

OVARIAN AND UTERINE ACID PHOSPHATASE OF LACTATING FEMALES OF *PTEROPUS GIGANTEUS GIGANTEUS* BRÜNNICH (MEGACHIROPTERA : MAMMALIA)

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ABSTRACT

The histochemical site and distribution of acid phosphatase (AcPase) in the ovary and uterus of lactating females of frugivorous megachiroptera—*Pteropus giganteus giganteus* Brünnich has been described.

No quiescence or anoestrous condition in the ovarian histo-architecture was discerned. A fully developed, healthy Graafian follicle with distinct chromatin figure was observed. The uterine components displayed conspicuous proliferative (pre-implantation) changes.

AcPase activity in the ovary and uterus showed varying intensities of reactions in the various histological constituents. Intense AcPase reaction was seen in the degenerating and vesicular follicles with antra. Atretic follicles exhibited moderate enzyme activity. The interstitial stroma exhibited high AcPase activity. Maximum AcPase reaction was observed in the endometrial components and relatively less in the myometrium.

Uninterrupted folliculogenesis and proliferative state of uterine endometrium are suggested to represent 'silent' heat in this species during lactation. The role of AcPase in folliculogenesis, controlled degradation of follicles and release of metabolites along with their transfer, etc., is discussed.

INTRODUCTION

**I**NHIBITORY effects of lactation on follicular development are indicated by the substance of many reports for several mammalian species. Thus, in lactating females of guinea pig and mouse delay in the resumption of follicular growth and ovulation are believed to be due to absence of estrogen in significant quantities<sup>1-2</sup>. Litter-size and the consequent intensity of suckling stimulus to the lactating mother has a varying degree of suppressive effect on the estrous cycle of mouse and rat<sup>3</sup>. In myomorph rodents, lactational anoestrous and delayed implantation of blastocyst after post-partum mating are usual<sup>4-5</sup>. On the other hand, in females of cotton-tail rabbit and vole neither lactation nor suckling has any effect on folliculogenesis and ovulation<sup>5-6</sup>. Thus, although the effects of lactation and suckling on the ovarian structure of small number of mammalian species studied so far seem to indicate a broad spectrum of species—specific characteristics, the concomitant changes in the uterus have not been adequately studied.

Activities of several classes of anabolic and catabolic enzymes in the ovary and uterus are known to undergo significant changes during various phases of oestrous/menstrual cycle, pseudopregnancy, pregnancy and after parturition<sup>7-11</sup>. Very little is known about the ovarian and uterine enzymatic profiles in lactating mammalian females and there is no report for any chiropteran species. The present report concerns the histochemical site and distribution of acid phosphatase (AcPase) in the ovary and uterine tissues of lactating females of *Pteropus giganteus giganteus*.

MATERIALS AND METHODS

Ten lactating females with suckling litters clinging to their body were netted (shot during April/June from their roosting sites on the mango tree) where a herd of 80–200 individuals per tree were noted. Parturition in this species occurs in late March and as such the suckling young were one to three months old. The female genitalia were quickly dissected out surgically under aseptic conditions; freed off of blood and connective tissues and fixed in chilled neutral formalin (10% at 4° C) for 6–8 h.

Frozen sections of ovary and uterus were cut at 10  $\mu$ m. AcPase was determined histochemically using the method of Gomori (1950). The sections from these two organs were incubated in a substrate solution containing  $\beta$ -glycerophosphate (pH 5.2) at 37° C for 20–30 min. A black precipitate of lead sulphide indicated the presence of AcPase. Suitable controls were run simultaneously.

For histological orientation, the tissues were fixed in aqueous Bouin, washed off of fixative, dehydrated, cleared; infiltrated and embedded in wax. Sections were cut at 5–7  $\mu$ m and stained with haematoxylin and eosin.

Enzyme activity was visually scored in the various histological constituents of the ovary and uterus as intense (++++), high (+++), moderate (++) , slight (+) and negative (0). Integrated tables were prepared to record these observations.

RESULTS

The left and right ovary (see Plate II : Figs. 7–9 and 10–12 for comparison) and uterus (Plate I :



Figs. 1-3 and 4-6) of lactating females of *Pteropus giganteus giganteus* displayed little difference of shape, size and histoarchitecture: although they displayed spatial asymmetry.

