



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2022; 10(1): 395-402

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Received: 13-11-2021

Accepted: 17-12-2021

Ekeolu Oyetunde Kazeem

Lecturer I, Head of Department
Department of Veterinary
Anatomy, Faculty of Veterinary
Medicine, University of Benin,
Benin city, Edo state, Nigeria

Olukole Gbadebo Samuel

Senior Lecturer, Head of
Department, Department of
Veterinary Anatomy, Faculty of
Veterinary Medicine, University
of Ibadan, Ibadan city, Oyo
state, Nigeria

Oke Olusiji Bankole

Professor, Department of
Veterinary Anatomy, Faculty of
Veterinary Medicine, University
of Ibadan, Ibadan city, Oyo
state, Nigeria

Corresponding Author:**Ekeolu Oyetunde Kazeem**

Lecturer I, Head of Department
Department of Veterinary
Anatomy, Faculty of Veterinary
Medicine, University of Benin,
Benin city, Edo state, Nigeria

Reproductive cycle in the male African fruit bat (*Epomops franqueti*)

Ekeolu Oyetunde Kazeem, Olukole Gbadebo Samuel and Oke Olusiji Bankole

DOI: <https://doi.org/10.22271/j.ento.2022.v10.i1e.8959>

Abstract

Epomops franqueti is common in Sub-Saharan Africa. Unlike micro chiropteran, information on the reproductive activity of this bat is scanty. The morpho-physiology of the testis and epididymis of *E. franqueti* was investigated to determine the seasonal reproductive activity.

Sixty adult male *E. franqueti* were captured at the University of Ibadan, Nigeria. Histomorphometry of the testis and epididymis was investigated. Serum hormonal assays were carried out using ELISA. Concentrations of testosterone, FSH, and LH were least (0.25, 5.80, and 7.01 IU/L, respectively) and highest (3.80, 12.03 and 14.04 IU/L, respectively) during late dry and late wet seasons, respectively, being significantly different.

The early and late wet seasons had the highest gonadosomatic (0.20%) and epididymal mass (0.08%) indices, respectively. The least values were recorded during the early dry season for both gonadosomatic (0.15%) and epididymal mass (0.06%) indices.

Epomops franqueti may be a seasonal breeder.

Keywords: African fruit bat, *Epomops franqueti*, seasonal breeder, gonadosomatic index, histomorphometry

Introduction

The reproductive biology in bat species varies widely. This wide range of differences depends on temperature, rain, optimal humidity, and food availability. These factors vary in degree across bat habitats^[1]. Seasonal environmental factors and the availability of food determines the production of sex hormones which affects the reproductive cycle^[2]. Adequate food and energy enhance the regulation of the reproductive cycle^[3]. Changes in reproductive behaviour are related to changes in gonadal activity. There are seasonal differences in the testicular and epididymal parameters reported in common vampire bats^[4]. Wide species-specific differences in respect to the duration of gonadal activities have been reported in non-fruit-eating species of bat. Non-fruit-eating bats such as *Myotis nigricans* in the neotropical region have been reported to show two periods of low reproductive activities, which matches the variation in the secretion of sex hormone^[5].

Reproductive cycle regarding seasons has been reported mostly in insectivorous bats^[6, 7, 8]. Also, there are reports on neotropical fruit-eating bats, *Artibeus lituratus* and another micro chiropteran whose reproductive cycle varies regionally with seasonal factors^[9, 10, 11, 12]. However, there is little or no information on the seasonal changes in the reproductive cycle of adult male *Epomops franqueti*, a megachiropteran and pteropodid^[39], widely distributed in the West African region, including Nigeria^[14]. This species of African fruit bat (AFB) has been implicated in viral and bacterial zoonotic diseases^[15, 16]. AFB is hunted for its nutritious meat in the rural areas. AFB helps in seed dispersal through its flight zones therefore helping to balance the ecosystem by regenerating and conserving the vegetation^[17, 18].

In order to link up the information gap between the insectivorous and African fruit bats regarding their reproductive cycles, this study was carried out during the wet and dry seasons in Ibadan, Nigeria. The knowledge of the reproductive cycle will be helpful in the conservation of this fruit bat and its population control. It is also, providing basic data for further research work. *E. franqueti* is crucial because this fruit bat has been mentioned in recent times to be a symptomless vector of deadly viruses such as Ebola and Coronaviruses which may have predilection sites in the fruit bat reproductive tracts^[19, 20, 21].

Hence, reproductive activities of the testis and epididymis of *E. franqueti* in wet (early and late rain) and dry (early and late dry) seasons were investigated from the histological and hormonal profile approach.

