Vespertilio 9–10: 175–181, 2006
ISSN 1213-6123

Histochemical pattern of ovarian 5'-nucleotidase in *Rhinopoma microphyllum* during different reproductive states

Seema Trivedi[1,3] & Suresh Bihari Lall[2]

[1] *Department of Zoology*, *J. N. Vyas University*, *Jodhpur* (*Rajasthan*) *India*
[2] *Department of Zoology*, *M. L. Sukhadia University*, *Udaipur* (*Rajasthan*) *India*
[3] *Madan Niwas*, *104*, *near Lodha Complex*, *Shastri Marg*, *Udaipur* (*Rajastan*) *313 001*, *India*;
trivedi_seema@rediffmail.com; *svtrived@hotmail.com*

Abstract. Histochemical shifts in the pattern of 5'-Nucleotidase were observed in the ovaries of non--pregnant, pregnant and lactating microchiropteran *Rhinopoma microphyllum kinneari*. In the ovary of non-pregnant bats, 5'-Nucleotidase activity was observed to be intense in the granulosa cells of different follicle types. Extroverted corpus luteum; in the ovulating ovary of pregnant bats; and the follicle types of both ovaries showed moderate, though differential staining pattern. In lactating bats, all follicle types of both ovaries manifested enzyme activity of varying intensity. Stroma in all phases was 5'-N positive and granulosa cells were markedly differential in 5'-N activity. These differences relate with the functional status of the ovary and are commensurate with the reproductive state, thus correspond to change in the metabolic requirements of ovary with changing reproductive state.

Chiroptera, corpus luteum, follicles, 5'-nucleotidase, ovaries

Introduction

The reproductive strategies of chiropterans display a wide spectrum of unique and characteristic patterns. Some bats have functionally equivalent ovaries, alternating in function annually; others show dextral or sinistral dominance (Wimsatt 1975, 1979, Gopalakrishna & Choudhari 1977, Kitchener 1973, Krutzsch & Crichton 1985, Jaroli & Lall 1987, Trivedi & Lall 1989, 2004). The present study was undertaken to monitor shifts in the histochemical pattern of 5'-Nucleotidase (5'-N) enzyme – substrate relationship to delineate aspects of bio-energetics of folliculogenesis in *Rhinopoma microphyllum* ovaries during different reproductive stages. 5'-N is involved in catabolism of nucleic acids and NADP (Dixon & Webb 1964) and may have a role in regulation of glycolysis (Reiss 1951). However, the role of 5'-N in the ovarian functions remains enigmatic and therefore, shifts in patterns of 5'-N in the contralateral ovaries of different reproductive phases viz. non-pregnant, pregnant and lactation stages were studied in this bat.

Materials and Methods

R. m. kinneari females of non-pregnant, pregnant (early pregnancy) and lactation (within a week after parturition) phases were used for the study. Five bats per reproductive state were sacrificed; the ovaries were excised under semi-sterile conditions and weighed individually on a mono-pan electrical balance. These ovaries were then fixed in 10% neutral (buffered) formalin at 4 °C for 12–14 hrs. Cyosections (7–10 mm thickness) were incubated in substrate medium and processed for histochemical detection of 5'-N as per the lead staining method of Wachstein & Meisel (1957) as described by Pearse (1968). Appropriate controls were run in substrate deficient media. Enzyme activities in cell types of contra-lateral ovaries were visually appraised.

Table 1. 5'-N profile in ovarian components during different reproductive states; reaction: +++ very strong, ++ moderate, + weak to low
Tab. 1. Profil 5'-nukleotidázy komponentoch ovárií v rôznych reprodukčných štádiách; reakcia: +++ veľmi silná, ++ mierna, + nevýrazná až nízka

follicle types	non-pregnant		pregnant		lactation	
	ovary		ovary		ovary	
	left	right	ovulating	non-ovulating	left	right
primary follicles	+	+	+	+ to ++	+	+ to ++
secondary follicles	+++	+++	+	++	++ to +++	+++
tertiary follicles	+++	+++	+	+++	+++	+ to +++
pre-antral follicles	+++	+++		++		
antral follicles	+++	+++		++ to +++		+++
corpus luteum			+			
stroma	++	+++		+	+ to ++	+ to ++

Though the ovaries are functionally equivalent, we sought differences if any, in the ovaries of two sides in all the three phases. For the sake of clarity we have referred to the ovaries of the two sides as the contralateral ovary instead of referring to them always as the left ovary and the right ovary. This however, does not imply that only the left ovary or only the right ovary is being described. The reaction product [Lead sulphide granules: black or dark brown granules as a result of enzyme reactions] colour intensity was visually scored (see Table 1).

