Changes in the morphology and behaviour of spermatozoa between copulation and fertilization in the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*

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Summary. Live spermatozoa were first stored in the colliculus tubaricus of the uterotubal junction (UTJ) on both sides, Spermatozoa then ascended to the caudal isthmus just before ovulation. Spermatozoa with intact acrosomes in the caudal isthmus, which had their heads orientated towards the cephalic isthmus, were seen amongst groups of epithelial cilia in parallel to the epithelium; on the other hand, acrosome-less spermatozoa that were not attached to the epithelium were orientated irregularly in the lumen. A few acrosome-intact spermatozoa were found in the left ampulla at the time of ovulation of the single egg on the left side. Most of the spermatozoa failed to reach or survive in the ampulla of the right oviduct and leucocytic phagocytosis was restricted to this side.

Introduction

Investigations on sperm ascent through the oviduct in mammals, e.g. rat, mouse, hamster, guinea-pig and rabbit, have been extensive (see Overstreet & Katz, 1977), but there have been few such studies for wild mammals. The aim of the present study was to investigate the morphology and movement of spermatozoa in the oviduct of the Japanese long-fingered bat, *Miniopterus schreibersii fuliginosus*. This bat has a unique reproductive pattern; autumn copulation in mid-October is followed closely by ovulation and fertilization (Mōri & Uchida, 1981a, b), and the single ovum, discharged always from the left ovary, remains in diapause in the right uterine horn during winter (Uchida, 1957).

Materials and Methods

Adult females were collected at Ibarayama, an abandoned mine in the Fukuoka Prefecture, and Ohse-dō Cave in the Kumamoto Prefecture in mid-October during 1970–1977. The 15 mated animals were kept healthy in captivity for 0.5–4 days before use in this study.

Adult females were maintained in a cluster in a dark wooden box (25 × 25 × 15 cm) in which sufficient water and diet (boiled egg yolk, cheese, banana and vitamins) were placed, at a temperature of 15–16°C. The opportunity to cluster appeared to minimize any stress.

After the females were perfused with 3% glutaraldehyde in 0.2 M-phosphate buffer at pH 7.4 under ether anaesthesia, the oviducts were removed and further fixed for 3 h in cold 1.3%

osmium tetroxide buffered with the same buffer, dehydrated with acetone and embedded in Epon 812. Thick serial sections (1.5 μm) for light microscopy were cut with glass knives on a Porter–Blum MT-2B microtome, applied to a glass slide heated to 60°C, and stained with 0.5% toluidine blue; the number of sperm heads was counted in the left oviduct (ovulation side) and the right oviduct. Thin sections (~60 nm) were then taken from suitable thick sections mounted on the facet of another prepared Epon block by the method of Ōura & Usuku (1976), and doubly stained with uranyl and lead acetate before examination in an Hitachi HS-9 electron microscope (75 kV). In an additional 18 females only the left ampulla containing an egg was examined.

Results

The Japanese long-fingered bat has an asymmetric bicornuate uterus (Pl. 1, Fig. 1). The oviduct was divided into the UTJ (colliculus tubaricus and intramural part), isthmus (caudal and cephalic parts), ampulla and infundibulum (Text-fig. 1). The changes in number and morphological features of spermatozoa in the oviducts of both sides are described separately according to the reproductive stage of the female.

Text-fig. 1. Diagram of the ventral view of the left oviduct in the Japanese long-fingered bat.

Copulatory stage

Bats 1–9 had a Graafian follicle with a primary oocyte in the left ovary. Vast numbers of spermatozoa were stored in the left and the right colliculus tubaricus, but no spermatozoa were seen in the oviduct (Table 1). At the inner part of the slits formed by mucosal folds of the colliculus tubaricus, clumps of spermatozoa with parallel orientation rested between microvilli of the epithelial cells, being nearly perpendicular to the epithelium (Pl. 1, Fig. 2 and inset). A few of the spermatozoa near the entrances to the slits had a swelling acrosome cap (Pl. 1, Fig. 3) or had discarded the acrosome, but most of the spermatozoa had an intact acrosome.

Preovulatory stage

Bats 10 and 11 each had a Graafian follicle containing the secondary oocyte just before ovulation; vast numbers of spermatozoa were present in the colliculus tubaricus (both sides), and some had reached the caudal isthmus (Table 1). Although the situation and morphology of

spermatozoa in the intramural part of the UTJ were similar to those of the spermatozoa in the colliculus tubaricus, the spermatozoa in the upper region had their heads orientated towards the caudal isthmus.

