



Seasonal variations in histomorphology and histochemistry of vesicular gland of buffalo bull

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Received: 30 October 2020; Accepted: 26 August 2021

ABSTRACT

The present study was conducted to study the seasonal changes in the histomorphology and histochemistry of the vesicular gland of buffalo bull. The vesicular gland of buffalo bull was collected from the abattoir immediately after sacrifice during winter and summer season. The tissue sample were fixed in 10% neutral buffered formalin (NBF) and were processed for paraffin blocks preparation by acetone-benzene schedule and paraffin sections of 3–5 µm were obtained on glass slides with rotary microtome. The paraffin sections were stained with various stains to study the histomorphological and histochemical parameters. The results showed that the vesicular glands were compound tubulo-alveolar type of gland in buffalo and were composed of four layers, i.e. lamina epithelialis, propria submucosa, tunica muscularis and tunica adventitia. In winter, the lumen of acini became wider than summer. The secretory material was observed during winter but could not be seen in summer season both in glandular acini and ducts. The connective tissue of stroma was less in winter and increased in summer which may be the reason for reduced lumen of glandular acini in summer. The tunica muscularis layer was thicker in winter than in summer season. The micrometrical data revealed that the average epithelial height and acinar diameter significantly decreased from winter to summer. The activity of neutral mucopolysaccharides, basic proteins, lipids and phospholipids were more in winter than summer season. The acid mucopolysaccharides were more in summer than winter. The results indicate that the vesicular gland of buffalo bull was more functionally active during winter season as compared to summer season.

Keywords: Buffalo, Histochemistry, Histomorphology, Seasonal, Vesicular gland

Tremendous demands are focussed on buffaloes for their strength and resistance against different diseases and ecological conditions (Rahman *et al.* 2010). Efficient reproduction in male animals is dependent to a great extent on the effective functioning of the genital glands (Elhamd *et al.* 2013). The accessory sex glands in mammals are comprised of paired vesicular glands, prostate gland (corpus and disseminate parts), bulbourethral and ampullary glands (Sarangi *et al.* 2020b).

The seminal glands are elongated, multi-lobulated, compact gland which appear like the cluster of grapes having one excretory duct that joins with ductus deferens. The seminal vesicles produce viscid, yellowish secretion containing fructose, citrate, inositol, prostaglandins, flavin, riboflavin, sorbitol, inositol, ascorbic acid, phosphorylcholine, ergothionin and several proteins which act as a source of energy for the sperm motility (Eurell and Frappier 2006).

The age-related studies have been reported on accessory sex glands of goat (Pathak *et al.* 2012) and Bangladeshi

bull (Adhikary *et al.* 2015). Buffalo has a seasonal mating cycle (short-day breeder) like goat and sheep and become sexually active in response to decreasing day length in the late summer to early autumn (Scala and Maruccio 2012). Seasonal variation of vesicular gland has been reported in Gaddi goat and Gaddi sheep (Suri 2007). But the information regarding the seasonal variation in the histomorphology and histochemistry of vesicular glands of buffalo is scanty. Keeping this fact in view the present research work was carried out.

MATERIALS AND METHODS

Fixation and processing: The tissue samples of vesicular gland were collected in 10% neutral buffered formalin (NBF). The samples were processed further by acetone-benzene schedule and paraffin sections of 3–5 µm were obtained on glass slides with rotary microtome. The paraffin sections were stained with various stains to study the histomorphological parameters, viz. morphology, distribution of collagen, elastic and reticular fibres. Histochemical moieties (acidic and neutral mucopolysaccharides and basic proteins) were localized on paraffin sections whereas lipids and phospholipids on cryostat sections. Micrometrical observations were recorded on

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haematoxylin and eosin stained sections and were subjected to statistical analysis (Sarangi *et al.* 2020a).

RESULTS AND DISCUSSION

Histomorphology: The paired vesicular glands were compound tubulo-alveolar type of gland in buffalo. The lobar and lobular pattern was well distinct and drained by intralobular secretory ducts and main excretory duct. Rahman *et al.* (2010) also found that the tubuloalveolar glandular units of vesicular gland were compact and lobulated in Bangladeshi bull.

