

Growth of ovarian follicles in the Natal clinging bat

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Growth and atresia of the ovarian follicles of *Miniopterus schreibersi natalensis* (A. Smith, 1834) were studied from females collected on the southern Transvaal Highveld. In this hibernating subspecies no storage of sperm or delayed ovulation occur and females enter hibernation in a pregnant condition. Only one Graafian follicle develops, which is characterized by a large antrum with the ovum-bearing mass of cells occupying only a relatively small portion of the antrum. Many follicles develop to a medium size before undergoing regression. Basically two types of regression occur; usually destruction of the stratum granulosum takes place before the oocyte is affected. The reverse condition was also observed but was very uncommon. Corpora lutea atretica resulting from the luteinization of medium-sized Graafian follicles were commonly found.

S. Afr. J. Zool. 14: 111–117 (1979)

Die groei en atresie van die ovariumfollikels van *Miniopterus schreibersi natalensis* (A. Smith, 1834) is bestudeer by wyfies wat versamel is op die Suid-Transvaalse Hoëveld. In hierdie hibernerende subspesie vind geen storting van sperms of vertraagde ovulering plaas nie, en wyfies is alreeds dragtig met die aanvang van hibernasie. Slegs een Graaffollikel ontwikkel en dit word gekenmerk deur 'n groot antrum en relatief klein eierdraende selmassa wat slegs 'n relatief klein gedeelte van die antrum in beslag neem. Baie follikels ontwikkel tot 'n mediumgrootte voordat hulle regressie ondergaan. Twee tipes regressie is waargeneem: Gewoonlik vind vernietiging van die stratum granulosum eers plaas voordat die oöset beïnvloed word. Die omgekeerde proses is ook waargeneem, maar dit kom baie selde voor. Corpora lutea atretika wat ontstaan as gevolg van die luteïnisering van mediumgrootte Graaffollikels het algemeen voorgekom.

S.-Afr. Tydskr. Dierk. 14: 111–117 (1979)

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Accepted 30 January 1979

Guthrie and Jeffers (1938a, b) working mainly on *Myotis lucifugus*, gave a thorough account of ovarian structure and growth patterns of the primary and secondary follicles; the development of the Graafian follicles in the same species was traced by Wimsatt (1944).

A common feature of Graafian follicles in hibernating bats appears to be a small antrum and a many-layered cumulus (Wimsatt 1944; Racey 1972) which together represent a physiological adaptation to the survival of the follicle throughout hibernation (Wimsatt 1949). These surviving follicles persist virtually without change throughout the hibernation period, with pre-ovulatory growth, maturation of the oocyte and ovulation occurring at the end of this period (Wimsatt & Parks 1966). This probably occurs in the majority of hibernating Microchiroptera where copulation takes place during autumn, and is followed by storage of spermatozoa throughout hibernation with ovulation and fertilization taking place in spring.

However, it has been known for some time (Richardson 1977) that temperate latitude members of the genus *Miniopterus* deviate from this pattern of delayed ovulation and sperm storage. In *Miniopterus* ovulation, copulation and fertilization occur before hibernation and females hibernate in a pregnant condition. The present study examines follicle growth and atresia in *M. s. natalensis*.

Methods

All bats (144) were collected in caves on the southern Transvaal Highveld from 1972 to 1976. The majority were collected in Schurveberg Cave No. 1, Schurveberg Mountain, District Pretoria, (25°48'S, 28°01'E) and few were also taken at Long One Cave, Uitkomst, (25°54'S, 27°46'E) and brought alive to the laboratory where they were killed with chloroform. The uterus with both ovaries attached was severed just behind the bladder and fixed in Bouin's fluid or occasionally in Susa. The uterine horns of non-pregnant females and others during early pregnancy tended to curl and become folded when immersed in the fixative. This tendency was overcome by sticking the specimen to a dry square piece of glass cut from standard microscope slides before fixing. The fixed specimen was later scraped from the glass. Standard histological techniques were used; sections were cut at 5 µm and 8 µm

In this study four stages in the development of the follicles are recognized: Primordial, primary, secondary and Graafian follicles. For convenience the latter are divided into small, medium and ripe Graafian follicles.

