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The quality of Bali bull sexed sperms at different incubation time using egg white sedimentation method

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Abstract. The success rate of Artificial Insemination (AI) can be increased by using semen from superior bulls of the desired sex. For meat production purposes, sexed semen used is the male sex, but for milk production, then the female sex is used. This study aimed to determine the effect of incubation time for sexing the sperms using egg white albumin sedimentation method on the quality of Bali bull semen. This study was using a completely randomized design (CRD) with three different incubation time treatments; 25 (T1), 30 (T2) and 35 minutes (T3) with three replications. T0 was used fresh semen as a control without incubation time. The variables measured in this study were the motility and concentration of the sperms before and after sexing. The results of this study showed that the incubation time did not affecting the sperms motility and concentration ($P > 0.05$). However, motility of the X sexed sperms in T2 is likely higher than in T1 and T3. Contrary, motility of Y sexed sperms in T1 had higher than in T2 and T3. It can be concluded that the incubation time during separation of X and Y sperms using egg albumin sedimentation had no significant effect on sperms motility and concentration.

1. Introduction

Efforts to increase the population of Bali cattle in Indonesia are still continue in various ways, one of them is the application of reproductive technology particularly the use of artificial insemination (AI) in cattle. This AI is a reproductive technology that is most widely applied in Indonesia to accelerate the growth rate of the cattle population. Several things that can affect the success rate of AI include the skills of the



inseminator, detection of heat, the condition of the female reproductive organs and the quality of the semen used. The success rate of AI is greatly influenced by the quality of semen to be used [1].

The success rate of AI can be increased by using semen from superior bulls of the desired sex. For meat production purposes, sexing semen is used with the male sex, but for milk production, then the female sex is used. The selection of semen with different sex can be chosen when it is available. The availability of sexed sperms is only present when the producer separated the sperms before using a certain method.

One method of sexing spermatozoa that can be used is the sedimentation of egg white albumin [2]. This method is based on differences in the motility of spermatozoa X and Y as an implication of the difference in mass and size of spermatozoa Y which is smaller than spermatozoa X, so that Y sperms move faster or have high penetration power to enter a solution such as egg albumin (egg white) [3]. This method is very commonly used because the materials used are relatively cheaper and the process is easier. Garner and Hafez [4] stated that the success rate of sexing spermatozoa can be influenced by the medium used and the incubation time. Therefore, the aim of this study was to determine the effect of incubation time for sexing the sperms using egg white albumin sedimentation method on the quality of Bali bull semen.

2. Materials and methods

2.1. Materials

This study was conducted at the Samata Integrated Farming System (SIFS) for semen collection and the Laboratory of Animal Reproduction; semen processing unit, Faculty of Animal Science, Hasanuddin University, Makassar. In the present study, one Bali bull was used to collect the semen twice a week. The bull was housing in the barn all the time, except after semen collection, the bull was exercise outside the barn. The equipment used in the study consisted of artificial vagina, micropipettes, electric scale, microscope with Computer-Assisted semen analysis (CASA) software, and spectrophotometer. The other supporting materials were vaseline, 70% alcohol, litmus paper, andromed, aquabidest, egg yolk, egg albumin, tris (Hydroxymethyl aminomethane), penicillin, streptomycin, glucose, and citric acid.

2.2. Methods

2.2.1. Preparation of extender. Tris egg yolk (TEY) was used as an extender after collecting and evaluating the semen. Extender preparation was performed by weight out 1,422 gr of tris (Hydroxymethyl aminomethane), 1,500 gr of lactose, and 0.677 gr of citric acid, then put into a volumetric flask and add 80 ml of aquabidest. The materials were homogenized for 15 minutes using vortex-mix. All the materials that have been homogenized were heated to a boil and then cooled to room temperature.

The next step was to prepare 20 ml of egg yolk which has been removed from the vitellin membrane. Then mix with 80 ml of tris solution that has been made previously with egg yolk, then stir for 30 minutes until homogeneous. Furthermore, 0.100 g of penicillin and streptomycin were added, then homogenized for 15 minutes.

2.2.2. Collection of semen. Collecting semen was carried out in the morning twice a week using an artificial vagina. For collecting the bull semen, a cow teaser was used to increase the libido of the bull. The teaser was placed in the collecting semen place before the bull approaching the teaser.

2.2.3. Evaluation of semen. The quality of semen was examined macroscopically including volume, color, pH, and viscosity. Furthermore, microscopic evaluation was conducted for concentration, mass, individual and progressive motilities.

2.2.4. Sexing the sperms. The fresh semen that has been obtained during collection was subjected to macroscopic and microscopic examination, then diluted with TEY in a ratio of 1: 1 [5]. The next step was to make a sexing medium consisting of upper and lower fractions. The medium fraction was prepared by mixing egg whites at 10% (top fraction) and 30% (bottom fraction) into the TEY extender. Furthermore, three medium fractions according to the incubation time treatment was made; 25 (T1), 30 (T2), and 35 minutes (T3), respectively. T0 was used fresh semen as control without incubation time. Each treatment medium consisted of upper and lower fractions of 2 mL each to form a gradient with a total volume of 4 mL. For each medium that has been made fractions, then 1 mL of diluted semen was added and finally, it consisted of three layers with the total volume of each medium was 5 mL. After making the medium in three layers, then incubated according to the treatment.

