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Research article

Effect of age and season on fresh semen quality of Bali bulls in Indonesia

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Abstract

Importance of the work: In tropical regions, the success of artificial insemination (AI) programs relies on the use of locally adapted breeds with strong reproductive performance. Bali cattle, as a native Indonesian breed, possess valuable traits such as fertility and heat tolerance, yet remain under-researched in the context of AI.

Objectives: To investigate the impacts of age, collection season and their interaction on the quality of fresh semen from Bali bulls in Indonesia.

Materials and Methods: Data were collected from 353 ejaculates during the rainy and dry seasons from 15 Bali bulls divided into three age groups: pubertal, adult and senior. The evaluation was performed on fresh semen based on a standard operating procedure in the Singosari National Artificial Insemination Centre (SNAIC), Indonesia. Two-way analysis of variance and values of Pearson correlation coefficient (r) were used to determine the effects on fresh semen quality of age, season and their interaction. Data were analyzed using the IBM SPSS Statistics for Windows software.

Results: There was a significant interaction between age and collection season on sperm concentration ($p < 0.05$). Age significantly influenced semen pH, concentration and abnormalities of sperm ($p < 0.05$), while the season affected semen volume, motility, concentration and abnormalities of sperm ($p < 0.05$). There was a positive correlation between age and sperm concentration ($r = 0.42$) and abnormality ($r = 0.15$); however, there was a negative correlation with semen pH ($r = -0.15$). Additionally, the season was positively correlated with semen volume ($r = 0.12$) and sperm abnormalities ($r = 0.24$) but negatively correlated with motility ($r = -0.20$) and concentration ($r = -0.11$) of sperm.

Main finding: Age, season and their interaction significantly affected the quality of fresh semen from Bali bulls. For commercialization, bulls older than 7 yr were the best because they had a higher sperm concentration. Semen collection is still recommended in both the rainy and dry seasons because the potential production straw was the same, based on semen volume and sperm concentration.

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Introduction

Artificial insemination in tropical regions relies heavily on the adaptability and reproductive performance of locally bred animals. Local breeds exhibit critical traits such as heat tolerance, disease resistance, and the ability to thrive on low-quality feed, which support successful AI programs (FAO, 2007). In Indonesia, continuous efforts are being made to improve the genetic quality of cattle. Superior bulls from farmers and government-owned breeding institutions are selected to collect semen, which is then disseminated throughout Indonesia for artificial insemination (AI) programs (Sutarno and Setyawan, 2015; 2016).

Bali cattle is one of the cattle breeds whose quality continues to be improved through AI. Bali cattle are native to Indonesia and have adapted to tropical climates, can survive on limited feed, and are very highly fertile (Widyas et al., 2017). The implementation of an AI program using Bali cattle is an appropriate and efficient strategy to disseminate to and reach all regions in Indonesia where ich are the distribution areas of Bali cattle are raised. The offspring of AI have higher pre-weaning daily weight gain and live weight than natural-mating cattle (Warmadewi and Bidura, 2021).

The success of the AI program is inseparable from the quality of the semen produced. Semen quality must be evaluated continuously to obtain good results in breeding programs (Prastowo et al., 2019). Semen quality can be influenced by various factors, including bull age (Majić Balić et al., 2012), total ejaculation (Murphy et al., 2018), collection interval (Kanthiya et al., 2020), genetics (Carvalho et al., 2023) and season (Filipčík et al., 2023). The influence of these factors has been widely observed in Indonesian beef cattle, with several studies showing that age and season affect the semen quality of cattle breeds derived from *Bos indicus* (Sitanggang, 2018) and *Bos taurus* (Konenda et al., 2020) with high variation and contradictory results. In addition, there is still limited information on the quality of fresh semen from Bali bulls (*Bos javanicus*), as a native Indonesian breed, especially on how age, season and their interaction affect the quality of fresh semen of Bali bulls.

The effect of age and season on the quality of fresh Bali bull's semen at the Singosari National Artificial Insemination Centre (SNAIC), one of the leading frozen semen producers in Indonesia, should be thoroughly investigated as analysis of these factors could provide a scientific basis for developing efficient management strategies, especially regarding reproductive management and AI for beef cattle development in Indonesia and the region's tropical climate. Therefore, the current study aimed to provide a deep understanding of the interaction between age and season and their effects on the quality of bull semen, especially Bali bulls.

