

## Ultrastructure of the epithelium lining of cauda epididymidis in mongrel dogs<sup>1</sup>

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**ABSTRACT.**- Schimming B.C., Pinheiro P.F.F., Vicentini C.A. & Domeniconi R.F. 2012. **Ultrastructure of the epithelium lining of cauda epididymidis in mongrel dogs.** *Pesquisa Veterinária Brasileira* 32(Supl.1):32-36. Departamento de Anatomia, Universidade Estadual Paulista, Botucatu, SP 18618-970, Brazil. E-mail: [bruno@ibb.unesp.br](mailto:bruno@ibb.unesp.br)

The epithelium lining of cauda epididymidis in mongrel dogs was examined by transmission electron microscopy. The epididymal epithelium is pseudostratified with stereocilia and is composed predominantly of principal and clear cells. Therefore, exist basal and apical cells. The principal and clear cells show features suggesting that they may be preferentially involved in absorptive and secretive functions. These results are compared with previously published data on the cauda epididymidis of other mammalian species, in order to understand the significance of the epididymis in sperm maturation.

INDEX TERMS: Cauda epididymis, dogs, morphology, ultrastructure.

**RESUMO.**- [Ultraestrutura do epitélio de revestimento da cauda epididimária em cães sem raça definida.] O epitélio de revestimento da cauda epididimária em cães sem raça definida foi examinado através da microscopia eletrônica de transmissão. O epitélio epididimário é pseudoestratificado com estereocílios na borda luminal e é composto principalmente por células principais e claras. Além destes tipos, foi observado algumas células basais e apicais. As células principais e claras apresentaram características ultra-estruturais que sugerem que as mesmas estão envolvidas com funções absorptivas e secretórias. Os resultados foram comparados com estudos prévios realizados na cauda do ducto epididimário de outros mamíferos, com o objetivo de melhor entender o papel do epidídimo na maturação espermiática.

TERMOS DE INDEXAÇÃO: Cauda epididimária, caninos, morfologia, ultra-estrutura.

### INTRODUCTION

The epididymis represents the main segment of the excurrent ducts in mammals. According to Bedford (1966) and

Orgebin-Crist (1967), the epididymis stores spermatozoa and also matures them. This outlines the main functions of the epididymal duct. Thus, mammalian spermatozoa leaving the testis undergo a series of structural, functional and biochemical changes during their descent through the epididymis by they gain the ability to move and to fertilize eggs. The mammalian epididymis synthesizes and secretes numerous proteins into its lumen (Roberts et al. 2002). Maturation of spermatozoa takes place in the proximal and middle regions of the epididymis in most species. This process probably involves interactions between spermatozoa and epididymal secretion leading to changes in sperm surface proteins, in addition to interactions between spermatozoa and the apical membrane of the epididymal epithelium (Cooper 1998).

The spermatozoa are kept in an immotile state in the cauda epididymidis (Hermo et al. 1992). In the cauda region the sperm is stored until the moment of ejaculation. Thus, the cauda epididymidis is a place for long-term storage of mature spermatozoa. Depression of their metabolism and motility as well as prevention of the acrosome reaction occur in this region (Goyal & Williams 1991).

The epithelium lining of the epididymis of mammals, because of its role in providing a suitable milieu for the maturation of spermatozoa, has been the object of numerous cytological and histochemical investigations (Robaire & Hermo 1988, Robaire & Viger 1995, Schimming & Vicentini 2005, Lorenzana et al. 2007, Alkafafy et al. 2012).

The epididymal epithelium contains several cell types: principal, basal, clear, narrow, halo, and apical cells (Serre

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& Robaire 1998). The principal, basal, and halo cells appeared throughout the epididymis. The clear cells are not present in the initial segment of the rat epididymis (Moore & Bedford 1979). Apical cells have been previously described in the initial segment in the adult rat epididymis. Narrow cells has been found only in the initial segment too (Adamali & Hermo 1996).

The aim of this paper was to describe the ultrastructural aspects of the cauda epididymidis in the dog and to describe its cellular population considering the morphological and physiological complexity of the epididymis in mammals.

## MATERIALS AND METHODS

Samples of epididymal tissues were obtained from five adult and sexually mature mongrel dogs (*Canis familiaris*), during castration surgery performed at the Small Animal Hospital of the Veterinary Medicine School of UNESP, Botucatu, São Paulo. Small pieces were collected from the cauda epididymidis and destined for transmission electron microscopy (TEM). The tissue samples were fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3, for 24 h at 4°C. Following primary fixation, the pieces were washed three times in the same buffer, post-fixed for 2 h in 1% osmium tetroxide ( $OsO_4$ ) in 0.1 M phosphate buffer, dehydrated through a graded series of ethanol solutions and embedded in Araldite resin.

