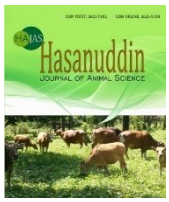

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Effect of Ejaculation Frequency on Semen Quality of Bali Cattle

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ABSTRACT

Ejaculation frequency is an important factor influencing semen quality in breeding bulls. This study aims to evaluate the effect of two consecutive ejaculations on the fresh semen quality of Bali cattle at the Pucak Maros Artificial Insemination (AI) Center. Secondary data from semen production records of five Bali bulls were analyzed, focusing on semen volume, sperm motility, and sperm concentration. The results showed that the frequency of ejaculation generally increased semen volume but significantly reduced sperm motility and concentration in several bulls ($P<0.05$). Correlation analysis revealed a negative correlation between semen volume and both motility and concentration, while motility and concentration were positively correlated. In conclusion, the second ejaculation with a short interval increases semen volume but reduces sperm motility and concentration. Optimizing ejaculation frequency is therefore essential to ensure high-quality semen for AI programs in Bali cattle.

Keywords: Ejaculation frequency, Bali cattle, semen volume, sperm motility, sperm concentration

INTRODUCTION

The livestock sector plays a crucial role in meeting animal protein needs, with Bali cattle among Indonesia's most important local genetic resources [1]. Bali cattle are widely used for their

adaptability, reproductive efficiency, and contribution to meat production. To support population growth and genetic improvement of Bali cattle, the Artificial Insemination (AI) program remains a key reproductive strategy [2]. AI also improves the genetic quality of livestock, streamlines bull use, and helps prevent disease transmission. The initial step in this program is the provision of high-quality semen [3].

The effectiveness of an AI program depends heavily on semen quality. High-quality semen is crucial for achieving optimal fertilization rates and reproductive efficiency. Semen quality is influenced by factors such as individual bull characteristics, semen collection method, diluent type and composition, storage conditions, and ejaculation frequency. Among these factors, ejaculation frequency plays a crucial role in determining semen volume, sperm motility, and concentration [4] [5].

Previous studies have shown that ejaculation frequency significantly affects semen characteristics across species, including cattle, sheep, horses, and dogs [6]. Purwantara et al. [7] reported that in Bali cattle, ejaculation frequency influences semen volume and sperm concentration, though results vary by collection interval and individual bulls. Short intervals between consecutive ejaculations can deplete the epididymal sperm reserve, potentially affecting semen quality. To achieve optimal semen production, semen collection procedures must preserve sperm quality.

Therefore, this study aimed to evaluate the effects of two consecutive ejaculations on fresh semen quality, specifically semen volume, sperm motility, and sperm concentration, at the AI Center Pucak Maros. These findings are expected to provide practical recommendations to improve semen collection efficiency and the performance of artificial insemination programs for Bali cattle.

MATERIALS AND METHODS

Research materials

This study used secondary data from records of fresh semen quality collected from five Bali bulls aged 10-11 years, with a frequency of two ejaculations from routine semen collection activities at the AI Center Pucak Maros. Semen collection procedures were performed by trained personnel in accordance with the AI Center's standard operating procedures (SOPs) and national animal welfare regulations. No additional experimental treatment or invasive procedures were applied to the animals.

Research procedures

Semen collection and quality inspection procedures are carried out by the AI center in accordance with the center's standard operating procedure (SOP). Semen collection was performed using the artificial vagina method, and samples were collected in graduated tubes. The first and second ejaculations were performed on the same day and collected separately, with a 5-10-minute interval between collections. The collected semen was then evaluated macroscopically and microscopically. Semen quality parameters in this study include semen volume, individual motility, mass motility, and spermatozoa concentration.

Semen volume

The semen volume observation procedure is performed by reading the number on the collecting tube for the total ejaculate volume of the male [8].

Spermatozoa motility

This observation is performed by placing a semen sample on a glass slide, covering it with a cover slip, and examining it under a microscope at 400x magnification [9]. Motility assessment is performed subjectively by counting the number of spermatozoa that move forward in a straight line (progressive), with a standard assessment ranging from 0 to 100%.

Spermatozoa concentration

The procedure for measuring spermatozoa concentration using a spectrophotometer involves mixing semen and a physiological NaCl solution at a 1:10 ratio, then placing the mixture in a cuvette and measuring the total spermatozoa concentration [10].

Data analysis

The data obtained in this study were analyzed using an independent-samples t-test in IBM SPSS Statistics for Windows, Version 16.0 (IBM Corp., NY, USA) to compare the means of the data groups.

