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# SEASONAL CIRCULATING SERUM TESTOSTERONE CHANGES AND REPRODUCTIVE PATTERN IN THE WILD CAUGHT MALE BAT, *TAPHOZOUS KACHHENSIS* (DOBSON).

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**ABSTRACT-**
The seasonal pattern of body weight and testes weight of male bats were recorded and correlate with circulating serum testosterone in the wild caught bat, *Taphozous kachhensis* (Dobson). A gradual increase in the body weight as well as testes weight were recorded from the month of October which was the recrudescence period of the testicular cycle (preparation of testes for spermatogenesis) where as marked decline was apparent from April till July (the phase of regression). The body weight and testes weight reached a peak level in December which is the breeding phase when vigorous spermatogenesis occurs followed by mating in late November. The peak of testosterone coincided with the peak of the body weight and testes weight. A highly significant correlation was noted between body weight –testosterone ($r= 0.61$, $p<0.001$), testes weight-testosterone ($r= 0.72$, $p<0.001$) and even between body weight-testes weight ($r=0.79$, $p< 0.001$). Thus peak level of testosterone and testicular activity concludes the existence of close correlation with the reproductive activity which leads to the successful annual breeding cycle of the present bat.

**Key words-:** Testosterone, Spermatogenesis, Body weight

## INTRODUCTION:

Bats are only the successful mammal in the world with the capacity of flight. They are second only to the rodents in number of species (about 960 species in the world) and are represented on all major land masses. With at least 109 species in India has an incredible diversity of bats (Bates and Harrisson, 1997). Reproduction in Chiroptera is of special interest since they show numerous adaptive radiation or specializations. Adaptive specialization can be observed with regards to their breeding and reproductive habits, structure and mechanism (Krutzsch, 1979; Racey, 1982; Rasweiler, 1993). Earlier literatures on the breeding biology of the chiropterans have been reviewed by various workers (Gopalkrishna and Rao, 1977). It has been suggested that the closely related species exhibit divergent breeding habits and that different species inhabiting a particular biotope with similar environmental conditions show different reproductive patterns (Gopalkrishna, 1955, Gopalkrishna and Chaudhari, 1977).

Racey (1974) recorded that in *Nyctalus noctula* there is a thirteen-fold increase in the weight of testes, due to active process of spermatogenesis. Spermatozoa were released in the August and September. The Leydig cells were never abundant, with no clear changes in the volume of their cytoplasm. He further observed that the testosterone content of the testes rise with the progress of spermatogenesis. Testosterone levels were maintained during autumn, when most mating took place, but decreased during subsequent winter period of sperm storage.

*Taphozous kachhensis* is small bat, found in colonies with variable size, which varies during different periods of the year and during different phases of reproductive cycle. These bats are found to inhibit cold and humid places, usually dark. The *Taphozous kachhensis* is monotocous bat, breeding once in a year. With the vigorous spermatogenesis and according to the reproductive status body weight and testes weight also varies. The peak of testosterone coincided with the peak of the body weight and testes weight. Thus the present study describes the seasonal changes in circulating testosterone and their correlation with the body weight and testes weight.

## MATERIAL AND METHODS:

The specimens for present study were collected from Ambai-Nimbai, District-Chandrapur in Maharashtra (India) (20038'39.08"N and 79035'30.99"E), throughout the year representing different reproductive cycle during (April 2007 to March 2009). They were sexed after collection by their external genitalia. The males are easily identified by deep black or brownish beard on ventral side of the lower jaw. The animals were weighed before sacrificing by decapitation or with chloroform. Testes from different reproductive phases were excised under semi-sterile condition, weighed. Then testes are processed for histological observation.

**Hormone assay:**

For the determination of serum testosterone level in blood, 2 ml of blood was allowed to clot at room temp for half an hour. The clotted blood was then used for measurement of serum testosterone by ELISA as follows (Wisdom, 1976). Minimum noise, human exposure and handling were employed to minimized capture stress and excitement during the blood collection.

