Research Article

Characteristic and Kinematics of Bali-Polled Bull Sperms

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Abstract | The purpose of this study was to determine the characteristics and kinematics of Bali-polled bull sperm to identify reproductive potential. Semen collection of Bali-polled bull was conducted once a week using an artificial vagina. The semen was proceeded until the frozen semen. Parameters were motility, motility progressive, kinematics sperms, viability, abnormality membrane integrity, and acrosome integrity. The Bali-polled bull semen was analyzed by using CASA with the Sperms Vision Version 3.7.5 program. The results in this study showed that sperms motility in Bali-horned bull was significantly lower (P<0.01) than Bali-polled bull, Brahman, Limousine, and Simmental. The sperms viability in Bali-horned and polled bull was significantly lower (P<0.01) compared to other bull breeds. The membrane integrity and acrosome integrity of Bali-polled bull sperms was significantly higher (P<0.01) than in Bali-horned bull. DCL, DAP, and DSL of Bali-polled bull were significantly higher (P<0.01) than Bali-horned bull. VCL, VAP, and VSL of Bali-horned bull were significantly lower (P<0.01) than Bali-polled, Simmental, Limousine, and Brahman bull. Based on the results of this study, it can be concluded that Bali-polled bull had good sperm characteristics and kinematics compared with various breeds so that the Bali-polled bull can have the potential to become superior bulls to contribute to the strategies for developing Bali bull as a national beef bull contributor.

Keywords | Bali-polled bull, Characteristic, Kinematics, Sperms

Received | April 14, 2022; **Accepted** | May 30, 2022; **Published** | July 20, 2022
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Citation | Diansyah AM, Yusuf M, Toleng AL, Dsgong MIA (2022). Characteristic and kinematics of bali-polled bull sperms. Adv. Anim. Vet. Sci. 10(8): 1787-1796.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.8.1787.1796
ISSN (Online) | 2307-8316

INTRODUCTION

The global demand for beef as a food source has increased significantly, and Indonesia is no exception, due to the increasing Indonesian population. The increasing demand for beef is not being fulfilled by an increase in domestic beef production, so the national beef supply remains insufficient. In 2019, national beef production was 515,600 tons, while beef consumption was 683,294 tons (Ditjenpkh, 2020). As a consequence, the Indonesian government focused more on the development of national beef bull to support the fulfillment of beef needs.

The development of the Bali bull is now leading to the development of the Bali-polled bull (Zulkarnaim, 2017). The polled bull has several advantages, including a lower risk of injury, which is common in bull breeders (McConnachie et al., 2019), and polled bull productivity is higher than horned bull productivity (Sarika et al., 2010). Animal reproductive biotechnology today has grown and is a great opportunity to explore the potential reproductive performance, increase in population, and the genetic quality of livestock. Artificial insemination is the first generation of reproductive technology that aims to efficiently utilize superior bulls, prevent the spread of reproductive diseases

and improve the genetic quality of livestock (Said, 2020). In Indonesia, artificial insemination technology has the potential to be used for the development of Bali-polled bulls. As a direct consequence, choosing a polled bull is essential, especially in modern livestock management (Zulkarnaim, 2017).

The motility and kinematic sperms are important factors in assessing the quality of spermatozoa to support the fertilization process. Kinematic of sperms can be evaluated using Computer Assisted Sperm Analysis (CASA) based on the values of curvilinear velocity (VCL), linearity (LIN), amplitude of lateral displacement (ALH) (Hinrich and Loux, 2012), total motility and progressive motility (Verstegen et al., 2002). Spermatozoa assessment using CASA can assess in detail and a high degree of accuracy (Verstegen et al., 2002; Shojaei et al., 2012).

Livestock reproductive biotechnology has now developed and opened great opportunities to explore the potential for reproductive performance, increasing population and genetic quality of livestock. The phenomenon polled trait in Bali bull should have a scientific basis to explain the validity of the type of breed. The results of the early assessment show that the Bali-polled bull is still the same as Bali bull in general (Baco et al., 2020). However, research on the reproduction of Bali-polled bull is still limited, especially on the characteristics and kinematics sperms. The purpose of this study was to determine the characteristics and kinematics of Bali-polled bull sperm to identify reproductive potential.

