Chalaza Journal of Animal Husbandry
Url: http://usnsj.id/index.php/CJAH
Email: chalaza.usnsj@gmail.com

The Effect of Centrifugation Time on Sexing Spermatozoa with Bovine Serum Albumin Media on the Morphology of X-Y Spermatozoa in Simmental Cattle

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ARTICLE INFO

p-ISSN: 2548-5504
e-ISSN: 2548-3803
Vol. 7, No. 1, June 2022
URL: https://dx.doi.org/10.31327/chalaza.v7i1.1706

Abstract

Sexing and centrifugation of spermatozoa is one way to improve the quality of spermatozoa. This study aimed to obtain the right centrifugation time for sexing spermatozoa with Bovine Serum Albumin media on the morphology of X-Y spermatozoa in Simmental cattle. This study was carried out from October 2021 to November 2021 at the Laboratory of Animal Reproduction and Health at the Breeding Center for Superior Livestock and Forage, Sembawa. The design used in this study was a completely randomized design (CRD) with 3 treatments and 4 replications. Centrifugation treatments for sexing spermatozoa with BSA media P1: centrifugation time 4 minutes, P2: centrifugation time 8 minutes, and P3: centrifugation time 12 minutes. The parameters observed were the morphology of the X and Y spermatozoa. The results showed that the centrifugation time for sexing spermatozoa—which was different with Bovine Serum Albumin (BSA) media—indicates that the X spermatozoa abnormalities had no significant effect ($P>0.05$) but the Y spermatozoa abnormalities had a significant effect ($P<0.05$). The result of the right time use was in the P1 treatment with a centrifugation time of 4 minutes where the abnormalities value in the lower layer Y was 28.79%.

Keywords: bovine serum albumin, centrifugation, morphology

A. Introduction

Simmental cattle (*Bos taurus*) are cattle that have superiority such as fast growth, easy adaptation to the environment—especially in Indonesia, and the ability to convert feed into good meat so that it can be used for beef cattle. Aidilof (2015) stated that Simmental cattle are favored by breeders because they have superiority, namely relatively fast body growth, high fertility, and easy calving. Artificial insemination (AI) in Simmental cattle can also be increased in value in producing superior breeds based on the sex of the calves according to the purpose of rearing with sexing technology on spermatozoa.

BSA is believed that this technology can add value to AI applications because it can produce excellent calves of the desired sex and maintain breeding objectives. The BSA gradient from sperm purification techniques is impossible to manipulate sperm too much, in addition to exposing the sperm to BSA media, which is also often added to the sperm Therefore it is expected to prevent a decrease in sperm quality after division Procedure. Afiati (2004) reported that the proportion of sperm is caused by the sex ratio of the albumin gradient possible X chromosome carrier 80.88 n Y 58.82 and mobility after sexual intercourse Reaches 75.00%.

The advantage of sexing technology is that it is the right choice to support increased productivity of the cattle business, either for beef or dairy cattle. Various methods of separating X and Y spermatozoa have been carried out, such as the use of Bovine Serum Albumin (BSA) as a medium and centrifugation to separate spermatozoa from sperm plasma. Sexing spermatozoa with BSA media in principle is a combination of concentrations between the upper and lower fractions to separate X and Y chromosome spermatozoa. The level combination of BSA concentrations can be used as diluted media centrifugation because it is considered capable of maintaining sperm membranes from damage caused by the sexing process and improving the level of abnormalities in spermatozoa. Based on the description above, it is necessary to carry out a study to get the right length of centrifugation time of sexing spermatozoa with Bovine Serum Albumin (BSA) media on the morphology of X-Y spermatozoa in Simmental cattle.

B. Methodology

1. Material

The material in this study was one Simmental cattle, aged 3 years whose semen was collected once a week on Wednesday in the morning.

2. Method

This study was carried out at the Breeding Center for Superior Livestock and Forage (BPHPT) Sembawa, Banyuasin, South Sumatra, and the Laboratory for Animal Reproduction and Health at BPHPT, Sembawa. This study was carried out from October to November 2021. The

tools used were a clamp cage, teaser, artificial vagina (thin rubber cylinder, thick rubber cylinder, and rubber funnel), semen storage tube, water bath, measuring tube, measuring cup, Erlenmeyer, drying and sterilization oven, spectrophotometer, test tube, centrifugation tube, centrifuge, Trinocular microscope, electric scale, micropipette, tweezers, tube rack, microscope, object glass, cover glass, table paper, stationery (pens, labels), tissue, straw, automatic straw filling, and printing straw. Meanwhile, the materials used were Simmental cow semen, Bovine Serum Albumin (BSA), sexing medium, NaCL 0,9 %, Tris (Hydroxymethyl) aminomethane, citric acid, fructose, aquabides, egg yolk, glycerol, penicillin, streptomycin, Vaseline, fresh semen, aquabidetilata, eosin-nigrosine, and hot water.

