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Bull Spermatozoa Morphometry Characteristic on Different Breeds and Ages

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ABSTRACT

This study was conducted to determine the morphometric characteristics of bull spermatozoa of different breeds and ages. This study used a Completely Randomized Design with a Factorial pattern with factor A consisting of A1 (Simmental bull) and A2 (Limousin bull), and factor B consisting of B1 (2-4 years), B2 (5-7 years), and B3 (8-10 years). The variables observed in this study included the area of the spermatozoa head, length of the spermatozoa tail, and size of the spermatozoa body. This study's findings indicate an interaction ($p < 0.05$) between the breed and age of the bull on the area of the spermatozoa head. Still, there is no interaction between the breed and age of the bull on the length of the spermatozoa tail and the size of the spermatozoa body. This study shows that Simmental bulls have higher spermatozoa morphometric characteristics than Limousin bulls. The results of the study of spermatozoa morphometric characteristics showed that the highest spermatozoa head area was in Limousin bull aged 5-7 years, namely 35.79 ± 3.91 µm2, the highest spermatozoa tail length was in Simmental bull aged 5-7 years, namely 49.28 ± 2.38 µm, the highest spermatozoa body length was in Simmental bull aged 5-7 years, 73.02 ± 4.32 µm. The findings of this study indicate that morphometric characteristics increased at the age of 5-7 years and decreased again at the age of 8-10 years.

Keywords: Morphometry, sperm body, sperm head, sperm tail.

INTRODUCTION

Applying Bull semen through artificial insemination (AI) can increase productivity and rapid distribution of Bulls with superior genetic quality and optimal function (Kaya & Memili, 2016). In addition, combining AI with the strict selection of bulls in livestock breeding programs plays a strategic role in producing high-quality offspring with better performance (Baharun et al., 2021). Proper characterization of Bull semen is essential in evaluating Bull fertility. Semen quality evaluation is the primary tool for assessing the level of male fertility because it is related to the success of Artificial Insemination (AI) in most livestock (Valverde et al., 2016). One of the parameters used to evaluate semen quality is the morphometric characteristics of spermatozoa. Sperm morphometry is an essential clinical parameter in cattle that is used to estimate fertilization capacity in cattle (Garcia-Herreros & Leal, 2014) several factors, including the breed and age of the male, influence the morphometry of spermatozoa.

Different breeds of Bull also have differences in sperm morphometric characteristics. Felton-Taylor et al. (2020) found differences in morphometrics in other cattle breeds, including Brahman, Braford, Belmont, Red Charbray, Droughtmaster, and Santa Gertrudis. The increasing age of bulls is associated with a significant decrease in several semen parameters, including morphometric characteristics (Kipper et al., 2017). There are many potential mechanisms known to change with age, including the decreased function of the accessory reproductive glands, cellular and physiological changes, including the reduced capacity to repair cellular and tissue damage, decreased germ cell and androgen levels, and structural changes in male reproductive anatomy, including narrowing of the seminiferous tubules, vascular insufficiency, and systemic diseases associated with aging (Halvaei et al., 2020). Sperm quality is generally described as increasing from young to adult and decreasing from adult to old. Abah et al. (2023) Stated that spermatozoa can develop better flagellar movement from young to adult age, resulting in better sperm kinematics and increasing the chances of successful fertilization.

The relationship between sperm morphometric characteristics and male fertility is interrelated. The relevance of sperm morphometric evaluation is related to its indicative role in the presence of deviations in spermatogenesis and sperm maturation, and correctly observed morphometry remains an essential tool in the assessment of bull fertility (Felton-Taylor et al., 2020). Kondracki et al.