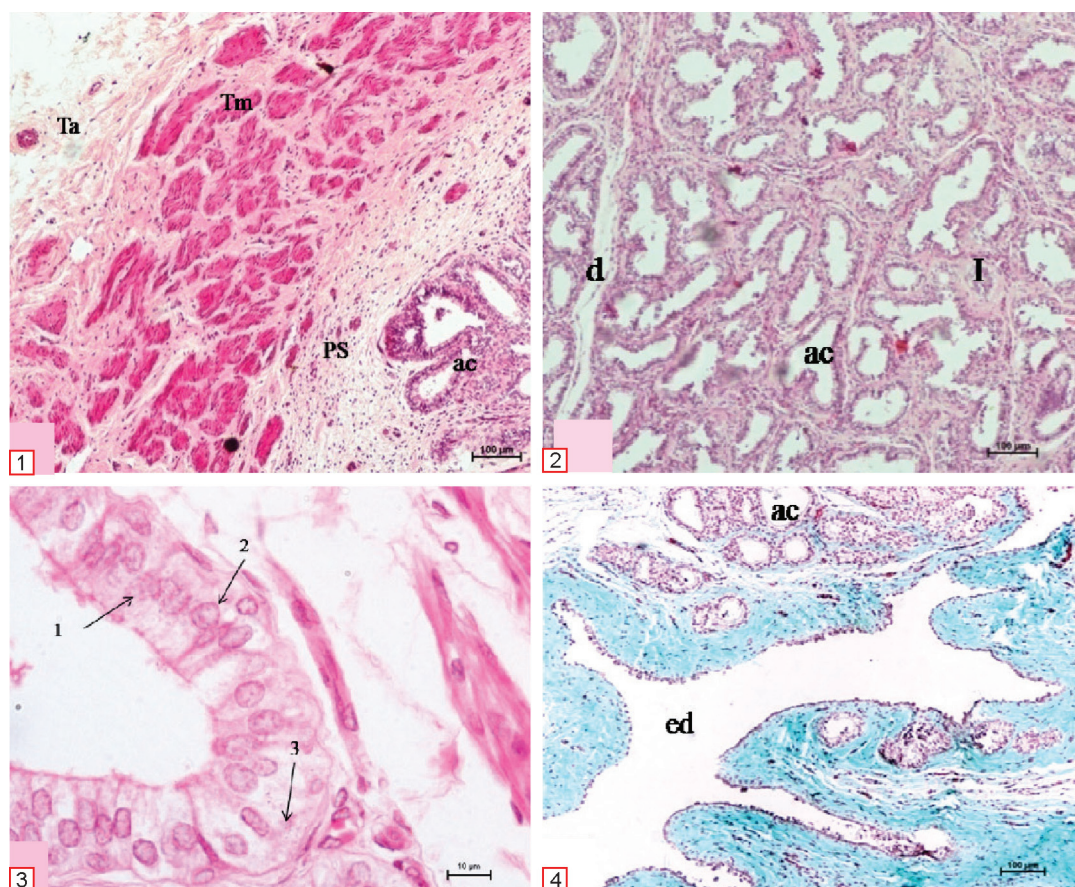
The vesicular gland was composed of four layers, i.e. lamina epithelialis, propria submucosa, tunica muscularis and tunica adventitia (Fig. 1). Similar findings were reported by Roy *et al.* (2006) and Eurell and Frappier (2006) in buffalo bull.

