

Original Research Paper

Relationship of Scrotal Circumference with Spermatozoa Production in Various Breed of Indonesian Local Bulls

¹Trinil Susilawati, ²Nisa'us Sholikah, ¹Sri Wahjuningsih,
³Enniek Herwiyanti, ¹Kuswati and ¹Aulia Puspita Anugra Yekti

¹Animal Science Faculty, Brawijaya University, Indonesia

²Animal Husbandry Faculty, Islam Malang University, Indonesia

³Artificial Insemination Centre of Singosari Malang East Java, Indonesia

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Corresponding Author:

Trinil Susilawati
Animal Science Faculty,
Brawijaya University
Malang, Indonesia
Email: tsusilawati@ub.ac.id

Abstract: Bulls for artificial insemination must qualify as a superior bull that has good genetic quality and semen production. Therefore, the selection process for bulls must be carried out accurately. The study aims to determine the relationship between scrotal circumference with semen production in the Ongole crossbred, Bali, Madura and Brahman bulls. The research materials were four breeds of bull Ongole crossbred, Bali, Madura and Brahman, which are each breed consist of ten bulls. The research methods were measuring scrotal circumference volume, spermatozoa concentration and individual motility of spermatozoa, Total Spermatozoa per ejaculate (TS) and Total Motile Spermatozoa per ejaculate (TMS). The observations performed as much as ten replications for each bull. Data were analyzed descriptively and statistically by using Analysis of Variance (ANOVA) with randomized group design. Furthermore, the relationship between scrotal circumferences with semen production was analyzed by using Pearson correlation and the effect of the scrotal circumference with semen production was calculated by linear regression. The results showed the breed of cattle influences the production of spermatozoa in Ongole crossbred, Bali, Madura and Brahman bulls. The largest scrotal circumference was the highest semen production in Brahman bull, 3.682 Cm with total spermatozoa 8.000,24+2.289 million/ml and total motile spermatozoa 4,667.60+2,289 million/ml. Ongole crossbred bull have a scrotum circumference of 34.50±4.80 cm with total spermatozoa 6,192.55±2,102.88 million/ejaculate and total motile spermatozoa 4,225.75±1,465.56 million/ejaculate. Bali bull scrotum circumference 32.50±2.65 cm, total spermatozoa 7,033.19±2,024.98 million/ejaculate and 4,908.36±1,494.30 total motile spermatozoa 4,908.36±1,494.30 million/ejaculate. Madura bull have a scrotum circumference of 33.88±2.17 cm, 5,349.24±2,183.97 million/ejaculate and the total motile spermatozoa are 3,460.48±1,414.20 million/ejaculate. In conclusion, scrotal circumferences of the bull have a contribution to the production of spermatozoa, where the bulls with the largest of scrotal circumferences have the highest semen production. Moreover, there is no correlation between scrotum circumferences with spermatozoa production.

Keywords: Scrotal Circumference, Ongole Crossbred Bull, Bali Bull, Madura Bull and Brahman Bull

Introduction

The success of the AI program depends on the fertility of the bull used to produce semen. Therefore, the superior bull used for the production of frozen semen should be strictly selected by looking at the genetic potential, disease conditions, reproductive health, libido

and sexual behavior and the potential for semen production (Shukla, 2011).

Currently, the best way to evaluate the breeding potential of bulls is to conduct a Bull Breeding Soundness Evaluation (BBSE) Supriatna (2004). BBSE requires that bulls must meet minimum standards in four categories, namely general physical or phenotypic,

reproductive examination, scrotal circumference indexed according to age and sperm motility and sperm morphology. Therefore, bulls must pass through all four categories to be classified as potential livestock (Alexander, 2008).

Susilawati *et al.* (2019), Sholikah *et al.* (2018a; 2018b) stated that Indonesian local cattle consisting of Madura, Bali, Ongole Crossbred. Those cattle are known to be able to adapt well to tropical temperatures and high humidity, ready to consume low-quality feed and resistant to ectoparasites and endoparasites. Therefore, Bali, Madura and Ongole Crossbred are suitable for cattle in the tropics. According to the opinion of Garner and Hafez (2008).

Genetic factors affect Semen production, so that semen production in different breeds will show differences as well. This is supported by the opinion of Akhter *et al.* (2013), differences in the volume of semen caused by different breeds, whereas according to Yates *et al.* (2003), bulls are a source of significant variation in the total ejaculation volume, concentration and motility of spermatozoa.

The size of the testes is following the size of the bull body, the testes consist of more than 90% of the seminiferous tubules, whereas Spermatozoa are formed in the seminiferous tubules, therefore the higher the testis, the more spermatozoa it produces (Susilawati, 2011).

The purpose of this study was to determine the relationship between scrotal circumference and spermatozoa production, which includes total spermatozoa and total motile spermatozoa.

