



Testicular biometry and seasonal variations in semen parameters of Black Bengal goats

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ABSTRACT

Body weight, growth rate and testicular biometry of Black Bengal bucks (19) belonging to different age groups were studied. Further, this study was extended to delineate the histological changes in the testes with advancement of ages and to evaluate the seasonal variations in the important semen parameters. The body weight differed significantly across the age groups but the body weight gain (g/d) was higher in the age groups of 0–4 and 17–20 months. The mean scrotal circumference increased with the advancement of age and attained maximum circumference at the age of 12 months. The mean testicular diameter increased significantly till 10th month of age. All these biometrical parameters were highly correlated with each other, and with age and body weight. The histological examination of testes revealed an effect of age on the seminiferous tubules and stages of the spermiogenesis. At 3.5 months of age, lumen of seminiferous tubules became distinct with the abundance of spermatozoa. These observations along with testicular biometry indicated the age at which bucks attained puberty. Significant effect of the seasons (e.g., spring and summer) was observed on the semen parameters with the improvement in the progressive motility, percentage of live spermatozoa, chromatin integrity and a drop in membrane integrity during summer. These results will provide an important impetus in selection of breeding bucks of Black Bengal breed.

Key words: Acrosome integrity, Histology of testis, HOST, Membrane integrity, Progressive motility, Semen parameter, Testis

The major limitations of goat production are the lack of good breeding bucks and the seasonal nature of semen production and quality (Kridli *et al.* 2007). Black Bengal breed of goats is known for its prolificacy and excellent meat and skin quality in India (Singh *et al.* 1991). The acute shortage of genetically superior buck is one of the major constraints for the efficient propagation of this breed. Body weight and growth rate may serve as a useful predictor of live weight since Black Bengal goats are being reared primarily for meat production (Islam 2001). The most accurate way to test the genetic worth of a sire is to perform breeding soundness evaluation (BSE) i.e., fertility potential of a buck based on an examination that includes tests for physical soundness, testicular size, semen quality, and in some cases libido/mating ability (Okere *et al.* 2011). In the

present study we reported the testicular biometry and the change in seminiferous epithelium of testis at different ages and the seasonal variations in semen parameters of Black Bengal goat.

MATERIALS AND METHODS

Animals: Male Black Bengal goats (19), maintained in the experimental goat farm of the laboratory at the Institute, were included in the study. All the measurements (scrotal and testicular biometry) were initiated at the birth and continued till 20 months of age. For biometrical studies, animals were classified into 6 different age groups: A, at birth; B, up to 4 months; C, 5 to 8 months; D, 9 to 12 months; E, 13 to 16 months; and F, 17 to 20 months. To commensurate the results of biometry, testes of 4 male kids of different age groups were subjected to histological examination. For studying the semen parameters, adult bucks (3) of 2 years of age were included.

Recording of age and body weight (BW), Scrotal and testicular biometry: The age of goats were calculated from the date of birth. The body weight (kg) of each animal was recorded at 30 days interval. Scrotal circumference (SC) was measured with a measuring tape in centimeters (cm) and the testicular diameter (mm) was measured with a vernier caliper (Raji *et al.* 2008). All these measurements

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were taken on the same day when body weight was recorded.

Evaluation of semen parameters: Ejaculates were collected from the trained bucks (3) using an artificial vagina (AV). To assess the effects of seasons on semen parameters, semen was collected during two consecutive seasons: spring (February) and summer (May). Semen was evaluated for different parameters. To assess progressive motility, semen was diluted with sodium citrate glucose buffer (1:10). One drop of the diluted semen was placed on a glass slide and after placing a cover slip it was immediately examined high power (40×) objective of phase contrast microscope. To calculate the live/dead percentage of sperms, 2 methods namely, Eosin-Nigrosin (Rodríguez-Martínez 2000) and carboxyfluorescein diacetate/propidium iodide (CFDA/PI; Peđa *et al.* 1998) based differential staining were used. Acrosomal integrity was determined by using both Giemsa (Kumar and Singh 2003) and Fluorescein isothiocyanate coupled with peanut agglutinin (FITC-PSA; Sukardi *et al.* 1997) based staining methods. Hypo-osmotic swelling test (HOST) was used to assess the functional integrity of the sperm tail membrane (Jeyendran *et al.* 1984). Sperm chromatin structure assay was performed using chromomycin A₃ according to Bianchi *et al.* (1996).

