

Correlation Between Testosterone Concentrations with Scrotal Circumference, and Semen Characteristics in Aceh Bulls

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Abstract. This study was conducted to examine testosterone concentrations its relationship with the scrotal circumference and physical characteristics of semen in aceh bulls. Semen samples were collected weekly from jugular vein of three aceh bulls aged 4-5 years old for 10 weeks. Testosterone concentration was measured by *enzyme-linked immunosorbent assay* (ELISA) method. Semens were collected by using artificial vagina and evaluated for physical characteristics namely ejaculatory volume, pH, and sperm motility, concentration, and abnormalities. Data were analyzed using correlation-regresion test. Testosterone concentrations showed a positive correlation with scrotal circumference ($r = 0.799$), number of sperm ($r = 0.703$), sperm motility ($r = 0.857$) and sperm abnormalities ($r = -0,877$). No correlation, however, was found between testosterone concentrations with semen volume ($r = 0.038$) and pH ($r = 0.418$). It can be concluded that testosterone concentrations correlated positively with scrotal circumference, numbers of sperm, sperm of motility and sperm of abnormality.

Keywords: Aceh bulls, testosterone concentration, scrotal circumference and semen characteristics

1 Introduction

Aceh cattle is one of Indonesian beef cattle germplasmas native to Indonesia, that have been bred by farmers in Aceh province for meat production and workforce [1]. According to Abdullah [2], Aceh cattle belong to small beef cattle breed genetically have unique genes and different from other native Indonesian cattle pools such as Bali cattle, Madura cattle, Pasundan cattle and Ongole cattle. Although having growth rate smaller than crossbred cattle, Aceh cattle have been well adaptive to Indonesian environment that is warm, humid, and having insufficient food and water, and prevalent of parasitic diseases [3]. Population numbers and genetical quality of Aceh cattle, however, tend to decrease [1].

Efforts to increase population and genetic quality of Aceh cattle have been done by optimizing reproduction efficiency by the application of artificial insemination (AI). Application of AI technology is not only able to increase productivity and accelerate distribution of cattle populations have good genetic quality, but also is expected to be able to optimize function of a bull [4,5]. The success of AI program in animal is closely related to quality of sperms ejaculated by a bull. Effort to select a bull has good performance and high fertility. This

condition is related to the ability of a bull to produce high fertility spermatozoa [6]. One of the ways to determine fertility rate of a bull is by investigating capacity of spermatozoa production that can be indirectly predicted by measuring serum testosterone and scrotum circumference. This is because spermatozoa were formed by epithelial cells called spermatogonia that are located inside the seminiferus tubules of testes. Testes productivity in producing spermatozoa is dependent upon the numbers of spermatogonia cells performed mitosis and meiosis cleavages [5], and testes ability to produce testosterone [7]. The higher testosterone concentration the better production of substrates are responsible for regulating and performing spermatogenesis activity [8,9,10]. Some studies have analyzed the relationship between serum testosterone concentration, sperms quality and fertility of bulls, and some of them reported a strong correlation [11,12,13], but other could not find any association [10].

Data on the level of testosterone hormone in the serum, scrotal circumference, and semen characteristics (semen volume and pH as well as sperms motility, concentration and abnormality) of Aceh bulls have been not reported so far. This condition becomes a problem in developing Aceh cattle breeding program. Therefore, it is

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necessary to conduct a study that able to contribute to basic information about serum testosterone level, scrotal circumference, and semen quality of Aceh bulls in the UPTD-BIBD of Saree, Aceh Besar.

2 Materials and Methods

2.1 Research design

This study was a field observational research used three adult, healthy Aceh bulls aged 4.0-5.0 years old and had body weight ranged from 300-350 kg, all of which were kept at the Technical Implementation Unit and Local Artificial Insemination Agency (UPTD-BIBD) of Saree, Aceh Besar. The bulls were kept in individual close cages equipped with feeding and drinking facilities. Animals were fed with concentrate (3.0 kg/head/day) and fresh grass (30-35 kg/head/day). The concentrate was given in the morning whereas fresh grasses were provided *ad libitum* in the midday and afternoon. Measurement of scrotal circumference, collection of whole blood and semen samples were done every week from 10 consecutive weeks.

2.2 Measurement of scrotal circumference

Scrotal circumference (cm) was measured at a week interval by measuring tape had one tenth accuracy. Prior to every measurement, testes were palpated and slowly pressed downward to allow them enter scrotal cavity. Measurement tape was circled at the neck of scrotum and moved downward until reaching the biggest scrotal curvature and recorded.

