



Relationship Between the Amount of Subcutaneous Fat, Testicular Morphometry, Epididymis and Some Sperm Parameters in *Leptonycteris yerbabuenae* Bat Before, During and After Mating

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Abstract: Studies in reproductive age mammals show that the increase in fat content is inversely related to sperm quality in response to a decrease in testosterone levels. In turn the decrease in androgen concentration is related to morphophysiological involution in the testis and epididymis. However, the bat *Leptonycteris yerbabuenae* increases the body fat content previous the mating period, contrary to what was reported in most mammals. Therefore the aim of this study was to determine the importance of variation in fat content in *L. yerbabuenae* in relation to testicular and epididymal cycle; and sperm parameters. The results clearly show an increase in the fat content as it approaches the mating season. Also, there is an increase in the size of the testis and epididymis. In the post-mating months, both: fat and testis, decrease and the sperm cells were not presents. *Leptonycteris yerbabuenae* has a different behaviour to most mammals, accumulate fat while producing, transit and sperm storage could be happening. So, in this model, we must do studies that meet these processes emerging from that reported in other mammals.

Keywords: Fat, Testis, Epididymis, Sperm, Bat Reproduction

1. Introduction

Leptonycteris yerbabuenae is a nectarivorous bat, inhabits desert and tropical deciduous forest in the northern hemisphere environments, from southern USA, through México and reaches the Salvador in Central America. In this area the species is composed of two groups or population with different breeding and behavioral period [1-2]. Populations distribution above parallel 21°N behave as a latitudinal migrant and births occur during the spring [1, 3, 4], while populations below that parallel are local residents or regional migrant and births occurs at autumn [12, 5]. Members of the family Phyllostomidae are restricted to the neotropics, except *Macrotus waterhousii*, *Leptonycteris nivalis* and *L. yerbabuenae*, species that reach to the Southern United States, in a subtropical area [6]. So far it is

not known that a phyllostomid bat hibernates, therefore, it's interesting that *L. yerbabuenae* has subcutaneous fat accumulation. Originally it was proposed that the accumulation of fat by *L. yerbabuenae* was related to the latitudinal migration that some populations of this species make to breed in Northern Mexico and the southern United States [3, 7] however it was curious that both sexes accumulate fat, even when it know that only females perform the migration journey to northern Mexico and the southern United States for reproductive purposes, while males movements is unknown [3,6]. Another interesting fact registered, at least in populations of Central and Southern of Mexico, is that the males accumulate more fat than females [4, 5]. Martínez-Coronel et al. [6] found that in a resident colony of *L. yerbabuenae* of Chiapas, México, the fat accumulated by males is used for breeding and not for

migration [1, 3, 6]. It was also found that compared to other species of bats that accumulate fat, in *L. yerbabuenae*, the increase of fat coincides with the increase in the size of the testis, and considering that increase testicular could be an indicator of spermatogenesis [7], then these observations are contradictory to those reported for most of other species of bats [8] but *Pteropus poliocephalus* and *P. scapulatus* [9, 10].

It has been reported the functions of adipose tissue as energy reserve, but also as endocrine organ, having an influence on hormones and adipokines derived from adipose tissue that relate to the reproductive organs and its hormones [11]. However, the total and intra-abdominal subcutaneous fat, is associated with low testosterone levels and high levels of free and total estradiol [12-13]; altering and reducing the sperm concentration [14], and erectile dysfunction causing an increase in scrotal temperature [15].

For example, in the *Scotophilus heathii* bat has been reported that serum leptin levels are positively correlated with body fat and negatively related to reproductive function [16-17].

Not only that, it is important to note that the testis and epididymis functions in most mammals, is chaired by androgen, since in the absence, a decrease in testicular and epididymal weight is observed, is induces apoptosis in the epididymis starting with the head region and progressively moving up the caudal region of the same [17-18].

Due to the different effects that causes fat accumulation in mammalian reproduction and that in *L. yerbabuenae* has been observed a different behavior than other species analyzed in this aspect, this paper was aimed at evaluating the accumulation of fat, body morphometry, testicular and epididymal sperm parameters of this species during the previous period, during and after mating.

