Assiut Vet. Med. J. Vol. 39 No. 78, July 1998.

Assiut University
Faculty of Veterinary Medicine
Dept of Anatomy & Histology

ULRASTRUCTURAL OBSERVATIONS ON EPITHELIAL PHAGOCYTOSIS OF SPERMATOZOA IN BOVINE EJACULATORY DUCT

(With 10 Figures)

By

A. ABOU-ELMAGD

(Received at 27/6/1998)

مشاهدات ميكروسكوبية دقيقة لالتهام الحيوانات المنوية بواسطة النسيج الطلائى لقناة القذف فى الأبقار

أحمد أبو المجد أحمد

أستخدم فى هذه الدراسة عدد عشرة من قنوات القذف لعجول الأبقار بالغة النمو والتى تتمتع بصحة جيدة ، حيث تم فحص النسيج الطلائى باستخدام الميكروسكوب الإلكتروني النافذ. تم تتبع خطوات التهام الحيوانات المنوية بواسطة النسيج الطلائى المبطن لقناة القذف. وقد وجد أن الغشاء البلازمي السطحي للحيوانات المنوية داخل قناة القذف به مناطق ارتباط بطبقة من بقايا السائل المنوى على شكل شبكة دقيقة تغطى سطح النسيج الطلائى. وقد شوهدت هذه الحيوانات المنوية فى درجات متفاوتة من التحلل مثل انفصال الغشاء البلازمي والقلنسوة والغشاء النووي على أبعاد مختلفة من سطح النسيج الطلائى. كما لوحظ وجود نشاط كبير لامتصاص والتهام لطبقة السائل المنوي المغطية لسطح النسيج الطلائى. وقد لوحظ أن خلايا الامتصاص التى يقترب منها الحيوان المنوي أو أجزاء منه يظهر على سطحها ارتفاع سيتوبلازمى مخروطي الشكل وتقل الزوائد السيتوبلازمية الدقيقة فى الارتفاع والعدد. ثم يندفع السيتوبلازم على شكل زوائد كبيرة ليحيط بالحيوان المنوي ليغوص تدريجيا داخل جسم الخلية. وعند نهاية عملية التهام الحيوانات المنوية ، يصبح السطح الخارجي لخلايا الامتصاص أملس مع وجود القليل من الزوائد الدقيقة والقصيرة ويظهر داخل الخلايا العمادية المكونة للنسيج الطلائى حيوانات منوية فى مراحل هضم مختلفة داخل حويصلات مختلفة الحجم. كما لوحظ وجود أ جسام هاضمة ثانوية ذات محتويات مختلفة الشكل والكثافة الإلكترونية. وتشير هذه الدراسة إلى أن الخلايا العمادية المبطنة لسطح قناة القذف تقوم بعمليات امتصاص للسائل المنوي ثم يتبع ذلك تغير فى سطح الخلية لتقوم بالتهام الحيوانات المنوية.

SUMMARY

In the present study 10 ejaculatory ducts from sexually mature healthy bovine bulls were used. The collected materials were prepared for ultrastructural examination. The process of sperm phagocytosis by the lining epithelial cells of the ejaculatory duct was examined. The spermatozoa appeared mostly embedded within a layer of fine particles of seminal plasma over the surface of an absorptive epithelium with well-developed microvilli. These particles of the seminal plasma formed a fine delicate network. The spermatozoa attached with the network by darkly stained points of attachment on their surface. The spermatozoa showed different degrees of degeneration such as the separation of plasma membrane, acrosomal cap and nuclear envelope. These spermatozoa were observed at different levels over the absorptive epithelial surface, which may be a result of absorptive and pinocytotic or phagocytic activity of the covering layer. In the areas of close contact between spermotozoa and epithelium, the absorptive epithelial cells form cone-shaped elevations of cytoplasm above the cell surface. At the areas of cytoplasmic protrusions, the slender-shaped microvilli became short and few in number. Other absorptive cells showed cytoplasmic processes protruded over the surface and surrounded the spermatozoon that located in contact with the cell surfaces. At the end of phagocytic process, the absorptive epithelial cells demonstrated changes in the morphology of their apical surface and the contents of their cytoplasm. The apical border became smooth with few short microvilli. Within the cell body, engulfed spermatozoa at different stages of ingestion, and several lysosomal bodies with heterogenous contents were observed. It can be concluded that spermiophagy in bovine ejaculatory duct is undertaken by the lining columnar cells through morphological transformation from the absorptive phase into the phagocytic phase.

