

AN ANATOMICAL PERSPECTIVE: DOES THE MALE GREATER CANE RAT (*Thryonomys swinderianus*) HAVE A SCROTUM?

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ABSTRACT

We evaluated the anatomy of the "scrotal" skin pouch in an attempt to answer the question of whether (or not) there exist true scrotum in the greater cane rat (*Thryonomys swinderianus*). The "scrotal" skin folds from ten (10) matured male cane rats were morphologically and morphometrically studied with samples routinely processed for histology. Grossly, the pouch has features like fine hair on thin skin and longitudinal raphae typical of scrotum in other species. The histoarchitecture of the "scrotal" skin in the cane rat follows the normal scrotal pattern and the arrangements of the cutaneous strata, hair follicles and glands were similar in animals with perineal staining and those without perineal stain. The cane rat scrotal skin, though different in conformation from the scrotum of other mammalian species, has all the properties of a true scrotum thereby establishing the presence of scrotum in the greater cane rat.

Key words: Scrotum, Greater cane rat, Skin Pouch, Perineal staining

INTRODUCTION

The greater cane rat (*Thryonomys swinderianus*), popularly known as Grasscutter, is an African hystricomorphic herbivore that is constantly hunted and exploited for food in parts of West Africa (Addo *et al.*, 2007). In enhancing its captive-rearing and domestication, several studies have been carried out on the functional and structural reproductive biology (Adebayo *et al.*, 2015) with more research still ongoing especially on the male gender of this rodent (Adebayo *et al.*, 2016).

In most male mammalian species, the testes which is the major reproductive organ, descends into a skin pouch called the scrotum. While the scrotum is absent in some animals like the elephant and hyraxes (Sisson 1975, Carvendish 2010), in species where present, it varies in forms and location (Dyce *et al.*, 2002). Studies have shown that beyond being a pouch for the testes, the scrotal structure and conformation actually play vital roles in testicular functions in particular and in reproduction generally. For instance, Machado Junior *et al.*, (2011) have

shown that the scrotal bipartition affects daily sperm production and sperm cell populations in goats.

Information on the structure of the scrotum is generally scarce and reports on the scrotum of the greater cane rat are confusing. Addo *et al.*, (2003) and Opara (2010) reported that there is no true scrotum in the cane rat based on the location of the testes in the abdomen but without any anatomical consideration of the cutaneous pouch found at the perineal region. Adu and Yeboah (2003) have reported the presence of perineal staining with no mention of scrotum in the greater cane rat. It is therefore necessary to anatomically evaluate the cutaneous pouch that house the testes and examine whether it can be considered a scrotum or not. In this study, the perineal skin pouch was morphologically and morphometrically evaluated with the view to answer the "scrotal question" in the greater cane rat.

Submitted 4th March 2019. Published online 14th May 2019. To cite: Adebayo AO, Adegbesan ZA, Okandeji ME, Mustapha OA, Olude MA, Akinloye AK. 2019. An anatomical perspective: does the male greater cane rat (*Thryonomys swinderianus*) have a scrotum? Anatomy Journal of Africa. Vol 8 (2): 1523 - 1530.

MATERIAL AND METHODS

Ten (10) sexually mature male greater cane rats, raised in captivity with known medical and reproductive history were used in this work. All the animals had the perineal skin folds with most (n=7) having the brownish 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 while elephant grass and water were given *adlibitum*. The experimental protocol is in line with the ethical codes for animal research adopted by the University of Ibadan Animal ethics and experimentation committee.

Each animal was weighed, anaesthetized and perfuse-fixed transcardially using the Karnovsky's fixative which is phosphate buffered 2% paraformaldehyde – 2.5% glutaraldehyde fixative at pH 7.4 sacrificed after anaesthesia with chloroform in a closed container. The perineal pouches were carefully examined, measured, photographed and sampled for histology. The abdominal wall was then dissected open through a mid-ventral abdominal incision and the ischiatic arch was completely disarticulated to expose the testes. Testes and epididymidis were also dissected out, grossly examined, measured and sampled for histology and histometry.

Samples of the scrotum and testis were further fixed in Karnovsky's fluid for 72 hours. The tissue samples were passed through graded concentrations of alcohol at 50%, 70%, 90%, and absolute alcohol (100%) in order to achieve dehydration. The tissues were cleared in two jars of xylene for one hour and then embedded after passing through the four

changes of paraffin wax at 60^oc. Paraffin sections of 5µm thick were obtained on a microtome. These were mounted on clear albuminized slides after floating on a warm water bath and then dried in an oven and stained in haematoxylin and eosin (H&E) dyes. All slides were examined under the light microscope.

