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Biochemical estimation of glycogen in the epididymis during the reproductive cycle of Rhinolophid bat *Hipposideros speoris* (Schneider).

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ABSTRACT

Spermatogenesis commenced in September and by October spermiogenesis is observed and sperms are slowly transported to the cauda epididymis. Peak spermatogenesis and spermiogenesis occur in testis in November-December and thus enormous numbers of sperms accumulate in the cauda epididymis. Regression sets in the testis by mid January and complete regression or involution of the testis occurs by March. Although the testis undergoes regression the sperms are stored in cauda epididymis until mid-April. Estimation of Glycogen during the reproductive cycle in the epididymis of Leaf nosed bat *Hipposideros speoris* was examined. Fructose was estimated throughout the year, with the commencement of breeding in September the quantity of epididymal fructose is highest during January, February, March (56.55 ± 10.72, 59.72 ± 3.01 and 59.36 ± 20.97 mg/g of tissue) and in April the level is slightly lower than these three months 43.79 ± 29.01 mg/g of tissue. The epididymal fructose level declines from May (24.33 ± 4.1 mg/g of tissue) and reaches minimum value during July 9.52 ± 4.71 mg/g of tissue).

KEYWORDS

Bat;
Biochemistry;
Glycogen;
Reproductive cycle;
Testis;
Epididymis.

INTRODUCTION

The breeding biology of Indian bats has been reviewed by Gopalakrishna and Sapkal (1986) and Gopalakrishna and Badwaik (1993). Some information on the breeding habits of Indian bats is also known through the works of Bhat *et al*, (1980), Sandhu (1984); Karim and Banerjee (1989); Dominic and Krishna (1989). Family Rhinolophidae comprises two subfamilies: Rhinolophinae and Hipposiderinae, the latter comprises 09 genera and 65 species. Details of breeding biology, early development implantation and placentation is known for the following species: *Hipposideros bicolor pallidus* (Gopalakrishna and Moghe, 1960) *Hipposideros ater* (Gopalakrishna and Madhavan, 1978; Inamdar, 1986) *Hipposideros fulvus fulvus* (Madhavan *et al.,* 1978; Karim, 1976; Bhiwgade, 1990; Kothari and Bhiwgade, 1992) *Hipposideros lankadiva lankadiva* (Sapkal and Bhandarkar, 1984; Bhiwgade, 1979; Kothari and Bhiwgade 1992; Khan 1996). Some information is available on fur colour and the social behaviour of *Hipposideros galeritus brachyotis* (Brosset, 1962).

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). Amongst the tropical species the phenomenon is reported for *Hipposideros speoris* (Gopalakrishna and Bhatia, 1980, 1983) *Rhinopoma kinneari* (Anand Kumar, 1965) *Rhinopoma hardwickei hardwickei* (Banerjee

and Karim, 1986) *Cynopterus sphinx* (Krishna and Dominic, 1984) *Taphozous longimanus* (Singh, 1997).

Although storage of spermatozoa in the epididymides has been reported in *Hipposideros speoris* (Gopalakrishna and Bhatia, 1980), no information is available on the biochemical concentration of any substrate as well as enzymes in the epididymis in any Indian rhinolophid bat. Thus it will be interesting to study the role of substrates like glycogen biochemically in the epididymis of Leaf-Nosed bat *Hipposideros speoris* (Schneider), Order- Chiroptera, Mammalia.

The present report comprises the biochemical estimation of glycogen as an essential substrate for male reproductive physiology of *Hipposideros speoris.*

MATERIALS AND METHODS

The males of Leaf-Nosed bat *Hipposideros speoris* collected from underground delapidated dark rooms from Ballarshah, Chandrapur, India, throughout the year. The collections were made once a month (mid-monthly) and during the breeding activity of the male, the collections were made twice monthly (1st week and last week of the month). For biochemical observations, bats were decapitated and the testis were separated, cleared off their adnexa, rolled on filter paper and weighed on a metlar balance. 10% homogenate was prepared for biochemical analysis. The weight of testis was recorded and the tissues homogenized with the help of electric homogenizer. Biochemical estimation of glycogen in the testis was carried out Glycogen concentration biochemically was estimated by Anthrone method.

