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SPERMATOGENESIS AND POSTINSEMINATIONAL ALTERATIONS OF SPERM STRUCTURE IN A SARCOPTID MITE, NOTOEDRES CATI (HERING) (ACARI, ACARIDIDA, SARCOPTIDAE)

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Summary: Ultrastructural features of sperm formation and their postinseminational alterations in a sarcoptid mite, Notoedres cati, were described. In the testes, the spermatogonia, spermatocytes, spermatids as well as spermatozoa are distributed in a sequence from the dorsal to the ventral region. Primary spermatocytes show the presence of a peripheral spongy layer of cytoplasm formed by ER cisternae which grow into the cytoplasm during spermatocyte division to separate the newly formed spermatids. At the spermatid stage the chromatin condenses in a form of threads, cisternae of the spongy layer become electron-dense, anastomosing ER tubules appear in the cytoplasm and the mitochondria loose their normal structure and are transformed into double piles of mitochondrial derivatives. As spermiogenesis proceeds, the thickness of the chromatin threads increases and the ER tubules become electron-dense.

Spermatozoa found in the male genital tract contain thick chromatin threads freely embedded in the cytoplasm, ertron-dense ER tubules, and mitochondrial derivatives, but are devoid of the acrosomal complex.

Spermatozoa observed in the seminal receptacle of the female genital tract show similar structure but are less condensed, their mitochondria reveal a more typical structure and the cytoplasmic face of the plasmalemma is incrusted with regularly distributed filaments. Contact zones can be observed between the adjacent spermatozoa.

Spermatozoa characterized by long cytoplasmic protrusions and different levels of electron density were found penetrating the ovarian somatic cells.

Résumé : On décrit les facteurs ultrastructuraux de la formation des spermatozoïdes et leurs transformations après l’insémination chez les sarcoptidés Notoedres cati. La spermatogenie, les spermatocytes et les spermatozoïdes sont situés successivement dans le nucléus, de la région dorsale jusqu’à la ventrale. Les spermatocytes primaires possèdent une zone spongide de cytoplasme périphérique, formé de cîtènes ER qui pendant la division du spermatocyte s’incarne en cytoplasme en dispersant les spermatides récemment formées. Dans le stade de spermatide, la chromatine soumise à la condensation se forme en filaments, les cîtènes de zone spongide deviennent...
électrons-denses, les tubes ER ramifiés apparaissent dans le cytoplasme et les mitochondries perdent leur structure normale et se transforment en doubles piles d’origine mitochondriale. Durant le processus de spermiogenèse, l’épaisseur des filaments de chromatine augmente, et les tubes ER deviennent électrons-denses.

Les spermatozoïdes dans les trajets génitaux mâles possèdent les longs filaments de chromatine dispersés dans le cytoplasme, les tubes ER électrons-denses, ainsi que les dérivés mitochondriaux, mais sont privés du complexe acrosomal.

Les spermatozoïdes observés dans le receptaculum seminis des trajets génitaux femelles possèdent une structure semblable, mais sont moins condensés, leurs mitochondries montrent une structure plus typique et la face cytoplasmique du plasmalemme est incrustée par les filaments régulièrement dispersés. On peut observer les zones de contact parmi les spermatozoïdes adjacents.

Dans la sphère des cellules somatiques ovarienes, on a trouvé des spermatozoïdes se caractérisant par de longues protrusions cytoplasmatiques et par différents niveaux de densité de cytoplasme.

INTRODUCTION

Sarcoptid mites are ectoparasites which infest mostly birds and mammals. *Notoedres cati* attacks mainly domestic cats but also rabbits, lynxes and coatimundis (Krantz, 1978) to produce mange symptoms in the host. Female mites burrow in the epidermis and form numerous, perpendicular channels which terminate on the border with connective tissue. This results in a considerable thickening of the epidermis and loss of hair. In massive infestation *N. cati* can lead to a heavy disease of the host.

The structure of spermatozoa has been investigated only in one sarcoptid mite, *Psoroptes equi* (Alberti, 1984). The obtained data indicate a significant similarity between spermatozoa of that parasitic mite and those of non-parasitic species belonging to Acaridida such as pests of stored food (*Acarus siro, Caloglyphus anomalus, Tyrophagus putrescentiae*) (Reger, 1971; Alberti, 1980b; Witaliński et al., 1986). Sperm cells in both groups share such characters as undefined shape, thread-like chromatin, electron-dense lamellae, mitochondria with a poorly defined structure, as well as absence of acrosomal complex and nuclear envelope.

