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Histochemical localization of two oxidative enzymes in the Leydig cells of microchiropteran bat *Hipposideros speoris* (Schneider) during the annual testicular cycle

M.S. Sastry and Sushmita Choudhury
Department of Zoology, RTM Nagpur University, Nagpur-440033, India

SUMMARY

Hipposideros speoris is an insectivorous bat which breeds once in a year. The adult males show peak in their testicular activity from November-December, corresponding to the mid-December ovulation in the females. The testis of this bat was investigated for histochemical localization of succinic dehydrogenase and glucose-6-phosphate dehydrogenase in the Leydig cells. Specific changes in the enzyme reactions were observed correlative to the reproductive status. Thus, during the quiescent phase (April-August) proliferation of the atrophied Leydig cells was observed, along with a moderate activity of both the enzymes. With the approach of maturity (recrudescent phase: September-October) an enhancement in the enzyme activities was noticed. The onset of breeding period (November-December) brought about an abundance of Leydig cells and increase in the intensity of the enzyme reactions. During the post-mating phase (mid-December) the Leydig cells were not only regressed but decreased in abundance, with corresponding decrease in the localization of the enzymes. During the regression phase (January-March) there was a dramatic decrease in the abundance of the cells as well as intensity of reaction of the enzymes. It is concluded that G-6-PDH and SDH would generate NADPH that is needed for hydroxylation of steroids during steroidogenesis and the histochemical demonstration of these two enzymes provides an additional evidence for the steroid generating capacity of the Leydig cells, which follows a seasonal pattern.

Key words : Bat, *Hipposideros speoris*, Leydig cells, G-6-PDH, SDH.

Introduction

The mammalian testicular interstitial cells of Leydig have been shown to be steroidogenic sites (Akingbemi et al., 1998; Lejeune et al., 1998). The localization of mitochondrial oxidative enzymes, succinic dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) belonging to the tricarboxylic acid cycle and pentose phosphate pathway respectively, in the Leydig cells is an indication of energy-dependent metabolic processes probably involved in absorptive functions and in steroidogenesis (Blackshaw and Samisoni, 1966; Johnson, 1970; Akingbemi et al., 1998; Kishore et al., 2007). The metabolism of Leydig cells and the localization of these enzymes have not been extensively studied in chiropterans in relation to the annual reproductive cycle except for a few species such as *Myotis lucifugus* (Baillie et al., 1966) and *Vesperugo pipistrellus* (Saidapur, 1976). Hence, the present work was undertaken to find the localization of these mitochondrial enzymes through the annual 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 of RTM University, Nagpur, Maharashtra, India. In each calendar month three bats were trapped from the natural population inhabiting abandoned mines in Khapa, Nagpur, Maharashtra (20°92′′N 78°95′′ E) with the help of a mist net. The reproductive cycle of *H. speoris* is divided into five phases: (i) quiescence (April-August), the resting stage; (ii) recrudescence (September-October), preparation of testis for breeding; (iii) breeding (November-early December), peak of spermatogenesis; (iv) post-mating (mid-December), decline in spermatogenesis; and (v) regression (January-March), spent testis. The sucrose-fixed testis tissue was cut in a freezing microtome at -20°C and the sections 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 Baillie

Correspondense to be addressed to: Dr. M.S. Sastry, Ph.D., Email: sastry_nidhi@rediffmail.com

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et al. (1966) was employed to demonstrate G-6-PDH activity. The reaction medium 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 were graded visually on an arbitrary scale (from + = low, ++ = moderate, +++ = high, ++++ = intense).

Results

Hipposideros speoris breeds once in a year. Adult males show peak in 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 stages as in the table 1, and the corresponding histochemical profiles of enzymes are depicted.

Quiescence phase: This is the resting phase, which begins with the end of regression period (January-March). A renewal in the testicular activity and, therefore, in the Leydig cells was marked by prominence in their development. The reaction intensity of SDH and G-6-PDH was low to 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 (Fig. 2). However, with the advancement of quiescence period the pattern of distribution of the enzymes appeared more or less identical (Figs. 3, 4).

Recrudescent phase: An increase in the activities of both the enzymes was indicated (Figs. 5-8).

Active phase: The interstitium was particularly prominent at this time, and the Leydig cells increased in abundance. The SDH reaction was deeply intense, as revealed in the deposition of closely clustered coarse formazan granules coincident to the hydroxylation during steroidogenesis (Fig. 9). The G-6-PDH reactivity was more or less similar to that of SDH (Fig. 10).

