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Display of fresh semen quality, frozen semen production, and field fertility of Bali bulls (*Bos sondaicus*) in different season

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ABSTRACT

Bali cattle is one of Indonesia's local cattle that has high economic potential. The quality of the semen used plays an important role as a factor in the success of artificial insemination (AI) programs. Climate change, including temperature variations, rainfall, and length of solar exposure, can affect the quality of fresh semen produced by bulls. Differences in environmental conditions can affect semen characteristics, such as spermatozoa concentration, motility, and morphology, which in turn have an impact on the field fertility rate. The aim of this study is to explore the differences in male fertility based on environmental conditions that change throughout the seasons. The research method used in this study is non-experimental, using secondary data on the quality of fresh semen and frozen semen production collected from the Regional AI Center, Bali Province. In addition, this study also used data from the big data of the National Animal Health Information System (iSIKHNAS) to calculate field fertility represented by conception rate. Climate data was obtained from the Indonesian Agency for Meteorological, Climatological and Geophysics (BMKG) Bali Province, the data collected was climate data in Tabanan Regency. The differences in the appearance of fresh semen quality, frozen semen production, and field fertility were analyzed using the Independent T-Test. In this study, there was no significant difference in fresh semen quality, frozen semen production, and field fertility (P>0.05) in all parameters, but the average reproductive performance of Bali bulls was higher in the rainy season than in the dry season.

Keyword: Bali bull, conception rate, fertility, season, semen quality

1. Introduction

Bali cattle (*Bos sondaicus*) are one of Indonesia's local cattle that have high economic potential, especially in the livestock sector. The advantages possessed by Bali cattle, such as good carcass quality, adaptability to diverse environments, and their ability to digest low-quality feed [1], make them a strategic option for breeding programs and increasing livestock productivity in Indonesia. To increase the population of Bali cattle, artificial insemination (AI) technology has been massively applied. AI allows for faster and wider genetic spread, and can improve efficiency in livestock production [2].

One of the key factors in the success of the AI program is the quality of the semen used. The quality of fresh semen greatly affects the success rate of the AI program because the frozen semen processing often leads to a decrease in quality. Therefore, it is important to ensure that the fresh semen used in this process meets good quality standards and is in accordance with the Indonesian National Standard (SNI) before being further processed into frozen semen. Bulls with good quality semen are expected to produce high field fertility, which is crucial in the success of the AI program.

Bull fertility, especially in relation to AI program, can be predicted using various methods, one of which is the conception rate (CR). CR is calculated by comparing the number of pregnant cows in the first AI service with the total number of cows who receive AI services. CR is an important indicator in evaluating the field fertility success of bulls, which is the basis for the selection of superior bulls for the breeding program.

Several studies state that climate contributes to affecting semen quality and fertility in cattle and goats [3-5]. Climate change, including temperature variations, precipitation, and length of solar exposure, can affect the quality of fresh semen produced by bulls. Differences in environmental conditions that occur in different seasons can affect semen characteristics, such as spermatozoa concentration, motility, and morphology, which in turn have an impact on the field fertility rate. Research related to the effect of seasons on semen quality in various cattle breeds has been carried out a lot, including Madura cattle [6], Limousin [7], Holstein Friesian X Sahiwal crosbred dairy bulls [8], and Fleckvieh-Simmental bull [9]. However, although many studies have focused on the quality of fresh semen, few have discussed in depth the variation in male fertility in the field that is affected by seasonal differences. This indicates the need for further studies that explore the differences in male fertility based on environmental conditions that change throughout the seasons.

2. Methods

This research was carried out in a non-experimental using secondary data on the quality of fresh semen and frozen semen production collected from the Regional Artificial Insemination Center, Bali Province. In addition, this study also uses data from the big data of the National Animal Health Information System (iSIKHNAS) to calculate field fertility represented by the conception rate (CR). Climate data was obtained from the Indonesian Agency for Meteorological, Climatological and Geophysics (BMKG), Bali Province, the data collected was climate data in Tabanan Regency.

