Jurnal Sain Peternakan Indonesia
Available at https://ejournal.unib.ac.id/index.php/jspi/index
DOI: https://doi.org/10.31186/jspi.id.17.3.159-169

P-ISSN 1978-3000
E-ISSN 2528-7109
Volume 17 Issue 3 July-September 2022

Relationship between Scrotal Circumference and Quality of Semen Production in Bulls: A Meta-Analysis Review

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ABSTRACT

This meta-analysis review was conducted to determine the relationship between scrotal circumference and semen production quality in bulls. The research method includes article search, selection, and assessment stages based on the specified inclusion criteria. The results of the selection and assessment of articles obtained 38 articles for meta-analysis. Data analysis was performed using the CMA program using a random-effects model to estimate the correlation coefficient. The results of the meta-analysis showed that overall scrotal circumference was significantly correlated ($p < 0.05$) with semen volume ($r = 0.441$), semen concentration ($r = 0.328$), and spermatozoa motility ($r = 0.411$). The relationship between scrotal circumference and semen quality was positive in the moderate category. Analysis based on the variation of bull breeds showed that the relationship between scrotal circumference and semen volume and concentration was not significantly different in *Bos taurus*, *Bos indicus*, and *Bos sondaicus* cattle ($p > 0.05$). Scrotal circumference and spermatozoa motility were significantly different in the *Bos taurus*, *Bos indicus*, and *Bos sondaicus* cattle ($p < 0.05$). This meta-analysis confirms that scrotal circumference can be a parameter in determining superior bulls because the scrotal circumference is positively related to the quality of semen production.

Keywords: meta-analysis, scrotal circumference, semen concentration, semen volume, spermatozoa motility

INTRODUCTION

The superior bulls selection is one of the efforts to improve the quality of cattle through natural mating succession and artificial insemination technology (Muthiapriani *et al.*, 2019). Cattle productivity as superior bulls can be considered through their reproductive potential and production performance (Dakhlan *et al.*, 2021). Superior bulls must have genetic potential, a history of disease and health, and sexual behavior in good condition. Through BBSE (Bull Breeding Soundness Evaluation), superior bulls' requirements are the scrotal circumference index based on age and sperm quality (Susilawati *et al.*, 2020).

Scrotal circumference is a physical parameter that is easily measured and known to select superior bulls. The bull reproduction potential through the quantity and quality of semen produced is related to the scrotal circumference size (Anwar & Jiyanto, 2019; Jiyanto & Anwar, 2019). Saputra *et al.* (2017) reported that scrotal circumference was significantly correlated with semen volume and concentration and spermatozoa motility. The scrotal circumference size reflects the testes size in cattle. Tissue development in the seminiferous tubules increased in line with the development of the scrotal circumference, followed by the absolute number of Leydig cells and Sertoli cells (Penitente-Filho *et al.*, 2018).

The semen production quality can be evaluated macroscopically or microscopically. Mahdi *et al.* (2021) stated that three essential parameters must be considered: the volume and concentration of semen and the motility of spermatozoa. Semen quality reflects the health condition of the seminiferous tubules, epididymis, and accessory gland systems (Jain *et al.*, 2008). Furthermore, the seminiferous tubules account for about 90% of the testes (Susilawati *et al.*, 2020). Therefore, testicular biometry, including scrotal circumference, is one of the indicators to see the reproductive potential of bulls.

Many studies investigating the relationship between scrotal circumference and semen quality have been carried out on livestock, including bulls (Gopinathan *et al.*, 2018; Indriastuti *et al.*, 2020; Rashid *et al.*, 2015; Viu *et al.*, 2015). Mahendra *et al.* (2022) reported that scrotal circumference had a low correlation with volume and motility but a moderate correlation with the semen concentration of *Bos taurus* cattle. In contrast to the *Bos indicus* cattle reported by Perez-Osario *et al*. (2016), the correlation coefficient is almost perfect. Mahdi *et al*. (2021) revealed a moderate correlation between scrotal circumference and semen concentration, and sperm motility of *Bos sondaicus* cattle. Likewise, Saputra et al. (2017) showed a moderate correlation between scrotal circumference and semen concentration, and volume of *Bos sondaicus* cattle. The correlation results indicate variations in the relationship between scrotal circumference and bull semen production quality. Therefore, this study was conducted through a meta-analysis study to determine the correlation coefficient and significance between scrotal circumference and overall semen production quality. Furthermore, previous studies' moderator analysis of each breed of cattle revealed differences in these correlations.

