The Microstructures of the Ductus deferens in the African fruit bat, *Epomops franqueti* (TOMES, 1860), from Histological and Immunohistochemistry Perspective

[1*]EKEOLU O.K. and LANIPEKUN D.O.
[1]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Benin, Benin City Edo State.
[2]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State.
e-mail: oyetunde.ekeolu@uniben.edu

Abstract
Mammalian ductus deferens has been described as being a mere conduit for the expulsion of spermatozoa through the ejaculatory duct, prostatic urethra and penile urethra. Reports on the ductus deferens in bats were relatively scanty compared to other mammalian species. There exists scarcity of information on the knowledge of the microscopic structural make up in the bat ductus deferens, essential for propulsion of the spermatozoa required for conservation of bat vis-a-vis fertilization. Therefore, the need to investigate the structures of the ductus deferens in bat. Ten adult male *Epomops franqueti* captured during the peak of the raining season in Ibadan (between June and September) were used for this work. Routine (H&E) and differential immunohistochemistry staining of the ductus deferens were carried out. Cytokeratin AE1 and α-smooth muscle actin were also used. Light microscopy of the paraffin sections revealed that the ductus deferens in *E. franqueti* had three layers: mucosa, muscular and adventitia layers. The mucosa was lined by numerous pseudostratified columnar cells and few basal cells. The epithelium rested on a basal lamina propria. The epithelium of the ductus deferens was positive to Cytokeratin AE1 but nonreactive to α-smooth muscle actin. The smooth muscle of the ductus deferens was positive to α-smooth muscle actin but nonreactive to Cytokeratin AE1. The structure of the ductus deferens in the *E. franqueti* suggested its vital role in the survival of the spermatozoa beyond their propulsion in the course of ejaculation during coitus. *E. franqueti* ductus deferens was typically mammalian.
Key words: *Epomops franqueti, ductus deferens, Stereociliated pseudostratified columnar epithelium, H&E, Cytokeratin AE1, α-smooth muscle actin*

Introduction.

The ductus deferens is a tubular structure and component of the spermatic cord at the region of the inguinal canal. It is enclosed by spermatic cord fascia and caudal to the urinary bladder (Gurung *et al*., 2025). The satellite structures of the ductus deferens includes: deferential artery, testicular artery, pampiniform plexus, genitofemoral and sympathetic nerve (Narasimman *et al*., 2024). The ductus deferens extends from the cauda epididymis to continue with the urethra within the pelvis (Bieth *et al*., 2021). The terminal part of the ductus deferens is referred to as the ampulla in rodent studied by Kennedy and Heidger (1979), forming the ejaculatory duct where it merges with the accessory gland duct in the bat (Ekeolu *et al*., 2020). Therefore, the ductus deference serves as a passage way for spermatozoa from the epididymis to the exterior (Snyder *et al*., 2010). The ductus deferens comprises three layers: the tunica muscularis, tunica mucosa and tunica adventitia (Yang *et al*., 2020). Spermatozoa are equipped with flagella for locomotion, however the ductus deferens have been reported to assist in the propulsion of the sperm cells (Elfgen *et al*., 2018).

The microscopic structures of the ductus deferens that aid in the movement and survival of spermatozoa during ejaculation have been investigated in man, monkey, goat and rat (Murakami *et al*, 1982, Marettová, and Maretta, 2018), available information on the mammalian (Chiropteran) ductus deferens are still not adequate (Hermo *et al*., 2002), including detailed information on the ductus deferens in African fruit bats, although the previously investigation into the adult male reproductive tracts in the *Epomops franqueti* have been carried with the exception of the ductus deferens (Ekeolu *et al*., 2020).

However, in the African fruit bats such as *Eidolon helvum, Roussetus aegyptiacus* and *Epomops franqueti*, the gross description of the paired ductus deferens have been fairly mentioned, (Ekeolu *et al*., 2020, Danmaigoro *et al*., 2014, Fard and Ghassemi, 2017). In the investigation carried out on *Artibeus lituratus*, Oliveira and Oliveira (2011) were silent on the anatomy of ductus deferens other than mere mentioning as a component of the male bat reproductive tract. Also, reports on the ductus deferens of chiropteran in the temperate bat has shown that the viability of ductus deferens microenvironment is important to breeding because without a viable ductus deferens, spermatozoa are depleted (Racey, 1979). The availability of bat is therefore crucial to the increasing interest in using bats as scientific experimental animals (Eisenstein, 2018). Therefore, with only few works done on the ductus deferens of bats generally, the need to investigate the ductus deferens in the *Epomops franqueti* is imperative to add and to bridge the gap of knowledge in the male reproductive tracts of the fruit bats which will provide data for comparative anatomy of the ductus deferens of the tropical fruit bats and temperate bats already studied.

