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Effects of natural environmental conditions on testicular Histometric Dynamics of Bats (*Pipistrellus kuhlii*)

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Abstract
The present study was aimed to elucidate the actual period when male bats are reproductively active and determine its high and low spermatogenic activity. Total of seven specimens of *Pipistrellus* bats were captured from four different localities of Faisalabad District of Punjab, Pakistan. Histological sections were prepared by paraffin tissue preparation technique and stained by hematoxylin and eosin (H&E). Morphometric analysis of specimens was performed by Image J® analysis software. Statistical data showed significantly (P<0.001) higher values of studied parameters of testis in peak spermatogenic phase (September) than in low (December) spermatogenic phase. Histomicrographs showed that males, collected in August, had large number of spermatids with very few or no spermatozoa in the epididymis. Testes showed no testicular activities in the winter hibernation period. The weight of testes was relatively higher in August which declined gradually from December to February. It is conceivable from the present data that season has a substantial impact on the testicular activity in bats (*Pipistrellus kuhlii*). There is an annular testicular cycle which is followed by the bats. The testicular activity showed a peak during August and September which declines gradually from December to February. Testes showed no testicular activities in winter hibernation.

Keywords: Bats, spermatogenesis, testes, histometry, season

1. Introduction

Bats (Order Chiroptera) constitute one of the most successful and the most diverse mammalian order, almost 87% of the bats belong to suborder Microchiroptera which has a worldwide distribution and exhibit a most dietary niche being insectivorous, frugivorous, nectarivorous, carnivorous and omnivorous [1]. Bats are present mostly in moderate and tropical region, avoid extreme cold and polar zones and from some remote island [2]. Almost 1250 living bat species constitute about twenty percent of all well-known living species of mammals and comprise the 2nd most species group of after rodents. The number of bat species is greater than the combined sum of all the remaining mammal species in a few hot and humid region of the world [2]. *Pipistrellus kuhlii* ranges from southern Europe to India; also most of Africa, from Morocco to Egypt and South to South Africa [3]. In Pakistan, Muzaffargarh, Rajanpur, Lyallpur, [4] Multan, Baluchistan, Panjgur and Darzi chach. Sind: Kashmor, Hydrabad, Mirpur and Sukkur little is known about this species in the Indian subcontinent. It is geographically more wide spread and abundant then *P. tenuis mimus* and *P. ceylonicus* in the Indus plain and being best adopted in areas of the open desert and is not depend on trees and not endemic subspecies [4].

Bats use number of reproductive strategies in order to regulate their physiological conditions to the different environmental and ecological conditions [5]. Delayed ovulation and fertilization such as in *Pipistrellus ceylonicus* [6], delayed implantation in *Rhinolophus rouxi*, and embryonic diapauses in *Artibeus jamaicensis* are the reproductive strategies that are used by bats to face all extremes of environment [7].

Sperm storage by mammals is almost completely limited to the members of the Chiroptera. Some of the bat species can store sperm for few days to several months [8]. Many species of genus Pipistrellus and family Vespertilionidae have been found to store up the spermatozoa in their reproductive tracts. These species include *Pipistrellus subflavus, P. rusticus, P. savii, P. coreensis, P. endoi* [7]. *Pipistrellus kuhlii* has been recorded from different area in Middle East Africa, Kashmir and southwest Europe [9]. This vast division may conceal many climatic regions. About no data is accessible on biology of reproduction of *pipistrellus kuhlii* [10].Studies have neither revealed that of bats species that store sperm are entirely torpid all through during sperm storage period nor these bats hibernate. Other few studies have recommended that fructose, carnitine and glucose act as energy source for the storage of sperm. The fine control of the processes of exhibiting the storage of sperm might be hormonal [11]. Pipistrellid species which were ovariectomized during the period of hibernation represents highly harmful effects on the growth of sperm that stored when compared with the control group. A little information about the hormonal function during the period of sperm-storage is available. Circulating the levels of hormone (androgens), that may be needed for the maintenance of sperm existence and feasibility in male, are found for only some species of bats [11,7]. Low level of testosterone had been measured in some bats that stored sperm during the period of sperm storage as compared with pre-hibernation level [7]. Even though sperm storage process has been represented in a number of bats species, the process has not been completely explained up till now [8]. The *Pipistrellus kuhlii* is a species of bat distributed (cosmopolitan) from Western Europe to Afghanistan, Kazakhstan, Turkmenistan and up to the North Africa [12].

