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## Gross and histological observations of the testis and epididymis of adult male African fruit bat (*Epomops franqueti*)

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### Abstract

Biometrical and histometrical studies were carried out on the testis and epididymis of fifteen adult male bat (*Epomops franqueti*). The basic data generated could be useful in the comparative regional anatomy of the male reproductive organs of *E. franqueti* and other bat species. The average body weight of the experimental bats was  $97.64 \pm 5.90$ g. The mean relative testicular weights were  $(0.089 \pm 0.0031)$  g and  $(0.083 \pm 0.0038)$  g for right and left respectively while the epididymal weights were  $(0.037 \pm 0.0012)$  g and  $(0.033 \pm 0.001)$  g for right and left respectively. The testis was covered by a testicular capsule. The seminiferous tubules were lined, successively, with germs cells at different stages of cell division. The diameter of the tubules compared to those of the lumen and germinal epithelium of the testis, the epididymal ductal diameter, luminal diameter and epithelial height of the *E. franqueti*, were similar to previous reports on other chiropteran species.

**Keywords:** Biometry, epididymis, *Epomops franqueti*, histomorphometry, testis, Peak of rain

### Introduction

The African fruit bat (*Epomops franqueti*) belongs to the order Chiroptera and of the suborder Megachiroptera, family Pteropodidae<sup>[1]</sup>. The species of bats under the Pteropodidae family include the cave-dwelling Egyptian fruit bat: *Rousettus aegyptiacus*, and African fruit bats: *Myonycteris torquata*, *Micropteropus pusillus*, *Hypsignathus monstrosus*, *Epomops dobsonii* and *Epomops franqueti*<sup>[2, 3]</sup>. *Epomops franqueti* is widely distributed in the forest regions of West Africa, including Nigeria<sup>[4]</sup>. *Epomops franqueti* possess white patches at the base of the ear. The adult male displays secondary sexual characteristic by exhibiting white hair tufts on the shoulder this is a glandular pouch called the epaulette<sup>[5, 6]</sup>.

African fruit bats have been incriminated in several viral zoonotic diseases such as rabies in the case of *Eidolon helvum*<sup>[7]</sup>. Beside the transmission of Nipah virus to human via contact with bat faeces<sup>[8]</sup>, fruit bats may be a natural reservoir of filo viruses<sup>[9, 10]</sup>. Also, *Epomops franqueti* has been implicated in the direct or indirect transmission of Ebola virus to human<sup>[11]</sup>. African fruit bats are hunted for their meat<sup>[12]</sup>. These bats are pest to farmers<sup>[13]</sup> as well as agents of seed dispersal<sup>[14]</sup>.

Unlike tropical fruit bats, the reproductive biology of bats of the temperate region especially Microchiroptera, have been widely studied. These include the biometric and histometrical observations of the male reproductive organs of *Molossus molossus*,<sup>[15]</sup>; comparative study of the reproductive organs of the netropical bats: *Eumops glaucinus* and *Molossus molossus*;<sup>[15,]</sup> *Myotis levis*<sup>[16]</sup>, *Sturnira lilium*<sup>[17]</sup>, Yellow house bat, *Scotophilus heathii*<sup>[18]</sup> and *Myotis nigricans*<sup>[19]</sup>. The reproductive biology of the large fruit-eating phyllostomid bat, *Artibeus lituratus* has been documented<sup>[20]</sup>.

With the exception of preliminary investigations on the reproductive biology of the African fruit bats *Eidolon helvum* and *Rousettus aegyptiacus*<sup>[21, 22]</sup>, existing research documentation on this African fruit bat had mainly focused on its conservation, nutrition and history of migration<sup>[23]</sup>.

There is hardly any research report on the reproductive anatomy of adult male African fruit bat *Epomops franqueti*. This study was therefore designed to investigate the gross and microscopic anatomy of the testis and epididymis of the *E. franqueti* with the aim of generating basic data that could be useful in the comparative regional anatomy of the male reproductive organs of the different chiropteran species.

