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Characteristic of Bali Bulls Sperms Assessed Using Computerized Assisted Semen Analysis (CASA)

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Abstract

The objective of this study was to know the characteristics of Bali bull sperms assessed using computerized assisted semen analysis (CASA). Three selected Bali bulls aged 3-5 years and one Bali cow as a teaser was used in the study. The study was conducted in university farm and Laboratory of Animal Reproduction Faculty of Animal Science Hasanuddin University, Makassar, Indonesia from May – June 2015. The bulls were confined in tie-stall barn for 24 hours, except several hours for exercise at the day of semen collection. Semen collection was conducted twice a week using artificial vagina and examined macroscopically and microscopically. Concentration of the sperms was measured using photometer SDM5. Mass motility, viability and abnormality were observed in the slide under microscope. CASA was used to characterize the DAP, DCL, DSL, VAP, VCL, VSL, STR, Straightness (VSL/VAP), LIN, Linearity (VSL/VAP), WOB, Wobble (VAP/VCL), ALH, and BCF of the sperms. The results of this study showed that there was no difference concentration of the sperms was found among the three bulls ($803.6x10^6 \pm 109.2x10^6$ cells per mL). Viability of the fresh semen was 74.7% in average. Bull A had higher ($P<0.05$) viability in comparison to bull B (79.3% vs. 74.9%). The average of sperms abnormalities at different bulls was 8.8%. Bull C had higher the incidence of sperms abnormalities in comparison to bull A and B (11.11% vs. 7.71% and 7.51%).

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The average (±SD) of distance and velocity achieved by the sperms for DAP, DCL, and DSL were 22.00±3.76 µm, 39.14±8.58 µm, and 14.84±1.81 µm, respectively, VAP, VCL and VSL were 52.43±8.24 µm/sec. 92.84±19.4 µm/sec. and 35.48±3.81 µm/sec., respectively. In conclusion, the VCL value of Bali bulls' semen was 92.84±19.4 µm/sec. and can be categorized as non-hyperactive; approaching hyperactive.

Keywords: Bali bull; Sperms; CASA; fertility.

1. Introduction

A normal spermatozoon is a critical point to achieve high fertility in cattle breeding farms as well as the use of artificial insemination (AI) for breeding program. So far, AI has widely been used in livestock industry as one of the methods for mating as well as to improve the animals genetic. For this AI program, the quality of sperms is a critical factor in determining success rate of AI [1]. Therefore, it is necessary to assess the sperms including concentration, motility, and morphology as basic parameters before being use for AI or in vitro fertilization. Generally, a criterion in selecting semen at the most of AI station over the world is motility of the sperms, concentration and morphology [2]. This criterion has become a common use at any AI station to assess the sperms for subsequently determining whether the semen would be processed or not for freezing for future use for AI.

Basically, the quality of semen depends upon the genetic of the bulls and environment as well as the interaction between genetic and environment. Furthermore, the quality of semen is differed at different bulls, age, breed, environment, etc. Therefore, to achieve high performance and successful of breeding program by the use of AI technology is required high quality semen. Experientially, bull semen including Bali bull does not continuously have good fertility at each ejaculation, even in a good management applied. Several ejaculations during his life has poor quality of semen that caused by variety of problems. Therefore, assessing semen periodically is one of the methods to prevent the use of poor quality semen as well as to achieve high success rate of AI program.

In South Sulawesi Province, Indonesia, the most cattle breed raised by the farmers is Bali cattle [3]. These Bali cattle have long been traditionally spread out through the region as one of the farmers' activities to increase their income. One factor in maintaining their effort to raise these cattle depends upon the fertility of both bulls and cows. Several studies in this region have shown variety the fertility of Bali cattle with unknown causes [4,5]. Therefore, we hypothesized that low fertility at some of Bali bulls is one of the main causes. In order to prove the problems, it is necessary to characterize the Bali bulls' semen. One of methods for assessing the semen is by the use of computerized assisted semen analysis (CASA).