The lamina epithelialis was composed of glandular tissue and stroma. Tubular epithelial invaginations extended into propria submucosa (Fig. 2). The secretory acini were round or oval in shape and presented wide variation in size (Fig. 2) in winter season which became irregular and narrow in summer season. The glandular epithelium of acini varied from pseudostratified tall columnar epithelium to tall columnar with small, spherical and often sparse basal cells

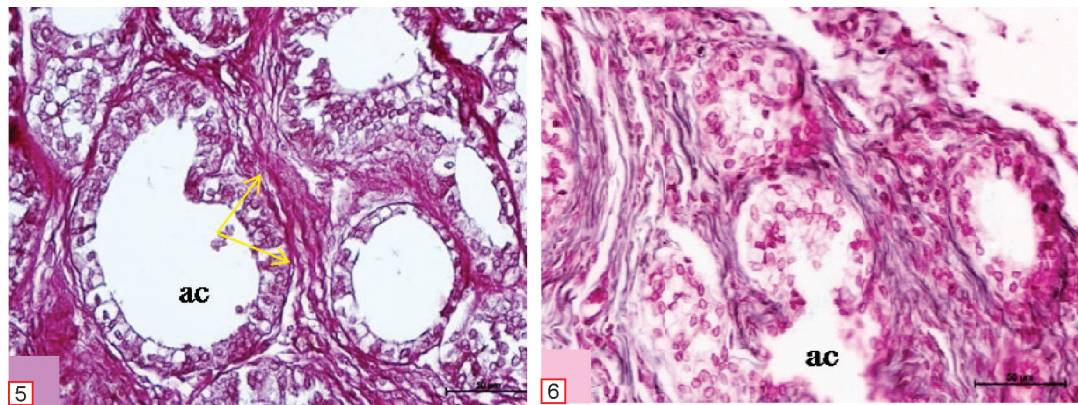
(Fig. 3). In summer, the height of epithelial cells decreased. Similarly, the alveoli of vesicular glands were lined by pseudostratified columnar epithelium in buffalo bull (Anurag 2000) and Gaddi goat and Gaddi sheep (Suri 2007).

The small intralobular ducts were lined by tall columnar cells but in the larger ducts epithelium changed gradually to pseudostratified columnar type (Fig. 4). The cytoplasm of ductular epithelium was dark with eosinophilic granulations in the supranuclear regions with sparse vacuolations in infranuclear region (Fig. 3). No clear-cut difference in the ductular epithelium was found between the seasons. Similar findings have been reported by Anurag (2000) in buffalo bull and Eurell and Frappier (2006) in bull. Whereas Samuelson (2007) reported that a single central area was present where the secretions were accumulated in equine. Contrary to the present study, Gofur (2015) reported simple cuboidal epithelial lining in the duct system of buck.

The stroma was made up of loose connective tissue and contained smooth muscle fibres, collagen fibres and few reticular fibres (Figs 4 and 5). A thin layer of reticular fibres appearing as a common basement membrane surrounded the epithelium of the acini (Fig. 6). The interstitial connective tissue significantly increased in summer. The



Figs 1–4. 1. Secretory units (ac), propria submucosa (PS), tunica muscularis (Tm) and the tunica adventitia (Ta). Hematoxylin and Eosin $\times 100$; 2. Distribution of tubular glands (ac), ducts (d) and interstitial tissue (I) during winter season. Hematoxylin and Eosin $\times 100$; 3. Pseudostratified columnar epithelium of the glandular end piece having tall columnar cell (1), basal cell (2) with myoepithelial cell (3) surrounding the acini. Hematoxylin and Eosin $\times 1000$; 4. Cuboidal epithelium of excretory ducts (ed) and glandular unit (ac). Masson's Trichrome $\times 400$.



Figs 5–6. **5.** Distribution of elastic fibres (arrow) surrounding basement membrane of epithelial cells of the acini (ac) during winter season. Verhoeff's $\times 400$; **6.** Distribution of reticular fibres in connective tissue surrounding the acini (ac) during winter season. Gridley's $\times 400$ in vesicular gland of buffalo bull.

highly vascularized loose connective tissue of propria submucosa was continuous with the dense muscular connective tissue septae. Eurell and Frappier (2006) stated that the interlobular septa were fibro-muscular in nature and was mainly composed of connective tissue elements and less amount of smooth muscles in equines. Whereas Nissar *et al.* (2014) reported that the coarse collagen and fine reticular fibres were present in capsule which gave rise to septae and dispersed into glandular parenchyma in buck.

