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## POSTNATAL DEVELOPMENT OF THE EPIDIDYMIS IN THE SHEEP

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### ABSTRACT

The ovine epididymis undergoes several histological changes during its development, these changes are occurred in type of lining epithelium which begins with ciliated low columnar then with age changed to ciliated pseudostratified columnar epithelium, this differentiation is initiated firstly in tail then body and head regions. There are three stages in development, pre-puberty stage, maturity stage and adulthood stage. In first two stages, the epididymis is divided into head, body and tail while in adulthood stage is divided into six regions three in head, two in body and last part in tail.

**Keywords:** Postnatal, Development, Epididymis, Sheep, Histology.

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### 1. INTRODUCTION

The epididymis is a dynamic organ in which maturational changes of spermatozoa occur (Axner, 2006), and has been recognized as a potential site for purpose of regulating fertility as spermatozoa undergo a process of maturation during their passage through the epididymis (Prakash et al. 1980). Testicular sperms lack the ability to move and to fertilize an ovum. However, they acquire these properties during their passage through the epididymis (Bedford, 1975). Based on histological, histochemical and ultrastructural characteristics, the epididymis of most mammalian species can be subdivided into several segments, the number and distribution of these segments is species-specific (Wrobel, 1998). In this study, postnatal development of epididymis was evaluated by showing histological changes occurring in the epididymis of ram, also ultrastructural observation was done on mature ram.

### 2. MATERIALS AND METHODS

This study was conducted on 15 clinically healthy rams, from 1 to 12 months age were divided into three groups, first group started from 1-5 months age, second group started from 6-8 months and third group started from 9-12 months. The epididyma were collected directly by castration till fifth month, then from slaughter-house in mature ages. The tissue pieces were collected from head, body and tail of the epididymis and rapidly fixed in 10% neutral buffered formalin and Bouin's fluid then dehydrated, cleared and embedded in paraffin. Sections at 4-6 micrometer thick were taken and stained with several histological stains including harris haematoxylin and eosin, PAS and toluidine blue stains. All these fixations and staining methods were done after Bancroft and Cook (1994). The ultrastructure study was done on head, body and tail epididymis. 1mm<sup>3</sup> samples from epididymis were collected directly after slaughter from elkanater elkheiria slaughter house and were used for transmission electron microscope study. Samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, and postfixated for 2 h in 1% osmium tetroxide buffered to

pH 7.4 at 4°C, Then were dehydrated in a graded series of ethyl alcohol and embedded in epoxy resin embedding medium prior to sectioning. The ultrathin sections were prepared with ultracut microtome, stained with uranyl acetate and lead citrate (Chiu, Schmidt, and Prasad, 1993), and examined by a Jeol JEM-100S transmission electron microscope (25KV) in national cancer institute.

### 3. RESULTS

#### *3.1. Epididymis of ram from first to fifth month (pre-puberty stage)*

The epididymis parenchyma was composed of tubules their lining epithelium was changed with advance of age. The tubules of head epididymis were lined by simple ciliated low columnar epithelium with oval to elongated centrally located nucleus some tubules lined by ciliated columnar cells with oval centrally located nucleus with some patches of ciliated pseudostratified columnar epithelium at fifth month from age (Fig. 1). The tubules in body region at fifth month were lined with simple columnar epithelium with elongated centrally located nucleus, some columnar cells produce some secretions into the lumen, with some patches of pseudostratified columnar epithelium (Fig. 2) and those in the tail epididymis from first month were lined by ciliated pseudostratified columnar epithelium composed of ciliated columnar cells with elongated centrally located nucleus and basal cells with small round nucleus (Fig. 3). All the tubules were surrounded by few layers of smooth muscle fibers. No spermatozoa were observed in lumen of epididymal tubules till the end of this stage (Figs. 1-3).

#### *3.2. Epididymis of ram at 6-8 month (puberty stage)*

Spermatozoa could be easily seen in all tubules of epididymal duct. The epithelium lining epididymal tubules of head epididymis was high and composed of ciliated pseudostratified columnar epithelium which had four types of cells are principle cells, apical cells, holocrine cells and basal cells. principle cells were columnar cells with long stereocilia, acidophilic cytoplasm and oval basely located nucleus, apical cells are type of principle cells with apically located spherical nucleus, holocrine cells, which resemble in appearance goblet cell were clup shaped cells with oval or round basely located nucleus and clear cytoplasm, the basal cells were small basely located with round small nucleus. The lining epithelium was rest on basement membrane and surrounded by few layers of smooth muscle fibers (Fig. 4). While in body epididymis the epithelium was higher with higher cilia than those in head epididymis. The types of cells in epithelium were like that of head epididymis except no holocrine cells, some intraepithelial macrophages were observed at basal region (Fig. 5). The tubules in the tail epididymis were characterized by wider lumens, lower epithelial height, shorter cilia than body epididymis the principle cell nucleus was central, elongated and darkly stained, also some intraepithelial macrophages and lymphocytes were seen at basal region (Fig. 6). The epididymal tubules were covered by connective tissue capsule formed mainly of collagen fibers and contain blood vessels; also collagen fibers present in-between epididymal tubules (Fig. 7). There were toluidine blue positive cells (mast cells) were observed in between epididymal tubules at tail epididymis (Fig. 8).