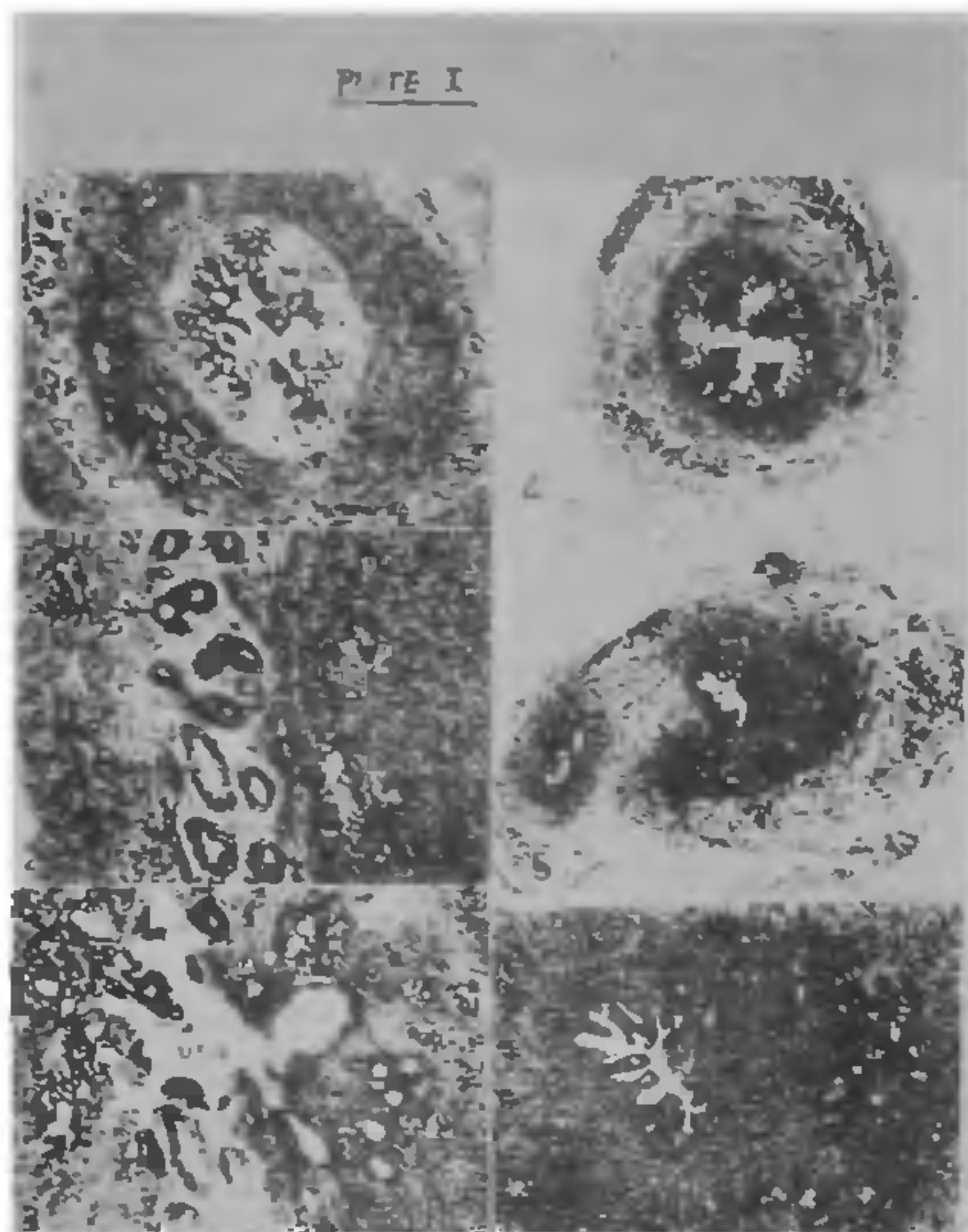


PLATE I: Acid phosphatase (AcPase) activity in the ovary and uterus of lactating females of *Pteropus giganteus giganteus*. Fig. 1. Frozen section ( $10\ \mu\text{m}$ ) of the uterus. Note the proliferative endometrial and myometrial zones with differential AcPase activity ( $28\times$ ). Fig. 2. A portion of the endometrium enlarged to show the details of enzyme activity in the various histologic constituents ( $70\times$ ). Fig. 3. A portion of endometrial and myometrial interface showing comparative distribution of enzyme activity ( $70\times$ ). Figs. 4-6. Implantational changes and AcPase activity along the length of the uterus: Fig. 4 is a section taken from the base of the uterus ( $28\times$ ); Fig. 5 displays the section taken from near the uterine junction (note the openings of the right and left uterine cornu ( $28\times$ ); Fig. 6 is a magnified view of Fig. 5 ( $70\times$ ).

A number of follicles in various stages of development was seen in the substance of the ovary. Degenerating and atretic follicles were also observed. In the healthy follicles the granulosa cells were loosely arranged with fluid-filled spaces between them. Corpus luteum of pregnancy was not observed but lutein cells with pycnotic nuclei dispersed in the interstitium were clearly seen in the left ovary. Healthy looking corpora lutea with well-developed lutein cells mostly with vesicular nuclei were also seen (Plate II: Figs. 7-9). A single, large, healthy Graafian follicle showing a

distinct chromatin figure in metaphase along with corpora radiata and antrum was observed in the right ovary (Plate II: Fig. 12).

