IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

Movement patterns of sperms at different bull breeds using computer-assisted sperm analysis (CASA)

IOP Conf. Series: Earth and Environmental Science **788** (2021) 012137 doi:10.1088/1755-1315/788/1/012137

Movement patterns of sperms at different bull breeds using computer-assisted sperm analysis (CASA)

M Raafi[1], M Yusuf[2], A L Toleng[2], A M Diansyah[3], Surahman[1] and Sahiruddin[2]

[1]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia
[2]Laboratory of Animal Reproduction Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia
[3]Graduate Student of Science and Technology of Animal Science, Hasanuddin University, Makassar

Email: myusuf@unhas.ac.id

Abstract. Bull plays an important role in the implementation of artificial insemination (AI), especially as a producer of spermatozoa. The movement pattern of sperms itself determines bull fertility. This is very important for the process of capacitation in the female reproductive tracks. The purpose of this study was to determine the differences in the characteristics of the motility of frozen semen at various breeds of bulls. This study was conducted in May 2020, at the Processing Semen Laboratory, Faculty of Animal Science, Hasanuddin University. This study was designed with 6 breeds of different bulls and 3 straws for replications. The results of the study were analyzed statistically using the one-way ANOVA. The results showed that the LIN and WOB values of frozen semen of Bali had significantly higher ($P<0.05$) than the other breeds. Likewise, STR values in Bali sperms had significantly higher ($P<0.05$) than Simmental, FH, Angus, and Brahman. VAP, VCL, VSL, and ALH values in Simmental had significantly higher ($P<0.05$) than the other breeds. The parameter distance achieved by Simmental sperms; DAP had significantly higher ($P<0.05$) in comparison to Bali and FH. Simmental had DCL highest value, and significantly ($P<0.05$) higher than Bali, FH, Angus, and Brahman. While Simmental had a significantly higher DSL value ($P<0.05$) than those Bali and FH.

1. Introduction

The beef cattle business in Indonesia requires special attention in relation to efforts in maintaining and supporting an increase in a population where appropriate technology in the field of reproduction and feed should be applied easily and efficiently. Increasing reproductive efficiency in efforts to optimize the use of Artificial Insemination (AI), among others, is to ensure that each cow is able to produce calves every year in order to maximize livestock productivity [1].

One of the ways to increase the productivity and reproduction of cattle is the use of AI technology. This AI is an effective technology that can be used to increase cow productivity by utilizing the potential of superior bulls to mate more cows [2]. AI has been well known as one of the appropriate technologies to increase the population and livestock production qualitatively and quantitatively by using high-quality bull semen that is disease-free and has high genetic quality [3]. Genetically qualified bull will make a major contribution to the offspring because quality semen is the main requirement that must be met for

AI application. Therefore, the selection is needed to select bulls with good performance. This is related to the ability of bulls to mate some cows or heifers, produce high-quality sperms, and high fertility [4].

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 will increase the productivity of beef cattle, but using AI can also eliminate the nature of the local breed and reduce calving interval [5]. In practice, AI consists of a collection of semen, a process of freezing and deposition of semen in the female reproductive organs, thus allowing sperms to undergo fertilization as a natural process [6].

Bulls play an important role in the implementation of AI, especially as a producer of sperms. According to Yimer et al. [7] stated that bull fertility is more important than cows. The testes are the place for sperms production. The testes are covered by a scrotum that reflects the size of the testes and states the amount of tissue or seminiferous tubules that function to produce spermatozoa. The quality and production of semen are influenced by several factors, such as genetics, feed, temperature, season, frequency of ejaculation, age, and bodyweight of bulls and breeds [8,9]. Good quality semen starts with the quality of fresh semen produced by bulls in AI station centers which is then processed into frozen semen to meet the minimum standards for AI.

The low sperm quality of the bulls can reduce conception rates, reduce pregnancy rates, and calving rates, consequently, it can reduce population numbers which can affect meat availability, which in turn can affect food security. Fertility is a complex process that is influenced by many factors such as physiology, nutrition, management, and the environment [10]. According to Sarastina et al. [11], there is no single test that can predict bull fertility. In general, spermatozoa quality testing can be carried out easily in AI centers or the field, such as sperm motility testing. The sperm motility test is an important parameter that can be used as a basis for information on the fertilization ability of the sperms. Low-quality sperms are one of the causes of the failure of AI, this is supported by the study of Toleng et al. [12] where the conception rate of cows was 23.0% on the large-scale farm and 38.3% on a small-scale farm. Service per conception of the cows from the two farm scales above 4.4 and 2.6 respectively which state that although there was no effect of the bull on the conception rate, the conception number above was low. Therefore, this study was carried out to compare the motility characteristics of frozen semen of various breeds in South Sulawesi Province.