(2012) also stated that the sperm morphometric structure is a factor that ensures the success of spermatozoa transportation to the oocyte and egg cell activation. The success of spermatozoa reaching the fertilization site is determined by several factors, including the morphometric characteristics of spermatozoa; the morphometric characteristics have functional relevance to ensure a more significant potential for spermatozoa to fertilize eggs. (Garcia-Vazquez et al., 2016). Previous researchers have reported a positive correlation between spermatozoa morphometric characteristics and other sperm quality parameters. (Kondracki et al., 2012), who noted that higher spermatozoa concentrations had larger head circumferences than spermatozoa with low concentrations. Another study by (Garcia-Herreros & Leal, 2014) Reported that the morphometric characteristics of spermatozoa have a positive correlation with the viability of bovine spermatozoa.

Balai Pengembangan Teknologi dan Sumber Daya (BPTSD) Tuah Sakato Payakumbuh is one of the Regional Technical Implementation Units (Unit Pelaksana Teknis Daerah/UPTD) of the Animal Husbandry and Animal Health Service of West Sumatra Province. The main task and function of the Tuah Sakato Technology and Resources Development Center is to carry out the production and marketing of frozen semen of superior seeds as well as the application and development of reproductive technology. The highest frozen semen straw product at UPTD BPTSD Tuah Sakato is frozen semen straw from Simmental and Limousin cattle. Based on this description, this study was conducted to determine the morphometric characteristics of bull spermatozoa in different breeds and ages.

MATERIALS AND METHODS

This research was conducted at UPTD BPTSD Tuah Sakato and the Animal Reproduction Laboratory of the Faculty of Animal Husbandry, Andalas University, from August to September 2024. It used frozen semen from six bulls consisting of two different breeds, Simmental and Limousin, in three age groups: 2-4 years, 5-7 years, and 8-10 years.

Observation of Sperm Morphometrics

Observation of spermatozoa morphometric characteristics was carried out with the following steps:

1. Thawing of frozen semen was carried out at 37°C for 20 seconds.
2. Thawed semen was dripped onto a smear preparation with a ratio of 1: 2 (1 semen and 2 eosin 2%).
3. Smear preparations were made for each nation and age group with as many as 6 preparations so that a total of 144 preparations were used.
4. The smear preparation was made as thin as possible to make the spermatozoa more clearly visible when observed.
5. The preparations that had been made were then put into the oven.
6. The preparations that have been oven-dried were observed for spermatozoa under an OptiLab microscope (OptiLab®Sigma 4K, Made In Indonesia) with a magnification of 1,000 times by observing 2,400 spermatozoa cells.
7. Morphometric measurements were carried out using the ImageJ application by standardizing the lines on the measurements on the ocular with the scale on the object of observation; after standardization, observations were made on the morphometry of the spermatozoa.

Research Design

This study used a Completely Randomized Design with a Factororial pattern. The treatments consisted of two factors, namely factor A (bull breed) and factor B (age). Factor A consists of A1 (Simmental bull) and A2 (Limousin bull). Factor B consists of B1 (2-4 years), B2 (5-7 years), and B3 (8-10 years). Each treatment was repeated four times.

Observed Variable

The variables observed in this study included:

a. Sperm Head Area, the spermatozoa head area is calculated using the formula: Head Area = (0.8988 x Head Length x Head Width) – 1.63 (0.8988: correction factor, 1.63: regression constant value) (Situmorang et al., 2014).
b. Sperm Tail Length: the length of the spermatozoa tail is measured from the middle to the tip of the spermatozoa tail (Figure 1).
c. The overall length of spermatozoa is measured from the head's tip to the spermatozoa tail's tip (Figure 1).

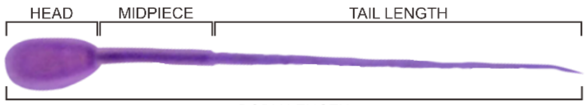

Figure 1. Measuring Sperm Morphometrics.

Data Analysis

Research data was analyzed using ANOVA and further DMRT test if there were differences ($p<0.05$) using IBM SPSS v.25.

RESULTS AND DISCUSSION

Spermatozoa Head Area

This study's findings indicate an interaction ($p<0.05$) between the breed of Bull and age on the area of the spermatozoa head. The results of observations of the average area of the spermatozoa head can be seen in Table 1.