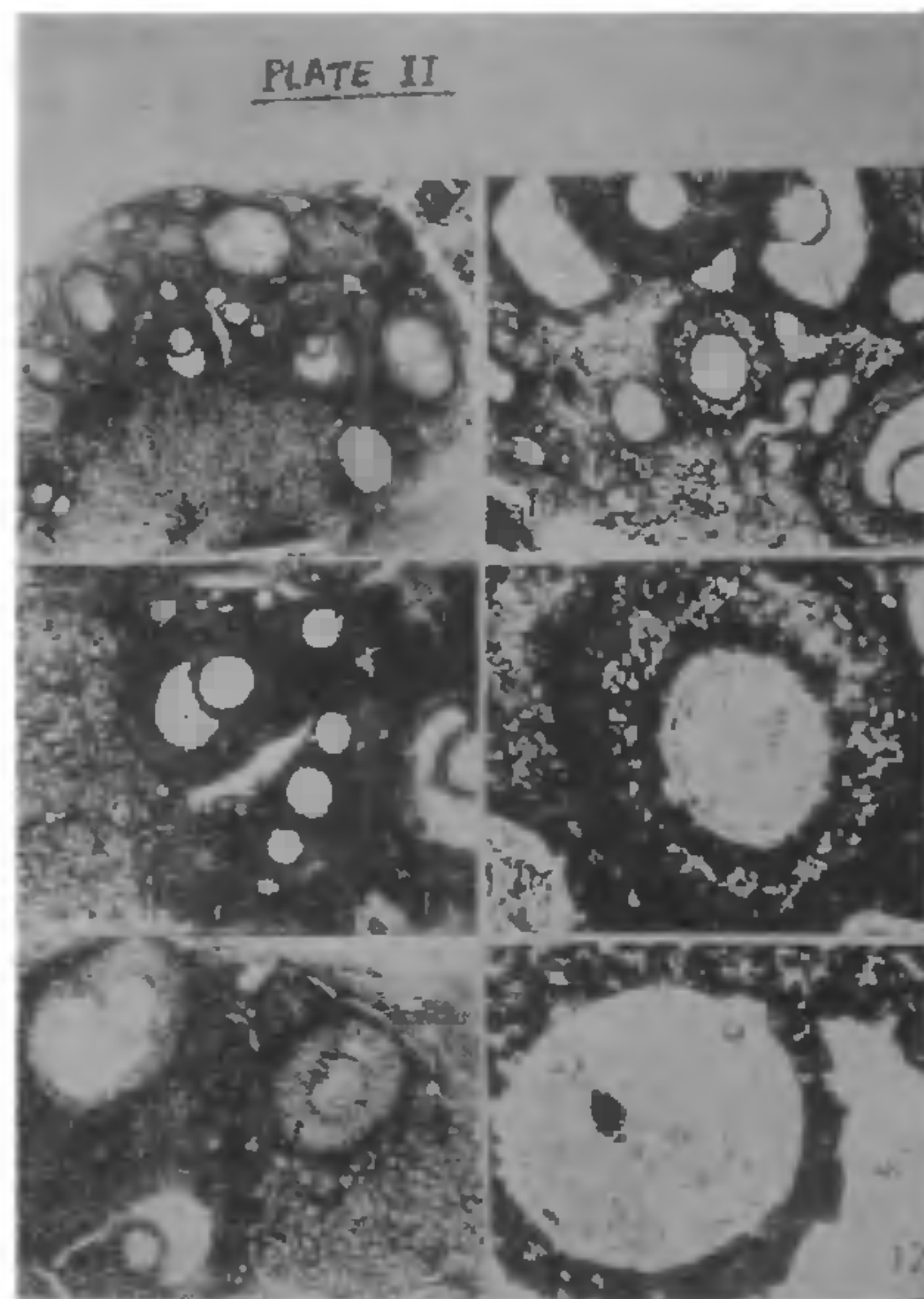


PLATE II: Acid phosphatase (AcPase) activity in the ovary and uterus of lactating females of *Pteropus giganteus giganteus*. Figs. 7-9. Sections of the left ovary showing histoarchitecture and differential AcPase profile. Note the atretic follicles, antral follicles and developing follicles ( $20\times$ ,  $70\times$ ,  $70\times$ ). Figs. 10-12. Sections through the right ovary showing variable AcPase reaction in the ovarian stroma, follicles and other constituents ( $70\times$ ,  $280\times$ ,  $280\times$ ). Fig. 12 shows the large, healthy Graafian follicle enlarged to show enzyme reaction in various components. Note the distinct metaphase chromatic figure showing positive AcPase reaction.

(DGF—Degenerating follicles; ATF—Atretic follicles; AN—Antrum; OV—Ooplasm; End—Endometrium; UTL—Lumen; UTGL—Uterine gland; EP—Epithelium.)

Intense AcPase activity was seen in the healthy follicles. The enzyme activity was high in the degenerating follicles while atretic follicles displayed moderate AcPase reaction. Lutein cells exhibited high AcPase activity. In the single, large healthy Graafian follicle intense AcPase activity was observed in the granulosa cells and undifferentiated theca. The distinct metaphase chromatin figure of this follicle showed positive enzyme activity at pH of 5.2. The ooplasm showed