RESULTS AND DISCUSSIONS

Semen quality is a crucial factor in successful fertilization and animal breeding programs [11]. Semen quality directly affects herd reproductive efficiency and farm profitability [12]. Semen quality parameters measured in this study include sperm volume, motility, and concentration. Table 1 presents data from a study on the effect of ejaculation frequency on the quality of Bali cattle semen at the Pucak Maros AI Center.

Table 1. Effect of ejaculation frequency on semen quality of Bali cattle at Pucak Maros AI Center

Bull Code	Semen Volume (ml)		Sperm Motility (%)		Sperm Concentration ($x10^6$ cells/ml)	
	Ejaculation 1	Ejaculation 2	Ejaculation 1	Ejaculation 2	Ejaculation 1	Ejaculation 2
11522	4.2±2.2	7.4±0.4	53.3±23.6	56.7±18.9	821.0±383.5	753.7±337.2
11521	3.7±0.5	3.9±1.9	70.0±0.0	70.0±0.0	1008.2±139.8	857.2±118.3
11442	4.4±1.9^a	7.1±1.9^b	70.0±0.0	56.7±19.4	1110.4±142.0^a	701.9±311.5^b
11434	3.5±1.3^a	7.1±0.7^b	70.0±0.0	57.5±9.4	1687.3±276.6^a	663.5±207.3^b
11539	4.5±1.5	5.3±0.3	70.0±0.0^a	25.0±5.0^b	1149.0±182.0^a	266.0±49.0^b

Description: Different superscripts in the same row indicate significant differences ($P<0.05$)

Based on the results in Table 1, the frequency of ejaculation was statistically significantly different ($P<0.05$) for semen volume in males 11442 and 11434, but not different ($P>0.05$) in males 11522, 11521, and 11539. Semen volume at the second ejaculation of each male studied tended to be higher than at the first. Bahadur et al. [13] conducted a similar study with 2 ejaculations on the same day, with a 30-40-minute interval between the first and second. The study found that the first semen volume was 2.9 ml, while the second was only 1.1 ml. Ayad et al. [14] reported the relationship between semen volume and ejaculation frequency in daily periods across several studies, showing a significant increase in semen volume with increased ejaculation frequency.

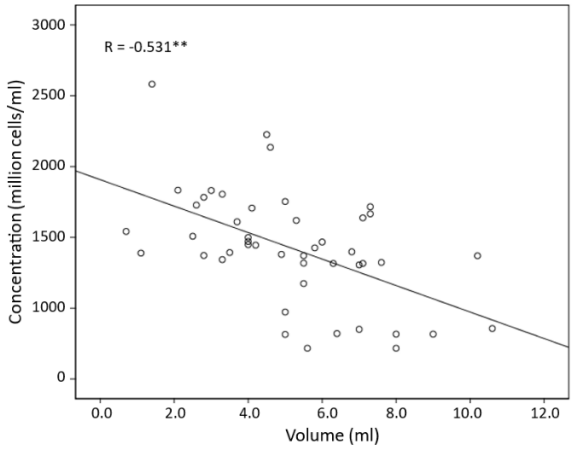

Figure 1. Correlation between semen volume and sperm concentration in Bali Cattle semen collected at the AI Center Pucak, Maros. A significant negative correlation was observed (P<0.01), indicating that sperm concentration decreases as semen volume increases.

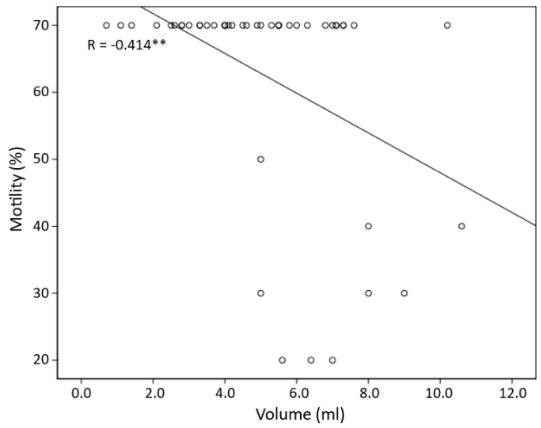

Figure 2. Correlation between semen volume and sperm motility in Bali Cattle semen. The analysis showed a significant negative correlation (P<0.01), indicating that motility decreases as semen volume increases.