Linear measurements of the length, circumference and weight of the scrotum as well as the length, volume and weight of the testis were taken using Mettler Toledo weighing balance and rope. Microscopic measurements of the scrotal epithelial and dermal height as well as the seminiferous epithelial heights and tubular diameters were taken. The scrotal epithelial and dermal heights and tubular diameter were measured at X10 magnification while the seminiferous epithelial height was measured at X40 using Microscope Eyepiece camera (AmScope TouView 3.7). Ten tubular profiles that were round or nearly round were chosen randomly and measured for each animal. The epithelial height was obtained in the same tubular sections utilized to determine tubular diameter.

Statistical analysis was done using SPSS 15.0 packages. Data were expressed as means ± standard deviation; values were subjected to Pearson's correlation analysis to determine the relationships between the different parameters and tested using the t-test. *P*-value less or equal to 0.05 was taken as significant.

RESULTS

A horizontal perineal skin fold situated caudal to and extending about 5.38 ±0.34cm (Table 1) from the penis to the anus was observed in the greater cane rat (Fig. 1). Though the skin is generally wrinkled, the degree tends to vary under different conditions being more when the animal is either excited or restrained. It has few short, fine hairs that are distinct from that which covers the animal body. In most of the adult animals the 'scrotal' skin is brown coloured as a result of the brown pigment secreted at the perineal region (Fig 1). However, those without the pigment were reproductively active with viable offspring. Grossly the 'scrotum' is divided into

indistinct compartments by a longitudinal raphae that extends from the prepuce to the anal opening (Fig 1). The left and right compartments are apparently symmetrical.

A strong relationship was observed between the 'scrotal' circumference and testicular weight and volume while there was no significant relationship between the testicular parameters and 'scrotal' height. The epididymal parameters showed strong correlation with 'scrotal' circumference. Whereas there was no significant relationship between the

'scrotal' parameters and seminiferous parameters (epithelial height and ductular diameter). There was strong relationship between the 'scrotal' parameters and the epididymal parameters (epididymal epithelial height and tubular diameter) in the greater cane rat (Table 2).

Histologically, the 'scrotum' in the greater cane rat reveals histoarchitecture typical of any thin skin. The stratum corneum and stratum lucidum are minimal

relative to the other strata. The stratum granulosum is made up of few layers with flat cells that characteristically stain basophilic. The cell cytoplasm in this layer contains some kerato-hyaline granules (Fig 2A). The stratum spinosum is the most extensive, having irregular polyhedral cells (Fig 2B). The uppermost layer contains few cells with a lot of odland bodies. The stratum germinativum is made up of a single layer of cuboidal cells with round to oval shaped nucleus.

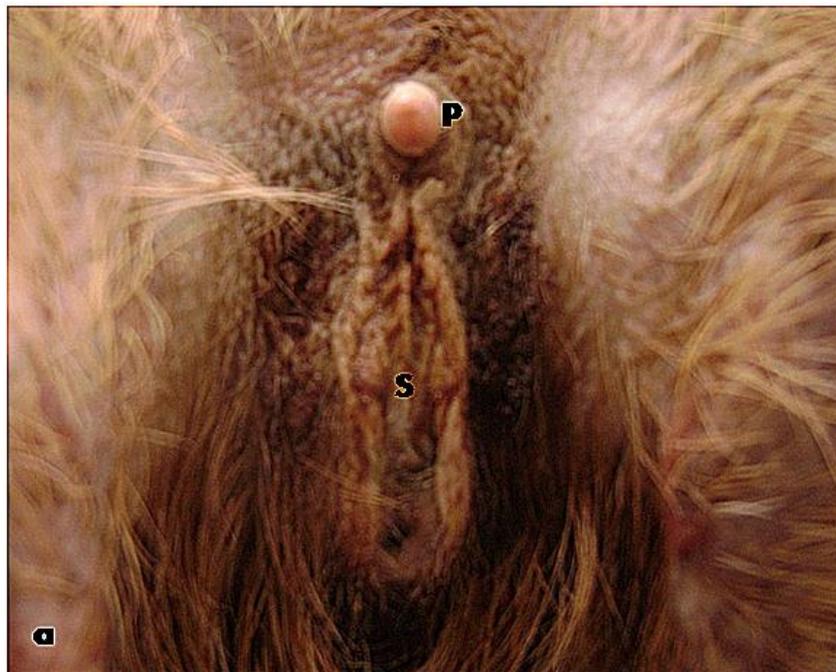


Fig. 1: Photograph of the perineal area of the male greater cane rat showing the scrotum and penis. Note the perineal staining on the scrotum (S) and the penis (P). [Mag X5]

