Correlation between Semen Quality, Libido, and Testosterone Concentration in Bali Bulls

Iskandar H1, Sonjaya H2, Arifiantini RI3, Hasbi H2

1Agricultural Science Study Program, Graduate School Hasanuddin University, Makassar, Indonesia
2Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia.
3Department of Veterinary Clinic, Reproduction and Pathology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
E-mail: arifiantini@apps.ipb.ac.id

(received 29-12-2021; revised 20-05-2022; accepted 24-05-2022)

ABSTRACT

Indonesia has two National Artificial Insemination Centers (AIC) and more than 15 Regional Artificial Insemination Centers (RAIC) spread across several provinces. Bulls in the AIC must have a high libido and produce good quality semen. This study examines the correlation between libido with semen quality and testosterone concentration to determine potential frozen semen production from Bali bulls in South Sulawesi RAIC. Ten Bali bull were used in this study. Semen collection was carried out twice a week with semen evaluation following the RAIC protocol. At the same time, blood samples and libido measurements were carried out from each bull. The frozen semen production potential was calculated by multiplying the semen volume, motility, and sperm concentration. The results showed that the quality of fresh semen and testosterone concentrations did not differ between high and low libido of Bali bulls. Libido has a positive correlation with semen volume (r = 0.52) and sperm motility (r = 0.62), while testosterone concentration has a negative correlation with semen volume (r = -0.65), sperm motility (r = -0.60), and libido (r = -0.48). Bulls with high and low libido have good frozen semen production potential, ranging from 19,755 – 21,640 straws per year. Bali bulls in RAIC have fresh semen quality and testosterone concentrations under normal conditions, with high potential for frozen semen production, although only 60% of Bali cattle have high libido and 40% have low libido.

Key Words: Bali Bull, Frozen Semen Productivity, Libido, Semen Quality, Testosterone Concentration

INTRODUCTION

Bali cattle are known to have high environmental adaptability and good reproductive efficiency. Bali cattle are also resistant to low feed conditions, resistant to parasites, with carcasses 52.72-57.6% and good meat quality (Alwiyah et al. 2016; Gunawan et al. 2016; Jakaria et al. 2017). Mating these cattle is mainly done by artificial insemination (AI), using frozen semen produced by the AI centre (AIC). Indonesia has two National AICs, and more than 15 Regional Artificial Insemination Centers (RAIC) spread across several
provinces. Bulls in the AIC must have a high libido and produce high semen quality. Thus, bulls in AIC and RAIC have to be superior, so they can make a good quality frozen semen to support the success of AI in the field and improve the quality of Bali cattle in the area. The bull fertility rate is related to sperm motility (Zubair et al. 2015), libido (Chenoweth 2021), sperm production capacity, and testosterone hormone (Singh et al. 2014).

The problem in the selected Bali bull is their libido. Libido is vital in bull because it will speed up the process of semen collection and also accelerate the process of semen freezing. Libido, or desire to mate, is manifested in the sexual behaviour of bulls and is naturally more active in approaching in estrus females (Abell et al. 2017). With various physiological mechanisms, bull desires mediate libido responses and experiences acquired by bulls (Kowalczyk et al. 2021). Bos taurus has a high libido (Le Danvic et al. 2015), while Bos indicus have a lower libido (Manegassi et al. 2021) compared to the Crossbreed (Mukhopadhyay et al. 2010). Bali cattle (Bos javanicus) are native Indonesian breeds with a lower libido than Maduras cattle (Susilawati 2011). For collecting semen purposes in AIC and RAIC, they use a live bull or a dummy. Therefore, the bull libido in AIC is essential, and libido is known to be influenced by the testosterone hormone (Perumal et al. 2020).

Testosterone is the primary androgen required for spermatogenesis in the testes and is responsible for maintaining secondary sexual characteristics and libido (Senger 2012). Testosterone is produced by Leydig cells (Kowalczyk et al. 2021) under the influence of luteinising hormone (LH) from the anterior pituitary (Wang et al. 2021). The testosterone concentration in Bos taurus aged 6 - 7 years is 35.16 ng/mL (Baharun et al. 2021), and in Bos indicus, 14.86 ng/mL (Littlejohn et al. 2017). Information on testosterone concentrations in native and local cattle has been reported by Gholib et al. (2020) in Aceh cattle (4.39 ng/mL), Kuantan cattle 2.82±1.99 ng/mL (Anwar & Jiyanto 2019), Ongole crossbreeds 6.14 ng/mL (Widyaningrum et al. 2015) and Bali cattle ranging from 4.57-4.79 ng/mL (Syarifuddin et al. 2017). Male fertility can be determined by analysing the testosterone hormone (Singh et al. 2014).

