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Occurrence of polyovular follicles and its possible significance in the ovary of the bat, *Scotophilus heathi*

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Seasonal changes in the number of polyovular follicles were estimated in a Vespertilionid bat, ***Scotophilus heathi****. The largest number of polyovular follicles was observed during September, which coincided with the period of maximum development of intra-ovarian rete cells cord. It has been suggested that interaction between rete cells cord and follicle may be responsible for giving rise to a number of polyovular follicles in the medullary region of the ovary. Most of the polyovular follicles observed were biovular and they grow maximum up to the late preantral stage. Polyovular follicles with four or more oocytes were observed during September and they do not grow beyond stage I of the follicular development. The polyovular follicles were generally short lived and may contribute to the extensive development of the interstitial cells in* ***S. heathi****.*

Key terms: *bat, ovary, polyovular follicles,* ***Scotophilus heathi***

INTRODUCTION

Polyovular follicles have been reported in several mammalian species particularly in prepubertal mammals, and they have been observed occasionally in women (Gougeon, 1981). It has been proposed that biovular follicles may be the cause of inherited dizygotic twinning and even of the histogenesis of cystic teratomas (Scherrer *et al*, 1977; Plachet *et al*, 1977; Gougeon, 1981). The factor(s) causing the formation of polyovular follicles in the ovary is not yet known.

The subtropical vespertilionid bat, *Scotophilus heathi*, has been shown to exhibit a number of unique features, such as delayed ovulation, extensive development of interstitial gland cells, presence of well developed rete ovarii in adult ovary and structurally modified Graafian follicles (Krishna & Singh, 1992; Abhilasha & Krishna, 1996). The extensively developed interstitial gland cells in the ovary of *S. heathi* have been shown as the major source of high circulating androstenedione, which may be responsible for suppression of ovulation leading to the phenomenon of delayed ovulation in *S. heathi* (Abhilasha & Krishna, 1996). During an investigation of reproductive cycle of *S. heathi*, we have noticed a large number of polyovular follicles in the ovary. To find out the possible factor(s) responsible for the development of polyovular follicles, changes in the ovary of the bat during the time of their first appearance during reproductive cycle were studied. A close association between rete ovarii and polyovular follicles was observed and results are reported here.

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MATERIALS AND METHODS

Bats were trapped alive in every calendar month from the Banaras Hindu University campus and adjacent area. Animals were sacrificed as soon as they were brought to the laboratory. Ovaries were dissected out, fixed in Bouin's fluid, embedded in paraffin wax, and serially sectioned at 5 µm. The sections were stained by haematoxylin and eosin. The follicles were classified according to the number of granulosa layers, as follows:

Stage I: One or two layers of granulosa cells (primary follicle).

Stage II: Three or four layers of granulosa cells (early preantral).

Stage III: Five or six layers of granulosa cells (late preantral).

The detailed histological and quantitative procedures are described elsewhere (Krishna & Singh, 1992). During each calendar month the number of polyovular follicles, the number of oocytes present in each polyovular follicle, and the stage of polyovular follicle development were estimated in the serial ovarian sections. A sampling frequency of every fifth serial section was used.

RESULTS

Seasonal changes in the number and developmental patterns of polyovular follicles.

Changes in the number of polyovular follicles in the ovary of adult females during different months are shown in Table I. Maximum number of polyovular follicles were observed during September-October (recrudescence phase) as compared with other months of the year (Fig 1). Most of the females collected during these months showed polyovular follicles in their ovaries mainly in the medullary region. During subsequent months, the ovary contained mostly uniovular follicles, and polyovular follicles were observed only occasionally. The number of polyovular follicles containing 2, 3 or more oocytes per ovary was also estimated and shown in Table II. It was found that polyovular follicles with more than 3 oocytes were observed only during September. As many as seven or more oocytes were found in some follicles (Fig 2). Polyovular follicles with more than three oocytes were observed from September to November.

Table I

Monthly changes* in the number of polyovular follicles** in different stages of follicular development in the ovary of *S. heathi*.

Month	N° of ovaries observed	N° of ovaries containing polyovular follicles	Stages of follicular development Follicular stages ***		
			I	II	III
September	12	9	276 (1565)****	78 (566)	—
October	12	7	96 (1872)	14 (88)	5 (30)
November	12	4	20 (1228)	4 (24)	—
December	12	3	22 (1668)	15 (288)	14 (104)
January	12	3	9 (1272)	5 (156)	6 (24)
February	12	3	16 (1222)	6 (204)	9 (86)
March	12	2	20 (996)	6 (157)	4 (24)
April	12	1	8 (1596)	—	—

* No polyovular follicles seen during May to August.
** Values are total numbers of polyovular follicles.
*** Follicular types based on number of layers of granulosa cells.
**** Numbers within parentheses, total healthy follicles examined.