This study found that the average head area of Simmental Bull spermatozoa was larger ($P<0.05$) than that of Limousin Bull sperm. The average head area of spermatozoa was found in 5-7-year-old bulls higher ($p<0.05$) than in 2-4-year-old and 8-10-year-old bulls. This study's findings indicate that spermatozoa's head area increased from 2-4 years to 5-7 years and decreased at 8-10 years. The highest head area of spermatozoa was found in 5-7-year-old Limousin Bull spermatozoa at 35.79 μm^2, and the lowest head area was found in 2-4-year-old Limousin Bull spermatozoa at 30.93 μm^2 (Table 1). The findings of this study are lower than those of (Jalius et al., 2023) Found the average spermatozoa head area of Simmental Bull was 41.96±11.38 μm^2, and the spermatozoa head area of Ongole Bull was 42.57±23.35 μm^2.

The spermatozoa head is one of the essential parameters in the assessment of spermatozoa morphometry. Sperm considered dead after cryopreservation showed a smaller head area than surviving spermatozoa, indicating that spermatozoa head morphometry could be a valuable tool to detect changes related to spermatozoa quality. (Garcia-Vazquez et al., 2016). Maroto-Morales et al., (2016) stated that the width of the sperm head can play an important role by making sperm more hydrodynamically efficient, which, in turn, can affect the fertilization ability of sperm. The sperm head also plays a role in spermatozoa transportation in the female reproductive tract; as stated by Garcia-Vazquez et al. (2016), spermatozoa with elongated heads may move faster in the female reproductive tract. Previous researchers have also confirmed the importance of spermatozoa head morphometry to fertilization success. (Mashiko et al., 2017). The findings of this study indicate that the head area of spermatozoa of Simmental and Limousin bull at different ages is still within the normal range. This refers to (Susilawati, 2014), who stated that minimum head area of the spermatozoa is 32 μm^2.

This study found that the head area of Simmental bull spermatozoa at 2-4 years old is smaller than 5-7 years old, the head area of Simmental cattle spermatozoa at the age of 5-7 years is more significant than 8-10 years old. The head area of Limousin bull spermatozoa at 2-4 years old is smaller than 5-7 years old, and the head area of Limousin bull spermatozoa at 5-7 years old is more significant than 8-10 years old. It is well known that the increasing age of bulls is associated with a significant decrease in semen quality parameters, including spermatozoa morphometry. (Abah et al., 2023). The increase in sperm head area is related to the development of reproductive organs, including the testes. Baharun et al., (2021) also confirmed that spermatozoa morphological quality decreases with increasing age of bulls. Sperm abnormalities increased in bulls collected between 21 and 30 months of age compared to the reference age (<20 months) (Felton-Taylor et al., 2020).

Another study by (Kipper et al., 2017) conducted research on three age groups of Nalore cattle consisting of young bulls (1.8-2 years), adult bulls (3.5-7 years), and old bulls (8-14.3 years), also reported the same results as this study, where there was an increase in morphometric characteristics of spermatozoa in young to adult bulls and decreased again at old age. It was also confirmed by Trevizan et al. (2018) that sperm quality decreases after more

Table 1. Average area of spermatozoa heads (μm^2).

A (Breeds)	B (Age)			Average
	B1	B2	B3	
A1	35.45	35.08	34.02	**34.85 ^{a}**
A2	30.93	35.79	31.80	**32.84 ^{b}**
Average	**33.19 ^{a}**	**35.43 ^{b}**	**32.91 ^{a}**	

Note: A1 (Simmental Bull), A2 (Limousin Bull), B1 (2-4 years), B2 (5-7 years), and B3 (8-10 years). ^{ab} Means within rows or columns with different superscript letters differ significantly at p<0.05.

than seven years. The decline in spermatozoa morphometry as males reach old age is associated with a decrease in testosterone hormone concentration in males. Furthermore, FSH and LH hormones affect spermatogenesis and sperm maturation. LH regulates testosterone synthesis in Leydig cells and aromatizes estradiol in Sertoli cells (Baharun et al., 2021). Dasrul et al. (2020) support the idea that high and low testosterone concentrations can affect the formation of sperm morphology (normal or abnormal). Increasing male age causes a decrease in testosterone hormone levels, causing physiological changes in the male reproductive tract and changes in spermatozoa morphometry.