2. Methodology

2.1. Study Area

The cave "Los Laguitos" is located 4 km NW (16° 46'42" N, 93°08'55" W), Tuxtla Gutierrez, Chiapas, 781m a.s.l. The average temperature of the cave is 32.3°C and relative humidity is 95.4%, which is classified as a "hot cave" [20]. The local climate is warm humid with summer rains, with rainfall less than 60 mm in the driest month, with winter rain less than 5% of annual and annual temperature around 24.7 °C [21]. The vegetation surrounding the cave corresponds to a Tropical Deciduous Forest [22]. During the study period the cave was inhabited by *Mormoops megalophylla*, *Pteronotus davyi*, *P. parnelli*, *P. personatus*, *Artibeus jamaicensis*, *Glossophaga soricina*, *L. yerbabuenae* and *Natalus stramineus*.

2.2. Ethics Statement

L. yerbabuenae is not listed as an endangered species in the Norma Oficial Mexicana NOM-059-ECOL-2010 SEMARNAT-2010 for the protection of native wild species of México (SEMARNAT, 2010). All protocols for the capture and

handling of the live animals as well as for the euthanasia procedure used in this study were conducted in strict accordance with the guidelines established by the American Society of Mammologists for the use of wild mammals in research [23] and were approved by the Ethics Committee of our institution. The capture of specimens studied was approved by the scientific collector's license FAUT-0070 issued to Yolanda Hortelano Moncada by the Dirección General de Vida Silvestre de México (an agency of SEMARNAT).

2.3. Fieldwork

Three adult males of *L. yerbabuenae* were captured every month from May to October 2014 using an expand hand-net (Bioquip Tropic net) in a cave. During transport to the laboratory, bats were fed, ad libitum, with fruit puree, brand: "Gerber, Nestlé" apple and tropical fruit diluted in water. The age of the bat was estimated on the fusion of the cartilaginous epiphysis of the metacarpal-phalangeal joint of the 4th digit [24].

2.4. Laboratory Work

Bats were sacrificed by decapitation to obtain serum (under study), the rest of the body was dissected to remove the testis and epididymis. From each organ, wet weight and size, was measured. Subcutaneous fat was also removed and its weight recorded. Under a stereoscopic microscope each epididymis was sectioned in its three principal regions: head, body and tail, and the weight of each region was recorded.

Both epididymal segments of the same region (right and left caput; corpus, and cauda) were pooled and minced with scissors followed by gentle stirring in 1 ml of Ringer solution at 34°C to provoke the output of sperm [25]. Subsequently, the minced material was collected with a Pasteur pipette and filtered by passage through a 20- μ l diameter weft lycra mesh (98% Nylon/Polyamide, 2% Lycra/elastane), and the fluid was collected in a 2-ml Eppendorf tube [26]. The sperm suspensions were washed twice by centrifugation at 500 \times g for 5 minutes. The supernatant was removed, and the cell pellet was resuspended with 1 ml of Ringer solution. Determinations of sperm number, viability, and morphology were carried out using standard microscopic procedures.

2.5. Statistical Analysis

The Pearson index was applied to determine the correlation between body mass, fat, testicular mass and epididymal mass.

3. Results

3.1. Body Mass and Amount of Fat

The changes that showed the individuals mass over time, were related to the amount of stored fat ($r = 0.90$; $R^2 = 0.82$; $F_{1, 17 \text{ d.f.}} = 74.78$; $P = 0.0000$). The presence of subcutaneous fat was evident in March and increased at the same time of body mass until late May when start the mating period,

during June, fat content and body mass decreased sharply in the following months the two variables continued to decline (Figure 1).

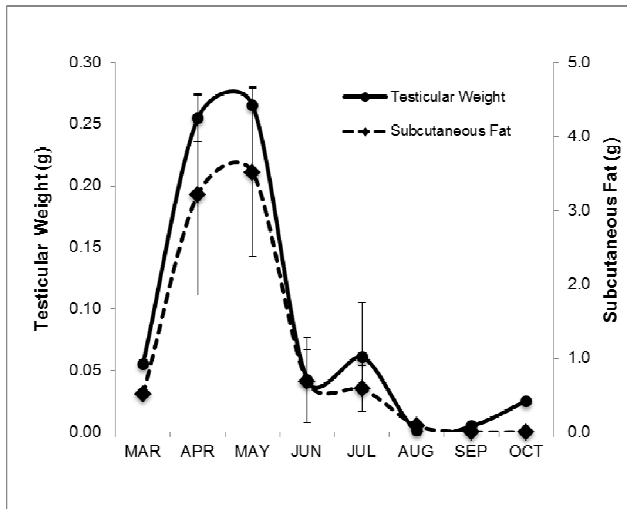


Figure 1. Temporal variation in body mass and subcutaneous fat accumulation of *L. yerbabuenae* males in the cave "Los Laguitos", Chiapas, Mexico.

