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Research Article

HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE PRENATAL DEVELOPMENT OF EPIDIDYMIS IN GOAT (CAPRA HIRCUS)

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ABSTRACT

The study was conducted on the epididymis of 70 goat foeti ranged from 23 days to full term. At 23 days of gestation, the duct was lined by high simple cuboidal to columnar cells. At 78th day of gestation, the supranuclear zone of columnar cells was highly eosinophillic while the deeper zone was lightly eosinophillic. The duct became more tortuous at this stage. Few basal cells were encountered at 108th day of gestation. The basement membrane and luminal border of epithelial cells exhibited intense PAS and mild to moderate acid mucopolysaccharide reaction. The nuclei showed moderate Feulgen reaction.

Key words: Histology, epididymis, goat

The mammalian epididymis is an important organ in which spermatozoa undergo morphological, physiological and maturational changes (Bedford, 1975). Lot of work has been carried out on the postnatal development and regional histology of epididymis of goat (Goyal and William, 1991; Islam *et al.*, 2002; Archana, 2006) but very little literature is available on its prenatal development in goat. Therefore, the present work was planned to study the histology of prenatal development of epididymis in goats.

MATERIALS AND METHODS

A study was conducted on 70 foeti ranging from 23 days to full term which were collected from a local abattoir. The approximate age was computed after measuring the crown rump length and according to the formula derived by Singh *et al.* (1979). These foeti were divided into five groups viz. group I (0-30 days), group II (31-60 days), group III (61-90 days), group IV (91-120 days) and group V (121 days till term) with weight of foeti as nil, 0.86-18.9, 56-200, 284-435.8 and 675-1720.3g, respectively.

The foeti were dissected by giving ventral abdominal incision to expose the developing epididymis. Thin pieces of tissues were collected from the epididymis and put into the 10% neutral buffered formalin and processed. The paraffin sections (5-7 μ) were cut and stained with Haematoxylin and Eosin (Luna, 1968) for routine fibrocellular architecture, Mallory' for collagen fibres, Wilder's reticulum stain for reticular fibers and Weigert's resorcin fuchsin stains (Luna, 1968) for elastic fibers, PAS with and without saliva as well as diastase (Davenport, 1960) for polysaccharides and glycogen, Alcian blue at pH 2.5 (Luna, 1968) for acid mucopolysaccharides (AMPS), Gomori's alkaline phosphatase cobalt method for alkaline phosphatase (Davenport, 1960), Modified lead nitrate method after Takeuchi and Tanoue (Pearse, 1968) for acid phosphatase and Sudan Black-B for lipids and Feulgen reaction (Davenport, 1960) for DNA demonstration. Micrometrical measurements were recorded with the help of a calibrated ocular micrometer. Data were subjected to standard statistical analysis (Snedecor and Cochran, 1994) for interpretation.

RESULTS AND DISCUSSION

Capsule: In group I (0-30 days), developing epididymis (mesonephric duct) was covered by 2-3 layers of mesenchymal cells just below the germinal epithelium of mesonephros at 23rd day of gestation. In group II (31 to 60 days), the developing capsule, the future tunica

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albuginea was located just below the germinal epithelium of mesonephros. It contained mesenchymal cells, immature RBCs and few reticular fibres. The germinal epithelium was lined by simple cuboidal epithelium which became simple squamous at either end of mesonephros. The cytoplasm was eosinophilic and nuclei were vesicular in nature. Connective tissue showed mild reaction for PAS.

In group III (61 to 90 days), the thickness of capsule ranged from 20-25 µ. The septae emanated from the capsule, invaginated into underlying duct and divided the duct into incomplete different lobules. In group IV, the tunica albuginea was 20 to 30 µ thick and became fibrous containing compactly arranged fibroblasts, collagen fibres and reticular fibres and blood vessels running parallel to the free surface. In group V, the thickness of capsule ranged from 30 to 40 µ. In addition to above structures, fine elastic fibres appeared in blood vessels and become highly fibrous and thicker than previous stages.