Primordial and primary follicles

Primordial follicles were situated on the perimeter of the ovarian cortex where they were tightly packed together. Because of this it was not always possible to observe any epithelium around them and many of the oocytes appeared to be naked. The mean diameter of these naked oocytes was $14,8 \pm 1,1 \mu\text{m}$ (13,8—15,7; $n = 8$). When they had a mean diameter of $18,9 \pm 1,2 \mu\text{m}$ (17,6—21,1; $n = 17$) they were surrounded by irregular spindle-shaped cells. All these oocytes were ovoid in form with eccentric nuclei. This eccentric position was maintained throughout the growing period. Amongst these primordial follicles and especially deeper into the cortex, many early primary follicles occurred. Here the oocytes were surrounded by a layer of flattened to low cuboidal cells (Fig. 1 C). The mean diameter of these early primary follicles was $34,4 \pm 5,8 \mu\text{m}$ (24,3—45,9; $n = 25$) and that of their oocytes $22,3 \pm 3,5 \mu\text{m}$ (17,0—29,6; $n = 25$). From the early primary follicle stage onwards the form of the oocytes varied from round to mostly ovoid. Because of the ovoid form in the majority of oocytes, the majority of follicles were also ovoid in form. The older primary follicles (the diameter of which ranged in size from 33,8—101,6 μm ; $n = 28$) were situated deeper into the cortex. As these follicles got older and still further increased in size, the cuboidal follicular cells progressively became more columnar with their nuclei moving to the outside or apex of the cells (Fig. 1D). In ovoid follicles however, it was found that all the follicular cells did not become columnar simultaneously as in round follicles. Here the cells at the opposite poles of the ovoid follicles were slightly in advance of those on the sides (Fig. 1D), and in many the cells at the opposite poles were already dividing by the time those on the sides became columnar (Fig. 1E).

Secondary follicles

The transition between primary and secondary follicles was not always very clear, but in general, secondary follicles were recognized from the time that the first divisions in the single layer of follicular cells commenced, until a multicellular condition was reached. The size of the secondary follicles ranged from 91,2—138,4 μm ($n = 27$). In round follicles divisions of the follicular cells occurred at various places around the oocyte (Fig. 1F), which usually resulted in a clear two-layered secondary follicle stage (Fig. 2A). The mean diameter of these two-layered secondary follicles was $112,4 \pm 5,2 \mu\text{m}$ (102,4—120,2; $n = 13$). Such a two-layered condition was never as obvious in ovoid follicles, because divisions usually started at the two poles and stayed slightly in advance of those on the sides, resulting in these areas being slightly thicker than the rest of the layer (Fig. 2B). In the secondary follicles the follicular theca, already visible from the late primary follicle stage, became conspicuous and a very distinct basement membrane occurred (Fig. 2B).

At what stage the zona pellucida in *M. s. natalensis* starts forming is not certain, but it appeared to be during the late primary follicle stage. However, during the late secondary follicle stage this layer was present as a clear blue ring (with

Mallory staining) around the oocyte. In many sections it was full of vacuoles. In some of the Graafian follicles it radiated between the cells of the zona radiata (Fig. 2C). It is doubtful whether this is a natural phenomenon in *M. s. natalensis* as it was found only in a few of the Graafian follicles.

Graafian follicles

Small Graafian follicles were those where the first signs of cavities between the follicular cells could be detected. Small cavities varied from one to several that developed at various levels between the follicular cells (Fig. 2D). The mean diameter of these follicles was $181,8 \pm 26,5 \mu\text{m}$ (140,8—221,8; $n = 14$). In the majority of cases the oocyte, with eccentric nucleus, was still ovoid in form and situated in the middle of the follicle (Fig. 2D). The various cavities gradually increased in size and started to unite with each other to form a single lumen or antrum which rapidly enlarged to form the medium and ripe Graafian follicles respectively. As the antrum grew the oocyte was progressively pushed more to one side of the follicle (Fig. 2E). At this stage the theca folliculi as well as the zona pellucida were very conspicuous. Outside the zona pellucida there is a layer of radially elongated follicular cells constituting the corona radiata (Patten 1948). In *M. s. natalensis* the corona radiata was not very distinct from the cells of the surrounding discus proligerus. They did not give a distinct radiated appearance and were not composed of very high columnar cells. The margin between them and the discus proligerus was also not very distinct (Fig. 2C). In medium Graafian follicles the oocyte was pushed well to the one side of the follicle with the cumulus oophorus cells attached to the surrounding stratum granulosum over a relatively broad and low surface (Fig. 2E).

