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Anatomy and histomorphometry of accessory reproductive glands of the Black Bengal buck

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SUMMARY

The Black Bengal buck has a notable goat genetic resource. Seminal fluids from male accessory reproductive glands contain nutrients and other substances necessary for the optimum motility and fertility of spermatozoa, and act as a buffer against excess acidity of the female genital tract. This study was conducted to understand the anatomy and histomorphometry of accessory reproductive glands of the Black Bengal buck of Bangladesh. Anatomical study was performed immediately after euthanasia, and histomorphometrical study was performed by Hematoxylin and Eosin staining. There was a difference in biometric values between right and left accessory reproductive glands within the same buck. The average length, width and weight were 1.653 ± 0.025 cm, 1.146 ± 0.015 cm and 1.486 ± 0.010 gm, respectively for the vesicular glands, and 1.045 ± 0.037 cm, 0.918 ± 0.042 cm and 0.537 ± 0.029 gm respectively for the bulbourethral glands. The secretory units of the vesicular glands were lined by pseudostratified columnar epithelium consisting of tall columnar type cells and short basal type cells. The vesicular secretion was stored in the lumen of secretory units. We also found the myoepithelial cells around the secretory cells of glandular end pieces of vesicular gland in all bucks that helped in the excretion of vesicular secretion. All the bucks in the present study had only pars disseminata of prostate gland, and located in the propria-submucosa of the dorsal wall of the pelvic urethra. The secretory units and intraglandular ducts of the prostate gland were lined with simple cuboidal to columnar epithelium with basal cells. The bulbourethral glands were surrounded by the bulboglandular muscle, and the secretory units were lined with tall simple columnar epithelium and occasional basal cells. Most of the columnar cells were of the mucous type, with the nuclei basally placed and the cytoplasm contained the secretion granules in Black Bengal bucks.

Key words: Anatomy – Histomorphometry – Accessory reproductive glands – Black Bengal buck

INTRODUCTION

The Black Bengal goat has a notable position amongst the world goat genetic resources (Kabiraj, 2011). IAEA (International Atomic Energy Agency, 2014) claimed that among the world's developing countries, Bangladesh is home to one of the richest treasures, prized Black Bengal goats (http:www.iaea.org). The Black Bengal goat is used as a tool to promote sustainable livelihoods in rural Bangladesh. Since 1998, the Bangladesh Livestock Research Institute has been attempting to improve the Black Bengal goat through selective breeding (Faruque et al., 2010). Recently artificial insemination (AI) of goats has become popular in order to improve the genetics of the goat herd (http:www.uky.edu/Ag/Animal Sciences/goats). Male reproduction is one of the important aspects in the livestock. Acute shortage of genetically superior bucks throughout the country is one of the

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major constraints of goat production in Bangladesh (Husain, 2004). Superior buck selection seems to be a very important and alternative approach to boast up the production potential. Therefore, during the selection of breeding bucks special attention should be given to age, body weight, soundness of the reproductive organs and quality of ejaculated semen (Kabiraj, 2011). Male accessory reproductive glands secrete additional fluids, which, being combined with the sperm and other secretions from the epididymis, form the semen. They contribute greatly to the fluid volume of semen. Their secretions are a solution of buffers, nutrients and other substances needed to assure optium motility and fertility of spermatozoa (McDonald, 1980; Bone, 1988), and act as a buffer against excess acidity of the female genital tract (Cunningham, 2002; Frandson and Spurgeon, 1992). The secretory capacity of the accessory glands has relevance mainly to the blood concentration of testosterone. Although there are some research reports on bovine testicular measurements (Coulter and Foote, 1979), the biometry of bull testes (Gofur et al., 2007), the development of testes in the Assam goat (Baishya et al., 1987), and the histology and histochemistry of the testes of the Black Bengal goat (Kabiraj, 2011), no substantial study has yet been undertaken with accessory reproductive glands of Black Bengal buck. Therefore, the present study has been carried out in order to understand the anatomy and histomorphometry of accessory reproductive glands of the Black Bengal buck which will provide valuable information to the anatomists, pathologists, theriogenologists and cell biologists.

