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Relationship between serum testosterone levels and morphometric indices in Ongole bulls

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Abstract
The study evaluated the relationship between serum testosterone concentration and morphometric traits in Ongole breeding bulls. Six breeding bulls were included in the study. Serum testosterone (nmol/L) was quantified *via* ELISA, while scrotal circumference, body girth and body length were measured; body weight was derived using Shaffer's formula. Testosterone demonstrated statistically significant correlations with age (r=0.918) and scrotal circumference (r=0.915), while body girth (r=0.622) length (r=0.586) and calculated body weight (r=0.566) showed non-significant moderate trends. With regression analysis it was elucidated that age (R^2=0.843) and scrotal circumference (R^2=0.837) independently accounted for the variation in testosterone while scrotal circumference emerged to be a more practical selection trait. Inversely, scrotal circumference depends on the testosterone concentration, establishing bi-directional relationship. The findings suggest that scrotal circumference might be considered as a useful morphometric biomarker for testosterone, thereby for evaluating the endocrine and reproductive potential of breeding bulls.

Keywords: Ongole bulls, testosterone, scrotal circumference, morphometry, fertility

1. Introduction

Reproductive efficiency of breeding bulls dictates the fertility soundness of the cattle herd. Semen quality is critical for cryopreservation (Sai Prasanna *et al.*, 2025) [1] and for breed improvement through artificial insemination in cattle and buffalo breeds (Praveen *et al.*, 2024) [2]. Testosterone regulates the semen quality (Budhiyadnya *et al.*, 2012) [3] and reproductive growth in males (Carreau *et al.*, 2011) [4]. It helps in skeletal growth and metabolic health in bulls (Surabhi *et al.*, 2022, Archana Sagarika *et al.*, 2025) [5, 6]. Body conformations and scrotal circumference are regarded as selection criteria in breeding bulls (Silva *et al.*, 2017) [7]. While body weight and girth mostly rely on nutritional status and genetics of the bull (Praveen *et al.*, 2024) [8], scrotal circumference indicates testicular mass and sperm output (Chandler *et al.*, 2013, Bindu *et al.*, 2024) [9, 10]. This study is envisaged to assess the interrelationship between serum testosterone and morphometric indices in Ongole bulls which in turn might emphasize their relevance in fertility assessment and selection strategies.

2. Materials and Methods

For the present study Six Ongole breeding bulls were selected from the Livestock Research Station, Lam, Guntur, Sri Venkateswara Veterinary University, Andhra Pradesh, India. All the bulls selected were clinically sound and were maintained under similar management conditions. Bulls were fed 20-25 kg green fodder, 5-6 kg dry fodder and 2-3 kg concentrate on daily basis with free access to clean drinking water. Morphometric indices like body length (inches), girth (inches) and scrotal circumference (cm) were measured as detailed in Table 1. Blood samples were collected aseptically from the jugular vein from each bull using sterile serum separator vacutainers in the morning hours before feeding. After a brief period of incubation at room temperature, all the samples were subjected to centrifugation at 3,000 rpm for 15 minutes to collect the serum. Serum testosterone (nmol/L) was assessed by chemiluminescence method (Toishi *et al*., 2018) [11]. Pearson correlation and linear regression analyses were conducted. Simple regression graphs with testosterone as dependant (outcome) variable and morphometric traits as independent (predictor) variables were plotted. Inverse regression graphs with testosterone as predictor were also explored.

3. Results

Associations were drawn between serum testosterone and morphometric traits using correlation and regression analyses in six breeding bulls. Correlation analysis using Pearson correlation coefficient (r) (Table 3) showed strong associations of testosterone with both age (r=0.918; $p<0.05$) and scrotal circumference (r=0.915; $p<0.05$), indicating that these traits were statistically significant predictors of testosterone. In contrast, body length (r=0.586; $p>0.05$), girth (r=0.622; $p>0.05$) and weight (r=0.566; $p>0.05$) showed only moderate and non-significant correlations. Among the morphometric traits, age with scrotal circumference (r=0.873; $p<0.05$); body length with girth (r=0.986; $p<0.001$); body weight with both length (r=0.963; $p<0.001$) and girth (r=0.990; $p<0.0001$) were significantly correlated.

