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HISTOCHEMICAL CHANGES IN LEYDIG CELLS AND SEMINIFEROUS TUBULES OF MICROCHIROPTERAN BAT *HIPPOSIDEROS SPEORIS* (SCHNEIDER) DURING TESTICULAR CYCLE

M. S. SASTRY AND SUSHMITA CHOUDHURY*
Department of Zoology, RTM Nagpur University, Nagpur - 440 033, INDIA
E-mail: sushmita.chaudhury7683@gmail.com

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***Corresponding author**

ABSTRACT
Hipposideros speoris is an insectivorous bat and breeds once in a year. For the demonstration of oxidative enzymes namely succinic dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) throughout the annual reproductive cycle the testis were histochemically studied. Correlative to the reproductive status specific changes in the enzymatic reaction were observed. Thus during quiescense (April-August) a moderate activity in the Leydig cells whereas very low reactivity in the seminiferous tubules for both the enzymes was observable. With the approach of maturity (recrudescence: September-October) an enhanced activity reflecting high intensity in the Leydig cells whereas in the seminiferous tubules similar intensity expressed by granular formazan deposition. Massive deposition of formazan granules characterized this period (breeding period: November-December), however, there appeared differential distribution of the granules being even all over the seminiferous tubule while concentrated at one spot in Leydig cells. The characteristic features of the regression period (January-March) were prominent decrease in the intensity of enzymes which was almost negligible in the seminiferous tubules. From the foregoing, it is concluded that the annual reproductive cycle and enzymic activity go vis-à-vis.

INTRODUCTION

The mammalian testis consists of two compartments: seminiferous tubules and interstitium which fills the space between the seminiferous tubules. The interstitium contains blood and lymphatic vessels, cell types including fibroblasts, macrophages, monocytes, mast cells and the steroid secreting Leydig cells (Akingbemi and Ren-Shanbe Hardy, 1998; Soder, 2003; Johnson, 2007). The localization of mitochondrial oxidative enzymes namely succinic dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) belonging to tricarboxylic acid cycle and pentose phosphate pathway is an indication of energy-dependent metabolic processes probably involved in absorptive functions and in steroidogenesis (Blackshaw and Samisoni, 1966; Johnson and Gomes, 1970; Akingbemi and Ren-Shanbe Hardy, 1998; Kishore *et al.,* 2007). The metabolism of the Leydig cells along with seminiferous tubules and localization of these enzymes have not been extensively studied in Chiropterans in relation to the annual reproductive cycle except for few species such as *Myotis lucifugus* (Baillie *et al.,* 1966) and *Vesperugo pipistrellus* (Saidapur, 1976). Hence the present work was undertaken to find the reaction pattern of these enzymes throughout the reproductive cycle in the Indian leaf- nosed bat *Hipposideros speoris* (Schneider).

MATERIALS AND METHODS

All experiments were conducted in accordance with the principles and procedures approved by the departmental research committee at RTM University, Nagpur, Maharashtra, India.Three bats were trapped in each calendar month with the help of a mist net from the natural population inhabiting abandoned mines in Khapa, Nagpur, Maharashtra (20º92′ N 78º95′ E). The reproductive cycle of *H. speoris* was studied in the following four phases: (1) Quiesence (April-August): The resting stage (2) Recrudescence (September-October): Preparation of testis for breeding (3) Breeding (November early December): Peak of spermatogenesis (4) Regression (January-March): Spent testis. The sucrose fixed tissues were cut on freezing microtome at 20ºC and were histochemically stained. The method of Nachlas *et al.* (1957) was employed to demonstrate SDH activity. The incubation medium for SDH consisted of phosphate buffered (pH 7.6) disodium-succinate as substrate and nitroblue tetrazolium. The method of Ballie *et al.,* (1966) was employed to demonstrate G-6-PDH activity. The reaction medium for G-6-PDH consisted of phosphate buffered (pH 7.4) glucose-6-phosphate as substrate, nicotinamide adenine dinucleotide phosphate (NADP) and nitroblue tetrazolium. Control incubation was performed without the substrate. Histochemical reaction and reaction intensity was graded visually on an arbitrary scale (from = negligible, + = low, ++ = moderate, +++ = high, ++++ = intense).

