

Studies on abnormal buffalo bulls with reference to scrotal circumference, semen characteristics, seminal plasma hormones and their association with testicular and epididymal histopathology

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ABSTRACT

This study was carried out on six buffalo bulls (*Bubalus bubalis*) for a one-year period. Four bulls selected had good and two bulls (No. 321 and 323) had poor semen quality. All bulls were aged from 6-10 years. Scrotal circumference (SC) and sperm characteristics of both bulls were lower than healthy bulls, while dead sperm percentage and total sperm abnormalities were high. Overall seminal plasma testosterone was lower in these bulls, while oestrogen was lower in bull 323 and higher in 321. Histopathological studies of testes of bull 323 showed a 100% loss of germinal epithelium (DGEL) in all three regions of the right testis; however, it was 89.96% in left testis. DGEL in bull 321 was 35.88 % in right and 31.70% in left testis, with higher DGEL in the ventral part in both testes. Total and lumen diameter was greater ($P < 0.01$) in the caudal region of the left epididymis. Epithelial height in the caput region of the left epididymis was higher ($P < 0.05$) in bull 323 while in the corpus of the right epididymis in bull 321 DGEL correlated negatively ($P < 0.001$) with sperm concentration ($r = -0.98$), progressive motility ($r = -0.88$) and oestrogen ($r = -0.87$), and correlated positively ($P < 0.001$) with dead sperm percentage ($r = 0.89$), total sperm abnormalities ($r = 0.99$) and testosterone ($r = 0.98$). Epithelial height showed a negative correlation ($P < 0.001$) with DGEL ($r = -0.88$) and seminal plasma testosterone ($r = -0.87$), while it was positive ($P < 0.001$) with oestrogen ($r = 0.89$).

Key words: sperm abnormalities, epididymis, testis, histopathology, testosterone,

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Introduction

Scrotal circumference (SC) is a reliable predictor of puberty, semen production, semen quality (MADRID et al., 1988), testicular weight and pathological conditions leading to sub-fertility or infertility (OTT, 1991). Infertility in sexually mature bulls with small testes is often associated with testicular degeneration and/or hypoplasia (MCENTEE, 1970). Decrease in SC may occur because of extremely hot or cold ambient temperature, systemic infections, trauma, nutritional factors, genetic predisposition or other causes (OTT, 1991). It has generally been observed that testicular size is associated with gonadotropic activity (LAND, 1985) and small testes at puberty are 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 was observed that exogenous oestradiol produced an increased number of abnormal spermatozoa, particularly with looped or bent tails (CUPPS and BRIGGS, 1965).

Correlation of SC to other seminal characteristics and seminal plasma hormonal profiles, along with testicular pathology in Nili-Ravi buffalo bulls, have not been comprehensively studied to date. The present study was carried out to investigate the relationship between the SC, semen characteristics, seminal plasma hormones (testosterone and oestrogen) and histopathology of testes and epididymis in abnormal bulls.

Materials and methods

This study was conducted at the Semen Production Unit (SPU), Qadirabad, District Sahiwal, on six buffalo bulls (*Bubalus bubalis*) for a period of one year. Among these, two bulls (bulls N^o 321 and 323) were abnormal on their initial examination of semen characteristics, while four bulls selected were normal and of the same age. All bulls were kept under identical conditions of management, feeding and watering. Semen from all bulls was collected early in the morning, before sunrise at fortnightly intervals

for one year. A total of two ejaculates collected from each bull were pooled and evaluated for total volume, mass activity, motility, pH, dead and morphological abnormal spermatozoa. A total of 24 observations were made over a one-year period on these bulls with reference to above mentioned parameters and hormonal studies, as shown in Table 1. Hormonal studies on seminal plasma obtained from the same samples were conducted, including testosterone and oestrogen, at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, by using Radio-immunoassay kits (ICN Biomedicals Inc., Diagnostic Division, Costa Mesa, CA 92626).

