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PRENATAL DIFFERENTIATION OF EPIDIDYMAL DUCTAL EPITHELIUM IN THE CAMEL (*CAMELUS DROMEDARIUS*)

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SUMMARY

The prenatal differentiation of the camel's epididymal ductal epithelium was investigated at the light microscopic level. The epididymal analage, the mesonephric duct, was first identified at 1.5 cm CRVL fetus and was lined by a single layer of undifferentiated cuboidal cells. The epididymal ductal epithelium was nearly differentiated and had been found to include at least five distinct cell types namely principal, basal, apical, clear and halo cells in 115 CVRL. Steriocilia-like processes were evident at the apical surface of the principal cells at 69 cm CVRL. In conclusion, the epididymal ductal epithelium of camel during the prenatal period had been found to undergo gradual differentiation to various distinctive cell types with modifications on the apical surface of the principal cells.

Key Words: Epididymal duct - Prenatal - Camel - Light microscopy.

INTRODUCTION

It is well established that mammalian epididymis has both secretory and absorptive functions that are believed to be related to the maturation and storage of sperms (Sonnenberg - Riethmacher et al., 1996 and Tzeng et al., 1996). The active secretory and absorptive functions of the epididymis are primarily conducted by a differentiated epididymal epithelium that has been found to include five distinct cell types namely, principal, apical, clear, basal, and halo cells (Bedford, 1975; Hamilton, 1975; Sun and Flickinger, 1979; Yeung et al., 1996; Bendahmane & Abou-Haila, 1997).

The histology, histochemistry and ultrastructure of the adult epididymis have been taken into consideration by several investigators (Nicander and Glover, 1973; Suzuki and Races, 1976; Setty and Jehan, 1977; Nwoha, 1996; Asada-Kubota et al., 1996; Cyr et al., 1996; Sonnenberg-Riethmacher et al., 1996; Fisher et al., 1997; Olson et al 1997; Goyal et al., 1997; Smithwick and Young, 1997).

Elucidation of the sequential histology of the epididymis is preliminary to determining the role of each region of epididymal duct in the process of sperm maturation. Regional differences based on histological and / or histochemical observations have been reported in adult rat (Erkmann, 1977), camel (Moniem, 1972; Moniem and Glover, 1972; Tingari and Moniem, 1979), and chimpanzy (Smithwick and Young, 1997).

Despite the importance of cellular relationships and regional differences in epididymal function, the developmental events leading to the definitive adult state of the epididymal epithelium are incompletely understood from the available literature.

Despite intensive investigation of the adult mammalian epididymis, only few studies have been devoted to elucidate the prenatal differentiation of the mammalian epididymis were conducted. To our knowledge, studies of the prenatal development of epididymis of the camles have not been made so far.

The present study was therefore undertaken to highlight the prenatal differentiation of the camel's epididymmal ductal epithelium and the determine the time at which different cell types and rgional differences appear.

MATERIAL AND METHODS

The material used in this study was obtained from 60 male camel embryos and fetuses measured between 1.5 - 115 cm CVRL. The sex of the embryos and fetuses was determined from the external genitalia except for those at early stages of development that could not be differentiated grossly. Just after slaughtering, embryos and fetuses were collected. The entire fetal epididymis were removed and tissue pieces representing the cross-sections of the initial, middle, and terminal segments were fixed in 10 % neutral buffered formalin or Bouin's solution. The embryos up to 5 cm CVRL were cut either crossly or sagittally and transferred to the fixative solution. The fixed specimens were processed for paraffin embedding. Serial and step serial sections of 4µm thick were prepared and stained with haematoxylin and eosin; Crossman trichrome; Gomori's reticulin; Van Giseson's Weigert elastic stain; periodic acid schiff (PAS), and alcian blue-PAS (Carson, 1990).