Tunica muscularis was made up of thick layer of circularly arranged smooth muscle fibres and collagen fibres. It was continuous with connective tissue septae extending from tunica adventitia dividing the gland into lobes and lobules (Fig. 7). Neves *et al.* (2013) and Gofur (2015) stated that the tunica muscularis layer was composed of inner circular and outer longitudinal layer with variable thickness in ram and buck, respectively.

Tunica adventitia comprised of loose irregular connective tissue with few smooth muscle fibres, blood vessels and nerves (Fig. 7). Similarly, Ghonimi *et al.* (2014) found that tunica serosa or tunica adventitia lined the gland externally in buffalo bull. The loosely arranged collagen, reticular and elastic fibres meshwork of adventitia layer was composed of many blood vessels, nerve fibres and

autonomic ganglia in bull (Adhikary *et al.* 2015).

Micrometry: The micrometrical data revealed that the average epithelial height and acinar diameter were found to be $26.7 \pm 1.14 \mu\text{m}$ and $70.05 \pm 3.03 \mu\text{m}$ in winter which decreased significantly to $14.95 \pm 0.58 \mu\text{m}$ and $48.03 \pm 3.01 \mu\text{m}$ in summer respectively. Whereas, Anurag (2000) measured the diameter of largest alveoli as $472 \pm 14.82 \mu\text{m}$ in buffalo bull. Similarly, the tubular diameter and epithelial height was significantly higher in autumn season than summer season in Gaddi goat and sheep (Suri 2007). The average thickness of tunica muscularis was found to be $306.74 \pm 12.53 \mu\text{m}$ in winter and $347.98 \pm 10.32 \mu\text{m}$ in summer, indicating no significant difference in thickness of tunica muscularis with respect to seasons. Gofur (2015) found the average thickness of tunica muscularis was $117.00 \pm 36.16 \mu\text{m}$ in bucks.

Histochemistry: A weak to moderate PAS positive reaction was observed in the acinar epithelium whereas reaction was weak in ductular epithelium of vesicular glands during winter and summer seasons. Moderate to strong PAS reaction was seen in the apical part of principle cells. The basement membrane of secretory acini showed strong PAS activity in winter which became moderate in summer. The basement membrane of ducts showed moderate PAS activity in winter which became weak to moderate in summer (Figs 8 and 9). The interstitial connective tissue and smooth muscles of tunica muscularis showed moderate to strong PAS positive reaction in both the seasons. Whereas, tunica adventitia showed weak activity in winter and negligible activity in summer (Fig. 8). These findings suggested that basement membrane of secretory acini and principal cells contained more glycogen, which serves as an energy source for ejaculated spermatozoa in the domestic animals (Eurell and Frappier 2006). Similar findings have been reported in vesicular gland by Nissar *et al.* (2014) in buck, Anurag (2000) and Ghonimi *et al.* (2014) in buffalo bull, Sudhakar *et al.* (2005) in yak. Badia *et al.* (2006) and Nagasailaja *et al.* (2016) also reported presence of neutral mucosubstances in the apical cytoplasm of secretory cells in vesicular gland of boar. Pathak *et al.* (2012) found in buck that the PAS reaction was uniform in the cytoplasm and more granular