#### *3.3. Epididymis of ram at 9-12 month (adulthood stage)*

The epididymis divided into six regions, the first three segments were present in

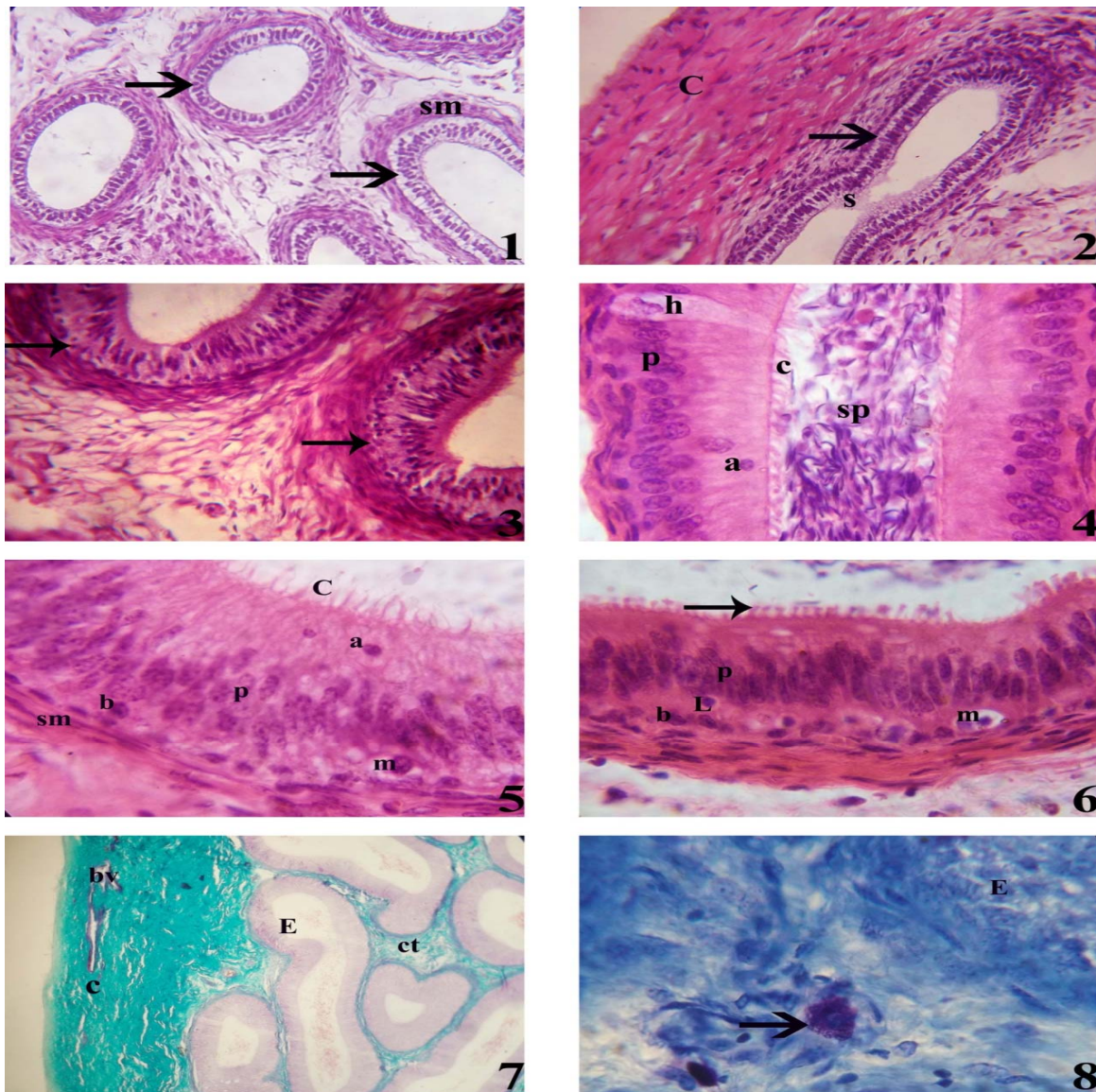


Fig. (1): Photomicrograph of head epididymis of 4 months old ram showing ciliated low columnar epithelial cells with some patches of pseudostratified epithelium line epididymal duct (arrow) and surrounded by few layers of smooth muscle fibers (sm). (H&E stain x100). Fig. (2): Photomicrograph of body epididymis of four months old ram shows connective tissue capsule (C), epididymal duct lined by ciliated simple columnar epithelial cells, with some secretory non ciliated columnar cells (arrow) and lumen contain secretions (s). (H&E stain x40). Fig. (3): Photomicrograph of tail epididymis of two months old ram shows epididymal duct lined by ciliated pseudostratified columnar epithelium and surrounded by few layers of smooth muscle fibers (arrows). (H&E stain x40). Fig. (4): Photomicrograph of head epididymis of 8 months old ram shows epididymal tubule composed from ciliated pseudostratified epithelium with some apical cells (a), principle cell (P) contain cilia (C), holocrine cell (h) and lumen filled with spermatozoa (sp). (H&E stain x100). Fig. (5): Photomicrograph of body epididymis of 8 months old ram shows stereocilia (C), principle cell (P), apical cell (a), intraepithelial macrophage (m) and surrounded by few layers of smooth muscle fibers (sm). (H&E stain x40). Fig. (6): Photomicrograph of tail epididymis of 8 months old ram shows microvilli (arrow) principle cell (P), basal cell (b), intraepithelial macrophage (m) and lymphocyte (L). (H&E stain x40). Fig. (7): Photomicrograph of body epididymis of 8 months old ram shows presence of connective tissue capsule (c) composed of collagen fibers and contain blood vessels (bv) also collagen fibers (ct) present inbetween epididymal tubules(E). (Crossmon's stain x40). Fig. (8): Photomicrograph of body epididymis of 8 months old ram shows presence of mast cells (arrow) in connective tissue between epididymal tubules (E). (Toluidine blue stain x100).