Materials and Methods

Experimental Animals

Sixty adults male *E. franqueti* (five every month of the year) were captured and used for this work. A mist net was used to trap the bats as described by Ekeolu *et al.* [22]. The average body parameters were all measured using a Draper® 115mm vernier caliper and metric tape. The bodyweight of the bats was measured with the aid of Microvar® weighing balance. They were anaesthetized with ketamine HCl at 25mg/kg body weight intramuscularly, through the thigh muscle. The testes and epididymides were harvested. Their weights were measured with the aid of Digital Microvar® weighing balance. The anatomical nomenclature used in this work is in accordance with the report of Fard and Ghassemi [22, 23].

$$\text{GSI or EMI} = \frac{\text{Weight of organs (g)} \times 100}{\text{Weight of animal (g)}}$$

Samples were fixed in Bouin's fluid, embedded in paraffin blocks and sectioned to 4mm thickness, staining with Haematoxylin and Eosin [24] for light microscopy. Testicular and epididymal parameters were measured using TSview software.

Hormonal assay

In accordance with the procedure described by Olukole *et al.* [25], the blood sample from each bat was collected in a universal bottle and allowed to coagulate at 25 °C. It was then centrifuged for 15minutes at a speed of 5thousand revolutions and the serum was stored in an Eppendorf tube at a temperature of 4 °C. The serum hormonal profile for testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormones (LH) were assayed for each of the five animals for each month of the year using specific Kits and well labelled ELISA microplate immunoassay and microplate reader for the three hormones. Commercial ELISA kits were used to quantify the serum hormones abiding strictly by the manufacturer's guides.

Statistical Analysis: The data obtained every month were expressed as means with standard error of mean using the Graph Pad Prism version 5.00 for Windows, GraphPad Software (GraphPad Prism, 2003), Analysis of variance was performed using two-way ANOVA and significance was reported at $P < 0.05$.

Results and Discussion

The testicular colour was milky white irrespective of the season (Fig. 1). The difference in the seasonal body weight obtained in the wet and dry seasons was significant ($P < 0.05$). The average weight of the African fruit bat used in this study falls within the range reported by Nowak and Walker [26] on bats from the temperate region. Still, it is larger than the average body weight recorded for the common vampire bat, *Demondus rotundus* [27]. The values of the average body weight of the bat were higher in the wet season than the values obtained in the dry season (table 1). Also, the average weights obtained for the testis across the seasons vary significantly at $P < 0.05$ (table 1). The mean GSI for in the

early rainy and early dry seasons were 0.201% and 0.154% respectively (table 1). The seasonal GSI varied significantly ($P < 0.05$). During the long period of rain, the highest average testicular weight of $0.172 \pm 0.001\text{g}$ were recorded (table 1). The gonadal tissue investment had the highest value during the wet season. However, this value was relatively low compared to that which was reported for *Desmondus rotundus* [27], suggesting that this bat may be monogamous in nature with a lower GSI (0.2%) compared to the GSI (0.46%) of *Molossus molossus*, that is harem in nature [28]. The GSI (0.20%) recorded during the wet season was also close to the GSI (0.27%) of *Sturnira lilium* [29], higher than the GSI (0.13%) of a bull [30], supporting the hypothesis that small-sized mammals invest more in spermatogenesis than large ones [31].

There was a significant difference in the seminiferous tubular and luminal diameters. The germinal epithelial heights of the testis during wet and dry seasons showed significant variations at $P < 0.05$ (table 2). During the wet season, the germinal epithelial height increased with numerous spermatocytes, spermatids and fewer spermatozoa (in the late wet season), anchoring on the Sertoli cells as the spermatogonia replenishes the differentiating spermatocytes but low reproductive activities during the dry season, with few spermatocytes, presence of spermatogonia and Sertoli cells. Remnant of spermatozoa is seen within the lumen of the seminiferous tubules. The Leydig cells were intact and their nuclei appeared vesicular and foamy, with prominent blood vessels in the interstitium during the wet season as against its appearance in the dry season (Fig. 2 A, B, C & D). The spermatogonial stem cell undergoes division to replenish itself and the other undergoes mitotic division, then meiotic division and differentiation. This complex process was observed in the germinal epithelium of *Epomops franqueti*, similar to the report of Beguelini *et al.* in *Myotis spp* [12] and *Sturnira lilium* [29]. During the meiotic phase, the spermatocyte gradually migrates toward the adluminal compartment corresponding with the reports of Fard and Ghassemi [22] and Morais *et al.* [27]. Spermatogenesis peaked when there was maximum rainfall, unlike in the cave bat, *Myotis velifer*, documented by Krutzsch [32]. The epithelial height of epididymis during the dry season decreased. The spermatozoa in the lumen were more in the long period of the wet season but scanty in the dry season. The relative epididymal mass index (EMI) value was higher during the wet season. The parameters measured for the epididymis showed significant differences during wet and dry seasons (table 2, Fig 4&5). This is similar to reports in *Taphozous longimanus* [33] and vampire bat [34] in their breeding seasons. The scanty spermatozoa observed in the lumen of the epididymis during the dry season suggests that the bat may be a seasonal breeder, unlike in sheath tailed bat [35]. The morphological, morphometrical, and histological characteristics of the testis and epididymis of *Epomops franqueti* suggest seasonal variation given by the differences in the weight of the testis and epididymis. The least values of the testicular and epididymal parameters were recorded during the late dry season conforming to the previous study on the seasonal breeding bat, like the Tree-roosting bat [8]. Extreme temperature affects reproductive activities negatively and this occurs between the months of January and February when also fruits are not available, in Ibadan. An increase in daylight with moderate temperature is excellent for reproductive activity [35]. The principal cell, which was the predominant cell in the epididymis of *Epomops franqueti*, exhibited a vesicular lightly stained oval nucleus during the