very mild AcPase reaction confined to the periphery, while the antral fluid was AcPase negative (Plate II: Figs. 10-12, Table I). The uterus showed distinct signs of initiation of pre-implantation changes all along the length as manifested by the development of endometrial stroma, uterine glands and myometrium. These changes were observed both in the left and the right uterine cornu (Plate I: Figs. 1-3 and 4-6).

AcPase activity in the uterus showed varying intensities of reaction in the serosa, myometrium, endometrial stroma, uterine glands, uterine epithelium and lumen (Plate I: Figs. 1-6).

TABLE I

*Acid phosphatase (AcPase) in the various histologic components of the ovary of lactating females of Pteropus giganteus giganteus*

Sl. No.	Ovary	Enzyme activity
1. Growing follicles		++++
2. Degenerating follicles		++
3. Atretic follicles		+
4. Healthy Graafian follicle		++++
5. Lutein cells		+++
6. Interstitial cells		++

Intense (++++), High (+++), Moderate (++) , Slight (+), Negative (0).

TABLE II

*Acid phosphatase (AcPase) in the various histologic components of the uterus of lactating females of Pteropus giganteus giganteus*

Sl. No.	Uterus	Enzyme activity
1. Serosa		++
2. Myometrium		+++
3. Endometrial stroma		+++
4. Uterine gland		++++
5. Uterine epithelium		+++
6. Lumen		0

Intense (+++), High (+++), Moderate (++), Slight (+), Negative (0).

#### DISCUSSION

The present study highlights (a) uninterrupted folliculogenesis with concomitant proliferative changes in the right and the left uterine horn and (b) differential

AcPase profile in the histological constituents of the ovary and the uterus of lactating females of *Pteropus giganteus giganteus*.

