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The Relationship between Spermatozoa and Epithelium of the Female Genital Tract during Sperm Storage in the Greater Yellow Bats (*Scotophilus heathi*): The Light and Electronmicroscopic Observations

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ABSTRACT

The present paper describes the relationship between spermatozoa and the epithelium of the female genital tract during sperm storage in greater yellow bats, *Scotophilus heathi*. All the female bats collected from mid-January till ovulation in early March showed the presence of spermatozoa with their heads orientated towards the epithelial lining of the female genital tract. During this period, the epithelial cells of the uterine horn showed extensive endoplasmic reticulum (ER), numerous mitochondria and well developed Golgi complexes. In females inseminated during December, the spermatozoa were not arranged linearly and showed signs of degeneration. During December, the uterine epithelial cells contained only a few small mitochondria and less developed rough ER. The leucocytes and Langerhans cells were frequently seen in the epithelial lining during the course of sperm storage especially at the site of phagocytised sperm heads.

Key Words: spermatozoa; sperm storage; bat; female genitale tract; EM; Langerhans cell; uterus.

I. Introduction

The coincidence of sperm storage and hibernation led earlier workers to believe that torpor was necessary for sperm to retain their fertilizing capacity over a prolonged period (Racey, 1975). Discovery of the sperm-storage phenomenon in tropical vespertilionids indicated that a special physiological mechanism was involved in sperm storage (Krishna and Dominic, 1978; Crichton *et al.*, 1981; Dominic and Krishna, 1989). Several temprate-zone species have been investigated in detail to ascertain the mechanism (Racey, 1975; Uchida and Mori, 1987); in contrast, only a few tropical species have been investigated. In *Scotophilus heathi*, a tropical vespertilionid bat, previous observations have shown that sperm are stored in the uterus and oviduct, where they are orientated with their heads towards the epithelium (Krishna and Dominic, 1978). Another study on the same species found "swarms" of spermatozoa in the female genital tract (Gopalakrishna and Madhavan, 1978). The present paper reports a further investigation of the relationship between spermatozoa and their storage organ in *S. heathi*.

II. Materials and Methods

A total of 30 adult greater yellow bats (*S. heathi*) were collected at Banaras Hindu University and in adjacent areas during a period which extended from the beginning of the breeding phase (September) to just before ovulation (early in March) during 1988-89 (Table 1). The animals were sacrificed soon after their arrival in the laboratory by perfusion through the dorsal aorta with 1% glutaraldehyde and 1% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4). One hundred *iu* heparin (Liquemin 100 R, Hoffmann La Roche, Grenzach-Whylem, Germany) per 100 gm body mass were injected *i.p.* under anaesthesia 15 min before the perfusion was begun.

Table 1. Details of the Bats Studied for the Occurrence of Spermatozoa in the Female Tract of *Scotophilus heathi*

Reproductive stage	Date captured	Number of bats	Sperm in female genital tract
Quiescence (ovary does not contain antral follicles)	30 September, 1988	5	-
Recrudescence (ovary contains antral follicles)	24 November, 1988	5	-
Winter dormancy (sperm seen in genital tract, ovary contains antral follicles)	18 December, 1988	6	+
Breeding (mating reported, sperm seen in genital tract, ovary contains antral follicles)	20 January, 1989	6	+++
Breeding (sperm in genital tract, ovary contains antral follicles)	10 February, 1989	4	++
Post-Ovulatory (ovary contains corpus luteum)	8 March, 1989	4	-

The thoracic and abdominal cavities were opened along the mid line of the body, and a blunt cannula fitted to a silicone tube was introduced into the left ventricle and slowly pushed into the ascending aorta. After fixing the cannula with one clamp and interrupting the large cervical blood vessels with other clamps, the remaining blood circulation was rinsed using 50 ml Ringer's solution and a perfusor. The speed of the perfusor was adjusted to 300 ml/hr. After rinsing, 50 ml fixative, 1% gluteraldehyde and 1% paraformaldehyde in phosphate buffer (pH 7.4) was injected. The reproductive tissues were promptly placed in cold fixative for 4 hrs. After being thoroughly rinsed with the buffer, the tissues were post-fixed in 2% osmium tetroxide in 0.1 *M* phosphate buffer and 3% sucrose. After dehydration in a graded series of ethanol and polypropylenoxide, the tissues were embedded in araldite resin. Thick sections (1.5 μm) were stained with 0.5% toluidine blue and examined using a light microscope. Ultrathin sections from selected areas were then cut on a LKB ultramicrotone, mounted on copper grids, stained with uranyl acetate and lead citrate, and examined in a Phillips EM 400.