METHOD

A meta-analysis study was conducted to examine the results of studies related to the relationship between scrotal circumference and the quality of semen production (volume, concentration, and motility). The study was conducted on articles published in the last 15 years in the Google Scholar database in April 2022. The keywords used were "correlation" "scrotal circumference" "semen" and "bulls" both in English and Indonesian, yielding 2,723 search results. The selection and assessment of articles are carried out as shown in Figure 1.

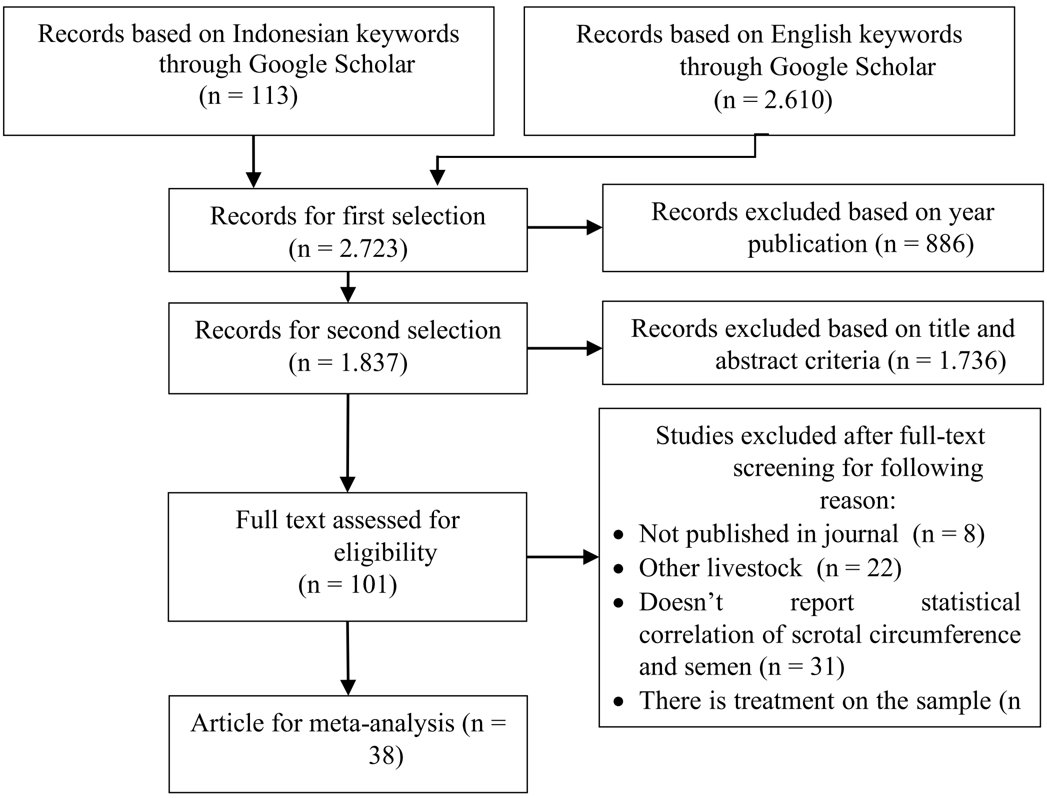

Figure 1. Selection and assessment of articles

The initial selection stage was carried out in two stages. The first selection stage was to remove searches based on year criteria, leaving 1,837 articles. The second selection stage reviewed the title and abstract containing all the search keywords, leaving 101 articles. The following assessment criteria: 1) correlational studies and no treatment between samples, 2) conducted on bulls, 3) measuring the scrotal circumference and semen quality on volume, concentration, and motility parameters, 4) reporting statistical values of scrotal circumference correlation and semen quality that are sufficient for significance testing, and 5) published on the journal. The final results showed that 38 articles met the criteria for the meta-analysis process.

