ULTRSTRUCTURAL STUDY ON EFFERENT DUCTULE OF BUFFALO FOETII

Anil Sharma[1], Neelam Bansal[2], Varinder Uppal[2] and Devendra Pathak[2,*]

ABSTRACT

The present study was conducted on 10 buffalo foetii ranging from 13.5 cm CVRL to 98 cm CVRL (89 to 295 days). After collection the tissue samples were processed for transmission electron microscopy. The efferent ductules of buffalo foetii were lined with simple columnar epithelium with ciliated and non-ciliated cells. The ciliated cells were few in number in comparison to the non-ciliated cells. The ciliated cells were characterized by presence of cilia on apical surface with typical 9+2 arrangement and their basal bodies just beneath the apical plasma lemma. Whereas the non-ciliated cells were devoid of cilia. The supra-nuclear cytoplasm of non-ciliated cells contained many coated vesicles. In the later stage of development some migratory intraepithelial and peritubular leukocytes were also observed. The tight junctions found to be poorly developed in the epithelial lining of efferent ductules at 13.5 cm CVRL (89 days) and were well developed at 19.5 cm CVRL (116 days) onwards. In early stage of development efferent ductules were surrounded with the mesenchymal tissue which after condensation differentiated into smooth muscle cells in later stage of development.

Keywords: ultra structure, efferent ductules, buffalo foetii, prenatal development

INTRODUCTION

Reproductive efficiency in sexually mature animals can be influenced by various environmental and physiological factors. Such factors may influence normal embryological differentiation of the reproductive system which later may influence reproductive performance (Gier and Marion, 1969). Thorough knowledge of the development of reproductive system is a prerequisite to understand the structure and function of this system. Efferent ductules are series of small tubules that connects the rete testis and epididymis and to be considered as a component of head of epididymis (Eurell and Frappier, 2006). The efferent ductules are involved in fluid reabsorption and secretion of glycopeptides which play a very critical role in sperm maturation. In bovines the efferent ductules originated as a new set of secondary mesonephric tubules those grew out from the dorsal aspect of the mesonephric giant corpuscle and were not formed by transformation of primary mesonephric tubules (Wrobel, 2001). In the available literature, most of the research on the efferent ductules had been reported during postnatal life in buffalo

[1]Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Junagadh, Agricultural University (JAU), Gujarat, India

[2]Department of Veterinary Anatomy, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Punjab, India, *E-mail: drdevendra@gmail.com

(Goyal and Dhingra, 1975; Singh, 1989; Abdou *et al*., 2007). Prenatal histological work is reported mainly in dog (Gier and Marion, 1969), rhesus monkey (Alexander, 1972), rat (Marshall *et al*., 1979), human (De Miguel *et al*., 1998), cat (Arrighi *et al*., 1993), bovine (Mohamed, 2005) and mouse (Joseph *et al*., 2009). So keeping in view the paucity of available literature on prenatal ultrastructural studies especially in buffalo, the present study was conducted to observe the ultrastructure of efferent ductules during prenatal life of buffalo.

MATERIALS AND METHODS

Sample collection

The present study was conducted on epididymis from 10 Indian buffalo foetii. The samples were collected from slaughter house and from the Veterinary Clinics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The age of foetii was determined by measuring the CVRL 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 as described by Edward (1965). The approximate age of the foetii was calculated by using following formula given by Soliman (1975).

$Y = 28.66 + 4.496\ X$ (CVRL < 20 cm)

$Y = 73.544 + 2.256\ X$ (CVRL ³ 20 cm)

Where Y is the gestational age in days and X is the CVRL in cm.

As per the above mentioned formula the gestational age of buffalo foetii used in present study was ranging from 89 to 295 days (13.5 cm CVRL to 98 cm CVRL). The sample from undescended epididymis was collected by dissecting the abdominal cavity and from the descended epididymis were collected after dissecting the scrotum.