Key words: *Spermatophagy–Ultrastructure–Ejaculatory duct–Bovine bull.*

INTRODUCTION

Spermatophagy is the intracellular ingestion and degradation of altered sperms or non-ejaculated spermatozoa within the male genital system. It also occurs within the female genital system (Aumüller and Riva, 1992). Phagocytosis of spermatozoa has been reported along the

excurrent duct system of the male genital system in human-being, many laboratory animals and bovine bull. Moreover, sperm phagocytosis was observed in testicular seminiferous tubules, transitional zone and tubuli recti as well as rete testis (Dym, 1974; Burgos and Cavicchia, 1975; Holstein, 1978; Nykänen, 1979; Goyal, 1982; Murakami and Yokoyama, 1989) and in ductuli efferentis, vas deferens and seminal vesicle (Riva et al., 1981; Goyal, 1982; Cossu et al., 1983; Aumüller and Riva, 1992). The sperm phagocytic activity of the lining epithelial cells, in ampulla of the vas deferens and ejaculatory ducts of different laboratory animals (Murakami and Yokoyama, 1989) and man (Riva et al., 1981), is performed not only by epithelial cells but also mostly by macrophages. In addition, Murakami et al. (1984 & 1985) reported that in vas deferens the luminal epithelial cells and the macrophages are involved in phagocytosis of spermatozoa and also capable of actively taking up latex beads administrated intraluminal.

Although in ejaculatory ducts of bovine bulls two types of adluminal epithelial cells; absorptive and phagocytic columnar epithelial cells, have been described by Abou-Elmagd and Wrobel (1990), it is still unclear; whether these two adluminal cell-types are specified to perform two separate functions. Additionally, if the absorptive cells of the ejaculatory ducts contribute to the spermatophagy or not. Accordingly, this study was performed to clarify the phagocytic process of spermatozoa within the bovine ejaculatory duct using transmission electron microscope.

MATERIAL and METHODS

For the present study, ten ejaculatory ducts obtained from sexually mature and apparently healthy bovine bulls were used. The material was collected from Assiut slaughterhouse. After slaughtering, the ejaculatory ducts together with the pelvic urethra were separated from the animals and fixed as quickly as possible using a vascular perfusion technique through the prostatic arteries. The vascular perfusion began by injection of rinsing buffer solution (0.1M phosphate buffer, pH 7.4), and followed by the fixation solution. The fixation solution is formed of a mixture of paraformaldehyde and gluteraldehyde as described by Karnovesky (1965). After fixation, the ejaculatory ducts were cut into small pieces and immersed in the same fixative for about 4 hours at 4 °C in refrigerator. Then the samples of ejaculatory ducts were washed by 0.1M phosphate buffer (pH 7.4) several times and osmicated by 1%

Osmium tetroxide for one hour. After washing by 0.1M phosphate buffer, the samples were dehydrated by ascending grades of ethanol and embedded in a mixture of epon and araldate (Mollenhauer, 1964). Semithin sections were cut and stained with methylene blue – azur II for light microscopical examination to determine the places of ultrathin sections. Ultrathin sections were mounted on copper grids, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined by JEM 100CXII transmission electron microscope in the Unit of Electron Microscope of Assiut University.

RESULTS

Ultrastructurally, the lining epithelium of the bovine ejaculatory duct was composed of tall columnar cells. In between them, basally located small rounded or oval–shaped cells with rounded nuclei (Fig.1a). In the lumen of ejaculatory duct and in between the mucosal folds (Figs.1&2), many spermatozoa or fragments representing the different regions of spermatozoon; head, middle piece and tail, were observed. They were found at different levels from the luminal surface of the lining epithelium. The head region of spermatozoa was formed of elongated and compact electron dense central nucleus. It was surrounded by acrosome composed of homogenous and low electron dense substance. The head regions showed frequently signs of degeneration. The acrosomal cap began to separate from the compact nucleus by vacuolation of the inner most layer of acrosome (Fig. 2). Also separation of plasma membrane from the acrosomal cap was demonstrated (Fig. 4). These spermatozoa were embedded in a layer of seminal plasma covering the epithelial surface. This covering layer was composed of slightly electron dense material. The components of the covering layer formed a fine delicate network (Fig.3). Within the plasma membrane of the spermatozoa, points of attachment were demonstrated, which represent anchoring sites of the fine short filamentous components of the layer of seminal secretion over the epithelial surface (Fig.3). At higher magnification, the anchoring sites appeared as darkly stained points within the plasma membrane of the spermatozoa, particularly in the separated plasma membrane around the head region (Fig. 3a and 3b). On the outer surface of the spermatozoal tail, the attaching fine and short filamentous material were also observed (Fig.3c).