RESULTS

At 1.5 cm CVRL, the epididymal duct anlage, the mesonephric duct, was represented by a comparatively large irregular tubular structure located in the ventral region of the mesonephros (Fig. 1). It was lined by a single layer of cuboidal cells resting on a thin discontinuous basal lamina. Two distinct cell types were differentiated within the epithelium of the mesonephric duct. One cell type was encountered in the ductal wall located close to the coelomic mesothelium. These cells were comparatively smaller, with spherical centrally located nuclei, some cells were darkly basophilic while few others were lightly basophilic. The cytoplasm was scanty and acidophilic (Fig. 1). The other cell type located close to the mesonephric tubules. The cells were comparatively large with large spherical or ovoid lightly stained nuclei. Their cytoplasm was abundant and acidophilic (Fig. 1).

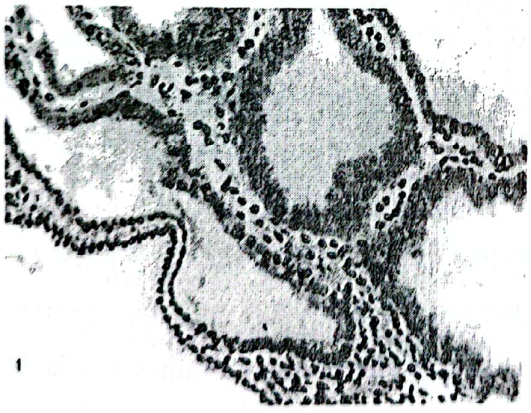

Figure 1: Photomicrograph of a section of 1.8 cm CVRL camel fetus showing the two different epithelial types (curved arrow and straight arrow) lining the mesonephric duct. H & E X 400.

At 7 cm CVRL, the epididymal duct was nearly straight with few undulation evidenced from the few ductal profiles. It was lined by a single layer of undifferentiated cuboidal cells with spherical centrally located lightly stained nuclei. The ductal lumen enclosed desquamated cells.

At 8 cm CVRL, the primitive epididymal duct had few convolutions. The ductal epitheliocytes were densely packed, cuboidal to columnar in shape (Fig. 2). Their large, ovoid nuclei were lightly stained with prominent nucleoli. Their cytoplasm was homogenous and acidophilic.

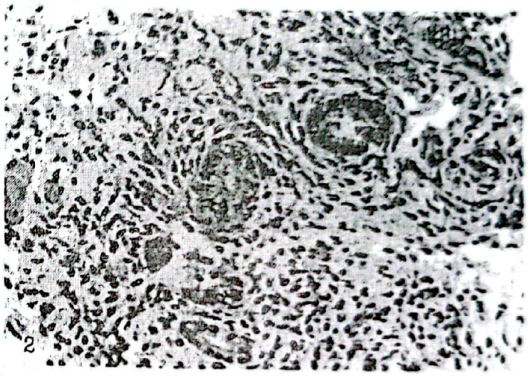

Figure 2: Photomicrograph of the epididymis of 8 cm CVRL camel fetus showing the densely packed, cuboidal to columnar ductal epitheliocytes (arrow). H & E X 250.

At 10.5 cm CVRL, the epididymal duct had a slightly wider lumen and showed few undulations along its course. The ductal epitheliocytes were mostly of cuboidal to columnar shapes with large ovoid lightly stained nuclei containing prominent nucleoli (Fig. 3).

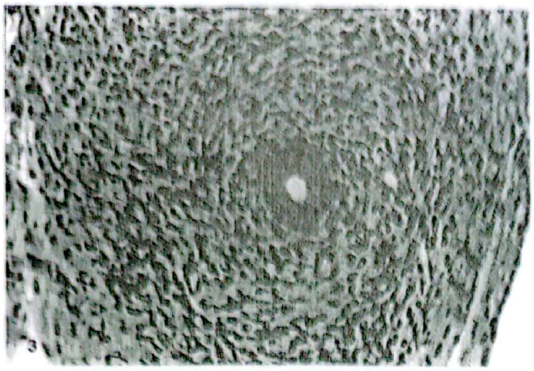

Fig 3: Photomicrograph of a section of the epididymis of 10.5 cm CVRL camel fetus showing cuboidal to columnar ductal epitheliocytes (arrow) with ovoid lightly stained nuclei. H & E X 250.