The meta-analysis was carried out using the CMA (Comprehensive Meta-Analysis) program. The calculation of the effect size uses the correlation coefficient in each study. The heterogeneity test was performed first to determine the meta-analysis model. The overall effect size significance test was performed with 95% confidence intervals. The final analysis was carried out based on the type of cattle using a moderator analysis (Fernández-Espínola *et al.*, 2020; Saiphoo *et al.*, 2020).

RESULTS AND DISCUSSION

Test of heterogeneity between studies

The heterogeneity test was conducted to determine the model in the meta-analysis to be used. Table 1. shows the heterogeneity test results of the analyzed studies.

Table 1. Test results for heterogeneity between studies

Relationship	Q	df	p-value	I-squared
SC – Vol	159,598	25	0,000	84,34
SC – Con	377,308	24	0,000	93,64
SC – Mot	1.639,037	28	0,000	98,29

SC: scrotal circumference, Vol: semen volume, Con: semen concentration, Mot: spermatozoa motility

Based on Table 1, the analyzed studies were heterogeneous regarding the relationship between the scrotal circumference and the volume, concentration, and motility ($p < 0.05$). Heterogeneity between studies is high, 84.34% - 98.29%. Thus, a random-effect model was used to estimate the overall effect size. Heterogeneity between studies has the potential to be investigated in the analysis of moderator variables. In this study, the moderator variable investigated was the type of cattle: *Bos taurus*, *Bos indicus*, and *Bos sondaicus*.

Test of overall correlation coefficient significance

The overall correlation coefficient significance test results using the random-effects model are shown in Table 2.

Table 2. Overall estimation results with random effects

Relationship	N	r	Confidence Intervals 95%	Test of Hypothesis	
				Z-value	p-value
SC – Vol	26	0,442	0,281 – 0,579	5,007	0,000
SC - Con	25	0,328	0,192 – 0,453	4,543	0,000
SC - Mot	29	0,411	0,271 – 0,534	5,405	0,000

Based on Table 2, the correlation coefficient between the two variables manifests the overall effect size. All correlation coefficient values are within the moderate category and indicate a significant relationship ($p < 0.05$).

Moderator analysis by bull type

The moderator analysis aims to determine whether there is a difference in the relationship between scrotal circumference and semen quality for each bull type, as shown in Table 3.

Table 3. Correlation coefficient analysis on each bull type as moderator

Relationship	Type	N	r	Confidence interval 95%	Test of Heterogeneity	
					Q	p-value
SC – Vol	*Bos taurus*	17	0,416	0,224 – 0,578	2,243	0,326
	Bos indicus	6	0,741	0,287 – 0,923		
	Bos sondaicus	3	0,374	-0,049 – 0,683		
SC – Con	*Bos taurus*	15	0,282	0,125 – 0,425	2,070	0,355
	Bos indicus	7	0,592	-0,012 – 0,879		
	Bos sondaicus	3	0,469	0,133 – 0,708		
SC - Mot	*Bos taurus*	10	0,198	-0,227 – 0,560	6,590	0,037
	Bos indicus	16	0,550	0,387 – 0,680		
	Bos sondaicus	3	0,196	-0,091 – 0,453		

Based on Table 3, the relationship between scrotal circumference and semen volume and concentration was not significantly different in the three types of cattle ($p>0.05$).

Meanwhile, the relationship between scrotal circumference and spermatozoa motility was significantly different ($p < 0.05$), showing heterogeneous results in the three bull types.