The testes of the two bulls (321 and 323) showing abnormal semen quality were removed through open castration and palpated for any gross abnormalities. Tissue pieces of about 5-7 mm thickness were taken for histopathological examination of the testes from the dorsal, middle and ventral regions and of epididymides from caput, corpus and cauda. These were fixed and tissue sections of 5 μ m thickness were cut and stained with Harris haematoxylin and eosin stain for histological examination. Stained sections of testes were examined under a microscope at 400 X, and degree of germinal epithelial loss (DGEL) for each part (dorsal, middle and ventral) of testes was studied. Randomly selected tubules (n = 200) from each part were classified into one of nine different grades defined after VEERAMACHANENI et al. (1986). Epididymal sections were studied for total diameter (TD), lumen diameter (LD), epithelial height (EH) and muscle layer thickness (MLT) by using an ocular micrometer.

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

Results

The SC of bull N^o 323 decreased from an initial 26.40 \pm 0.55 to 25.00 \pm 0.00 cm at the end of the year with a lower than overall SC of healthy bulls (34.41 \pm 0.10 cm). The SC of bull N^o 321 was initially low (25 cm) but thereafter increased, apparently due to uniform swelling of the scrotum. The sperm characteristics, including mass activity, progressive motility and sperm concentration, were low, while dead sperm percentage

and total sperm abnormalities were higher in these two bulls than in healthy bulls (Table 1). Head abnormalities were higher in bull 321 and there were tail abnormalities in bull 323 (Table 1).

Overall testosterone was relatively lower in seminal plasma of the two bulls, while oestrogen was lower in bull 323, but higher in 321 than in healthy

Table 1. Means \pm sd of body mass, scrotal circumference, sexual behaviour semen characteristics including seminal plasma hormones of bull number 321, 323 and healthy bulls

