Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Fresh Semen Quality of *Bos taurus*, *Bos indicus* and *Bos sondaicus* Bulls in the Tropical Condition

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Article Info

Received: 03.02.2023
Accepted: 15.06.2023
Online published: 15.09.2023
DOI: 10.29133/yyutbd.1244506

Keywords

Abnormalities,
Age,
Concentration,
Motility,
Season

Abstract: This study aimed to assess the effect of genetic and seasonal interaction on semen quality in the Artificial Insemination Center. A total of 36,754 ejaculation records were evaluated. The dependent variable was semen quality which consisted of volume, pH, concentration, abnormalities, and motility of fresh semen. The independent variables consisted of age, season, and number of ejaculation. The mixed procedure with Tukey–Kramer multiple comparison test was used to analyze the effect of interaction between the variables. Seasonal factors significantly affected concentration, fresh semen motility, and abnormality, but did not affect pH and volume. Age factor had a significant effect on all semen quality variables. The ejaculation factor significantly affected pH, volume, concentration, and fresh semen motility, but did not affect abnormality. The species factor significantly affected pH, concentration, and fresh semen motility, but did not affect volume and abnormality.

To Cite: Wijaya, F M P, Sutopo, S, Samsudewa, D, Setiyono, A, Setiaji, A, 2023. Fresh Semen Quality of *Bos taurus, Bos indicus,* and *Bos sondaicus* Bulls in the Tropical Condition. *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(3): 420-428.
DOI: https://doi.org/10.29133/yyutbd.1244506

1. Introduction

Artificial insemination (AI) is still one of the technologies that play a very important role in optimizing the birth rate to fulfill the need for cattle commodities in Indonesia. Semen quality is one of reproductive potency which is the main factors in frozen semen production, where good quality semen also greatly influences the success rate of AI (reproductive performance) (Febriana et al., 2022).The quality of semen can be influenced by genetic and non-genetic factors including the environment and the technical aspects of semen storage. The age of the bull is one of the factors that has been frequently studied in various breeds of cattle (Snoj et al., 2013; Murphy et al., 2018; Konenda et al., 2020). Older bulls have semen with better quantity and quality than younger bulls (Ramajayan et al., 2023), although several studies have stated that there is an optimal point of semen quality at a certain age for bulls in each breed (Snoj et al., 2013). The effect of season on semen quality has been extensively studied in

several previous studies (Malama et al., 2017; Murphy et al., 2018; Ramajayan et al., 2023), but the results presented are contradictory.

Climatic conditions in each region are different, as well as the season, so season-based research needs to be carried out in certain areas with certain livestock. Livestock in the tropics is often characterized by variations in fertility for both males and females. This has been attributed to several external factors such as environmental factors, as well as internal factors such as genotype and their interactions (Landaeta-Hernández et al., 2020; Ramajayan et al., 2023; Tripathi et al., 2023). These factors are rarely considered to contribute to seasonal variations in male fertility (Landaeta-Hernández et al., 2020). Climatic conditions are not only different in each region but they are also known to change from time to time (Lee et al., 2016). Rainfall caused by climate change has increased, especially in the tropics. Increased rainfall intensity causes a longer rainy season period, resulting in a delay in the change of seasons. However, bulls in the tropics experience significant changes in semen quality as the seasons change. In the tropics, bulls with different genotypes also have different semen quality in each season (Landaeta-Hernández et al., 2020).

The mixed model in this study is a statistical model that has fixed effects (age and season) and random effects (name of the bulls). This model is useful in repeated measurements performed on the same statistical unit (longitudinal study), or in groups. Non-genetic and genetic factors have been studied both simultaneously and separately. Genetic factors can be seen in different breeds or species of cattle. In general, breeds represent certain characteristics at the lowest taxonomic level above individual genetic variation, while species include several breeds of cattle that have the same general characteristics and are located or originating in a particular region. Research that has been carried out generally focuses on one breed (Bhakat et al., 2014; Prastowo et al., 2019) and several breeds in the same species (Snoj et al., 2013; Konenda et al., 2020). Cattle commodities in Indonesia consist of three different species (*Bos sondaicus, Bos taurus, and Bos indicus*), and each species has different characteristics based on the environment of origin. The subject of this study was to assess the effect of genetic and seasonal interaction on semen quality in the Artificial Insemination Center.