Mucosa: In group I (0 to 30 days), the mesonephric duct at 23rd day of gestation was located just beneath the tunica albuginea lined by 7.5 to 9 µ high simple cuboidal to columnar epithelium. The cytoplasm was moderately eosinophilic with vesicular nuclei. As reported by Singh (1970) in foetal camel calves, the basement membrane was discontinuous and highly acidophilic in nature (Fig. 1). The diameter of the duct was 30-70 µ with a mean of 45 µ.

In group II (31 to 60 days), the mesonephric duct at 48th day of gestation was mainly lined by simple columnar cells. However, at places, the clusters of undifferentiated cells were also noticed. The nuclei of the cells showed mild to moderate Feulgen reaction (Table 1). At 56th day of gestation, the infra nuclear zone was almost vacuolated while supranuclear zone was lightly eosinophilic. At places, the process of canalization was still in progress (Fig. 2)

In this group, the diameter of duct and luminal diameter ranged from 19.5 to 95 µ and 52.5 to 117.8 µ, respectively. The height and width of epithelial cells measured 6 to 8 µ and 4.5 to 6 µ while the respective height and width of nuclei were 4.5 to 6 µ and 3 to 4.5 µ, respectively (Table 1). The cytoplasm of epithelial cells showed moderate reaction for PAS and acid mucopolysaccharides in supranuclear zone. The luminal border of epithelial cells exhibited intense and mild reaction for alkaline and acid phosphatase, respectively. Lipid granules were observed in the cytoplasm of epithelial cells (Table 1).

In group III (61 to 90 days) at 66th day of gestation, the ductus epididymis was lined by simple columnar epithelium. At places, patches of more than one layer of cells were also observed in the same tubule. Some of the tubules were still solid at this stage whereas the lumen in some tubules contained eosinophilic mass. In this group, the height and width of epithelial cells were 12 to 16.5 µ and 6 to 7.5 µ, respectively (Table 1). At 70th day, the supranuclear zone of the cytoplasm contained two-sub zones, on the basis of staining characters. The superficial zone towards the luminal border was strongly eosinophilic. The deeper zone, situated in between the nuclei and the superficial zone (close to nuclei) was relatively less eosinophilic and granular in nature. Infranuclear zone was vacuolated and less eosinophilic as compared to supranuclear zone. At 70th day, the cell boundaries were distinct. The nuclear chromatin was evenly distributed. The epithelium became tall columnar type at 78th day of gestation. The superficial zone had similar cytoplasmic characters as observed on 70th day except that the cytoplasm was more granular than previous stage. Infranuclear zone was slightly vacuolated.

At 88th day of gestation, the epididymal duct was well developed. Most of the sections of the duct had circular lumen. The duct became more tortuous as revealed by many sections of the duct in cross sectional area. The cytoplasm of columnar cells was lightly eosinophilic than the 78th day. Few narrow columnar cells were also observed. The nuclei of the epithelial cells showed moderate Feulgen reaction. In this group, the diameters of duct and lumen measured 48 to 60 µ and 9 to 15 µ, respectively. Cytoplasm of epithelial cells showed moderate reaction for acid mucopolysaccharides. The supra nuclear zone of epithelial cells exhibited moderate reaction for PAS. The luminal border and

basement membrane showed moderate to intense reaction for PAS. The luminal border of epithelial cells showed intense reaction for alkaline and acid phosphatase. Fine lipid granules were observed in different part of cytoplasm of epithelial cells.