Parameters studied	Initial			Final			Overall		
	Bulls			Bulls			Bulls		
	321	323	Healthy	321	323	Healthy	321	323	Healthy
BM (kg)	692.80 \pm 1.64	761.20 \pm 1.10	706.65 \pm 8.65	691.20 \pm 5.54	769.80 \pm 2.86	728.55 \pm 8.65	694.86 \pm 6.09	768.00 \pm 4.96	717.25 \pm 3.87
SC (cm)	25.00 \pm 2.87	26.40 \pm 0.55	35.07 \pm 0.24	29.20 \pm 0.45	25.00 \pm 0.00	33.82 \pm 0.24	30.41 \pm 0.91	25.54 \pm 0.60	34.41 \pm 0.10
Libido	2.40 \pm 0.55	2.20 \pm 0.45	2.55 \pm 0.11	2.00 \pm 0.71	2.80 \pm 0.45	2.90 \pm 0.11	2.36 \pm 0.58	2.35 \pm 0.56	2.78 \pm 0.05
MB	4.40 1.34	3.60 \pm 0.89	4.40 \pm 0.20	4.20 \pm 1.09	5.40 \pm 0.55	5.40 \pm 0.20	4.45 \pm 1.01	4.46 \pm 1.07	5.18 \pm 0.09
Time (min.)	10.60 \pm 9.29	8.80 \pm 6.34	11.25 \pm 1.77	22.00 \pm 10.10	11.80 \pm 1.48	10.45 \pm 1.77	12.41 \pm 10.29	11.61 \pm 5.89	10.61 \pm 0.79
Volume (mL)	5.80 \pm 2.02	6.60 \pm 1.47	4.82 \pm 0.31	3.90 \pm 1.02	3.00 \pm 0.35	5.71 \pm 0.33	4.70 \pm 1.99	4.36 \pm 1.55	4.96 \pm 0.14
pH	7.05 \pm 0.40	7.18 \pm 0.34	6.40 \pm 0.09	7.05 \pm 0.28	6.68 \pm 0.49	6.61 \pm 0.09	6.94 \pm 0.45	7.08 \pm 0.41	6.45 \pm 0.04
Colour	1.00 \pm 0.00	0.60 \pm 0.89	0.95 \pm 0.13	0.40 \pm 0.55	0.00 \pm 0.00	1.05 \pm 0.15	0.68 \pm 0.57	0.23 \pm 0.59	1.00 \pm 0.06
MA	1.60 \pm 0.89	1.40 \pm 1.52	3.15 \pm 0.21	1.40 \pm 0.55	0.20 \pm 0.45	3.00 \pm 0.23	1.41 \pm 0.85	0.77 \pm 1.07	2.94 \pm 0.09
Motility (%)	44.00 \pm 5.48	34.00 \pm 27.93	61.50 \pm 2.44	40.00 \pm 7.07	26.00 \pm 23.98	59.70 \pm 2.44	43.64 \pm 13.29	25.77 \pm 21.89	59.50 \pm 1.09
Conc. (10 ⁶ / μ L)	0.92 \pm 0.34	0.79 \pm 0.75	1.12 \pm 0.10	0.65 \pm 0.32	0.10 \pm 0.07	1.08 \pm 0.10	0.70 \pm 0.43	0.41 \pm 0.21	1.05 \pm 0.04
Dead (%)	30.27 \pm 16.38	46.95 \pm 31.26	13.08 \pm 2.40	19.42 \pm 5.20	34.23 \pm 13.48	16.09 \pm 2.40	23.75 \pm 14.09	37.97 \pm 21.01	13.96 \pm 1.07
Head (%)	10.81 \pm 7.70	5.15 \pm 2.74	1.87 \pm 1.11	13.45 \pm 5.85	3.26 \pm 1.14	1.47 \pm 1.11	10.25 \pm 6.59	6.54 \pm 2.47	2.58 \pm 0.49
Tail (%)	12.69 \pm 6.48	17.01 \pm 9.28	22.44 \pm 3.26	22.33 \pm 3.21	39.11 \pm 18.23	10.52 \pm 3.26	20.85 \pm 11.02	31.84 \pm 22.16	14.79 \pm 1.43
MP (%)	0.67 \pm 0.77	1.02 \pm 0.72	0.26 \pm 0.31	0.85 \pm 1.40	1.06 \pm 0.63	0.65 \pm 0.31	0.49 \pm 0.82	0.93 \pm 1.05	0.61 \pm 0.14
Total (%)	24.17 \pm 11.70	23.17 \pm 10.39	24.57 \pm 3.45	36.63 \pm 3.41	43.39 \pm 18.24	14.38 \pm 3.45	31.53 \pm 15.34	39.31 \pm 21.14	22.25 \pm 1.54
Testost. (ng/mL)	0.63 \pm 0.06	0.67 \pm 0.26	1.28 \pm 0.17	1.50 \pm 0.06	0.44 \pm 0.03	1.88 \pm 0.84	0.71 \pm 0.20	0.94 \pm 0.28	1.55 \pm 0.21
Oestro. (pg/mL)	36.15 \pm 12.32	20.20 \pm 9.80	56.72 \pm 18.60	42.93 \pm 12.21	7.42 \pm 4.12	13.78 \pm 4.87	48.60 \pm 21.60	23.58 \pm 6.52	43.50 \pm 12.80

The overall values in each row with different capital letters are statistically different ($P < 0.05$). Each figure represent mean \pm standard deviation.

bulls (Table 1).

Oestrogen showed positive correlation with tail abnormalities ($r = 0.40$ and $r = 0.62$; $P < 0.05$), while testosterone showed positive correlation with head abnormalities ($r = 0.46$ and $r = 0.56$; $P < 0.05$) in bull 323 and 321, respectively. However, the effects of these hormones on other sperm abnormalities were indifferent.