Histological study: Male kids (4) of different ages (e.g., 3 days, 1, 3.5 and 7 months) were included. Under local anesthesia, one of the testes was removed. Tissue sections were fixed and stained with haematoxylin and eosin. Slides were examined under 10× and 40× magnifications of a compound microscope.

Statistical analysis: Mean and standard error for body weight and testicular biometric data was analyzed using SPSS software program (ver. 16). Testicular biometry with relation to age was studied by performing one-way ANOVA analysis. Duncan multiple range test (DMRT) was done to observe significant difference among mean values. Pearson's correlation analysis was performed to study correlation among different traits. To study the seasonal difference in semen characteristics t-test was applied.

RESULTS AND DISCUSSION

Body weight and growth rate: Mean body weights (Table

1) increased steadily from birth till 20 months of age. The body weight also differed significantly across the age groups ($P < 0.01$). However, the body weight gain (g/d) was significantly high in the age group of 0–4 and 17–20 months and almost same in remaining age groups. The body weight of Black Bengal bucks significantly increased with the advancement of age which is in agreement with previous studies (Rahman 2007, Kabiraj *et al.* 2011) in Black Bengal bucks.

Scrotal circumference: The mean SC increased significantly with the advancement of age ($P < 0.01$; Table 2) and attained maximum value after 12 month of age. The SC is an indirect measurement of testicular weight and a reliable indicator of testicular growth and spermatogenic capacity of the testis. Scrotal circumference, being the most heritable component of fertility, is proposed to be included in breeding soundness evaluations (Bailey *et al.* 1996). It was extensively used in predicting the reproductive capacity of male domestic animals. Shamsuddin *et al.* (2000) and Kabiraj *et al.* (2011) observed the significant increase in SC of Black Bengal bucks also with the advancement of age.

Testicular diameter: The mean testicular diameter increased till 10th month of age and did not increase further significantly (Table 1). The testicular diameters of age group B to E were significantly higher than that of group A ($P < 0.01$).

Correlation coefficients (r) between age, BW, SC and average testicular diameter: All the biometrical parameters studied were significantly correlated with each other and age ($P < 0.01$; Table 2). Body weight showed high (> 0.8) correlation with age, SC and testicular diameter. In the present study, all the parameters of testicular biometry

Table 2. Correlation coefficients (r) between age, body weight, scrotal circumference and testicular diameter in Black Bengal buck (19)

Parameter	Age	Body weight	Scrotal circumference
Body weight	0.838**	–	–
Scrotal circumference	0.763**	0.784**	–
Testicular diameter	0.681**	0.827**	0.923**

** $P < 0.01$.

Table 1. Comparative studies of testicular biometry of Black Bengal bucks with respect to age and body weight (mean±SE)

Age group	No. of kids	Body weight (kg)**	Average scrotal circumference (cm)**	Average testicular diameter (mm)**	Body weight gain (g/d)*
Group-A (At birth)	11	1.86 ^a ±0.11	5.50 ^a ±0.23	8.55 ^a ±0.38	–
Group- B (0 to 4 m)	19	6.23 ^b ±0.32	11.71 ^b ±0.59	24.1 ^b ±1.30	32.57 ^b ±3.58
Group- C (5 to 8 m)	13	8.20 ^c ±0.53	16.90 ^c ±0.41	34.1 ^c ±1.08	19.79 ^a ±1.7
Group- D (9 to 12 m)	11	10.56 ^d ±0.46	18.29 ^{cd} ±0.26	36.4 ^c ±1.18	19.79 ^a ±2.0
Group-E (13 to 16 m)	11	12.86 ^e ±0.41	18.67 ^d ±0.28	37.0 ^c ±0.93	20.83 ^a ±1.5
Group-F (17 to 20 m)	11	15.31 ^f ±0.79	–	–	27.97 ^b ±3.8

Means with different superscripts within the column differ significantly (** $P < 0.01$, * $P < 0.05$); SE, standard error.

showed a significant correlation among themselves as well with the age and body weight. Similar findings were reported in Black Bengal (Kabiraj *et al.* 2011) and other breeds (Raji *et al.* 2008) of goat.