Figure 1 shows a negative correlation between semen volume and sperm concentration (R = -0.531). This means that the higher the semen volume, the lower the sperm concentration. This pattern is due to differences in the composition of semen plasma. The first ejaculation tends to contain less plasma but more total spermatozoa than the second. According to [7], high ejaculate volume is not always positively correlated with overall semen quality and is often associated with lower concentrations. Bahadur et al. [13] reported that a high volume of semen collected reduces the concentration of spermatozoa, whereas a lower volume increases it.

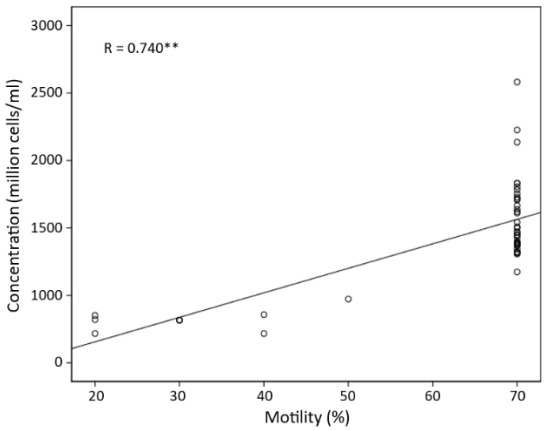

Figure 3. Correlation between sperm motility and concentration in Bali Cattle semen. A significant positive correlation was found (P<0.01), indicating that higher sperm concentration is associated with higher motility

Semen is an organic mixture containing spermatozoa and plasma, with components from accessory glands [15]. The process by which the gel-like ejaculate liquefies is called semen liquefaction. Semen liquefaction is a proteolytic process in which the gel-like ejaculated semen becomes watery. Liquefaction requires proteins present in the acellular fraction (seminal plasma) that are crucial for sperm motility [16]. Kirton et al. [17] examined sperm composition and plasma nutrient components in the semen of bulls with an ejaculation frequency of 4 collections. The study found that semen volume and spermatozoa concentration in the first ejaculation were higher and gradually decreased in subsequent ejaculations. Likewise, the nutritional composition of semen plasma, including amino nitrogen, citric acid, and protein, also gradually decreased with each ejaculation.

In their study, Mayorga-Torres et al. [18] reported that the frequency of ejaculation per day affects semen volume and spermatozoa concentration, with both decreasing. Semen volume on the first day tends to be higher than on the following day. On the first day, semen volume was 2.5 ml with a concentration of 121.3×106. On day 2, 1.8 ml and 80.3×10^6; day 4, 1.8 ml and 72.1×10^6; day 6, 1.6 ml and 66.1×10^6; day 9, 1.9 ml and 75.1×10^6; day 11, 1.7 ml and $80.4\times10^{6;}$ and day 13, 1.9 ml and 80.3×10^6. This decrease is attributed to reduced reserves stored in the epididymis. As the frequency of ejaculation increases, these reserves are depleted, and the total number of spermatozoa decreases relative to daily spermatogenic production.

Motility is a key factor in determining semen quality and serves as a reliable predictor of successful AI [19]. The motility percentages in Table 1 show that the first ejaculation differed significantly (P<0.05) from the second in male 11539, but not significantly (P>0.05) in males 11522, 11521, 11442, and 11434. In this study, motility tended to be higher during the first ejaculation. This pattern is due to the frequency of ejaculation over a short period. In the first ejaculation, the sperm that comes out has high motility, and optimal semen plasma components help sperm maintain motility and viability. The first ejaculation releases most of the mature, motile sperm cells and the semen plasma reserves containing optimal nutrients. This leaves the sperm cells that come out in the second ejaculation with low motility, due to rapid, unoptimized sperm production and reduced concentrations of semen plasma components that support

motility. According to Tunner and Howards [20], the frequency of ejaculation can affect osmolarity and fructose concentration in semen. The content of these nutrients affects sperm motility and fertility more in the first ejaculation than in subsequent ejaculations.

Figure 2 shows a negative correlation between semen volume and sperm motility (R = -0.414). This means that the higher the semen volume, the lower the percentage of motile spermatozoa. The results of this study showed that semen volume in the first ejaculation tended to be lower than in the second, whereas sperm motility in the first was higher than in the second. Spermatozoa that come out during the first ejaculation have long been accommodated in the epididymis, so they have high motility because they have gone through a series of maturation processes. Research conducted by Ayad et al. [14] found that the higher the volume of semen, the lower the motility. According to James et al. [21] and Gervasi and Visconti [22], sperm cells are essentially non-functional and non-motile. Chakraborty and Saha [23], however, state that spermatozoa undergo maturation in the epididymis and acquire motility during epididymal transition. This maturation process causes the spermatozoa collected in the cauda epididymis to have high motility at the time of first ejaculation and to contain various components that protect sperm from exposure to free radicals.

