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Light microscope observations on the epididymis of paca (*Agouti paca*)

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Summary

The features of paca epididymis, based on its appearance in light microscope, is described in this paper. The cellular population of the epithelial lining comprises principal cells, basal cells, apical cells, narrows cells, and hallo cells. The epididymis is divided in five distinct and continuous regions, Zone I, or initial segment, and zone II, are both localized into the head. Zone III comprises the distal head and all the body. Zones IV and V are restricted to the tail, in the proximal and distal *cauda epididymis* respectively. Each zone can be readily distinguished on the basis of morphological characteristics. The height of epididymal epithelium is greater in zone I. There is a progressive increase in the diameter of the tubular lumen through the different areas, with the maximum in the zone V. The presence of a high epithelium, and the virtual absence of sperm in zone I suggest fast transit of spermatozoa in this region. Zone V comprises the distal tail, has smaller epithelial lining, greater luminal diameter, shorter stereocilia than the other zones, and contains spermatozoa packed inside the lumen, that characterizes this zone as a place of sperm storage. The findings are compared with other reports in rodents and other domestic animals, to contribute to the understanding of epididymal morphophysiology.

Key words

Epididymis; paca; morphology; rodents.

Introduction

The paca is a typical animal of the neotropics, the second largest rodent in these regions. They are medium sized animals, which encourages and facilitates their management (Mondolfi, 1972; Bentti, 1981, Matamoros, 1982).

In addition, the paca has a high potential as a livestock because its meat is highly appreciated for consumption. Pacas and agoutis are cited as one of the main items in the diet of rural populations in the tropics, and consequently, the small mammals are hunted over these regions. Paca produces high quality animal protein (Kleiman et al. 1980).

In working towards a rational zootechnical exploration, it is important to know the reproductive system in pacas. There are few reports on the male reproductive system in this species. Matamoros (1981) described the anatomy of the reproductive sys-

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tem of the paca (*Cuniculus paca*) found in Central America. This same author, in 1982, made some observations on the biology of this species bred in captivity. Studies have been devoted to the histology of the seminiferous epithelium and the identification of the stages of spermatogenesis (Pashov and Matamoros, 1984). Costa et al. (2010), described the spermatogenesis and its chronology in the pacas.

Pashov and Matamoros (1994) presented a brief description of the ultrastructure of the testis and epididymis of the paca of Central America. For these authors, the epididymis presents principal cells, basal cells and clear cells, but they did not cite apical cells through the different epididymal regions. There are other ultrastructural studies on the epididymis (Orsi et al., 2009a), and vas deferens of the paca (Orsi et al., 2006, 2009b). Although there are reports on the ultrastructure of the epididymis of pacas, there is a lack of studies on the level of light microscopy. For another species of rodent, the agouti, for example, there are descriptions of the arterial supply to the penis (Carvalho et al., 2008), and of the morphology of the accessory sex glands of the male (Mollineau et al., 2009).

Given the importance that the paca may have to the poorest populations, where it is a low-cost source of proteins and considering the scarcity of information on the reproductive morphology of these animals, the aim of this study was to describe the morphology at the light microscopy level, as a premise to future investigations and the zootechnical rational exploitation of these rodents.

Material and methods

Five adults, sexually mature pacas (*Agouti paca*), were obtained from the breeding centre for wildlife animals at the Department of Animal Science, Faculty of Agricultural Sciences and Veterinary, Jaboticabal, Universidade Estadual Paulista. These animals had to be discarded from the colony for the selection and maintenance of the breeding balance between males and females.

The male paca remains reproductively fertile throughout the year (Dubost et al., 2005). Thus, we did not care about the time of collection. After necropsy, the animals had the epididymis dissected and isolated from the testis. The samples were fixed in a solution of 10% buffered formalin and Mc Dowell solution for 24 hours. The ducts were cut through in longitudinal planes with inclusion *in totum*, to provide a sequential description of the various regions of the epididymis. Afterwards, they were dehydrated and embedded in historesin (Leica-Historesin, Germany). Cross sections, 3 μm thick were stained with hematoxylin and eosin (H/E) and Masson's trichrome.

Round, i.e. transversally cut tubules, obtained from 30 sections of different epididymal regions, were used to measure the height of epithelium and diameter of lumen. From the average values for each animal and its distinct epididymal zones, arithmetic average and standard deviation (SD) were calculated for each histological epididymal zone.