2. Material and Methods

2.1. Ethical approval

Approval from the committee on the care and use of animals was not sought because no treatment and field experiments were conducted on animals.

2.2. Data collection

A total of 36,754 ejaculation records of 143 bulls were carried out by the Singosari National Artificial Insemination Center (SNAIC) of Indonesia. Five breeds included were Simmental, Limousine, Brahman, Ongole, grade, and Bali Cattle. The period of collecting data was between January 2018 and December 2021. The detail of the data used in this study is presented in Table 1.

The five parameters in the study were:

1). Volume (VOL) is the amount of semen in one ejaculation, calculated using a measuring cup with units of ml.

2). The pH is a degree of acidity for each ejaculation, tested by using Bromothymol Blue (BTB) indicator pH paper.

3). Concentration (CON) is the number of spermatozoa cells contained in semen, calculated by a spectrophotometer.

4). Abnormality (ABN) is the percentage of sperm cells that are considered not to have normal morphology.

5). Fresh Semen Motility (FSM) is the percentage of sperm cells that can move independently with a pattern a straight line or a large circle in fresh semen.

ABN and FSM were measured using Computer-Assisted Sperm Analysis (CASA).

2.3. Categories and Statistical Analysis

The bulls were grouped into three species: *Bos taurus* (Simmental and Limousine; n = 105), *Bos indicus* (Brahman and Ongole Grade; n = 17), and *Bos sondaicus* (Bali; n = 21). Age is the lifespan of

bulls to the time of semen collection, expressed in years. The age of bulls was categories to six groups: A1 (<3 years; n = 7848), A2 (≥3-<4 years; n = 10612), A3 (≥4-<5 years; n = 9208), A4 (≥5-< 6 years; n = 4621), A5 (≥6-<7 years; n = 1344), and A6 (≥7 years; n = 3121). The season was grouped based on the precipitation: low (rainfall 0 – 50 mm; n = 14259), medium (rainfall 51 – 150 mm; n = 13662), and high (rainfall 151 – 300 mm; n = 8833). The number of ejaculation was the frequency of taking semen from the same bull on the same day. The ejaculation used was the first ejaculation (n = 25137) and the second ejaculation (n = 10,151). The data was analyzed by using a mixed procedure of SAS On Demand for Academic (SAS, 2021). Species, age, season, and ejaculation were tested as fixed effects, then bulls were treated as a random effect. Tukey-Kramer multiple comparison was applied with a 5% significant level.

3. Results

The descriptive statistics for fresh semen quality from 36 715 ejaculates are presented in Table 1. The average of pH, VOL, CON, FSM, and ABN were 6.49; 5.77 mL; 1.16 10^9/mL; 77.12 and 5.39%, respectively. Bulls among species showed different quality of pH, CON, and FSM.

Table 1. Descriptive statistics of the data used in the study

Variable	Number	Mean	SE	Min.	Max.
pH	36715	6.49	0.01	6.00	7.60
VOL (mL)	36686	5.77	0.01	0.20	20.20
CON (10^9/mL)	35493	1.16	0.01	0.25	1.98
FSM (%)	35211	77.12	0.05	55.50	98.90
ABN (%)	24224	5.39	0.02	0.50	20.40

VOL, Volume; CON, Concentration; FSM, Fresh Semen Motility; ABN, Abnormality.

The significant values of bull traits can be seen in Table 2. The pH, CON, and FSM were affected by species. Age factor had a significant effect on all semen quality traits. Seasonal factors had a significant effect on CON, FSM, and ABN. The ejaculation factor had a significant effect on pH, VOL, CON, and FSM. The interaction between factors significantly affected semen quality traits, except pH, and ABN were not affected by the interactions species with the season, and species with ejaculation numbers, respectively.