At 16 cm CVRL, the epididymal duct had a slightly larger diameter and more convolutions. At least, two distinctive cell types were identified within the ductal epithelium; principal and apical cells (Fig. 4). The principal cells had constituted the predominant cell type. They were columnar with elongated oval lightly stained nuclei that were oriented vertical to the underlying basal lamina. The cytoplasm was acidophilic, alcian blue and PAS negative. The apical cells represented a small percentage of the ductal epitheliocytes. THey had an ovoid lightly stained nuclei and chromophobic cytoplasm (Fig. 4).

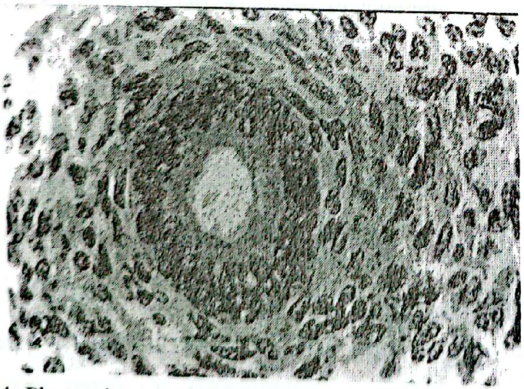

Fig 4: Photomicrograph of a section of the epididymis of 37 cm CVRL camel fetus showing the differentiation of the ductal epitheliocytes into principal (straight arrow) and apical cells (curved arrow) H & E X 1000.

At 25 cm CVRL, segmental differentiation could be encountered. The epididymal duct in the initial and middle segments was lined by the aforementioned two cell types (Fig. 5). In the terminal segment, pseudostratified columnar epithelium was noticed to line small areas of the epididymal duct.

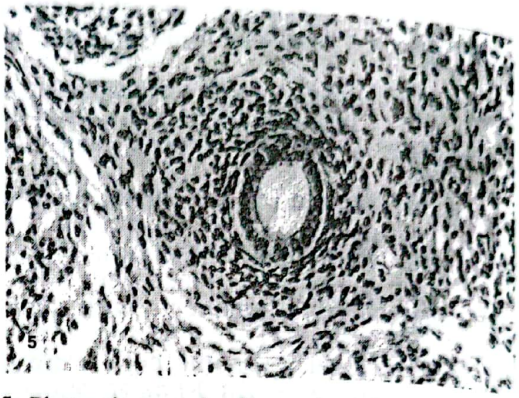

Fig 5: Photomicrograph of a section of the initial segment of the epididymis of 25 cm CVRL camel fetus showing ductal profile lined by a single layer of cuboidal to columnar cells (arrow). H & E X 400.

As camel fetus reached 35 cm CVRL, the epididymal duct in the initial and middle segments was more convoluted with a slightly wider lumen. The apical surface of the predominant principal cells had a well-defined bleb-like protrusions (Fig. 6). At the terminal segment, the epididymal duct was lined in some parts by pseudostratified columnar cells, besides the predominant principal simple columnar type that lined other parts.

At 42 cm CVRL, small areas of pseudostratified

columnar epithelial type were observed among the ductal epitheliocytes lining both initial (Fig. 7) and middle segments. Besides the principal and apical cells, clear cells were also recognized among the ductal epitheliocytes of the initial segment. The clear cells had comparatively large spherical or ovoid nuclei and relatively clear pale

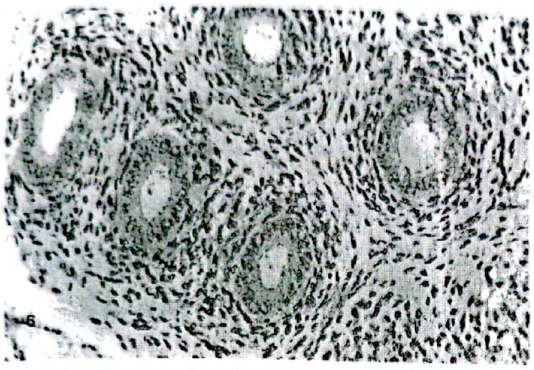

Fig 6: Photomicrograph of a section of the initial segment of the epididymis of 35 cm CVRL camel fetus showing more ductal profiles with a slightly wider lumina. Note the simple cuboidal to columnar ductal epitheliocytes (thick arrow) and the bleb-like protrusions on the apical surface of cells (small arrow) H & E 250.