RESULTS

Hipposideros speoris breeds once in a year. Adult males show peak in their testicular activity from November-December corresponding to the mid-December ovulation in the female (Gopalakrishna *et al.,* 1991). With respect to the reproductive pattern the annual cycle is divided into following stages and all the histochemical changes in the enzymatic profile are

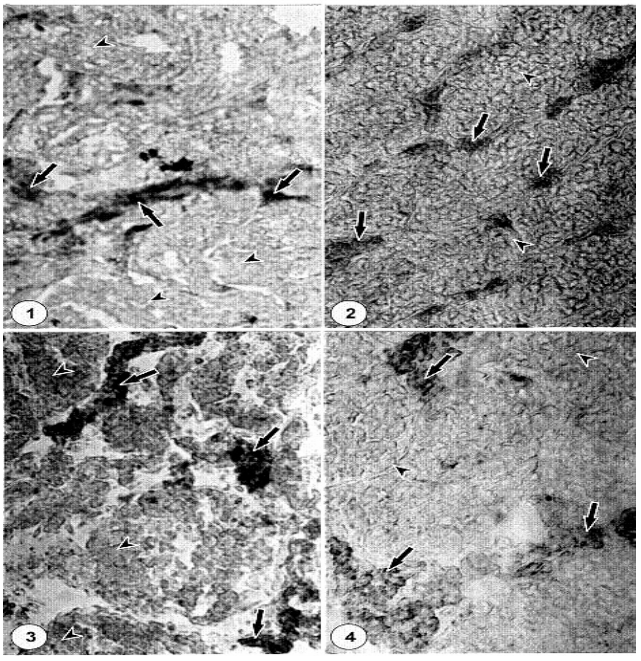


Figure 1, 2, 3 and 4: (1) Less proliferated Leydig cells showing patchy and moderately stained formazan granules during early, quiescence (arrow). Please note primary and secondary spermatocytes in seminiferous tubules where the reaction intensity of SDH is low (arrow head) X 100; (2) Identical staining profile for G-6-PDH during quiescence. Note diffusely distributed smaller granules both in the Leydig cells (arrow) and spermatogenic elements (arrow head) X 100; (3) Arrow marks further increment in number of Leydig cells and in intensity of SDH during late quiescence (arrow). Note diffuse but scattered formazan granules in the seminiferous tubules (arrow head) X 100; (4) Mirrors the distribution pattern and staining characteristic of G-6-PDH in Leydig cells (arrow) as shown in Fig.3. but of a little lesser degree distributed in all components of the germinal epithelium (arrow head) X 100

