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GONADAL AND EXTRAGONADAL SPERM PARAMETER PROFILES IN THE AFRICAN FRUIT BAT (*EPOMOPS FRANQUETI*, TOMES, 1860).

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ABSTRACT

This study investigated the profile of testicular (gonad) and epididymal (extragonadal) sperm characteristics and spermiogram in Epomops franqueti bat caught during the peak (late wet season) of reproductive activities. Fifteen adult male African fruit bats (E. franqueti) trapped using Mist net at University of Ibadan, Nigeria (07° 26′N 03° 54′ E) were used for this experiment. Bats were anaesthetized using ketamine HCl (25mg/kg), incised via ventral abdomen to exteriorize testes and epididymides needed for the evaluations of sperm morphology, motility, concentration, livability ratio and morphometry. Morphologically, the spermatozoa head of E. franqueti under the light microscope were oval with the apex of the sperm head being conical. The entire length of the spermatozoa measured approximately 58.11 ± 1.84 µm. Generally, the head measured 8.97± 0.38µm in length and 5.91 ± 0.34 µm in diameter which varied significantly (P< 0.05). The relationship between the entire sperm length and the head length (r=0.566), the mid piece (r=0.283) and the tail (r=0.886) showed positive correlation. The epididymal sperm motility, livability and concentration were markedly increased compared to the testicular parameters. Also, both testicular and epididymal sperm abnormalities were strikingly lower than the range reported for most mammals. The set of data evaluated in this study provided the basis for the elaborate breeding potential of this bat species.

Keywords: *Sperm morphology, Spermiogram, Testis, Epididymis, Epomops franqueti*

INTRODUCTION

The mature spermatozoon is actively motile as it is a free-swimming cell in the seminal plasma (Talluri *et al*., 2017). The spermatozoon consists of the head and tail. The tail can be further divided into four segments: the neck, middle piece, principal piece, and end piece. All the segments are only slightly different in thickness under the light microscope (Bloom and Fawcett, 1975). Some authors reported three major segments in the mature sperm, each with its subsegment, which are head, mid-piece, and tail. The sub segments for the head are the acrosomal cap and the post acrosomal region while the tail is made up of the principal piece and the end or terminal piece (Oyeyemi and Babalola, 2006; Oyeyemi *et al*., 2009).

Generally, the mammalian spermatozoon head is flat and oval (ref). The sperm head in most rodent is hook shaped and called apical hook (Leblond and Clermont, 1952). The outline of the human sperm head is ovoid in frontal view and pyriform when observed on the edge, being thicker at the base near the neck and tapering towards the apex or tip. The head is 4-5µm in length and 2.5-3.5 µm in thickness (Bloom and Fawcett, 1975). The greater part of sperm head consists of the nucleus, whose chromatin has become greatly condensed to reduce its volume for greater mobility and protects its genome from damage during its transit to the egg for fertilization (Bloom and Fawcett, 1975).

Previous spermatozoa morphometric investigation conducted by Ogwuegbu *et al*. (1985) on the sperm cells of goats revealed a total length of 58.2 µm; while, the head, mid-piece and tail were: 8.2, 12.8 and 37.2 µm, respectively. Also, the head width is 3.8 µm. (Ogwuegbu *et al*., 1985). Massanyi et al. (2003) to be 34.9 µm. in whole length and 5.4, 6.8 and 23.4 µm. for the head, mid-piece and tail length, respectively. The functional relevance of the sperm cell mid piece length in competitive fertilization have been reported to influence its swimming and velocity (Firman and Simmons, 2010).

The principal piece of spermatozoon is in mammal is about 45 µm. in length and about 0.5 µm. thick at the base and tapered progressively towards the end piece (Bloom and Fawcett, 1975). This portion of the sperm cell helps in spermatozoa movement. The details of the mechanism of sperm tail movement have not been unraveled. However, it has been associated that the microtubules of the axoneme produce bending by a sliding mechanism comparable to that of

skeletal muscle, and the end piece is essentially identical to a simple flagellum or cilium.