The mean diameter of these follicles was $245,8 \pm 17,5 \mu\text{m}$ (206,4—270,9; $n = 12$). Although the majority of oocytes were still ovoid in form the follicles tended to have a more spherical appearance. This was due to the increase in antrum size, pressing the follicular cells more equally to the outside.

In ripe Graafian follicles the oocyte was surrounded by a clear zona pellucida followed by an indistinct corona radiata. This layer in turn was surrounded by a narrow layer of cumulus cells which was never found to display any particular hypertrophy (Fig. 2F). According to Wimsatt (1944) this ovum-bearing mass is much more prominent in bats than in other mammals, and occupies a relatively larger proportion of the follicular cavity. In *M. s. natalensis* however, this ovum-bearing mass of cells was eccentric and occupied only a relatively small proportion of the antrum in ripe Graafian follicles.

Although the cells of the corona radiata were not clearly distinguishable from the cumulus cells, they did appear slightly more columnar and narrower. The cells of the cumulus were more loosely organized and roundish with big round nuclei, which could be either centrally or peripherally located. The mean diameter of the ripe Graafian follicles was $478,6 \pm 64,3 \mu\text{m}$ (396,7—587,0; $n = 13$).

From the primordial follicles up to the secondary follicles there appeared to be an almost linear relationship between oocyte and follicle growth, both increasing at a relatively even rate (Fig. 3). However, from the small Graafian follicles onwards there was no longer any significant

