

Prolonged Sexual Rest on Fresh Semen Quality Across Different Age Groups in Holstein Friesian Bulls

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Abstract. Sexual rest and age of bulls affect semen quality. This study aimed to evaluate fresh semen quality prior to and following a long period of sexual rest in six Holstein Friesian bulls at the Lembang Artificial Insemination (AI) Center. The bulls were categorized into age groups of 3, 5, and 9 years. Secondary data from 2024–2025 on the semen volume, pH, sperm concentration, total sperm count (TSC), and sperm motility were analyzed. Bull libido, sperm viability, and abnormality were also evaluated on the first semen collection following sexual rest. The results indicated that bulls generally exhibited relatively good libido following sexual rest. Nevertheless, prolonged sexual rest decreased semen volume, sperm concentration, TSC, and sperm motility, with TSC and motility also significantly influenced by age. Sperm abnormality was elevated in the first collection following sexual rest. Notably, older bulls (9 years) exhibited a more marked decline in sperm motility, indicating a significant age effect. This was likely due to their reduced ability to maintain an optimal epididymal microenvironment as a consequence of prolonged sexual rest. In conclusion, regular semen collection intervals are crucial for maintaining optimal semen quantity and quality, which maximizes the efficiency of semen production.

1 Introduction

Milk production from Holstein Friesian (HF) cattle in Indonesia currently accounts for only about 20% of the national demand. This situation highlights the need to enhance cattle population and productivity through the use of assisted reproductive technologies, particularly artificial insemination (AI). One of the key factors influencing AI success and overall dairy productivity is bull fertility [1]. Bull fertility plays a crucial economic role in the AI industry, as it determines herd reproductive efficiency, given that a single sire has the potential to impregnate thousands of dams.

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The reproductive potential of bulls is commonly assessed through semen quality evaluation, since sperm are essential for fertilization and early embryonic development [2]. The Lembang AI Center holds a strategic role as a national hub for the production and distribution of frozen semen to support Indonesia's AI program. The large-scale production of HF bull semen at the Lembang AI Center requires each sire to maintain consistent and efficient reproductive performance. This is reflected in an estimated stock of 808,241 doses of frozen semen, with 146,820 doses distributed in 2024. However, once the frozen semen storage capacity has been reached, semen collection is temporarily suspended until additional stock is required. Semen quality following sexual rest, a prolonged period without sexual activity, often exhibits variation. Although sexual rest represents a physiological adaptation that is naturally reversible, it has been reported to cause a decline in sperm quality [3].

HF bulls maintained at the Lembang AI Center also vary in age, which may influence their physiological responses and semen quality after such periods. Age-related declines in fertility are closely associated with reduced sperm quality, as advancing age compromises cellular defense and repair mechanisms against free radical-mediated damage, resulting in oxidative stress and physiological decline [4]. Although several studies have investigated the effects of age on semen quality, research examining how prolonged sexual rest influences semen parameters in HF bulls of different age groups remains limited, particularly under the management and collection systems implemented in semen production units such as the Lembang AI Center. Therefore, this study aimed to analyze changes in semen quality prior to and following a prolonged period of sexual rest. The findings are expected to provide valuable insights for maintaining reproductive efficiency and semen quality in AI programs.

2 Materials and Methods

This study conducted a comparative analysis of fresh semen quality in six Holstein Friesian bulls prior to and following sexual rest, using secondary data obtained from the Lembang AI Center between 2024 and 2025. Prolonged sexual rest was defined as a period without semen collection lasting more than six months. The dataset comprised bulls that had not undergone semen collection for at least six months (6.67 ± 0.41 months) and were categorized into three age groups of 3, 5, and 9 years, with two bulls in each group. The bulls aged 3 years had not undergone semen collection for 6.50 ± 0.0 months, those aged 5 years for 7.00 ± 0.71 months, and those aged 9 years for 6.50 ± 0.0 months. The limited number of bulls per age group was primarily constrained by the availability of bulls in Lembang AI Center, which maintains only selected elite bulls with proven genetic merit.

All bulls included in the study had been qualified through the Breeding Soundness Examination (BSE) and were routinely used for AI. The bulls were maintained according to the Standard Operating Procedures for bull management at the Lembang AI Center. In total, twelve semen collections, six collections prior to and six collections following sexual rest period were analyzed in this study.

Semen was collected twice a week with an artificial vagina. The evaluated semen parameters included semen volume, pH, sperm concentration, total sperm count, and sperm motility. All samples were analyzed according to the standard laboratory procedures of the Lembang AI Center. Semen volume was evaluated directly in the collection tube, pH was measured using a pH meter, and sperm concentration was assessed with a Photometer SDM 6. The total sperm count was determined by multiplying the concentration by the semen volume. Sperm motility was assessed in semen diluted at a 1:4 ratio with physiological saline. A single drop of diluted semen was placed on an object glass, covered with a cover glass, and examined under a light microscope at $400\times$ magnification. Sperm motility was assessed on a scale of 0–100%.