MATERIALS AND METHODS

Five Black Bengal bucks (8 months old, apparently healthy) were used in this study. The study was conducted in the Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Bangladesh during the period from January to September, 2014.

Anatomical study

After euthanasia, the accessory reproductive glands (vesicular glands, prostate glands and bulbourethral glands) were collected. The location and shape were observed, and the length, width and weight of them were measured and preserved in 10% formaline immediately after collection.

Histological study

The selected samples were processed in the laboratory for histological studies following standard histological techniques, and the paraffin sections then cut at 6 µm thickness using microtome. After cutting, the sections were floated on lukewarm water in a floatation bath at 37°C for stretching, and then the sections were attached on cleaned glass slides using egg albumin and dried on a hot plate of slide warmer boxes. The sections then were stained with routine Hematoxylin and Eosin stain (Gridley, 1960) for histomorphological study. After staining, the sections were rehydrated in descending grades of alcohol, cleared in xylene and mounted with “DPX”. The stained sections of vesicular glands, prostate glands and bulbourethral glands were studied thoroughly under compound microscope using 4, 10, 40 and 100 objectives.

The capsular thickness of bulbourethral glands, the thickness of lamina muscularis and adventitia of vesicular gland, and the histological cross sectional length and width of the glandular secretory units of vesicular gland and prostate glands were measured (at 10X) using calibrated scale by oculometer (12.5X). The photographs of this study were taken from the studied slides with the help of OPTICA photo-microscope (B-350), Italy. All data were analyzed with the help of the SPSS Statistics version 20.

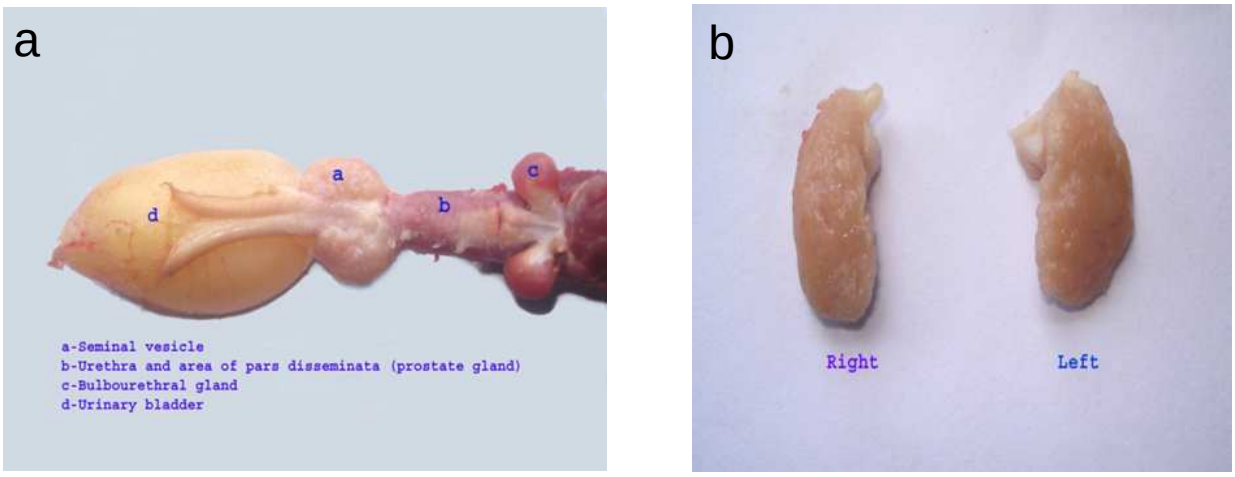


Fig 1. (a) Accessory reproductive glands of the Black Bengal buck. **(b)** Vesicular glands of the Black Bengal buck.

RESULTS AND DISCUSSION

Anatomical study

Vesicular gland

Vesicular glands were the paired accessory sex glands in bucks.

