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The morphology and morphometry of the epididymis in the greater cane rat (*Thryonomys swinderianus* Temmincks)

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The structure and morphometry of the epididymis in the greater cane rat were studied in this work. In assessing the morphology and characterising the morphometric values, a total of 15 adult male greater cane rats, bred and raised in captivity, were used. All the animals had brownish perineal staining, which was taken as index of sexual maturity in male cane rats, and they were maintained on elephant grass stems with water given ad libitum. *From this work, the epididymis of the greater cane rat was observed to have a mean weight of 0.0365 ± 0.091 g, forming about 0.016% of the total body weight and an average volume of 0.36 ± 0.08 mL. There was a positive correlation between the epididymal weights, testicular weight, and the body weight in this animal. However, the gross divisions of the epididymis into head, body, and tail were not conspicuous in the cane rat; instead it had two divisions — the cranial and the caudal divisions. In addition, based on the histological and histomorphometric analyses, five zones were observed in the epididymal epithelium of this animal. This preliminary information on the epididymis will serve as a basis for further research on the epididymis of the greater cane rat and will contribute to the knowledge of the its reproductive biology, which will subsequently aid in the captive rearing and domestication of this animal.*
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Key words: domestication, wild rodent, reproductive biology

INTRODUCTION

The greater cane rat (*Thryonomys swinderianus*, Temmincks) is a prolific, herbivorous wild rodent of the order *hystricomorpha*, which is vigorously hunted and exploited in most areas, particularly in West Africa south of Sahara [4, 6]. It is currently undergoing domestication and captive rearing in this region, and a recent trend in its farming is towards increased stock levels and intensification of production practices [2]. Therefore, a good understanding of the reproductive biology, particularly of each segment of the male reproductive tract, is vital [25].

The mammalian epididymis is no longer regarded as a mere conduit pipe for spermatozoa from the testis to the exterior [26], but equally serves a critical function in preparing the male germ cells for fertilisation. The epididymis as an epithelial tube is folded into a highly organised structure comprised of many segments that can be grouped into roughly five gross anatomical segments, namely: *initial*

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segment, caput, corpus, cauda, and *vas deferens*, with each having distinct morphology and function [15]. So, in assessing the contributions of the different segments in providing an appropriate environment for sperm maturation, the knowledge of its structure is vital.

Species variations in the structures and specialisations along the epididymal duct have been reported by several authors [12, 17, 18]. For instance, different species display various zones of the epididymis. In the rat, Reid and Cleland [22] reported six zones, while Nicander [16] reported eight zones in the rabbit and six zones in the stallion, ram, and bull. Holstein [9] observed eight zones in man. In the African giant rat, five zones were observed [18]. However, no structural difference was found throughout the length of the epididymal epithelium in the monkey [21], and that of the greater cane rat is yet to be described. Though the significance of these species differences is not yet fully understood, it is inconceivable that the structural differences have no functional significance or relevance peculiar to each species.

Therefore, this work seeks to describe the gross and histological appearance, as well as characterise the histomorphometry and the various zones, of the epididymis in the greater cane rat (*Thryonomys swinderianus*, Temmincks). The data might provide the basis for future research as well as contribute to the knowledge of the reproductive biology of the greater cane rat.

MATERIAL AND METHODS

Subjects

A total of 15 adult, male greater cane rats, bred and raised in captivity, were used in the study. They aged between 13 and 41 months and weighed between 1.4 and 4.01 kg (1400–4010 g). All the animals had brownish perineal staining, which was taken as an index of sexual maturity in male cane rats, as reported by Adu and Yeboah [3]. They were maintained on elephant grass stems and water was given *ad libitum*

Sample collection

Each animal was weighed alive and sacrificed after anaesthesia with chloroform in a closed container. The abdominal part was then dissected open through a mid ventral abdominal incision. The ischiatic arch was completely removed to expose the reproductive organs. The testis and epididymis were dissected out, collected, and examined to evaluate the organs grossly. Each epididymis was separated from the testis, weighed (using a Microwa analytical balance) and the volume found by water displacement method to determine the gross morphometry of the organs.

Histological procedure

Samples of the epididymis were fixed in Bouin's fluid for 72 hours, dehydrated in graded alcohol cleared in two jars of xylene (Needham Market, Suffolk, England), and embedded in paraffin wax. Sections 5 μ thick were stained with haematoxylin and eosin (H & E). All the slides were examined under an Olympus BX 50 light microscope to evaluate the histological features.

