

Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal buffalo bulls and their relationship with other semen parameters

Muhammad Tariq Javed^{1*}, Ahrar Khan¹, and Mumtaz Ali²

¹*Department of Veterinary Pathology, University of Agriculture, Faisalabad, Pakistan.*

²*Radio-immunoassay Laboratory, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.*

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ABSTRACT

Sixteen buffalo bulls (*Bubalus bubalis*) were divided into four age groups, four bulls in each. Bulls in three groups had good semen quality while in one group it was poor. The study was undertaken for a one-year period divided into five seasons. The study revealed an overall seminal plasma testosterone concentration of 0.97 ± 0.12 ng/ml. It was higher ($P < 0.05$) in the 6-10-year-olds, less than 5 and older than in 11-year-old bulls. Testosterone showed a non-significant difference between seasons. Overall seminal plasma oestrogen concentration was 43.67 ± 7.62 pg/ml and showed a non-significant difference between healthy groups and between healthy and abnormal bulls. Oestrogen was higher ($P < 0.05$) in winter than in other seasons in 6-10 and older than 11-year-old bulls, including abnormal bulls. Testosterone in healthy bulls showed significant positive correlation ($P < 0.05$) with sperm concentration ($r = 0.223$) and time between two ejaculates ($r = 0.242$), while there was a significant ($P < 0.05$) negative correlation with semen pH ($r = -0.293$). Oestrogen showed significant ($P < 0.01$) positive correlation with semen pH ($r = 0.350$) and negative ($P < 0.05$) with time between two ejaculates ($r = -0.296$) and sperm head abnormalities ($r = -0.262$). Both hormones were negatively correlated ($P < 0.05$) with each other ($r = -0.277$).

Key words: buffalo bull, *Bubalus bubalis*, seminal plasma, testosterone, oestrogen, season

Introduction

It has generally been recognised that testicular size is the end product of gonadotropic activity (LAND, 1985). Small testes at puberty are

* Contact address:

Dr. Muhammad Tariq Javed, Department of Veterinary Pathology, University of Agriculture, Faisalabad 38040, Pakistan. Phone: 92 41 625834; Fax: 92 41 624607; E-mail: mtjaved@fsd.paknet.com.pk

associated with deficiency of gonadotropins (TURNER and BLOODWORTH, 1968) that are necessary for initiation and maintenance of spermatogenesis (PARVINEN, 1982). Oestrogen synthesis and secretion by the testes (AMANN and GANJAM, 1976), its presence in the seminal and epididymal fluids (EILER and GRAVES, 1977) and binding to spermatozoa (SCHAFFENBURG and McCULLAGH, 1954), suggest its importance in male reproduction. It has been reported that oestradiol-17 β inhibits testicular development and function by blocking gonadotropin release at the hypothalamic level in the male buffalo (SHAHAB et al., 1993) resulting in decreased levels of androgens. The latter may disturb spermatogenesis in bulls older than five years (HOOKER, 1944). Correlation between plasma testosterone, body weight and scrotal circumference suggests its application in the evaluation of testicular function (ANDRADE et al., 1992).

Much work has been done on blood plasma hormones and their relationship with many semen parameters (THUN et al., 1980; AHMAD et al., 1984; SIDIBE et al., 1992; SOUSA et al., 1996; WOLF, 1996). Although the presence of testosterone and oestrogen in seminal fluid has been reported in cattle bulls, their relationship with other semen parameters, and with respect to age and season, have not been a particular subject of study in buffalo bulls. The present study was therefore carried out to observe the effect of age and season on seminal plasma testosterone and oestrogen in apparently healthy, and abnormal, Nili-Ravi buffalo bulls, and to discover their relationship with semen characteristics.

Materials and methods

The study was conducted at the Semen Production Unit, Qadirabad, District Sahiwal, Pakistan, on 16 buffalo bulls (*Bubalus bubalis*) for one year. The bulls were divided into four age groups, four bulls in each group. The first three age groups, i.e., less than 5, 6-10 and older than 11 years, comprised bulls with good quality semen. Bulls in the abnormal group (6-10 years of age) had poor semen quality, with 50% progressive motility or less at their initial examination; they also had a history of deteriorating semen quality.

The year was divided into five seasons on the basis of temperature and humidity records (Table 1). All bulls were kept under identical conditions of management, feeding and watering throughout the study period.