3.2. Body and Testicular Mass

Like the fat content, the testicular mass showed changes related with the body mass of the individuals ($r = 0.73$; $R^2 = 0.53$; $F_{1,18 \text{ d.f.}} = 19.75$; $P = 0.0004$). The testes increase from March to May, when they reached their highest value, then declined sharply in June and continued to decline in the coming months (Figure 2).

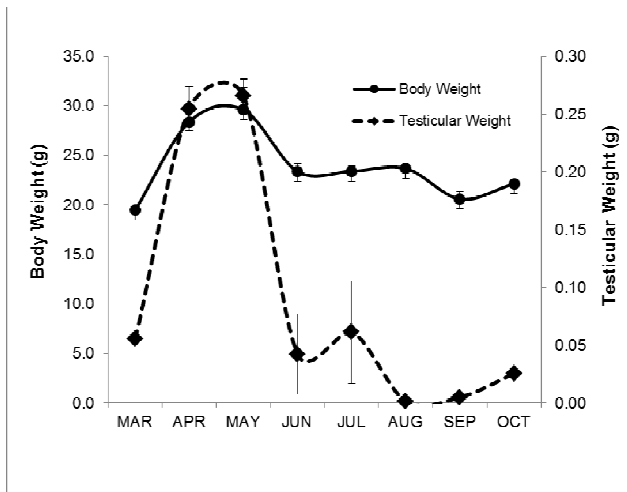


Figure 2. Temporal variation in body mass and testicular mass of *L. yerbabuenae* in the cave "Los Laguitos", Chiapas, Mexico.

3.3. Testicular Mass and Epididymal Mass, and the Subcutaneous Fat Quantity

These three variables showed similar changes over time, prior to mating, the three variables increased their value and peaked in May, when start the mating period. During June, at the time of mating, declined sharply observed this trend until

October (Figures 3, 4). The correlation between testicular mass and subcutaneous fat was high ($r = 0.75$; $R^2 = 0.57$; $F_{1,17 \text{ d.f.}} = 21.82$; $P = 0.003$) as well as, testicular and epididymal mass ($r = 0.97$; $R^2 = 0.94$; $F_{1,17} = 297.86$; $P = 0.0000$) were high.

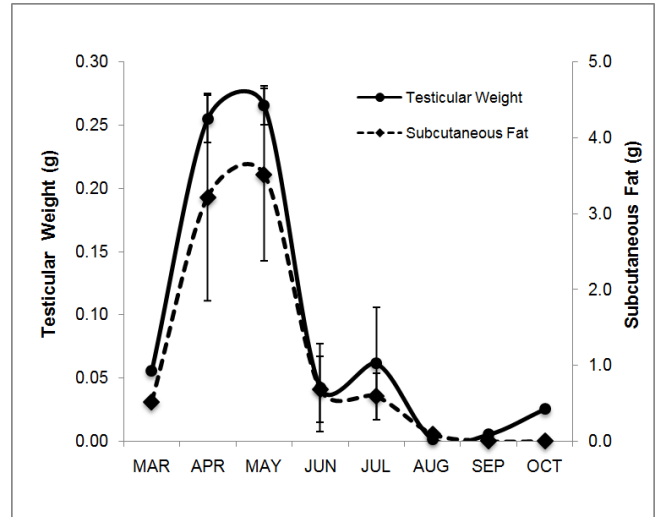


Figure 3. Temporal variation in testicular mass and subcutaneous fat accumulated by the males of *L. yerbabuenae* in the cave "Los Laguitos", Chiapas, Mexico.