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## Materials and Methods

Fifteen adult male *E. franquet* were used for the study. They were captured using mist net between the months of July and September, 2017 from their roost at the Faculty of Arts University of Ibadan, Nigeria. The animals were kept in cages and stabilized for three days prior to the investigations carried out. They were fed *ad libitum* with Almond fruits obtained from their roost on daily basis. The anatomical nomenclature used in this research work was in accordance with the reports of Makanya and Mortola [24]. The protocol for this research was approved by the Research and Ethics Committee of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria (UI-ACUREC/19/0001). The body weight of the fruit bat was taken with the aid of a Microvar® weighing balance. The fruit bats were restrained and anaesthetized using ketamine HCl at 25mg/kg body weight intramuscularly using the thigh muscle. The animals were sacrificed by cervical decapitation. The skin was dissected to expose the superficial muscles and the lineal alba which was incised to further expose the deep structures of the abdominal cavities of the *E. franqueti*. The right and left testes and epididymides were then dissected out and immediately put in Petri dishes containing normal saline before morphometric investigations were carried out. The weight of the testes and epididymides were measured with the aid of Digital Microvar® weighing balance. Gross observations of the testis and epididymis were carried as earlier reported by Olukole *et al.* [25]. Biometrical investigations were carried out metric tape and venier caliper. For histological analysis, harvested samples of the testis and epididymis were fixed in Bouin's fluid and embedded in paraffin blocks. Sections of 4-5 µm thick were stained with haematoxylin and eosin. Then slides of testis and epididymis were observed under the light microscope. The measurements taken under the light microscope were: the height of the germinal epithelium, the luminal diameter of seminiferous tubule, the seminiferous tubular diameter, epididymal tubular diameter, epididymal luminal diameter and epididymal epithelial height. TS View software was used.

## Statistical analysis

The data obtained were recorded as means with the standard deviation. It was subjected to Student's t test and correlation analysis using the Graph Pad Prism version 5.00 for Windows. Statistical significance was reported at  $P < 0.05$

## Results and Discussion

The mean body weight of bats used in this work as well as the dimensions of the testis and epididymis are given in table 1. The epididymis was attached to the medial surface of the testis and it extended from the cranial pole to the caudal pole of the testis. The anatomy of bats reproductive organs is different across species [16, 26].

The testis is located in the pelvic region on the ventral aspect (Fig.1). In *E. franqueti* the testes were either intra-abdominal or scrotal, lateral to the penis, therefore could be termed as being migratory (Fig 2 A&B). This is similar to what was observed in *Eumops glaucinus* and *Molossus molossus* by Beguelini *et al.* [19] but contrary to the findings of Karim and Banerjee [28] from their work on Rhinopomatidae (Indian tailed-bat: *Rhinopoma hardwickii*) where the testicles were strictly abdominal. Generally, members of Pteropodidae and Rhinolophidae have their testes only located in the scrotum [29]. However, the orientation of the testis in this present study was horizontal as seen in the study of Krutzsch and Crichton

[27]. The scrotal area is darker than the surrounding skin (Fig. 2B). The darkish colour of the scrotum, creamy white colour, oval shape and composite cells of the testis and epididymis observed in *E. franqueti* were similar to earlier reports on the male reproductive organs of African fruit bat, *Eidolon helvum* and *Rosettus aegyptiacus* [21, 22]. The testis was richly vascularized (Fig 5) in line with the observation of Fard and Ghassemi [22] made on the testis of *Rosettus aegyptiacus*.

Within the pelvic cavity, the testis, with the epididymis attached to the orchido-epididymal surface (medial border of the testis), was located between the colon and the urinary bladder. The epididymis was highly convoluted and extended from the cranial to the caudal pole but lacked bulky epididymal fat unlike *Rousettus aegyptiacus* [22]. It continued caudally with the ductus deferens but lacked ampullary gland (Fig. 6 &7). The difference between the right and left testicular parameters at ( $P > 0.05$ ) was not significant. Similarly, there was no significant difference between the left and right epididymal parameters (Tables 1 and 2). Although our statistical findings revealed that the right testis was bulkier in size than the left testis, the difference was not significant. This observation differs from the investigation carried out on *Rosettus aegyptiacus* in Iran [22]. The pattern of gonadosomatic index observed for this AFB (*Epomops franqueti*) is similar to those of *Sturnira lilium* [17]. Therefore, there was low positive correlation between the weight of the adult male fruit bats and the weight of both right and left testes and there was low positive correlation between the weight of the bats and the circumference of both right and left testes (Table 3). The mean relative testicular and epididymal weights of the African fruit bats used for this work were: 0.2% and 0.07%, respectively. The slight positive correlation between the body weight of the bats and weights of the gonads and epididymis showed that the body weight increment and the testis-epididymal weight increase were slightly associated. This report is in accordance with the findings of Hosken [31]. The anatomical relations of the reproductive organs with the visceral structures were consistent with reports on other fruit bats [21, 22, 32].

