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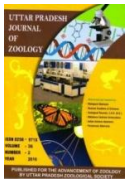

STUDIES ON THE HISTOMORPHOMETRIC ANALYSIS OF REPRODUCTIVE ORGANS OF FIVE STRIPED SQUIRRELS (*Funambulus pennanti*) UNDER NATURAL ENVIRONMENTAL CONDITIONS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author AMC conducted research work, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AMC and FD managed the analyses of the study and managed the literature searches. Authors ASQ and MUH designed the study and supervised research work. All authors read and approved the final manuscript.

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ABSTRACT

The present study was designed to investigate morphological and histometrical changes in the testes of five striped squirrels (*Funambulus pennanti*) in different breeding seasons. Sixteen specimens were collected from 3 different locations of Chiniot district, Punjab, Pakistan during different months to collect their testes samples. Body weight and external body measurements were recorded. Two adult males were euthanized and autopsied to obtain their male reproductive organs (i.e. testes, epididymis, penis) in intact form. Size and shape of reproductive organs were determined during three reproductive phases i.e quiescent phase (November), recrudescence phase (December) and peak reproductive phase (January to June). After euthanasia, the tests were excised washed with normal saline solution and fixed in Bouin's solution, dehydrated, embedded in paraffin, cut into 5µm sections and stained with hematoxylin and eosin, for the examination under light microscopy. Statistical analysis revealed highly significant ($P<0.05$) values of all histological parameters of tests during the peaks spermatogenic phase in January-June when compared with the low spermatogenic phase in November and December. Significantly ($P<0.05$) higher values of testes parameters were observed during peak spermatogenic phase (January-June) as compared to others. Finally,

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this study shows that different stages of sexual activities are controlled by seasonal variations. These variations are according to different physical factors which influences anatomical and histological changes of testes and spermatogenesis of male five-striped northern palm squirrel (*Funambulus pennanti*) for adjustment in breeding phenology.

Keywords: Five striped palm squirrels; testes; spermatogenic activity; breeding seasons.

1. INTRODUCTION

Squirrels (Mammalia: Rodentia) belong to family scuiridae which is one of the largest and widely dispersed families of mammals [1]. Sciurids contains a diverse and abundant group of species. The five-striped palm squirrels are included in genus *Funambulus* that includes five species. These include *F. layardi, F. palmarum, F. sublineatus, F. tristiatus and F. pennantii* [2]. *Funambulus pennanti* which is also known as the northern palm squirrel, is widely distributed ranging from Southeast Iran, through Afghanistan, Pakistan, northern and central India to Nepal [3]. This species was introduced to the Andaman, Nicobar Islands, Sydney, Western Australia and New South Wales [4].

Rodents, including squirrel play an important role in the animal food chain and in the ecosystem. Squirrels hair coat mainly caudal tails hairs are used in the making of brushes for hair and for cleaning electrical goods. These squirrels also play role in the dispersal of seed and help in pollination, provide food for birds of prey. *Funambulus pennanti* could be hunted and if needed to be used as a food source for humans [5]. But day by day their population is decreasing due to extensive hunting for furs, skin, meat and teeth. In recent years many rodents are included in endangered species, and some have also gone extinct due to shifting agriculture, human settlements, hunting for meat, deforestation and also by the development of infrastructure in forest areas [6].

Funambulus pennanti is a seasonal breeder and its breeding period ranges from February to August and male squirrels were found to be reproductively active that had large and scrotal testes. From late September to November the gonads regress. In November and December there is a short period of reproductive quiescence [7]. In this rodent species it was observed that gonads regress due to the short period (decrease day length) and gonads were active during long photoperiods [8]. Experiments have shown that during short days the mammalian pineal gland produces a substance which is generally supposed to inhibit neuroendocrine gonadal function [9].

Squirrel activities were reduced in winter when there is reduced day length, but, from April to July as the day length increased their activities are also increased. Their activities were also ceased due to rainfall. During winter and autumn maximum time spent in sleeping due to a lower temperature, reduced photoperiod and non-availability of food items. In all the four seasons sleeping percentage was higher in female squirrels than males, whereas, feeding ratio was more in males as compared to females. In summer and autumn, grooming percentage was also high in male but in winter it was same in both males and females [10].

