Haryana Vet. (December, 2016) **55 (2)**, 214-219

Research Article

MICROANATOMICAL AND HISTOCHEMICAL STUDIES ON THE PENIS OF PRENATAL GOAT (*CAPRA HIRCUS*)

M.M. FAROOQUI*, C.P. SHARMA, PRABHAKAR KUMAR, VARSHA GUPTA and AJAY PRAKASH
Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry
DUVASU, Mathura- 281 001, India

Received: 27.07.2016; Accepted: 22.10.2016

ABSTRACT

A study was conducted on the penile urethra and penis of goat foeti from 0 day of gestation to till term. The foeti were divided into five groups viz. Group I (0-30 days), II (31-60 days), III (61-90 days), IV (91-120 days) and V (121-till term). The urethral plates and the groove on ventral aspect of tubercle were observed at 16 mm stage. At 25th day of gestation, microscopically the primordia of the genital tubercle consisted of mass of mesenchymal cells which were arranged in clusters as well as in linear fashion. At 48th day of gestation, the penis consisted of corpus cavernosum penis surrounded by tunica albuginea. The mesenchymal cells from the tunica albuginea started invaginating into underlying mesenchyme to form future septae. Formation of venous spaces began at this stage. The venous spaces increased in number and size as the age of foetus advanced. The shape of the corpus cavernosum penis varied from oval to rectangular with concavity facing ventrally towards the glans region. The connective tissue septae became thicker as the age of foetus advanced. In initial stages of gestation, tunica albuginea contained inner densely arranged, outer loosely arranged zones. The outer zone contained mesenchymal cells and blood capillaries while the inner zone contained compactly arranged mesenchymal cells, differentiating fibroblasts and blood capillaries along with reticular fibres. In late stage of gestation tunica albuginea became fibrous and thicker. The epithelium of glans penis was fused with prepuce epithelium till term.

Key words: Goat, foetus, histogenesis, histochemistry, penis

The work on the anatomy and histology of prenatal development of penis in domestic animals is very scanty. However, few reports are available in swine (Patten, 1948), cattle (Jost *et al.*, 1972), bovines (Inomata *et al.*, 1982) and mammals (Noden and de Lahunta, 1985). Understanding of normal embryonic and foetal development is necessary to understand the consequences of harmful influences at various stages of gestation (Evans and Sack, 1973). Apparently, no work has been reported on the stages of differentiation and the development of the penis in this species, hence the present study was planned.

MATERIALS AND METHODS

The present study was conducted on 70 goat foeti ranged from 0 day to full term collected from the gravid uteri of apparently healthy goat obtained from a local abattoir. Each foetus was weighed and crown rump length (cm) was measured with the help of a nylon tape (Harvey, 1959). The approximate age was computed using the formula derived by Singh *et al.* (1979) after interpolation suggested by Hugget and Widdas (1951) in mammals. These embryo/foeti were divided into five groups (I-V) based on the stage of development 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-till term). The sex of foeti was determined by the appearance of genital swelling, anogenital raphae in group I whereas, in other groups by the development of penis and scrotal sac. The complete male genitalia were collected and the histogenesis of penis was recorded at various stages. The tissues of penis were fixed in 10% neutral buffered formalin and were processed by routine paraffin embedding technique. The section of 5-6 µ were stained with hematoxylin and eosin, Wilder's reticulin stain for reticular fibres, Weigert's resorcin fuchsin stain for elastic fibres, PAS for glycogen, Alcian blue for acidic mucopolysaccharides (Luna, 1968), Mallory's triple stain (Crossman's modification, 1937) for collagen fibres, Modified Gomori's calcium method for alkaline phosphatase and Gomori's lead method for acid phosphatase demonstration (Bancroft and Stevens, 1971).

*Corresponding author: mmfarooqui64@gmail.com

RESULTS AND DISCUSSION

Penile Urethra

Urethral Plate: At 25th day of gestation, the penile urethra was represented by a urethral plate (Fig. 1). The cells of cloacal epithelium invaginated into the urethral plate from its anterior wall. Glenister (1956) observed the urethral plate at 11 mm stage of pig embryo which developed as a lamellar out growth from fused anterior wall of cloaca and urogenital sinus. Basal lamina started appearing in patches. The cytoplasm of the urethral plate

cells was less eosinophilic than the surrounding mesenchyme. The nuclei were spherical to ovoid. Their nuclear chromatin was concentrated towards periphery and few chromatin clumps were noticed. Eccentrically placed nucleoli were intensely acidophilic.