As shown by the present study, the spermatozoa of *N. cati* have a slightly different structure. This paper also includes data on spermatogenesis and postinseminational fate of the sperm cells, providing clues to the origin of structural modifications found in mature spermatozoa and on the alterations in their structure during the passage through the female genital tract.

MATERIAL AND METHODS

In order to collect the material, small pieces of cat skin were removed from the scab areas. The males and females of *Notoedres cati* (Hering) were collected from the skin surface and terminal portions of the epidermal channels, respectively, placed on a sticky tape and covered with droplets of 3 % glutaraldehyde in 0,09 M cacodylate buffer, pH 7,4. The anterior part of the body was cut off with a razor blade. After fixation for 1,5 h at room temperature, the material was washed and postfixed with 1,4 % osmium tetroxide in 0,45 M saccharose. After removal from the sticky tape the mites were dehydrated in ethanol and propylene oxide, and embedded in Epon 812. Ultrathin sections were collected on formvar-coated grids, contrasted with uranyl acetate and lead citrate (Venable and Coggeshall, 1965), and examined in a Tesla BS 500 electron microscope at 60 kV.

RESULTS

*N. cati* males possess two testes, interconnected by a narrow bridge and located symmetrically in
FIG. 1: Cross section of testis. Germ cells: spermatogonia (Sg), primary spermatocytes (Sc), dividing spermatocytes (Sc'), spermatids (Sd) and spermatozoa (Sz) are arranged in a sequence from dorsal part of the testis (top) to the ventral one (bottom). White asterisk indicates an electron-dense, flocculent material. The testis is surrounded by a thin amorphous layer or basal lamina (black asterisk). × 5,000.

FIG. 2: Spermatogonium. Nucleus (N) with nucleolus (asterisk) is surrounded by mitochondria (M) containing dense bodies. × 20,500.

FIG. 3: Fragment of spermatogonium with the Golgi apparatus (asterisk), mitochondria, and ER cisternae (arrow). × 20,700.
the posterior part of hysterosoma. Each testis show a regular distribution of the germ cells: spermatogonia are grouped in the dorsal region, the central part is occupied by spermatocytes and spermatids, whereas the ventral area is filled with sperm cells (Fig. 1). Somatic cells are only occasionally visible. The testis is surrounded by a thin, amorphous layer (basal lamina). The intertesticular bridge, as well as part of intercellular spaces are filled with an electron-dense, flocculent material.

**Spermatogonia**

Spermatogonia (Figs. 1-3) have a diameter of about 3.0-4.0 μm, and frequently adhere to the basal lamina. The nuclei are relatively large, devoid of larger patches of condensed chromatin and contain distinct nucleoli. The nuclear envelope shows the presence of a few pores. Mitochondria with dense bodies, free ribosomes, the Golgi apparatus, and occasionally ER cisternae, can be observed in the cytoplasm (Figs. 2 and 3).

**Spermatocytes**

Spermatogonia entering the spermatocyte stage increase in diameter to 7.0-7.7 μm, part from each other and move towards the central part of testis. The nucleus of spermatocyte is large and spherical in shape, the cytoplasm contains numerous free ribosomes, microtubules, scarce rough endoplasmic reticulum (RER), and mitochondria with still visible dense bodies (Fig. 4). At the cell periphery, two or three layers of parallel cisternae form the zone of spongy cytoplasm. Terminal parts of some cisternae turn towards the centre of the cell, and form racket-shaped profiles with the cytoplasmic side of membranes showing higher electron density (Fig. 5).

The spermatocyte division was not observed in detail, except in one case: a large primary spermatocyte devoid of nuclear envelope but possessing intracytoplasmic chromosome-like densities associated with numerous microtubules (Fig. 6).

During the course of cell division mitochondria aggregate into several clusters and, later, the cisternae of the spongy layer separate such completed spermatids (Fig. 7). The electron microscopic observations have not allowed to determine precisely the number of spermatids; the simple comparison of cell volumes indicates, however, that 6-8 spermatids are being formed in this process from a single spermatocyte.