II. Results

S. heathi exhibits a sharply defined annual breeding season. The first sign of recrudescence of the ovary was observed in females collected during late September and early October. In November, the ovary contained several large antral follicles and the testes showed active spermatogenesis. The females remained sexually quiescent during the peak winter months from late December to mid-January. The second phase of reproductive activity was seen during mid-January. Large numbers of spermatozoa were found in the epididymis from November until March. Ovulation occurred during the first week of March.

Bats collected until early December were not yet inseminated. Two out of 6 bats collected on December 18th appeared to have mated recently as their uteri contained large clumps of spermatozoa. In these females, the spermatozoa were not arranged linearly and showed signs of degeneration, characterized by fusion of the plasma and outer acrosomal membrans in the cap region. These bats generally seemed to mate again during January. All the females collected after mid-January showed the presence of spermatozoa, mainly in the caudal oviduct, utero-tubal junction and distal uterine horn, arranged linearly with their heads orientated towards the epithelial lining (Figs. 1 and 2). This situation persisted consistently until immediately after ovulation in March. During this period, the heads of the spermatozoa made oblique and vertical contact with microvilli of the epi-thelial cells (Fig. 3). Some spermatozoa were arranged perpendicular to the non-ciliated epithelial cells and had close contact with microvilli (Fig. 3). Spermatozoa which remained free in the lumen were seldom found. Females collected during late January showed the prersence of many spermatozoa in the uter-

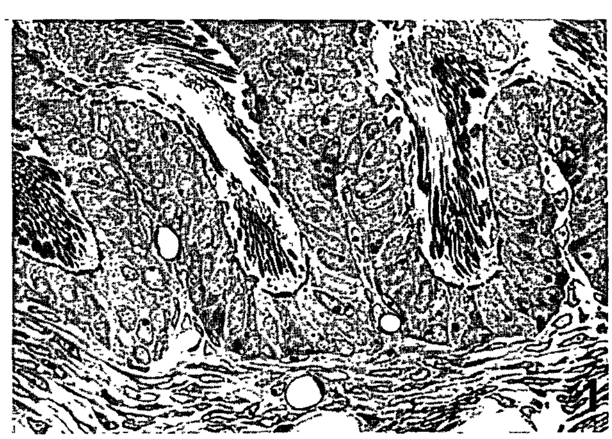

Fig. 1. Transverse section of the oviduct in January showing storage of spermatozoa.

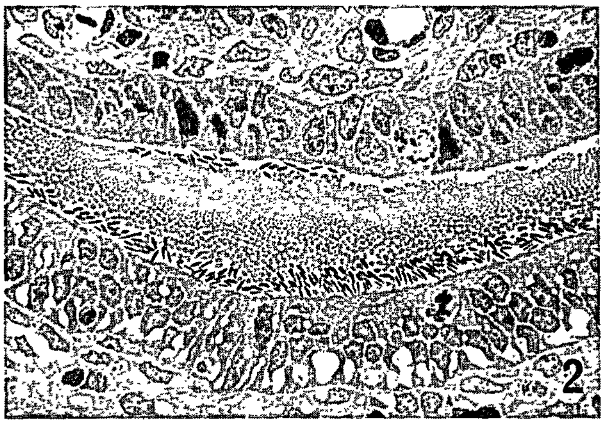

Fig. 2. Transverse section of the uterus in January showing the arrangement of the spermatozoa with their heads orientated towards the uterine epithelum.

Fig. 3. Electron micrograph of the uterus in February showing close contact of spermatozoa with microvilli of the ciliated epithelial cells (arrow). Spermatozoa are seen in close contact with non-ciliated epithelial cells (broken arrow).

ine glands.

