J Endocrinol Reprod 9(1) (2): 37-42 (2005)
JER 57

CYCLICAL CHANGES IN THE EPITHELIAL CORDS OF THE OVARY OF A MICROCHIROPTERAN BAT, *HIPPOSIDEROS SPEORIS* (SCHNEIDER)

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SUMMARY

The ovaries of *Hipposideros speoris* were observed throughout the reproductive cycle from July 2001 to 2002. The interstitial cells or so called "epithelial cords" showed variations in their distribution, morphology and association with other ovarian structures. These cords appear to be formed in the ovarian cortex by the transformation of granulosa cells of primordial follicles and small preantral follicles whose ova regress and disappear. Mostly, these cords were conspicuous, hypertrophied, abundant and in clusters or in zones occupying major portion of the cortex during 4-5 months of gestation and also during lactation period. The accumulation of sudanophilic lipid droplets in these cords suggests their role in steroid biosynthesis, most probably the progestins. An increase in lipid droplets parallels the development of alkaline phosphatase reactivity. The origin and the fate of the cords were not determined but their absence or reduction in number suggests their transformation into stroma during non-pregnancy.

Key words : Lipid droplets; Progestins ; Reproductive cycle of bat ; Epithelial cords.

INTRODUCTION

Primordial oocytes form the constant component of the ovary during all phases of reproduction, until they undergo regression or enter into next stage of development. Two types of atresia were prevalent among the ovarian follicles-first type characterized by the degeneration of the granulosa layers before the oocyte degeneration; second type characterized by the oocyte degeneration before any atretic changes in the stratum granulosum becomes extensive among primordial and small preantral primary follicles, where the unaffected granulosa layers form the epithelial cords.

Although the presence of epithelial cords was well confirmed in the ovaries of several species of mammals belonging to a number of different orders (1-14), no account is documented in the order Chiroptera except a small note on *Myotis grisescens* (10). The present paper gives an account of the origin, distribution, nature or function, as well as cyclical changes in the epithelial cords of the ovaries of *H. speoris* (Schneider) during the complete reproductive cycle. Such observations may help in understanding the functional significance of the epithelial cords in cycling ovaries.

MATERIALS AND METHODS

Specimens of *H. speoris* were collected twice every calendar month throughout the year with the help of a mist net from the natural population inhabiting abandoned mines in Khapa, Nagpur, Maharashtra.

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For histological studies, the ovaries were fixed quickly in Bouin's fixative, dehydrated in ethanol and embedded in paraffin wax. The sections cut at 5 µm were stained with haematoxylin and eosin.

For histochemical studies, calcium-formol and buffered formalin fixed tissues were cut on freezing microtome at -20°C and were stained by Chiffile and Putt method (15) as follows. Cut frozen sections were washed in water for 2-5 min to remove formaldehyde, dehydrated for 3-5 min in Pure propylene glycol by moving sections, at intervals, transferred to the dye solution (0.7g of Sudan black B dissolved in 100 ml propylene glycol at 100-110°C) for 5-7 min with occasional agitation. Then, differentiated in 85% propylene glycol for 2-3 min, washed in distilled water for 3-5 min and mounted in glycerol jelly. Lipids appeared black or bluish black.

