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Ultrastructure and morphometric features of epididymal epithelium in *Desmodus rotundus*

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ABSTRACT

The blood-feeding behavior of *Desmodus rotundus* made this bat a potential vector of rabies virus and a public health issue. Consequently, the better understanding of its reproductive biology becomes valuable for the development of methods to control its population. In this study, we described morphological aspects of epithelial cells in *D. rotundus*' epididymis using light and transmission electron microscopy methods. The duct compartment was the main component of initial segment (83%), caput (90%), corpus (88%) and cauda (80%) regions. The epithelium lining the duct presented a progressive decrease in its height from initial segment to cauda regions. Moreover, the morphology of each cell type was the same along the entire duct. Similarly to rodents, columnar-shaped principal cells were the most abundant cell type throughout the epididymis, followed by basal and clear cells. Differently in rat and mice, the frequency of clear cells did not increase in the epididymis cauda, whereas the proportion of principal and basal cells was greater in this region. Furthermore, *D. rotundus* presented goblet-shaped clear cells with the nucleus located in the apical portion of the epididymal epithelium. This cellular portion also presented electron-lucid vesicles of different sizes that may correspond to vesicles enriched with proteins related to proton secretion. In addition to the findings regarding clear cells' structural organization, basal cells presented scarce cytoplasm and no axiopodia. Taken these findings together, we suggest that the mechanism of luminal acidification may have other pathways in *D. rotundus* than those described in rodents.

1. Introduction

Bats from subfamily Desmodontinae are intriguing by their hematophagous feeding habits. Particularly, *Desmodus rotundus* is one of the three desmodontine species that feeds exclusively on mammal blood, especially cattle (Kotait et al., 2007; Peracchi et al., 2007). The proximity between livestock and *D. rotundus* has stimulated its geographic range expansion and bat population growth. Consequently, there was a greater incidence of rabies in cattle, since vampire bats may be one of the main transmitters of rabies virus (Delpietro et al., 1992; Schneider et al., 2009; Lee et al., 2012).

Little is known about the reproductive biology of *D. rotundus*. Apparently, there is no influence of either temperature or day length or food availability on reproductive events in *D. rotundus* when compared to other Neotropical bats (Wilson and Findley, 1970; Krutzsch, 1979; Altrigham, 1998; Neuweiler, 2000). This species may breed year-round due to the abundant feeding resources, and a promiscuous pattern of mating system (Wilkinson, 1985; Taddei et al., 1991; Alencar et al., 1994; Crichton and Krutzsch, 2000; Freitas et al., 2006).

Recently, studies have described the testicular morphometry and spermatogenesis in *D. rotundus* (Morais et al., 2017) and Neotropical bats (Beguelini et al., 2009; Duarte and Talamoni, 2010; Morais et al., 2012; Morais et al., 2013; Beguelini et al., 2014; Morais et al., 2014). In those animals, however, research focused on the epididymis histophysiology is still scarce (Beguelini et al., 2010; Oliveira et al., 2013; Castro et al., 2017). It is known that spermatozoa produced by testes are immature and unable to fertilize an egg. Thereby, they need to pass through the epididymis to acquire their motility and fertilizing capacity. This male reproductive organ is a long, single, and convoluted duct lined by an epithelium composed of several cell types. Epithelial cells, in turn, are responsible for creating an adequate luminal environment for sperm maturation and storage (Cornwall, 2009; Robaire and Hinton, 2015).

Previously, Castro et al. (2017) identified three cell types along the

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entire length of *D. rotundus*' epididymis by immunocytochemistry. Aquaporin 9, V-ATPase subunit B1, and cytokeratin 5 were used as cell-specific markers of principal, clear, and basal cells, respectively (Castro et al., 2017). Those authors reported that pencil-shaped V-ATPase-rich cells (narrow cells) were not detected in the initial segment of the bat epididymis, unlike in rodents (Robaire and Hinton, 2015; Castro et al., 2017). While principal cells are responsible for fluid exchange and secretion of different luminal proteins (Cornwall, 2009), clear cells are involved in luminal acidification (Da Silva et al., 2007a). The activities of those cell types might be controlled by basal cells, which regulate electrolyte and water transport in principal cells via paracrine signaling (Leung et al., 2004), and control the V-ATPase-dependent proton secretion by clear cells (Shum et al., 2008).

Despite principal, basal and clear cells have been identified in *D. rotundus* (Castro et al., 2017), the histology and ultrastructure of those cell types cells have not been assessed yet. Therefore, we aimed to describe the morphology, morphometry and ultrastructure of epithelial cells in *D. rotundus*' epididymis. We associated light microscopy with transmission electron microscopy in order to create a baseline for future studies in epididymis morphology of vampire bats. This knowledge may generate information about its reproductive biology and, in turn, may support the development of methods to control *D. rotundus* population.

2. Material and methods

2.1. Animal capture

Adult males (Fig. 1A) were captured using mist nets, positioned near a cave in Itamarati de Minas, Minas Gerais State, Brazil (21°23′S and 42°51′W; 585 m altitude), in October 2014. Permits for field collection were provided by Chico Mendes Institute for Biodiversity Conservation (ICMBio; number 40629-1). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All the experimental procedures were reviewed and approved by the Committee on the Ethics and Use of Animal Experiments of UFV (CEUA process number 55/2013).

