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Ultrastructure of sperm development in the free-living marine nematode *Enoplus anisospiculus* (Enoplida: Enoplidae)

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Summary - The development of sperm in testes of the free-living marine nematode *Enoplus anisospiculus* was studied with electron microscopy. The spermatogonia are undifferentiated polygonal cells with a large nucleus surrounded by a small cytoplasm. The cytoplasm of spermatocytes is filled with numerous Golgi bodies, cisternae of the RER, ribosomes, and mitochondria and it forms membranous organelles (MO). The development of MOs proceeds along two parallel lines that are characteristic of two different types of spermatocyte. In the first type, MOs begin as a system of cisternae; in the second type, MOs first appear as large vesicles filled with osmiophilic material. Later in the development of spermatocytes, all MOs are bipolar because of a large eccentric dense body associated with the system of cisternae. The nuclei of spermatids have a distinct nuclear envelope. During the collapse of the nucleus, mitochondria and MOs become closely associated with the nuclear envelope. In older spermatids, mitochondria form a layer at the future anterior end of the nucleus, all MOs are positioned posteriorly, and fibrous bodies with a marked radial orientation appear first between the anterior layer of the mitochondria and the nucleus. This cluster of organelles is retained in the immature sperm after detachment of the residual body. The distinctly external cytoplasm (ectoplasm) of the immature sperm is devoid of organelles. © Orstom/Elsevier, Paris

Résumé - ***Étude ultrastructurale du développement des spermatozoïdes chez le nématode libre marin*** **Enoplus anisospiculus** ***(Enoplida : Enoplidae)*** - Le développement des spermatozoïdes dans le testicule du nématode libre marin *Enoplus anisospiculus* a été étudié en microscopie électronique. Les spermatogonies se présentent comme de grandes cellules polyédriques pourvues d'un gros noyau entouré d'une faible quantité de cytoplasme. Le cytoplasme des spermatocytes contient de nombreux corps de Golgi, des saccules du réticulum endoplasmique, des ribosomes et des mitochondries, formant des organites membraneux (MO). Ces MO se développent concurremment selon deux procédures, caractéristiques de deux types différents de spermatocytes. Dans le premier type, les MO sont initialement formés comme un système de saccules ; dans le second, les MO se présentent comme de grandes vésicules emplies d'un matériel osmiophile. Chez les spermatocytes plus âgés, les MO assument une condition bipolaire uniforme par suite de la présence d'un important corps dense, excentrique, associé au système des saccules. Les noyaux des spermatides possèdent une enveloppe nucléaire distincte. Au cours de la disparition des noyaux, les mitochondries et les MO s'associent étroitement à l'enveloppe nucléaire. Chez les spermatides âgées, les mitochondries forment une couche à l'endroit de la future extrémité antérieure du nucléus, tous les MO se situent postérieurement, et des corps fibreux nettement orientés radialement apparaissent, en premier entre la couche antérieure de la mitochondrie et le noyau. Cette grappe d'organites se maintient dans le spermatozoïde mature après séparation du corps résiduel. Le cytoplasme distinctement externe (ectoplasme) des spermatozoïdes immatures est dépourvu de ces organites. © Orstom/Elsevier, Paris

Keywords: *Enoplus anisospiculus*, nematode, spermatogenesis, ultrastructure.

Spermatogenesis and ultrastructure of nematode sperm have been studied mainly in animal and plant-parasitic species (Foor, 1983; Bird & Bird, 1991). Literature concerning the sperm structure of free-living marine nematodes includes four papers only. Three of them deal with the sperm ultrastructure of the members of the order Enoplida, subclass Enoplia (Wright *et al.*, 1973; Baccetti *et al.*, 1983; Yushin & Malakhov, 1994); the fourth one (Noury-Srairi *et al.*, 1993) deals with the development of *Sphaerolaimus hirsutus* belonging to the order Monhysterida of the subclass Chromadoria.

As found earlier, the spermatozoa of most of the nematodes studied are characterized by the absence of a nuclear envelope (Foor, 1983). The nucleus of the enoplid sperm has a distinct nuclear envelope, which is clearly a primitive feature (Baccetti *et al.*, 1983; Yushin & Malakhov, 1994).