This study found that spermatozoa head area on age 2-4 years of Simmental bull spermatozoa was more significant than that of Limousin bull spermatozoa, the head area on age 5-7 years of Simmental bull spermatozoa was smaller than Limousin bull spermatozoa, and the spermatozoa head area on age 8-10 years of Simmental bull was more significant than that of Limousin bull spermatozoa. Previous researchers have also reported differences in Bull breeds to impact spermatozoa morphometry. (Felton-Taylor et al., 2020). Attia et al., (2016) also reported varying morphological differences between male breeds. It is further explained that Bos indicus genetics shows that this genotype is more susceptible to abnormalities in spermatozoa morphology. (Felton-Taylor et al., 2020) When compared to other breeds. This susceptibility can be caused by these bulls in stressful environmental conditions. (Callaghan et al., 2016). Hapsari et al., (2022) Simmental cattle had better resistance than Limousin cattle to climatic conditions, evidenced by better semen quality in Simmental cattle. Stress is thought to cause a larger sperm head area in Simmental Bull than in Limousin Bull. It can be further explained that the decrease in testosterone production from Leydig cells after increased cortisol caused by stress reduces LH circulation. (Felton-Taylor et al., 2020). Decreased testosterone production disrupts the male reproductive organs, so semen production is not optimal.

The morphometry of the bull spermatozoa head has also been identified as being related to the acrosome status of the spermatozoa. This is especially true when the spermatozoa undergo the capacitation process. During this process, spermatozoa undergo physiological changes, which are fundamental prerequisites for acquiring functional sperm competence to undergo the acrosome reaction and thus fertilize the oocyte (Maroto-Morales et al., 2016). Disturbances or defects in the spermatozoa head result in disruption of the capacitation process, or even the spermatozoa cannot undergo the capacitation process, which will impact male fertility. This was also confirmed by Yániz et al. (2015) that the spermatozoa head consists of the nucleus, plasma membrane, and acrosome; the acrosome status is closely related to the fertility of the ejaculate, so various acrosome morphological defects must be considered about bull fertility.

Spermatozoa Tail Length

This study's findings indicate no interaction ($p>0.05$) between the Bull breed and age on the length of the spermatozoa tail. The results of observations of the average length of the spermatozoa tail can be seen in Table 2. This study found that the average tail of Simmental Bull spermatozoa was longer ($P<0.05$) than Limousin Bull spermatozoa. The average length of spermatozoa tail was found in bulls aged 5-7 years higher ($p<0.05$) than in bulls aged 2-4 years and 8-10 years. The findings of this study indicate that the length of the spermatozoa tail increased from 2-4 years to 5-7 years and decreased at the age of 8-10 years. The highest tail length of spermatozoa was found in spermatozoa of Simmental Bull aged 5-7 years at 49.28 µm and the lowest tail length was found in spermatozoa of Limousin Bull aged 8-10 years at 45.34 µm (Table 2).

Table 2. Average length of spermatozoa tail (µm).

A (Breeds)	B (Age)			Average
	B1	B2	B3	
A1	46.71	49.28	46.86	**47.62 ^{a}**
A2	46.19	46.52	45.34	**46.02 ^{b}**
Average	**46.45 ^{a}**	**47.90 ^{b}**	**46.10 ^{a}**	

Note: A1 (Simmental Bull), A2 (Limousin Bull), B1 (2-4 years), B2 (5-7 years), and B3 (8-10 years). ^{ab} Means within rows or columns with different superscript letters differ significantly at $p<0.05$.

