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Prolonged Survival of the Graafian Follicle Accompanied with Sperm Storage and the Subsequent Early Development in the Female Greater Tube-nosed Bat, *Murina leucogaster**

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In the greater tube-nosed bat, *Murina leucogaster*, after the mating season in autumn, the ovary maintained the Graafian follicle during hibernation until spring, while the ovum was in prophase of meiosis I, and a considerable amount of glycogen and lipids as nutrient sources was characteristic of the follicle cells. On the other hand, the principal site of sperm storage was the uterotubal junction (UTJ), where their heads oriented towards the non-ciliated epithelial cells established a close association with well-developed microvilli. After ovulation, in the UTJ where non-ciliated epithelial cells lost almost all the microvilli, clusters of stored spermatozoa had been detached from the epithelium, and some dead sperm were engulfed by the non-ciliated cells. The block to polyspermy seemed to reside in the zona pellucida, because no spermatozoa could be detected in the perivitelline space of 3 fertilized ova examined. At the preimplantation stage, uterine glands of the mesometrial wall increased in number, length and thickness, and the glandular cells became high and were characterized by accumulation of massive glycogen particles. All microvilli of the endometrial epithelial cells lengthened and were covered by a great amount of glycocalyx.

INTRODUCTION

The greater tube-nosed bat (*Murina leucogaster*), belonging to the subfamily Murininae of Vespertilionidae, occurs widely in Northeastern India, Altai, China, Korea, Ussuri, Sakhalin and Japan, ect., but is rather rare. This beautiful species is characterized by the tubular nose, long golden hairs on the back and brilliant silver hairs on the belly. There have been few ultrastructural studies on the reproductive phenomena of the female greater tube-nosed bat, except for an abridged description on sperm storage (Uchida and Mōri, 1987).

The aim of the present study was to examine in detail with electron microscope the early development of ova, and the principal site of sperm storage and changes in sperm situation in the sperm storage organs of the females concerned from insemination to ovulation, and to discuss adaptations of the Graafian follicles for their prolonged survival, polyspermy block and uterine environments at the preimplantation stage in mammals including bats.

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MATERIALS AND METHODS

Three adult female greater tube-nosed bats (M. *leucogaster*) used were divided into two control bats (Group C, not injected with hormones) and an experimental bat (Group H, injected with gonadotrophins). Bat Cl in torpor was collected at an abandoned mine in Ochoun, Youngdock-gun, Kyungsangbuk-dō, Korea on 22 November 1983 and killed 3 days after. Bats C2 and H1 were captured at the Obaga-ana Cave on the Akiyoshi-dai Plateau, Yamaguchi Prefecture, Japan on 11 April 1983. Bat C2 was sacrificed on the next day : in Bat H1, to induce ovulation, a subcutaneous injection of 5 i. u. PMSG (Teikoku-zōki Pharmaceutical Co., Ltd, Tokyo) was administered on the day of capture, and 2 i. u. hCG (Mochida Pharmaceutical Co., Ltd, Tokyo) were also injected subcutaneously on 14 April ; on 16 April the bat was examined (see Table 1).

All bats were killed by decapitation under ether anaesthesia, their reproductive organs were removed, and then the tissues were promptly placed in cold 3% glutaraldehyde in 0.2 M-phosphate buffer (pH 7.4) for 4 hr. After being thoroughly rinsed with the same buffer, the tissues were post-fixed with 1.3% osmium tetroxide in the same buffer for 3 hr, dehydrated with acetone and embedded in epoxy resin. Thick serial sections (1.5 μm) for light microscopy were stained with 0.5% toluidine blue. Thin sections (-60 nm) were double stained with uranyl and lead acetate. In order to detect the quantity of glycocalyx covering epithelial microvilli of the endometrium, the PFP staining method (Fujioka and Ogawa, 1979), in which oxidation of epoxy resin sections by per-formic acid (PF) was done prior to the phosphotungustic acid (PTA) staining, was applied to a part of the thin sections concerned. All the thin sections were examined with an Hitachi HS-9 electron microscope (75 kV).

RESULTS

The situations of ova and stored spermatozoa in the three females are described separately according to the following three reproductive stages-postcopulatory, fertilized one-celled and cleavage stage (Table 1).

1. Postcopulatory stage in hibernation

Bat Cl had two Graafian follicles, containing an oocyte with a germinal vesicle at the dictyotene stage in prophase of meiosis I (resting stage) and abundant large vacuoles, only in the left ovary : each of the follicles possessed a large, ill-defined cumulus oophorus and a few small antra (Fig. 1, insets a and b). The ovum ectoplasm was crowded with numerous spherical mitochondria with a few cristae and was scattered with small electron-dense cortical granules (Fig. 1). The moderately hypertrophied corona radiata cells and cumulus cells near the egg environs were characterized by a considerable amount of glycogen, and the glycogen content was maximal in the former cells and decreased nearer the granulosa layer (Fig. 2). On the other hand, the granulosa cells and the cumulus cells facing the antra contained abundant lipid droplets and well-developed rough endoplasmic reticulum (rER), and the lipid amount was maximal in the granulosa cells and decreased towards the egg environs (Fig. 3). That is, the gradients of the distribution of glycogen and lipid within the follicle cells

Table 1. Prolonged survival of the Graafian follicle accompanied with sperm storage and the subsequent early development in female greater tube-nosed bats.

Reproductive stage	Bats*	Developmental stage of ovum	No. of spermatozoa counted in: †			
			Uterus	UTJ	Isthmus	Ampulla
Postcopulatory (in hibernation)	Cl	Two ova in prophase of meiosis I (germinal vesicle), found in the Graafian follicles of the left ovary alone	+	++	−	
Fertilized one-celled (at arousal)	c2	One ovum in the pronucleus stage, found in the left and right ampullae each	+	+++	ttt	+
Cleavage (after arousal)	H1	One ovum in the 8-celled stage, found in the left uterine horn; one undetected ovum because of preparation failures	−	−	−	

*Group C and Group H represent control bats and a hormone-treated bat, respectively. Bat Cl captured on 22 November 1983 and Bat C2 collected on 11 April 1983 each was killed on 25 November 1983 and on 12 April 1983. Bat H1 captured on 11 April 1983 was sacrificed on 16 April 1983 after injections of gonadotrophins.

†The symbols of −, +, ++ and +++ show no, l-10, 11-100 and >101 spermatozoa/section, respectively.

were reversed.

Many intact spermatozoa were stored in the uterotubal junction (UTJ), where their heads oriented towards the non-ciliated epithelial cells established a close association with well-developed microvilli or shallow indentations (Fig. 4), although a few dead spermatozoa were engulfed by non-ciliated cells (Fig. 4, inset).

The endometrium of the symmetrically bicornuate uterus at this stage was generally shallow and compact, and the glands were not much developed on all sides of the uterine wall (Fig. 5a). The endometrial epithelial cells, containing an infinitesimal amount of glycogen (Fig. 6), had short microvilli (-0.6 μm) covered by only a little glycocalyx (Fig. 6, inset). The columnar gland cells were still low in their height (-11 μm) and had a basally placed nucleus and an appreciable quantity of glycogen : the endometrial stroma exhibited a compact appearance (Fig. 7). A few spermatozoa were irregularly scattered in the uterine lumen (Fig. 6), and some of them were phagocytised by infiltrated polymorphonuclear leucocytes (Fig. 8).