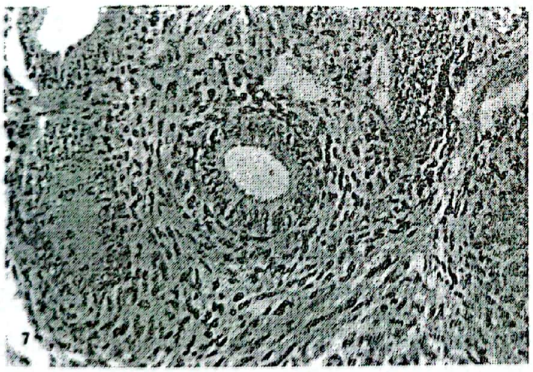

Fig 7: Pathomicrograph of a section of the initial segment of the epididymis of 42 cm CVRL camel fetus showing small areas of psedostratified epithelium (arrows) among the ductal epitheliocytes H & E X 250.

stained cytoplasm (Fig. 8). At the terminal segment, a considerable area of the epididymal duct was lined by pseudostratified columnar epithelium.

Fig 8: Pathomicrograph of a section of the initial segment of the epididymis of 42 cm CVRL camel fetus showing a clear cell (arrow) among the ductal epitheliocytes H & E X 1000.

At 57 cm CVRL, convolution of the epididymal duct continued as evidenced of the comparatively large number of ductal profiles per each sectional area (Fig. 9). The ductal epithelium of both initial and middle segments was composed mainly of simple columnar cells with small areas of pseudostratified type (Fig. 10). Many bleb-like protrusions were seen on the apical surface of the principal cells. Besides the principal, apical and clear cells, few basal cells were encountered among the ductal epitheliocytes. The basal cells were smaller with smaller dense nuclei located close to the basal lamina. Their apical surfaces were insinuated in-between the principal cells and did not reach the lumen (Fig. 10). At the terminal segment, the epididymal duct was lined in most of its parts by pseudostratified columnar epithelium.

The most significant change during the period from 69 up to 73 cm CRVL was the progressive differentiation of the ductal epitheliocytes of the three epididymal segments into pseudostratified

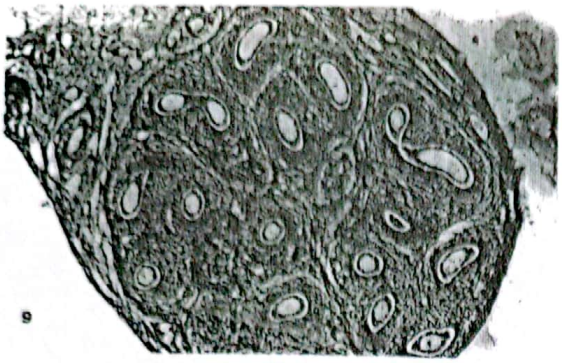

Fig 9: Photomicrograph of a section of the middle segment of the epididymis of 57 cm CVRL camel fetus showing widely spaced ductal profiles with a comparatively larger amount of an intervening mesenchyme. Gomori's reticulin X 100.

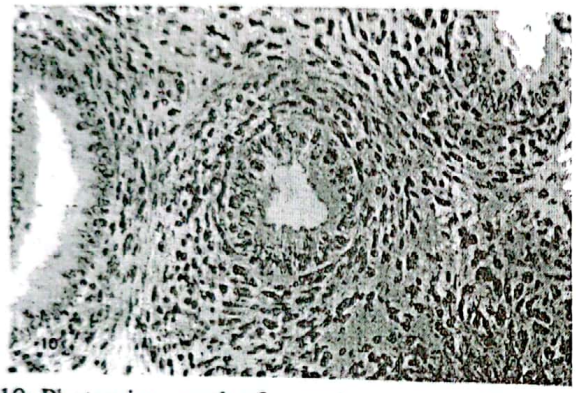

Fig 10: Photomicrograph of a section of the initial segment of the epididymis of 57 cm CVRL camel fetus showing small areas of pseudostratified epithelium (thick arrows) among the simple columnar cells. Note also few basal cells (long arrows). H & E X 400.