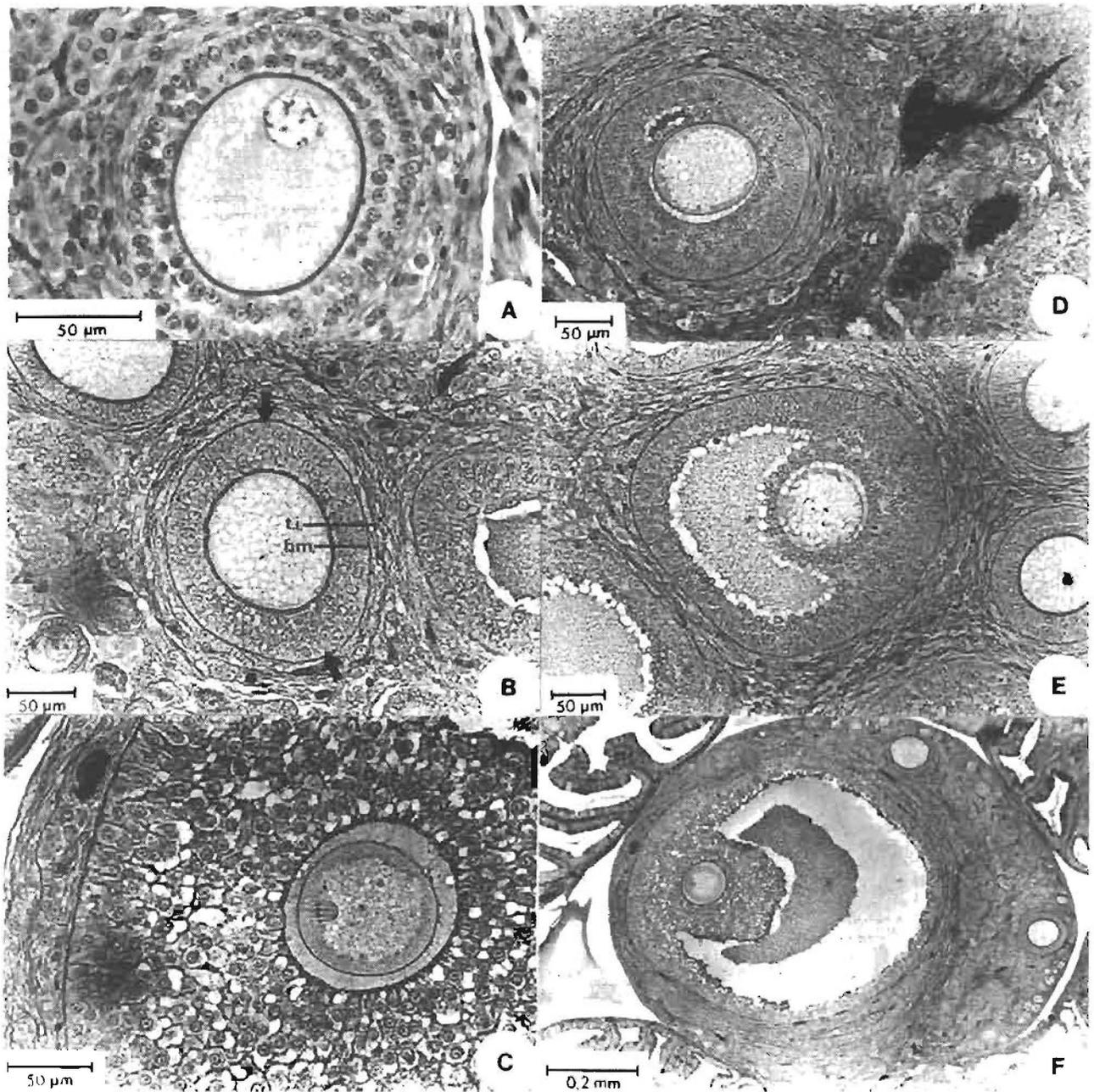


Fig. 2 A Secondary follicle where the oocyte is surrounded by two layers of follicular cells. Bouin, 5 μ m, H&E.

B Secondary follicle indicating accelerated division of the follicular cells at the opposite poles (indicated with arrows), causing these two areas to be thicker than the rest of the layer on the sides. In this figure the theca interna (Li.) and basement membrane (b.m.) are also clearly visible. Bouin, 5 μ m, Mallory.

C Section through the ovum-bearing mass of cells in a big Graafian follicle prior to the formation of the first polar body (first meiotic division). In section striations from the zona pellucida running inbetween the basis of the corona cells are clearly visible (for the latter see text). Bouin, 5 μ m, Mallory.

D Small Graafian follicle showing the first signs of the antral cavity appearing between the follicular cells. Bouin, 5 μ m, Mallory.

E Medium Graafian follicle (busy regressing) with single moderate antrum and oocyte more eccentric. The cumulus oophorus is broad and low where attached to the stratum granulosum. Bouin, 5 μ m, Mallory.

F Ripe Graafian follicle, illustrating the small size of the ovum-bearing mass relative to the big antrum of the follicle. Bouin, 5 μ m, Mallory.

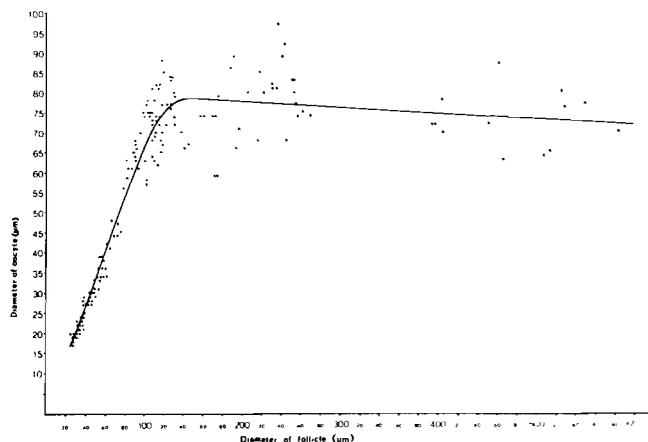
correlation in the growth of the oocyte and the follicle. This was due mainly to the formation and expansion of the antrum, causing a rapid enlargement of the follicle. However, when the diameter of the oocytes is plotted as a percentage of follicle diameter (Fig. 4) a highly significant ($P < 0,001$) negative exponential relationship was found ($r = 0,95$; $n = 143$) with the equation $y = 81,54e^{-0,003x}$ (where y = diameter of the oocyte as a percentage of the follicle diameter and x = diameter of the follicle). From this it can be seen that the diameter of the ovum, expressed as a

percentage of the diameter of the follicle, decreases from 76% in follicles with a diameter of 20 μ m to 11% in follicles with a diameter of 580 μ m.

From Fig. 3 the impression emerges that the oocytes reach full size during the late secondary and small Graafian follicle stage (i.e. follicles from about 120,0—150,0 μ m). However, the highest mean oocyte diameter was found only during the medium sized Graafian follicle stage, suggesting the termination of oocyte growth only at that stage (Table 1.)