2. Fertilized one-celled stage at arousal

In Bat C2, two ova were discharged, one from each of the left and right ovaries where an ovulation point of the ruptured follicle had began to heal by obliteration of the original opening with luteinizing granulosa cells, and both were in the pronucleus stage in the upper part of the ampulla (Fig. 9 and inset). Shortly after sperm penetration, one egg was accompanied with two polar bodies, a chromatin mass of the female pronucleus divided into sub-areas by lamellar structures (Fig. 10 and inset a), a swelling male pronucleus (not shown because of technical failures) and a principal

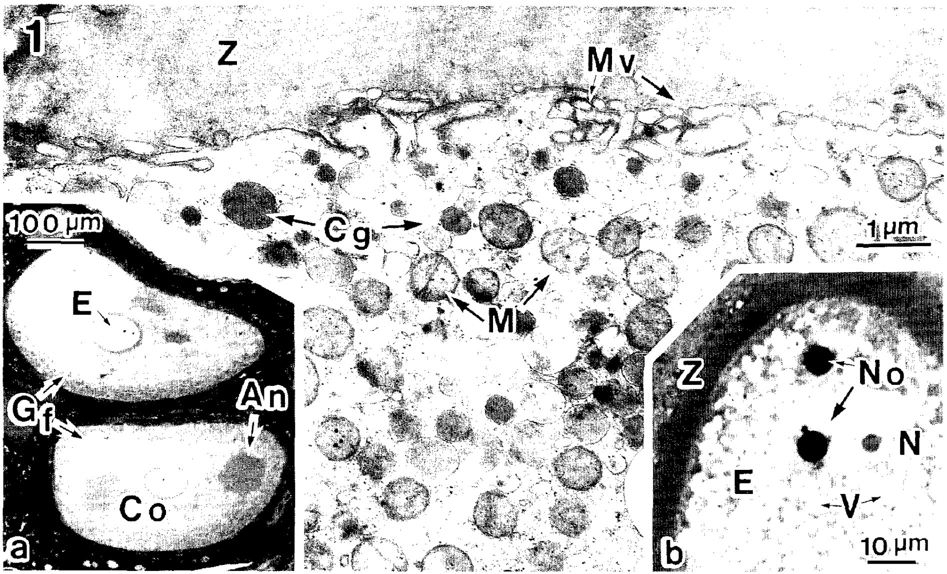
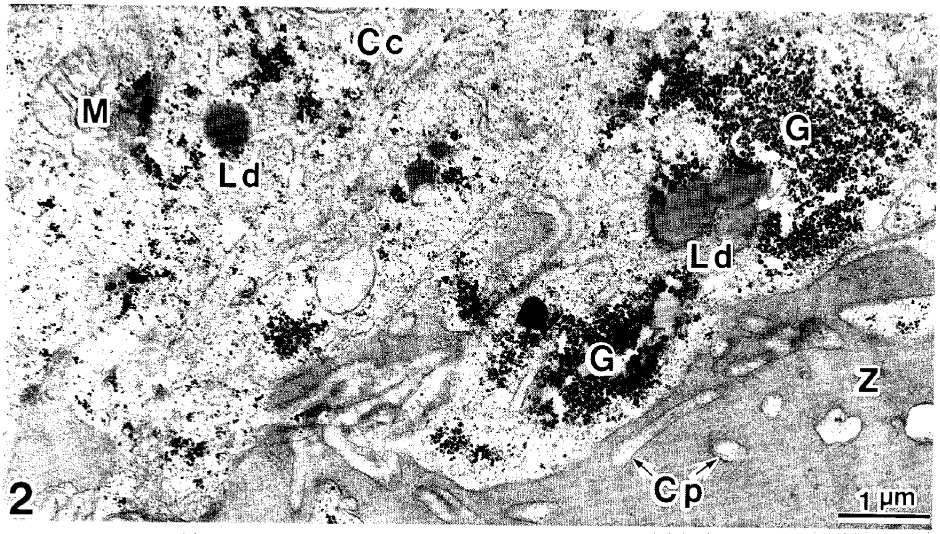


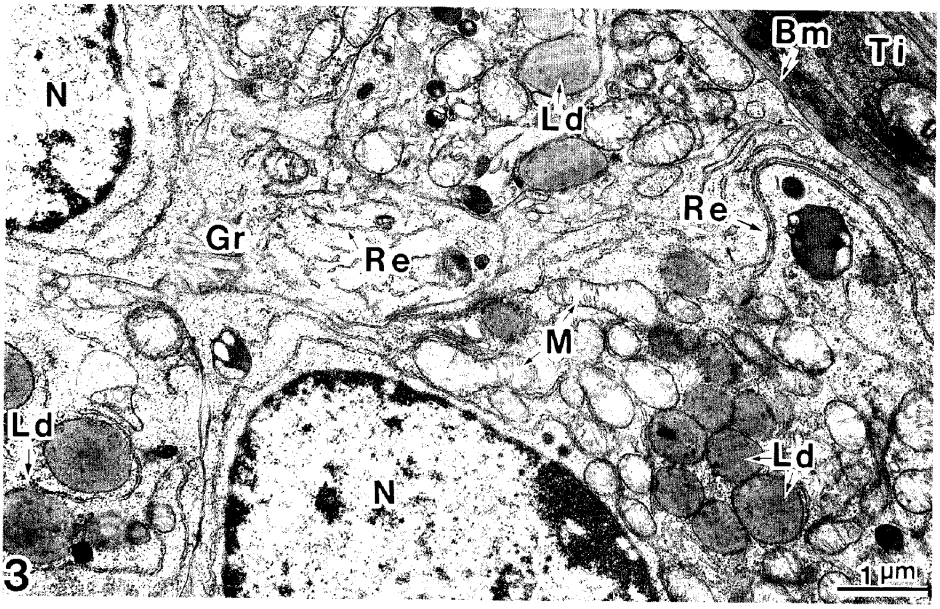
Fig. 1. Electron micrograph of an ovum in a Graafian follicle at the postcopulatory stage, showing its ectoplasm with abundant mitochondria (M) and scattered cortical granules (Cg) (Bat Cl). Mv, microvilli ; *Z*, zona pellucida. *Inset a :* light micrograph showing two Graafian follicles (Gf) with the ill-defined cumulus oophorus (Co) and small antra (An) in the left ovary (Bat Cl). E, egg. *Inset b :* light micrograph showing the egg (E) with many vacuoles (V) in the prophase of the meiosis I (resting stage) (Bat Cl). N, nucleus; *No,* nucleolus; *Z,* zona pellucida.

piece of a spermatozoon (Fig. 10, inset b). Abundant mitochondria were still concentrated in the ectoplasm but vacuoles had decreased in number and in size at this stage ; although the cortical reaction had occurred, some cortical granules still remained beneath the egg plasma membrane (Fig. 10). The other egg was in a more advanced stage of pronucleus formation, its pronuclei contained a few electron-dense, round nucleoli (Fig. 11, insets a and b), mitochondria were spread throughout the ooplasm and all cortical granules had disappeared (Fig. 11). Notwithstanding careful observations of serial sections, a second spermatozoon was not detected in either the zona pellucida or the perivitelline space of the above two ova, although many acrosome-intact spermatozoa ascended to the caudal isthmus with a few to the ampulla (Fig. 12).

In the UTJ where non-ciliated epithelial cells lost almost all the microvilli and consequently their luminal surface became smooth, clusters of stored spermatozoa had been detached from the epithelium, and some sperm were engulfed by the non-ciliated cells (Fig. 13 and inset).

At this stage, the glands on all sides of the uterine wall somewhat elongated and increased, together with a thickening of the endometrium (Fig. 5b ; cf. Fig 5a). Although the endometrial and glandular epithelial cells looked the same as those at the postcopulatory stage in general appearance and in glycogen content, some microvilli of the former cells commenced to lengthen (-1.4 μm) and became heavily covered by





Figs. 2 and 3. Electron micrographs showing the reverse gradient of the distribution of glycogen and lipid within the follicle cells (Bat Cl). Fig. 2. A corona radiata cell (Cc) containing a considerable amount of glycogen particles (G) and some lipid droplets (Ld). Cp, corona radiata process; M, mitochondrion; Z, zona pellucida. Fig. 3. The granulosa cells (Gr) characterized by abundant lipid droplets (Ld) and well-developed rough endoplasmic reticulum (Re). Bm, basement membrane; M, mitochondrion; N, nucleus; Ti, theca interna.

