Seasonal variations in histomorphology and histochemistry of the prostate gland of buffalo bulls

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Received: 16.08.2019 • Accepted/Published Online: 06.01.2020 • Final Version: 10.02.2020

Abstract: The prostate glands of buffalo bulls were collected from an abattoir immediately after slaughter and were fixed in 10% neutral buffered formalin for paraffin block preparation by an acetone-benzene schedule. Paraffin sections of 3–5 µm were obtained on glass slides with a rotary microtome and were stained with various histomorphological and histochemical stains. The distributions of glandular acini and ducts were variable throughout the length of the pelvic urethra. The disseminate part of the prostate gland was derived from the epithelium of the pelvic urethra and was a compound tubulo-alveolar type of gland. The glandular acini comprised three types of cells, i.e. principal (serous cells), mucous, and narrow dark cells. In winter, the lumen of the acini became wider than in summer. The amount of glandular tissue decreased from winter to summer, whereas a reverse pattern was observed in the stroma. The prostatic concretions were larger and centrally placed during winter but became peripheral in summer. The average epithelial height of prostatic acini varied significantly between seasons. The activity of neutral mucopolysaccharides, basic proteins, lipids, and phospholipids was higher in winter than summer, whereas that of acid mucopolysaccharides was higher in summer than winter.

Key words: Prostate, histomorphology, histochemistry, seasonal, buffalo

1. Introduction
Buffalo (Bubalus bubalis) is widely spread over all of Asia and has been an integral part of the livestock economy for over 5000 years, providing draft power, milk, meat, and hides [1]. Efficient reproduction in male animals is dependent to a great extent on the effective functioning of the genital glands [2]. The accessory sex glands in mammals comprise paired vesicular glands, the prostate gland (corpus and disseminate parts), and bulbourethral and ampullary glands [3].

The prostate gland is a singular gland consisting of a body present at the junction between the bladder and urethra and a disseminate part forming a glandular layer in the wall of the pelvic urethra enclosed by a tough fibrous capsule. The prostate gland contributes 4%–6% of the seminal fluid in ruminants and contains acid phosphatase, citric acid, zinc, and spermine [3]. The acid phosphatase enzyme hydrolyzes phosphoric acid esters at acidic pH, citric acid provides the energy for sperm metabolism, zinc is bactericidal and prevents destruction of the DNA of spermatozoa, and spermine is involved in growth regulation [4].

Seasonality appears to influence sexual function, either through the photoperiod or through changes in ambient temperature [5]. Seasonal variation of the prostate gland has been reported in donkey [2] and Gaddi goat and Gaddi sheep [5]. However, information regarding seasonal variation in the histomorphology and histochemistry of accessory glands of buffalo is limited. Keeping this fact in view, the present research work was carried out.

2. Materials and methods
2.1. Fixation and processing
To study the histomorphology of the prostate gland, tissue samples from the body and disseminate part of the prostate gland were fixed in 10% neutral buffered formalin. After the fixation of tissues, the tissue samples were processed for paraffin block preparation by an acetone-benzene schedule [6] and paraffin sections of 3–5 µm were obtained on glass slides with a rotary microtome. The paraffin sections were stained with hematoxylin and eosin for routine morphology, Masson’s trichrome for collagen fibers, Alcian blue for acid mucopolysaccharides [6], Gridley’s stain for reticular fibers, Verhoeff’s for elastic fibers, periodic acid–Schiff (PAS) for neutral mucopolysaccharides, bromphenol blue for basic protein [7], Sudan black B for lipids, and acid hematin for...
phospholipids [8] to study the histomorphological and histochemical details.

2.2. Micrometry
The micrometrical observations were recorded from hematoxylin and eosin stained sections with the help of ImageJ software. Micrometrical observations were performed on the diameters of acini and epithelial heights of acini of the prostate glands of buffalo bulls during summer and winter seasons.

2.3. Statistical analysis
Arithmetic mean, range, standard error, and coefficient of variation for micrometric measurements were computed and statistically analyzed for their significance [9].