Histo-morphometric procedure

Linear measurements of the epididymal tubular diameter and epididymal epithelial heights were taken at 100× magnification (that is, 10× from the objective and 10× from the eyepiece) using an ocular micrometer calibrated with a stage micrometer (Graticules Ltd., Tonbridge, Kent, England). The ocular micrometer used was fixed on the Olympus BX 50 light microscope, which measured at 40×, 100×, 400×, and 1000× magnifications. The choice of 100× magnification was because this is the best for epithelial height and tubular diameter measurements and the most commonly used [5, 7, 25]. Ten tubular, round or nearly round profiles were chosen randomly and measured for each animal. The epithelial heights were obtained in the same tubule sections utilized to determine tubular diameters.

Statistical analysis

The data were subjected to correlation analysis to examine the relationship between and within the data using Microsoft Excel® data analysis tools.

RESULTS

Morphology of the epididymis

Gross appearance. The epididymis consists of a single duct that runs from the efferent ductules to the vas deferens. The inverted S shaped duct was loosely attached to the medial border of the testes by a thin connective tissue and could easily be separated from the testes. Grossly, two subdivisions were observed (Fig. 1), namely the cranial and the caudal parts.

The pinkish coloured cranial part that communicates directly with the efferent duct corresponded to both the initial and middle segments. This part, which extended to about one-third of the length of the testes (Fig. 1), differed from the caudal part in the degree of convolution of the duct. The cream

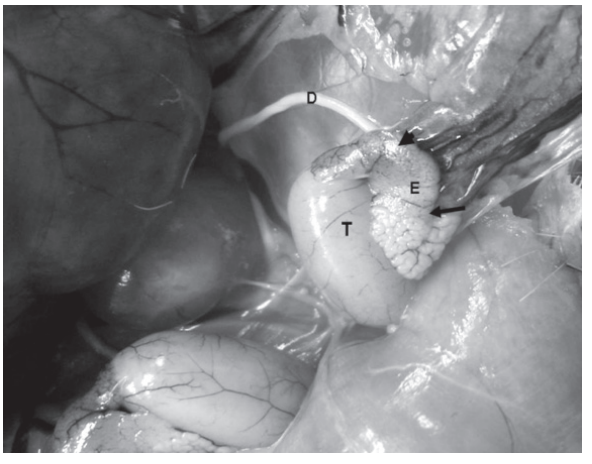

Figure 1. Photograph of epididymis (E) of the greater cane rat. Note the cranial part (arrow head) and the caudal part (arrow). The inverted S-shaped epididymis is loosely attached to the testis (T), while the cauda epididymis continues as the deferent duct (D) covered by the spermatic cord in this photograph.

Table 1. Histo-morphometric data of the epididymis in the greater cane rat (*Thryonomys swinderianus*)

Parameters	Mean	Standard error of mean
Epididymal tubular diameter [μm]		
Zone 1	212.18	± 24.05
Zone 2	226.76	± 14.17
Zone 3	248.78	± 24.78
Zone 4	411.38	± 48.37
Zone 5	678.75	± 71.67
Epididymal epithelial height [μm]		
Zone 1	75.93	± 9.26
Zone 2	57.07	± 4.99
Zone 3	38.63	± 4.97
Zone 4	20.35	± 1.37
Zone 5 (*folded part*)	42.00	± 7.48

coloured caudal part, which corresponded to the cauda epididymis, was relatively less convoluted, wider, and lighter in colour than the cranial part. The caudal part also had two parts: the proximal caudal and the distal caudal parts. Its proximal part was situated at about the middle of the testes and then bent craniad to become the distal caudal part, which remained on the lateral part without extending to the caudal pole of the testes (Fig. 1).

The coiled epididymal duct gradually straightened out and continued as the deferent duct.

Histological appearance. The epididymal duct in the greater cane rat was observed to be lined by pseudo-stratified columnar epithelium, surrounded by a small amount of loose connective tissue and circular smooth muscle fibres. The number of fibres increased progressively towards the terminal segment of the duct. Histological analysis of the epithelium revealed five distinct zones (zones 1–5) based on the epithelial height, conformation, and cell types. The characteristic features of these zones can be used to determine their relative placement within the conventional regions (initial, middle, and terminal segments) of the epididymis.