Relationship between scrotal circumference and semen volume

The overall correlation coefficient results of the scrotal circumference and semen volume are in the moderate category ($r = 0,442$; $p < 0,05$). Dakhlan *et al.* (2021) and Gopinathan *et al.* (2018) reported a moderate positive relationship between scrotal circumference and semen volume. In contrast to Indriastuti et al. (2020), which found a negative correlation. Perez-Osario et al. (2016) revealed that bulls with large scrotal circumferences would produce more semen volume in healthy conditions. Figure 2 shows the correlation coefficients of the analyzed studies grouped by bull type.

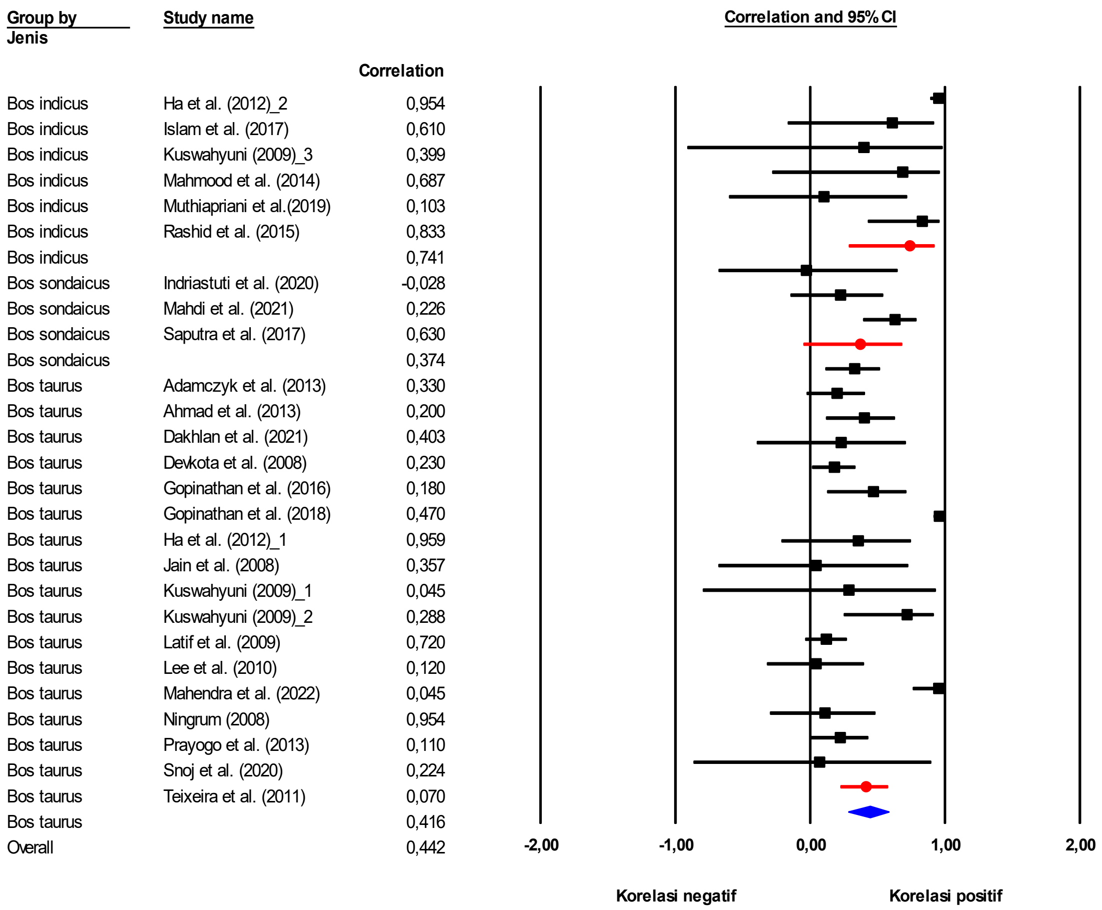

Figure 2. Forest plot relationship between scrotal circumference and semen volume

The scrotal circumference size of bulls is directly related to the testis's size. Khairi (2016) revealed that the scrotal circumference size indicates the number of seminiferous tubules, affecting the number of sperm and seminal plasma produced. These two components are directly related to semen volume production. An increase in scrotal circumference is consistently associated with an increase in the total mass of sperm-producing tissue (Kushwaha et al., 2018). In addition, the volume of semen is also influenced by the fluid secreted by the sex glands outside the testes (Heinrich & DeFalco, 2020).