The *E. franqueti* testis was encapsulated by two layers of connective tissue: the tunica vaginalis and tunica albuginea. The tunica albuginea of this bat is thicker than the tunica vaginalis. This consisted of dense connective tissue which is rich in blood vessels, fibrocytes and collagen fibers. The thin tunica vaginalis is composed of a single layer of simple epithelium having elongated nuclei and resting on a basement membrane. The basement membrane separates it from the inner tunica albuginea (Fig. 8 A). The histological features of the thick tunica albuginea and thin tunica vaginalis of the *E. franqueti* also corresponds with those reported on other fruit bats [38]. The tunica albuginea of this chiropteran had dense connective tissue rich in blood vessels, fibrocytes and collagen fibres, however in Iranian fruit bat, the connective tissue was loose [17].

The histology of the testis of the *E. franqueti* shows tunica albuginea extending into the testicular tissue proper in the form of septa (septula testis) so that the testis is divided into lobules (lobuli testis). The histological components of the testicular capsule of the *E. franqueti* in this study was in accordance with the findings of [17]. Unlike some Pteropodids there was no visible brown and white fat cells in the tissue [22]. One lobule contained one or more seminiferous tubules and the interstitium separated adjacent seminiferous tubules from one another (Fig. 8 B). Within the interstitium were Leydig

cells and blood vessels. These cells were polygonal in shape and was uniform with most of the histological sections observed in this study.

The seminiferous tubules seen in the sections were of various sizes and irregular shapes, mainly spherical (Fig. 10 A&B). The seminiferous tubules were highly convoluted and formed the major part of the testicular tissue of this fruit bat. Each of the seminiferous tubules have their basement membrane lined with germ cells arranged in successive layers indicating different stages of cell division (spermatogenesis: mitosis and meiosis) and differentiation (spermiogenesis) as the fully matured cells migrate into the lumen of the tubules. From the basement membrane to the lumen of the seminiferous tubules, the cells recognized were in this order: spermatogonia, primary spermatocyte, secondary spermatocyte (few in number), round and elongated spermatids; and spermatozoa. The Sertoli cell was identified by its abundant nuclear-cytoplasmic ratio. However, the outline and details of the Sertoli cells identified were not clearly seen. Myoid cells were found at the basement membrane surrounding each of the seminiferous tubule (Fig. 10D).

The histomorphometry study carried out on the testis and epididymis of *E. franqueti* is similar to those of straw coloured fruit bats [22]. This finding also resembles the observations reported for rodentia [35, 36, 39, 41]. The diameter of the seminiferous tubules of the *E. franqueti* is however lower to those reported in *E. helvum* [21]. The forms and organization of the seminiferous tubules with various sizes and irregular shapes, including spherically shaped tubules presented in the testis of *E. franqueti* have been widely reported in other chiropteran species [21, 22, 37, 38]. Also, the epithelial organization of the cells of the spermatogenic series as well as the location of the Sertoli cells in the seminiferous tubules of *E. franqueti* is similar to those reported in other bat species [15, 39] while the mediastinum testis was centrally placed (Fig. 9) unlike the peripherally position in man [40]. Within the mediastinum testis, is located the rete testis. The relationship among the transitional zone, the seminiferous tubules and the rete testis within the mediastinum testis as observed in the *E. franqueti* is similar to the observations of Shende and Kanhobaji, [37]. The rete testis connected the seminiferous tubules to the efferent ducts. The efferent duct of *E. franqueti* was lined by pseudostratified columnar epithelium as earlier reported in other species of bats [20, 34]. The transitional zones within the mediastinum connected the seminiferous tubules with the straight tubules (Fig. 10B).

*E. franqueti* epididymis can be grossly divided into head, body and tail as in seen in other mammals and its luminal diameter, epithelial height and epithelial ductal diameter across the caput, corpus, and caudal part of the epididymis, varied in similar patterns as seen in the dimensions reported for the head, body and tail of the Indian bat [37]. In this study, the low positive correlation recorded between the diameter of the duct of epididymis and its epithelial height indicates that with an increase in epithelial height, the increase in ductal