Zone 1. This was the first portion of the cranial part that communicates with the efferent ductules and could not be easily distinguished grossly but constitutes part of the initial segment of the duct. The epithelium of this zone was thrown into folds with an irregular height measuring an average of 75.93 ± 9.26 μm and ductular diameter of 212.18 ± ± 24.05 μm (Table 1). Due to the folded epithelium, the lumen of this zone becomes irregularly shaped with a sparse content of spermatozoa (Fig. 2A). Basically, two cell types can be distinguished in the epithelium of this zone under light microscope: the principal cells and the basal cells.

The principal cells extended from the basal lamina to the narrow lumen into which their stereocilia projected. The nuclei of these cells were oval to elongate in shape and were situated within the basal half of the cell (Fig. 2B). The basal cells which lie on the basal lamina were almost equal in number to the principal cells in this zone. The nuclei of these cells were relatively large, highly indented, and round or elongated in shape. A thin rim of cytoplasm surrounds the nuclei of these cells (Fig. 2B).

Zone 2. The epithelium in this zone was observed to be less folded and the luminal outline was smooth (Fig. 3). Although this zone showed a lower epithelial height of 57.07 ± 4.99 μm, the ductular diameter of 226.76 ± 14.17 μm was almost similar to that in zone 1 (Table 1).

The main cells in this zone were the principal cells and the basal cells although truncate-looking cells were also present. The nuclei of the principal cells in this zone were located at about the same level. Also, there was the presence of vacuoles at the apical part of the cytoplasm. The basal cells were fewer relative to the principal cells in this zone. They also had a thin cytoplasm surrounding the round or horizontally elongated nucleus.

The truncate looking cells were distinct from the principal cells in that they had darker stained nuclei that were situated above the level of the principal cells (Fig. 3).

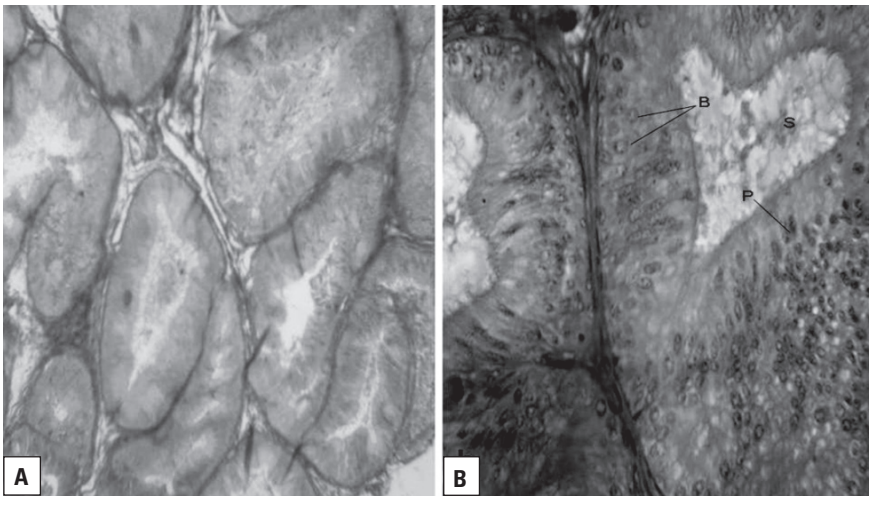

Figure 2. Photomicrographs of zone 1 segment of the epididymal duct in the greater cane rat at different magnifications, H & E 100× (**A**) and 400× (**B**). Note in (**A**) the folded epithelium and in (**B**) the principal cell (P), numerous basal cells (B) as well as sparse spermatozoa content (S).

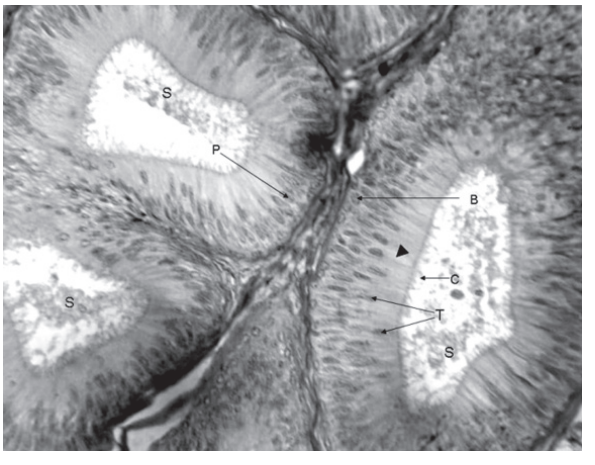

Figure 3. Photomicrograph of zone 2 segment of the epididymal duct in the cane rat. Note the truncate-like cell (T), principal (P), basal cells (B), and the cilia (C) projecting into the lumen, which contained some sparse, loosely packed spermatozoa (S). H & E 400×.