Semi-thin sections (0.5µm) were stained with azure-II and methylene blue (Sigma, New York, USA), for light microscopy. Ultrathin sections (50 nm), from selected areas of the cauda epididymidis, were mounted on uncoated copper grids, and uranyl acetate and lead citrate were used as contrast (Reynolds 1963, Venable & Coggeshall 1965). The sections were then examined with a Philips EM-301 and CEM-100 transmission electron microscope (Philips, Eindhoven, the Netherlands) in the Electron Microscope Center of Institute of Biosciences of Botucatu, Unesp. The anatomical terms used in this study are in accordance with the International Committee on Veterinary Gross Anatomical Nomenclature (2005).

## RESULTS

The lining epithelium of the cauda epididymidis of the mongrel dogs is pseudostratified and is made up of four cell types: principal cells, basal cells, clear cells and apical cells. Apical cells were rarely. Basal cells were observed to contact the basement membrane and were rather poor in organelles (Fig.1A,B).

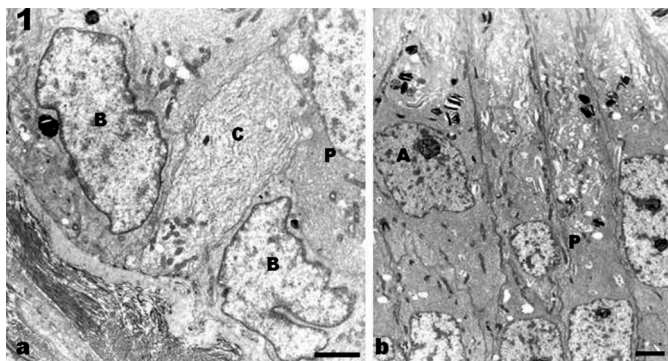


Fig.1. Epithelial lining of the dog cauda epididymidis. Principal (P), basal (B), clear (C) and apical (A) cells are showed. Bar: 1µm.

Principal cells, the major cell type of the epithelium, are tall, cylindrical cells which extend from the basal lamina to the lumen (Fig.1B). In the electron microscope, principal cells contained many basally located mitochondria and some profiles of endoplasmic reticulum (ER) (Fig.2A). In the surrounding cytoplasm of the perinuclear and infranuclear region, numerous and developed vesicular and lamellar ER were also evident such as mitochondria. The lamellar rough ER present profiles of long cisterns, disposed parallelly to the nuclear envelope in which they apparently have origin or direct continuity (Fig.2B).

In the supranuclear region, multivesicular bodies (MVB), numerous lysosomes with a moderately or densely stained matrix are visible. Several endosomes, small uncoated vesicles and mitochondria were found adjacent to the MVB and lysosomes. The Golgi apparatus located in the supranuclear region was extensive and composed of many stacks of saccules that were scattered. Profiles of ER were scattered nearly the Golgi complex (Fig.2C).

A variable number of apically located endosomes were presented in the apical cytoplasm of the principal cells. They were of various sizes. MVB and mitochondria appear in the apical region of the cell. Numerous smaller vesicular elements with no apparent content were also evident such as small coated and uncoated vesicles. The apical surface is covered by numerous stereocilia. Coated electrodense ve-