Histology of testes: The histological examinations (Fig. 1) revealed the effect of age on the seminiferous tubules (ST) and stages of the spermiogenesis. Increase in tubular

convolution and decrease of inter-tubular space was marked as the age of the animals advanced. Transverse section of the testes from 3– day old kid showed smaller sized ST amidst connective tissue (Fig.1a), which were lined by undifferentiated germ cells or spermatogonial stem cells interspersed with primitive polygonal or cone-shaped sertoli cells (Fig.1a). The lumens of the ST were indistinct

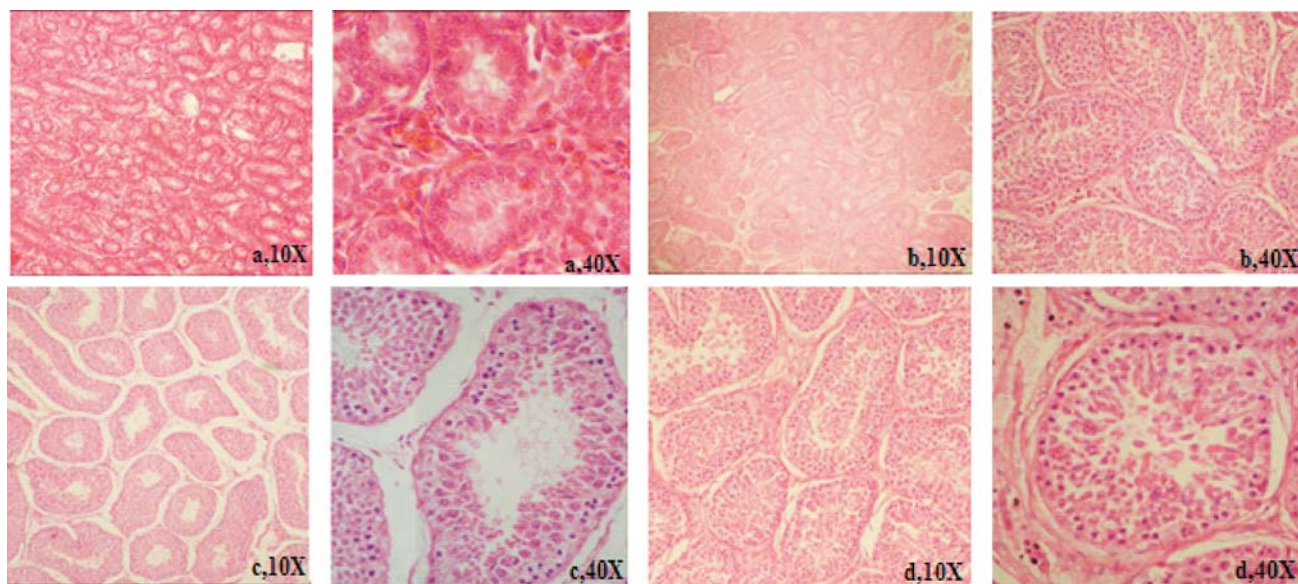


Fig. 1. Photomicrograph (H & E stain; at 10× and 40×) of testis of Black Bengal bucks at different ages: (a) 3rd day; (b) 1 month; (c) 3 months; and (d) 7 months.