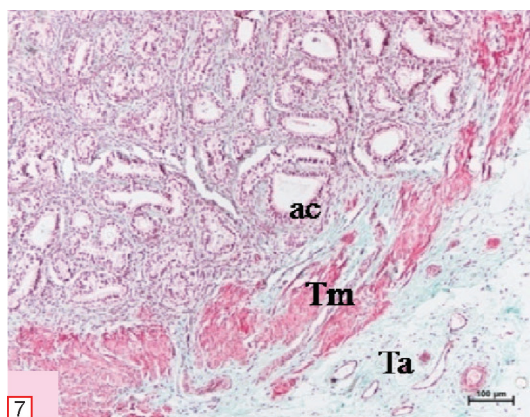
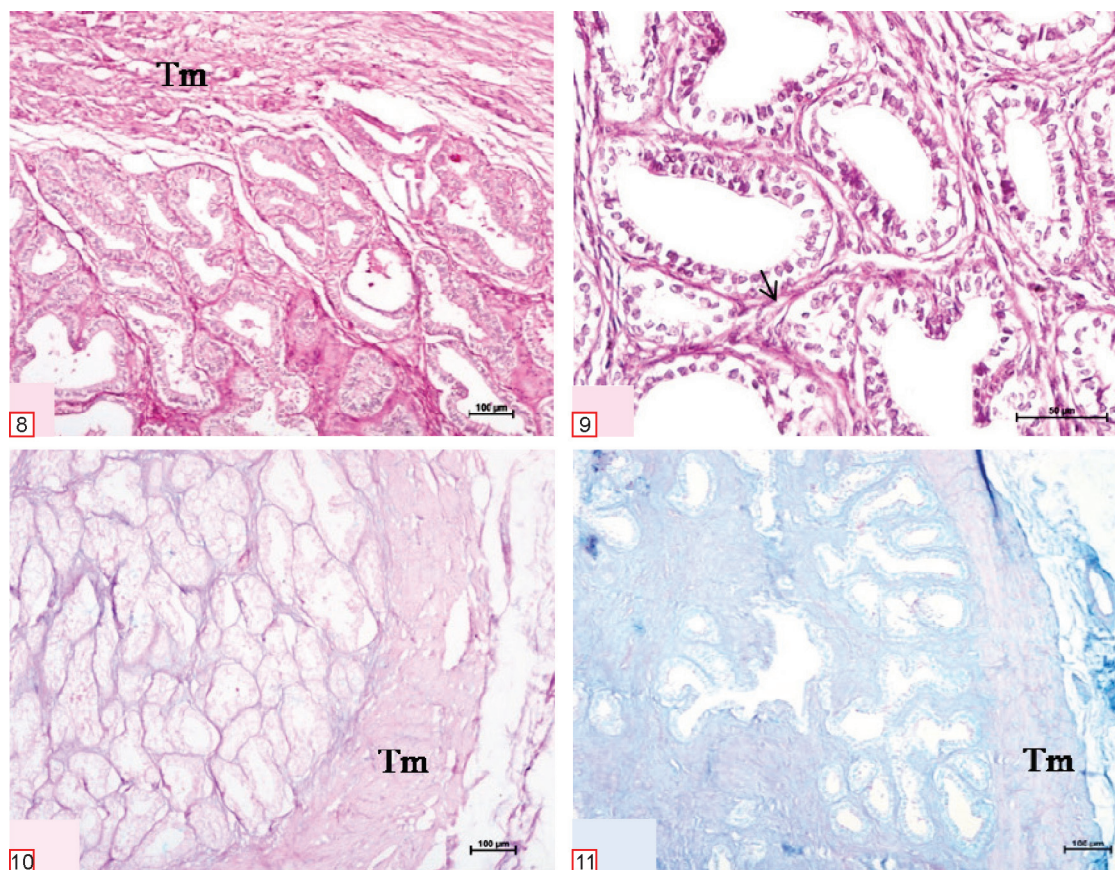


Fig. 7. Secretory unit (ac), circular layer of muscle fibres in tunica muscularis (Tm) and the tunica adventitia (Ta) in summer season. Masson's Trichrome $\times 100$.



Figs 8–11. **8.** Moderate PAS positive activity in the tunica muscularis (Tm) in winter season. Periodic acid Schiff $\times 100$; **9.** Moderate PAS positive activity in the basement membrane (arrow) around the epithelium in summer season. Periodic acid Schiff $\times 400$; **10.** Moderate AB/PAS positive activity in the tunica muscularis (Tm) during winter season. Alcian Blue/Periodic acid Schiff $\times 100$; **11.** Photomicrograph of paraffin sections of vesicular gland of buffalo bull showing Moderate to strong AB/PAS positive activity in the tunica muscularis (Tm) during summer season. Alcian Blue/Periodic acid Schiff $\times 100$.

in the apical part which suggested that the material was produced in the cytoplasmic region closer to basement membrane and migrates from there to the luminal border. Pinki (2013) showed a mild PAS reaction in the tunica serosa, whereas intense to mild reaction was observed in the lamina propria and tunica muscularis of vesicular gland of buck. Mokhtar *et al.* (2016) showed PAS positive reaction throughout the gland in the basement membrane, acini, secretory columnar cells, blebs and secretions in both castrated and non-castrated bucks.

