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Zonal Changes in the Ultrastructure of the Epididymal Principal cell of the Greater Cane Rat (*Thryonomys swinderianus*)

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Key words: Epididymis, Ultrastructure, Greater cane rat, Principal cell

ABSTRACT

This study presents the varying ultrastructural characteristics of the principal cell along the different zones of the epididymis of the greater cane rat. Ten sexually mature male cane rats with known medical and reproductive history were perfusion-fixed using Karnovsky fixative (phosphate buffered 2% paraformaldehyde – 2.5% glutaraldehyde fixative at pH 7.4). Epididymal samples were then obtained, processed for ultrastructural analysis and examined under the transmission electron microscope. Our findings showed that the cane rat epididymis has four distinct regions – initial segment, caput, corpus and cauda epididymides. These regions are further subdivided into discrete subregional zones based on the cytological characteristics of the principal cell in each region. The principal cell in the proximal initial segment showed abundant rough endoplasmic reticulum and extensive Golgi apparatus arranged in a peculiar whorl shape predominantly in the apical cytoplasm with small vesicles, dense granules and multivesicular bodies scattered in and around the whorls. The principal cell of the distal initial segment is characterized by multivesicular bodies, small and coated vesicles as well as numerous flat and whorl-shaped cisternae of rough endoplasmic reticulum and dense-core mitochondria. In the caput epididymis, the principal cell contains more of lipid vacuoles, while in the corpus; it has prominent dense bodies of various sizes at its supranuclear area. These dense bodies tend to increase in the principal cells of the cauda epididymis. The transition between regions was however observed to be gradual with overlapping of morphological characteristics between two adjacent regions. These ultrastructural diversities exhibited by the epididymis along its length point to a corresponding functional diversity and do dictate the overall intra-luminal environment necessary for sperm maturation in the cane rat.

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1. INTRODUCTION

The structure of the Epididymis in relation to its functions has been extensively studied in several mammalian species including mouse (Soranzo et al., 1982), hamster (Flickinger et al., 1978), rat (Robaire and Hermo, 1988), guinea pig (Hoffer and Greenberg, 1978), giant rat (Oke et al., 1988), dog (Connell and Donjacour, 1985), goat (Goyal and Williams, 1991), ram (Marengo and Amann, 1990), Primate (Ramos and Dym, 1977; Smithwick and Young, 2001) and even human (Holstein, 1969; Arroteia et al., 2012). In almost all the studied species, the epididymis, which is a single and highly coiled tubular duct, can be divided into anatomical regions based on its gross appearance, epithelial height, luminal diameter and thickness of the muscle wall (Goyal and Williams, 1991; Smithwick and Young, 2001). According to Arroteia et al, (2012), on the basis of the cytological characteristic of the epididymal epithelial cells, several sub-regional zones have been identified along the epididymal length (Franca et al., 2005), with the number varying from one species to another (Robaire et al., 2006; Turner, 2008). Although the entire epididymal epithelial lining is predominantly composed of principal, basal and apical cells, the peculiarity of each zone stems primarily from the number, histological and ultrastructural characteristics of the principal cells (Marengo and Amann, 1990; Arroteia et al., 2012). In the same vein, the contributions of the principal cell in each zone are said to be majorly responsible for the

distinctive zonal intra-luminal environment which produces the morphological, biochemical, physiological and functional alterations to the structures of the spermatozoa, converting it into cells capable of fertilization (Toshimori, 2003; Gatti et al., 2004). Hence, the functional differences between the zones are a reflection of the varying ultrastructure of the principal cells along the epididymal length (Marengo and Amann, 1990).

The ultrastructural characteristics of the principal cell along the epididymal length have been described in several mammalian species (Smithwick and Young, 2001; Robaire *et al*., 2006; Turner, 2008; Arroteia *et al.,* 2012). With the variation in the number of epididymal zones among species, differences in the ultrastructural features of the organelles within the principal cells have also been observed among mammalian phylogenies (Dacheux et al., 2005; Arroteia et al., 2012). Therefore, in an attempt to understand the structural-functional relationship of the epididymal principal cell in sperm maturation and storage particularly in wild rodents that are undergoing domestication, information on the ultrastructure of this cell is imperative.