Fixation and tissue processing for transmission electron microscopy

Immediately after collection tissue samples were thoroughly washed in phosphate buffer saline (pH 7.4) solution and subsequently trimmed of 1 mm^3 size. These samples were fixed for 2 h in 2.5% gluteraldehyde fixative and then secondary fixation was done for 3 h in 2% OsO_4. Subsquently tissue samples were subjected to dehydration in ascending grades of acetone (30 to absolute). The dehydration in dry acetone was done at room temperature. The clearing of the tissue samples were carried out by treatment with toluene. Subsequent infiltration was carried out and the tissues were embedded in pure embedding media using beam capsule. After polymerization the blocks were prepared and trimmed by block trimmer (Reichert TM 60). Semi thin sections (0.5 to 2.0 μm) were cut to scan the tissues under optical microscope for selection of area for ultrathin sectioning. The ultrathin sections (70 to 90 nm) were cut and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50 Aº thickness. The grids were then stained with uranyl acetate (15 minutes) followed by lead citrate (10 minutes). The grids thus prepared were examined under TEM (Morgagni) for detailed study and required photographs were taken.

RESULT AND DISCUSSION

The efferent ductules developed as small and round luminized and non luminized set of secondary mesonephric tubules. Present

ultrastructural study on efferent ductules of buffalo foetii revealed that the efferent ductules were lined by simple columnar epithelium. At 13.5 cm CVRL (89 days) the epithelium of efferent ductules contained two types of columnar cells *viz*; ciliated and non-ciliated cells. These cells rested on a highly undulating basement membrane which displayed an irregular border to the surrounding stroma. The nuclei of these ciliated and non-ciliated cells located in basal half of cell, ovoid in shape and contained one or two nucleoli. Some nuclei had slightly undulated contour and some had deeper invaginations (Figure 1). Moustafa and Hafez (1971) noticed in bovine fetus between 70 and 150 days of gestation that efferent ductules were lined by an irregular cuboidal epithelium supported by a prominent basement membrane, whereas Rüsse and Sinowatz (1991) observed that the epithelium of ductuli efferentes contained ciliated and nonciliated cells at the fourth month of gestation in bovine fetus. Wrobel and Schimmel (2001) observed in bovine fetuses that cytological differentiation of these ductules proceeds in a proximo - distal direction. At about 50 to 60 days post conception the proximal portion of efferent ductules were lined by the simple columnar epithelium consisting of reabsorptive principal cells and ciliated cells. The distal part of efferent ductules was lined by cuboidal epithelium.

The ciliated cells were few in number in comparison to the non-ciliated cells and were characterized by presence of cilia on the apical surface of cell. A number of ciliary basal bodies with rootlets were arranged just beneath the apical plasma lemma. In a mature male the cilia of efferent ductules helps in transit of sperms towards the ductus epididymidis (Ilio and Hess, 1994). But at prenatal stage function was not clear; it is may be a part of differentiation process of ciliated cell of efferent ductules. The supra-nuclear cytoplasm of ciliated cells contained many clear vacuoles, and free ribosomes, whereas the infra-nuclear cytoplasm contained mainly free ribosomes and some mitochondria.

The non-ciliated cells were more in number. The supra-nuclear cytoplasm had large clear areas, some mitochondria and free ribosomes, whereas the infra-nuclear cytoplasm had clear or less electron dense areas. Pelliniemi *et al.* (1983) also observed such type of clear area in some epithelial cells of efferent ductules of early stage human embryo and stated that these less electron dense areas in infranuclear cytoplasm was due to the accumulation of glycogen particles. The luminal surface of some non-ciliated cells had large apical protrusions which were attached with narrow neck to the cell and supra-nuclear cytoplasm of these non-ciliated cells also contained many coated vesicles (Figure 2). Our findings were supported by the findings of Ilio and Hess (1994) who reported similar apical protrusions on luminal surface of non-ciliated epithelial cells in efferent ductules of dog in postnatal life. At early stage of development of efferent ductules, the junctional complex was poorly developed and sealed the intercellular cleft against the ductular lumen. The efferent ductules at this stage were surrounded by undifferentiated mesenchymal cells with poorly developed boundaries having oval nuclei. The proliferating mesenchymal cells arranged in several concentric layers at about day 85 post conception and were considered to be the precursors of the periductular musculature in bovine fetuses (Wrobel, 2001).