Results

Very intense 5'-N reaction product staining in all follicle types of both ovaries of non-pregnant phase was observed. Staining was seen in granulosa cells and the thecal cells of all follicle types. Contralateral differences in the staining pattern were not significant. The stroma also manifested high 5'-N profile (Figs. 1, 2).

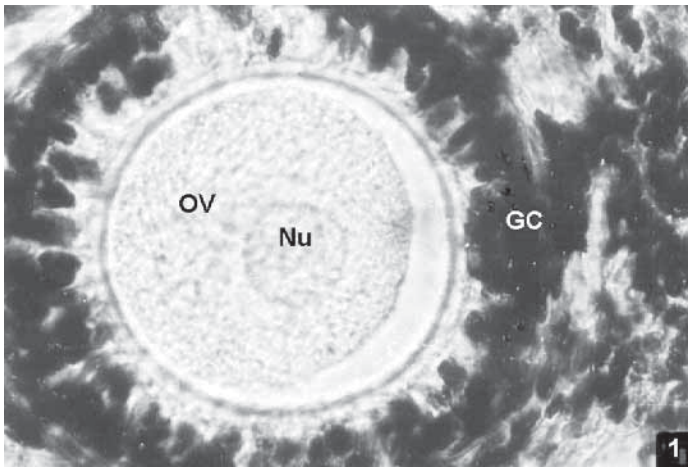
Moderate 5'-N enzyme reaction product was seen in both the ovaries of pregnant bats. The lutein cells also displayed 5'-N activity. Contralateral differences were visible. The ovary bearing the corpus luteum displayed slightly lower reaction product staining (Fig. 5) as compared to the one that did not ovulate (Figs. 3, 4).

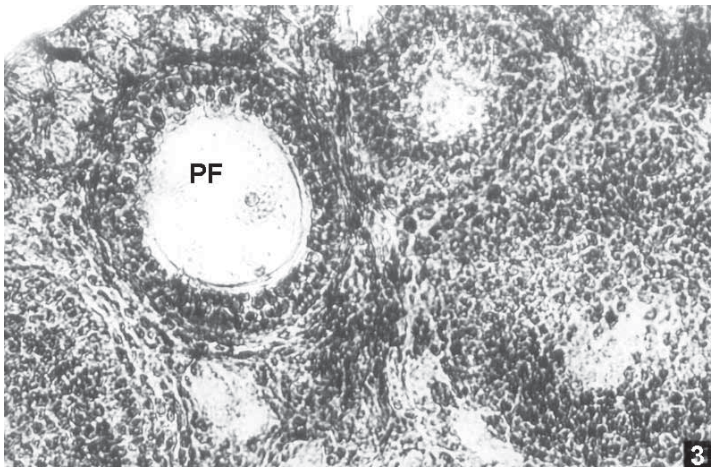
Higher intensity of 5'-N reaction product could be seen in the different follicle types of lactating bats. The contralateral differences were not significant. Different follicle types displayed intense staining. Granulosa cells were markedly differential in reaction staining in most follicle types (Figs. 6, 7).