**Spermatids**

Spermatids (Figs. 7 and 8) usually occur in groups composed of several closely apposed cells resulting from the spermatocyte division. The early spermatid is characterized by the absence of the nuclear envelope. The nuclear material is organized in a form of fine threads spread throughout the cytoplasm. At the periphery of the nuclear material, tubules of ER, piles of regularly arranged mitochondrial derivatives and, most externally, cisternae of spongy layer are present. Piles of mitochondrial derivatives (Fig. 8, inset) are formed by superimposed pairs of mitochondria-derived flattened bodies which no longer show the presence of cristae and dense bodies. As the spermiogenesis proceeds, the chromatin threads become thicker and show a tendency to assemble. The rest of cytoplasm is filled with tubular ER cisternae of a moderate electron density. Cisternae and vesicles of the spongy layer become increasingly electron-dense.

**Spermatozoa**

Testicular spermatozoa with an average size of 1.5-3.5 × 3.8-5.8 μm closely adhere to each other (Fig. 9). They are triangular or tetragonal in sections. The chromatin threads, 70-85 nm thick, are located in the central part of the cell, while the rest of cytoplasm is occupied by electron-dense tubules of ER, 55-60 nm in diameter. In the vicinity of plasmalemma, piles of mitochondrial derivatives can be found. The long axes of such derivatives are parallel to the cell surface. The subplasmalemmal cytoplasm contains more or less swollen, empty-looking spaces. The acrosomal complex is absent.

**Spermatozoa in the female reproductive tract**

The inseminated spermatozoa were observed in the seminal receptacle, and in the ovary. The
FIG. 4: Primary spermatocyte. Externally located cisternae form the peripheral spongy layer (SL). Some endings of their cisternae are bent centripetally (arrow). GA, Golgi apparatus; N, nucleus; Sd, spermatids. × 22,300.

FIG. 5: Primary spermatocyte. A racket-shaped terminal fragment of spongy layer cisterna. Note an electron-dense layer (arrows) localized on cytoplasmic side of the membrane. × 26,400.

FIG. 6: Central part of primary spermatocyte during division. Numerous microtubules associated with chromosome-like densities (asterisks) are visible. × 25,400.
Fig. 7: Group of newly formed spermatids separated by cisternae (asterisks) of the spongy layer. Aggregated mitochondrial derivatives (M) can be seen. Sc, primary spermatocyte; Sz, spermatozoon. × 17,700.

Fig. 8: Two spermatids with fine chromatin threads in the central part of cytoplasm, ER tubules (arrowheads), and peripherally located mitochondria-derived aggregation (M). Cisternae of the spongy layer are electron-dense (arrows). × 25,600. Inset: Cross-sectioned (right) and longitudinally sectioned (left) piles of the mitochondrial derivative. × 20,300.
clustered spermatozoa present in the lumen of seminal receptacle differ in appearance from the testicular sperm cells (Fig. 10). They are polygonal in cross-sections and tightly adhere to each other. The cytoplasm of spermatozoa shows different levels of condensation. In the less condensed cells the internal structure can be better seen. The chromatin threads, 69-83 nm in diameter, are located in the centre or slightly eccentrically in the cell. They are embedded in a flocculent cytoplasm penetrated by ramified ER tubules, and containing poorly defined groups of vesicular mitochondria with occasionally visible mitochondrial cristae. The peripheral cytoplasm is devoid of subplasmalemmal cisternae; however, in the most condensed spermatozoa such cisternae, corresponding to the ones observed in the testicular sperm, can be present. The cytoplasmic side of plasmalemma is incrusted with regularly distributed, parallel electron-dense filaments, 18-20 nm thick (Fig. 10, inset).

The sperm cells develop contact zones composed of two apposed membranes which are separated by an electron-lucent gap containing a simple electron-dense layer (Fig. 10, inset). In places where contact zones are absent, the sperm cell membranes show irregular undulations and the intercellular space is devoid of the electron-dense layer.

The ovary of *N. cati* is composed of four elements: (1) a giant central cell to which (2) other germ cells (oogonia and oocytes) are connected via intercellular bridges, (3) somatic cells filling the spaces between germ cells, and (4) an outer thin basal lamina. The identification of spermatozoa in ovarian tissue was based on the presence of subplasmalemmal filaments, chromatin threads, or both. In the ovary, sperm cells were found exclusively in the deep invagination formed by the cell membrane of somatic cells. Such spermatozoa (Fig. 11) were characterized by different levels of cytoplasm condensation. Highly condensed spermatozoa possessed long and ramose protrusions (Figs. 11 and 12). In their cytoplasm only chromatins were clearly visible. The protrusions of moderately condensed sperm cells were very short and the cytoplasm contained electron-dense granules, 0.3-0.4 µm in diameter, as well as ER tubules (Fig. 13). Spermatozoa with long protrusions, "empty" cytoplasm containing a network of fine filaments, and chromatin threads coalescing to form irregular electron-dense bodies were also found in the somatic cells (Fig. 11). In many cases the spermatozoa were located very close to the central cell, the oogonia or the oocytes; however, they have never been observed to penetrate those cells to form junctions with their membranes.