Table 1: Morphometric parameters of the Scrotum, Testis and Epididymis in the greater cane rat

| PARAMETERS | MEAN (n=10) | STANDARD DEVIATION |
|--|-------------|--------------------|
| Body weight(g) | 1670.0 | 0.19 |
| Scrotal circumference (cm) | 13.62 | 1.68 |
| Scrotal height (cm) | 5.38 | 0.84 |
| Testicular weight (cm) | 1.27 | 0.68 |
| Testicular length (cm) | 3.10 | 0.56 |
| Testicular volume (cm ³) | 1.18 | 0.62 |
| Epididymal weight (g) | 0.27 | 0.08 |
| Epididymal volume (cm ³) | 0.23 | 0.08 |
| Epididymal length (cm) | 3.63 | 1.56 |
| Seminiferous epithelial height (SE) (µm) | 188.96 | 23.50 |
| SE Ductular diameter (µm) | 505.09 | 75.80 |
| Epididymal epithelial height (µm) | 163.30 | 28.60 |
| Epididymal tubular diameter (µm) | 580.43 | 104.60 |

| | Body weight (g) | Scrotal circumference (cm) | Scrotal height (cm) | Testicular weight (g) | Testicular volume (cm ³) | Epididymal weight(g) | Epididymal volume (g) | Epid.epith. height(μm) | Epid. Tubular diameter (μm) | SE. epith. height (μm) | SE. ductular diameter (μm) |
|--------------------------------------|-----------------|----------------------------|---------------------|-----------------------|--------------------------------------|----------------------|-----------------------|------------------------|-----------------------------|------------------------|----------------------------|
| Body weight (g) | 1 | | | | | | | | | | |
| Scrotal circumference (cm) | 0.11 | 1 | | | | | | | | | |
| Scrotal height (cm) | 0.33 | 0.68 | 1 | | | | | | | | |
| Testicular weight (g) | 0.42 | 0.37 | 0.24 | 1 | | | | | | | |
| Testicular volume (cm ³) | 0.16 | 0.64 | 0.12 | 0.58 | 1 | | | | | | |
| Epididymal weight(g) | 0.14 | 0.47 | 0.47 | 0.16 | -0.22 | 1 | | | | | |
| Epididymal volume (g) | 0.4 | 0.84 | 0.56 | 0.4 | 0.41 | 0.76 | 1 | | | | |
| Epid.epith. height (μm) | 0.28 | 0.41 | 0.81 | 0.38 | -0.21 | 0.74 | 0.51 | 1 | | | |
| Epid.Tubular diameter(μm) | 0.28 | 0.73 | 0.68 | 0.3 | 0.07 | 0.93 | 0.91 | 0.77 | 1 | | |
| SE. Epith. height (μm) | 0.77 | -0.18 | 0.01 | 0.68 | 0.02 | 0.19 | 0.19 | 0.32 | 0.17 | 1 | |
| SE. Ductular diameter (μm) | 0.61 | 0.043 | 0.31 | 0.83 | 0.07 | 0.31 | 0.27 | 0.62 | 0.34 | 0.9 | 1 |

The dermis of the scrotum of the greater cane rat is made up of the thin papillary layer and the thick reticular layer (Fig 3). The papillary layer which consists of loose connective tissue forms the dermal papilla with the epidermis while the reticular layer,

composed of dense irregular connective tissue, contains the hair follicles, sebaceous and apocrine sweat glands. The arrangement of hair follicle was observed to be both singly and in clusters of over six follicles surrounded by dense connective tissues and multiple lobes of the sebaceous glands (Fig 4).