As supporting data, bull libido, as well as sperm viability and abnormality, were assessed only in the first semen collection following sexual rest. These parameters were not assessed using secondary data. Libido was assessed using a scoring scale ranging from 1 to 10 [5]. Reaction time was recorded in minutes as the interval between the bull's arrival at the semen collection area and the first mount on the teaser, while libido duration was measured from the bull's approach to the teaser until the first ejaculation.

Sperm viability and abnormality were determined using the eosin–nigrosine staining technique, with at least 200 sperm observed under a light microscope at 400× magnification. The semen-to-eosin-nigrosine ratio was 1:4. The semen and eosin-nigrosine were mixed on a glass slide and then smeared to a microscopic slide. Live sperm were identified by unstained or transparent heads, while dead sperm had pink-stained heads. Abnormalities were evaluated in the head, midpiece, and tail regions, without differentiating between primary and secondary abnormalities.

Data were analyzed using IBM SPSS 25. As data were normally distributed, parametric tests were applied throughout the analysis. Repeated-measures general linear models (GLM) with Bonferroni pairwise comparisons were used for data from the same bulls, while generalized estimating equations (GEE) with Bonferroni pairwise comparisons were applied specifically for sperm motility. The within-subject effect represented the effect of sexual rest, the between-subject effect represented age group, and the interaction term (time × age) indicated whether the effect of sexual rest differed among age groups. Pearson or Spearman correlation on difference scores were also performed. Bull libido, sperm viability, and abnormality were presented descriptively, as statistical analysis was not possible due to the limited sample size and single measurements per individual.

3 Results and Discussion

Libido characteristics of HF bulls on the first semen collection following a prolonged sexual rest showed that all age groups had libido scores ranging from 7 to 9 (Table 1). All bulls were able to ejaculate following a prolonged period of sexual rest, with the number of mountings varying among individuals. Some bulls showed higher sexual activity by ejaculating more than once; therefore, they were assigned a score of 9 [5].

Table 1. Bull libido characteristics during the first semen collection following sexual rest

Age	Score	Reaction time	Duration
3 years	7–9	2.00±2.12	5.00±1.41
5 years	7–9	7.1±8.34	9.50±9.19
9 years	7–9	3.59±2.00	10.50±4.95

The results showed reaction time varied among age groups, with bulls aged 3 years exhibiting the shortest average reaction time, bulls aged 5 years showing a higher and more variable reaction time, and bulls aged 9 years displaying an intermediate reaction time (Table 1). Libido duration also appeared to increase with age. Bulls aged 3, 5, and 9 years exhibited libido durations ranging from 5.00±1.41 to 10.50±4.95 minutes after sexual rest, with some values exceeding the 10-minute threshold associated with reduced libido according to Jackson and Cockroft [6]. The older bulls required a longer time to ejaculate, likely due to age-related limb impairments in the bulls. Overall, bulls exhibited relatively good libido following sexual rest.

An interesting finding was observed in one of the 5 years old bulls, which exhibited a reaction time of 13 minutes and libido duration of 16 minutes, resulting in a high standard deviation. Libido level is believed to be influenced by various factors, including hereditary

traits, psychogenic disorders, endocrine imbalance, management, and environmental factors [7]. Bulls with weak libido are not recommended for use in breeding programs, as they may disseminate undesirable genes associated with poor libido.

The mean values of semen characteristics prior to and following a sexual rest across different age groups were presented in Table 2. Secondary data on the semen color of HF bulls prior to and following sexual rest generally showed that it varied from cream to milky white. The semen color of 3-year-old bulls tended to become milky white after sexual rest, compared to the previous cream to milky white appearance. The observed semen color was within the normal range, since healthy bull semen is generally cream in color. In some cases, bull semen may appear yellowish due to the presence of riboflavin [8].