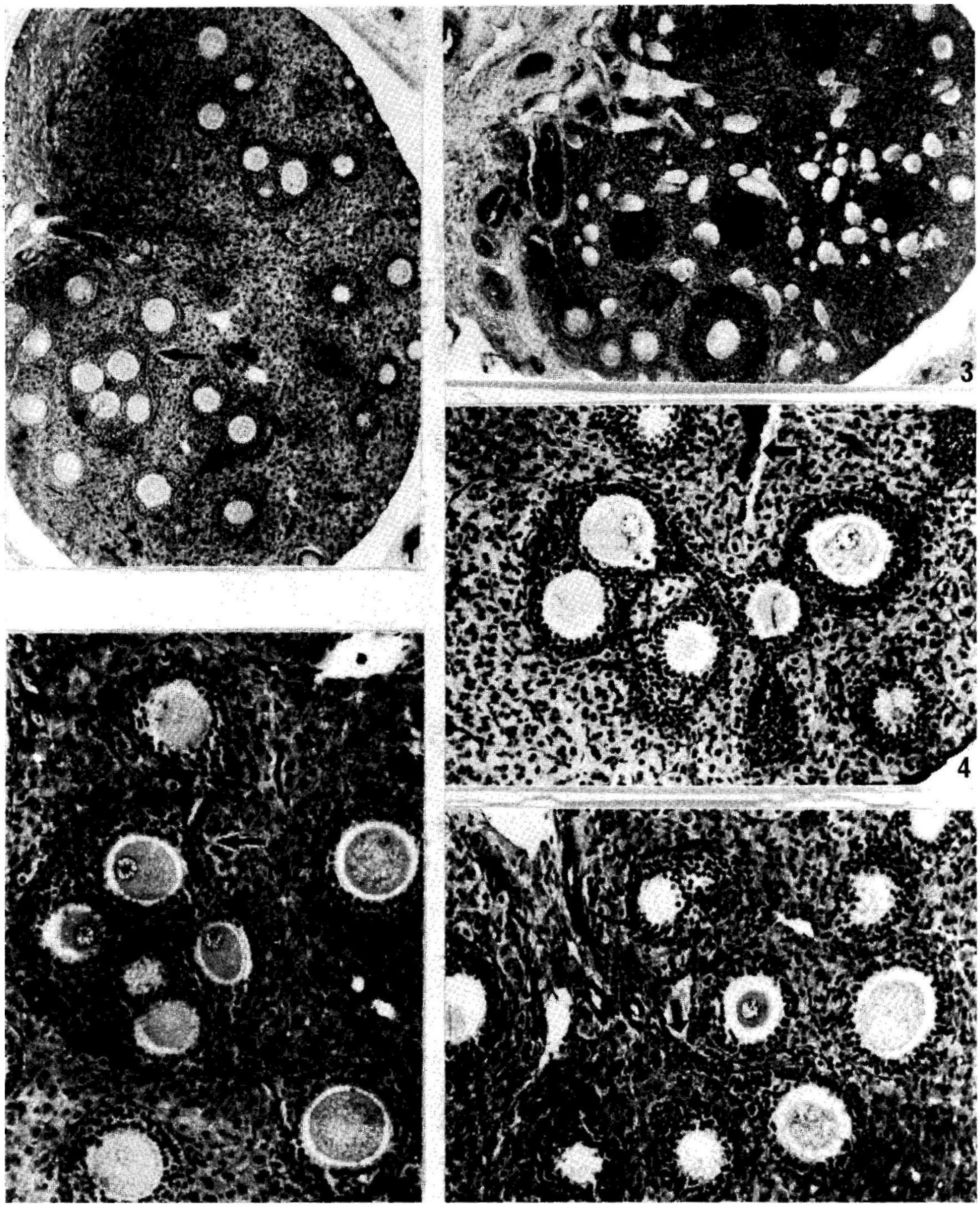

Fig 1. Transverse section of the ovary (September) showing large numbers of polyovular follicles (arrow). X 90.

Fig 2. Transverse section of the ovary (September) showing a single polyovular follicle containing six oocytes (arrow). X 145.

Fig 3. Transverse section of the ovary (October) showing large number of atretic follicles mainly in medullary region. X 90.

Fig 4. Transverse section of the ovary (September) showing direct connection of rete cells with developing follicle (arrow). X 145.

Fig 5. Transverse section of the ovary showing polyovular follicle connected with rete cells (arrow). X 145.

Table II

Changes in the number of polyovular follicles* in ovaries of adult *S. heathi* according to the number of oocytes.

Month **	N° of ovaries examined	N° of oocytes		
		Two	Three	More than three
September	12	157 (5)***	60 (3)	24 (2)
October	12	89 (3)	24 (3)	—
November	12	27 (2)	6 (1)	—

* Values are total numbers of polyovular follicles in all ovaries examined.
** During remaining months only biovular follicles were found
*** Values in parentheses, number of ovaries containing polyovular follicles.

Present data also showed an inverse relationship between the number of oocytes per follicle and the extent of their development (Table III). Polyovular follicles containing more than three oocytes soon undergo atresia as they never attained stage II of development (Table III). Biovular follicles initially grow normally, but they did not grow beyond follicular stage III. The majority of these polyovular follicles undergo atresia during October (Fig 3).