Surface Epithelium: The cells of the surface epithelium were tightly packed and had two layered thick stratified squamous to cuboidal epithelium. Cytoplasm of these cells was finely granular and more acidophilic than urethral plate epithelial cells. Fine chromatin granules were evenly distributed. Few of the surface epithelial cells started invading towards the urethral plate. Glenister (1956) and Felix (1912) observed the disintegration of thickened portion of urethral plate resulted into formation of urethral groove. In the urethral plate few intercellular spaces were encountered to form urethral groove (Fig.1).

The penile urethra was composed of mucosa, corpus cavernosum urethrae and outer most tunica albuginea.

Mucosa: At 48th day of ge station in group II, urethral lumen consisted of solid mass of cells in the glans and terminal part of the body while in the remaining part of the body and root few of the centrally placed epithelial cells started degeneration to form the lumen. Compactly arranged spherical to cuboidal basal cells of the solid mass contained highly acidophilic cytoplasm. The spherical nuclei of these cells were placed centrally and had acidophilic nucleoplasm. The cells of the remaining layers were less eosinophilic and relatively loosely arranged than the basal cells (Fig. 2). At 56th day, lumen formation further enhanced (Fig. 3). The basal cells of epithelium had similar tinctorial characters as observed on 48th day.

The cytoplasm of the basal cells and developing luminal border showed strong acid mucopolysaccharides and PAS reactions.

In group III, the fully developed lumen of the penile urethra was lined by 20 to 35 µ high transitional type of epithelium. At 66th day of gestation, the basal cells were spherical or cuboidal in shape (Fig. 4). Their cytological characters were similar to that described in group II. The cells in the middle layer were polygonal in shape with distinct cell boundaries. The cytoplasm of these cells was less eosinophilic than the basal cells. The nuclei of the cells were eccentrically placed and nucleoplasm was lightly stained and nuclear chromatin was evenly distributed. Few degenerating cells in the middle layer were also encountered. At 88th day of gestation, the mucosa became folded containing 5 to 7 layers thick transitional epithelium. The cytological characters were similar to those observed on 66th day of gestation.

In group III, the basal cells and luminal borders showed strong PAS and acid mucopolysaccharides reaction. The luminal border and basal cells showed moderate to intense acid and alkaline phosphatase reactions. The cells of the remaining layers exhibited moderate reactions for both enzymes. In group IV (91 to 120 days), the penile urethra was lined by stratified squamous to transitional epithelium (Fig. 5). Cytological characters resembled to those observed in group III. The luminal border and the basal cells showed strong acid phosphatase reaction (Fig. 10). In group V, the mucosa was lined by transitional epithelium (Fig. 6). At places, patches of stratified squamous epithelium were also observed. However, in glans region at 144th day, highly longitudinally folded mucosa was lined by 12 to 18 µm thick transitional epithelium (Figs. 7 and 9). The basal layer of epithelium contained cuboidal to columnar cells having highly eosinophilic cytoplasm with spherical, ovoid or elongated, densely stained nuclei. The cytoplasm of the middle layer cells was less eosinophilic as compared to the basal cells. Their ovoid or elongated nuclei contained evenly distributed and relatively lightly stained chromatin than the basal cells. Few cells of this layer showed degenerative changes. The superficial cells had pear shaped structure with distinct cell boundaries with eosinophilic cytoplasm and flat, elongated or ovoid nuclei which showed moderate Feulgen reaction.

The cytoplasm of basal cells and luminal border exhibited intense reaction for PAS and moderate reaction for acid mucopolysaccharides, respectively. Middle layer cells showed mild to moderate reaction for PAS. Corpus cavernosum urethrae showed intense reaction for PAS. The luminal border and basal cells exhibited moderate to intense alkaline and acid phosphatase reactions.