Two ejaculates were collected from each bull at fortnightly intervals, and were pooled. Semen was evaluated for total volume, mass activity,

motility, pH, sperm concentration and morphologically abnormal spermatozoa. Hormonal studies on seminal plasma samples were conducted after separating the latter by centrifuging the semen samples at 3000 rpm for 10 minutes. Hormones, including testosterone and oestrogen, were determined in the seminal plasma by using the radio-immunoassay technique at the Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan, and by using RIA kits (ICN Biomedicals Inc., Diagnostic Division, Costa Mesa).

Table 1. Means±SD of temperature (°C) and relative humidity (%) as recorded during different seasons of the year in Sahiwal district, Pakistan

Seasons		Temperature		Humidity
		Maximum	Minimum	
Summer	Dry (May 1-June 30)	42.54±3.89	25.12±2.47	38.46±1.58
	Humid (July 1-Sep. 15)	35.04±2.72	26.49±2.22	69.68±2.08
Autumn		32.14±3.13	18.84±5.98	57.38±3.51
Winter		21.22±2.75	6.54±2.16	69.79±9.04
Spring		27.17±4.47	12.88±4.85	56.76±5.54

Data was subjected to one-way analysis of variance by using SPSS (7.5.1), a computer software package (ANONYMOUS, 1996). Correlation coefficients among different parameters were also worked out.

Results

The overall mean of testosterone in seminal plasma of healthy buffalo bulls during the present study was 0.97±0.12 ng/ml (Table 2). Overall mean testosterone was higher ($P<0.05$) in 6-10 year-old bulls than <5 and >in 11-year-old bulls (Table 2). However, the difference between groups in each season was not significant. Overall mean testosterone between seasons was also not significant. However, it was higher ($P<0.05$) in autumn and humid summer than in winter in bulls more than 11 years old (Table 2). Overall mean seminal plasma oestrogen in healthy bulls was 43.67±7.62 pg/ml (Table 2). A non-significant difference in seminal plasma oestrogen between age groups was observed (Table 2). The difference between seasons, however, was significant, with higher ($P<0.05$) concentration in winter than in other seasons in the two age groups and abnormal bulls, except those <5 years of age (Table 2).

The overall mean of seminal plasma testosterone of abnormal bulls was lower ($P<0.05$) than in healthy bulls of the same age, but no difference was observed in oestrogen. However, the latter was relatively lower in abnormal bulls (Table 2). As with the healthy bulls, testosterone showed no difference between seasons in abnormal bulls, while oestrogen was higher ($P<0.05$) in the winter season (Table 2).

Table 2. Comparison of testosterone and oestrogen concentration in seminal plasma between buffalo bulls of different ages in various seasons of the year and in abnormal bulls

	Age groups (years)	Summer		Autumn	Winter	Spring	Overall mean
		Dry	Humid				
Testosterone (ng/ml)	<5	0.60±0.34 ^{Aa}	0.75±0.29 ^{Aa}	1.04±0.09 ^{Aa}	0.66±0.23 ^{Aa}	0.51±0.12 ^{Aa}	0.71±0.11 ^A
	6-10	1.28±0.17 ^{Aa}	1.71±0.86 ^{Aa}	1.84±0.65 ^{Aa}	1.06±0.68 ^{Aa}	1.88±0.84 ^{Aa}	1.55±0.21 ^B
	>11	0.63±0.18 ^{Aab}	0.97±0.28 ^{Aab}	0.92±0.13 ^{Aa}	0.16±0.13 ^{Aa}	0.59±0.11 ^{Aab}	0.65±0.11 ^A
	Abnormal	0.91±0.27 ^{Aa}	1.62±0.70 ^{Aa}	0.93±0.35 ^{Aa}	0.56±0.12 ^{Aa}	0.72±0.31 ^{Aa}	0.95±0.18 ^A
	Overall mean	0.83±0.16 ^a	1.14±0.32 ^a	1.27±0.23 ^a	0.63±0.25 ^a	0.99±0.33 ^a	0.97±0.12
Oestrogen (pg/ml)	<5	27.20±16.05 ^{Aa}	10.48±3.98 ^{Aa}	9.23±2.75 ^{Aa}	82.87±11.10 ^{Aa}	87.13±51.10 ^{Aa}	43.38±12.60 ^A
	6-10	56.72±18.6 ^{Aa}	8.34±2.00 ^{Aa}	10.83±5.04 ^{Aa}	128.22±35.30 ^{Ab}	13.38±4.87 ^{Aa}	43.50±12.80 ^A
	>11	24.31±15.10 ^{Aa}	9.70±2.58 ^{Aa}	7.20±1.47 ^{Aa}	152.41±40.40 ^{Ab}	27.05±7.70 ^{Aa}	44.14±14.80 ^A
	Abnormal	31.90±6.81 ^{Aa}	11.50±2.86 ^{Aa}	13.84±5.25 ^{Aa}	79.27±20.20 ^{Ab}	21.66±9.08 ^{Aa}	31.63±7.12 ^A
	Overall mean	36.07±11.20 ^a	9.51±1.57 ^a	9.08±1.84 ^a	121.17±10.70 ^b	42.52±18.40 ^a	43.67±7.62

Overall mean in a row represents values of all healthy buffalo bulls.