Simple linear regression (Table 4; Figure 1) confirmed that age (R^2 = 0.843; $p<0.01$) and scrotal circumference (R^2 = 0.837; $p<0.05$) each independently explained more than 80% of the variance in testosterone, whereas length (R^2 = 0.343; $p>0.05$), girth (R^2 = 0.386; $p>0.05$) and body weight (R^2 = 0.321; $p>0.05$) contributed far less. Inverse modelling (Table 5; Figure 2) showed that testosterone could reliably estimate scrotal circumference.

4. Discussion

4.1 Correlations between testosterone and morphometric traits

The present study confirmed that serum testosterone concentration in Ongole bulls was strongly associated with age and scrotal circumference, whereas body girth and body weight showed comparatively weaker relationships. Age and scrotal circumference individually showed high correlation coefficients and statistically significant associations with serum testosterone, suggesting possible role as physiological indicators of reproductive status in bulls. These findings are in agreement with previous reports in *Bos indicus* bulls, where scrotal dimensions were shown to reflect testicular development and Leydig cell function and there by production of testosterone (Chandler *et al*., 2013; Silva *et al*., 2017) [9, 7].

Body girth and body weight exhibited only moderate, statistically non-significant correlations with testosterone, indicating that general body conformation contributes less directly to endocrine variation. In acceptance with the present findings, Hafizuddin *et al.,* (2025) [12] noted that testosterone exhibited strong positive and significant correlation with scrotal circumference but non-significant correlations with morphometric traits like body height and pelvic circumference in Aceh bulls.

4.2 Correlations among morphometric traits

In the present study, age with scrotal circumference; body length with girth; body weight with both length and girth showed significant correlations. The present study was in agreement with the finding of Perumal (2014) [13], who found that with age, scrotal circumference was increased in *Bos indicus* bulls. In contrast, from our institute, a study in Ongole bulls by Rao *et al.,* (2010) [14] reported non-significant correlation of scrotal circumference with age who attributed the differences in scrotal biometry between various reports to variations in age, selection intensity and genetics. In support to the findings of the present study, is the report of Dakhlan *et al.,* (2024) [15], which noted great correlation between length and girth to the body weight in Ongole cattle.

4.3 Interdependence among morphometric traits and testosterone

Regression analysis reinforced that scrotal circumference is accountable for the most of the explainable variation in testosterone. Given that age is not a trait subject to selection, scrotal circumference remains the more practical field-level indicator of reproductive maturity, as also put forth by Perumal (2014) [13] in Tho Tho (*Bos indicus*) bulls.

The inverse regression model, wherein testosterone predicted scrotal circumference, further confirmed the bidirectional physiological relationship - larger testes produce more testosterone, and higher testosterone supports testicular maintenance. This observation of the present study is in support of the previous workers, Smith and Walker (2014) [16] who put forth that testosterone is required to maintain spermatogenesis and testicular structure; and D'Occhio *et al*., (2021) [17] whose cattle-specific experiment showed that suppression of GnRH/testosterone (immunocastration) produces testicular atrophy in bulls. On the contrary, testosterone held only weak predictive control over other morphometric traits.

Among all the morphometric traits studied in the present study, scrotal circumference remains the most useful phenotypic marker as it is easy to measure with high heritability and established correlation with semen quality and early puberty in offspring (Rao *et al*., 2015; Ramesh *et al*., 2019) [18, 19]. Though determining testosterone concentration offer proper physiological insight, the cost of the ELISA test and lab dependency make it difficult in field applications. Therefore, scrotal circumference can be used an indicator of reproductive performance in Ongole bulls. The present study was limited to a small sample size and so the results should be interpreted cautiously and must be validated in larger cohorts.