Testis

Histopathological studies of testes of bull 323 showed 100% loss of germinal epithelium (DGEL) in all the three regions of the right testis. However, DGEL was 79.50, 92.08 and 98.29% in dorsal, middle and ventral

Table 2. Grading of testes of bull 323 on the basis of degree of germinal epithelial loss (DGEL) in the seminiferous tubules and grade 4+ (G4+) tubules

Part of testis	Percentage of tubules graded as:									DGEL (%)	G4+ tubules (%)
	0	1	2	3	4	4a	5	6	7		
BULL 321											
<i>Right testis</i>											
Dorsal	18.35	22.50	42.14	13.21	2.4	-	-	-	1.4	40.41	3.80
Middle	45.75	30.72	21.57	1.96	-	-	-	-	-	19.95	0.00
Ventral	1.71	23.43	62.04	9.72	3.1	-	-	-	-	47.29	3.10
TOTAL	21.94	25.55	41.92	8.30	1.38	-	-	-	0.47	35.88	2.30
<i>Left testis</i>											
Dorsal	33.33	39.39	26.52	0.76	-	-	-	-	-	23.68	0.00
Middle	18.99	31.28	46.94	2.79	-	-	-	-	-	33.38	0.00
Ventral	22.16	21.35	41.92	11.38	3.19	-	-	-	-	38.03	3.19
TOTAL	24.88	30.67	38.46	4.98	1.06	-	-	-	-	31.70	1.06
<i>Combined testes of 321</i>											
TOTAL	23.41	28.11	40.19	6.64	1.22	-	-	-	0.23	33.79	1.68
BULL 323											
<i>Right testis</i>											
Dorsal	-	-	-	-	-	100.0	-	-	-	100.0	100.00
Middle	-	-	-	-	100.0	-	-	-	-	100.0	100.00
Ventral	-	-	-	-	-	95.0	5.0	-	-	100.0	100.00
TOTAL	-	-	-	-	33.33	65.0	1.67	-	-	100.0	100.0
<i>Left testis</i>											
Dorsal	0.98	5.85	17.07	26.34	30.24	9.76	9.27	0.49	-	79.50	49.76
Middle	-	4.19	7.91	3.26	9.77	74.87	-	-	-	92.08	84.64
Ventral	-	-	1.95	2.93	85.37	5.85	3.90	-	-	98.29	95.12
TOTAL	0.33	3.35	8.98	10.84	41.79	30.16	4.39	0.16	-	89.96	76.50
<i>Combined testes of 323</i>											
TOTAL	0.16	1.68	4.49	5.42	37.56	47.58	3.03	0.08	-	94.98	88.25

Tubules are graded as 0, 1, 2, 3, 4, 4a, 5, 6, 7, on the bases of DGEL (see materials and methods for detail)

regions of the left testis, respectively, with an overall DGEL of 89.96% (Table 2). The vacuolated Sertoli's cell-only (grade 4) tubules were 33.33% and Sertoli's cell-only tubules (grade 4a) were 65.0% in the right testis (Table 2; Fig. 1). The left testis of bull 323, however, showed spermatogenic activity, and 30.16% were grade 4a tubules (Table 2). However, DGEL was high in three regions of left testis, as was the percentage of grade 4+ tubules (Table 2). Among grade 4+ tubules in the left testis, sperm stasis was observed in 0.16% tubules (Fig. 2)

Bull 321 showed spermatogenic activity; and DGEL and was 35.88% in the right and 31.70 per cent in the left testis, with higher DGEL in the ventral part in both testes (Table 2). There were only 0.23 per cent grade 4+ tubules with some tubules having sperm stasis in them (Table 1, Fig. 3).

DGEL in these bulls showed negative correlation with sperm concentration ($r = -0.98$; $P < 0.001$) and progressive motility ($r = -0.88$; $P < 0.001$) and positive correlation with dead sperm percentage ($r = 0.89$; $P < 0.001$) and total sperm abnormalities ($r = 0.99$; $P < 0.001$). DGEL and

Table 3. Comparison of different areas/components (mm) in caput, corpus and cauda of left and right epididymides of bull 321 and 323, using one-way analysis of variance