In the same area of ejaculatory duct, where the spermatozoa were observed in the lumen and away from the epithelial surface, the apical

borders of the adluminal columnar cells showed marked ultrastructural features of absorptive activities (Fig.2). The cells were characterized by well-developed finger-shaped microvilli and numerous pinocytotic coated pits located at the base of the mivcrovilli. Also pinocytotic vesicles, multivesicular bodies and dense granules scattering in the apical cell cytoplasm could be seen.

The spermatozoa, which were found within the lumen in position away from the surface of the absorptive epithelial cells, appeared intact, but some of them showed signs of separation of the covering plasma membrane particularly at the head region (Fig. 2). The spermatozoa, which were observed near the epithelial surface of the absorptive cells, demonstrated pronounced separation of plasma membrane with the underlying thin layer of acrosomal cap in the head region. This separation of plasma membrane appeared very clear in the spermatzoon lie between two absorptive mucosal surfaces, where the separated plasma membrane formed a large vacuole containing the condensed nucleus of the spermatozoon (Fig. 4). In addition, nuclei of some degenerated spermatozoa have lost both the surrounding plasma membrane and the acrosomal cap, and located in close position to the epithelial absorptive surface. Their nuclear envelope showed few areas of separation from the compact chromatin substance on the nuclear surface facing the absorptive epithelium (Figs.6&7). At each separation, the nuclear envelope displayed fine threads of electron dense substance connecting between it and the surface of the microvilli of the absorptive epithelial cells (Fig.7b). The separated nuclear envelope located in position between the chromatin substance and the epithelial surface showed a zigzag –like appearance (Fig.6). It appeared still in contact with the nuclear poles of the condensed chromatin substance of the sperm head. Also, it attached with the epithelial surface by fine electron dense filamentous material.

In the areas of spermatozoa-epithelial contact, the apical cytoplasm of the absorptive cells appeared protruded above the epithelial surface forming a cone-shaped cytoplasmic structure and supported by central core of cytoplasm. These cone–shaped cytoplasmic elevations possessed an irregular luminal borders (Figs.4&8). The supporting core of the cytoplasm was formed of a fine electron dense filamentous material similar to that filling the cores of microvilli (Fig.8a). At the base of the cone –shaped cytoplasmic elevation, constrictions were demonstrated at the level of the zonulae adhaerens. Bundles of electron dense filaments were also observed extending between the apical zonulae adhaerens (Fig. 8b). The microvilli on the free surface of the absorptive

cells appeared very short on the surface of the cone-shaped cytoplasmic elevation facing the luminal spermatozoa. From the protruded apical cone-shaped cytoplasmic portion, processes were developed and surrounded the spermatozoon (Fig.9). The engulfed spermatozoa sinked in the apical cytoplasm and appeared as fragments of degenerated spermatozoa within phagocytic vacuoles. In addition, active pinocytosis was observed in cells engaged in phagocytosis of spermatozoa. These cells demonstrated few short microvilli on the free surface, and small vesicles or coated pits in the apical cytoplasm (Fig.9). At the end of the phagocytic process, the epithelial cells displayed ingested desintegrated spermatozoa in different stages of digestion and formation of endosomal complexes. Lysosomes filled with heterogenous contents of variable electron densities were frequently observed (Fig.10). Also, chromatin residues, dense bodies or lipid droplets could be seen within the cytoplasm. The apical surface appeared smooth and carried few, short micovilli. In addition, phagocytosis of spermatozoa by intraluminal macrophages in bovine ejaculatory duct could not be seen.

DISCUSSION

In the present study, steps of the phagocytic process of spermatozoa by the adluminal epithelial cells have been described in the bovine ejaculatory duct. The sperms are undergoing degenerative processes accompanied by alteration in their entire structure. The fine particles of the seminal fluid, which surround the sperms in the lumen and form a layer over the luminal epithelial surface, are probably the main cause of spermatozoal degeneration after ejaculation. The alteration of sperm structure may allow the epithelium to begin a spermiophagy activity. Metz et al. (1968) and Davis (1973) reported the presence of fine particles in seminal plasma of rabbit, bull and man. These particles originate from accessory genital glands and epididymis and contain highly active group of enzymes (Agrawal & Vanha-Pattula, 1987). Additionally, some authors stated that these enzymes and other components of seminal fluid can influence the spermatozoa with deleterious effects (Eliasson & Lindholmer, 1975; Dott et al., 1979; Mann & Lutwak-Mann, 1981; Baas et al. 1983 and Agrawal & Vanha-Pattula, 1987).

