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Spermatozoa Quality of Bali Cattle Following Sexing using Egg Yolk Tris Medium at Different Duration

Viny Aprilia Kesumanengsih, Rahim Aka, Takdir Saili*

Faculty of Animal Science, Universitas Halu Oleo
Kampus Hijau Bumi Tridharma, Jl. H.E.A. Mokodompit, Anduonohu,
Kecamatan Kambu, Kota Kendari, Sulawesi Tenggara 93232, Indonesia

*Corresponding author: takdir69@uho.ac.id

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ABSTRACT

The length of sexing duration may affect the quality of Bali cattle spermatozoa. This study aims to examine the effect of sexing duration on viability, motility and abnormalities of Bali cattle spermatozoa. This research has been carried out at Regional Technical Implementation Unit (RTIU) Center for Breeding and Animal Feed, Department of Food Crops and Livestock, Southeast Sulawesi Province. The material used was a bull of Bali cattle as a source of semen and spermatozoa to be evaluated. In this study, a completely randomized design (CRD) was used with 3 treatments and 6 replications. The treatments consisted of three different length of sexing duration namely 40 min. (P1), 50 min. (P2), and 60 min. (P3). The variables evaluated in this study included semen quality (volume, color, odor, consistency and pH) and spermatozoa (mass movement, concentration, percentage of motility, viability and abnormalities). The data obtained were analyzed using analysis of variance (ANOVA) and continued with the Least Significant Difference test (LSD test). The results showed that the average volume of Bali cattle semen obtained was 5,38 ml/ejaculate, semen color was milky white, odor was specific, consistency was viscous, pH was 6,3, spermatozoa concentration was 1,445 million/ml and mass movement was +++. Whereas the motility of spermatozoa in each treatment following sexing were 76,67% (P1), 68,23% (P2), and 60,00% (P3). The viability of spermatozoa after sexing were 93,42% (P1), 84,42% (P2), and 79,83% (P3). The abnormality of spermatozoa after sexing were 2,58% (P1), 2,67% (P2), and 2,67% (P3). Furthermore, the results of variance analysis showed that the length of sexing duration had a significant effect ($P<0.05$) on the viability and motility of the Bali cattle spermatozoa, but did not significantly affect the sperm abnormalities of Bali cattle. The 40 min. (treatment P1) and 50 min. (treatment P2) of sexing duration resulted in better spermatozoa viability and motility of Bali cattle compared to 60 min. (treatment P3) of sexing duration.

Keywords: abnormality, motility, sexing, spermatozoa, viability

INTRODUCTION

Bali cattle are native cattle in Indonesia which are the result of direct domestication of wild banteng. Bali cattle have a fairly varied production performance and high reproductive capacity (Hikmawaty et al., 2014). Bali cattle breed either by natural mating or through the application of artificial insemination technology.

Artificial Insemination (AI) is a technology that can provide opportunities for superior males to maximize their offspring. The use of males in natural mating is less effective in increasing the livestock population, because each ejaculation can only fertilize one female. The advantages achieved in the AI program include improving genetic quality, efficient use of bulls, opening up opportunities to use superior bulls widely, preventing disease transmission, reducing excessive physical disturbances to female cows during mating, and saving costs (Hoesni, 2015). The implementation of

artificial insemination (AI) program activities in the field is urgently needed for the availability of semen, both in the form of liquid semen as well as frozen semen. The production process of both types of semen requires the presence of a diluent.

The diluent is very influential on the life of spermatozoa during preservation. The diluent used for semen freezing varies widely but must meet the following requirements, including being able to provide nutrients for the needs of spermatozoa during storage, must allow spermatozoa to move progressively, be non-toxic, act as a buffer, and can protect spermatozoa from cold shock either on the semen frozen or liquid semen. In addition, the diluent must also be able to maintain the motility of spermatozoa after the preservation process (Solihati & Kune, 2009).

