HISTOMORPHOCHEMICAL STUDIES ON MEDIASTINUM TESTIS OF BUFFALO FETUS

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ABSTRACT

Histomorphochemistry was conducted on the mediastinum testis of buffalo fetuses collected from a local abattoir immediately after the sacrifice of buffalo. The results revealed that mediastinum testis was comprised of rete-testis, blood and lymph vessels, differentiating fibroblasts and mesenchymal cells at 15.5 cm curved crown rump length (CVRL). At 20 cm CVRL, the rete tubules were luminated lined by simple cuboidal to columnar type of epithelium. The connective tissue surrounding the rete-testis contained abundance of collagen fibres, few nerve fibres, elastic fibres and reticular fibres. The reaction of acid and neutral mucopolysachharides, sudanophilic lipids and basic proteins were moderate to strong in the mediastinum testis. The activity of phosphatases and dehydrogenases was weak to moderate in the mediastinum testis. The presence of various histochemical and histoenzymic activites in the mediastinum may be correlated with developmental changes during early fetal life.

Keywords: Buffalo fetus, Histology, Histochemistry, Histoenzymology, Mediastinum testis

INTRODUCTION

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. In fetal testis, the central most cords of blastemal cells, organized into an inter-connecting network of cords that do not contain germ cells were termed as rete testis (Dellmann, 1993). The present work was planned due to the availability of scanty information on the histomorphology and histochemistry in prenatal testis of buffalo.

MATERIALS AND METHODS

The present study was conducted on six male buffalo fetuses obtained from pregnant non-descript buffalo slaughtered at a local abattoir that were ranging between 7.5-20 cm curved crown rump length (CVRL). The foetuses were, Meerut. The CVRL was measured as a curved line in cm using an inelastic thread along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity. The approximate age of the fetus was calculated by using formula viz. Y= 28.66 + 4.496X (CVRL <20 cm), where Y is the age in days and X is the CVRL in cm. Both the testis from all the fetuses were collected in 10% neutral buffered formalin and were processed for paraffin blocks preparation by acetone benzene. 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 pH2.5 and pH1.0 for the demonstration of neutral and acid mucopolysaccharides, and bromphenol blue for basic proteins. 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.

RESULTS AND DISCUSSION

Histological studies of 7.5 cm CVRL buffalo fetus revealed mediastinum testis as an area devoid of sex cords. At 11.5 cm CVRL, the mediastinal area was darkly stained than testicular parenchyma and contained non-luminated tubules lined by simple columnar epithelium.

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Although these tubules appeared in clusters but these were lined by a distinct basement membrane and separated from each other by mesenchymal tissue (Figure 1). At 15.5 cm CVRL, some of the mesenchymal cells were located in the vesicular zone to form solid clusters that are considered as forerunners of rete testis, thus, the formation of rete testis was observed at this stage. First of all, vacuolation was seen in the centre of these tubules indicating the formation of lumen. Earlier, the formation of rete testis was reported at 21.6 cm CVRL in buffalo fetus (Kaur *et al.,* 2011). At 20 cm CVRL, the mediastinum was comprised of rete-testis, blood and lymph vessels, differentiating fibroblasts and mesenchymal cells. At this stage, the rete tubules were luminated, rounded or elongated in shape, surrounded by a distinct basement membrane and lined by simple cuboidal to columnar epithelium (Figure 3). Similar observations were reported earlier in bovine embryos (Abd-Elmaksoud, 2005). In the testis, the cords of outer zone would form permanent seminiferous tubules, whereas the inner thin cords established the direct connection between the future seminiferous tubules and rete-testis. The channels of rete testis were connected with the testicular parenchyma by straight tubules whose terminal part started canalization leading to lumen formation of rete testis (Figure 2). The concept of origin of straight tubules from two parts, i.e. the basal lamina originated from seminiferous tubules, but thin lining epithelium originated from rete testis was reported earlier (Kaur, 2006). At 15.5 cm CVRL, the connective tissue trabeculae originated from tunica albuginea migrated through the testicular parenchyma to reach up to the mediastinum testis and contained abundance of collagen fibres surrounding the rete-testis (Figure 4). It also contained few nerve fibres, elastic fibres and few reticular fibres around the rete-testis. Similar findings were reported previously in buffalo fetus (Kaur, 2006).