3. Results

3.1. Histomorphology
The prostate gland of buffalo bull was composed of two parts: a corpus prostate and pars disseminate. The corpus prostate remained at the neck of the bladder, whereas the disseminate prostate was derived from the epithelium of the pelvic urethra and found in the propria submucosa of the pelvic urethra. The disseminate prostate extended from the colliculus seminalis to the ischial arch up to the level of the bulbourethral glands. It remained covered by the urethralis muscle throughout the length of the pelvic urethra. The thickness of this muscle varied on dorsal, lateral, and ventral sides along the length of the pelvic urethra from cranial to caudal side.

The prostate gland was a compound tubulo-alveolar type of gland (Figures 1A and 1B). The dorsal wall of the urethra contained more glandular acini than the ventral and lateral parts. The number of glands was highest in the middle of the pelvic urethra (Figures 1C–1F). The glandular acini were lined with simple columnar epithelium while the tubules and intraglandular ducts were lined with simple cuboidal to low columnar epithelium in both seasons (Figures 2A and 2B). The latter changed to transitional epithelium towards the terminal portion of the ducts. The glandular acini and ducts were surrounded by loose connective tissue and comprised smooth muscle cells, collagen fibers, reticular fibers and blood vessels, and scanty elastic fibers in both seasons (Figures 2C and 2D).

The disseminate part of the prostate was surrounded by a distinct fibromuscular capsule, which was further surrounded by the striated urethral muscles. The capsule was dense irregular connective tissue containing an abundance of collagen fibers with few reticular fibers. The large trabeculae originating from the capsule divided the disseminate part into individual lobules (Figures 2C and 2D). The glandular acini comprised three types of cells, i.e. principal (serous cells), mucous, and narrow dark cells. The nuclei of principal cells were round, euchromatic, and close to the basement membrane. The supranuclear part showed basophilia. These cells possessed bleb-like protrusions. The mucous cells were lightly stained with distinct cell boundaries. Dark narrow cells were very few, slender cells with darkly stained elongated nuclei. The myoepithelial cells could not be demonstrated in buffalo bulls (Figure 2E and 2F).

3.2. Micrometry
The average epithelial height was measured to be 16.25 ± 0.73 µm in winter and 9.46 ± 0.35 µm in summer. The values for average epithelial height showed a significant decrease from winter to summer in both left and right glands at 1% and 5% levels of significance. This indicated that the epithelial height of prostatic acini varied significantly between seasons. The average acinar and ductule diameters were found to be 79.31 ± 3.78 µm and 210.09 ± 19.67 µm in winter and 64.25 ± 4.13 µm and 147.15 ± 17.08 µm in summer, respectively, but these changes were statistically insignificant at 1% and 5% levels of significance. This showed that there was no significant difference in acinar and ductule diameters between seasons (Figure 3).

3.3. Histochemistry
In the disseminate part of the prostate gland, a weak PAS-positive reaction was observed in the serous acini and moderate to strong in the mucous acinar epithelium during winter. However, the PAS reaction was slightly decreased in the summer season. The apical part of the acini contained more PAS-positive granules in winter than summer. Prostatic concretions were seen in tubules and as well as in acini (Figure 4A). The latter showed intense PAS-positive reactions in winter and moderate in summer. The interstitial connective tissue showed moderate PAS-positive reaction in winter and weak in summer. A weak to moderate PAS-positive reaction was observed in the smooth and skeletal muscles during winter and summer seasons (Figures 4B and 4C; Table).

In the disseminate part of the prostate gland, serous acini were devoid of AB/PAS reaction, whereas moderate to strong reaction was seen in the mucous acinar epithelium during winter. Some mucous acinar cells had PAS-positive reactions in the apical parts, whereas the other cells had evenly distributed AB/PAS-positive material in the cytoplasm. These AB-positive cells in the mucous acini may be present individually or in groups (Table). The prostatic concretions in the acini showed moderate AB/PAS-positive reaction in winter and intense in summer. The interstitial connective tissue showed moderate AB/PAS-positive reaction in summer and weak in winter (Figures 4D–4F). A weak to moderate AB/PAS-positive reaction was observed in the smooth and skeletal muscles during the winter and summer seasons (Table).