Lamina Propria: In group II (31 to 60 days), a layer of differentiating fibroblasts encircled the basal layer of penile urethra. The cytoplasm of these cells was highly eosinophilic and nuclei were relatively darkly stained. Few RBCs were present on the periphery of epithelial mass. It contained loosely arranged mesenchymal cells, which varied in shape from fusiform, spherical to stellate. Few of the flat mesenchymal cells arranged in a roughly circular manner and enclosing a space, had one to two RBCs, indicated the development of corpus cavernosum urethrae. These capillaries were of relatively larger in size as compared to corpus cavernosum penis of the same stage. The connective tissue of lamina propria showed moderate acid mucopolysaccharides and PAS reactions.

In group III (61 to 90 days), lamina propria contained loosely arranged reticular fibres, mesenchymal cells, fibroblasts and cavernous spaces (Fig. 4). It was thick ventrally and thin towards the dorsal side. Cavernous spaces increased in size and numbers at this stage towards ventral side of urethral lumen. At 88th day, lamina propria submucosa contained loosely arranged circularly directed collagen and reticular fibres along with cavernous spaces (Fig. 5). The cavernous spaces were smaller in size around the urethral lumen and became larger towards periphery. Connective tissue of lamina propria exhibited moderate reaction for PAS and acid mucopolysaccharides. The cavernous spaces showed moderate to strong alkaline and acid phosphatase activity. Connective tissue of lamina propria exhibited mild to moderate reaction for both enzymes.

In group IV, cavernous spaces increased in size and numbers. Numerous longitudinally oriented collagenous fibres were observed. The connective tissue was loosely arranged than the previous stages. Connective tissue of lamina propria showed mild to moderate reaction for neutral and acid mucopolysaccharides. The connective tissue of lamina propria exhibited mild to moderate reaction for acid phosphatase and the cavernous spaces showed moderate reaction for acid phosphatase (Fig. 10). Cavernous spaces exhibited moderate to strong alkaline phosphatase reaction. In group V, lamina propria contained mesenchymal cells, fibroblasts, wavy collagenous and reticular fibres. Cavernous spaces were connected with each other (Fig. 6) and surrounded by elastic fibres. The connective tissue of lamina propria showed mild to moderate PAS and AMP reactions (Fig. 7). Cavernous spaces showed intense reaction for PAS. It exhibited mild to moderate reaction for both enzymes.

Tunica Albuginea: In group II, at 48th day of gestation the tunica albuginea contained mesenchymal cells, differentiating fibroblasts, nucleated RBCs and reticular fibres. At 56th day of gestation tunica albuginea of urethra was thinner and cells were loosely arranged as compared to the tunica albuginea of the penis. It was slightly reactive to PAS while the cavernous spaces showed strong reaction for PAS. In group III, it contained 2 to 3 layers of circularly arranged differentiating fibroblasts, reticular and collagenous fibres along with blood vessels (Fig. 4). In groups IV and V, it became thicker than the previous stages. It was 10-25 µ thick and contained fibroblasts, collagen and reticular fibers along with blood vessels. Collagenous fibres were directed circularly.

Penis: In group I, at 25th day of gestation, the primordium of genital tubercle consisted of mass of mesenchymal cells. In group II from 48th day onwards, the penis in prenatal male goat, consisted of corpus cavernosum penis surrounded by tunic a albuginea. At 48th of gestation, the outer zone contained loosely arranged mesenchymal cells and blood capillaries while the inner zone contained compactly arranged mesenchymal cells, differentiating fibroblasts and blood capillaries along with fine reticular fibres (Fig. 2). The tunica albuginea was covered by loose connective tissue containing dilated capillaries and developing nerve bundles. At one to two places, the mesenchymal cells from the tunica albuginea started invaginating into underlying developing mesenchymal corpus cavernosum penis, the future septae (Fig. 2).

Corpus cavernosum penis mainly consisted of mesenchymal cells, which were spherical, ovoid and stellate shape. The cytoplasm was highly eosinophilic. At places, nucleated RBCs and clusters of mesenchymal cells were noticed. Arey (1966) described that during initial stage of development, developing RBCs had nuclei with presence of hemoglobin in their cytoplasm in mammals and their nuclei were lost during the process of development. At one or two places flat mesenchymal cells were arranged in circular manner and enclosed a narrow space, future lumen of developing capillaries indicated the beginning of the formation of venous space.

