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Morphophysiology of the Epididymis of the African Sideneck Turtle (*Pelusios castaneus*): Histological, Microstereological and Ultrastructural Approach

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Abstract

This study was carried out to describe the morphophysiology of the epididymis of the adult African sideneck turtle using histological, microstereological and ultrastructural methods. The epididymal duct lies within a relatively thin sheath of connective tissue, and is lined by pseudostratified columnar epithelium. Unlike luminal diameter and stereocilial height, epithelial height, as well as the population of principal cells, decreased from the proximal to posterior segment. The clear cells of the turtle epididymis are limited to the posterior segment of the duct. Basal and apical cells as well as intra-epithelial lymphocytes are all distributed across the three segments of the epididymis while macrophage-like cells are absent throughout the length of the duct epithelium. The structure of the African sideneck turtle epididymis demonstrates, as in most mammals and few reptiles studied to date, obvious regional differentiation of the duct epithelium with evidences of secretory and endocytotic abilities as demonstrated by the contents of highly developed endoplasmic reticulum and secretory blebs in the principal and basal cells as well as clear cells, believed to be concerned with endocytosis. The outcome of the study is expected to be useful in the comparative structural and functional anatomy of turtle epididymis.

Keywords: African sideneck turtle; epididymis; ultrastructure; microstereology; principal cell.

Introduction

Turtles of the genus *Pelusios* belong to the family *Pelomedusidae* which are characterised by their inability to fully withdraw their head into their shell but instead, draw it to the side and fold it beneath the upper edge of the shell. For this reason, they are called sideneck turtles (Broadley and Boycott, 2009).

The African sideneck turtle (*Pelusios castaneus*) is a freshwater turtle, widely distributed in West Africa, from Guinea and Senegal to northwestern Angola (Kirkpatrick, 1995). It is small to medium in size, with relatively extensive plastron that may have a hinge present between the pectoral and abdominal scutes (Olukole and Oke, 2014).

The epididymal duct is a channel that transports, concentrates and stores spermatozoa which, on leaving the testis, are immotile, immature and unable to fertilize an oocyte (Yanagimachi et al., 1985; Flesch and Gadella, 2000). Under androgen control, the epididymal epithelium secretes proteins into the intra-luminal compartment that assist in the maturation of spermatozoa (Hermo et al., 1994, Hermo et al., 1998; Sullivan, 2004). This luminal compartment stores the spermatozoa until ejaculation and specifically prepares the sperm for fertilization by providing the essentials in terms of temperature, oxygen tension, pH and available energy substrate (Dacheux et al., 2005).

While studies abound on the structure, function, and regulation of the duct of the epididymis of mammals and birds (Oke et al., 1987, 1989; Gist et al., 2001; Massanyi et al., 2003; Aire, 2007; Rheubert et al., 2010a), there is, however, limited information on the morphology of the epididymis of reptiles (Sever, 2009, Rheubert et al., 2010b; Sever et al., 2013). Reports on the gross anatomy of turtles have been documented by Wyneken (2001) as well as Kellner and Schwanke (2001). However, there is scarcity of information on the histology and ultrastructure of turtle epididymis**.** In addition to providing a better understanding of the functions of the epididymis, the histological and ultrastructural details of the epididymis of the African sideneck turtle would be useful in the comparative cytology of turtles and reptiles.

Preliminary studies on the gross anatomy of the epididymis of this turtle described proximal, middle and posterior segments attached to the lateral aspect of the caudal border of the testis and extending caudally to about 2- 3 cm before transiting into the ductus deferens (Olukole et al., 2014a). To the best of our knowledge, the current study, being the first

of this nature performed in any freshwater turtle species, aims to describe the structure of the epididymis of the African sideneck turtle on the basis of the aforementioned proximal, middle and posterior segments using histological, microstereological and ultrastructural methods.

Materials and Methods

Experimental Animals

Twelve adult male African sideneck turtles (*Pelusios castaneus*) with an average bodyweight of 0.68kg, were sampled in August, a period of peak spermiogenesis (Olukole et al., 2014b). The turtles were collected from river drainages in Ibadan, Nigeria. Carapacial and plastral characteristics were used in the determination of adulthood in the turtles (Kirkpatrick,1995). The turtles were anaesthetized by intramuscular injection of ketamine-HCl (25 mg/kg body-weight) and sacrificed by cervical decapitation. The epididymis was removed after separating the plastron from the carapace and grossly divided into its proximal, middle and posterior segments as earlier described (Olukole et al., 2014a). All procedures were carried out according to the guidelines for the care and use of experimental animals, National Institute of Health (NIH), USA.

Ethical Statement

The study was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UIACUREC: 12/13/05).

Light Microscopy and Microstereology

Epididymal samples from each segment were fixed in neutral buffered formalin prior to paraffin techniques, sectioned (2-4 µm thick) and stained with Haematoxylin and Eosin (H&E), as well as Periodic Acid Schiff, PAS (Rao and Shaad, 1985). The slides were then studied under a light microscope (Olympus BX63 with a DP72 camera). Quantitative histomorphometric measurements of the three segments of the epididymis were taken from the H&E- stained sections, using a stereological module of computer-assisted digital image analyser (CellSens® dimension software version 1.6) attached to a computer.

