



Membrane Integrity Test for Spermatozoa in Simmental Bull Liquid Semen Using the Addition of Gentamycin and Sweet Orange Essential Oil in the Extender

Sukma Aditya Sitepu¹ and Julia Marisa^{2*}

¹Department of Animal Husbandry, Faculty of Science and Technology, Pembangunan Panca Budi University, Medan, Indonesia.

²Department of Agrotechnology, Faculty of Science and Technology, Pembangunan Panca Budi University, Medan, Indonesia.

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.

Article Information

DOI: 10.9734/AJARR/2020/v8i430206

Editor(s):

(1) Dr. Fagbadebo Omololu Michael, Durban University of Technology, South Africa.

Reviewers:

(1) Dennis Amaechi, University of Abuja, Nigeria.

(2) O. Oluwole Olufunke, Obafemi Awolowo University, Nigeria.

(3) Popoola Mustapha Ayodele, Nigeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55100>

Received 28 December 2019

Accepted 04 March 2020

Published 12 March 2020

Original Research Article

ABSTRACT

Aims: This study aimed to determine the quality of Simmental Bull liquid semen through laboratory tests, including the percentage value of membrane integrity Spermatozoa with the addition of gentamycin and sweet orange essential oil to tris yolk extender.

Methodology: The material used in this research is Simmental Cattle bull fresh semen, tris yolk extender, gentamycin, and sweet orange essential oil. Tris yolk extender was prepared with Tris (hydroxymethyl aminomethane) (3.32 g), citric acid (1.86 g), fructose (1.37 g), glycerol (6 ml), egg yolk (20 ml), aquades (100 ml). The experimental design used in the study was a nonfactorial Complete Randomized Design with five treatments and five replicates. The treatment given is the addition of sweet orange essential oil 0%, 0.25%, 0.5%, 0.75% and 1%. The parameter observed was the evaluation of membrane integrity before equilibration and after equilibration.

*Corresponding author: Email: juliamarisa@pancabudi.ac.id;

Results: The results showed that the percentage of membrane integrity in Simmental bull liquid semen continues to increase with the addition of sweet orange essential oils. The results showed that the addition of sweet orange essential oil as much as 0% produced a membrane integrity percentage after equilibration of 63%, adding 0.25% resulted in 68%, adding 0.5% yielded 70%, adding 0.75% yielded 75% and adding 1% resulted in 79%.

Conclusion: The best percentage of membrane integrity in Simmental bull liquid semen was the addition of gentamicin and sweet orange essential oil in the tris yolk extender at the level of 1%.

Keywords: Essential oil; gentamicin; liquid semen; membrane integrity; sweet orange; simmental.

1. INTRODUCTION

Indonesia has not been able to meet the protein needs for human consumption. Beef cattle farms have many obstacles in achieving production, and productivity is low. To increase the population and productivity of local cattle can be done by utilizing reproductive technologies, such as Artificial Insemination. Artificial insemination can optimize the use of a superior bull to inseminate many cows. Simmental cattle are one of the superior cattle in the world that are very suitable to be used to improve the genetic quality of local cattle.

One of the success factors of Artificial Insemination is liquid semen quality. Sperm quality after storage will decrease if not used immediately. Undiluted fresh semen will decrease the fertility of spermatozoa. Therefore to maintain the quality of spermatozoa during storage can be done by adding extenders. An important requirement for sperm extender is to protect against cold shock that occurs during freezing and as a buffer to maintain pH stability and contain antibiotics to inhibit bacterial growth [1].

The cause of the low quality of liquid semen has removed the development of bacteria in Simmental Cattle liquid semen, which can cause damage to the membrane of spermatozoa. Spermatozoa with damaged membrane integrity cannot fertilize the ovum. The higher percentage of membrane integrity will cause fertility in liquid semen of Simmental cattle to increase. Bacteria can damage the membrane of spermatozoa. Membrane integrity is a must-have good spermatozoa because the plasma membrane plays an important role in regulating all biochemical processes that occur in spermatozoa cells.

Inhibition and killing of bacteria can be done with the addition of antibiotics. The addition of antibiotics such as streptomycin to the semen extender is common. But its use is still

considered unfavorable so many attempts to suppress bacterial growth. Gentamicin is active against bacteria, especially with the gram-negative and positive bacteria [2]. Sweet orange essential oil containing limonene and linalool are toxic to bacteria [3].

The results of previous studies by adding a combination of penicillin and essential oils have been shown to increase the percentage of membrane integrity in Simmental spermatozoa [4]. In this research, the combination of gentamicin and the sweet orange essential oil is expected to further increase the percentage of whole spermatozoa membrane integrity in Simmental liquid semen.