2.1. Research Materials

This study uses data obtained from the ejaculation of productive Bali bulls with an age range of 5-10 years. There are 5 bulls that are used as a source of research material. The identities of the five bulls are shown in Table 1. The data used in this study is semen production data in 2020-2021, while the big data of iSIKHNAS is an AI program and pregnancy check data in Bali Province in 2020-2022.

Table 1. Identity of Bali bulls studs raised at UPTD BIBD PTHPT Bali Province

Bull ID	Age (year)	Manufactured Straw Codes	
		2020	2021
11544	5	T001 – T056	U042 – U048
11443	6	T001 – T048	U001 – U056
11341	7	T001 – T062	U001 – U066
11240	8	T001 – T016	U001 – U032
11037	10	T001 – T037	U001 – U030

2.2. Data Collection

This study uses secondary data reported by the Regional AI Center (UPTD BIBD PTHPT) Bali Province every month. The data collected includes data on the results of fresh semen quality inspections observed macroscopically and microscopically. The parameters used in this study include: a). macroscopic examination consisting of volume, color, and consistency; and b). microscopic examination consisting of mass motility, individual motility, and spermatozoa concentration. In addition to fresh semen quality data, data that is also used from the results of the monthly report of UPTD BIBD PTHPT Bali Province is frozen semen production data. The data used includes straw codes, production months, and the number of straws produced in the batch.

The big data of iSIKHNAS is data collected from all over Indonesia, in this study using data on the implementation of the AI program and pregnancy check in Bali Province. The data collected are cows inseminated with frozen semen produced in 2020 (straw coded T) and 2021 (straw coded U). The data that has been collected is then tabulated based on the production straw code and calculated the CR in each bull.

2.3. Data Grouping

Data on the quality of fresh semen and frozen semen production, data from the Bali Province AI program that has been obtained, are then tabulated and grouped based on climate determination. The determination of climate in the study is the rainy season and the dry season. The determination refers to the amount of monthly rainfall. According to the BMKG Bali Province report, the dry season is determined based on rainfall <150 mm/month, while the rainy season is determined based on rainfall ≥ 150 mm/month. The following is data on the number of days and monthly rainfall in 2020-2021 in Tabanan Regency, Bali Province (Table 2).

Table 2. Data on the number of days and monthly rainfall in 2020-2021 Tabanan Regency, Bali Province

Month	2020		2021	
	Rainy Day (day)	Rainfall (mm)	Rainy Day (day)	Rainfall (mm)
January	22	333	29	445
February	19	505	26	434
March	28	638	31	449
April	19	291	16	102
May	21	181	10	182
June	14	50	16	186
July	13	50	6	35
August	14	42	17	193
September	12	118	20	154
October	19	349	18	222.2
November	18	345	26	781
December	27	349	23	614

The data is then calculated on average, and based on the average value obtained, this study uses data on fresh semen and frozen semen production in January, February, March, and April to represent the rainy season and June, July, August, and September to represent the dry season

2.4. Data Analysis

The data that has been collected is then tabulated and grouped based on different seasons. The differences in the appearance of fresh semen quality, frozen semen production, and field fertility were analyzed using the Independent T-Test. Data analysis was carried out using SPSS software version 26.0. The analyzed data is presented in the form of an average±standard error mean (SEM).

3. Results and Discussion

Evaluation of the quality of fresh semen is very necessary to determine the feasibility of ejaculation to be further processed into frozen semen. Fresh semen that meets quality standards not only has the potential to produce good offspring, but can also be diluted and processed into frozen semen that is suitable for insemination. According to SNI 4869-1:2024 Frozen Semen – Part 1: Bull, fresh semen that can be processed must have an individual motility of >70%. This motility parameter is a key indicator of spermatozoa viability, indicating their ability to move well towards the egg [10].

3.1. Quality of Fresh Semen

Some of the criteria examined in the evaluation of the quality of fresh semen at UPTD BIBD PTHPT Bali Province include:

3.1.1. Volume of Semen

The volume of fresh semen is one of the crucial parameters in the evaluation of ejaculation quality, as it provides important information about the reproductive potential of male animals. This volume refers to the total amount of ejaculation produced during collection, and the ideal amount varies depending on the species. According to Hafez and Hafez [11], the normal volume of fresh semen in bulls generally ranges from 5-8 mL. Based on the results of the study shown in Table 3, the average volume of Bali bulls is in the normal range.