Table 1: Morphometric traits measured

Parameter	Measurement Method	Unit
Scrotal Circumference	Measured at the widest point using flexible measuring tape after lowering the testicles into the scrotum with left hand (Rao *et al.,* 2010) [14].	cm
Body Girth	Measured behind hump around thoracic cavity using measuring tape	inches
Body Length	Shoulder to ischium using measuring tape	inches
Body Weight	Estimated using Shaffer's livestock formula (Wangchuk *et al.,* 2017) [20]	kg

Table 2: Pearson Correlation Coefficients (r) with corresponding p-values

Variable (Mean ± S.E)	Testosterone (nmol/L)	Age (years)	Scrotal Circumference (cm)	Length (inches)	Girth (inches)	Body Weight (Kg)
Testosterone (6.73±2.48)	1.000	0.918* (p=0.0097)	0.915* (p=0.0106)	0.586 (p=0.2194)	0.622 (p=0.1877)	0.566 (p=0.2415)
Age (7.5±1.06)	0.918* (p=0.0097)	1.000	0.873* (p=0.0227)	0.566 (p=0.2415)	0.595 (p=0.2055)	0.520 (p=0.2894)
Scrotal Circumference (36.08±1.16)	0.915* (p=0.0106)	0.873* (p=0.0227)	1.000	0.769 (p=0.0748)	0.762 (p=0.0774)	0.705 (p=0.1202)
Length (60.41±0.88)	0.586 (p=0.2194)	0.566 (p=0.2415)	0.769 (p=0.0748)	1.000 (—)	0.986** (p=0.0002)	0.963** (p=0.0012)
Girth (74.33±1.41)	0.622 (p=0.1877)	0.595 (p=0.2055)	0.762 (p=0.0774)	0.986** (p=0.0002)	1.000 (—)	0.990*** (p<0.0001)
Body Weight (507.91±25.79)	0.566 (p=0.2415)	0.520 (p=0.2894)	0.705 (p=0.1202)	0.963** (p=0.0012)	0.990*** (p<0.0001)	1.000 (—)

p < 0.05 → * p < 0.01 → ** p < 0.001 → ***

Table 3: Simple Linear Regression Models Predicting Testosterone (n = 6) [Morphometric Traits as predictor (independent variable): Testosterone as outcome (dependant variable)]

Predictor → Testosterone	Intercept (β_0)	Slope (β_1)	R^2	p-value
Age	-9.454	2.158	0.843	0.0097
Scrotal Circumference	-63.620	1.950	0.837	0.0106
Length	-22.842	1.754	0.343	0.2194
Girth	-74.847	1.097	0.386	0.1877
Body Weight	-20.951	0.055	0.321	0.2415

Table 4: Inverse Regression Models Predicting Morphometric Traits from Testosterone (n = 6) [Testosterone as predictor (independent variable): Morphometric Traits as outcome (dependant variable)]

Testosterone → Outcome	Intercept (β_0)	Slope (β_1)	R^2	p-value
Scrotal Circumference	33.195	0.429	0.837	**0.0106**
Body Weight	468.325	5.881	0.321	0.2415
Girth	68.970	0.259	0.386	0.1877
Length	58.093	0.125	0.343	0.2194
Age	4.163	0.285	0.843	0.0097