## Postnatal development of the epididymis in the sheep

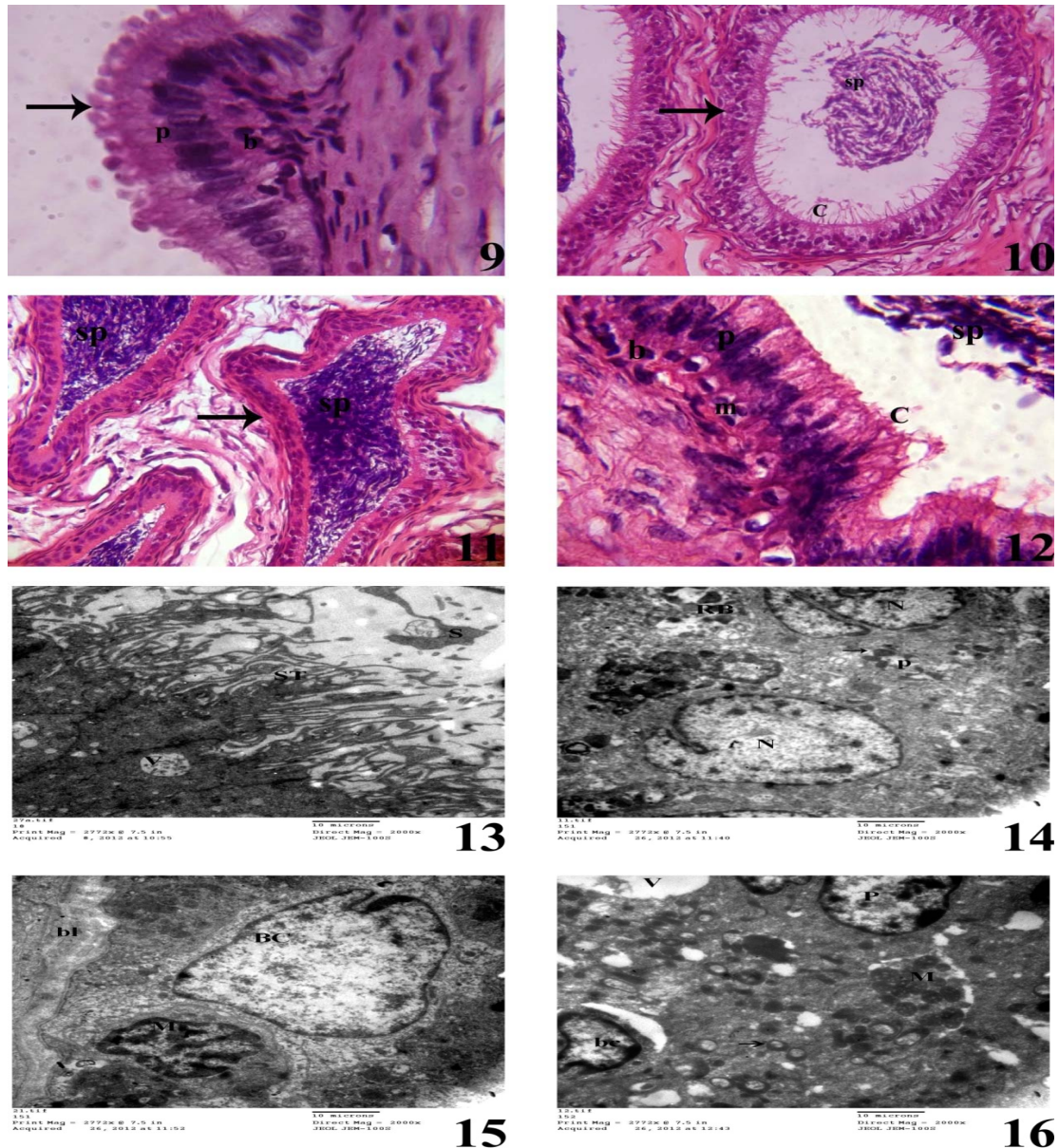


Fig. (9): Photomicrograph of segment one of epididymis of 12 months old ram shows epithelium composed from columnar cells with elongated nucleus (P), their free border contain apical blebs (arrow) and basal cell (b). (PAS stain x100). Fig. (10): Photomicrograph of body epididymis segment 4 of 12 months old ram shows presence of oval, shaped lumina lined by pseudostratified epithelium (arrow), with long cilia (C) also the amount of spermatozoa (sp) became more concentrated. (H&E stain x40). Fig. (11): Photomicrograph of body epididymis segment 5 of 12 months old ram shows epididymal duct with star shaped lumen and lined by ciliated pseudostratified columnar epithelium (arrow) and lumen filled with spermatozoa (sp). (H&E stain x40). Fig. (12): Photomicrograph of segment six of epididymis of 12 months old ram shows principle cell (p), contain few and short cilia (C), basal cell (b) and macrophage (m). (H&E x100). Fig. (13): Photomicrograph of epididymis at 1 year age shows apical region of epithelial cells containing columnar cell with many multivesicular bodies in cytoplasm (v), the free border contain stereocilia (st) and lumen contain secretions (E.M. x 2000). Fig. (14): Photomicrograph of on body epididymis segment 5 at 8 months age show principle cells (p), with lobulated nucleus (N) and cytoplasm contain few mitochondria (arrow) and