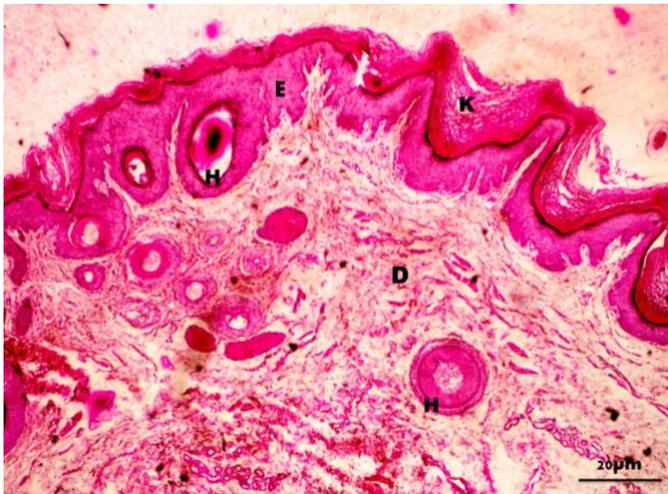


Fig. 2A: Photomicrograph showing histology of the scrotal skin of greater cane rat. Note the Epidermis (E), Dermis (D), the keratin (K) and the hair follicle (H). [H&E, Scale bar =20μm]

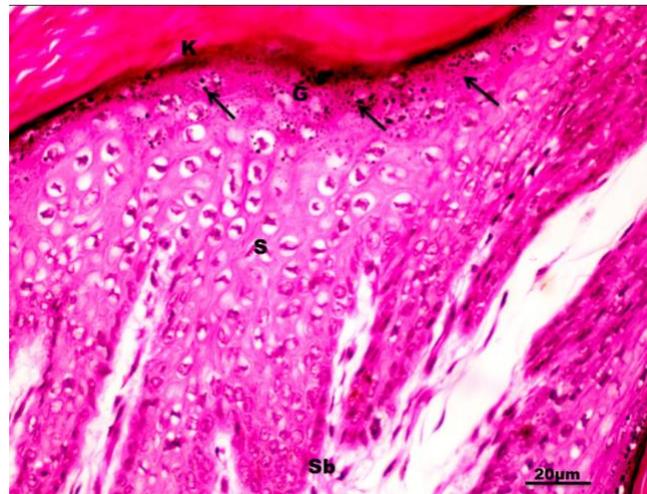


Fig. 2B: Photomicrograph showing all the layers of the epidermis in the scrotum of the greater cane rat. Note stratum basale (Sb), stratum spinosum (S), stratum granulosum (G), keratin (K) and the kerato-hyaline granules (Arrows). H&E, Scale bar =20μm

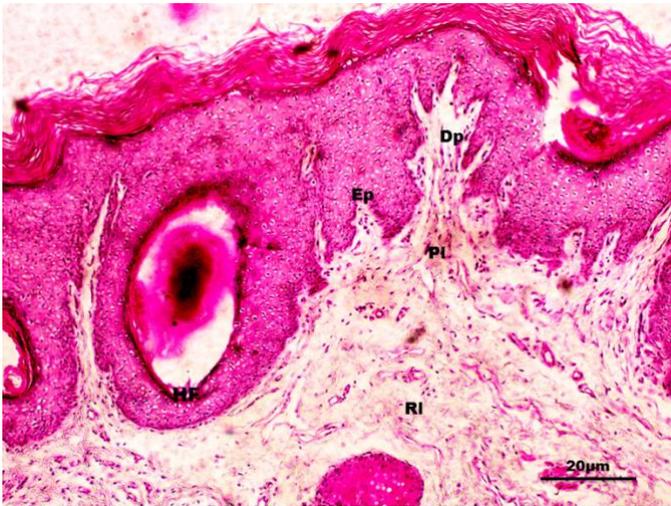


Fig. 3: Photomicrograph showing the thin papillary (PI) and the thick reticular (RI) layers of the dermis in the scrotal skin of greater cane rat. Note the dermal papilla (Dp) and the epidermal papilla (Ep).[H&E, Scale bar =20µm]

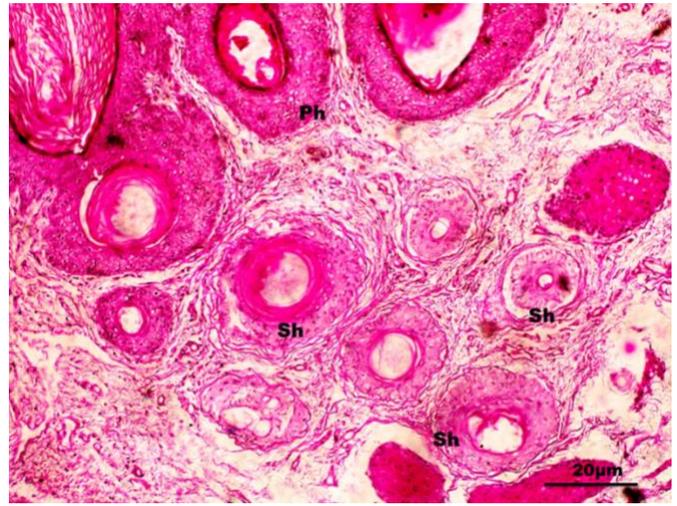


Fig. 4: Photomicrograph showing hair follicles in the scrotal skin of the greater cane rat. Note primary hair follicle (Ph) and secondary hair follicle (Sh). [H&E, Scale bar =20µm]