Squirrels are widely distributed in South and Southeast Asian region but on squirrel few scientific publications are generated by this region and there is lack of knowledge. Most endangered squirrels found in tropical regions due to a high disforestation rate. So increased effort should be made to study their biology and status [11]. In Pakistan information on the breeding habits of five-striped palm squirrel is not well documented. Histological changes in the testes have not been studied so far. Therefore, the present study was designed to observe histological changes in the reproductive organs of male five-striped palm squirrel inhabiting croplands of Chiniot, Punjab, Pakistan.

2. MATERIALS AND METHODS

2.1 Experimental Design

The study was conducted in Chiniot district, Punjab, Pakistan to investigate spermatogenesis in male five striped palm squirrel, *Funambulus pennanti* (Fig. 1). Three locations in Chiniot district Pakistan were selected for collection of squirrels. Squirrels were collected alive by local made one-way cage traps. These were wire cages and grains, walnut, almond and peanut were added in them to attract squirrels. From October 2013 to June 2014 at different sampling localities, these cages were placed and occasionally checked if any squirrel trapped. The trapped squirrels were then transferred to a comfortable natural environment before autopsy. Chloroform was put drop by drop on nose of each squirrel until they anaesthetized. Then picked him and a swab of chloroform was put on nose until death (Fig. 2). A total of sixteen adult male specimens were collected during this study from three different locations.

Fig. 1. Male *Funambulus pennanti* captured from Chiniot district

Fig. 2. Male five-striped palm squirrel, *Funambulus pennanti* (ventral view showing scrotal testes)

2.2 Morphological Assessment

The exact species of each captured specimens was identified on the basis of external morphological features. The body measurements like head and body length, ear length, fore and hind limb length and different skull measurements of each specimen were taken before autopsy.

2.2.1 Body and cranial measurements

The skull of each specimen was examined for obtaining necessary data. The eye balls, tongue and excessive flesh were removed from each skull. Most of the brain tissue was macerated and removed (using forceps and cotton) before cranial cavity were washed with a jet of water. Skulls thus cleaned were kept overnight in a dilute solution (0.2%) of potassium hydroxide (KOH). After being thoroughly washed with tap water, the skulls were kept in absolute alcohol for a night before being transferred to acetone for another night. Each of the dry and stored skulls was stored in a properly labelled vial padded with cotton. The following skull measurements were taken, BL-basal length; CBL-condylobasal length; GLS-greatest skull length; NL-Nasal length; ZOB-zygomatic breadth; PL-palatal length; ML-mandible length; IOB-interorbital breadth; POC-postorbital

constriction; MDTR-mandible toothrow; MXTR-maxillary toothrow; NB-nasal breadth; BOB-biorbital breadth; PB-palatal breadth. The Vernier caliper, and thread with measuring tape were used for measurements in mm. and weight in g was measured with weighing machine. The data thus obtained was analyzed using SPSS 13 to find out variations of morphometrics of the five-striped palm squirrel. The left and right testes, epididymis and penis from individual squirrel were obtained and measured in centimeter.

2.3 Histometrical Analysis

Testes were washed with normal saline solution and fixed in Bouin's solution. Different slides were prepared using tissue paraffin technique as described by Suvama et al. [12], staining was done by hematoxylin and eosin (H&E). Photomicrographs of tests were captured using Nikon Optiphot 2 microscope at 200X. These photos were used to determine the length (μm) of seminiferous tubules, width of seminiferous tubules (μm) and lumen area of seminiferous tubules (μm^2) with the help of automated image analysis system Image J® version 1.43n (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

2.4 Statistical Analysis

The means of all parameters were compared with One-way analysis of variance (ANOVA) and least significance (LSD) test applied to compare the group means at 5% level of significance.

3. RESULTS

3.1 Morphological Measurements

Total sixteen specimens of adult male five striped palm squirrel were collected during this study period from November 2013 to June 2014. External body measurements and Cranial measurements of these specimens are represented in Table 1.

Body weight ranged from 102.5 g in November to 128 g in June. There was not marked variation present in penis length during different breeding phases, it ranges from 0.47 cm in November to 0.57 cm in June. Epididymis lengths during different breeding phases of this study ranged from 2.35 cm in November to 3.35 cm in June. Testes had scrotal position and during different months of study, testes weight showed marked variations. During November, testes were in quiescent state and their weight was as minimum as 0.235 g. In December, testes were in redevelopment state and their weight increased up to 0.810 g. During breeding season from January to June testes had shown maximum weight that was 0.919 g in January, 1.040 g in February, 1.001 g in March, 1.065 in April, 0.954 g in May and 1.216 g in June. Body weight, testis weight and their position during different breeding seasons are depicted in Table 2.

