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Sperms motility, viability, and abnormality of the frozen semen at different bull breeds

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Abstract. The quality of semen is one of the most important things in the reproductive process. The aim of this study was to determine differences in the quality of frozen semen at various breeds of bull. This study was conducted for two months from May to June 2020, at the Laboratory of Animal Reproduction, Faculty of Animal Science, Hasanuddin University. The materials used in the study were frozen semen of bull at different breeds; Angus (AG), Brahman (BM), Fries Holland (FH), Simmental (SM), Limousine (LM), and Bali (BL), that were provided from the Livestock and Animal Health Services of South Sulawesi Province. Supporting materials were warm water at 40°C, alcohol 70%, liquid nitrogen. A completely randomized design one-way ANOVA using six treatments and three replications was used in the present study. The treatments in this study were a different breed of bulls. The results of this study showed that sperms' motility at different breeds of the bull differed significantly ($P < 0.05$). Sperms motility of AG and BM had significantly ($P < 0.05$) higher than sperms motility of FH and BL (58.8% and 56.8% vs. 41.1% and 44.0%), and had a tendency higher than in SM and LM (59.7% and 49.2%). The viability of the sperms among breeds had relatively similar each other, ranging from 46.9% to 60.8%, however, BL viability had significantly ($P < 0.05$) lower than the other breeds. Likewise, sperms abnormality was relatively similar among breeds, however, the highest abnormality was found in BL. It can be concluded that there were differences in motility, progressive motility, viability, and abnormality of frozen semen among the different bulls breed; in which Simmental had better quality than the other breed.

1. Introduction

Animal husbandry products are one of the most important in world agriculture, most of the world's population needs meat, fat, milk, dairy products, eggs, and wool, therefore livestock products need to be continuously increased [1]. Increasing livestock production requires various technologies and the integration of several other agricultural elements [2]. Artificial Insemination (AI) in dairy cows has been carried out in the Asia Pacific more than half a century ago and currently, it is predicted that more than 75% of dairy cows have used this reproductive technology. AI which is carried out continuously using



superior bulls' semen will increase the productivity of cattle, but using AI can also eliminate the nature of local livestock and reduce calving interval [3].

Artificial insemination in practice consists of semen collection, freezing, and deposition of semen on the female reproductive organs, thus allowing the sperms to fertilize the ovum as natural processes [4]. Frozen semen experience damage to the plasma membrane and acrosome membrane of the sperms, resulting in the death of sperms around 20-80% or an average of 50% [3]. The breed, age, environment, and rearing system of the bulls will affect the quality of semen, as well as the method of extending and freezing will affect the quality of semen after freezing [5]. The quality of semen determined by the Indonesian National Standard (SNI) [6] for fresh semen that can be frozen is >70% and the percentage of motility is >2+. The Ungaran Regional Artificial Insemination Centre produces frozen semen from various breeds of cattle, so the existing data can be used to determine the quality of frozen semen in various beef cattle breeds. Semen quality is one of the most important things in the reproductive process. The success rate of AI is determined by several factors, one of them is the quality of the semen used. One of the factors that influence the quality of semen is the breed of the bull.

The distribution of frozen semen from the National Artificial Insemination Centre to the Provinces as well as to the regency/city must also receive proper attention to handling the semen, including monitoring the level of liquid nitrogen in the container in order to maintain the quality of semen during transportation or storage. Komariah et al. [7] stated that color, volume, pH, consistency, individual motility, mass motility, and concentration of the sperms varied widely. This is influenced by the health condition of the bulls, age, environmental conditions, management, the type of feed, and the breed of cattle used. This study aimed to determine the differences in the quality of frozen semen in various breeds of bulls. The purpose of this study was as a development material in the advancement of science and technology, information material for cattle breeders and inseminators in developing cattle breeding businesses, and information material for researchers and related institutions regarding the characteristics of the motility, viability, and abnormalities of spermatozoa from various breeds of bulls.