CASA) is a powerful tool for the objective assessment of sperm motility and it is frequently used for evaluating the quality of semen from man and animals [6]. This quantitative evaluation is based on kinematic parameters of individual spermatozoa that are reconstructed merely from the movements of their heads [7]. Therefore, the objective of this study was to know the characteristics of Bali bull sperms that assessed using CASA.

2. Materials and Methods

2.1. Bali bulls and herd management

This study was conducted in university farm and Laboratory of Animal Reproduction Faculty of Animal Science Hasanuddin University, Makassar, Indonesia from May – June 2015. A total of three selected Bali bulls (A, B, C) aged 3-5 years and one Bali cow as a teaser were used in the study. The bulls were confined in tie-stall barn for 24 hours, except several hours for exercise at the day of semen collection. All bulls were fed with natural grass or rice straw and 4kg of concentrate per day with protein content of 16%.

2.2. Collection and measurement of fresh semen

Semen collection was conducted twice a week (Monday and Thursday) during a period of two months using artificial vagina. After collection, the semen was macroscopically and microscopically examined. The volume and pH of semen were recorded soon after the collection. The color of semen was observed with naked eye. The consistency of semen was observed by inclining and moving the collection vial [8]. Concentration of the sperms was measured using photometer SDM5. Mass motility was observed in the slide under microscope. Likewise, Viability and abnormality of the sperms were measured in the slide covered glass under microscope.

2.3. CASA analysis

The sperms movement of fresh semen of 20µL was microscopically visually using CASA and repeated five times. Parameters measured in this stage were the distance and motility which consisted of DAP = Distance Average Path; DCL = Distance Curvilinier; DSL = Distance Straight Line; VAP = Velocity Average Path; VCL = Velocity Curvilinier; VSL = Velocity Straight Line; STR = Straightness (VSL/VAP); LIN = Linearity (VSL/VAP); WOB = Wobble (VAP/VCL); ALH = Amplitude of Lateral Head Displacement; BCF = Beat Cross Frequency.

2.4. Statistical analyses

All data obtained were presented as average ± standard deviation (SD). Concentration of the sperms, motility, progressive motility, viability, and abnormality were compared using ANOVA. Likewise, differences in DAP = Distance Average Path; DCL = Distance Curvilinier; DSL = Distance Straight Line; VAP = Velocity Average Path; VCL = Velocity Curvilinier; VSL = Velocity Straight Line; STR = Straightness (VSL/VAP); LIN = Linearity (VSL/VAP); WOB = Wobble (VAP/VCL); ALH = Amplitude of Lateral Head Displacement; BCF = Beat Cross Frequency were also compared using ANOVA. All calculations were performed with the help of statistical software package (SPSS 15 for Windows).

3. Results and Discussion

Macroscopic and microscopic characteristic of Bali bull semen are shown in Table 1 and 2.

Table 2 shows the microscopic characteristic of three Bali bulls semen. The average concentration (±SD) of sperms for the three bulls was $803.6x10^6 \pm 109.2x10^6$ cells per mL. There was no difference concentration of the sperms was found among the three bulls. This indicated that the bulls occupied in the present study were relatively similar. Likewise, mass movement of the sperms among the bulls was also relatively similar (Table 2).

Table 1: Macroscopic characteristic of the sperms at different Bali bulls semen.

Parameter		Bull		
		A	B	C
Volume (mL)		3.07	3.62	3.18
Color (%)				
a.	Creamy	72.7	96.0	100.0
b.	White-creamy	27.3	4.0	0
Consistency (%)				
a.	Thick milky	66.7	88.5	90.9
b.	Slightly thick	23.8	-	9.1
c.	Watery	9.5	11.5	-
pH		7	7	7

Table 2: Microscopic characteristic of the sperms at different Bali bulls semen

Parameter		**Bull**		
		A	**B**	**C**
Concentration ($x10^6$/mL)		817.7	688.0	905.1
Mass motility (%)				
a.	+++	78	68	71
b.	++	22	12	24
c.	+	6	20	5

It has been stated that sperm motility is the single most important criterion in determining fertilization rates, and normal sperm motion characteristic parameters are imperative to determine the successful fertilization of tested spermatozoa [1].