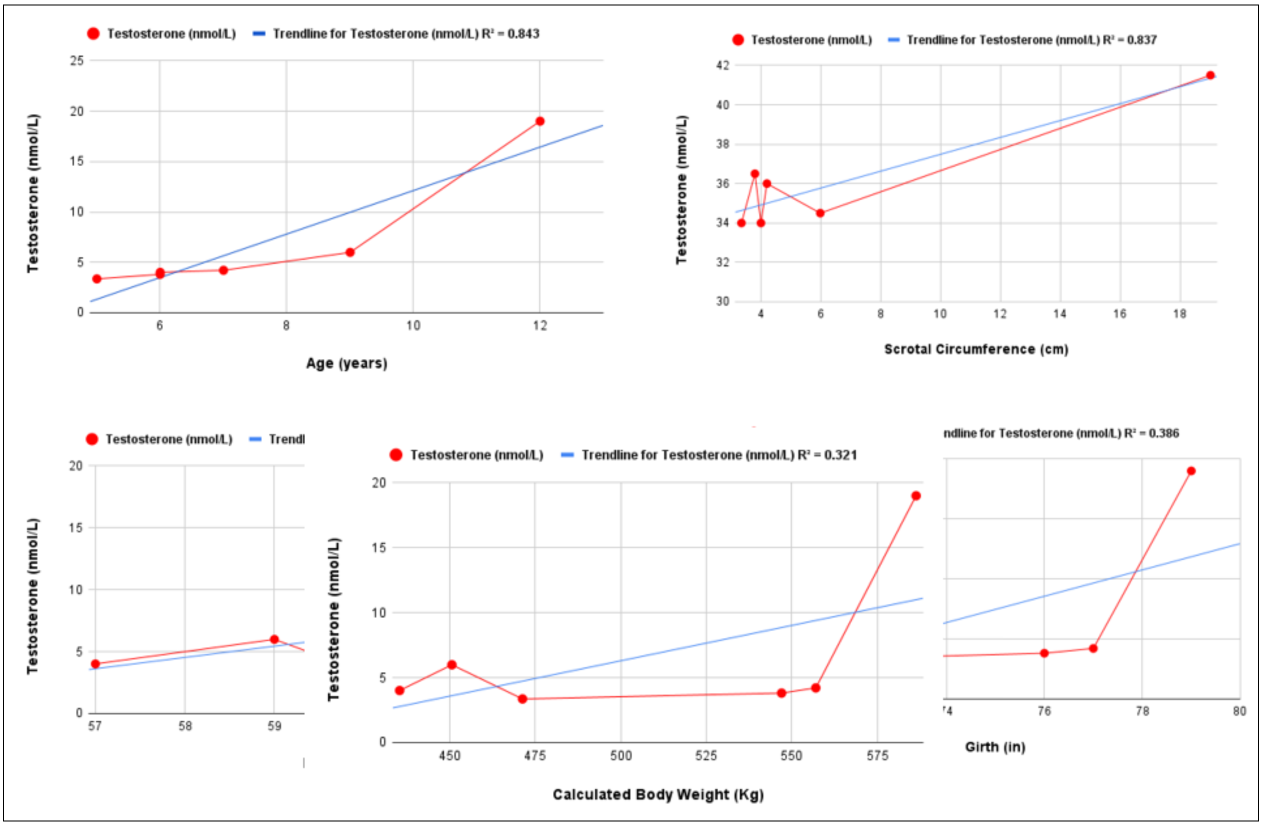

Fig 1: Simple Linear Regression Models Predicting Testosterone (n = 6) [Morphometric Traits as predictor (independent variable): Testosterone as outcome (dependant variable)]