After the incubation period was completed, the three layers were taken out with a micropipette and transferred to different tubes of 1 mL (top layer), 2 mL each (middle and bottom layer). Furthermore, each of these layers was subjected to centrifugation at 2500 rpm for 5 minutes. The result of supernatant centrifugation was discarded and the prescription part was then added with TEY extender until it reaches 2 ml. Furthermore, observations were made to measure the motility and concentration of sexed sperms.

3. Data analysis

The data obtained were analyzed using SPSS with a completely randomized design (CRD). The Least Significant Difference Test (LSD) was conducted to determine whether or not the difference was significant in each treatment.

4. Results and discussion

4.1. The quality of Bali bull fresh semen

In table 1 shows that fresh semen from Bali bull used in this study had a good category and met the requirements for use. This is in accordance with the opinion of Garner and Hafez [4] which stated that the volume of bull semen ranged from 5-8 mL/ejaculate. Normal (fertile) bulls should have individual motility of 40% - 75% progressively active sperms, concentrations of more than 2000 million per ml of semen and mass movement ++ / +++ [6].

Table 1. The quality of Bali bull fresh semen.

Parameter	Score
Volume (ml/ejaculate) (\pm SD)	6.63 \pm 1.11
Color	White – Cream
Consistency	Slightly thick - Thick
Acidity pH (\pm SD)	6 \pm 0.00
Massa Motility	Very Good (+++)
Individual Motility (%) (\pm SD)	83.67 \pm 3.72
Concentration (10^9 /mL) (\pm SD)	1.610 \pm 0.550
Viability (%) (\pm SD)	81.30 \pm 6.19

4.2. Motility of Bali bull sexed sperms

The motility of Bali bull sexed sperms, both X and Y sperms that incubated at different times are shown in table 2. The motility of the sperms at different treatments (T1, T2, and T3) did not show any significant difference ($P > 0.01$). However, the motility of the X sexed sperms in T2 is likely higher than in T1 and T3. Contrary, the motility of Y sexed sperms in T1 had higher than in T2 and T3.

Based on table 2, it can be seen that the percentage of spermatozoa motility decreases due to spermatozoa metabolism that occurs during the incubation process. This is in line with the study of Susilawati [6,7], which stated that the average motility of spermatozoa resulting from separation has decreased compared to the motility of spermatozoa before separation or fresh semen. This decrease in the percentage of motility is reasonable because spermatozoa have undergone treatment starting from the washing process which requires a lot of energy to maintain physiological conditions. The energy needed by sperm during the sexing process comes from the process of cell metabolism which produces ATP (adenosine triphosphate). Furthermore, Afiati [1] stated that the energy used for the movement of spermatozoa is stored in the form of ATP compounds.

Table 2. Motility of Bali bull sexed sperms at different incubation time.

Motility (%)	Treatment			
	T0	T1	T2	T3
Spermatozoa X	88.74 ± 6.11 ^a	61.47 ± 8.95 ^b	67.55 ± 10.23 ^b	59.96 ± 3.28 ^b
Spermatozoa Y	88.74 ± 6.11 ^a	58.48 ± 7.26 ^b	45.68 ± 11.56 ^b	53.20 ± 6.73 ^b

^{a,b} Different superscripts that follow the numbers in the same column show significantly different ($P < 0.05$).

4.3. Concentration of sperms after sexing

Based on the data analysis, it shows in table 3 that the incubation time treatment had no significant effect ($P > 0.01$) on the sperms concentration after sexing.

Table 3. Percentage of sperms concentration after sexing.

Concentration (10^9 /ml)	Treatment			
	T0	T1	T2	T3
Spermatozoa X	0.348 ± 0.14	0.379 ± 0.03	0.426 ± 0.09	0.420 ± 0.07
Spermatozoa Y	0.348 ± 0.14	0.615 ± 0.25	0.393 ± 0.07	0.623 ± 0.16

Table 3 shows that there was a decrease in spermatozoa concentration in all treatments when compared to fresh semen before incubation. This decrease indicates that the spermatozoa separation process has occurred in the two medium fractions. Some spermatozoa were in the upper fraction layer and some were able to penetrate to the lower fraction layer. Spermatozoa that cannot penetrate the two medium fractions will remain on the surface of the tube. This is in accordance with the opinion [8] that the ratio of the total concentration of fresh semen before and after separation is decreased, this is due to the presence of sperm left in the column and some of it is wasted when removing the media between the upper and lower fractions.

5. Conclusion

This study concluded that the incubation time during the separation of X and Y sperms using egg albumin sedimentation had no significant effect on sperms motility and concentration.

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