Analysis of the present morphological findings indicates that there is a one cell-type, columnar cell, showing apical conformational changes to perform absorptive function then spermartophagy. The well-

developed microvilli are obviously very important for absorption of seminal fluid from the lumen, while formation of cone-shaped apical cytoplasmic protrusion accompanied by involution of the microvilli is necessary to engulf the spermatozoa. Particularly, in this study intraluminal macrophages sharing in spermatophagy have not been recorded. Spermiophagy by the modified Sertoli cells in the terminal segment of bovine seminiferous tubules (Wrobel et al., 1982) and also by the epithelial lining of bovine straight testicular tubules and rete testis (Sinowatz et al., 1979) has been interpreted as a selective removal of degenerated or abnormal spermatozoa. While Aumüller and Riva (1990) defined the spermatophagy as a process of ingestion and degradation of altered sperms or non-ejaculated spermatozoa. On the other hand, the described spermatophagy in the ejaculatory duct may be considered as a completely different process. A selective removal of abnormal or altered spermatozoa is unlikely explanation for spermatophagy in this part of excurrent duct system. The spermatophagy in the ejaculatory duct is probably a process for clearing the lumen from sperm cells, which remain after ejaculation or passively flow from the vas defferens, and it mostly performed by the adluminal epithelial cells. This suggestion is inconsistent with the observations of Abou-Elmagd & Wrobel (1990) in bovine bull and Abou-Elmagd et al. (1994) in buffalo bull.

The importance of the absorptive function of the lining epithelium is not only in removing of seminal plasma, but also in the process of spermatophagy. The separation of the plasma membrane, the acrosome and the nuclear envelope toward the absorptive surface have been morphologically demonstrated at different levels from the microvilli. These observations may suggest that the strong absorptive activities of epithelial cells are responsible for the movement of the sperms toward the epithelial surface. This function is necessary to bring the sperm–epithelial contact and to initiate the phagocytic process of spermatozoa. Alberts et al. (1989) stated that the phagocytosis is a triggered process in which activated receptors transmit signals to the cell interior to initiate the response. Therefore, the present work indicates that the absorption and spermatophagy in ejaculatory duct are two complementary successive processes in one adluminal cell, at least to remove the passively flowing of seminal plasma and spermatozoa or those staying after ejaculation. The absorptive function of the microvilli is necessary to draw the sperms to come in contact with the free surface of the absorptive cell. The response of the absorptive epithelial cells against the sperms begins with morphological transformation of the apical portion

into cone-shaped cytoplasmic protrusion, which develops under the effect of the contraction of the apical actin filaments. The microvilli decrease in height and number. Then cytoplasmic processes develop and embrace the spermatozoon that attaches to the surface by fine filamentous network. These observations confirm the previous findings of Murakami et al. (1984) and Aumüller and Riva (1990), who mentioned that apical cytoplasmic processes arise over the cell-surface during spermatophagy in man.

The formation of the apical cytoplasmic protrusions and then the development of cytoplasmic processes in the absorptive cells take place probably as a result of contraction of the actin filaments, those attaching to zonulae adhaerens. This explanation is inconsistent with the previously mentioned statement of Alberts et al. (1989), who reported that for some purposes, small bundles of actin filaments project outword to form a core of cell surface extension. While in the absorptive phase the free cell border carries well-developed finger-shaped microvilli, it becomes a smooth surface with few short microvilli in phagocytic phase. This morphological transformation may be attributed to a large proportion of plasma membrane used in formation of many phagocytic vacuoles and endosomal bodies during phagocytosis of spermatozoa.

REFFERENCES

Abou-Elmagd A.; Wrobel K.-H. (1990): The epithelial lining of the bovine ejaculatory duct. Acta Anat 139: 60-65.

Abou-Elmagd A., Salem A.O., Abdel-Rahman Yousria A., Kelany A.M. (1994): Ultrastructural evidence for resorption, spermiophagy and renewal in the epithelium of buffalo ejaculatory duct. 6th Scientific Congress, Fac. Vet. Med., Assiut University, pp. 183-195.

Agrawal Y., Vanha-Perttula T (1987): Effect of secretory particles in bovine seminal vesicles secretion on sperm motility and acrosome reaction. J. Reprod. Fert. 79:409-419.

Alberts B., Bray D., Lewis J., Raff M., Robberts K., Watson J. (1989): Molecular Biology of the cell. 2nd ed. Garland Publishing, Inc. New York & London, pp. 630.

Aumüller G., Riva A (1990): Morphology and functions of the human seminal vesicle. Andrologia 24: 183-196.

