome integrity is related to the low percentage of motility, viability, and membrane integrity [15]. Physiologically there is a relationship between motility and integrity of the plasma membrane and also spermatozoa viability. Besides, extenders or diluents used should always be isotonic with the seminal plasma for normal function. Damage to the plasma membrane will cause the loss of enzymes needed [16]. As a result of leakage.

The results showed that the damage caused by the equilibration process would be lower if sweet orange essential oil is added to the semen diluent. Sweet orange contains antioxidant compounds [17]. Acrosome damage to spermatozoa that occurs during the cryopreservation process could be reduced by the addition of antioxidants [18].

4. CONCLUSION

The higher addition of sweet orange essential oil to the tris yolk extender perhaps increased the percentage of acrosome integrity in Simmental bull liquid semen, with the best treatment revealed asP_4 which is recorded 73%.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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