RESULTS

Estimation of Glycogen during the reproductive cycle in the epididymis of *Hipposideros speoris* was examined. The amount of epididymal fructose is expressed as mg/g of tissue. The amount of fructose was estimated in the epididymis throughout the year with the variations given in Table and Histogram. Spermatogenesis sets in the testis in September and by October spermiogenesis is observed and sperms are slowly transported to the cauda epididymis. Peak spermatogenesis and spermiogenesis occur in testis in November-December and thus enormous numbers of sperms accumulate in the cauda epididymis. Regression sets in the testis by mid January and complete regression or involution of the testis occurs by March. Although the testis undergoes regression the sperms are stored in cauda epididymis until mid-April. The level of epididymal fructose is highest during January, February, March (56.55 ± 10.72, 59.72 ± 3.01 and 59.36 ± 20.97 mg/g of tissue) and in April the level is slightly lower than these three months 43.79 ± 29.01 mg/g of tissue. Fructose provides nourishment to the stored spermatozoa in the epididymis and coincidently the level of fructose is high from November - until April. The epididymal fructose level declines from May (24.33 ± 4.1 mg/g of tissue) and reaches minimum value during July 9.52 ± 4.71 mg/g of tissue).

Table 1: Biochemical estimation of glycogen in the epididymis during the reproductive cycle of *H. speoris.* (Glycogen – mg/g of tissue).

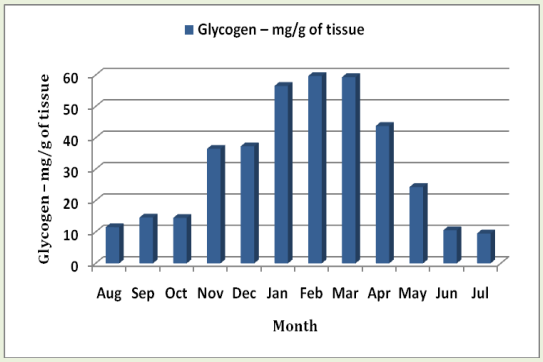
Sr. No.	Month	Epididymis
1	August	11.5251 ± 5.7507
2	September	14.5985 ± 3.6135
3	October	14.4670 ± 4.5663
4	November	36.4963 ± 11.6278
5	December	37.3073 ± 6.3323
6	January	56.5529 ± 10.7249
7	February	59.7213 ± 3.0100
8	March	59.3673 ± 20.9708
9	April	43.7956 ± 29.0101
10	May	24.3309 ± 4.1837
11	June	10.5433 ± 5.0768
12	July	9.5207 ± 4.7133

DISCUSSION AND CONCLUSSIONS

Present observation on the biochemical estimation of glycogen in the epididymis throughout the year with the variations given in Table 1 and Histogram 1, of *Hipposideros speoris* finds similarity with the observations by Janbandhu and Patil (2013) in his work on glycogen in the testis of *Hipposideros speoris* and Racey (1975) in his review on the prolonged survival of spermatozoa in bats observed that reproductive tracts of several species of vespertilionid and rhinolophid bats store sperms in the uterus or oviducts of females and in the cauda epididymides

and ductus deferens of males. He further observed that histochemical survey of the sperm - storing organs of male and female pipistrelle bat, *Pipistrellus pipistrellus* have shown that the linning epithelia secrete substances which may be of nutritive value to the sperm.