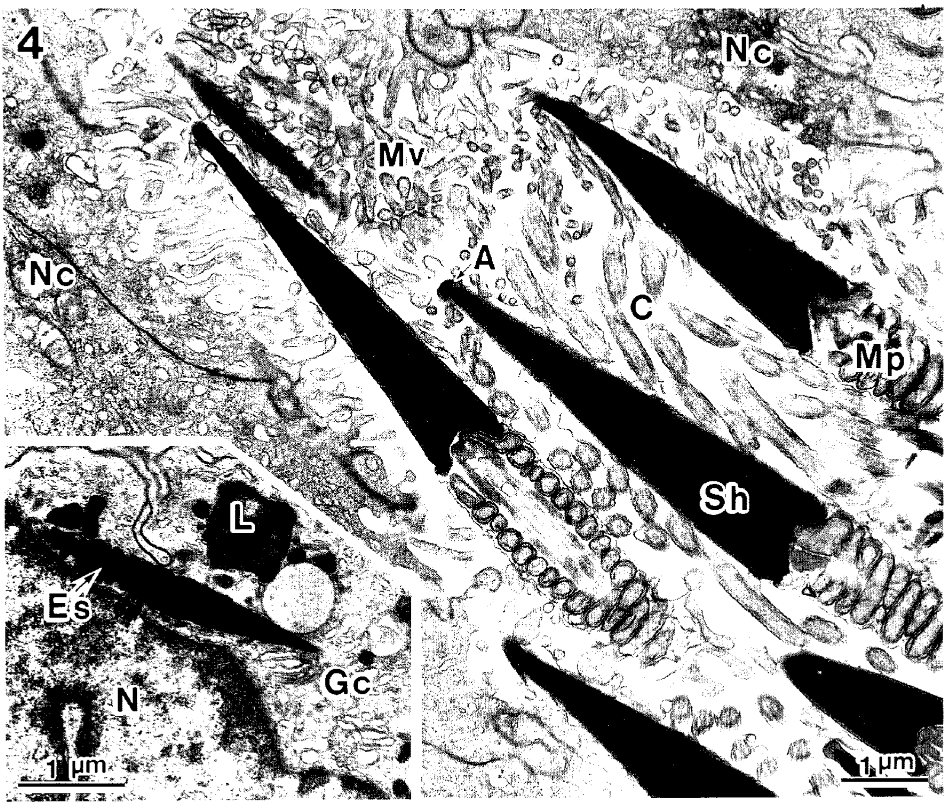


Fig. 4. Electron micrograph showing intact spermatozoa with parallel orientation resting between microvilli (Mv) of the non-ciliated epithelial cells (Nc) in the utero-tubal junction (Bat Cl). A, acrosome; C, cilia ; Mp, middle piece ; Sh, sperm head. *Inset :* an engulfed spermatozoon (Es) in a non-ciliated epithelial cell (Bat Cl). Gc, Golgi complex ; L, lysosome ; N, nucleus.

glycocalyx (Fig. 14 and inset ; cf. Fig. 6 and inset). A few degenerating spermatozoa still remained free in the uterine lumen where leucocytic infiltration occurred.

3. **Cleavage stage after arousal**

Although Bat H1 treated with PMSG and hCG had 2 young corpora lutea in the left ovary alone (Fig. 15), only an ovum in the 8-celled stage was found in a recess of the uterine lumen between the outer wall of the colliculus tubaricus and facing endometrial epithelium (Fig. 15, inset). A second spermatozoon was not found in either the zona pellucida or the perivitelline space of the ovum, despite careful examinations with the electron microscope. The other egg was lost because of preparation failures.

Uterine glands of the mesometrial wall increased conspicuously in number, length and thickness, and consequently the functional layer of the endometrial lamina propria became more and more edematous at this stage (Fig. 5c ; cf. 5a and b) : the columnar

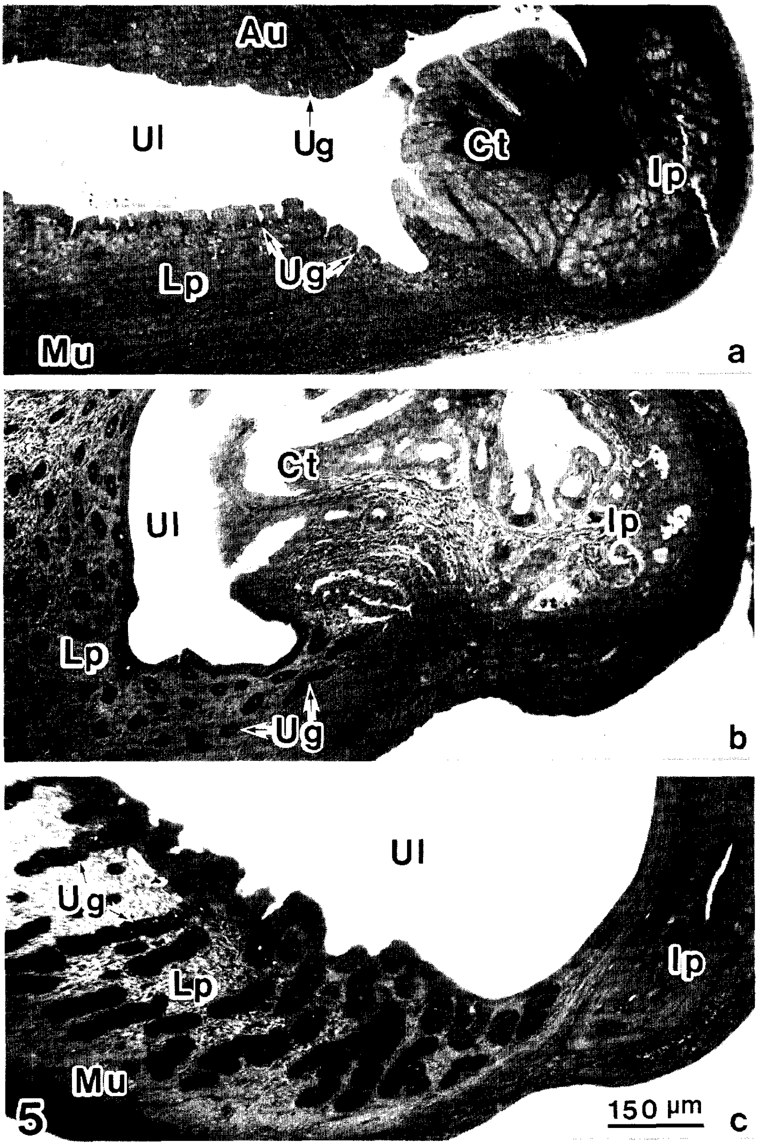


Fig. 5. Light micrographs showing changes in the glandular development and endometrial thickness of the uteri with the reproductive stage. a) The shallow and compact endometrium with undeveloped uterine glands (Ug) at the postcopulatory stage (Bat Cl). b) The slightly hypertrophied endometrium at the fertilized one-cell stage (Bat C2). c) The hypertrophied endometrium with well-developed uterine glands (Ug) at the cleavage stage. Note the edematous endometrial lamina propria (Lp) (Bat H1). Au, antimesometrial uterine wall ; Ct, colliculus tubaricus ; Ip, intramural part ; Mu, mesometrial uterine wall ; Ul, uterine lumen.

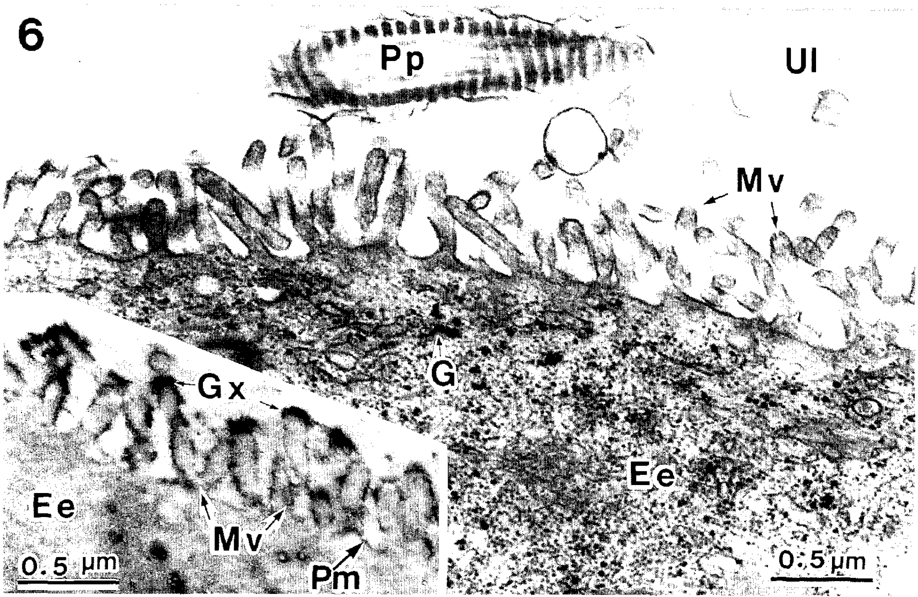


Fig. 6. Electron micrograph showing a free spermatozoon in the uterine lumen (Ul) and an endometrial epithelial cell (Ee) with short microvilli (Mv) and a few glycogen particles (G) (Bat Cl). Pp, principal piece. Inset : microvilli (Mv) of the endometrial epithelial cell (Ee), showing a little glycocalyx (Gx) covering them (Bat Cl). Pm, plasma membrane. PFP staining.

gland cells abruptly became high ($\sim$21 μm) and were characterized by accumulation of massive glycogen particles in the great part of the cytoplasm (Fig. 16 ; cf. Fig. 7). All microvilli of the endometrial epithelial cells lengthened (-2.1 μm) and were covered by a large amount of glycocalyx (Fig. 17 and inset ; cf. Fig. 14 and inset). Spermatozoa had been completely eliminated from the whole reproductive tract.