The relationship between testosterone with sperm quality and sperm motility has not been proven (Rajak et al. 2014a; Rajak et al. 2014b). Bali cattle producing frozen semen's productivity is essential and closely related to the value of semen volume, sperm motility, and sperm concentration (Nugraha et al. 2022). Information regarding the correlation of semen quality, libido, and testosterone concentration in Bali bulls is limited. Bulls in RAIC varied in libido and semen quality. Some bulls show a high libido with medium semen quality; on the other hand, some bulls exhibit low libido but have excellent semen quality. There are also bulls with low libido and low semen quality. Libido and semen quality are essential in RAIC. Therefore, this study aims to evaluate semen quality, libido, and testosterone concentration and examine the relationship between these three variables in Bali bulls’ reproductive performance.

**MATERIALS AND METHODS**

**Ethical approval**

The Animal Ethics Commission, Hasanuddin University, has approved this research number 302/UN4.6.4.5.31/PP36/2021.

**Study period and location**

This study was conducted from October 2020 to July 2021. This semen and blood samples were obtained from RAIC South Sulawesi. Sperm motility, volume, and concentration were evaluated at the South Sulawesi RAIC. Blood sample plasma preparation was performed at the South Sulawesi RAIC. The testosterone concentration was measured using the enzyme-linked immunosorbent assay (ELISA), performed according to the manufacturer's protocol at the Primate Research Center, IPB University.

**Research animal**

This study used ten Bali bulls aged 5-10 years belonging to South Sulawesi RAIC. All bulls were reared following the Standard Operating Procedures (SOP) of RAIC. The RAIC kept Bulls in 2.5x2 m cages equipped with feed and drink containers. Feeding in fresh forage 10% of body weight and concentrate as much as 2 kg per day given twice a day, in the morning and evening, drinking water provides ad libitum.

**Semen collection and evaluation**

Fresh semen was collected twice a week using an artificial vagina by the RAIC bull master. Semen collection is carried out between 06.00-10.00 AM. The collected semen was immediately brought to the laboratory for macroscopic and microscopic evaluation, referring to Arifiantini (2012). The data displayed is only semen volume, sperm motility, and sperm concentration. These three variables are the data needed to calculate the number of frozen semen produced by each bull.
Semen volume was measured visually on a semen collection tube with mL units. The sperm motility assessment by mixing 10 µL of semen with 40 µL of saline solution. After homogenising, 5 µL was taken, dripped onto the object glass, and covered with a cover glass. The evaluation was done using a binocular microscope (Olympus CX31 RTSF). Calculation of sperm concentration using a photometer SDM6 (Minitub, Germany).

**Libido assessment**

This study evaluates bull libido during semen collection and records the reaction time of the bull. The mounting enthusiasm was scored during mounts and service. Mounting enthusiasm was a score from -2 to +2 (Table 1).

**Table 1. Mounting enthusiasm of Bali bulls at South Sulawesi RAIC**

| Score | Criterion                          | Libido |
|-------|------------------------------------|--------|
| -2    | Bull does not mount                | Low    |
| -1    | Bull mounts by sliding              | Low    |
| 0     | Bull mounting between sliding and jumping | Moderate |
| +1    | Bull mounts by jumping              | High   |
| +2    | Bull jumps with great enthusiasm    | High   |

*Modification from Hoffack et al. (2006)*

**Testosterone concentration assessment**

Approximately 3-5 mL of blood samples were collected using a 5 mL vacutainer blood collection containing EDTA (three fingers, USA) through the vena jugularis region of the bulls. The blood was centrifuged at 3000 pm at room temperature for 10 minutes. Blood plasma was collected, put into a microtube, and stored at -20°C until analysis. Testosterone analysis was carried out using the Bovine testosterone ELISA kit method (Signalway antibody, #EK0019). Blood plasma was diluted in a ratio of 1:4 using distillate water. Standard solutions with concentrations ranging from 0.2 to 16 ng/mL. Samples and standard solutions were transferred (25 µL each) into ELISA microplate wells (Duplo performed), then added with conjugate enzymes (except blanks) and covered with cling film.

