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Semen Quality of Bali Bulls Produced by The South Sulawesi Regional Artificial Insemination Center in The Dry And Rainy Seasons

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Abstract

Background: Bali cattle are one of the most prominent local cattle breeds widely raised by the people of Indonesia. **Purpose:** This study aimed to investigate the semen quality of Bali cattle produced during the dry and rainy seasons at the South Sulawesi Regional Artificial Insemination Centre (RAIC). **Methods:** The data of this study were obtained from secondary data from the production records taken by purposive sampling of 5 Bali bulls for 12 months, categorized into the rainy season (May - October 2019) and the dry season (November 2019 - April 2020). The study parameters included semen volume (ml), sperm concentration (10×10^6 /ml), sperm motility (%), and post-thawing motility (%). **Results:** The results indicated that semen volume was not significantly affected by seasonality ($P > 0.05$). However, sperm concentration, sperm motility, and post-thawing motility were significantly higher during the dry season than in the rainy season ($P < 0.05$). It can be concluded that the quality of Bali bull semen is superior during the dry season compared to the rainy season. **Conclusion:** The findings of this study can be applied to the management of Bali cattle breeding, particularly in organizing semen collection schedules and improving reproductive quality through the management of environmental factors and feed.

Keywords: Bali bulls; dry season; rainy season; semen quality

INTRODUCTION

Bali cattle are one of Indonesia's indigenous cattle breeds, possessing several advantages, including a relatively greater body height compared to other local cattle breeds. They are well known for their strong adaptability to the local environment and adequate productivity (Baco et al., 2020). However, to enhance the genetic potential and productivity of Bali cattle, one crucial aspect that must be considered is male reproduction and the application of artificial insemination (AI) technology. AI technology facilitates genetic improvement in livestock through strict selection and the

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use of semen from superior bulls. This process begins with a comprehensive semen evaluation to ensure optimal motility, concentration, and other quality parameters, ultimately producing superior breeding stock that meets established quality standards. Consequently, the proper implementation of AI technology is expected to improve the production and quality of the Bali cattle population in Indonesia, contributing to the sustainability and development of the local livestock sector.

In addition to enhancing feed quality, disease prevention, and livestock production in a sustainable and integrated manner, increasing the productivity of Bali cattle must strongly emphasize the selection of males for breeding stock. Through improved genetic quality and effective management with well-planned, integrated, and sustainable programs, Bali cattle productivity can be further enhanced. Bali cattle, one of Indonesia's indigenous breeds, originated in Bali Island and have since spread to nearly every part of the country, including South Sulawesi. Several factors influence semen quality, including age, breed, genetics, environmental conditions, nutrition, and type of extender used. Unfavourable environmental conditions can locally and systematically affect hormone regulation and impact sperm metabolism in Sertoli cells.

Indonesia has two distinct seasons: the rainy season and the dry season. Seasonal variations are believed to influence both the quantity and quality of fresh semen. During the rainy season, the availability of forage feed increases, allowing Bali cattle to attain higher yearling weights. Seasonal factors are closely linked to forage availability, which directly affects cattle productivity. If the feed provided meets the nutritional requirements of the cattle, their overall production can improve. Mandal et al. (2022) argue that heat stress significantly affects mammalian reproduction, including its role in disrupting spermatogenesis. Therefore, maintaining fresh semen quality is essential for ensuring optimal fertility. Previous studies have suggested that high temperatures and humidity during the rainy season may negatively affect reproductive performance by disrupting hormone regulation and sperm metabolism. Heat stress has been shown to impair testicular function, leading to reduced sperm concentration and motility. However, most previous studies have focused on other breeds or broader climatic regions, providing limited specific insight into Bali bulls raised under the tropical conditions of South Sulawesi. Additionally, data on the post-thawing motility of frozen semen across seasons remain scarce. Therefore, this study aims to fill these gaps by investigating the influence of seasonal variations on the semen quality parameters of Bali bulls. The findings will provide new insights into optimal conditions for AI programs and livestock breeding in tropical climates. However, there is currently limited data on how seasonal changes affect the semen quality of Bali bulls reared at the South Sulawesi Regional Artificial Insemination Centre (RAIC). Hence, this study was conducted to examine semen quality under different seasonal conditions.