At 56th day, the process of formation of venous spaces was further progressed. Mesenchymal cells of the tunica albuginea, at few places, dipped down in different directions. The cytoplasm of mesenchymal cells was less eosinophilic than the 48th day of gestation. Vesicular nuclei contained fine chromatin granules (Fig. 3). The mesenchymal cells enveloping the developing capillaries further elongated and their nuclei were flat, the future endothelial cells as described by Arey (1966). In between he mesenchymal cells open labyrinthine spaces were observed which were filled with ground substance. The ground substance was like semi fluid jelly, which might be serving as packing material between the emerging sinusoids and developing connective tissue fibres as also reported by Copenhaver *et al.* (1978). The connective tissue of corpus cavernosum penis was mildly positive for PAS and moderately positive for acid mucopolysaccharides. Venous spaces exhibited moderate PAS and AMP reactions.

In group III from the tunica albuginea, developing fibroblasts formed 2-3 cells thick chains, which arranged in a perpendicular fashion, the future connective tissue

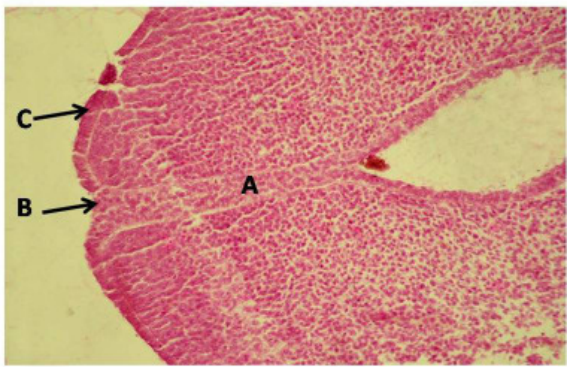
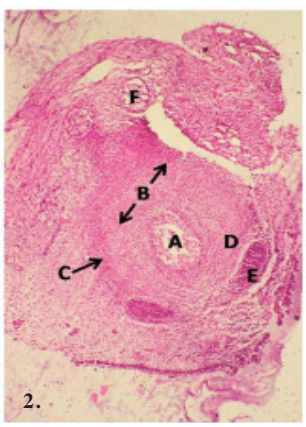


Fig 1. Photomicrograph of cross section through cloacal region of 25 day old goat embryo showing urethral plate (A), primary urethral groove (B) and surface epithelium (C).H&E x 200



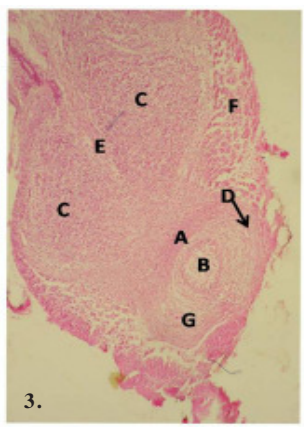
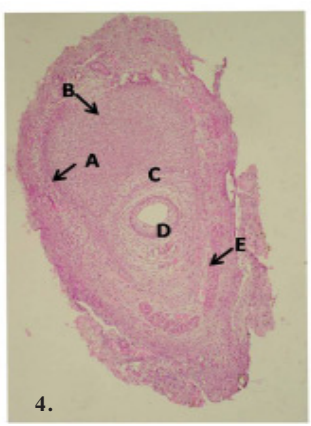


Fig 2. Photomicrograph of penis from a 48 day old goat foetus showing lumen of penile urethra (A), corpus cavernosum penis (B), tunica albuginea of penis (C), lamina propria of penile urethra (D), smooth muscle (E) and nerve bundle (F). H&E x 100

Fig 3. Photomicrograph of root of penis from a 56day old goat foetus showing tunica albuginea of urethra (A), penile urethra with canalization (B), corpus cavernosum penis (C), corpus cavernosum uraethrae (mesenchymal mass) (D), developing septum (E), muscle (F) and lamina propria (G). H&E x 100