Materials and Methods

Ethics statements

The Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia approved this research with the number 00018/EC-FKH/EKs/2021.

Location and environmental conditions

This research was conducted at SNAIC in Malang, East Java, Indonesia. SNAIC is one of the largest government institutions (a public service agency) in Indonesia, with the main task of producing, marketing, testing and monitoring the quality of superior livestock semen and preparing and strengthening AI methods. The bulls used at SNAIC are the best bulls selected from the breeding center of the Denpasar and the Livestock Embryo Center Cipelang (BBIB Singosari, 2022).

Located at 7°50'34.3" S and 112°38'56.4" E in Toyomarto village, Singosari subdistrict, Malang district, SNAIC is an ideal location for keeping bulls. Data were collected from April 2022 to March 2023. The collection season consisted of the dry (April–September) and rainy (October–March) seasons. Usually, the dry season has rainfall of less than 50 mm/10 d for at least 30 d consecutively, whereas in the rainy season, the usual rainfall is equal to or greater than 50 mm/10 d for at least 30 d consecutively (BMKG, 2024). Environmental data were obtained from secondary data owned by the East Java Meteorology, Climatology and Geophysics Agency Class II Climatology Station in Malang Regency based on daily data observed by the observation station, which were subsequently averaged every month during the dry and rainy seasons. These data were used in the calculation of the temperature humidity index (THI), as shown in Equation 1 (Isnaini et al., 2020):

$$THI = (0.8 \times AT) + (RH / 100) \times (AT - 14.4)) + 46.4 \quad (1)$$

where AT is the average temperature measured in degrees Celcius and RH is the relative humidity measured as a percentage.

Bull management and data collection

In total, 353 ejaculates were used to analyze the quality of fresh semen from 15 Bali bull studs. The bulls used were selected based on purposive sampling with the criteria being actively collected semen from April 2022 to March 2023 and in the specified age category. The bulls were categorized into three age groups: AG-I (pubertal, less than 7 y, $n = 5$); AG-II (adult, 7–9 y, $n = 5$); and AG-III (senior, more than 9 y, $n = 5$), according to Abah et al. (2023). The ejaculation frequency of each group was AG-I = 120, AG-II = 115 and AG-III = 118. Based on the season, the frequency was 145 in the rainy season and 208 in the dry season. Details of the total ejaculation from each bull can be seen in Table 1.

The bulls were reared in an intensive system and placed in individual pens with a moored system. Daily care included the sanitation of the bulls and their pens, rotational exercise in the morning, with monthly weighing and body condition score assessment. The bulls were fed a mixed feed or total mixed ratio of forage (90%) and concentrates (10%), with *ad libitum* access to drinking water. Various measures were implemented to prevent and control diseases such as quarantine for adaptation (14 d), biosecurity, sanitation, disinfection and vaccination. Health checks were conducted daily, while disinfection was conducted weekly. Additionally, nail clipping and deworming were performed every 6 mth.

Professional technicians at SNAIC collected semen from the Bali bulls every Wednesday during 0700–1030 hours. Before semen collection, bulls were sexually stimulated using a bull teaser. Collection was performed using an artificial vagina after the bull had completed at least three false mounts (Furqon et al., 2021).

After collection, the fresh semen samples were sent to the laboratory for evaluation of macroscopic parameters—volume (mL), color and determining acidity (pH)—and microscopic parameters—motility (%), abnormality (%) and concentration (measured in millions of cells per milliliter, 10^6 cells/mL) of sperm (Tanga et al., 2021). The semen quality assessment was conducted indoors at 18–22°C with no more than 55% RH. In the macroscopic evaluation, the semen volume was determined using a scaled semen collection tube after collection. The degree of acidity (pH) was measured by placing a BTB pH paper on the semen, observing the resulting color changes, and comparing the change with the indicator (Wijaya et al., 2023).

Motility and abnormality were evaluated using a computer-assisted sperm analyzer (Hamilton Thorne; IVOS II; Beverly; USA), according to Miraz et al. (2022). Sperm abnormality criteria were bent tail, coiled tail, distal mid-piece reflex, and distal droplets. The concentration test was performed using a photometer (Minitube SDM6; Tiefenbach; Germany). In brief, a sample of 0.035 mL of semen was mixed with 3.5 mL of physiological NaCl solution and homogenized for 5–10 s. Then, the mixture was placed into a cuvette for reading using the photometer (Suyadi et al., 2020; Prastiya et al., 2023).