Previous studies have shown a relationship between scrotal circumference and the level of testosterone produced (Dasrul et al., 2020; Santoso et al., 2021). Testosterone is a hormone that plays a role in the process of spermatogenesis. Anwar & Jiyanto (2019) revealed that the rate of testosterone synthesis is influenced by the developmental activity of the testicular structure. Scrotal circumference is related to testicular development and indirectly associated with the absolute number of Sertoli cells (Filho et al., 2018). Therefore, the results of this study are in line with the criteria for determining the superior bulls that the scrotal circumference size is related to the semen production quality parameter in the form of volume.

Based on Figure 2 different bull types have different correlation coefficients. However, with a 95% confidence interval, the results were not significantly different (homogeneous; p > 0.05). This result could be due to the dissimilarity in the number of studies reporting for each type of cattle and the relatively small number of samples used. Semen volume in each bull type is not only influenced by the scrotal circumference (Mota et al., 2019), but there are external factors that also contribute. Snoj et al. (2020) show that environmental management, including season/climate, pollution, and stress affect livestock fertility. Furthermore, feeding determines the semen production quality in bulls (Muthiapriani et al., 2019). The addition of age and body weight of bulls is directly related to the testes, affecting the semen volume (Jain et al., 2008; Putri et al., 2019).

Relationship between scrotal circumference and semen concentration

The overall correlation coefficient results of the scrotal circumference and semen volume are in the moderate category (r = 0,328; p < 0,05). This result is in line with Devkota (2008), Adamczyk *et al.* (2013), Prayogo *et al.* (2013), Muthiapriani *et al.* (2019), and Mahendra *et al.* (2022), who reported that there was a positive relationship between scrotal circumference and semen concentration with moderate category. However, unlike the research of Teixeira et al. (2011), Gopinathan et al. (2016), and Indriastuti et al. (2020), which found a negative relationship between the two variables. According to Indriastuti et al. (2020), a low negative correlation may be influenced by many factors, such as the potential for spermatozoa output, which depends on the function of the total testicular mass (germ cell activity). In addition, sperm production may not only be affected by the scrotal circumference but also by the testicular shape. Bailey et al. (1996) found that testicular shape should be considered to predict sperm production. Figure 3 shows the correlation coefficients of the analyzed studies grouped by bull types.