In the disseminate part of the prostate gland, the activity of bromophenol blue was moderate in the acinar and
Figure 1. Photomicrograph of paraffin sections of prostate gland of buffalo bull. A) Cross-section showing the urethra (U), urethralis muscle (UM), and distribution of prostatic ducts and secretory units in the propria submucosa (PS) of the pelvic urethra. Hematoxylin and eosin, 20×. B) The distribution of loose connective tissue (CT) around the acini (ac). Masson’s trichrome, 100×. C) Cross-section at the neck of the bladder showing the lumen of urethra (LU) and the sparse distribution of prostatic ducts (d) and glandular units (ac) in the propria submucosa (PS) of the pelvic urethra. Hematoxylin and eosin, 100×. D) Cross-section at 3 cm from the neck of the bladder showing the urethralis muscle (UM) and the distribution of prostatic ducts (d) and glandular units (ac) in the propria submucosa (PS) of the pelvic urethra during summer season. Hematoxylin and eosin, 100×. E) Cross-section at 8 cm from the neck of the bladder showing the urethralis muscle (UM) and the distribution of prostatic ducts (d) and glandular units (ac) in the propria submucosa (PS) of the pelvic urethra during winter season. Hematoxylin and eosin, 100×. F) Cross-section at 10 cm from the neck of the bladder showing the urethra (U), urethralis muscle (UM), and distribution of prostatic ducts (d) and glandular units (ac) in the propria submucosa (PS) of the pelvic urethra during winter season. Hematoxylin and eosin, 20×.
Figure 2. Photomicrograph of paraffin sections of pars disseminate of buffalo bull. A) Increased epithelial height and number of secretory units (ac) and decreased interstitial tissue (I) during winter season. Hematoxylin and eosin, 400×. B) Decreased epithelial height of secretory units (Se) and increased interstitial tissue (I) during summer season. Hematoxylin and eosin, 400×. C) Distribution of branched tubulo-acinar glandular units (ac) in propria submucosa (PS) and urethralis muscle (UM) in winter season. Masson's trichrome, 100×. D) Elastic fibers (arrow) in tunica intima of blood vessels during summer season. Verhoeff’s stain, 400×. E) Mixed acinus containing mucous cells (1), serous cells (2), and narrow dark cells (3) and prostatic concretions (PC). Hematoxylin and eosin, 1000×. F) The serous epithelial cells containing basophilic material (1) in the supranuclear cytoplasm and narrow dark cells (2). Masson's trichrome, 1000×.
ductule epithelium during winter and weak to moderate in summer. The cytoplasm of serous acini showed strong reactions in the supranuclear part, whereas moderate reaction was seen in the mucous acinar epithelium in winter. Moderate reaction was observed at the boundaries of serous and mucous cells as well as in the prostatic concretions in winter, which decreased in summer. The cytoplasm of mucous acinar cells had a foamy appearance of basic proteins (Table). The interstitial connective tissue showed moderate bromphenol blue reaction in both seasons. A weak to moderate reaction was observed in the smooth and skeletal muscles in winter and summer (Figures 5A and 5B).

In the disseminate part of the prostate gland, the activity of sudanophilic lipids was weak, whereas phospholipids showed moderate to strong reactions in the acinar and ductule epithelium in both seasons. Fine sudanophilic droplets were seen in the mucous acinar cells but this was very weak in the serous acini in winter, which significantly decreased in summer (Figures 5C–5F). The phospholipids were uniformly distributed in the serous and mucous acini. The interstitial connective tissue and skeletal muscles showed weak to moderate reactions, but smooth muscles were devoid of lipids and phospholipids in both seasons (Table).

4. Discussion

The urethralis muscle was uniform in thickness up to the middle of the pelvic urethra, but near the penile urethra it became thin dorsally and thick laterally and ventrally. Similar findings have been reported in buffalo bulls [3]. The results showed that the distributions of glandular acini and ducts were variable throughout the length of the pelvic urethra. Similar to the present findings, glandular tissues were present in the dorsal wall of the cranial portion of the pelvic urethra, whereas the distribution was less than half of the pelvic urethra caudally [10]. On the contrary, the distribution of glandular units in the propria submucosa was more so in the lateral and ventral wall than the dorsal wall of the urethra in buffalo [4].