MATERIALS AND METHODS

The study was conducted in August-November 2021 at the Laboratory of Animal Reproduction, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia. A bull was used in this study from the Faculty of Animal Science Hasanuddin University, Makassar, Indonesia. All procedures performed in this study were approved by the Animal Ethics Committee, Hasanuddin University. The bull was confined in an individual stall barn and fed elephant grass (80%) and concentrate (20%) given in the morning and evening. Semen Collection of Bali-polled bull was conducted once a week using an artificial vagina. Frozen semen of various breeds was taken from the Department of Animal Husbandry and Animal Health of South Sulawesi.

Evaluation of Semen

The evaluation of sperm quality was analyzed by macroscopic (volume, pH, color, odor, and consistency) and microscopic (motility, progressive motility, kinematic, concentration, abnormality, viability, membrane integrity, and acrosome integrity).

Macroscopic Evaluation

The macroscopic evaluation included volume, color, odor, consistency, and pH. Volume was evaluated by looking at the scale on the reservoir tube. Color was evaluated by visually observing the color from milky white to cream. Consistency was evaluated by tilting the collection tube and then straightening it again and judged by the speed at which the semen returns to the bottom of the tube. The assessment was based on three categories, namely the watery category (semen quickly returns to the bottom of the tube), medium (semen slowly returns to the bottom of the tube and leaves some on the tube wall), and thick (semen very slowly returns to the bottom of the tube and leaves some on the tube wall). pH was evaluated using pH indicator paper in the pH range of 6.0 – 8.0.

Microscopic Evaluation

Sperms Motility, Progressive Motility and Kinematics: The sperms motility, progressive motility, and kinematics were calculated by making 10 µl spermatozoa spot on the object glass. Spermatozoa were then analyzed using CASA Sperms (Vision Version™ 3.7.5 program Minitub, Germany) (Diansyah et al., 2020).

Sperms Concentration: Sperms concentration were evaluated using a photometer SDM 6 (Minitub, Germany). The cuvette containing 3 ml of physiological NaCl solution was inserted into the device with the line facing forward and then the zero button was pressed. The cuvette was removed and then replaced with a cuvette containing a physiological NaCl solution to which 30 µl of fresh semen was added and then the result button was pressed, the concentration of spermatozoa would be obtained in the amount per ml.

Sperms Viability and Abnormality: The sperms viability and abnormality were evaluated using the formula: Sperms viability were calculated by mixing 10 µl of semen and 10 µl of Eosin 2% above the object glass. Then, observed using a trinocular microscope (Primo Star, Zeiss, Germany) at 400x magnification with Indomicro View 3.7 software. Dead spermatozoa was red and live spermatozoa was colorless. Abnormal spermatozoa observed were severed tails, broken tails, and abnormal head shape. At least 200 sperms cells per observation. (Diansyah et al., 2020).

Membrane Integrity

The evaluation of membrane integrity was carried out microscopically. The membrane integrity was observed by adding HOST solution (0.179g NaCl in 100 ml of aquabides) into a 10 µl semen, then incubated for 30 minutes at 37°C in the oven. Sperms with membrane integrity were characterized by a circular tail and damaged sperms were characterized by a straight tail. Evaluation was carried out with a 400x magnification using a trinocular microscope

(Primo Star, Zeiss, Germany) by counting 200 spermatozoa cells (Diansyah et al., 2020).

Acrosome Integrity

Acrosome integrity were observed by mixing the semen to be tested with a formol-saline solution in a ratio of 1: 4 into an Eppendorf tube, left for a while and dripped on an object glass and then covered with a cover glass. The examination was carried out with trinocular microscope (Primo Star, Zeiss, Germany) using 400x magnification as many as 10 randomized fields of view with a minimum number of 200 spermatozoa. The percentage of acrosome integrity that has integrity acrosomal hoods is marked by a black head tip when exposed to a formol-saline solution with a minimum of 200 cells evaluated by microscope with a magnification of 10 x 40 (Rizal, 2006).