Component/ Region	Bull No. 321		Bull No. 323	
	Right	Left	Right	Left
TOTAL DIAMETER				
Caput	495.30±58.20	543.30±45.20 ^A	366.60±39.50	444.40±16.76 ^A
Corpus	501.30±53.00 ^a	846.20±6.68 ^{ABb}	417.70±28.40	401.35±32.20 ^A
Cauda	358.30±25.60 ^a	1361.00±238.00 ^{Bb}	376.10±46.90	749.93±17.90 ^B
LUMEN DIAMETER				
Caput	286.10±64.00	324.30±38.50 ^A	179.60±43.50	199.98±8.48 ^A
Corpus	229.10±62.70 ^a	627.70±27.80 ^{ABb}	173.30±42.20	145.82±25.20 ^A
Cauda	139.80±22.40 ^a	1052.70±272.00 ^{Bb}	190.50±34.40	562.91±17.70 ^B
EPITHELIAL HEIGHT				
Caput	77.77±3.21 ^A	79.16±4.30	64.81±3.10 ^a	103.70±4.90 ^{Cb}
Corpus	109.71±7.65 ^{Bb}	62.96±6.68 ^a	83.33±6.33	83.33±2.27 ^B
Cauda	71.29±2.65 ^A	66.66±4.54	62.22±10.10	51.85±3.70 ^A
MUSCLE THICKNESS				
Caput	26.85±1.71 ^A	29.86±1.46 ^A	28.70±1.71 ^b	18.52±1.85 ^{Aa}
Corpus	26.39±3.49 ^{Aa}	46.29±6.68 ^{ABb}	37.78±3.24	44.44±4.54 ^B
Cauda	37.96±2.23 ^{Ba}	87.50±17.20 ^{Bb}	30.56±4.92	50.00±0.10 ^B

Values of each component in each row with different small letters and in each column with different capital letters are statistically different ($P < 0.05$). Each figure represent mean±standard deviation

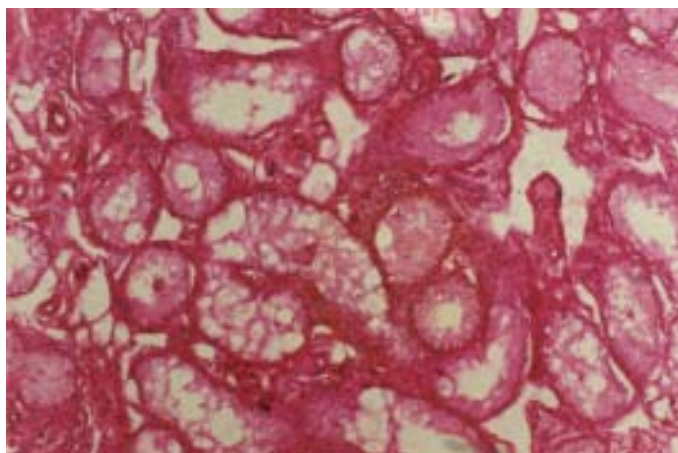


Fig.1. Photomicrograph of left testis (dorsal region) of bull No. 323 showing seminiferous tubules lined with Sertoli's cells with or without vacuolation. H&E; ×400; scale bar = 85 μm

G4+ tubules correlated positively with testosterone ($r = 0.98$, $r = 0.99$; $P < 0.001$) and negatively with oestrogen ($r = -0.87$, $r = -0.88$; $P < 0.001$).

Table 4. Comparison of total diameter, lumen diameter, epithelial height and muscle thickness (μm) of left and right epididymides of bull No.321 and 323 using one-way analysis of variance

Component/ Region	Right Epididymis	Left Epididymis	Both Epididymides
Total diameter			
Bull No. 321	445.40±30.80 ^a	821.40±111.00 ^{Bb}	627.40±64.80 ^B
Bull No. 323	383.30±25.30 ^a	518.80± 52.50 ^{Ab}	427.00±26.30 ^A
Lumen diameter			
Bull No. 321	217.00±32.30 ^a	579.20±107.00 ^{Bb}	392.30±62.80 ^B
Bull No. 323	183.30±21.80	287.20± 61.50 ^A	216.80±25.70 ^A
Epithelial height			
Bull No. 321	83.30±4.59 ^B	72.60±3.32	78.10±2.98
Bull No. 323	68.00±5.30 ^{Aa}	80.00±6.99 ^b	71.90±9.29
Muscle thickness			
Bull No. 321	30.90±1.90 ^a	48.50±7.81 ^b	39.40±4.16
Bull No. 323	31.80±2.55	38.30±4.72	33.90±2.32

Values of each component in a row with different small letters and in a column with different capital letters for two bulls are statistically different at $P < 0.05$. Each figure represent mean±standard deviation.