**DISCUSSION**

The present study on the spermatozoa in *N. cati* provided data concerned with two successive processes: development of the sperm cells leading to the formation of spermatozoa in the male reproductive system, and the ultrastructural alterations of the inseminated spermatozoa prior to their fusion with the oocytes in the female reproductive tract. Spermatozoa in Acaridida are multiform, devoid of acrosome, their chromatin forms characteristic threads freely located in the cytoplasm, whereas mitochondria undergo changes leading to the disappearance of their usual structure. Furthermore, the cytoplasm contains a variable amount of electron-dense lamellae. Data concerned with Acaridida were obtained in studies of four species: *Caloglyphus anomalus* (Reger, 1971), *Acarus siro*, *Tyrophagus putrescentiae* (Witaliński et al., 1986), and *Psoroptes equi* (Alberti, 1984). The former three species belong to the supercohort Acaridides, whilst the latter one to Psoroptides, similarly to *N. cati*, described in this study. Generally, the morphological aspects of spermiogenesis in *N. cati* are similar to those observed in the species mentioned above. Differences include the absence of electron-dense lamellae as well as alterations of mitochondria. The electron-dense lamellae, probably originating from the cell membrane (Reger, 1971) or ER cisternae (Witaliński et al., 1986) were observed in the spermatozoa of all the acarid mites investigated so far. The species differ only in their amount and distribution: in *C. anomalus* they form 1-2 subplasmalemmal layers, in *A. siro* and *T. putrescentiae* they are less numerous and can be located closer to
Fig. 9: A group of testicular spermatozoa sectioned on different levels. Chromatin threads (Ch), electron-dense tubules (arrows), mitochondrial derivatives (M), and peripheral dilated cisterna-like spaces (asterisks) are visible. × 19,200.

Fig. 10: Group of inseminated spermatozoa from the seminal receptacle. Chromatin threads (Ch), electron-dense tubules (arrows), and clusters of vesicular mitochondria (M) can be seen. An asterisk indicates a spermatozoon with condensed cytoplasm. × 23,400.

Inset: Higher magnification of a tight contact between the apposed sperm plasmalemmea incrusted with particle-like cross sections of filaments. Cross sectioned electron-dense tubules are indicated with arrows. × 53,100.
Fig. 11: Fragment of ovary. In the somatic cell (SC) located between the central cell (CC) and oocyte (Oc), two sperm cells showing different levels of condensation are present. The spermatozoon processes are marked by asterisks. In decondensed cytoplasm of left spermatozoon the network of fibrous material is visible. Arrows and arrowheads indicate obliquely sectioned subplasmalemmal filaments and electron-dense tubules, respectively. Ch, chromatin. × 21,900.

Inset: The ramifying processes of spermatozoon (asterisks) are covered by plasmalemma of the somatic cell (arrowheads). × 28,800.

Fig. 12: Spermatozoon in a close vicinity to the central cell (CC). Sperm cell contains chromatin threads (Ch), electron-dense tubules (arrowheads), and electron-dense granules (asterisks). BL, basal lamina separating the ovary from the surrounding tissue. × 24,100.

Fig. 13: Schematic drawing showing organization of sperm cell in male of *N. cati*. Ch, chromatin threads; M, mitochondrial derivatives; T, electron-dense tubules.
the chromatin threads, whereas in a sarcoptid mite, *P. equi*, they are numerous and form parallel complexes in the regions of cytoplasm not occupied by the chromatin threads.

Spermatids of *N. cati* show the presence of anastomosing tubular ER elements, instead of cisternae. In the course of spermiogenesis the interior of those tubules becomes increasingly electron-dense, suggesting that the anastomosing tubules are homologous to electron-dense lamellae observed in the other species, and can be considered as a synapomorphy. The role of both structures is unknown.