The structure of mature sperm cells of *Enoplus* spp. from the female gonoduct is close to the basic type of nematode spermatozoon (Yushin & Malakhov, 1994). This type was described as a bipolar amoeboid cell with anterior pseudopod and posterior main cell body (MCB). The MCB has a condensed nucleus with a nuclear envelope surrounded by mitochondria and so-called 'membranous organelles' (MO). MOs are unique organelles that are characteristic of most nematode sperm cells (Foor, 1983). As previously

observed in many nematodes, MOs derive from Golgi bodies and appear as parts of cytoplasmic complexes. Each MO includes a crystalline 'fibrous body' (FB) associated with membranous cisternae (Noury-Srairi *et al.* [1993] call this the 'MO-FB complex'). During spermatogenesis, these MO-FB complexes dissociate into: *i*) MOs, which attach to the sperm plasma membrane and open to the exterior via pores, and *ii*) free FBs, which transform during sperm maturation into ectoplasmic filaments or into the cytoskeleton of the pseudopod (Foor, 1983; Wright, 1991). However, this scheme is not supported by studies of representatives of several nematode groups including four related orders of the subclass Enoplia: Dorylaimida, Mermithida, Trichurida, and Dioctophymida (Foor, 1970, 1983; Neill & Wright, 1973; Kruger, 1991; Poinar & Hess-Poinar, 1993).

In this paper, we describe sperm development from spermatogonia through immature sperm to mature sperm in the testes of the free-living marine nematode *Enoplus anisospiculus* (Enoplia, Enoplida). The fate of nuclear envelope and the origin of MOs and FBs were of special interest in this study.

Materials and methods

Adult males of *Enoplus anisospiculus* Nelson, Hopper & Webster, 1972 were collected from the druses of bivalve *Crenomytilus grayanus* at 'Vostok' Marine Biological Station of the Institute of Marine Biology (Vladivostok, Russia) located in Vostok Bay, Sea of Japan. Live animals were cut into 0.4-0.5 mm long pieces containing different parts of testis. These specimens were fixed for TEM in 2.5 % glutaraldehyde in 0.05 M cacodylate buffer with 21 mg/ml NaCl, then postfixed in 2 % osmium tetroxide in a similar buffer with 23 mg/ml NaCl. Postfixation was followed by en bloc staining for 12 h in 1 % uranyl acetate, then the specimens were dehydrated in ethanol and acetone series and embedded in Araldite. Thin sections were cut with a Reichert Ultracut E ultratome, stained with lead citrate, and examined with JEOL JEM 100B and Philips EM 300 electron microscopes. Wholemount specimens of males were examined with a Reichert Polyvar microscope. The testes of four individuals of *E. anisospiculus* were examined for the study reported here.

Results

General anatomy of the testes as revealed by light microscopy

The male reproductive system of *E. anisospiculus* comprises two (diorchic) opposed testes (Fig. 1). Each testis is a long (about 2 mm) epithelial tube filled with germ cells. This tube is subdivided into several regions according to the stages of sperm development. The distal tip of the testis contains spermatogonia (germinal zone) followed by growing spermatocytes that fill the long growth zone. The region of