Baas J.W., Molan P.C., Shannon P. (1983): Factors in seminal plasma of bulls that affect the viability and motility of spermatozoa. J. Reprod. Fert. 68: 275-280

Burgos M.H., Cavicchia J.C. (1975): Phagocytic activity in the epithelium of the rete testis. In: Proc. 10th. Int. Congr. Anat. Tokyo,Science Council of Japan, pp. 444.

Cossu M., Marcello M.F., Usai E., Testa-Riva F., Riva A. (1983): Fine structure of the epithelium of the human ejaculatory duct. Acta. Anat. 116: 225-233.

Davis B.K. (1973): Occurrence of vesicles in rabbit seminal plasma. Experientia 29: 1484-1487.

Dott H.M., Harrison R.A.P., Foster G.C.A. (1979): The maintenance of motility and the surface properties of epididymal spermatozoa from bull, rabbit and man in homologous seminal and epididymal plasma. J. Reprod. Fert 55: 113-124.

Dym M. (1974): The fine structure of monkey Sertoli cells in the transitional zone at the junction of the seminiferous tubules with the tubuli recti. Amer. J. Anat. 140: 1-26.

Eliasson R., Lindholmer C. (1975): Human seminal plasma and sperm survival and transport. In *The biology of spermatozoa*, pp. 83-92. Eds. Hafez E.S.E. & Thibault C.G., Karger, Basal.

Goyal H.O. (1982): Light microscopic and ultrastructural evidence of epithelial phagocytosis of sperm in rete testis and ductuli efferentis in the bull. Amer. J. Vet. Res. 43: 785-790.

Holstein A.F. (1978): Spermatophagy in seminiferous tubules and excurrent ducts of the testis in rhesus monkey and in man. Andrologia 10: 331-352.

Karnovsky M.J. (1965): A formaldehyde-gluteraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27: 137A-137B.

Mann T., Lutwak-Mann C. (1981): Male reproductive function and semen. Springer-Verlag, Berlin.

Metz C.B., Hinsch G.W., Anika J.L. (1968): Ultrastructure and antigens or particles from rabbit semen. J. Rep. Fert. 17: 195-198.

Mollenhauer H.H. (1964): Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39: 111-114.

Murakami M., Nishida T., Iwanga S., Shiromoto M. (1984): Scanning and transmission electron microscopic evidence of epithelial phagocytosis of spermatozoa in the terminal region of the vas deferens of cat. Experienta 40: 958-960.

Murakami M., Nishida T., Shiromoto M., Inokuchi T. (1985): Phagocytosis of spermatozoa and latex beads by epithelial cells of ampulla vasis deferentis of rabbit: A combined SEM and TEM study. Archv. Histol. Jap. 48: 269-277.

Murakami M., Yokoyama R. (1989): SEM observations of the male reproductive tract with special reference to epithelial phagocytosis. In: Developments in ultrastructure of reproduction, pp. 207-214. Ed.Motta PM, AR Liss, New York.

Nykäenen M. (1979): Fine structure of the transitional zone of the rat seminiferous tubule. Cell Tissue Res. 198: 441-454.

Reynolds E.G. (1963): The use of lead citrate at high pH as an electron – opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

Riva A., Cossu M., Usai E., Testa-Riva F. (1981): Spermatophagy by epithelial cells of the seminal vesicle and the ampulla ductus deferentis in man: A scanning and transmission EM study. In: Oligozoospermia. Recent progress in andrology, pp. 45-53. Eds. G. Frajese, E.S.E. Hafez, C. Conti and A. Fabbrini Raven press, New York,.

Sinowatz F., Wrobel K.-H., Sinowatz S., Kugler P. (1979): Ultrastructural evidence for phagocytosis of spermatozoa in bovine rete testis and testicular straight tubules. J. Reprod. Fertil. 57: 1-4.

Wrobel K.-H., Sinowatz F., Mademann R. (1982): The fine structure of the terminal segment of the bovine seminiferous tubule. Cell Tissue Res. 225: 29-44.

LEGENDS

Fig. 1(a,b,c):

The lining epithelium of bovine ejaculatory duct is composed of columnar and small basal cells (Bc) in 1a. The epithelial free surface is covered by fine electron dense substance. Degenerated spermatozoa appear at different levels from the epithelial surface in 1a, 1b and 1c. x5100,

Fig. 2:

Spermatozoa are embebbed within a layer of seminal plasma covering the epithelial surface (asterisk). This layer is composed of fine particles and appeared as a delicate network. The apical portion of the absorptive columnar cell shows well-developed finger-shaped microvilli (mv) and pinocytotic vesicles (arrow) at the base of microvilli. Notice separation of the compact nucleus from the surrounding acrosome. x20.000.

