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

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

ABSTRAK

Iskandar H, Sonjaya H, Arifiantini RI, Hasbi H. 2022. Korelasi antara kualitas semen, libido dan konsentrasi testosteron pada pejantan sapi Bali. JITV 27(2):57-64. DOI: http://dx.doi.org/10.14334/jitv.v27i2.2981.

Indonesia memiliki dua Balai Inseminasi Buatan (BIB) Nasional dan lebih dari 15 Balai Inseminasi Buatan Daerah (BIBD) yang tersebar di beberapa provinsi. Pejantan unggul di BIB dan BIBD, harus memiliki libido yang tinggi dan menghasilkan semen yang berkualitas. Penelitian ini bertujuan untuk mengevaluasi dan menguji korelasi antara libido dengan kualitas semen dan konsentrasi hormon testosteron, serta potensi produksi semen beku dari pejantan sapi Bali di BIBD Sulawesi Selatan. Sepuluh ekor pejantan sapi Bali milik BIBD Sulawesi Selatan digunakan dalam penelitian ini. Koleksi semen dilakukan dua kali setiap minggu dengan evaluasi semen mengikuti protokol BIBD, bersamaan dengan itu dilakukan pengambilan sampel darah dan pengukuran libido dari masing-masing pejantan. Potensi produksi semen beku dihitung dengan mengalikan volume semen, motilitas dan konsentrasi sperma. Hasil penelitian menunjukkan bahwa kualitas semen segar dan konsentrasi hormon testosteron tidak berbeda antara pejantan sapi Bali libido tonggi dan rendah. Libido memiliki korelasi positif dengan volume semen (r= 0,52) dan motilitas sperma (r= 0,62), sedangkan konsentrasi testosteron berkorelasi negatif dengan volume semen (r= -0,65), motilitas sperma. Pejantan sapi Bali libido tinggi dan rendah memiliki potensi produksi semen beku yang baik yaitu berkisar antara 19.755 – 21.640 straw per tahun. Pejantan sapi Bali di BIBD memiliki kualitas semen segar dan konsentrasi hormon testosteron dalam kondisi normal dengan potensi produksi semen beku yang tinggi, meskipun hanya 60% sapi Bali yang memiliki libido tinggi dan 40% memiliki libido rendah.

Kata Kunci: Sapi Bali, Produksi Semen Beku, Libido, Kualitas Semen, Konsentrasi Testosteron

ABSTRACT

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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. 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 Hoflack 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 $1000x10^6$ to $1500x10^6$ 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			
	Semen volume (mL)	Sperm Motility (%)	Sperm concentration (× 106 mL)	Testosterone hormone concentration
High	6.83±0.44[a]	72.49±0.93[a]	1365.90±76.65[a]	4.87±1.27[a]
Low	6.71±0.22[a]	73.74±1.42[a]	1247.70±69.28[a]	2.99±0.33[a]

Same superscript letters following numbers in the same column indicate non-significant difference (P< 0.05)

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 (Masoumi 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 (Baharun 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).

Table 4. Libido and productivity of Bali bulls at South Sulawesi RAIC ordered from highest to the lowest production

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

Furthermore, teasing also stimulates the secretion of the bulbourethral gland, which secretes an alkaline fluid, cleaning the urogenital tract of the bull. As we all know, urine and semen come out in the same channel; therefore, the alkaline fluid secreted by the bulbourethral gland cleans the urogenital tract, previously passed by urine. This condition is proved by good sperm motility. Mahmood et al. (2014) reported that the age of the bull had a significant correlation with semen volume (r= 0.93) negative correlation with sperm concentration (r= -0.87). All bulls in this study are at the productive age from five to ten years. Availability of sufficient testosterone will cause an increase in testosterone accumulation. As a result, the area activates the metabolism brain and regulates libido to be active (Indrayanto 2021). Andersson (1992) reported a significant correlation between testosterone concentration and fertility in Ayrshire bulls, followed by high sperm motility and sperm concentration. On the other hand, Chacur et al. (2018) reported an increase in serum testosterone levels, testes' growth and bodyweight of young Brahman bulls at 12 and 14 months.

This result exhibit a high negative correlation between semen volume and testosterone concentration. In contrast, Dasrul et al. (2020) reported a relationship between testosterone and semen volume in Aceh bull. In small ruminants such as Bligon goats and Anglo-Nubian x Etawah Grade, Rachmawati et al. (2014) and Hafizuddin et al. (2020) reported similar to Dasrul et al. (2020). One of the testosterone hormone functions is to regulate the work of the accessory glands, which produce seminal plasma (Senger 2012). Testosterone concentration is related to semen quality (Malik et al. 2018), which is plasma testosterone concentrations remain high during active semen production

The testosterone concentration negatively correlates with sperm motility (-0.60). According to Ansari et al. (2007), testosterone did not correlate with motility and sperm concentration in buffalo. Moreover, Souza et al. (2011) state that mass movement and sperm motility had low relationship with testosterone concentration in Simental bull aged 33-41 months. In this study, testosterone and sperm concentrations were negatively very low correlated (-0.02). This condition was also reported by Rajak et al. (2014) that testosterone concentration was not related to sperm concentration of crosses bulls. Testosterone plays a role in spermatogenesis, especially at the stage of spermiogenesis. Furthermore, sperm concentration is 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 (Schallenberger 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

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 year. 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.

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