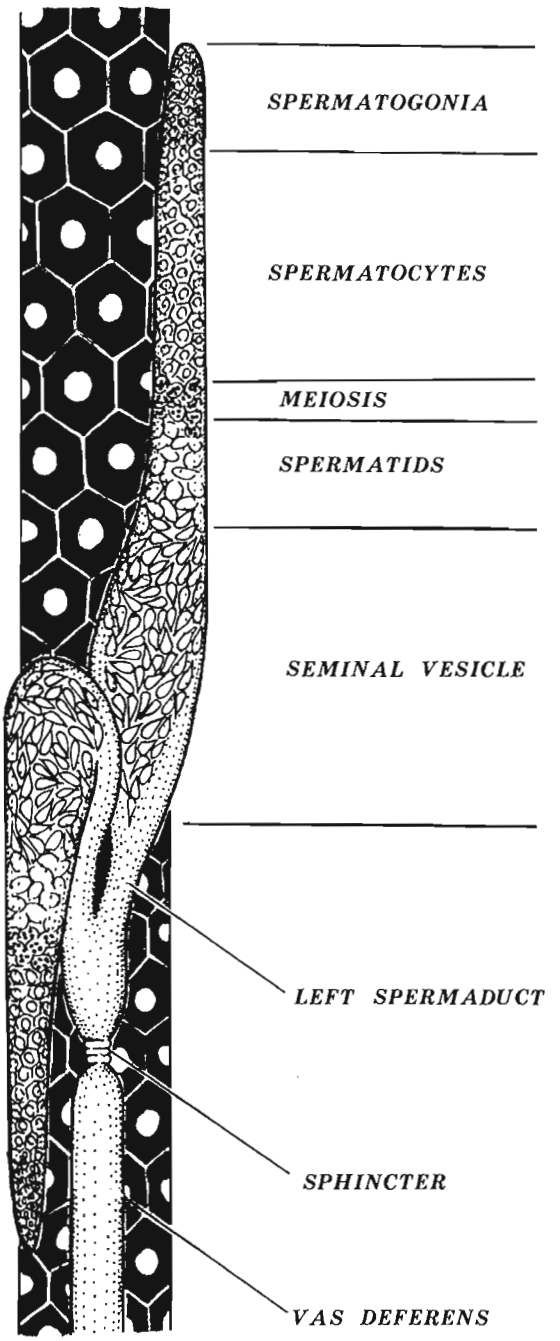

Fig. 1. *Schematic representation of the male gonads in* Enoplus anisospiculus.

meiosis marks the boundary between spermatocytes and spermatids, but this region often is not visible in wholemount specimens and semithin sections. There is no clear boundary between the adjacent spermatid and spermatozoon zones. The slightly dilated sperm-filled proximal part of the testis may be called the seminal vesicle. This seminal vesicle opens into a glandular spermaduct. The two spermaducts fuse into a single tube before the sphincter-like constriction of the gonoduct. The gonoduct is followed by a long glandular *vas deferens*.

Ultrastructure of sperm development

Spermatogonia

Spermatogonia are formed at the distal tip of the testis as undifferentiated polygonal cells, about 10 µm