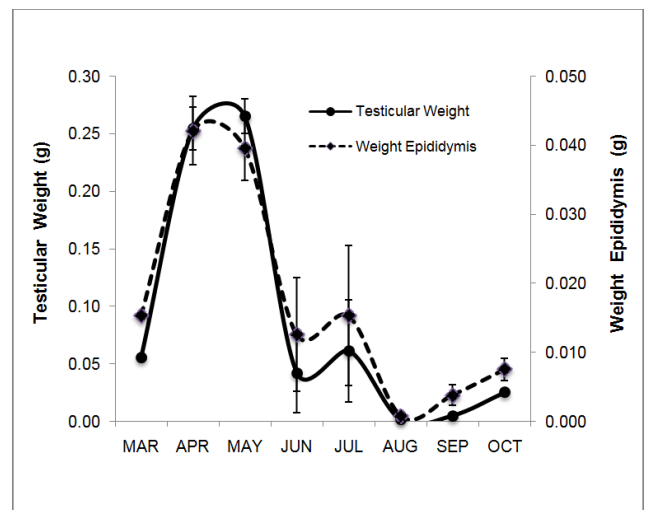


Figure 4. Temporal variation in testicular mass and epididymis of *L. yerbabuenae* in the cave "Los Laguitos", Chiapas, Mexico.

3.4. Sperm Parameters

The presence of sperm in epididymis was only detected in specimens of April and May (Table 1), before and just at the beginning of the breeding season. In some specimens of June 30 and July 30 the presence of sperm was detected, but it was impossible to count because they were isolated, and which were observed degenerates with necrotic appearance. The highest sperm concentration in April and May were found in the tail of the epididymis (108.50×106), followed by the body (75.24×106) and head (55.73×106). Also, the greater amount of alive sperm was found on epididymal cauda (79.83%), while the highest mortality was found to occur in

the body (33.33%).

Table 1. Estimated average sperm concentration ($\times 10^6$) stored on different parts of the epididymis, and the percentages of live (V) and dead sperms (M) for each region

	Caput	Body	Cauda	CAPUT		BODY		CAUDA	
				V	M	V	M	V	M
April	81.67	118.75	176.67	67.66	32.33	60.67	39.33	84.67	15.33
May	29.80	31.73	40.33	72.00	28.00	72.67	27.33	75.00	25.00
Average	55.73	75.24	108.5	69.83	30.17	66.67	33.33	79.83	20.17

4. Discussion

Several studies have investigated the relationship of the increased in body fat with conventional sperm parameters according to the WHO, i.e. concentration, motility and sperm morphology [27-29]. Finding a negative effect on these parameters, i.e. sperm count decreased, motility and a DNA fragmentation index increased.

Some of the mechanisms that are involved in male infertility due to a fat quantity increase are: changes in the hormones concentration such as reduction of inhibin-B and androgen levels, accompanied by estrogen elevated levels [15]. This is because the total fat, intra-abdominal and subcutaneous is associated with low levels of free and total testosterone in men. This decrease is explained by overactivity of the enzyme cytochrome P 450 aromatase expressed at high levels in white adipose tissue and it is responsible for being a key in the biosynthesis of estrogens [12].

The integrity and physiology of the testis and epididymis are androgen dependent and is observed that in the absence of these, in testis and epididymis loss weight occurs, affects seriously the gene expression and induce to apoptosis [18-19]. Based on this, it would expect that if a male mammal begins to accumulate fat, the testis would show minimal activity, and the sperm cells would be of poor quality. However, in *L. yerbabuenae* happens that while fat accumulates, it also increases the size of the testis and epididymis, as well as the quantity and quality of sperm stored in the epididymis, prior to the mating period that start in late May and through June [6]. This seasonal phenomenon of fat accumulation and testicular activity increase, in mammals, is not new, was described originally for primates [30], and is known as "fatted male phenomenon", has also been reported in *Pteropus poliocephalus* [10] as well as in large mammals like ungulates [31], pinnipeds [32] and ursids [33], among others. However, here is the first time that the ratio between fat and sperm parameters was studied.

In *L. yerbabuenae*, body mass is reduced in June, and is related to reproductive activity [6], and not with migration as other authors proposed [1, 3], because males and females make up a mating colony inside the cave and individuals do not go out to forage at night, mainly males [6], and in the case of the last point mentioned, energy expenditure can be associated with spermatogenesis, courtship and for defense.