The state of folliculogenesis in the left and the right ovary and the endometrial growth all along the uterine substance is at considerable variance with the reports on monkey<sup>13</sup>, hamster<sup>14</sup>, and *Notomys*<sup>15</sup>, in these species lactation causes extensive follicular atresia or acts as a positive deterrent to follicular growth, development and maturation. However, in *Pteropus giganteus giganteus*, lactation does not seem to affect folliculogenesis nor the suckling stimulus has any adverse effect on the process. It seems that the differences of ovarian response to lactation and suckling stimulus are species-specific characteristics.

In the ovary the biologic importance of several enzymes is not fully understood. AcPase activity has been considered as marker for lysosomes<sup>16</sup>. Such organelles are abundant in cells involved in digestive activity as well as in cells undergoing autolysis. AcPase activities in the ovarian follicles, corpora lutea and interstitial tissues show considerable variation. These differences may be related to variable hydrolytic activities of this enzyme. Thus, it may generate metabolites by its hydrolytic action and may also facilitate their transfer in growing follicles. In degenerating and atretic follicles, it may promote autolysis. In the single large Graafian follicle of *Pteropus giganteus giganteus*, AcPase activity may be related to sustained production and transfer of metabolites ensuring maintenance and survival of the follicle. Our results are in disagreement with the observations made in the ovary of sow<sup>19</sup>, rat<sup>17</sup> and women<sup>18</sup>.

The proliferative status of the uterus and the contained variable AcPase activity in the histological constituents as observed in the present study clearly indicate the functional preparedness for accepting the product of fertilisation, when mating takes place.

Intense AcPase activity in the endometrium may be involved in glycogen degradation which is believed to be an important source of utilizable energy during the proliferative changes. Controlled degradation of tissues and generation of a characteristic biochemical milieu interior may be the other roles which AcPase may effectively play.

Our results are in total conflict with the observations in *Hemitechnus*<sup>8</sup>, *Suncus murinus suncus*<sup>21</sup>, and women<sup>22</sup>.

Thus, from the results of ovarian and uterine structure and AcPase distribution, it is tempting to suggest that in lactating females of *Pteropus giganteus giganteus*, folliculogenesis and proliferative changes in the uterus remain uninterrupted. Lack of post-partum pregnancy in this species is not due to inhibition of folliculogenesis or inhibition of release of ova from the ovaries. The proliferative changes in the uterus

are an additional indication of functional preparedness for accepting the conceptus. Therefore, factors other than stated above such as suckling young clinging to the mother's body, etc., may be responsible for reducing the attractiveness of the female, so that her chances of successful mating are reduced.

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## CHANGES IN THE OXYGEN CONSUMPTION OF THE MATERNAL ANIMAL, AND MATERNAL AND EMBRYONIC TISSUES DURING THE GESTATION PERIOD OF THE VIVIPAROUS SCORPION, *HETEROMETRUS FULVIPES*

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#### ABSTRACT

The respiratory metabolism of the maternal animal, and maternal and embryonic tissues of *Heterometrus fulvipes* is studied at different stages of development. The embryonic metabolism is almost similar to the maternal metabolism. From the metabolic compatibility observed between the embryo and the maternal animal, it is suggested that the development proceeds without any obligatory sacrifice on the part of the mother.

#### INTRODUCTION

THE pattern of respiratory metabolism during gestation period is known only for mammals<sup>1</sup>. It is desirable to extend similar studies to other animals, invertebrates in particular, where viviparity exists, for understanding the metabolic relationships between the maternal animal and the growing embryos during gestation period. *Heterometrus fulvipes* is a vivi-

parous scorpion with a long gestation period of about 11 months<sup>2</sup>. An attempt is made here to study the respiratory metabolism of the maternal animal and maternal and embryonic tissues at eight different stages of development of *H. fulvipes*.

#### MATERIALS AND METHODS

Scorpions were collected and maintained as reported by Subburam and Reddy<sup>3</sup>. Hepatopancreas, muscle and the embryos were obtained from gravid females at appropriate stages of the gestation period as reported

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