Table 1. Occurrence of spermatozoa in the left oviduct of the Japanese long-fingered bat

Bat	Reproductive stage	No. of spermatozoa counted in:							
		Uterotubal junction		Isthmus		Ampulla	Cumulus cells	Zona pellucida	Perivitelline space
		Colliculus tubaricus	Intramural part	Caudal part	Cephalic part				
1-9	Copulatory	++++	—	—	—	—	—	—	—
10 11	Preovulatory	++++	+++	++	—	—	—	—	—
12 13	Postovulatory	++++	+++	++	1(1) —	4(2) 10(8)	2 —	2 —	— —
14 15	Fertilized	++++ +	++ +	— +	— —	4(1) 2	— 11	— 10	2 102

The symbols of —, +, ++, +++ and ++++ show no, 1-10, 11-50, 51-100 and >101 spermatozoa/section, respectively; values in parentheses indicate the numbers of spermatozoa with an intact acrosome.

Acrosome-intact spermatozoa in the caudal isthmus were seen amongst groups of long epithelial cilia (7.5 µm) which streamed towards the UTJ, obliquely (Pl. 2, Fig. 4) or in parallel (Pl. 2, Fig. 5) to the epithelium. In these spermatozoa the side of the outer dense fibre 8 was regularly orientated to the epithelium, i.e. the X-Y (dorsoventral) axis of the spermatozoon was perpendicular (Pl. 2, Fig. 6), whilst almost all the spermatozoa which were not attached to the epithelium were orientated irregularly in the lumen and had lost the acrosome cap (Pl. 3, Fig. 7).

Postovulatory stage

Bat 12 had an unfertilized egg in the middle portion of the left ampulla. There were vast and moderate numbers of spermatozoa in the UTJ and caudal isthmus respectively, a free spermatozoon with an intact acrosome was recognized in the cephalic isthmus, and 4 unattached spermatozoa (2 with an intact acrosome) were scattered between the ampullary-isthmic junction and the unfertilized egg. Two spermatozoa were counted in the cumulus cell layer and 2 in the zona pellucida (Table 1). In the right ampulla, there were a few spermatozoa without an acrosome and phagocytosis by polymorphonuclear leucocytes was confirmed (Pl. 3, Fig. 8).

Bat 13 also contained an unfertilized egg. Of the 10 unattached spermatozoa in the left ampulla, 8 had an intact acrosome (Table 1 and Pl. 3, Fig. 9). On the right side, a small number of spermatozoa was present in the UTJ and the caudal isthmus, and 5 intact spermatozoa were counted in the ampulla (Pl. 3, Fig. 10).

Fertilized stage

The left oviduct of Bat 14 contained an ovum showing sperm fusion in the middle portion of the ampulla, although vast numbers of spermatozoa were still present in the colliculus tubaricus. Of the 4 free spermatozoa in the ampulla, one had an intact acrosome; 2 spermatozoa were seen in the perivitelline space (Table 1). No spermatozoa were present in the isthmus and ampulla of the right oviduct although a few spermatozoa were found in the UTJ.

In the left oviduct of Bat 15 there was an ovum showing pronucleus formation in the middle portion of the left ampulla. Only a small number of spermatozoa was present in the UTJ and caudal isthmus, 2 free spermatozoa were seen in the ampulla, 11 in the cumulus cell layer, 10 in the zona pellucida and 102 in the perivitelline space (Table 1). Many spermatozoa were seen in the perivitelline space of 18 additional fertilized ova. There were no spermatozoa in the right oviduct other than a few in the UTJ and caudal isthmus.

Discussion

In Japanese long-fingered bats, most of the semen ejaculated into the female genital tract forms the vaginal plug (Mōri & Uchida, 1981b), and the cervix does not play any major barrier in limiting sperm ascent (unpublished observations). All the spermatozoa remaining in the uterus degenerate (Uchida & Mōri, 1974; Mōri & Uchida, 1980), while live spermatozoa are first stored in the colliculus tubaricus and then ascend to the caudal isthmus via the intramural part of the uterotubal junction which can be considered as a secondary storage site and temporarily prevent sperm ascent.

