Relationship between physical parameters and seminal plasma testosterone levels in Aceh Bulls

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Abstract. In Aceh bulls, physical traits linked to testosterone levels are hypothesized to indicate reproductive potential and aid in selecting superior sires for breeding programs. Therefore, this study aimed to investigate the relationship between select physical parameters and seminal plasma testosterone levels in Aceh bulls. The study was conducted at BPTU-HPT Indrapuri Aceh Besar, Indonesia, using five Aceh bulls aged 3-5 years with a body weight range of 300-450 kg. Fresh semen was collected using the artificial vagina technique, and seminal plasma testosterone levels were measured using the enzyme-linked immunosorbent assay (ELISA). Pearson's correlation test was used to assess the relationships between variables. The mean (± SD) scrotal circumference, body height, and pelvic circumference were 29.8 ± 2.86 cm, 120.3 ± 6.05 cm, and 171.0 ± 7.96 cm, respectively. Testosterone levels showed a strong, positive, and significant correlation with scrotal circumference ($r = 0.955^*$, $p < 0.05$), but non-significant correlations with body height ($r = 0.785$) and pelvic circumference ($r = 0.408$). In conclusion, seminal plasma testosterone level exhibits a strong positive correlation with scrotal circumference in Aceh bulls, suggesting that scrotal circumference may serve as a useful indicator of reproductive potential in this breed.

1 Introduction

The measurement of physical parameters in breeding bulls, combined with reproductive hormone profiling, serves as a valuable predictive tool for assessing overall genetic merit and breeding soundness [1]. This multimodal evaluation enables the identification of biologically significant biomarkers that correlate strongly with reproductive performance, including morphometric characteristics (body condition score, scrotal circumference), seminal

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parameters, and endocrine profiles [2]. Ultimately, this approach enhances breeding programs by improving the likelihood of successful insemination and overall fertility rates in the herd.

Besides physical parameters, testosterone levels are crucial for sperm development and function. Testosterone contributes to the process of spermatogenesis, which is essential for the production of viable sperm cells [3]. This hormone influences various semen characteristics, including sperm motility and morphology, which are essential for successful fertilization. Monitoring testosterone levels in plasma and seminal blood is essential to understand how this hormone interacts with semen quality. Research has shown that optimal testosterone levels are necessary to maintain healthy sperm production and overall reproductive health in bulls. Low testosterone levels can lead to decreased sperm production and impaired fertility, highlighting the importance of this hormone in male reproductive physiology [4]. Furthermore, fluctuations in testosterone levels can affect not only sperm quality but also the overall reproductive performance of bulls and other livestock [5]. Therefore, assessing testosterone levels in conjunction with physical parameters may provide a more comprehensive understanding of bull fertility and effectively guide breeding programs.

Among the critical factors influencing male fertility are the physical condition of the bulls, the quality of semen they produce, and the biochemical composition of their seminal plasma, including testosterone [2]. Previous studies have established the importance of these factors in other cattle breeds, highlighting their potential impact on reproductive success [6]. However, specific data pertaining to Aceh cattle are sparse, underscoring the need for targeted research.

This study focuses on Aceh cattle, a local breed in Indonesia renowned for its adaptability to tropical environments. Aceh cattle have significant potential to enhance meat production in the region. Further research has been conducted on cows, especially for improving reproductive performance [7], and treating reproductive disorders [8]. However, there are still few studies discussing the reproductive performance of Aceh bulls. Therefore, a comprehensive understanding of the relationship between physical parameters and testosterone levels in seminal plasma in Aceh cattle is essential. This knowledge is important to support a more effective and sustainable Aceh cattle reproduction program.

2 Methods

2. 1 Animal samples

The research involved five Aceh bulls, aged between 24 and 34 months, which were randomly chosen from the Center for Superior Animal Breeding and Forage (BPTU HPT) Indrapuri, affiliated with the Ministry of Agriculture of the Republic of Indonesia. All selected bulls exhibited a favorable body condition score, varying from 3 to 4 on a scale of 5.

2. 2 Seminal plasma collection and preparation

Semen was collected using an artificial vagina in the morning. Semen from each bull was then centrifuged at 3,500 rpm for 10 minutes. This process separated the seminal plasma (supernatant), which was carefully transferred into Eppendorf tubes and stored in the freezer until testosterone measurements were performed.

2. 3 Measurement of testosterone concentration

Testosterone concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) with the DRG ELISA kit EIA-1559 (DRG Instruments GmbH, Germany), following the manufacturer's instructions. A volume of 25 µl of seminal plasma or standard solution was dispensed into the wells of a microplate, followed by the addition of 200 µl of HRP-conjugate solution to each well. The microplate was then sealed with plastic film, gently mixed, and incubated for 60 minutes at room temperature (21-23 degrees Celsius). After incubation, the wells were emptied and washed three times with wash buffer, with a 10-second interval between washes to ensure thorough removal of unbound substances. Residual buffer was aspirated, and the plate was inverted onto absorbent paper to dry. Subsequently, 50 µl of substrate solution A and 200 µl of substrate solution B were added to each well. The plate was covered and incubated for 20 minutes in the dark. The reaction was stopped by adding 100 µl of stop solution to each well, followed by gentle mixing. Finally, the optical density (OD) was measured at a wavelength of 450 nm using a microplate reader.

2. 4 Data analysis

The data obtained in this study were analyzed using the Pearson correlation test.

3 Results and discussions

3. 1 Physical parameters and seminal plasma testosterone levels in Aceh Bulls

The seminal plasma testosterone concentrations varied significantly across all sampled Aceh cattle, consistent with findings reported by Kenny and Byrne [9] regarding the influence of age, photoperiod, ambient temperature, and nutritional status. further substantiated that testosterone levels in livestock are modulated by age, thermal conditions, and dietary factors. The observed testosterone concentrations and physical parameters in Aceh cattle from the Center for Superior Animal Breeding and Forage (BPTU HPT) Indrapuri were presented in Table 1.