Data analysis

The IBM SPSS Statistics for Windows software (version 25; IBM Corp.; Redmond, CA, USA) and the Microsoft Office Home and Student software 2021 (version 2402 ; Microsoft.; Redmond, WA, USA) were used for statistical analysis and graphing of data. The semen parameters were tested for a normal distribution. Two-way analysis of variance was conducted to analyze all data. Data that showed significant differences were further analyzed using Duncan's multiple range test. Pearson correlation (r) analysis was used to test for correlation between semen variables. Data were presented as mean ± SD values. Significant differences were tested at $p < 0.05$.

The statistical model used to determine the effects of age and collection season on the quality of the semen of the Bali bulls was based on Kaps and Lamberson (2004), as shown in Equation 2:

$$yijk = \mu + Ai + Sj + (AS)ij + \varepsilon ijk \quad (2)$$

i = 1,2,3, (a); j = 1,2, (b); k = 1,2,3,4,5,6, (n)

Table 1 Ejaculation frequency per bull at different age groups

Age group I (<7 yr)		Age group II (7–9 yr)		Age group III (>9 yr)	
Bull Id	Total Ejaculate	Bull Id	Total Ejaculate	Bull Id	Total Ejaculate
116133	21	114110	31	110113	14
116135	16	11293	8	11082	27
115130	13	11292	33	111117	31
115101	32	114108	24	11095	28
116132	38	11289	19	110115	18

Id = identification number.

where yijk is observation k in level i of factor A and level j of factor S; μ is the overall mean; Ai is the effect of level i of the factor age; Sj is the effect of level j of the factor season; (AS)ij is the effect of the interaction of level i of factor A with level j of factor S; εijk is the random error, with mean 0 and variance σ^2; a is the number of levels of factor A; b is the number of levels of factor S; and n is the number of observations for each A × S combination.

Results

Environmental conditions

A comparison of the environmental conditions between the dry and rainy seasons is presented in Table 2, while the monthly data on environmental conditions are presented in Figs. 1. There was a downward trend in the temperature in the Malang Regency area between the dry and rainy seasons, with the warmest temperature during April (25.50°C) and the coldest month being during July (Fig. 1A). There was no significant difference between the average temperature during the dry and rainy seasons (Table 2); in contrast there were significant differences in RH . There was an upward trend in humidity from the dry to the rainy seasons, with the most humid air conditions occurring in November, with an average humidity of 85.07% (Fig. 1B). During the dry season, there was a downward trend in the THI (Fig. 1C). However, there was no significant difference between THI in the dry and rainy seasons (Table 2).

Table 2 Climatic conditions in Malang Regency during collection seasons

Variables	Season		*p* value
	Dry	Rainy	
Temperature (°C)	24.15 ± 0.87	24.08 ± 0.28	0.87
Relative humidity (%)	78.04 ± 1.72^{b}	82.26 ± 2.61^{a}	0.01
THI	73.33 ± 1.43	73.62 ± 0.27	0.63

THI = Temperature humidity index.
Mean±SD in the same row followed by different lowercase superscript letters indicate significant (*p* < 0.05) differences.