DISCUSSION

This is the first report, except for a brief description of sperm storage (Uchida and Mōri, 1987), on the reproduction of the greater tube-nosed bat (*Murina leucogaster*). Since the November sample (Bat Cl) with the Graafian follicles in the ovary had abundant spermatozoa stored in the uterotubal junction (UTJ) and the April samples (Bats C2 and H1) each freshly had ovulated, it may safely be said that the bat belongs to the 'prolonged sperm storage' type which is attended with prolonged survival of the Graafian follicle. Our discussion takes the focus on the following three topics.

1. Adaptations of the Graafian follicle for its prolonged survival in bats

In most mammals, the Graafian follicle ruptures soon after its formation, thus being unable to live for a long time. In such a Graafian follicle, the cumulus oophorus is small, consists of non-hypertrophied cumulus cells with little energy-rich substances in their cytoplasm, protrudes into a large antrum and is attached to the granulosa layer

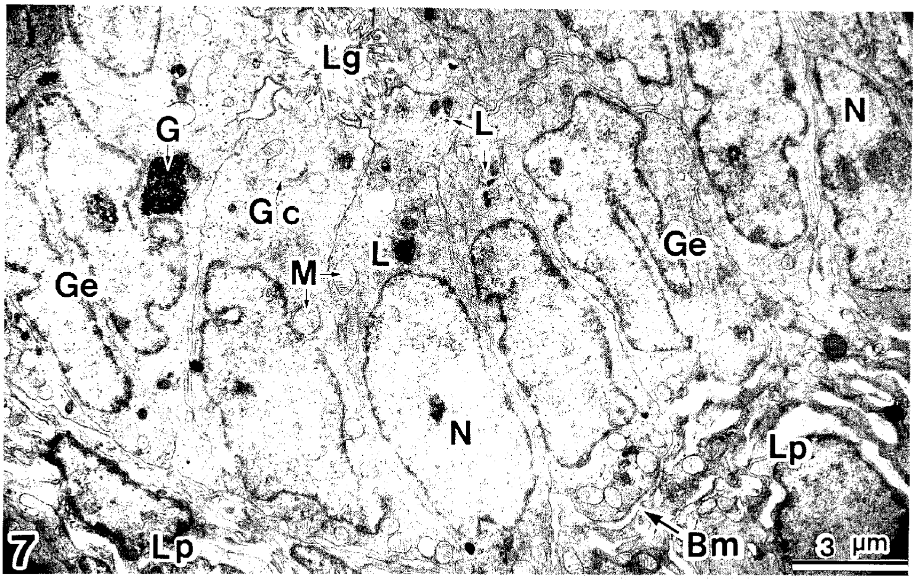


Fig. 7. Electron micrograph of the uterine gland, showing columnar but low glandular epithelial cells (Ge) with a basally placed nucleus (N) and an appreciable amount of glycogen particles (G), and the compact endometrial lamina propria (Lp) (Bat Cl). Bm, basement membrane; Gc, Golgi complex ; L, lysosome; Lg, lumen of uterine gland ; M, mitochondrion.

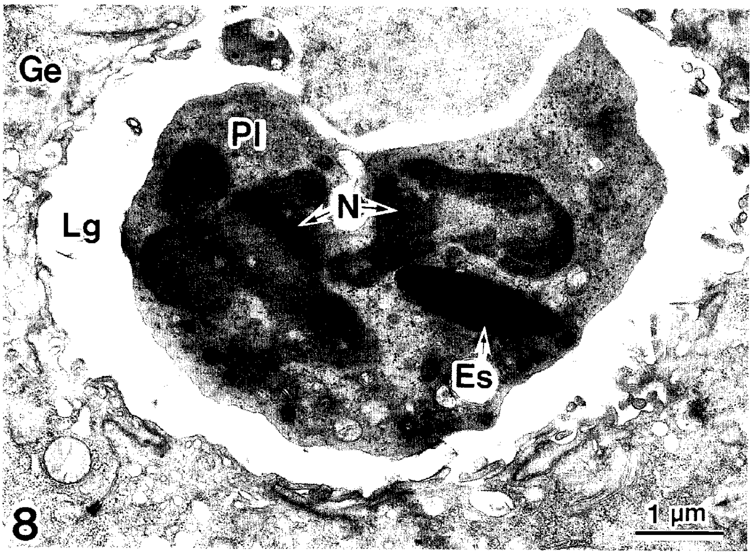


Fig. 8. Electron micrograph showing a polymorphonuclear leucocyte (PI) with an engulfed spermatozoon (Es) in the lumen of the uterine gland (Lg) (Bat Cl). Ge, glandular epithelial cell ; N, nucleus.

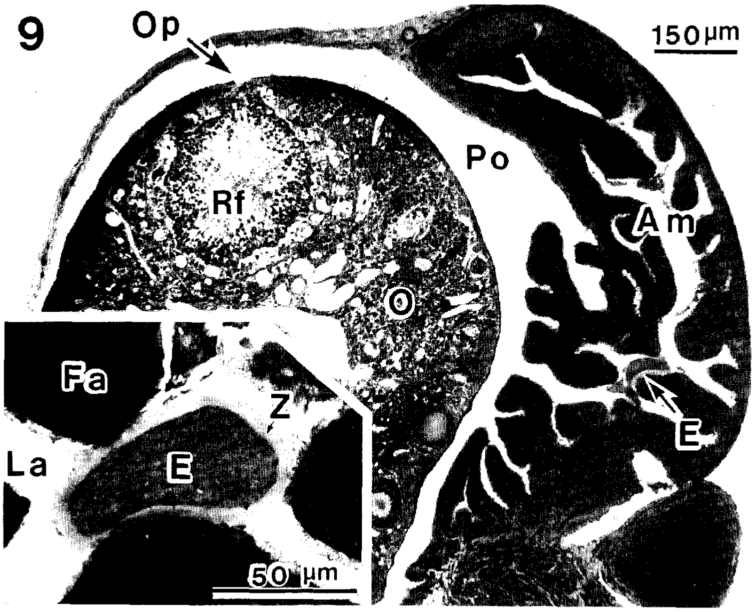


Fig. 9. Light micrograph showing the left ovary (0) containing a ruptured follicle (Rf) with an ovulation point (Op) and a fertilized one-celled egg (E) in the ampulla (Am) (Bat C2). Po, periovarian space. *Inset :* the egg (E) surrounded by the zona pellucida (Z) in the lumen of the ampulla (La) (Bat C2). Fa, fold of ampulla.

along one side ; and consequently the oocyte is markedly eccentric in position (the standard type, hereinafter referred to as the Type S), except for some lagomorphs with the 'spider' type Graafian follicle (Pincus and Enzmann, 1937 for the rabbit ; Mossman and Duke, 1973 for pikas) and some insectivores with the Graafian follicle which is devoid of the antrum or has only a slit-like antrum (Strauss, 1938 for tenrecs; Pearson, 1944 and Dryden, 1969 for shrews).

On the other hand , almost all the temperate-zone hibernating vespertilionid and rhinolophid bats examined so far copulate in autumn and ovulate in spring, but in the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus* copulation is followed closely by ovulation (Mōri and Uchida, 1982). It seems therefore that they have adapted their reproductive pattern for circumstances, together with their metabolic regulation, which may be of the 'prolonged sperm storage' (Wimsatt, 1944a ; Racey, 1979 ; Uchida and Mōri, 1987) or 'delayed implantation' type (Kimura and Uchida, 1983 for *M.* s. *fuliginosus*). In bats with the former reproductive pattern, the Graafian follicle has morphological and/or chemical peculialities in relation to its prolonged survival, even in some tropical non-hibernating bats, as follows.