Table 1. Body and cranial measurements (mm; Mean ± SEM) and range of the male five striped palm squirrel, *Funambulus pennanti* captured from Chiniot district Pakistan (n=16)

Parameters	Mean ± SEM	Range
HBL	149.57±3.49	146.11-153.09
T	137.5±4.84	132.66-142.34
HF	40.25±1.77	38.48-42.02
E	17.69±1.25	16.44-18.94
BL	32.97±1.25	31.72-34.22
CBL	31.58±1.49	30.09-33.07
GLS	36.92±0.53	36.39-37.45
NL	10.61±0.49	10.12-11.1
ZOB	20.19±0.67	19.52-20.86
PL	18.43±1.21	17.22-19.64
ML	22.45±1.71	20.74-24.16
IOB	10.86±0.56	10.30-11.42
POC	16.42±0.58	15.84-17
MDTR	7.04±0.43	6.61-7.47
MXTR	7.31±0.47	6.84-7.78
NB	4.65±0.39	4.26-5.04
BOB	16.75±1.88	14.87-18.63
PB	5.81±0.58	5.23-6.39

Abbreviation: HBL-Head and body length; T-Length of tail; HF-Length of hindfoot; E-Ear; BL-Basal length; CBL-Condylobasal length; GLS-Greatest skull length; NL-Nasal length; ZOB- Zygomatic breadth; PL-Palatal length; ML-Mandible length; IOB-Interorbital breadth; POC-Postorbital constriction; MT-Mandible toothrow; MXTR-Maxillary toothrow; NB-Nasal breadth; BOB-Biorbital breadth; PB-Palatal breadth

3.2 Histological and Histometrical Evaluation of Testes

Using image J® analysis computer software, different parameters of testes including (a) length of testes (b) width of testes (c) area of seminiferous tubules (e) length of seminiferous tubules (f) width of seminiferous tubules were studied (Figs. 3 - 5). Statistical analysis showed the highly significant values $P< 0.005$ of all parameters like diameter, length of testes (cm), length of seminiferous tubules (μm), width of seminiferous tubules (μm) and area of seminiferous tubules (μm^2) during different spermatogenic phases. Table 3 represents the mean monthly variations in different testes parameters of male five-striped palm squirrel, *Funambulus pennanti.*

Table 2. Mean monthly body and testes weight and position, penis and epididymis length the adult male specimens of five striped palm squirrel, *Funambulus pennanti* captured from Chiniot district – Pakistan (n=16)

Months	N	Body weight (g)	Testes weight (g)	Penis length (cm)	Epididymis length (cm)	Testes position
November (2013)	3	102.5	0.235	0.47	2.35	Scrotal
December (2013)	3	107.5	0.810	0.49	2.55	Scrotal
January (2014)	3	123	0.919	0.56	3	Scrotal
February	3	120.5	1.040	0.55	3.15	Scrotal
March	3	128	1.001	0.57	3.05	Scrotal
April	3	102.5	1.065	0.52	3.35	Scrotal
May	3	109	0.954	0.54	3.10	Scrotal
June	3	125.5	1.216	0.57	3.35	Scrotal

Table 3. Mean monthly variation (mean ± SEM) in different testes parameters of male five-striped palm squirrel, *Funambulus pennanti*

Breeding Phases	Quiescent phase (November)	Recrudescence phase (December)	Peak phase (January to June)	P value
Testes width (cm)	0.55± 0.057	0.79± 0.014	0.971± 0.138	0.02
Testes length (cm)	1.15± 0.071	1.95± 0.071	2.288± 0.192	0.0002
Area of seminiferous tubules (μm^2)	9606.92± 853.94	15429.94± 1145.725	28629.325± 2987.288	0.0001
Length of semi-niferous tubules (μm)	225.46± 20.93	403.500± 5.44	783.79± 116.75	0.0004
Width of semi-niferous tubules (μm)	188.50± 14.66	262.88± 42.180	408.382± 181.77	0.000