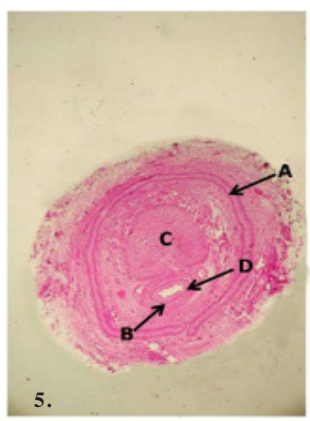


Fig 4. Photomicrograph of body of penis from a 66 day old goat foetus showing tunica albuginea of penis (A), venous spaces (B), tunica albuginea of urethra (C), urethral epithelium (D) and smooth muscle (E). H&E x 100

Fig 5. Photomicrograph of glans of penis from a 118 day old goat foetus showing fused epithelium of glans and prepuce (A), lamina propria (B), corpus cavernosum penis (C) and epithelium of penile urethra (D). H&E x 40

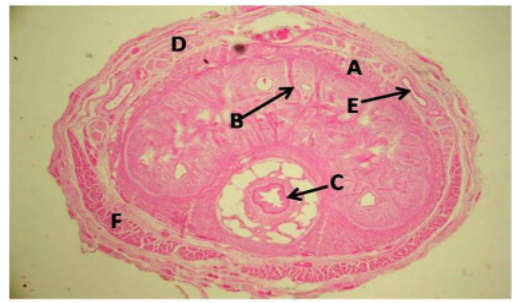


Fig 6. Photomicrograph of body of the penis near the glans from a 125 day old goat foetus showing tunica albuginea (A), corpus cavernosum penis containing trabeculae (B), epithelium of penile urethra (C), loose mesenchyme (D), blood vessels (E) and smooth muscle (F).

H&E x 40

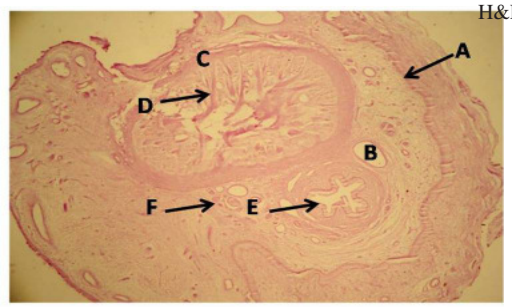


Fig 7. Photomicrograph of glans of penis from a 135 day old goat foetus showing epithelium of glans (A), venous spaces (B), outer tunica albuginea (C), corpus cavernosum penis containing septae (D), epithelium of penile urethra (E) and lamellated nerve ending (F).

:E x 40

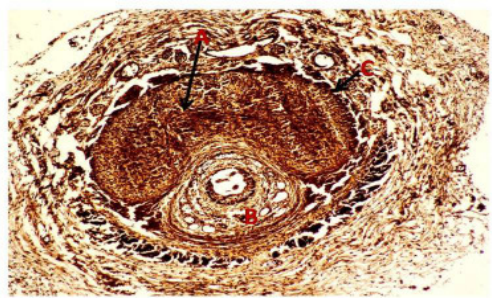


Fig. 8 Photomicrograph of penis from a 69 day old goat foetus showing, reticular fibres in corpus cavernosum penis (A), lamina propria of penile uretha (B) and outer layer of tunica albuginea (C).

Wielder's reticulum stain x 100

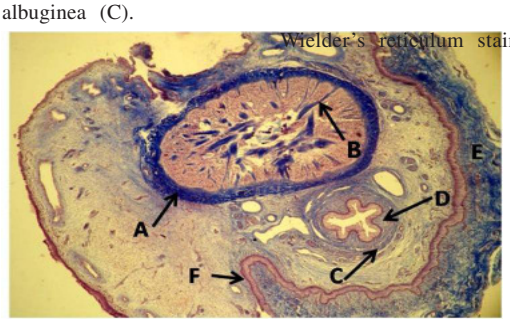


Fig. 9 Photomicrograph of glans of penis from a 144 day old foetus showing collagen fibres in tunica albuginea of penis (A), trabeculae (B), tunica albuginea of penile urethra (C), lamina propria of penile urethra (D), loose connective tissue of prepuce (E) and epithelium of glans (F). Mallory's triple stain x 40