wet season, indicating high reproductive activity.

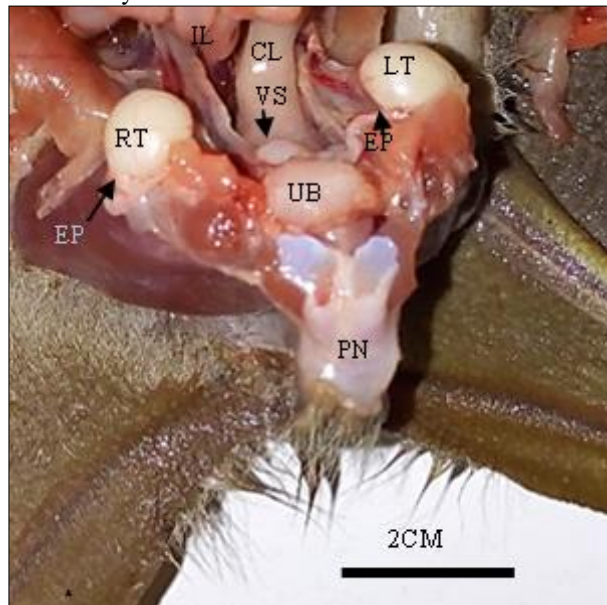


Fig 1: The pelvic and lower abdominal cavities of adult male *E. franqueti* at the dorso-ventral view showing the relationship of the ileum (IL); colon (CL) with the reproductive organs: the right (RT) and left (LT) testes, the right (RV) and left (LV) vesicular glands, urinary bladder (UB) and the penis (PN) Note: the milky colour of the testis. There was no change in testicular colour through the wet and dry seasons.