The greater cane rat (*Thryonomys swinderianus*) is a hystricomorphic wild rodent found only in Africa. In the West Africa sub-region, it is vigorously hunted and exploited for food because of its excellent meat taste and high nutritive value (Addo et al.., 2007). Although efforts and resources are now being deployed to its domestication and captive-rearing in this part of Africa, information on the reproductive biology that will enhance these processes are still scanty. Thus, the need to understand the structure and ultrastructure of each segment of the male reproductive tract is essential. While information on the histological zones of the epididymis, and the ultrastructure of the accessory glands of the cane rat have earlier been provided (Adebayo and Olurode, 2010; Adebayo et al., 2015), this study attempts to describe the distinguishing ultrastructural characteristics of the principal cell along epididymal zones in this animal.

2. MATERIALS AND METHODS

2.1. ANIMALS

A total of ten (10) captive-reared, sexually matured male greater cane rats, with known reproductive and medical records were used in this work. All the animals have brownish perineal staining which is usually used as index of sexual maturity in the male cane rat (Adu and Yeboah, 2003). The animals were fed commercial cane rat feed, elephant grasses and water was given *ad libitum.* The experimental protocol followed the ethical principles in animal research adopted by the University of Ibadan Animal ethics and experimentation committee.

2.2. Sample collection

Each animal was weighed, anaesthesized and perfusion-fixed transcardially using the Karnovsky's fixative (phosphate buffered 2% paraformaldehyde – 2.5% glutaraldehyde fixative at pH 7.4). After opening the abdominal and pelvic cavities, the epididymis was separated from the testis for gross tissue assessment while samples were taken for electron microscopy.

2.3. Tissue Processing for Electron microscopy

The samples of each epididymal region were further fixed in Karnovsky's fixative, post-fixed in 1% osmium tetraoxide for 1hour, dehydrated in an increasing ethanol series, infiltrated and embedded in Epon-Araldite resin. Semi-thin and Ultrathin sections were cut with an Ultracut S ultramicrotome (Reichert-Jung, Austria). While the semi-thin sections were stained with Toluidine blue-Pyronin Y mixture and examined under Axioskop 2 plus, Carl Zeiss light microscope (Germany), the ultrathin sections were mounted on copper grids, double-contrasted with uranyl acetate and lead citrate and observed in a Phillips CM10 transmission electron microscope (TEM).

3. RESULTS

The epididymis of the greater cane rat was divided into four regions – initial segment, caput, corpus and cauda epididymides with each region differing in cell types, epithelial height, tubular diameter and luminal shapes. These regions were further subdivided into discrete sub-regional zones on the basis of the differences in cytological characteristics of the epithelium, predominantly the principal cells, in each region. While the initial segment showed three zones – proximal initial segment (PIS), middle initial segment (MIS) and distal initial segment (DIS), the caput, corpus and cauda epididymides presented a zone each (Fig. 1, 2, 3& 4). Transition between regions was however observed to be gradual with overlapping of morphological characteristics between two adjacent regions.

The ultrastructure of the principal cell in the PIS revealed characteristic presence of abundant rough endoplasmic reticulum and extensive Golgi apparatus arranged in a peculiar whorl shape predominantly in the apical cytoplasm (Fig. 5a) with small vesicles,