1) Temperate-zone hibernating bats

In most hibernating vespertilionid bats (Vespertilionidae) belonging to the 'prolonged sperm storage' type, the Graafian follicle can survive during the long hibernation period as can spermatozoa in the female reproductive tract (Racey, 1979 ; Uchida

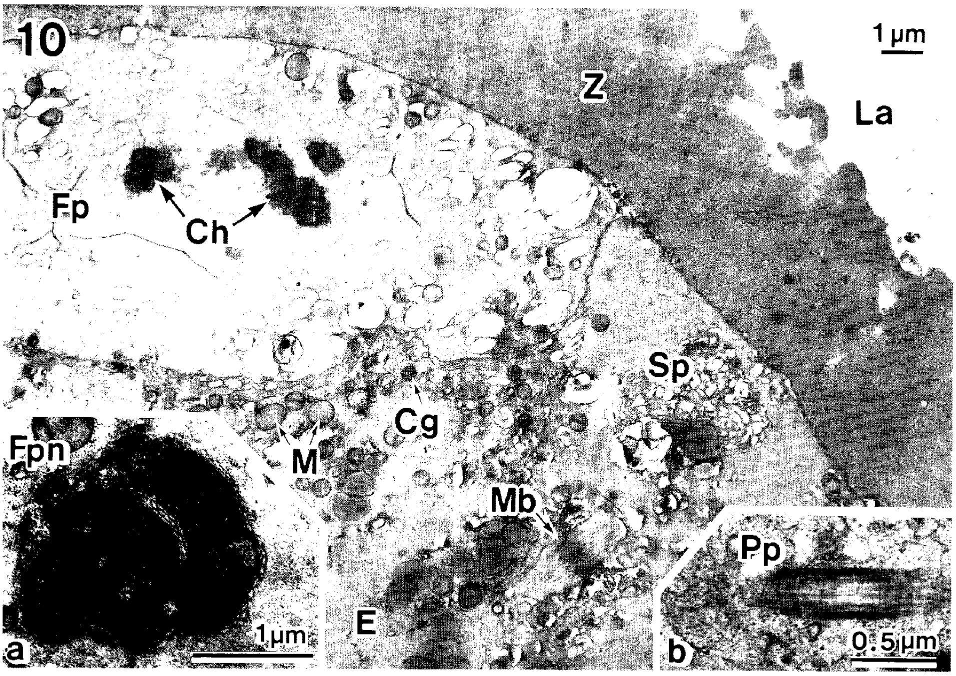


Fig. 10. Electron micrograph of the same egg (E) at the early stage of pronucleus formation as in Fig. 9 and inset, showing the first polar body (Fp) containing chromatin (Ch) and the second polar body (Sp) accompanied by the mid body (Mb) (Bat C2). Cg, cortical granule ; La, lumen of ampulla ; M, mitochondrion ; Z, zona pellucida. *Inset a :* the female pronucleus (Epn) containing lamellar structures (Bat C2). *Inset b :* the sperm prinicipal piece (Pp) (Bat C2).

and Mōri, 1987). In vespertilionine bats (Vespertilioninae), the Graafian follicle differs from that of most other mammals in the relative smallness of the antrum, the large size and central position of the cumulus oophorus, and consequently the almost concentric position of the ovum in the follicle (the modified type, referred to as the Type M) ; the conspicuous enlargement of the cumulus oophorus is extreme (Wimsatt, 1944b for *Myotis lucifugus* ; Sluiter and Bels, 1951 for *Myotis myotis* and *M. emarginatus* ; Pearson *et al.*, 1952 for *Plecotus townsendii* [*Corynorhinus rafinesquei*] ; Uchida, 1953 for *Pipistrellus abramus*), in comparison with that of most mammals, being due to accumulation of enormous quantities of glycogen particles within the fully hypertrophied cumulus cells (Wimsatt, 1949a, Wimsatt and Kallen, 1957, Wimsatt and Parks, 1966 for *M. lucifugus* ; Uchida and Mōri, 1977 for *P. abramus* ; Oxberry, 1979 for *Antrozous pallidus* ; Son *et al.*, 1987 for *Myotis formosus tsuensis* ; Son *et al.*, 1988 for *Pipistrellus savii velox*. In this respect, our observations revealed that the Graafian follicle of the greater tube-nosed bat (Murininae) resembled the appearance of the Type M, and the moderately hypertrophied cumulus cells contained a considerable amount of not only glycogen but also lipid droplets.

On the other hand, although hibernating rhinolophid bats (Rhinolophidae) display

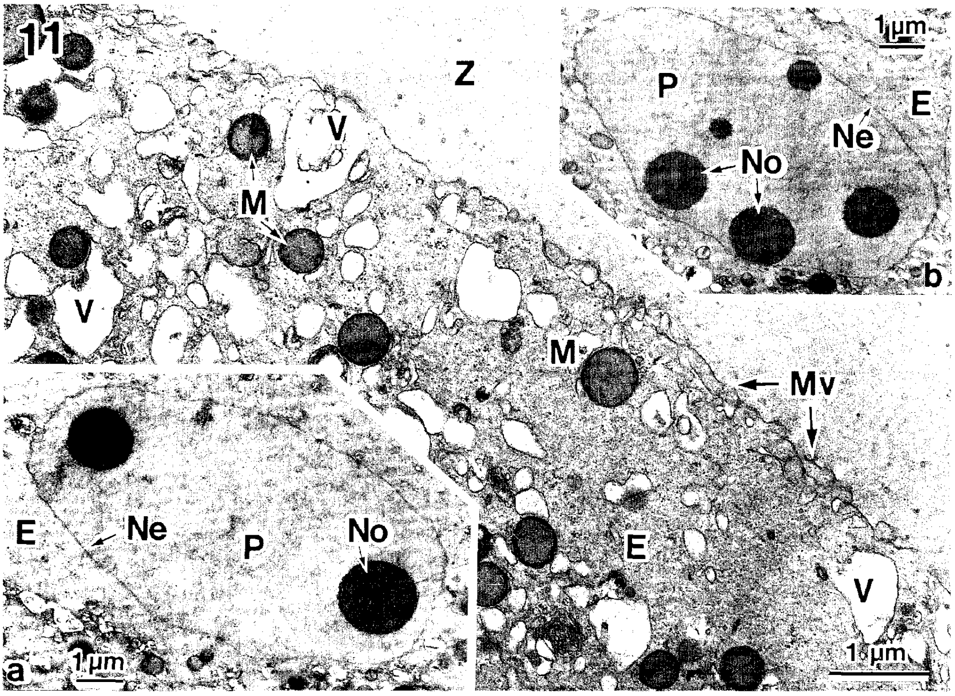


Fig. 11. Electron micrograph of another egg (E) at the more advanced stage of pronucleus formation containing many mitochondria (M) spreading throughout the ooplasm (Bat C2). Mv, microvilli ; V, vacuole. *Insets* a and *b* : the two pronuclei (P) with a few round nucleoli (No) (Bat C2). E, egg ; Ne, nuclear envelope.

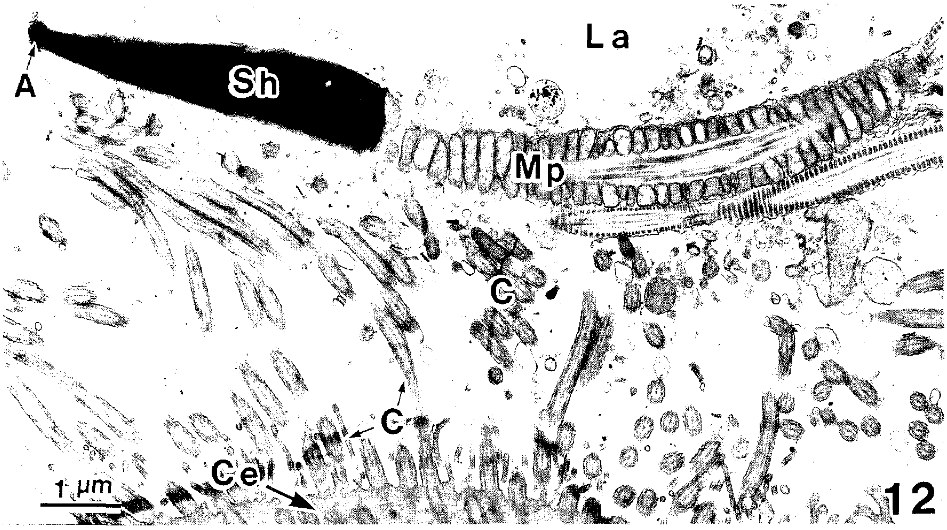


Fig. 12. Electron micrograph of a free acrosome-intact spermatozoon in the lumen of the ampulla (La) (Bat C2). C, cilia ; Ce, ciliated epithelial cell ; Mp, middle piece ; Sh, sperm head.