2.3 Blood collection and preparation

Whole blood samples were withdrawn from jugular vein of three Aceh bulls weekly for 10 consecutive weeks at 07.00-08.00 am using plain collection tubes (BD Vacutainer, Franklin Lakes, NJ, USA). Tubes containing sample were put at slanted position at 4-5 °C for 4-5 hours. Serums formed were separated by centrifugation at 1,500xg for 15 minutes and stored at -20 °C until used in next experiment.

2.4 Measurement of serum testosterone levels

Serum testosterone was measured by using a commercial Testosterone ELISA kit (Cat. No. EIA-1559, DRG Instrument GmbH, Germany). This assay has been validated for Aceh bulls [14] and other animals [15,16]. In brief, all serums were diluted 1:4 using aquabidestilata. Standard solutions having concentration ranged from 0.2-16 ng/ml were prepared. Samples and standard solutions, 25 µl each, were transferred in triplicate into wells of ELISA *microplate*, added with conjugate enzyme (except for *blank*) and covered with a *cling film*. Mixtures were homogenized by shaking it gently for 10 seconds and incubated at room temperature for 60 minutes. Microplate wells were washed 3-4 times with mashing 300 µl of washing solution, added with 200

µl of substrate, and incubated at room temperature for 15 minutes. Reaction was stopped by the addition of 100 µl of stop solution (0.5 M H₂SO₄) to each well. Absorbance was read using ELISA reader at 450 nm.

2.5 Semen collection and evaluation

Semen samples were collected weekly from 3 Aceh bulls for 10 consecutive weeks at 08.00 am by using artificial vagina 42-45 °C. Semens were evaluated macroscopically (volume, color, odor, pH and consistency) and microscopically (spermatozoa concentration, motility and abnormality). Semen volume was measured by scaling reaction tube whereas its pH was determined using a digital pH-meter. Spermatozoa concentration was counted by using a Haemocytometer as described by [17]. Spermatozoa motility was evaluated by observed a drop of semen sample onto a deck glass using a light microscope (Olympus). To evaluate spermatozoa morphology, semen samples were fixed using a formalin solution (99 ml sodium citrate 2.9% + 1 ml of 37% formaldehyde).

2.6 Data analysis

The relationships between testosterone level and scrotal circumference and semen characteristics were evaluated by using correlation analysis tested followed by a regression analysis.

3 Results and Discussions

Average measurement results of serum testosterone level, scrotal circumference and fresh semen characteristics (semen volume and pH, spermatozoa concentration, motility and abnormality) of Aceh bulls evaluated are presented in Table 1.

Table 1. Serum testosterone, the scrotal circumference of semen characteristics of Aceh cattle bulls kept at the UPTD-BIBD of Saree, Aceh Besar

Parameter	Mean ± SD	Normal range
Serum testosterone (ng/ml)	7.95 ± 2.85	5.14 – 13.06
Scrotal circumference (cm)	28.02 ± 2.23	24.80 – 32.80
Semen volume (ml/ejaculate)	4.70 ± 0.61	3.70 – 5.80
Semen pH	6.98 ± 0.15	6.80 – 7.20
Spermatozoa number (10 ⁹ /ml)	1.40 ± 0.72	1.12 – 1.69
Spermatozoa motility (%)	77.00 ± 3.40	70.00 – 80.00
Spermatozoa abnormality (%)	9.60 ± 2.04	7.00 – 14.00

3.1 Serum testosterone levels in Aceh bulls

Serum testosterone levels of Aceh bulls examined in this study ranged from 5.14 – 13.06 ng/ml with the average of 7.95 ± 2.85 ng/ml. Average serum testosterone level found in this study was relatively similar to those reported in Ongole crossbred cattle, 7.0 ± 0.8 ng/ml [18], but slightly

higher than those found in Simmental bull 6.1 ± 0.4 ng/ml [18], Friesian Holstein (FH) bull aged 3 - 4 years old 2.07 ± 1.12 ng/ml [13], and adult FH bulls 5.66 ± 1.08 ng/ml [10]. Variation of blood testosterone levels might caused by many factors such as breed, climate, rations and age [19,20,21,22] as well as sensitivity and accuracy of measurement methods used [23].