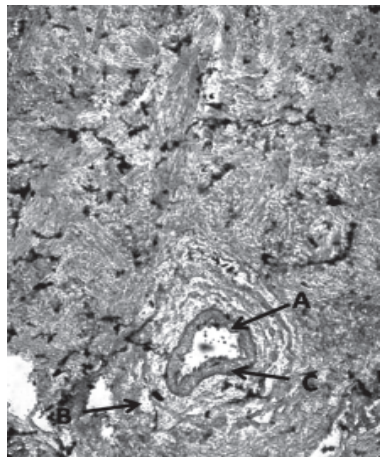


Fig. 10 Photomicrograph of body of penis from a 115 day old goat foetus showing alkaline phosphatase reaction in lumina border of urethra (A), venous spaces (B) and basal layer of cells (C).
Modified Gomori's calcium method x 200

septae (Fig. 4). These cells were fusiform in shape. The cytoplasm of these cells was less eosinophilic and the nuclei were densely stained.

Reticular fibres were abundant in corpus cavernosum penis while coarse reticular fibres were seen in the tunica albuginea (Fig. 8). Fine collagen fibrils appeared in the tunica albuginea and outer fascia. Venous spaces of corpus cavernosum penis further increased in number and size. These spaces were separated from each other by mesenchymal tissue with fine reticular fibres at 66th day of gestation. At 88th day of gestation, the septae contained compactly arranged fibroblasts running in different directions inside the corpus cavernosum penis.

In group III, the connective tissue septae of corpus cavernosum penis showed mild to moderate reaction for acid mucopolysaccharides. The venous spaces exhibited moderate reaction for acid mucopolysaccharides and intense PAS reactions.

The tunica albuginea showed mild reaction for alkaline phosphatase. The connective tissue septae showed moderate reaction for alkaline phosphatase. The venous spaces showed moderate to intense reaction for alkaline phosphatase. The tunica albuginea and connective tissue septae showed mild and moderate reactions for acid phosphatase, respectively. The venous spaces at few places exhibited moderate reaction for acid phosphatase.

The shape of corpus cavernosum penis varied greatly in different regions. In root region it appeared oval in shape. In the body region it was almost flat or rectangular, below the sigmoid flexure it showed concavity ventrally facing towards urethra (Figs. 6, 8). Towards the glans region it was deeply grooved. Gupta *et al*. (1980) reported that there was continuous and gradual recession in the cross sectional area of the body of the penis from the level of the root towards the glans in neonatal buffalo calves. In the glans region it did not form the groove and the penile urethra was placed ventrally and appeared pushed to one corner near term (Fig. 9).

At 93rd day of gestation in group IV, the connective tissue septae contained reticular and collagen fibres with fibroblasts. The branching and anastomosing reticular fibres along with wavy collagen fibres formed thick bundles. At 108th day, these septae increased in thickness and were directed in various directions. These structures were anastomosing in centre of corpus cavernosum penis with the septae originating from ventral side and formed a connective tissue band running longitudinally. Cavernous spaces further increased in number and size. At 116th day, septae became thicker and gave the subsequent branches and divided the corpus cavernosum penis. In this group, the tunica albuginea and connective tissue septae showed moderate reaction for acid mucopolysaccharides while cavernous spaces exhibited relatively more reaction for acid mucopolysaccharides. The tunica albuginea and connective tissue septae showed moderate reaction for alkaline and acid phosphatase. Venous spaces exhibited moderate to intense reaction for acid phosphatase (Fig. 10).

In group V, the thickness of tunica albuginea further increased and became highly fibrous (Fig. 6). The connective tissue septae became fibrous and thicker than group IV. These septae contained collagen fibre bundles (Fig. 9) along with reticular fibres and fibroblasts. The mesenchymal cells decreased in number. Cavernous spaces further increased in number and size. The connective tissue septae showed moderate while tunica albuginea exhibited mild reaction for acid mucopolysaccharides. Histoenzyme reactions were similar to those described in group IV. The tunica albuginea and connective tissue septae showed moderate to intense reaction for PAS. The tunica albuginea was surrounded by loosely arranged connective tissue containing blood vessels, smooth muscles of retractor penis and nerve bundles (Fig. 6) as also observed by Trautman and Fiebiger (1957).