Materials and Methods

Ten adult male bats, 98-112g b.w., with pubic bone synostosis (Ekeolu and Ozegbe, 2012) were captured from their habitat at the Faculty of Arts, University of Ibadan using mist net. The animals were transported in a

cage to the Department of Veterinary Anatomy where they were kept for 48hours for acclimatization being exposed to potable water and fed Almond fruits. The bats were anaesthetized with ketamine HCl at 25mg/kg b.w., through intramuscular administration on the medial aspect of the thigh. The samples of vas deferens harvested were fixed in 4% neutral buffered formalin and processed for paraffin sectioning (Pinkert-Leetsch *et al*., 2022). The paraffin embedded ductus deferens tissues were sectioned, using the microtome, to a thickness of 5µm, and stained with hematoxylin and eosin for histological study. The immunological study was carried out by pretreating the slides with polylysine before mounting the sections. They were deparaffinized using changes of xylene, and rehydrated using changes of alcohol. They were heat-treated in citrate buffer for 20minutes in order to retrieve the antigen. To reduce endogenous peroxidase interactions, the sections were incubated for 5 minutes in hydrogen peroxide (3% in distilled water), and the blockage of non-specific binding sites was carried out by rinsing the slides in a 0.01M phosphate buffered saline solution (PBS, pH 7.4), that contains bovine serum albumen (BSA). This was carried out in 5 minutes. Immunostaining of slides were carried out for 1 hour at room temperature, using the LSAB-plus kit (Dakocytomation, Glostrup, Denmark) monoclonal antibodies against Cytokeratin AE1, S-100 and α-smooth muscle actin at dilutions of 1:100; 1:400 and 1:400 respectively. Subsequently, the slides were rinsed in PBS. They were then incubated for 15 minutes in link antibody (Biotinylated secondary antibody, LSAB-plus kit; Dakocytomation), and then in peroxidase-labelled streptavidin. This was followed by the addition of 3,3'-diaminobenzidine tetrachloride solution (DAB) from the LSAB+® kit to visualize antigen localization. The ductus deferens tissues were then counterstained with hematoxylin for 30 seconds, washed in water, dehydrated through graded ethanol, cleared in xylene and mounted with DPX permanent mounting media (Sigma-Aldrich, St. Louis, MO, USA). Finally, sections were examined under a light microscope (Olympus BX63 with a DP72 camera) (Ekeolu *et al*., 2023).

Results and discussion

Histological observations

Reports on the histology of ductus deferens in Chiropteran, bats were almost lacking thus the discussion of our findings was centered around other mammalian species and few chiropterans that have been investigated. Light microscopy of the histological study of the ductus deferens in the *Epomops franqueti* revealed three separate layers: tunica mucosa, tunica muscularis and tunica adventia. The mucosa comprised the epithelium and lamina propria, and muscularis mucosae was absent. The epithelium type identified was pseudostratified columnar epithelium with numerous principal cells. These observations were similar to the findings in *Azara's agouti* (Schimming *et al*., 2021), whereas in other species of animal such as in the rat, the epithelial type lining the mucosa of the ductus deferens were stereo ciliated pseudostratified epithelium (Maynard and Downes, 2019). Also, the epithelium of the ductus deferens in *Dromedarius camelus* was presented with pseudostratified columnar with brush borders (Abdelmohaimen *et al*., 2020) while Sohn *et al*. (2021) mentioned the epithelial lining of the acini of the terminal part of the vas deferens in the greater horseshoe Bat, *Rhinolophus ferrumequinum*, to be pseudostratified epithelium, they were silent about the epithelium type lining the other part of the vas deferens which may be different from the observations reported in the *Epomops franqueti*, a frugivorous bat. Therefore, it further established the phylogeny variation in the ductus deferens among the different species of bats.

The tunica mucosa was thrown into longitudinal mucosal folds ranging from 2 to 12, and mostly flat, with the lumen containing spermatozoa. The presence of the folds was suggestive of the ability of the ductus deferens to expand during ejaculation similar to the findings in *Eidolon helvum*, (Danmaigoro *et al*., 2014). The mucosa folds in other mammalian species such as the goat and rabbit have been documented to be simple and complex, and also phylogenetically characteristic (Marettová and Maretta, 2018).