Transmission Electron Microscopy

Tissues from the three segments of the epididymis were fixed in glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 4 hours at 4^0 C. The samples were processed as reported by Olukole et al. (2017). Briefly, samples were then thoroughly washed in the same buffer, post-fixed in 1% osmium tetroxide, and subsequently dehydrated in graded series of ethanol. Thereafter, they were then cleared with propylene oxide, infiltrated with a 1:1 solution of propylene oxide:epoxy resin, 1:2 solution of propylene oxide:epoxy resin, and then placed in 100% epoxy resin for 36 hours under vacuum. They were thereafter embedded in fresh epoxy resin and cured at 60^0 C for 48 hours. Semi-thin sections were stained with toluidine blue and observed under the light microscope (Olympus BX63 with a DP72 camera). Ultra-thin sections (70-80 nm) were cut with a diamond knife on an ultramicrotome (Ultracut- Reichert, Austria), and then double-stained with uranyl acetate and lead acetate. The copper grids were examined under a transmission electron microscope (Philips CM 10 TEM) operating at 80kv. Representative micrographs of different segments of the epididymis were taken using a Gatan 785 Erlangshen *digital* camera (GatanInc, Warrendale, PA). Analysis and assembling of composite micrographs were carried out using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA).

Results

Histological and Ultrastructural Observations

In all the three segments, the ductus epididymis lies within a relatively thin sheath of connective tissue and is lined by a pseudostratified columnar epithelium. The ductal diameter, luminal diameter and epithelial height varied from proximal to its posterior segment (Table 1), with the epithelial height being highest at the proximal segment and lowest at the posterior segment. However, luminal diameter was highest at the posterior segment and lowest at the proximal segment.

Table 1: Microstereology of the Epididymis of *P. castaneus* (n=12).

Parameter	Epididymal Segments		
	Proximal	Middle	Posterior
Ductal Diameter (µm)	521 ± 30.6 [a]	548 ± 42.7 [a]	643 ± 65.2 [b]
Luminal Diameter (µm)	386 ± 22.6 [a]	496 ± 36.7 [b]	560 ± 50.5 [c]
Epithelial Height (µm)	84.2 ± 8.1 [a]	62.5 ± 7.5 [b]	40.3 ± 5.4 [c]
Stereocilia height (µm)	7.2 ± 0.4 [a]	11.6 ± 1..2 [b]	18.5 ± 1.4 [c]

[a,b,c] Means with different superscript within rows differ significantly (P<0.05).

Proximal segment

The epithelial height, ductal and luminal diameters of the proximal segment of the epididymis of the African sideneck turtle are shown in table 1. This segment has the highest epithelial height as well as the lowest luminal diameter when compared to the other two segments. The cell types in the epithelium of the proximal segment are the principal and basal cells, very few apical cells, and intra-epithelial lymphocytes (Figs. 1A, 2A and 3A).

The *lamina propria* consists of loose connective tissue with smooth muscles (Fig. 1A). The principal cells in the proximal segment form about 80% of the total epithelial cell population. They are long cylindrical, columnar cells, ranging from 50-60 µm in length, extending from the basement membrane to the adluminal surface with their stereocilia projecting as far as 7.2 ± 0.4 µm into the duct lumen (Table 1, Fig. 1A). Their vesicular nuclei possess one or two nucleoli The supranuclear cytoplasm of the principal cells in this region displays numerous mitochondria, rough endoplasmic

reticula, free ribosomes and tight junctions with a few intercellular vacuoles (Fig. 4A). The few apical cells encountered in this segment have a characteristic apically located spherical nucleus but do not contact the basement membrane.

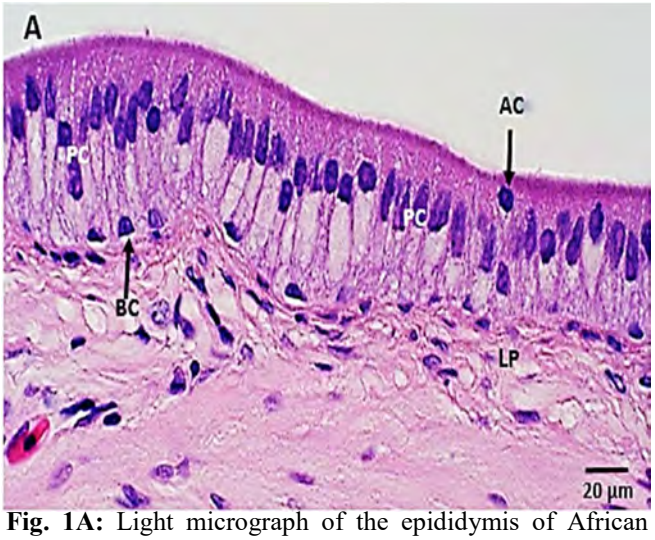

Fig. 1A: Light micrograph of the epididymis of African sideneck turtle (Proximal segment), PC: principal cell; BC: basal cell; AC: apical cell; LP: *lamina propria* (H&E).

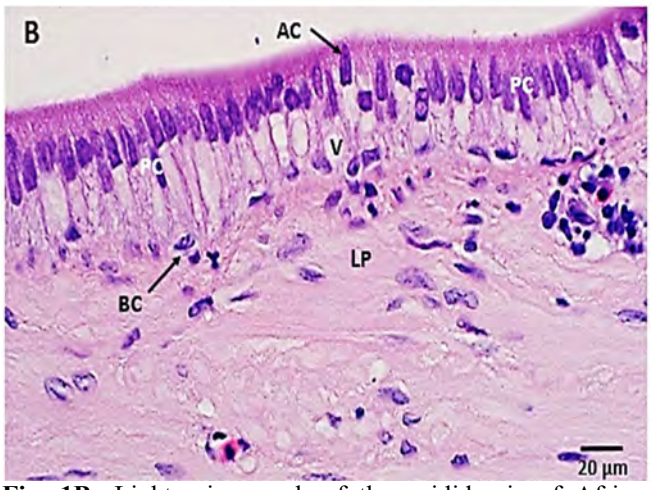

Fig. 1B: Light micrograph of the epididymis of African sideneck turtle (Middle segment), PC: principal cell; BC: basal cell; AC: apical cell; LP: *lamina propria*; V: vacuole (H&E).