diameter is low. And, the significant negative correlation recorded between epithelial height and lumen diameter indicates that decreasing height of the epithelium, the lumen increased significantly as it was observed at the caput of the epididymis. This may be attributed to the functional differences as the caudal part of the epididymis has wider lumen which helps in storage of spermatozoa while the caput does more of secretion into the lumen. Thus, the reason why more spermatozoa mass was found in the caudal portion of the epididymis of the *E. franqueti* than was seen in the corpus and caput of the epididymis. The epididymal epithelium consisted of principal, apical and basal cells and stereocilia projected into the lumen that contained spermatozoa (Fig. 10 E&F). The epididymal tubular and luminal diameter increased progressively from caput to the caudal epididymis while the epithelial height decreased progressively from caput to caudal epididymis (Table 3). This our findings correspond with the previous documentation on the histology of the epididymis of other fruit bat and Microchiroptera [33]. And also, similar to earlier reports on other mammalian species [34, 35, 36]. The basal cells were located at the basement membrane of the epithelium of the epididymis. The principal cells were more in numbers, also oriented towards the basal region of the duct. And, few apical cells were found very close to the luminal surface with smooth muscles beneath the basement membrane of the epididymal epithelium, corresponding to the reports on other species of bats [15, 20, 37].

### Conclusion

In conclusion, this research work shows that biometrical observations, histology and histomorphometry of the testis and epididymis of the *E. franqueti* are similar to earlier reports on chiropteran. This work also points to the fact that the gross and micro anatomy of *E. franqueti* is not only similar to both Mega and Microchiroptera but to other mammals including man. Many of the microbats that have been worked on especially in the temperate region suggest that they are seasonal breeders. Therefore, seasonal studies on these observations are recommended to further elucidate more details on the reproductive biology of the male *E. franqueti*. Thus, the findings of this work provide baseline data on the dimensions of the testis and epididymis in the *E. franqueti*, thereby making available useful research data in comparative regional anatomy within Megachiropteran bats.

### Acknowledgements

The authors would like to thank Dr. Oluwadamilare Olanipekun of the (Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan), for his assistance during the sample collection stage of this work.

PS: For the statistics, weights of the testes (right and left) were compared using Student t test while the length, circumference of the testes (right and left) were compared separately.

Table 1: Mean and SD values of the testicular biometrical parameters of *E. franqueti*

WAO(g)	WRT(g)	WLT(g)	GSI (%)	LRT	LLT	CRT	CLT
97.64±5.90	0.089±0.0031 <sup>a</sup>	0.083±0.0038 <sup>a</sup>	0.20	0.65 ± 0.025 <sup>b</sup>	0.63±0.029 <sup>b</sup>	1.3±0.04 <sup>c</sup>	1.4± 0.04 <sup>c</sup>

Means with different superscripts differ significantly (P<0.05). WOA – Weight of the animal. WRT – Weight of the right testis. WLT – Weight of the left testis. GSI- Gonosomatic Index. LRT – Length of the right testis. LLT – Length of the left testis. CRT – Circumference of the right testis. CLT - Circumference of the left testis Table 2: Mean and SD values of the testicular biometrical parameters of *E. franqueti*

**Table 2:** Mean and SD values of the epididymal biometrical parameters of *E. franqueti*

WAO(g)	WRE(g)	WLE(g)	EMI (%)	LRE	LLE
97.64±5.90	0.037±0.0012 <sup>a</sup>	0.033±0.001 <sup>a</sup>	0.07	0.72±0.04 <sup>b</sup>	0.65±0.02 <sup>b</sup>

Means with different superscripts differ significantly ( $P < 0.05$ ). WOA – Weight of the animal. WRT – Weight of the right epididymis. WLT – Weight of the left epididymis. EMI–Epididymal Mass Index. LRT – Length of the right epididymis. LLT – Length of the left epididymis.

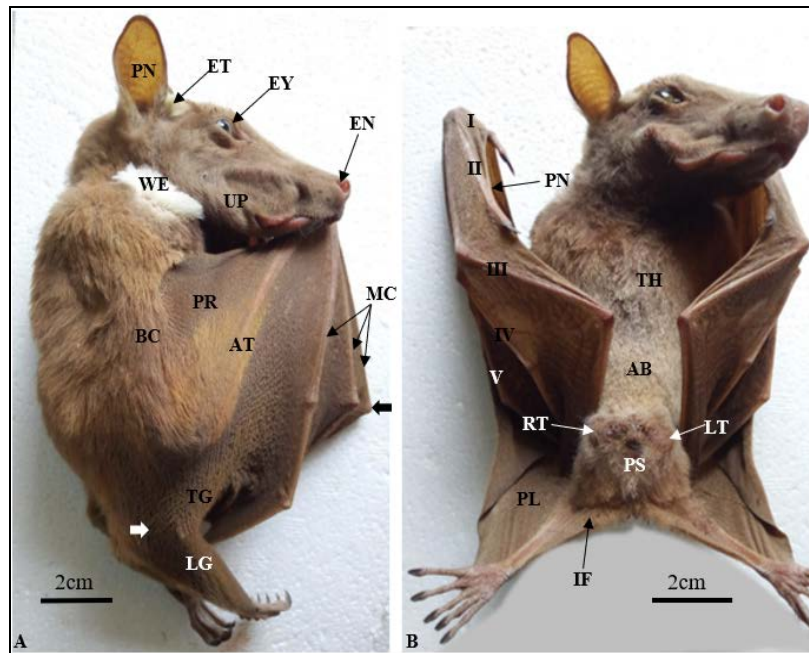
**Table 3:** Mean and SD values of the epididymal histo-morphometrical parameters of *E. franqueti*