Results

In the paca, the epididymal duct is lined by a pseudostratified columnar epithelium with stereocilia on the apical surface. This epithelium is surrounded by a lamina

propria and a peritubular muscle coat (Fig. 8). The cellular population of the epididymal epithelium is comprised of principal (P) cells, basal (B) cells, apical (A) cells and narrow (N) cells. The P cells, predominant in number, were observed in all zones of the epididymis. They are tall cells, which extend from the basal lamina to the lumen, and are covered with stereocilia. The B cells lie next to the basal lamina, and do not reach the tubular lumen. The A cells have a slender shape, and are characterized by a wide cytoplasm in the apical part of the cell and a narrow body; the nucleus is more apically located than in P cells. The N cells were frequently found in some zones (I-III) of the duct; they were tall with darkly stained cytoplasm and elongated nuclei (Figs. 1-8). In addition to these cell type, halo (H) cells were frequently found in the epithelium of the terminal regions (Figs. 6, 8).

Light microscopic studies revealed that the ductus epididymis duct in the paca is divided in five distinct regions, continuous with each other. Zone I or the initial segment, is located between the efferent ducts and the flexure of the head; the zone II is the proximal epididymal head; zone III corresponds to the distal part of the head and practically the entire epididymal body; zone IV corresponds to the proximal part of the epididymal tail; zone V corresponds to the distal part of the epididymal tail (Figs. 1-8). It should be noted that transition between zones is gradual, not abrupt. Hence, there is always an overlapping of morphological features between two adjacent regions.

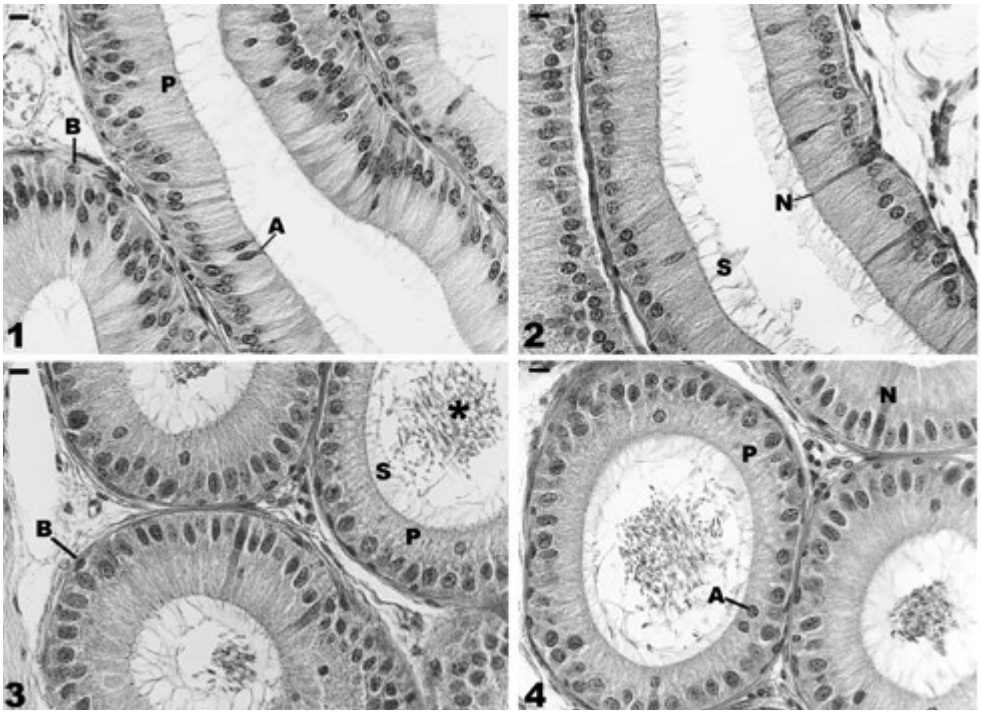
Zone I is characterized by a high epithelium and an irregular lumen devoid of spermatozoa. The epididymal epithelium in this zone consists of P, B, A and N cells. Principal cells show a spherical to elongated nucleus, located in the lower third of the cell, with one or more distinct nucleoli. A large number of long stereocilia projects from the luminal surface of P cells into the lumen. B cells, with a small ovoid nucleus, were observed next to basal lamina. A and N cells show the classical features for these cell types (Figs. 1, 2).