**Statistical analysis:**

The available data were analyzed by statistical method. Data is expressed as standard Deviation (SD), correlation and mean ± SEM The degree of relationship between two attributes were determined by calculating a coefficient called correlation coefficient expressed by 'r'

**RESULTS:**

Seasonal variations of body weight, testes size and serum testosterone levels in male *T. kachhensis* are depicted in the table-1 and lined in diagrams (Graph-1, 2 & 3)

**Body weight and Testes weight:**

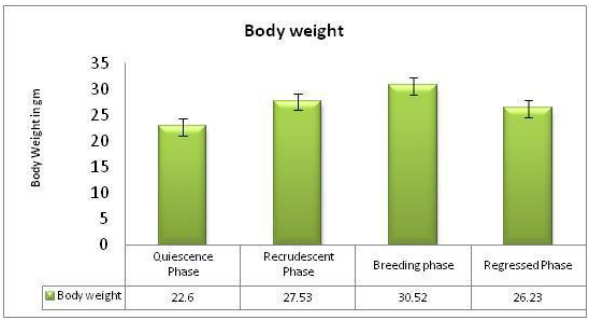
A gradual increase in the body weight along with testes weight were recorded from the month of October which was the recrudescent reproductive phase of testicular cycle (Preparation of testes for the process of spermatogenesis) whereas marked decline was apparent from February to July (the regression Phase). The body weight and testes weight reach a peak level during breeding phase which is in December where vigorous spermatogenesis occurs followed by mating in mid December and during quiescence phase the weight of body and testes were almost static.

**Changes in serum testosterone:**

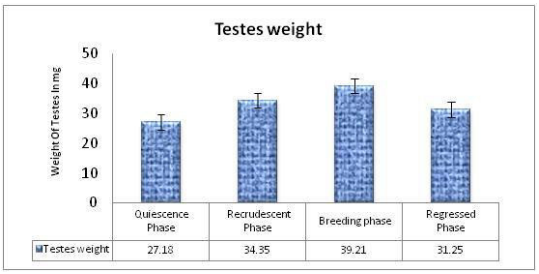
In this bat spermatogenesis starts in month of October and shows peak value in December and January and become slightly reduced in February and successive months. Sexually quiescence bat shows low level of testosterone (2.54 ng /mL). Progressively increased in the value of hormone (6.38 ng/mL) when testes show initiation of spermatogenetic activity. During the breeding phase when testes show vigorous spermatogenesis and the testosterone attends peak value (9.85 ng /mL). In successive months, (February to July) after the completion of breeding phase there is slight reduced in the level of serum testosterone i.e. (7.12 ng /mL to 4.29 ng /mL)

**Table-1** Changes in body weight, testes weight serum testosterone level in the male *T. kachhensis* (Values are mean ± SEM)

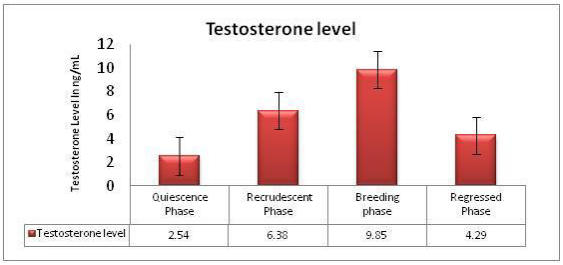
| Reproductive period | Months | Body weight (gm) | Testes weight (mg) | Testosterone (ng/mL) |
|---|---|---|---|---|
| Quiescence Phase | August-September | 22.60 ±0.49 | 27.18±0.36 | 2.54 ±0.06 |
| Recrudescent Phase | October-November | 27.53±0.19 | 34.35 ±0.13 | 6.38 ±0.02 |
| Breeding Phase | December-January | 30.52 ±0.67 | 39.21±0.70 | 9.85 ±0.08 |
| Regressed Phase | February-July | 26.23±0.18 | 31.25 ±0.56 | 4.29 ±0.09 |




**Graph- 1**: Annual changes in body weight of male *T. kachhensis* in gm (mean± SEM)




**Graph- 2**: Annual changes in testicular weight of male *T. kachhensis* in mg (mean± SEM)




**Graph- 3**: Annual changes in level of testosterone of male *T. kachhensis* in ng/mL (m ean± SEM)

The peak of testosterone coincided with the peak of body weight and testes weight and highly significant correlation was noted between body weight-testosterone (r= 0.61, p<0.001), testes weight-testosterone (r= 0.72, p<0.001) and even between body weight-testes weight (r=0.79, p< 0.001).

**DISCUSSION:**

Male reproductive cycle in Indian bats was reviewed by Gopalkrishna and Badwaik (1993a, b) and Sapkal and Bhandarkar (1984). The reproductive pattern and breeding habits in Emballonurid bats, *Taphozous melanopogon* (Sapkal and Khamre, 1984), *Taphozous kachhensis* (Sapkal and Deshmukh, 1985), and *Taphozous georgianus* (Kitchener, 1973; Jolly, 1990) has a sharply restricted annual sexual cycle. The reproductive cycle in family Emballonuridae varies with changes in latitude, and this was so even in same species (Singh, 1997). The examination of few species of Indian bats among nearly hundred species inhabiting this country reveals a variety of breeding patterns and adaption for successful reproduction.