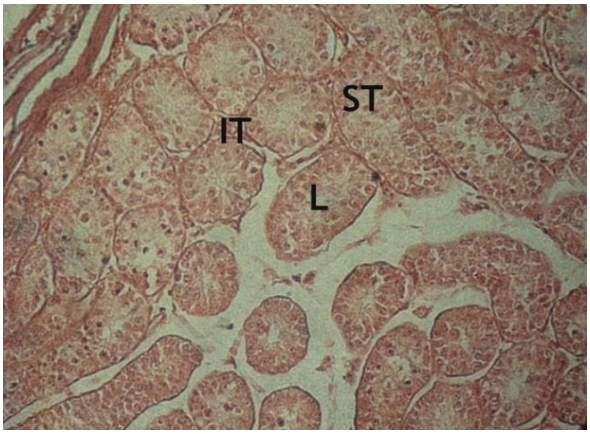

Fig. 3. Photomicrograph of testes of the five striped palm squirrels (*Funambulus pennanti*) during the quiescence breeding phase of annual cycle showing decreasing activity of seminiferous tubules (ST), absence of spermatids in lumens (L) and not distinguishable interstitial tissue (IT). Hematoxylin and eosin (H&E) 200X

4. DISCUSSION

The present study was focused to conduct the histomorphometric analysis of reproductive organs of five striped squirrels (*Funambulus pennanti*) in Chiniot district of Pakistan under natural environmental conditions. The results revealed that that adult male five striped palm squirrels (*Funambulus pennanti*) captured from Chiniot district of Pakistan were seasonal breeders, their spermatogenic activity continued from January to July and testicular weight was found maximum during this period, whereas from August to October, testes were regressed and minimal weight of testes were recorded. These squirrels were in quiescent phase during November. Redevelopment of testes occurred in

December while in January reproductive activity was completely restored when testes weight attained the breeding condition. These findings are in line with the results of Reddi and Prasad [13] and Sivashankar and Prasad [14]. According to Haldar and Saxena [15], five striped palm squirrel (*Funambulus pennanti*) showed seasonal testicular cycle. In November when daylength and temperature were reduced, a short phase of sexual inactivity was showed by these squirrels with arrest of spermatogenesis. In December when daylength and temperature were minimum and humidity was changing a phase of sexual recrudescence occurred. Rest of the year testes remained sexually active.

Microscopic data obtained in this study showed that spermatogenic activity started in January which continued till June. This assumption was made on changes observed in the testes size and weight during different months of this study. Maximum size and weight of testes were observed in specimens of the *Funambulus pennanti* during their peak spermatogenic activity and during this phase, straight blood vessels were present on testes. Testes had scrotal position in all squirrels which were studied. No relation was found between body weights and testicular activity during different months of our study. Length of penis and epididymis also did not show marked variation during different months.

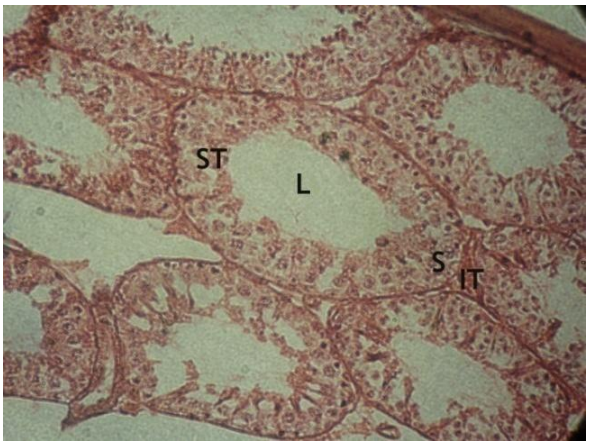

Fig. 4. Photomicrograph of testes of the five striped palm squirrels (*Funambulus pennanti*) during the recrudescence breeding phase of annual cycle showing developing activity of seminiferous tubules (ST), presence of spermatogonia cell (S), absence of spermatids in lumens (L) and developing interstitial tissue (IT). Hematoxylin and eosin (H&E) 200X

Fig. 5. Photomicrograph of testes of the five striped palm squirrels (*Funambulus pennanti*) during the peak breeding phase of annual cycle showing larger diameter of seminiferous tubules (ST), presence of mature spermatids (SP) in lumens (L) and large interstitial cells in interstitial tissue (IT). Hematoxylin and eosin (H&E) 200X

Using image J® analysis computer software, different parameters of testes including length of testes, width of testes, area of seminiferous tubules, length of seminiferous tubules, width of seminiferous tubules were studied. This analysis clearly showed that testes were in quiescent phase in November, and minimum values of these parameters were found during this period. Seminiferous tubules width and length were small and only spermatogonia cells were observed in them. Lumen of seminiferous tubules is filled with cellular debris. Whereas, in December these parameters values began to increase. High testicular activity was examined during peak breeding season from January to June. Vigorous spermatogenesis was observed in the seminiferous tubules and lumen of these tubules were filled with tail of sperms.

