



Histomorphochemical changes in the mediastinum testis of buffalo bull in different seasons

BEENISH ASLAM¹, NEELAM BANSAL², VARINDER UPPAL³ and ANURADHA GUPTA⁴

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

The present study was conducted on the mediastinum testis of 24 adult buffalo bulls collected from the abattoir, Dera Bassi (Chandigarh) immediately after sacrifice during summer, autumn, winter and spring seasons. The tissue samples were fixed in 10% neutral buffered formalin, processed by acetone benzene technique and stained for histological and histochemical studies. For histoenzymic studies, fresh tissue samples were subjected to cryostat sectioning and incubated in different substrates to demonstrate the sudanophilic lipids and various enzymes. The results revealed that the mediastinum testis comprised rete testis and large blood and lymph vessels. The rete tubules were lined by simple cuboidal to columnar type epithelium in all the seasons except in summer where it showed stratification. There was decrease in the tubular size of rete testis but the collagen, elastic and reticular fibres and neuronal elements were more during autumn and winter than summer and spring. The reaction of acid and neutral mucopolysaccharides, Sudanophilic lipids and basic proteins were moderate to strong in the mediastinum testis during winter and autumn, moderate in spring and weak in summer seasons. The activity of phosphatases and dehydrogenases was weak to moderate during winter and weak in summer. The alterations of various histochemical and histoenzymic activities in the mediastinum testis may be correlated with the reproductive status of the animal with respect to the season.

Key words: Buffalo, Histology, Histochemistry, Histoenzymology, Mediastinum testis, Season

Dhingra (1977) stated mediastinum testis as intratesticular reflection of the tunica albuginea. Its extent and position in different animals is variable. In ruminants, the mediastinum testis occupies the central part of testis along the longitudinal axis. It is made up of rete testes, blood vessels and lymph vessels (Dellmann 1993). In the available literature, the studies have been conducted on the rete testis of adult goat (Kakade and Singh 1990, Pathak *et al.* 2014), buffalo (Singh 1996) and pig (Lambate 2012), but scanty information is available on the histomorphology and histochemistry of mediastinum testis during different seasons. Therefore, the present work was planned to elucidate the histomorphology and histochemistry of buffalo testis during different seasons of the year.

MATERIALS AND METHODS

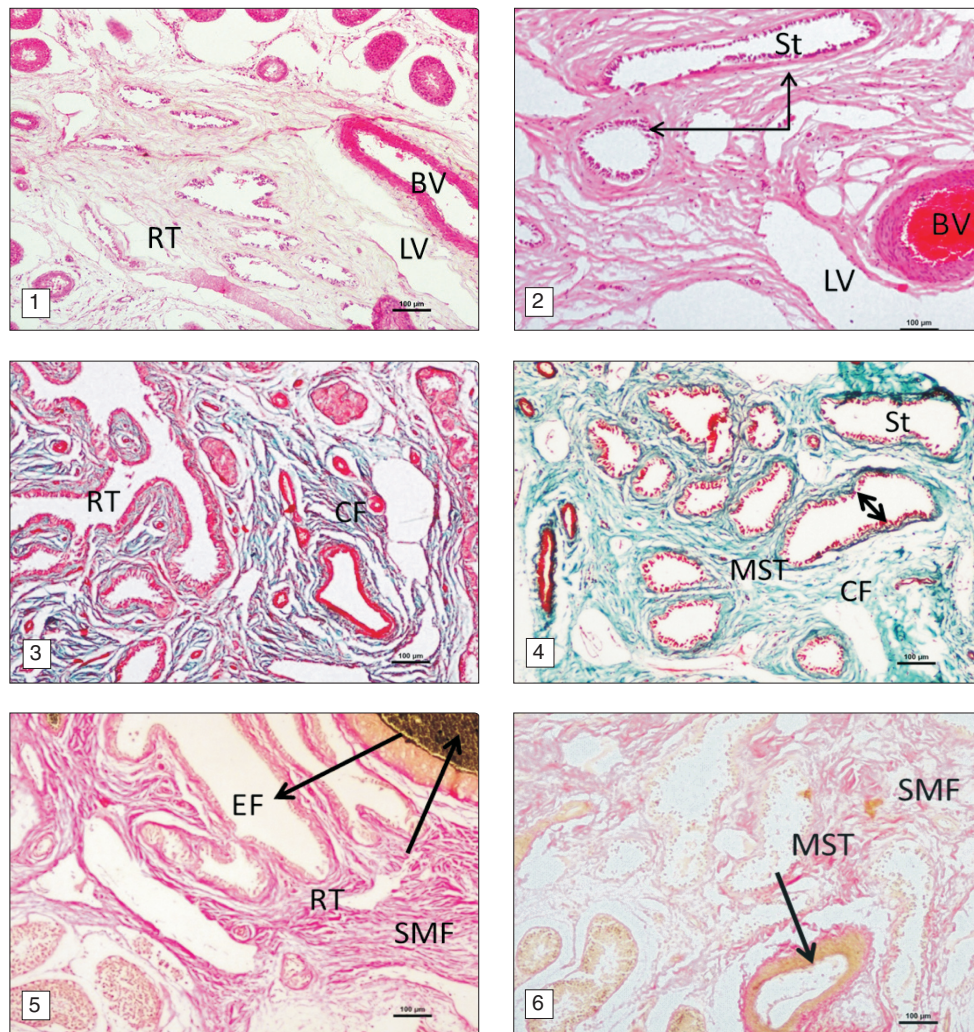
The present study was conducted on testis of 24 adult buffalo bulls divided into 4 groups, viz. group 1 (spring: February-April), group 2 (summer: May-July), group 3 (autumn: August-October) and group 4 (winter: November-January). The left and right testes were collected from the animals in the slaughter house located at Dera Bassi