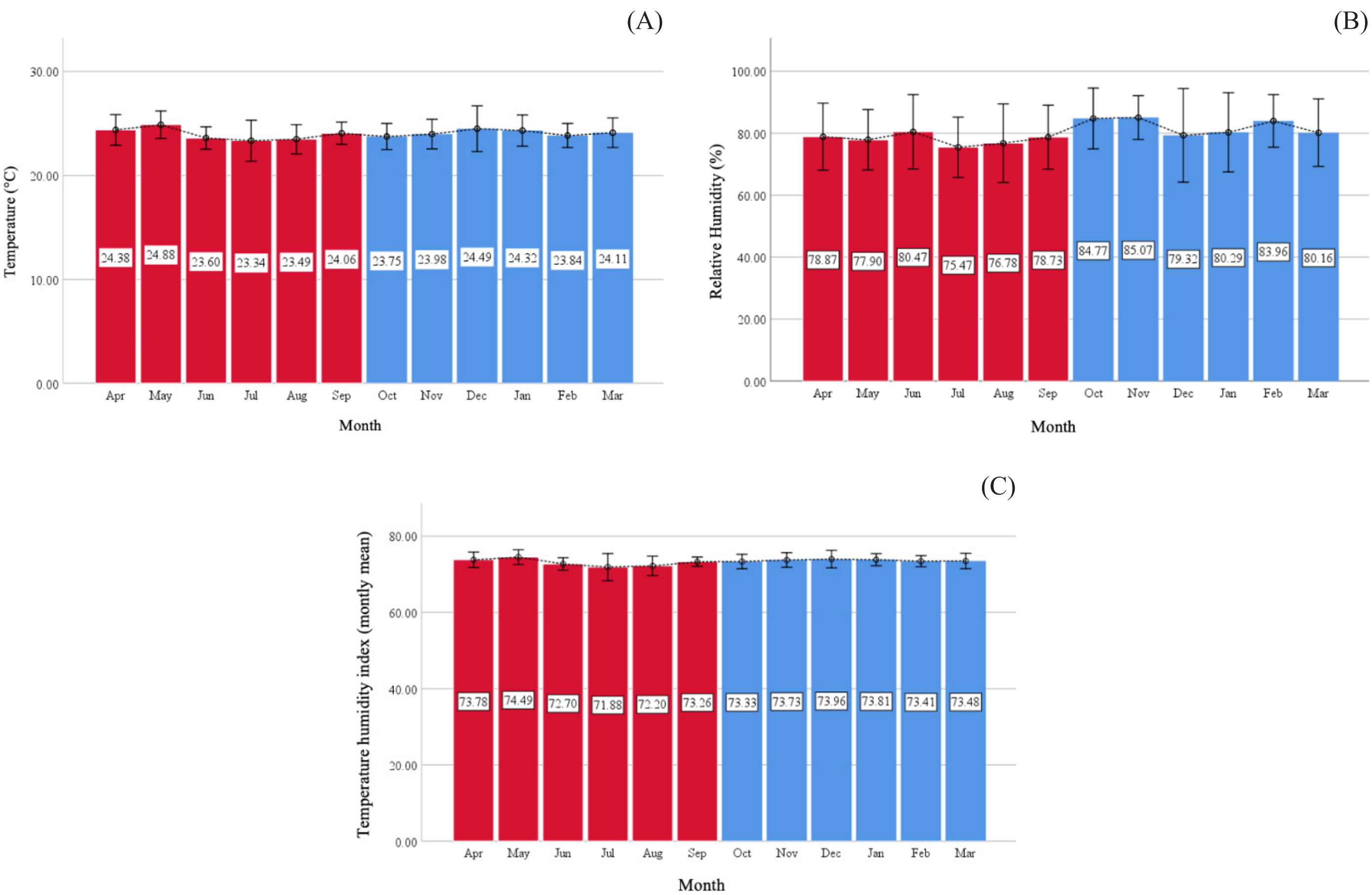

Fig. 1 Fluctuations in temperature humidity index during the study period, where error bars = ± SD .

Semen quality

The effects of bull age, collection season and their interaction on the quality of fresh Bali bull semen can be seen in Table 3. The fresh Bali bull semen was milky white-to-yellowish white. The age of the Bali bull significantly affected semen pH, sperm concentration, and abnormality. The bulls in AG-III had the lowest semen pH ($p < 0.05$), and those in AG-I and II had high semen pH (Table 3). The semen concentration increased with the age of the bulls, with bulls in the AG-I class having the lowest sperm concentration among the age groups. A similar pattern was observed for sperm abnormalities, with these abnormalities being lower in AG-I bulls than in AG-II and AG-III bulls.

Season had a significant impact on semen volume, concentration, motility, and abnormality of sperm. Specifically, sperm motility and concentration were higher during the dry season than the rainy season ($p < 0.05$), while semen volume and sperm abnormality were higher in the rainy season ($p < 0.05$). This increase was in line with the increase in rainfall and relative humidity during the rainy season. Additionally, there was a significant interaction effect between bull age and season of collection for sperm concentration. Bulls in the AG-III class had notably higher sperm concentration during the dry season compared to the other age and season combinations ($p < 0.05$), as shown in Table 3.