Table 2. Type 3 tests of fixed effects for fresh semen quality

Factors	df	Traits									
		pH		VOL		CON		FSM		ABN	
		F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Species	2	10.42	<.0001	0.93	0.3947	10.84	<.0001	32.14	<.0001	1.43	0.2385
Age	5	7.48	<.0001	42.81	<.0001	43.86	<.0001	113.25	<.0001	7.93	<.0001
Season	2	0.45	0.6398	1.44	0.2376	27.43	<.0001	44.31	<.0001	6.38	0.0017
Ejaculation	1	37.71	<.0001	28.27	<.0001	175.34	<.0001	17.08	<.0001	1.34	0.2468
Species x Age	10	2.17	0.0168	3.40	0.0002	13.82	<.0001	15.62	<.0001	2.84	0.0024
Species x Season	4	2.09	0.0795	4.11	0.0025	2.60	0.0339	18.74	<.0001	2.74	0.0270
Species x Ejaculation	2	21.49	<.0001	11.33	<.0001	203.47	<.0001	159.03	<.0001	2.72	0.0659

VOL, Volume; CON, Concentration; FSM, Fresh Semen Motility; ABN, Abnormality.

Seasonal factors affected CON, FSM, and ABN ($p<0.01$), but did not affect pH and VOL. Age factor had a significant effect on all fresh semen quality. The ejaculation factor had a significant effect on pH, VOL, CON, and FSM.

As the age of bulls increased, the pH was declined but not statistically different across species. The pH of semen for *Bos taurus* was affected by season. pH was different among ejaculation in *Bos sondaicus* and *Bos taurus* bulls. pH of the first ejaculation was slightly lower than in the second one (Table 3).

Table 3. Interactions between species and age, between species and season, and between species and number of ejaculations for pH (mean ± SE)

Factors	Mean ± SE	Species		
		Bos indicus	***Bos sondaicus***	***Bos taurus***
Age				
A1	6.51 ± 0.01	6.48 ± 0.02^{bx}	6.63 ± 0.05^{cy}	6.51 ± 0.01^{bx}
A2	6.51 ± 0.01	6.47 ± 0.01^{bx}	6.53 ± 0.02^{by}	6.52 ± 0.01^{cy}
A3	6.50 ± 0.01	6.44 ± 0.01^{ax}	6.54 ± 0.02^{bcy}	6.51 ± 0.01^{ay}
A4	6.49 ± 0.01	6.42 ± 0.02^{ax}	6.53 ± 0.02^{by}	6.50 ± 0.01^{ay}
A5	6.48 ± 0.01	6.42 ± 0.02^{ax}	6.49 ± 0.01^{ay}	6.50 ± 0.01^{ay}
A6	6.48 ± 0.01	6.41 ± 0.05^{ax}	6.48 ± 0.01^{ay}	6.49 ± 0.01^{ay}
Season				
Low	6.50 ± 0.01	6.44 ± 0.01^{x}	6.52 ± 0.01^{y}	6.51 ± 0.01^{by}
Medium	6.49 ± 0.01	6.44 ± 0.01^{x}	6.53 ± 0.01^{z}	6.50 ± 0.01^{ay}
High	6.50 ± 0.01	6.44 ± 0.01^{x}	6.54 ± 0.01^{y}	6.51 ± 0.01^{by}
Ejaculation				
1	6.47 ± 0.01	6.44 ± 0.01^{x}	6.52 ± 0.01^{az}	6.47 ± 0.01^{ay}
2	6.52 ± 0.01	6.44 ± 0.02^{x}	6.54 ± 0.02^{by}	6.54 ± 0.01^{by}

^{a,b,c} Mean with different superscripts within a column are different ($P < 0.05$).
^{x,y,z} Mean with different superscripts within a row are different ($P < 0.05$).

The age of bulls had a significantly effect on VOL that increased with increasing Age. VOL in the season with low rainfall has the highest value compared to other seasons, except for *Bos sondaicus* which has a high VOL in the season with moderate rainfall. The first ejaculation tended to have a higher VOL than the second ejaculation, although in *Bos sondaicus* the difference was not significant. There was no significant interaction between species and the other factors for VOL (Table 4).