Sufficient volume can indicate good reproductive health and increase the chances of successful fertilization, as it is directly related to spermatozoa concentration. When semen volume is below standard, it usually signals the presence of a possible health problem, such as an infection or hormonal disorder, that can affect the quality of spermatozoa [12, 13]. In addition, adequate volumes allow insemination officers to obtain more spermatozoa, thus increasing the amount of semen produced. According to Rahmawati et al. [14], the amount of frozen semen refers to the volume of semen. Therefore, it is important to regularly measure and analyze the volume of fresh semen, as well as consider factors that can affect it, one of which is the factor of seasonal differences. The average value of the semen volume of Bali bulls in this study is presented in Table 3.

Table 3. Average value of semen volume of Bali bulls at UPTD BIBD PTHPT Bali Province in the rainy and dry seasons

Bull ID	Season	
	Rainy (mL)	Dry (mL)
11544	5.98±1.07	6.25±1.12
11443	6.43±1.67	6.14±1.42
11341	4.85±1.05	4.69±1.05
11240	6.43±1.96	6.57±2.09
11037	5.98±1.67	5.94±1.49
Total average	5.94±1.48	5.92±1.43

In the rainy season, UPTD BIBD PTHPT managed to collect 346 ejaculates from 5 Bali bulls during 2020-2021. Based on Table 2, there was no significant difference in volume of semen ($P>0.05$) between the rainy season (5.94±1.48 mL) and the dry season (5.92±1.43 mL). This is in accordance with the research of Komariah et al. [6] and Rahmawati et al. [14], who observed the difference in the average volume of fresh semen of bulls in the rainy and dry seasons. However, based on a study conducted by Bhakat et al. [15], the seasons in a country of four seasons have a significant difference in semen volume ($P<0.05$). Seasonal differences can affect the production of hormones, one of which is FSH. When the sun is excessive, the secretion of the hormone FSH is reduced, thus inhibiting the spermatogenesis process [16]. In addition, heat stress factor also affects the production of FSH [17], so that in summer, the volume of fresh semen is lower. This is because the changes in temperature, humidity, and duration of solar irradiation in the four seasons are very different between the seasons, while in countries with two seasons, the difference is not significant.

In the rainy season, Bali bull with ID 11443 and 11240 have a higher average semen volume than other bulls, with an average of 6.43±1.67 mL and 6.43±1.96 mL respectively, while Bali bull with ID 11240 has the highest average semen volume with 6.57±2.09 mL in the dry season. Bali cattle are known for their adaptable nature [1], so the difference in volume is not significant. In addition, weather changes, especially in Indonesia, do not have much impact on the reproductive performance of Bali cattle. Based on Table 2, the Bali bull with ID 11240 has the highest average semen volume in both seasons. This phenomenon is thought to be due to the effect of individual variation [18].

3.1.2. Color of Semen

The color of fresh semen is an important factor in determining the overall quality of the semen. The normal color of bull semen usually ranges from milky white to beige [19-21]. Discoloration can be an indication of health problems in male animals. Semen that is cloudy, red, or brown in color may indicate the presence of contamination, infection, or even bleeding [22], which can affect the viability of spermatozoa and the ability to fertilize. Therefore, semen color checks not only help in rapid visual assessment, but also serve as an early detection tool for more serious health problems. In this study, it is known that the processed semen is milky white and beige semen. The color of the semen of Bali bulls both in the rainy and dry seasons is dominated by milky white. The appearance of semen color from ejaculates collected in the rainy and dry seasons is shown in Table 4.

Based on Table 4, the average score of fresh semen color of Bali bulls in the rainy season (2.49±0.43) and dry season (2.40±0.47) there was no significant difference ($P>0.05$), although the average total score was higher during the rainy season than in the dry season.

Reproductive performance in livestock is also influenced by individual factors. According to Indriastuti et al. [18], individual variations can affect the characteristics of semen. Based on Table 4, among the 5 bulls, the bull with ID 11341 has the highest average color score of fresh semen in both the rainy and dry seasons, which means that this semen has a higher viscosity. According to Komariah et al. [6], the cloudy color of the semen of bulls indicates the number of spermatozoa.