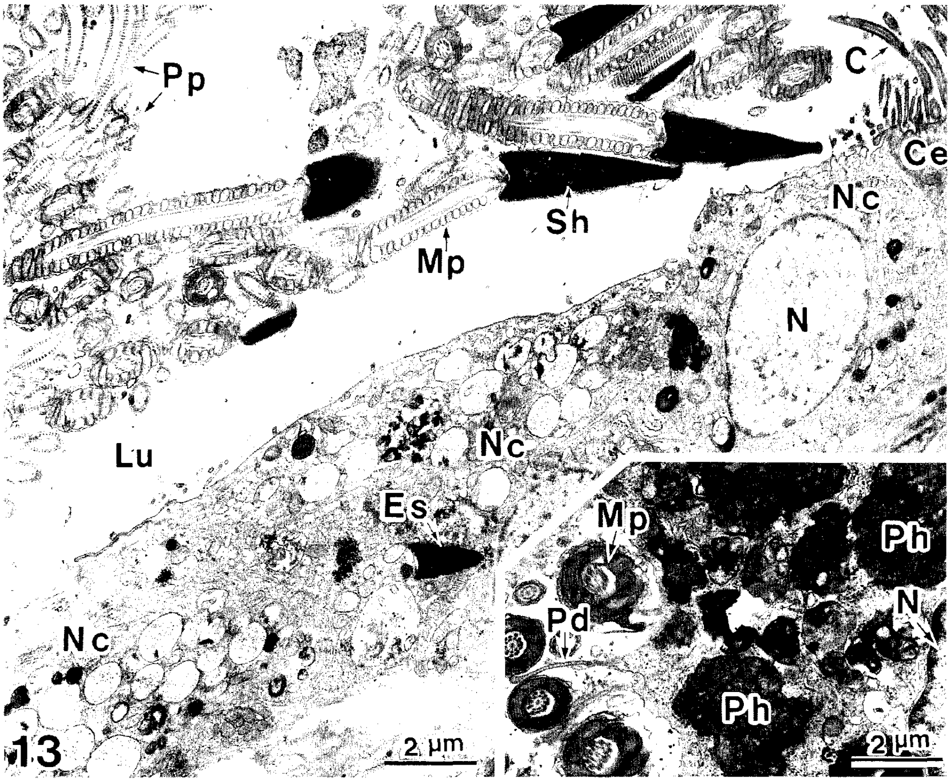


Fig. *13.* Electron micrograph showing sperm clusters detached from the smooth surface, almost completely lacking in microvilli, of the non-ciliated epithelial cells (Nc) in the uterotubal junction (Bat C2). C, cilia; Ce, ciliated epithelial cell; Es, engulfed spermatozoon ; Lu, lumen of UTJ ; Mp, middle piece; N, nucleus; Pp, principal piece ; Sh, sperm head. *Inset :* engulfed spermatozoa in a non-ciliated epithelial cell (Bat C2). Mp, middle piece ; N, nucleus ; Pd, pseudopodium ; Ph, phagosome.

prolonged suvival of the Graafian follicle accompanied by sperm storage, the Graafian follicle differs from that of both vespertilionine and murinine bats in that it exhibits the features of the Type S (Matthews, 1937 for ***Rhinolophus fewmequinum insulanus*** and ***R. hipposideros minutus*** ; ***Oh et al.,*** 1985 for ***R.*** *f. nippon*), and in that a large amount of lipid droplets is stored up in both the ovum and the non-hypertrophied cumulus cells in the last bat.

As mentioned above, in *M.* s. *fuliginosus* (Miniopterinae, Vespertilionidae), in spite of being a hibernator, copulation and fertilization occur in quick succession in autumn, and consequently the Graafian follicle does not show unusual longevity (Mōri and Uchida, 1981a, b). Such phenomena are compatible with the fact that this hibernating bat has the same Type S Graafian follicle as in most other mammals.

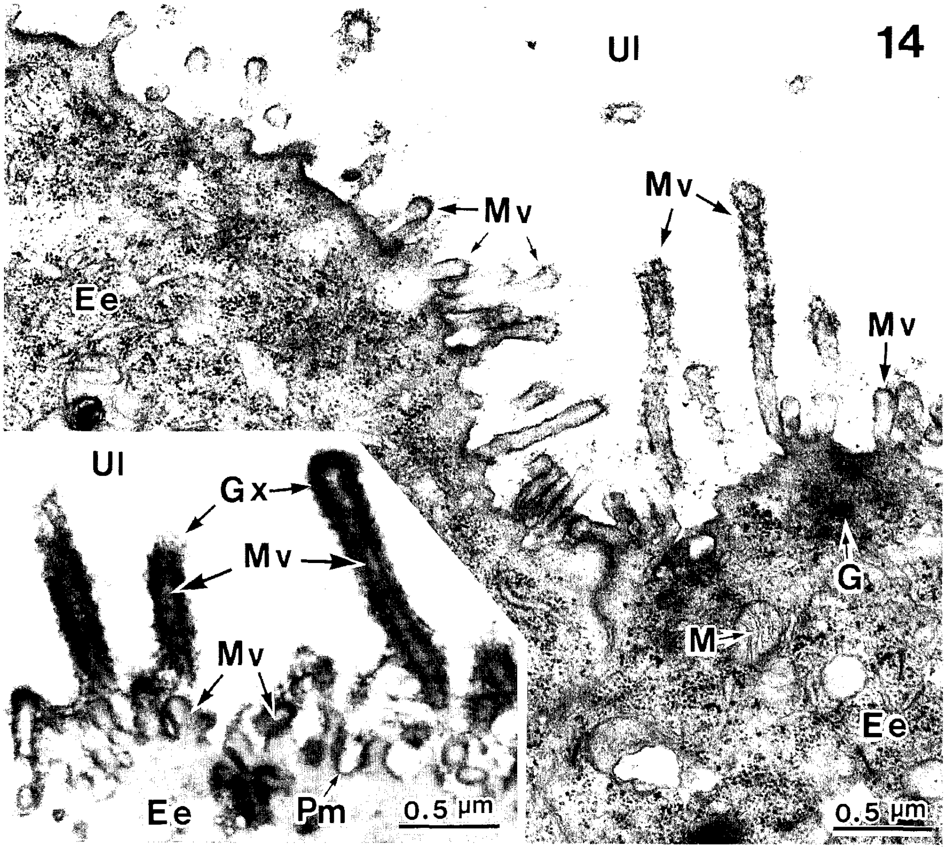


Fig. 14. Electron micrograph showing endometrial epithelial cells (Ee) with long microvilli (Mv) compared with those in Bat C1 (Fig. 6) and a few glycogen particles (G) (Bat C2). M, mitochondrion; Ul, uterine lumen. *Inset :* microvilli (Mv) of the endometrial epithelial cell (Ee), showing heavy glycocalyx (Gx) covering them (cf. Fig. 6, inset) (Bat C2). Pm, plasma membrane; Ul, uterine lumen. PFP staining.