dense granules and multivesicular bodies scattered in and around the whorls. While the inner Golgi cisternae were dilated with pale materials, the outer cisternae were flat and surrounded by endoplasmic reticulum. Large sized multivesicular bodies were seen at the supranuclear area below the whorl (Fig. 5b). The cells had microvilli on their apical surfaces, while others had apical blebs. Tight junctional complexes held the apical ends of the cells together (Fig. 5b). Small vacuoles were however present at either the subapical cytoplasm or blebs of the cells. Numerous mitochondria, some of which appear in clumps were seen scattered more at the apical cytoplasm (Fig 5a&b). The euchromatic nucleus of the principal cell is indented and contains multiple nucleoli. The infranuclear cytoplasm contained cisternae of rough endoplasmic reticulum and some round to rod-shaped mitochondria. While PIS and MIS differ in the amount and ultrastructure of apical cell, the fine structure of the principal cell in both zones are similar (Fig. 5a,b & 6a,b). The fine structure of the principal cell in the DIS showed that, the apical surfaces are covered by numerous unbranched microvilli and the apical cytoplasm was laden with pale vacuoles of different sizes. At the supranuclear region, multivesicular bodies, small and coated vesicles as well as flat cisternae of Golgi apparatus and abundant tubular mitochondria were observed (Fig 6a). The infranuclear cytoplasm was packed with numerous flat and whorl-shaped cisternae of rough endoplasmic reticulum and dense-core mitochondria. Dense vacuoles that are suggestive of lysosomes are also present in this part of the cell (Fig. 6b).

The electron micrograph of the principal cell in the caput epididymis reveals that the apical and supranuclear parts not only contain vacuoles but also multivesicular bodies, secretory vesicles, coated vesicles and lipid vacuoles with Golgi cisternae at the Golgi zone. While the nucleus showed several deep indentations and contained nucleolus, the microvilli were branched and several secreted vesicles and granules were seen in the lumen (Fig.7a). At the infranuclear part, there was the presence of both dilated and flat cisternae of the Golgi apparatus surrounded by secretory and coated vesicles (Fig. 7b).
Apart from the reduced height, the major histological feature of the principal cell in the corpus epididymis was the presence of prominent dense bodies of various sizes at the supranuclear part of the cell (Fig. 3). Ultrastructurally, the principal cell nucleus in this region was euchromatic, highly indented and irregular in outline. The supranuclear dense bodies were of various shapes, sizes and electron densities and extend to the apical areas where some flat and curved Golgi cisternae were present (Fig. 8). Vacuoles of various sizes were also present at the subapical part while the luminal surfaces of the cells were covered by short microvilli (Fig. 8). The ultrastructural differences between of the principal cell in the corpus and that in the cauda epididymis were increase in the amount of dense bodies at the supranuclear and apical cytoplasm as well as presence of less dense vesicles of various sizes around the rough endoplasmic reticulum at the infranuclear cytoplasm of the cell in the cauda epididymis (Fig. 9).

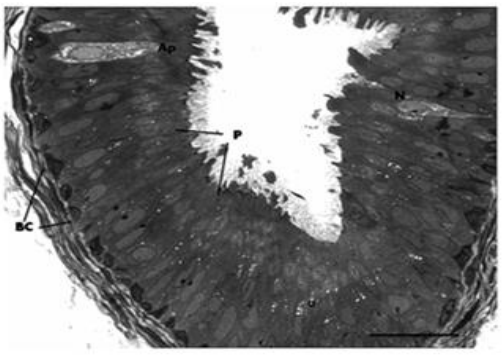

Figure 1a: Photomicrograph of the epithelium of PIS of the epididymis in the greater cane rat. Note the few apical (Ap) and narrow (N) cells wedged in between the principal cells (P) with the basal cells (BC) attached to the epithelial basement membrane. Toluidine blue grayscale, Scale bar = 20µm.

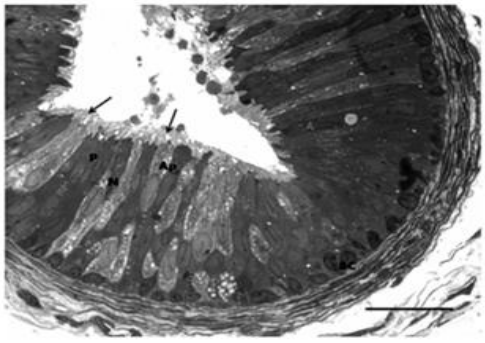

Figure 1b: Photomicrograph of the epithelium of MIS of the epididymis of the greater cane rat. Note the increase in the number of apical (Ap) and narrow (N) cells wedged in-between principal cells (P). Secretory blebs from these cells were also shown (arrows) with the basal cells (BC) abutting the epithelial basement membrane. Toluidine blue, Scale bar = 20µm