Apical cells of this segment bear cellular debris, numerous mitochondria, intercellular vacuoles as well as microvilla (Fig. 4A)**.** Basal cells were found resting on the basement membrane without reaching the ad-luminal surface (Figs. 1A, 2A and 3A). They possess pear-shaped nucleus and thin attenuated processes that extend along the basement membrane from their main hemispherical cell body (Figs. 3A and 5A)**.** The prominent organelles found within the basal cells were abundance of mitochondria, ribosomes and rough endoplasmic reticula (Fig. 5A). Intra-epithelial lymphocytes were found in-between the principal cells in this segment, having round to spherical nuclei of about 5 µm in diameter (Figs. 3A and 6A). The cytoplasm of the intra-epithelial lymphocytes of this segment is composed of mitochondria, numerous free ribosomes as well as rough endoplasmic reticula (Fig. 6A).

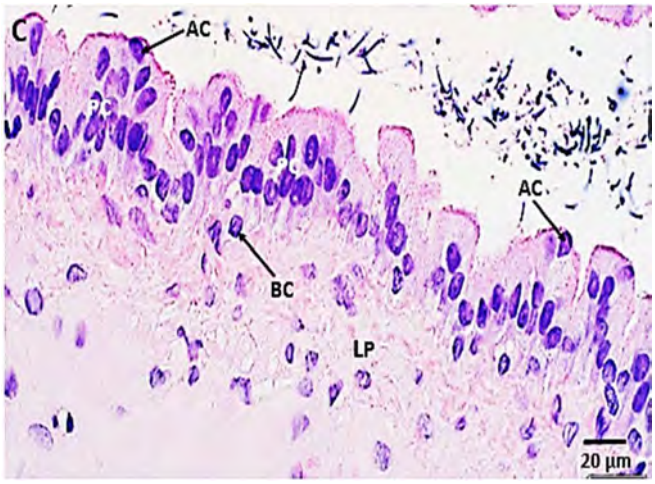

Fig. 1C: Light microscopy of the epididymis of African sideneck turtle (Posterior segment) PC: principal cell BC: basal cell; AC: apical cell; LP: *lamina propria*; V: vacuole; SC: stereocilia (PAS).

The supranuclear cytoplasm of the principal cells in this region displays numerous mitochondria, rough endoplasmic reticula, free ribosomes and tight junctions with a few intercellular vacuoles (Fig. 4A). The few apical cells encountered in this segment have a characteristic apically located spherical nucleus but do not contact the basement membrane. Apical cells of this segment bear cellular debris, numerous mitochondria, intercellular vacuoles as well as microvilla (Fig. 4A)**.** Basal cells were found resting on the basement membrane without reaching the ad-luminal surface (Figs. 1A, 2A and 3A). They possess pear-shaped nucleus and thin attenuated processes that extend along the basement membrane from their main hemispherical cell body (Figs. 3A and 5A)**.** The prominent organelles found within the basal cells were abundance of mitochondria, ribosomes and rough endoplasmic reticula (Fig. 5A). Intra-epithelial lymphocytes were found in-between the principal cells in this segment, having round to spherical nuclei of about 5 µm in diameter (Figs. 3A and 6A).

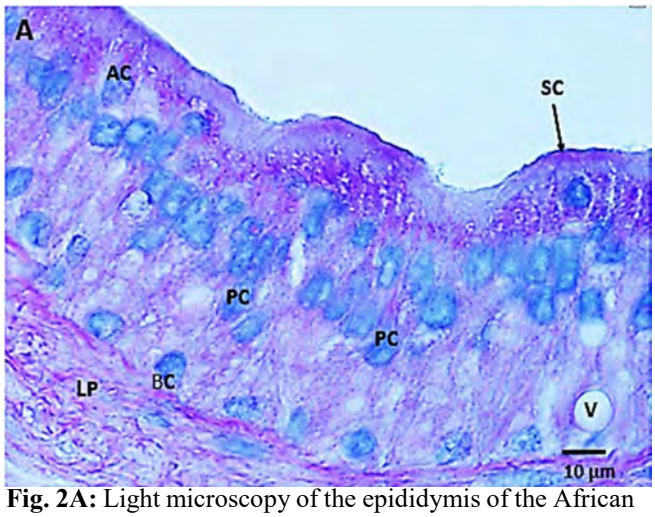

Fig. 2A: Light microscopy of the epididymis of the African sideneck turtle (Proximal segment). PC: principal cell; BC: basal cell; AC: apical cell; LP: *lamina propria*; V: vacuole; SC: stereocilia (PAS).

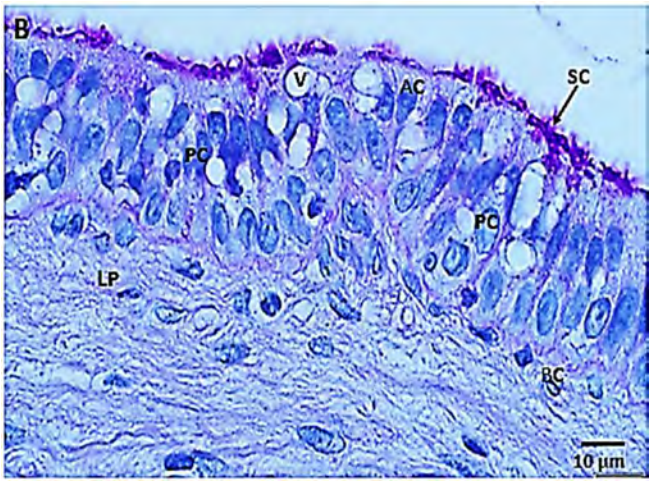

Fig. 2B: Light microscopy of the epididymis of the African sideneck turtle (Middle segment), PC: principal cell; BC: basal cell; AC: apical cell; LP: *lamina propria*; V: vacuole; SC: sterocilia (PAS).