2) Tropical non-hibernating bats

In tropical non-hibernating bats, the Graafian follicle displays two types according to whether or not the bat stores spermatozoa in the female genital tract. The Graafian follicle of the following bats which do not store or seem not to store spermatozoa, in which copulation is quickly followed by fertilization, is recognized as the Type **S** as in most mammals : *Desmodus rotundus murinus* (Wimsatt and Trapido, 1952) ; *Pteropus giganteus*, *Cynopterus sphinx gangeticus*, *Rhinopoma kinneari*, *Megaderma lyra*, *Rhinolophus rouxi*, *Hipposideros bicolor pallidus*, *H. speoris*, *Taphozous longimanus* and *Scotophilus wroughtoni* (Gopalakrishna and Moghe, 1960 ; Gopalakrishna *et al.*, 1974) ; *Macrotus californicus* with embryonic diapause after implantation, belonging to so-called 'delayed development' type (Bradshaw, 1962 ; Bleier, 1975) ; *Glossphaga soricina* (Rasweiler, 1972) ; *Noctilio albiventris* (Rasweiler, 1977) ; *Peropteryx kappleri*

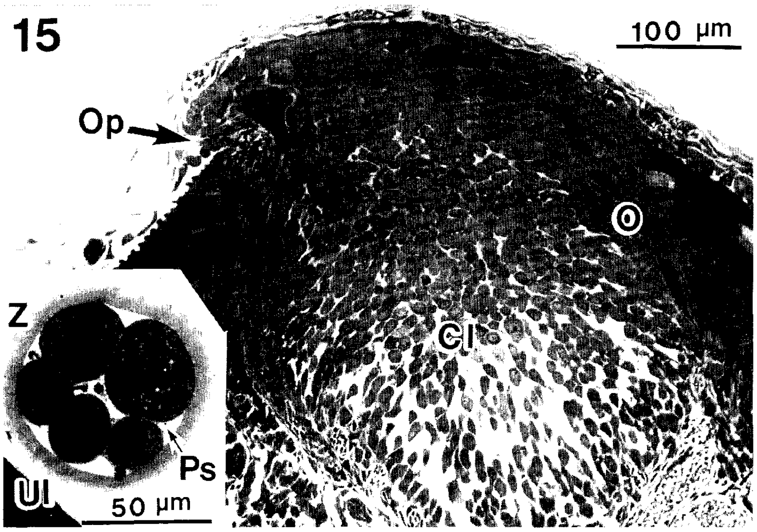


Fig. 15. Light micrograph showing a part of the left ovary (0) containing 2 young corpora lutea (Cl) with an ovulation point (Op) (Bat H1). *Inset* : an ovum surrounded by the zona pellucida (Z) at the g-celled stage in the uterine lumen (Ul) (Bat H1). Ps, perivitelline space.

(Rasweiler, 1982) in which copulation appears to take place just before ovulation. In this context, it is known that little glycogen is stored by the follicle cells in the above both C. s. *gangeticus* and *R. rouxi* (Modak and Kamat, 1968), the latter of which belongs to the 'delayed implantation' type (Ramakrishna and Rao, 1977 ; Gopalakrishna and Rao, 1977), and such lack of glycogen is consistent in most mammals.

On the other hand, the following eight tropical non-hibernating bat species are recognized as the 'prolonged sperm storage' type : *Pipistrellus ceylonicus chrysothrix* (Gopalakrishna and Madhavan, 1971) ; *Tylonycteris pachypus* and ***T. robustula*** (Medway, 1972 ; Racey ***et al., 1975)*** ; *Lasiurus* ***ega, Eptesicus furinalis*** and *Myotis albescens* (Myers, 1977) ; *Scotophilus heathi* (Krishna and Dominic, 1978 ; Gopalakrishna and Madhavan, 1978 ; Madhavan, 1981) ; *Nycticeius schlieffenii* (van der Merwe and Rautenbach, 1987). Of these, in the five species except for ***E.*** *furinalis,* ***M.*** *albescens* and *S. heathi,* the Type M Graafian follicle has been confirmed by Gopalakrishna ***et al.*** (1974) for *P.* c. *chrysothrix,* by Medway (1972) for ***T.*** *pachypus* and ***T.*** *robustula,* and by the authors concerned for the remaining two species. In addition, both *Pipistrellus mimus* and ***P.*** *dormeri* also have the Type M Graafian follicle (Gopalakrishna ***et al.,*** 1974), although it remains unclear if both continual asynchronous breeding species store spermatozoa in the female genital tract (Gopalakrishna ***et al.,*** 1975 ; Madhavan, 1978). All the above bats possess the Type M Graafian follicle which exhibits a striking similarity in appearance to that of temperate-zone hibernating bats (except *Miniopterus* and *Rhinolophus*). However, since neither histochemical nor ultrastructural study has been carried out in these bats concerned, the cause leading to the hypertrophy of the cumulus cells and to the enlargement of the cumulus oophorus is unfortunately not yet explained, although it is assumed that the cause is attributed to accumulation of

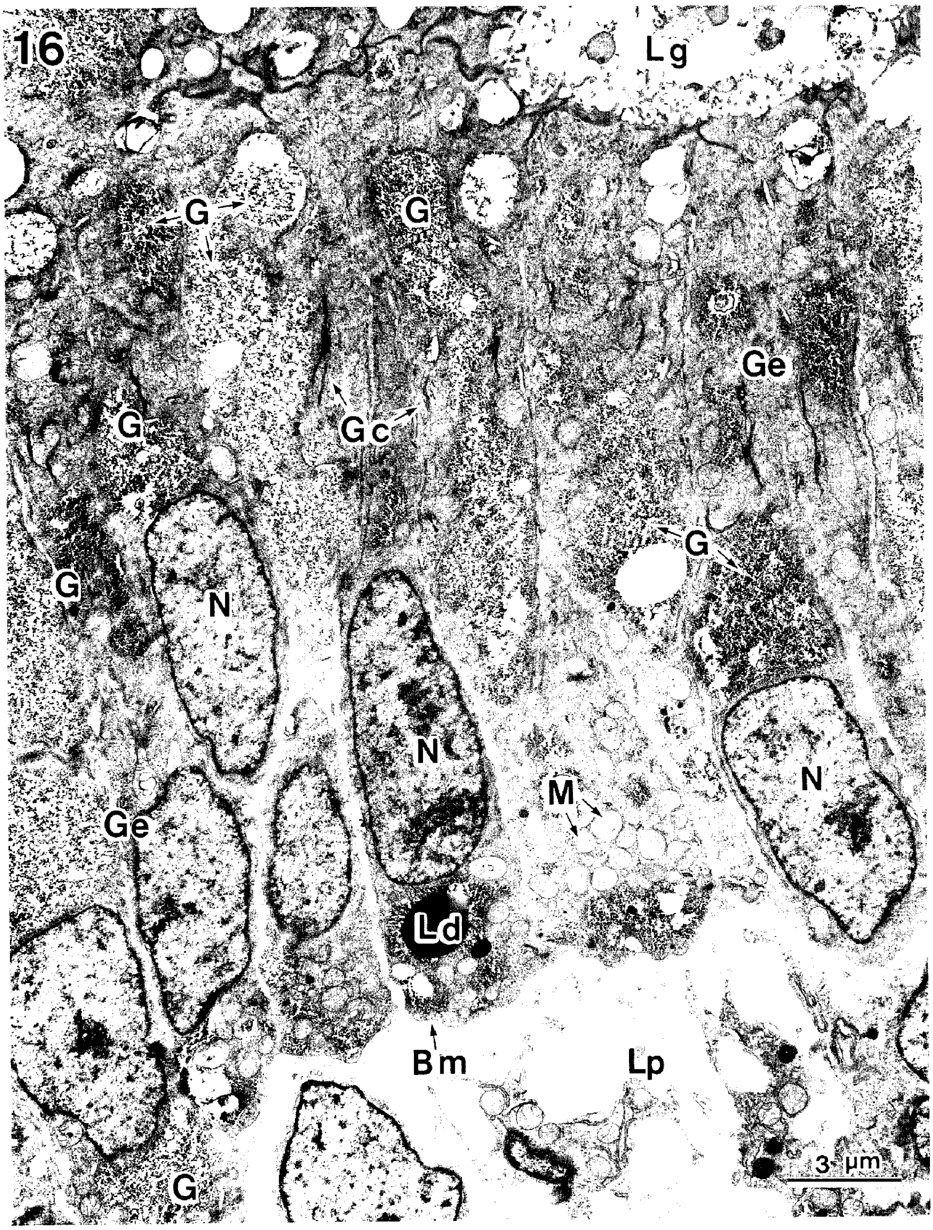


Fig. 16. Electron micrograph of the uterine gland consisting of very high columnar glandular epithelial cells (Ge), showing mass-produced glycogen particles (G) and the edematous endometrial lamina propria (Lp) (Bat H1) (cf. Fig. 7). Bm, basement membrance; Gc, Golgi complex ; Ld, lipid droplet ; Lg, lumen of uterine gland ; M, mitochondrion ; N, nucleus.