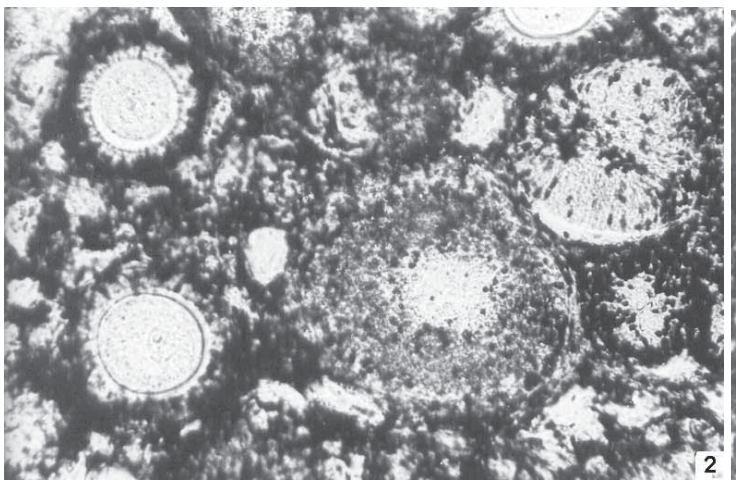
In general, granulosa cells towards the thecal cells displayed very high enzyme reaction product in most follicles. Thus, 5'-N profile displayed reproductive state specific changes, though the contralateral differences were negligible (Tables 1, 2).

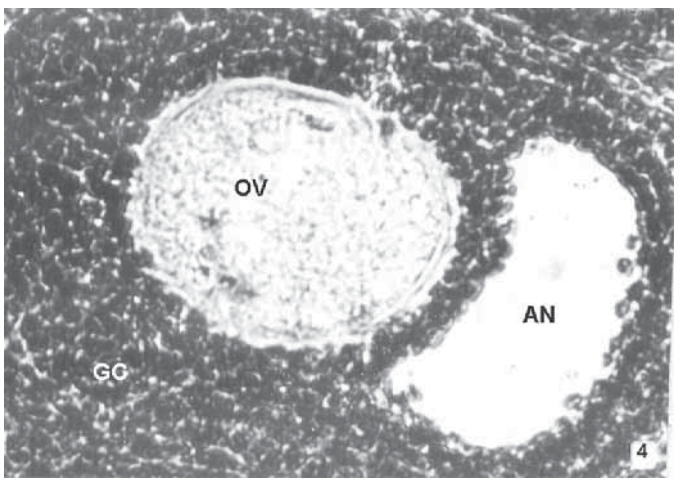
>>

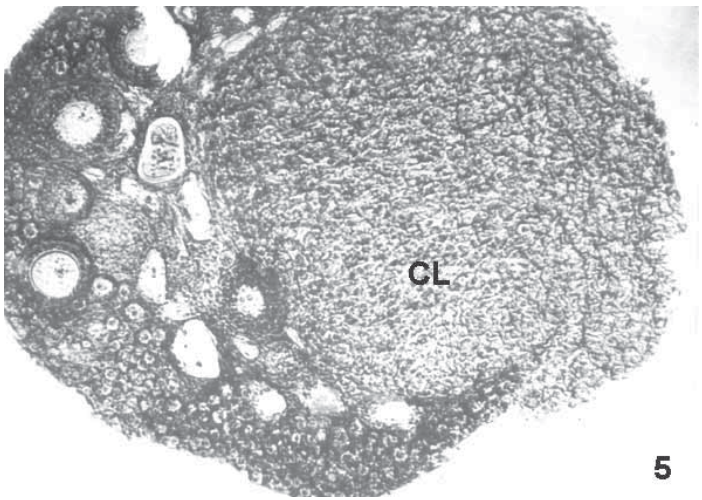
Figs. 1–4. 1 – A primary follicle in ovary of a non-pregnant bat; ×40. 2 – Contralateral ovary of non-pregnant bat showing different follicle types; ×10. 3 – Primary follicles and ovarian tissue in ovary of pregnant bat (non-ovulating side); ×10. 4 – An antral follicle in ovary of pregnant bat (non-ovulating side); ×40.
Obr. 1–4. 1 – Primárne folikuly vo vaječníkoch negravidného netopiera; ×40. 2 – oprotiležiaci vaječník negravidného netopiera s rôznymi typmi folikúl; ×10. 3 – Primárne folikuly a tkanivá vo vajačníkoch gravidného netopiera (neovulujúca strana); ×10. 4 – Antrálne folikuly vo vaječníkoch gravidného netopiera (neovulujúca strana); ×40.
Legend / legenda: AN – antrum, CL – corpus luteum, GC – granulosa cells / granulosové bunky, Nu – nucleus / jadro, OP – ooplasm / ooplazma, OV – ovary / vaječník, PF – primary follicle / primárny folikul.