Sperm morphology and morphometry are pivotal to theriogenologist as spermatozoa capacity to fertilize an ovum as well as their motility is all dependent on sperm head and tail parameters, respectively (Kolodzieyski and Danko, 1995).

Although there are reports on the sperm morphology and morphometry of several other mammalian species including man (Esteso *et al*., 2006, Kumar and Singh, 2015, Steinberg *et al*., 2019, Omirinde *et al*., 2019), there is paucity of reports on the gonadal and extragonadal sperm cell morphology and morphometry in bats, and especially in the African fruit bat, *Epomops franqueti*. The knowledge of the gonadal and extragonadal sperm morphology and morphometry is important to wildlife conservationist; therefore, this necessitated the investigation of sperm morphology and morphometry of the African Fruit Bat, *E. franqueti*

Materials and Methods

Experimental Animal

Fifteen adult male African fruit bats (*E. franqueti*) at the peak (late wet season) of reproductive activities were used for this experiment. The bats were trapped at University of Ibadan, situated at latitude 07° 26´N and longitude 03° 54´ E, using Mist net. The procedure of setting of the net and removal of trapped bats is in accordance with our previous report (Ekeolu *et al*., 2020). Briefly, a mist net of a mesh dimension 1´X 1´ with 50ft length and 35ft height was used. The net was suspended in a vertical and horizontal fashion to form a wall and a bed. The wall was made between two trees of 45ft apart. The bed was formed between two trees. The trees were 30ft apart so that the bed sags from the excess of 5ft. The wall was relatively taut compared to the bed. The wall of the trap was set across the regular flight path of the bats whose flight pattern was previously observed by (Ekeolu et al.,2020). When *E. franqueti* flew across the wall, it either got trapped (rarely) in the wall or fell into the bed (often). Once the wings got through the mesh, they got trapped. Wearing a tough leather glove, *E. franqueti* was immediately removed and put into a specially designed metal cage to avoid any form of injury to the animal. The bats were housed for 48hours in the metal cage with almond leaf-roof, mimicking its natural habitat. They were fed with almond fruits and water. They were anaesthetized using ketamine HCl at 25mg/kg body weight administered through the medial aspects of the

thigh muscle as described by Fard and Ghassemi (2014).

The animals were sacrificed by cervical decapitation. The right and left testes and epididymides were then quickly harvested and placed in Petri dishes that contain normal saline before carrying out the morphometric investigations. The weight of the testes and epididymides were measured with the aid of Digital Microvar® weighing balance. Some selected Petri dishes containing testis in normal saline were kept in the refrigerator for a period of 5minutes at a temperature of 4˚ C prior to staining of the sperm cells.

Sperm Morphometry

Spermatozoa morphometry was evaluated using the approach reported by Omirinde *et al*. (2019) in cane rat. Briefly, sperm morphometric parameters including head length (HLL), head diameter (HDD), mid piece length (MPL), Tail length (TLL) and Entire sperm length (ESL) were determined as follows;

HLL – is the vertical distance between the tip of the acrosome and the boundary with the neck of spermatozoa

HDD- is the longest horizontal distance between the two lateral edges of sperm head.

MPL – is the distance between the beginning and the end of the mid-piece

TLL - distance between the proximal end of the neck and the tip of the tail), sperm whole length TLL - is the distance between rostral tip of the sperm head and the pointed tip of the tail of spermatozoa.

The above stated morphometric parameters were analysed using Software from smear sections captured for morphological study. For each of the fifteen bats, 10 spermatozoa devoid of any morphological defects were selected totalling 150 spermatozoa.