The movement pattern of sperms itself greatly determines bull fertility. This is very important for the capacitation process in the tract of the female reproductive organs. The movement pattern and distance traveled by sperms in the female reproductive organs, to support high fertility must be able to reach the target site of fertilization and have the ability to fertilize the ovum [13]. Therefore, this study aimed to determine the differences in the motility characteristics of frozen semen at different breeds of the bull in South Sulawesi Province.

2. Materials and methods

2.1. Study period and materials

The study was conducted in May 2020 in the Laboratory of Animal Reproduction, Faculty of Animal Science Hasanuddin University, Makassar. The materials used in the present study were Computer-Assisted Sperm Analysis (CASA) Sperm Vision Ver 3.7.5 (LG®), ZEISS micro-tube 1.5 mL microscope (Onemed®), micropipette, tip, glass object, cover glass (Onelab®), container straw, warm plate (minitube®), cutter straw (minitube®), tweezers. The main material of this study was frozen semen from different bull breeds. The breeds were Bali, Brahman, Simmental, Limousine, Fries Holland (FH), and Brangus (Brahman-Angus) which were taken from the Animal Livestock and Animal Health Services of South Sulawesi Province. Supporting materials were warm water with a temperature of 40ºC, tissue, label paper, and liquid nitrogen.

2.2. Procedure and parameter of the study
The frozen semen of various bulls was thawed by dipping 1 straw of each breed in a thawing device filled with water with a temperature of 37ºC for 30 seconds. The straw was dried with a tissue, then both ends of the straw are cut and placed in a micro-tube. Subsequently incubated on a warm plate with a temperature of 37ºC, then subjected to a microscopic evaluation. The evaluation of semen was carried out microscopically which included: curved line distance (DCL), straight-line distance (DSL), average path distance (DAP), velocity curved line (VCL), velocity straight line (VSL), velocity average path (VAP), linearity (LIN), straightness (STR), wobble (WOB), beat cross frequency (BCF), the amplitude of lateral head displacement (ALH).

Those DCL, DSL, DAP, VCL, VSL, VAP, LIN, STR, WOB, BCF, and ALH were the parameters in the present study that were used to characterize the sperm's fertility of each breed. The movement pattern of the spermatozoa will show the motion pattern and/or velocity of the spermatozoa, as well as other more specific motion criteria.

2.3. Study design and data analysis
This study was designed to characterize the movement pattern of the spermatozoa using different breeds bull semen. This study was arranged using a completely randomized design with 6 breeds of bulls as treatment and 3 straws in each breed as a replication. The different treatments were analyzed using one-way ANOVA. A least significant difference was used between the treatments. The difference is considered significant if the probability value was less than 0.05 (P <0.05).

3. Results and discussion

3.1. Movement pattern of the sperms at different breeds of bull
The movement pattern of the spermatozoa is a measure that shows how fast the spermatozoa moves, which is expressed in units of micrometers per second (µm/sec). The speed of sperms movement semen spermatozoa from various breeds of bulls was calculated and analyzed using CASA is presented in table 1.

Table 1. Movement patterns of the sperms at the different breed of bulls.

Parameters	Breed					
	Bali	Brahman	Limousine	Simmental	Fries Holland	Brangus
VAP (µm/sec)	46.6^{a}	51.3^{b}	50.7^{be}	56.0^{c}	48.6^{ae}	50.4^{bde}
VCL (µm/sec)	77.3^{a}	93.9^{b}	92.0^{b}	108.4^{c}	92.3^{b}	90.2^{b}
VSL (µm/sec)	34.9^{a}	36,0^{b}	37.1^{b}	39.3^{c}	35.0^{a}	36.4^{ab}
STR (VSL/VAP)	0.7500^{a}	0.7200^{bc}	0.7333^{ab}	0.7000^{c}	0.7233^{bc}	0.7244^{bc}
LIN (VSL/VCL)	0.4533^{a}	0.3933^{bd}	0.4033^{b}	0.3633^{c}	0.3800^{cd}	0.4033^{b}
WOB (VAP/VCL)	0.6033^{a}	0.5433^{bd}	0.5533^{b}	0.5167^{c}	0.5257^{cd}	0.5600^{b}
ALH (µm)	4.8^{a}	5.7^{bc}	5.5^{bd}	5.8^{c}	5.5^{be}	5.23^{ed}
BCF (Hz)	22.1	21.4	22.3	22.0	22.1	21.53

Superscripts at the same row indicate differed significantly (P<0.05).