Materials and Methods

Four breeds of bulls from Bali, Madura, Ongole crossbred and Brahman, which consist of ten bulls in each breed, were used in this research. The bulls aged were 3-14 years old. Bodyweight ranges from 400 to 900 kg, has a good record of spermatozoa production during the last three months before the study. The bulls were housed on Artificial insemination Singosari center on the individual cages with the temperature around 22-27°C.

The method used in this study was a field observation. Sampling was performed by purposive sampling. Semen was collected for 1-2 times in a week for 10 replications. In addition, laboratory observations was conducted by observing semen quality to calculate the spermatozoa production including total spermatozoa and total motile spermatozoa. The variables observed in

this study were scrotal circumference and semen quality including semen volume, spermatozoa concentration, spermatozoa motility to obtain total spermatozoa and motile spermatozoa.

Measurement variables consist of:

1. Scrotal circumference
Scrotal circumference is measured by pushing the two testicles to the bottom of the scrotum with the thumb on one side and the other finger on the other hand of the scrotum and then measure the circumference at the maximum point using a measuring tape in centimeters (Shukla, 2011).
2. Individual Motility
Individual motility is observed visually using a light microscope with a magnification of 400 times. Assessment of individual motility by observing forward-moving or progressive spermatozoa and expressed in percent (Ax *et al.*, 2008; Susilawati, 2013).
3. Concentration
A spectrophotometer is a machine calibrated at 550 nm to determine spermatozoa concentrations. Spermatozoa concentration was observed using a spectrophotometer by taking 0.02 cc of semen using a socorex pipette, then placed into a tube containing 5 ml of sodium citrate 2.9% at 10% formalin per liter and homogenized using a thermomixer. The mixture is then put into a cuvet tube and then the next figure is seen in the table so that the concentration of spermatozoa per cc of semen is known (Susilawati, 2013).
4. Spermatozoa Production
Spermatozoa production is obtained by counting the total spermatozoa and total motile spermatozoa. Total motile spermatozoa are calculated by multiplying the semen volume, concentration and percentage of individual motility (Susilawati, 2013).

Results

Spermatozoa production was obtained by observing the quality and production of spermatozoa. Spermatozoa production includes total spermatozoa and total motile spermatozoa. Data collected from observations of semen volume, spermatozoa concentration and motility of individual spermatozoa are available in Table 1.

Table 1: Average of semen volume, concentration and individual motility of Ongole crossbred, Bali, Madura and Brahman Bulls

Breed of cattle	Volume (ml)	Concentration (milion /ml)	Individual Motility (%)
Ongole Crossbred	4,58±1,29	1.370,90±347,38	68±5
Bali	5,40±1,04	1.317,95±310,50	70±4
Madura	5,54±1,69	1.022,53±454,79	65±6
Brahman	5,65±1,18	1.389,23±443,27	59±13

The volume of semen in all-breed was varied with a range of 4,58 ml to 5,65 mL per ejaculation of bull. The concentration of spermatozoa on Ongole Crossbred, Bali and Brahman Bulls was not significantly different from around 1.317,95 million/ml to 1.389,23 million/ml. While on Madura Bulls, the sperm concentration was lowest at 1.022,53 million/ml. Individual motility of sperm Madura, Bali and Ongole crossbred was varied with a range 59% to 70% with the lowest individual motility was Brahman Bulls.

Total spermatozoa are obtained by multiplying the volume of semen with the concentration of sperm (Susilawati, 2013). The average value of total spermatozoa can be seen in Table 2, while the mean value of scrotal circumference to total spermatozoa and total motile spermatozoa in various cattle breeds can be seen in Table 3.

The results of correlation analysis and regression results obtained, Ongole crossbred and Brahman cattle showed a positive correlation between scrotal circumference with total spermatozoa, whereas in Bali and Madura cattle showed a negative correlation. The relationship between scrotal circumference and total spermatozoa in all cattle breeds was not significant ($P >$

0.05). The highest correlation coefficient (r) in Madura cattle is 0.58, while the lowest in Brahman cattle is 0.00. The relationship between scrotal circumference with total spermatozoa can be seen in Table 4.

In addition, The correlation coefficient (r) in Bali cattle is low at 0.34 with a coefficient of determination of 11.56%, but the correlation between scrotal circumference and total spermatozoa is not significant ($P > 0.05$). The form of relationship in Bali cattle is illustrated through the regression equation $y = 12077 - 220,6x$. No significant correlation between scrotal circumference and total spermatozoa ($P > 0.05$) was also found in Madura and Brahman cattle. In Madura cattle, the correlation coefficient is the same low as Bali cattle, which is 0.34 with a coefficient of determination of 11.56%, the form of the relationship can be seen in the regression equation $y = 12056 - 254x$. The correlation coefficient (r) in Brahman cattle is classified as moderate, namely 0.41, with a coefficient of determination of 16.81% and the regression equation obtained $y = 52141 - 1319x$. The results of correlation analysis and regression between scrotal circumference with total motile spermatozoa can be seen in Table 5.