Keeping in mind, the lack of knowledge regarding reproductive biology of bats, the present study was designed to provide baseline information of process of spermatogenesis in Kuhl's Pipistrellus (*Pipistrellus kuhlii).*

2. Material and Methods

2.1 Study Area

Faisalabad stands in the rolling flat plains of northern Punjab between longitude 73°74 East and latitude 30°31.5 North with an elevation of 184 meters (604 ft) above sea level. The city proper covers an area of approximately 1,230 square kilometers (470 sq mi), while the district covers more than 16,000 square kilometers (6,200 sq mi). The bats were collected from four localities of Faisalabad including (a) Fisheries research farm in University of Agriculture (b) Fish hatchery at Satiana Road (c) Wildlife park at Gutwala (d) Govt. Postgraduate College Saman abad. A total of twenty-four specimens were collected during the study period ranging from August 2012 to March 2013.

Fifteen specimens (13 males, 2 females) of three species collected from Fisheries Research Farm, University of Agriculture Faisalabad, in two seasons August and March. Out of these fifteen, three specimens were of *Pipistrellus kuhlii.* A total five specimens (3 males, 2 females) were collected from fish hatchery Satiana road, Faisalabad, in September that included three species. Two of them belonged to our desired specie i.e. *Pipistrellus kuhlii*. A total of four specimens of two species were collected from Wildlife Park Gutwala, Faisalabad, from October to November. Our desired specimens were two. None of the specimen was collected from Govt. Postgraduate college of Science Samanabad, Faisalabad.

2.2 Sampling strategy

Bat sound detector (Pettersson Ultrasound Detector D 1000X) was used to maximize chances for locating a bat roosts. Once located, the global position of each roost was determined using Garmin etrax H Global position system (GPS).

A total of twelve viz., 12 m (n = 1), 9 m (n = 2) and 6 m (n = 3) long high quality deep black UV stable at strong mist nets (Ecotone 716/6, 716/9 and 716/12) was used to capture bats. Each of these five shelved, 16×16 mm mesh sized and 2.5 m high net was erected either in "L" or "V" shape at strategic position on a pair of 3m long bamboo poles in such a way that the last shelf of each net remained one foot above the ground. The total mist net area in season was 120 $m^{2.}$ The nets were ready to operate half an hour before sunset. All the nets were opened simultaneously at sun set and continued to operate, on the weather condition for two hours after sunset nets were checked continuously to disentangle any captured bat. The sampling effort remained the same throughout the study period.

2.3 Morphological Assessment

2.3.1 External Body Measurements

Each disentangled bat was placed in a separate cotton bag during mist netting and at the completion of a netting session. Each bat was weighed using Pesola® balance 10050 (Swiss made), euthanized and preserved in a plastic bottle in absolute alcohol. Field number, sex, exact locality and district of capture of each bat were noted on the plastic bottle. The exact species of each captured specimens was identified on the basis of external morphology and dental formula. The standard and morphometric measurements of each specimen were taken before skinning, which included the parameters: head and body length, ear length, hind foot length, tibia length, forearm length, thumb length including claw. Fore limb studies included a) 3rd metacarpal (3mt), b) 4th metacarpal (4mt), c) 5th metacarpal (5mt), d) 1st phalanx on 3rd metacarpal (1 ph3mt), 2nd phalanx on 3rd metacarpal (2ph3mt), 1st phalanx on 4th metacarpal (1ph4mt) 2nd phalanx on 4th metacarpal (2ph4mt) and wing span.

2.3.2 Weight of Testes

The testes from individual bats were obtained and observed carefully for their gross features. The weight of each sample was recorded in grams (g) using an electrical weighing balance.

2.4 Histometrical Analysis

The tissues collected from each testis were processed by paraffin tissue preparation technique followed by Hematoxylin and Eosin staining (H&E) [13]. Photomicrographs of each testis captured using Nikon Optiphot 2 microscope at X100 and X400. These photos were used to determine diameter of testes, diameter of the seminiferous tubules, diameter of the seminiferous tubules lumen, width of seminiferous tubules, length of seminiferous tubules and area of interstitial cells of each testis with the help of automated image analysis system Image J® version 1.43n. Image J® is World's fastest image analysis software with a processing speed about 40 million pixels per second.

2.5 Statistical Analysis

To compare the means of parameters, one way analysis variance (ANOVA) was used and least significance difference (LSD) test to compare the group means at 5% level of significance.

3. Results

Mean ± SD values and ranges of 21 different morphological features of seven adult male bats (*Pipistrellus kuhlii),* captured from four different locations of Faisalabad district during different months of the year are presented in Table 1.