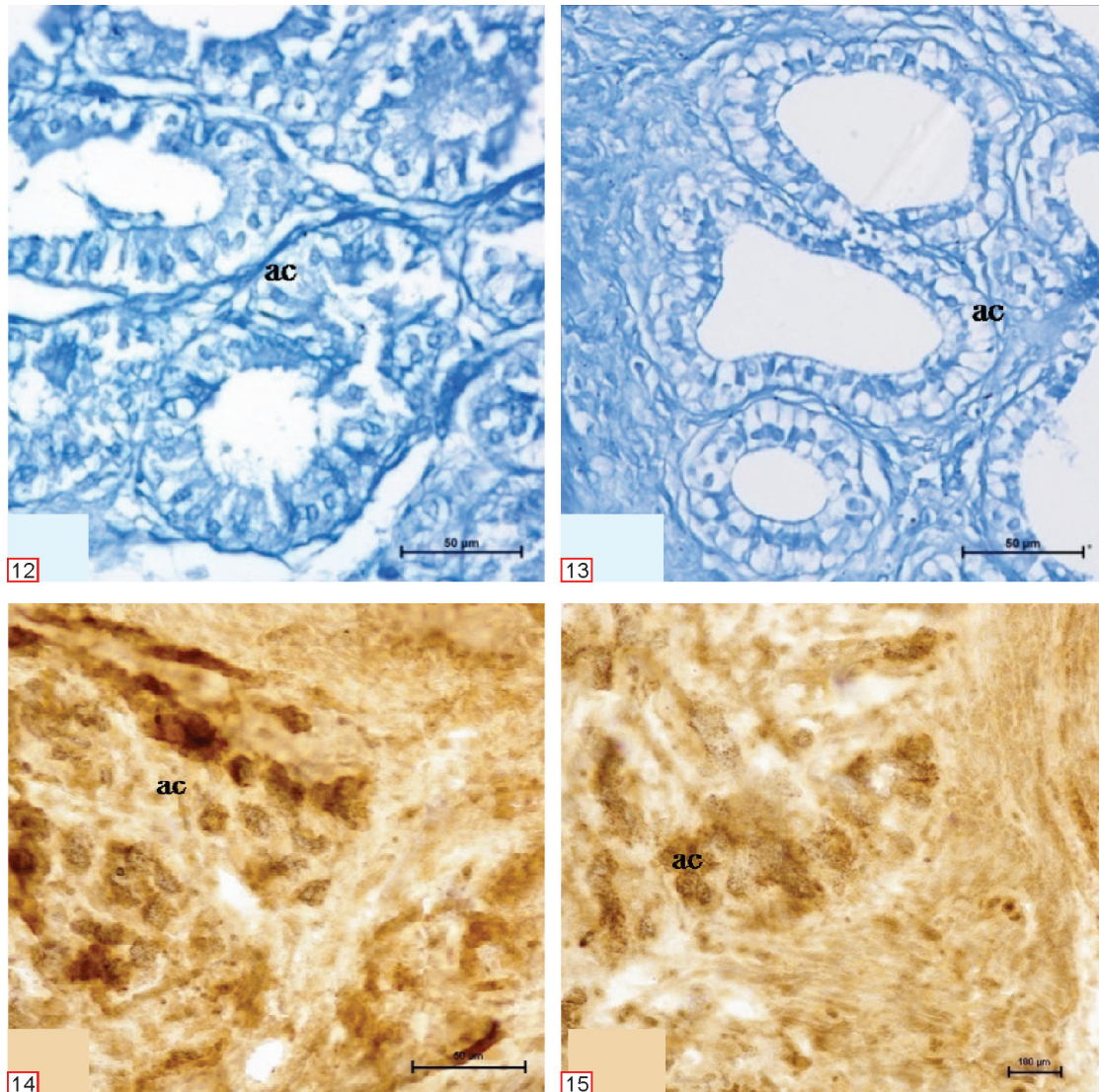
A weak to moderate alcinophilia was observed in the acinar epithelium of vesicular glands during winter and moderate in summer season. The basement membrane of secretory acini showed moderate PAS activity in winter, whereas moderate to strong AB/PAS reaction in summer (Figs 10 and 11). The ductular epithelium and basement membrane showed weak AB reaction during winter and weak to moderate in summer season (Figs 10 and 11).