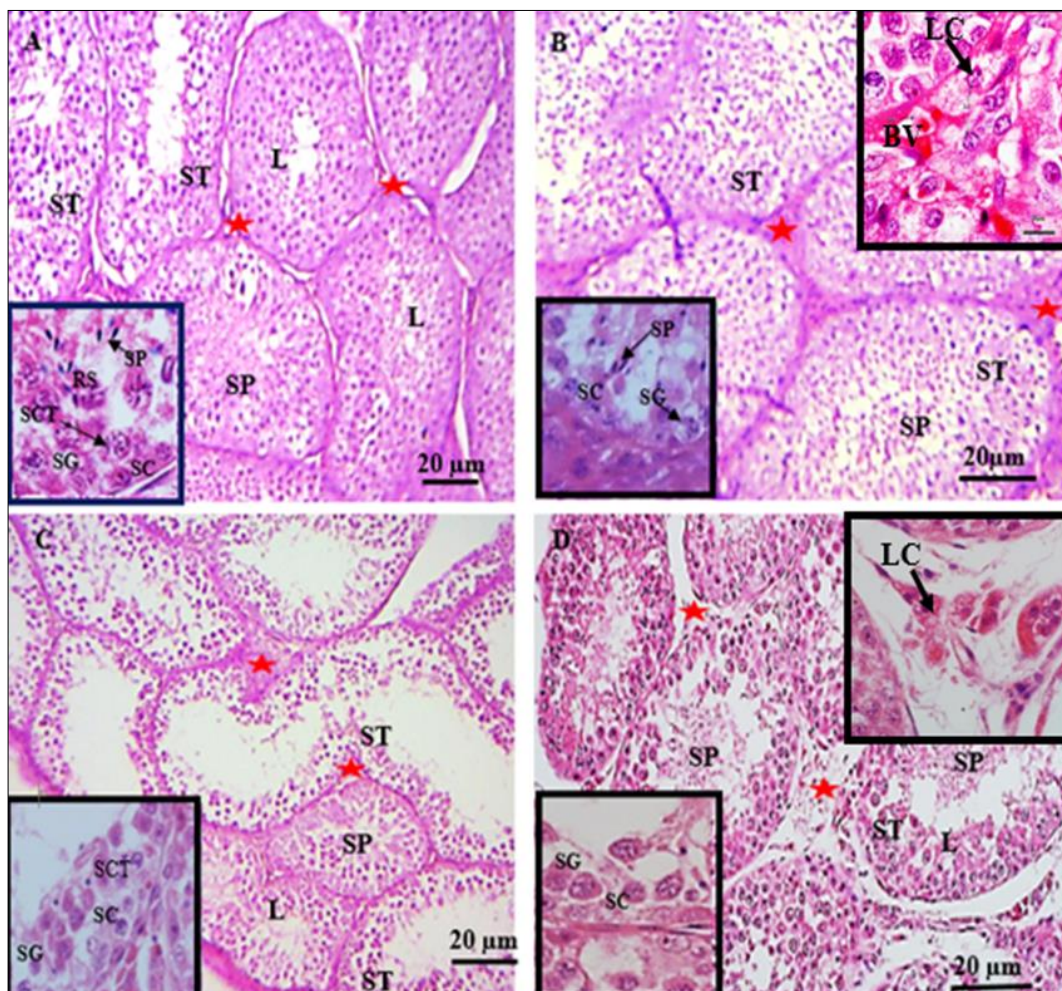


Fig 2: Photomicrograph of the testis of *E. franqueti* A&B: early and late rain seasons respectively. Note the full germinal epithelium of the seminiferous tubule (ST), lumen (L) with spermatozoa (SP). Insets show: Sertoli cell (SC), spermatogonia (SG), spermatocytes (SCT) and spermatids (SP). C&D, early and late dry seasons. Note the reduced germinal epithelium of the seminiferous tubules (ST), lumen (L) with scanty spermatozoa (SP). Insets shows: Sertoli cells, a few spermatogonia and few spermatocytes and abundant debris of spermatozoa in D. Inset: Note the foamy appearance of the Leydig cell nucleus in the wet season with intact interstitium and its disruption in the late dry season.

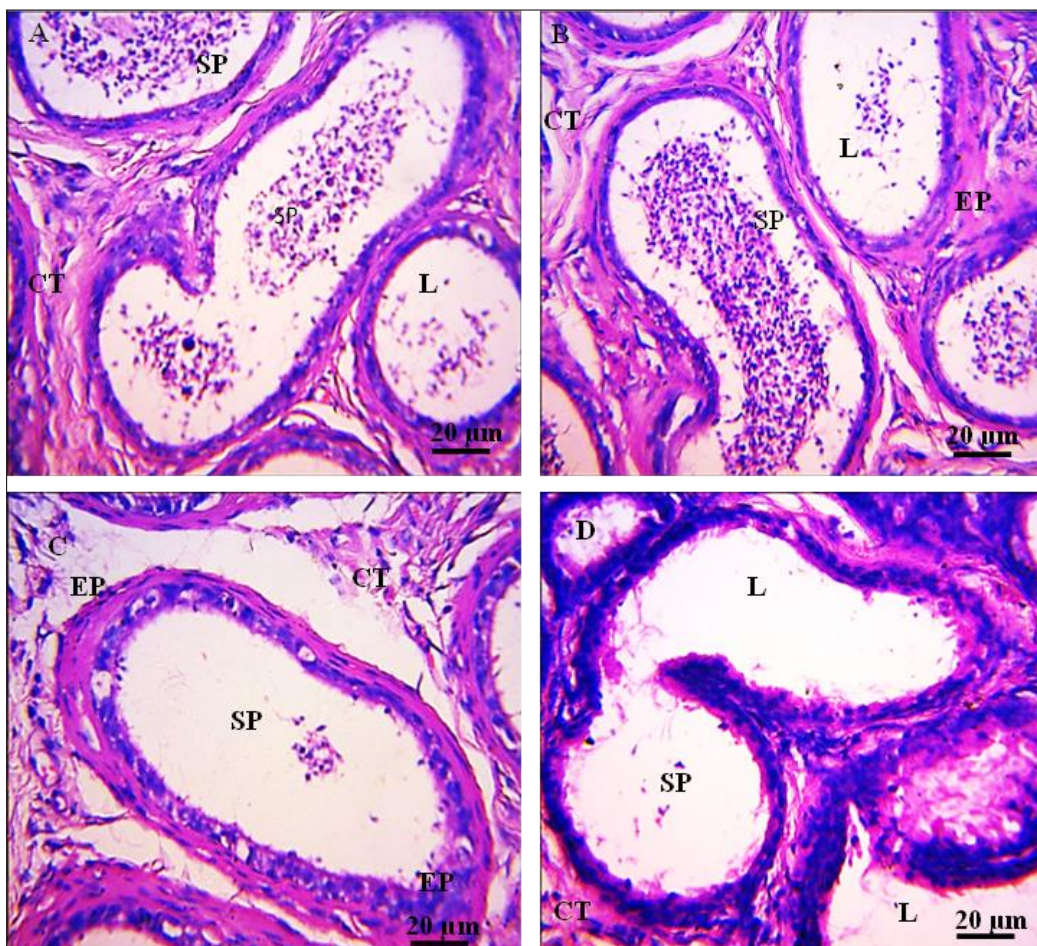
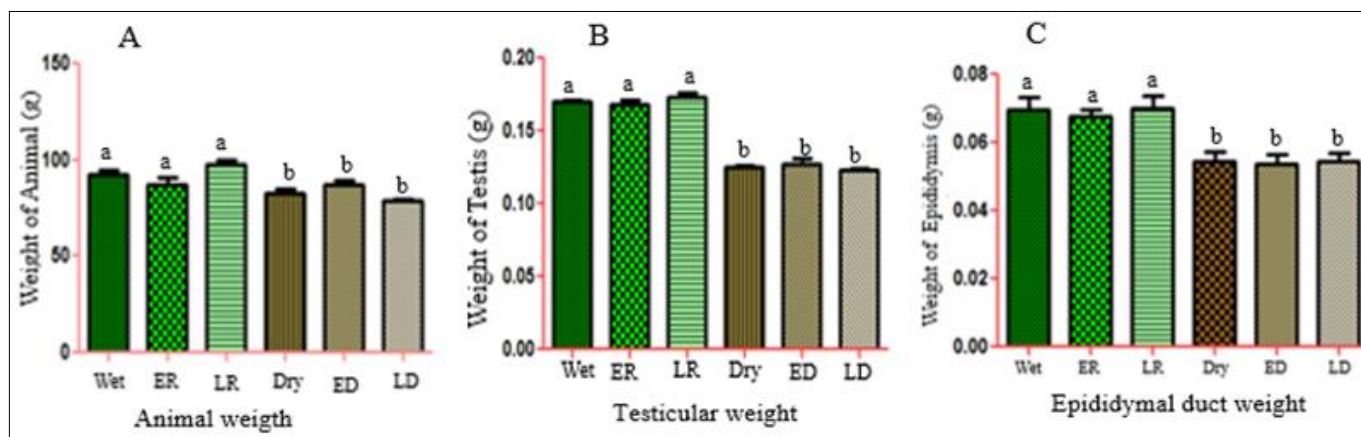