3.2 Scrotal circumference of Aceh bulls

Scrotal circumference is a central circumference of both testes determined by measuring the largest central part of scrotum after testes are pressed downward into scrotum cavity. Scrotal circumference of Aceh bulls kept at the UPTD-BIBD of Saree Aceh Besar ranged from 24.80-32.80 cm, average circumference was 28.02 ± 2.23 cm. Average scrotal circumference of Aceh cattle examined in the study is comparable to those reported by Fadhli [23] in Aceh e bulls aged 3-5 kept in intensive system (28.28 ± 2.20 cm) and Bali bulls aged 6-7 years old (28.70 ± 2.30 cm). Scrotal circumference of Aceh bulls recorded in this study was higher than that of reported by Toleng *et al.* [24] in Bali bulls aged 3-4 years old 23,2 cm. Among factors might infleuced were age, body weight, nutritional status and environment. Hight scrotal circumference of Aceh bulls may be caused by the age of bull examined had reached puberty and body maturation as reflected by their average body weight. Moreover, Aceh bulls examined are bulls kept for semen production so that they have passed selection process based on reproduction organ criteria. According to Ratnawati and Affandhy [25]improved body condition or bodyweight will give a response to enlargement of scrotal size. Khairi [26] also added that scrotal size is positively correlated with the cattle body size or weight.

3.3 Semen volume and pH

Semen volume (ml) of Aceh bulls examined in this study ranged from 3.70 – 5.80 ml/ejaculation, with average of 4.70 ± 0.61 ml/ejaculation. The pH of the semen ranged from 6.8 – 7.2., with average of adalah 7.01 ± 0.08 . Average semen volume and pH obtained were relative similar to those reported by Zaini *et al.* [27] at Aceh bulls aged 3-4 years old, 3.82 ± 0.47 ml and 6.84 ± 0.17 , respectively. The semen of Bali cattle also have relative

similar volume and pH namely 4.83 ± 1.40 ml and 6.51 ± 0.12 [28]. The volume of semen obtained, however, relatively higher than those of PO cattle aged 2-3 years old, 2.6 ± 1.5 ml. variation of semen volume reported might be related to the diferences in animal species, age, and body weight, as well as climate and raising management. Different average colume and pH of bovine semen are influenced by spesies, age, body weight, collection interval and environmental condition [5,28,29] and scrotal circumference [30]. In this study, all Aceh bulls used as semen sources have been trained to be a bull for semen collection..

3.4 Spermatozoa concentration, motility and abnormality

Evaluation on spermatozoa concentration, motility and abnormality is very important because these factors represent semen properties used as criteria for determining semen quality. Average spermatozoa concentration, motility and abnormality of Aceh bulls evaluated in this study were $1.40 \pm 0.72 \times 10^9$ sperms/ml, $77.00 \pm 3.40\%$ and $9.60 \pm 2.04\%$, respectively. Values reported are relatively similar to those reported in Bali cattle kept at the UPTD-BIB Maros i.e. 1.513×10^9 sperms/ml; $61 \pm 13.0\%$ and $10.8 \pm 1.,8\%$ [31] Spermatozoa numbers, motility and abnormality of Aceh bulls reported, however, are higher than those reported in Bali cattle namely $1.02 \pm 0.32 \times 10^9$ sperms/ml, $67.02 \pm 6.92\%$ and $3.89 \pm 1.43\%$ [28]. The differences in values reported might be related to species, age, body weight, ransom and environment of cattle used. These are in accordance to previous reports showing semen characteristics were influenced by age, fed quality, body weight, collection frequency and environment [27,28,29,32].

3.5 The relationships of serum testosterone level and scrotal circumference and semen characteristics of Aceh bulls

Results of correlation analysis on testosterone serum level and scrotal circumference and semen characteristics (volume and pH, and spermatozoa concentration, motility dan abnormality) of Aceh bulls in the UPTD-BIBD Saree Aceh Besar are listed in Table 2.

Table 2. Correlation coefficient values (r), determinant coefficient (r²) and linier formula of the relationship of serum testosterone levels and scrotal circumference and semen characteristics of Aceh bulls in the UPTD-BIBD Saree Aceh Besar

Parameter	Correlation coefficient (r)	Determinat coefficient (r ²)	P	Linier Formula
Scrotal circumference	0.799	0.639	0.000**	Y = 1.021 x – 20.67
Semen volume	0.038	0.001	0.872	Y = 0.178 x + 7.108
Semen pH	0.418	0.175	0.066	Y = 0.088 x + 7.069
Spermatozo concentration	0.703	0.494	0.001**	Y = 0.007 x – 2.212
Spermatozoa motility	0.857	0.734	0.000**	Y = 0.536 x – 32.534
Spermatozoa abnormality	-0.877	0.769	0.000**	Y = - 1.137 x + 19.318