Post-mating: The Leydig cells regressed and became sparse, and enzyme localization was from high to moderate (Figs.11, 12).

Regression phase: Further intensification of the regressive changes was marked by decline in the abundance of Leydig cells and their fibrotic appearance. The compactness of regressing Leydig cells resulted in an enormous increase in interstitial spaces facilitated by shrinkage of the seminiferous tubules. There was deterioration in the reactivities of the enzymes, corroborating the close relationship between enzyme and testicular activities (Figs. 13, 14).

Table – 1 : SDH and G-6-PDH profile in the Leydig cells of *Hipposideros speori*s

Reproductive stage	SDH	G-6-PDH
Quiescence	+ to ++	+ to ++
Recrudescence	++ to +++	++ to +++
Breeding	++++	++++
Post-mating	++	++
Regression	+	+

Discussion

Active spermatogenesis in *Hipposideros speoris* occurs during November-December and the testis is quiescent from April-August. This study was undertaken to demonstrate the pattern of activities of SDH and G-6-PDH in the Leydig cells through the annual reproductive cycle, since among the chiropteran mammals such studies are limited to only few 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 can be taken as indicating an increase in the metabolic activity of this tissue. The pattern recorded in the reaction intensity and Leydig cell development was different during the different phases of the annual reproductive cycle. The patterns of localization of these two oxidative enzymes were very similar and suggest that there is gradual and well-organized shift in the loci of the enzyme activities. Thus, during the quiescent phase (April-August) both the enzymes showed moderate to high activity accompanied by proliferation and development of Leydig cells. With the approach of maturity (recrudescent phase: September-October) an enhancement in the prominence of Leydig cells accompanied by a high intensity of enzymes was noticed. The onset of breeding phase (November-December) caused an elaboration of Leydig cells, and an

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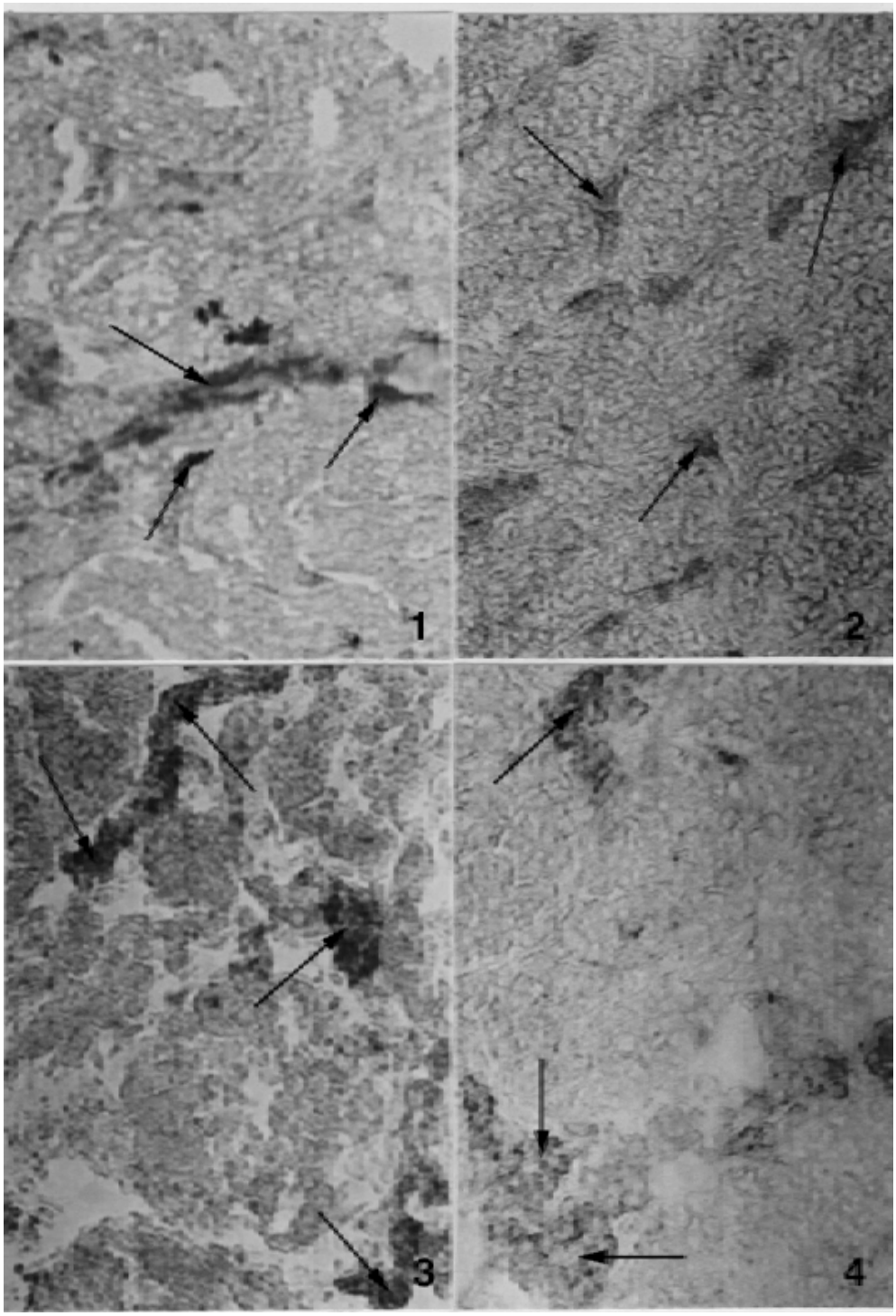