residual bodies (RB) (E.M. x 2000). Fig. (15): Photomicrograph of body epididymis segment 5 at 8 months age show basal lamina (bl) above it basal cell (BC) and intraepithelial macrophage (M) (E.M. x 2000). Fig. (16): Photomicrograph of tail epididymis at 8 months age show basal cell (bc) and principle cell (p) which contain vesicles (v), mitochondria (M) and lipid droplets (arrow) in their cytoplasm (E.M. x 2000).

head, the lining epithelium was pseudostratified columnar epithelium, which was high in first and second segments but lower in third segment, the epithelium was rested on irregular basement membrane lead to folding of epithelium, the epithelial lining first segment was composed of tall principle cells with elongated centrally located nucleus and their free border contained apical plebs which indicated apocrine mode of secretions (Fig. 9). There were two segment inside body epididymis (fourth and fifth segments of epididymal duct) differed in shape of tubules, size of lumen, epithelial height and amount of spermatozoa (Figs. 10 & 11). The tail epididymis form the sixth segment of epididymal duct, the height of epithelial lining was the lowest in comparison to other segments, the principle cell contained oval or elongated centrally located nucleus, cilia were few and short, obvious increase in number of intraepithelial lymphocytes was observed (Fig. 12). The ultrastructure observation of head epididymis at 1 year