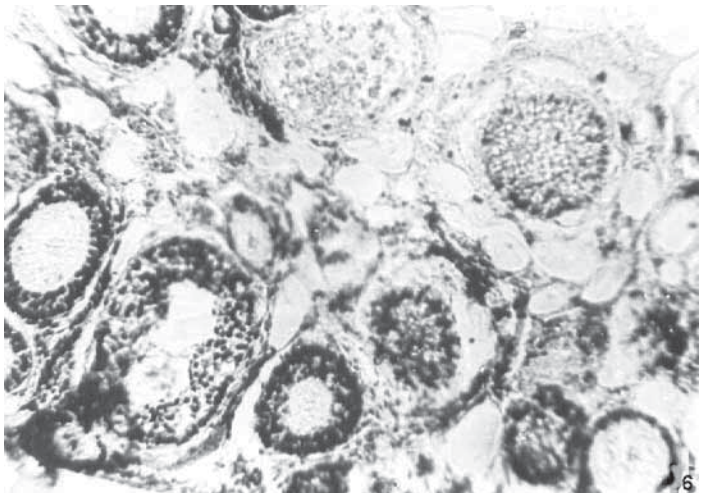

OV
Nu
GC
1

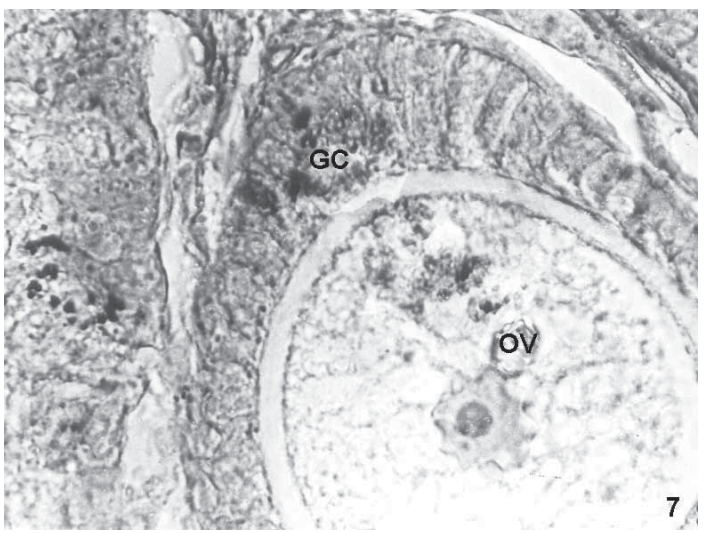

PF
3


2


OV
AN
GC
4







Figs. 5–7. 5 – Extroverted corpus luteum and follicle types in ovary of pregnant bat (ovulating side); ×10. 6 – Different follicle types and ovarian tissue in lactating bat; ×10. 7 – A secondary follicle in contralateral ovary of lactating bat; ×40. For a legend see Figs. 1–4.

Obr. 5–7. 5 – 5 – Extroverted corpus luteum a typy folikúl vo vaječníkoch gravidného netopiera (ovulačná strana); ×10. 6 – Rôzne typy folikúl a tkanív vaječníkov laktujúceho netopiera; ×10. 7 – Sekundárne folikuly v oprotiležiacom vaječníku laktujúceho netopiera; ×40. Legendu pozri na obr. 1–4.

Table 2. Comparison of 5'- N profile in different reproductive states; +++ very strong; ++ moderate; + low
Tab. 2. Porovnanie profilu 5'-nukleotidázy v rôznych reprodukčných štádiách; +++ veľmi silná; ++ mierna; + nízka

non-pregnant		pregnant		lactating	
left ovary	right ovary	ovulating side	non-ovulating side	left ovary	right ovary
+++	+++	+	++	++	++