The treatments given in this study were based on the difference in sexing centrifugation time at the same speed of 1500 rpm, using a Completely Randomized Design (CRD) with 3 treatments and 4 replications:

P1: Centrifugation time 4 minutes
P2: Centrifugation time 8 minutes
P3: Centrifugation time 12 minutes

3. Spermatozoa Morphological Determination Analysis Coloring

The method of coloring spermatozoa with eosin-nigrosine, spermatozoa with color red is deat and spermatozoa with white color is live**.** The morphology of spermatozoa was observed by looking at the abnormalities of the spermatozoa which was as many as 200 cells with a magnification of 400x (Noakes et al., 2009). Spermatozoa morphology abnormalities (no tail, abnormal head, abnormal tail shape, abnormal tail shape with proximal cytoplasmic droplets, and abnormal tail shape with distal droplets)

$$Abnormalities = \frac{Abnormalities}{Abnormalities + normal} \times 100\%$$

4. Data analysis

The data obtained were then analyzed based on the design used and if there was an effect between treatments, further testing with Duncan's Multiple Range Test (DMRT) would be carried out (Steel & Torrie, 2002).

C. Result and Discussion

XY Abnormalities of Semen Sexing by Centrifugation

The results of the analysis of variance showed that the centrifugation time of sexing spermatozoa with Bovine Serum Albumin (BSA) media had no significant effect (P>0.05) on the abnormalities of the upper layer (X) after centrifugation. The results are presented in Table 1.

Table 1. The average value of X-Y spermatozoa abnormalities in the upper and lower layers in Simmental cattle by centrifugation

Treatment	Spermatozoa abnormalities in the upper layer	Spermatozoa abnormalities in the lower layer
P1	$20.91^{a} \pm 5.80$	$25,34^{a} \pm 2,38$
P2	$28,79^{b} \pm 7,92$	$26.51^{b} \pm 4.61$
P3	$31.22^{c} \pm 7.92$	$35.30^{c} \pm 3.09$

Note: The numbers followed by the same letter in the same column show no significant difference at the 5% level (P < 0.05). P1 = centrifugation time 4 minutes, P2 = centrifugation time 8 minutes, P3 = centrifugation time 12 minutes.

On the other hand, the results of the analysis of variance showed that the centrifugation time of sexing spermatozoa with Bovine Serum Albumin (BSA) media had a significant effect (P <0.05) on the abnormalities of the lower layer (Y) after centrifugation. The results of further testing with Duncan's Multiple Range Test (DMRT) on the lower layer showed that the P1 treatment was significantly different (P<0.05) compared to the P2 and P3 treatments—and the P2 and P3 treatments were also significantly different (P<0.05) on the abnormalities value of sexing spermatozoa after centrifugation. Based on (Table 1), it is shown that the abnormalities increase in each layer, both in the upper and lower layers, which is related to the average value of the abnormalities decreasing in each treatment—both in the upper and lower layers.

This is presumably due to the increase in the percentage of abnormalities caused by friction and collision during centrifugation so it can damage the spermatozoa membranes—which then

have an impact on increasing X-Y abnormalities in spermatozoa resulting from sexing. This is to the statement by Sujoko et al. (2009); Choudhary et al. (2010); Fatahillah et al (2016) who reported that centrifugation resulted in friction between the spermatozoa—or between the spermatozoa and the medium or the tube wall, causing damage to the spermatozoa membrane and an increase in the percentage of abnormalities.

The results of observations in the 12-minute treatment showed that the average abnormalities in the lower layer of spermatozoa were higher than the upper layer, while in the 4-minute treatment, the average abnormalities of the upper layer of spermatozoa were lower than that of the lower layer. This is presumably because during the 12-minute centrifugation process, the separation between the upper and lower layers didn't go well—so in the upper layer there was still a lot of remaining spermatozoa population that should have gone down to the lower layer, while in the 4-minute treatment, it was possible to get enough centrifugation time for the separation of the upper and lower layers so that the population of spermatozoa in the upper layer has gone down to the lower layer according to the size of the spermatozoa (Jeyendran et al., 1984).

The lighter spermatozoa will be in the upper layer, while the heavier spermatozoa—or, the spermatozoa with a larger size will be in the lower layer and if centrifugation is carried out they tend to form deposits more quickly (Hafez, 2008). The centrifugal force during centrifugation causes the spermatozoa to be attracted to the lower layer and form more deposits compared to the upper layer. Hafez & Hafez (2008) stated that semen with an abnormalities value above 20% was not suitable for further processing. In this study, the highest percentage of abnormalities of spermatozoa sexing in the upper layer was found in the P3 treatment with a percentage of abnormalities of 31.22%, while in the lower layer was also found in the P3 treatment with the percentage of abnormalities of 35.30%. the quality of sperm had to more get attention after sexing and thawing (Mahfud, et al., 2019)

D. Conclusion

Based on the results of the study, it can be concluded that the centrifugation time for sexing spermatozoa—which was different with Bovine Serum Albumin (BSA) media—indicates that the X spermatozoa abnormalities had no significant effect ($P>0.05$) but the Y spermatozoa abnormalities had a significant effect ($P<0.05$). From the results of the centrifugation time use, the right centrifugation time use was in the P1 treatment with a centrifugation time of 4 minutes where the abnormalities value in the lower layer Y was 28.79%.

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