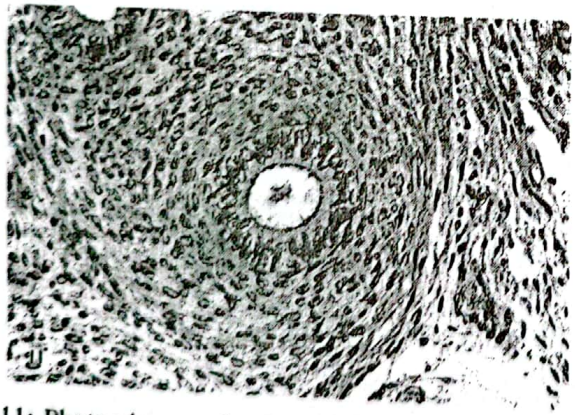

Fig 11: Photomicrograph of a section of the middle segement of the epididymis of 79 cm CVRL camel fetus showing the arrangementof ductal epithelium into simple columnar with small area of pseudostratified columnar type (curved arrow). Note also few stereocilia-like processes (small arrow) on the apical surface of the principal cells. H & E X 400.

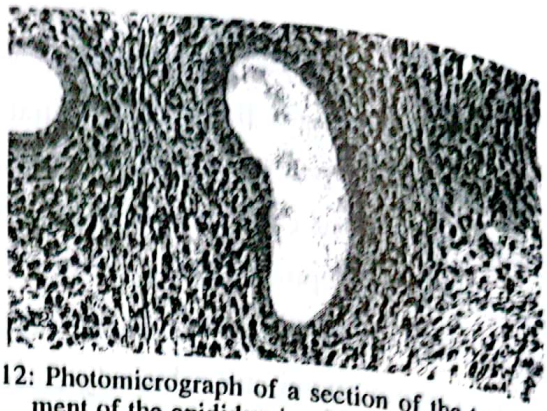

Fig 12: Photomicrograph of a section of the terminal segment of the epididymis of 79 cm CVRL camel fetus showing the more ductal profiles lined by simple columnar. H & E X 400.

type. Few stereocilia-like processes were evident on the apical surfaces of the tall principal cells (Fig. 11, 12). Mitotic figures wee found both in the apical and basal regions of the epithelium.

The major developmental events during the period from 79 up to 115 cm CVRL included progressive coiling and undulation of the epididymal duct so as at 115 cm CVRL fetus, the epididymis was composed primarily of large numbers of epididymal duct profiles separated by a comparatively small amounts of intervening connective tissues (Fig. 13). The ductal epitheliocytes of the initial and middle segments wee mostly arranged into simple columnar type. Besides the principal, apical, basal and clear cells, small spherical cells with densely stained nuclei surrounded by light cytoplasm (halo cells) were first recognized at 87 cm CVRL fetus (Fig. 14). Within the terminal segment, the ductal epitheliocytes were arranged mainly into pseudostratified type with comparatively smaller areas of simple columnar type. The apical surface

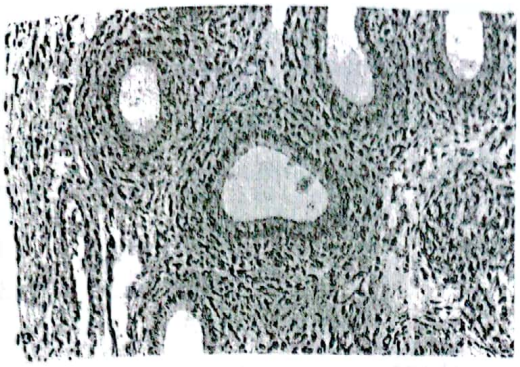

Fig 13: Photomicrograph of a section of the middle segment of the epididymis of 105 cm CVRL camel fetus showing large number of comparatively wider ductal profiles lined by simple columnar cells (curved arrow) with small areas of pseudostratified type (arrows). H & E 400.