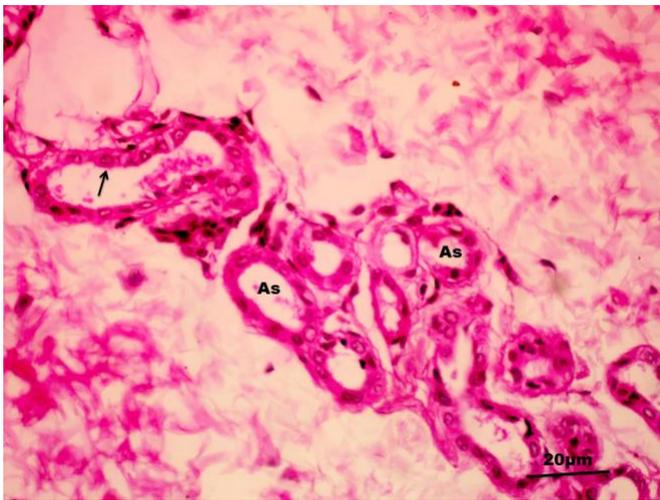


Fig. 5. Photomicrograph showing apocrine sweat glands in the scrotal skin of the greater cane rat. Note the apocrine sweat glands (As), myoepithelial cells with the apical bleb (Arrow). [H&E, Scale bar =20µm]

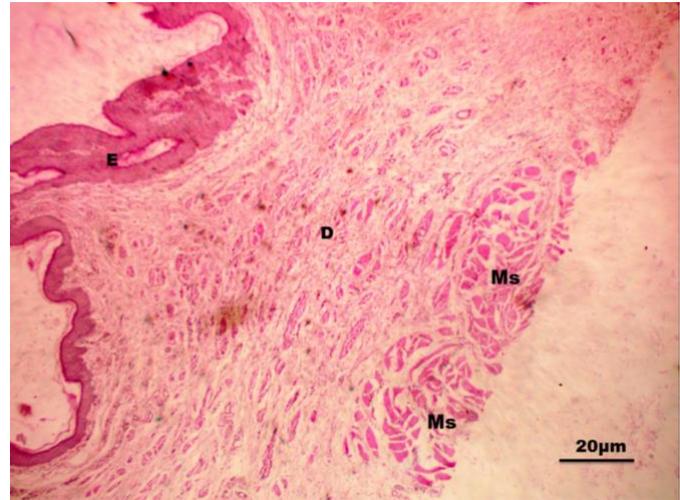


Fig. 6a: Photomicrograph of the scrotal skin showing the subcutaneous muscle tissues in the cane rat with no perineal stain. Note epidermis (E), the dermis (D) and the subcutaneous muscle tissue (Ms). [H&E, Scale bar =20µm]