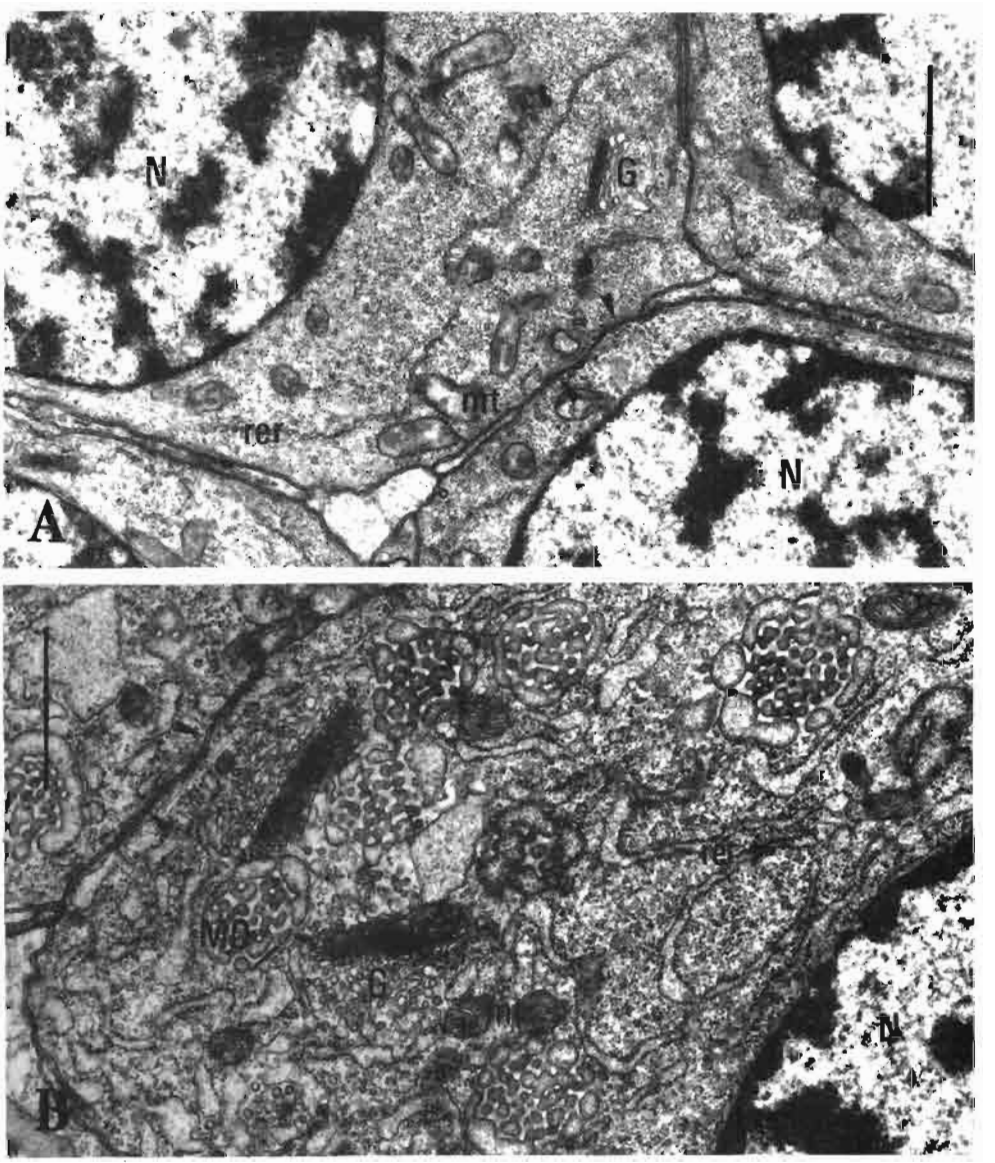

Fig. 2. *A: Two neighbouring spermatogonia; B: Cytoplasm of the young spermatocyte (type 1) with newly formed MOs. (Scale bar = 1 µm. Arrowhead: intercellular space between spermatogonia [A] and spermatocytes [B]. (Abbreviations: CH = nuclear chromatin; cy = cytoplasm; ep = ectoplasm; FB = fibrous body; fl = fibrous layer; G = Golgi body; mcb = main cell body; MO = membranous organelle; mt = mitochondrion; N = nucleus; np = nucleoplasm; RB = residual body; rer = rough endoplasmic reticulum; v = osmiophilic vesicle).*

in diameter, with a large spherical or slightly irregular nucleus about 8 μm in diameter. Distinct images of mitosis are rarely available from this region. The interphase nucleus of the spermatogonium has a nucleolus and small patches of condensed chromatin (Figs 2A; 9A). The cytoplasm contains mitochondria, ribosomes, occasional Golgi bodies, and cisternae of the rough endoplasmic reticulum (RER). The organelles seem to be concentrated in certain areas of the cell.

The wall of the testis in the germinal zone is made of a thin basal membrane and a layer of somatic cells. These cells have large irregular or triangular nuclei with condensed chromatin and electron-transparent cytoplasm containing rare mitochondria, Golgi bodies, and bundles of filaments. The somatic cells develop long narrow protuberances that penetrate between the spermatogonia and can surround individual or clustered spermatogonia. Both spermatogonia and spermatocytes are closely packed together and separated by narrow intercellular spaces 40-50 nm wide.

Spermatocytes

We failed to observe meiotic divisions anywhere along the testes of the individuals studied. Therefore, we were unable to precisely distinguish the primary and secondary spermatocytes. This part of the description concentrates on the cytoplasmic development of interphase germ cells.

In the region of completion of the mitotic divisions, organelles begin to proliferate in the cytoplasm of the germ cells (young primary spermatocytes). The cytoplasm become filled with numerous Golgi bodies, cisternae of the RER, ribosomes, and mitochondria (Figs 2B; 9B). The spermatocytes grow to a diameter of about 20 μm. Their nuclei grow to 10 μm in diameter and contain eccentric nucleoli and small patches of condensed chromatin.

When the organelles of young spermatocytes start to proliferate, young MOs appear in the cytoplasm. They are formed from the terminal cisternae of Golgi bodies closely associated with an extensive RER. Numerous mitochondria are also concentrated in the regions of this cytoplasmic activity. MOs increase in number during spermatocyte development, so that these organelles become the most conspicuous cytoplasmic component of secondary spermatocytes.