Comparison of testis weight of adult male specimens of bats (*Pipistrellus kuhlii)* captured during different months of the year is presented Fig. 1. Testicular weight showed a significant ($P<0.05$) increase in testicular weight during months of August and September which started to decline in October and this trend continued till December. Comparison between increased testicular weight from August to September and the decreased values in December showed a fivefold difference (Fig. 1).

The testicular activity in different months of male bats (*Pipistrellus kuhlii)* determined on the basis of different parameters including (a) Testes weight and body weight. (b) Length of testes (c) Width of testis (d) Width of seminiferous tubules (e) Length of seminiferous tubules (e) Area of interstitial cells and (f) Diameter of the seminiferous tubule lumen is presented in Table 2. Statistical analysis revealed that length and width of testes and seminiferous tubules along with the lumen area were found significantly ($P\leq0.005$) decreased during the low spermatogenesis phase from October to December. The mean (±SEM) values of these parameters were statistically non-significant ($P>0.05$) during the different months of same phase (Table 2). Histological plates (Fig. 2-4) witness different trends of spermatogenic activity during peak (August -September) and low (December) reproductive phases.

Table 1: Mean (±SEM) and range (mm) of body measurements of *Pipistrellus kuhlii* captured from Faisalabad District.

Body parameters	Mean ± SD	Range
HBL	38.32±1.239	36.17-39.57
E	10.93±0.49	10.14-11.74
TRH	5.50±0.45	5.1-6.13
FA	35.25±0.73	34.16-35.96
TH+C	4.39±0.49	3.9-543
2MT	29.51±0.63	28.67-30.37
1PH2MT	2.76±0.61	2.19-3.99
3MT	31.50±0.92	30.42-32.72
1PH3MT	10.37±0.78	9.05-11.25
2PH3MT	10.09±0.81	9.09-11.09
3PH3MT	7.91±0.80	6.57-8.52
4MT	29.62±1.44	27.62-31.62
1PH4MT	11.48±0.53	11.01-12.31
2PH4MT	8.80±0.51	8.05-9.15
5MT	25.38±1.02	23.56-56.56
1PH5MT	11.00±0.78	10.12-12.42
2PH5MT	5.86±0.72	5.1-6.97
WS	218.42±1.13	217.1-220.2
TIB	11.93±0.62	10.75-12.75
HF	7.45±o.75	6.1-8.15
T	31.78±3.33	30.1-39.27

Abbreviations: HBL-head and body length; E- ear; TRH- tragus height; FA-forearm; TH+C- thumb + claw; 2MT- 2nd meta carpel; 1PH2MT- length of 1st phalange on 2nd metacarpal; 3MT- length of 3rd metacarpal; 1PH3MT- length of 1st phalange on 3rd metacarpal; 2PH3MT- 2nd phalange on 3rd metacarpal; 3PH3MT- length of 3rd phalange on 3rd metacarpal; 4MT- length of 4th metacarpal; 1PH4MT- 1st phalange on 4th metacarpal; 2PH4MT- 2nd phalange on 4 metacarpal; 5MT- length of 5th metacarpal; 1PH5MT- 1st phalange on 5th metacarpal; 2PH5MT- 2ndphalange on 5th metacarpal; WS- wing span; TIB- length of tibia; HF- length of hind foot; T- length of tail

Table 2: Major histological parameters of testes of bats (*Pipistrellus kuhlii)* during peak (September) and low (December) spermatogenic phases.

Spermatogenesis Phase	Testes (µm)		Seminiferous tubules (µm)		
	Length	Width	Length	Width	Lumen Area
Peak	264.16±40.56^{A}	195.23.±32.19^{A}	152±25.04^{A}	141.1±14.21^{A}	Filled with spemato-genic cells
Low	143.21±32.34^{B}	111.±20.45^{B}	78.34±11.45^{B}	67.34±09.14^{B}	98.48±9.346

Means sharing different superscript are statistically significantly different at ($P<0.05$)