Fig.1. Scattered Leydig cells showing dispersed and moderately stained formazan granules of SDH during the early quiescent phase (arrows). x100.

Fig.2. Similar profile of G-6-PDH during the quiescent phase. Note diffusely distributed smaller granules (arrows). x100.

Fig.3. Increase in abundance of Leydig cells and fairly high SDH activity during the late quiescent phase. Note diffuse but scattered formazan granules (arrows). x100.

Fig. 4. The distribution pattern of Leydig cells and staining characteristic (arrows) of G-6-PDH during the late quiescent phase. x100.

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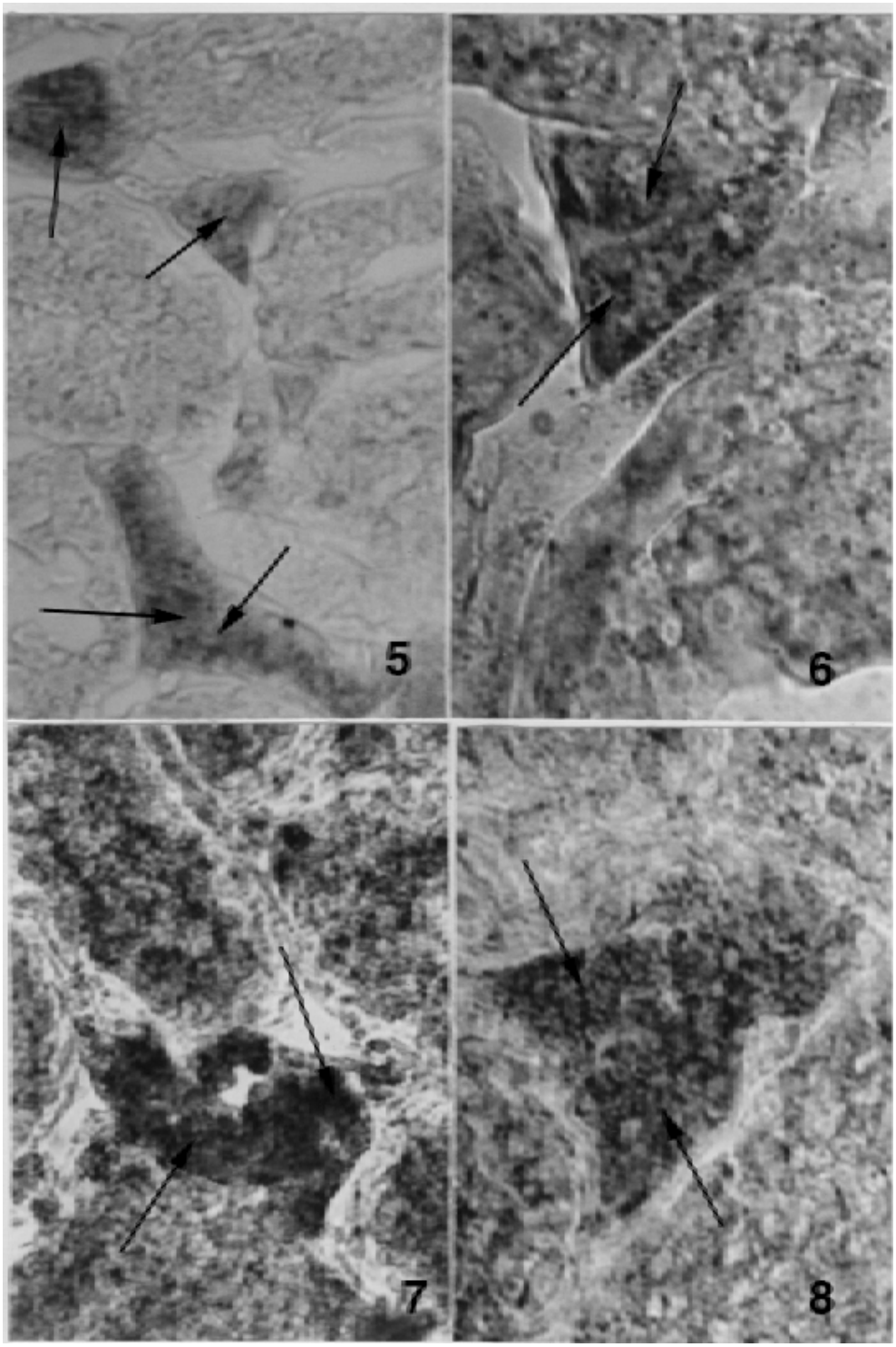