Fig 3: Photomicrograph of the cauda epididymis of *E. franqueti* in A and B, early and late rainy seasons showing lumen (L) filled with spermatozoa. Early and late dry seasons show the lumen (L) filled with very few spermatozoa in C and D. Note that the lumen of the epididymis contains more spermatozoa in the period of wetness than in dryness, with a period of late rain having the most abundant. H&E

Table 1: Seasonal variation in Reproductive parameters of the testis of *E. franqueti*

Seasons	WOA (g)	WOT (g)	WOE (g)	GSI	EMI
ER	86.89 ± 3.15	0.168 ± 0.001	0.068 ± 0.002	0.201	0.0690
LR	97.64 ± 5.90 ^a	0.172 ± 0.001	0.070 ± 0.002	0.176	0.0797
WET	92.67 ± 4.52	0.170 ± 0.001 ^a	0.069 ± 0.001	0.189 ^a	0.0743 ^a
ED	87.19 ± 4.76	0.127 ± 0.003	0.053 ± 0.003	0.154	0.0614
LD	78.76 ± 5.73 ^b	0.123 ± 0.006	0.055 ± 0.008	0.155	0.0741
DRY	82.98 ± 5.25	0.125 ± 0.005 ^b	0.054 ± 0.005	0.155 ^b	0.0678 ^b

*Means with dissimilar superscripts within columns varied significantly ($P < 0.05$), WOA: Weight of Animal, WOT: Weight of Testes (combined), GSI: Gonadosomatic Index, WOE: Weight of Epididymis (combined), EMI: Epididymal mass index, ER: Early rain season, LR: Late rain season, ED: Early dry season, LD: Late dry season