Sperm motility

The surface of the testis was incised and a drop of semen from the testis was mixed with warm, 2.9% Sodium citrate buffer on a clean slide with cover slip placed on top. The slide and cover slip were warm before use. A percentage of progressive forward motile spermatozoan with unidirectional rectilinear motion was estimated by rapid observation of 8-10 low power microscope fields under X10 objective (Zemjanis, 1970).

Live/Dead Ratio

To estimate live-dead ratio, Omirinde *et al*. (2019) was adopted. Briefly, a drop of semen was placed on a warm slide and mixed with a drop of warm Eosin-Nigrosin stain

and therafter was examined under light microscope at X40 objective. Dead sperm cells picked up the stain and appeared purple while live sperm cells did not pick up the stain and appeared clear cells. Live/Dead count was determined from total count of 600 spermatozoa in smears stained with Eosin-Nigrosin.

Sperm Count

Sperm concentration was determined using improved Neubauer Haemocytometer (deep 1/10mm, LAMBART, Germany) in accordance with the method by Pant and Scrivastava (2003).

Morphological characteristics

From each of the testis and epididymis kept in the refrigerator for 5 minutes at a temperature of 4˚ C, smears were made and stained with Wells and Awa stain (0.2g of Eosin and 0.6g of Fast green prepared in distilled water and ethanol in ratio 2:1). Thereafter normal and abnormal spermatozoa were recorded. Also, site of defects in the abnormal spermatozoa (head, neck/mid-piece, tail) were observed and further classified as reported by Bloom (1973) and Parkinson (2001).

Statistical Analysis

The data obtained were analyzed using the GraphPad Prism version 5.0 for windows, GraphPad Software (GraphPad Prism, 2003). Differences between testicular and epididymal spermiogram parameters was established using Paired t- test. The relationship between the morphometric parameters was revealed with correlative coefficient tests. Results were expressed as Means and Standard Error of Mean (M ± SEM). The level of significance for all the analyses was set at $p<0.05$.

Results

Morphological Characteristics and Morphometry of Spermatozoa in the Epomops franqueti

Spermatozoa Morphology and Morphometry in the *Epomops franqueti* bat

The morphology of *Epomops franqueti* spermatozoa investigated under light microscopy is like that of human with an oval shaped head that is conical at the apex (Figures 1 & 2). The entire length of the spermatozoa measured approximately 58.11 ± 1.84 µm (Table 1 & Figure 2B) while the head measured 8.07± 0.38µm in length and 5.91 ± 0.34 µm in diameter being insignificantly different while other mean values were significantly different at $P<0.05$ (Table 1 & Figure 2B).

The relationship between the entire sperm length and the head length

(r=0.566), the mid piece (r = 0.283) and the tail (r = 0.886) was a positive correlation. Also, there was a positive correlation (r = 0.341) between the length of the head of the spermatozoa and the diameter of the head of the spermatozoa. However, there was a negative correlation (r = -0.174) between the tail and midpiece length (Table 2).

Testicular (Gonadal) and Epididymal (Extragonadal) Sperm Parameters

The percentage sperm motility was significantly higher ($p<0.05$) in the epididymis than in the testis. Also, the percentage live to dead spermatozoa, sperm concentration and count were significantly increased ($p<0.05$) in the epididymis of *Epomops franqueti* when compared to their testicular counterparts (Table 3).

The difference in the total abnormal sperm cells in both the testis (3.4%) and epididymis (5.6%) were insignificant, and, also, there was no significant difference in the total normal sperm cells in the testis (96.6%) and epididymis (94.4%) at $P<0.05$ (Table 4).

Table 1: Morphometric parameters of the spermatozoa of *E. franqueti*

HLL (µm)	HDD (µm)	MPL (µm)	TLL (µm)	ESL (µm)
8.07± 0.38[a]	5.91 ± 0.34[a]	4.07 ± 0.40	36.30 ±1.40[b]	58.11 ± 1.84[c]

Means with different superscripts within row are significantly different at $P<0.05$

HLL- Head length, HDD- Head diameter, MPL- Mid piece length, TLL- Tail length, ESL- Entire sperm length.