Chaudrey and Beg [16], examined a total of 348 squirrels were examined during a fourteen-month study conducted in Faisalabad Pakistan and reported that squirrels were reproductively active from January to October. Pregnancies were recorded from April to September. During active breeding seasons testes became large and scrotal.

5. CONCLUSION

It is conceivable from the current data that squirrels follow an annular reproductive cycle. They remain reproductively active from January to July and quiescent phase falls in November and December when testes are in the regeneration phase. Regression phase spans from August to October.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Herror MD, Castoe TA, Parkinson CL. Sciruid phylogeny and the paraphyly of Holarctic ground squirrels (Spermophylus). Mol Phylogenet Evol. 2004;31:1015-1030.
2. Moore JC, Tate GHH. A study of diurnal squirrels, Sciuridae, of the Indian and Indochinese subregions. Fieldiana, Zool. 1965; 48:1-351.
3. Molur S, Srinivasulu C, Srinivasulu B, Walker S, Nameer PO, Ravikumar L. Status of South Asian non-Volant small mammals: Conservation assessment and management plan (C.A.M.P.) Workshop Report. Zoo Outreach Organization /CBSG-South Asia, Coimbatore, India. 2005;618.
4. Long JL. Introduced mammals of the world: Their history, distribution and influence. CABI Publishing, Oxon. 2003;589.
5. Nameer PO, Molur S. *Funambulus pennanti*. In: IUCN 2013. IUCN Red List of threatened species. Version 2018. 2008;2.
   Available:www.iucnredlist.org
6. Kumawat MM, Singh KM, Debashish S, Tripathi RS. Threatened rodent species of Arunachal Pradesh. Int J Agric Environ Biotechnol. 2013;6:657-668.
7. Vidhu S. Control of male reproductive cycles of Indian palm squirrel, *Funambulus pennanti*. Role of pineal organ, male hormones, and environmental factors. Ph.D. Thesis, Banaras Hindu University, Varanasi, India; 1992.
8. Haldar-Misra C, Srivastava M. Effect of pinealectomy on photoperiodic gonadal response of Indian Palm squirrel, *Funambulus pennanti*. Can J Zool. 1987;65:833-836.
9. Turek FW, Campbell CS. Photoperiodic regulations of neuroendocrine gonadal activity. Biol Reprod. 1979;20:32-50.
10. Kour DN, Sahi DN, Sharma R. Sex related seasonal patterns of time and activity allocation in the Northern Palm Squirrel, *Funambulus pennanti* in Jammu (Jammu and Kashmir), India. Int J Curr Microbiol App Sci. 2014;3: 309-314.
11. Koprowski JL, Nandini R. Global hotspot and knowledge gaps for tree and flying squirrels. Curr Sci. 2008;95:857-865.
12. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques, 7th ed. Livingstone Churchil, New York, USA; 2013.
13. Reddi AH, Prasad MRN. The Reproductive cycle of the male Indian Palm Squirrel, *Funambulus pennanti* wroughton. J Reprod Fert. 1968;17:235-245.

14. Sivashankar AK, Prasad MRN. Seasonal Variations in cholesterol concentration in the testis, adrenal, liver and plasma of the Indian Palm Squirrel *Funambulus pennanti* (Wroughton). Gen Comp Endocrinol. 1968;10: 399-408.
15. Haldar C, Saxena N. Eco-factors and Effect of Pinealectomy in regulation of seasonal testicular cycle in the Indian Palm Squirrel, *Funambulus pennanti.* J Interdiscipl Cycle Res. 1990;21:141-151.
16. Chaudrey MA, Beg MA. Reproductive cycle and population structure of the Northern Palm Squirrel, *Funambulus pennanti*. Pakistan J Zool. 1977;9:183-189.