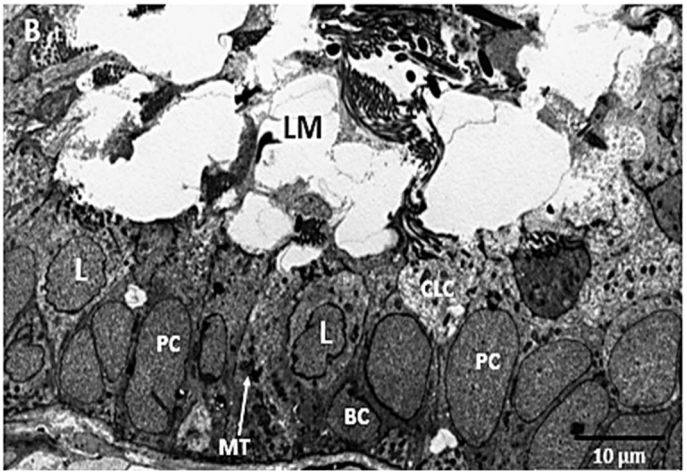

Fig. 3B: Transmission Electron Micrograph of the epididymis of the African sideneck turtle showing (Middle segment), CLC: ciliated cell; PC: principal cell; BC: basal cell; L: lymphocyte; LM: lumen; MT: mitochondria

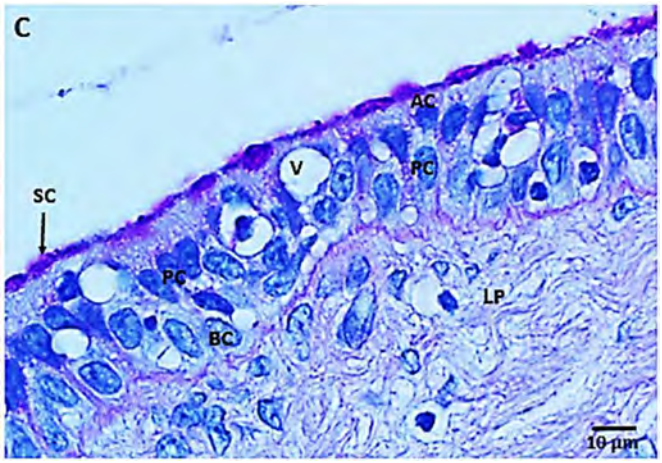

Fig. 2C: Light microscopy of the epididymis of the African sideneck turtle (Posterior segment), PC: principal cell; BC: basal cell; AC: apical cell; LP: lamina propria; V: vacuole; SC: stereocilia. (PAS).

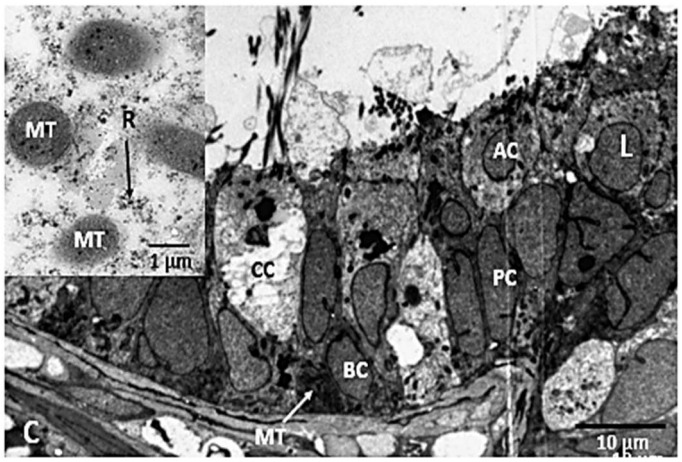

Fig. 3C: Transmission Electron Micrograph of the epididymis of the African sideneck turtle showing (Posterior segment), AC: Apical cell; CC: clear cell; PC: principal cell; BC: basal cell; L: lymphocyte; MT: mitochondria. Inset: Cytoplasm of clear cell. MT: mitochondria; R: free ribosomes

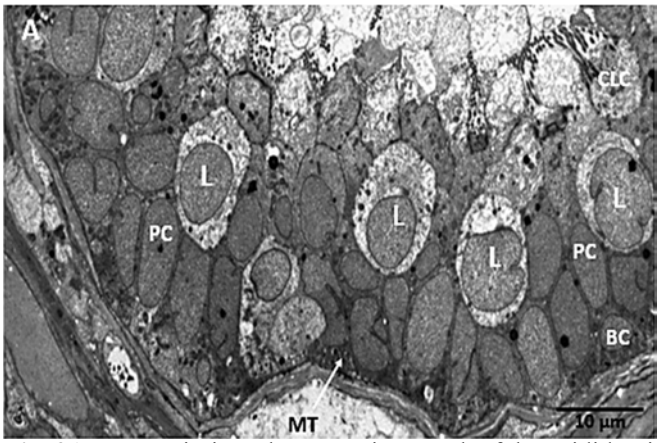

Fig. 3A: Transmission Electron Micrograph of the epididymis of the African sideneck turtle showing (Proximal segment), CLC: ciliated cell; PC: principal cell; BC: basal cell; L: lymphocyte; MT: mitochondria

The cytoplasm of the intra-epithelial lymphocytes of this segment is composed of mitochondria, numerous free ribosomes as well as rough endoplasmic reticula (Fig. 6A).