In the present study, the average of fresh semen motility of the three bulls was 86.7% (Table 3). There was no difference (P=0.795) among the three bulls. Likewise, progressive motility did not differ (P=0.789) among the bulls with average of 68.5%.

Table 3: Motility and progressive movement of the sperms at different Bali bulls semen.

Parameter	**Bull**			**Average**	**P Value**
	A	**B**	**C**		
Motility					
Average (%)	89.4	87.1	84.6	86.7	=0.795
Standard Deviation	7.6	12.7	19.2		
Confidence interval 95%	82.3 - 96.5	78.6 - 95.6	71.7 - 97.5		
Minimum	73.6	60.1	35.2		
Maximum	96.5	97.7	97.4		
Progressive motility					
Average (%)	72.3	69.1	65.4	68.5	=0.789
Standard Deviation	13.5	19.3	25.9		
Confidence interval 95%	82.3 -96.5	56.2 - 82.1	47.9 - 82.8		
Minimum	45.2	28.5	9.8		
Maximum	83.7	90.2	89.9		

Table 4: Viability and abnormality of the sperms at different Bali bulls semen.

Parameter	Bull			Average	P Value
	A	B	C		
Viability					
Average (%)	79.3[a]	70.0[b]	74.9[ab]	74.7	=0.064
Standard deviation	3.1	0.5	1.3		
Confidence interval 95%	65.7-92.8	67.8-72.2	58.1-91.7		
Minimum	75.6	69.3	73.6		
Maximum	85.5	70.9	76.2		
Abnormality of the sperms					
Average (%)	7.71[a]	7.51[a]	11.11[b]	8.8	=0.025
Standard deviation	1.51	2.61	4.45		
Confidence interval 95%	6.63-8.80	5.64-9.37	7.92-14.29		
Minimum	5.75	3.85	4.35		
Maximum	10.20	11.49	19.30		

[a,b] within a rows, differed significantly (P<0.05).

Table 4 shows viability and abnormality of fresh semen of the three Bali bulls. Viability of the fresh semen was 74.7% in average. The viability of the three Bali bulls differed significantly (P=0.064). Bull A had higher (P<0.05) viability in comparison to bull B (79.3% vs. 74.9%). However, there was no significant difference

between bulls B and C (70.0% vs. 74.9%).

Generally, it is necessary to have high viability of the sperms during collecting semen, processing semen for extending and freezing, as well as at the time of thawing for inseminating the cows. The importance of sperms viability is related to bull fertility; ability of the sperm to fertilize and activate ovum in supporting early embryogenesis [9]. Furthermore, they stated that adequate number of sperms produced with normal motility and morphology, several bulls are also affected by lower fertility whereas the molecular mechanism has not been well understood. Consequently, it would affect the reproductive performance of the bulls.

Reproductive performance and fertility of the bull is affected by many factors, including testicular growth, quality of the plasma semen, mating ability, and physical [10]. Direct impact that could be observed the factors mentioned above is abnormality of the sperms morphology. These abnormalities usually occurred in the sperms cell and even up to cell death as a result of DNA damaged before and after spermatogenesis [11]. This incidence might result in development of sperms abnormality [12].

The average of sperms abnormalities in the present study at different bulls was 8.8% (Table 4). This results indicated that sperms abnormalities of the fresh semen affected by different bulls (P=0.025). Bull C had higher the incidence of sperms abnormalities in comparison to bull A and B (11.11% vs. 7.71% and 7.51%). While between Bulls A and B, the incidence of sperms abnormalities did not show any difference (7.71% vs. 7.51%). However, the rates of sperms abnormalities in the present study were still below 20% and can be categorized as normal. Therefore, this semen was further possible for freezing and subsequently for inseminating the cows.

Basically, the rate of sperms abnormalities affects the fertility of the bull in fertilization process in the reproductive tracts of the cow. However, beside this abnormality, movement pattern of the sperms is also affecting the fertility of bull especially during capasitation. Movement pattern and distance achieved by the sperms in the female reproductive tract support high fertility of the bull in order to achieve the site of fertilization and ability to fertilize the ovum.