Cryopreservation

The semen of Bali-polled bull was frozen using commercial extender (Andromed, Germany) with reference to the SOP of the AI center for the process of frozen-semen production. Semen was equilibrated in a cool top (5C) for 4 hours and then filling and sealing. The cryoperservation process was carried out in a liquid nitrogen vapor above using Styrofoam box for 15 minutes. The frozen semen was then stored in a liquid nitrogen container (-196C).

Thawing

The thawing of frozen semen was conducted by dipping the straw in waterbath with temperature of 37°C for 30 seconds. Straw was dried with a tissue paper, then both ends of the straw were cut and placed in a microtube (Diansyah et al., 2020).

Statistical analysis

All data for semen characteristics of sperm were presented as means ± SD (standard deviation) and analyzed by one-way analysis of variance (ANOVA) using SPSS Version 25. Differences statistically highly significant at $P<0.01$ by Fisher LSD analysis.

RESULTS

The Characteristic of Bali-Polled Bull Fresh Semen

The characteristic of Bali-polled Bull fresh semen are shown in Table 1.

Table 1: The Characteristics of Bali-Polled Bull Fresh Semen

Parameters	Means SD	95% Confidence Interval
Macroscopic Volume (ml)	5.52 0.91	4.39 – 6.65
pH	6.22 0.35	5.79 – 6.65
Color	Cream	
Odor	Specific	
Consistency	Moderate	
Microscopic Concentration (million/ml)	1358 36.82	90.8 – 181.52
Motility (%)	94.94 2.75	91.52 – 98.35
Progressive Motility (%)	85.54 4.53	79.92 – 91.16
Viability (%)	95.73 2.15	93.06 – 98.40
Abnormality (%)	4.37 1.12	2.98 – 5.77
Membrane Integrity (%)	96.35 1.48	94.52 – 98.19
Acrosome integrity (%)	96.36 1.67	94.29 – 98.44

The fresh semen of Bali-polled bulls in this study was considered as normal category according to standard of *Bos taurus* (Parthipan et al., 2017).

The Kinematics of Bali-Polled Bull Fresh Semen

The kinematics of Bali-polled bull fresh semen are shown in Table 2.

Table 2: The Kinematic of Bali-Polled Bull Fresh Semen

Parameters	Means SD	95% Confidence Interval
DCL (μm)	55.19 4.75	49.30 – 61.09
DAP (μm)	28.80 2.28	25.97 – 31.64
DSL (μm)	18.59 1.62	16.58 – 20.60
VCL (μm/s)	133.62 10.08	121.10 – 146.14
VAP (μm/s)	70.03 4.85	64.01 – 76.04
VSL (μm/s)	45.50 3.61	41.02 – 49.99
LIN (%)	0.34 0.02	0.32 – 0.36
STR (%)	0.65 0.02	0.62 – 0.68
WOB (%)	0.54 0.06	0.48 – 0.61
BCF (Hz)	22.80 1.30	21.19 – 24.42
ALH (μm)	6.77 0.73	5.87 – 7.68

The Characteristic of Bali-Polled Bull Post-Thawing

The Characteristic of Bali-polled bull frozen semen with various breeds are shown in the Table 3.

Table 3: The Characteristic of Bali-Polled Bull Post-Thawing with various Breeds

Parameters	Breeds (Means SD)				
	Bali-horned	Bali-polled	Brahman	Limousine	Simmental
Motility (%)	47.200.73[a]	49.740.79[b]	52.272.21[c]	52.41 0.24[c]	52.29 1.21[c]
Viability (%)	48.460.25[a]	50.480.60[a]	53.532.99[b]	53.30 0.71[b]	53.170.93[b]
Abnormality (%)	15.280.58	14.680.20	13.990.90	14.99 0.57	14.791.52
Membrane Integrity (%)	48.830.63[a]	51.300.43[b]	52.300.76[bc]	52.991.35[bc]	53.680.61[c]
Acrosome Integrity (%)	48.900.24[a]	52.371.69[b]	52.070.32[b]	50.791.09[ab]	51.810.21[ab]

Note : Different superscript at the same row indicate differed significantly P<0.01).