Table 2. HF semen characteristics prior to and following sexual rest

Semen characteristics	Age	Sexual rest ($\bar{x}\pm$ SD)		Between-effect <i>p</i> -value	Interaction
		Before	After		
Color	3 years	Cream to milky white	Milky white	-	-
	5 years	Cream to milky white	Cream to milky white		
	9 years	Yellowish to milky white	Cream to milky white		
Volume (mL)	3 years	6.65±1.45 ^{aA}	4.87±2.35 ^{bA}	0.545	0.634
	5 years	7.13±2.34 ^{aA}	6.12±3.38 ^{bA}		
	9 years	6.63±1.66 ^{aA}	5.79±2.07 ^{bA}		
Within-effect <i>p</i> -value 0.008					
pH	3 years	6.64±0.08 ^{aA}	6.59±0.07 ^{aA}	0.150	0.023
	5 years	6.69±0.15 ^{aA}	6.62±0.02 ^{aA}		
	9 years	6.57±0.07 ^{aA}	6.66±0.12 ^{aA}		
Within-effect <i>p</i> -value 0.721					
Sperm concentration (10 ⁶ /mL)	3 years	1328.42±70.66 ^{aA}	1109.50±293.56 ^{bA}	0.080	0.080
	5 years	761.42±166.09 ^{aA}	707.17±398.39 ^{bA}		
	9 years	1309.08±89.55 ^{aA}	1103.67±363.86 ^{bA}		
Within-effect <i>p</i> -value 0.000					
Total sperm count (10 ⁶ /ejaculate)	3 years	8816.98±1917.87 ^{aA}	5221.99±2620.11 ^{bA}	0.007	0.080
	5 years	5283.29±1713.68 ^{aB}	4373.92±2914.63 ^{bB}		
	9 years	8690.81±2278.87 ^{aA}	6366.22±3219.52 ^{bA}		
Within-effect <i>p</i> -value 0.000					
Motility	3 years	74.25±1.95 ^{aA}	73.33±2.46 ^{aA}	0.000	0.004
	5 years	71.25±2.26 ^{aB}	71.25±4.33 ^{aAB}		
	9 years	74.17±1.95 ^{aA}	65.42±8.11 ^{bB}		
Within-effect <i>p</i> -value 0.001					

*Values within the same column or row with different superscripts differ significantly ($p < 0.05$). Lowercase superscripts (a, b) indicate significant differences between sexual rest periods within the same age group ($p < 0.05$). Uppercase superscripts (A, B) indicate significant differences among age groups within the same sexual rest period ($p < 0.05$).

Sexual rest significantly affected semen volume, sperm concentration, and total sperm count, whereas age had a significant effect on total sperm count. A significant interaction between sexual rest and age was observed for semen pH and motility. Interestingly, the semen volume, sperm concentration, and total sperm count of HF bulls significantly decreased after

sexual rest across all age groups. This finding is noteworthy, as sexual rest is generally expected to increase ejaculate volume, sperm concentration, and, in turn, total sperm count [3]. Spermatogenesis is a continuous process, and sperm are continuously transported through the ductus deferens during both sexually active and inactive periods. Sexual rest leads to the accumulation of sperm in the epididymis and vas deferens; however, most unreleased sperm are gradually eliminated via excretion into the urine [7]. Since semen volume is closely associated with the secretory function of the accessory sex glands, prolonged sexual rest may lead to reduced gland activity and, consequently, a lower ejaculate volume.

Nonetheless, the results remained within the normal range for bull semen volume and pH, which is 5–8 mL and 6.4–7.8 [7]. Similarly, the sperm concentration of 3 years and 9 years old bulls were within the normal range, approximately $800\text{--}2000 \times 10^6$ sperm/mL [7]. In contrast, the sperm concentration of 5 years old bulls was categorized as low. Although sperm concentration does tend to decrease with increasing bull age, this value is still considered low, as bulls of this productive age generally have a minimum concentration of 1100×10^6 sperm/mL [9]. Interestingly, bulls in this age group demonstrated a higher semen volume, but a lower total sperm count compared to the other age groups. This finding should be emphasized, as it likely reflected an excess of seminal plasma rather than efficient sperm production at this age. The maintenance of bulls with such reproductive characteristics requires consideration, as the high ejaculate volume was not accompanied by an adequate number and quality of sperm. This condition may negatively affect frozen semen productivity and the effectiveness of the AI program [7].

Furthermore, total sperm count following sexual rest showed the greatest decrease in 3 years old bulls compared to 5 years and 9 years old groups. This finding indicates that bulls at 3 years old produce markedly fewer sperm per ejaculate than bulls in the 5 years and 9 years old age groups following sexual rest. Total sperm count is associated with the sperm production capacity of the testis. This is closely associated with testicular volume, as it reflects the amount of seminiferous epithelium, which is the primary site of spermatogenesis and a key determinant of sperm production [10]. However, the marked reduction in total sperm count observed in younger bulls after sexual rest may reflect a transient disruption in spermatogenic efficiency due to physiological adaptation to the period of sexual inactivity.

This study also showed that semen production tended to fluctuate following sexual rest period, particularly in terms of volume, concentration, and total sperm count, as indicated by the high standard deviation values. This finding may be attributed to the recovery phase of the bulls following the sexual rest period. Semen characteristics typically return to normal after approximately six weeks of sexual rest [7].