The zone II shows a sharp decrease in epithelial height and cellular exfoliation in the lumen, which is filled with sperm cells. In this zone, a transverse section of the duct reveals a regular contour. One could observe P cells, B cells, A cells and N cells. The P cells posses an ovoid nucleus with granular heterochromatin. Cells in zone II are similar, in appearance, to those found in the zone I. The stereocilia continued to be elongated (Figs. 3, 4).

Zone III shows increased epithelial height and luminal diameter. This zone is characterized by a regular profile of the duct section. The lumen contains cellular exfoliation and spermatozoa. The epithelium contains the same cell types as other zones. P cells are the major cell type, and posses slightly shorter, irregular stereocilia. A cells, B cells and N cells are similar to those of preceding zones. Moreover, in this zone one could see another cell type, H cells (Figs. 5, 6).

The most striking feature of the zone IV is the folded epithelium. The lumen contains many spermatozoa and cellular exfoliation (Fig. 7). This zone shows all cellular types, but unlike the previous zones it contains short stereocilia.

Several histological features differentiate the zone V from the other epididymal zones in the paca. It is characterized by lower epithelium, greater luminal diameter, greater sperm concentration, denser and shorter stereocilia, and a thicker smooth-muscle wall, compared with other epididymal zones. The epithelial lining is 11.4 ± 2.78 μm high. The P cells are the most common epithelial cell type found in this zone. Their nucleus is round and situated in the lower half of the cell. Because of the small



Figures 1-4 – Light micrograph of the paca epididymis. Note the different appearance of zone I (figs. 1 and 2) and zone II (figs. 3 and 4) of the epididymis. Principal cells (P), basal cells (B), apical cells (A), narrow cells (N), stereocilia (S) and spermatozoa (asterisks) are visualized. (bar = 1 µm H&E).

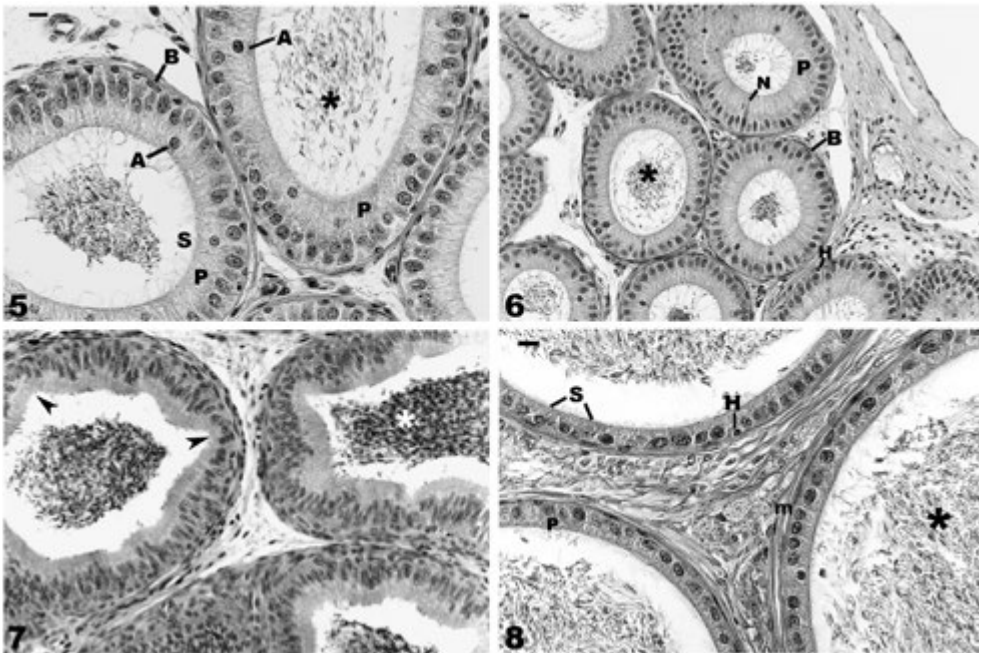
size of the P cells, nuclei rest near the basement membrane. B cells, A cells and H cells are scarce. N cells were not observed in the zone V. The stereocilia still are regular and shorter resembling a brush border (Fig. 8).