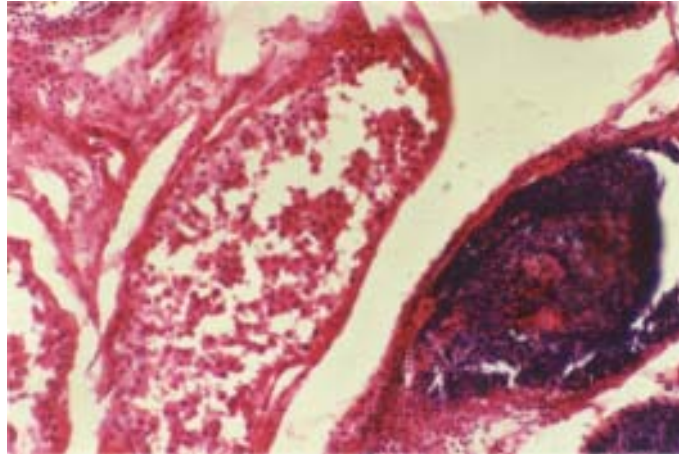


Fig.2. Photomicrograph of left testis (dorsal region) of bull No. 323 showing seminiferous tubules with sperm stasis (right side). H&E stain, $\times 600$; scale bar = $43 \mu\text{m}$

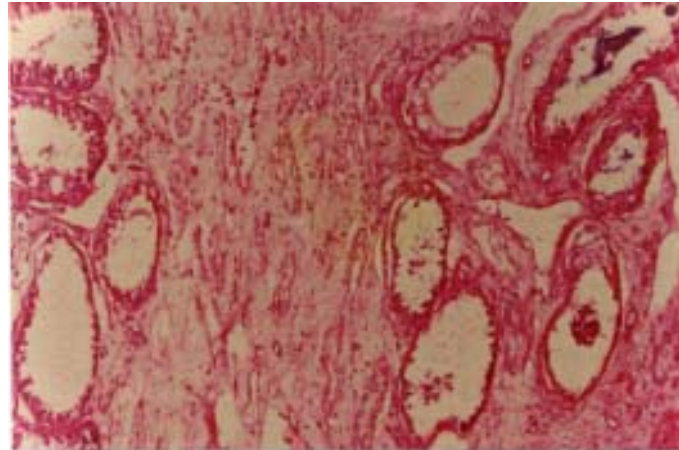


Fig.3. Photomicrograph of right testis (dorsal region) of bull No. 321 showing collapsed tubules (center) replaced by fibrous tissue. Tubules on sides are showing advanced degree of DGEL with vacuolated Sertoli's cells lined tubules (upper right side) and most of other tubules are lined with single layer of germinal epithelium. H&E; $\times 400$; scale bar = $85 \mu\text{m}$

Epididymis

Total and lumen diameter in epididymis was significantly ($P < 0.05$) greater in the left epididymis in both bulls, except for lumen diameter in bull 323 (Table 4). Overall total and lumen diameter was greater ($P < 0.05$) in bull 321 than in bull 323 (Table 4). Total and lumen diameter was greater ($P < 0.01$) in the caudal region of the left epididymis compared with other regions of the same sides, and its right counterpart in both bulls (Table 3). Both total and lumen diameter was negatively ($P < 0.05$) correlated with DGEL ($r = -0.40$ and $r = -0.39$, respectively).

Epithelial height (EH) in caput region was greater ($P < 0.05$) in the left side of bull 323, while it was greater ($P < 0.05$) in the corpus region of the right epididymis in bull 321 (Table 3). Overall epithelial height in the left epididymis was greater ($P < 0.05$) than in the right epididymis in bull 323. The EH showed a negative ($P < 0.001$) correlation with seminal plasma testosterone ($r = -0.87$) and positive ($P < 0.001$) with oestrogen ($r = 0.89$).

