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BLOOD SERUM TESTOSTERONE LEVEL AND ITS RELATIONSHIP WITH SCROTAL CIRCUMFERENCE AND SEMEN CHARACTERISTICS IN NILI-RAVI BUFFALO BULLSM. SAJJAD, S. ALI, N. ULLAH¹, M. ANWAR¹, S. AKHTER AND S. M. H. ANDRABI¹Department of Zoology, University of Arid Agriculture, Rawalpindi,¹ Animal Reproduction Programme, Animal Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan**ABSTRACT**

This study was aimed at determining the blood serum testosterone level and its relationship with scrotal circumference and physical characteristics of semen in Nili-Ravi buffalo bulls. Semen samples were collected weekly from three buffalo bulls of 14 years age for 12 weeks and were evaluated for physical characteristics i.e. ejaculatory volume, sperm motility, sperm concentration, pH and sperm abnormalities. Jugular blood samples were collected from each bull at weekly intervals and analyzed for serum testosterone concentrations. Mean (\pm SE) blood serum testosterone level (ng/ml), scrotal circumference (cm), semen volume (ml), progressive sperm motility (%), sperm concentration ($10^6/\mu\text{l}$), semen pH and total sperm abnormalities (%) observed were 0.69 ± 0.12 , 34.6 ± 0.9 , 3.59 ± 0.41 , 51.53 ± 2.23 , 0.99 ± 0.07 , 7.01 ± 0.08 and 11.67 ± 0.90 , respectively. Positive correlations between testosterone level and scrotal circumference ($r=0.414$) and ejaculatory volume ($r=0.348$) were observed. However, no correlation of testosterone level with sperm motility ($r=0.145$), sperm concentration ($r=0.264$), semen pH ($r=-0.208$) and total sperm abnormalities ($r=-0.242$) was found. Similarly, ejaculatory volume did not show any correlation with sperm motility percentage ($r=0.115$), sperm concentration ($r=0.045$), semen pH ($r=-0.015$) and total sperm abnormalities ($r=-0.135$). Sperm motility percentage had positive correlation with sperm concentration ($r=0.347$) and negative correlation with semen pH ($r=-0.670$). Sperm concentration was negatively correlated with semen pH ($r=-0.501$). It was concluded that in 14 years old buffalo bulls the level of serum testosterone and scrotal circumference and ejaculatory volume were positively correlated. The other semen quality parameters including sperm motility, sperm concentration, semen pH and sperm abnormalities were not related with serum testosterone level.

Key words: Buffalo bulls, testosterone level, semen characteristics, scrotal circumference.**INTRODUCTION**

The average maturity age of buffalo bulls in Pakistan is 25 months (Ahmad *et al.*, 1984). At the time of puberty, the values for minimal body weight, testicular volume and sperm concentration of buffalo bulls are 335 Kg, 134 cm^3 and $70 \times 10^6/\text{ml}$ per ejaculate; however, testosterone concentration in buffalo bulls raised to $3.3 \pm 1.2 \text{ ng/ml}$ at the age of 18 months (Ahmad *et al.*, 1989).

As puberty sets in, the bulls start improving sexual performance and maintain it for a long time before reaching old age. The best quality of semen from buffalo bulls has been obtained at 3-4 years of age. It has further been postulated that the age of the bull and season of the year significantly affect semen characteristics (ejaculatory volume, sperm motility and concentration). Variations in semen quality, however, exist even in the same season and at same age at different localities (Saeed, 1988). Although information on testosterone levels, scrotal circumference and semen quality of young and adult buffalo bulls is available

(Ahmad *et al.*, 1984; Javed *et al.*, 2000a, b), the relationship between blood testosterone levels, scrotal circumference and semen quality parameters in buffalo bulls of 14 years age has not been reported. Hence, this study was designed to study the relationship between blood testosterone levels, scrotal circumference and semen quality parameters in Nili-Ravi buffalo bulls of 14 years age.

MATERIALS AND METHODS

Three Nili-Ravi buffalo breeding bulls of 14 years age were used for semen collection in this experiment. A total of 12 semen samples were collected from each bull in 12 weeks with the help of an artificial vagina at 42°C . The semen collected from each bull was immediately transferred to laboratory for the evaluation of volume (ml), motility (%), sperm concentration ($10^6/\mu\text{l}$), pH and sperm morphological abnormalities. Sperm concentration was measured by spectrophotometer. Motility percentage of spermatozoa was assessed by examining a drop of semen sample on a glass slide

under light microscope. The pH was measured with a digital pH meter. For morphological study of spermatozoa, the semen sample was fixed using formal-citrate solution (99 ml of 2.9% sodium citrate + 1 ml of 37% formaldehyde). Sperm morphology (%) was studied at 1000x under oil immersion. One hundred spermatozoa were counted for each semen sample. Scrotal circumference (SC; cm) was measured at weekly intervals with a measuring tape.