Table 4. Interactions between species and age, between species and season, and between species and number of ejaculations for VOL (mean ± SE)

Factors	Mean ± SE	Species		
		Bos indicus	***Bos sondaicus***	***Bos taurus***
Age				
A1	5.30 ± 0.14	5.34 ± 0.31^{a}	5.30 ± 0.50^{a}	5.28 ± 0.11^{a}
A2	5.59 ± 0.14	5.73 ± 0.30^{b}	5.40 ± 0.34^{a}	5.58 ± 0.11^{b}
A3	6.05 ± 0.14	6.49 ± 0.30^{c}	5.77 ± 0.34^{b}	6.01 ± 0.11^{c}
A4	6.24 ± 0.14	6.78 ± 0.31^{d}	6.21 ± 0.34^{c}	6.18 ± 0.12^{cd}
A5	6.22 ± 0.15	6.69 ± 0.32^{cd}	6.24 ± 0.33^{c}	6.11 ± 0.13^{d}
A6	6.27 ± 0.15	6.88 ± 0.36^{d}	6.28 ± 0.33^{c}	6.20 ± 0.14^{d}
Season				
Low	5.97 ± 0.14	6.44 ± 0.30^{b}	5.79 ± 0.33^{a}	5.92 ± 0.12^{b}
Medium	5.94 ± 0.14	6.29 ± 0.30^{a}	5.94 ± 0.33^{b}	5.88 ± 0.12^{a}
High	5.93 ± 0.14	6.22 ± 0.30^{a}	5.86 ± 0.33^{ab}	5.89 ± 0.12^{ab}
Ejaculation				
1	6.13 ± 0.14	6.44 ± 0.29^{b}	5.88 ± 0.33	6.09 ± 0.11^{b}
2	5.76 ± 0.14	6.19 ± 0.31^{a}	5.85 ± 0.33	5.70 ± 0.12^{a}

^{a,b,c,d}Mean with different superscripts within a column are different ($P < 0.05$).
^{x,y,z} Mean with different superscripts within a row are different ($P < 0.05$).

CON increased significantly with age in *Bos indicus* ($p<0.05$), whereas in *Bos sondaicus*, the highest CON was in the cattle group A5 and CON was highest for *Bos taurus* in the age group A6. The three species had significantly high CON in the season with low rainfall ($p<0.05$). CON in ejaculation 1 tended to be higher than in the second ejaculation, although in *Bos indicus* it was not significantly different. *Bos sondaicus* has a relatively lower CON when compared to *Bos indicus* and *Bos taurus* (Table 5).

Table 5. Interactions between species and age, between species and season, and between species and number of ejaculations for CON (mean ± SE)

Factors	Mean ± SE	Species		
		Bos indicus	***Bos sondaicus***	***Bos taurus***
Age				
A1	1.06 ± 0.02	1.15 ± 0.06^{a}	1.03 ± 0.08^{a}	1.09 ± 0.02^{b}
A2	1.01 ± 0.02	1.22 ± 0.05^{by}	1.09 ± 0.05^{abxy}	1.04 ± 0.02^{ax}
A3	1.09 ± 0.02	1.34 ± 0.05^{cy}	1.04 ± 0.05^{ax}	1.11 ± 0.02^{cx}
A4	1.16 ± 0.02	1.44 ± 0.06^{dz}	1.03 ± 0.05^{ax}	1.19 ± 0.02^{dy}
A5	1.21 ± 0.02	1.52 ± 0.06^{ey}	1.17 ± 0.04^{cx}	1.21 ± 0.02^{dx}
A6	1.25 ± 0.03	1.64 ± 0.07^{fz}	1.15 ± 0.04^{bcx}	1.31 ± 0.03^{ey}
Season				
Low	1.15 ± 0.02	1.42 ± 0.05^{by}	1.12 ± 0.04^{bx}	1.18 ± 0.02^{bx}
Medium	1.12 ± 0.02	1.36 ± 0.05^{ay}	1.07 ± 0.04^{ax}	1.15 ± 0.02^{ax}
High	1.12 ± 0.02	1.38 ± 0.05^{ay}	1.06 ± 0.04^{ax}	1.14 ± 0.02^{ax}
Ejaculation				
1	1.26 ± 0.02	1.40 ± 0.05^{y}	1.10 ± 0.04^{bx}	1.30 ± 0.02^{by}
2	1.00 ± 0.02	1.38 ± 0.06^{y}	1.07 ± 0.05^{ax}	1.02 ± 0.02^{ax}

^{a,b,c,d,e,f} Mean with different superscripts within a column are different ($P < 0.05$).
^{x,y,z} Mean with different superscripts within a row are different ($P < 0.05$).