Middle segment

The epithelial height, ductal and luminal diameters of the middle segment of the epididymis of the African sideneck turtle are shown in Table 1. The epithelium is shorter in this segment compared to that of the proximal segment. Stereocilia projecting from the apical portions of the epithelium into the lumen of the duct are longer than those of the proximal segment (Table 1). The typical cell types in the epithelium of the middle segment are the principal and basal cells, very few apical cells and intra-epithelial lymphocytes (Figs. 1B, 2B and 3B). The *lamina propria* consists of loose connective tissue with fewer smooth muscle cells than those of the proximal segment (Figs. 1B and 2B). Principal cells in the middle segment form about 75% of the total epithelial cell population. They are also long cylindrical, columnar cells, ranging from 30-45 µm in length but with fewer nuclei compared to those of the proximal segment. The Principal cells of this segment

have spherical to vesicular nuclei with one or two nucleoli (Figs. 1B, 2B and 3B). The supra-nuclear cytoplasm of this segment, displays more mitochondria, rough endoplasmic reticula and free ribosomes compared to the proximal segment (Fig. 4B). This segment bears apical secretory cells that contain numerous mitochondria (Fig. 4B). Basal cells in this segment did not differ from those of the proximal segment in terms of their position and structure. The prominent organelles found within the basal cells were abundance of mitochondria, ribosomes and rough endoplasmic reticula (Fig. 5B).

Also, intra-epithelial lymphocytes were found in-between the principal cells in this segment. The dimensions and structure of these lymphocytes did not differ from those encountered at the proximal segment (Figs. 3B and 6B). The cytoplasm of the intra-epithelial lymphocytes of this segment, like that of the proximal segment, is composed of mitochondria, numerous free ribosomes as well as rough endoplasmic reticula (Fig. 6 B).

Posterior segment

The epithelial height, ductal and luminal diameters of the posterior segment of the epididymis of the African sideneck turtle are shown in table 1. The epididymal epithelium in this segment is the shortest of the three segments but has the widest lumen (Table 1). Stereocilia projecting from the epithelial surface of this segment was the tallest of the three segments, being significantly different from those of the proximal and middle segments (Table 1). The cell types constituting the epithelium of the posterior segment are the principal, apical and basal cells, as well as intra-epithelial lymphocytes and clear cells, the latter being resident only in the posterior segment (Figs. 1C, 2C and 3C). The spermatozoa content of the duct in this segment is compact (Fig. 1C). The principal cells in this segment are shorter than those of both proximal and middle segments, constituting about 65% of the total epithelial cell population. The structures of the principal, basal and intra-epithelial lymphocytes of this segment did not differ from those of the proximal and middle segments. Apical cells were more frequently encountered in this segment than the other segments (Fig. 1C). The main feature of this segment which clearly distinguishes it from the other segments is the clear cell (Fig. 3C). It is a tall "flask-shaped" cell extending from the basement membrane to the duct lumen (Fig. 3C). The cytoplasm of the clear cell is composed of numerous mitochondria and free ribosomes (Fig. 3C).

Basal cells in this segment did not differ from those of the proximal and middle segments in terms of their position and structure (Figs. 1C, 2C and 3C). The prominent organelles found within the basal cells were abundance of mitochondria, ribosomes and rough endoplasmic reticula (Fig. 5C). However, this segment had more mitochondria, ribosomes and rough endoplasmic reticula than the two segments.

Also, like in the other two segments, intra-epithelial lymphocytes were found in-between the principal cells in the posterior segment. The dimensions and structure of these lymphocytes did not differ from those encountered at the proximal and middle segments (Figs. 3C and 6C). The cytoplasm of the intra-epithelial lymphocytes of the posterior segment, like those of the proximal and middle segment, is composed of mitochondria, numerous free ribosomes as well as rough endoplasmic reticula (Fig. 6C).