Table 4: The Kinematics of Bali-Polled bull Post-Thawing with Various Breeds

Parameters	Breeds (Means SD)				
	Bali-horned	Bali-polled	Brahman	Limousine	Simmental
DCL (µm)	26.462.49[a]	32.761.31[b]	42.072.46[d]	40.222.26[cd]	36.860.51[c]
DAP (µm)	15.870.53[a]	18.431.50[b]	21.180.81[c]	21.060.52[c]	18.650.52[b]
DSL (µm)	11.420.17[a]	12.970.63[b]	13.650.46[bc]	14.470.54[c]	12.940.50[b]
VCL (µm/s)	63.836.84[a]	90.182.00[b]	102.635.99[d]	97.516.33[cd]	91.571.30[bc]
VAP (µm/s)	38.501.78[a]	46.052.78[b]	48.805.72[b]	51.431.75[b]	46.780.79[b]
VSL (µm/s)	27.760.79[a]	32.630.90[b]	33.851.49[bc]	35.530.92[c]	32.760.85[b]
LIN (%)	0.440.01[a]	0.400.02[ab]	0.330.1[c]	0.370.03[bc]	0.360.03[bc]
STR (%)	0.720.15[a]	0.710.23[a]	0.650.17[b]	0.690.36[ab]	0.700.01[a]
WOB (%)	0.610.04[a]	0.570.03[ab]	0.510.02[c]	0.530.02[bc]	0.510.02[c]
BCF (Hz)	19.820.67[a]	20.160.91[a]	20.730.28[a]	21.141.10[a]	20.810.38[a]
ALH (µm)	4.300.07[a]	5.210.02[b]	5.760.14[c]	5.542.66[c]	5.660.07[c]

Note : Different superscript at the same row indicate differed significantly (P<0.01).

All the recorded data on the characteristics of post-thawing at Bali-polled bull with various breeds were good characteristics according SNI 01-4869.1-2005 (2005). It showed that the motility spermatozoa Bali-horned bull was significantly lower (P<0.01) than Bali-polled bull, Brahman, Limousine, and Simmental. Each breeds has a value of > 40%. The viability in Bali-horned bull and polled spermatozoa were not significantly difference (P<0.01). The abnormality of spermatozoa showed that there was no difference in each breeds. The membrane integrity spermatozoa in Bali-polled bull was significantly higher (P<0.01) than Bali-horned bull. Bali-polled bull was not significant difference (P<0.01) compared to Brahman and Limousine bull. The acrosome integrity spermatozoa in Bali-polled bull was significantly higher (P<0.01) than Bali-horned bull, and Bali-polled bull not significant (P<0.01) difference compared to other bulls.

Kinematics of Bali-Polled Bull Post-Thawing

The kinematic of Bali-polled bull post-thawing with various breeds are shown in Table 4.

The distance traveled by spermatozoa in DCL, DAP, and DSL Bali-polled bull were significantly higher (P<0.01) than Bali-horned bull. Meanwhile, the velocity that can be reached by spermatozoa in VCL, VAP, and VSL Bali-horned bull were significantly lower (P<0.01) than Bali-polled, Simental, Limousine, and Brahman bull. LIN, STR, and WOB in Bali-polled bull were not significantly different (P<0.01) with Bali-horned bull. BCF in Bali-polled bull was not significantly different (P<0.01) compared to other bull. However, ALH in Bali-polled bull was significantly higher (P<0.01) than Bali-horned bull.

