

Reproductive biology of bats and the musk shrew (Insectivora)

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1. Sperm storage in bats

Sperm storage in the epididymis at the copulatory stage (late October), followed by spermatogenesis in September up to the ovulatory stage (late April of the next year), is seen in *Pipistrellus abramus* as well as *Pipistrellus pipistrellus*, where live spermatozoa almost never make contact with stereocilia of the epithelial cells.

In a few vespertilionine bats, spermatozoa retain their fertilizing ability over a period of 6 months in the female reproductive tract. The principal site of sperm storage is limited to the caudal isthmus of the oviduct in *Rhinolophus ferrum-equinum nippon* and *Pipistrellus endoi*, to the uterotubal junction in *Myotis formosus tsuensis*, *Murina leucogaster*, *Pipistrellus savii velox*, and to both the uterus and the uterotubal junction in *P. abramus*, *Pipistrellus ceylonicus* and *Scotophilus heathi*.

A high percentage of *P. abramus* spermatozoa covered with fuzz filaments of the endometrial epithelial cells survive even after prolonged storage. Thus, adherence of the sperm to the endometrial epithelial cells may promote the unusual longevity of spermatozoa, especially since the spermatozoa remaining free in the lumen had degenerated. In the uterotubal junction of *P. abramus*, all viable spermatozoa appear to adhere to the epithelial cells by simple apposition, but dead spermatozoa were engulfed by these cells. Moreover, *in vitro*, cationized ferritin was carried into the phagocytotic vacuoles of the epithelial cells. Thus, in addition to its capacity for sperm storage, the uterotubal junction seems able to react to dispose of foreign materials.

2. The form and behaviour of gametes in the musk shrew, *Suncus murinus*

Because the Insectivora display a number of primitive characteristics, they are considered to represent existing links to the progenitors of eutherian mammals. The musk shrew was examined in regard to coitus-induced ovulation and the role of the vaginal copulation plug, and to post-coital transport and behaviour of gametes and embryos within the female tract. At ejaculation, < 1 million spermatozoa were incarcerated in the anterior vagina by a firmly-lodged sperm free copulation plug for 6-7 h, by which time a ~maximal population of several hundred became established as small group of free languidly moving spermatozoa in posterior storage crypts of the isthmus of the Fallopian tube. Post-ovulatory transport was characterised by an unusual parsimony of spermatozoa in the ampulla, the site of fertilization. Generally, only one or two had gained the ampulla by 4-5h, and often no more than ten had done so by 5-13 h after ovulation. Consequently, few eggs were penetrated during the first 5h with a delay often of 10-13h before most eggs were fertilized.

The cumulus oophorus, ovulated as a hyaluronidase-insensitive ball of closely apposed cells stabilized by gap junctions ca. 15.5 h after mating of hCG, remained for relatively long periods of ca. 14 h around fertilized, and for 24 h around unfertilized eggs. Soon after ovulation, however, the corona radiata retreated progressively from the zona pellucida, creating a large peri-zona space within the cumulus oophorus. Generally, a majority of the ampullary spermatozoa were located within the cumulus and specifically within that peri-zona space. Moreover, all of those moving within the cumulus and the few seen adhering to the zona of unfertilized eggs had shed the acrosome, whereas the acrosome was intact among the sparse population of free ampullary spermatozoa, and in those adhering to the zonae of naked eggs recovered after delayed mating. The cumulus in *Suncus* may therefore function both to sequester spermatozoa, and as an essential mediator of fertilization-probably in induction of the acrosome reaction.