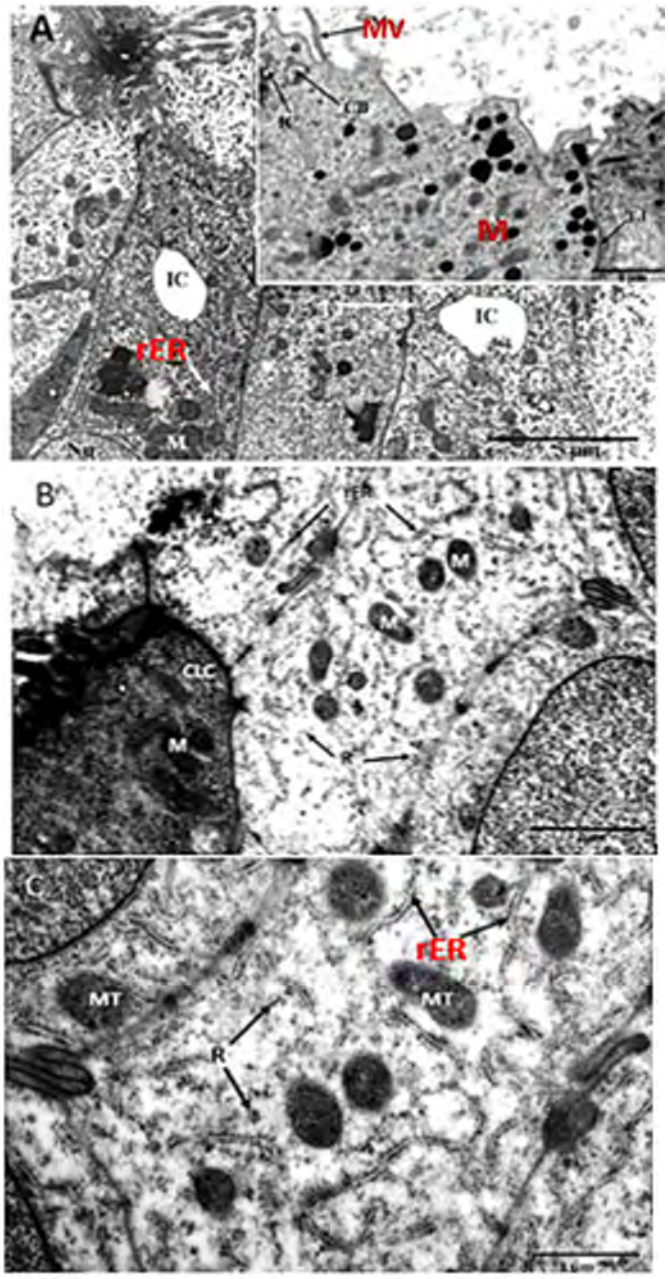

Fig. 4: Transmission Electron Micrograph of the supra-nuclear region of the principal cell of the epididymis in the African sideneck turtle. (**A**) Proximal segment, IC: intercellular vacuoles; M: mitochondria; Nu: nucleus of the principal cell; rER: rough endoplasmic reticulum; TJ: tight junction. Inset: CB: cellular debris; CF: collagen fibres; IC: Intercellular vacuoles; M: mitochondria; MV: microvillus; TJ: Tight junction. (**B**) Middle segment, M: mitochondria; Nu: nucleus of the principal cell; rER: rough endoplasmic reticulum; R: free ribosomes; CLC: ciliated cell. (**C**) Posterior segment, MT: mitochondria; rER: rough endoplasmic reticulum; R: free ribosomes.

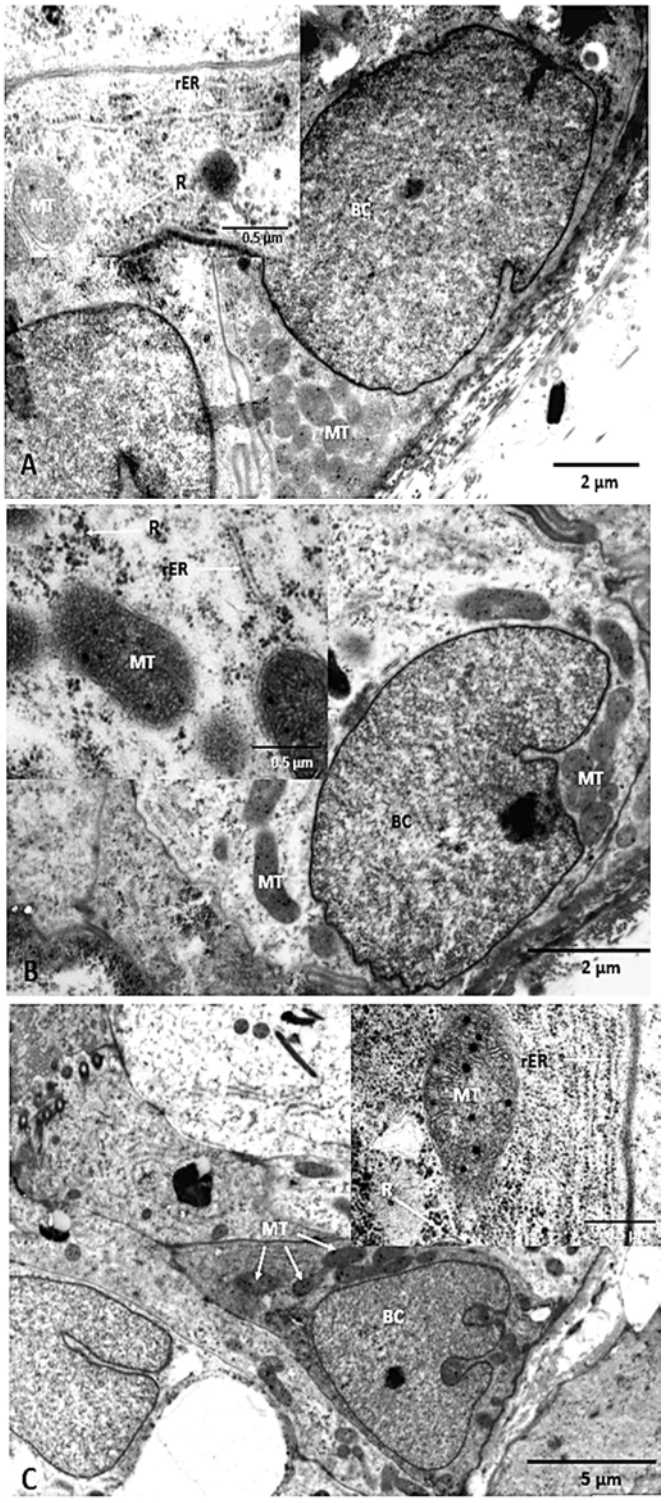

Fig. 5: Transmission Electron Micrograph of the basal cell of the epididymis of the African sideneck turtle (**A**) Proximal segment, BC: basal cell; MT: mitochondria. Inset: Cytoplasm of basal cell. MT: mitochondria; R: free ribosomes; rER: rough endoplasmic reticula. (**B**) Middle segment, BC: basal cell; MT: mitochondria. Inset: Cytoplasm of basal cell. MT: mitochondria; R: free ribosomes; rER: rough endoplasmic reticula. (**C**) Posterior segment, BC: basal cell; MT: mitochondria. Inset: Cytoplasm of basal cell. MT: mitochondria; R: free ribosomes; rER: rough endoplasmic reticula.