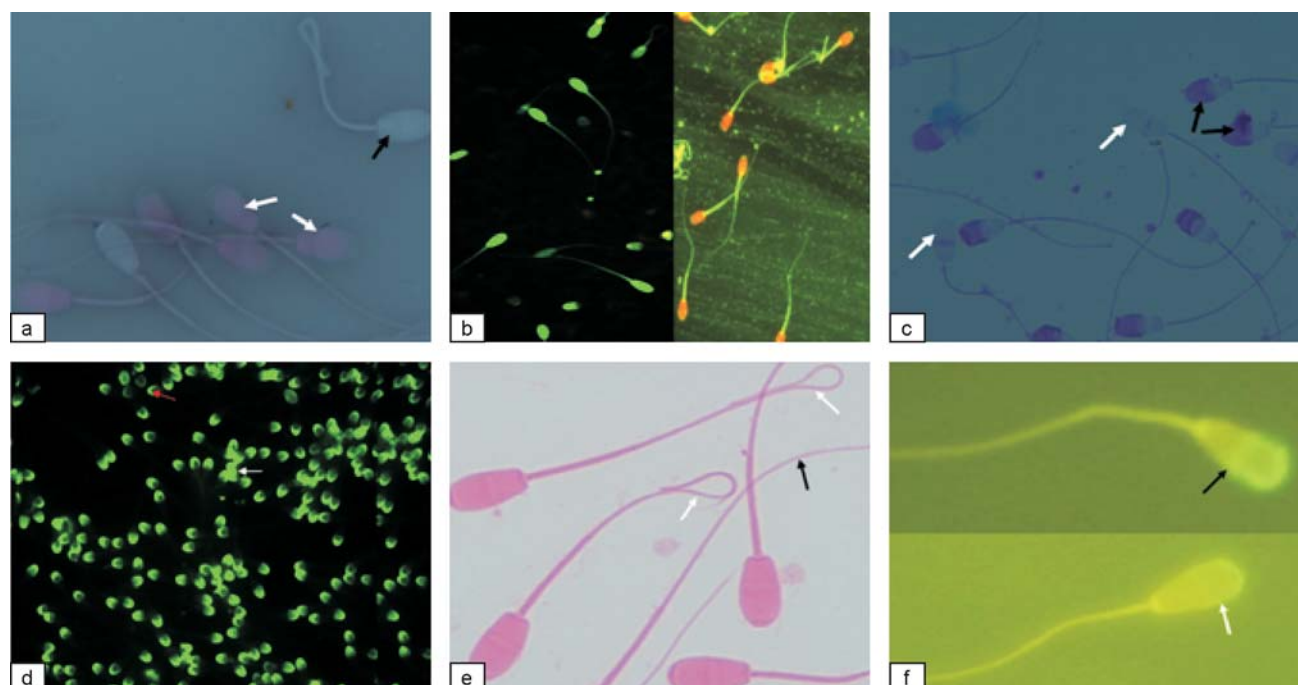


Fig. 2 (a-f). Assessment of semen parameters in Black Bengal bucks: Live and dead count (%) of spermatozoa using (a) Eosin-nigrosin method (black and white arrows indicate live and dead sperm, respectively); (b) CFDA/PI method (live and dead sperms stained green and red, respectively); acrosome integrity using (c) Giemsa staining (black and white arrows indicate intact and reacted acrosome, respectively); (d) FITC-PSA method (white and red arrows indicate intact and reacted acrosome, respectively); membrane integrity of sperm using (e) HOST (white arrow indicate HOST+ve and black arrow indicate HOST-ve sperm); sperm chromatin integrity using (f) chromycin A₃ (CMA₃) assay (black and white arrows indicate CMA₃+ve and -ve sperm, respectively).