The thickness of outer zone of the tunica albuginea ranged 10 to 15, 20 to 28.0, 34.2 to 60.8 and 63 to 78 µ in groups II, III, IV and V, respectively. The thickness of inner zone of the tunica albuginea ranged from 4 to 6, 10 to 15.2, 20.9 to 26.6 and 21.28 to 38.5 µm in groups II, III, IV and V, respectively. The data indicated that the thickness

of capsule increased gradually from group II to group V.

Covering Epithelium of Glans Penis: The epithelium of the glans penis was fused with the prepuce epithelium till term (Figs. 5, 6, 9). The epithelium was indistinguishable-stratified type. The basal cells were cuboidal in shape with spherical or elongated nuclei. The cell boundaries were distinct and the cytoplasm of the basal cells was highly eosinophilic than the other layer cells. The nuclear chromatin was evenly distributed. The distinct basement membrane was highly eosinophilic. Many venous spaces were encountered in the loose connective tissue layer located immediately beneath the epithelium. The epithelium showed mild to moderate reaction for acid mucopolysaccharides and moderate PAS reaction (Fig. 7). The endothelium of venous spaces located below the epithelium showed intense PAS reaction (Fig. 7). The venous spaces increased in size and number from II to V group. Lamellated nerve endings were observed in the loose connective tissue beneath the glans epithelium near the penile urethra (Fig. 7).

REFERENCES

Arey, L.B. (1966). Developmental Anatomy - A Text Book and Laboratory Manual of Embryology. (7th edn.). W.B. Saunders Company, Philadelphia and London.

Bancroft, J.D. and Stevens, A. (1971). Theory and Practice of Histological Technique. Churchill Livingston, New York.

Copenhaver, W.M.; Kelly, D.E. and Wood, R.L. (1978). Bailey's Text Book of Histology. (17th edn.). The Williams and Wilkins Company, Baltimore.

Crossman, G.A. (1937). A modification of Mallory's connective tissue stain with discussion of principles involved. *Anatomical Rec.* **69**: 33-38.

Evans, H.E. and Sack, W.O. (1973). Prenatal development of domestic and laboratory mammals. *Zbl. Vet. Med.(c) Anatomia Histologia Embryologia* **2**: 11-45.

Felix, W. (1912). The Development of the Urogenital Organs. In: Human Embryology. Keibel and Mall, J.B. (edts.). Lippincott Company, Philadelphia and London.

Glenister, T.W. (1956). The development of penile urethra in the pig. *J. Anat.* **90**: 461-477.

Gupta, A.N., Dhingra, L.D., Singh, Y. Singh and Sharma, D.N. (1980). Postnatal development of the penis in Murrah buffalo – gross anatomical study. *Haryana Vet.* **19(1)**: 12-18.

Harvey, E.B. (1959). Aging and Foetal Development. In*:* Reproduction in Domestic Animals (Eds.), Cole, H.H. and Eupps, P.T. (edts.). (Ist edn.). Vol. I, Academic Press Inc., New York.

Hugget, A. St. G. and Widdas, W.F. (1951). The relationship between mammalian foetal weight and conception age. *J. Physiol.* **114**: 306-317.

Inomata, T., Eguchi, Y., Yamamoto, M., Asari, M. and Kano, Y. (1982). Development of the external genitalia in bovine fetuses. *Japan J. Vet. Sci.* **44**: 489-96.

Jost, A., Vigier, B. and Prepin, J. (1972). Freemartins in cattle: The first steps of sexual organogenesis. *J. Reprod. Fert.,* **29** : 349-379.

Luna, L.G. (1968). Manual of Histological Staining Methods of the Armed Forces Institute of Pathology. (3rd edn.). Mc-Graw Hill Book Company, New York.

Noden, D.M. and de Lahunta, A. (1985). The Embryology of Domestic Animals. (Ist edn.). The Williams and Wilkins Company, Baltimore, U.S.A.

Patten, B.M. (1948). Embryology of the Pig. McGraw Hill Book Company, New York.

Singh, Y., Sharma, D.N. and Dhingra, L.D. (1979). Morphogenesis of the testis in goat. *Indian J. Anim. Sci.* **49(11)**: 925-931.