Location: The vesicular glands (seminal vesicle) of all bucks were situated on the caudodorsal aspect (near to the neck) of the bladder and the initial part of the pelvic urethra, lateral to the ampullae of the ductus deferens (Fig. 1a). The present observation was similar to the Getty (1975), Dyce et al. (2002), Pineda and Dooley (2003) and Youngquist and Threlfall (2007). It was also found that the left vesicular gland was situated 2-3 mm caudal than the right one (Fig. 1a).

Size and weight: The vesicular glands of the same species were somewhat unsymmetrical in size (in length, width and weight) (Fig. 1a-b). Such a comment was made by Getty (1975) and Khalaf and Merhish (2010) in goats. In the present observation, the average length, width and weight of the left vesicular glands were higher than the right glands (Table 1). These were similar to the findings of Archana et al. (2009) in gaddi goats.

Shape: The vesicular glands were grossly lobulated, irregular and more or less solid. The present study identified the vesicular glands easily by their knobby, or mulberry, or glandular appearance (Fig. 1a-b). The observation has an agreement with Athure et al. (1996), Dyce et al. (2002) and Archana et al. (2009), and a similarity to the anatomical description of vesicular gland in bull by McDonald (1980) and Bearden and Funguay (2000).

Prostate gland

The prostate gland was the unpaired accessory reproductive glands in bucks. Although generally the prostate gland consists of two portions — the compact or external portion (corpus prostate) and the disseminate or internal portion (pars disseminata) — it consists of only pars disseminata in goats, which surrounds the pelvic urethra (Bacha and Wood, 1990; Getty, 1975; Dyce et al., 2002; Dellmann and Eurell, 1998; Pathak et al., 2012). The present observation was the similar to the test, as the corpus prostate was not found in any bucks of this study. This indicated that the prostate gland of bucks consisted only of pars disseminata. Hence, it was impossible for gross anatomical study, but the samples from the dorsal portion of the pelvic urethra were collected for histological study.

Bulbourethral gland

The paired bulbourethral glands are present in all domestic mammalian species except the dog (Dyce et al., 2002; Getty, 1975). They consist of right and left club-shaped independent lobes in all bucks of present study, which lie on the dorsal surface of the caudal part of the pelvic urethra at the level of the ischial arch under the covering of a layer of fibromuscular tissue and was closely related to the bulb of penis (Fig. 1a). The bulbourethral glands are well visible and somewhat round bodies. The current observation is consistent with the description in domestic animals by Getty (1975), Ghosh (1995), Dyce et al. (2002) and Pineda and Dooley (2003). The present study found a significant difference on anatomical parameters between right and left bulbourethral glands. The length and weight of left bulbourethral glands were significantly ($p<0.05$) higher than the right bulbourethral glands in Black Bengal buck (Table 1).

Histological study

Vesicular Gland

Histologically, the vesicular gland of Black Bengal bucks was a lobulated compound tubuloalveolar gland similar as described by Kundu (1980), Sudhakar et al. (1986) and Dellmann and Eurell (1998). Each lobule of the gland showed folded tunica mucosa (comprising of lamina epithelialis and lamina propria), tunica propria submucosa, tunica muscularis and tunica adventitia (Fig. 2a -b). A similar description has been given by Sudhakar (1982) and Mollineau et al. (2009) in other domestic animals. The mucosa was folded and lined with pseudostratified columnar epithelium containing tall columnar cells and small, spherical, often sparse basal cells. They contained eosinophilic cytoplasm and their nuclei were ovoid in shape (Fig. 2b). The present findings were similar

Table 1. Length, width and weight of Black Bengal buck vesicular gland and bulbourethral gland (mean ± SD).