DISCUSSION

The volume obtained from the fresh semen collection of Bali-polled bull in this study was a means of 5.52±0.91 ml/ejaculate (Table 1). The data shows that the means semen of Bali-polled bull obtained was higher than Aceh bull (3.8±0.47 ml) (Zulyazaini et al., 2016), Bali-horned bull (4.48±0.91 ml) (Prastowo et al., 2018), Madura bull (3.0±0.38 ml) (Romadhoni et al., 2014). The means semen volume of Bali-polled bull is comparable to that of Simental bull (5.81±2.36 ml) (Priyanto et al., 2015), while it was relatively lower than compared with Limousine bull, Ongole bull, and Brahman bull with a means of 6.83±0.58 ml, 8.20±0.75 ml, and 6.00±1.67 ml, respectively. The difference is probably due to differences in the type and age of bull used. This is consistent with the statement of Melita

et al. (2014) that the semen properties are affected by the age of bulls and the interaction between age and reservoir interval.

The pH of Bali-polled bull fresh semen on the examination of this study showed that the pH of semen was a means of 6.22±0.35 (Table 1). These results are relatively lower than in Aceh bull semen (6.84±0.17) (Zulyazaini et al., 2016), Bali-horned bull semen (6.51±0.12) (Prastowo et al., 2018), and Madura bull (7.0±0.0) (Romadhoni et al., 2014). The pH means for Bali-polled bull is relatively the same as for Limousine, Ongole, Brahman, and Simental bull with a means of 6.51±0.03, 6.47±0.16, 6.53±0.04, and 6.56±2.92, respectively (Priyanto et al., 2015). This is following the opinion of Dewi et al. (2012) that age and breeds do not affect pH. Sunami et al. (2017) argue that the high and low pH value of semen is related to the state of sperms concentration, high sperms concentration will have an impact on the pH of the semen which tends to be acidic in the normal range. According to Zulyazaini (2016) that high concentrations of sperms usually have a slightly acidic pH.

The color and odor of fresh semen Bali-polled bull were cream and typical (Table 1). This was similar to the semen of Aceh bull (Zulyazaini, 2016), Bali-horned bull (Prastowo et al., 2018), Madura bull (Komariah et al., 2020) semen for Limosin, Ongole, Brahman and Simental bull (Priyanto et al., 2015). Lestari et al. (2013) argued that the cloudier the color of the semen, the greater the number of sperms per milliliter of semen. Feradis (2010) said that in general, the odor of semen is categorized as a distinctive smell.

The results of the observation that the means sperms concentration of Bali-polled bull obtained in this study was 1358±0.06 million/ml (Table 1), the means sperms concentration sperms of Bali-polled bull obtained in this study was relatively the same compared to the concentration sperms of Aceh bull, which was 1194±52.25 million/ml (Zulyazaini et al., 2016), in Bali-horned bull the concentration is higher, which is 1700 million/ml (Juyanto, 2011), in Madura bull, it is 1814±2.97 million/ml (Romadhoni et al., 2014). The concentration of Bali-polled bull is relatively the same as Limousine, Ongole, Brahman, and Simental bull with a means of 1180±204.17 million/ml, 1578±80.00 million/ml, 1340±242.49 million/ml, and 1290±496 million/ml (Priyanto et al., 2015). The concentration of sperms is influenced by age and type of bull (Benson et al., 2012). Campbell et al. (2003) that the concentration of spermatozoa in adult bulls normally ranges from 800-1200 million/ml. The assessment of concentration sperms is very important because it is this factor that describes the properties of the semen used as one of the criteria for determining the quality of semen Ax et al. (2008) argue that the normal motility of spermatozoa is 70-90% with rapid movement. The motility value of bovine sperms ranges from 70 to 80% (Garner and Hafez, 2016). Many factors influence the differences in sperm motility values, including age, breed, maturity of sperm, and quality of sperm plasma (Bhakat et al., 2014). Motility is very important; when females are inseminated with spermatozoa exhibiting different levels of motility, those spermatozoa with poor motility are found in the backflow after only 15 min (Hernandez-Caravaca et al., 2015). This indicates that an initial sperm selection process within the genital tract is biased in favor of highly motile spermatozoa (García-Várquez et al., 2022). The value of progressive motility is an important criterion because it plays a role in assessing the fertility of bulls (Abavisani et al., 2013). According to Morrell (2019) progressive motility of sperms is required to enter into cumulus oophorus and move inside to be able to reach the oocyte.