#### 4. DISCUSSION

The tail epididymis was lined by ciliated pseudostratified columnar epithelium while other regions lined by simple columnar epithelium which indicate that differentiation of epithelium starts from tail toward head, this result disagrees with (Naidu and Pattabiraman, 2001) in red ram lambs and (Jiang *et al.* 1994) in rat and agrees with (Abdel-Raouf, 1960) in bulls, Wrobel and Fallenbacher (1974) in boars and (Nilnophakoon, 1978) in rams. At 6-8 months age, the epithelium in head is high and composed from ciliated pseudostratified columnar epithelium containing principle cells, apical cells, basal cells and holocrine cells, this result disagree with Arrighi *et al.* (1986) in cat, Arrighi *et al.* (1993) in *equine* and Hoffer and Greenberg (1978) as they did not describe the presence of holocrine cells, while

age revealed presence of long stereocilia in the apical border of principle cells; some nonciliated cells contain vesicles produce secretions into the lumen (Fig. 13). At 8 months age the epithelium rest on multilayered basal lamina above it principle cells which contained irregular lobulated nucleus, the mitochondria occur in small group and some residual bodies were present beside nucleus (Fig. 14). The basal cell rested on basal lamina and contained euchromatic centrally located nucleus, beside it macrophage was present contained heterochromatic nucleus (Fig. 15). The principle cells of tail epididymis at 8 months age contained polymorphic nuclei, with some prominent patches of heterochromatin. The cytoplasm contained large number of lipid droplets and loose or tightly packed groups of mitochondria were also present. Also the cytoplasm characterized by large vacuoles contained flocculent material, these vacuoles were often larger than the nuclei and more than one vacuole may be present in the same cell at any level (Fig. 16).

Piomboni (1997) found that both light and electron microscopic studies of the mammalian ductus epididymis revealed presence of holocrine cells in addition to the previously mentioned cells. In body epididymis, the types of cells in epithelium resemble that of head epididymis except no holocrine cells are seen in body epididymis this result disagree with Goyal (1985) in *bovine* and Goyal and Williams (1991) in the *caprine* as they described that holocrine cells are not found in the first epididymal segment but found in the corresponding segment of the epididymis. As recorded in previous investigations, intraepithelial leukocytes are found mainly near the basal membrane and occasionally at higher levels, this result in accordance with Goyal and Williams (1991) in goat, Nicander (1958) in the ram, Sinowatz (1981) and Goyal (1985) in *bovine*, as they found intraepithelial lymphocytes throughout the

entire length of the epididymis. At one year age, the epididymis is divided on the basis of histological and histochemical characteristics into six segments, the head contains from segment one to three, the body contains both fourth and fifth segments and the tail contains segment six this classification depends on shape of the tubules, luminal diameter, height of epithelial cells and amount of spermatozoa this result disagrees with Kishore et al (2012), Oke and Clermont, (1989), Abdou et al (1985) in *buffalo*, Goyal and Williams (1991) in *goat* and Ariyaratna et al (1996) in the pre-pubertal *swamp buffalo*. But agrees with Alkafafy (2005) in bull in distribution of segments. There are two segments inside body epididymis (fourth and fifth segments of epididymal duct) are differed in shape of tubules, size of lumen, epithelial height and amount of spermatozoa, this result is reported by Alkafafy (2005), while Kishore et al (2012) who found that fourth segment is very long and constituted the whole of the corpus epididymis till it is joined the fifth segment in the cauda epididymidis. The lumina of the tubules of the fifth segment are narrower than those of the fourth one. Moreover, the lumina of this segment are filled with numerous spermatozoa; this result is also reported by Sinowatz (1981) and Goyal (1985). In accordance with Alkafafy (2005) in bull the tail epididymis forms the sixth segment of epididymal duct. In accordance to Oke et al. (1989) in African giant rat and Sun and Flickinger (1980) the principle cells have several long stereocilia and contain several multivesicular bodies filled with electron dense granules usually seen in apical part of the cells, this result confirm secretory activities of principle cells. The ultrastructure observations of body epididymis were in agreement with Oke et al. (1989) on the other hand Flickinger et al (1978) in hamster suggested that macrophage-like cells have not been reported in the epididymal epithelium. The ultrastructure of epithelium of tail

epididymis in accordance with Oke et al. (1989).

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## تطور البربخ في الخراف بعد الولادة

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## الملخص العربي

بربخ الخراف يمر بتغيرات هستولوجية عديدة أثناء تطوره بعد الولادة هذه التغيرات لوحظت باستخدام الميكروسكوب الضوئي وقد تم تجميع العينات في أعمار مختلفة بداية من عمر شهر حتى اكتمال البلوغ عند عمر سنة تكون التغيرات في نوع النسيج الطلائي الذي يبدأ من عمودي قصير مزود بأهداب ومع زيادة العمر يتحول الي عمودي عديد الطبقات كاذب مزود بأهداب. هذه التغيرات تبدأ في البداية من ذيل البربخ ثم الجسم ثم الرأس. في الخراف كاملة البلوغ يقسم البربخ الي ستة اجزاء ثلاثة في الرأس واثنين في الجسم والجزء الأخير في الذيل ولكن في الخراف الصغيرة هذا التقسيم غير موجود.

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