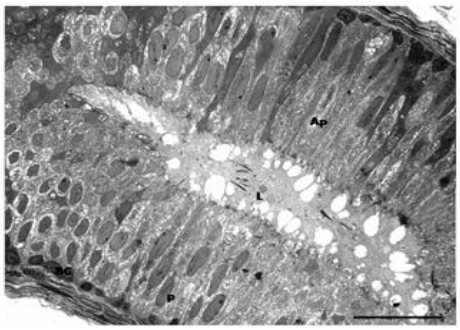

Figure 1c: Photomicrograph of the epithelium of DIS of epididymis in the greater cane rat. Note the increase in the number of apical (Ap) cells and changes in the structure of principal cells (P). Secretory blebs released into the lumen (L) were conspicuously shown with the basal cells (BC) attached

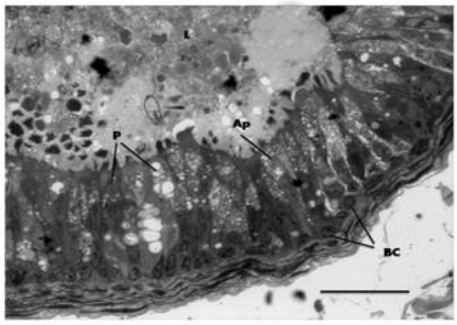

Figure 2: A semi-thin section of the caput epididymal epithelium in the greater cane rat. Note the presence of the vacuoles of various sizes in the apical (Ap) and principal (P) cells. Secretory blebs from these cells also occupy the lumen (L) and the basal cells (BC) abutting the epithelial basement membrane. Toluidine blue. Scale bar = 20µm.

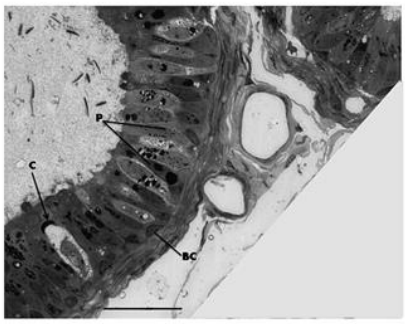

Figure 3: Photomicrograph of the corpus epididymal epithelium in the greater cane rat. Note the presence of the clear (C) and principal (P) cells containing dense bodies. The basal cells (BC) were attached to the basement membrane. Toluidine blue, Scale bar = 20µm.Scale bar = 20µm.

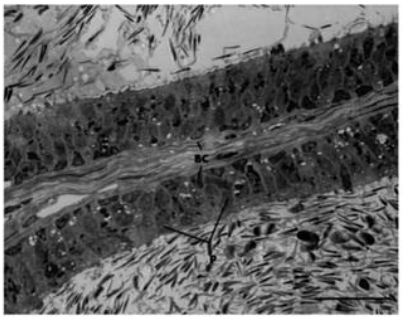

Figure 4: Photomicrograph of the epithelium in the cauda epididymis of the greater cane rat. Note the low cuboidal principal (P) cells containing dense bodies. The basal cells (BC) were attached to the basement membrane and sperms (Sc) fill the tubular lumen. **Toluidine blue, Scale bar = 20µm**.

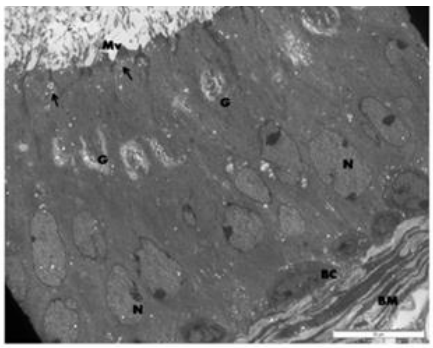

Fig. 5a: Ultrastructure of the epithelium of PIS of the epididymis in the greater cane rat. Note the presence of microvilli (Mv), prominent Golgi complexes (G), subapical vacuoles (arrows) and euchromatic nucleus (N) in the principal cell. The basal cells (BC) were anchored to the basement membrane (BM). Scale bar = 10µm.