Table 1 shows that the value of LIN and WOB on the movement pattern of Bali bull sperms had the highest value which is significantly different (P<0.05) from the other bulls, namely 0.4533 and 0.6033, respectively while Limousine had a value of 0.4033, and 0.553, Simmental 0.3633 and 0.5167, FH 0.3800 and 0.5257, Brangus 0.4033 and 0.5600 and Brahman 0.3933 and 0.5433, respectively. The LIN values shown from the six bull breeds mentioned above have a lower value than the results stated by Hufana-Duran [14] on buffalo sperm which has a value of 0.5333. BCF of the sperms through the mean flow rate did not show a significant difference in all of the bulls used as suggested by Perchec et al. [15].

The low frequency of movement can be caused by decreasing temperature and ending progressive motility. The STR in Bali bull had the highest value, significantly differ (P<0.05) with Simmental, FH, Angus, and Brahman bulls. The STR values of Bali, Limousine, Simmental, FH, Brangus, and Brahman bulls were 0.8, 0.7, 0.7, 0.7, 0.7, and 0.7, respectively. The STR values shown from the six breeds of bulls above have a lower value than the results stated by Hufana-Duran [14] for buffalo sperm which has a value of 0.85. Sarastina et al. [11] stated that the LIN and STR values can be used as indicators of progressive motility and swimming patterns. Meanwhile, the ability to fertilize has a correlation with the parameters of VSL and VCL which contribute to the characteristics of spermatozoa function [16].

The VAP and VSL values for FH and Bali bulls had the lowest values which were significantly different (P <0.05) from the values of other breeds. The VAP and VSL values for Bali bull were 46.6 µm/s, and 34.9 µm/s, FH was 48.6 µm/s, and 35.0 µm/s, significantly different from the Limousine, Simmental, Brangus, and Brahman, whereas the VAP and VSL values of Limousine were 50.7 µm/s and 37.1µm/s; Simmental was 56.0 µm/s and 39.3 µm/s; Brangus were 50.4 µm/s and 36.4 µm/s, and Brahman were 51.3 µm/s and 36.0 µm/s. The VAP value of frozen semen showed that spermatozoa have progressive motility as stated by Suzuki et al. [16] that, VAP values> 25.0 µm/s indicates the progressive motility of spermatozoa.

In table 1, it states that the lowest VCL value is Bali bull (77.36 µm/s) and the highest is Simmental (108.46 µm/s), which is significantly different (P <0.05) with the Limousine (92.06 µm/s), FH (92.36 µm/s), Brangus (90.26 µm/s), and Brahman (93.9 µm/s), respectively. The VCL results obtained in this study were lower than the study of Sarastina et al [11], these different results were might be due to the use of different bulls, and as for the same bulls might be caused by differences in age, feed, genetics, and environment and how to maintain bulls, subsequently will show different results. According to Yendraliza [17], the quality and quantity of semen are influenced by several factors such as feed, feed constituents, temperature and season, frequency of ejaculation, and libido.

ALH values as shown in table 1 indicate that Bali bull has the lowest value which is significantly different (P<0.05) from other bull breeds. Bali bull has an ALH value of 4.8 µm, which is significantly (P<0.05) lower than ALH values of Limousine (5.5 µm), Simmental (5.8 µm), FH (5.5 µm), Angus (5.23 µm), and Brahman (5.7 µm), respectively. According to Susilawati [18] states that in general, there are three groups of spermatozoa motility patterns that can be analyzed using CASA. Those are hyper-activation group which has a value of VCL = 100 µm/sec, LIN <60% and ALH = 5 µm/s, the non-hyper-activated group if the value of VCL = 40 µm/s, LIN = 60% and ALH <5 µm/s and the transition group has a value between the two groups. Based on the group of spermatozoa movement patterns above, Simmental is categorized as a hyper-activation group, and other breeds of bulls are in the transition group. The fertility rate in the hyper-activated group had a higher success rate than the non-hyper-activated group. It is stated that evaluating the hyperactivity motility pattern using CASA can be a good way to predict the fertilization ability of the sperms. Susilawati et al. [19] also stated that spermatozoa hyper-activation is required just before the acrosome reaction; the movement in the oviduct during fertilization.