Note: ** in coloum shown a significant effect (p<0.01), while tn shown not significant effect (p>0.05)

Based on result of analysis presented in Table 2, concentration of serum testosterone positively correlated with scrotal circumference in Aceh bulls (r = 0.799) as

shown by a regression formula Y = 0.178x + 7.108. This means that higher serum testosterone level proportional to larger scrotal circumference. This finding was in

agreement to those reported by Fadli [22] who found strong correlation between serum testosterone and scrotal circumference of Aceh bulls aged 3-5 years old kept in intensive system ($r = 0.719$). Other study in Simelue buffalo aged 4-5 years old shown relative similar results, $r = 0.954$ [21]. These results showed that adult Aceh cattle already have completely matured and fully functional reproductive organs and accessory glands to allow adequate serum testosterone and spermatozoa. Some researchers previously reported that Leydig cells in the testes of adult cattle active in synthesizing testosterone.

Results of this study also showed that serum testosterone level of Aceh bulls positively correlated with semen volume with a correlation coefficient (r) of 0.038 and regression formula $Y = 0.178x + 7.108$, also on semen pH with a correlation coefficient (r) 0.418 and regression formula of $Y = 0.088x + 7.069$. Correlation property between testosterone concentration and semen volume and pH of Aceh bulls could be categorized as very low and medium. This result showed that increased testosterone concentration did not influence semen volume and pH in Aceh bulls. These findings were different from those reported by Rachmawati *et al.* [33] in Bligon goats that serum testosterone concentration significantly associated with semen volume ($P < 0.01$), $r = 0.65$. Blood testosterone level plays a role in determining spermatozoa concentration and motility rate of cattle [1-10], and Bligon and Kejobong goats [33].

Serum testosterone level of Aceh cattle strongly and significantly correlated with spermatozoa concentration ($r = 0.703$) and motility ($r = 0.857$). These mean that higher serum testosterone level will be followed by higher spermatozoa numbers and motility percentage in Aceh bulls. These results are consistent with Rachmawati *et al* [33], who reported positive correlation between testosterone concentration and spermatozoa numbers in Bligon goats ($r = 0.72$). This condition might be caused by testosterone role in stimulating spermatogenesis so that spermatozoa concentration in the semen also increased. Testosterone is a hormone involved in spermatogenesis process, especially at spermatogenesis and spermiogenesis phases of spermatozoa maturation (Sulisbury and VanDemark, 1985). Adequate testosterone concentration will result in faster differentiation of spermatogonia cell into spermatocyte and transformation of spermatid into spermatozoa, resulting in more spermatozoa to ejaculate [5]. Determination coefficient of 0.703 found between serum testosterone level and spermatozoa concentration indicated that serum testosterone concentration only 70.3% influenced spermatozoa concentration, and the rest was associated with other factors. Correlation coefficient values obtained between serum testosterone level and spermatozoa concentration in Aceh cattle supported results of study by Rachmawati *et al* [33] that serum testosterone level positively correlated ($r = 0.59$) with spermatozoa concentration in goats. Higher coefficient correlation of serum testosterone level and spermatozoa concentration in Aceh bulls included in this study compared to those reported Sajjad *et al.* [7], suggested that serum testosterone concentration was not significantly affect the

spermatozoa concentration of buffalo with a correlation coefficient of 0.264.

Results of this study also found that serum testosterone negatively associated with spermatozoa abnormality in semen ($P < 0.05$), $r = -0.877$. This result gave evidence that higher serum testosterone level resulted in less spermatozoa abnormality. This finding is in agreement to that reported by previous researchers that shown negative correlation of testosterone level with spermatozoa abnormality in adult Kejobong goats [9], buffaloes [12], and FH crossbred [10], suggested that healthy adult produce better and higher testosterone hormone than young animals. More testosterone hormone available to be used in spermatogenesis due to matured reproductive organs and sex glands will result in higher quality of semen compared to that produced by young animals. High testosterone may increase spermatozoa concentration, motility and viability in semen [9,10,12,21].

4 Conclusions

Serum testosterone level strongly correlated with scrotal circumference, sperm concentration and sperm motility of Aceh bulls whereas semen volume and pH, and sperm abnormality were not associated with serum testosterone levels.

Further research needs to do in order to investigate the relationship between serum testosterone concentration with libido, semen quality and spermatozoa fertility rate of Aceh bulls after freezing.

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