2. Materials and methods

2.1. Study period and materials

The study was conducted in the Laboratory of Animal Reproduction, Faculty of Animal Science Hasanuddin University, Makassar for a period of two months from May to June 2020. The frozen semen from different bull breeds were used in the study. They were Angus (AG), Brahman (BM), Fries Holland (FH), Simmental (SM), Limousine (LM), and Bali (BL) which were provided by the Animal Livestock and Animal Health Services of South Sulawesi Province. The other supporting materials used in this study were Computer-Assisted Sperm Analysis (CASA) Sperm Vision Ver 3.7.5 (LG®) ZEISS, micro-tube 1.5 mL, trinocular microscope, micropipette, tip, glass object, cover glass (Onelab®), container straw, warm plate (minitube®), cutter straw (minitube®), tweezers. Warm water with a temperature of 40°C, tissue, label paper, and liquid nitrogen was also used.

2.2. Study design

This study was arranged for six treatments and three replication. The treatments were different frozen semen from bull breeds; Angus (AG), Brahman (BM), Fries Holland (FH), Simmental (SM), Limousine (LM), and Bali (BL). The origin of frozen semen was the same place, management for rearing, feeding system, and environment.

2.3. Procedure and parameter of the study

The straw of frozen semen from different bulls was thawed using a thawing device at a temperature of 37°C for 30 seconds. The straw was then dried with a tissue, followed by cutting both ends of the straw and placed in a micro-tube. The semen was then placed on a warm plate with a temperature of 37°C before subjected to the microscopic evaluation. Microscopic assessment of semen included motility, viability, and abnormalities.

The motility of the sperms was calculated by first making the semen spot of 10 μ L on the glass object. The sperms were then analyzed using CASA with the Sperm Vision Version 3.7.5 program by observing 6 times the field of view. The viability of sperms was carried out by evenly mixing 20 μ L of semen and 20 μ L of Eosin 2% over the glass object, smeared, and observed using a trinocular microscope. The dead spermatozoa will absorb red color and the viable sperms were colorless, at least 200 sperm cells per observation were counted to determine the percentage of viable sperms. The percentage of viable sperms was calculated using the formula below.

$$A = [P/(P+Q)] \times 100\%$$

A = Percentage of viable sperms

P = Number of viable sperms

Q = Number of dead sperms

The sperms abnormality was observed using the same glass object that was used for the viability parameter. The observed abnormal sperms such as an abnormal head, body, and tail in which at least 200 sperm cells per observation were counted to determine the percentage of abnormal sperms under a trinocular microscope. The percentage of abnormal sperms was calculated using the formula below.

$$X = [Y/(Y+Z)] \times 100\%$$

X = Percentage of abnormality sperms

Y = Number of abnormal sperms

Z = Number of normal sperms

2.4. Data analysis

Data collected in this study were tabulated and analyzed in the Excel program for windows. This study was designed using a completely randomized design with 6 treatments at different bull breeds and three straws as a replication. One-way ANOVA was used to differentiate the treatments, followed by least significance difference analyses at significant treatment. A significant difference was considered if the probability value was less than 0.05 ($P < 0.05$).

3. Results and discussion

3.1. Motility and progressive motility of the sperms at different breeds of bull

Table 1 shows the motility and progressive motility of the sperms from six different semen straw breed bulls. It shows that the motility and progressive motility of FH and BL breeds semen had the lowest value which is significantly different ($P < 0.05$) compared to other semen bull breeds. Based on the results of the study, the motility of SM was 59.7%, AG 58.8%, BM 56.8%, LM 49.2%, BL 44.0%, and FH 41.1%, respectively. The progressive motility of AG was 45.9%, SM 45.5%, BM 39.6%, LM 38.3%, BL 34.4% and FH 31.6%, respectively. This is in accordance with the study of Mostari et al. [8] stated that the percentage of sperms motility in different breeds of bulls shows a significant difference. Coulter et al. [9] also stated that the difference in sperms motility can be caused by different heritability and repeatability.

Table 1. Motility and progressive motility of the sperms at different breeds of bull.

Parameter	Breed					
	AG	BM	FH	SM	LM	BL
Motility (%)	58.8 ^{ac}	56.8 ^{ac}	41.1 ^b	59.7 ^c	49.2 ^{ab}	44.0 ^b
Progressive motility (%)	45.9 ^{ac}	39.6 ^{ab}	31.6 ^c	45.5 ^b	38.3 ^c	34.4 ^c

^{abc}Superscripts at the same row indicate differed significantly ($P < 0.05$).