Table 2: Correlation coefficients of the morphometric parameters of the Spermatozoa of *E. franqueti*

	ESL	HLL	HDD	MPL	TLL
ESL	1.000				
HLL	0.566	1.000			
HDD	0.278	0.341	1.000		
MPL	0.283	0.042	0.031	1.000	
TLL	0.886	0.076	0.255	-0.174	1.000

HLL- Head length, HDD- Head diameter, MPL- Mid piece length, TLL- Tail length, ESL- Entire sperm length

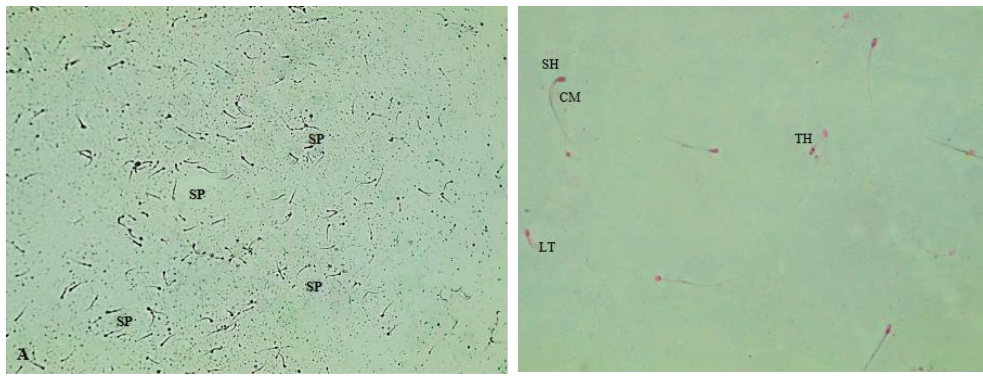

Figure 1: *Photomicrograph of the spermatozoa in the epididymis of E. franqueti showing the various morphological characteristics. CMP: Curved mid piece; TH: Tailess head; SH: Swollen head; LT: Looped tail. Magnifications: X 40 (A) and X400 (B); Stain: Eosin-Nigrosin)*

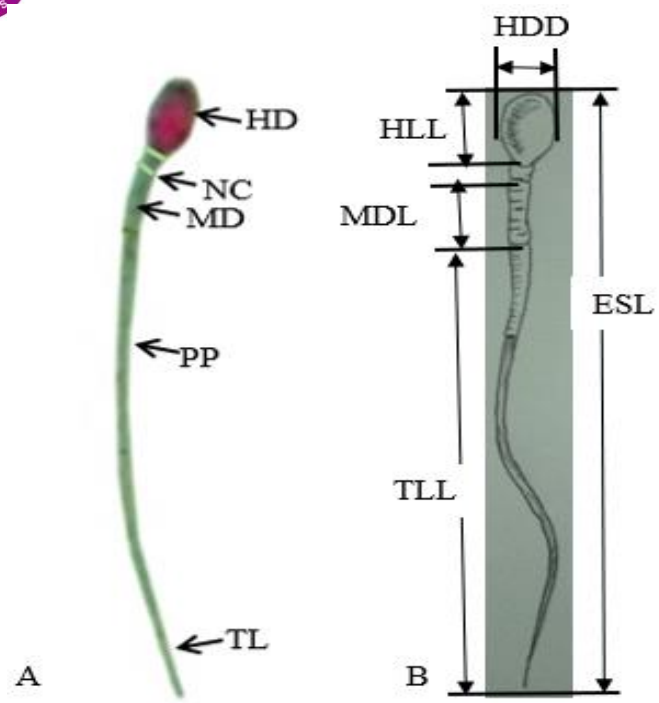

Figure 2: *The morphological appearance of E. franqueti spermatozoa. A – the sperm cell of E. franqueti stained with eosin and nigrosine showing the basic components: the head (HD), the neck, (NC), the mid piece (MD), principal piece, (PP) and the tail (TL). X400. B – Schematic illustration of E. franqueti spermatozoa. ESL - Entire sperm cell length, HLL - Sperm cell head length, MDL - Midpiece length, TLL - Tail length, HDD - Sperm cell head diameter*