Table 2: Average Total Spermatozoa in Ongole Crossbred, Bali, Madura and Brahman bull

Breed of cattle	Total Spermatozoa (milion/ejaculate)	Total Spermatozoa Motil (milion/ejaculate)
Ongole Crossbred	6.192,55±2.102,88 ^{ab}	4.225,75±1.465,56 ^b
Bali	7.033,19±2.024,98 ^{bc}	4.908,36±1.494,30 ^b
Madura	5.349,24±2.183,97 ^a	3.460,48±1.414,20 ^a
Brahman	8.000,24±2289,33 ^c	4667,60±2289,33 ^b

Note: different notations in the same column show very significant differences ($P < 0.01$)

Table 3: Scrotum circumference of total spermatozoa and total motile spermatozoa in the ongole crossbred, bali, madura and brahman bulls

Breed of Cattle	Scrotum Circumference (cm)	Total Spermatozoa (milion/ejaculat)	Total Motile Spermatozoa (milion/ejaculat)
Ongole Crossbred	34,50±4,80	6.192,55±2.102,88	4.225,75±1.465,56
Bali	32,50±2,65	7.033,19±2.024,98	4.908,36±1.494,30
Madura	33,88±2,17	5.349,24±2.183,97	3.460,48±1.414,20
Brahman	36,00±0,82	8.000,24±2289,33	4.667,60±2289,33

Table 4: Analysis of the relationship between scrotal circumference and total spermatozoa

Breed of cattle	r	R ²	Regression Equation	Information
Ongole Crossbred	0,18	0,0324	$y = 3275 + 84,6x$	$P > 0,05$
Bali	-0,38	0,1444	$y = 17941 - 336x$	$P > 0,05$
Madura	-0,58	0,3364	$y = 20061 - 434x$	$P > 0,05$
Brahman	0,00	0,00	$y = 7909 + 3x$	$P > 0,05$

Table 5: Analysis of the Relationship between Scrotal circumference and Total Motile Spermatozoa

Breed of Cattle	R	R ²	Regression Equation	Information
Ongole Crossbred	0,10	0,01	$y = 3090 + 32,9x$	$P > 0,05$
Bali	-0,34	0,1156	$y = 12077 - 220,6x$	$P > 0,05$
Madura	-0,34	0,1156	$y = 12056 - 254x$	$P > 0,05$
Brahman	-0,41	0,1681	$y = 52141 - 1319x$	$P > 0,05$

Discussion

Spermatozoa Production

The volume of semen in all-breed is classified as normal according to the results of research by Barrios *et al.* (2005) which states the volume of semen each collection varies with a range of 1-15 ml or 5-8 ml per ejaculation of bull. Spermatozoa concentration varies according to the opinion of Lemma and Shemsu (2015) Garner and Hafez (2008) that there are variations in the concentration of beef cattle between 1000-1800 million spermatozoa per milliliter. While the percentage of individual motility in the Madua and Brahman cattle is low and varied, while in Ongole crossbred and Bali, they are classified as good and can be processed. The results of the study Kusumawati *et al.* (2019) Quality of Ongole crossbred cattle semen with ten replications produced a volume of 7+0 mL, concentration $1.758+137,66 \times 10^6$ /mL and Progressive motility 70+0%. While the results of the study of Muhammad *et al.* (2019) in filial Ongole or Ongole Crossbred cattle volume 4+1.41 mL, concentration $1.245+184,30 \times 10^6$ /mL and individual motility 71,25+2,5%. Ratnawati *et al.* (2018) resulted in Ongole crossbred, Bali and Madura cattle for semen volume was 4.7+1.9 mL, 3,4+0,7 mL and 5,7+0,5 mL respectively. While, concentration was $1.286+230 \times 10^6$ /mL, $1.228+265,2 \times 10^6$ /mL and $1.076,0+127,6 \times 10^6$ respectively. In addition, progressive motility was 71,0+2.2%, 71,0+2,2% and 66,0+6,5% respectively.

The results of the analysis of variance showed that the total spermatozoa in the various breed of cattle gave a very significant difference ($P < 0.01$). It is consistent with Garner and Hafez (2008) that differences between breeds affect the quality of semen produced. Fuerst *et al.* (2004) explained that livestock handling officers have a significant effect on semen volume and total spermatozoa in each ejaculation. Research results by Ratnawati *et al.* (2018) Total motile spermatozoa in cattle of Ongole Crossbred, Bali and Madura are $4.349,0+2.139,7 \times 10^6$ /ml, $2.979+947,7 \times 10^6$ /ml and $4.020,9+758,9 \times 10^6$ /ml. Muhammad *et al.* (2019) revealed that the total motile spermatozoa in Ongole crossbred is $886.75+129,85 \times 10^6$ /ml. The results of research using CASA by Susilawati *et al.* (2018) Bali ejaculated semen and given diluter Tris Aminomethan Egg yolk are Motility 95.8+35% and Progressive motility 79.3+4.31%.