Development of MOs in spermatocytes

Close inspection of longitudinal sections through the distal halves of the testes suggests that the development of MOs proceeds along two parallel lines, which are characteristic of two different types of spermatocytes. These types are easily differentiated, even in semithin sections.

The first type of spermatocytes has Golgi bodies filled with an electron-transparent substance. These Golgi bodies form vesicles and cisternae that congeal into stable spherical bodies (young MOs), 0.8-0.9 μm in diameter (Figs 2B; 9B; 10A). At this stage, MOs have a complicated internal system of cisternae. In the sections, they appear as round porous plates. An electron-dense amorphous material is deposited on the membranes of the cisternae during development. MOs become darkly stained but retain membrane-bound compartments (Figs 3A; 10A). The content of the cisternae condenses greatly and sometimes appears as dense dots in sections through the cisternae (Figs 3B; 10A). In older spermatocytes, these cisternae appear to fuse and form a large eccentric electron-dense body that gives a distinct bipolarity to MOs (Figs 3C; 10A).

In the second type of spermatocytes, the cisternae of Golgi bodies are filled with an osmiophilic substance. The Golgi bodies form large vesicles, in which this electron-dense material is initially concentrated into an amorphous mass surrounded by an electron-transparent matrix (Figs 4A; 9C; 10B). Later, this material condenses into a spherical body, 0.5-0.9 μm in diameter, surrounded by a unit membrane. At the next stage of development, membranous cisternae (probably derived from Golgi bodies) form in an eccentric position inside developing MOs (Figs 4B; 10B). This gives bipolarity to these MOs, which thus become indistinguishable in structure from the MOs of the first line of development (Fig. 10C, D).

We saw no evidence of the formation of FBs in the spermatocytes of both types. No inclusions resembling FBs were found, either associated with MOs or free in the cytoplasm. The wall of the testis in the spermatocyte, spermatid, and immature sperm regions is made of flattened epithelial cells attached to the basal lamina.

Spermatids

The spermatid stage is limited to the period from the last meiotic division to the detachment of the residual body (Shepherd, 1981). Young spermatids are polygonal or slightly elongated cells, 20 μm in diameter, with spherical or oval nuclei without nucleoli. The nucleus has a distinct nuclear envelope that formed after the last meiotic division. The chromatin is condensed into randomly distributed dense particles. The cytoplasm is filled with Golgi bodies, cisternae of the RER, mitochondria, ribosomes, and MOs. The development of the spermatid includes three simultaneous and interrelated processes: *i*) collapse of the nucleus; *ii*) polarization and elongation of the cell; *iii*) segregation and detachment of the residual body.

When the nucleus begins collapsing, the cell elongates and becomes ovoid in outline. Initially, the

