Phagocytosis of Spermatozoa and Latex Beads by the Epithelial Cell of the Cat Oviduct: Combined SEM and TEM Study

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Summary. The adult cat oviduct was viewed by SEM and TEM as spermatozoa of the same species and inert latex beads were injected into its lumen. The present study demonstrates that nonciliated epithelial cells in extensive areas of the oviduct as well as luminal macrophages are capable of actively taking up both the spermatozoa and the latex beads in the manner of phagocytosis. The epithelial cells are thus able to eliminate foreign bodies including degenerating spermatozoa in order to scavenge the lumen, though the cellular mechanisms involved in their phagocytotic activity remain to be elucidated.

Since the beginning of this century, the presence of spermatozoa within the mucosal epithelium of the oviduct after mating has been reported in a variety of mammalian species. However, the validity of this finding obtained from light microscopic studies with limited resolution on the problem as to whether it is real or artefactual has long been debated (see; Austin, 1959, 1960).

Recent electron microscopic investigations have clearly shown that, following mating, spermatozoa can be readily phagocytosed by nonciliated epithelial cells of the oviduct in the reproductive tract of several species including the mouse (Oura et al., 1970; Chakraborty and Nelson, 1975) and bat (Mori and Uchida, 1974). However, it remains undetermined as to whether such epithelial spermiophagy is a universal phenomenon in mammalian species or an event peculiar to the above-mentioned animals.

This experiment was undertaken to examine whether or not the cat oviductal epithelial cells can also be responsible for elimination of spermatozoa of the same kind as well as inert particles as latex beads, standing on our previous confirmation that the epithelial cells in the vas deferens of the cat are able to actively phagocytose both spermatozoa and latex beads injected into the lumen (Murakami et al., 1984a, b).

MATERIALS AND METHODS

Four mature female cats, weighing 3–3.5 kg, which were fed within individual cages in our Animal Center for at least 3 months were used.
Under Nembutal anesthesia, the abdomen was opened and the oviducts of both sides were exposed. In two cats, 0.5 ml of physiological saline suspension of spermatozoa which were collected by flushing from the cauda epididymidis of healthy mature male cats immediately before the initiation of the experiments was injected through the abdominal ostium of the oviduct into its lumen. A majority of the spermatozoa exhibited vigorous movement under the microscope. In the remaining two animals, 0.5 ml of physiological saline containing polyvinyl toluene latex beads (0.3 μm or 1 μm in diameter) was introduced to the lumen of the oviducts in a similar manner. Three hours after administration of either the spermatozoa or latex beads suspension, all the experimental animals were fixed by vascular perfusion with 2.5% glutaraldehyde and 2% formaldehyde in a cacodylate buffer (pH 7.2). The entire length of the oviduct was removed, cut into small blocks, placed in the same fixative for an additional 2 hr and postfixed in 1% OsO₄ buffered with cacodylate (pH 7.2) for 1 hr. For TEM (transmission electron microscopy), the tissue specimens were dehydrated in an ascending series of alcohol and embedded in epoxy resin. Thin sections were cut with a diamond knife on a Reichert-Yung ultramicrotome and examined with a JEM 2000 EX-TEM. For SEM (scanning electron microscopy), the samples were dehydrated similarly in an alcohol series, critical-point-dried in liquid CO₂ coated with gold-palladium in an ion sputter coater, and viewed with a Hitachi HFS-2 SEM.

Organic reagents such as propylene oxide and isoamyl acetate were not used in specimen preparation because they can completely or partially dissolve the latex beads used.

Fig. 1. Low magnification of the luminal surface of the ampullary region of the cat oviduct. Showing complicated anastomosis of mucosal folds. Dot-like structures distributed on the mucosal surface represent ciliated cells. ×110
RESULTS AND DISCUSSION

The present observations were mainly confined to the area from the ampullary to the isthmic region of the oviduct.

The mucosal lining of the cat oviduct revealed extensive foldings throughout its whole length. The folds in the ampullary region were divided in a complex manner into branches of second and third orders separating the lumen into a labyrinthine diverticula of various sizes and shapes. In contrast, the folds in the area approaching the isthmic region gradually became lower and less prominent. The epithelium covering the mucosa is made of ciliated cells with long bush-like cilia and nonciliated cells with short stubby microvilli. The ciliated cells were fewer and interspersed among the nonciliated cells, though the number ratio between both types of cells possibly varies with the stage in sexual cycle (Fig. 1).

The luminal surface of the nonciliated cell was flat or slightly bulgy and often revealed a hexagonal cell boundary. Spermatozoa introduced in the oviduct were found scattered here and there on the epithelial surface. Noteworthily, some of them thrust their heads vertically or obliquely into the nonciliated cells (Fig. 2). The formation of a flap-like pseudopod of the epithelial cells for accepting spermatozoa is not always apparent by SEM in the cat oviduct.