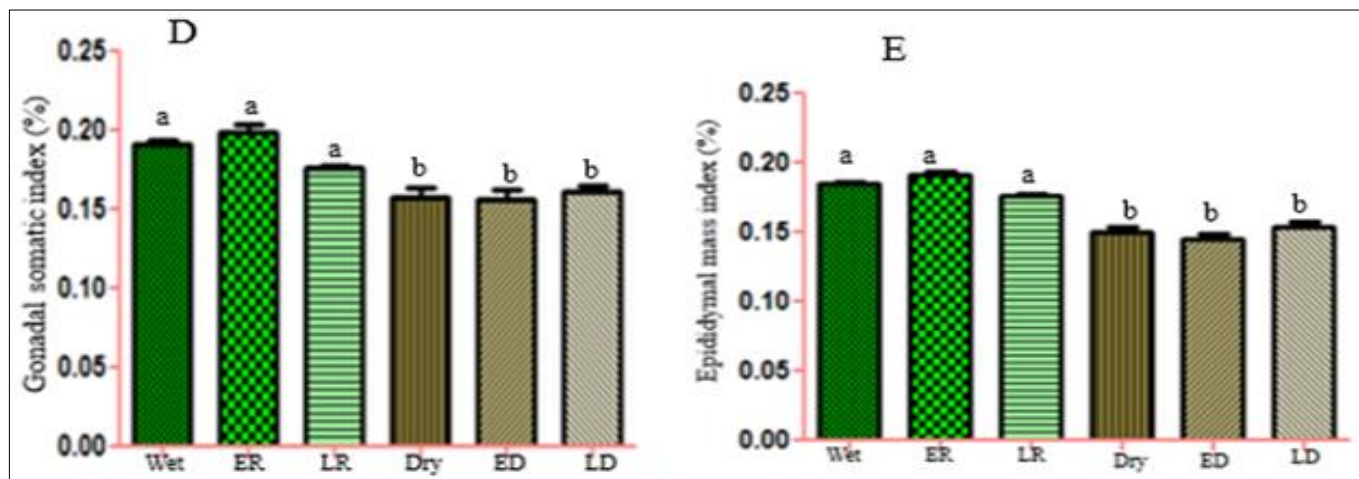


Fig 4: Chart illustrating seasonal variation in the animal weight (A), testicular weight (B), epididymal duct weight (C), gonadal somatic index (D) and epididymal mass index (E) of *Epomops franqueti* during the wet (early, late rainy) and dry (early and late dry) seasons of the year as seen in table 1. Bars with different alphabets are shown to be significantly different at $P < 0.05$

Table 2: Seasonal variation in Reproductive parameters of the testis of *E. franqueti*

Season	STD (μm)	SLD (μm)	GH (μm)	EDD (μm)	ELD (μm)	EH (μm)
ER	238.3±9.09	99.83±15.32	135.5±12.82	322.2 ± 17.18	212.6 ±34.02	109.3±19.31
LR	297.7±7.40	97.8 ±11.13	198.8 ±20.32	365.2 ± 32.27	278.8 ±20.13	85.1± 12.20
WET	268.0 ±8.23 ^a	98.1±16.18 ^a	166.7±18.53 ^a	343.7± 24.73 ^a	247.6 ±17.23 ^a	92.6± 14.05 ^a
ED	186.1±6.19	122.4±11.62	63.7±13.69	249.5 ± 15.23	197.5± 20.03	48.1±10.32
LD	173.8 ± 6.0	125.1±13.36	48.6± 15.13	222.2 ± 22.06	167.3±16.3	54.4±11.06
DRY	179.9 ± 6.09 ^b	123.8±15.04 ^b	56.1±16.07 ^b	235.9± 18.65 ^b	189.3±13.07 ^b	49.1±16.21 ^b

*Means with different superscripts within columns are significantly different ($P < 0.001$), STD: Seminiferous tubular diameter, SLD: Seminiferous tubule luminal diameter, GH: Germinal epithelial height, EDD: Epididymal Ductal diameter, ELD: Epididymis luminal diameter, EH: Epithelial height, ER: Early rain season, LR: Late rain season, ED: Early dry season, LD: Late dry season