Table 3: The distribution of sperm parameters in the testis and epididymis of *E. franqueti*

	Testis	Epididymis
Progressive motility (%)	Not motile	68.25±3.62
Percentage Live Spermatozoa	49.06±5.01^{a}	78.06±0.15^{b}
Sperm Concentration (X10^{6}/ml)	52.06±4.12^{a}	72.14±0.39^{b}
Sperm count (X10 ^{6})	39.06±3.02^{a}	63.06±2.12^{b}

Means with different superscripts within row are significantly different at P<0.05

Table 4: Mean percentage values of the sperm morphological abnormalities in the testis and epididymis of *E. franqueti*

	WT (g)	BTL (%)	CMP (%)	THD (%)	CTL (%)	HTL (%)	BMP (%)	LTL (%)	RTL (%)	SHD (%)	TAS (%)	TNS (%)
T	0.170 ± 0.001	0.41	0.49	0.41	0.57	0.57	0.41	0.41	0.25	0.33	3.4[a]	96.6[b]
E	0.069 ± 0.001	0.67	0.58	0.65	0.37	1.34	0.67	0.22	0.45	0.52	5.6[a]	94.4[b]

Means with different superscripts within row are significantly different at $P<0.05$

T-Testis, E-Epididymis, BTL-Bent tail, CMP-Curved mid piece, THD-Tailless Head (Normal head without tail), CTL-Curved tail, HTL-Headless Tail (Normal tail without head), BMP-Bent mid piece, LTL-Looped tail, RTL-Rudimentary tail, SHD-Swollen head, TAS-Total abnormal sperm, TNS-Total normal sperm.

Discussion

The sperm cell of *E. franqueti* is similar to those reported in the primates (Steinberg *et al*., 2019), therefore this light microscopy study of *E. franqueti*'s sperm cell has been able to establish strong morphological resemblance of the sperm cell to that of other mammals, especially man. Although, study conducted by Beguelini *et al*. (2011) on chiroptera (*Platyrrhinus lineatus*) sperm morphology and morphometry contrarily showed small arrow like head whereas that of *E. franqueti* is oval. This observation suggests the existence of phylogenetic differences as *E. franqueti* is a megachiropteran while *P. lineatus* is microchiroptera.

Comparing with small mammals, the sperm cell heads of *E. franqueti* unlike in most rodents (Breed *et al*., 2014) lack hooked-like structure. However, this finding corroborates the report of Olukole *et al*. (2014) on the African greater cane rat. Also, our study showed that the head length of the sperm cell of *E. franqueti*, 8.07± 0.38µm, falls within the range of most mammalian sperm cell head length, 4-12µm (Forman *et al*., 1989). However, when compared with other chiroptera, the sperm head length of *E. franqueti* is larger than that of *Pipistrellus subflavus* (Forman, 1968) but almost half the length of Bulldog bat, 15.70µm, reported by Forman *et al.* (1989). This African fruit bat has a sperm head length that is twice the sperm head length, 4.48 µm of *Artibeus jamaicensis*, Jamaican fruit bat (Forman and Genoways, 1979). The volumetric

morphology of sperm head has effect on the sperm cell velocity (Gomendio *et al.*, 2011). The sperm head length of this African fruit bat is however approximately equal to the sperm head length of other pteropodids: *Pteropus conspicillatus*, *Pteropus neohibernicus* and *Dobsonia praedatrix* which are 7.70µm (Rouse and Robson, 1986), 7.58µm and 7.83µm (Forman *et al*., 1989), respectively. This indicates their phylogenetic similarities as megabats.