Measurement	Vesicular gland			Bulbourethral gland		
	Right	Left	General	Right	Left	General
Length (cm)	1.636 ± 0.015	1.667 ± 0.031	1.653 ± 0.025	1.01 ± 0.044	1.09 ± 0.036*	1.045 ± 0.038
Width (cm)	1.136 ± 0.015	1.160 ± 0.017	1.146 ± 0.015	0.887 ± 0.076	0.973 ± 0.078	0.918 ± 0.042
Weight (gm)	1.461 ± 0.041	1.511 ± 0.025	1.486 ± 0.012	0.490 ± 0.027	0.570 ± 0.043*	0.537 ± 0.017

*p<0.05

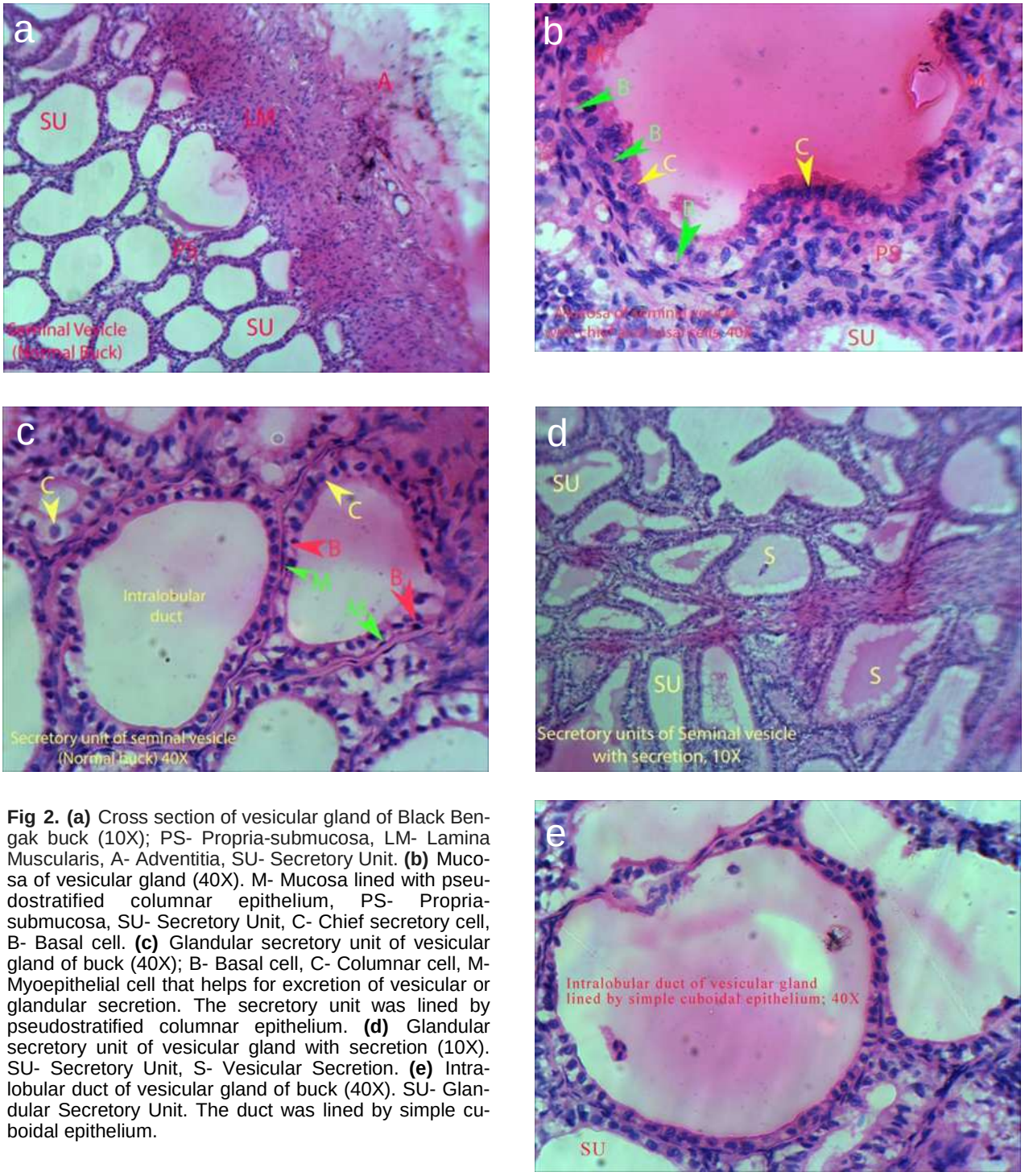
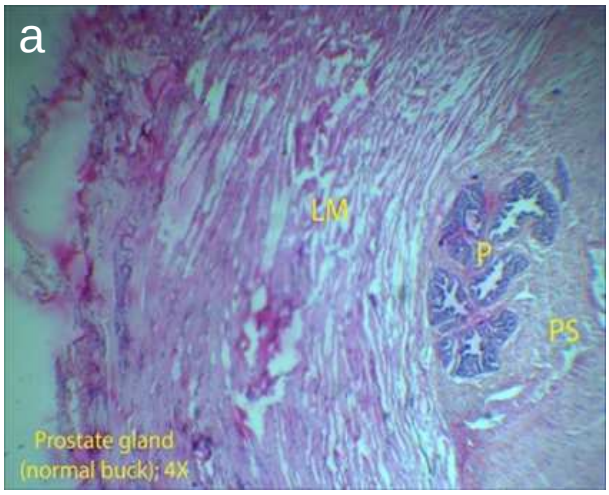
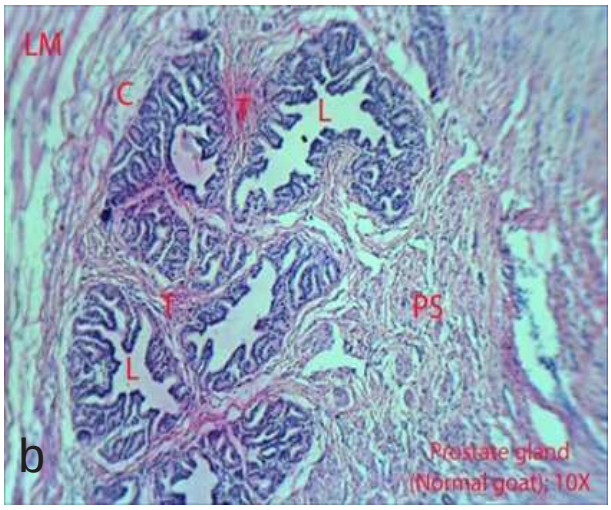