Values in each row with different small letters and in each column with different capital letters are statistically different ($P<0.05$).

Each figure represents mean±SE

Correlation

Testosterone in healthy bulls showed a significantly ($P<0.05$) positive correlation with sperm concentration ($r=0.223$) and time between two ejaculates ($r=0.242$), while there was a significantly ($P<0.05$) negative correlation with semen pH ($r=-0.293$). Oestrogen in healthy bulls showed a significantly ($P<0.05$) negative correlation with time between two ejaculates ($r=-0.296$) and sperm head abnormalities ($r=-0.262$), while there was a significantly ($P<0.01$) positive correlation with semen pH ($r=0.350$). Both hormones in healthy bulls showed a significantly ($P<0.05$) negative correlation with each other ($r=-0.277$).

Testosterone in abnormal bulls showed a significantly ($P<0.05$) negative correlation with sperm tail abnormalities ($r=-0.411$) and total sperm abnormalities ($r=-0.431$). Oestrogen, however, showed a non-significant correlation with all sperm parameters. Both hormones showed a negative ($r=-0.198$) but non-significant correlation with each other.

Table 3. Correlation coefficients of seminal plasma testosterone and oestrogen with different semen parameters in healthy and abnormal bulls

Semen parameters	Healthy bulls		Abnormal bulls	
	Testosterone	Oestrogen	Testosterone	Oestrogen
Sperm concentration	0.223*	-0.200	-0.137	-0.055
Mass activity	0.158	-0.128	-0.095	-0.225
Motility	0.184	-0.174	0.220	-0.355
Semen pH	-0.293*	0.350**	0.015	0.338
Total sperm abnormality	0.064	0.115	-0.411*	0.104
Sperm head abnormality	0.035	-0.262*	0.090	0.028
Sperm tail abnormality	0.056	0.164	-0.431*	0.098
Testosterone	1.000	-0.277*	1.000	-0.198
Semen volume	0.055	-0.143	0.092	0.005
Time between two ejaculates	0.242*	-0.296*	0.071	-0.161

Figures with * indicate significant at $P<0.05$ and with ** at $P<0.01$

Discussion

Testosterone concentration in seminal plasma observed during the present study on healthy buffalo bulls was lower than that of blood plasma values (1.58 ± 0.32 ng/ml) in buffalo bulls (Murrah breed) reported by GUPTA et al. (1984). Lower testosterone (one-third) in seminal than in blood plasma in cattle bulls has already been reported (EILER and GRAVES, 1977). The present findings also suggest that as with blood (PERERA et al., 1979; CHANTRAPRATEEP et al., 1981), semen of the buffalo bull contains a lesser volume of testosterone than does the cattle bull (1.15 ± 0.26 ng/ml) EILER and GRAVES (1977).

Significantly ($P<0.05$) higher testosterone in bulls of 6-10 years of age agreed with the findings of WOLF (1996) in 6-9 years than in <2-year-old German Black Pied bulls. This suggests that as with blood (VASHCHENKIN and KRIVOPUSHKINA, 1995; ALLAM and SHEHATA, 1996), semen testosterone increases with age up to around 9-10 years, and starts decreasing with further increase in age. This decrease is probably due to senility. Lower testosterone in seminal plasma in winter was almost

similar to the findings of BARNABE et al. (1995) in blood of buffalo bulls in Brazilian conditions. This could be due to the effect of cold and shorter day length on the hypothalamus-pituitary axis, causing its depression. The winter is also known for being a non-breeding season in buffalo under local conditions.