WAO (g)	STD (µm)	STL (µm)	STE (µm)	CAP (µm)	COR (µm)	CAU (µm)	CAL (µm)	COL (µm)	CUL (µm)	CAE (µm)	COE (µm)	CUE (µm)
97.64±5.90	300.7±7.60 <sup>a</sup>	106.5±9.67 <sup>b</sup>	92.01±7.80 <sup>c</sup>	271.9±17.17 <sup>a</sup>	334.9±14.26 <sup>b</sup>	503.8±36.05 <sup>c</sup>	77.90±10.02 <sup>d</sup>	202.3±14.98 <sup>e</sup>	443.6±23.63 <sup>k</sup>	76.07±6.146 <sup>x</sup>	40.67±3.71 <sup>y</sup>	36.04±1.87 <sup>z</sup>

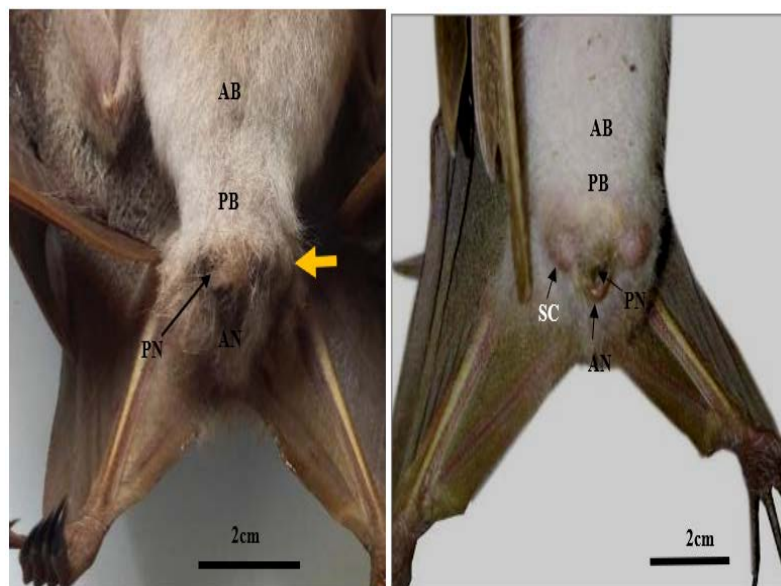
Means with different superscripts differ significantly ( $P < 0.05$ ). WOA – Weight of the animal.

STD –Diameter of seminiferous tubules. STL–Luminal diameter of seminiferous tubules. STE–Epithelial height of seminiferous tubules.

CAP –Diameter of caput epididymis. COR –Diameter of corpus epididymis. CAU–Diameter of cauda epididymis. CAL –Luminal diameter of caput epididymis. COL –Luminal diameter of corpus epididymis. CAU–Luminal diameter of cauda epididymis. CAE –Epithelial height of caput epididymis. COE – Epithelial height of corpus epididymis. CAE– Epithelial height of cauda epididymis