The interstitial connective tissue showed moderate AB/PAS positive reaction in winter and strong in summer season. A very weak reaction of acidic mucopolysaccharides was observed in the tunica muscularis and tunica adventitia during winter which became moderate during summer (Figs 10 and 11). Similar findings have been

reported by Nissar *et al.* (2014) in buck, Anurag (2000) and Ghonimi *et al.* (2014) in buffalo bull, Sudhakar *et al.* (2005) in yak. Badia *et al.* (2006) and Nagasailaja *et al.* (2016) in boar. Pinki (2013) also showed mild reaction for acid mucopoly-saccharides in the lamina propria and tunica muscularis of vesicular gland in buck.

The activity of bromphenol blue was moderate to strong in the acinar and ductular epithelium of vesicular glands during winter and weak to moderate in summer season. The basement membrane of secretory acini and tunica muscularis showed moderate to strong reaction for basic proteins in winter, whereas moderate reaction was seen in summer. The secretory material present in the lumen showed moderate to strong activity in winter, but could not be demonstrated in summer. The interstitial connective tissue showed moderate amount of basic proteins in winter, which decreased in summer season (Figs 12 and 13). The tunica adventitia showed weak activity in winter and negligible to weak in summer. Similar findings have been reported by Anurag (2000) in buffalo bull.

The activity of sudanophilic lipids, phospholipids and fat was weak to moderate in the acinar and ductular epithelium of vesicular glands during winter and weak in summer season. The basal cells of secretory acini showed



Figs 12–15. **12.** Moderate to strong activity of basic proteins in the secretory unit (ac) during winter season. Bromophenol blue \times 400; **13.** Weak to moderate activity of basic proteins in the secretory unit (ac) during summer season. Bromophenol blue \times 400; **14.** Moderate to strong amount of phospholipids in the glandular units (ac) during winter season. Acid hematin \times 400; **15.** Weak to moderate amount of phospholipids in the glandular units (ac) during summer season. Acid hematin \times 100.

moderate to strong reaction in winter, whereas moderate reaction was seen in summer (Figs 14 and 15). The secretory material present in the lumen contained fine lipid droplets in winter, but could not be demonstrated in summer. The interstitial connective tissue showed moderate amount of sudanophilic lipids and weak to moderate reaction for phospholipids in winter, which decreased in summer season.

Moderate to strong activity of lipids and phospholipids was observed in the tunica muscularis during winter which became weak to moderate during summer. The tunica adventitia showed weak activity in winter and negligible to weak in summer. Similar findings have been reported in the vesicular gland of buffalo (Anurag 2000) and Yak (Sudhakar *et al.* 2005). Whereas Selvinjose *et al.* (2009) reported absence of lipids in the vesicular glands of Red sheep. Rahman *et al.* (2010) also reported that 50% of the lipid material was cholesterol and its esters, whereas triglycerides and phospholipids contributed approximately

25% and 10% respectively in bull. Nagasailaja *et al.* (2016) found diffuse cytoplasmic reaction with Oil red O suggesting the presence of lipid materials in the secretory epithelial cells of vesicular gland in boar. Mokhtar *et al.* (2016) showed large number of lipid droplets and glycogen granules were found in the castrated buck.

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