The mixture was homogenised using a vortex for 10 seconds and incubated at room temperature for 60 minutes. Afterwards, the microplate wells were washed 3-4 times with 300 µL of washing solution each, added 200 µL of the substrate, and incubated for 15 minutes (room temperature). The reaction stopped by adding 100 µL of stop solution to each well. The absorbance was read using an ELISA reader at 450 nm (Dasrul et al. 2020; Hafizuddin et al. 2020).

**Data analysis**

Independent-Sample T Test was applied to the differences between treatments (P<0.05), which were considered statistically significant. The data on fresh semen quality were analysed descriptively. The Pearson correlation test analysed the correlation between semen quality, libido, and testosterone concentration. Data were analysed using SPSS version 20 software.

**RESULTS AND DISCUSSION**

The average semen volume of bulls with high libido was 6.83±0.44 mL and bulls with low libido was 6.71±0.22 mL. The average sperm motility of bulls with high libido was 72.49±0.93 and bulls with low libido was 73.74±1.42. The sperm concentration of bulls with high libido was 1365.90±76.65 and bulls with low libido was 1247.70±69.28 (Table 2). This study showed only three variables of semen quality associated with frozen semen production. Semen volume, sperm motility, and sperm concentration were not significantly different between bulls with high and low libido (P>0.05). The high and low percentage of motility sperm in semen is affected by age, individuals, season, and temperature. Semen volume, sperm motility, and sperm concentration are considered essential indicators of sperm quality and fertility. Ismaya (2014) reported that temperatures influence the motility of sperm: cold temperatures will inhibit motility, while hot temperatures will increase motility. Increasing the sperm concentration could improve the chances of conception by increasing the number of normal sperm until the required threshold for conception is reached (Morrell et al. 2018). Murphy et al. (2013) reported that a higher concentration of fresh bull semen might cause an increase in oxidative stress. The semen volume in cattle, according to Ax et al. (2000), is 7-10 mL per ejaculate, with sperm concentration ranging from 1000x10⁶ to 1500x10⁶ sperm/mL. Individual influences on the fresh semen quality of Bali bulls have been reported by Indriastuti et al. (2020). Moreover, the sperm motility of the Bali bull at South Sulawesi RAIC is below sperm motility at Baturiti RAIC (Indriastuti et al. 2020). However, it still meets the quality requirements of fresh semen state of the Minister.
Table 2. The quality of the fresh semen samples and testosterone hormone concentration from Bali bulls at South Sulawesi RAIC

| Libido | Variable                      | Value               |
|--------|-------------------------------|---------------------|
|        | Semen volume (mL)             | 6.83±0.44 ±0.742    |
|        | Sperm Motility (%)            | 72.49±0.93 ±0.742   |
|        | Sperm concentration (× 106 mL)| 1365.90±76.65 ±0.742|
|        | Testosterone hormone concentration | 4.87±1.27 ±0.742 |

Table 3. Correlation between semen quality, libido, and testosterone hormone concentration of Bali bulls

| Variable                      | Semen volume | Sperm motility | Sperm concentration | Libido | Testosterone hormone concentration |
|-------------------------------|--------------|----------------|---------------------|--------|-----------------------------------|
| Semen volume                  | 1            |                |                     |        |                                   |
| Sperm motility                | 0.107        | 1              |                     |        |                                   |
| Sperm concentration           | -0.012       | 0.194          | 1                   |        |                                   |
| Libido                        | 0.525*       | 0.629*         | 0.109               | 1      |                                   |
| Testosterone hormone concentration | -0.659*    | -0.602*        | -0.026              | -0.485 | 1                                 |

*Shows a significant relationship between pairs of variables (P<0.05)

of Agriculture of Republic of Indonesia Number 10/Permentan/PK.210/3/2016 in 2016). The Indonesian National Standard for frozen bovine semen is as the SNI number: 4869.1:2017 (BSN 2017) also states that sperm motility of fresh semen to be processed into frozen semen must have sperm motility >70%. Semen quality is influenced by several factors such as age (Hapsari et al. 2018), season and temperature (Soren et al. 2016), semen collection interval (Sankhi et al. 2019), and individual variations (Indriastuti et al. 2020), and genetics (Mohammed & Ahmed 2017).