The use of tris egg yolk media is not only used as a diluent but also as a medium for sexing spermatozoa in Bali cattle. Sexing spermatozoa is a sex separation technology in livestock that is applied to produce calves of a certain sex (Saili et al., 2017). Based on the description on the background, it is necessary to conduct research on the quality of Bali cattle spermatozoa on egg yolk tris medium at different duration.

MATERIAL AND METHOD

Research Location and Time

This research has been carried out at the Regional Technical Implementation Unit (RTIU) of the Center for Animal Breeding and Feed, the Food Crops and Livestock Service Office of Southeast Sulawesi Province in Morome Village, Konda Sub District, South Konawe Regency. The research has been carried out for two months starting from January to February 2021.

Research Material

The materials used in this study were bovine semen obtained from a 3-year-old male Bali cattle with a body weight of ±300 kg. The feed given to the bulls was field grass as much as ± 30 kg per day (10% of body weight) and fortifying feed (concentrate) as much as 4-5 kg per day. The medium of tris egg yolk tris used as a semen diluent was composed of tris aminomethane, citric acid, fructose, and egg yolk as well as physiological NaCl. Egg yolk tris medium, apart from being used as a diluent, is also used as a sexing spermatozoa medium.

A set of artificial vaginal apparatus used to collect semen. The tube used in the sexing spermatozoa process is a test tube with a diameter of one centimeter and a height of ten centimeters. To make and store the medium used measuring cups, in various sizes. Cover glass and object glass were used to make spermatozoa preparations for various observation purposes. Haemocytometer is used as a tool to calculate the concentration of spermatozoa. In addition, water baths, timers and others are also used.

Research Design

This study used a completely randomized design (CRD) with 3 treatments and 6 replications. Data collection includes vitality, motility and abnormalities carried out at each stage of evaluation of the quality of spermatozoa. The treatments used in this study were as follows:

P1 = The Length of Sexing Duration 40 min.
P2 = The Length of Sexing Duration 50 min.
P3 = The Length of Sexing Duration 60 min.

Research Procedure

Semen collection is done 2 times a week. Bali cattle semen was collected using the artificial vaginal (AV) method. The initial stage is preparation of an artificial vagina. The Bali cattle that will be accommodated for semen are brought to a holding cage that has been provided by an angler female cow. Semen is collected when ejaculation occurs, which is characterized by a strong push from a perfectly erect penis in the artificial vagina (Apriyanti, 2012). The collected semen (fresh semen) was immediately evaluated including the volume, color, odor, consistency and pH of the semen as well as the concentration and mass movement of spermatozoa.

Semen that meets the requirements is then diluted until the concentration of spermatozoa in it reaches $200x10^6$ per ml using a physiological NaCl diluent (0.9%). The process of sexing spermatozoa begins with placing 1 ml of diluted semen onto egg yolk tris medium (2 ml) in a sexing tube. The sexing tube is mounted on a tube rack and the sexing process is carried out at room temperature (27℃) with different durations based on the treatment. After the sexing process, the upper part of the sexing medium was removed as much as 1 ml and the lower part was taken to evaluate the quality of the spermatozoa which included the percentage of viability, motility and percentage of abnormalities.

Evaluation of spermatozoa viability was carried out by dripping one drop of semen onto a glass object, then adding two drops of eosin dye and homogenizing it. Dead spermatozoa will absorb the color (red color) while the live ones will not absorb the color (transparent). Spermatozoa motility was evaluated by dripping 20 µl of diluted semen onto a glass slide and the movement of spermatozoa was observed under a microscope using a 40 x objective lens in five fields of view. Ratings are given from zero percent (no spermatozoa move forward) to 100 percent (all spermatozoa move forward).

Data Analysis

Data were collected and tabulated, then analyzed using variance. The treatments that had a significant effect on the observed variables were further tested to determine the differences between treatments using the Least Significant Difference Test (LSD test) on the IBM SPSS Statistics 24 program.