In the majority of animals, mitochondria undergo significant transformation during spermiogenesis. As far as mites are concerned, the spermiogenesis-associated mitochondrial transformations are particularly pronounced in two suborders from the order Actinotrichida: Oribatida and Acaridida (*Alberti* 1980a,b; *Witaliński*, 1982; *Witaliński et al.*, 1986), in which they are manifested by disappearance of typical structure (mainly cristae) and by specific translocation inside the cell. The mitochondrial changes observed in *N. cati* slightly differ from those described in non-parasitic acaridid mites. The spermatagonia and spermatocytes of *N. cati* possess typical mitochondria with cristae and dense bodies, both structures, however, disappear during spermiogenesis and the resulting degraded mitochondrial derivatives move towards the plasmalemma to form regular, double piles, what is rather unusual among Acari. Regrettably, there are no data concerned with the spermiogenesis in other representatives of parasitic acaridid mites.

The functional significance of the spongy layer and the origin of its cisternae are still unclear. It has been recently suggested (*Witaliński et al.*, 1986) that in Acaroidae the formation of spongy layer might represent a specific form of (1) elimination of superfluous cytoplasm and/or (2) formation of new plasmalemma. As observed in this study, cisternae of the spongy layer appear between the newly formed spermatids, which not only confirms their participation in the production of new plasmalemma (at least in the regions of spermatid separation) but also suggest their importance in the cytokinesis.

Cisterna-like, often diluted spaces were observed beneath the plasmalemma of spermatozoa in the male reproductive system of *N. cati*. Such structures can result from unperfect fixation, although they were not found in the spermatozoa present in seminal receptacle of identically fixed females.

According to Prasse (1968) and Witaliński *et al.* (1986), in Acarida the penetration of the spermatozoa into the oocyte occurs very early, i.e., before the egg envelopes have been formed. This can explain the absence of acrosome in the spermatozoa.

In order to fuse with the oocyte, spermatozoa must (1) undergo capacitational alterations, which occur in the female reproductive system under influence of factors derived from female reproductive organs, and (2) reach the direct vicinity of the oocyte. In *N. cati* spermatozoa clustered in the seminal receptacle differ in their structure from those located in the male reproductive system: they have more regular polygonal shape, show a decrease in the electron density of cytoplasm, and reconstruct, partially at least, the normal structure of mitochondria from the mitochondrial derivatives. Such spermatozoa develop regular contact zones, similarly to the spermatozoa of *A. siro* and *T. putrescentiae* (*Alberti*, 1980b; *Witaliński et al.*, 1986), and periodically arranged dense filaments appear beneath their plasmalemma.

In *N. cati*, similarly to other acarid mites (*Griffiths* and *Bočzek*, 1977; *Krantz*, 1978) insemination takes place via a special channel (bursa copulatrix) which connects the copulatory aperture situated in vicinity of anal orifice with the seminal receptacle. Single spermatozoa pass from the seminal receptacle to the ovary. They can be passively transported, e.g., with the stream of fluid, but at least in the ovary some active movement is required to pass through the somatic cells surrounding the oocytes. Since the spermatozoa of Acari have no flagella (*Breucker* and *Horstmann*, 1968; *Reger*, 1974; *Alberti*, 1980a,b) it seems that they perform some kind of amoeboid movements, similarly to the nematode spermatozoa (*Wright* and *Sommerville*, 1984) which produce pseudopodia equipped with an abundant filamentous cytoskeleton. Although the movement of spermato-
zoa has not been observed in Acari (with the exception of ticks), such possibility is very probable and supported by the presence of numerous appendages formed by ovary-penetrating spermatozoa. In N. cati and other acarid mites the only structure which can be related to cell motility are filamentous materials clearly visible in cytoplasm of decondensed sperm cells (Fig. 11), as well as subplasmalemmal filaments, 18-20 nm in diameter. Interestingly, similar subplasmalemmal filaments were observed in female-located spermatozoa of several other groups of mites. Thickness of the filaments measured on figures was as follows:

- Tetanychus urticae (Actinedida, Tetranychidae) - 15-17 nm (Mothes and Seitz, 1981, Fig. 13), Trisetacus juniperinus (Actinedida, Eriophyidae) - 13-15 nm (Nuazzaci and Solinas, 1984, Fig. 8), Acarus siro and Tyrophagus putrescentiae (Acarida, Acaridae) - 16-18 nm (Witalinski et al., 1986). However, it remains unclear whether the subplasmalemmal filaments in mites other than ticks are involved in the motility of spermatozoa, as suggested by Mothes and Seitz (1981) for spider mite, T. urticae, and by Witalinski et al. (1986) for Acarida. Further detailed studies are necessary to elucidate this problem.

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