This finding is lower than (Saini et al., (2018), who found the tail length of FH Bull spermatozoa ranged from 60.83-61.37 µm and the tail length of Murrah buffalo spermatozoa 55.72-56.33 µm and Kondracki et al., (2012) who researched FH, Simmental, Limousin, Piemontese, and Charolais Bull, found the tail length of spermatozoa ranged from 61.12-61.61 µm. This finding is not much different from the report by Jalius et al., (2023) Found that the average tail length of Simmental Bull spermatozoa was 47.68±6.45 µm and the tail length of Ongole Bull spermatozoa was 47.96±4.70 µm.

The absence of interaction between age and breed of bulls is thought to be because tail length tends to be the same, namely higher in Simmental cattle in all age groups, increasing from 2-4 years to 5-7 years and decreasing at 8-10 years in both bulls breeds. Flagella or spermatozoa tails are essential in the transportation process in the female livestock reproductive tract. The level of spermatozoa competition in the female reproductive organs is relevant to its dimensions, where spermatozoa with longer tails have been shown to have stronger flagella (Garcia-Vazquez et al., 2016). The morphometric condition of spermatozoa is one of the determining factors during spermatozoa migration through the female reproductive tract towards the fertilization site. (Hernández-Caravaca et al., 2015). The environment in the cervix and uterus is hostile to spermatozoa, spermatozoa must swim actively to move forward along the upper part of the female reproductive tract. The first type of spermatozoa to be excluded are probably spermatozoa with defects in the midpiece or tail that interfere with motility (Garcia-Vazquez et al., 2016). Factors that determine the speed of spermatozoa to the site of fertilization are the sperm swimming speed, the condition of the tail and the force generated by the relative size of the spermatozoa tail (Garcia-Vazquez et al., 2016). The findings of this study indicate that the tail length of spermatozoa of Simmental and Limousin cattle at different ages is still within the normal range. This refers to (Prastiya et al., 2021), who stated that the length of the spermatozoa tail ranges from 35–45 µm. Normal conditions are conditions in which spermatozoa can perform their functions optimally, which, if this size does not meet the standard, will impact the performance of spermatozoa in the female reproductive tract.

This study found that the tail length of Simmental bull spermatozoa at 2-4 years old is smaller than 5-7 years old, the tail length of Simmental cattle spermatozoa at the age of 5-7 years is more significant than 8-10 years old. The tail length of Limousin bull spermatozoa at 2-4 years old is smaller than 5-7 years old, and Limousin bull spermatozoa at 5-7 years old is more significant than 8-10 years old. Roy, (2014) also reported the same thing: there was a decrease in the quality of spermatozoa in old bulls. Baharun et al., (2021) state that primary spermatozoa abnormalities were higher in the group of bulls aged 8-10 years (Baharun et al., 2021). In addition, this increase is influenced by dysfunction or decreased performance of the seminiferous tubules, which play an essential role in spermatogenesis (Baharun et al., 2021). Dysfunction of the epididymis and accessory sex glands, which can affect sperm morphometry, decreased germ cell count and androgen levels, decreased ability to repair cell and tissue damage as a result of cellular and physiological changes, systemic and genital diseases associated with aging, and changes in male reproductive anatomy, such as decreased blood supply to the testes and narrowing of the seminiferous tubules (Halvaei et al., 2020), have been identified as causes of reduced sperm tail length when bulls are 8-10 years old. In addition, decreased sperm tail length with increasing age of bulls can also be caused by hormonal influences. Heinz et al., (2015) reported that adiponectin concentrations in bulls in the older group were significantly higher than in the younger group. In other words, as bulls age, adipose tissue is more susceptible to hypertrophy, which causes increased adiponectin concentrations (Yu et al., 2019). Adiponectin concentration is correlated with testosterone concentration, where increasing adiponectin concentration causes decreased testosterone concentration (Baharun et al., 2021), affecting spermatozoa formation in the male reproductive organs.