Zone 3. This zone had a uniform epithelium with a ductular diameter of 248.78 ± 24.78 μm and height of 38.63 ± 4.97 μm (Table 1) indicating lower ductular epithelium with increasing luminal dia-meter than the preceding zones (zones 1 and 2) (Fig. 4A, B). In fact, there was a consistent increase in luminal diameter from this zone down the length of the epididymal duct. In addition, the lumen was filled with loosely packed spermatozoa.

The basic cell types observed in this zone were the principal and basal cells. The nuclei of the principal cells were mostly elongated or roughly oval in shape, centrally located, with centrally placed nucleoli. Also, there was the presence of some sub-apical vacuoles in the cytoplasm of the principal cells (Fig. 4B). The basal cells in this zone were relatively few compared to those found in the previous zones.

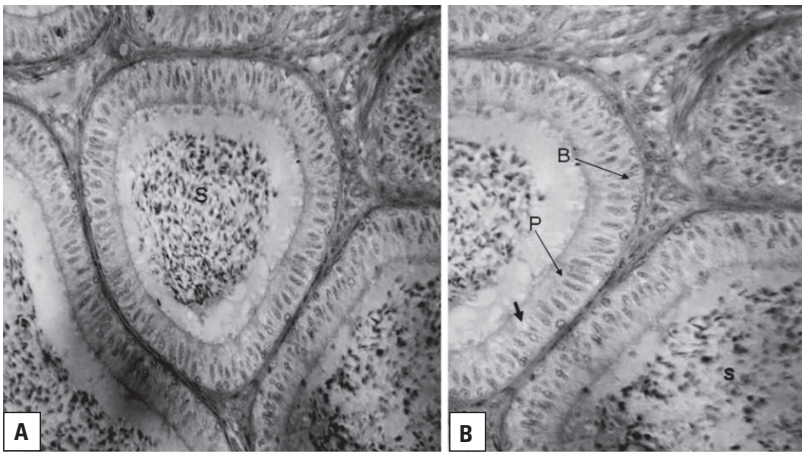

Figure 4. Photomicrographs of zone 3 segment of the epididymal duct in the cane rat. H & E 400× (**A**) and 600× (**B**). Note in (**A**) the lumen with loosely packed spermatozoa (S). Note in (**B**) the principal (P) with sub-apical vacuoles (small arrow) and basal (B) cells.

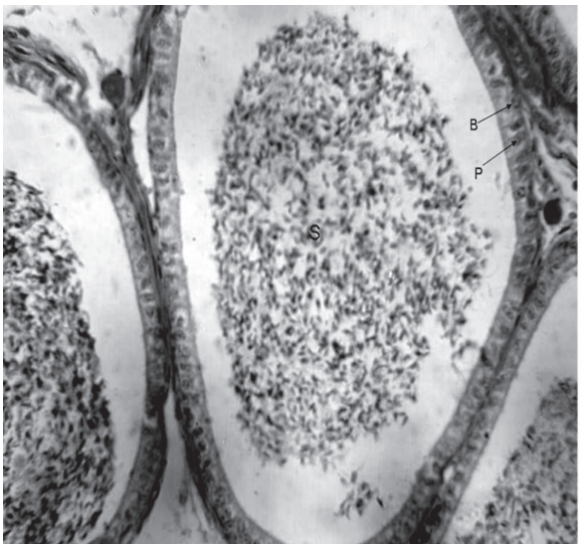

Figure 5. Photomicrograph of zone 4 segment of the epididymal duct in the cane rat. Note the presence of the more loosely packed spermatozoa (S), principal cells (P) with nuclei at almost the same level, and relatively few basal cells (B). H & E 400×.