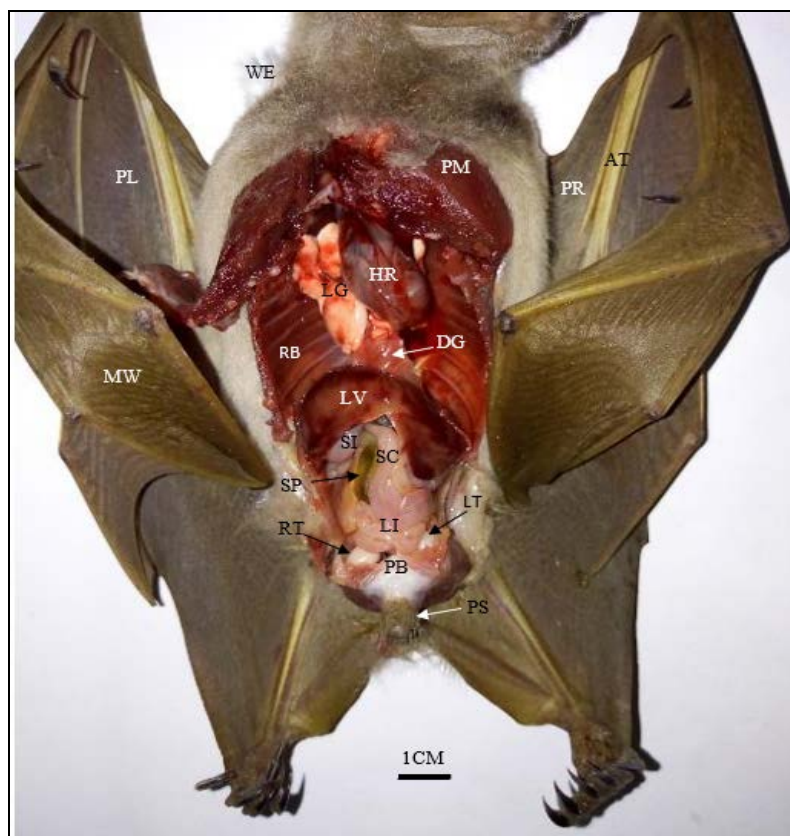
**Fig 1:** The surface structures of Adult male *E. franqueti* at the right lateral view in (A) showing the: simple oval pinna (PN); folded upper lip (UP); simple eye (EY) located rostrally and the external nares (EN); brachium (BC); Ante-brachium (AT); thigh (TG); leg (LG). Note the white epaulette (WE) which is a unique secondary sexual character. Black arrow points to the meta-carpo-phalangeal joint while white arrow points to the femoro-tibial joint. In (B), at the dorso-ventral view, shows the: right and left testes (RT and LT); penis (PS); robust thorax (TH); slim abdomen (AB). Note: the first-fifth digits (I-IV); plagiopatagium (PL); inter femoral membrane (IF) and, the propatagium (PR) in (A).



**Fig 2:** A: The gross picture of adult male *E. franqueti* at the dorso-ventral view showing the: scrotum (SC); the penis (PN); anus (AN) and the pubic bone (PB) just below the abdomen (AB). Note the dark coloration of the scrotal skin with sparse hair in (B), and yellow arrow points to position of the intra-abdominal testis in (A).



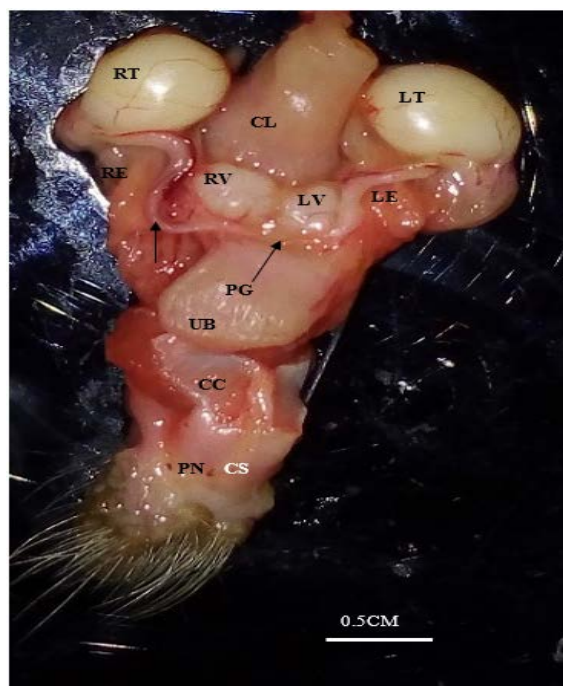
**Fig 3:** The superficial structures of Adult male *E. franqueti* at the dorso-ventral view showing the: simple oval pinna (PN); shoulder epaulette (WE); carpus (CP); Ante brachium (AT); propatagium (PR) pectoral muscle (PM); reflected skin (RS); xyphoid bone (XP); sternal ribs (SR); wing: (MW); external Abdominal muscles (AB); lineal alba (LA) that runs from the xyphoid to attached to the pubic bone (PB); stout penis (PS); ischiocavernosus muscle (IM); Vestigial tail (VT); plagiopatagium (PL) and the leg (LG).