Immunohistochemistry observations

Our immunohistochemical findings revealed that the epithelium stained positive to cytokeratin AE1. The reactivity for the stains in the epithelium varies; the apical region of the epithelial cells intensity of staining was high while the basal part of the epithelium stained moderately with cytokeratin AE1. The lateral membrane that form the intercellular contacts between adjacent cells also stained moderately with cytokeratin AE1. The bundles of smooth muscle cells that form the tunica muscularis revealed a low staining intensity for cytokeratin AE1. Generally, cytokeratin, have been demonstrated in the epithelium of the ductus deferens in the adult male goat (Marettová and Maretta, 2018) however, literature search of cytokeratin staining of the ductus deferens in bats wass scanty. Cytokeratin staining was observed in the apical region of the pseudostratified columnar epithelium, the basal region around the basal cells. This observation and pattern of immunostaining distribution across the ductus deferens in the *Epomops franqueti* was similar to the report on the differential aquaporin immunostaining in the big fruit-eating bat *Artibeus lituratus* (Oliveira *et al*., 2013) indicating the ductus deferens absorptive and secretory roles as the epithelium has also been reported to be involved in water transport (Andonian and Hermo, 1999) while the filament helps in maintaining the cell structure during the movement of cell organelles, for sperm cells to thrive and survive within the lumen of the ductus deferens. Also, the sparingly presence of cytokeratin AE1 at the lateral borders of the epithelial cells suggested cell-cell contact integrity for optimum environment for sperm cell survival within the lumen of the ductus deferens of *Epomops franqueti*, perhaps as a result of selective

transportation of molecules within and out of the lumen by junctional complexes (Alberts *et al*., 2002). The pattern of slight differential distribution of cytokeratin AE1 in the tunica muscularis of the ductus deferens was suggestive of the filaments regulating the contraction of the smooth muscles surrounding the ductus deferens during ejaculation. The tunica muscularis of the ductus deferens in the *Epomops franqueti* was generally pronounced in thickness. Three layers of smooth muscles were identifiable within the tunica muscularis based on the orientation of the cells of the muscle fibers. The inner layer of the tunica muscularis was thin with smooth muscle cells that were circularly arranged. The middle layer had bundles of smooth muscle cells that were obliquely arranged. The outer most layer of the tunica muscularis had the smooth muscle cells longitudinally arrange. The adventitia was mainly made of loose connective tissue that blended with the surrounding structures of the ductus deferens in the bat (Figure 1 A&B). The loose connective cells of the tunica adventia of the ductus deferens in the *Epomops franqueti* revealed a high intensity of staining with cytokeratin AE1 as observed in the apical part of the epithelial cells that lined the tunica mucosa. The cells of the bundles of smooth muscle of the tunica muscularis stained poorly to cytokeratin AE1 but was able to distinguished the layers of the tunica muscularis with thin inner layer of circularly oriented muscle surrounded by a thicker middle layer of obliquely oriented muscle, then an outer layer of longitudinal muscle. This orientation of muscle layers was rather different from the arrangement in the vas deferens in man (Koslov and Andersson, 2013).

The myofibroblast of the lamina propria underlying the tunica mucosa of the ductus deferens in the *Epomops franqueti* stained positive to α smooth muscle actin and blended with the increased intensity of the α smooth muscle actin to the bundles of smooth muscle cells in the tunica muscularis. As an important component of the cytoskeletal proteins, α smooth muscle actin stained positive to smooth muscular tissues (Gunst and Zhang, 2008). This observation corroborated the reports on the ductus deferens in the goat (Marettová and Maretta, 2018). However, the layers of the tunica muscularis positive reactions to α smooth muscle actin was uniformly pronounced unlike in the report of Marettová and Maretta (2018) but the staining of the connective tissue cells of the tunica adventitia was slightly less intense in comparison to intensity of α smooth muscle actin in the tunica muscularis. The endothelial cells of the deferential blood vessels were positively stained to α smooth muscle actin which suggested its role also in the regulating the contraction of the smooth muscle cells (Donadon and Santoro, 2021) were located within the connective tissue of ductus deferens in the bat.

The reactivity for α smooth muscle actin in the bundle of smooth muscle cells in the tunica muscularis was positive, with high degree of staining intensity. The connective tissue cells of the adventitia layer also revealed a positive reaction for α smooth muscle actin. The reaction for α smooth muscle actin in the epithelial cells of the ductus deferens in the *Epomops franqueti* was negative (Figure 1 C&D). Also, the blood vessels around the ductus deferens and stained positive to cytokeratin AE1 and α smooth muscle actin (Figure 2)

Conclusion

The ductus deferens in *Epomops franqueti* is essentially similar to other mammalian species with little variation which may be as a result of phylogenetic specificity. The information on a detailed microscopic anatomy on vas deferens in chiropteran was generally not adequate. This work may further be a point of reference to further research in the vas deferens of bats.