The decrease in the total number of motile spermatozoa in ejaculates obtained over a short period is due to a combination of a decrease in total sperm count and an increase in the percentage of motile sperm that does not fully compensate [24]. Spermatozoa undergo many changes during production in the seminiferous tubules and maturation in the epididymis, involving complex interactions between epididymal secretions and spermatozoa [25]. These changes include the release and absorption of fluids, ions, antioxidants, and exosomes known as “epididymosomes” [25]. A short ejaculation time will affect sperm kinematics because sperm have not fully adapted to their physical environment until ejaculation finally occurs [26].

Spermatozoa concentration is one of the indicators that determine male fertility [21]. Spermatozoa concentration in Table 1 shows that the frequency of the first ejaculation was significantly different ($P<0.05$) from the second ejaculation in males 11442, 11434, and 11539, but not significantly different ($P>0.05$) in males 11522 and 11521. This was due to the reduced number of sperm cells ejaculated during the second ejaculation, as most had been expelled during the first. In their study, Alipour et al. [26] reported that semen volume and sperm concentration in the first ejaculation were higher than in the second. Only about 50% of the spermatozoa in the cauda epididymis are available for ejaculation, so the decrease in sperm concentration and total sperm count in the ejaculate collected in a short period in the second ejaculation is due to the underdeveloped sperm reservoir capacity and inadequate time for the sperm to transfer from the more proximal parts of the epididymis to the cauda and vas deferens.

Figure 3 shows a positive correlation between sperm motility and concentration (R=0.740). This indicates that higher spermatozoa concentration in the semen is associated with increased spermatozoa motility. The results of this study showed that spermatozoa concentration and motility in the first ejaculation tended to be higher. Similarly, the second ejaculation showed that the concentration and motility of spermatozoa tended to be lower, due to the low total number of motile spermatozoa in the semen. According to Lagu et al. [27], spermatozoa motility is positively correlated with the total spermatozoa content in semen.

The low concentration of spermatozoa in the second ejaculation, performed after a short interval, is due to the lack of mature spermatozoa reserves remaining in the epididymis after the

first ejaculation. This leaves the body unable to replenish the reserves of spermatozoa ready for ejaculation, and it requires time to produce motile, mature spermatozoa again. Daily spermatozoa production in cattle is correlated with the number of Sertoli cells in the seminiferous tubules, where spermatogenesis takes place. The duration of spermatogenesis in bulls is 61 days, divided into three phases: spermatocytogenesis (21 days), meiosis (23 days), and spermiogenesis (17 days). The cycle occurs at constant intervals. The seminiferous epithelium is composed of several generations of germ cells that are continuously produced in the process of spermatogenesis in the seminiferous tubules, without waiting for the previous generation to complete its development and disappear as spermatozoa in the tubule lumen [28] [29] [30].

The relatively small number of bulls used in this study is a limitation. However, the five Bali bulls included were proven breeding males routinely used for semen production at the AI Center Pucak Maros. Such limitations are common in AI center-based studies because elite bulls are scarce. Despite this, the data provide valuable practical insights into semen quality patterns under real-world operational conditions.

CONCLUSIONS

Ejaculation frequency significantly affects semen volume, sperm motility, and concentration in Bali cattle. The first ejaculation generally yields semen with higher motility and sperm concentration, whereas the second, when collected after a short interval, tends to increase semen volume but reduce overall sperm quality. These findings suggest that limiting consecutive ejaculations within short intervals is advisable. AI centers are recommended to optimize the frequency of ejaculation and collection intervals to maintain high semen quality and improve the efficiency of Bali cattle artificial insemination programs.

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AUTHORS' CONTRIBUTIONS

The study was conducted by AAT, MY, SS, AMD, MM, and ATN, and all authors contributed equally. All authors above consented to be responsible for all parts of the work and participated in its preparation, drafting, and revision. They also granted final approval for the published version.

COMPETING INTERESTS

The authors declared that there is no conflict of interest

ETHICAL CLEARANCE

The authors declare that, in this study, they did not directly use animals as research subjects (using secondary data). The entire research process was conducted in accordance with the ethical principles of scientific research, and no additional procedures were performed beyond routine procedures at the artificial insemination (AI) center.

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