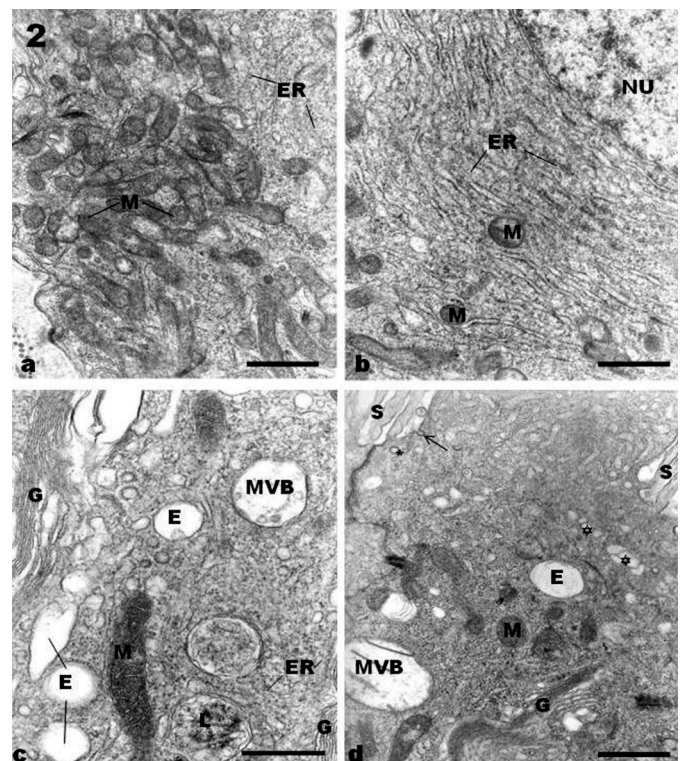


Fig.2. (a) Basal cytoplasm, (b) infranuclear and perinuclear region of the cytoplasm, (c) supranuclear region of the cytoplasm, and (d) apical cytoplasm of the principal cells of the dog cauda epididymidis. Note mitochondria (M), cisternae of endoplasmic reticulum (ER), nucleus of the principal cell (NU), Golgi complex (G), endosomes (E), multivesicular bodies (MVB), vesicular elements (star), stereocilia (S), coated vesicles (asterisks) and pits (arrow). Bar: 2µm.

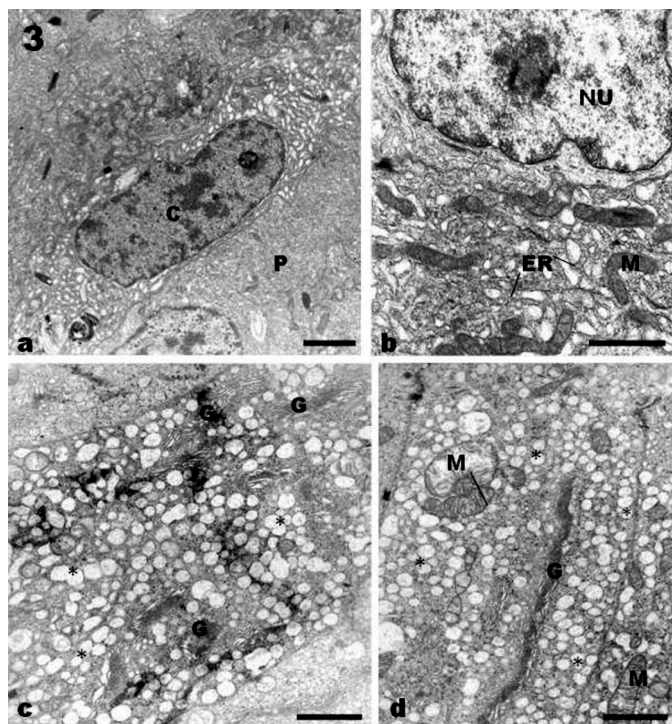


Fig.3. (a) Clear cells of the dog cauda epididymidis: clear (C) and principal (P) cells; (b) Infranuclear cytoplasm, (c) supranuclear, and (d) apical cytoplasm of the clear cells showing nucleus of the clear cell (NU), numerous vesicular cisternae of endoplasmic reticulum (ER), mitochondria (M), Golgi complex (G) and vesicular elements (asterisks) that appears as abundant vesicles and vacuoles. Bar: 1 $\mu$ m.

sicles, with presumable origin from the Golgi complex and interrelationship among the Golgi stacks of saccules and ER cisterns are observed (Fig.2D).

The clear cells, arranged between the principal cells, are characterized by the presence of abundant vesicular elements and larger vacuoles in the apical cytoplasm (Fig.3A). Clear cells display a high cytoplasmic vesicular pattern, distributed uniformly through their cytoplasm (Fig.3A-D). Generally, the nucleus is situated in the mid-region of the cell. Many mitochondria and numerous dilated and flattened cisternae of endoplasmic reticulum occupied the infranuclear region of the cell (Fig.3B). Numerous profiles of Golgi complex and vesicles of several size were distributed in the supranuclear region (Fig.3C).

Apical region of a clear cell of the cauda epididymidis in the dog containing numerous small vesicles and several large, membrane-bound bodies of different sizes, called to as endosomes. The vesicles and vacuoles are bound by the Golgi complex and mitochondria. Round mitochondria apparently have close relation to the ER lamellae (Fig.3D).

## DISCUSSION

The fine structure of the epididymis of the mammals has been previously described (Hamilton 1975, Goyal & Williams 1991, Orsi et al. 1994, Schimming & Vicentini 2001, Lorenzana et al. 2007, Beu et al. 2009). The lining epithelium of cauda epididymidis in the mongrel dogs contains four

cell types: principal, clear, basal and apical cells. The apical cells were rarely. Similarly, a few apical cells have been reported in the tail region of the cat epididymis (Arrighi et al. 1986, Viotto et al. 1996). Basal cells lie on the basal lamina and are characterized by scanty organelles as observed in the goat epididymis (Goyal & Williams 1991). Dadoune (1981) reported that the basal cells function is to renew the principal cells in the epididymis. Although their function is to great extent unknown (Alkafafy et al. 2012).