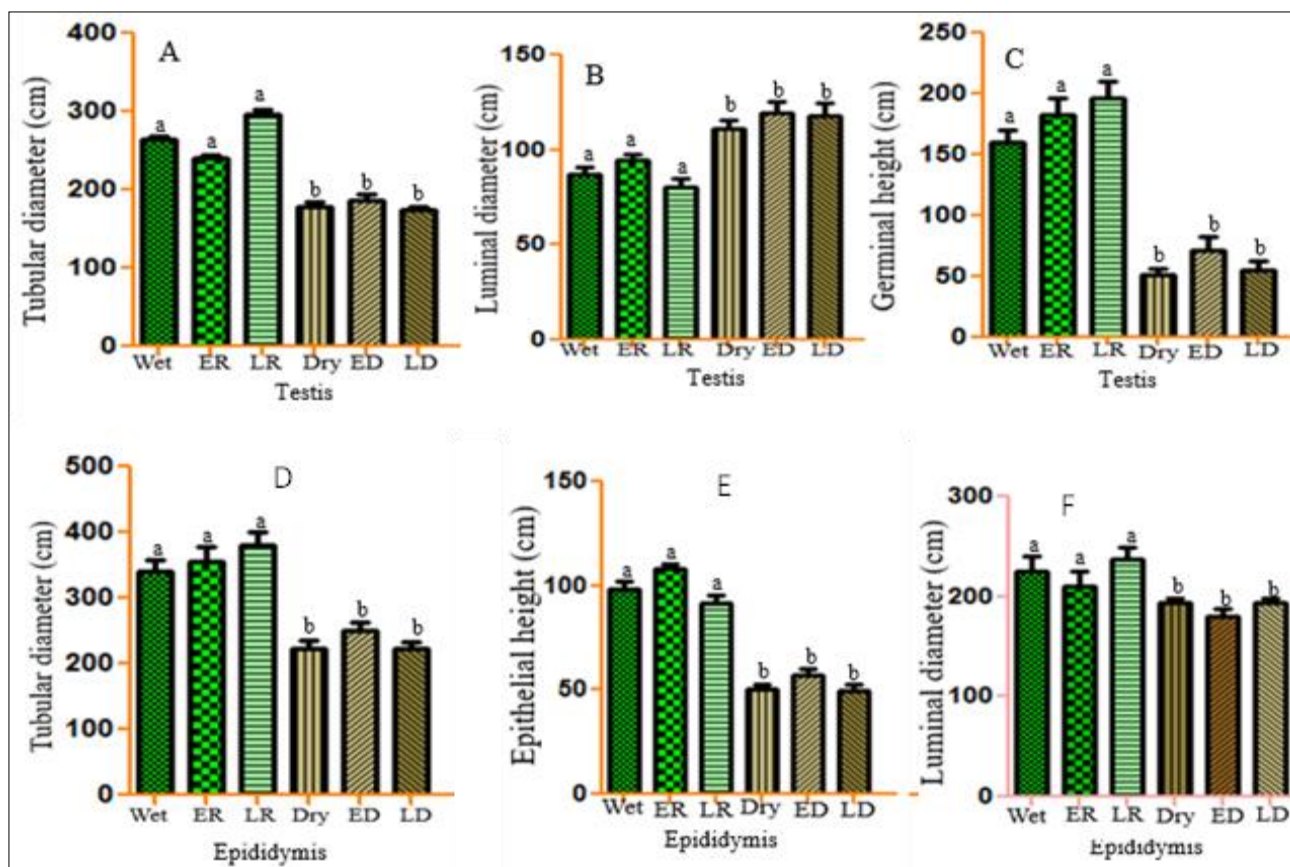


Fig 5: Chart illustrating seasonal variation in the seminiferous tubular diameter (A), the diameter of seminiferous tubules lumen (B), germinal height (C), the diameter of epididymis tubules (D), the diameter of epididymis lumen (E) and epithelial height of the epididymis of *E. franqueti* during the wet (early, late rainy) and dry (early and late) seasons of the year as seen in table 2. Bars with different alphabets varied significantly at $P < 0.001$

Sex Hormonal Profiles of the African Fruit Bat (*Epomops franqueti*) during the Months (seasons) of the year

The mean serum testosterone concentration of *E. franqueti* increased steadily up to March after and declined in May. From May, testosterone increased steadily to reach its peak in July. Then declined continuously up to October. From October to December, the testosterone concentration formed a plateau (Fig 6 A). *E. franqueti* shows seasonal variation in sex hormone production, which is pivotal to the morpho-physiology of the reproductive organs, being influenced by the availability of reproductive energy as demonstrated in *Molossus molossus*,^[35]. In this work, it was observed that the sex hormone profile followed a seasonal variation pattern. Testosterone (T) concentration in this fruit bat declines to the minimum level during the dry season in February with a low degree of sexual activities. Therefore, suggesting a low level of steroidogenesis and spermatogenesis in congruence with the report of Barros *et al.*^[36]. The mean serum concentration of luteinizing hormones of *E. franqueti* declined briefly from January to February with the least

luteinizing hormones recorded in February. It then increased steadily from the month of February to reach its peak in August. After that, it declined in October. It however increased up till December (Fig 6 B). The mean serum concentration of follicle-stimulating hormone of *E. franqueti* had the least value in the month of February as it declined briefly from the month of January. After that it increased slightly in February to form a plateau up till May. From May it increased sharply to reach its peak in July. Thereafter it declined in October then it increased, forming a plateau between November and December (figure 6 C). The serum concentration of luteinizing (LH) and follicle-stimulating hormones (FSH) reached their peak in the wet season when there was maximum rainfall and abundant food. This implies high reproductive activity. This trend is similar to the report of Kawamoto *et al.*^[37] in *Rhinolophus ferrumequinum*. The peak production of testosterone during the period of maximum wetness corresponds to the reports of Kawamoto *et al.*