MATERIALS AND METHODS

Study Location and Climatic Conditions

The study was carried out at the South Sulawesi RAIC, Indonesia, which is 100 meters above sea level and situated at 5°09'07" South Latitude and 119°40'41" East Longitude. The Maros Meteorology, Climatology, and Geophysical Agency reports that Pucak Village has two distinct seasons: a rainy period from November to April and a dry period from May to October. Less than 150 mm of precipitation per month

is considered the dry season, whereas more than 150 mm per month is considered the rainy season. Table 1 shows the precipitation and ambient temperature based on information from the Maros Meteorology, Climatology, and Geophysical Agency.

Table 1. Precipitation and Ambient Temperature in Pucak Village, Maros District, South Sulawesi

Months	Precipitation (mm)	Ambient Temperature (°C)
Dry Season		
May 2019	47	28.0
June 2019	95	27.0
July 2019	5	28.7
August 2019	< 0.5	27.2
September 2019	No rain	28.0
October 2019	< 0.5	28.9
Rainy Season		
November 2019	160	28.6
December 2019	308	27.9
January 2020	557	26.3
February 2020	564	27.0
March 2020	339	26.9
April 2020	157	26.7

Data collection

Data on semen production during the 2019-2020 period for this study were gathered from the South Sulawesi RAIC logbook. Bulls were selected based on age (5-7 years), health status (free from reproductive diseases), and proven fertility history. The semen collection procedure was performed twice a week using an artificial vagina. Immediately after collection, semen volume was measured in milliliters (ml) using a semen collection tube. To determine sperm concentration, 0.0035 ml of a semen sample was mixed with 3.5 ml of a 0.9% NaCl solution. After homogenizing the solution for 5 to 7 seconds, it was transferred into a cuvette, allowing the photometer to measure the concentration. Sperm motility was assessed by placing a semen sample onto a glass slide, covering it with a cover slip, and observing it under a microscope at 400x magnification. Motility was evaluated subjectively by counting the number of sperm moving in a linear (progressive) direction. Post-thawing motility (PTM) was analyzed by thawing the semen at 37°C for 15 seconds. Subsequently, semen quality was examined, including the percentage of motile sperm. Motility was subjectively assessed by counting the number of sperm moving forward (in a straight direction). Finally, semen quality, specifically the motility percentage, was evaluated.

Statistical Analysis

The data were compared between Bali cattle samples from the rainy and dry seasons using the independent sample t-test. The statistical analysis was conducted using IBM SPSS Statistic Version 25.

RESULTS AND DISCUSSION

One of the most important factors in Bali bull semen collection is semen quality. Data on the duration of production and frequency of semen collection of Bali bulls at

RAIC South Sulawesi are presented in Table 2, while the semen quality of Bali bulls is shown in Table 3.

Table 2. Duration of Production and Frequency of Semen Collection for Bali Bull at South Sulawesi RAIC

Bali Bull	Duration of Production (Months)		Frequency of Semen Collection (Times)	
	Season			
	Dry	Rainy	Dry	Rainy
Sinyo	6	6	13	17
Lewa	6	6	15	19
Rewa	6	5	13	6
Dewa	6	5	12	6
Singo	5	5	10	12
Average	5.8 ± 0,45	5.4 ± 0,55	12.6 ± 1,82	12 ± 6,04

Based on Table 2, in the dry season, the duration of Bali bull production in dry seasons was recorded at 5.8 ± 0.45 months, while in the rainy season, it was 5.4 ± 0.55 months. The average frequency of semen collection 12.6 ± 1.82 times in dry season and 12 ± 6.04 times in the rainy season.

Table 3. Semen Quality Parameters of Bali Bulls

Bali Bull	Semen Volume (ml)		Sperm Concentration (10 ⁶ /ml)		Sperm Motility (%)		Post Thawing Motility(%)	
	Season							
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
Sinyo	6.7	5.3	2,917	983	58,2	53,3	48,2	43,3
Lewa	4.5	4.6	1,898	1,041	58,5	52,2	48,5	42,2
Rewa	6.2	6.7	2,345	868	58,5	46	48,5	36.0
Dewa	5.6	10.2	1,365	1,280	58,5	44,6	48,5	34,6
Singo	6.6	1.7	2,114	0.3	58.0	58,5	48	48,5
Rata-rata	5.92±0.90	5.7±3.12	2,128±570 ^a	923±310 ^b	58,34±0.23 ^a	50.92±5,68 ^b	48.34±0.23 ^a	40,92 ± 5,67 ^b