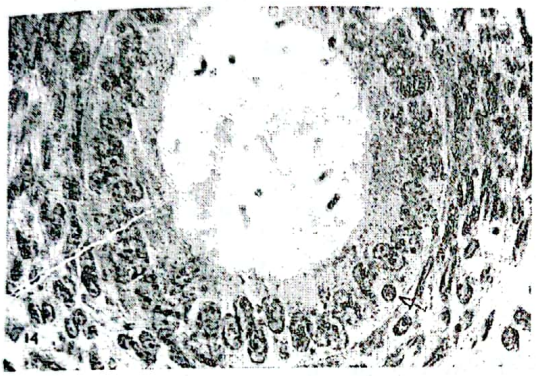

Fig 14: Photomicrograph of a section of the initial segment of the epididymis of 105 cm CVRL camel fetus showing halo cell (arrow) with small spherical darkely stained nucleus and light cytoplasm. H & E 1000.

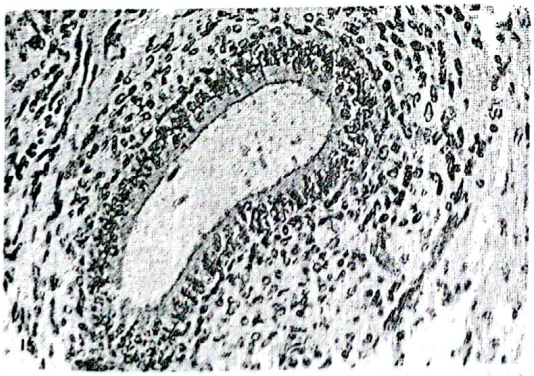

Fig 15: Photomicrograph of a section of the terminal segment of the epididymis of 105 cm CVRL camel fetus showing differentiation of ductal epithelium into pseudostratified type (thick arrow). Note also stereocilia-like processes (open arrows) on the apical surfaces of the tall principal cells. H & E X 400.

of the principal cells appeared to have a well-developed stereocilia (Fig. 15).

DISCUSSION

The epididymal anlage of the camel, the mesonephric duct, was first recognized in 1.5 cm CVRL camel fetuses. In human, the anlage of the epididymal canalicular system appeared in 13.0 - 17.0 long embryos (Krutsiak and Kumka, 1988). At 7 cm CVRL, the primative epididymal duct was lined by a single layer of undifferentiated cuboidal cells that had close morphological similarities to the ductal epitheliocytes lining the mesonephric ducts. The close morphological similarities between the two epithelial types might led us to suggest that the camel epididymis is generally derived from the mesonephric duct. Such findings were consistent with those of Deringer and Heston (1956) and Hamilton et al (1975) who mentioned that the mesonephric or Wollffian duct is generally thought to be the anlage of the mammalian epididymis. Based on histochemical assays, Marshall et al., 1979 revealed marked regional differences in the epididymis and suggested that the ductuli efferentes and caput epididymis of the rat seemed to be derived from the mesonephric tubules rather than the mespnephric duct. The aforementioned assumption concerning the dual origin of the mammalian epididymis both from mesonephric duct and tubules might gain additional supports from the clinical observations of Girgis et al

(1968) and Landing et al (1969) who reported that in some patients with congenital absence of the ductus deferens, a portion of the caput epididymidis remains. These clinical findings suggest that the caput epididymidis may be derived from structures other than the mesonephric duct.