This study found that spermatozoa head area on age 2-4 years of Simmental bull spermatozoa was more significant than that of Limousin bull spermatozoa, the head area on age 5-7 years of Simmental bull spermatozoa was larger than Limousin bull spermatozoa, and the spermatozoa head area on age 8-10 years of Simmental bull was larger than that of Limousin bull spermatozoa. The difference in the length of the sperm tail between the two male breeds in this study is thought to be caused by the ability of the males to adapt to environmental conditions. (Felton-Taylor et al., 2020) Stated that changes in spermatozoa morphology are often found in summer, indicating that excessive heat may be a factor causing stress and affecting the hormonal status of males. The testes are very sensitive to high environmental temperatures, which cause degenerative changes characterized by a reduction in testicular size, consistency, and scrotal circumference (Perumal et al., 2017). Previous research by Hapsari et al., (2022) the scrotal circumference of Simmental and Limousin cattle decreased in the dry season. Still, Simmental cattle had a larger scrotal circumference than Limousin cattle in different climate conditions in Indonesia. Higher environmental temperature severely affects these seminiferous tubules, resulting in smaller testicular volume and size; thus, smaller scrotal circumference is observed in summer. (Perumal et al., 2017). Heat promotes the formation of reactive oxygen species that damage sperm DNA, and heat can inhibit the production of antioxidants that usually protect sperm from oxidative attack. (Morrell, 2020). The factor of adaptability to the environment between the two different breeds of Simmental and Limousin Bull is thought to cause the difference in the length of the sperm tails in these two breeds of bulls.

Spermatozoa Body Length

This study's findings indicate no interaction (p>0.05) between the Bull breed and age on the length of the spermatozoa body. The results of observations of the average length of the spermatozoa body can be seen in Table 3.

Table 3. Average length of spermatozoa body (µm).

A (Breeds)	B (Age)			Average
	B1	B2	B3	
A1	69.64	73.02	69.61	**70.75 [a]**
A2	68.79	69.36	68.38	**68.84 [b]**
Average	**69.22**	**71.19**	**69.00**	

Note: A1 (Simmental Bull), A2 (Limousin Bull), B1 (2-4 years), B2 (5-7 years), and B3 (8-10 years). [ab] Means within rows or columns with different superscript letters differ significantly at p<0.05.

This study found that the average body length of Simmental Bull spermatozoa was longer (P<0.05) than that of Limousin Bull spermatozoa. The average body length of spermatozoa was found in 5-7 year old males, which is higher than 2-4 year old and 8-10 year old males. This study's findings indicate that spermatozoa's body length increases from 2-4 years to 5-7 years and decreases at 8-10 years. The highest body length of spermatozoa was found in 5-7-year-old Simmental Bull spermatozoa at 73.02 µm, and the lowest body length was found in 8-10-year-old Limousin Bull spermatozoa at 68.38 µm (Table 3). This finding is not much different from the previous report by Kondracki et al., (2012), who researched FH, Simmental, Limousin, Piemontese, and Charolais Bull; they found the length of the spermatozoa body ranged from 70.79-71.27 µm and Jalius et al., (2023) who found the average length of the spermatozoa body of Simmental Bull was 70.92±6.78 µm and the length of the spermatozoa body of Ongole Bull was 71.28±5.42 µm.

The absence of interaction between age and breed of bulls is thought to be because body length tends to be the same, namely higher in Simmental cattle in all age groups and increasing from 2-4 years to 5-7 years and decreasing at 8-10 years in both bulls breeds. Sperm length has a positive correlation with the speed of spermatozoa transport. Sperm undergo selection within the female reproductive tract after both natural and AI mating, and it has been suggested that different spermatozoa morphometric dimensions are responsible for the ongoing adjustment of male fertility. (Garcia-Vazquez et al., 2016). The findings of this study indicate that the length of spermatozoa from Simmental and Limousin Bull from the survey was in the normal range, according to the statement of (Prastiya et al., 2021) that the overall length of animal spermatozoa ranges from 50–70 µm.