The correlation analysis highlighted the diverse relationships among the parameters (Fig. 2). Age had a positive correlation with sperm concentration (r = 0.42) and sperm abnormality (r = 0.15), but a negative correlation with semen pH (r = -0.15). Season was positively correlated with semen volume (r = 0.12) and sperm abnormalities (r = 0.24), but negatively correlated with semen concentration (r = -0.11) and motility (r = -0.20). Semen pH was negatively correlated with sperm concentration (r = -0.25). Furthermore, sperm abnormalities were negatively correlated with both sperm concentration (r = -0.12) and motility (r = -0.18).

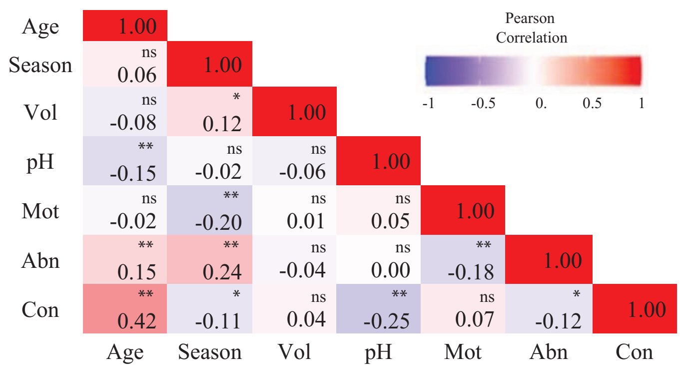

Fig. 2 Matrix of Pearsons correlation coefficients, where * = significant ($p < 0.05$), ** = highly significant ($p < 0.01$) and ns = not significant ($p > 0.05$) differences and Vol = volume, Mot = motility, Abn = abnormality and Con = concentration

Table 3 Effect of age and season and their interaction on quality of fresh semen quality from Bali bulls

Effect and level	N	Volume (mL)	pH	Motility (%)	Concentration (10^6/mL)	Abnormality (%)
Age						
AG-I	120	7.25±1.95	6.53±0.18^a	76.26±9.28	1,134.02±299.84^c	4.52±2.40^b
AG-II	115	6.96±1.59	6.55±0.17^a	74.81±9.09	1,226.48±265.11^b	5.73±2.49^a
AG-III	118	6.92±1.73	6.47±0.13^b	75.82±8.49	1,445.13±264.93^a	5.39±2.15^a
p value		0.20	< 0.01	0.52	< 0.01	< 0.01
Season						
Dry	208	6.87±1.68^b	6.52±0.17	77.13±9.98^a	1,296.69±313.96^a	4.73±2.37^b
Rainy	145	7.29±1.87^a	6.51±0.16	73.50±6.71^b	1,227.17±290.59^b	5.90±2.27^a
p value		0.02	0.75	< 0.01	0.01	0.01
Age × Season						
AG-I Dry	76	7.03±1.75	6.54±0.18	77.19±10.57	1,128.34±301.60^c	3.85±2.37
AG-I Rainy	44	7.62±2.24	6.50±0.18	74.65±6.25	1,143.82±299.99^c	5.70±1.99
AG-II Dry	66	6.99±1.50	6.54±0.17	75.97±10.03	1,301.50±249.80^b	5.26±2.27
AG-II Rainy	49	6.93±1.72	6.56±0.17	73.24±7.47	1,125.43±253.51^c	6.36±2.65
AG-III Dry	66	6.57±1.75	6.46±0.13	78.22±9.23	1,485.74±277.68^a	5.21±2.22
AG-III Rainy	52	7.36±1.62	6.47±0.14	72.78±6.33	1,393.58±240.62^{ab}	5.63±2.07
p value		0.16	0.35	0.38	0.03	0.06

AG-I = age group I (<7 yr); AG-II = age group II (7–9 y); AG-III = Age group III (>9 Y); N = Total ejaculates.
Mean ± SD in the same treatment and column, followed by different lowercase superscript letters indicate significant ($p < 0.05$) differences.

Discussion

The current comprehensive study investigated the effect of age, collection season, and the interaction of both on the quality of Bali bull semen maintained in the most prominent artificial insemination center in Indonesia with tropical climate conditions. Regular evaluation of fresh bull semen quality is crucial, as semen quality contributes 20–25% to overall fertility performance (Iskandar et al., 2022). Thus, this quality evaluation could support the success of AI programs (Hoque et al., 2018).