Table 4. Average score of fresh semen color score of Bali bulls at UPTD BIBD PTHPT Bali Province in the rainy and dry season

Bull ID	Season	
	Rainy	Dry
11544	2.31±0.47	2.35±0.48
11443	2.40±0.50	2.36±0.49
11341	2.92±0.28	2.69±0.47
11240	2.63±0.50	2.33±0.48
11037	2.21±0.42	2.27±0.45
Total average	2.49±0.43	2.40±0.47

Note: Color score: (1) clear, (2) milky white, (3) beige

3.1.3. Consistency of Semen

Good quality semen generally has a thick consistency but is not too sticky, allowing spermatozoa to move freely and efficiently. Good quality semen has a slightly thicker viscosity than milk, while poor quality semen, both in terms of color and viscosity, is similar to coconut juice [16]. In this study, it is known that the consistency of fresh semen processed into frozen semen is the one that has a moderate and high consistency, while fresh semen whose consistency is diluted will be disqualified. In UPTD BIBD PTHPT Bali Province, the consistency of fresh semen is dominated by moderate consistency. The average consistency score of fresh semen is shown in Table 5.

Table 5. Average of the consistency score of fresh semen of Bali bulls at UPTD BIBD PTHPT Bali Province in the rainy and dry season

Bull ID	Season	
	Rainy	Dry
11544	2.29±0.45	2.35±0.48
11443	2.30±0.47	2.32±0.47
11341	2.92±0.28	2.67±0.47
11240	2.56±0.51	2.38±0.50
11037	2.11±0.32	2.27±0.45
Total average	2.44±0.41	2.40±0.47

Note: Consistency score: (1) diluted; (2) moderate; (3) thick

Based on the results of the study (Table 5), the average consistency score of fresh semen of Bali bulls in the rainy season (2.44±0.41) and dry season (2.40±0.47) there was not significantly different (P>0.05), although the average was higher in the rainy season than in the dry season. The highest average is owned by bulls with ID 11341 in both the rainy and dry seasons. This is influenced by the season, body weight, and age [23, 24]. The thicker and more consistent, the more turbid the color of the fresh semen will be, and the concentration of spermatozoa will be higher [25, 26, 21, 6].

3.1.4. Spermatozoa Concentration

The concentration of semen in Bali bulls was very diverse in this study. The average concentration of fresh semen of Bali bull for each individual is shown in Table 6. Based on these data, the average spermatozoa concentration score of Bali bulls in the rainy season (1,015.48±143.46x106/mL) and dry season (1,000.80±164.64X106/mL), there was no significant difference (P>0.05), although the average total score

was higher during the rainy season than in the dry season. Similar results were also obtained from previous studies, where the sperm concentration of Bali bulls had a value of about 1,057±3.2x106/mL) [25]. A similar result was also shown in the study of Zulyazaini et al. [18], where the average concentration of spermatozoa in Aceh bulls semen were 1,194 ±52.25x106/mL. The milky white color generally indicates a thick consistency and high concentration, while the clear white color indicates a lower concentration of spermatozoa [27].

Table 6. Average value of fresh semen concentration of Bali bulls in UPTD BIBD PTHPT Bali Province in the rainy and dry seasons

Bull ID	Season	
	Rainy (x10$^{6/}$ mL)	Dry (x10$^{6/}$ mL)
11544	941.31±88.89	957.63±144.26
11443	952.73±133.90	965.92±173.03
11341	1,294.75±243.17	1,177.21±240.30
11240	1,025.81±171.39	958.50±118.16
11037	862.80±79.94	944.67±153.43
Total average	1,015.48±143.46	1,000.80±164.64

According to Ko's study [17], the hot season has a negative correlation with the concentration of spermatozoa. Feradis [20] also stated that erratic changes in environmental temperature will affect the male reproductive organs such as in the thermoregulatory function of the scrotum can be disturbed which adversely affects the process of spermatogenesis, an increase in air temperature due to high humidity can lead to failure in the formation and decrease of spermatozoa production.