Discussion

R. m. kinneari is an insectivorous microchiropteran, having an annual breeding cycle (Anand Kumar 1965, Lall 1986, Trivedi 1991, Trivedi & Lall 2004). The profile of enzyme substrate relationship, in ovaries during different reproductive stages in chiroptera has not been widely studied. This study was undertaken to have an understanding of altering metabolic demands of the ovarian tissue during different reproductive phases in these animals. 5'-N cleaves H_3PO_4 from ribo- and deoxyribonucleoside-5-phosphate and has been localized in the ovaries of many mammals (Bjersing 1977), guinea pigs (Adams et al. 1966), mouse (Hardonk 1968), rat (Hadjisky et al. 1969), rabbit (Hadjisky 1970), pig (Mayner 1966) and man (Hertig & Adams 1967). Dixon & Webb (1964) suggest involvement of 5'-N activity in catabolism of nucleic acids and NADP. Reiss (1951) argued that this enzyme may regulate glycolysis.

Though, the role of 5'-N in ovarian functions remains enigmatic, from the present study one may infer that this enzyme might be involved in the metabolic activities of ovaries of different reproductive stages as reproductive stage specific alteration in pattern of 5'-N was evident. It was found that in the non-pregnant animals, the enzyme profile was elevated and no contralateral differences were observed. In pregnant bats, the contralateral difference was evident as the ovary bearing the corpus luteum had milder enzyme profile as compared to the non-ovulating ovary of the same animal. This is similar to the profile observed with Succinate dehydrogenase (SDH) and Lactate dehydrogenase (LDH) in *R. m. kinneari* (Trivedi & Lall 2004). In the lactating bats, again no contralateral differences were seen and enzyme intensity observed was somewhere in between that of non-pregnant and pregnant phases. On comparison of 5'-N with SDH and LDH profile, it is evident that 5'-N enzyme reaction product show higher intensity than SDH and LDH activity during lactation state (Trivedi & Lall 2004). A peculiar feature was that of marked differential staining of 5'-N in the granulosa cells of the healthy follicles. The granulosa cells near the thecal cells showed higher enzyme staining as compared to the cells that were towards the ova. The reason behind this feature is not clear and needs further investigation. The reasons behind the differences in the ovaries of pregnant phase are also elusive. Enzyme intensity was seen in the stroma, granulosa cells and the thecal cells in ovaries of all phases.

Similarities of 5'-N reaction product with SDH and LDH profiles indicate that these three enzymes are probably synergistic in function at least for ovarian functions in this bat. The comparison of 5'-N activity in the chiropteran ovary with the profile of the same enzyme in ovaries of other mammals indicates that this enzyme is participating in the metabolic activities in all orders of class mammalia. Presence of similar ovarian metabolic activity in Chiroptera indicates conservation of certain metabolic reactions in the course of evolution even in higher orders of class mammalia.

Súhrn

Histochémia vaječníkovej 5'-nukleotidázy u *Rhinopoma microphyllum* počas rôznych štádií reprodukcie. Pozorované boli histochemické zmeny na základe 5'-nukleotidázy vo vaječníkoch negravidných,

gravidných a kojacich samíc *Rhinopoma microphyllum kinneari*. Vo vaječníkoch negravidných netopierov bola intenzívna aktivita 5'- nukleotidázy pozorovaná v granulosových bunkách roznych typov folikúl. Otvorené *corpus luteum*; v ovulujúcich vaječníkoch gravidných netopierov; a folikuly types oboch vaječníkov ukazovali mierny, aj keď rôzne škvrnitý charakter. U laktujúcich netopierov, všetky typy folikúl oboch vaječníkov prejavovali enzýmovú aktivity rôznej intenzity. Stróma bola 5'-N pozitívna vo všetkých fázach; granulosové bunky mali významne rozdielnu aktivitu 5'-nukleotidázy. Tieto rozdiely súvisia s funkčným stavom vaječníkov a porovnateľne reprodukčný stav teda korešponduje so zmenami metabolických potrieb vo vaječníkoch v rámci zmien reprodukčného stavu.

Acknowledgements

We are thankful to University Grants Commission, India, for the financial assistance for the study.

References

Adams E. C., Hertig A. T. & Foster S., 1966: Studies on guinea-pig oocytes. II. Histochemical observations on some phosphatases and lipid in developing and atretic oocytes and follicles. *Am. J. Anat.*, **119**: 303–340.