The ultrastructural features of the principal cells of the epithelium lining the cauda epididymidis in the mongrel dogs are related with secretion and absorption, similar to the epithelial cells of the epididymis in other mammals (Hermo et al. 1991, Hermo 1995, Orsi et al. 1998, Schimming & Vicentini 2008). The principal cells contain morphological features, such as a well-developed Golgi complex and numerous profiles of smooth and rough endoplasmic reticulum, that are suggestive of their ability to synthesize and secrete proteins/glycoproteins. Golgi apparatus associated with secretory vesicles, scattered mitochondria, and numerous dilated and flattened cisternae of endoplasmic reticulum (Hermo et al. 1991, Hermo 1995), are features evident in principal cells of the cauda epididymidis in the dog. The rough endoplasmic reticulum and free ribosomes observed are characteristics of protein synthesis (Hamilton 1975, Flickinger et al. 1978, Goyal 1985, Robaire & Hermo 1988, Goyal & Williams 1991, Schimming & Vicentini 2001).

The apical region of epididymal principal cell of the dog showed an endocytotic apparatus with endosomes, multi-vesicular bodies, lysosomes, coated and uncoated pits and vesicles. These features are common to the stereociliated cells and morphological peculiarities of a cell type that is involved in an absorption process (Schimming & Vicentini 2008).

In addition to the above cells, there is another type, the clear cell, which has been reported in the tail regions of the rat (Hamilton 1975, Brown & Montesano 1981, Sun & Flickinger 1982, Hermo et al. 1988, Serre & Robaire 1998), mouse (Soranzo et al. 1982, Orsi et al. 1994), hamster (Flickinger et al. 1978, Beu et al. 2009), and gerbils (Domeniconi et al. 2007). However, clear cells were not described in stallion, ram, and bull (Nicander 1957, Goyal 1985), monkey (Ramos & Dym 1977), pigs (Orsi et al. 1985); cat (Arrighi et al. 1986), goat (Goyal & Williams 1991), and donkey and dromedary camel (Alkafafy et al. 2012). Chandler et al. (1981) did not report these cellular type in the dog epididymis, although, Schimming & Vicentini (2001) observed clear cells in the canine epididymis. These reported suggested that the presence and distribution of clear cells in the epididymis seem to be species specific.

The clear cell has been referred to as a typical element at the terminal segment or tail region (Brown & Montesano 1981, Martínez-García et al. 1995). The presence of abundant vesicles and vacuoles in the clear cells is correlated with sites of fluid absorption, of greater endocytotic activity (Brown & Montesano 1981).

According to Hermo et al. (1988), the clear cells account for the endocytosis of the cytoplasmic droplet contents. As

the cytoplasmic droplet releases from the flagellum of the spermatozoon, it quickly breaks off, releasing its contents into the lumen fluid. This is endocytosed by the clear cells, and is subsequently, presumably, degraded by secondary lysosomes. The presence of clear cells in the cauda epididymidis of the dog seems to corroborate the theory of Hermo et al. (1988), that it is in this segment that the cytoplasmic droplet content is found spread in the epididymal lumen.

Studies using cellular markers as horseradish peroxidase have demonstrated that these cells are able to take up large amounts of the marker. In the cauda epididymidis, horseradish peroxidase was taken up predominantly by the clear cells rather than the principal cells. Therefore, this contention that the clear cell is specialized for absorption is supported by its fine structure (Moore & Bedford 1979). The ultrastructural characteristics of clear cells in the dog epididymis, such as the large number of vesicles, vacuoles and lysosomes, indicate that these cells actively participate in absorptive and endocytic process, in agreement with observations in rats (Moore & Bedford 1979, Hermo et al. 1988), and golden hamster (Beu et al. 2009).

On the basis ultrastructural observations of the epithelial lining cells of the cauda epididymidis in the mongrel dogs, mainly in principal and clear cells, suggest that cauda epididymidis in the dog exerts other morphological roles than storage of spermatozoa. Active processes of uptake and release of substances among the cells and the luminal content could be proposed. Perhaps, processes of cellular resorption of water, salt ions and macromolecules as well as protein secretion occurred in this segment, according to its ultrastructural characteristics and with previous support in other morphological studies in mammalian epididymis (Flickinger 1983, Arrighi et al. 1993). Alkafafy et al. (2012) reported that the distal regions of camel epididymis may possess some secretory potential. According to Beu et al. (2009), the cauda epididymidis is the site where spermatozoa acquire their fertilization potential. Marengo (2008) reported that cauda epididymidis is site of final maturation and storage of quiescent sperm. The cauda may also be responsible for the safe processing of degenerating or defective sperm (Jones 2004). This is a logical function because some attrition of sperm is likely during storage and the release of enzymes from dead sperm could damage the remaining sperm (Marengo 2008).

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