RESULT AND DISCUSSION

Characteristics of Fresh Bali Cattle Semen

Fresh semen obtained from male Bali cattle ensured to determine the semen before proceeding further. The results of the evaluation of Bali cattle semen in this study are presented in Table 1. The volume of fresh semen of Bali cattle obtained in this study was (5.38±1.80). The volume of fresh semen of Bali cattle obtained in this study was still within the normal range. The volume of semen varies between 5-8 ml for cattle, 0.8-1.2 ml for sheep, 150-200 ml for pigs, and 60-100 ml for horses (Feradis, 2010). The difference in semen volume shown is influenced by differences in each individual male, including differences in body weight and scrotal circumference (Fazrien et al., 2020). The color of Bali cattle semen obtained in this study was milky white. The color of normal bulls semen is milky white and only 10% is cream (Toelihere, 1985). Bali cattle semen obtained in this study has a characteristic odor of spermatozoa.

This indicates that the semen is normal and there is no contamination. Semen with normal conditions generally has a distinctive odor (Kusumawati et al., 2020). In addition, bulls semen is said to be normal if it is not contaminated with pus caused by infection in the reproductive tract (Setyani et al., 2017). Furthermore, if there are no other odors such as rancid and rotten, it indicates that the semen of the male Bali cattle is in clean condition and is not contaminated with feces or bacteria that cause bad odors. The consistency of Bali cattle semen obtained in this study was thick, so it was classified as good or normal quality semen. Normal semen of bulls has a medium to thick consistency (Feradis, 2010). The thicker the semen produced, the higher the concentration of spermatozoa and the darker the color (Sujoko et al., 2009). The average pH value of the semen obtained in this study was (6.3±0.08). The pH value of this semen was classified as a slightly acidic pH. Bali cattle semen has an optimum pH ranging from (5.9-7.3) (Nirwana & Suparman, 2017). So, the pH of fresh semen of Bali cattle obtained in this study can be said to be normal. The lower or higher pH value of a semen than the normal pH value can cause spermatozoa to die more quickly (Zulyazaini et al., 2016). Concentration assessment is very important because it is used to determine the amount of semen dilution. The average concentration of spermatozoa obtained in this study was 1.445±164.08 million/ml. The concentration of spermatozoa in the semen of bulls is influenced by the size of the testes and the frequency of semen collection carried out (Komariah et al., 2020). The mass motion of Bali cattle spermatozoa obtained in this study was +++ with the characteristic visible thick clouds indicating that these spermatozoa were still in the normal category.

Table 1. Average characteristics of fresh Bali cattle semen

Obervation Variable	Observation Result
Volume (ml)	5,38±1,80
Color	Milky white
Odor	Typical spermatozoa
Consistency	Thick
Acidity/pH	6,3±0,08
Concentration (million/ml)	1,445±164,08
Mass movement	+++

Spermatozoa Evaluation

The results of the evaluation of the percentage of motility, viability and abnormalities of spermatozoa in Bali cattle after sexing can be seen in Table 2. The results of the analysis of variance showed that the length of sexing duration had a significant effect ($p<0.05$) on the percentage of sperm motility in Bali cattle. Furthermore, the results of the Least Significant Difference test (LSD test) showed that the sperm motility of Bali cattle sexing in treatment P3 (60.00%) was significantly lower than the motility of spermatozoa in treatment P1

(76.67%). The results of this study indicate that the longer the sexing duration used, the lower the motility of spermatozoa. This is probably because the spermatozoa run out of energy to move down through the egg yolk tris medium layer. Spermatozoa require greater energy at every distance traveled to penetrate the highest density, while insufficient energy is available for spermatozoa and insufficient energy requirements can reduce motility (Luzardin et al., 2020). Sexing in this study aims to obtain the motility of spermatozoa that carry male sex characteristics rather than spermatozoa that carry female sex characteristics.