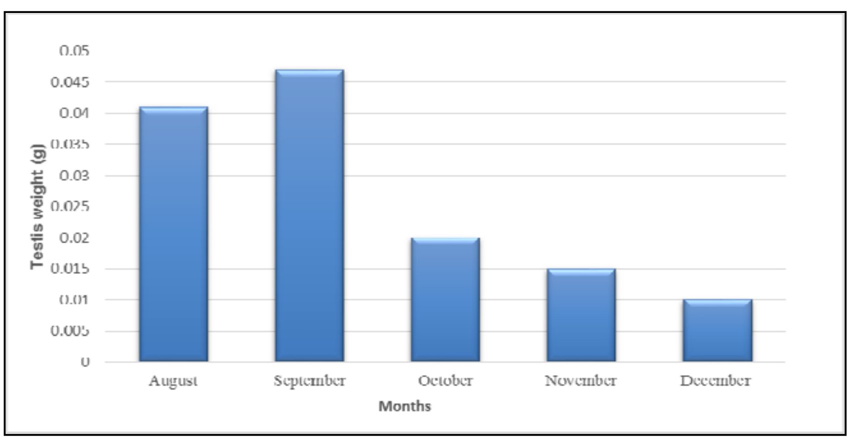

Fig 1: Trends in the testis weight of adult male specimens of *Pipistrellus kuhlii* captured from Faisalabad district during different months.

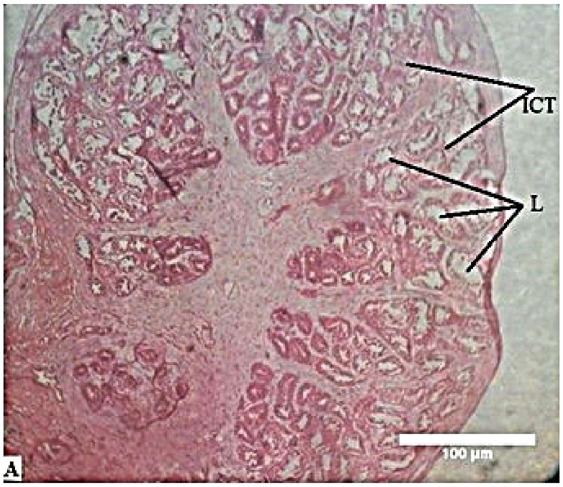

Fig 2: Histomicrograph of *kuhlii's pipistrellus* testis during low spermatogenic activity (December): Showing small lumen of seminiferous tubules (L) and low area of interstitial connective tissue (ICT). H&E; 100X.

Fig 3: Histomicrograph of *kuhl's pipistrellus* testis during low spermatogenic activity (December): Showing small and empty lumen of seminiferous tubules (L) with basement membrane (B) and low area of interstitial connective tissue (ICT). H&E; 400X.

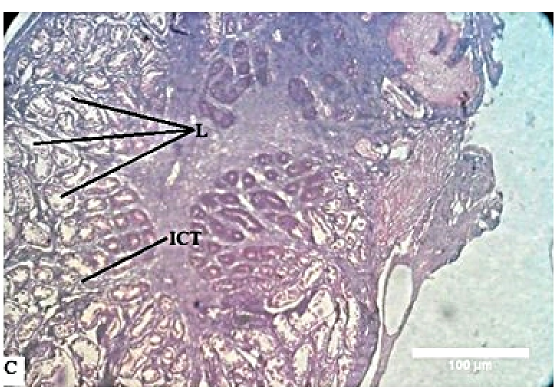

Fig 4: Histomicrograph of *kuhl's pipistrellus* testes during peak spermatogenic activity (September): Showing large lumen (L) of seminiferous tubules having spermatids and interstitial connective tissue (ICT). H&E; 100X.

Fig 5: Histomicrograph of *kuhl's pipistrellus* testes during peak spermatogenic activity (September): Showing large lumen (L) of seminiferous tubules having spermatids, basement membrane (B) and interstitial connective tissue (ICT). H&E; 400X

4. Discussion

In the present study, the impact of different months of year on histomorphometrical characteristics of testes in bats (*Pipistrellus kuhlii*), captured from different locations of Faisalabad during August 2012 to March 2013 was examined. Different parameters of testes including (a) diameter of testes (b) length of testes (c) number of seminiferous tubules (d) diameters of seminiferous tubules (e) area of interstitial cells (f) diameter of the seminiferous tubule's lumen were measured by using image J® analysis software.

Histological and histometric data collected in this study revealed that peak spermatogenesis occurs in bats during late summer and early autumn months (August and September) in Pakistan followed by a decline in October which continued till December. The same seasonal reproductive pattern of *Scotophillus heathii* was reported in earlier literature [14, 15].