Jugular blood samples of 6 ml collected from each bull were centrifuged at 3000 rpm for 15 minutes. The serum was collected and stored at -20°C until measurement of testosterone. Concentrations of testosterone in each sample were determined in duplicate by radioimmunoassay kit (Immunotech SA France). Specificity of the assay was 100% and sensitivity was 0.025 ng/ml. Intra and inter assay coefficients of variation were 8.5 and 13%, respectively. For measuring intra and inter assay coefficients of variation, two known standards were placed three times in a batch of 100 samples. Data of experiment were analyzed by using correlation techniques using software Minitab (version 11.12 32 Bit).

RESULTS AND DISCUSSION

The data depicting values of blood serum testosterone, scrotal circumference and semen characteristics of Nili-Ravi buffalo bulls are given in Table 1. Testosterone concentration in blood serum of Nili-Ravi buffalo bulls of the present study was 0.69 ± 0.12 ng/ml which is comparable to earlier studies of Javed *et al.* (2000b) from buffalo bulls of 12-15 years of age. Higher testosterone values (1.58 ± 0.32 ng/ml) were reported in young buffalo bulls of Murrah breed by Gupta *et al.* (1984). The decrease in blood testosterone levels in buffalo bulls associated with senility has also been reported by other workers (Allam and Shehata, 1996).

Table 1: Blood serum testosterone, scrotal circumference and semen characteristics of Nili-Ravi buffalo bulls

Parameters	(Mean \pm SE)
Serum testosterone (ng/ml)	0.69 ± 0.12
Scrotal circumference (cm)	34.60 ± 0.90
Ejaculatory volume (ml)	3.59 ± 0.41
Sperm motility (%)	51.53 ± 2.23
Sperm concentration ($10^6/\mu\text{l}$)	0.99 ± 0.07
Semen pH	7.01 ± 0.08
Sperm abnormalities (%)	11.67 ± 0.90

Values are mean of 36 observations.

The SC of Nili-Ravi buffalo bulls observed during the present study was 34.6 ± 0.9 cm which is comparable to young bulls (with normal semen picture) reported by Anzar *et al.* (1993), Younis (1996) and Javed *et al.* (1998). However, the SC values of bulls of this study were less than those reported by Ahmad (1987). These findings indicate that increasing age has little effect on the SC of buffalo bulls. SC in buffalo bulls at various ages, however, has been affected by level of nutrition and environment.

The ejaculatory volume (ml) observed during the present study (3.59 ± 0.41) was similar to that observed by Malik *et al.* (1974) and Ahmad *et al.* (1984). Javed *et al.* (2000b) recorded semen volume as 4.67 ± 1.62 ml in Nili-Ravi buffalo bulls of 12-15 years of age, while El-Wishy (1978) reported 4.10 ml for Iraqi buffalo bulls. Variation in semen volume reported for buffalo bulls of different origins can be due to differences in breeds, reproductive health condition of bulls, age of bulls, frequency of collection, pooled volume, nutrition, season and management (Nazir, 1988). Javed *et al.* (2000b) observed no significant difference in the volume of semen between buffalo bulls of various age groups. However, it was relatively high in adult bulls (8-9 years of age), followed by old bulls (12-15 years of age). Nordin *et al.* (1990) and Younis (1996) also reported higher ejaculatory volume in adult and old bulls than in young buffalo bulls. This indicates that buffalo bulls produce more volume of semen in older age as compared to younger age due to unexplained phenomenon.

The progressive sperm motility (%) in the buffalo bulls of the present study (51.53 ± 2.23) was lower than in the bulls of Murrah breed of buffalo (Vyawanare *et al.*, 1989; Suryaprakasam and Rao, 1993) and Nili-Ravi adult buffalo bulls (Younis, 1996). However, it was close to the findings of Javed *et al.* (2000b) in Nili-Ravi buffalo bulls of 12-15 years of age (56.89 ± 0.65). The difference in sperm motility in various reports could be due to variations in the judgement of motility, number of bulls studied, or difference of season of studies and age of the bulls. The same trend of decreasing sperm motility in older buffalo bulls compared to young buffalo bulls has been observed by Younis (1996).