In this study, Bali bulls at south Sulawesi RAIC show that 60% have a high libido, while 40% show a low libido. The libido of Bali bulls at South Sulawesi RAIC differs due to environmental conditions, nutrition, age, and experience of bulls. Factors influencing libido and mating ability include the clump, number of estrus females, the environment (Hastono & Praharani 2014), and bull age (He et al. 2014). Libido is also influenced by genetic and management factors, environmental conditions, and bull age. The libido of the Brahman cross and Friesian Holstein cattle is higher than the Red Chittagong breed (Islam et al. 2018). Older bulls are more likely to have physical or pathological conditions that reduce libido (Masouni et al. 2011).

Testosterone hormone concentrations of Bali bulls with high and low libido showed not significant different (Table 2), the blood collection was carried out simultaneously. The bull’s reaction to riding the female is naturally related to the presence of stimulation from the female. The testosterone concentration in this study was within normal ranges. Therefore, semen quality was also categorised as good. Bull libido level is affected by testosterone concentration. Testosterone concentrations and semen quality vary throughout the year (Chacur et al. 2013). Furthermore, testosterone concentrations are higher during early animal growth (Gulia et al. 2010) and accelerated physical and testicular growth (Chacur et al. 2018). Testosterone concentration in Holstein bulls aged 8-9 months is 0.49 ng/mL (Gholami et al. 2010); in Aceh bulls, cattle aged 4-5 years are 5.14-13.06 ng/mL (Dasrul et al. 2020). Simmental bull 8-10 years old were 13.39 to 23.27 ng/mL (Baharan et al. 2021). Low libido can be caused by low testosterone levels (Rajak et al. 2014a; Rajak et al. 2014b).

The correlation between semen volume, sperm motility, sperm concentration, and testosterone hormone concentration in Bali bulls is presented in Table 3. In this study, sperm motility has a low positive correlation with semen volume and a low negative correlation with sperm concentration. Bull's libido positively correlates with semen volume, sperm motility, and low correlation with sperm concentration. However, semen volume with testosterone concentration had a strong negative relationship (-0.65). During semen collection, the bull master performs several teases. Teasing stimulates the secretion of seminal plasma, mostly from vesicular glands, which consists of 75% of semen volume (Garner & Hafez 2016).
eans the urogenital tract. Bulls' age may affect semen quality. Hafizuddin et al. (2020) reported a relationship between semen volume and testosterone concentration, which is plasma testosterone concentrations determined by mitosis at the time of spermatocytogenesis. Spermatocytogenesis is controlled by Follicle Stimulating Hormone (Senger 2012).

Testosterone concentration and libido had a moderately negative correlation (-0.48). This moderate negative relationship means that the higher the testosterone concentration, the lower the libido. Previous studies have reported that testosterone concentrations are not associated with libido (Schallenger et al. 1991; Sekasiddhi & Buban 1997), semen quality (Souza et al. 2011) and only have a low association with mass movement and sperm motility (Santos et al. 2004; Souza et al. 2011). Other studies reported that the libido of bulls is significantly related to semen quality and fertilization rate (Singh et al. 2019; Kowalczyk et al. 2021). The testosterone concentration varies within each species, individual bull, age, season, and environment (Rajak et al. 2014b). Bulls' age may also influence this result, the range of bulls' age in this study was 5 to 10 years old, and each had a different testosterone concentration between bull with high and low libido.

This study shows that libido has a moderate relationship with semen volume (0.52) and a strong relationship with sperm motility (0.62). Several studies reported that libido scores were strongly associated with semen volume, sperm motility, and sperm concentration in Brahman, Friesian Holstein, Red Chittagong bulls (Islam et al. 2018), and Sahiwal bulls (Singh et al. 2015). This study showed that bulls with high libido react faster to mount and ejaculation than bulls with low libido. During teasing and courtship, the accessory glands will secrete a seminal plasma.

Semen volume, sperm motility, and sperm concentration, if each variable is seen separately, cannot describe the potential of each bull. The multiplication of these three variables will explain the productivity of

| Libido | Number of motile sperm per ejaculate (x10^6)* | Number of straws per ejaculate (pieces)** | The potential of frozen semen production annually (pieces)*** |
|--------|-----------------------------------------------|------------------------------------------|-------------------------------------------------------------|
| High   | 6762.66                                       | 270.50                                   | 21640.00                                                   |
| Low    | 6173.56                                       | 246.94                                   | 19755.20                                                   |