No morphological modifications of the spermatozoa were found during the period of sperm storage. The epithelial lining at the sperm storage site consisted of both ciliated and non-ciliated cells. Ultrastructural features of the uterine epithelium before and during the period of sperm storage showed some differences. During January when linear arrange-

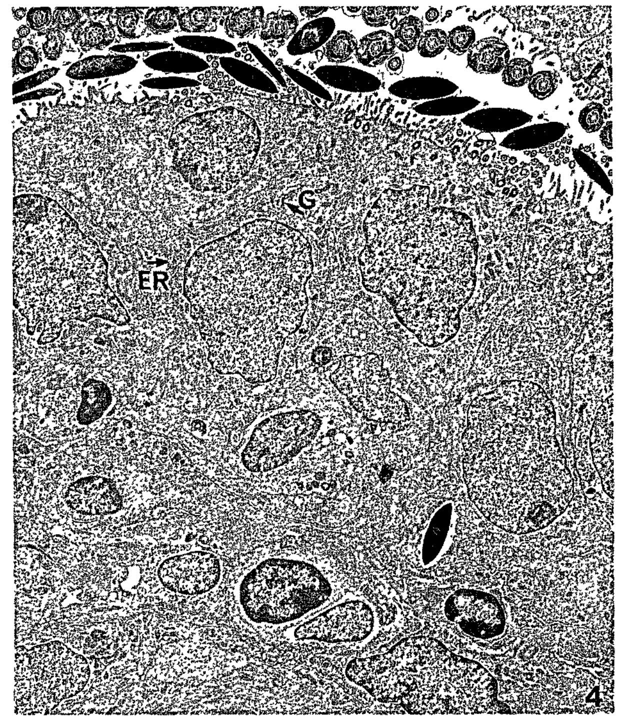

Fig. 4. Electron micrograph of the uterus in February showing linear arrangement and intimate contact of sperm with the epithelium. Note the presence of extensive rough endoplasmic reticulum (ER), numerous mitochondria (M) and large Golgi complexes (G). Further, note the presence of a phagocytised sperm head at the base of the epithelial cell (arrow).

ment and intimate contact of sperm with the epithelium were noted, the epithelial cells of the uterine horn showed extensive rough endoplasmic reticulum (ER), numerous mitochondria with well-developed cristae and large Golgi complexes (Fig. 4). During December when linear arrangement and intimate contact of sperm with the epithelium were not observed, the epithelial cells contained only a few small mitochondria and less developed rough ER (Fig. 5).

Even in bats which had not ovulated, some spermatozoa in the storage sites degenerated and were finally engulfed by the epithelial cells (Figs. 4 and 6). Pieces of sperm head surrounded by the cytoplasmic pseudopodia on the luminal surface were frequently observed. Phagocytised sperm heads were also seen within the epithelial cell cytoplasm. The heads of spermatozoa later reached the base of the epithelial cells. The region beneath the endometrial basal lamina was filled with numerous leucocytes. Several leucocytes were observed passing through the basal lamina into the epithelial lining. Although leucocytes were frequently seen in the epithelial lining during the course of sperm storage, they never infiltrated into the lumen of the genital tract. Another

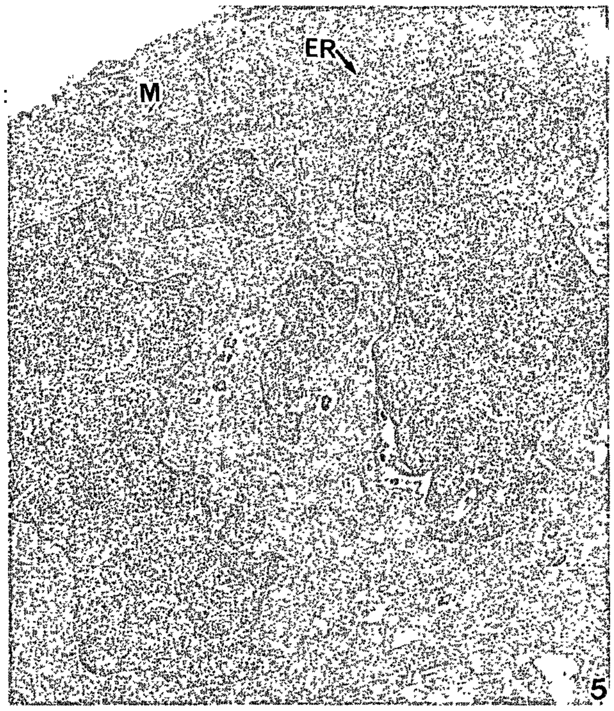

Fig. 5. Transverse section of the uterus in December. Note the epithelial cell containing only a few small mitochondria (M) and less developed rough endoplasmic reticulum (ER).