Present findings of much higher oestrogen concentration in semen than in blood (SHAHAB et al., 1993) is similar to the findings of EILER and GRAVES (1977) in cattle bulls. The finding of WOLF (1996) of higher oestrogen concentration in 1.5-2 years than in 6-9 years old bulls was dissimilar to the present observations. It may be possible that bulls of 1-2 years of age have a higher oestrogen level than bulls of 4-5 years of age. Higher oestrogen in winter ($P < 0.05$) was the reverse of testosterone concentration in the same season. This shows that with an increase in one hormone, the other decreases, and vice versa ($r = -0.277$). However, in male goats oestrogen is higher in May-June and in October than in other months, contrary to buffalo bulls.

The present study, and those of TEGEGNE et al. (1994), VASHCHENKIN and KRIVOPUSHKINA (1995) and WOLF (1996) suggest that higher testosterone levels in semen and blood has a good relationship with semen quality (sperm concentration, pH, motility and mass activity) in bulls of all ages, except those of less than 24 months of age (AHMAD et al., 1984; SOUSA et al., 1996). However, semen volume is independent of testosterone concentration in both blood (SOUSA et al., 1996; D-OCCHIO and ASPDEN, 1996) and semen (present finding). The significantly ($P < 0.05$) lower testosterone level, while relatively lower in oestrogen in abnormal than normal bulls is similar to the findings of VEERAMACHANENI et al. (1986) of lower testosterone in abnormal than normal bulls. This may be related to pathology of the testis or lower spermatogenic activity.

The correlation suggests that bulls having a higher oestrogen level have lower sperm head defects ($P < 0.05$), while an increase in sperm defects occurs due to exogenous administration of oestrogen (CUPPS and BRIGGS, 1965) and zeranol, a compound having an oestrogen-like activity (DESCHAMPS et al., 1987), causing the increase in bent and coiled tails. However, it appears that sperm abnormalities are closely associated with semen testosterone ($P < 0.05$) in abnormal, but not in normal, bulls, while exogenous administration of testosterone in normal bulls produces semen of low quality (MEINECKE and McDONALD, 1996).

The shorter time between two ejaculates ($P < 0.05$) in bulls having higher oestrogen as opposed to testosterone ($P < 0.05$) suggest the role of oestrogen in revival of sexual activity after ejaculation. The oestrogen level

is directly related to pH of semen both in normal ($r=0.350$, $P<0.05$) and abnormal bulls ($r=0.338$).

Conclusions

It can be concluded that semen testosterone is higher in adult bulls, while oestrogen is higher in the winter season in both adult and old bulls, while both testosterone ($P<0.05$) and oestrogen is lower in abnormal bulls. Further, testosterone is associated with semen quality, while oestrogen is associated with revival of sexual activity. The pH of semen can be used as indicator of oestrogen level in semen.

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SAŽETAK

Šesnaest bivolskih bikova (*Bubalus bubalis*) podijeljeno je u 4 dobne skupine s po 4 bika. Bikovi iz 3 skupine imali su dobru kvalitetu sjemena, a iz jedne lošu. Istraživanje je provedeno tijekom jedne godine podijeljene u 5 godišnjih doba. Opće koncentracija

testosterona u sjemenskoj plazmi bila je $0,97 \pm 0,12$ ng/ml. Ona je bila viša ($P < 0,05$) u dobnoj skupini od 6-10 godina nego u bikova mlađih od 5 i starijih od 11 godina. Testosteron nije pokazao značajne razlike po godišnjim dobima. Opća koncentracija estrogena u sjemenskoj plazmi je bila $43,67 \pm 7,62$ pg/ml i nije bilo značajne razlike između zdravih skupina bikova niti između zdravih i nenormalnih bikova. Estrogen je bio viši ($P < 0,05$) zimi nego u drugim godišnjim dobima u 6-10 godišnjaka i u bikova starijih od 11 godina uključujući i nenormalne bikove. Testosteron je u zdravih bikova pokazao značajnu pozitivnu korelaciju ($P < 0,05$) s koncentracijom spermija ($r = 0,223$) i vremenom između dvije ejakulacije ($r = 0,242$), dok je bila značajna ($P < 0,05$) negativna korelacija sa pH sjemena ($r = -0,293$). Estrogen je pokazao značajnu ($P < 0,01$) pozitivnu korelaciju sa pH sjemena ($r = 0,350$) i negativnu ($P < 0,05$) s vremenom između dvije ejakulacije ($r = -0,296$) i s abnormalnostima glave spermija ($r = -0,262$). Oba su hormona bila u negativnoj međusobnoj ($r = -0,277$) korelaciji ($P < 0,05$).

Ključne riječi: bivolski bik, *Bubalus bubalis*, sjemenska plazma, testosteron, estrogen, godišnje doba