The results in this study (Table 1) are higher than the percentage of bull semen motility Aceh is 77.28±3.17 with a range of 72.70 to 81.30% (Zulyazaini et al., 2016) and reported by Dewi et al. (2012) who found the percentage of sperms motility of Bali-horned bull in Indonesia was 74.50±3.69. In Madura bull, the percentage of motility was 73±2.58 (Romadhoni et al., 2014). The progressive motility of semen from Bali-polled bull in this study was 85.54±5.22. This result is higher than that reported by Sarastina et al. (2007) on Bali-horned, Madura, Limousine, Simental, Brahman, and Ongole bull. This difference in the percentage of motility is caused by differences in species, age, feed, frequency of collection semen, storage techniques, and maintenance management (Gordon, 2017).

The means percentage of viability of semen from Bali-polled bull in this study was 95.73±2.47 (Table 1). This result is relatively high with that reported by Yekti et al. (2018) where the means percentage of live sperms in Bali bull is 89.94±2.84. This result is also relatively higher than the percentage of sperms Aceh bull is 86.76±2.87 with a range between 81.40 to 91.00 (Zulyazaini et al., 2016). In Madura bull, the percentage of viability low as 90.98±3.13 (Yekti et al., 2018). The mean percentage of viability of Bali-polled bull in this study was relatively higher than that of Limousine, Ongole, Brahman, and Simental bull with a means of 83.93±6.81, 82.17±2.19, 79.92±2.23, and 78.63±6.12 respectively (Priyanto et al., 2015). The percentage of viability in Bali-polled and horned bull are higher than percentage of motility. Sukmawati et al. (2014) stated that the percentage of viability sperm value higher than the percentage motility, because that viable is not motile sperms were progressive, but still alive so it is not exposed at the time of fixation.

The means sperm abnormalities in this study was 4.37±1.12 (Table 1). The means percentage of sperm abnormalities in Aceh bull is relatively the same, namely 5.98±1.77 (Zulyazaini et al., 2016), also relatively the same as the percentage of abnormal sperms in Bali-horned bull kept in Indonesia, which is 3.57±1.19 (Yekti et al., 2018). In Madura bull, it is lower, namely 4.5±1.88 (Romadhoni et al., 2014). This shows that the percentage of abnormalities in Bali-polled bull semen can be used for artificial insemination. This is following the opinion of Ax et al. (2008) which states that semen that has an abnormality of 15% cannot be used for artificial insemination (AI). According to Barth and Oko (1989) spermatozoa abnormalities are influenced by various factors, such as stress, genetics, and disorders of the seminiferous tubules. The percentage of spermatozoa abnormalities in bulls is different for each researcher (Riyadhi et al., 2012).

The means membrane integrity in this study has 96.35±1.70 (Table 1). The mean percentage of the intact plasma membrane for Bali-horned bull is relatively the same, namely 90.16±1.42 (Marawali et al., 2019), also relatively the same as Aceh bull at 85.42±1.78 (Hidayat et al., 2018). However, it is relatively higher than Madura bull, namely 78.83±10.61 (Romadhoni et al., 2014). The membrane integrity of Bali-polled bull was relatively higher than for Limousine, Ongole, Brahman, and Simental bull with a means of 85.76±4.57, 79.50±4.86, 74.25±13.47, and 74.92±7.25 respectively (Priyanto et al., 2015). The membrane integrity is an absolute thing that sperms must have. The plasma membrane serves as the cell's first defense from the outside environment that can damage cells (Nofa et al., 2017). According to Anwar et al. (2014), the membrane integrity has a positive effect on the active movement of sperms. The membrane integrity has a relationship with sperm motility, more the membrane integrity, the more active a progressive sperms (Azzahra et al., 2016).

The acrosome integrity in this research has a means of 96.36±1.91 (Table 1). The mean percentage of the acrosome integrity of Bali-polled bull is higher than Bali-horned bull with a means of 90.12±0.26 (Marawali et al., 2019). The results of this study were also higher than the Limousine, Ongole, Brahman, and Simental bull with an means of 84.67±3.40, 85.42±5.86, 81.17±5.96, and 83.67±7.16 respectively (Priyanto et al., 2015). The acrosome plays an important role in the fertilization process. The success of artificial insemination must be accompanied by good quality sperms, good quality is not only seen in the progressive motility of the sperms but also the integrity of the sperms acrosome hood (Anwar et al., 2015).