3.2. Distance achievement of the sperms during movement at different breeds of bull

Sperms movement distance can be defined as the ability of the sperms to move from one point to another point or the process of moving precisely to the egg. The distance traveled by the movement of sperms from various cattle breeds of bull, calculated and analyzed using CASA is presented in table 2.

Table 2 shows that the DAP and DCL values in Bali cattle are the lowest and significantly differ (P <0.05) from other bull breeds. It can be seen that the distance traveled (DAP) of Bali bull was 19.1 µm and the curve path (DCL) was 31.7 µm. The other breeds such as Brahman has a value of 20.8 µm and 38.3 µm, Limousine were 21.1 µm and 38.6 µm, Simmental was 23.5 µm and 45.8 µm, FH was 19.8 µm and 37.9 µm, and Brangus were 20.3 µm and 36.5 µm. These results were lower than that achieved by Sarastina et al. [11]. Differences between these two results are might be due to the use of different breed bulls. Furthermore, as for the same breed of bull that is shown differences might be caused by differences in the way they are raised. The straight line (DSL) of Simmental has the highest value in comparison to the others and significantly different. The DSL value of Simmental was 16.5 µm, which was significantly (P<0.05) higher than the other bulls.

Table 2. Distance achievement of the sperms at different breed of bulls.

Parameters	Breed					
	Bali	Brahman	Limousine	Simmental	Fries Holland	Brangus
DAP (µm)	19.1^{a}	20.8^{bd}	21.1^{b}	23.5^{b}	19.8^{c}	20.3^{ab}
DSL (µm)	14.3^{a}	14.8^{a}	15.4^{ac}	16.5^{bc}	14.2^{d}	14.6^{a}
DCL (µm)	31.7^{a}	38.3^{bf}	38.6^{b}	45.8^{bc}	37.9^{d}	36.5^{be}

Superscripts at the same row indicate differed significantly (P<0.05).

It is expected that the measurement of some of the above parameters can provide an assessment of bull fertility. An assessment of this fertility is needed, as stated by Clay and McDaniel [20] that each character can be measured to identify low fertility so that it is possible to reject or avoid the use of the bull, or vice versa, high fertility bulls can be used in the herd, so as to increase the fertility rate in the herd.

Bull fertility has a very important role in the reproductive process of cattle [21]. It is further stated that the intensity of selection in the selection of bulls for the mating program, both in the beef cattle industry and dairy cattle, is aimed at producing as many young as possible for the number of cows. Sub-fertile bulls can prolong the mating season due to delayed pregnancy, and further increase the number of cows' rejections [22].

4. Conclusions

Based on the results and discussion can be concluded that sperms of Simmental bull have a higher movement pattern than the other bulls. This indicated from the values of VAP, VCL, VSL, and ALH were better than the other five bull breeds. Likewise, the distance traveled by the sperms of Simmental was also better than the others; indicated by higher values of DAP, DCL, and DSL than the other five breeds.

Acknowledgment

The authors are thanks to the Livestock and Animal Health Services of South Sulawesi Province for providing straws at different breeds. The first author is a student of Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia, and this article is dedicated to his final project.