3.1.5. Mass Motility

Mass motility is the movement of spermatozoa that is assessed by colonies in an observation. The movement of these colonies is assessed by the scoring method, where (+++) is rated as a colony with excellent performance, (++) is rated as a good colony, and (+) is rated as a poor colony, and (0) is a bad colony [10]. The average motility value of the mass of fresh semen of Bali bulls is shown in Table 7.

Table 7. Average value of movement score/mass motility of fresh semen of Bali bulls at UPTD BIBD PTHPT Bali Province in the rainy and dry season

Bull ID	Season	
	Rainy	Dry
11544	2.40±0.50	2.35±0.48
11443	2.30±0.47	2.32±0.47
11341	2.92±0.28	2.67±0.47
11240	2.56±0.51	2.38±0.50
11037	2.16±0.38	2.27±0.45
Total average	2.47±0.43	2.40±0.47

Note: movement score: (+ or 1) diluted; (++ or 2) moderate; (+++ or 3) thick

The results of this study showed that no significant difference (P>0.05) in the mass motility score of the fresh semen of Bali bulls between the two seasons (2.47±0.43 vs 2.40±0.47), although the average in the rainy season was higher than in the dry season. In this study, it was known that the mass motility of Bali bulls was relatively good, compared to the study conducted by Komariah et al. [5] where the mass motility in each individual did not reach (++). However, the results of this study are in accordance with Dzulqarnain et al. [25] which stated that the mass movement in the fresh semen of Bali bulls resembles a thick cloud and moves quickly with a score (+++). Mass motility is affected by various factors, including individual variation, season, heat stress, libido, and bull activities [23, 28]. Exercising bulls have better motility [23].

3.1.6. Individual Motility

Individual motility is a fundamental parameter that is often used to determine the feasibility of fresh semen for further processing. According to SNI 4869-1:2024, fresh semen can be processed into frozen semen with an individual motility of at least 70%. Individual motility checks can be performed by two methods, namely conventional and Computer Assisted Sperm Analysis (CASA). In this study, the method used is a conventional method with a microscope. The average individual motility value of fresh semen of Bali bulls is shown in Table 8.

Table 8. Average individual motility value of fresh semen of Bali bulls at UPTD BIBD PTHPT Bali Province in the rainy and dry season

Bull ID	Season	
	Rainy (%)	Dry (%)
11544	70.64±3.35	70.00±0.00
11443	70.00±0.00	70.00±0.00
11341	70.67±2.52	70.00±0.00
11240	70.37±1.05	70.00±0.00
11037	70.08±0.34	70.00±0.00
Total average	70.35±1.45	70.00±0.00

Based on the results of the study, there was no significant difference in individual motility of fresh semen of Bali bulls (P>0.05) between the rainy season (70.35±1.45) and the dry season (70.00±0.00). The motility of individual spermatozoa is closely related to the presence of seminal plasma that serves as an energy source [7]. In semen, there are organic materials that can be used directly by spermatozoa as an energy source for the survival and motility of spermatozoa, these materials are fructose, sorbitol, and *glyceryl phosphoryl choline* (GPC) [29]. In addition, individual motility is also influenced by various factors such as heat stress, nutrition, health status, and age [30-32].

3.2. Frozen Semen Production

Fresh semen that is accommodated at UPTD BIBD PTHPT Bali Province after meeting the inspection standards will be processed into frozen semen. The straw packaging for frozen semen of Bali bulls contains about 25 million spermatozoa. The frozen semen produced will be evaluated again, only frozen semen with a post-thawing motility (PTM) of >40% is suitable for distribution to the farmer (SNI 4869-1:2024). As for Table 9, showing the average value of frozen semen production in both season groups.

