INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
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www.vmsindia.org

Correlative Studies on The Body Weight, Testis Weight, Histological and Hormonal Profiles of *Rousettus leschenaulti*

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Introduction:

The switching on and off of reproductive functions during the annual breeding cycle of bats is the most striking example of photoperiodically induced process. (Krutzsch and Crichton, 1990; Gopalkrishna and Badwaik., 1993; Begulliani et al., 2009). Intraspecific variation has been reported, not just in the timing of reproduction, but also in the periodicity of reproduction in different environments and across the geographic range of the species (Vivier and Merve, 1996). It is therefore often impossible to characterize a specific pattern of reproduction within species with a wide distribution (Bernard and Cumming, 1997). The occurrence of varied reproductive patterns appears to be generally related to major differences in latitude.(Bernard and Cumming, 1997).

Considering the worldwide distribution and immense diversity exhibited by members of the order Chiroptera, remarkably limited attention has been given to reproduction in the male. In the present investigation attempts has been made to assess annual reproductive cycle of male *Rousettus leschenaulti* by correlating hormonal profile, histoloogical parameters with testis weight and body weight.

Materials and Method:

The specimens of *Rousettus leschenaulti* from roosting site of an underground tunnel of Kandri Mine, Nagpur in Maharashtra State, India. On the same day, these live animals, were bought to the laboratory and specimen anesthetized by petroleum ether and weighted on sensitive spring balance. Male animal were dissected out to collect the testis from the scrotal sacs. Fixed in alcoholic Bouin's fluid for 24 hrs. Dehydrated in upgrade series of alcohol, infiltrated in paraffin wax and blocks were prepared. Sectioning carried out on rotory microtome for getting ribbon of 5µm thickness. Ribbons having sections of testis and epididymis were affixed on the slides. Staining of sections carried out with the help of double staining of haemotoxyline and eosin. Sections were cleared in xyline and mounted in DPX. Sections were photographed with the help of image capturing device of 'Labomed make' attached to the compound microscope. Microscopic measurements of different parameters were also taken with help of inbuilt software of image capturing device. The results were expressed as MEAN ± standard error of mean (± SEM). Correlation coefficient between different parameters assessed by using ANOVA.

Results:

The variations in the histomorphological parameters and plasma hormonal profile were used to assess the different periods during the annual reproductive cycle of are shown in the Table.1. Table.2. shows correlation coefficient indices for body weight, testis weight and mean plasma testosterone concentration.

The adult testes of *Rousettus leschenaultia* shows great variations in their weight during different months of annual reproductive cycle. The maximum weight of testis is found during Feb-April (1.64 gm ±0.42) which coincides with the mean body weight of the animal which is progressively increases to 105gm (SEM ±0.83). After this period the weight of the testis sharply decreases and the minimum weight observed during the months of May-July (0.21 gm ±0.36) which was reflected in the drop in the mean body weight to 80 gm (SEM ±0.74). During the Au, mean testes shows slight increase in weight to 1.56 gm (SEM ± 0.39) with increase in the body g-Oct. weight of bat.

On the basis of presence of sperms in the seminiferous tubules and epididymis and mean plasma concentration of testosterone hormone the reproductive cycle was divided into three different periods, preparatory, breeding and regressed. (Table 1 and 2)

During the preparatory period the testis begins to enlarge in size throughout the month of September, early signs of spermatogenesis initiation were seen. An intertubular space between the seminiferous tubules were greatly reduced showing bunch of hypertrophied Leydig cells.

During an active breeding period, testis shows, vigorous spermatogenesis. Intense spermatogenesis starts from October onwards. In late October the seminiferous tubules shows

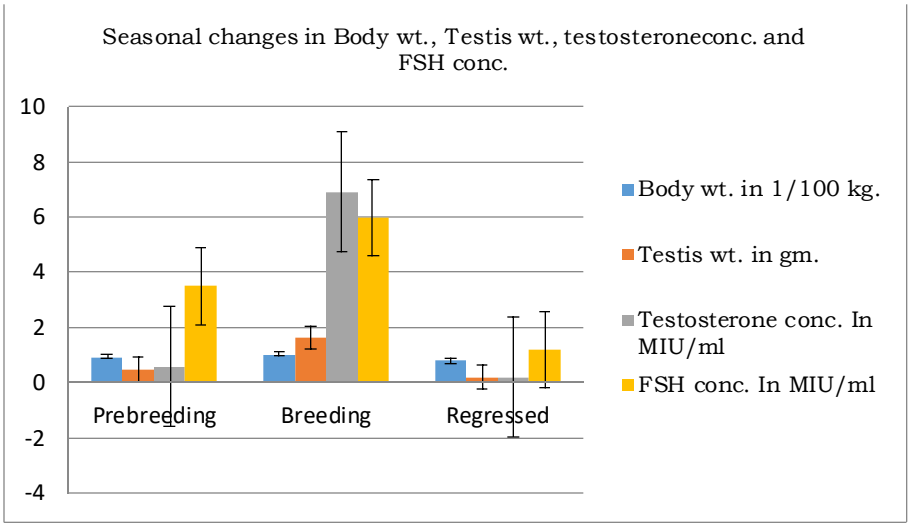
almost no lumen and this trend continues during the month of November. From early December onward upto the month of May testis were regressed and spermatogenetically iactive.
Hormonal profile of plasma testosterone has been analysed by hormonal assay during months of the annual reproductive of *Rousettus leschenaultia* were shown in the Table.1. The peak of circulating plasma testosterone concentrations were observed during Oct-Nov (±0.26 SEM 6.9 ng/mL).