Fig 1: Biochemical concentration of glycogen in the epididymis during the reproductive cycle of *H. speoris*



Mokkapati and Dominic (1976) investigated, the sites of production of fructose and citric acid in the accessory reproductive glands of three species of male microchiropterans viz. *Cynopterus sphinx*, *Taphozous longimanus* and *Scotophilus heathi* and have shown that the prostate of the three species secreted both fructose and citric acid, fructose in greater amount than citric acid. The seminal vesicles of *Cynopterus* and *Taphozous* produced both fructose and citric acid but citric acid content was more abundant than fructose. They also showed that the ampullary glands of *Scotophilus* secreted only fructose while those of *Taphozous* produced both fructose and citric acid.

Crichton *et al.,* (1981) carried out biochemical estimation of fructose in ampullary glands, seminal vesicles, prostate gland and cauda epididymides of *Myotis lucifugus* and Myotis velifer. They showed that *M. lucifugus* exhibited a fructogenic cycle in the accessory sex glands (ampullary, prostate and seminal vesicles) that varied seasonally. They observed that the ampullary gland was the major fructose producing sex organ, the maximum level (almost 5.0 mg fructose/g of tissue reaching in (September-October) at the onset of hibernation and the fructose level remained elevated until male bats emerged from hibernation (April). Following the return of bats to an active metabolic state, ampullary gland fructose content decreased markedly (1.4 mg fructose/g tissue during May and June) in keeping with the involution of the entire gland. The prostate gland also showed an annual fructose cycle - not as dramatic, however, as the ampullary gland, and furthermore, the organ produced less sugar. They showed a rise in the fructose content of the prostate at the beginning of hibernation (2.2 mg fructose/g tissue in September) and with some fluctuation, remained higher throughout the sperm storage period than at other times in the year. The seminal vesicles also elaborated fructose, but at even lower levels (0.5 - 1.5 mg fructose/g of tissue) and with less seasonal fluctuation than the ampullary and prostate glands. When the differences in seasonal levels of fructose in either the prostate or seminal vesicles, were analyzed, no significance was recorded ($P<0.05$). In *M. velifer* they recorded a small elevation in fructose in the prostate and ampullary glands, from trace levels during July and August to 2.0 - 3.0 mg fructose/g tissue at the onset of hibernation in October. They observed that the levels were reduced in May (1.5 mg fructose/g tissue). The seminal vesicle fructose values, though much lower, demonstrated a similar though less well marked trend in annual cyclicity. An analysis of the differences between monthly means revealed no significance ($P<0.05$). The authors could not identify fructose in the cauda epididymides in both M. lucifugus and M. velifer.

Gadegone and Sapkal (1983) studied the male accessory sex glands of *Pipistrellus dormeri* and reported that the seminal vesicles elaborated neutral mucins and protein bound sialomucins. The prostate showed the presence of glycogen, neutral mucins and protein bound sialomucins and the Cowpers glands elaborated neutral sialo and sulfomucins.

Agrawal (1984) studied the biochemical composition of epididymides in Scotophilus heathi and five other mammals. They estimated total protein, sialic acid, glycogen, phospholipid, glyceryl phosphorylcholine (GPC) and acid and alkaline phosphatase in the caput, corpus and cauda epididymis and found that in the majority of the species the proximal regions of caput and corpus exhibited higher level of total protein, glycogen, phospholipid and acid phosphatase as compared to the distal cauda region. Sialic acid and GPC levels were higher in the distal region as compared to the proximal region.

Krutzsch and Crichton (1986) observed that *Pipistrellus subflavus* stored epididymal spermatozoa throughout hibernation, when the testes were involuted but the accessory gland activity was maintained. They also observed that the epididymal and testicular spermatozoa persisted longer and the weights of the accessory glands were not strongly differentiated between winter and spring/summer. They observed that this bat lacked a seminal vesicle. They estimated the fructose content in the accessory sex glands (ampullary glands, prostate and Cowper's glands) and reported that fructose was present in the prostate at all seasons but occurred at highest levels during the period of greatest prostate hypertrophy (late October : 19.0 g/prostate) which follows the onset of testicular involution. The fructose content of the prostate was shown to be lowest in the summer (July: 1.0 - 3.0 g/fructose). They showed the presence of fructose in the ampullary glands at all seasons showing lowest level in mid-July (4.0 g fructose/gland). A rise in fructose level with increase in ampullary gland weight in between late July (10.00 g fructose/gland) was noticed by them. This interval overlapped testicular involution and corresponded to the late summer and autumn period of preparation for breeding before hibernation (October). High fructose values were also recorded in December (38.0 g fructose/gland). The Cowper's gland according to them showed little seasonal change in their secretory state and gross size. Chemical analyses for fructose of homogenates prepared for this gland were negative.