The sexing technology of spermatozoa makes it possible to regulate the birth of livestock according to the desired sex (Saili et al., 2017). This technology aims to answer the high demand of farmers for beef calves or calves because the selling price is higher when compared to female calves. The motility of spermatozoa below 40% indicates a poor semen value, because the range of sperm motility percentage suitable for fertilization is 50-80% of progressively motile active spermatozoa (Feradis, 2010). Individual motility in spermatozoa becomes very important, because it is very influential in the process of transporting spermatozoa while in the female reproductive tract. In addition, the motility of individual spermatozoa is also very influential in the ability of the fertilization process (Setyawan et al., 2019).

The results of the analysis of variance showed that the length of sexing duration treatment had a significant effect ($p<0.05$) on the viability of spermatozoa in Bali cattle after sexing. This is probably because the sexing medium used contains egg yolk tris where the egg yolk media contains lipoproteins and phospholipids that can protect the integrity of the spermatozoa membranes of Bali cattle, so as to minimize the death of spermatozoa due to the sexing process. The percentage of sperm viability of Bali cattle in the P3 treatment (60 minutes) was 79.83%, a significant decrease compared to the P1 treatment (40 minutes) which was 93.42%. The decrease in the percentage of spermatozoa viability after sexing in P2 and P3 treatments in this study was probably due to damage to the spermatozoa plasma membrane after sexing treatment. The longer the sexing treatment is carried out, the damage to the plasma membrane of spermatozoa also increases. The decrease in the percentage of spermatozoa viability is caused by the condition of the plasma membrane that has been damaged (Setyawan et al., 2019). This is probably caused by the ongoing cell metabolism and changes in the environmental pH of the egg yolk tris media used. The process of spermatozoa metabolism takes place continuously which will result in the accumulation of lactic acid. The greater the amount of lactic acid, there will be an increase in membrane damage, thereby reducing metabolic processes which will affect the energy produced. The accumulation of lactic acid will inhibit the metabolic process and the respiration process of spermatozoa so that it will decrease viability more quickly and experience death. Increased damage to the spermatozoa membrane will decrease the metabolic process so that the energy produced will decrease (Hafez, 2004).

The results of the analysis of variance showed that the length of sexing duration treatment had no significant effect ($p<0.05$) on the percentage of spermatozoa abnormalities in Bali cattle. The number of spermatozoa abnormalities obtained in this study ranged from 2.58-2.67% with a general average of 2.64%. This value is below the abnormality rate required in the AI program, which is 10%. The percentage of spermatozoa abnormalities below 10% is in accordance with the Indonesian National Standard for spermatozoa that are still suitable for use in AI (Ghopa et al., 2019) and spermatozoa abnormalities should not exceed 20% (Susilawati, 2013). Spermatozoa abnormalities are structural abnormalities of spermatozoa from normal structures which can be caused by several factors, namely environmental, genetic or a combination of both (Chenoweth, 2005).

Table 2. Average percentage value of motility, viability and abnormality of spermatozoa in Bali cattle after sexing

Variable	Treatment			General Average
	P1	P2	P3	
Motility	76.67^{a}±5.16	68.33^{ab}±7.53	60.00^{b}±8.94	68.33±9.85
Viability	93.42^{a}±4.42	84.42^{ab}±6.40	79.83^{b}±5.62	85.89±7.80
Abnormality	2.58±0.74	2.67±1.33	2.67±1.40	2.64±1.12

Note Numbers followed by different letters in the same row indicate significant differences ($p<0.05$), P1 = the length of sexing duration 40 min, P2 = the length of sexing duration 40 min, P3 = the length of sexing duration 40 min

CONCLUSION

It can be concluded that the length of sexing duration has a significant effect on the motility and viability of spermatozoa, but not significantly on the spermatozoa abnormalities of Bali cattle. The highest motility rate was obtained at 40 minutes of the length of sexing duration, while the highest viability was obtained at 40-50 minutes.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the article.

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