The present study clarified that the epididymal duct was lined first by densley packed, undifferentiated simple cuboidal to columnar epithelium. As development proceeded, small areas of pseudostratified epithelial type were encountred in-between the simple undifferentiated ductal epitheliocytes. At 79-115 cm CVRL, The ductal epitheliocytes of the initial and middle segments were mostly arranged into simple columnar. However, the ductal epitheliocytes within the terminal segment were arranged mainly into pseudostratified columnar type with comparatively smaller areas of simple columnar type. The present findings were in harmony with those of Raja and Rao (1983) in bulls who had demonstrated that the degree of transformation of epithelial linning of the ductus epididymidis from simple columnar to pseudostratified type varied between the three different regions. Pseudostratification of the epithelial cells was completed earliest in the terminal segment, later in the middle segment and last in the initial segment. The different speed of transformation into pseudostratified type among the three distinct epididymal regions could be explained either on the basis of the different functional roles related to each region, a concept which might be supported by Marshal et al., (1979) who demonstrated differences in enzymatic activity between the caput epididymidis and the remainder of the epididymis with three different enzymes. Nicander and Glover (1973) suggested that histologically and functionally, the mammalian epididymis can be divided into an initial segment where sperm maturation takes place, a terminal segment where sperms are stored and an intermediate or middle segment where transit between the two regions in most but not all species is accomplished.

The nearly differentiated epididymal epithelium, revealed in the present study, included at least five distinct cell types namely principal, basal, clear, apical and halo cells.

The morphological criteria of the different cell types that had been revealed in the present study were similar to those previously described for postnatal epididymis (Sun and Flickinger, 1979). On the other hand, the present findings were conflicting with those of Sun and Flickinger (1979) in rat who reported that the first sign of differentiation of the epididymal epithelium was detected during the third week after birth. Although the functional implication of the various cell types during the prenatal period is not fuly understood, the different distribution pattern of these cells among the different

epididymal regions might lead us to speculate that the ductal epitheliocytes of the camel epididymis were nearly differentiated during the prenatal period, and the differentiation of the ductal epitheliocytes was independent on androgen level, sperm arrival or the arrival of testicular fluid as was postulated earlier by Calandra, et al. (1974).

The present study had clarified that apical cells were first recognized among the epitheliocytes lining the initial segment of the epididymal duct at 16 cm CVRL. They had comparatively large spherical apically located nuclei. The term apical cell was used by Reid and Cleland (1957) to describe a population of cells visualized by light microscope which had apically placed nyclei and were present in substantial number in the initial segment of the adult rat epididymis. In subsequent studies of the rat epididymis, all cells in the initial segment with an apically placed nucleus have usually been collectively termed apical cells (Hoffer and Greenberg, 1978; Cohen, et al., 1976). Sun and Flickinger (1980) reported that there were many cytological similarities between the apical cell and the principal cell. They suggested that the apical cell is simply a form of principal cell and not a separate cell type. The apical cells might represent principal cells in that have lost their contact with the basal lamina. This view is consistent with the previous suggestion that apical cells are produced by division of principal cells (Reid and Cleland, 1957). The functional implication of the apical cells was suggested by Cohen et al (1976) who demonstrated that cells with apical nuclei in the initial segment of the rat epididymis contain carbonic anhydrase, and they have suggested that these cells may possess a mechanism of acidic secretions. In accord with this notion, analysis of epididymal fluid obtained via the micropuncture technique has shown that acidification occurs in the caput epididymis (Levine and Marsh, 1971). Recently, Adamali and Hermo (1996) reported that the apical and narrow cells differ not only from each other but also from principal and basal cells in their structure and relative distribution. They also express different proteins within the distinct epididymal regions, indicating that they perform different functions. The localization of cathepsin D and beta-hexosaminidase A within apical cells suggests these cells may be involved in the degradation of specific proteins within their lysosomes. Although the functional significance of the apical cells during the prenatal development of the camel epididymal duct is not understood, their early appearance during the prenatal differentiation might substantiate our current view that the epididymal epithelium reached its pull differentiation earlier.