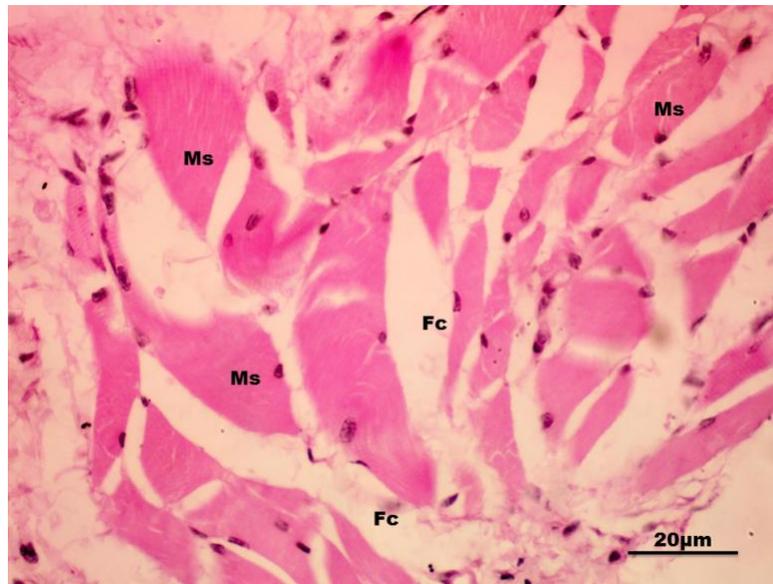


Fig. 6b: Photomicrograph showing the subcutaneous muscle tissues in the scrotal skin of the cane rat. Note the smooth muscle fibres (Ms) surrounded by fibroelastic connective tissue (Fc). [H&E, Scale bar =20µm]

The observed apocrine gland was also made up of a single layer of myoepithelial cells with apical bleb on the laminal surface (Fig. 5). Within the subcutaneous tissue is a layer of smooth muscle and fibroelastic connective tissue that forms the tunica dartos in the scrotum of the greater cane rat (Fig 6A&B). The histology of the scrotal skin was observed to be

similar in animals with perineal staining and those without the perineal staining. The presence of well associated spermatogenic cells in the testis and the presence of sperm cells in the epididymal lumen were also observed in the cane rats that has no brownish perineal staining.

DISCUSSION

The morphology and morphometry of the scrotum in any mammalian species reflect its role, not just as a cutaneous pouch that houses the testis and epididymis but also betray its involvement in the normal functioning of the male gonad (Machado Junior, 2011). Generally, the positions of the scrotum which determine the orientation of the testes vary from one species to another (Dyce *et al*, 2002). In the same vein, scrotal heights are often more than scrotal circumference in most mammalian species (Machado Junior, 2011). The scrotum of the greater cane rat is peculiar in that the scrotal circumference is greater than the scrotal height. With the observed strong relationship between the scrotal circumference and testicular weight/volume coupled with the significant relationship between the testicular parameters and the scrotal height, there is the possibility that the scrotal positioning will affect the positioning of the testes in the scrotum of this animal. More so that we have earlier observed the

horizontal positioning of testes even in the abdominal cavity of the greater cane rat (Adebayo *et al*, 2009). It can therefore be inferred that, though the scrotal conformation does not follow the typical mammalian pattern, it sure contributes to the orientation and consequently the normal functioning of the testes in the cane rat.

Scrotal structure has been reported to affect testicular functions and spermatogenic processes in goats (Ugwu, 2009; Machado Junior, 2011). While thin skin with short fine hair and the longitudinal raphae observed in the greater cane rat are characteristic of scrotum in all mammalian species, the amount of pigmentation on the skin varies with species and breed (Monteire-Riviere, 2006). With the evidence of normal spermatogenic processes with or without the brown perineal staining, this work confirms the report of Adu and Yeboah (2003) that the brownish perineal pigmentation observed on the scrotal skin may have no effect either on the

reproductive efficiency or sexual maturity of male greater cane rats.

The histo-architecture of the scrotal skin in the greater cane rat is generally consistent with that in other rodent species (Samuelson, 2007) except for some differences in the arrangement of hair follicle and appearance of the sebaceous and sweat glands. The follicular arrangement in the greater cane rat is similar to that in goat which has single primary hair follicle occurring with three to six secondary hair follicles associated with it (Ugwu, 2009). While the appearance of multilobular sebaceous gland resembles that in the horse, the apocrine sweat gland has a close semblance to that in the dog having a layer of myoepithelial cells and apical secretory blebs (Montiere-Riviere, 2006). According to Seyle (2005), the presence of smooth muscle and fibroelastic connective tissue within the subcutaneous tissue of the scrotal skin play a significant role in keeping the testis away from the body during the summer and close to the body

during winter. Also, the numerous sebaceous and sweat gland as well as the varied appearance, the degree of contraction and the level of wrinkle of the scrotal skin are important in testes temperature regulatory mechanism (Dyce *et al*, 2002). With the structural description of the cane rat scrotal skin, it can be inferred that this skin also plays a vital role in the testicular temperature regulation in this animal. Therefore, contrary to Addo *et al.*, (2003) and Opara (2010) who reported the absence of true scrotum in the greater cane rat, this work establishes the presence of scrotum in the greater cane rat.

In conclusion, the gross and histological appearance of the cane rat scrotal skin presented in this work showed that, though different in conformation from the scrotum of other mammalian species, it has all the properties of a true scrotum.

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