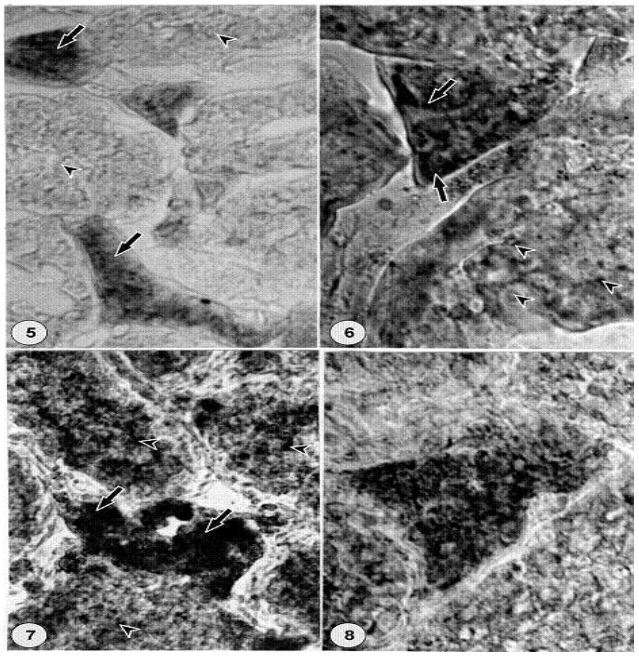


Figure 5, 6, 7 and 8: During recrudescence period (September) the development of Leydig cells (arrow) as well as spermatogenic (arrow head) activity and enzyme reactivity of SDH spurts. Note moderately strong activity in Leydig cells when compared with tubular elements X 450; (6) Cross-section of testis at recrudescence emphasizing similar intensity for G-6-PDH enzyme (arrow and arrow head) X 450; (7) Note deeply stained clustered formazan granules of variable size in the Leydig cells (arrow) as well as seminiferous tubules (arrow head) X 450; (8) Similar reaction intensity supports the earlier results shown in Figure 7. X 450.

depicted in Table 1.

Quiescence phase

This is the resting phase which comes with the end of regressive period (January-March). A renewal in the testicular activity and therefore in the Leydig cells was marked by prominence in their development. The kinetics of spermatogenesis appeared to be differed upto either primary or secondary spermatocytes in the less circularly designed seminiferous tubules mostly lacking or ill-developed lumen.The reaction intensity of SDH and G-6-PDH was moderate during early quiescence as evidenced by their patchy concentration in the SDH stained sections (Fig. 1) and further confirmed by the diffusely distributed smaller granules in the G-6PDH stained sections in the Leydig cells (Fig. 2). The nuclei of the Leydig cells and fibroblasts were mildly reactive and identifiable in the interstitial area. Whereas low intensity for the enzymes was notified in the seminiferous tubules (Figs.1 and 2). However, with the advancement of quiescence period the pattern of distribution of the enzymes appeared more or less identical in the Leydig cells but of a little lesser degree, evenly distributed in all components of the germinal epithelium in the seminiferous tubule (Figs. 3 and 4).

Recrudescence phase

In comparison to the previous stage the seminiferous tubules were characterized by further differentiation of spermatogenic stages upto long spermatids.The Leydig cells of the interstitium displayed moderately strong activity when compared with the tubular elements (Figs. 5 to 8).

Active phase

The seminiferous tubules were fairly large and occupied approximately 90% of the fields examined, and the small tubular lumina were lined with spermatozoa. The activity for

Table 1: SDH and G-6-PDH reaction intensity in the leydig cells and seminiferous tubules

	Leydig cell	Seminiferous tubule	Leydig cell	Seminiferous tubule
Quiescence	+ +	+	+ +	+
Recrudescence	+ + to + + +	+ + to + + +	+ + to + + +	+ + to + + +
Breeding	+ + + +	+ + + to + + + +	+ + + +	+ + + to + + + +
Regression	Early	+ +	+ +	+ + + +
	Late	+	-	+ -