FSM was significantly higher with increasing age ($p<0.05$) and then reached a maximum number in A6. FSM was significantly higher with increasing rainfall conditions ($p<0.05$) in *Bos sondaicus* and *Bos taurus*, whereas in *Bos indicus* FSM was not different among the season. FSM in the first ejaculation was higher except for *Bos taurus* where the second ejaculation was higher. Overall the highest FSM is *Bos taurus*, and the lowest is *Bos indicus* (Table 6).

Table 6. Interactions between species and age, between species and season, and between species and number of ejaculations for FSM (mean ± SE)

Factors	Mean ± SE	Species		
		Bos indicus	***Bos sondaicus***	***Bos taurus***
Age				
A1	70.32 ± 0.52	68.29 ± 1.23^{ay}	59.91 ± 2.66^{ax}	73.06 ± 0.40^{az}
A2	75.69 ± 0.52	73.00 ± 1.16^{by}	66.39 ± 1.54^{bx}	78.56 ± 0.40^{bz}
A3	77.04 ± 0.52	75.74 ± 1.17^{cy}	70.98 ± 1.48^{cx}	79.73 ± 0.40^{cz}
A4	77.73 ± 0.52	79.50 ± 1.23^{dy}	75.43 ± 1.52^{dx}	80.00 ± 0.42^{cy}
A5	80.88 ± 0.57	81.22 ± 1.35^{ey}	74.16 ± 1.44^{dx}	83.75 ± 0.53^{dy}
A6	85.38 ± 0.60	82.01 ± 1.63^{ex}	80.82 ± 1.40^{ex}	87.35 ± 0.64^{ey}
Season				
Low	77.10 ± 0.51	76.34 ± 1.19^{y}	68.99 ± 1.44^{ax}	79.88 ± 0.42^{az}
Medium	77.99 ± 0.51	76.94 ± 1.19^{y}	72.04 ± 1.44^{bx}	80.44 ± 0.41^{bz}
High	78.43 ± 0.51	76.59 ± 1.19^{y}	72.82 ± 1.44^{cx}	80.90 ± 0.42^{cz}
Ejaculation				
1	76.61 ± 0.50	77.77 ± 1.13^{by}	73.14 ± 1.42^{bx}	78.87 ± 0.41^{ay}
2	79.07 ± 0.51	75.48 ± 1.26^{ay}	69.42 ± 1.46^{ax}	81.95 ± 0.42^{bz}

^{a,b,c,d,e} Mean with different superscripts within a column are different ($P < 0.05$).
^{x,y,z} Mean with different superscripts within a row are different ($P < 0.05$).

ABN was significantly lower with age in *Bos indicus* and *Bos taurus* ($p<0.05$), whereas in *Bos sondaicus* they were not significantly different. ABN was lower in seasons with high rainfall in *Bos indicus* and *Bos taurus*, while there was no significant difference in *Bos sondaicus*. The ejaculation had no significant effect on ABN in *Bos indicus* and *Bos sondaicus*, while in *Bos taurus*, ABN in the second ejaculation was significantly lower ($p<0.05$). ABN between species did not differ significantly (Table 7).