Table 1 Mean diameter and standard deviations of oocytes from the late secondary to ripe Graafian follicle stage

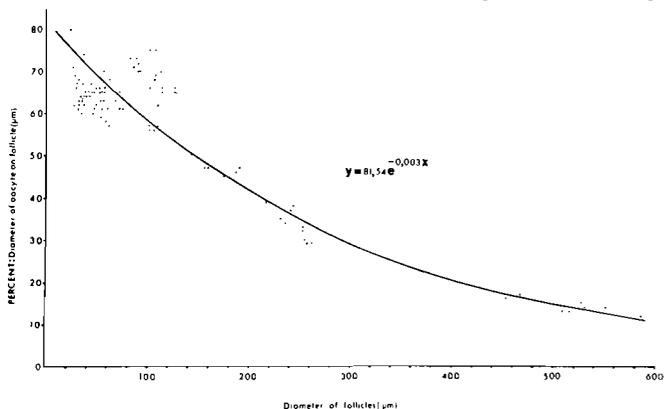
	Mean diameter of oocytes (μm)	Range (μm)	Number (n)
Late secondary follicles	75,4 \pm 4,8	67,2 – 83,5	14
Small Graafian follicles	75,3 \pm 7,2	65,9 – 89,4	14
Medium Graafian follicles	78,7 \pm 5,6	68,0 – 89,6	12
Ripe Graafian follicles	72,8 \pm 6,8	63,4 – 87,2	13

**Fig. 3** Scatter diagram for *M. s. natalensis* illustrating oocyte and follicle growth where the diameter of the oocytes is plotted against that of their respective follicles.

In Figs. 3 & 4 follicles between the medium and ripe Graafian follicle stages, i.e. follicles with sizes between 280,0—390,0 μm are conspicuously absent. It appears that in *M. s. natalensis* there is a gradual increase in follicle size from small to medium Graafian follicles. At this stage the majority of medium Graafian follicles become atretic with the exception of one (occasionally two) that are destined to become ripe Graafian follicles. This explains the relative abundance of medium Graafian follicles in both ovaries of *M. s. natalensis* as opposed to a single ripe Graafian follicle (occasionally two) in mainly the left ovary. Growth from the medium to ripe Graafian follicle stage must be a rapid process, making the chances of sampling material with follicles between 280,0—390,0 μm extremely rare.

Follicular degeneration

Atresia is the process whereby oocytes are lost from the ovary other than by ovulation (Ingram 1962). In *M. s. natalensis* atretic follicles were common up until the medium Graafian follicle stage. The majority of medium Graafian follicles underwent atresia as only one (and very