Angus (AG), Brahman (BM), Fries Holland (FH), Simmental (SM), Limousine (LM), and Bali (BL).

The motility of various breeds of bulls used in the present study is possible to be inseminated because it has a value above 40%. This is in accordance with SNI [6], which states that in order to distribute and inseminate, the percentage of post-thawing sperm motility must be at least 40%.

3.2. Viability of the sperms at different breeds of bull

The viability of the sperms at different breeds of the bull in the present study is shown in figure 1. The viability of Bali bull sperms had significantly ($P < 0.05$) lower in comparison to the other bull sperms. A good percentage of viability was found in Simmental, Brahman, Angus, Limousine, and FH. This is in accordance with the statement of Toelihere [10], that the standard of good frozen semen, which has a viability percentage of more than 50%. Partodiharjo [11] stated that immotile sperms are not necessarily categorized as dead so they do not absorb the color. Spermatozoa that live and do not move, accompanied by defects in the cell wall can absorb color so that under the microscope it is considered dead while other interpretations are considered alive.

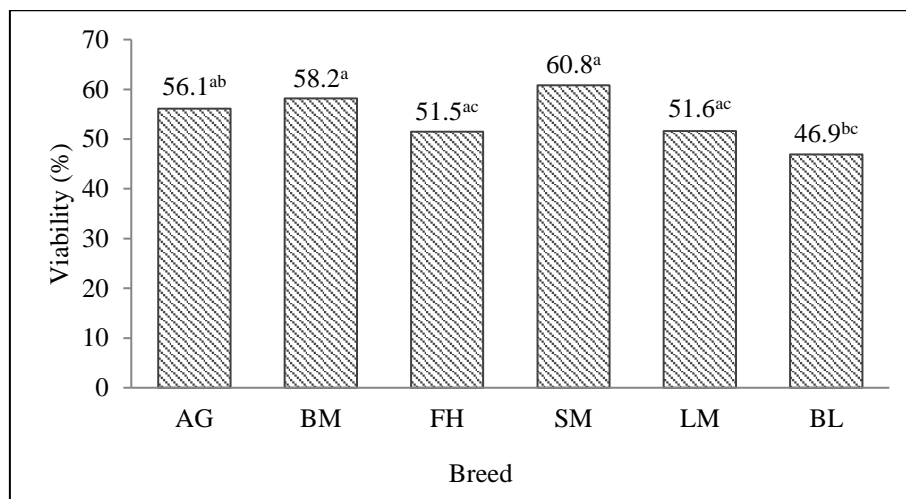


Figure 1. Viability of the sperms at different breeds of bull. ^{abc}Superscripts at the same row indicate differed significantly ($P < 0.05$). Angus (AG), Brahman (BM), Fries Holland (FH), Simmental (SM), Limousine (LM), and Bali (BL).



Figure 2. The difference between dead and viable sperms, (a) dead; (b) viable.

In figure 2, it can be seen that the heads of the dead sperm are colored while the heads of the viable sperm are colorless. This is in line with the statement of Mulyono [12] which stated that sperm that is dyed or colored red means that the sperm is dead, while those that are not colored or are not stained means that sperm is alive. The discoloration of the dead sperms is caused by the damage to the plasma membrane in the spermatozoa so that the dye in the eosin is absorbed by the spermatozoa.

3.3. Abnormality of the sperms at different breeds of bull

The abnormality of the sperms at different breeds of the bull in the present study is shown in figure 3. Abnormality in Bali bull semen had significantly ($P < 0.05$) higher compared to other bull breeds semen. The difference in sperms abnormalities is thought to be due to genetic factors in a breed. Basically, the preservation of semen in liquid nitrogen did not affect the abnormality of the sperms. A study by Malik et al. [13] stated that for storage up to 3 years, the sperms abnormality did not differ, however, the abnormality increased significantly from 4 to 6 years storage.