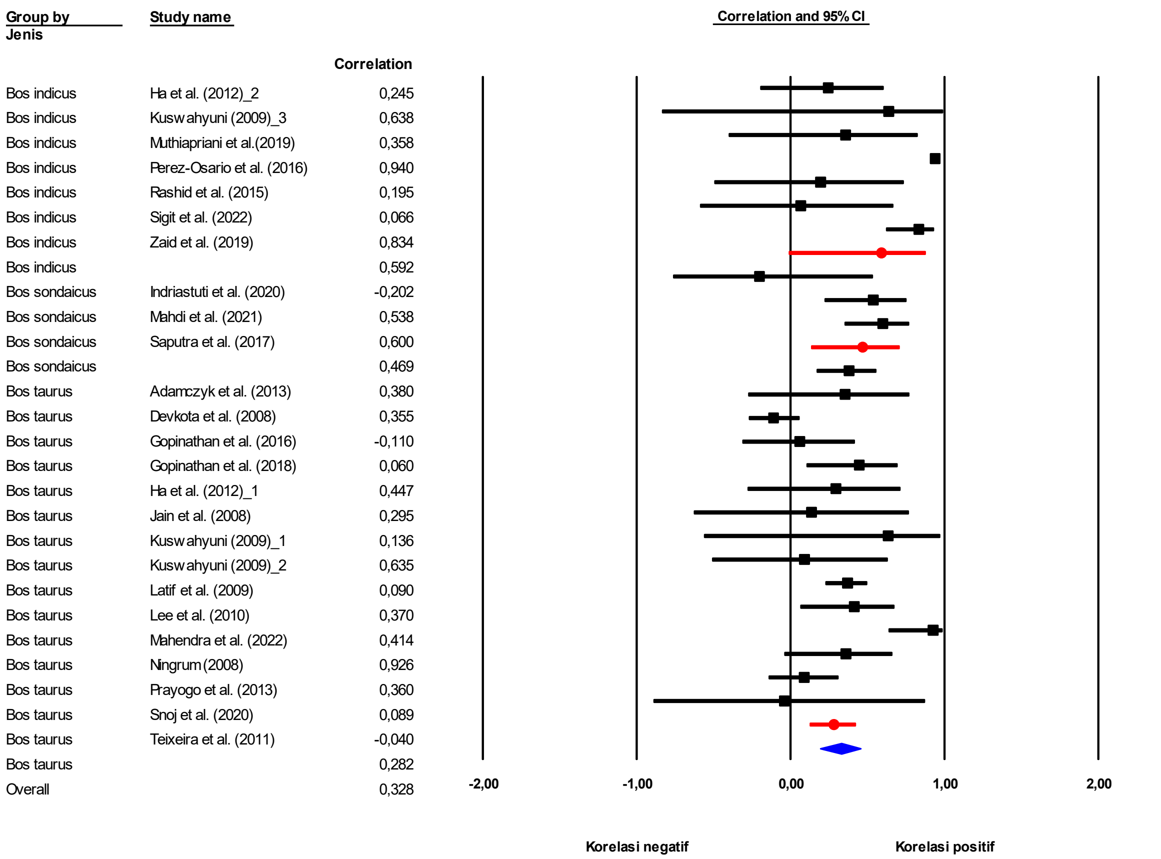

Figure 3. Forest plot relationship between scrotal circumference and semen concentration

Scrotal circumference reflects the size of the testes. As many as 80% of the weight of the testes are seminiferous tubules that function to produce spermatozoa (Saputra et al., 2017). This affects the number of spermatozoa produced in one mL of ejaculate. Dasrul et al.(2020) explained that increasing the size of the scrotal circumference increases the testosterone hormone produced by the testes. Anwar & Jianto (2019) confirmed a positive correlation between testosterone and scrotal circumference. Testosterone plays an essential role in spermatogenesis, both in spermatozoa formation and accessory gland fluid production. High testosterone can increase semen concentration production (Azzahra *et al*., 2016). Therefore, the results of this study are in line with the criteria for determining the superior bulls that the scrotal circumference is related to the semen production quality parameter in the form of concentration.

Based on Figure 3, different bull types have different correlation coefficients. However, with a 95% confidence interval, the results were not significantly different (homogeneous; $p > 0.05$). This result could be due to the dissimilarity in the number of studies reporting for each type of cattle and the relatively small number of samples used. Novianti et al. (2020) stated that the type of livestock affected the semen concentration of bulls. This difference in results is made possible by the difference in libido factors possessed by each breed of cattle. Bali cattle have more aggressive characteristics when compared to Limousin and Simmental cattle. According to Saputra et al. (2017), semen quality is influenced by male sexual libido. Repeated stimulation can increase the gonadotropin hormone and induce the testosterone hormone for optimum spermatogenesis. High testosterone can increase the semen concentration.

Susilawati et al. (2020) suggested that the relationship between scrotal circumference and spermatozoa concentration was also influenced by age. In the research of Kuswahyuni et al. (2009), the bulls used have a close age range, namely Limousin and Simmental (*Bos taurus*) 3 years while Brahman (*Bos indicus*) 2.5 years. Limousin and Simmental have reached puberty in this age range, while Brahman is still growing towards adulthood. Thus, it is suspected that after reaching puberty, Brahman can produce higher semen than Limousin and Simmental males. Sugeng (2001) also states that European cattle (*Bos taurus*) reach maturity at three years while tropical cattle (*Bos indicus*) get four years.