Table 7. Interactions between species and age, between species and season, and between species and number of ejaculations for ABN (mean ± SE)

Factors	Mean ± SE	Species		
		Bos indicus	***Bos sondaicus***	***Bos taurus***
Age				
A1	6.22 ± 0.27	6.93 ± 0.62^{e}	-	5.88 ± 0.20^{d}
A2	6.12 ± 0.25	6.04 ± 0.53^{d}	6.60 ± 0.82^{b}	5.84 ± 0.19^{d}
A3	5.87 ± 0.25	5.54 ± 0.52^{c}	5.49 ± 0.72^{a}	5.62 ± 0.19^{c}
A4	5.52 ± 0.25	5.24 ± 0.54^{bc}	5.99 ± 0.69^{ab}	5.24 ± 0.20^{b}
A5	5.13 ± 0.27	4.66 ± 0.59^{bx}	6.33 ± 0.70^{by}	4.67 ± 0.26^{ax}
A6	4.71 ± 0.32	3.72 ± 0.72^{ax}	5.82 ± 0.65^{aby}	4.48 ± 0.43^{ax}
Season				
Low	5.76 ± 0.24	6.08 ± 0.52^{c}	5.81 ± 0.48	5.86 ± 0.20^{c}
Medium	5.68 ± 0.24	5.58 ± 0.53^{b}	5.92 ± 0.49	5.76 ± 0.20^{b}
High	5.34 ± 0.24	5.14 ± 0.53^{a}	5.90 ± 0.50	5.34 ± 0.20^{a}
Ejaculation				
1	5.76 ± 0.24	5.81 ± 0.49	5.71 ± 0.46	5.78 ± 0.19^{b}
2	5.43 ± 0.24	5.39 ± 0.58	6.04 ± 0.54	5.53 ± 0.20^{a}

^{a,b,c,d,e} Mean with different superscripts within a column are different ($P < 0.05$).
^{x,y,z} Mean with different superscripts within a row are different ($P < 0.05$).

4. Discussion

This study used a large number of ejaculation records regarding the evaluation of fresh semen from bulls of different species groups in one of the Artificial Insemination centers in Indonesia. Indonesia is a country that has a tropical climate. The average pH of fresh semen (6.49) in this study was in range of the previous studies reported pH of Bali Cattle ranged 6.47 – 6.57 (Prastowo et al., 2019) and 5.95 – 6.49 for Limousin cattle (Konenda et al., 2020), Affandhy et al. (2022) reported higher pH of fresh semen in Ongole cattle ranged 6.93 – 6.96.

The pH in this study was significantly influenced by age and tends to decrease in older bulls. This result is agreed with a previous study that reported the age of the bulls was affected the pH of fresh semen (Prastowo et al., 2019). Seasonal differences in this study did not affect the pH of fresh semen. This is in accordance with the explanation in the study of Konenda et al. (2020). Bhakat et al. (2015) reported that seasonal differences in pH may occur due to changes in feeding in certain seasons.

There was an increase in the VOL of semen produced along with the age of the bulls in each of the studied species. This is consistent with the results of studies on Limousine bulls (Konenda et al., 2020), Friesian Holstein (Murphy et al., 2018), and Czech Fleckvieh (Paldusová et al., 2016). The results of a study on Murrah buffalos showed that the best semen (including ejaculate volume) was produced by bulls over 12 years of age (Ramajayan et al., 2023). The increase in age associated with the increase in scrotal circumference and the volume of semen produced (Mahmood et al., 2014) can also be caused by an increase in testosterone (Konenda et al., 2020). The decrease in VOL that **is** influenced by the frequency of ejaculation is following the study by Sitanggang et al. (2020) that the frequency and interval of ejaculation greatly affect the characteristics of semen in Bali cattle.

Based on the results of this study, age had an effect on CON in *Bos indicus*. The increase in CON along with the age of the bulls contradicts the research conducted by Konenda et al. (2020) which showed that the highest concentration was found in bulls aged 2 years and the lowest in bulls aged 8 years. Increasing bulls' age has a negative correlation with spermatozoa concentrations (Mahmood et al., 2014), which may be possible due to the reduced function of bull reproductive organs in spermatogenesis (Vince et al., 2018). This study showed that there was a positive correlation between age and sperm concentration in *Bos indicus*. The CON in *Bos sondaicus* and *Bos taurus* in this study was in line with (Murphy et al., 2018) which shows CON after more than 1 year of age was constant, while the total number of spermatozoa increases with age. The increase in the total number of spermatozoa was driven by an increase in VOL of ejaculation until the age of 4 years.