At 19.5 cm CVRL (116 days), the type of epithelial lining of efferent ductules was similar to that of earlier stage. The epithelial cells were resting on a well-developed and regular basal lamina (Figure 4). The ciliated cells were few in number. The

supra nuclear cytoplasm of ciliated cells contained few electron dense bodies, mitochondria, cisternae of rough endoplasmic reticulum and numerous free ribosomes. Well-developed cilia emerged from the apical surface and had a typical 9+2 arrangement. The apical surface also had short microvilli which intermingled with cilia (Figure 5). Arrighi *et al.* (1994) also observed similar type of intermingling of microvilli with cilia in adult equine efferent ductules. Some ciliated cells also contained apical protrusions in between two microvilli. The infra-nuclear cytoplasm of ciliated cells contained mitochondria and rough endoplasmic reticulum (rER). The supra-nuclear cytoplasm of non-ciliated cells contained some mitochondria, coated vesicles and few profiles of rER, whereas some cells also had endocytic vesicles just beneath the apical plasma lemma (Figure 3). The characteristics of infra-nuclear cytoplasm were similar to that of ciliated cells. At 19.5 cm CVRL the junctional complex (tight junction) were well developed and sealed the intercellular cleft against the ductular lumen. Wrobel and Schimmel (2001) also reported the presence of well-developed junctional complex and a number of desmosomes in between the adjacent cells of ductular epithelium of bovine fetuses. The junctional complex between two adjacent epithelial cells, near the lumen contained zonula occludens and zonula adherence which played a role in development of epididymis blood barrier. Lopez *et al.* (1997) noticed in stallion efferent ductules that the zonula occludens is the principal structural component of the blood-epididymis barrier. At this stage, the peritubular area of efferent ductules contained undifferentiated mesenchymal cells and developing connective tissue. The nuclei were elongated and showed mitotic divisions at places.

At 31 cm CVRL (143 days), the lining epithelium of efferent ductules were simple columnar consisting of randomly distributed few ciliated cells and more numerous non-ciliated cells. The oval nuclei of both cell types were placed in basal half of cells, but the nuclei of ciliated cells were more heterochromatic. It was also observed that the cytoplasm of ciliated cells were more electron dense than the non-ciliated cells (Figure 6). Similar findings were also observed by Arrighi *et al.* (1994) in equine efferent ductules. The supranuclear cytoplasm of ciliated cells contained more rER cisternae and free ribosomes and few vesicles; while that of non-ciliated cells contained few rER profiles, many membrane bound vesicles and large Golgi bodies. The narrow infranuclear cytoplasm of ciliated cells had more longitudinal mitochondria and rER as compared to non-ciliated cells. Wrobel and Schimmel (2001) stated that the embryonic cells appeared less mature and showed absolute characteristics for their identification as efferent ductular epithelium. They also reported that the bovine efferent ductular epithelium displays two periods of high cytological differentiation, i.e. a prenatal one when the fetal Leydig cell population is well-developed and a postnatal one depending apparently on the pubertal rise in androgens. Bentvelsen *et al.* (1995) also reported the presence of androgen receptors in efferent ductules of fetal rats. The peritubular mesenchymal cells, near the epithelial basement membrane differentiated earlier and acquire appearance similar to that of smooth muscle cells (Figure 7); whereas the outer most ones were still less differentiated. The intertubular stroma consisted of blood capillaries, mesenchymal cells and leukocytes. Similar observations were made by Mohamed (2005) in bovine fetuses at 24 cm CRL.

At 75 cm CVRL (243 days), the characteristic of epithelial lining were similar to epithelium at 31 cm CVRL (143 days) buffalo foetii.