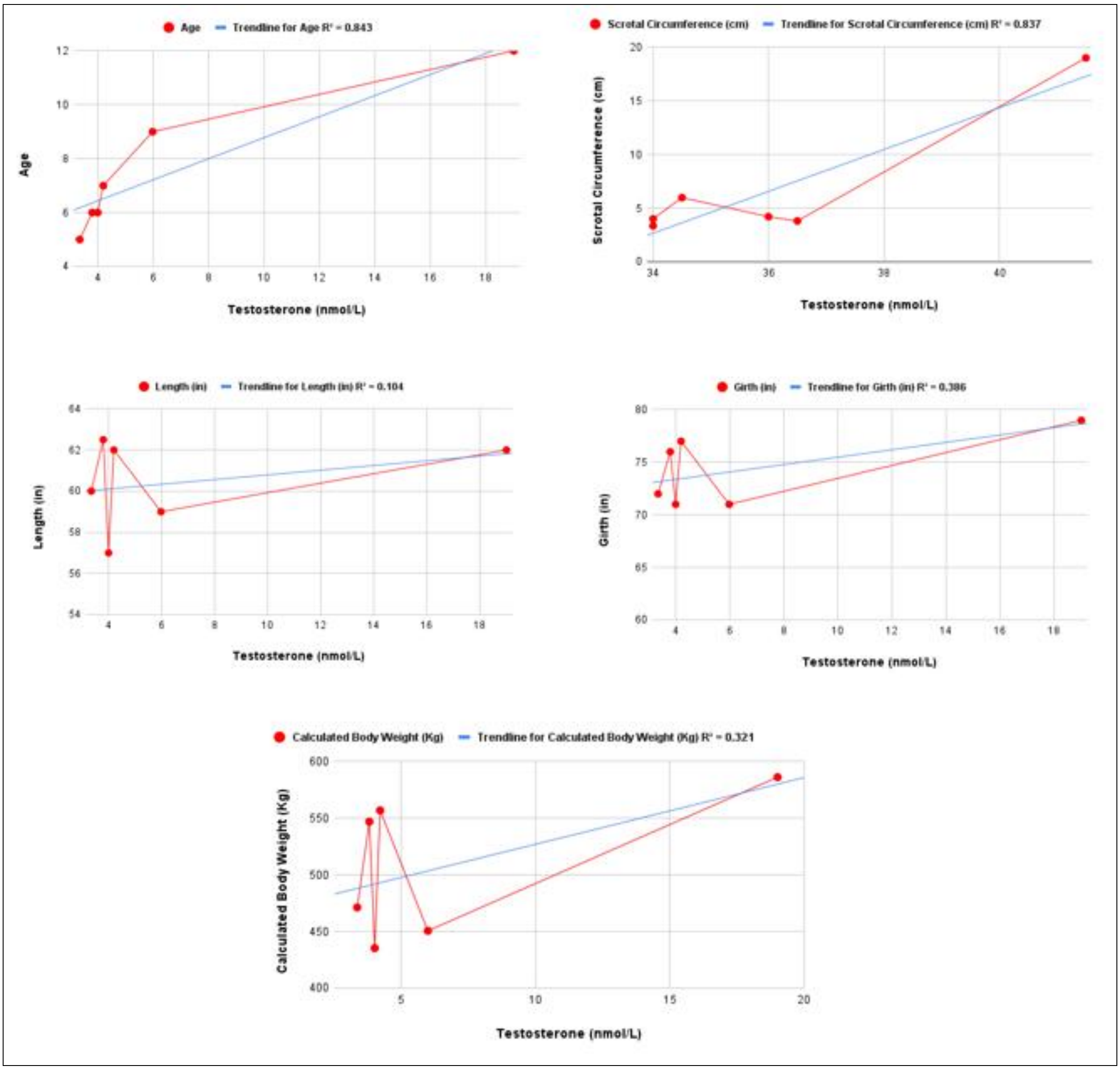

Fig 2: Inverse Regression Models Predicting Morphometric Traits from Testosterone (n = 6) [Testosterone as predictor (independent variable): Morphometric Traits as outcome (dependant variable)]

5. Conclusion

Among the attributes studied, scrotal circumference and age showed strong positive correlation with serum testosterone concentration in Ongole bulls. Regression analysis confirmed scrotal circumference as the most reliable and practical predictor of testosterone status, supporting its application as a field-level fertility marker in Ongole bulls. These findings reinforce the use of scrotal circumference as a selection criterion in Ongole cattle breeding programs.

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7. Conflict of Interest

Not available.

8. Financial Support

Not available.

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