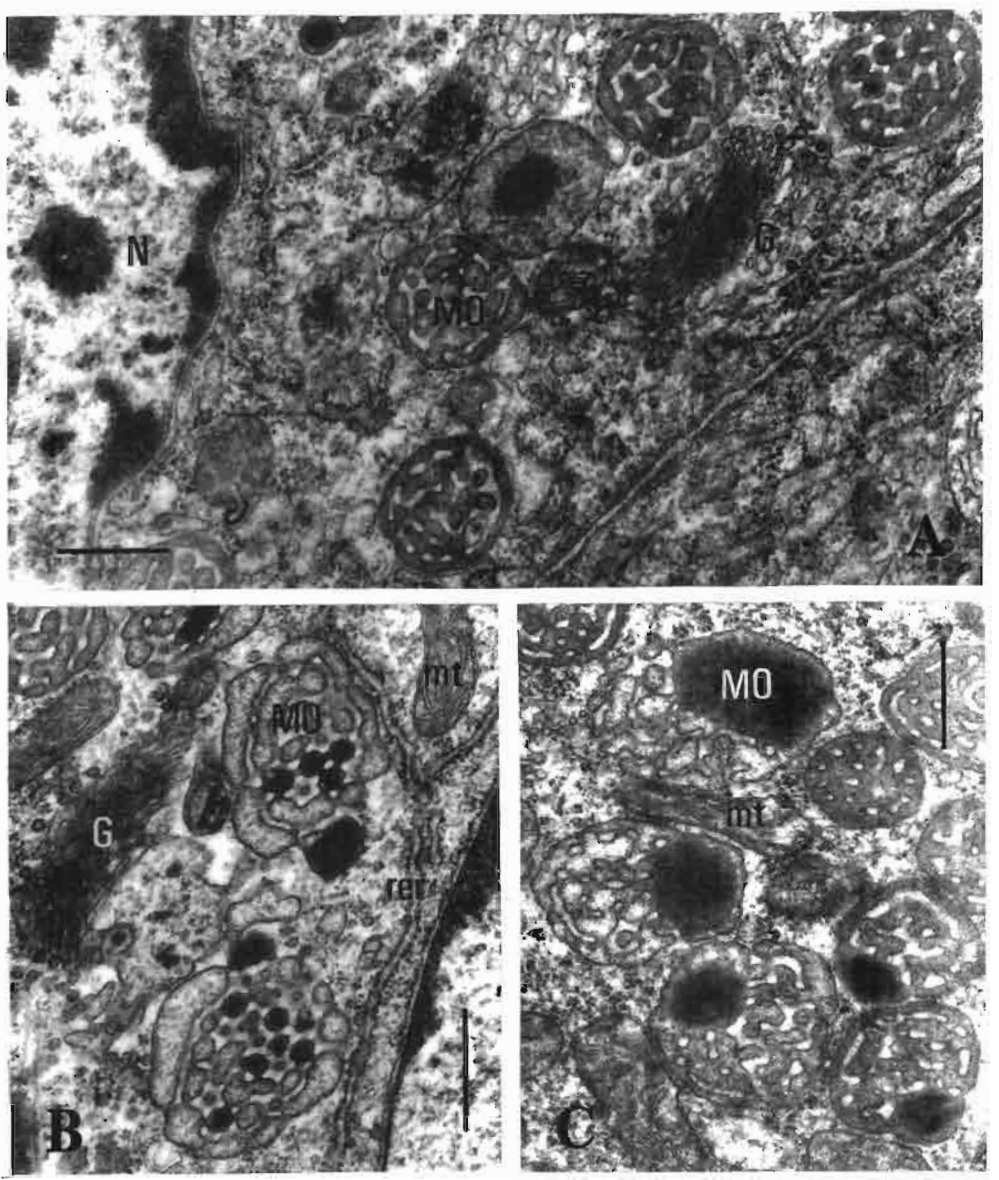

Fig. 3. *Development of MOs in type 1 spermatocytes. A: The electron-dense material is deposited inside the MO* cisternae*; B: Condensation of the MO content; C: The dense cisternae fuse to form a large eccentric body inside MOs. (Scale bar = 0.5 μm. For abbreviations see Fig. 2).*

nuclear chromatin is integrated into an irregular amorphous mass (Figs 4C; 5A; 9D) that later collapses to form a large electron-dense body extending along the longitudinal axis of the cell. At the beginning of the collapse, the voluminous electron-translucent nucleoplasm surrounding the nuclear material is bounded by a distinct nuclear envelope with irregular contour (Fig. 5 A). In young spermatids, the nuclear envelope is a clear double membrane that bounds the transparent perinuclear space. As the nucleus shrinks, the nuclear envelope transforms into an electron-dense lamina without a perinuclear space (Fig. 5B).

The collapse of the nucleus occurs together with a regrouping of MOs and mitochondria at the centre of the cell, around the nucleus. Most mitochondria move to the side of the nucleus that faces the future anterior