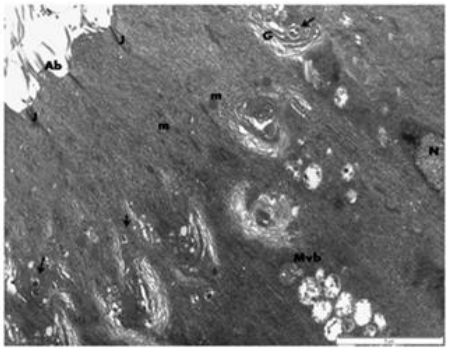

Fig. 5b: Ultrastructure of the apical part of the principal cells of the PIS of the epididymis of the greater cane rat. Note the apical bleb (Ab), tight junctional complexes (J), dilated cisternae of Golgi complexes (G) with small coated vesicles and dense granules (arrows) emanating from it, abundant mitochondria (m) of various shapes and presence of multivesicular bodies (Mvb) as well as euchromatic nucleus (N) of the principal cell. Scale bar = 5µm

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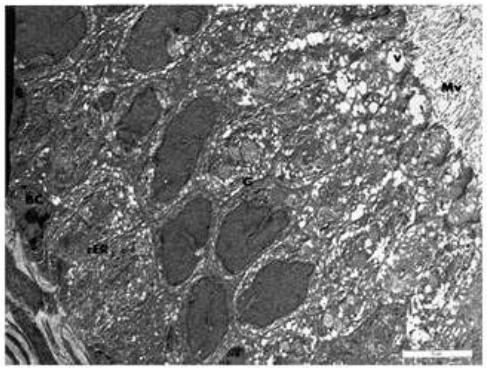

Figure 6a: Ultrastructure of the epithelium of DIS of epididymis in the greater cane rat. Note the microvilli (Mv), prominent Golgi complexes (G) and apical vacuoles (V) in the principal cell. The basal cells (BC) were also shown Scale bar = 5µm.

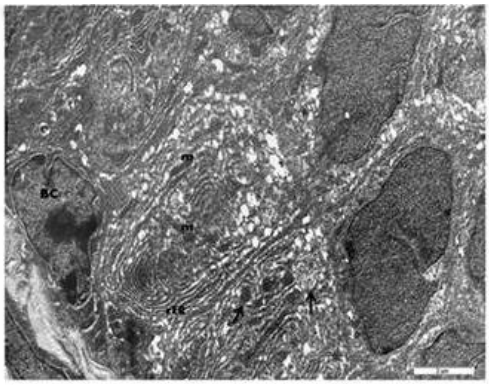

Figure 6b: Ultrastructure of the basal part of principal cells of DIS of epididymis in the greater cane rat showing cisternae of rough endoplasmic reticulum (rER) and abundant mitochondria (m) as well as vesicles of variable densities and sizes (arrows). The basal cells (BC) with its indented nucleus and few organelles were also shown. Scale bar = 2µm.

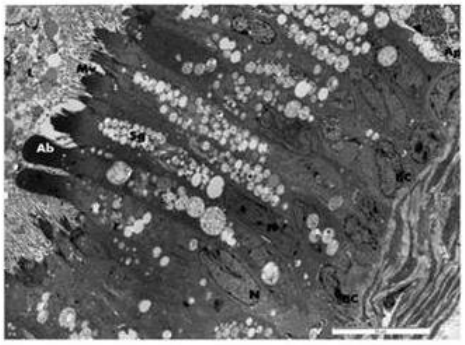

Figure 7a: Ultrastructure of the epithelium of the caput epididymis in the greater cane rat showing the principal cells with microvilli (Mv) and apical blebs (Ab), vesicles (Sg) at the apical cytoplasm and some in the lumen (L) and euchromatic nucleus (N).The apical (Ap) and basal (BC) cells were also present. Scale bar =