In group IV (91 to 120 days) at 98th day, the duct was lined by tall columnar epithelium. The height and width of columnar cells measured 12 to 18 µ and 6 to 8.0 µ, respectively. Their nuclear height and width measured 6 to 9 µ and 4.5 to 6 µ, respectively. At 108th day, the epithelium of duct contained few spherical basal cells. Their nuclei were spherical in shape and vesicular in nature and showed moderate Feulgen reaction. At 116th day, the cytoplasm of epithelial cells became less eosinophilic and clearer than the previous stages. The luminal border of these cells was highly

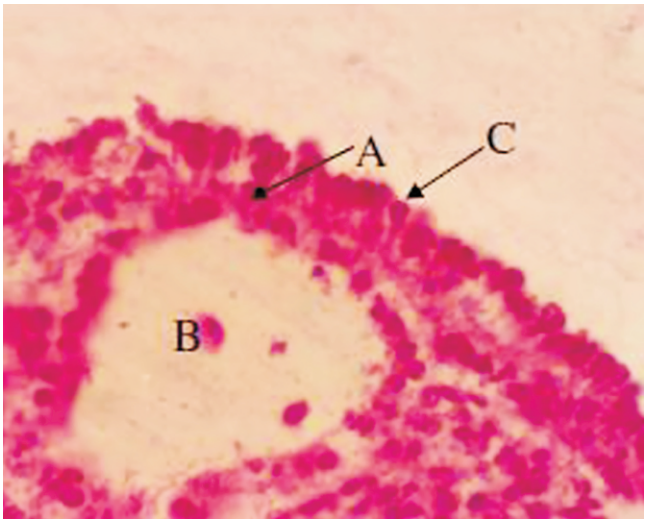

Fig 1. Photomicrograph of mesonephric duct from a 23 day old goat foetus showing columnar cells (A), nucleated RBCs in lumen (B) and germinal epithelium (C). (H. & E. x 400)

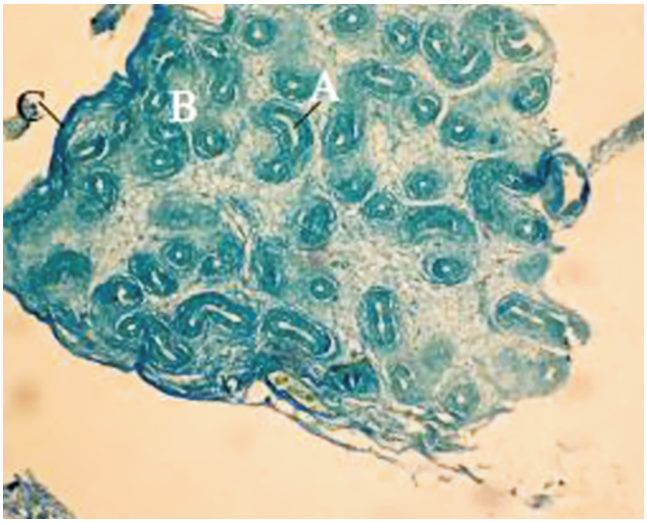

Fig 3. Photomicrograph of epididymis from a 144 day old goat foetus showing PAS reaction in luminal border (A), basement membrane (B) and interductular tissue (C).
(PAS method x 400)

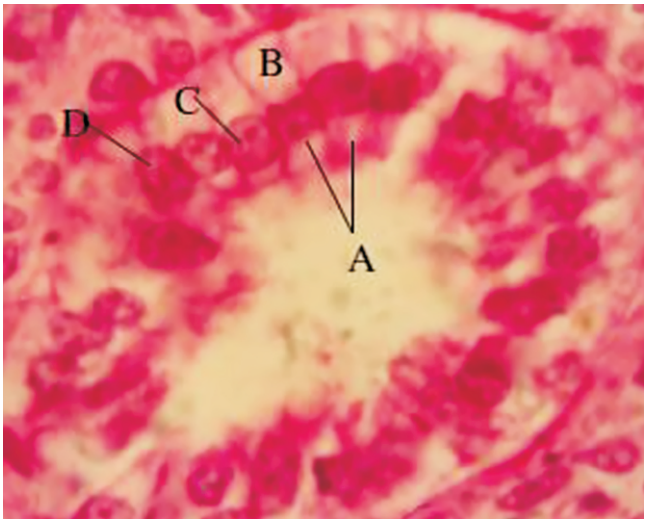

Fig 2. Photomicrograph of mesonephric duct from a 56 day old goat foetus showing columnar cells (A), vacuoles in infranuclear zone (B), spherical nuclei (C) and elongated nuclei (D). (H. & E. x 400)