Similar to the current study, the prostate gland was composed of numerous secretary end pieces of serous and mucous nature in buffalo bulls [11]. The ducts were lined by pseudostratified columnar epithelium, which transformed into a transitional type towards the urethral opening in rams [12]. However, the epithelial lining of the duct was mostly simple columnar or pseudostratified columnar epithelium in bulls [10]. The disseminate portion of the pelvic urethra was surrounded by a capsule made up of collagenous, elastic, reticular, and smooth muscle fibers, which was ensheathed by the urethralis muscle in buffalo [3,11]. The epithelial height was 15.20 µm and the average diameter of acini was 44.22 µm in goats [13]. Similarly, the tubular epithelium was highest in autumn and lowest in summer, and the difference was statistically significant in Gaddi goat but nonsignificant in Gaddi sheep [5].

The present study showed that the amount of neutral mucopolysaccharides secreted by the prostate was greater in winter than summer. Similar to the present study, works in buffalo [3,11,14,15] and in ram [12] reported that the glandular acini were positive for neutral mucopolysaccharides within the cells and lumen in the corpus prostate. The luminal border and subapical cytoplasm gave strong positive reactions for neutral mucopolysaccharides, whereas the reaction was mild in the rest of the cytoplasm in the corpus prostate of camel [16]. The cytoplasm of all the secretory end pieces was strongly PAS-reactive in Gaddi goat [13], which was in accordance with the present study.

The present study showed that alcianophilic reactions were stronger in summer than winter. This may be due to the increase in hyaluronic acid, sulfated glycosaminoglycans,
Figure 4. Photomicrograph of paraffin sections of prostate gland of buffalo bull. A) Increased epithelial height of ducts (d), decreased interstitial tissue (I) during winter season. Hematoxylin and eosin, 400×. B) Strong PAS-positive activity in the secretory units (ac) and prostatic concretions (PC) during winter season. Periodic acid–Schiff, 400×. C) Moderate PAS positive activity in the secretory units (ac) during summer season. Periodic acid–Schiff, 400×. D) Weak to moderate AB/PAS-positive activity in the propria submucosa (PS) and urethralis muscle (UM) during winter season. Alcian blue/periodic acid–Schiff, 20×. E) Moderate to strong AB/PAS-positive activity in the propria submucosa (PS) and urethralis muscle (UM) during summer season. Alcian blue/periodic acid–Schiff, 100×. F) Strong AB/PAS- and weak PAS-positive activity in the epithelium (arrow) and basement membrane (BM) during summer season. Alcian blue/periodic acid–Schiff, 400×.
and sialic acid [17]. Similar findings have been reported in the disseminate part of the prostate gland of buffalo [3,11,14] and ram [12]. The glandular acini were positive for acidic mucopolysaccharide within the cells and lumen in the corpus prostate of prepubertal buffalo [15]. The cell cytoplasm generally exhibited a mild reaction for acid mucopolysaccharides but the luminal mass and the apical border of the lining cells exhibited intense affinity for Alcian blue staining. The Alcian blue reaction was strongly positive in certain lobules [18], as seen in summer in the present findings. In pubertal animals, the cytoplasm of all the secretory end pieces showed very strong Alcian blue reactions at the luminal border [13].

Similar to the current study, in buffalo bulls [3] and in bucks [18] researchers found mucoprotein and glycoprotein in the serous alveoli. Mild to intense reaction for protein and DNA was found in the glandular epithelium of bucks [19]. The epithelial cells in secretory units showed strong positive reactions for protein in bucks [13].

In the disseminate part of the prostate gland of buffalo [3,11], ram [12], and camel [16], similar results were reported. Lipid granules were found in the secretory epithelial cells of the corpus prostate in prepubertal and postpubertal buffalo bulls [15]. Diffuse sudanophilia was reported in the parenchymatous components of the pars disseminate in bucks [13]. This is in agreement with the current findings.

The lumen of the acini became wider in winter than summer, whereas the connective tissue of the stroma was less in winter than summer, which may be due to the reduced lumen of the glandular acini in summer. The prostatic concretions were larger and centrally placed during winter but became peripheral in summer. The average epithelial height and acinar diameter were greater in winter than summer. The activities of neutral mucopolysaccharides, basic proteins, lipids, and phospholipids were higher in winter than summer, whereas the acid mucopolysaccharides was more more in summer than winter in all the glands.