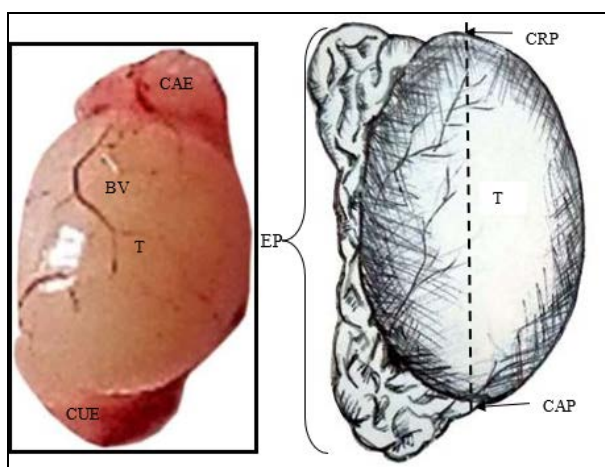
**Fig 4:** The deep structures of Adult male *E. franqueti* at the dorso-ventral view showing the: simple oval pinna (PN); shoulder epaulette (WE); Ante brachium (AT); propatagium (PR); wing: (MW); deep pectoral muscle (DM); and the visceral organs: the heart (HR); lungs (LG); vertebral ribs (VR); diaphragm (DG); liver (LV); gall bladder (GB); long slim, spindle shaped spleen (SP). Note the relation of the small intestine (SI) to the right (RT) and left (LT) testes respectively; stout penis (PN).



**Fig 5:** The pelvic and lower abdominal cavities of Adult male *E. franqueti* at the dorso-ventral view showing the relationship of the ileum (IL); colon (CL) with the reproductive organs: the right (RT) and left (LT) testes. The testis is ovoid in shape. Note: the ileum is cranial to the testes while the colon is dorsal to the testes. The right (RV) and left (LV) vesicular glands are medial to the testes. Also, note that the testes and the vesicular glands are located between the colo-rectum and the urinary bladder (UB). The penis (PI) is stout and extends from the pubic bone.



**Fig 6:** The reproductive organ of adult male *E. franqueti* and its relationship with the colon (CL). Note the vesicular gland (VG) at the neck of the urinary bladder (UB). In (B), note the right (RT) and left (LT) testes with blood vessels. Black arrow points to the vas deferens (VD) which continues from the epididymis (RE) to the neck of the urinary bladder (UB). The urinary bladder was reflected to show the prostate glands (PG) and the right (RV) and left (LV) vesicular glands. Also, note the penis (PN) and the corpus cavernosum (CC) corpus spongiosus (CS)



**Fig 7:** The gross picture and diagrammatic representation of the testis of adult male *E. franqueti* showing the cranial and cauda poles (CRP and CAP) and the epididymis (EP). Observed that the epididymis extends from the cranial pole to the caudal pole of the testis

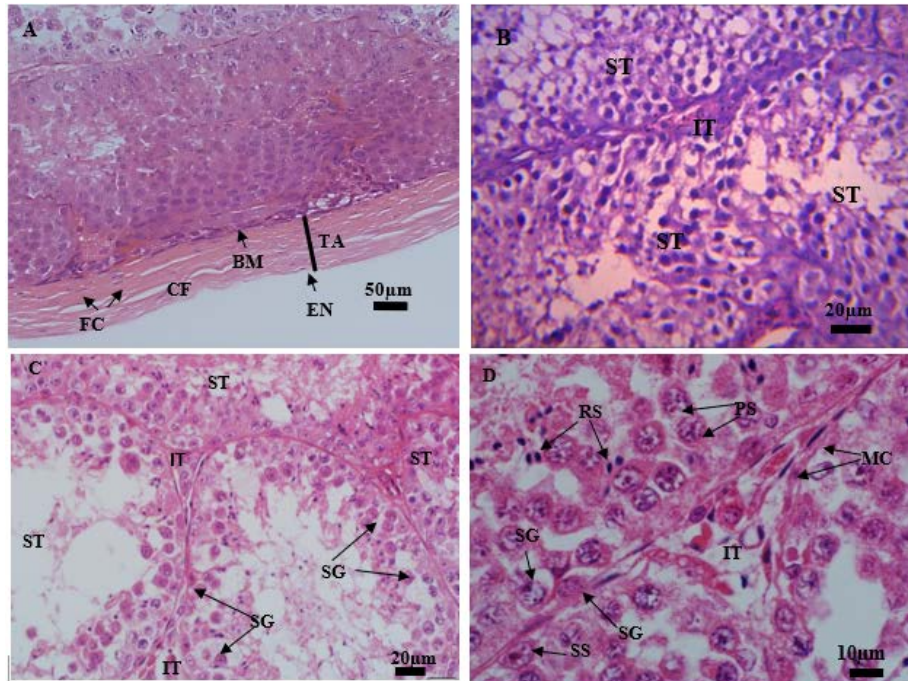
Parameters	Coefficient of correlation (r)
WOA (g)	1.000
WRT (g)	0.593
WLT (g)	0.504
LRT (cm)	0.670
LLT (cm)	0.500
CRT (cm)	0.002
CLT (cm)	0.002
WRE (g)	0.378
WLE (g)	0.322
LRE (cm)	0.679
LLE (cm)	0.533