Table.1. Showing variations in different parameters during annual reproductive cycle of *Rousettus leschenaulti*

SN	Parameters	Preparatory (Sept.)	SEM (n=5)	Breeding (Oct-Nov.)	SEM (n=5)	Regressed (Dec-June)	SEM (n=5)
1	Body wt.	96gm	± .21	105 gm	± 0.33	80gm	± 0.34
2	Testis wt.	0.49 gm	±.39	1.64 gm	± 0.42	0.21 gm	± 0.36
3	Testosterone	0.6 MIU/ml	±.26	6.9 MIU/ml	± 0.28	0.2 MIU/ml	± 0.23
4	FSH	3.5 MIU/ml	± 0.30	6.0 MIU/ml	± 0.29	1.2 MIU/ml	± 0.25
5	LH	8.5 MIU/ml	± 0.29	1.5 MIU/ml	± 0.32	0.5 MIU/ml	± 0.35
6	Semini. Tubule Diameter	210 µm	± 0.35	235 µm	± 0.25	135 µm	± 0.28
7	Semini epithe. Height.	60 µm	± 0.26	80 µm	± 0.32	34 µm	± 0.36
8	Leydig Cell diameter	13.1 µm	± 0.25	14.1 µm	± 0.45	11.2 µm	± 0.37
9	Leydig cell nucleus	6.1 µm	± 0.31	8.2 µm	± 0.43	5.0 µm	± 0.28

Table 2: Matrix chart showing correlation index for different parameters during testicular cycle of *Rousettus leschenaulti* FSH-Follicle stimulating Hormone, LH- Luteinizing Hormone, DHEAS-Dihydroepiandrosterone, STD - Seminiferous Tubule Diameter, SEH-Seminiferous Epithelium Height, LCD- Leydig Cell Diameter, LCN- Leydig Cell Nucleus

Parameter	Body wt.	Testis wt.	Testosterone	FSH	LH	S.T D SEH.	LCD	LCN
Body wt.	1							
Testis wt.	0.8785	1						
Testosterone	0.9871	0.9434	1					
FSH	0.9830	0.9511	0.9020	1				
LH	0.2717	0.220	-0.3479	0.0907	1			
S TD	0.9927	0.8145	0.7307	0.9538	0.3857	1		
S E H.	0.9963	0.9159	0.8548	0.9950	0.1889	0.9788 1		
LCD	0.9998	0.8702	0.7975	0.9797	0.2881	0.9946 0.9947	1	
LCN	0.9431	0.9873	0.9577	0.9880	0.0635	0.8962 0.9679	0.9373	1



Histogram.1. Showing correlation between body weight, testis weight and plasma testosterone conc. annual reproductive cycle of *Taphozous kachhensis*

Discussion

During the present investigation, the cyclic variations in the body weight and testis weight throughout the testicular cycle in were in consonance with the hormonal fluctuations during annual testicular cycle. The correlation between seasonal fluctuations in these parameters with that plasma testosterone can be inferred from the strong positive correlation in both the bats, as shown in table no.1 and 2. Body and testes weight of *Rousettus leschenaulti* fluctuates throughout the year, values of which coincides with an availability insects on which these bats feeds and subsequent stimulation of spermatogenesis by an interplay between the gonadotrophins and testosterone. These results in the present investigation expressed in graphical and tabular presentations find its parallel in the similar studies conducted on the number of chiropteran species by many workers. (Bernard., 1986).

In some species fluctuation of body weight can indicate times of food abundance and scarcity (Bhasin et al., 1997). According to McGuckin & Blackshaw (1991), assessment of changes in testicular weight in individuals provided information on the cyclic seasonal changes. The positive correlation between the body weight, testes weight and plasma testosterone concentration has been identified by Singh (1997); Singh and Krishna, (2000) in *Rousettus leschnaulti* which experiences two peaks of testosterone in a single testicular cycle (Sastry and Masram, 2007).

In the present study attempt has been made to correlate the body weight, testicular weight and plasma testosterone conc. with histological parameters like seminiferous epithelium and Leydig cell. Since androgen is a potent stimulant of nitrogen retention (Bhasin et al., 1997), causes an increase in the body weight due to an increased serum concentration of potassium (Turner and Bagnara, 1976). In addition, testosterone also modulates growth and metabolism in several peripheral tissues that contain androgen receptors such as skeletal muscle (Sauerwein and Meyer, 1989). In muscular tissue, adrogens stimulate cell hypertrophy to glycolytic white muscle cells possibly via reduction in glucocorticoid sensitivity (Sauerwein et al., 1991) and reduced proteolytic enzyme activities. (Blottener et al., 1996) The dramatic variations in plasma concentrations of steroid binding protein observed in the adult male little brown bats also confirm a seasonal variations of testosterone since the steroid binding proteins are the carriers of this hormone (Gustafson and Damassa, 1985; Gustafson, 1987). Physiologic increases or decreases in circulating steroid binding protein levels would be expected to influence the availability and therefore action of androgens (Gustafson and Damassa, 1985).

The data on mean plasma concentrations of *Rousettus leschnaulti* during breeding and post-breeding season suggest that the large increase in peripheral testosterone during the mating period is due, at least in part, to increased testicular production. Leydig cells appear fully active, histologically, suggesting that further stimulation occurs resulting in increased production of testosterone. Changes in stimulus could occur in response to many factors including environmental zeitgebers, female pheromones or stimulation of central nervous system associated with mating or territorial behavior (McGuckin and Blackshaw, 1991).

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