2.2. Tissue collection

Five animals were euthanized using intraperitoneal administration of sodium pentobarbital (40 mg/kg), followed by guillotining (Castro et al., 2017). The entire epididymides were removed, dissected (Fig. 1B), and weighed. The epididymal somatic index (ESI) was obtained by computing the ratio between epididymis weight (EW) and body weight (BW), where ESI = EW/BW x 100. The left epididymis was used for histology, while the right was used for transmission electron microscopy analysis. In addition, testes were collected for evaluating the sexual maturity of each animal.

2.3. Histological procedure for light microscopy

Testis (n = 5) and epididymides (n = 5) were immersed in Karnovsky fixative solution (Karnovsky, 1965) for 24 h. Then, they were dehydrated in a crescent ethanol series (70%, 80%, 90%, and 100%), and embedded in 2-hydroxyethyl methacrylate (Historesin®, Leica Microsystems, Nussloch, Germany). Sections with a thickness of 3 μm were stained with toluidine blue-sodium borate (1%), and qualitatively analyzed. Other histological sections were stained with periodic acid Schiff (PAS) reaction for identifying neutral glycoproteins, neutral carbohydrates and glycogen. We performed PAS reaction with amylase digestion and PAS reaction without periodic acid solution for negative controls. All sections were qualitatively analyzed using light microscope (Olympus CX40, Tokyo, Japan).

Histological sections of testis were used for identifying sexual maturity of the captured bats. Thus, we evaluated seminiferous tubules regarding the presence of seminiferous epithelium in the Stage VIII of spermatogenic cycle, as well as spermatozoa in the lumen.

For morphometric analysis, digital images of the four epididymal regions, initial segment, caput, corpus, and cauda (Fig. 1C), were obtained using a light microscope (Olympus BX53, Tokyo, Japan) equipped with a digital camera (Olympus DP73, Tokyo, Japan) and analyzed with Image-Pro Plus® 4.5 (Media Cybernetics, Silver Spring, MD, USA) software. The mean duct diameter of each epididymal region was obtained by randomly measuring 20 tubular cross sections per animal. These sections were also used to measure the luminal diameter, which is the distance taken from the lamina propria to the lumen. The epithelium height was estimated as the average of four diametrically opposed measurements (Souza et al., 2016).

The volumetric proportion of four epididymal regions was obtained by counting 2660 points projected onto 10 images captured from histological slides per animal. Coincident points were registered in duct components (epithelium and lumen) and interduct components (blood vessels and connective tissue). The percentage of points in each component was calculated using the formula: volumetric proportion (%) = (number of points in the component/2660 total points) x 100 (Souza et al., 2016).

The relative distribution of epithelial cell types in the four regions was estimated by cell count in different sections per region (Fig. 1C). Cells were counted in 10 sections per region per animal. The result of the crude cell count was corrected by applying Amann's formula (Amann, 1962), according to Beu et al. (2009).

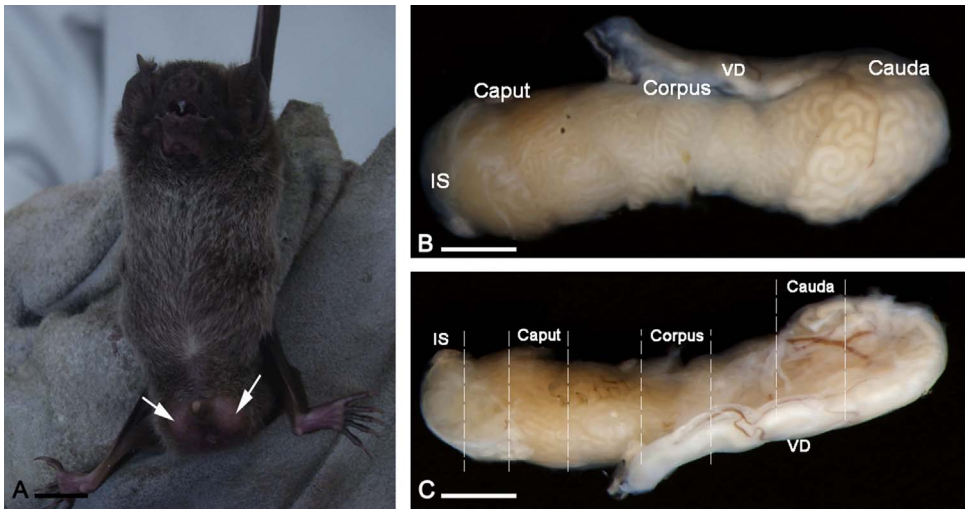

Fig. 1. (A) Adult male of *Desmodus rotundus*. Note the scrotal area (*white arrow*) out of the abdominal cavity. Dorsal (B) and lateral (C) views of epididymis show four anatomical regions, initial segment (IS), caput, corpus and cauda. VD, vas deferens. *Scale bars* (A) 1 cm; (B–C) 1 mm. *Photos* Mariana Machado-Neves (A), and José Lino Neto (B-C).