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Seasonal changes in plasma testosteron and leydig cell activity during the male reproductive cycle of *Hipposideros speoris* (Schneider).

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ABSTRACT

Males of *H. speoris* exhibit defined breeding season and seasonal spermatogenesis (September-January). Spermatogenesis commences in September and peak spermatogenesis and spermiogenesis occur during November-December and becomes slightly subdued in January. Regression sets in the testis by mid-January and the testes becomes completely regressed by March. The Leydig cells are observed in the testis throughout the year with variation in the number of cells per group. The plasma testosterone levels are low during the non-breeding period (July-August). Spermatogenetic activity commences in September-October and thus there is an increase in the plasma testosterone level. Peak spermatogenesis and spermiogenesis occur during November-December when there is considerable elevation in plasma testosterone concentration. Most adult females undergo copulation in the last week of December. Few adult females which miss copulation in December or females born in the previous year (especially July) are sexually immature in December. They copulate until the middle of March and since the testes does not produce sperms; the sperms stored in the cauda epididymides are viable and are able to fertilize the ovum released by these females.

Keywords- Testosterone, Leydig cell, Testis, Accessory gland, Cauda epididymis, Spermatogenesis.

INTRODUCTION

The first time sperm storage in the cauda epididymides for three to four months in a nonvespertilionid, *Hipposideros speoris*. Spermatogenesis commenced in September and continued until the second week of January, after which the testes underwent regression and there was complete cessation of spermatogenetic activity. However, the cauda epididymis was full of healthy spermatozoa until about the first week of April after which there was a sudden fall in their population in the cauda epididymis, and most underwent degenerative changes.

They reported that the male accessory glands- the seminal vesicles, prostate and bulbo-urethral glands came to activity in October and remained in an active state until the beginning of April. Since the sperms were not produced in the testes after the middle of January and since conception commenced during January to the middle of March in the few non-pregnant females, which had either missed copulation or which were sexually immature in December, it is evident that the ova released by these females must have been fertilized by the sperm stored in the cauda epididymis of the males, and which were received during copulation during January to March. They emphasized that the active status of the accessory glands and the healthy condition of the epididymal sperms in this bat are physiologically linked together, and these appeared to be independent of the factors which maintained spermatogenetic activity of the testis. Alternately the factors which triggered the onset of sexual activity in the males of *Hipposideros speoris*, continue to operate on the accessory glands even after the cessation of spermatogenetic activity in the testes. *H. speoris* has developed the mechanism to overcome the disadvantages of low fecundity in the females, to ensure that all adult females do conceive during the breeding season (Gopalakrishna and Bhatia 1980).

The biochemical estimation of fructose in ampullary glands, seminal vesicles, prostate gland and cauda epididymides of *Myotis lucifugus* and *Myotis velifer* (Crichton *et al*. 1981). While describing the ultra structural changes in the Leydig cells of *Rhinolophus capensis* observed that in this bat spermatogenesis occurs in summer, sperm are released to the epididymides in April and copulation occurs in August and September. The changes in Leydig cell ultra structure coincided with spermiogenesis (which began in March) and secretory activity of the seminal vesicles, supporting the suggestion that the changes may be associated with testosterone production (Quibell and Bernard 1983).

The annual variations in plasma sex steroid-binding protein and testosterone concentrations in the adult male little brown bat in relation to the asynchronous recrudescence of the testis and accessory reproductive organs (Gustafson and Damassa 1985).

The seasonal changes in plasma testosterone concentrations and Leydig cell and accessory gland activity in *Rhinolophus capensis* and have shown that the reproductive cycle is characterized by reactivation of the seminiferous tubules in early summer (October) after a 4-month (June to September) period of winter inactivity. Spermatogenesis was shown to occur between January to April, and spermatozoa were released to the epididymides in April and May. Spermiogenesis was shown to be associated with Leydig cell activity and increase in plasma testosterone concentrations, parallel to this the accessory glands showed increasing secretory activity. During winter Leydig cells were shown to be secretorily inactive and plasma testosterone concentrations dropped, but component of the accessory complex remained active. The author observed a second period of Leydig cell secretory activity with peak plasma testosterone values in late winter/early summer which may be associated with copulation or the initiation of new cycle of spermatogenesis (Bernard 1986).