Fig 2. (a) Cross section of vesicular gland of Black Bengak buck (10X); PS- Propria-submucosa, LM- Lamina Muscularis, A- Adventitia, SU- Secretory Unit. **(b)** Mucosa of vesicular gland (40X). M- Mucosa lined with pseudostratified columnar epithelium, PS- Propria-submucosa, SU- Secretory Unit, C- Chief secretory cell, B- Basal cell. **(c)** Glandular secretory unit of vesicular gland of buck (40X); B- Basal cell, C- Columnar cell, M- Myoepithelial cell that helps for excretion of vesicular or glandular secretion. The secretory unit was lined by pseudostratified columnar epithelium. **(d)** Glandular secretory unit of vesicular gland with secretion (10X). SU- Secretory Unit, S- Vesicular Secretion. **(e)** Intralobular duct of vesicular gland of buck (40X). SU- Glandular Secretory Unit. The duct was lined by simple cuboidal epithelium.

to those of Dellmann and Eurell (1998) and Mifune et al. (1986). It can be concluded that the tall columnar cells are the chief secretory cells and basal cells are the basal reserve cells. Similar contentions regarding these cells have been held by earlier authors (Cons, 1957; Chandrapal, 1976; Gupta, 1989; Sudhakar, 1982) in different domestic animals.