Note: ^{a,b}Mean with different superscripts within a row are different ($P < 0.05$)

Semen Volume

Table 3 presents the average semen volume of Bali bulls collected at South Sulawesi RAIC, which was recorded 5.92 ± 0.90 ml during the dry season and 5.7 ± 3.12 ml during the rainy season. Statistical analysis indicated that semen volume was not significantly affected by season ($P > 0.05$). However, the semen volume in the dry season was slightly higher than in the rainy season. This variation may be attributed to factors such as species differences, age, body weight, and diet. The finding aligns with Saragih et al. (2023), which stated that different cattle breeds and age groups exhibit variations in semen quality, particularly in semen volume. Additionally, annual and seasonal differences influence the semen volume of Bali and Ongole cattle during the rainy and dry seasons. Brilliantti et al. (2021) further explained that semen volume refers to the total amount of semen ejaculated in a single collection, which can vary depending on several factors, including age, body weight, health status, reproductive condition, feed quality, scrotal circumference, and semen collection frequency. Furthermore, the technique and method used during semen collection,

including equipment preparation, also play a crucial role in determining semen volume. Fazrien et al. (2020) added that variations in age, particularly between 4 and 6 years, contribute to increased semen volume in Bali cattle.\

Llamas-Luceño et al. (2020) reported that semen volume in bulls is influenced by several factors, including age, season, temperature, and humidity. An increase in temperature due to climate change can negatively affect livestock reproduction, primarily through an increase in the Temperature Humidity Index (THI), which is a combination of temperature and humidity used to assess livestock comfort (Sukandi et al., 2023). A higher THI value indicates greater heat stress in cattle, which can ultimately impair reproductive performance.

However, the findings of this study indicate that there was no statistically significant difference in semen volume produced by Bali bull during the rainy and dry seasons ($P > 0.05$). This result aligns with previous studies that also found no seasonal differences in semen volume (Suyadi et al., 2020). Nevertheless, the semen volume observed in this study was higher than that of Bali bull in the RAIC of West Nusa Tenggara, which produced 4.32 ± 0.37 ml in the rainy season and 3.91 ± 0.38 ml in the dry season (Tulus et al., 2022). These findings suggest that other factors, such as management practices, feed quality, and cattle adaptation to environmental conditions, may play a more dominant role in determining semen volume than seasonal variations.

Sperm Concentration

Table 3 shows that the sperm concentration of Bali bulls is significantly influenced by the season ($P < 0.05$). The availability of high-quality feed during both the rainy and dry seasons may contribute to variations in sperm concentration. Additionally, the age of Bali bulls affects changes in sperm concentration during semen collection. These fluctuations are also influenced by reproductive hormones, such as testosterone and FSH, which regulate spermatogenesis, as well as physiological factors and reproductive management (Nugraha et al., 2019).

The average sperm concentration of Bali bulls at the South Sulawesi RAIC was $2,128 \pm 0.57$ million/ml in the dry season and 923 ± 310 million/ml in the rainy season. This finding indicates that sperm concentration tends to be higher during the dry season. The Indonesian National Standard (Badan Standardisasi Nasional, 2021) states that the normal sperm concentration in bulls ranges from 300 to 2,500 million/ml, confirming that the values in this study fall within the normal range. Variations in sperm concentration among individuals can be attributed to factors such as testicular size and semen collection frequency.

Compared to previous studies, the sperm concentration of Bali bulls at the South Sulawesi RAIC was higher than that reported by Komariah et al. (2020), who found an average sperm concentration of $1,222.94 \pm 364.82$ million/ml in Madura bulls during the rainy season, and Adiputra et al. (2022), who recorded an average sperm concentration of $1,244 \pm 0.29$ million/ml in Bali bulls. Bull fertility can be influenced by humidity and heat stress. The testes and scrotum have thermoregulatory mechanisms to maintain optimal sperm development despite fluctuations in temperature and humidity. However, prolonged exposure to high environmental temperatures, especially when accompanied by high humidity, can negatively impact semen quality (Morrell, 2020).