Present address: ¹M.V.Sc Student (beenish.khan20@gmail.com), ^{2,3,4}Professor (bansal.neelam@rediffmail.com, v.uppal@yahoo.com, anugadvasu@gmail.com), Department of Veterinary Anatomy.

(Chandigarh), fixed in 10% neutral buffered formalin and were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968). The paraffin sections of 5–6 µm were obtained on glass slides with the help of rotary microtome and were stained with haematoxylin and eosin for general morphology, Periodic acid Schiff and alcian blue at pH 2.5 and 1.0 for demonstration of neutral and acid mucopolysaccharides, and bromphenol blue for basic proteins (Chayen *et al.* 1969). Fresh tissue samples were collected from testis and subjected to cryostat sectioning at 10 µm thickness. These sections were incubated in different substrates to demonstrate the sudanophilic lipids and various enzymes (Pearse 1972).

RESULTS AND DISCUSSION

Histology: The mediastinum occupied a central position along the longitudinal axis of the testis and extended from the proximal extremity to about three-fourths of the distance to the distal extremity. The central location of mediastinum was also reported in dog, pig and bull by Johnson *et al.* (1970), in buffalo by Goyal (1970), in Spiti pony by Sudhakar *et al.* (1991) and in sheep by Kishore (2006). The mediastinum testis contained the rete testis and large blood and lymph vessels which were more prominent during autumn and winter season than spring and summer (Figs 1 and 2). The anastomosing channels of rete testis were



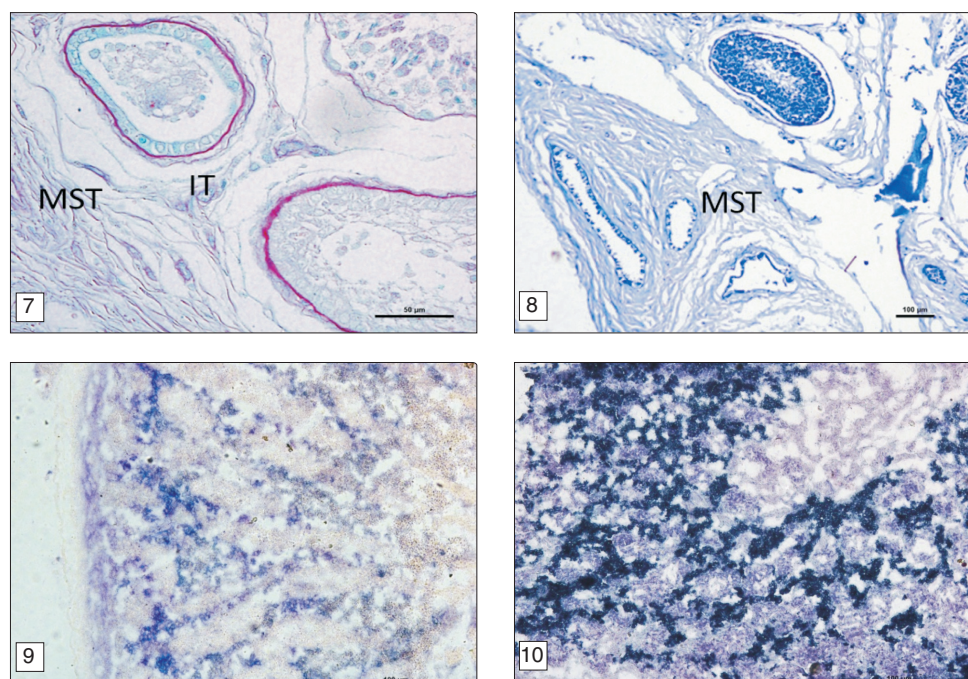
Figs. 1–6. **1.** Mediastinum testes comprised of rete testis tubules (RT), blood vessels (BV) and lymph vessels (LV) during summer season. Hematoxylin and Eosin stain $\times 100$; **2.** Increase in size and number of blood (BV) and lymph vessels (LV) and of straight tubules (St) during autumn season. Hematoxylin and Eosin stain $\times 100$; **3.** Abundant amount of collagen fibres (CF) in the rete tubules (RT) during spring season. Masson's Trichrome stain $\times 100$; **4.** Simple columnar epithelium (arrow) in the straight tubules (St) of mediastinum testis (MST) and abundant quantity of collagen fibres (CF) in between these tubules during winter season. Masson's Trichrome stain $\times 100$; **5.** Elastic fibres (EF) in the tunica intima (arrow) of blood vessel and smooth muscle fibres (SMF) in the rete testis (RT) during spring season. Verhoeff's stain $\times 100$; **6.** Less amount of elastic fibres (arrow) in the blood vessels and smooth muscle fibres (SMF) in the mediastinum testis (MST) during autumn season. Verhoeff's stain $\times 100$.