The patterns of Leydig cells and LH gonadotroph activity and plasma testosterone concentrations in *Miniopterus schreibersii* (Bernard *et al*. 1991).

The seasonal and experimental reactivation of Leydig cells of the bat, *Tadarida brasiliensis* and noted that Leydig cells in this bat exhibit two well defined periods of secretory activity that are intimately associated to the reproductive cycle (Aoki 1997).

After complete cessation of spermatogenesis the sperms are stored in the caudae epididymides of bats inhabiting temperate climate (Racey 1979); (Krutzsch *et al*. 1982) and also in the South African bats (Bernard and Cumming 1997).

Although storage of spermatozoa in the epididymides has been reported in *Hipposideros speoris* [Gopalakrishna and Bhatia (1980)], no information is available on plasma testosterone level during the different phases of the testis cycle, particularly during the storage of spermatozoa in the epididymides.

MATERIALS AND METHODS-

The males of Leaf-Nosed bat *Hipposideros speoris* were collected from Chandrapur district, India, throughout the year. For plasma testosterone collection, live male adult bats were brought to the laboratory from Chandrapur and they were anesthetized with mild clinical anesthesia. The blood was drawn by an insulin syringe and centrifuged at 3000 rpm for 20 minutes. The determination of plasma testosterone was done by Radio Immunoassay at the pathology laboratory.

OBSERVATION-

Plasma Testosterone concentration during the reproductive cycle :

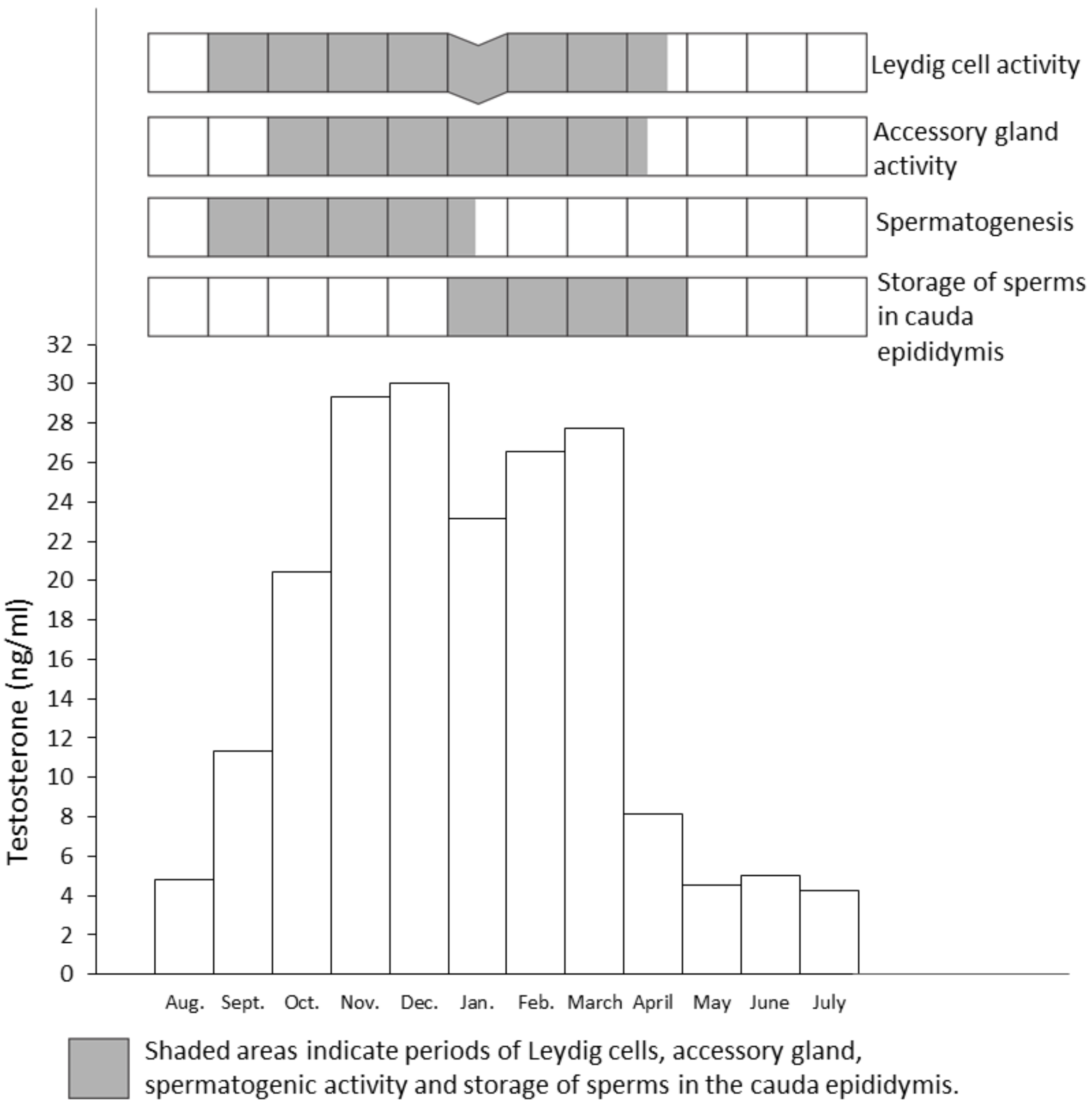
Table-1 and Histogram-1 gives the testosterone levels and Leydig cell activity during the male reproductive cycle of *H. speoris*.