Anand Kumar T. C., 1965: Reproduction in the rat-tailed bat *Rhinopoma kinneari*. *J. Zool., Lond.*, **147**: 137–155.

Bjersing L., 1977: Ovarian Histochemistry. Pp.: 303–391. In: Zukerman L. & Weir B. (eds.): *The Ovary. Vol. I, Second edition*. Academic Press, New York, 517 pp.

Dixon M. & Webb, E. C., 1964: *Enzymes*, *Longman*. New York & London, 1116 pp.

Gopalakrishna A. & Choudhari P. M., 1977: Breeding habits and associated phenomena in some Indian bats. *J. Bombay Natur. Hist. Soc.*, **74**: 1–16.

Hadjisky P., 1970: Functions histoenzymatiques lors de l'evolution et de la luteolyses du corps jaune de la lapine. *Histochemie*, **22**: 125–135.

Hadjisky P., Georgiewa Y. & Wegmann R., 1969: Etudes cyto-enzymatiques de l'ovaire de la ratte durant l'ontogenese et le cycle oestral. Enzymes du metabolisme enegetique. *Ann. Histochem.*, **14**: 19–32.

Hardonk M. J., 1968: 5'-Nucleotidase. I. distribution of 5-Nucleotidase in tissues of rat and mouse. *Histochemie*, **12**: 1–7.

Hertig A. T. & Adams E. C., 1967: Studies on the human oocyte aand its follicle. I Ultrastructural and histochemical observations on the primordial follicle stage. *J. Cell Biol.*, **34**: 647–675.

Jaroli D. P. & Lall S. B., 1987: Comparison of histoenzymological and histochemical changes in the ovary and uterus of non-pregnant and pregnant females of bat *Tahozous melanopogon melanopogon* Temmnick (Microchiroptera Mammalia) displaying absolute dextral dominance of the genital tract. *Acta Anat.*, **129**: 140–148.

Kitchener D. J., 1973: Reproduction in the common Sheath-tailed bat, *Taphozous georgianus* (Thomas) (Microchiroptera: Emballonuridae), in western Australia. *Aust. J. Zool.*, **21**: 375–389

Krutzsch P. H. & Crichton E. G., 1985: Observations on the reproductive cycle of female *Molossus fortis* (Chiroptera: Molossidae) in Puerto Rico. *J. Zool. Lond.*, **207**: 137–150.

Lall S. B., 1986: Folliculogenesis in *Rhinopoma kinneari* Wroughton (Microchiroptera: Mammalia). *Myotis*, **23–24**: 37–44.

Mayner D.A., 1966: Enzymatic histochemistry of the ovary, testis and epididymis of the fetal pig. *Anat. Rec.*, **154**: 384.

Pearse A.G.E., 1968: *Histochemistry: Theoretical and Applied. Vol. I. Third Edition*. Churchill, Livingstone, 759 pp.

Reiss J., 1951: The specificity of phosphomonoesterases in human tissues. *Biochem. J.*, **48**: 548–551.

Trivedi S., 1991: *Seasonal histochemical and histoenzymological alterations in the ovary and uterus of certain chiroptera* (*Mammalia*) *of Rajasthan*. Ph.D. Thesis. ML Sukhadia University, Udaipur, India, 227 pp.

Trivedi S. & Lall S. B., 1989: Histological and histochemical alterations in the ovary of nulliparous and parous bat: *Megaderma lyra*, exhibiting absolute sinistral dominance of genital tract. Pp.: 153–160. In: Hanák V., Horáček I. & Gaisler J. (ed.): *European Bat Research 1987*. Charles Univ. Press, Praha, 720 pp.

Trivedi S. & Lall S. B. 2004: Ovarian dehydrogenases of the non-pregnant, pregnant and lactating *Rhinopoma microphyllum kinneari* (Chiroptera: Rhinopomatidae). *Vespertilio*, **8**: 105–112.

Wimsatt W. A., 1975: Some comparative aspects of implantation. *Biol. Reprod.*, **12**: 1–40.

Wimsatt W. A., 1979: Reproductive asymmetry and unilateral pregnancy in Chiroptera. *J. Repr. Fertil.*, **56**: 345–357.

received 15. 7. 2005