In thin sections, the epithelium consisted mostly of simple columnar cells, but
Fig. 3 and 4. Legends on the opposite page.
occasional basal cells were present adjacent to the basal lamina and intercalated between the ciliated and nonciliated columnar cells. The nonciliated cells contained a few tubular elements of rough endoplasmic reticulum, scattered mitochondria, a small Golgi apparatus and a few lysosomal dense bodies. Oval and slightly indented nuclei with patches of heterochromatin occupied the major part of the cytoplasm. The dense granules suggesting secretory activity as reported in the nonciliated cells of the oviduct of the estrogen-treated cat by Bareither and Verhage (1981) were not observed.

Stages of phagocytosis of a spermatozoon could be identified in the cytoplasm of nonciliated cells. Spermiphagy seemed to be initiated either by extension of a pseudopod of the nonciliated cell or by invagination of the surface plasma membrane which ultimately engulfed the spermatozoon. Probably all the spermatozoa were incorporated along their long axis into the cytoplasm of the nonciliated cells before self-disintegration (Fig. 3). This result differed from the findings by Phillips and Mahler (1977) who reported using TEM that the spermatozoan tails were often severed from the heads and not ingested by epithelial cells in the rabbit vagina.

Fragments of the ingested spermatozoa were frequently found enclosed by phago-

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**Fig. 3.** Longitudinal section through the epithelium of the ampullary region showing a spermatozoon vertically entering the cytoplasm of a nonciliated cell. Note the disruption of the plasmalemma and acrosomal membrane of the spermatozoon head. ×6,800

**Fig. 4.** Longitudinal section of a nonciliated cell in the ampullary region. In the cytoplasm several fragments of disintegrated spermatozoa such as heads and tails are visible. ×11,300
Fig. 6 and 7. Legends on the opposite page.
cytic vacuoles or associated with lysosomal bodies in the cytoplasm (Fig. 4). The fate of the spermatozoa phagocytosed by the nonciliated cell was basically similar to that of spermatozoa described in the oviduct of the mouse (Chakraborty and Nelson, 1975) and the bat (Mori and Uchida, 1974). Some of the spermatozoa being phagocytosed appeared morphologically intact, and it seemed likely that not only dead or degenerating spermatozoa but living ones as well could be taken up by the nonciliated cells.

A number of macrophages were scattered in the lumen of the ampullary and isthmic region, either singly or in groups. Most of them were found to be actively involved in spermatozoan phagocytosis (Fig. 5, 6). A similar spermiophagy by luminal macrophages and leukocytes recently been confirmed to occur along almost the whole length of the female reproductive tract of several mammals including the human by means of TEM and SEM (Yanagimachi and Chang, 1963; Moyer et al., 1965; Thompson et al., 1975; Hafez, 1976; Mori and Uchida, 1980).

When latex beads of large (1 μm) and small (0.3 μm) sizes were injected into the lumen of the oviduct, they could be observed to be at various stages of phagocytosis by the nonciliated cells. In these cells the beads were seen singly or in a cluster, being either trapped by flange-like pseudopods of the plasma membrane or enclosed by phagocytic vacuoles or lysosomal dense bodies in the cytoplasm (Fig. 7). The mode and process of the latex bead phagocytosis were essentially the same as described for the epithelium of the vas deferens of the cat (Murakami et al., 1984b) and the rabbit (Murakami et al., 1985). This phenomenon seems to be not characteristic of sexually matured animals as we have confirmed the presence of a similar active uptake of latex beads by the oviductal epithelium in a three-month-old kitten in which ciliation of the epithelium was not yet visible (unpublished observation).

In the cat the phagocytosis of spermatozoa and latex beads by nonciliated cells occurs over an extensive area from the ampullary to the isthmic region of the oviduct as described above. This is at variance with findings in the mouse and bat in which epithelial spermiophagy has been reported in a restricted portion of the isthmic region near the uterotubal junction (Mori and Uchida, 1974; Chakraborty and Nelson, 1975). It is not known, however, whether this discrepancy is related to a species difference or other factors.

From the present study, it is apparent that the nonciliated cells of the cat oviduct possess the ability to phagocyte injected spermatozoa or latex beads. The significance of this phagocytic activity by the epithelial cell is not clear, but it is conceivable that the epithelial cells normally act to eliminate foreign bodies including surplus or degenerated spermatozoa in the lumen, though the cellular mechanism involved in phagocytosis may be different between spermatozoa and latex beads.

On the other hand, the ciliated cells were characterized by prominent cilia, abundant basal bodies and relatively sparse organelles, and did not reveal any morphological evidence for phagocytosis of either spermatozoa or latex beads.

**Fig. 6.** A thin section seen through the lumen of the ampullary region. The cytoplasm of luminal macrophages is filled with fragments of phagocytosed spermatozoa. × 4,000

**Fig. 7.** Longitudinal section through the epithelium of the ampullary region, showing phagocytosis of latex beads by nonciliated cells. Latex beads are either enveloped by pseudopods extending from the cell or enclosed, singly or in clusters, by phagocytic vacuoles within the cytoplasm. ×6,500. **Insert:** A latex bead being enveloped by a flap-like pseudopod of a nonciliated cell. At the lower left are the ciliated cell. ×16,000
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