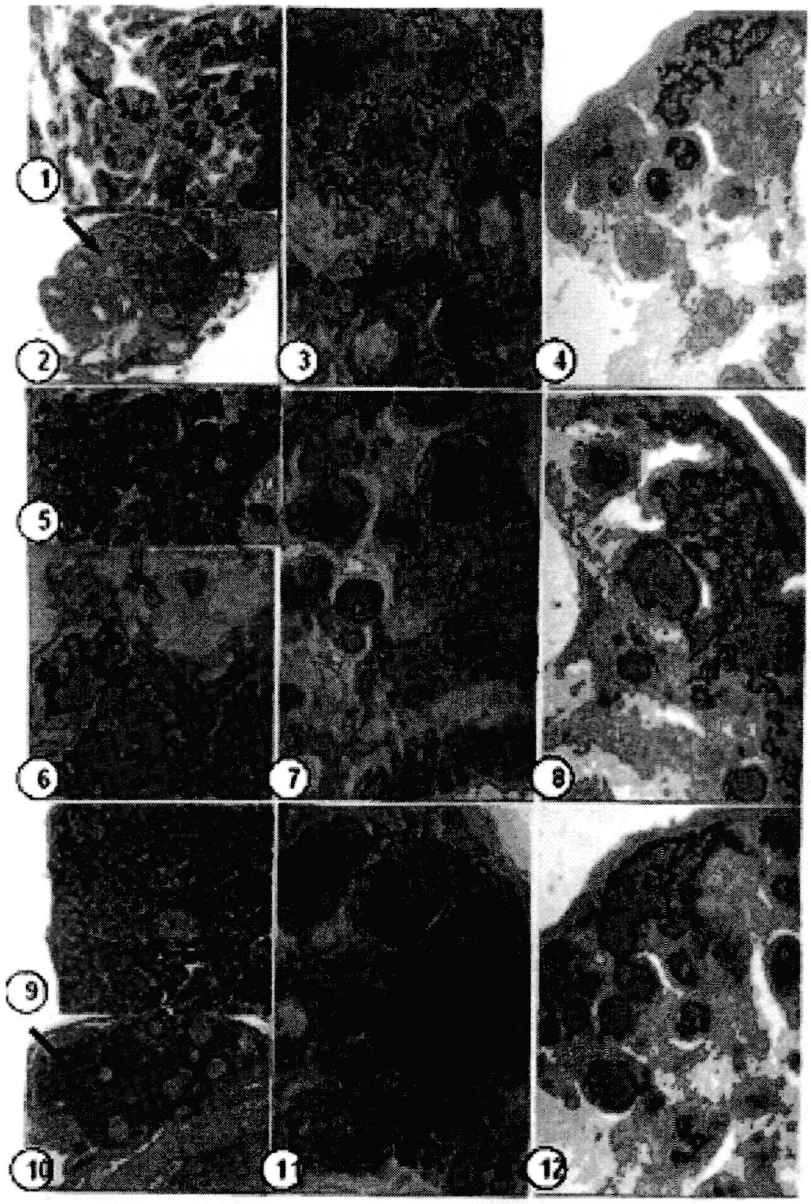
Alkaline phosphatase was demonstrated by Gomori's method, The sections after washing with distilled water were incubated in Gomori's medium for 3 h at 37°C, washed with distilled water, then treated with 2% solution of cobalt nitrate for 2-3 min, again washed with distilled water and treated with dilute ammonium sulphide for 1 min, washed in distilled water and mounted in glycerine jelly. Brownish black deposits in the section confirmed alkaline phosphatase activity.

RESULTS

H. speoris is a monovular and monotocous bat, breeding once in a year from December to May and the gestation lasts for 135 ± 5 days starting from second week of December upto last week of April. Thus, during the early pregnancy (January), the epithelial cords were found to be scattered in the cortex either in groups of 2-3 or singly, each cord appearing smaller in size with few cells (Fig.1). The early pregnancy was marked by an extrovert corpus luteum (Fig. 2) which was fully functional, with an implanted bilaminar blastocyst in the uterine cornua. Lipid droplets and alkaline phosphatase intensity was discrete and diffuse (Figs. 3 and 4).

At the neural groove stage of embryo (nearly February), the newly formed placenta and the corpus luteum both were functional. Also at this stage, the epithelial cords were extensive and were highly hypertrophied, large in size, coiled and clustered, each cord was demarcated by separate basal lamina and was lined by a single layer of 20-30 cells (Fig. 5). The cells of these cords appeared pale, translucent with slightly eosinophilic cytoplasm. Some of them were shifted towards the cortico-medullary parts of the ovary and were richly vascularized as judged by the presence of blood capillaries in their vicinity (Fig. 6). During this period, the cells were deeply stained with Sudan black B (Fig. 7) and displayed high alkaline phosphatase activity (Fig. 8).

The epithelial cords were at their peak of development, more or less occupying the complete peripheral cortex, were distributed into zones or patches, each showing the presence of highly hypertrophied 30-40 cells at the limb-bud stage of embryonic development during March (Fig. 9) when the placenta was functional but the corpus luteum was regressed (Fig. 10). Many of them were in the medullary region in close association with the capillary loops.