Discussion

The epididymis of the African sideneck turtle differs from that of mammals in that it does not have the conventional head, body and tail regions as reported (Oke *et al.,* 1987; Setchell et al., 1993, Olukole and Obayemi, 2010) in mammals. This finding is in conformity with earlier reports on the male reproductive organs of freshwater turtles (Kellner and Schwanke, 2001; Wyneken, 2001). In place of this is the gross sub-division of the duct into proximal, middle and posterior segments attached to the lateral aspect of the caudal border of the testis (Olukole et al., 2014a). Nevertheless, the morphometric relationships found in this study for the luminal diameter, epithelial height and epithelial ductal diameter across the proximal, middle and posterior segments of the epididymis, all follow similar patterns as in the caput, corpus and cauda epididymides of mammals in that the proximal segment had the highest epithelial height while the posterior segment had the widest lumen (Oke *et al.,* 1987; Massanyi et al., 2003; Olukole et al., 2009; Olukole and Obayemi, 2010).

The epithelial type observed across the segments of the epididymis confirms the reports of Wyneken (2001) on reptilian epididymis. The pseudostratified columnar ciliated epithelium of the epididymis tallies with the reports on the epididymis of the snake, lizard, turtle, crocodile, birds and mammals (Djakiew and Jones, 1982; Kellner and Schwanke, 2001; Holmes and Gist, 2004; Aire 2007; Akbarsha et al., 2007; Sever, 2009).

Across the segments investigated in this study, the principal cell is the most abundant cell type encountered and decreased in population from the proximal to posterior segments of the epididymis. Mammalian studies on the epididymis have established that principal cells constitute approximately 80% of the total epithelial cell population in the initial segment and that the number of principal cells gradually decreases to 65% of the total epithelial cell population in the cauda epididymis (Robaire and Hermo, 1988). It has been reported that principal cells are responsible for the bulk of the proteins that are secreted into the lumen and are directly involved in the control of luminal protein concentrations as evidenced by the blebs of cytoplasm emanating from the apical cell surface (Robaire et al., 2006; Cornwall, 2009). Also, the rough endoplasmic reticulum and free ribosomes observed within the supranuclear regions of the principal cells of the African sideneck turtle are features of a protein synthesising cell. These are in conformity with previous reports on the principal cells of the epididymis in dogs and goats (Robaire and Hermo, 1988, Goyal and Williams, 1991; Schimming and Vicentini, 2001; Schimming et al., 2012).

The presence of tight junctions in the apical aspects of the epididymal epithelium of the African sideneck turtle is similar to earlier reports in reptiles by Sever, 2009 and Rheubert et al., 2010b where the role of the formation of a blood-epididymis barrier was ascribed to them. The same has also been reported for principal cells in mammalian epididymis (Robaire et al., 2006; Cornwall, 2009). Tight junctions seal the apical aspects of the epithelium but do not occur basally, which may allow transport of materials externally through intercellular canaliculi (Rheubert et al., 2010b). In this study, small vesicles were observed along the canaliculi as well as the basal membrane. The dilated intra-cytoplasmic spaces between cells