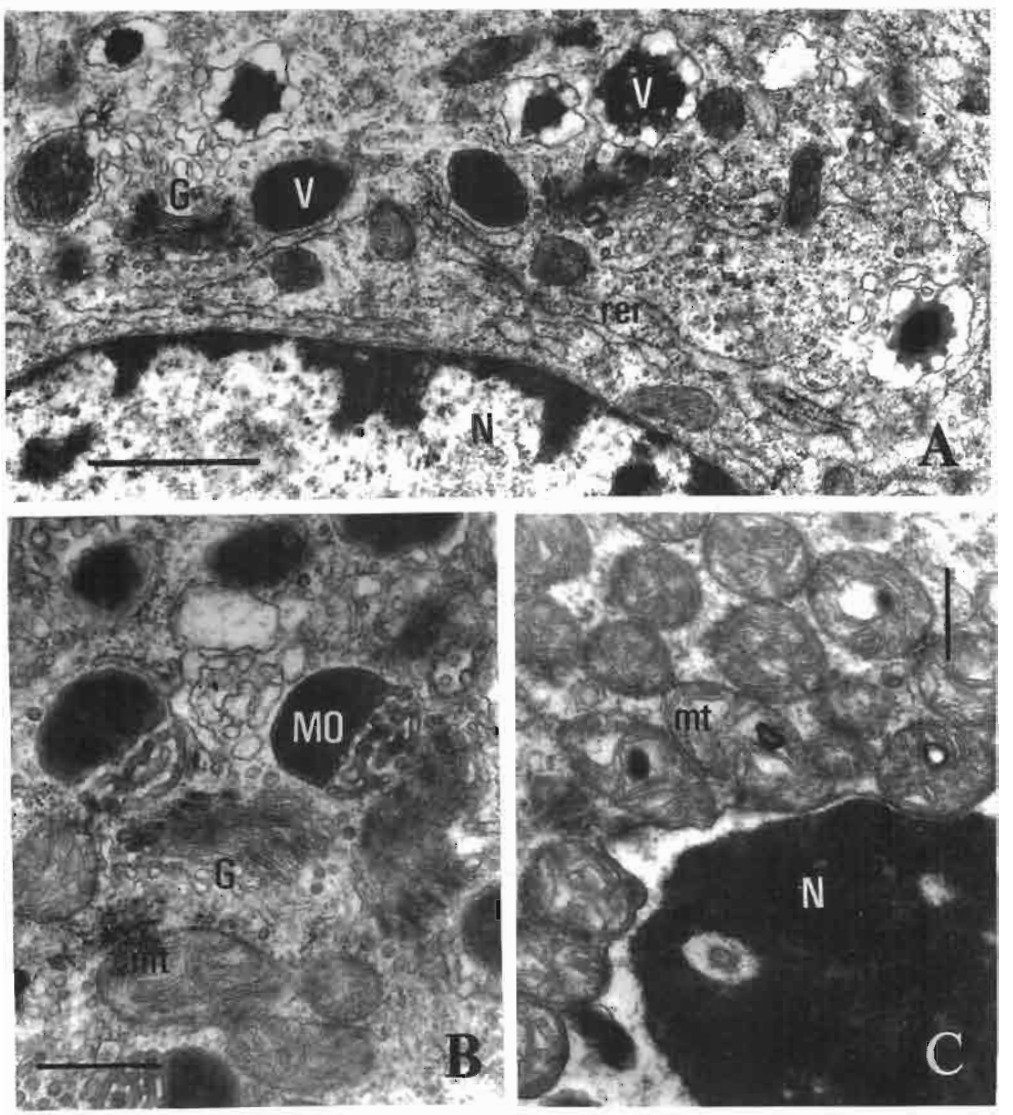

Fig. 4. *Type 2 spermatocytes. A: Cytoplasm of young spermatocyte, Golgi bodies form large vesicles (MO precursors) filled with electron-dense substance; B: System of MO* cisternae *formed in close association with Golgi bodies; C: The young spermatid, mitochondria are grouped at the anterior end of the nucleus. (Scale bars: A, C = 1 μm; B = 0.5 μm. For abbreviations see Fig. 2).*

pole of the cell (Figs 4C; 9D). This occurs at the same time as the wrinkling of the nuclear envelope and the displacement of the electron-translucent nucleoplasm. Mitochondria and MOs come into close contact with the nucleus (Figs 4C; 5B). Moreover, most MOs appear totally within the nuclear envelope (Figs 5C; 6A). Vacuoles containing one or several MOs are immersed into the nucleoplasm. In older spermatids, mitochondria and MOs form a compact mass of organelles anchored at the centre of the cell by the nuclear envelope (Fig. 9D). The voluminous external cytoplasm, with Golgi bodies, ribosomes, and the remnants of RER, surrounds this central complex of organelles.