PLATES 1–3

Abbreviations: A, acrosome; C, cilia; Ec, epithelial cell; L, leucocyte; La, lumen of ampulla; Lci, lumen of caudal isthmus; M, microvilli; Mp, middle piece; O, ovary; Oam, outer acrosomal membrane; Ov, oviduct; P, principal piece; Pas, postacrosomal sheath; Sh, sperm head; Spm, sperm plasma membrane; U, uterus; V, vagina; W, wall of tubal ampulla; X–Y, dorsoventral axis of sperm; ➡, ovarian end of the oviduct.

PLATE 1

Fig. 1. Ventral view of an adult female reproductive organ of the Japanese long-fingered bat, showing the asymmetric bicornuate uterus.

Fig. 2. Electron micrograph showing clumps of spermatozoa, nearly perpendicular against the epithelium, with parallel orientation between microvilli of the epithelial cells at the inner part of the slit of the left colliculus tubaricus (Bat 11). *Inset:* many microvilli in contact with the acrosome of spermatozoa.

Fig. 3. A spermatozoon near the entrance of the slit of the left colliculus tubaricus has a swelling acrosome cap (Bat 12).

PLATE 2

Figs 4–5. Electron micrographs of the acrosome-intact spermatozoa ascending the lumen of the left caudal isthmus. The spermatozoa orientated towards the cephalic isthmus lie obliquely (Fig. 4) or in parallel (Fig. 5) to the epithelium (Bat 11).

Fig. 6. Transection of spermatozoa ascending the left caudal isthmus, showing their regular orientation with the X–Y (dorsoventral) axis perpendicular to the epithelium (Bat 11).

PLATE 3

Fig. 7. Electron micrograph of spermatozoa detached from the epithelium of the left caudal isthmus, showing irregular orientation of the spermatozoa and loss of the acrosome cap (Bat 11).

Fig. 8. Light micrograph showing a small number of spermatozoa without acrosomes and polymorphonuclear leucocytes engulfing them in the lumen of the right ampulla (Bat 12).

Fig. 9. Electron micrograph of a free spermatozoon in the left ampulla, showing the intact acrosome (Bat 13).

Fig. 10. Light micrograph showing a free spermatozoon with an intact acrosome in the right ampulla (Bat 13).

(Facing p. 26)

Although the UTJ was considered to be part of a sperm reservoir in the pig (Rigby, 1966), the spermatozoa appear to be concentrated in the isthmus (Hunter, 1975), as in the hamster (Yanagimachi & Chang, 1963), mouse (Zamboni, 1972) and guinea-pig (Yanagimachi & Mahi, 1976). The isthmus also acts as a sperm reservoir in sheep (Quinlivan & Robinson, 1969) and rabbits (Harper, 1973; Overstreet & Katz, 1977), although these have an efficient cervical barrier to sperm ascent.

In rats (Braden & Austin, 1954; Shalgi & Kraicer, 1978), hamsters (Yanagimachi & Chang, 1963), rabbits (Harper, 1973; Overstreet, Cooper & Katz, 1978; Overstreet & Cooper, 1979), mice (Stefanini, Ōura & Zamboni, 1969; Zamboni, 1972), cattle (Thibault, Gerard & Heyman, 1975) and guinea-pigs (Yanagimachi & Mahi, 1976), only a few spermatozoa have reached the ampulla by the time of ovulation and the present observations on Japanese long-fingered bats gave similar results.

Spermatozoa were stored in the colliculus tubaricus and intramural part of the UTJ, resting between microvilli of the epithelial cells with a nearly perpendicular orientation against the epithelium, as reported first for the pipistrelle bat, *Pipistrellus pipistrellus* (Racey & Potts, 1970). The spermatozoa ascending the caudal isthmus appeared to be aligned along the long cilia of the epithelium with their heads towards the cephalic isthmus. During ascent through the caudal isthmus the numbers of spermatozoa were much reduced, and very few intact spermatozoa reached the cephalic isthmus and ampulla. These spermatozoa were free in the oviduct lumen (see Mōri & Uchida, 1981a).

It has been suggested that, in the rabbit, entry of the products of ovulation into the oviduct stimulates an increased rate of migration of spermatozoa from the caudal isthmus to the site of fertilization (Harper, 1970, 1973). In this bat, however, a few spermatozoa with an intact acrosome were found at the time of ovulation in the left ampulla on the side of ovulation and in the right ampulla. Nevertheless, in Bat 15 which had a pronuclear egg, many spermatozoa were present in the perivitelline space in the left ampulla but none was observed in the right ampulla. The products of ovulation may therefore stimulate more spermatozoa to ascend into the left ampulla.

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