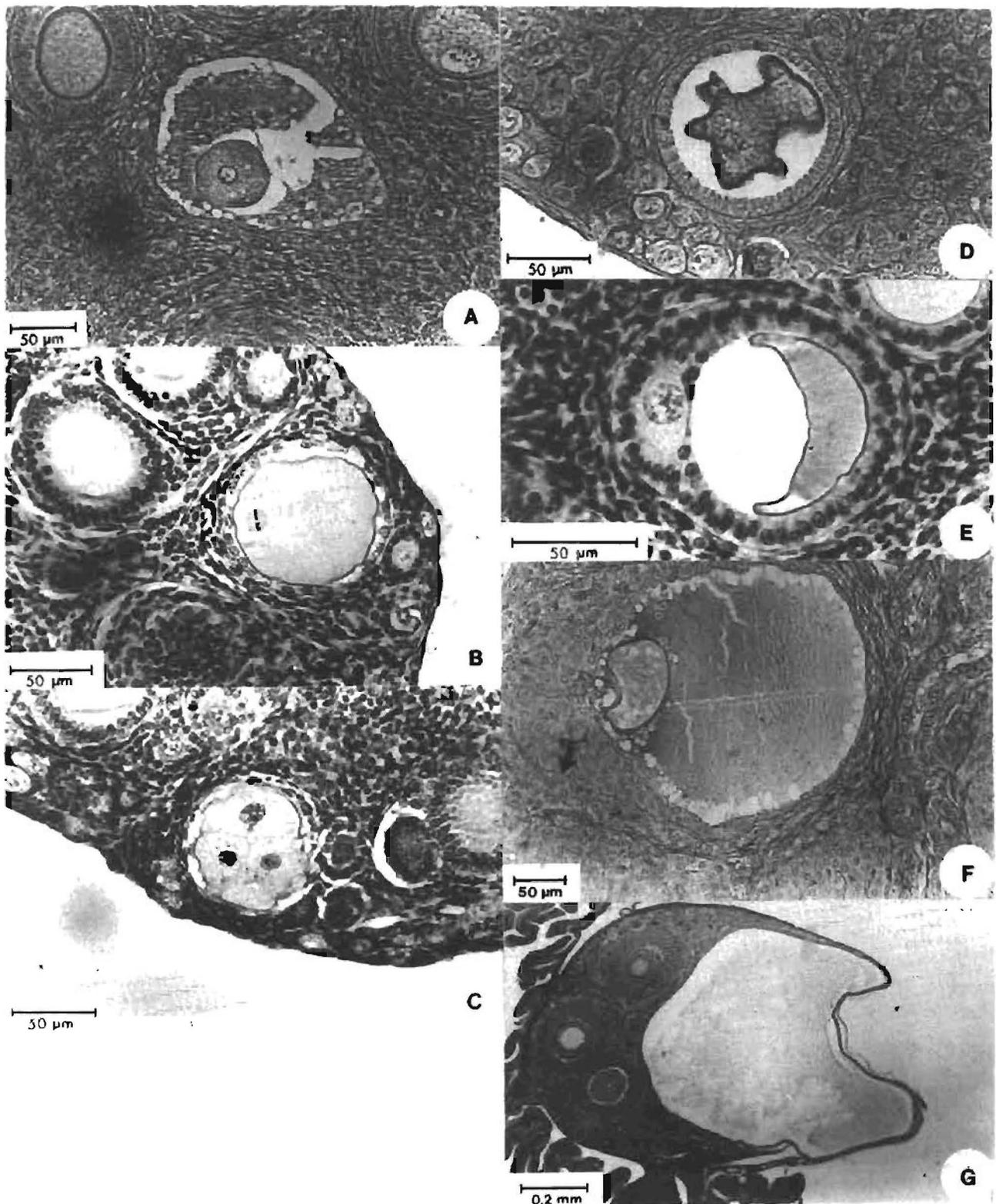
**Fig. 4** Scatter diagram for *M. s. natalensis* where the diameter of the oocytes is expressed as a percentage to those of their respective follicles.

occasionally two) developed further.

Guthrie and Jeffers (1938a) recognized two types of regression in the follicles of *Myotis lucifugus* and *M. grisescens*. The most common type of regression found, which also commonly occurred in *M. s. natalensis* was where the stratum granulosum almost completely disappeared before the oocyte began to degenerate (Fig. 5A). In secondary follicles and small to medium Graafian follicles this type of regression was very obvious. Where an antrum existed the stratum granulosum fragments invaded the antrum (Fig. 5A). The cumulus oophorus also degenerated and some follicles were found in which the cumulus and stratum granulosum remains were resorbed, leaving the oocyte with zona pellucida to float freely in the antrum. The eventual destiny of these oocytes appeared to be fragmentation and absorption. In the ovaries of nulliparous females containing many regressing primary follicles, various types of 'destruction' of the oocytes were observed. In some of these oocytes clear maturation divisions were seen (Fig. 5B). In others a multi-nuclear condition was present, whereas in yet others mitotic divisions and fragmentation of the oocyte were found. This resulted in a multi-cellular stage with clearly defined cells containing a clear nucleus (Fig. 5C).

The second type of regression, which was very seldom seen, was also described by Guthrie and Jeffers (1938a). Here the oocyte was absorbed first with the stratum granulosum and theca still intact. This type was very uncommon in *M. s. natalensis* and in some of the primary and early secondary follicles it looked more like a distortion of the oocyte (Fig. 5D & E). Atresia of medium and large Graafian follicles frequently leads to the formation of a nodule of tissue closely resembling a corpus luteum, and is known as a corpus luteum atreticum (Brambell 1956). Ingram (1962) mentioned corpora lutea atretica being formed by the luteinization of follicles still retaining their oocytes. These descriptions are the best explanation available at present to account for the common occurrence of 'small corpora lutea' in the ovaries of *M. s. natalensis*. They were quite common in some females and could easily be confused with small corpora lutea of ovulation. In some ovaries two or more of these 'small corpora lutea' occurred. They all were the size of medium Graafian follicles, leading to the conclusion that mainly medium Graafian follicles were undergoing this extensive luteinization. One corpus luteum atreticum was found in the initial stage where the oocyte was only partly destroyed, and the antrum partly infiltrated with luteinized cells (Fig. 5F). In this follicle it was the theca cells that proliferated and hypertrophied. Observing the area directly around the oocyte, and especially at the pole opposite to the oocyte, it was apparent that the stratum granulosum had disappeared. The proliferating cells on the side of the oocyte, which could be mistaken for stratum granulosum cells, must therefore have been theca cells that were pushing through the basement membrane.