Usually, bull fertility examined traditionally to know that a bull is possible for mating the cows. However this examination has a lot of bias in measuring the ability of the bull. In the last decades, many studies conducted and focus on the quality of semen using CASA (computer assisted semen analysis)]13], although this technology has limited [14].

Table 5 shows sperms movement pattern of the fresh semen at different Bali bulls analyzed using CASA. This movement was based on velocity and distance achieved by the sperms.

ANOVA indicated that there was no significant difference movement pattern of the sperms at different bulls, both velocity and distance achieved by the sperms. Table 5 shows that the average (±SD) of distance achieved by the sperms at difference bulls for DAP, DCL, and DSL were 22.00±3.76 µm, 39.14±8.58 µm, and 14.84±1.81 µm, respectively. While the velocity of the sperms for VAP, VCL and VSL measurements were 52.43±8.24 µm/sec. 92.84±19.4 µm/sec. and 35.48±3.81 µm/sec., respectively. Generally, there are three groups of movement pattern of the sperms; hyperactive, non-hyperactive, and transition [15]. Therefore, the results

obtained in the present study can be categorized as non-hyperactive; approaching hyperactive, whereas the VCL value was 92.84±19.4 µm/sec. (>40 µm/sec.).

Table 5: Movement patterns of the sperms at different Bali bulls semen.

Parameter	Average	SD	95 % CI	Min-Max	P Value
DAP (µm)	22.00	3.76	20.58 - 23.44	13.88-27.7	=0.865
DCL (µm)	39.14	8.58	35.88 - 42.40	21.35-51.26	=0.822
DSL (µm)	14.84	1.81	14.15 - 15.52	11.29-17.87	=0.883
VAP (µm/sec.)	52.43	8.24	42.29 - 55.56	35.7-64.03	=0.899
VCL (µm/sec.)	92.84	19.4	85.47 - 100.21	53.65-119.86	=0.838
VSL (µm/sec.)	35.48	3.81	34.03 - 36.93	28.7-41.92	=0.936
STR (VSL/VAP)	0.68	0.05	0.66 - 0.69	0.64-0.81	=0.698
LIN (VSL/VCL)	0.39	0.06	0.37 - 0.41	0.33-0.54	=0.608
WOB (VAP/VCL)	0.57	0.04	0.55 - 0.58	0.51-0.66	=0.600
ALH (µm)	6.32	0.86	5.99 - 6.65	4.41-7.55	=0.881
BCF (Hz)	19.56	1.58	18.96 - 20.16	14.56-21.34	=0.084

DAP = Distance Average Path; DCL = Distance Curvilinier; DSL = Distance Straight Line; VAP = Velocity Average Path; VCL = Velocity Curvilinier; VSL = Velocity Straight Line; STR = Straightness (VSL/VAP); LIN = Linearity (VSL/VAP); WOB = Wobble (VAP/VCL); ALH = Amplitude of Lateral Head Displacement; BCF = Beat Cross Frequency

The average (±SD) of straightness (STR), linearity (LIN; VSL/VCL) and WOB (VAP/VCL) of the sperms movement pattern of three Bali bulls fresh semen were 68.0±5.0%, 39.0±6.0%, and 57.0±4.0%, respectively. No significant difference at different bulls was found for the three parameters mentioned above. This means that movement pattern of three Bali bulls both velocity and distance relatively similar at each Bali bull. Therefore, measurement as above is hoped to give assessment on bulls' fertility. This is in line the statement of Clay and McDaniel [16] that every character that can be measured to identify lower fertility of the bull is possibly to cull or avoid the use of bull. Contrary, the bull with high fertility can be use in the group of cows that can increase the fertility in the group of animal. Bull fertility has an important role in reproductive process in cattle [13]. Furthermore, intensity selecting of bull for mating program in livestock industry aims to produce as many as possible offspring relatives to the cows. Sub-fertile bull prolongs breeding/mating season as well as prolongs pregnancy and subsequently increase culling rate for the cows [17]. In conclusion, the VCL value of Bali bulls' semen was 92.84±19.4 µm/sec. and can be categorized as non-hyperactive; approaching hyperactive.

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