**Acknowledgment**

This work was funded by Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.
Figure 5. Photomicrograph of paraffin sections of prostate gland of buffalo bull. A) Strong activity of basic proteins in the urethralis muscle (UM) in winter season. Bromophenol blue, 400×. B) Strong activity of basic proteins in the secretory unit (ac) and prostatic concretions (PC) in winter season. Bromophenol blue, 400×. C) Weak sudanophilic lipid activity in the propria submucosa (PS) and urethralis muscle (UM) and strong in the prostatic concretion (PC) during winter season. Sudan black B, 100×. D) Weak sudanophilic lipid activity in the propria submucosa (PS) during summer season. Sudan black B, 100×. E) Moderate amount of phospholipids in the secretory units (ac) during winter season. Acid hematin, 100×. F) Much lower amount of phospholipids in the secretory units (ac) during summer season. Acid hematin, 400×.
References

1. Taneja VK, Birthal PS. Role of buffalo in food security in Asia. Asian Buffalo Magazine 2004; 1 (1): 4-13.
2. Abou-Elhamd AS, Salem AO, Selim AA. Histomorphological studies on the prostate gland of donkey Equus asinus during different seasons. Journal of Histology 2013; 2013: 643287.
3. Chopra R. Post-natal development of the prostate gland in buffalo (Bubalus bubalis): A histomorphochemical study. MVSc, Punjab Agricultural University, Ludhiana, Punjab, India, 1998.
4. Eurell J, Frappier BL. Dellmann's Textbook of Veterinary Histology. 6th ed. New York, NY, USA: Wiley-Blackwell; 2006.
5. Suri S. Gross, Histological and histochemical changes in male accessory genital glands of Gaddi goat and Gaddi sheep - A seasonal study. PhD, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Himachal Pradesh, India, 2007.
6. Luna LG. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. New York, NY, USA: McGraw-Hill Book Company; 1968.
7. Sheehan DC, Hrachak BB. Theory and Practice of Histchemistry. Saint Louis, MO, USA: CV Mosby Co.; 1973.
8. Chayen J, Butcher RG, Bitensky L, Poulter LW. A Guide to Practical Histochemistry. Edinburgh, UK: Oliver and Boyd; 1969.
9. Snedecor GW, Cochran WG. Statistical methods. 9th ed. Ames, IA, USA: Iowa State University Press; 1994.
10. Adhikary GN, Begum MIA, Islam MN, Islam KM, Rauf SMA. Morpho-histometric evaluations of pre-pubertal, pubertal and post pubertal prostate glands of indigenous bulls (Bos indicus) of Bangladesh. International Journal of Biological & Pharmaceutical Research 2015; 6 (8): 586-592.
11. Sudhakar LS. Histological and histochemical changes in the male accessory genital glands of Murrah buffalo during postnatal development. PhD, Haryana Agricultural University, Hisar, India, 1982.
12. Roy KS, Pawar HS, Saigal RP. Histomorphological, histochemical and histoenzymological studies on prostate gland of ram (Ovis aries). Indian Journal of Animal Science 1985; 55 (12): 983-986.
13. Pathak A, Katiyar RS, Sharma DN, Farooqui MM, Prakash A. Gross anatomical, histological and histochemical studies on the postnatal development of the prostate gland of Gaddi goat. International Journal of Morphology 2012; 30 (2): 731-739.
14. Chandramouly KN. Studies on the histology of prostate in the Indian buffalo (Bos bubalis). Mysore Journal of Agricultural Sciences 1971; 5: 32-38.
15. Chandrapal S. Gross histological and histochemical studies on the male genital system of buffalo (Bubalus bubalis). PhD, Agra University, Agra, Uttar Pradesh, India, 1976.
16. Sambyal RS. Histomorphological and histochemical studies on the accessory genital glands of male camel (Camelus dromedarius). MVSc, Haryana Agricultural University, Hisar, India, 1989.
17. Guraya SS. Biology of Ovarian Follicles in Mammals. Berlin, Germany: Springer Verlag; 1985.
18. Gupta AN, Singh Y. Histological and histochemical studies on the prostate gland of goat. Indian Journal of Animal Science 1982; 52: 82-89.
19. Pyne SK, Sinha RD, Chauhan HVS. Histochemical study on the bulbo-urethral gland of normal and vasectomized goats (Capra hircus). Indian Veterinary Journal 1991; 68: 145-147.