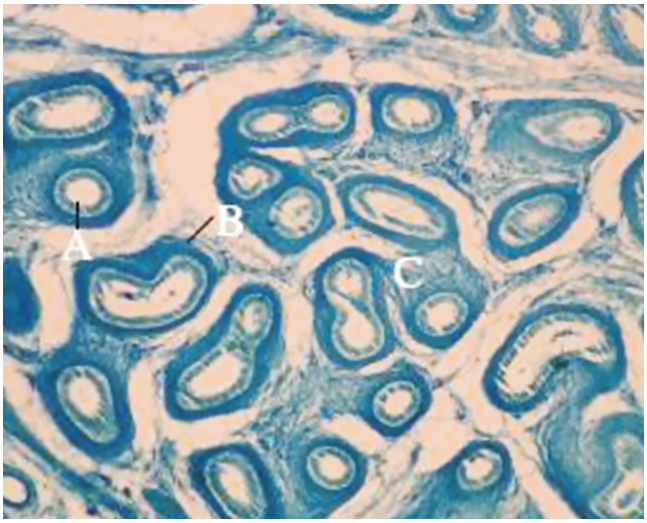

Fig 4. Photomicrograph of epididymis from a 144 day old goat foetus showing presence of AMP in columnar cells (A), basement membrane (B) and periductular tissue (C).
(AMP method x 200)

eosinophilic than the previous stage and showed striations. The basement membrane and luminal border exhibited intense PAS and mild to moderate acid mucopolysaccharides reactions, respectively. The supranuclear zone of cells showed mild PAS reaction. The lumen of the few ducts contained eosinophilic mass. The diameters of the duct and its lumen measured 52.5 to 81.0 µ and 18 to 48 µ, respectively (Table 1). The luminal border of cells exhibited intense and moderate reactions for alkaline and acid phosphatase, respectively. Lipid granules were observed in different parts of cytoplasm (Table 1).

In group V (121st day to till term), the supranuclear zone of the epithelial cells was lightly eosinophilic while infra nuclear zone was almost unstained. The nuclei of the cells showed basophilic nucleoplasm at 144th day. These nuclei exhibited moderate Feulgen reaction (Table 1). The height and width of the epithelial cells were 12.0 to 22.5 µ and 6 to 8 µ, respectively. The height and width of their nuclei measured 4.5 to 8.5 µ

Table 1

Histological and histochemical variations in the epithelium lining the goat epididymis at various stages of gestation

Gp	Type of Epithelium	Diameter		Columnar cells		Cytoplasmic Characters					Nuclear Character	
		Duct	Lumen	Height	Width	PAS	AMP	ALKP	AcP	Lipids	Shape & Size Height & Weight (µ)	Feulgen reaction
II	Simple columnar	19.5-95 µ	52.5-117.8 µ	6-8	3-4.5	++	++	++	+	+	Spherical ovoid or elongated 4.5 to 6x 3 to 4.5	+to++
	Periductular tissue	-	-	-	-	+	++	–to+	+to++	+	-	++
III	Simple columnar to high columnar	48-60 µ	9-15 µ	12-16.5	6-7.5	L. Border +++ cyto++	++	++	L.Border +++ Other part +	+ +	Elongated 4.5 to 9x 3 to 6	++
	Periductular tissue	-	-	-	-	+	+to++	++	++	-	-	++
IV	Tall columnar	52.5-81 µ	18-48 µ	12-18	6-8	L. border +++ cyto+	L. border +++ cyto+ to++	+to ++	L. border ++ Other part +	+	Spherical (basal) Col-elongated 6 to 9x 4.5 to 6	++
	Periductular tissue	-	-	-	-	++	++	+	+	-	-	++
V	Tall Columnar to cuboidal	48-97.5 µ	25-60 µ	12-22.5	6-8	L. border +++ cyto++	L. border +++ cyto++	++	L. border +++ epithelial cells+	+	Spherical (basal) elongated 4.5 to 6.5x 3 to 5.7	++
	Periductular tissue	-	-	-	-	++	++to +++	+	+	-	-	++