References

[1] Pamungkas D, L Affandhy, D B Wijono, A Rasyid and T Susilawati 2004 Kualitas spermatozoa sapi po hasil sexing dengan teknik sentrifugasi menggunakan gradient putih telur dalam beberapa imbangan tris-buffer semen *Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner* (Malang: Fakultas Peternakan, Universotas Brawijaya) pp 37 – 40

[2] Susilawati T 2013 *Pedoman Inseminasi Buatan Pada Ternak* (Malang: Universitas Brawijaya Press)

[3] Suteky T, Kadarsih S and Yanti F 2007 Pengaruh pengencer kuning telur dengan air kelapa dan lama penyimpanan terhadap kualitas semen kambing nubian *Jurnal Sain Peternakan Indonesia* **2** 65–71

[4] Kuswahyuni 2009 Pengaruh lingkar skrotum dan volume testis terhadap volume semen dan konsentrasi sperma pejantan Simmental, Limousin, dan Brahman *Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner* (Semarang: Universitas Dipenogoro)

[5] Thornton P K 2010 Livestock production: recent trends, future prospects. *Philos. Trans. R .Soc. B: Biol. Sci.* **365** 2853–867
[6] Hopkins S M and Evans L E 2003 *Veterinary Endocrinology and Reproduction* Fifth Edition (State Avenue, USA: Blackwell Publishing Company)
[7] Yimer N Y, Rosina N, Wahid A A, Saharee K C, Yap P, Ganesamurti and Fahmi M M 2011 Trans-scrotal ultrasonography and breeding soundness evaluation of bull in a herd of dairy and beef cattle with poor reproductive performance *J. Trop. Agric. Sci.* **34** 217–222
[8] Mathevon M, Buhr M and Dekkers J C M 1998 Environmental management and genetic factors affecting semen productionin Holstein bulls *J. Dairy Sci.* **81** 3321–30
[9] Coulter G H, Cook R B and Kastelic J P 1997 Effect sof dietary energy onscrotal surface temperature, seminal quality and spermproduction inyoung beef bulls *J. Anim. Sci.* **75** 1048–52
[10] Anggraeni A 2007 Indeks reproduksi sebagai faktor penentu efisiensi reproduksi sapi perah: fokus kajian pada sapi perah bos taurus *Prosiding Semiloka Nasional Prospek Industri Sapi Perah Menuju Perdagangan Bebas* 2020 **6** 61–74
[11] Sarastina T, Susilawati G and Ciptadi 2007 Analisa beberapa parameter motilitas spermatozoa pada berbagai bangsa sapi menggunakan computer assisted sperm analysis (CASA) *Jurnal Ternak Tropika* **6** 1–12
[12] Toleng A L, Sonjaya H, Yusuf M and Hamid A 2001 The use of progesterone RIA to increase efficiency and quality of artificial insemination of beef cattle in South Sulawesi, Indonesia, radio-immunoassay and related techniques to improve artificial insemination programmes for cattle reared under tropical and sub-tropical condition *IAEA-TECDOC* **1220** 37–43
[13] Haryati R 2017 *Rasio gradien putih telur optimal pada sexing spermatozoa dalam upaya meningkatkan proporsi spermatozoa Y pada semen sapi Bali* Disertasi (Makassar: Sekolah Pascasarjana Universitas Hasanuddin)
[14] Hufana-Duran D 2020 Predicting bull fertility by fourier harmonic analysis Laporan Kuliah Bioteknologi Reproduksi Peternakan (Makassar: Universitas Hasanuddin)
[15] Perchec G, Jeulin C, Cosson J, Andre F and Billiard R 1955 Relationship between sperm ATP content and motility of carp spermatozoa *J. Cell Sci.* **108** 747–53
[16] Suzuki K, Geshi M, Yamaguchi N and Nagai T 2008 Functional changes and motility characteristic of Japanese black bull spermatozoa separated by percoll *Anim. Reprod. Sci.* **77** 157–72
[17] Yendraliza 2008 *Inseminasi Buatan pada Ternak* (Pekanbaru: Suska Press)
[18] Susilawati T 2011 *Spermatology* (Malang: Universitas Brawijaya Press)
[19] Susilawati T 2000 *Teknologi Preservasi dan Kriopreservasi Spermatozoa dan Ova* Tesis (Malang: Program Pasca Sarjana Fakultas Peternakan Universitas Brawijaya)
[20] Clay J S and McDaniel B T 2001 Computing mating bull fertility from DHI Non-return Data *J. Dairy Sci.* **84** 1238–45
[21] Pepper-Yowell A R 2011 The use of computer assisted semen analysis to predict fertility in Holstein bulls. PhD Dissertation (Fort Collins, Colorado: Colorado State University)
[22] Kastelic J P and Thundathil J C 2008 Breeding soundness evaluation and semen analysis for predicting bull fertility *Specal Issue 16th: International Congress on Animal Reproduction* **43** 368–73