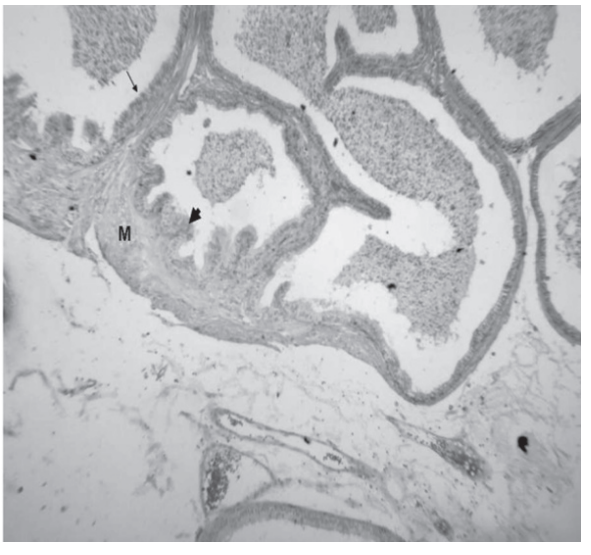

Figure 6. Photomicrograph of zone 5 segment of the epididymal duct, H & E 400×. Note the presence of the folded (arrow head) and the smooth parts (arrow). The increase in the smooth muscle layer (M) is also prominent.

The nuclei were more rounded with few horizontally elongated ones (Fig. 4B).

Zone 4. The epithelium observed in this zone was low and uniform with a mean height of 20.35 ± ± 1.37 μm and mean tubular diameter of 411.38 ± ± 48.37 μm (Table 1). The amount of spermatozoa present in the lumen was greater and it was loosely packed in this zone compared to the previous zones. The thickness of the smooth muscle that surrounded the epithelium was about the same with that in zone 3 (Fig. 5).

The principal and basal cells were also the only cells found in this zone. However, the nuclei of the principal cells were located at about the same level close to the centre of the cells. They were oval to irregular in shape with nucleoli that were irregularly placed. The nuclei of the basal cells were more oval than elongated in the basal part of the cells (Fig. 5).

Zone 5 (the transitional zone). This zone had almost the same epithelial height as zone 4, but the luminal diameter of 678.75 ± 71.67 μm was greater in this zone (Table 1). The smooth muscle layers in this zone increased more than in the previous zones (Fig. 6).

Another prominent feature of the epithelium of this zone is that part of it was folded having both principal and basal cells, while the other part was smooth. The folded part had an epithelial height of 42 ± 7.48 μm (Table 1). The nuclei of the principal cells of this part were elongated to oval in shape and the basal cells were few (Fig. 7A). The lumen had loosely packed spermatozoa content. The epithelial cells of the smooth part had very low columnar cells with an epithelial height of 20.35 ± 1.37 μm. The nuclei were round and centrally located (Fig. 7B). This zone represented the transition from epididymal duct to deferent duct, histologically. Grossly the epididymis at the tail end appeared straight, and after a short distance it bent sharply to the left and continued as the deferent duct.

Morphometry of the epididymis

The epididymis of the greater cane rat was observed to have a mean weight of 0.0365 ± ± 0.091 g forming about 0.016% of the total body weight. It also had an average volume of 0.36 ± ± 0.08 mL. There was low correlation ($r^2 = 0.435$, $p < 0.05$) between the average weights of the epididymis and age in this animal (Fig. 8), but a positive correlation existed between the weight of the epididymis, testicular weight, and the body weight (Table 2). The histo-morphometric values of the different zones of the epididymis are summarised in Table 1.

DISCUSSION

The epididymis of the greater cane rat presents unique morphologic characteristics that are peculiar to this animal. The gross divisions of the epididymis into head, body and tail as observed in rat

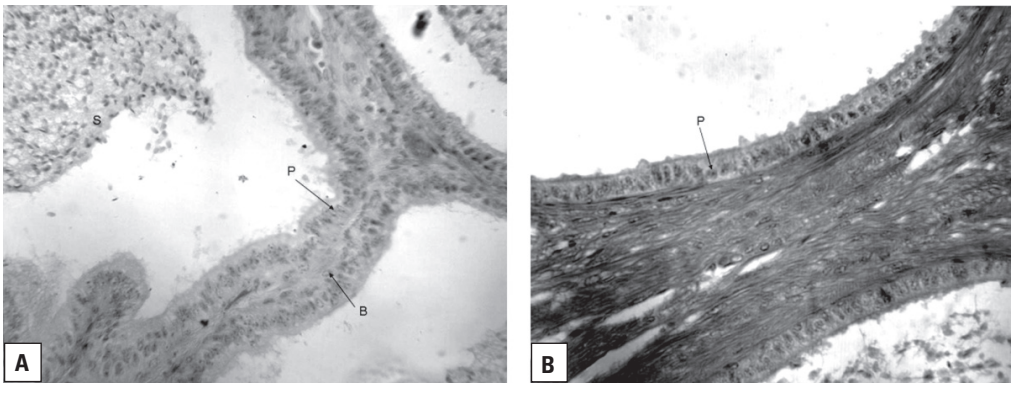

Figure 7. A. Photomicrograph of zone 5 segment of the epididymal duct in the cane rat showing the folded part. H & E 1000×. Note the principal cells (P) and the few basal cells (B) as well as the loosely packed spermatozoa (S); **B.** Photomicrograph of zone 5 segment of the epididymal duct in the cane rat showing the smooth part. H & E 1000×. Note the principal cells (P) with the centrally located round nucleus.