Histochemistry for carbohydrates revealed a weak to moderate PAS positive reaction at 9.5 cm CVRL and strong reaction at 15.5 cm CVRL in the mediastinum testis. At 19 cm CVRL and onwards, a

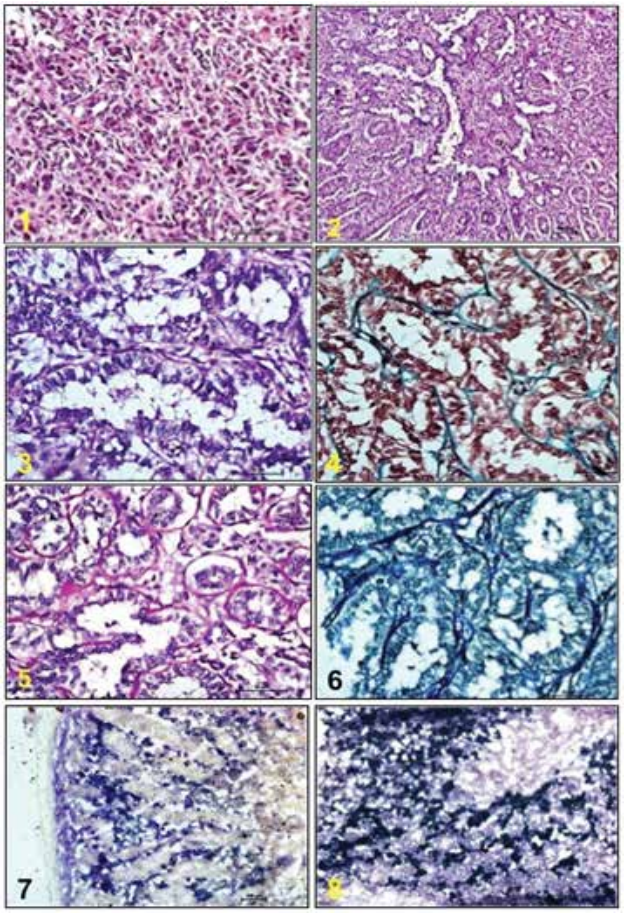

Figure: 1) Paraffin section of 11.5 cm CVRL buffalo fetus showing non-luminated rete cords surrounded by basement membrane (H&E, x400); Paraffin section of 20 cm CVRL fetus showing, 2) connections of semniferous tubules with luminated rete tubules by straight tubules (H&E, x100), 3) luminated rete tubules lined by simple columnar type epithelium and surrounded by a distinct basement membrane (H&E, x400), 4) large amount of collagen fibres in connective tissue separating rete cords and tubules (Masson's Trichrome x400), 5) moderate to strong PAS positive reaction in basement membrane surrounding rete cords and tubules (Periodic Acid Schiff x400), and 6) moderate to strong basic proteins in basement membrane and connective tissue surrounding rete cords and tubules (Bromphenol Blue Stain x400); Cryostat section of 20 cm CVRL fetus showing, 7) weak to moderate AKPase activity (Azodye Method x100), and 8) weak NADPH activity (Nitro BT Method x100) in mediastinum testis.

strong to intense PAS reaction was observed in the basement membrane of the rete testis and straight tubules (Figure 5). Also, a weak to moderate reaction of acid mucopolysacchrides was observed in the mediastinum testis that corroborated well with the earlier observations (Kaur *et al.,* 2008). In case of lipids, a moderate to strong reaction of sudanophilic lipids was observed in the mediastinum testis at 16 cm CVRL onwards, which was also reported earlier in buffalo fetal testis (Kaur *et al.*, 2008). The reaction of basic proteins was strong in the connective tissue components and basement membrane lining the channels of rete testis at 20 cm CVRL (Figure 6), as reported previously in the buffalo fetus (Kaur *et al.,* 2008).

Histoenzymological studies indicated that the activity of alkaline phosphatase and G-6-Pase was weak and granular in the mediastinum testis of buffalo fetus. A weak to moderate granular AKPase reaction was observed in the basement membrane and cellular components of the rete cords (Figure 7). However, no reaction was observed in their lumen. A weak to moderate AKPase activity was also documented in the mediastinum testis (Bansal *et al.,* 2013) and moderate activity in the testicular parenchyma (Singh *et al.,* 2015) in buffalo fetus. The localization of AKPase at the basement membrane and the cellular components of the sex cords may be related with the transportation of ions across the membrane. With regard to oxidoreductases, the mediastinum testis showed a weak activity of SDH, LDH and GLD, but the activity of G-6-PD was moderate to strong. A weak reaction of NADPHD was observed in the mediastinum testis, however, NADH showed more reaction at peripheral part than the centre of rete tubules (Figure 8). Similar activity was reported in buffalo fetus (Bansal *et al.,* 2013). The variation in the activity of various enzymes is correlated with the proliferation of different cell types in buffalo fetus.

The present observations indicated that channels of rete testis showed connections with the testicular parenchyma by straight tubules in early fetal life in buffalo. The presence of various histochemical moieties in the mediastinum testis may be required for transportation of sperms into the excurrent duct channels.

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