Table III

Extent of development of polyovular follicles containing different numbers of oocytes*.

Follicular stages **	N° of oocytes		
	Two	Three	More than three
I	176	40	20
II	89	30	—
III	28	7	—

* Values, total number of polyovular follicles from three ovaries, each taken from September to February.
** Follicular stages based on number of layers of granulosa cells.

Changes in the ovary during the time of recruitment of polyovular follicles.

During September, the time of first appearance of polyovular follicles, the ovary of *S. heathi* showed extensive proliferation of intra-ovarian rete cords extended up to the medullary region. During August (before the beginning of the ovarian recrudescence) intra-ovarian rete was small and confined to the hilar region. Folliculogenesis is reported to begin in this species following ovarian quiescence during early September (Krishna & Singh,1992). Simultaneously with the initiation of folliculogenesis, several cell cords grow rapidly from the rete into the medullary region of the ovary and become contiguous with the developing follicles (Figs 4, 5). During this time most of the follicles found in the medullary region were either connected with each other or with rete cords (Fig 6). Soon rete cords and growing follicles were enclosed by common basement membrane. Thereafter the follicles get detached, the intra-ovarian rete become restricted to the hilar region and the ovary contained several polyovular follicles in the medullary region (Fig 4).

Fig 6. Transverse section of the ovary (September) showing rete cells connected with several follicles (arrow). X 90.

Follicles in the peripheral region of the ovary never had any contact with rete cords. Several growing uniovular follicles were observed in this region of the ovary. Extensive development of rete cells cord –as seen during September– was not found during other months. This may be correlated with lesser occurrence of polyovular follicles in the ovary.

DISCUSSION

Two different patterns of follicular development were observed during September (early recrudescence phase) in the ovary of *S. heathi*. This is supported by the observation of two types of primary follicles, uniovular and polyovular, in the ovary during this month. In the medullary region of the ovary, rapidly growing rete cords develop contacts with germ cells or follicles and this correlate with the appearance of a number of polyovular follicles. This pattern of follicular development was observed only during September. In the periphery of the ovary, where developing follicles had no contact with the rete cords, several uniovular follicles were observed. This indicates that a close contact between proliferating rete cord and follicles may be required for the development of polyovular follicles. This was further supported by our observation of only a few or no polyovular follicles in most of the ovaries during other months, when the intra-ovarian rete cord was also small and restricted to the hilar region.

The close relationship between the germ cell and rete ovarii has earlier been described mainly in the prepubertal ovaries of several species (Byskov, 1975). The marked similarities between rete cells (Fig 7) and granulosa cells support the hypothesis that rete cells give rise to pregranulosa cells (Byskov & Hoyer, 1988; Zamboni, 1974). The presence of rete cells surrounding the developing germ cells in prepubertal ovary has also been shown in *S. heathi* (Krishna & Singh, 1993). The frequently observed connection between rete tubules and polyovular follicles shown in the present study also suggests that polyovular follicles arise in *S. heathi* as a result of the interaction between the oocyte and rete system during early folliculogenesis. How does the interaction between rete cells and germ cells give rise to polyovular follicles is not clear from the present study. But it may be hypothesized that oocytes lying within or in contact with rete tubules can easily be entangled by the rapidly proliferating rete granulosa cells and thus form polyovular follicles. The observation of a remarkable increase in rete cells cord, for a brief period during September, supports this hypothesis.

Fig 7. Transverse section of the ovary (September) showing a single tubule of intra-ovarian rete (RE). The tubules are lined with cylindrical epithelial cells having narrow lumen (arrow). X 145.

Other striking finding of the present study was the observation of an inverse relationship between the number of oocytes per follicle and the extent of their development. This suggests that oocytes are much less viable in the polyovular condition and they soon undergo atresia. The atretic polyovular follicles composed of granulosa cells derived from rete cells may then give rise to secondary interstitial tissue. Rete cells as a source of secondary interstitial cells was earlier suggested (Guraya & Greenwald, 1977). It may be noted that the ovary of *S. heathi* also contains extensive interstitial gland cells and their area increased significantly during November (Abhilasha & Krishna, 1997). The large number of atretic follicles noticed in the medullary region during October may have been responsible for an increased area of interstitial gland cells (Fig 2).

In conclusion, our observations support the earlier hypothesis of Hirshfield (1992) that not all growing follicles are created equally. Follicles differ with respect to the cell lineage of their granulosa cell population. Polyovular follicles may form due to granulosa cells derived from the rapid proliferation of rete cells. These follicles are short lived and may contribute to the extensive development of interstitial tissue which was shown to be a major source of abnormally high circulating concentration of androstenedione in *S. heathi* (Abhilasha & Krishna, 1996). The development of polyovular follicles in this species thus contribute indirectly to the phenomenon of delayed ovulation in *S. heathi*.

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