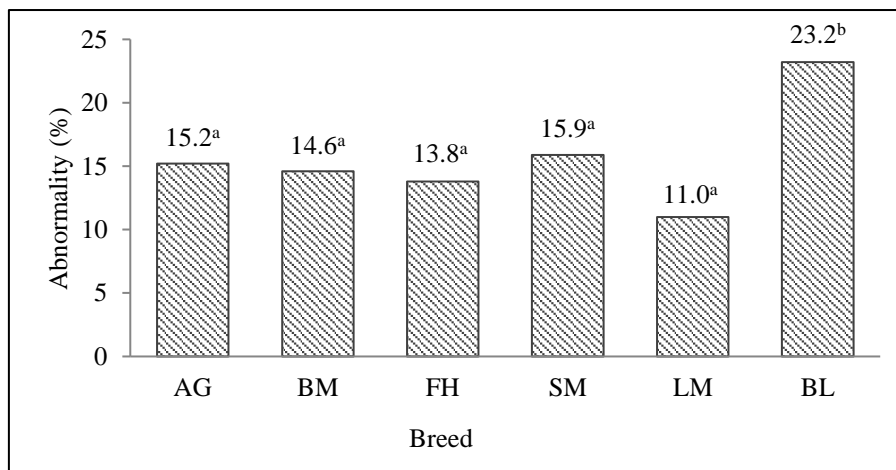


Figure 3. Abnormality of the sperms at different breeds of bull.
^{abc}Superscripts at the same row indicate differed significantly ($P < 0.05$).

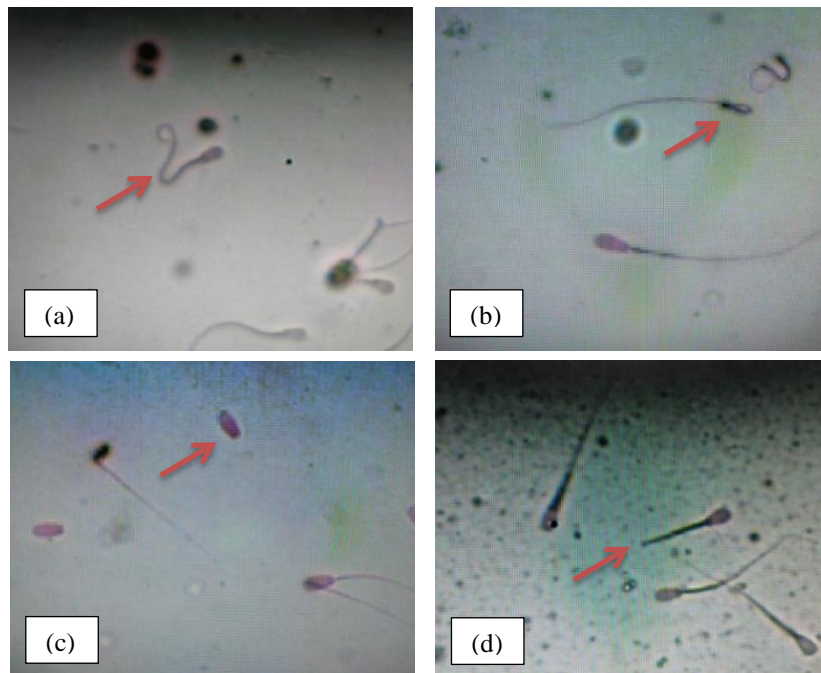


Figure 4. Sperms abnormalities; (a) Curved bodies; (b) Small head; (c) The head is cut off; (d) The tail is cut off.

Figure 4 showed some of the abnormalities in spermatozoa morphology (arrows) found in this study, such as primary abnormalities; curved spermatozoa bodies, and small spermatozoa heads. As stated by Toelihere [10], that the primary abnormality includes a head that is too large (macrocephalic), a head that is too small (microcephalic), a short head widened, flat elongated and piriformis; double head, double tail; the middle part folds, bends, enlarges, piriformis; or linked abaxially at the base of the head; and a coiled tail. Secondary abnormalities were also found, such as the severed head of the spermatozoa and the tail of the spermatozoa. It is in line with Toelihere [10] that secondary abnormalities include a broken tail, a head without a tail, a folded center, the presence of proximal or distal protoplasmic granules, and detached acrosomes. Furthermore, preservation could also be one of the causes of sperms abnormality [16]. Therefore, it is necessary to maintain the quality of the semen in order to reduce the abnormalities of the sperms by introducing an active compound in the extender [17].

4. Conclusions

Based on the results and discussion, it can be concluded that there were differences in motility, progressive motility, viability, and abnormality of frozen semen among the different bulls breed; in which Simmental had better quality than the other breed.

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