The present study revealed that clear cells were first recognized among the ductal epitheliocytes at 42 cm CVRL where they represented a small percentage than the principal cells. Epididymal clear cells differ from principal cells on the basis

of their morphology, a greater endocytotic activity (Moore and Bedford, 1979), and their glycoprotein content, as detected by immunocytochemical methods (Lea, et al., 1978). Although the role of clear cells is unknown, some evidence suggested that they are related to the apical cells of the initial segment (Brown and Montesana, 1980). Clear cells (also called foamy cells, light cells) are found only in the pididymal epithelium of rats (Reid and Cleland, 1957; Hamilton, 1975) and hamster (Nicander and Glover, 1973). It should be pointed out, however, that in addition to being absent from the guinea pig (Hoffer and Greenberg, 1978), they also have not been obsrved in the epididymis of the mouse (Hamilton, 1975), rabbit (Nicander, 1957), stallion, ram, bull (Nicander, 1957). The hypothesis that clear cells are part of a holocrine cell secretory cycle and the source of epididymal glycerylphosphorylcholine (Martan and Risley, 1962) had been disproved (Clermont and Flannery, 1970; Hamilton, 1975). The functional significance of the clear cells remains obscure and their limited distribution among mammalian epididymes suggests that their importance to epididymal physiology should be re-evaluated. Recently, Hermo, et al., (1997) had revealed that beta-hexosaminidase which is an important lysosomal enzyme was localized to clear cells throughout the epididymal duct. The positive reactivity of the clear cells to hexosaminidase might substantiate the previous concept of Moore and Bedford (1979) that clear cells had a well pronounced endocytotic activity. In this respect, Averal, et al., (1996) mentioned that impairment of the function of the clear cells of the cauda epididymidis was associated with impairment of epididymal function, particularly concerning endocytotic removal of the contents of the cytoplasmic droplets and dead sperm. Hermo, et al., (1997) revealed that beta-hexosaminidase was predominantly present in lysosomes in Sertoli and epididymal cells. The cellular and regional specificity of beta-hexosaminidase immunolocalization suggest an important role for the enzyme in testicular and epididymal functions.

Another interesting feature of the prenatal development of the camel epididymal epitheliocytes was the absence of halo cells throughout the early developmental stages. The halo cells were first recognized among the ductal epithiliocytes at 87 cm CVRL fetus. Studies comparing the fine structure of halo cells and leukocytes have suggested that halo cells in the adult are leukocytes that have infiltrated the epididymal epithelium (Hoffer, et al., 1973; Dym and Romrell, 1975). Other features such as the lack of junctional complexes with other cells and blunt pseudopod-like processes also indicate that the halo cells is a wandering leukocytes (Hoffer, et al., 1973). The entire absence of halo cells during the early developmental period that have revealed in the present study might favour the assumption that this cell type does not appear to

be a true epithelial cell of the epididymis. Moreover, their occurance at the late developmental period might led us to favour the suggestion that they represent a wandering leukocytes. Relevant to this notion, Flickinger et al (1997) concluded that the epididymal epithelium of the Lewis rat contains many T lymphocytes, which may correspond to halo cells and that leukocytes predominate in all regions of the epididymis. The same author added that the interstitium may function as a reservoir of leukocytes for the epithelial compartment and the epididymis is not normally a site for local immunoglobulin synthesis.

The present study demonstrated that the epididymal duct of camel during prenatal life comprised at least three morphologically distinct zones. The characteristics which differentiated one zone from the other included variations in cell types, regional differences in their distribution, and changes in cell height and tubular diameter. Zonation of the epididymal duct had also been reported in camels (Tingari and Moniem, 1979), rabbits (Nicander, 1957), stallions, bulls and rams (Nicander, 1958), rats (Reid and Cleland, 1957; Hamilton, 1975), mice (Allen and Slater, 1957, 1961), hamsters (Nicander and Glover, 1973), man (Holstein, 1969) and champenzy (Smithwick & Young, 1997). Thus in all mammalian species examined to date, the epithelial lining of the epididymal duct is not uniform along its length but consisted of a number of different regions with different cytological characteristics. The functional correlates of these differencs are unknown in most instances. The results of the present study could also be discussed with the statement of Hoffer and Greenberg (1978) who stated that studies that are based on the designation of only three regions of the epididymis do little to meet the need for precise correlation of function with identified segments of the duct or specific cell types. In this respect, Smithwick & Young (1997) had illucidated that the epididymal duct of the adult male chimpanzee present at least 16 histologically distinct epithelia and their transitional forms. Such diversity of epithelia suggesta a corresponding diversity of function.

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