Based on the results of the current study, the older the bull, the higher the sperm concentration per milliliter of semen, which was consistent with the result of Wijaya et al. (2023) for *Bos indicus* (Brahman and Ongole Grade bulls) and *Bos taurus* (Simmental and Limousine bulls). However, in contrast Sahiwal bulls did not have a reported increase in sperm concentration with increasing age of the bull (Bhakat et al., 2011). In the current study, the average sperm concentration of the Bali bulls was in the range 1,125–1,485 × 10^6 cell/mL, which was lower than the sperm concentration of Sumba Ongole bulls (1,848.33 × 10^6 cell/mL; Maulana et al., 2022), Munshiganj bulls (1,510 × 10^6 cell/mL; Das et al., 2023), and Red Chittagong bulls (1,648 × 10^6 cell/mL; Hossain et al., 2022).

The low pH of the adult and senior bull semen samples in the current study contrasted with the semen pH findings in Aceh bulls, where older bulls had an increased pH in their semen (Isnaini et al., 2022)Click or tap here to enter text.. The high concentration of sperm produced by old bulls was the cause of the decrease in semen pH. Spermatozoa activity strongly influences the pH of fresh semen in breaking down fructose, with the greater the activity of decomposing energy sources derived from fructose, the higher the lactic acid production and the more acidic the semen (Fazrien et al., 2020). However, the semen pH of the three age groups in the current study was still in the normal range for semen pH in mammals of 6.0–7.5 (Lagu et al., 2020).

Assessment of sperm morphological abnormalities is essential to identify and exclude suboptimal semen samples before cryopreservation. The highest sperm abnormalities in the semen from the bulls aged 7–9 yr (5.73%) were in accordance with the sperm abnormalities reported in Jersey bulls aged 8–9 yr (19.59%) by Sankhi et al. (2019). However, the tendency for increasing sperm abnormalities in older bulls in the current study was in contrast to Menon et al. (2011). In some cattle breeds, sperm abnormalities tend to decrease with bull age. For example, consistent with the current findings, sperm abnormalities were positively correlated with age in Simmental bull semen (Baharun et al., 2021). The increase in sperm abnormalities was due to a decrease in the performance of the seminiferous tubules with the increasing age of the bull (Mandal et al., 2010). In addition, increasing age causes an increase in the abnormalities in the seminiferous tubule epithelium, disrupting the spermatogenesis process (Tesi et al., 2020). In the current study, all semen produced by the various age groups of bulls was suitable for processing into frozen semen, based on the Indonesian National Standard with number: No.4869-1:2021 for fresh semen that is suitable for the freezing process, namely having sperm abnormalities < 20%.

Significant seasonal effects on semen quality have been widely reported. In the current study, the significant increase in semen volume from the dry season to the rainy season in Bali bulls was consistent with the semen volume produced by *Bos indicus, Bos taurus, Bos taurus* composites, and *Bos taurus* × *Bos indicus* crossbreds reared in Venezuela, which has a tropical climate (Landaeta-Hernández et al., 2020). Several findings that seasonality affected semen quality were reported for the semen volume of Gir bulls and Mehsana buffaloes bulls; however, these findings were inconsistent, with Mehsana bulls producing the highest amount of semen in the rainy season, while in contrast, Gir bulls produced the highest amount of semen in the dry season (Bhave et al., 2020). In American Brahman and Sahiwal Bulls, semen volume was unaffected by collection season differences (Nongbua et al., 2020). In tropical countries, RH was reported to be significantly correlated with the volume of semen produced (Kimsakulvech et al., 2022).

In the current study. the collection season significantly affected motility as was also reported for Simmental and Limousin bulls raised in Indonesia, although the results differed because in Simmental and Limousin bulls, the highest sperm motility was in the rainy season (Hapsari et al., 2022), while in Bali bulls, the highest motility was in the dry season. However, for Pasundan (Isnaini et al., 2021), swamp buffalo (Das et al., 2017), and Swedish red dairy (Valeanu et al., 2015) bulls, seasonal differences did not significantly affect the motility of sperm produced. Although the sperm motility of Bali bulls in the current study differed considerably between seasons, this should not have a commercial impact (Llamas-Luceño et al., 2020). As long as the motility and abnormalities of the sperm satisfy the requirements for fresh semen based on regulations in Indonesia, it may be further processed into frozen semen for use in artificial insemination. The main benchmarks in terms of semen commercialization are semen volume and sperm concentration because they affect the dose of frozen semen produced (Prastowo et al., 2018; Popović et al., 2024).