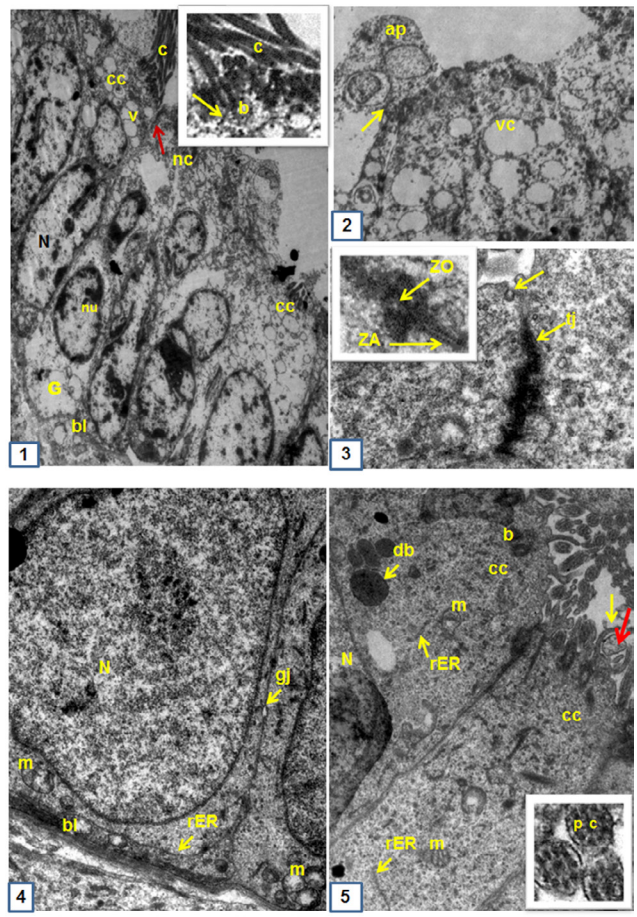

Figure 1. Simple columnar epithelium of efferent ductules showing Ciliated (cc) and non-ciliated (nc) cells, undulating basal lamina (bl), nuclei (N) had nucleoli (nu), vacuoles (v), cilia (c), large non staining areas (G). Poorly developed junctional complex (arrow) at 13.5 cm CVRL (89 days). X 1000. [Inset: Ciliary basal bodies (b) with rootlets (arrow) arranged just beneath the apical plasma lemma. X 2000.

Figure 2. Luminal surface of non-ciliated cells had large apical protrusions (ap) attached with a narrow neck (arrow). Supranuclear cytoplasm contained many coated vesicles (vc) at 13.5 cm CVRL (89 days). X 2500.

Figure 3. Supra nuclear cytoplasm of non-ciliated cells with endocytic vesicle (arrow) just beneath the apical plasma lemma. Well developed junctional complexes (tj) observed at 19.5 cm CVRL (116 days). X 6300. [Inset: The tight junction containing zonula occludens and zonula adherence].

Figure 4. Epithelial cells resting on a regular basal lamina (bl), the infra nuclear cytoplasm contained mitochondria (m) and rough endoplasmic reticulum (rER). Gap junctions (gj) observed in between adjacent cells at 19.5 cm CVRL (116 days). X 3200.

Figure 5. Supra nuclear cytoplasm of ciliated cells (cc) contained electron dense bodies (db), mitochondria (m), rough endoplasmic reticulum (rER). The apical surface had Cilia (c) emerged from basal bodies (b), intermingled microvilli (mv) and Some apical protrusions (arrow) at 19.5 cm CVRL (116 days). X 3200. [Inset: cilia with typical 9+2 (nine peripheral (p) microtubule doublets surrounding a pair of central (c) microtubules) arrangement. X 8000].