2. MATERIALS AND METHODS

The study was conducted experimentally with a quantitative or objective approach. Experimental research carried out by making some treatments using graded levels of sweet orange essential oil and gentamicin then compare it to without giving sweet orange essential oil. Activities in the experimental control aimed to assess the effect of adding sweet orange essential oil and determine the impact on giving it when compared to without giving sweet orange essential oil.

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

- P₀ = Gentamicin + Sweet orange essential oil 0%
- P₁ = Gentamicin + Sweet orange essential oil 0, 25%
- P₂ = Gentamicin + Sweet orange essential oil 0, 5%
- P₃ = Gentamicin + Sweet orange essential oil 0, 75%
- P₄ = Gentamicin + Sweet orange essential oil 1%

Data collection techniques used during the study were to observe the parameters, namely the

evaluation of Simmental cattle liquid semen before equilibration (After dilution of liquid semen) and after equilibration. Research methods that explain 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 continued with a multiple area difference test using the Duncan Multiple Range Test.

Evaluation is carried out on the integrity of the spermatozoa plasma membrane. The evaluation was done using methods hypoosmotic swelling test (HOST). Tests carried out by mixing 0.1 ml to 9.9 ml of the medium semen hypoosmotic. After mixing, the preparation was incubated in a 37°C water bath for 30 minutes [5]. The evaluation was done under a light microscope at 400 times magnification

$$\text{Membrane Integrity} = \frac{\text{sperm with a curved tail}}{\text{total sperm counted}} \times 100\%$$

3. RESULTS AND DISCUSSION

The results of Simmental bull liquid semen research before equilibration and after equilibration using a combination of antibiotic gentamycin and sweet orange essential oils on tris yolk extender with observations of membrane integrity can be seen in Table 1.

The results of the Simmental cattle Spermatozoa membrane integrity test before equilibration showed that the lowest percentage value was without treatment (P0), which was 70% while the highest was with the addition of 1% sweet orange essential oil (P4) which was 82%. From the data obtained, it can be seen that the addition of sweet orange essential oils increases the percentage value of membrane integrity spermatozoa of Simmental cattle spermatozoa before semen equilibration. The higher level of

sweet orange essential oil will further increase the percentage value of membrane integrity spermatozoa.

The results of the Simmental bull spermatozoa membrane integrity test before equilibration showed that the lowest percentage value was without treatment (P0), which was 70% while the highest was with the addition of 1% sweet orange essential oil (P4) which was 82%. From the data obtained, it can be seen that the addition of sweet orange essential oils increases the percentage value of whole intact spermatozoa of Simmental bull spermatozoa before cement equilibration. The higher the level of sweet orange essential oil will further increase the percentage value of membrane integrity spermatozoa.

The results of the Simmental cattle spermatozoa membrane integrity test after semen equilibration showed that the lowest percentage value was without treatment (P0), which was 63% while the highest was with the addition of 1% sweet orange essential oil (P4) which was 79%. From the data obtained, it can be seen that the addition of sweet orange essential oil increases the value of the Simmental Cattle membrane Integrity spermatozoa before semen equilibration.

The percentage value of membrane integrity spermatozoa of Simmental cattle after semen equilibration is higher than before equilibration. This shows a decrease in the quality of semen during the equilibration process. The highest percentage increase in membrane integrity of spermatozoa was shown in the treatment without the addition of sweet orange essential oil (P0), which was 7% while the lowest percentage increase was shown in the addition of 1% sweet orange essential oil treatment (P4), which was 3%.

Table 1. Average results of membrane integrity studies of simmental cattle semen before and after equilibration

Parameter	Treatment	Observation	
		Before equilibration	After equilibration
Membrane Integrity	0%	70±0.25	63±1.57
	0,25%	74±1.34	68±2.25
	0,5%	76±0.98	70±1.54
	0,75%	80±1.74	75±1.87
	1%	82±2.25	79±2.55

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

The results of the analysis of variance showed that the effect of the addition combination of gentamicin with sweet orange essential oil as an extender had a very significant effect ($P < 0.01$) on the membrane integrity of spermatozoa both before and after equilibration. The results of further tests showed that the highest membrane integrity was found in P4 treatment, which was 79% after the semen equilibration. The quality of liquid semen of Simmental cattle is one of the critical success factors for fertilization after Artificial Insemination [6]. The microscopic quality determinant of spermatozoa that is feasible to do Artificial Insemination is membrane integrity spermatozoa [7].