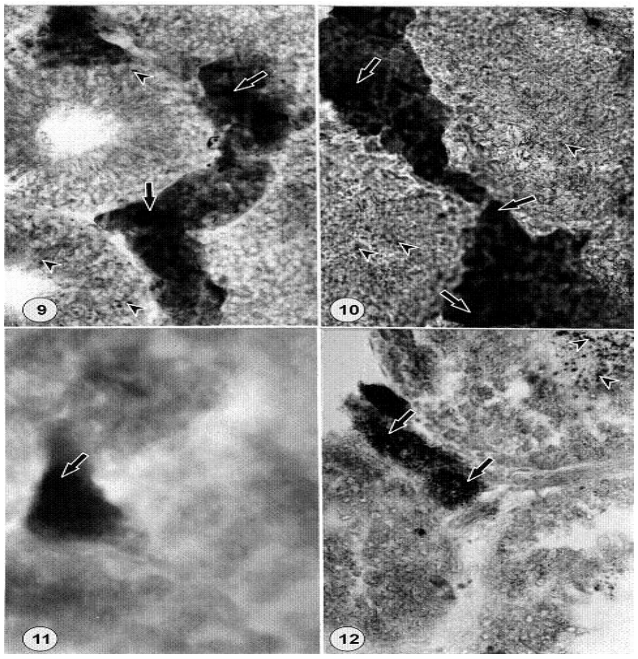


Figure 9, 10, 11 and 12: Interstitium prominent at this time (breeding phase: November-early December) and the Leydig cells remained numerous.Similarly the spermatogenic activity is at peak.The reaction appears intense with an increase in the deposition of formazan granules in interstitial (arrow) as well as tubular tissue (arrow head) X 450; (10) G-6-PDH displays a definite pattern of activity being extremely reactive in Leydig cells as marked by closely packed intense granules (arrow), however, intensity appeared distinct but is more in spermatogonia than in spermatocytes (arrow head) X 450; (11) Note regression in the number of testicular elements and moderate enzymity (arrow) when compared to active mating period in the spent testis X 450; (12) Coarser granules in the Leydig cells of G-6-PDH enzyme is a replica of the SDH enzyme (arrow). Since there is no synchrony in the reproduction the reactivity is evident in spermatozoa of other specimens but other elements of the seminiferous epithelium are meagerly stained (arrow head) X 450.

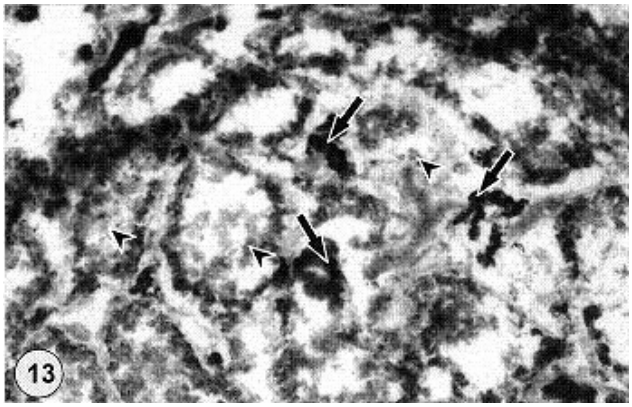
SDH and G-6-PDH was observed in all areas of the testis but was more in spermatogonia than in the spermatocytes, however the nuclei demonstrated marked reaction when compared to their cytoplasm. Moreover, tunica albuginea was inconspicuous due to negligible activity. Interstitium was particularly prominent at this time and the Leydig cells remained numerous. The reaction was fairly strong comprehended by the deposition of closely clustered coarser formazan granules coincident to the hydroxylations during steroidogenesis. On comparison it was noted that the reaction intensity of spermatocytes and Leydig cells were in close approximity (Figs. 9 and 10).

Regression phase

The infrastructure of the seminiferous tubules along with the spermatogenic elements displayed regressive changes hence as anticipated regression in the number of Leydig cells, spermatogonial cells of the tubular epithelium and Sertoli cells. Coincidently enzyme characteristic observed during active mating period declined from high to low in the testicular elements. The decrease in the triradius mesenchyme, fibrosis of Leydig cells and relative decline in the staining profile of the Leydig cells also confirmed the seasonality of the reproductive cycle (Figs.11 and 12). Thus during early regression SDH and G-6-PDH reactivity was evident in the spermatozoa but other elements of the seminiferous epithelium were meagerly stained. The presence of spermatozoa in the lumen of some species pointed to the asynchrony in reproduction in *H. speoris*. The compactness of regressing Leydig cells during late regression resulted into an enormous increase in interstitial spaces facilitated by simultaneous shrinkage of the seminiferous tubules. Due to above mentioned regressive changes there was a suppression in the staining characteristics in older spermatocytes and spermatids whereas the Sertoli cells showed a virtual absence of the reaction corroborating the close relation between enzymic and testicular activity (Figs.13 and14).