The lamina propria-submucosa comprised loose areolar connective tissue containing secretory end pieces with varying number of alveoli. The glandular end pieces (secretory units) of the vesicular glands were lined by pseudostratified columnar epithelium. Similar to the epithelial lining of the mucosa, the secretory units also consisted of tall columnar type cells and short basal type cells (Fig. 2c). The cytological characters of these cells and their nuclei were almost similar to those observed in the lining epithelium of tunica mucosa. The glandular or vesicular secretion was stored in the lumen of secretory units (Fig. 2d). It was also found that the myoepithelial cells were present around the secretory cells of the glandular end pieces of the vesicular gland in all bucks (Fig. 2c) that





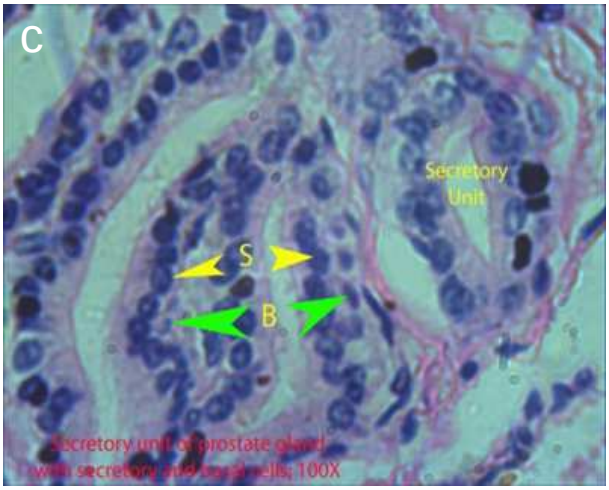


Fig 3. **(a)** Location of prostate gland (pars dessiminata) in buck (4X); PS- Propria-submucosa, LM- Lamina Muscularis, P- Prostate gland. **(b)** Cross section of prostate gland (pars dessiminata) of buck (10X); C- Capsule, T- Trabeculae, L- Lobule, PS- Propria-submucosa, LM- Lamina Muscularis. **(c)** Secretory unit of prostate glands (pars dessiminata) of Black Bengal buck (100X). S- Secretory cell, B- Basal cell. The secretory unit was lined by simple cuboidal epithelium.

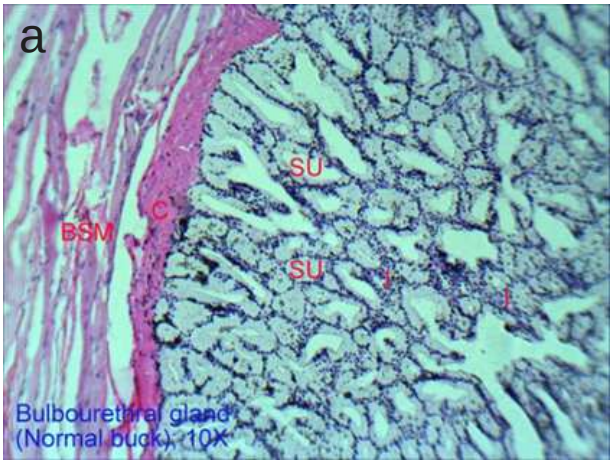
helped in the excretion of vesicular secretion. The intralobular and main secretory ducts were lined by a simple cuboidal epithelium (Fig. 2e). The highly vascularized loose connective tissue of the propria submucosa was continuous with the dense connective tissue trabeculae, which subdivided the organ into lobes and lobules. The interlobular septa were predominantly muscular, derived from the thick tunica muscularis, which was surrounded by a capsule of dense irregular connective tissue with a few smooth muscle cells. The results corroborate the findings of Dellmann and Eurell (1998), Hib (2003), Archana et al. (2009) and Neves et al. (2013).