Figures 1 to 4. Early pregnancy, blastocyst stage of development

Figure 1. Epithelial cords scattered in the cortex either in groups of 2-3 or singly. Note the smaller size of the cords with few cells in each cord (arrow) (X 50).

Figure 2. Note umbrella – shaped extrovert corpus luteum (arrow) in Hipposideros speoris (x 25).

Figure 3. Epithelial cords coinciding the implantation of blastocyst demonstrating diffuse lipids droplets along the cell outlines (x 250).

Figure 4. Epithelial cords displaying less alkaline phosphatase activity (x 250).

Figures 5 to 8 early pregnancy, neural groove stage of embryo

Figure 5. Aggregation of highly hypertrophied and functionally active epithelial cords consisting of 20- 30 cells (arrow) (x 250).

Figure 6. Epithelial cord with highly hypertrophied cells (arrow) in the vicinity of blood capillary (arrow head) (x 250).

Figure 7. Well developed epithelial cords deeply stained with Sudan black B (x 250).

Figure 8 . A part of the ovarian cortex demonstrating strong alkaline phosphatase reactivity (x 250).

Figures 9 to 12 mid-pregnancy, at limb-bud stage of embryo

Figure 9. Highly hypertrophied, closely clustered, coiled or elongated peripheral cortical zones of epithelial cords, each with 30-40 hypertrophied cells (x 250).

Figure 10. Regressing corpus luteum (arrow) during March merging into ovarian tissue (x 25).

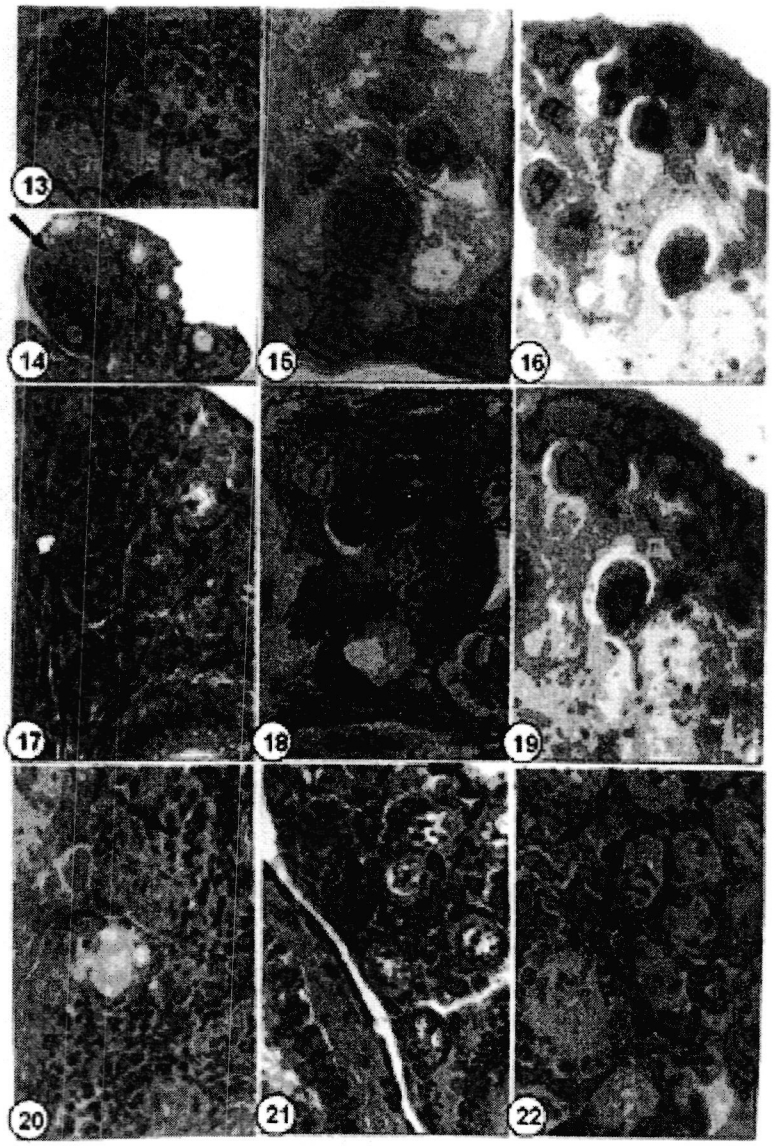
Figure 11. Note dense accumulation of sudanophilic lipids in the cords (x 250).