Epididymal muscle thickness was 39.40 ± 4.6 and 33.90 ± 2.32 μm in bulls 321 and 323, respectively. Muscle thickness was greater ($P < 0.05$) in the left epididymis of bull 321 (Table 4). Muscle thickness was significantly ($P < 0.05$) greater in the caudal region in the left epididymis in both bulls (Table 3).

Discussion

Lower values for semen parameters in buffalo bulls with a scrotal circumference of less than 30 cm were in line with those of VEERAMACHANENI et al. (1986) and MADRID et al. (1988) in cattle bulls. VEERAMACHANENI et al. (1986) reported more than 90 per cent as abnormal sperms in bulls with $SC < 30$ cm to be associated with testicular lesions and/or small testes. An increase in sperm abnormalities has also been reported by RAO and BANE (1985) in bulls with testicular degeneration. Lower testosterone in bulls with $SC < 30$ cm than in bulls with $SC > 30$ cm has also been reported by VEERAMACHANENI et al. (1986) in serum which they correlated with atrophy of Leydig's cells.

The negative correlation of SC with testosterone in abnormal ($r = -0.42$, $r = 0.61$; $P < 0.05$, in bull 323 and 321, respectively) than in healthy bulls was interesting. It has been reported that the relationship between

hormone concentration and parameters of testicular functions are quite variable, and abnormal spermatogenesis sometimes occurs concurrently with endocrine abnormalities (BLANCHARD et al., 1991). They further reported that concentration of hormones in the blood stream (particularly gonadotropin and testosterone) is likely to be abnormal, but it is unclear whether this is a secondary change that reflects testicular injury or a factor that contributes to further derangement of testicular function. Measurements of hormone concentrations in sub-fertile stallions have demonstrated that serum gonadotropins are sometimes abnormally low or high (BURNS and DOUGLAS, 1985).

Present findings of oestrogen correlation with tail abnormalities in buffalo bulls were similar to those produced by giving exogenous oestrogen in cattle bulls (CUPPS and BRIGGS, 1965). This was related to the effect of oestrogen on epididymal epithelium in the tail region of the epididymis (CUPPS and BRIGGS, 1965). The epithelial height in the tail region of epididymis of bull number 323, having higher sperm abnormalities, in the present study was also lower ($P < 0.05$) in the left, while numerically lower in the right epididymis (Table 3), which might be an indication of epididymal epithelium dysfunction. Higher oestrogen in bull 321 is probably related either with sperm concentration, higher abnormalities or inflammation/oedema of the scrotum.

Testis

The lack of vacuolation in grade 4a tubules indicated that germinal cells were never present (OTT, 1991) and are reported to be characteristics of hypoplastic tubules (CARROL and BALL, 1970), while the vacuolated Sertoli's cell-only tubules (grade 4) indicated a degenerative process in these tubules (VEERAMACHANENI et al., 1986; OTT, 1991). It has been reported that frequency of tubules with total germinal epithelial loss was greater in bulls having SC < 30 cm (VEERAMACHANENI et al., 1986) as was the case during the present study, particularly in bull 323.

Present findings suggest that loss of germinal epithelium causes lower sperm concentration and motility and higher sperm abnormalities, which were in line with those of VEERAMACHANENI et al. (1986) and ROB (1967). The poor motility seen in these bulls might be due to a defect that sperm acquire during spermatogenesis and/or during sperm maturation in epididymis, causing a higher proportion of tail abnormalities. The latter could

also be due to endocrine dysfunctions.