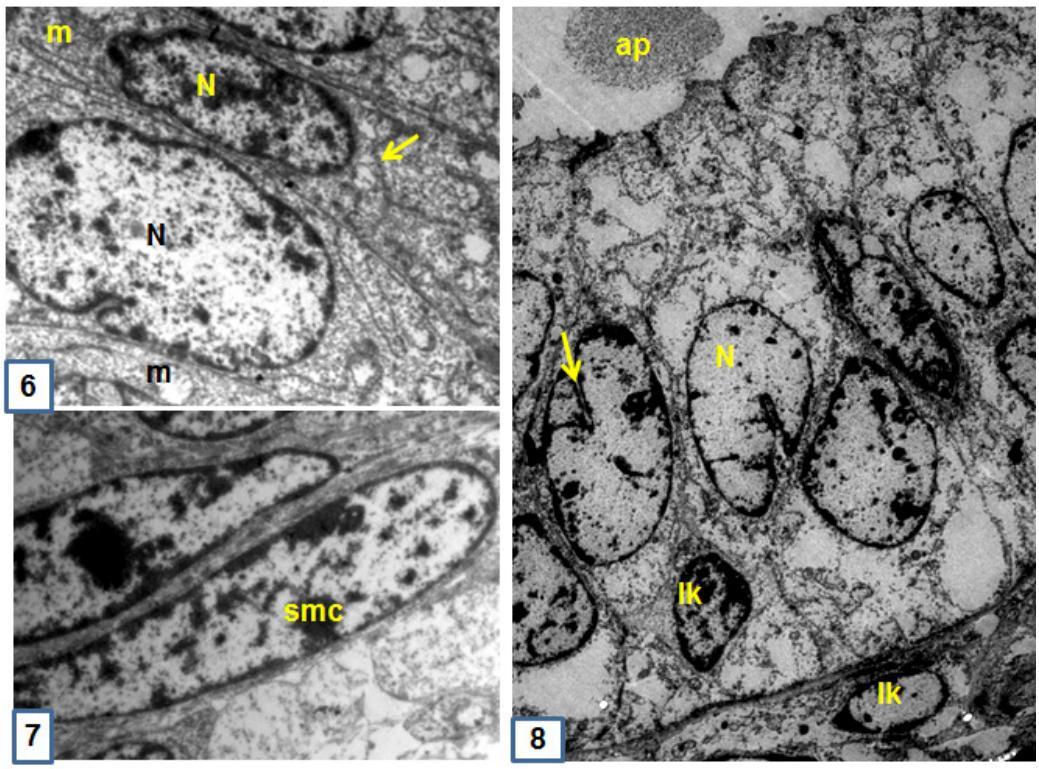

Figure 6. Nuclei (N) of ciliated cells (cc) was heterochromatic, the cytoplasm contained more rER and mitochondria (m) than the non-ciliated cells (nc) cells at 31 cm CVRL (143 days). X 1600.

Figure 7. Peritubular mesenchymal cells, near the epithelial basement membrane differentiated into smooth muscle cells (SMC) at 31 cm CVRL (143 days). X 2000.

Figure 8. Migratory intraepithelial and peritubular leukocytes (lk), Nuclei (N) have deeper invaginations (arrow) and apical protrusions (ap) on luminal surface at 75 cm CVRL (243 days). X 1000.

Some migratory intraepithelial and peritubular leukocytes were also observed at this stage (Figure 8). Similarly Arrighi *et al*. (1993) also noticed migrating lymphocytes throughout the epithelium of efferent ductules of horse. Nuclei have deeper invaginations at different locations and the supra-nuclear area of non-ciliated cells contained few Golgi apparatus which had forming face, maturing face and secretory vesicles. The apical protrusions were also observed at luminal surface of some non-ciliated cells.

ACKNOWLEDGMENTS

The authors thankful to the staff of electron microscopic facility, AIIMS, New Delhi, India for their sincere co-operation and technical help during processing and imaging on TEM.

REFERENCES

Abdou, M.S.S., M.H.G. Mouss, R.S.A. Ragab and A.A. El-Menoufy. 2007. On the regional

histology of the ductus epididymidis in the buffalo (*Bubalus bubalis*). *Anat. Histol. Embryol.*, **14**(3): 226-235.

Alexander, N.J. 1972. Prenatal development of the ductus epididymidis in the rhesus monkey. The effects of fetal Castration. *Am. J. Anat.*, **135**: 119-134.