Glycogen acts as one of the major readily available source of energy for various metabolic activities; hence the substrate is continuously supplied. Sperms begin to appear in the epididymis soon after the commencement of spermatogenesis in September-October and retained in it till their ejaculation. Remarkable changes in the diameter and cell height of epididymal tubular cells and varied PAS intensity in these cells suggests that they are vital centers for the supply of nutrition to the spermatozoa, thereby enriching them to survive, to gain viability, to improve fertilizing capacity and motility power. The remarkable ability of bat spermatozoa to survive for long periods in female and male reproductive tract without loss of fertilizing capacity has no known parallel among mammals. Fructose has been identified as nutritive substrate for the stored spermatozoa in the female reproductive tract of *Myotis lucifugus* and *M. velifer* during hibernation (Crichton *et al*, 1981,). Krutzsch *et al*., 1982; Krutzsch, 2009 has confirmed that there are few special morphological characteristics unique to bat sperm as most of their sites of storage which might serve to underwrite their prolonged survival. Sperm longevity may be conferred by special events in the reproductive environment, one of which may be regular supply of glycogen and its conversion into utilizable sugars as a source of energy. The orientation of spermatozoa in epididymal epithelium also plays significant role in transfer of nutrients. "Nutritional inter-relationship may more likely underline intimate sperm-uterine associations. Evidence giving credence to this speculation relates to the presence of glycogen in uterine (and epididymal) epithelium (Nakano, 1928; Wimsatt, 1949; Racey and Potts, 1970; Racey, 1975), while certain enzymes of glycolysis and the pentose and sorbitol pathways have been located in the spermatozoa as well as epithelia from both the epididymides and uterus (*Pipistrellus pipistrellus* - Racey, 1975; *Myotis lucifugus*- Wimsatt, 1969)".

Crichton *et al* (1981) finding that the annual fructogenic cycle correlates positively with accessory gland hypertrophy and fructose rises near the end of the spermatogenic phase and maintains maximum levels throughout the hibernation (sperm storage) in *Myotis lucifugus* and *M. velifer* resemble with present finding on *H. speoris* only in case of high glycogen levels during sperm storage phase but not at the time of regression of spermatogenic activity as suggested by Crichton *et al* (1981). In *H. speoris* there is close synchrony in the spermatogenesis and activity of accessory glands, the glycogen levels also show gradual increase in association with these events as well as sperm storage contrary to temperate zone *Myotis lucifugus and M. velifer* (Crichton *et al*, 1981). These authors have suggested that factors operating in the male to promote sperm survival may differ from those prevailing in the female, further they hinted at operation of different mechanism and added that fructose does not bear strong relationship with sperm storage period and it may only play a role in sperm survival.

In all the species energy for sperm motility is provided in the form of ATP which is produced as a

result of glycolysis and oxidative phosphorylation (Racey, 1979). Harrison (1977) has argued that spermatozoa are always able to acquire sufficient substrates for metabolism from the secretions with which they are in contact. PAS positive epididymal luminal secretions observed in *H. speoris* supports this view and further strengthened due to intense PAS concentration in sperm head. Sperm metabolism during storage depends upon acquisition of all the required metabolites. Unlike temperate zone bats, the storage period may not last more than two months during which maturity is attained and soon they are ejaculated during insemination. As the spermatogenetic process continues till mid-January, and glycogen titers are also high it is suggested that synchrony is also apparent in reproductive event and glycogen concentration which elevate and decline with these events in *H. speoris*.

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