*The result of multiplying semen volume x sperm concentration x % sperm motility; ** Represents the number of straws produced in one ejaculate, obtained by dividing the number of motile sperm in one ejaculate by 25 x10^6 (insemination dose of frozen bull semen); *** Represents the total straw production for one year, assuming one year is 40 weeks of collection, twice a week (40 x 2 = 80 ejaculates/year); The calculation is multiplying 80 by the number of straw production per ejaculate
each bull in producing frozen semen (Table 4). Table 4 shows that bulls with high and low libido at South Sulawesi RAIC are superior because they have a high frozen semen production, 19 to 26 thousand straws yearly. Semen production of native or local cattle in the Roadmap for Self-Sufficiency of Indonesian superior bulls (Ditjennak 2018), a minimum of 7500 straws per table. Table 4 shows bulls with high and low libido had good semen quality and high productivity in frozen semen production. Knowledge in evaluating bull libido and frozen semen productivity is fundamental to calculating the ideal population structure in each RAIC. The RAIC could determine their frozen semen production target per year to figure out the bulls needed to reach it.

CONCLUSION

This study concludes that Bali bulls in RAIC with high and low libido has no effect on semen quality and testosterone hormone testosterone. However, there is a positively correlates with semen volume and sperm motility, while testosterone hormone concentration negatively correlates with semen volume, sperm concentration, and libido

ACKNOWLEDGEMENT

This study is supported by the Ministry of Research, Technology, and Higher Education of Indonesia through the PMDSU Scholarship (letter of appointment number, 1421/D3/PG/2018;1345/D3/PG/2018). The authors thank South Sulawesi Regional Artificial Insemination Center for facilitating the research.

REFERENCES

[BSN] Badan Standarisasi Nasional. 2017. SNI Semen Beku-Bagian 1: Sapi. Jakarta (Indones): Badan Standardisasi Nasional.

[Ditjennak] Direktorat Perbibitan dan Produksi Ternak. 2018. Roadmap swasembada pejantan unggul 2018-2022. Jakarta (Indones): Direktorat Perbibitan dan Produksi Ternak.

Abell KM, Theurer ME, Larson RL, White BJ, Hardin DK, Randle RF, Cushman RA. 2017. Calving distributions of individual bulls in multiple-sire pastures. Theriogenology. 93:7–11. DOI: 10.1016/j.theriogenology.2017.01.010.

Alwiyah A, Naraini H, Agung P, Jakaria J. 2016. Polymorphism steeroyl-CoA desaturase (SCD) gene and association with characteristics meat in Bali cattle. J Indones Trop Anim Agric. 41:188. DOI: 10.14710/jitaa.41.4.188-195.

Andersson M. 1992. Relationships between GnRH-induced testosterone maxima, sperm motility and fertility in Ayrshire bulls. Anim Reprod Sci. 27:107–111. DOI: 10.1016/0378-4320(92)90050-N.

Ansari MS, Ali S, Ullah N, Anwar M. 2007. Blood serum testosterone level and its relationship with scrotal circumference and semen characteristics in Nili-Ravi Buffalo bulls. Pak Vet J. 27:63–66.

Anwar P, Jiyanto J. 2019. Identifikasi hormon testosteron sapi Kuantan plasma nutlah Riau sebagai penentu klasifikasi kriteria pejantan unggul. J Peternak Indones. 21:230. DOI: 10.25077/jpui.21.3.230-239.2019.

Arifiantini R. 2012. Semen collection and evaluation techniques in animals. Bogor (Indones): IPB Press.

Ax R, Dally M, Didion B, Lenz R, Love C, Vaner D, Hafez B, Bellin M. 2000. Artificial insemination. In: Hafez B, Hafez E, editors. Reprod Farm Anim. 7th ed. Philadelphia (US): Wiley-Blackwell.

Baharun A, Said S, Arifiantini RL, Karija NWK. 2021. Correlation between age, testosterone and adiponectin concentrations, and sperm abnormalities in Simmental bulls. Vet World. 14:2124–2130. DOI: 10.14202/vetworld.2021.2124-2130

Chacur M, Arikawa A, Oba E, Souza C, Roberto Gabriel Filho L. 2018. Influence of testosterone on body and testicular development in Zebu cattle in the tropical climate. In: Estrada M, editor. Adv Testosterone Action. London (UK): IntechOpen; p. 18.

Chacur MGM, Mizusaki KT, Filho LRAG, Oba E, Ramos AA. 2013. Seasonal effects on semen and testosterone in zebu and taurine bulls. Acta Sci Vet. 41.