Unlike *P. poliocephalus*, the males of *L. yerbabuenae* are not territorial, and they do not integrate harems, although it

was noted that they can spend hours waiting for the opportunity to copulate while showing agonistic behavior with other males [6, 9].

Traditionally, the testis size had taken as a sign of fertility, especially in ecological studies. This criterion is based that for sperm production there should be seminiferous epithelium proliferation, an increase that should be reflected in the overall size of the testis. Different studies such as those conducted in *L. yerbabuenae* [34], *Artibeus jamaicensis* [35] and *Myotis nigricans* Beguelini et al. [36] provide data, that until more testicular mass, spermatogenesis occurs. In the present study, there was not testis histology, but we found that before the start of the breeding season was increased testicular mass and storing sperm in the epididymis was confirmed in samples of the months April, May and June. After mating we observed the testis regress, while the degenerate sperm were observed in samples of June and July. These results agree with those reported in a histological study in a population of *L. yerbabuenae* of Chamela, Jalisco [34]. Also, in this study we found that the testicular mass varied seasonally similar to body mass, indicating a close relationship between spermatogenesis and the individual condition, as in *P. poliocephalus* [10].

Sperm storage is a strategy that, some bat species have developed, to ensure fertilization, associated with different reproductive strategies, especially in those with different synchrony of gametogenic cycle in females and males, and temperate distribution [37]. But so have some species of tropical distribution as *Lasiurus ega*, *Rhinopoma hardwickei*, *Scotophilus heathi* and *Hipposideros speoris* [37]. The results found in *L. Yerbabuenae* indicate no sperm storage in the epididymis because, as soon as ends the mating period (May-June), the regression of epididymal and testicular tissue is observed, and sperm cells degenerate. These results agree with those reported for a population of the same specie of Jalisco, Mexico [34] as well as general knowledge that exists for most tropical species [37]. There is also evidence that the sperm storage do not occur in females, because was found female pregnant in the first days of July, indicating that fertilization of ovum occurs immediately after the copula [6].

The concentration and sperm viability found in *L. yerbabuenae*, compared with reports for the cauda epididymis of *Artibeus jamaicensis* ($54.79 \pm 88.14 \times 10^6$) and *Sturnira lilium* ($51.80 \pm 25.93 \times 10^6$) [38] indicate that *L. yerbabuenae* has a higher sperm concentration ($108.50 \pm 93.94 \times 10^6$). These differences in members of the same family may result

from the different reproductive strategies that each species have, the first two species are bimodal polyestric, however *L. yerbabuenae* is seasonal monoestric [38].

Another consequence of the body fat increase, was the scrotal temperature increase [15], since fat is also deposited in the scrotal region, causing an increase in temperature in this region. A slight increase in scrotal temperature can disrupt spermatogenesis and cause fertility problems [39–42], caused in part by mitochondria degeneration, smooth endoplasmic reticulum dilatation and enlargement of the intercellular spaces between Sertoli cells and spermatids; directly associated with oxidative stress, followed by germ cell apoptosis [43].

However, the results in this study of fat amount, the size of testis, epididymis and presence of sperm in *L. yerbabuenae*, differ from those papers published in another mammals, because in fieldwork we registered higher scrotal temperatures ($\bar{x} = 38.84 \pm 0.23$, $n = 9$) in June 16th than body temperature ($\bar{x} = 36.21 \pm 0.09$, $n = 9$) during mating period. However, more studies are necessary, like the register of body and scrotal temperatures, during their annual cycle, and adaptive mechanisms that may be counteracting the scrotal hyperthermia, even in reproductive stages, among which may be acting: the system countercurrent heat exchange between pampiniform plexus and testicular artery; protection mechanisms against hyperthermia as the system "unfolded protein response" [44] and antioxidant mechanisms that protect against oxidative stress.

To achieve a better understanding of what happens in *L. yerbabuenae* in relation with the accumulation of subcutaneous fat and reproductive function, is necessary determine biochemical markers of obesity, and profound studies of testicular and epididymal function and sperm functionality.

5. Conclusions

The results obtained in *L. yerbabuenae* showed that in this species the fat accumulation is positively related with testis increase. These results open a number of questions that actually not been raised in relation to leptin-testosterone metabolic pathways proposed.

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