Fig.5. Recrudescent phase (September) : there is a spurt of development of Leydig cells and activity of SDH (arrows). x450.

Fig.6. Cross section of testis at the recrudescent phase showing similar intensity for G-6-PDH enzyme activity (arrows). x450.

Fig.7. Recrudescent phase. Note deeply stained and clustered formazan granules of variable sizes- SDH (arrows). x450.

Fig.8. Recrudescent phase. Similar reaction pattern of G-6-PDH (arrows). x 450.

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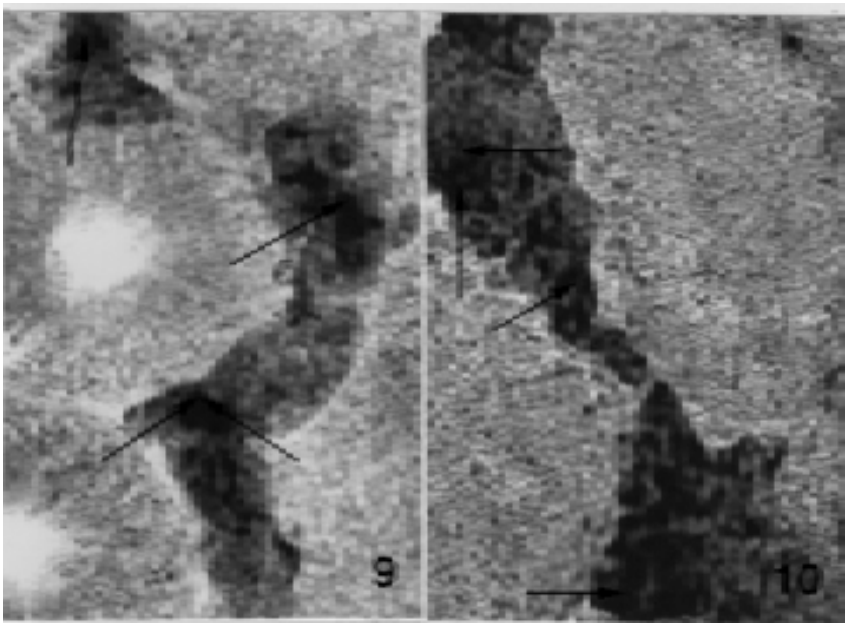

Fig.9. Active phase**.** The interstitium is particularly prominent at this time and the Leydig cells are numerous. The reaction for SDH appears intense with an increase in the deposition of formazan granules (arrows). x450.

Fig.10. Active phase. G-6-PDH in Leydig cells is very high as indicated in the closely packed and intense granules (arrows). x450.