Arrighi, S., M.G. Romanello and C. Domeneghini. 1993. Ultrastructure of epididymal epithelium in *Equus caballus*. *Ann. Anat.*, **175**: 1-9.

Arrighi, S., M.G. Romanello and C. Domeneghini. 1994. Ultrastructure of epithelium that lines the ductuli efferentes in domestic equidae, with particular reference to spermatophagy. *Acta Anat*, **149**: 174-784.

Bentvelsen, F.M., A.O. Brinkmann, P.Van der Schoot, J.E. Van der Linden, T.H. Van der Kwast, W.J. Boersma, F.H. Schroder and J.M. Nijman. 1995. Developmental pattern and regulation by androgens of androgen receptor expression in the urogenital tract of the rat. *Mol. Cell. Endocrinol.*, **113**: 245-253.

De Miguel, M.P., J.M. Mariño, F. Martínez-García, M. Nistal, R. Paniagua and J. Regadera. 1998. Pre- and post-natal growth of the human ductus epididymidis. A morphometric study. *Reprod. Fert. Develop.*, **10**(3): 271-277.

Edward, M.J. 1965. Observations on the anatomy of reproductive organs of cow. *New Zeal. Vet. J.*, **13**: 25.

Eurell, J.A. and B.L. Frappier. 2006. *Dellmann's Textbook of Veterinary Histology*, 6th ed. Blackwell publishers, Iowa, USA. p. 233-255.

Gier, H.T. and G.B. Marion. 1969. Development of mammalian testes and genital ducts. *Biol. Reprod.*, **1**: 1-23.

Goyal, H. O. and L. D. Dhingra. 1975. The postnatal histology of the epididymis in buffalo (*Bubalus bubalis*). *Acta Anat*, **91**(4): 573-582.

Joseph, A., H. Yao and B.T. Hinton. 2009. Development and morphogenesis of the Wolffian/ epididymal duct, more twists and turns. *Dev. Biol.*, **325**(1): 16-14.

Ilio, K.Y. and R.A. Hess. 1994. Structure and function of the ductuli efferentes: A review. *Microsc. Res. Techniq.* **29**: 432-467.

Lopez, M.L., P. Fuentes, C. Retamal and W. De Souza. 1997. Regional differentiation of the blood epididymis barrier in stallion (*Equus caballus*). *J. Submicr. Cytol. Path.*, **29**(3): 353-363.

Marshall, F.F., W.G. Reiner and B.S. Goldberg. 1979. The embryologic origin of the caput epididymidis in the rat. *Invest Urol*, **17**(1): 78-82

Mohamed, A. 2005. *Glycohistochemical, immunohistochemical and ultrastructural studies of the bovine epididymis*. Ph.D. Thesis, Ludwig-Maximilians-Universität, München, Germany.

Moustafa, L.A. and E.S.E. Hafez. 1971. Prenatal development of the bovine reproductive system. *J. Reprod. Med.*, **7**(3): 99-113.

Pelliniemi, L.J., P. Kellokumpu-Lehtinen and P.H. Anita. 1983. Glycogen accumulations in differentiating mesonephric ducts and tubuli in male human embryos. *Anat. Embryol.*, **168**(3): 445-453.

Rüsse, I. and F. Sinowatz. 1991. Harn-und Geschlechtsorgane. p. 304-337. *In Lehrbuch der Embryologie der Haustiere*. Verlage Paul Parey, Berlin, Germany.

Singh, B. 1989. *Histological and histochemical*

studies on Epididymis of Indian buffalo (*Bubalus bubalis*). M.V.Sc. Thesis, Punjab Agricultural University Ludhiana, India.

Soliman, M.K. 1975. Studies on the physiological chemistry of the allantoic and amniotic fluid of buffalo at various periods of pregnancy. *Indian Vet. J.*, **52**: 111-117.

Wrobel, K.H. and M. Schimmel. 2001. Establishment of the urogenital junction in the male bovine embryo: An ultrastructural study. *Anat. Embryol.,* **204**(3): 225-237.