Sweet orange essential oil functions as an antioxidant are very beneficial for the integrity of the spermatozoa membrane of a Simmental bull. The spermatozoa plasma membrane is rich in unsaturated fats, so it is susceptible to lipid peroxidation [8]. As a result of lipid peroxidation is the formation of lipid peroxides, which will react as free radicals and stimulate autocatalytic reactions, resulting in damage to the plasma membrane [9].

Membrane integrity is the integrity of spermatozoa, which is very instrumental in the process of fertilization for the success of Artificial Insemination. Spermatozoa with intact membranes will hold hypoosmotic fluid in the cell so that they are not damaged [10].

Spermatozoa, with a high percentage of life, show a high percentage of membrane integrity as well [11]. Spermatozoa, which have a high interest in life, indicates that the plasma membrane is physically intact so that spermatozoa cell organelles will be protected, the need for nutrients and ions for metabolic processes is available [12].

The higher level of essential oils will increase the percentage of membrane integrity. This might be due to the higher lactose concentration causing osmotic pressure changes in the extender towards hypertonic [13]. Hypertonic thinners indicate that there are more molecules or particles outside the cell than inside the cell [14]. As a result, there is an expulsion of water from inside the cell to thin the molecules outside the cell so that the cell will contract [15].

4. CONCLUSION

The results of the study with the best percentage of membrane integrity in Simmental bull liquid

semen were the addition of gentamicin and sweet orange essential oil in the tris yolk extender at the level of 1%.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hernández-Avilés CR, Serafini CC, Love SR, Teague KA, LaCaze SD, Lawhon D, Varner D. The Effects of antibiotic type and extender storage method on sperm quality and antibacterial effectiveness in fresh and cooled-stored stallion semen. *Theriogenology*. 2018;122:23-29.
2. Nattadiputra, S dan S. Munaf. Aminoglikosida dan beberapa antibiotika khusus, kumpulan kuliah farmakologi, 631, Jakarta, EGC; 2009.
3. Fisher K, Phillips CA. Potential antimicrobial uses of essential oils in food: Is citrus the answer?. *Trends in Food Sci and Technology*. 2008;19(3):156-164.
4. Sitepu SA, Marisa J. Percentage value of membrane integrity and acrosome integrity spermatozoa in simmental liquid semen with addition penicillin and sweet orange essential oil. In *IOP Conference Series: Earth and Environmental Science*. 2019; 327(1):012027. IOP Publishing.
5. Rodriguezgil JE, Montserrat A, Rigau T. Effects of hypoosmotic incubation on acrosome and tail structure on Canine Spermatozoa. *Theriogenology*. 1994;42: 815-29.
6. De Graaf S, Beilby KH, Underwood SL, Evans G, Maxwell WMC. Sperm sexing in sheep and cattle: The exception and the rule. *Theriogenology*. 2009;71(1):89-9.
7. El-Sheshtawy RI, Sisy GA, El-Nattat WS. Effects of different concentrations of sucrose or trehalose on the post-thawing quality of cattle bull semen. *Asian Pacific Journal of Reproduction*. 2015;4(1): 26-31.
8. Maxwell WMC, Watson PF. Recent progress in the preservation of ram semen. *Anim. Reprod. Sci*. 1996;42:55-65.
9. Sinha S, Deka BC, Tamulu MK, Borgohain BN. Effect of equilibration period and glycerol level in tris extender of quality of frozen Goat Semen. *Indian Vet. J*. 1992; 69:1107-1110.

10. Hafez ESE, dan B. Hafez. Reproduction in Farm Animal 7th Ed. Lippincott Williams and Walkins, South Carolina; 2000.
11. Singh VK, Singh AK, Kumar R, Atreja SK. Development of soya milk extender for semen cryopreservation of Karan Fries (crossbreed cattle). CryoLetters. 2013; 34(1):52-61.
12. Tambing SN, Sutama IK, Sariubang M. Efficacy of concentration of egg yolk in Tris extender with and without seminal plasma on frozen semen quality of Saanen bucks. Jurnal Ilmu Ternak dan Veteriner. 2009; 13(4):315-322.
13. Xu J, Chaubal SA, Du F. Optimizing IVF with sexed sperm in cattle. Theriogenology. 2009;71(1):39-47.
14. Bilaspuri GS, Bansal AK. Mn²⁺: A potent antioxidant and stimulator of sperm capacitation and acrosome reaction in crossbred cattle bulls. Archives Animal Breeding. 2008;51(2):149-158.
15. Baril G, Leboeuf B, Saumande J. Synchronisation of estrus in goat: The relationship between time of occurrence of estrus and fertility following artificial insemination. Theriogenology. 1993;40:621-628.

© 2020 Sitepu and Marisa; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/55100>