Fig. 7. Electron micrograph of the uterus in February showing stored sperm in close contact with the epithelial cells. Note the presence of dendrites of Langerhans cells (arrow) within epithelial cells. Morphologically, these dendrites are seen as radioopaque bodies containing very little rough ER (confirmed by ATPase staining).

Fig. 6. Electron micrograph of the uterus in February showing spermatozoa engulfed by the epithelial cell.

cell type prominently seen at the site of sperm storage during this period was dendrites of Langerhans cells (Fig. 7). Morphologically, these dendrites are seen as radioopaque bodies containing very little rough ER.

III. Discussion

Several studies have demonstrated the presence of a close relationship between spermatozoa and epithelial cells of the female genital tract (Uchida and Mori, 1987). Gopalakrishna and Madhavan (1978) have described "swarms" of spermatozoa in the female genital tract of *S. heathi*, which were not observed in most sperm storing bats. The present study confirmed our earlier observation that, in *S. heathi*, spermatozoa are arranged with their heads either orientated towards or in intimate contact with the epithelium of the genital tract during the period from early January to early March (Krishna and Dominic, 1978). Thus, intimate association between spermatozoa and female storage organs characterizes most sperm storing bats, including the present species, suggesting that such associations are an integral part of the mechanism of prolonged survival of spermatozoa (Uchida *et al.*, 1984;

Racey *et al.*, 1987; Uchida and Mori, 1987; Mori *et al.*, 1989). There have been several studies by different laboratories as well as by our laboratory to show the significance of close association between sperm and the female genital tract during prolonged storage (Racey, 1975; Krishna, 1984; Son *et al.*, 1988).

Although mating in *S. heathi* was observed since mid-December, the linear arrangement and intimate association of sperm with the female genital tract were not seen following insemination in December. Only clumps of degenerating sperm were observed in the female genital tract during this period. However, when mating occurred after early January, a close relationship between sperm and the uterine epithelium was established soon after insemination.

This may suggest that the uterine horns were not fully prepared for sperm storage during December. Ultrastructural features of the uterine horn (site of sperm storage) during January revealed an abundance of rough ER, free ribosomes and mitochondria and an active golgi apparatus as compared with those during December. These features suggest active secretary activity of the epithelial cells of the uterine horn during January, which might be required for linear arrangement and intimate association of sperm with uterine cells. The uptake of degenerating sperm heads by the epithelial cells of the genital tract of *S. heathi* by means of engulfment is not unique. Similar phenomena have been reported to occur in the oviduct and/or uteri of a variety of other mammals, including several bat species (Rasweiler, 1987). Interestingly, in *S. heathi*, a number of leucocytes and dendrites of Langerhans cells were observed within the epithelial cells especially at the site of phagocytised sperm heads. Leucocytes were never seen in the uterine lumen. Thus, it would be interesting to investigate the mechanism involved in controlling leucocyte migration into uterine epithelium cells and the roles which leucocytes and Langerhans cells play in the uterine function, especially during the period of sperm storage.

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大黃蝙蝠 (*Scotophilus heathi*) 雌性生殖道上皮細胞在精子儲存期與精子之交互關係：光學及電子顯微鏡的觀察

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摘　要

本篇報告描述大黃蝙蝠(*Scotophilus heathi*)之精子在雌性生殖道中儲存時期與上皮細胞之關係。所有於一月中旬至三月初排卵期所捕獲的雌性個體生殖道中，精子均排列整齊，其頭部朝向生殖道的上皮細胞。此時子宮角的上皮細胞內呈現眾多的內質網、粒線體，及完整的高爾基氏體。而在十二月時，受精的雌性個體中，精子排列不整齊並有退化現象。此時子宮的上皮細胞只含少量的粒線體及內質網。在精子儲存期，白血球及蘭氏細胞常出現於精子頭部被吞噬的上皮細胞附近。

ERRATUM

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In Abstract, 3rd line from the bottom:

"... heathy young adults ..." should read "... healthy young adults ..."