This study found that the body length of Simmental bull spermatozoa at 2-4 years old is smaller than 5-7 years old, and the body length of Simmental cattle spermatozoa at the age of 5-7 years is larger than 8-10 years old. The body length of Limousin bull spermatozoa at 2-4 years old is smaller than 5-7 years old, and Limousin bull spermatozoa at 5-7 years old is larger than 8-

10 years old. Studies involving older males (≥ 8 years) reported a decline in sperm quality from adult to old males (Carreira et al., 2017; Kipper et al., 2017; Trevizan et al., 2018). Changes in sperm morphology between young and adult Bulls signify sexual maturity and stability, while changes between adult and old Bulls are associated with disorders in the testes and epididymis (Carreira et al., 2017). Disorders of the testes and epididymis can be caused by a decrease in testosterone hormone concentration as the age of the Bull increases. Dasrul et al. (2020) stated that there is a correlation between testosterone concentration and semen quality (sperm morphology), as well as the report of Rajak et al. (2014) on Friesian Holstein×Tharparkar crossbred Bull. The effect of decreasing testosterone concentration on semen quality has also been reported by previous researchers that there is a correlation between increasing sperm abnormalities and decreasing testosterone and testosterone concentration decreases in Bull aged 8-10 years (Baharun et al., 2021). Low semen quality is associated with a lack of testosterone concentration (Gulia et al., 2010). Based on this, increasing the age of males is associated with decreasing testosterone levels, thereby reducing the length of the spermatozoa body.

This study found that spermatozoa body length on age 2-4 years of Simmental bull spermatozoa was more significant than that of Limousin bull spermatozoa, the body length on age 5-7 years of Simmental bull spermatozoa was larger than Limousin bull spermatozoa, and the spermatozoa body length on age 8-10 years of Simmental bull was larger than that of Limousin bull spermatozoa. The difference in sperm body length between Simmental and Limousin Bull is thought to be caused by the different adaptability of these two Bull breeds. Previous research by Chung et al., (2019) also reported that Jersey Bull showed better sperm quality compared to Limousin Bull, where this difference is thought to be influenced by the adaptability of the Bull. Furthermore, between Simmental and Limousin Bull, Nursita et al., (2022) The sweating rate was more significant in Limousin Bulls than in Simmental Bulls, where Limousin Bulls are more sensitive to increases in environmental temperature than Simmental. Heat stress is often exacerbated in tropical areas due to excessive humidity, which causes livestock to feel hotter than the actual temperature (Chung, et al., 2019). Heat stress affects fertility and inhibits the reproductive performance of animals through hormonal imbalance (Khan et al., 2023). Heat stress increases cortisol levels, ultimately reducing LH release (Fernandez-Novo et al., 2020). Heat stress primarily affects testosterone and LH levels, which were found to be decreased in males exposed to heat stress (Ngoula et al., 2020), leading to impaired spermatozoa production. In addition, differences in growth were also identified in these two bulls, where Simmental cows produced faster growth than Limousin cows. This difference is likely caused by genetic factors and differences in the availability of energy sources such as fructose, *glycerylphosphorylcholine* (GPC), and sorbitol, which tend to be higher in Simmental cows (Munawaroh et al., 2024). This difference also has an impact on the scrotal circumference where the scrotal circumference at the age of 365 days was found to be 32 cm in Simmental cows and 29 cm in Limousin cows (Brito, 2021), thus affecting the characteristics of the spermatozoa of the two bulls.

CONCLUSION

The difference in breed and age of bulls show different morphometric characteristics. Simmental bulls were found to have higher morphometric characteristics of spermatozoa than Limousin bulls, and morphometric characteristics increased from 2-4 years to 5-7 years and decreased again at 8-10 years.

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