Figure 12. Note the cord exhibiting rich alkaline phosphatase activity (x 250).

The cords histochemically exhibited rich sudanophilic lipid droplets (Fig. 11) and alkaline phosphatase activity (Fig. 12).

During the near term period, *i.e.* late April - early May, the epithelial cords were restricted only to some peripheral cortical portions. There was a decline in their number and appeared inactive. Even though there was a reduction in the size of clusters; only 2-3 cords formed a cluster, which was previously observed to be 10-12. The cells of each cord were also reduced in size and numbers when the placenta was well-established (Fig. 13) but the corpus luteum was completely resorbed (Fig. 14). The cords revealed lesser amount of lipids (Fig. 15) and declined alkaline phosphatase activity (Fig. 16).

During lactation (May), there was a pick up in the activities of epithelial cords (Figs. 17, 18 and 19), but some ovaries showed contrast activities of epithelial cords, as there is an asynchrony in the reproductive cycle from the same period.



Figures 13 to 16. Near term pregnancy

Figure 13. Note reduction in the size of cluster with small degenerating cells (arrow) (x 250).

Figure 14. Completely regressed corpus luteum as a scar (arrow) inside the ovary (x 25).

Figure 15. Reduced number of epithelial cords with lesser amount of lipid granules (arrow) (x250).

Figure 16. Note declined alkaline phosphatase activity (x 250).

Figures 17 to 19 during lactation

Figure 17. Note an increase in the size and number of epithelial cords (x 250).

Figure 18. Epithelial cords showing intensely accumulated sudanophilic lipid droplets (x 250).

Figure 19. Note intense alkaline phosphatase activity in the cords (x 250).

Figure 20. Arrow marks the regressed epithelial cords, with inactive cells during anoestrous (x 250).

Figure 21. Reduced but well-defined epithelial cords (arrow) at proestrous (x 250).

Figure 22. Epithelial cords (arrow) distributed individually between primordial follicles at oestrus (x 250).

During anoestrous, proestrus and oestrus period from July to November, the epithelial cords were mostly individual and irregularly distributed among the primordial follicles (Figs. 20, 21 and 22).

DISCUSSION

In the present study, the cyclic changes in the epithelial cords were correlated with the reproductive cycle of *H. speoris*. Thus, during early pregnancy, the epithelial cords were found scattered in the cortex either in groups of 2-3 or singly. The histochemical activities of lipid and alkaline phosphatase were low to moderate during early pregnancy. But during mid-pregnancy, there was a sudden burst in the histochemical activities. These activities were more enhanced during advanced pregnancy as evident by the occurrence of densely populated, highly hypertrophied zones in the cortex. Also during the lactational period, the cords were abundant and histochemically functional. synthesis as discussed earlier (3,7,13,14). An Increase in alkaline phosphatase activity during early and mid-pregnancy may be to interact with the lipid inclusions so that the substrates are made available for steroidogenesis (13). Their shift to the medullary region and association with the blood capillaries also suggested their role in the metabolism of steroidal compounds, particularly progestins (13, 14, 16).

The origin of the epithelial cords remains controversial. They were believed to be originating either by the invagination of the germinal epithelium (7,8,12) or as noticed in the present work from the granulosa cells of primordial follicles and small preantral follicles whose ova are lost by atresia (13,14).

In conclusion, the ovary of *H. speoris* shows extensive development of epithelial cords particularly during mid and advanced pregnancy and lactation. These epithelial cords present wide variations in morphology and histochemistry throughout the reproductive cycle. Both morphological and the histochemical studies revealed their significance as steroidogenic cells in *H. speoris*.

ACKNOWLEDGEMENT

The authors are thankful to Prof. Dr. D. B. Tembhare, Head of the Department, for providing laboratory facility.

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