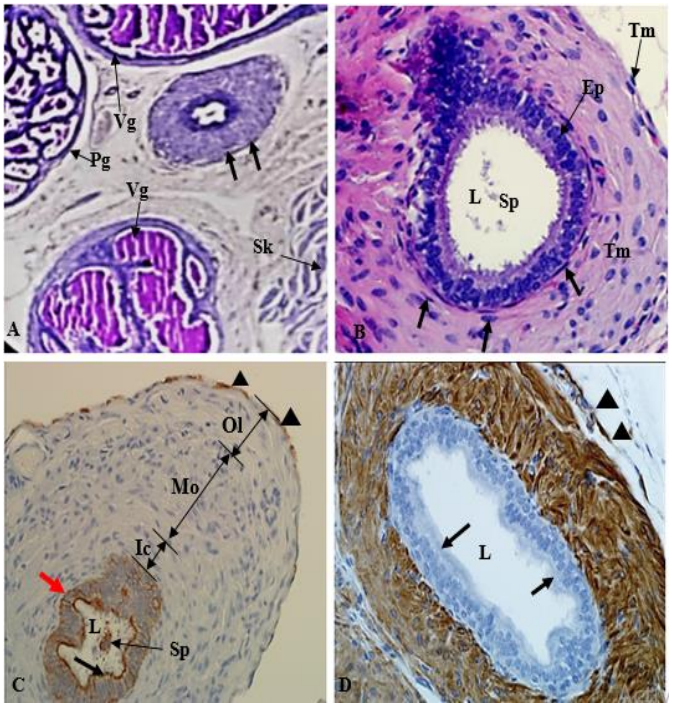

Figure 1. The micrograph of the histology of the ductus deferens in the *Epomops franqueti* in (A): arrow points to the ductus deferens at the ejaculatory duct surrounded by accessory glands (vesicular (**Vg**) and prostate glands (**Pg**)) and skeletal muscle (**Sk**). H&E, X4. In (B): the pseudostratified columnar epithelium (**Ep**), tunica muscularis (**Tm**), and tunica adventitia (**Ta)**. Black arrows point to the thin circular layer of bundles of muscle cells. H&E, X40. In (C): Cytokeratin AE1 positive staining of the ductus deferens with black arrow pointing to the apical part of the epithelium, while red arrow points to the basal part. Arrow heads point to the adventitia. The staining of the tunica muscularis shows low degree of intensity. Note the inner circular layer of muscle (**Ic**), middle oblique layer of muscle (**Mo**) and the outer longitudinal layer of muscle (**Ol**) X10. In (D): α smooth muscle actin positive staining of the bundles of muscle cells of the tunica muscularis. Arrow heads point to the tunica adventitia, showing positive reaction for α smooth muscle actin. The epithelium of the mucosa showed negative reaction. Black arrows points to the folds of epithelium. Observe that sperm cells (Sp) were found in the lumen (**L**) of the ductus deferens. X40

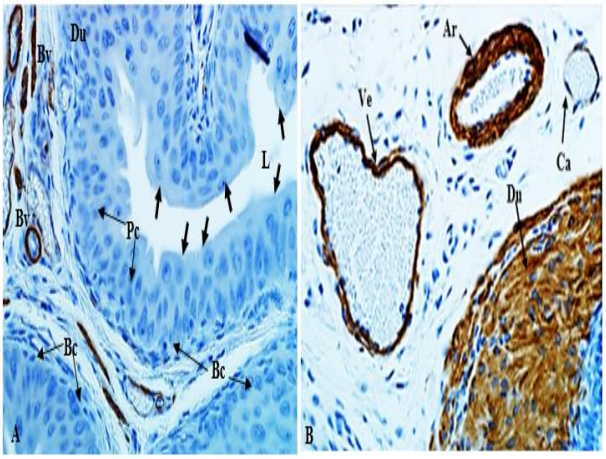

Figure 2. The micrograph of the ductus deferens (**Du**), and its blood vessels (**Bv**): vein (Ve), artery (Ar) and capillary (Ca) of *Epomops franqueti*, showing α smooth muscle actin positive reaction for the blood vessels as observed in the blood vessels. X40. Observe the mucosa folds (black arrows) into the lumen (**L**), the few basal cells with round nuclei (**Bc**) and the numerous principal cells with ovoid nuclei (**Pc**) in (A). X40.

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