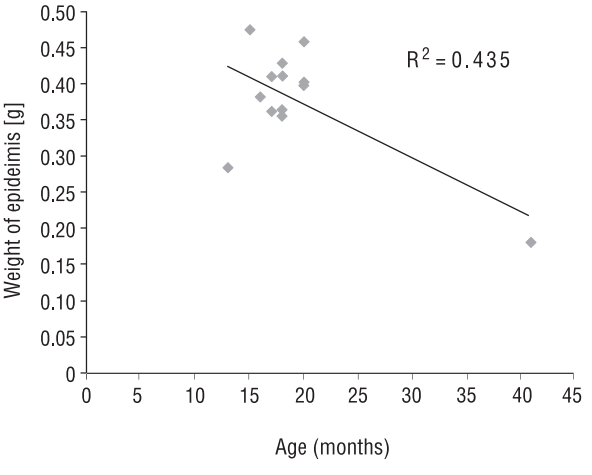

Figure 8. Scatter diagram showing the relationship between average epididymal weight and age in the greater cane rat.

Table 2. The correlation coefficients between weight of the epididymis and body weight as well as testicular weight in the greater cane rat *(Thryonomys swinderianus)*

	Body weight	Testis	Epididymis
Body weight	1.00		
Testis	0.50	1.00	
Epididymis	0.75	0.88	1.00

[10], rabbit [14], guinea pig [12], and giant rat [18] were not conspicuous in the cane rat. The division of the epididymis into different regions and segments has been a topic of considerable discussion in several reports [11, 15, 23]. While, conventionally, in most mammals three easily recognised regions or segments can be observed, namely: Head (caput), Body (corpus), and Tail (cauda) epididymis, Joseph et al. [15] reported that the epididymal tube can be grouped into roughly five gross anatomical segments: the Initial segment, Caput, Corpus, Cauda, and the Vas deferens. However, the epididymis of the cane rat does not conspicuously show any of these gross divisions.

Based on histological and histomorphometric analyses, five zones were observed in the greater cane rat. According to Oke [18], different species display various zones of the epididymis. The five zones seen in the cane rat were similar to those observed in the African giant rat but different from that of the rat, rabbit, bull, stallion, and man. The role and the contribution of each zone of the epididymis to the ultimate maturation of the spermatozoa will be better understood when histochemical and ultrastructural studies of this organ in the cane rat are carried out.

Histologically, apart from the principal and basal cells, no other cell types were observed in the epididymal epithelium of the greater cane rat, except the truncate-like cells found at the second zone. This is partly similar to the observation of Oke [18] in the giant rat. While apical cells are seen in the rat, macrophage-like cells have not been reported in the rat [9], guinea pig [12], or hamster [8]. However, macrophage-like cells have been observed in the epididymis of the monkey, bull, ram, and giant rat [13, 16, 18, 24].

The low correlation ($r^2 = 0.44$, $p < 0.05$) observed between the weight of the epididymis and age is similar to that observed between the age and gonadosomatic index (testis weight/body mass) ($r^2 = 0.41$, $p < 0.05$) in this animal [1]. The relationship between the testicular size, which is an index of the spermato-

genetic function [20], and the age in the cane rat has been previously discussed [1]. This shows that at sexual maturity, in the greater cane rat, there is little effect of age on the size of the epididymis, thereby suggesting a strong homogeneity of the epididymis at different ages in this animal. Although at 41 months there was reduction in the weight of the epididymis, the morphology and morphometric values were similar to those observed in the animals within sexually active ages. This reduction is probably due to reduced spermatogenic content in the epididymal lumen as it has been observed by Onadeko [19] that old male cane rats weighing as much as 4.46 kg (at 45 months) did not secrete any semen despite satisfactory reaction to electro-ejaculation.

CONCLUSIONS

In conclusion, this preliminary information on the epididymis of the greater cane rat will serve as a basis for further research into its reproductive biology, which will subsequently aid in the domestic rearing and domestication of this animal.

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