Males of *H. speoris* exhibit defined breeding season and seasonal spermatogenesis (September-January). Spermatogenesis commences in September and peak spermatogenesis and spermiogenesis occur during November-December and becomes slightly subdued in January. Regression sets in the testis by mid-January and the testes becomes completely regressed by March. Although the testis undergoes regression spermatozoa are stored in the cauda epididymis for a period of 2½ months. The male accessory reproductive organs- ampullae of Henle, Prostate, and Cowper's glands become active in October and remain secretorily active until the first week of April (Gopalakrishna and Bhatia 1980); (Pal 1977). The Leydig cells are observed in the testis throughout the year (Table-1) with variation in the number of cells per group, the diameter of the Leydig cells varying from 7.26 μ to 14.52 μ. The largest diameter of Leydig cells is observed during November-December the peak period of spermatogenesis and spermiogenesis.

Table- 1. Plasma testosterone concentration and Leydig cells of *H. speoris*

Months	**Testosterone (ng/ml)**	**Leydig cell diameter (μ)**	**No. of cells in each group**	**Structure of leydig cell**	**Testis cycle**	**Accessory gland**
Aug.	4.81	10.89	16-28	Prominent nucleus with clear nucleoli	Non-breeding period	
Sept.	11.32	10.89	32-48	Cell boundary not prominent, round nuclei	Spermatogenesis and spermiogenesis, release of sperms to the epididymis (Sept. to Mid of January) **[Copulation-** January to March]	
Oct.	20.47	12.7	17-34	Large, vacuolated with distinct cell boundary with round nucleus		**Secretorily active** from October to Mid of April (Based on Histology)
Nov.	29.36	14.52	24-37			
Dec.	30.06	14.25	22-28			
Jan.	23.15	10.89	18-20	Not vacuolated, cell boundaries not distinct		
Feb.	26.55	12.75	18-23	Large vacuolated with distinct cell boundaries, round nucleus	Regression of testis with storage of sperms in cauda epididymis (Second week of Jan to End of April)	
March	27.76	9.075	8-28	Cell boundaries not prominent, round nuclei		
April	8.12	7.26	20-32	Cell boundaries not distinct, pycnotic nuclei in an eosinophilic mass		
May	4.52	10.89	18-22	Cell boundaries not prominent, round, small nuclei	Inactive period	
June	5.02	9.075	12-43			
July	4.25	10.89	6-12		Non-breeding period	

Histogram-1, Plasma testosteron concentrations with spermatogenetic, accessory gland and leydig cell activity during reproductive cycle of *H. Speoris*