Table 1. Mean (±SD) testosterone concentrations and physical parameters in Aceh cattle at BPTU-HPT Indrapuri.

Parameter	Mean±SD	Range
Testosterone level (ng/mL)	5.80±1.52	3.73-7.98
Scrotal circumference (cm)	29.80±2.86	25.00-32.00
Body height (cm)	120.3±6.05	112.5-127.0
Pelvic circumference (cm)	171.0±7.96	164.0-183.0

The mean seminal plasma testosterone concentration was 5.80 ng/mL (range: 3.73-7.98 ng/mL). While all cattle received uniform management regarding the four aforementioned factors, age variation emerged as the predominant determinant of testosterone level fluctuations in the studied population at BPTU-HPT Indrapuri, Aceh Besar.

3. 2 Correlation Analysis

The results of the correlation test between physical parameters, namely scrotal circumference, height, and pelvic circumference with seminal plasma testosterone levels are illustrated in Figure 1.

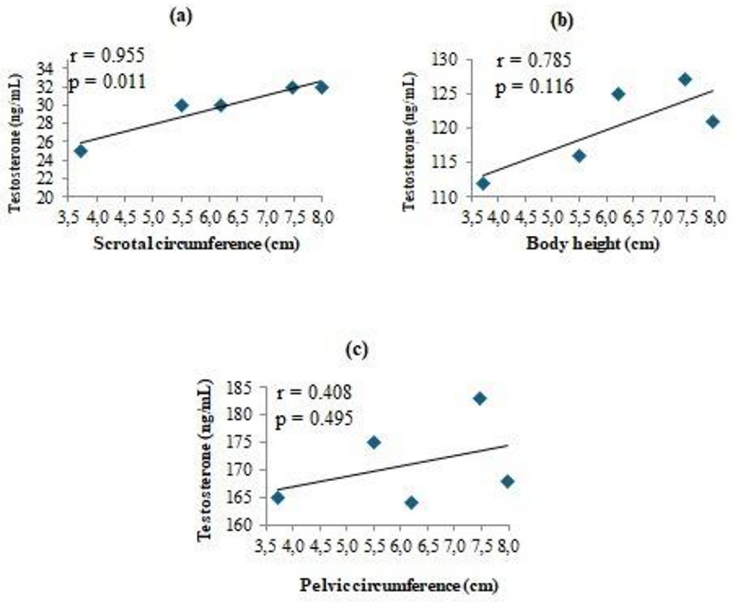

Fig 1. illustrates the correlations between seminal plasma testosterone and physical parameters: (a) Scrotal circumference exhibited a strong positive correlation ($r = 0.955$, $p < 0.05$); (b) Body height showed a moderate correlation ($r = 0.785$, $p > 0.05$); (c) Pelvic circumference displayed a weak correlation ($r = 0.408$, $p > 0.05$).

The average scrotal circumference (29.80 cm), measured at the maximal diameter after testicular manipulation, exceeded the 28.28 cm reported by Fadhli [10] in Aceh cattle. This variation likely reflects differences in pubertal status, body condition, and environmental factors among study populations. The observed dimensions align with expectations for semen-producing bulls that have undergone reproductive selection.

As established by Ajani et al. [11], scrotal circumference positively correlates with testicular mass, seminiferous tubule diameter, and daily sperm production. This relationship explains the significant association with testosterone levels, given the hormone's role in spermatogenic regulation.

The non-significant correlation between body height and testosterone ($p>0.05$) may reflect testosterone's dual role as both a reproductive hormone and growth regulator. While testosterone promotes musculoskeletal development (increasing muscle mass and bone density), these effects manifest over prolonged periods, potentially decoupling immediate hormonal concentrations from morphometric measurements.

Byrne et al. [12] documented that improved body condition enhances physical development, consistent with our observed trend between body height and testosterone levels. Optimal growth typically coincides with reproductive maturation, though this relationship may not achieve statistical significance in cross-sectional analyses.

Age-related variations in testosterone production exhibit a biphasic pattern, reflecting distinct physiological phases across the male reproductive lifespan. During the juvenile phase, the immaturity of reproductive organs results in reduced testosterone secretion and suboptimal semen quality. This is followed by a phase of prime maturity, characterized by peak reproductive performance and maximal testosterone output. Subsequently, in senescence, a gradual decline in gonadal function leads to decreased hormone levels and reproductive capability.

These developmental changes align with the findings of Picut and Remick [13], who documented significant age-related effects on male reproductive parameters. Additionally, Anawalt and Matsumoto [14] reported corresponding fluctuations in seminal plasma testosterone concentrations, further supporting the observed physiological transitions throughout aging.

While pelvic circumference showed minimal direct association with testosterone levels, Singh et al. [2] reported its strong correlation with scrotal dimensions (r=0.87), suggesting indirect reproductive implications. The interconnectedness of these physical parameters underscores their collective value in breeding soundness evaluations, though mechanistic relationships require further elucidation through longitudinal studies.

4 Conclusion

Among the physical parameters evaluated, scrotal circumference exhibited the strongest and most significant positive correlation with seminal plasma testosterone levels in Aceh cattle. In contrast, other physical traits, such as body height and pelvic circumference, also showed positive correlations but were not statistically significant. These findings indicate that scrotal circumference may serve as a more reliable phenotypic indicator of testicular endocrine function in this breed. Consequently, scrotal circumference could be a valuable trait for assessing reproductive potential and may aid in the selection of superior sires for breeding programs.

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