Chenoweth P. 2021. Bull behavior, sex-drive and management. In: Fields M, Sand R, editors. Factors Affect Calf Crop. Boca Raton: CRC Press; p. 319–330. DOI: 10.1201/9781003069119-23.

Le Danvic C, Gérard O, Selle S, Ponsart C, Chemineau P, Humblot P, Nagnan B, Belle M. 2000. Artificial insemination. In: Hafez B, Hafez E, editors. Reprod Farm Anim. 7th ed. Philadelphia (US): Wiley-Blackwell.

Le Danvic C, Gérard O, Selle S, Ponsart C, Chemineau P, Humblot P, Nagnan B, Belle M. 2000. Artificial insemination. In: Hafez B, Hafez E, editors. Reprod Farm Anim. 7th ed. Philadelphia (US): Wiley-Blackwell.

Le Danvic C, Gérard O, Selle S, Ponsart C, Chemineau P, Humblot P, Nagnan B, Belle M. 2000. Artificial insemination. In: Hafez B, Hafez E, editors. Reprod Farm Anim. 7th ed. Philadelphia (US): Wiley-Blackwell.

Le Danvic C, Gérard O, Selle S, Ponsart C, Chemineau P, Humblot P, Nagnan B, Belle M. 2000. Artificial insemination. In: Hafez B, Hafez E, editors. Reprod Farm Anim. 7th ed. Philadelphia (US): Wiley-Blackwell.

Le Danvic C, Gérard O, Selle S, Ponsart C, Chemineau P, Humblot P, Nagnan B, Belle M. 2000. Artificial insemination. In: Hafez B, Hafez E, editors. Reprod Farm Anim. 7th ed. Philadelphia (US): Wiley-Blackwell.
Gholib G, Wahyuni S, Akmal M, Hasan M, Agil M, Purwanta B. 2020. The validation of a commercial enzyme-linked immunosorbent assay and the effect of freeze-thaw cycles of serum on the stability of cortisol and testosterone concentrations in Aceh cattle. F1000Research. 8:1220. DOI: 10.12688/f1000research.19804.3.

Gulia S, Sarkar M, Kumar V, Meyer H, Prakash B. 2010. Divergent development of testosterone secretion in male zebu (Bos indicus) and crossbred cattle (Bos indicus x Bos taurus) and buffaloes (Bubalus bubalis) during growth. Trop Anim Health Prod. 42:1143–1148. DOI:10.1007/s11250-010-9538-x.

Gunawan I, Suwiti N, Sampurna P. 2016. The effects of mineral on the chest circumference, body length, and body height of male Bali cattle. Bul Vet Udayana. 8:128–134.

Hafizuddin H, Karja NWK, Praharani L, Setiadi MA. 2020. Adiponectin and testosterone level and their correlations with fertility in Anglo NubianXElwaah grade crossbred bucks. Trop Anim Sci J. 43:110–116. DOI:10.5398/tasj.2020.43.1.110.

Hapsari RD, Khalifah Y, Widyan S, Pramono A, Prastowo S. 2018. Age effect on post freezing sperm viability of Bali cattle (Bos javanicus). IOP Conf Ser Earth Environ Sci. 142:012007. DOI:10.1088/1755-1315/142/1/012007.

Hastoño H, Praharani L. 2014. Evaluation of libido and mating ability of bulls from Friesian Holstein and Ongole crossbreed. In: Pros Semin Nas. Bogor (Indones): Lembaga Ilmu Pengetahuan Indonesia.

He Z-X, Huang M-F, Wang A-K, Zhang J-C, Zhao G, Yuan X-P, Qu K-X, Huang B-Z. 2014. Patterns of plasma hormone concentrations in Mithun bulls under a semi-management. J Anim Vet Adv. 13:732–739.

Hoflack G, Van Soom A, Maes D, de Kruijff A, Opsomer G, Duchateau L. 2006. Breeding soundness and libido examination of Belgian Blue and Holstein Friesian artificial insemination bulls in Belgium and The Netherlands. Theriogenology. 66:207–216. DOI:10.1016/j.theriogenology.2005.11.003.

Indrayanto Y. 2021. Andropause. Surakarta (Indones): Sebelas Maret University.

Indriastuti R, Ulum MF, Arifiantini RI, Purwanta B. 2020. Individual variation in fresh and frozen semen of Bali bulls (Bos sondaicus). Vet World. 13:840–846. DOI:10.14202/vetworld.2020.840-846.