The plasma testosterone levels are low during the non-breeding period (July-August) being 4.25 ng/ml and 4.81 ng/ml respectively. Spermatogenetic activity commences in the testes in September-October and thus there is an increase in the plasma testosterone level which reads 11.32 ng/ml in September and 20.47 ng/ml in October. Peak spermatogenesis and spermiogenesis occur during November-December when there is considerable elevation in plasma testosterone concentration which reads 29.36 ng/ml and 30.06 ng/ml respectively. There is slight reduction in the production of sperms in the testis during January as compared to November-December and after mid-January regression sets in the testis, but the sperms are stored in the cauda epididymis until the second week of April. During January, February and March the testosterone level is 23.15 ng/ml, 26.55 ng/ml and 21.75 ng/ml respectively. Most adult females undergo copulation in the last week of December. Few adult females which miss copulation in December or females born in the previous year (especially July) are sexually immature in December.

They copulate until the middle of March and since the testes does not produce sperms; the sperms stored in the cauda epididymides are viable and are able to fertilize the ovum released by these females. The gestation period in this species is of 135-140 days and deliveries in the colony occur from the first week of May to the last week of July. The high testosterone level during January-March along with the sustained active state of the male accessory glands (October-April) is responsible for libido during January-March. The testosterone level falls during April (2nd week) to 8.12 ng.ml and remains low during the inactive period i.e. during May and June reading 4.52 ng/ml and 5.02 ng/ml respectively.

DISCUSSION

Table-1 incorporates observations on the testis cycle, Leydig cell, accessory gland activity and testosterone levels/ concentrations during the reproductive cycle of *Hipposideros speoris*. Correlative studies at the electron microscopic and biochemical level have established the fine structural characteristics related to active steroid synthesis and secretion in Leydig cells of a wide variety of mammals (Christensen and Gillim 1969); (Christensen 1975); (Neaves 1975); (Neaves 1977). To-date ultrastructural studies on Leydig cells are available for *Myotis lucifugus* (Gustafson 1975 b); (Gustafson 1979) *Rhinolophus capensis* (Quibell and Bernard 1983); (Bernard 1986) and *Miniopterus schreibersii* (Bernard *et al.*1991). The secretory activity of accessory glands at To-date ultra structural level is known only for *Rhinolophus capensis* (Sheppey and Bernard 1984); (Bernard 1986). Hence the control of reproductive processes are discussed only for these 3 species.

Studies on the control of male reproductive processes shows that the activity of the accessory glands and continued libido may be associated with either Leydig cell activity and high testosterone concentrations-Synchronous cycle eg. *Miniopterus schreibersii* (Bernard *et al*. 1991), or with inactive Leydig cells and low plasma testosterone concentrations Asynchronous cycle eg. *Myotis lucifugus* (Gustafson 1979); (Gustafson and Shemesh 1976); (Gustafson 1987), *Rhinolophus capensis* (Bernard 1986). A secondary elevation in plasma testosterone concentration in *Miniopterus schreibersii* occurs during reproductive inactivity (October) was not accompanied by any change in Leydig cell ultra structure, biological significance of this peak is unknown (Bernard *et al*. 1991). In *Rhinolophus capensis,* ultrastructurally two peaks of Leydig cell activity occurs one in summer-March-April and the other in late winter/ early summer- July to September. The Leydig cells are inactive during winter. The second period of Leydig cell activity and increasing testosterone level 3.2 ng/ml may be associated with copulation / libido (Bernard 1986) .In *Myotis lucifugus* copulation / libido occurs during winter hibernation associated with inactive Leydig cells and low testosterone level (Gustafson 1979).

The role of gonadotrophs in the control of male reproductive processes has been established for mammals (deKretser 1984); (Hall 1988). Limited data is available for bats (*Nyctalus noctula*, (Racey 1974); *Myotis lucifugus* (Anthony and Gustafson 1984) and *Miniopterus schreibersii* (Bernard *et al*. 1991) which indicate that activity of the gonadotrophs coincides with spermatogenesis.

Thus literature survey reveals that there is scope for further studies on the control of male reproductive processes in hibernating temperate zone bats and tropical non hibernating bats including *Hipposideros speoris* especially with regard to inter-relationship of hypothalamus-pituitary-gonad along with ultra structural studies on Leydig, Sertoli and accessory glands including the epididymis both caput and cauda and also testosterone concentrations throughout the male reproductive cycle.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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