The membrane integrity is a prerequisite for the survival of spermatozoa (Sharma et al., 2012). If the membrane integrity has been disrupted or damaged, it will result in anisosmotic conditions that cause intracellular leakage, which will affect the reshuffling of ATP and thus affect the motility of spermatozoa (Bohlooli et al., 2012). The low percentage of acrosome integrity is associated with lower percentage of membrane integrity, viability and motility (Sitepu et al., 2018). Damage to acrosome integrity can be caused by freezing. Ice crystallization in excessive cell dehydration can result in damage to acrosome integrity (Samsudewa et al., 2007).

According to SNI 01-4869.1-2005 (2005) which recommends sperm abnormalities below 20% and motility above 40%, indicated that frozen-thawed Bali-polled bull spermatozoa still suitable for AI. This is in accordance with Susilawati (2011) which states that frozen semen that has motility quality below the SNI 01-4869.1-2005 standard can still produce pregnancy with AI acceptors, which is 85-95% successful in getting pregnant. This indicated that frozen semen that has motility quality above 20% can be used for artificial insemination.

The assessment of semen quality concerning motility, velocity, swimming pattern, sperm head displacement and sperm abnormalities, may help in the selection of bulls for semen cryopreservation (Perumal et al., 2014). Computer-Assisted Sperm Analyzers (CASA) offer accurate information on different motion characteristics of spermatozoa (Amann and Waberski, 2014). CASA analysis proposed a potential indicator for accurate prediction of male fertility as compared to the traditional semen evaluation method (Broekhuijse et al., 2012). Motion characteristics of bull spermatozoa using CASA have shown correlation with oocyte penetration rate, in-vitro fertility, and field fertility (Kathiravan et al., 2008). Therefore, to optimize these results, the kinematic sperm needs to be further confirmed using the CASA.

The sperm value of Bali-polled bull fresh semen had good movement with a VCL value of 133.62 10.08 (Table 2). The kinematics of Bali-polled bull fresh semen are shown in the Table 2. The VAP and VSL values for Bali-polled bull have values of 70.03 4.85 and 45.50 3.61, respectively. VCL, VAP, and VSL values in Bali-polled bull have relatively higher values in comparison to Bali-horned, Limousine, Madura, Simental, Brahman and Ongole bull as the results of research by Sarastina et al. (2007). VCL is the velocity of sperm in one minute of the curve, VAP is the velocity sperms in one minute of the average path and VSL is the velocity sperms in one minute of a straight line (Sarastina et al., 2007). The results of DCL, DSL, and DAP analysis on Bali-polled bull have relatively the same value as the research of Sarastina et al. (2007). Distance Curve Line (DCL) is the distance that sperms can travel in one

minute on the curve path. Distance Straight Line (DSL) is the distance that sperms can travel in one minute on a straight line. Distance Average Path (DAP) is the distance that sperms can travel in one minute on the average path (Ratnawati et al., 2019). The LIN, STR, and WOB values for Bali-polled bull had values of 0.34 0.02, 0.65 0.02, and 0.54 0.06, respectively. These results are relatively the same as for Bali-horned, Limousine, Madura, Simental, Brahman and Ongole bull in the Sarastina et al. (2007) research. Udrayana (2009) states that LIN is a straight line of a linear curve. The LIN value is obtained by dividing VSL by VCL multiplied by 100 and expressed in %. The STR value is obtained by dividing VSL by VAP multiplied by 100 and expressed in %. STR is the average straightness of the spatial path. The WOB value is obtained by dividing VAP by VCL multiplied by 100 and expressed in %. The results of BCF analysis on Bali-polled bull have relatively lower values, but the ALH values are relatively the same as those of Bali-horned, Limousine, Madura, Simental, Brahman and Ongole bull in the Sarastina et al. (2007) research. ALH is the average deviation distance of each centroid from the average path (Setiyono et al., 2020).