Islam M, Apu A, Hoque S, Ali M, Karmaker S. 2018. Comparative study on the libido, semen quality and fertility of Brahman cross, Holstein Friesian cross and Red Chittagongg breeding bulls. Bangladesh J Anim Sci. 47:61–67. DOI: 10.3329/bjas.v47i2.40236.

Ismaya. 2014. Biotechnology of artificial insemination on cattle and buffalo. Yogyakarta (Indones): UGM Press.

Jakaria, Khasanah H, Priyanto R, Baihaqi M, Ulum MF. 2017. Prediction of meat quality in Bali cattle using ultrasound imaging. J Indones Trop Anim Agric. 42:59. DOI: 10.14710/jitaa.42.2.59-65.

Kowalczyk A, Gałęska E, Czersińska-Piątkowska E, Szul A, Hebda L. 2021. The impact of regular sperm donation on bulls’ seminal plasma hormonal profile and phantom response. Sci Rep. 11(1):11116. DOI: 10.1038/s41598-021-30630-8.

Littlejohn BP, Roberts MC, Bedenbaugh MN, Lewis AW, Neuendorff DA, Riley DG, Carroll JA, Vann RC, Amstalden M, Randel RD, Welsh TH. 2017. Evaluation of the influence of prenatal transportation stress on GnRH-stimulated luteinising hormone and testosterone secretion in sexually mature Brahman bulls1,2. J Anim Sci. 95:129–138. DOI: 10.2527/jas.2016.0445.

Mahmood S, Ahmad N, Rehman H, Zaneb H, Farooq U. 2014. A study on relationship among age, body weight, orhidometry and semen quality parameters in adult cholistani breeding bulls. J Anim Plant Sci. 2425:380–384.

Malik M, Jamil H, Qureshi Z, Meehoon A, Rizvi S, Ullah S, Dilshad S, Zaman A, Ullah N, Saifuddin S, et al. 2018. Investigation on relationship of hormonal profile and biochemical constituents of seminal plasma with physical characteristics of Damani buck semen. Pure Appl Biol. 7:684–691.

Manegassi S, Peripolli V, Canozzi M, Barcellos J. 2021. Libido and serving skills of Zebu cattle bulls. Semina. 42:3057–3063.

Masoumi R, Towhid A, Javaremi A, Nabizadeh H, Zandi M. 2011. Influence of PGF2α on semen quality and libido in Holstein bulls. Turkish J Vet Anim Sci. 35:1–6.

Minister of Agriculture of Republic of Indonesia. 2016. Peraturan Menteri Pertanian Republik Indonesia. Jakarta (Indones), Indonesia.

Mohammed A, Ahmed W. 2017. Pure Egyptian cattle bulls show both individual variation and different interaction with extender in the post-thawing sperm parameters. Andrology. 6:1000193.

Morrell JM, Valeau AS, Lundeheim N, Johannisson A. 2018. Sperm quality in frozen beef and dairy bull semen. Acta Vet Scand. 60:41. DOI:10.1186/s13028-018-0396-2.

Mukhopadhyay CS, Gupta AK, Yadav BR, Khate K, Raina VS, Mohanty TK, Dubey PP. 2010. Subfertility in males: An important cause of bull infertility. Asian-Australasian J Anim Sci. 23:450–455. DOI:10.5713/ajas.2010.90298.

Murphy C, Fahey AG, Shafat A, Fair S. 2013. Reducing sperm concentration is critical to limiting the oxidative stress challenge in liquid bull semen. J Dairy Sci. 96:4447–4454. DOI: 10.3168/jds.2012-6484.

Nugraha CD, Widodo N, Kuswati K, Suyadi S. 2022. The real potential of semen production of Bali Bull: over year observation at Singsosari National Artificial Insemination Center (SNAIC), Singsosari-Indonesia.Widodo E, Jayanegara A, Suyadı, Nurgiartiningingsih VMA, Ciptadi G, Iskandar et al. Correlation between semen quality, libido, and testosterone concentration in Bali bulls
Peramal P, Chang S, De AK, Baruah KK, Khate K, Vupru K, Mitra A. 2020. Slow release exogenous melatonin modulates scrotal circumference and testicular parameters, libido, endocrinological profiles and antioxidant and oxidative stress profiles in mithun. Theriogenology. 154:1–10.

Rachmawati L, Ismaya, Astuti P. 2014. Correlation between testosterone, libido and sperm quality of Bligon, Kejobong and Oktawa grade bucks. Bul Peternak. 38:8. DOI: 10.21059/buletinpeternak.v38i1.4598.