Sperm Motility

The data presented in Table 3 indicate a significant difference ($P < 0.05$) in the effect of season on sperm motility in Bali bulls. The results show higher sperm motility in the dry season ($58.34 \pm 0.23\%$) compared to the rainy season ($50.92 \pm 5.68\%$). However, these findings are lower than those reported by Bebas et al. (2021), who found that at Bali RAIC, sperm motility was $69.27 \pm 0.76\%$ in the dry season and $69.58 \pm 0.30\%$ in the rainy season.

Isnaini et al. (2021) and Baharun et al. (2023) stated that high rainfall can reduce sperm motility by affecting hormonal balance. Reduced light intensity inhibits GnRH secretion, leading to decreased production of FSH, LH, and testosterone, which in turn disrupts spermatogenesis and reduces sperm motility. In addition, Manehat et al. (2021) reported that average sperm motility can reach 80%, indicating variations across different studies.

Several factors contribute to variations in sperm motility, including age, breed, sperm maturity, and sperm plasma quality. This is consistent with the findings of Adiputra et al. (2023), who stated that semen quality and quantity in bulls are influenced by various factors, including age, nutrition, genetics, testicular size, health status, ejaculation frequency, and breed. Furthermore, Andini et al. (2023) emphasized that feed availability also plays a crucial role in determining semen quality.

Post-Thawing Motility

The evaluation of frozen semen quality is conducted after the thawing process. This evaluation includes assessing the individual movement of sperm. Table 3 shows that the season has an effect ($P > 0.05$) on post-thawing motility (PTM). During the dry and rainy seasons, the average PTM was $48 \pm 0.21\%$ and $40.92 \pm 5.6\%$, respectively. These findings are consistent with those reported by Gafur et al. (2024), who stated that the average PTM in Bali bulls ranges from 35% to 41%. The increase in temperature during thawing can affect PTM values. The temperature and duration of thawing play a crucial role in maintaining sperm integrity, where an optimal combination can minimize damage and enhance fertilization potential (Malinda et al., 2021).

The ability of sperm to rapidly adjust during the freezing stage accounts for the differences observed between the dry and rainy seasons. The decline in sperm motility after thawing is caused by an increase in liquid nitrogen vapor temperature, which affects metabolic function and disrupts sperm cell membrane integrity (Gafur et al., 2024). Furthermore, Diansyah et al. (2021) stated that PTM is a key parameter in determining the success of cryopreservation. This decline in motility is typically due to sperm membrane damage caused by the freezing and thawing process, further influenced by factors such as initial semen quality, the cryopreservation procedure, and storage conditions.

Previous studies (Chika et al., 2024; Isnaini et al., 2019) suggest that semen collection schedules in artificial insemination programs should be adjusted according to seasonal conditions. During seasons with optimal semen quality, collection can be conducted more intensively, whereas during less favorable seasons, frozen semen from more advantageous periods can be used to maintain the continuity of artificial insemination programs. Temperature is an environmental factor that significantly affects semen quality and production in bulls. The temperature of a region is directly influenced by the altitude of the breeding area. Variations in altitude contribute to

differences in temperature, humidity, and rainfall, which can indirectly induce stress in livestock and affect the availability of forage, ultimately impacting reproductive performance. Adiputra et al. (2025) also stated that environmental changes trigger stress, leading to decreased productivity in Bali cattle.

CONCLUSION

Seasonal variations significantly influence sperm concentration, motility, and post-thawing motility in Bali bull semen. Semen quality is generally higher during the dry season due to more favorable hormonal and metabolic conditions. These findings have practical applications for AI centers, where semen collection and storage practices can be optimized by prioritizing collection during the dry season. Additionally, improved management strategies, such as nutritional supplementation and environmental modifications, may help mitigate the adverse effects of the rainy season on semen quality. Future research should expand to larger-scale studies incorporating additional environmental factors, such as humidity, heat stress index, and nutritional intake, to develop comprehensive strategies for maintaining optimal semen quality year-round.

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AUTHORS' CONTRIBUTIONS

All authors participated equally to the manuscript's conceptualisation, data collection, analysis, interpretation, and writing. Each author reviewed the manuscript critically for essential intellectual content before approving the final version for submission. All authors agree to be responsible for all aspects of the work, including its integrity and correctness.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the publishing of this manuscript.

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