WOA – Weight of the animal. WRT – Weight of the right testis. WLT –Weight of the left testis. LRT – Length of the

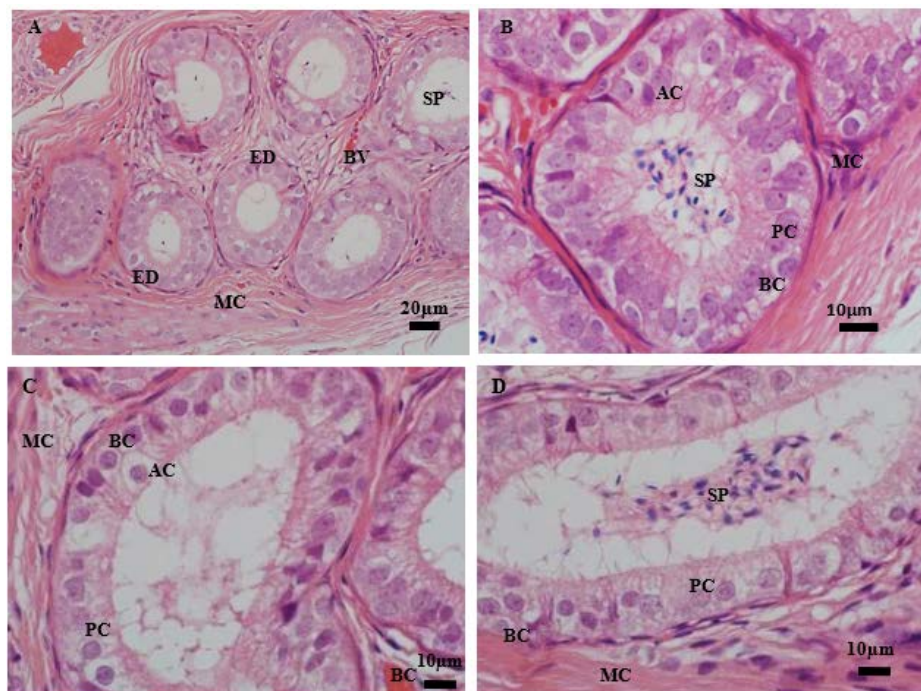
right testis. LLT – Length of the left testis. CRT – Circumference of the right testis. CLT - Circumference of the

left testis. WRE – Weight of the right epididymis. WLE– Weight of the left epididymis. LRE – Length of the right

epididymis. LLE– Length of the left epididymis



**Fig 8:** Photomicrographs of the testis and epididymis of the testis of the *E. franqueti* (A): The testicular capsule showing, BM: basal membrane of seminiferous tubule; CF: collagen fibers; EN: elongated nucleus of the epithelium of tunica vaginalis; FC: fibrocytes; TA: tunica albuginea; TV: tunica vaginalis. In (B): Testis showing ST: the seminiferous tubules. Note the interstitium wedged between three seminiferous tubules. In (C) and (D): Testis showing: Sertoli cell (SC); Spermatogonium (SG); Primary spermatocyte (PS); Secondary spermatocyte (SS); Round spermatid (RS); Spermatozoa (SP); Myoid cell (MC); Interstitial cell (IC) H&E



**Fig 9:** showing the epididymis of *E. franqueti*, showing the epididymal ducts with apical cell (AC); principal cell (PC); basal cell (BC); myoid cell (MC); spermatozoa (SP) in the lumen of the epididymis. Note in (A) the loose connective tissue transverse and surrounds each epididymal duct and the blood vessel (BV) within the connective tissue as seen in (C). Also, note that the corpus and the caudal epididymis in (C) and (D) have few apical cells and basal cells compared to the principal cells, and the presence of spermatozoa in (B) and (D). H&E

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