Rajak S, Tripathi U, Attupuram N, Boro P, Layek S, Aslam M, Kumaresan A, Mohanty T, Sreela L, Prakash M. 2014a. Relationship of blood and seminal plasma testosterone concentrations with semen quality in crossbred bulls. Indian J Dairy Sci. 67:162–167.

Rajak SK, Kumaresan A, Gaurav MK, Layek SS, Mohanty TK, Muhammad Aslam MK, Tripathi UK, Prasad S, De S. 2014b. Testicular cell indices and peripheral blood testosterone concentrations in relation to age and semen quality in crossbred (Holstein Friesian×Tharparkar) bulls. Asian-Australasian J Anim Sci. 27:1554–1561. DOI: 10.5713/ajas.2014.14139.

Sankhi S, Sapkota KR, Regmi B. 2019. Effect of age and frequency of collection on quality of Jersey bulls semen at National Livestock Breeding Center (NLBC), Nepal. Int J Appl Sci Biotechnol. 7:88–95. DOI: 10.3126/ijasbt.v7i1.23312.

Santos MD, Torres CAA, Ruas JR M, Guimarães JD, Silva Filho JM. 2004. Reproductive potential of Nelore bulls submitted to different bull:cow proportion. Arq Bras Med Vet e Zootec. 56:497–503.

Schallengerber E, Hartl P, Schams D, Lorrman W, Hahn R. 1991. Hormone profiles and fertility in bulls. Tierzuchter 43:402-403. Tierzuchter. 43:402–403.

Sekasidhi P, Buban S. 1997. Hormone profiles in low libido bulls. J Thai Vet Med Asso. 48:43–49.

Senger P. 2012. Pathways to pregnancy and parturition. 3rd ed. Redmond (USA): Current Conceptions. Inc.

Singh AK, Brar PS, Cheema RS. 2014. Relationships among frozen-thawed semen fertility, physical parameters, certain routine sperm characteristics and testosterone in breeding Murrah buffalos (Bubalus bubalis) bulls. Vet World. 7:644–651. DOI: 10.14202/vetworld.2014.644-651.

Singh K, Kumar A, Honparkhe M, Singh A. 2019. Quantification of Sexual Behaviour Traits in Presence of Teaser Male in Relation to Libido and Semen Quality in Buffalo Bulls. J Anim Res. 9:143–146. DOI: 10.30954/2277-940X.01.2019.19.

Singh S, Bhakat M, Mohanty TK, Kumar A, Gupta AK, Chakravarty AK, Singh P. 2015. Sexual behavior and its relationship with semen quality parameters in Sahiwal breeding bulls. Vet World. 8:745–749. DOI: 10.14202/vetworld.2015.745-749.

Soren S, Singh SV, Singh P. 2016. Influence of season on seminal antioxidant enzymes in Karan Friesbulls under tropical climatic conditions. Turkish J Vet Anim Sci. 40:797–802. DOI: 10.3906/vet-1603-76.

Souza LW de O, Andrade AFC, Celeghini ECC, Negrão JA, Arruda RP de. 2011. Correlation between sperm characteristics and testosterone in bovine seminal plasma by direct radioimmunoassay. Rev Bras Zootec. 40:2721–2724. DOI: 10.1590/S1516-3598201100120015.

Susilawati T. 2011. Spermatology. Malang (Indones): Universitas Brawijaya Press.

Syariifuddin NA, Toleng AL, Rahardja DP, Ismartoyo I, Yusuf M. 2017. Improving libido and sperm quality of Bali bulls by supplementation of Moringa oleifera Leaves. Media Peternak. 40:88–93. DOI: 10.5398/medpet.2017.40.2.88

Wang H-Q, Zhang W-D, Yuan B, Zhang J-B. 2021. Advances in the regulation of mammalian follicle-stimulating hormone secretion. Animals. 11:1134. DOI: 10.3390/ani11041134.

Widyaningrum Y, Luthfi M, Affandy L. 2015. Testosterone and Luteinizing hormone concentration of steer Ongolecrossbred in different housing models to accelerate puberty. In: Semin Nas Teknol Peternak dan Vet. Bogor (Indones): Pusat Penelitian dan Pengembangan Peternakan.

Zubair M, Ahmad M, Jumil H. 2015. Review on the screening of semen by hypo-osmotic swelling test. Andrologia. 47:744–750. DOI: 10.1111/and.12335.