DISCUSSION

Active spermatogenesis in *H. speoris* occurs during November-December but the testis is quiescent from April-August.This study has been undertaken to demonstrate the pattern of enzyme profile of SDH and G-6-PDH in Leydig cells as well as seminiferous tubules throughout the reproductive cycle since among Chiropterans such studies are limited to only few



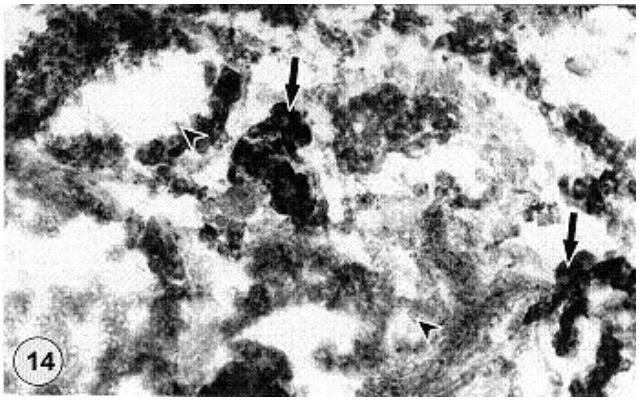


Figure 13 and 14: Illustrating the low reaction intensity of SDH in the reduced triradius mesenchyme with less number of fibrotic Leydig cells (arrow) during late regression period (January-March). Note simultaneous shrinkage of seminiferous tubules with very low to negligible intensity of SDH (arrow head) X 100; (14) low intensity of G-6-PDH in the Leydig cells (arrow) suggests its minimal utilization during regression. Note suppression in the staining characteristics in older spermatocytes and spermatids whereas the Sertoli cells shows virtual absence of the reaction (arrow head) X 100

species such as *Myotis lucifugus* (Baillie *et al.,* 1966) and *Vesperugo pipistrellus* (Saidapur, 1976).These two oxidative enzymes are key enzymes in the energy generating pathways (TCA and Pentose phosphate pathway) hence their localization in the Leydig cells and seminiferous epithelium be taken as an indication of an increase in the metabolic activity of these tissues. The patterns recorded in the reaction intensity and development of tubules as well as interstitial cells were variable during different phases of the reproductive cycle. It was also noted that the pattern of localization of these two oxidative enzymes were very similar. Thus, during quiescent phase (April-August) both the enzymes denoted low to moderate activity along with the proliferation in the development of Leydig cells and low activity in the seminiferous epithelium. With the approach of maturity (recrudescence period: September- October) high intensity of enzymes were notified due to an enhancement in the activity of Leydig cells and simultaneous approach of spermatogenic activity. The breeding period (November-December) revealed an elaboration in the morphological development of Leydig cells and the completion of spermatogenesis with intense reaction for both the enzymes in the interstitium and high to intense in the seminiferous epithelium, thereby indicating that the cells were sites of high metabolic activity thus provides additional albeit indirect evidence of steroidogenic potentiality of these cells, as the enzymes are known to generate NADPH needed for hydroxylations during steroidogenesis. With regression a prominent decrease in the infrastructure of Leydig, spermatogonial and Sertoli cells as well as intensity were noticeable. Similar pattern of reaction have also been described in Indian Gerbil for G-6-PDH (Chandrakala and Sarkar, 1980) during the complete reproductive cycle. However, the presence of these two enzymes has also been demonstrated in the testis of rat, ram, bull, fetal rat testis, human fetus (Wolfe and Cohen, 1964; Neimi and Ikonen, 1966; Blackshaw and Samisoni, 1967; Joanne and Judith, 1980; Kishore *et al.,* 2007) but not correlative to the reproductive cycle. From the foregoing it is concluded that in the bat *H. speoris* Leydig cells have a seasonal orientation expressed in the presence and variations in intensity of SDH and G-6-PDH in relation to the steroidogenic activity and localization of these two mitochondrial enzymes in the seminiferous epithelium is also an indication of energy dependent metabolic processes probably involved in absorptive functions needed for the process of spermatogenesis.

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