Table 9. Average value of frozen semen production of Bali bull in UPTD BIBD PTHPT Bali Province in the rainy and dry seasons

Bull ID	Season	
	Rainy (straw)	Dry (straw)
11544	152.00±37.45	160.44±35.72
11443	164.03±57.86	160.62±49.70
11341	163.38±34.50	146.73±44.69
11240	172.19±63.21	168.83±60.12
11037	135.68±36.97	151.10±44.85
Total average	157.46±45.00	157.54±47.02

The results of the study that have been carried out state that the average value of frozen semen production in the five individuals in the rainy season (157.46±45.00 straw) and dry season (157.54±47.02 straw) groups is not significantly different (P>0.05). Table 9 showed, the highest value of frozen semen production in both the rainy season (172.19±63.21 straws) and the dry season (168.83±60.12 straws) is owned by Bali bull with ID 11240. The production of frozen semen is produced through a dilution process whose amount is based on the

concentration and volume of ejaculation [6]. The higher the volume of ejaculation obtained, the higher the potential to produce frozen semen [29].

The freezing process generally causes a drastic decrease in temperature, which can cause cold shock in spermatozoa cells. This leads to a decrease in spermatozoa's quality. Cold shock can cause damage to the structure and function of spermatozoa cells and lead to an increase in reactive oxygen species (ROS), which ultimately impacts fertility [33].

3.3. Field Fertility

Evaluation of pregnancy after AI services needs to be carried out to evaluate the success of the service. There are various factors that affect the success of AI, such as the quality of semen, the management of AI implementation, including AI technicians, and female factors such as body condition and estrus time [34, 35]. In this study, field fertility was evaluated with the percentage of CR obtained from the big data of iSIKHNAS in 2020-2022. The percentage of CR obtained with AI services using frozen semen from each bull is shown in Table 10.

Table 10. Field fertility value (CR) in Bali bulls raised at UPTD BIBD PTHPT Bali Province in the rainy and dry season

Bull ID	Season	
	Rainy (%)	Dry (%)
11544	85.01	66.18
11443	72.07	74.66
11341	72.57	61.60
11240	64.60	56.72
11037	56.19	65.45
Total average	70.09±10.67	64.92±6.61

The results of the study that have been conducted stated that the average of CR value in the five bulls in the rainy season group (70.09 ± 10.67%) and the dry season (64.92 ± 6.61%) were not significantly different (P> 0.05), although the average value in the rainy season was higher than in the dry season. This is thought to be because Bali bulls are raised in the highlands, where the altitude decreases with temperature. According to Adnyani et al. [36], the highlands have low temperature and the lowlands have high temperature, thus affecting the heat balance in the body. Based on the research report conducted by Adnyani et al. [36], the heat balance of the livestock body can be influenced by external conditions, including air temperature, air humidity, and solar radiation, and internal conditions in the form of physiological processes, including metabolic processes. Baturiti, which is the location where this study was conducted, is one of the highland areas in Bali. Baturiti Village is a highland area with an altitude of 700-900 meters above sea level [37]. However, in general, in the dry season, cows will be exposed to heat stress, which can reduce the metabolic conditions in their bodies, which will have an impact on the decrease in CR values [38]. This is also supported by Duguma and Janssens [39] reported that natural grasslands are declining in availability and quality ahead of the dry season, and have an impact on low production and reproductive performance, and increase their susceptibility to disease and parasites. Apart from the stress factor, the nutritional factor also influences. The resumption of reproductive cycles after the postpartum period is essential for the effectiveness of breeding programs, and both nutrition and management during the transition period play a key role in determining the occurrence of the first ovulation and preventing BCS loss, thus it will increase pregnancy rate [40].

The results of the study were slightly different from the study by Komariah et al. [6], which stated that the quality of fresh semen and frozen semen production of madura bulls were relatively different in the rainy and dry seasons. Meanwhile, Asiah et al. [41] stated that in general the dry season gave better results than the rainy season. However, these results are consistent with the previous studies that reported by Bebas et al. [42], which stated that seasonal differences did not affect the semen quality of Bali bulls.

4. Conclusion

The results of this study indicate no significant difference (P>0.05) in the quality of fresh semen, frozen semen production, and field fertility in the rainy and dry seasons in Bali bulls semen. Nonetheless, all parameters observed in this study such as semen volume, color, consistency, spermatozoa concentration, mass motility, individual motility, frozen semen production, and field fertility showed higher performance in the rainy season than in the dry season. Based on the results of this study, it is recommended to conduct research on the effect of seasons in lowland areas where there are differences in heat during the rainy season and the dry season.

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