Table 3. Comparative study for seminal parameter in Black Bengal bucks (n=3) during spring and summer months

Descriptive	Seasons		Significance
	Spring	Summer	
Progressive motility (%)	65.00±1.17	70.55±1.30	**
Live (%), Eosin-Nigrosin method	61.11±0.60	64.44±1.33	*
Live (%), CFDA/PI method	49.44±1.23	49.44±1.75	NS
Acrosome integrity (%), Giemsa	84.72±0.27	83.33±0.33	NS
Acrosome integrity (%), FITC-PSA	88.05±0.55	86.11±1.38	NS
HOST positive (%)	82.77±0.27	76.66±2.35	*
Chromomycin A ₃ positive (%)	70.00±0.00	62.77±2.22	**

**P<0.01, * P< 0.05, NS, not significant.

because of long cytoplasmic process of the sertoli cells, referred as sex cords and there was no evidence of spermatogenesis (Fig.1a). However, in the testes of 1-month old kid, STs were larger in which abundant germ cells, prespermatogonia, started migrating towards the basement membrane of the sex cords and differentiating into primary/secondary spermatocytes and spermatids (Fig.1b). The lumens of the ST were distinct and filled with detached spermatids (Fig.1b). By the age of 3 months (Fig.1c) the spermatozoal bunches were prominently seen embedded in the cytoplasmic process of the sertoli cells and the lumen of the tubules was broader and contained moderate number of spermatozoa. In seven months, the ST have well developed series of different cell types and the lumen contained plenty number of spermatozoa along with few detached degenerated/apoptotic cells (Fig.1d). It was observed that undifferentiated germ cells (i.e., pre-spermatogonia) started migrating towards the basement membrane of the sex cords as early as 1 month of age (Sarma and Devi 2012). Further, increase in tubular convolution and decrease of intertubular space which is indicative of well formed reproductive stage of an adult animal was marked as the age of the animals advanced. These microscopic changes were well corroborated with the testicular morphometry. Histological examination and testicular biometry indicated that bucks attained puberty at 3–4 months age and became reproductively active at 7 months of age as indicated by the abundance of spermatozoa in the lumen of ST.

Effect of seasons on semen characteristics: Semen parameters like progressive motility, live/dead count and membrane and chromatin integrity varied significantly between the seasons (Table 3; Fig.2). The progressive motility improved ($P<0.01$) in spring to summer. The percentage of live spermatozoa, as estimated using eosin-nigrosin staining, also increased significantly ($P<0.05$) from spring to summer. The mean value of per cent spermatozoa with abnormal chromatin differed ($P<0.01$) between these 2 seasons while the integrity of membrane lowered ($P<0.05$)

during summer than that in spring. However, remaining parameters did not show any significant changes.

We evaluated different semen parameters of Black Bengal bucks using different laboratory staining techniques. In this study, in comparison to eosin-nigrosin staining, live count of spermatozoa was under estimated by 10% while using CFDA/PI. This difference between eosin-nigrosin and CFDA/PI might be due to the difference in time of exposure to the stain, which is only a few seconds for eosin-nigrosin and 10–30 min for PI (Brito *et al.* 2003). Acrosomal integrity provides an indicator for fertilizing capability of spermatozoa. The results obtained using FITC-PSA based staining was highly correlated with those obtained using Giemsa staining in the present study. Hypo osmotic swelling test (HOST) is used for identification of viable sperm in a non-destructive manner (Jeyendran *et al.* 1984). The chromatin integrity of spermatozoa was evaluated using chromomycin A₃ test, an indirect indicator of protamine deficiency and fertility status of spermatozoa (Foresta *et al.* 1992).

In the present study, a significant variation was observed in the semen parameters of Black Bengal bucks between the seasons. Others also reported significant seasonal variation in the semen production in goats (Webb *et al.* 2004, Zarazaga *et al.* 2009) and bulls (Sarder 2007). However, some studies could not observe any seasonal variation in the semen quality of Angora and Boer bucks (Greyling and Grobbelaar 1983) and buffalo bulls (Koonjaenak *et al.* 2007). In contrast to our result, Elsheikh *et al.* (2013) reported a decrease in livability of crossbred goat sperm in summer season.

In conclusion, we demonstrated a strong association of testicular biometry with age and body weight in Black Bengal breed of goat. All the parameters of testicular biometry increased significantly with the advancement of age. Histological studies also indicated that SC may serve as an accurate predictor of the puberty. Our results on testicular biometry and the seasonal variations of semen characteristics would be helpful in selection of breeding buck in Black Bengal goat.

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