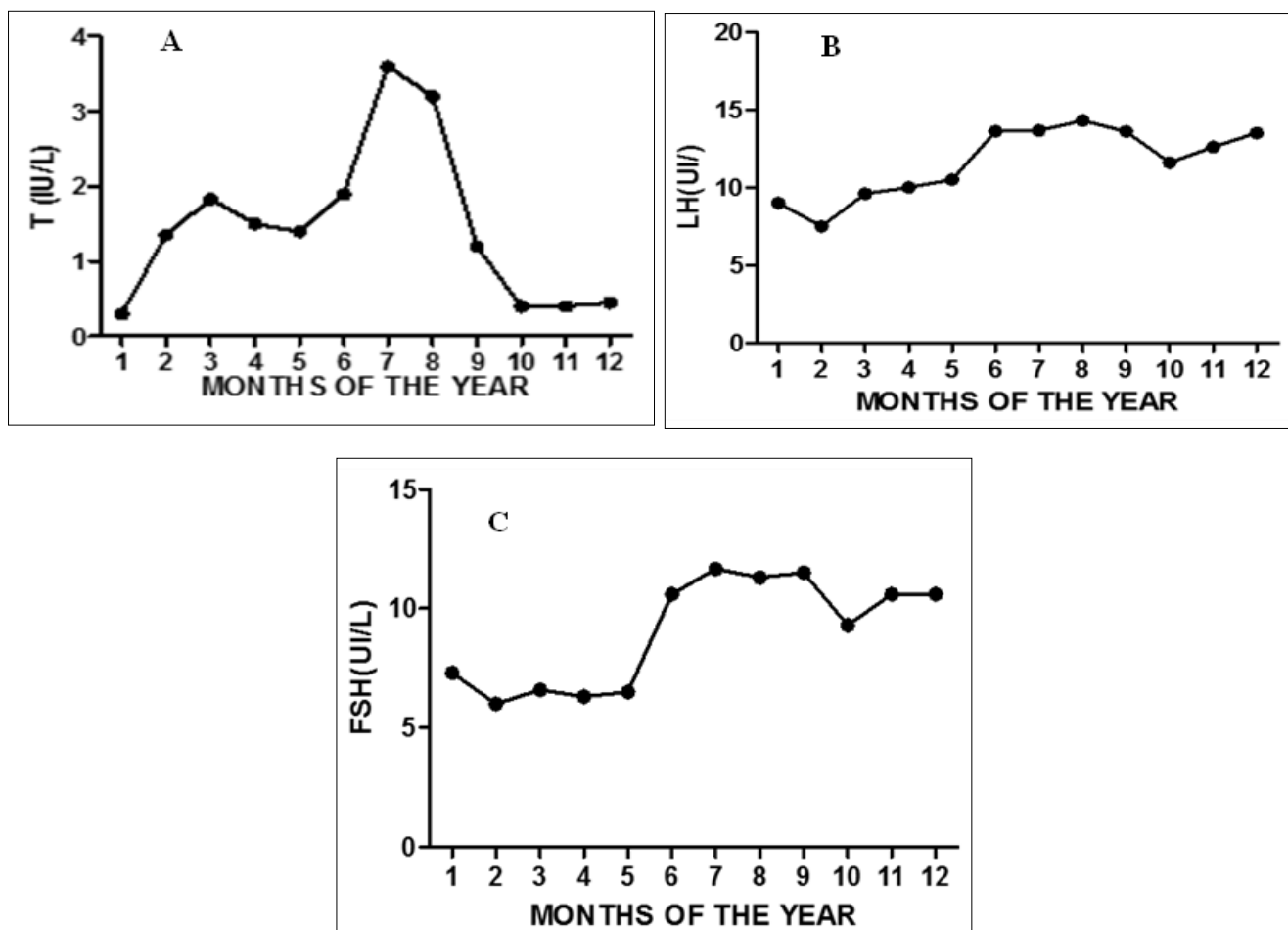


Fig 6: The mean serum concentration of sex hormones (A, testosterone, B, luteinizing and C, follicle-stimulating hormones) of *E. franqueti* during the months of the year.

Conclusion

This research work has been able to describe and show that: The late wet season had the highest gonadosomatic (0.20%) and epididymal mass (0.08%) indices, indicating that the testis is almost three times the size of the epididymis in the animal. Concentrations of testosterone, FSH and LH were least (0.25, 5.80, and 7.01 IU/L) and highest (3.80, 12.03 and 14.04 IU/L) during late dry and late wet seasons, respectively. The late wet season was therefore established as the peak

period of reproductive activities in the adult male *Epomops franqueti*. The reproductive biology of the *Epomops franqueti* is suggestive of a seasonal breeder.

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.

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