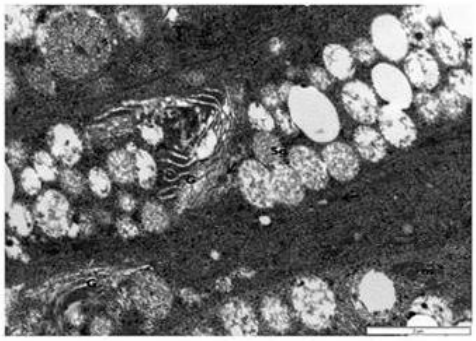

Figure 7b: Ultrastructure of the supranuclear cytoplasm of the principal cells in the caput epididymis showing the dilated cisternae of the Golgi complex (G), secretory granules (Sg) and coated vesicles (arrows) as well as mitochondria (m). Scale bar = 2µm.

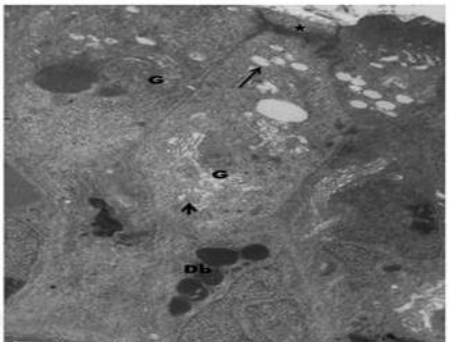

Figure 8: Ultrastructure of the apical cytoplasm of the principal cell in the corpus epididymis of the greater cane rat. Note the flat Golgi cisternae (G), secretory granules (arrow head), subapical vacuoles (arrow), coated pit (asterick) and the dense bodies at the supranuclear area. Scale bar = 2µm.

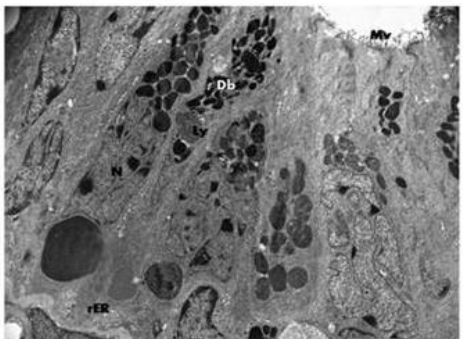

Figure 9: Ultrastructure of the epithelium of the cauda epididymis in the greater cane rat showing the principal cells with microvilli (Mv), and numerous dense bodies (Db), lysosomes (Ly) at the supranuclear cytoplasm as well as irregularly shaped nucleus (N) and rER cisternae at the basal cytoplasm. Scale bar = 5µm.

DISCUSSION

The structural and ultrastructural peculiarities of the principal cell along the epididymal duct have been studied in several domestic and laboratory species but few reports are available on wild species especially those undergoing domestication in West Africa (Oke et al., 1988; Aguilera-Merlo et al., 2005). This work presents the ultrastructural features of the principal cell along the epididymal length in the greater cane rat.

According to Arroteia et al, (2012) the four anatomical regions of the epididymal epithelium contain functionally distinct and cell-specific zones which represent discrete regulatory units. The precise numbers of these zones are species-specific, being four zones in the rat (Hamilton, 1975), five in mouse, hamster, African giant rat, goat and man (Flickinger et al., 1978; Takano, 1980; Oke et al., 1988; Goyal and Williams, 1991; Arroteia et al., 2012), seven in the guinea pig (Hoffer and Greenberg, 1978) and eight in the rabbit (Jones et al., 1979). The six zones in the cane rat can therefore be said to be characteristic of this species of animals. As reported by Goyal and Williams, (1991) and corroborated by Joseph et al, (2011), irrespective of the number of zones within any species, there is always an overlapping of morphological and functional characteristics between two adjacent zones and there are more structural diversities along the length of the proximal segment than along the length of the body and tail regions. The observed presence of three of the six zones in the initial segment alone and the close semblance in the ultrastructure of the epithelial cells in the body and tail regions confirms this report in the greater cane rat.