Relationship between scrotal circumference and spermatozoa motility

The overall correlation coefficient results of the scrotal circumference and spermatozoa motility are in the moderate category ($r = 0.411$; $p < 0.05$). This result is in line with the Devkota et al. (2008), Garmyn et al. (2011), Prayogo et al. (2013), Muthiapriani et al. (2019), and Mahdi et al. (2021). They reported a moderate positive relationship between scrotal circumference and spermatozoa motility. In contrast to Jain et al. (2008), Gopinathan et al. (2016), Gopinathan et al. (2018), and Susilawati et al. (2020) found a negative relationship between the two variables. Ismaya (2014) revealed that scrotal circumference, testicular weight, production, and fresh semen quality were positively related. However, it becomes negative if there is fat accumulation in the scrotal area, so testicular weight, production, and quality decrease (Mahendra et al., 2022). Figure 4. shows the correlation coefficients of the analyzed studies grouped by cattle breed.

Muthiapriani et al. (2019) stated that concentration, normal motility percentage, and spermatozoa morphology were affected by scrotal circumference. The large size of the testes assumes more seminiferous tubules to produce more sperm and seminal plasma. The increase in the motility of individual fresh semen spermatozoa is strongly related to seminal plasma, which functions as an energy source. The energy used for the motility of spermatozoa comes from the breakdown of Adenosine Triphosphate (ATP) in the mitochondria through its breakdown reaction into Adenosine Diphosphate (ADP) and Adenosine Monophosphate (AMP). The energy produced will be used as cell movement (mechanical energy) or as biosynthesis (chemical energy) (Khairi, 2016). In semen, fructose, sorbitol, glyceryl phosphorylcholine (GPC), and plasmalogen can be used for the survival and motility of spermatozoa (Toelihere, 1993).

Based on Figure 3, each type of cattle has a significantly different correlation coefficient (heterogeneous; $p < 0.05$). The average correlation in *Bos indicus* cattle was highest at 0.550 (high criteria), followed by Bos taurus and Bos sondaicus cattle, respectively, at 0.198 and 0.196 (low category). This result is presumably because the adaptability of *Bos indicus* cattle is better than other types of cattle. Good adaptability in livestock will affect the quality of semen. Brito et al. (2004) stated that the morphology of the TVC (testicular vascular cone) of *Bos indicus* larger than *Bos taurus* contributes to the resistance of cattle to environmental temperature and affects the ability of the testes to thermoregulate so that it affects the quality and production of sperm in bulls. Mussabekov et al. (2016) research show that national differences and characteristics between individuals directly affect semen quality.