Fig. 3(a,b,c):

Points of attachment (arrows) of the seminal plasma layer with the plasma membranes of the spermatozoa. They appear darkly stained and separated from each other by non-attachment less darkly stained areas. Head of sperm, x28.000 in 3a and x 20.000 in 3b. Tail of sperm x20.000 in 3c.

Fig.4:

Sperm lying between two absorptive surfaces. Notice detached plasma membrane (arrows) forms a large vacuole. Within the vacuole located the compact nucleus surrounded with the acrosome. Microvilli (mv) and protruded apical cytoplasm (pac). x 13.400.

Fig.5:

Head of sperm formed of condensed chromatin substance surrounded by acrosome. Irregularly detached plasma membrane (arrows) toward the epithelial microvilli (mv). x10.000.

Fig. 6:

A zigzag-like detached nuclear membrane still in connection with some areas on the surface of the compact chromatin. Arrows indicate to fine filamentous material connecting between the separated membrane and the epithelial surface. Notice disappearance of the long microvilli. Many pinocytotic vesicles (arrowhead). x20.000.

Fig. 7:

Detached nuclear envelope (arrows) facing the protruded apical cytoplasm (Pac) of absorptive cell surface covered by microvilli (mv) in 6a, x15.000. Higher magnification of the detached areas showing fine filamentous material (arrows) extending between the detached nuclear envelope and the apical microvilli (arrowheads) in 6b, x 40.000

Fig. 8 (a,b):

The absorptive cell shows a cone–shaped protruded apical cytoplasm (Pac) filled by electron dense filamentous material similar to that forming the cores of microvilli. In 8b, actin filaments (arrow) form a constriction at the base of the protruded apical cytoplasm. x14.000.

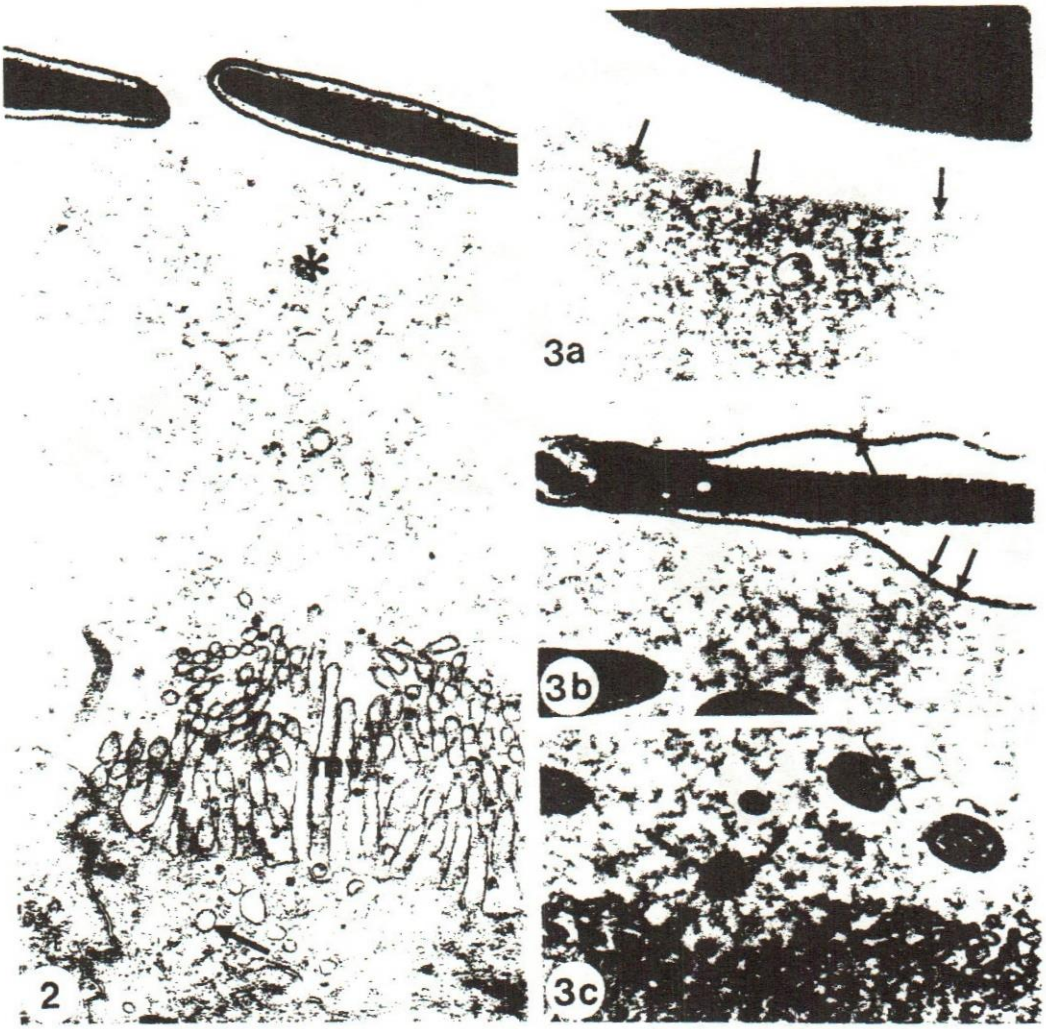
Fig. 9:

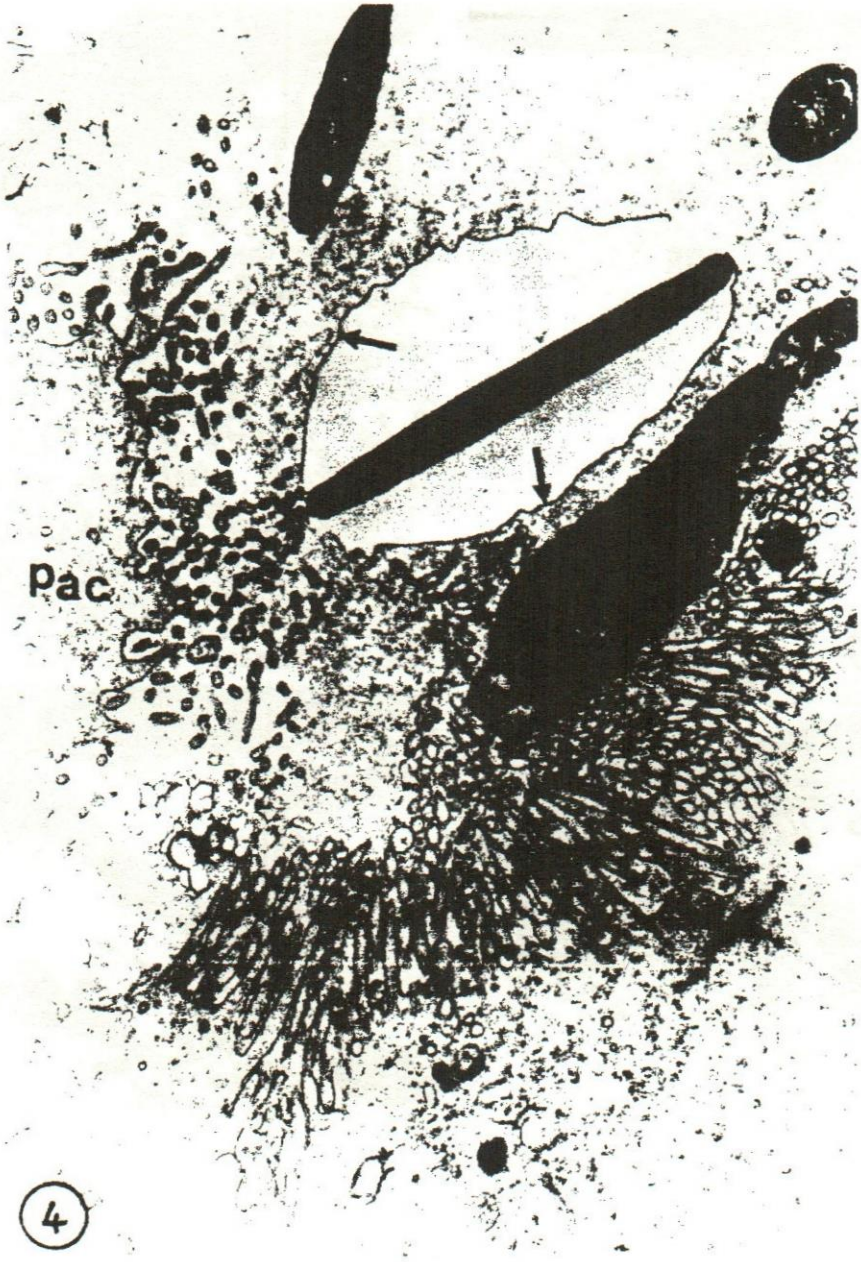
Cytoplasmic processes projected over the cell surface (arrows) and embrace the engulfed spermatozoon. Notice coated pinocytotic pit (arrowhead) and electron dense substance at the base of the cytoplasmic processes similar to that form the core of microvilli. x20.000.