In all mammalian species, the uniqueness of each zone stems from the fine structural features of the principal cell primarily and that of other cells in the epithelium (Goyal and Williams, 1991; Joseph et al., 2011). The ultrastructural appearance of the principal cell in the initial segment in the greater cane rat is comparable to that observed in other rodents (Hoffer and Karnovsky, 1981; Robaire and Hermo, 1988; Oke et al., 1988) in having prominent branched microvilli with the presence of large stacks of Golgi saccules, mitochondria and multivesicular bodies at the supranuclear area. The infranuclear area is also packed with rough endoplasmic reticulum. However the whorled pattern of arrangement of the dilated Golgi apparatus and the presence of clumps of mitochondria at the supranuclear area are peculiar features in the first zone of the initial segment in the cane rat. It is not impossible that these might affect the type of protein and steroid secreted by these cells. While the functional significance of these features are yet unknown, Oke et al, (1988) had reported the presence of mitochondria clumps in the same area in the giant rat.

In rat and man, the tight junctions between adjacent epithelial cells are said to form blood-epididymis-barrier which restrict passage of a number of ions, solutes, and macromolecules through the epididymal epithelium as well as serve as an extension of the blood-testis-barrier in these species (Robaire et al., 2006; Cornwall, 2009),. The observed tight junctional complexes found between adjacent principal cells and other cells might also serve the same purpose in the greater cane rat.

According to Robaire et al, (2006) and Joseph et al, (2011), the presence of endocytotic features such as microvilli, coated vesicles and pits and subapical vacuoles are sure evidences of absorptive function of the principal cell. In the same vein, while the presence of highly developed Golgi complexes and numerous cisternae of rough endoplasmic reticulum in the supranuclear and infranuclear areas are morphological correlates of protein synthesis, whorls of smooth endoplasmic reticulum, mitochondria with tubular cristae and lipid droplets are morphological correlates of steroid synthesis. The presence of these organelles in the principal cells of the different zones in the cane rat epididymis confirms they are involved in both absorptive and secretory functions with zone 1 being involved in more of absorptive functions; zones 2-4 in "net secretion" than "net absorption" and zones 5-6 being involved in "net absorption" rather than "net secretion". This trend has also been observed in ram, bull and goat (Amann et al., 1982; Pholpramool et al., 1985; Goyal, 1989). Moreover, since different kinds of epididymal secretory proteins and glycoproteins have been characterized in different species showing this trend (Goyal and Williams, 1991), this arrangement will surely impact on the different kinds of proteins secreted by the principal cell of different epididymal zones in the greater cane rat.

The characteristic presence of abundant vacuoles of various sizes and densities in the principal cell of zones 2 and 3 in the cane rat has been observed in mouse, hamster and guinea pig (Takano, 1980; Hoffer and Karnovsky, 1981) with the large vacuoles particularly similar to the lipid droplets found in the

guinea pig. These lipid droplets together with whorls of smooth endoplasmic reticulum and the mitochondria with tubular cristae in the principal cells of these zones in the cane rat suggest the possibility of steroid synthesis in this cell. While the possibility of *de novo* synthesis of testosterone in some mammalian species like mouse, rat, rabbit and ram had long been indicated by Hamilton (1971), morphological evidence of testosterone synthesis has only been shown in the principal cell of zone 2 in the guinea pig epididymis (Hoffer and Karnovsky, 1981). The very type of steroid that may be synthesized in the principal cell of these zones in the cane rat is yet to be reported.

In conclusion, these ultrastructural peculiarities of the principal cell along the epididymal duct can serve as basis for the functional interpretation of the distinct role played by each zone in creating appropriate epididymal intra-luminal milieu necessary for sperm maturation and storage in the greater cane rat and beyond.

4. Acknowledgements

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