Sperm motility is an important functional characteristic associated with fertilizing ability and may also serve as an indicator of other parameters, such as viability [11]. The results showed that both age and sexual rest significantly affected sperm motility. The significant effect of collection period ($p < 0.05$) indicated an overall difference in motility between the two collection periods, mainly due to changes in bulls aged 9 years. This suggested that sexual rest had a marked influence on sperm motility. A significant difference was also observed among age groups ($p < 0.05$), indicating that sperm motility varied with the bull's age. Bulls aged 3 years old showed slight decrease motility values, those aged 5 years remained unchanged motility values, and bulls aged 9 years showed markedly lower motility values. Furthermore, the significant interaction effect ($p < 0.05$) suggested that the influence of sexual rest on sperm motility differed among age groups.

The sperm viability and abnormality were also evaluated in the first collection following a prolonged sexual rest (Table 3 and Figure 1). The viability of HF bull sperm could be considered high, as sperm viability was the last parameter to be compromised during cellular damage [12]. Therefore, sperm viability should be higher than motility parameters. The

percentage of sperm abnormalities following sexual rest in all age groups was markedly elevated, exceeding the Indonesian National Standard threshold of 20%. This result was consistent with Udoekong et al. [3], who reported that sperm abnormalities in the first ejaculate following sexual rest were greater than 20%.

Table 3. Sperm viability and abnormality in the first semen collection following sexual rest

Age	Viability	Abnormality
3 years	91.8±7.4	27.4±10.5
5 years	77.2±21.7	32.4±5.8
9 years	94.0±0.2	33.4±1.2

Prolonged sexual rest may lead to the accumulation of sperm in the epididymis. Sperms that are not ejaculated become senescent, undergo structural and functional damage, lose their fertility, and eventually disintegrate [7]. It was evidenced by the high percentage of low sperm progressive motility and high sperm abnormalities. These changes induced by sexual rest promote the accumulation of toxic metabolic residues, while the ability of the epididymis to maintain an optimal microenvironment for sperm gradually declines with the duration of storage [13, 14]. Furthermore, prolonged sexual rest altered the proteomic profile of sperm and seminal plasma, which may be associated with premature acrosome reactions induced by oxidative stress [3]. Such reactions impair the fertilization process and accelerate sperm cell death. Younger animals have a more efficient physiological system than older ones. This was consistent with bulls achieving optimal semen production and highest fertility rates in 3–4 years old [15].

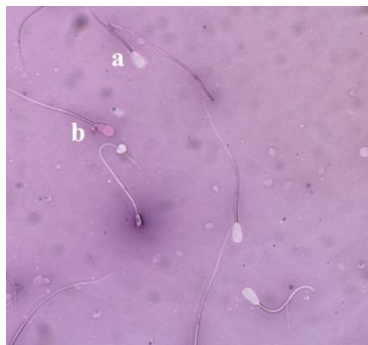


Fig. 1. Sperm viability using eosin nigrosine stain: live sperm appeared unstained or transparent (a), while dead sperm were stained pink (b)

The correlation between age and the changes in fresh evaluation prior and following sexual rest in HF bulls was presented in Table 4. The results observed a weak positive correlation between age and semen pH ($r = 0.359$), suggesting that pH tended to slightly increase with age, although this relationship was not statistically strong. Age had a significant negative correlation with the difference in sperm motility prior to and following sexual rest ($r = -0.466$).

This means that as bulls aged, the decline in sperm motility following sexual rest became more pronounced. In other words, older bulls experienced a greater reduction in motility compared to younger bulls following the rest period. As increasing age in bull results in a

decline in reproductive function [10, 15], a decrease in semen quality parameters may be expected in older individuals.

Table 4. The correlation between age and the differences in fresh semen evaluation parameters prior to and following sexual rest in HF bulls

Semen characteristics	Sexual rest				
	Volume	pH	Concentration	Total sperm count	Motility
Age	0.155	0.359	0.016	0.178	-0.466

Significance: $p < 0.05$ bold

It is recommended that regular semen collection intervals be maintained to preserve both semen quality and quantity, thereby maximizing production efficiency. Semen collected after a period of sexual rest should not be immediately processed into frozen semen until its quality has returned to optimal levels, as the decline in semen quality and quantity after sexual rest may reduce overall semen production. Therefore, an accurate diagnosis requires an individualized assessment to ensure that only ejaculates with optimal reproductive potential are selected for processing. This approach ensures that the distributed semen is of superior quality, facilitates the dissemination of desirable genetics, and supports sustainable milk production.

4 Conclusion

Based on the study, prolonged sexual rest affected most semen quality and quantity across all age groups. Notably, a significant decline in sperm motility was observed in older bulls following the resting period.

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