The height of the epididymal epithelium is greater in zone I, with 68.56 ± 6.80 μm, then falls abruptly in zone II (32.16 ± 3.74 μm), increases again in the zone III (47.06 ± 3.73 μm), and from this area, the epithelial height decreased progressively toward the zone V (11.4 ± 2.78 μm). Instead, there is a progressive increase in the diameter of the tubular lumen through the different areas, to a maximum in the region V (Table 1).

Discussion

In this paper the light features of the paca epididymis were demonstrated for the first time, that is important considering the scarcity of information on the reproductive morphology of this species.

The epididymis has been extensively studied in several mammals, and is know that its plays an important role in sperm maturation. Parillo et al. (2009) reported that in mammals, the ductus epididymis plays a pivotal role in sperm maturation. During



Figures 5-8 – The light features of zone III (figs. 5 and 6), zone IV (fig. 7) and zone V (fig. 8) in the paca epididymis. Principal cells (P), basal cells (B), apical cells (A), narrow cells (N), stereocilia (S), spermatozoa (asterisks), and lamina propria e peritubular muscle coat (m) may be observed. Noted the halo cells (H), that appeared in the terminal zones and folds of the epithelium in zone IV (arrowhead). (bar = 1 µm; figs. 5 and 6 H&E; figs. 7 and 8 Masson's trichrome).

Table 1 – Epithelial height, luminal diameter and cell types in different zones of ductus epididymis of the paca.

Zones	Epithelial height (μm)	Luminal diameter (μm)	Cell types[1]
I	68.56 ± 6.80	113.05 ± 3.67	P, B, A, N
II	32.16 ± 3.74	127.21 ± 6.80	P, B, A, N
III	47.06 ± 3.73	152.46 ± 3.66	P, B, A, N, H
IV	30.70 ± 4.38	199.74 ± 4.31	P, B, A, H
V	11.40 ± 2.78	497.77 ± 2.53	P, B, A, H

[1]P, principal; B, basal; A, apical; N, narrow; H, halo. Data are expressed as mean ± SD.

sperm transit through the epididymis duct, the testicular fluid is modified as a result of the absorptive and secretory functions of the epithelial cells lining the epididymal lumen (Dacheux et al., 2003; Srivastav et al., 2004; Tulsiani, 2006).

Ductus epididymis can be divided into segments or histological zones. The epididymal division into initial, middle and terminal segments was proposed by Glover and Nicander (1971). The epididymis of the rat (Robaire and Hermo, 1988;

Hermo et al., 1991), equines (Arrighi et al., 1993), cat (Axnér et al., 1999), and dog (Schimming and Vicentini, 2001) were divided based on this criterion. A second pattern was used in this study. The establishment of five histological zones in the epididymis of paca was based on morphological criteria, that is, analysis of the epithelial lining as a whole, of the different cell types and of the luminal contents.

Based on similar criteria the regional histology of epididymis was described in various species of laboratory or domestic mammals: rat (Reid and Cleland, 1957), stallions, rams and bulls (Nicander, 1958), hamster (Vicentini and Orsi, 1987), goat (Goyal and Williams, 1991), dog (Schimming et al., 1997), cat (Axnér, 2006), the endemic mexican rodent (Lorenzana et al., 2007), and roe deer (Schön and Blottner, 2009). The epididymis presents variability in structure and function along its length in all the species studied so far. The presence of different zones with different cytological features implies functional differences (Lorenzana et al., 2007).

The number of histological zones in the epididymis is species-specific. Five zones have been described in the epididymis of the hamster (Vicentini and Orsi, 1987), goat (Goyal and Williams, 1991), dog (Schimming et al., 1997), and roe deer (Schön and Blottner, 2009), similar to the paca. The epididymis comprises six different regions in the cat (Axnér, 2006) and seven zones in *Peromyscus winkelmanni* (Lorenzana et al., 2007).

The presence of a high epithelium and the virtual absence of sperm found in zone I of the epididymis of paca, was also described in other mammals (Vicentini and Orsi, 1987; Schimming et al., 1997). This zone exhibit the greatest epithelial height, associated to scarce spermatozoa, suggesting fast transit of spermatozoa in this region, similar reports were described for the hamster and cat epididymal initial segments (Vicentini and Orsi, 1987; Viotto et al., 1988). In the paca, zone I was a segment anatomically located between the efferent ducts and the flexure of the head, zone II corresponds to the proximal part of the epididymis head, and zone III to the distal part of the head and practically the entire epididymal body. Unlike, in *P. winkelmanni* the caput epididymis contains five zones (zones I-V) and the corpus epididymis consists of zone VI (Lorenzana et al., 2007). In the roe deer, caput epididymis consists of zones I, II and III, while zone IV was located in the corpus epididymis (Schön and Blottner, 2009).