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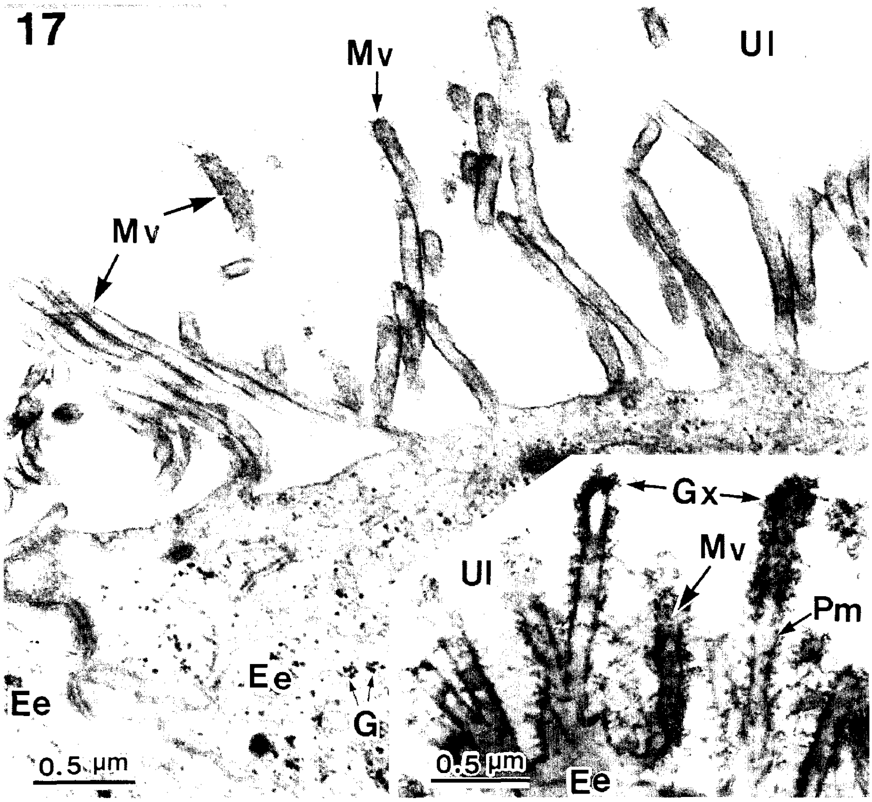

Fig. 17. Electron micrograph showing endometrial epithelial cells (Ee) with longer microvilli (Mv) than those in Bat C2 (Fig. 14) and a few glycogen particles (G) (Bat H1). Ul, uterine lumen. **Inset** : microvilli (Mv) of the endometrial epithelial cell (Ee), showing a large quantity of glycocalyx (Gx) covering them as in Bat C2 (Fig. 14, inset). Pm, plasma membrane ; Ul, uterine lumen. PFP staining.

some energy-rich chemical substances as in temperate-zone hibernating bats.

None of the Graafian follicles in most other mammals seem to exhibit the conspicuously glycogen- and/or lipids-laden cumulus cells so characteristic of surviving follicles in hibernating bats. Looking over the above, it may be concluded that such chemical peculiarities of the cumulus cells, which serve as the presumed source of energy-rich substances, are regarded as a physiological adaptation for the prolonged survival of the Graafian follicle in bats belonging to the ‘prolonged sperm storage’ type, regardless of hibernation.

2. **Polyspermy block in some mammals**

Mammalian ova seem to have two different devices to prevent polyspermy, i. e. the zona reaction and vitelline block. The mechanism has been made clear mainly in laboratory and domestic animals. The zona block is highly developed in sheep and dogs (Braden et al., 1954), golden hamsters (Austin and Braden, 1956; Barros and

Yanagimachi, 1971; Yanagimachi, 1977), cows (Brackett *et al.*, *1980)* and horses (Enders *et al.*, 1987), but in the last a spermatozoon is detected in the perivitelline space of only 1/26 recovered eggs (Betteridge et *al.*, *1982).* In the pig, spermatozoa are found in the perivitelline space of 24/208 eggs examined and polyspermy occurs in 6/143 fertilized ova (Hunter and Dziuk, 1968), indicating partial failure of both devices. The vitelline block is prominent only in the rabbit (Austin and Braden, 1956). Both mechanisms play a role in keeping out excess spermatozoa in the mouse and rat (Austin and Braden, 1956).

For wild mammals, so far as we know, there have been only a few such studies on bats alone. In the greater tube-nosed bat, no spermatozoon was detected in the perivitelline space of the two pronuclear-stage ova and one eight-celled stage egg : therefore, the block to polyspermy in this bat seems to reside in the zona pellucida, as in *Rhinolophus f. nippon* (Oh *et al.*, 1985), *Pipistrellus s. velox* (Son *et al.*, *1988)* and *P. abramus* (unpublished data). In *Peropteryx kappleri*, however, spermatozoa are noted in the perivitelline space of 4/28 tubal ova, indicating a partial zona block (Rasweiler, 1982). On the other hand, the vitelline block is confirmed only in *Miniopterus s. fuliginosus* (Mōri and Uchida, 1981a).

In *Murina leucogaster*, a few cortical granules still remained in close contact with the egg plasma membrane at the early pronuclear stage even after sperm penetration, as in *R. f. nippon* (Oh et *al.*, 1985) and the horse (Enders *et al.*, 1987) ; and then they had completely disappeared when the nucleolus became visible within the male and female pronuclei, as in *R. f. nippon* (Oh *et al.*, *1985)* and *P. s. velox* (Son *et al.*, 1988), suggesting that the complete disappearance of the cortical granules does not always synchronize with the cortical reaction.

3. Uterine environments at the preimplantation stage in some mammals

In the rat and/or mouse, the uterine columnar epithelial cells play an important role in interaction with the ovum during the initial stage of pregnancy (Tachi *et al.*, 1970), and their surface undergoes structural changes by ovarian hormones (Nilsson, 1966a, b ; Psychoyos and Mandon, 1971; Tachi *et al.*, *1974).* Before ovulation, the luminal surface of the endometrial epithelial cells contain large numbers of long and straight microvilli with glycocalyx under the influence of estrogen (Finn, 1977). During the pre-attachment stage, the uterine epithelium is affected first by progesterone, then at the attachment stage by progesterone and estrogen (Nilsson, 1966a). Microvilli of the epithelial cells are regular in appearance during the former stage, but become irregular during the latter stage (Nilsson, 1966a, b). Another important factor in the interaction between the ovum and endometrial cells may be glycocalyx on microvilli of both the cells. In the ferret, glycocalyx is extremely thick but is removed as contact between both the plasma membranes becomes established (Enders and Schlafke, 1972). In the mouse, the endometrial epithelium acquires RCA- I binding sites in thick glycocalyx during early pregnancy, whose thickness is greatly reduced at the time of implantation (Chavez and Anderson, 1985). In this connection, in the great tube-nosed bat, both elongation of microvilli and thickening of glycocalyx in the endometrial epithelial cells during preimplantation were thought to be part of the attachment reaction due to the influence of progesterone and/or estrogen, as in the above laboratory animals. For bats, however, much information on the nature of the endometrial cells is needed, because there have been few studies on the implantation reaction in bats.

The fluctuating glycogen-content of the endometrium also is under control of ovarian hormones. During delayed and early implantation, the epithelial cells of weasels (*Mustela frenata*) and badgers (*Taxidea taxus*) exhibit a transient engorgement with glycogen, but the glandular epithelial cells do not contain extensive glycogen stores (Schlafke et al., 1981). In humans, glycogen is found in the greatest amount in the endometrial epithelial cells after ovulation, but is absent in the glandular epithelium (Gordon, 1975).

In some bat-groups (pteropodids, emballonurids, noctilionids and phyllostomatids) the embryos develop to the blastocyst stage while still in the oviduct ; in *Pteropteryx happleri* (Rasweiler, 1982) and *Noctilio albiventris* (Rasweiler, 1977), both of which have such a pattern, glycogen deposits are more prominent in the oviducal epithelial cells than in the uterine epithelium. On the other hand, in other groups (megadermatids, rhinolophids and vespertilionids) the ova enter the uterus at the cleavage or morular stage (Rasweiler, 1979) ; in *Myotis lucifugus* with such a pattern, glycogen is abundant in the glandular epithelium during the preimplantation stage, but almost completely absent in the endometrium (Wimsatt, 1949b). With respect to this, it was interesting to note that glycogen loading was found in the glandular epithelium in *Murina leucogaster*, but whether or not the accumulation of glycogen was affected by PMSG and hCG stimuli is uncertain. Although the mechanisms of glycogen synthesis in the epithelium of the oviduct or uterus in bats remain open to speculation, such glycogen deposition has been regarded as evidence that this carbohydrate plays a source of energy for embryos during preimplantation.

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