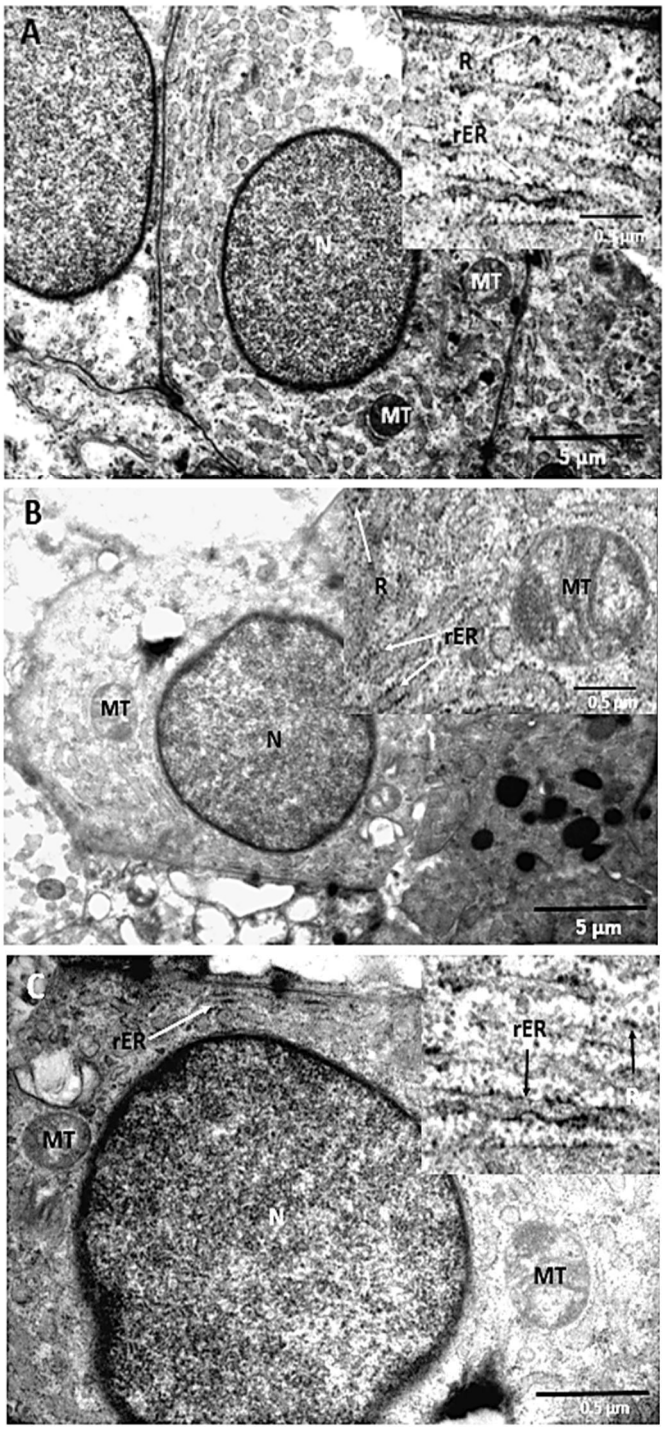

Fig. 6: Transmission Electron Microscopy of the intraepithelial lymphocyte of the epididymis of the African sideneck turtle. (A) Proximal segment. N: nucleus of lymphocyte cell; MT: mitochondria. Inset: Cytoplasm of lymphocyte. R: free ribosomes; rER: rough endoplasmic reticula. (B) Middle segment. N: nucleus of lymphocyte cell; MT: mitochondria.Inset: Inset: Cytoplasm of lymphocyte. R: free ribosomes; rER: rough endoplasmic reticula; MT: mitochondria. (C) Posterior segment, N: nucleus of lymphocyte cell; MT: mitochondria. Inset: Cytoplasm of lymphocyte. R: free ribosomes; rER: rough endoplasmic reticula.

are also suggestive of transport of water across epithelium (Hermo and Robaire, 2002).

The consistent position and structure of basal cells observed across the three segments of the epdididymis of the African sideneck turtle conforms with those of previous studies (Rheubert et al., 2010a; Schimming et al., 2012). Basal cells have been reported to be in close association with the overlying principal cells through the presence of cytoplasmatic extensions thereby forming an extensive cellular sheet surrounding the epididymal epithelium (Robaire et al., 2006; Cornwall, 2009). It has been suggested that basal cells may have a role within the processes of the epithelial immune system and in the regulation of electrolytes by principal cells (Cornwall, 2009).

The numerous mitochondria observed in the apical cells of the African sideneck turtle across the three segments of the epididymis are similar to those reported in the epididymis of the Geoffroy's side-necked turtle, *Phrynops geoffroanus* and the ground skink, *Scincella lateralis* (Cabral et al., 2011; Sever et al., 2013). Apical cells are clearly defined by the numerous mitochondria in the apical cytoplasm, the few microvilli at the luminal border and a nucleus that is located in the upper half of the cell cytoplasm (Adamali and Hermo, 1996; Robaire et al., 2006). Apical cells are related to sperm quiescence and to the regulation of the pH in the lumen through the production of enzymes of the carbonic anhydrase family (Hermo et al., 2005).

The intraepithelial lymphocytes reported to be found in the epididymis of *P. castaneus* in this study is at variance with the report of Rheubert *et al.* (2010a) in the Mediterranean Gecko even though intra-epithelial lymphocytes were reported for the rete testis of the same Mediterranean Gecko as resembling the ''halo cells'' of mammalian epididymis (Hermo and Robaire, 2002; Rheubert et al., 2010b). Intra-epithelial leukocytes have also been reported in the epididymis of lizards (Meeran et al., 2001). Hermo and Robaire (2002) described these leukocytes as either monocytes or T-lymphocytes. Intra-epithelial lymphocytes were also reported to be present in the epididymis of the African giant rat, *Cricetomys gambianus*, Waterhouse (Oke et al., 1987, 1989).

The posterior segment of the epididymis, like the cauda epididymis of mammals contained more spermatozoa than its proximal and middle portions. This is in conformity with previous reports on the epididymis of mammals (Oke and Aire, 1990; Massanyi et al., 2003; Olukole et al., 2009; Olukole and Obayemi, 2010) and reptiles (Sever, 2009, Rheubert et al., 2010b; Cabral et al., 2011; Sever et al., 2013). Ultrastructural observations of the epithelial lining cells of the posterior segment in the African sideneck turtle, suggest that the segment exerts other morphological roles than storage of spermatozoa. Active processes of uptake and release of substances among the cells and the luminal content has been proposed in mammals. It has been reported that the proteins and small molecules secreted by epithelium of the epididymis into the lumen interact with the transiting spermatozoa and directly or indirectly affect the spermatozoal surface and that these proteins alongside small molecules function as signaling molecules to induce activity in the other epididymal proteins (Gatti et al., 2004). Studies on mammalian epididymis had suggested that processes of cellular resorption of water, salt

ions and macromolecules as well as protein secretion, sperm final maturation and storage of quiescent sperm occur in this segment (Flickinger, 1983; Arrighi et al., 1993).

The presence of clear cells in the posterior segment of the epididymis of the African sideneck turtle is similar to the report on the epididymis of the Geoffroy's side-necked turtle, *Phrynops geoffroanus* (Cabral et al., 2011) as well as the ground skink, *Scincella lateralis* (Sever et al., 2013). Clear cells have been reported as endocytic cells which may be responsible for the clearance of proteins from the epididymal lumen, taking up the contents of the cytoplasmic droplets released by the spermatozoa as they transit through the duct (Hermo et al., 2005; Robaire et al., 2006). It can however, be inferred from this study that the posterior segment secretes the least protein into the duct lumen.

Conclusions

In conclusion, the proximal, middle and posterior segments of the epididymis of the African sideneck turtle has been found to contain principal, basal, apical cells and intra-epithelial lymphocytes, each with specific micro-anatomical characteristics. Clear cells are found mainly in the posterior segment, suggestive of structural evidence of basic endocytotic function. The apical and principal cells show evidence of both secretory and absorptive functions while the basal cells and clear cells have been postulated to have a role in the processes of epithelial immune system.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Author Contributions

SGO and BOO designed, executed and jointly wrote the manuscript.

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