During peak reproductive season (August and September), lumen of the seminiferous tubules was mostly filled but it showed spaces in December and January when their testicular activities were considered low. A similar trend was found in *Myotis macrodactylus* bat [16]. Histological evaluation showed that males collected in August have large number of spermatids with no or very few number of spermatozoa, were found in the epididymis. In contrast, the seminiferous tubules of testes showed merely one layer of darkly stained Sertoli cells and some large spermatogonia with prominent nucleoli during winter season. No sign of testicular activities was recorded in winter hibernation period. This finding is in line with the several other moderate zone Rhinolophid and the Vespertilionid bats like *Nyctalus noctula* [17] and *Pipistrellus pipistrellus* [18]. All of these bats however, undergo spermatogenic activity during different seasons like summer and autumn. The first change in the testes was seen in the March, spermatogonia were not divided until the May and June [17].

The pattern of testis weight followed the increasing trend in August and September might be due to development of spermatogenic cells up to the spermatid stage (mature sperm) and the position of testes was found scrotal [19]. Decreasing trend was found in October to December while position of testes was abdominal. Comparison between increased testicular weight in August and the decreased in December showed a fivefold increase in testis weight during high spermatogenic time period.

5. Conclusion
It is conceivable from the present data that season has a substantial impact on the testicular activity in bats (*Pipistrellus kuhlii*). There was an annular testicular cycle which is followed by the bats. The testicular activity showed a peak during August and September which declines gradually from December to February. Testes showed no testicular activities in winter hibernation.

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6. References

1. Nowak R. Order Chiroptera, in Walker's Mammals of the World. Ed 5, Baltimore: Johns Hopkins University Press, UK. 1991; 1:190-194.
2. Vaughan T, Ryan J, Czaplewski N. Mammalogy, Edn 4, Toronto: Brooks Cole. Canada, 2000.
3. Jane MW, Kunz TH. Day roosting activity budget of the eastern Pipistrelle bat, *Pipistrellus subflavus* (Chiroptera: Vesper tilionidae). Canadian Journal of Zoology. 1995; 74:431-441.
4. Robert TJ. The Mammals of Pakistan. Ernest Bem Ltd, 1977, 361.
5. Neuweiler G. The Biology of Bats. Oxford University Press, Oxford, UK, 2000.
6. Racey PA. The prolonged storage and survival of spermatozoa in Chiroptera. Jornal of Reproduction and Fertility. 1979; 56:391.
7. Crichtone EG, Krutzsch KH. Reproductive biology of bats. Sperm storage and fertilization. Edn 1, Academic Press, New York, 2000, 295.
8. Wang Z, Liang B, Racey PA, Wang YL, Zhang SY. Sperm storage, delayed ovulation, and menstruation of the female rickets big-footed bat (*Myotis ricketti*). Journal of Zoological Studies. 2008; 47:215-221.
9. Corbet GB, Hill JEA. World list of mammalian species. Edn 3, Oxford University Press, Oxford UK, 1991.
10. Sharifi M, Ghorban R, Akmali V. Reproductive cycle in *Pipistrellus kuhli*i. Mammalia. 2004; 68:323-327.
11. Hosken DJ, Blackberry MA, Stewart TG, Stucki AF. The male reproductive cycle of three species of Australian vespertilionid bats. Journal of Zoology. 1998; 245:261-270.
12. Dietz C, Helversen O, Nill D. Kuhl's Pipistrelle bat - *Pipistrellus kuhlii*. Bats of Britain, Europe and Northwest Africa, Black Publishers Ltd, London, 2009, 301-305.
13. Layton JD, Suvarna C, Bancroft's SK. theory and practice of histological techniques. Edn 3, London, Churchill Livingstone Elsevier. 2013, 303-320.
14. Orlandini G, Gatti R. Seasonal changes in the seminiferous tubules and leydig cell of hibernating bats. Acta Biomedica de I'Ateneo Parmense. 1990; 61(3-4):127-48.
15. Krishna A, Singh K. Changes in the thyroid gland during the reproductive cycle of the male vespertilionid bat, *Scotophilus heathi*. Revista Brasileira de Biologia. 1998; 58:1-12.
16. Lee H Mori. Annual cycle of seminiferous epithelium of Myotis macrodactylus. Journal of The Faculty of Agriculture. 2004; 49:355-365.
17. Racey PA. The prolonged survival of spermatozoa in bats in Duckett and Racey (eds), the biology of male gamete, Academic Press, London, 1974, 385-416.
18. Racey PA, Tam WH. Reproduction in male pipisttrellus (Mammalia: Chiroptera). Journal of Zoology. 1974; 172(1):101-122.
19. Muzafar S, Ghovrbani R, Fazali A, Holt W. Evidences of sperm storage in Pipistrellus kuhlii (chiroptera:vespertilinidea) in western iran. Journal of Folia Zoologica. 2004; 53:1-6.