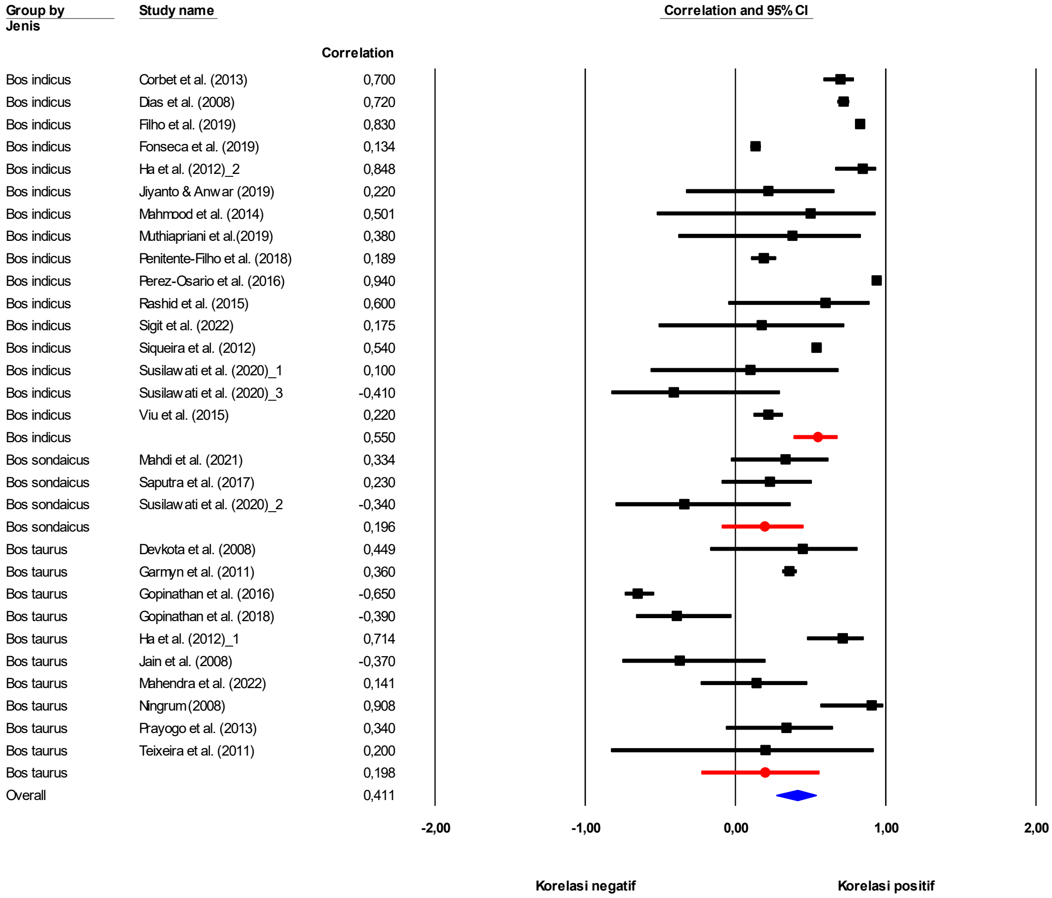

Figure 4. Forest plot relationship between scrotal circumference and spermatozoa motility

In addition to being affected by the temperature in the testes, sperm motility is also influenced by environmental conditions with low temperatures and high rainfall, changes in seasons, and duration of irradiation can inhibit FSH production and the process of spermatogenesis (Khairi, 2016). In addition, the length of treatment time after ejaculation also affects the motility of spermatozoa. This result is reinforced by Ismaya (2014) opinion that the first ejaculation after a long rest causes many spermatozoa cells to die and reduces the percentage of spermatozoa motility. Brito (2006) added that feeding with low nutritional content can decrease testosterone production. Azzahra et al., (2016) explained that the hormone testosterone plays a role in seminal plasma secretion produced by the accessory glands.

The results of this study indicate that scrotal circumference is a physical parameter that can be used to determine superior bulls. The scrotal circumference positively correlates with the semen production quality in all cattle breeds. However, this meta-analysis involved only 38 articles based on strict selection and assessment according to established inclusion criteria. Some studies could not be evaluated due to the limited information presented and methodological differences between studies. In addition, the moderator analysis in this study was only based on the type of cow. Therefore, meta-analysis studies need to be carried out with more varied inclusion criteria and moderators.

CONCLUSION

Based on the random-size effect calculation results, it shows that overall there is a positive relationship between scrotal circumference and semen volume, semen concentration, and spermatozoa motility in the moderate category. The scrotal circumference and semen volume and concentration were not significantly different in the *Bos taurus*, *Bos indicus*, and *Bos sondaicus* cattle breeds ($p > 0.05$). The scrotal circumference and spermatozoa motility were significantly different in the *Bos taurus*, *Bos indicus*, and *Bos sondaicus* cattle breeds ($p < 0.05$). This meta-analysis confirms that scrotal circumference can be a parameter in determining superior bulls because the scrotal circumference is positively related to the quality of semen production.

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