In the paca, zone IV corresponds to the proximal part of the epididymal tail and zone V, corresponds to the distal part of the epididymal tail. The same zones were found in the tail of epididymis in the hamster (Vicentini and Orsi, 1987) and dog (Schimming et al., 1997). Only one zone, zone VII, appeared in the cauda epididymis of the *P. winkelmanni* (Lorenzana et al., 2007) and roe deer (Schön and Blottner, 2009). The zones IV and V of the epididymis of the paca would correspond to the terminal segment of the epididymis described by Glover and Nicander (1971).

Zone V comprises the distal tail and is characterized by lower epithelial lining, greater luminal diameter, shorter stereocilia, packed spermatozoa inside the tubular lumen and a thicker smooth muscle wall, comparatively to the other histological zones in the paca epididymis. The ducts of the zone VII (cauda epididymis) *P. winkelmanni* show the largest diameter (Lorenzana et al., 2007). In the donkey and dromedary camel, the epithelium lining of the cauda epididymis was the smallest (Alkafafy et al., 2012). These morphological characteristics suggest that this area is a place of sperm storage. The distal cauda epididymidis has then a function as spermatozoa storage (Nicander, 1958; Robaire and Hermo, 1988; Goyal and Williams, 1991; Gloria et al., 2011). The specific luminal environment of the cauda allows spermatozoa to

survive for several weeks, primarily by maintaining metabolic quiescence and preventing premature sperm activation (Sostaric et al., 2008; Gloria et al., 2011).

The cellular populations of epididymal epithelial lining of paca I made of P cells, B cells, A cells, N cells and H cells. The distribution of cell types is different according to zones. The P, B, A, and N cells appear in zones I and II. Zone III, beyond these cell types, presented H cells. Zones IV and V showed no N cells, only P, B, A and H cells. Goyal and Williams (1991) found P and B cells in all regions of the epididymis of goat, and also intraepithelial lymphocytes/macrophages. Domeniconi et al. (2007) reported P, B, A and dark cells in the epididymis of gerbil. Alkafafy et al. (2012) consider the halo cells and lymphocytes as the same cells, and reported this cell type throughout the entire length of the epididymal duct in donkey and dromedary camel.

Based on ultrastructural studies it has been suggested that P cells may both secretory and absorptive roles (Moore and Bedford, 1979; Goyal and Williams, 1991, Schimming and Vicentini, 2001; Lorenzana et al., 2007; Alkafafy et al., 2012). The B cells are the second consistent cell population of the epididymis (Alkafafy et al., 2011). It is unlikely that B cells are either absorptive or secretory in function because most of their organelles are poorly developed (Goyal and Williams, 1991). Indeed, their function is yet to be clarified. The A cells are few in number in the paca epididymis and especially scarce in zone V. This cell type is the less common cell type in different mammalian species (Goyal, 1985; Alkafafy et al., 2011). A cells were called mitochondria-rich cells by Palacios et al. (1991) and Martínez-Garcia et al. (1995) because they contain a high amount of mitochondria in the apical cytoplasm. The exact functional significance of A cells is not yet clear (Martínez-Garcia et al., 1995).

Tingari (1989) called N cells as dark cells; their origin and physiological role have been the subject of controversy. The narrow cell cytoplasm touches the basement membrane, and the apical cytoplasm may bulge slightly into the lumen with numerous vacuoles, endocytic vesicles, lysosomes, and mitochondria (Joseph et al., 2011). This findings may suggest that these cells have a role in endocytosis.

Schimming and Vicentini (2001) and Domeniconi et al. (2007) reported the presence of clear cells in the dog and gerbil epididymis, respectively, while this cell type has not been observed in the paca epididymis. Clear (C) cells are also absent in stallion, ram, bull and goat (Nicander, 1958; Goyal, 1985; Goyal and Williams, 1991). Also C cells seem related to endocytosis (Hermo et al., 1988). Joseph et al. (2011) suggested that C cells are large endocytic cells interspersed between principal cells.

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