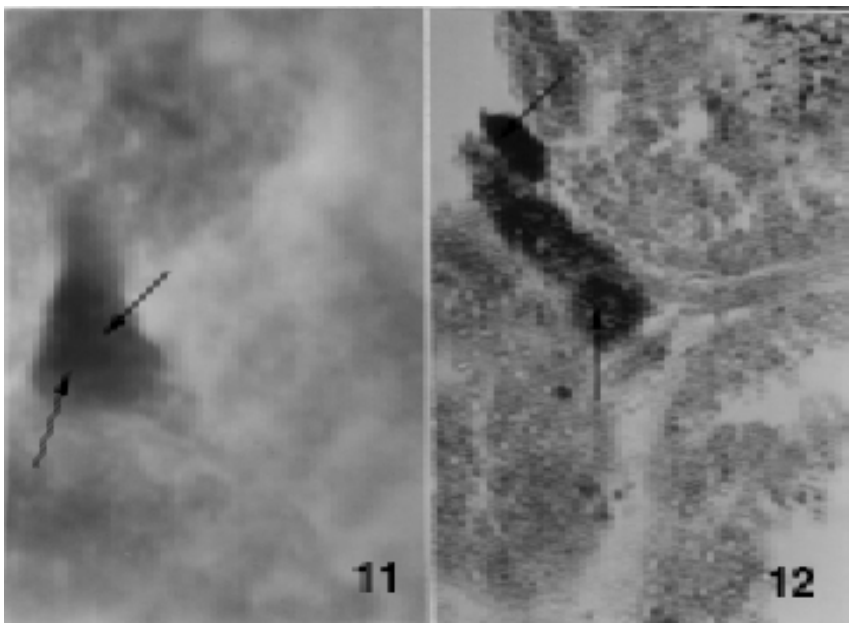

Fig.11. Post-mating**.** Note decrease in the abundance of Leydig cells and expression of SDH activity from high to moderate (arrows) when compared to the active mating period. x 450.

Fig.12. Post-mating**.** Coarse granules of G-6-PDH in the Leydig cells (arrows). x450.

intense reaction of the enzymes during this phase, indicating that these cells are sites of high metabolic activity. This provides additional, albeit indirect, evidence of steroidogenic potential of these cells, as the enzymes are known to generate NADPH needed for hydroxylation during steroidogenesis. During the post-mating phase (mid-December) the cells were regressing, with a moderate reactivity of the enzymes. With regression, a prominent decrease in the cells as well as intensity of the enzyme reaction was found. A similar pattern has been described

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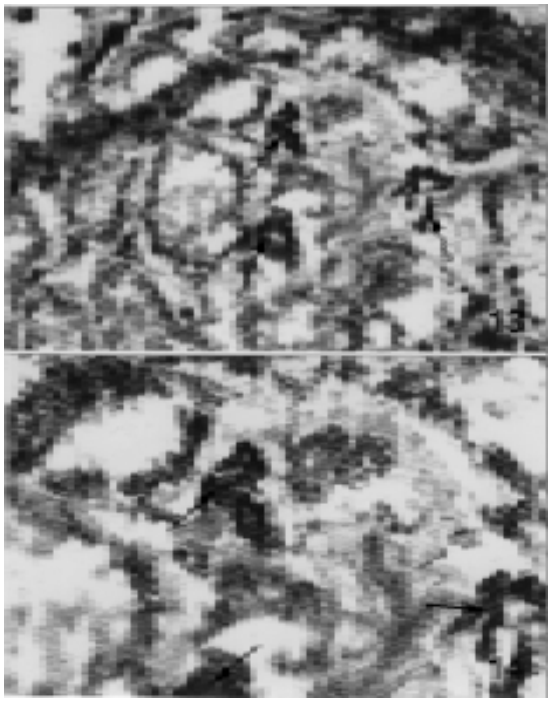

Fig.13. Regression phase**.** Low reaction intensity of SDH in the reduced number of fibrotic Leydig cells (arrows). x100.

Fig.14. Regression phase. Low intensity of G-6-PDH activity in the Leydig cells (arrows). x100.

in Indian Gerbil for G-6-PDH during the annual reproductive cycle (Chandrakala and Sarkar, 1980). The presence of these two enzymes has been demonstrated in the testis of rat, guinea pig, ram, bull, fetal rat, and human fetus (Neimi and Ikonen, 1962; Wolfe and Cohen, 1964; Blackshaw and Samisoni, 1967; Joanne and Judith, 1980; Kishore et al., 2007) but not in relation to the reproductive cycle. It is concluded that in the bat *H. speoris* the Leydig cells follow a seasonal pattern, as reflected in the abundance of Leydig cells as well as the activities of SDH and G-6-PDH in relation to the steroidogenic activity.

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