The effect of season on sperm concentration has been documented in *Bos indicus* cattle, where the highest concentration was in the dry season (Bhave et al., 2021). Furthermore, the current findings were in accordance with those of Sarkar et al. (2009) on the quality of Yak (*Poephagus grunniens L.*) sperm, where the sperm concentration was high during the dry season. In other breeds, such as dairy cattle in Tamil Nadu, India, the rainy season produced the highest sperm concentration compared to other seasons (Jeevan et al., 2024), while Chacur et al. (2013) reported that the sperm concentration of Simmental bulls was not affected by the collection season.

The high morphological abnormalities of sperm during the rainy season in the current study were different from the sperm abnormalities in the Murrah buffalo and Jaffarabadi buffalo, where the highest rates of abnormalities were identified in the dry season (Bhakat et al., 2015; Hirabhai et al., 2022). Furthermore, in Holstein bull semen, seasonal changes did not affect the abnormalities of the sperm produced (Al-Gebouri and Eidan, 2024). Sperm morphology is strongly related to fertility as abnormal sperm morphology affects sperm motility and can be the cause of low fertility (Sun et al., 2020). A healthy bull has sperm with a normal morphological count greater than 70% (Thundathil et al., 2016). Body condition score, age, and month of semen collection have been reported to affect sperm morphology (Sitali et al., 2016). Furthermore, abnormalities in spermatozoa are caused by the physiological process that takes place in the form of disturbances in spermiogenesis and maturation in the epididymis (Baharun et al., 2023).

Although there were no significant THI differences in the current study, a high THI can cause heat stress in livestock (Seifi-Jamadi et al., 2020). Heat stress hurts reproductive function in mammals due to increased reactive oxygen species (ROS) production in mitochondrial cells and tissues due to hypoxic conditions (Yadav et al., 2022). While ROS are a by-product of normal physiological processes, an imbalance in ROS production can damage sperm and impair its fertility (Sengupta et al., 2024). Elevated ROS levels can damage sperm plasma membranes, DNA, and other physiological processes. Moreover, excessive ROS can reduce sperm motility and fertilization capacity (Bansal and Bilaspuri, 2010). Thus, from several theories, it is suspected that the higher THI causes the low semen quality during the rainy season.

Sperm concentration and semen volume were affected by the interaction of age and season in Holstein Friesian bulls (Murphy et al., 2018). However, different findings were observed for the semen of Ongole Grade bulls in Indonesia and Murrah buffaloes in India, with no interaction between bull age and seasonal collection factors on either semen volume or sperm concentration (Suyadi et al., 2020; Kumar et al., 2024). The mechanisms influencing how season and age affect fresh semen quality include physiological changes, hormonal regulation, heat stress, direct impacts on the testes, fluctuations in photoperiod, and nutritional status (Surisetti et al., 2024).

The current study investigated the quality of fresh semen in different age groups and collection seasons before freezing. The findings should be of value for artificial insemination centers involved in frozen semen production because this information will help to optimize the age of productive bulls and the best season to produce the best quality fresh semen. However, the current study was limited to one artificial insemination center with a relatively limited number of replicates, so the results may not fully reflect conditions elsewhere. Therefore, further research is needed at various artificial insemination centers that process the semen of Bali bulls to obtain more representative data and strengthen these findings. In addition, research is needed on the physiological processes underlying the effects of season and age on semen quality and on specific feed supplementation to improve semen quality and reduce the impact of age and season of collection.

In conclusion, increasing the age of the Bali bulls increased the concentration and level of abnormalities of sperm produced, followed by a decrease in semen pH due to the increase in concentration. Changing from the dry to the rainy season produced a higher volume and level of abnormalities in the Bali bull sperm, while decreasing sperm motility and concentration. In addition, the interaction between age and holding season greatly affected sperm concentration. Bali bulls over 9 yr had the highest sperm concentrations of 1,485.74 × 10^6/mL (dry season) and 1,393.58 × 10^6/mL (rainy season). Based on the current results, the best age of bulls for semen commercialization was age was older than 7 yr because these bulls had a higher sperm concentration. Semen collection is still recommended in both seasons because although the dry season produced a low volume of semen, it was balanced by a high concentration, and *vice versa* in the rainy season. These results should be considered in the development of AI programs outside Indonesia, especially in countries with tropical climates.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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