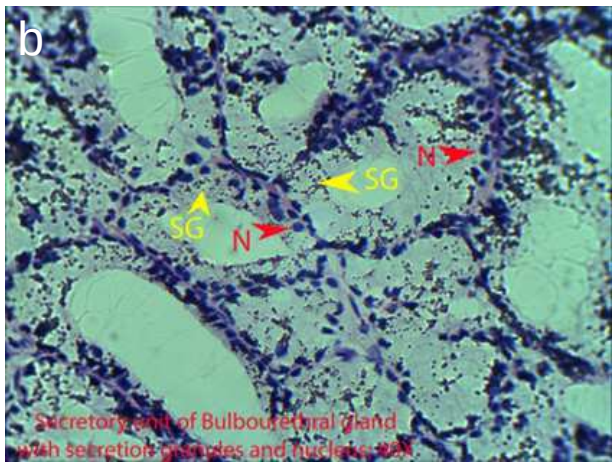
The tunica muscularis, of varying width and arrangement, was composed of a thick layer of interwoven (inner circular and outer longitudinal) smooth muscle fibers, surrounded the organ, followed by a tunica adventitia. The same pattern was observed in all Black Bengal bucks (Fig. 2a). The same architecture for the tunica muscularis has been encountered by earlier authors (Dellmann and Eurell, 1998; Hib, 2003; Archana et al., 2009) in different species of animals. The tunica adventitia comprised loosely arranged meshwork of connective tissue fibers (Fig. 2a) similar to as reported in sheep (Neves et al., 2013), buffalo (Sudhakar et al., 1986) and in men (Hib, 2003). Many blood vessels and nerve fibers were seen in the tunica adventitia.

The study measured the thickness of tunica muscularis and adventitia (together) and the length and width of glandular end piece (secretory units) of vesicular gland of Black Bengal bucks. The average thickness of tunica muscularis and adventitia was 117.00 ± 36.16 µm and the average length and width of secretory units were 164.00 ± 48.64 µm and 96.20 ± 24.76 µm respectively in bucks.

The Prostate Gland

Histologically, the prostate consisted of a varying number of individual tubuloalveolar glands derived from the epithelium of the pelvic urethra. Generally the prostate consists of two portions: the compact or external portion (corpus prostate), and the disseminate or internal portion (pars disseminate prostatae) in animals. Because of description of different text books, the corpus prostaticus does not occur in the small ruminants (Getty, 1975). Further, Kundu (1980), Gupta and Singh (1982) and Gupta (1989) described only the pars disseminata in the goats. In present observation, only pars disseminata was found in all groups of bucks (Fig. 3a-b). The result of present study corroborates with the findings of Kundu (1980), Gupta and Singh (1982), Dyce et al. (2002), Pineda and Dooley (2003), Youngquist and Threlfall (2007), Khalaf and Merhish (2010), Dellmann and Eurell (1998) and Pathak et al. (2012). Roy et al. (1985) also observed a small compact glandular mass





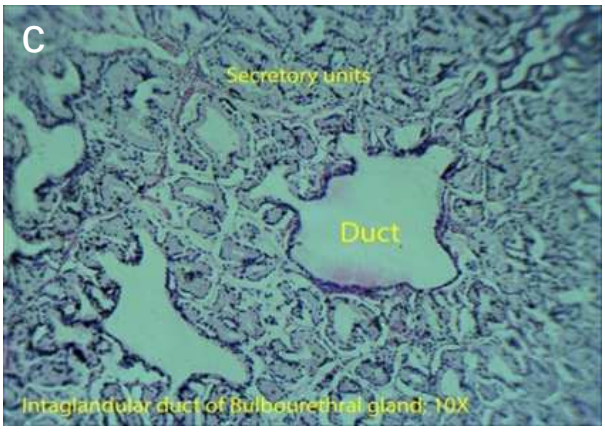


Fig 4. **(a)** Cross section of bulbo-urethral gland of Black Bengal buck (10X); BSM- Bulbospongiosus muscle, C- Capsule, I- Interstitium, SU- Secretory Unit. **(b)** Secretory unit of bulbo-urethral glands of buck (40X). N- Nucleus, SG- Secretion granules. The secretory unit was lined by tall simple columnar epithelium. The nucleus was placed basally and the cytoplasm contained the secretion granules. **(c)** Intraglandular duct of bulbo-urethral glands of buck (10X). The duct was lined by pseudostratified columnar epithelium.

embedded in the urethral muscle of ram and named it as corpus prostate. The external portion of the prostate gland is absent in small ruminants. The particularly well-developed internal portion encircles the urethra in bulls and bucks; in rams, it is U-shaped, and the midline of the ventral aspect of the urethra is free of glandular tissue (Bacha and Wood, 1990; Dellmann and Eurell, 1998).