Gp=Group; L. Border=Luminal border; Cyto=Cytoplasm; PAS=Periodic Acid Schiff; AMP=Acid mucopolysaccharides; ALKP=Alkaline phosphatase; AcP=Acid phosphatase; +=Mild reaction; ++=Moderate reaction; +++=Intense reaction; +=in traces; -=Negative reaction

and 3 to 5.75 μ, respectively. The diameter of the duct and its lumen measured 48.0 to 97.5 μ and 25 to 60 μ, respectively. The basement membrane and luminal border of cells showed intense and moderate reaction for neutral and acid mucopolysaccharides, respectively (Figs. 3, 4). The columnar cells of duct of epididymis exhibited moderate activity for alkaline phosphatase but the basement membrane exhibited intense reaction for alkaline phosphatase. The cell cytoplasm showed mild to moderate reaction of acid phosphatase. The endothelium of blood vessels showed intense reaction for acid phosphatase. Lipid granules were found in the different part of cytoplasm of cells of epididymal duct. Luminal border exhibited similar histoenzymic reactions as in group IV.

Periductular Tissue: In group II (31st to 60th days) at 48th day of gestation, the mesonephric duct was surrounded by 1-2 layer of differentiating mesenchymal cells (fibroblasts) which varied in shape from spherical, ovoid to fusiform. At 56th day, differentiating circularly arranged mesenchymal cells surrounded the duct (Fig. 2). Periductular tissue showed mild reaction for PAS and moderate acid mucopolysaccharides reactions.

In group III (61st to 90th days) at 66th day of gestation, epididymal ducts were surrounded by differentiating mesenchymal cells. Mesenchymal tissue was very compact and abundant which became somewhat loosely arranged at 69th day of gestation. At 70th day, the layer of fibroblasts encircling the duct was more concentrically arranged and their nuclei were darkly stained. These findings were in agreement with earlier reports of Singh (1970) in foetal camel calves. In the present study, fine reticular and collagenous fibres surrounded the basement membrane of lining epithelium. Gier and Marion (1970) reported that epithelium in foetal epididymis was surrounded by loose fibrocytic adventitia and was enclosed by a firm connective tissue sheath. The entire duct had thin muscular sheath. At 88th day of gestation, each section of duct was surrounded by two layers of differentiating fibroblasts followed by similar developing smooth muscle layers. The nuclei of fibroblasts and mesenchymal cells of peritubular tissue showed intense Feulgen reaction. Branching and anastomosing pattern of reticular fibres in between mesenchymal cells of periductular tissue was observed at 88th day of gestation. Periductular tissue and blood vessels showed moderate reaction of PAS and acid mucopolysaccharides.

In group IV (91st to 120th days), 5-8 layers of smooth muscle surrounding the ducts were circularly directed containing blood vessels in the outer layer. At 108th day, interlobular tissue was comparatively less. Connective tissue septae had similar structures as described on 88th day of gestation. At 116th day, the lobule formation was very much prominent and each lobule contained 4-6 sections. Similar findings were reported earlier by Gier and Marion (1970) in dog and cattle foeti. These authors reported that the epididymal tube elongated and resulted into secondary loops and subsequently complex coiling with lobules before birth was established in 110 days in cattle. However, the age of lobule formation was different in the present study. At 116th day of gestation, inter- and intra-lobular tissue was less in amount and relatively more loosely arranged than 108th day. Periductular tissue showed mild to moderate reaction for PAS and acid mucopolysaccharides

In group V (121st to till term), the duct became more tortuous. Increased in number of sections of duct indicated its complex coiling. There was decrease in amount of connective tissue. Fine elastic fibres were noticed in the blood vessels in this group (at 144th day). Gier and Marion (1970) reported that after inguinal passage, the duct showed complex coiling. Periductular tissue showed mild reactions for alkaline and acid phosphatase and moderate reaction for acid mucopolysaccharides.

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