Atresia may also be associated with the formation of cysts which may attain a considerable size (Ingram 1962). On four occasions cysts were found in the ovaries of *M. s. natalensis*. In three cases they were of moderate size, more or less the size of medium Graafian follicles. However, one of these cysts was much bigger than a ripe Graafian follicle (Fig. 5G).



- Fig. 5** A Medium Graafian follicle regressing, showing complete destruction of the stratum granulosum with its remains drifting in the antrum with the oocyte. Bouin, 5 μ m, Mallory.
- B Atretic follicle where the oocyte shows a typical meiotic spindle. Bouin, 5 μ m, H&E.
- C Atretic follicle containing four oocytes (one not clearly visible in this section) which are the result of mitotic divisions and fragmentation. Bouin, 5 μ m, H&E.
- D & E Follicles with oocytes showing regression of the second type (see text). In the polyovular follicle (E) only the biggest oocyte is undergoing regression. Note the unaffected condition of the follicular cells in both cases.
(D) Bouin, 5 μ m, Mallory.
(E) Bouin, 5 μ m, H&E.
- F Formation of a corpus luteum atreticum with the oocyte only partly destroyed. Here proliferation and hypertrophy occur only on one side of the follicle, but appear to be in the process of spreading to the other side. Parts of the basement membrane (indicated with arrow) can still be seen between the proliferating cells. Bouin, 5 μ m, Mallory.
- G Ovarian cyst occupying the greater part of the ovary. Bouin, 5 μ m, Mallory.

Polyovular follicles

In *M. s. natalensis* these were only found on three occasions; always in the peripheral layer of the ovary amongst the small follicles, and in each case two oocytes were enclosed within the same follicle (Fig. 5E). Polynuclear oocytes have only been observed in atretic follicles.

Discussion

The obvious decline in oocyte diameter from medium to ripe Graafian follicles may be the result of distortion of the oocyte in vesicular follicles due to the histological techniques employed as discussed by Wimsatt (1944).

In contrast to the situation occurring in other hibernating bat species (Wimsatt 1944; Racey 1972), the Graafian follicles in *M. s. natalensis*, and presumably all hibernating members of the genus *Miniopterus*, have no special adaptations for prolonged survival. Here the ovum-bearing mass of cells is not a prominent feature; it occupies only a relatively small portion of the antrum, and the cumulus cells display no particular hypertrophy.

The zona pellucida which encases the oocyte and separates it from the neighbouring follicular cells (Mossman & Duke 1973), is a transparent, non-cellular secreted layer which in some mammals exhibits radial striations (Patten 1948). Guthrie and Jeffers (1938a), working on *Myotis lucifugus* and *M. grisescens* mentioned that the inner ends of the corona cells seem to be embedded in the zona, which gave this layer a striated appearance. Although the same radial striations have been found in some of the ripe Graafian follicles of *M. s. natalensis*, these were apparently an artifact caused during preparation. The corona cells were perhaps slightly pulled away from each other, and fluid trapped between them during staining could have been responsible for this phenomenon.

The distension of the Graafian follicle is due to the secretion of the liquor folliculi into the antrum (Brambell 1956). Of the three types of liquor folliculi recognized by Robinson (1918) the secondary liquor folliculi is responsible for the rapid enlargement of the ripening follicle during the pre-ovulatory period. Wimsatt (1944) feels that the same fluid is also responsible for the detachment of the ovum-bearing mass of cells from the stratum granulosum. This mass of cells becomes entirely free during the late pre-ovulatory growth stage in *Myotis lucifugus* (Wimsatt 1944) and during late pro-oestrus in *Desmodus rotundus* (Wimsatt and Trapido 1952). In *M. s. natalensis* however, these ovum-bearing masses of cells were always found attached to the stratum granulosum, some very loosely, but never drifting freely in the antrum. It is possible that adequate material was not obtained to cover this specific phase in the late pre-ovulatory follicle.