This study showed a significantly higher CON in the season with low rainfall in each species. The results agreed with previous studies where sperm concentration and total sperm count were greater in the season with low rainfall (Snoj et al., 2013; Murphy et al., 2018). The ejaculation had a significant

effect on CON, where CON was higher in the first ejaculation than in the second ejaculation. These results were similar to a previous study, where the first ejaculation for bulls had a higher sperm concentration and total sperm count than the second ejaculation taken on the same day (Ramajayan et al., 2023).

FSM in this study tends to increase as increasing age of bulls in each species. Although the optimal point of sperm motility value has not been found in this study, several previous studies reported that the age factor had a significant effect on sperm motility (Boujenane & Boussaq, 2013; Sitanggang, 2018; Suyadi et al., 2020). The effect of seasons on FSM was consistent with previous studies on Simmental Bulls (Hapsari et al., 2022), Limousine Bulls (Konenda et al., 2020), Thapkar Bulls (Perumal et al., 2017), and Karan Fries Bulls (Bhakat et al, 2014). Other studies suggest a link between temperature and the continuity of spermatogenesis in terms of the effect of season on sperm motility (Konenda et al., 2020), where heat stress can reduce sperm motility (Selçuk et al., 2014). The increasing temperature resulted in a decrease in sperm motility when spermatozoa were in the epididymis (Konenda et al., 2020).

This study showed that the number of ejaculations has a significant effect on FSM where FSM in the first ejaculation was higher than in the second ejaculation. The process of spermatogenesis in the second ejaculation may not be perfect because of the difference in the short time lag when compared to the first ejaculation. Long intervals of semen collection resulted in better semen qualities (Boujenane & Boussaq, 2013; Murphy et al., 2018). A possible explanation for lower semen quality related to ejaculation frequency is the shorter meeting interval of the second ejaculation (Murphy et al., 2018).

ABN in this study was significantly influenced by season, where the highest ABN was found in seasons with low rainfall. This result agreed with the analysis of Quezada-Casasola et al. (2016) where the percentage of defective sperm was higher in European bulls during the summer and fall. Age significantly affects the ABN of *Bos indicus* and *Bos taurus*, where the abnormality tends to decrease with the increasing age of bulls. This result is in contrast to the results of the study where abnormalities increase with the increasing age of the bulls (Baharun et al., 2021). This is possible because this study used the age group from 3 to 7 years, whereas the results of the study by Baharun et al. (2021) stated that although abnormalities increased with age, semen abnormalities in the 4-5 and 6-7 age groups did not experience a significant increase. Based on this study, what might happen was as bulls mature or enter puberty, the scrotal circumference will also increase where Quezada-Casasola et al. (2016) agree, if bulls do not maintain their scrotal circumference, the result will be lower-quality sperm production.

Based on the results of this study, Bos sondaicus, which is a species native to Indonesia, showed a much higher pH than Bos indicus but was not significantly different from *Bos taurus. Bos indicus* has a much higher CON than other species, this may be because Bos indicus itself is a domestic species originating from the Indian continent which is a tropical area like Indonesia. *Bos indicus* has a higher concentration of semen, according to the results of a study by Landaeta-Hernández et al. (2020) when compared with Bos taurus. The highest FSM in this study was shown by the Bos taurus species with a significant difference when compared to other species. Despite originating from the subtropics, Bos taurus shows a high level of adaptation in the tropics (Landaeta-Hernández et al., 2020; Staiger et al., 2018). The result of this study showed that the concentration, abnormality, and motility of fresh semen were significantly affected by seasonal factors. The age factor significantly affects all semen quality variables observed in this study. The number of ejaculation significantly affects the pH, volume, concentration, and motility of fresh semen. The species factors present in this study showed a significant effect on pH, concentration, and motility in fresh semen.

Conclusion

This study shows that each bull with a different species gives a different response of semen quality to age, season, and ejaculation factors. Based on this study, species had a significant effect on the pH, concentration, and motility of fresh semen. These results indicate that genetic factors (species) also play a role in determining bull reproductive traits.

Acknowledgements

The authors thank to Singosari National Artificial Insemination Center of Indonesia for providing data set and technical assistance.

Declaration of Competing Interest

The authors have no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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