The results of the analysis of variance showed that the total motile spermatozoa of all breed cattle gave a very significant difference ($P < 0.01$) in Madura cattle. In contrast, the Ongole crossbred, Bali and Brahman bull showed insignificant differences ($P > 0.05$). Yates *et al.* (2003) explained that the breed is a source of significant

variation in the total ejaculation volume, concentration and motility of spermatozoa. In addition, according to total spermatozoa, The lowest total motile spermatozoa value was found in the Madura cattle breed with an average of $3,460.48 \pm 1,414.20$ million/ejaculate, Brahman cattle have a relatively high total motile spermatozoa of 4667.60 ± 2289.33 million/ejaculate. The size of the scrotal circumference likely influences this in Brahman cattle, which tends to be abundant among other cattle breeds, namely 36.00 ± 0.82 cm. The large scrotal circumference has more seminiferous tubules so that it will increase the number of spermatozoa produced. Besides that, it is supported by seminal plasma, which is also more numerous.

Relationship between Scrotal Circle and Spermatozoa Production

The scrotal circumference can indicate the ability of a bull to produce semen. The lowest scrotal circumference was Madura Bull, which is 32.50 ± 2.65 cm. Scrotum circumference in all Bull is classified as a good category according to SNI 7651 (Indonesian national standards) for local cattle breed requirements, the size of the scrotum must be at least 25 cm. According to Shukla (2011), bulls are included in the good category if they have a scrotal circumference > 34 cm at the age of 30 months more based on world standards. Therefore, the local cattle scrotal circumference is smaller than the norm for world Breeding Soundness Evaluation (BSE), because the dimensions of the body, as well as the quantitative measure of local cattle's body weight, is much smaller. The difference in scrotal circumference size is influenced by several factors, including breed, body weight, age and environment. According to Perumal (2014), scrotal circumference is an essential factor in determining the selection criteria for bulls, because it can describe the size of the testis. Westhuizen *et al.* (2002) states that the bulls aged 2-3 years experienced rapid development of reproductive organs and to increase the amount Leydig cells are organized and perfect, due to the influence of the hormone testosterone, causing the size of the testes to enlarge.

Brahman bulls, which have the largest scrotal circumference size have the highest total spermatozoa and total motile spermatozoa compared to other breeds. It can illustrate that the higher the size of the scrotal circumference, the more spermatozoa produced. This is consistent with the statement of Yekti *et al.* (2017) that testicular size correlates with semen production, the higher the size of the testis, then more semen production, this is because more than 90% of the contents of the testes are seminiferous tubules which are spermatozoa production sites.

Based on the results of correlation analysis, it is known that scrotal circumference has a positive correlation with total motile spermatozoa in Ongole crossbred cattle. In contrast, a negative correlation is found in Bali, Madura and Brahman cattle. In Ongole Crossbred cattle obtained a meager correlation value of 0.10 with a coefficient of determination of 1%, which can be interpreted that the total motile spermatozoa are affected by the scrotal circumference of 1% other factors influence the rest. If there is an increase in the scrotal circumference of one unit, it will increase the total motile spermatozoa in Ongole crossbred cattle by 32.9 plus the constant 3090.

The results showed scrotal circumference did not significantly influence spermatozoa production, allegedly because the measurement was only done once when the Bull was not in its infancy. In contrast, many other factors influenced collection the semen for ten replications. Shukla (2011) explains that scrotal circumference, testicular size and production have a high correlation, especially in young bulls.

Conclusion

Based on the results of this study concluded that:

1. Breed of cattle influences the production of spermatozoa in Ongole crossbred, Bali, Madura and Brahman bulls
2. Furthermore, the Bull, which has the largest scrotum circumference, produced the highest of semen
3. There is no correlation between scrotal circumference with spermatozoa production, including total spermatozoa per ejaculate and total motile spermatozoa per ejaculation

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Author's Contributions

Trinil Susilawati and Sri Wahjuningsih: Designed and coordinated the experiment.

Nisa'us Sholikhah: Performed the experiment.

Kuswati: Analyzed the data.

Enniek Herwiyanti: Bulls maintenance management.

Aulia Puspita Anugra Yekti: Analyzed and drafted the manuscript.

Ethics

This research and treatment were approved by the Brawijaya University with the number 042-KEP-UB-2020.

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