surrounded by loose connective tissue containing collagen, elastic and some reticular fibres. The rete testis was lined by stratified cuboidal epithelium during spring and summer; however it was simple columnar type in autumn and winter (Figs 3, 4).

The collagen fibres showed a continuation of connective tissue trabeculae from tunica albuginea then in between the testicular lobules and reaching upto mediastinum testis as reported earlier by Kaur *et al.* (2011) in buffalo foetal testis. The amount of collagen fibres were more during the spring season and autumn season as compared to other seasons (Figs 3, 4). The size of rete tubules was more in spring and summer season as compared to autumn and winter. The elastic fibres were also seen in tunica intima of blood vessels which were more in spring and winter season than autumn and summer (Figs 5, 6), however the amount of reticular

and neuronal elements were less in these seasons. Similar observations were recorded by Eurell and Frappier (2006) in domestic animals, Singh (1996) in buffalo, Kishore (2006) in sheep, Pathak *et al.* (2014) in goat and Lambate (2012) in pig.

Histochemistry: The mediastinum testis including rete testis channels and connective tissue revealed negligible to weak PAS reaction during summer and spring which became weak to moderate in autumn and winter. Moderate to strong PAS reaction was observed in basement membrane of rete tubules, weak to moderate AB reaction in the epithelium of rete tubules and interstitial tissue of mediastinum testis during spring season (Fig. 7). The present findings corroborates well with the observations of Pathak *et al.* (2014), who also demonstrated mild Alcian blue reaction in the tubuli recti of prepubertal and pubertal goats.



Figs 7-10. 7. Moderate PAS reaction in basement membrane of rete tubules, moderate AB reaction in the epithelium of rete tubules (arrow) and interstitial tissue (IT) of mediastinum testis (MST) during spring. Alcian Blue/Periodic Acid Schiff $\times 400$; 8. Weak activity for basic proteins in the mediastinum testis (MST) during winter. Bromophenol Blue stain $\times 100$; 9. Cryostat section of buffalo testis during winter season showing weak AKPase activity in the mediastinum testis (MST). Azodye Method $\times 100$; 10. Cryostat section of buffalo testis during winter showing weak to moderate NADPH activity in the mediastinum testis (MST). Nitro BT Method $\times 100$.

A moderate to strong reaction of sudanophilic lipids was observed in the mediastinum testis in autumn which became weak in winter season. Channels of rete testis and connective tissue of mediastinum testis revealed comparatively lesser reaction for sudanophilic lipids. Similar observations were reported by Singh (1996) in buffalo, Kishore (2006) in sheep, Pathak *et al.* (2014) in goat and Lambate (2012) in pig.

A moderate to strong reaction of basic proteins was observed in the mediastinum testis in autumn which became weak in winter season (Fig. 8). The presence of basic proteins indicate that the cell membranes are lipoprotein in nature. These findings corroborated well with the observations of Kaur *et al.* (2011) in buffalo, Kishore (2006) in sheep, Bordalai (1979) and Pathak *et al.* (2014) in goats and Sarma *et al.* (2014) in Assam goat.

Histoenzymology: The activity of alkaline phosphatase and G-6-Pase was weak and granular in the mediastinum testis of buffalo during summer. A weak to moderate granular AKPase reaction was observed in the mediastinum testis in autumn and winter. The localization of AKPase may be related with the transportation of ions across the membrane. A weak to moderate AKPase activity was also documented in the mediastinum testis of buffalo fetus (Bansal *et al.* 2013). Similarly, Pathak *et al.* (2014) was also able to demonstrate the alkaline and acid phosphatase activity in the mediastinum testis of Gaddi goat.

The mediastinum testis showed a weak activity of SDH, LDH and GLD, but the activity of NADPHD and NADH was found to be moderate to strong during winter, which

became weak in summer. The diaphorases showed more reaction at the peripheral part than the centre of the rete tubules. Similar activity has been reported in buffalo foetus (Bansal *et al.* 2013, Singh *et al.* 2015) and in neonatal buffalo calves (Singh 1996). The variation in the activity of various enzymes is correlated with the active spermatogenesis and proliferation of different cell types in buffalo testis.

It may be inferred from the present observations that components of mediastinum testis showed normal histoarchitecture during winter. The presence of various histochemical moieties in the mediastinum testis was more in the winter, which showed that buffalo bull has more reproductive efficiency in winter than other seasons.

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