In the present study, the pars disseminata lay mostly in the dorsal walls of pelvic urethra of all bucks (Fig. 3a-b). This was in conformity with the description of Getty (1975), Ghosh (1995), Dyce et al. (2002), Pineda and Dooley (2003), Youngquist and Threlfall (2007) and Khalaf and Merhish (2010) who described it mostly lies dorsal to the lumen whereas Gupta and Singh (1982) described it mostly located in the ventral and lateral walls with only a few small lobules in the dorsal wall of urethra of male goats and Dellmann and Wrobel (1976) described it occurs all around (all sides) of the pelvic urethra in the propria of small ruminants.

The disseminate portion, pars disseminata, was located in the propria-submucosa of the pelvic urethra (Fig. 3a-b). The pars disseminata lay in the urethral wall surrounded by its own capsule of loose connective tissue. A layer of skeletal urethral muscle surrounded the gland capsule from outside. Large trabeculae originated from the capsule and separated the parenchyma into individual lobules (Fig. 3a-b). The findings of the present study are similar to the findings of Dellmann and Eurell (1998) and Pathak et al. (2012) in goats. The thickness of trabeculae increased but interlobular and intralobular connective tissue decreased with increase in age (Pathak et al., 2012).

The parenchyma comprised cisternae and ducts with luminated secretory end pieces. The epithelium showed a great variation in different glands and alveoli and even in a single alveolus. The secretory tubules, alveoli and intraglandular ducts of the prostate gland were lined by a simple cuboidal to columnar epithelium with basal cells (Fig. 3c). The cytoplasm contained secretion granules. This observation was supported by Kundu (1980), Dellmann and Eurell (1998) and Pathak et al. (2012). Yao and Eaton (1954) however, observed pseudostratified columnar epithelium lining the pars disseminate of Phillipine goats.

The study measured the length and width of lobules and glandular secretory units of prostate glands of bucks. The average length and width of lobules were 392.00 ± 114.73 µm and 259.00 ± 65.42 µm respectively and the average length and width of glandular secretory units were 106.20 ± 32.83 µm and 43.00 ± 11.26 µm respectively in bucks.

Bulbourethral Glands

Histologically, the paired bulbourethral gland was a compound tubular gland in bucks. The findings were similar to earlier authors (Bacha and Wood, 1990; Dellmann and Eurell, 1998; Hib, 2003; Junqueira and Carneiro, 2008; Khalaf and Merhish, 2010; Neves et al., 2013), as they described that the bulbourethral glands are compound tubular in boars, cats, bucks; tubuloalveolar gland in bulls,

rams, stallions and in human and absent in dogs. The glands were surrounded by the bulboglandular (bulbospongiosus) muscle (Fig. 4a) that was consistent to other animals observed by Dellmann and Eurell (1998) and Neves et al. (2013).

The secretory portions (secretory units) of the gland were irregular in size and shape and lined with a tall simple columnar epithelium and occasional basal cells. Most of the columnar cells were of the mucous type, with the nuclei basally placed and the cytoplasm contained the secretion granules (Fig. 4b). The intraglandular ducts were lined by a pseudostratified columnar epithelium (Fig. 4c). The gland was ensheathed by a capsule composed by dense connective tissue containing a variable amount of striated skeletal muscle. Trabeculae, extending from the capsule, also consisted of dense irregular connective tissue and skeletal muscle fibers. These results corroborate the findings of Dellmann and Eurell (1998), Mollineau et al. (2009) and Neves et al. (2013) in other domestic animals. The study measured the capsular thickness of bulbourethral glands and it was 76.00 ± 12.81 µm in bucks.

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