In the present study the changes in body weight, testes weight and circulating serum testosterone level have been studied throughout the reproductive testicular cycle divided into different phases like sexually quiescence, sexually recrudescence, breeding and regression which affirms its similarity to other studied mammals and bats till now.

In accordance with earlier literature, biosynthesis of testosterone is the key hormone for the maintenance of body weight and testes weight, therefore in present study attempt is made to correlate the body weight, testicular weight and serum testosterone level as androgen is 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). The dramatic variations in plasma concentrations of steroid binding protein observed in the adult male little bats also confirms a seasonal variation of testosterone since the steroid binding proteins are the carriers of this hormone (Gustafson and Damassa, 1985). Physiologic increases or decreases in circulating steroid binding protein levels would be expected to influence the availability and therefore the action of androgens (Gustafson and Damassa, 1985). The values of testosterone must be supported by the plasma concentrations of steroid binding proteins.

In *T. kachhensis* the cyclic variations in the body weight throughout the testicular cycle are in consonance with the testosterone level as described by various authors in number of Chiropteran species as well as our observation since the cyclic alterations between and involution of testes is well known phenomenon and the seasonality of testes mass depending upon the testosterone levels.

Result of the present study shows two peaks of circulating plasma testosterone concentration in *T. kachhensis* during sexually recrudescence and breeding phase. The correlation between spermatogenesis and plasma testosterone concentrations has been widely reported in bat species such as *H. speoris* (McGuckin and Blackshaw,1991; Martin *et al.*, 1995; Singh and Krishna, 1996; Martin and Bernard, 2000; Chaudhury and Sastry, 2011), in *Pteropus giganteus giganteus* (Deshmukh and Dhamani, 2011) and mammals (Racey, 1974; Bernard, 1986; Racey and Tam, 1974; Krutzsch, *et al.*, 1976; Gustafson and Damassa, 1985) suggesting direct role of testosterone in spermatogenesis.

McGuckin and Blackshaw (1991) demonstrated in *Pteropus poliocephalus* that a large increase in plasma testosterone concentration occurs during the mating season associated with increased testicular function. The highest plasma testosterone concentration observed in Jun-July compared with that regressed testes correspond to the

changes observed in spermatogenetic activity McGuckin and Blackshaw (1987).

The bat, *Pteropus giganteus giganteus* showed two peaks of serum testosterone concentration (Deshmukh and Dhamani, 2011) supported the present study. In contrast to two peaks of serum testosterone concentration the annual reproductive cycle of *Taphozous longimanus* shows three peaks (Singh and Krishna, 2000). The serum plasma testosterone concentration peak during June-July is relatively lesser. The less significant peak during pre-breeding phase as compared to peak observed during November appears to be sufficient to stimulate secretary activity of accessory sex glands and mating in this species. The present study revealed dose dependent action of serum testosterone on spermatogenesis, secretary activity of accessory glands and mating behavior in *T. kachhensis*. Similarly Jolly and Blackshaw (1989) demonstrated in *T. georgianus* (Emballonuridae), Gustafson and Shemesh (1976) in *Myotis lucifugus lucifugus* (vespertilionid) and Deshmukh and Dhamani, (2011) in *P. giganteus giganteus* (Pteropodidae) that while maximum level of testosterone is needed for increased function of accessory sex gland, the spermatogenesis may be able to proceed to low serum testosterone concentration levels. Similar results are also reported by Razzoli *et al.,* (2005) in Mangolian gerbil.

The peak activities of testosterone in *T. kachhensis* as in other bats have been associated with an increase in the body weight and in the testicular size and weight. Thus two peaks of serum testosterone in the *T. kachhensis* coincide with different biological responses depending upon the size of peak. Lower peak of testosterone during August-September coincide with the inactive spermatogenesis only; while higher peak of testosterone during November-December coincide with the active spermatogenesis, secretary activity of accessory sex glands and mating behavior. A close association between changes in body weight and circulating serum plasma testosterone concentration may be responsible for the first peak during recrudescent phase. Similarly Reeder *et al.,* (1993) are of the opinion that body mass declines in *Pteropus vampyrus* may be tied due to declining testosterone levels; however, it is unclear whether the decreased body mass was due to fat or muscle loss. It is conclusive to state that our results are in confirmation with the above statement.

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