The sperm cell neck generally connects the head to the mid piece as reported for other mammals (Gottreich *et al*., 2001). Under light microscope, the details of the neck where it connects the head with the midpiece were not clear. Although the midpiece is cylindrical and continues with the tail, tapering to the end point with no visible surface features under light microscope. This midpiece light microscopic feature in *E. franqueti* corresponds to the report on other species of bat (Forman *et al*., 1989). The mid piece of *E. franqueti* is rather short. The sperm head length recorded in this study seemed to be twice the length of the mid piece. This suggests that *E. franqueti* sperm cell swim faster and thus validates the report of Malo *et al*. (2006). It however, contrasted the report of Forman *et al*. (1989) on *Noctilio albiventr* in which the mid piece was observed to be longer than the head.

The observed tail length (36.30 ± 1.40) µm in *E. franqueti* sperm cell investigated appeared to be close to ten-times its midpiece length (4.07 ± 0.40) µm. This finding substantiates the morphophysiological assumptions of being a source of high energy for propelling force when sperm cell swims in the female reproductive tracts earlier documented by Milki (2007). The value of the head length in this study is closer to what was reported for the yellow shoulder bat with head length of 4-24 µm while the big fruit-eating bat has a head length value of 3.80 µm which is less than those reported for *E. franqueti* (Cummins and Woodal, 1985). The value reported for tail length in *E. franqueti* is lesser than those reported for little red flying fox with a value of 76.0 µm (Cummins and Woodal, 1985) but higher than that of black flying fox with a value of 40.0 µm (Cummins and Woodal, 1985) even though they are phylogenetically similar to *E. franqueti*.

The strong positive correlation existing between the entire sperm length and its various divisions (head, mid piece and tail lengths) further indicates that the different divisions of sperm cell contribute to its overall structural and functional needs.

Sperm morphology is an important parameter that indicates the extent of normality and maturity of the spermatozoa population contained in an ejaculate and be associated with the fertility potential of an animal (Memon *et al.*, 1986). Thus, the remarkable reduction observed in the percentage abnormal sperm cells in both the testes (3.4%) and the epididymis (5.6%) of *Epomops franqueti* is apparently lower than the suitable percentage which is less than twenty percent (<20%) reported for most mammals (Moss et al., 1979; Wilde *et al*., 1999; Omirinde *et al*., 2019). In line with this, it could be inferred that the reduced percentage abnormalities might not elicit substantial effect on the breeding soundness of this *spps* of bat. The abnormalities of the head and mid-piece as well as tail have been classified as primary and secondary defects of spermatogenesis respectively (Schumacher and Moll, 2011), and arise during testicular or epididymal degeneration respectively (Bloom, 1950; Ajitkumar et al., 2011). These defects especially the primary defects are more likely to be associated with decreased fertility (Schumacher and Moll, 2011). Though, an insignificantly increased proportions of head (tailless head, headless tail and swollen head), midpiece (curved and bent) and tail (rudimentary tail) abnormalities were observed more in the epididymis than in testis of this bat; but, the population of the different category of the abnormal sperms are not significant enough to initiate fertility reduction.

The spermiogram (sperm motility, livability and concentration) parameters are essential in breeding and have been documented to positively correlate with the fertilizing capability of sperm cells (Oyeyemi and Ubiogoro, 2005; Oyeyemi and Okediran, 2007). Therefore, the significant elevation in the spermiogram values in the epididymis of *Epomops franqueti* bat relative to its testicular values further substantiates the assertion stated above.

This study has laid to bear profiles of sperm morphometry, morphology and spermiogram parameters in *Epomops franqueti* bat investigated. From the available data, epididymal sperm motility, livability and concentration were markedly increased compared to the testicular parameters. Also, both testicular and epididymal sperm abnormalities were strikingly lower than the range reported for most mammals. These measured parameters provided the basis for the elaborate breeding potential of this animal.

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