The Bali-polled bull had a good value of kinematic sperms (Table 4), according to the statement Krízková et al. (2017) VCL values can be divided into: fast (>90 µm/s), moderate (45-90 µm/s), slow (10-45 µm/s), and statis or immotile (<10 µm/s). VAP values> 25.0 µm/s are a good predictor of in vitro fertilization ability (Suzuki et al., 2003). Fertilization ability correlates with the VSL parameter by making an important contribution to the characteristics of sperms (Shibahara et al., 2003). VCL, VAP, and VSL parameter values only show the strength of sperm movement, but do not provide information on the motion of sperm (Perreault, 2002). Research by Shojaei et al. (2012) explains that the determination of sperms changes movement to hyperactive motility when the value of ALH > 7 µm, LIN < 65%, and VCL > 80 µm. The swimming pattern of sperms is determined by the LIN, STR, and WOB values (Ratnawati, 2019). LIN and STR are indicators of progressive motility and swimming patterns (Sarastina et al., 2007). The LIN value in sperms can indicate the characteristics of the direction of movement or swimming straightness of the sperms. LIN in sperms can indicate that there is excessive bending of the middle of the tail and indicates that the sperms are hyperactivated (El-Bahrawy, 2017). The ALH and BCF values between studies are highly dependent on the type of CASA used (Perreault, 2002). Susilawati (2011) states that there are three patterns of sperm motility, namely the hyperactivation group having VCL values ≥ 100 µm/s, LIN <60%, and ALH ≥ 5. AOC is the average degree of change in sperm head movement (Sarastina et al., 2007). ALH is the width of the head oscillation when the sperm are moving (Verstegen et al., 2002) and an indicator of the movement of the sperm flagellum (Suarez, 2008). According to Chatiza et al. (2012), changes in kinematic sperms are the activity of the flagellum which can occur because sperms experience capacitation. High VCL, ALH and low percentage of LIN can described spermatozoa experiencing hyperactivity (Bernecic et al., 2019). While the value of VCL, ALH and total motility are parameters that affect the ability of spermatozoa to penetrate through cervical mucus and the zona pellucida (Verstegen et al. 2002; Taberner et al. 2010).

The kinematics sperms of Bali-polled bull in this study was belongs to hyperactivation. The hyperactivation is a movement pattern seen in sperm at the site and time of fertilization in mammals. It may be critical to the success of fertilization, because it enhances the ability of sperm to detach from the wall of the oviduct, to move around in the labyrinthine lumen of the oviduct, to penetrate mucous substances and, finally, to penetrate the zona pellucida of the oocyte (Suarez and Ho, 2003).

The characteristics and kinematics sperms in this study can be an additional reference in contribute to the strategies for developing Bali-polled bulls as a national beef bull contributor. Hence to optimize the result, the characteristics and kinematics sperms is still necessary to investigate for further using Bali polled bull in some areas in Indonesia.

CONCLUSIONS

Based on the results of this study, it can be concluded that the Bali-polled bull had good characteristics sperms accordance SNI 01-4869.1-2005 and kinematics sperms belongs to good category so that the Bali-polled bull can have the potential to become superior bulls to contribute to the strategies for developing Bali bull as a national beef bull contributor.

ACKNOWLEDGMENTS

The first author is supported by PMDSU scholarship, Ministry of Higher Education, Culture, Research and Technology of Indonesia. Our sincere thanks are due to technical assistance from Indra Wahyudi Syarif, A. Andri Tamiyadi, Zahra Jinan Fadillah, Hasrin, Sahiruddin, Masturi and Mila.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interests.

NOVELTY STATEMENT

This is the first to report reproductive potential of Bali-polled bull, especially on the characteristics and kinematics of the sperms.

AUTHORS CONTRIBUTIONS

AMD: Conception and design the study, data analysis, writing-original draft, writing-review and editing. MY: Conception and design the study, data analysis, writing-original draft, writing-review and editing. ALT: Data analysis, correcting and editing draft. MIAD: Data analysis, correcting and editing draft.

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