Polyovular follicles are rare in bats (Guthrie & Jeffers 1938a) and have been witnessed on only three occasions in *M. s. natalensis* during the present study, except in atretic follicles where more than one oocyte may occur due to fragmentation. Polynuclear oocytes in *M. s. natalensis* have also been found only in atretic follicles. In some mammals, however, both these phenomena appear to be common. Hartman (1926) mentioned that polynuclear ova are the rule rather than the exception in the opossum and polyovular follicles are often found in astounding numbers in this species.

Most of the follicles that begin growth in the ovaries of mammals undergo regression (Guthrie & Jeffers 1938a), which is the process whereby oocytes are lost from the ovary other than by ovulation (Ingram 1962). Atretic follicles in both ovaries of *M. s. natalensis* were common but were most conspicuous from the secondary follicle stage onward. Of the two types of atresia observed by Guthrie and Jeffers (1938a) the first type where regression starts in the stratum granulosum and theca cells, eventually leaving the oocyte free in an open cavity, was most commonly observed in *M. s. natalensis*.

Acknowledgements

This paper is part of a doctoral thesis carried out under the supervision of Professor J.D. Skinner.

References

- BRAMBELL, F.W.R. 1956. Ovarian changes. In: *Marshall's physiology of reproduction*. Vol. 1: 397-542, ed. Parkes, A.S., Longmans, Green & Co. London, New York, Toronto.
- GUTHRIE, M.J. & JEFFERS, K.R. 1938a. A cytological study of the ovaries of the bats *Myotis lucifugus lucifugus* and *Myotis grisescens*. *J. Morph.* 62: 523-557.
- GUTHRIE, M.J. & JEFFERS, K.R. 1938b. Growth of follicles in the ovaries of the bat *Myotis lucifugus lucifugus*. *Anat. Rec.* 71: 477-496.
- HARTMAN, C.G. 1926. Polynuclear ova and polyovular follicles in the opossum and other mammals, with special reference to the problem of fecundity. *Am. J. Anat.* 37: 1-51.
- INGRAM, D.L. 1962. Atresia. In: *The ovary*. Vol. 2: 247-273, ed. Zuckerman, S., Academic Press, London.
- MOSSMAN, H.W. & DUKE, K.L. 1973. Comparative morphology of the mammalian ovary. University Press, Wisconsin.
- PATTEN, B.M. 1948. Embryology of the pig. 3rd edn. McGraw-Hill, New York.
- RACEY, P.A. 1972. Aspects of reproduction in some heterothermic bats. Ph. D Thesis, St. Bartholomew's Hospital Medical College, London.
- RICHARDSON, E.G. 1977. Biology and evolution of reproduction in *Miniopterus* (Chiroptera: Vespertilionidae) in eastern Australia. In: *Reproduction and evolution*. pp 213-214, eds Calaby, J.H. & Tyndale-Biscoe, C.H. Australian Academy of Science, Canberra.
- ROBINSON, A. 1918. The formation, rupture and closure of ovarian follicles in ferrets and ferret-polecat hybrids, and some associated phenomena. *Trans. R. Soc. Edinb.* 52: 303-362.
- WIMSATT, W.A. 1944. Growth of the ovarian follicle and ovulation in *Myotis lucifugus lucifugus*. *Am. J. Anat.* 74: 129-173.
- WIMSATT, W.A. 1949. Glycogen, polysaccharide complexes and alkaline phosphatase in the ovary of the bat during hibernation and pregnancy. *Anat. Rec.* 103: 564-565.
- WIMSATT, W.A. & TRAPIDO, H. 1952. Reproduction and the female reproductive cycle in the tropical American vampire bat, *Desmodus rotundus murinus*. *Am. J. Anat.* 91: 415-445.
- WIMSATT, W.A. & PARKS, H.F. 1966. Ultrastructure of the surviving follicle of hibernation and of the ovum-follicle cell relationship in the vespertilionid bat *Myotis lucifugus*. *Symp. Zool. Soc. Lond.* 15: 419-454.