Correlation of DGEL and testosterone suggests that with an increase in DGEL and G4+ tubules, testosterone concentration decreases, probably due to feed-back depression of the secretion of this hormone from Leydig's cells because of damage to Sertoli's cells. However, Leydig's cells hyperplasia around degenerated tubules has been reported (AOKI and FAWCETT, 1978; VEERAMACHANENI et al., 1986). VEERAMACHANENI et al. (1986) reported Leydig's cell atrophy in areas of testis where degeneration of tubules was severe, resulting in loss of Sertoli's cells. It may be possible that the testosterone produced by the Leydig's cell is not being transported to the tubular lumen, perhaps due to loss of receptors on Sertoli's cell or loss of the latter, or due to some other unknown reasons, resulting in lower concentration of testosterone in seminal plasma.

Epididymis

DGEL in bull 321 was low, which may correspond to higher lumen diameter and epithelial height in the epididymis in this bull. This suggests that with less testicular function there is a decrease in the lumen diameter of the epididymis. VEERAMACHANENI et al. (1986) reported that a decrease in epididymal weight would be expected in bulls with lower testicular function.

The present findings of EH were slightly higher than those reported by VEERAMACHANENI et al. (1986) of 57.00 to 67.5 cm in the caput region and 49.9 to 53.9 cm in the caudal region in cattle bull with SC < 30 cm. The variation in the two studies might be due to difference in species, or may be subjective.

Epithelial height in the present study showed a negative correlation with DGEL ($r = -0.88$; $P < 0.001$) while positive with oestrogen ($r = 0.89$; $P < 0.001$), which agreed with VEERAMACHANENI et al. (1986) who also reported a negative correlation of EH with DGEL in both caput ($r = -0.85$) and cauda ($r = -0.34$) epididymis. This probably indicates that the height of the epithelium and its function is mainly influenced and/or stimulated by the presence of oestrogen in semen, as oestrogen receptors are identified on epididymal epithelium in rabbits (DANZO and ELLER, 1979).

Conclusions

It can be concluded from the present study that buffalo bulls with testicular hypoplasia and degeneration have lower levels of testosterone in semen. However, oestrogen was higher in bulls with testicular degeneration. Total and lumen diameter of the epididymis is higher in the left caudal epididymis, and epididymal epithelium has a direct relation to DGEL and testosterone, but an inverse relation with oestrogen.

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SAŽETAK

Istraživanje je provedeno na šest bivola (*Bubalus bubalis*) tijekom jedne godine. U dva bivola (321 i 322) utvrđena je sperma loše kvalitete. U njih je također utvrđen manji opseg skrotuma i velik postotak uginulih i abnormalnih spermija. Koncentracija testosterona u njihovoj spermi bila je niža u odnosu na onu u zdravih životinja. Razina estrogena bila je niža u bivola 323, a viša u bivola 321. Histopatološkom pretragom tkiva testisa bivola 323 utvrđena je potpuna odsutnost germinativnog epitela u desnom testisu te 89,6 %-tna odsutnost u lijevom testisu. U bivola 321 gubitak germinativnog epitela iznosio je 35,88% u desnom i 31,70% u lijevom testisu, s većim stupnjem gubitka u ventralnim dijelovima testisa. Ukupni promjer i promjer lumena bio je veći u kaudalnom području lijevog epididimisa ($P < 0,01$). Visina epitelnih stanica na području glave lijevog epididimisa bila je veća ($P < 0,05$) u bivola 323. Gubitak germinativnog epitela u području glave desnog epididimisa bivola 321 bio je u negativnoj korelaciji ($P < 0,001$) s koncentracijom sperme ($r = -0,98$), progresivnom pokretljivošću ($r = -0,88$) i estrogenom ($r = -0,87$), a u pozitivnoj korelaciji ($P < 0,001$) s postotkom uginulih spermija ($r = 0,89$), abnormalnih spermija ($r = 0,99$) i testosteronom ($r = 0,98$). Visina epitelnih

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stanica bila je u negativnoj korelaciji ($P < 0,001$) sa stupnjem gubitka germinativnog epitela ($r = -0,88$) i testosterona ($r = -0,87$), a u pozitivnoj ($P < 0,001$) s razinom estrogena ($r = 0,89$).

Ključne riječi: sperma, epididimis, testis, histopatologija, testosteron, estrogen, bivol