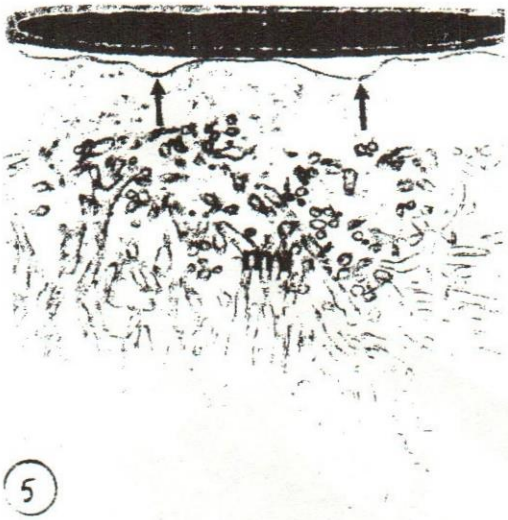
Fig. 10:

Adluminal epithelial cell at the end of the phagocytic process shows many phagocytic digestive vacuoles with heterogenous contents, smooth apical border with few short microvilli and pinocytotic vesicles (arrows). x5000.


1a
1b
1c

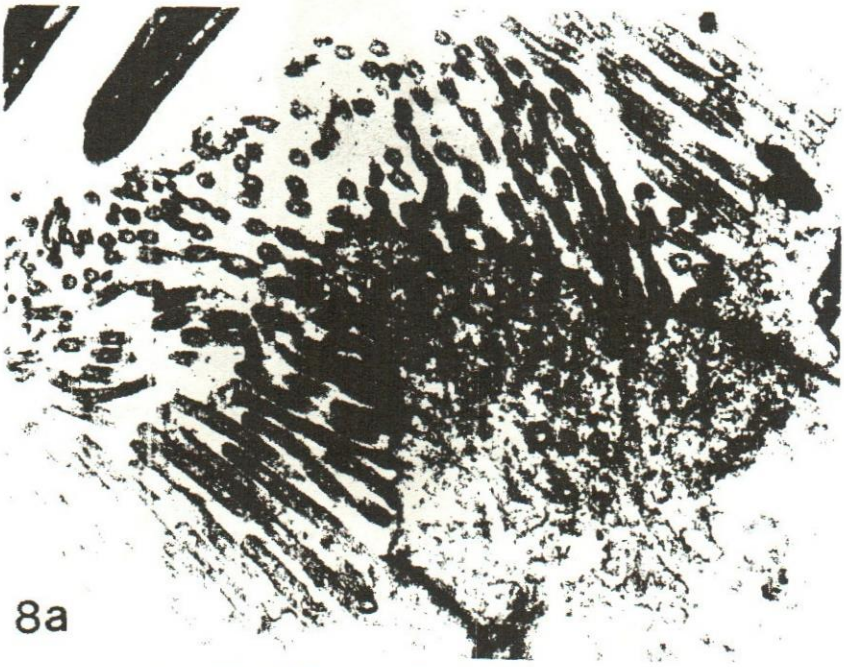

3a
3b
3c
2


pac
4



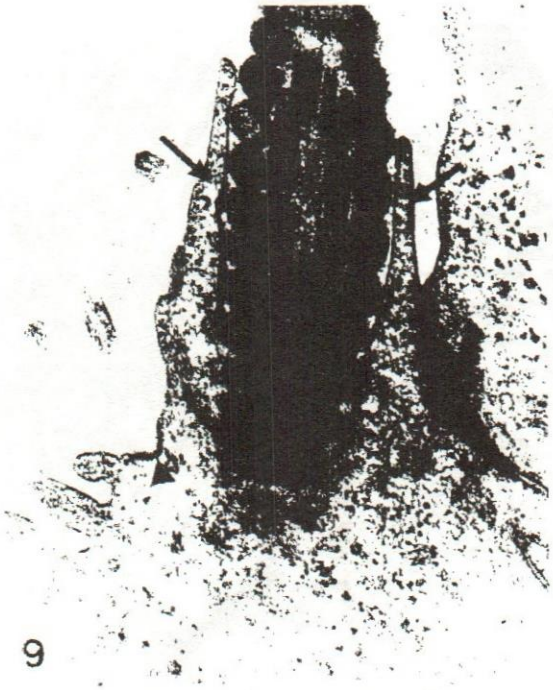
5

6



8a

8b



9

10