Sperm concentration ($10^6/\mu\text{l}$) in buffalo bulls of the present study was 0.99 ± 0.07 and was close to the findings of Javed *et al.* (2000b) in Nili-Ravi buffalo bulls, swamp buffalo bulls (Jainudeen *et al.*, 1982), Surti and Murrah buffalo bulls (Rahman *et al.*, 1991). El-Wishy (1978), Raizada *et al.* (1988), Nazir (1988) and Terezinha *et al.* (1991) reported comparable sperm concentration (1.65, 2.90, 1.15 and $1.33 \times 10^6/\mu\text{l}$, respectively) in buffalo bulls of younger age. It can therefore, be inferred from these reports that the buffalo

Table 2: Correlations between blood serum testosterone level, scrotal circumference and semen characteristics in Nili-Ravi buffalo bulls

Parameters	SC	T	VOL	MOT	CONC	pH	SA
Scrotal circumference (SC; cm)	1.000						
Serum testosterone (T; ng/ml)	0.414*	1.000					
Semen volume (VOL; ml)	0.181	0.348*	1.000				
Sperm motility (MOT; %)	0.112	0.145	0.115	1.000			
Sperm concentration (CONC; $10^6/\mu\text{l}$)	0.063	0.264	0.045	0.347*	1.000		
Semen pH	-0.115	-0.208	-0.015	-0.670*	-0.501*	1.000	
Sperm abnormalities (SA; %)	-0.245	-0.242	-0.135	-0.093	0.048	0.146	1.000

* = Significant at $P < 0.05$.

bulls upto the age of 15 years produce semen with a sperm concentration between 0.94 and $2.90 \times 10^6/\mu\text{l}$. Javed *et al.* (2000b) observed lower sperm concentration in older than in younger bulls. However, Younis (1996) reported a non-significant difference in sperm concentration between bulls of young, adult and old age groups. The lower sperm concentration in old bulls could be due to senility (Javed *et al.*, 2000b).

The semen pH observed in the buffalo bulls of present study was 7.01 ± 0.08 and was in close proximity of pH reported by Javed *et al.* (2000b) from the buffalo bulls of same age and breed. However, the same workers reported lower pH in semen from adult buffalo bulls compared to old bulls, the difference in pH value was attributed to a difference in the age group. Our findings are comparable to Terezinha *et al.* (1991) and Younis (1996), who reported relatively lower pH in adult bulls than old bulls.

The sperm abnormalities were recorded as 11.67 ± 0.90 percent during this study, which lie in the normal range reported by Saeed (1988). These observations indicate that sperm abnormalities in the semen from buffalo bulls of 14 years age remain in the normal range.

The values of correlation coefficients (r) between blood serum testosterone, scrotal circumference and semen characteristics of Nili-Ravi buffalo bulls are given in Table 2. Blood serum testosterone level of buffalo bulls had positive correlation ($r=0.414$; $P < 0.05$) with scrotal circumference and semen volume ($r=0.348$). However, D-Occhio and Aspden (1996) reported that semen volume was independent of testosterone concentration in blood of buffalo bulls.

Testosterone level had no correlation with percentage sperm motility ($r=0.145$) and sperm concentration ($r=0.264$), while Javed *et al.* (2000b) observed positive correlation of testosterone level with sperm concentration, and a negative correlation with semen pH and seminal plasma testosterone levels. No correlation was found between testosterone level and pH ($r=-0.208$); and testosterone levels and total sperm abnormalities ($r=-0.242$) in the semen of buffalo bulls

of this study. Higher testosterone levels in semen and blood had a good relationship with semen quality (sperm concentration, pH, motility and mass activity) in buffalo bulls of all ages, except those of less than 24 months of age (Ahmad *et al.*, 1984). Semen testosterone concentration was higher in adult buffalo bulls and was associated with semen quality parameters (Javed *et al.*, 2000a, b).

No correlation was observed between semen volume and sperm concentration ($r=0.045$) and similar observations were reported by Javed *et al.* (2000b) and Younis (1996) in their studies on buffalo bulls of different ages. Fields *et al.* (1979), however, observed a negative correlation between semen volume and sperm concentration in cattle bulls. Present findings also showed no correlation between semen volume and other semen parameters, including percentage motility ($r=0.115$), pH ($r=-0.015$) and total sperm abnormalities ($r=-0.135$). This suggested that in senile buffalo bulls, semen volume was not a good predictor of sperm concentration, sperm motility, semen pH and total sperm abnormalities. Sperm motility percentage in semen of buffalo bulls of this study had positive correlation ($r=0.347$; $P < 0.05$) with sperm concentration. Sperm motility percentage had negative correlation ($r=-0.670$; $P < 0.01$) with semen pH and these observations are in line with the findings of Javed *et al.* (2000b). No correlation ($r=-0.093$) was found between sperm motility percentage and total sperm abnormalities in the semen obtained from buffalo bulls of this study.

Sperm concentration had negative correlation ($r=-0.501$; $P < 0.01$) with semen pH and no correlation ($r=0.048$) with total sperm abnormalities. Javed *et al.* (2000b) also found negative correlation ($r=-0.37$) between semen pH and sperm abnormalities. No correlation ($r=0.146$) was found between semen pH value and sperm abnormalities.

Conclusions

In buffalo bulls of 14 years of age, the levels of blood serum testosterone were correlated with scrotal

circumference and semen volume. The other semen quality parameters including sperm motility, sperm concentration, semen pH and sperm abnormalities were not related with blood serum testosterone levels.

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