As the organelles completely replace the transparent nucleoplasm, the nucleus elongates and the cell reaches 25 μm in length. At this stage, MOs are released from the nuclear envelope that now rearrange into narrow radial or irregular outfoldings interspersed with organelles (Fig. 6B). At this point, the

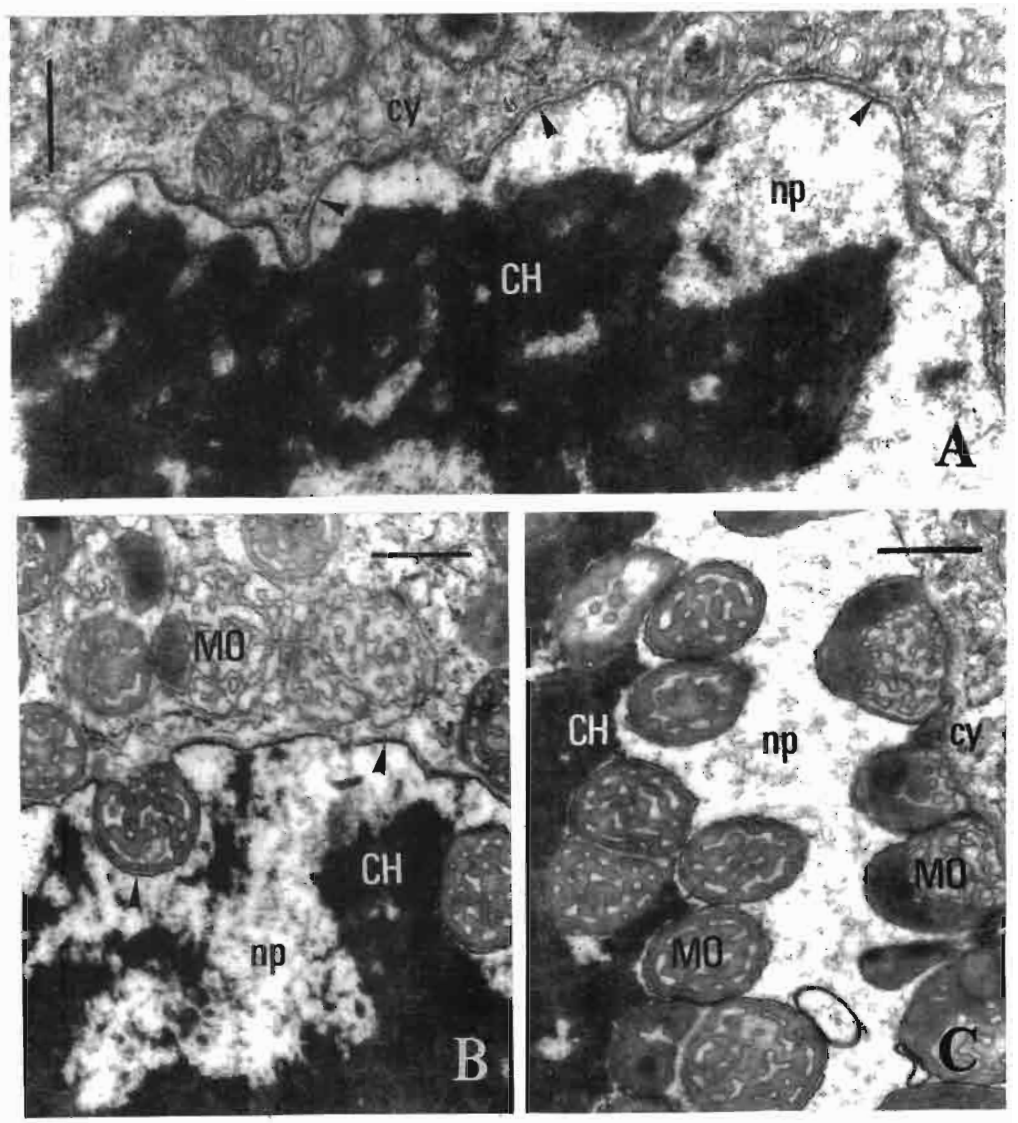

Fig. 5. *Young spermatids. A: Collapse of the nucleus, the double membrane of the nuclear envelope (arrowheads) is clearly visible; B: Occasional MOs come in contact with the nuclear envelope (arrowheads) and start to displace the nucleoplasm; C: Section showing the immersion of the MOs into the nucleoplasm, MOs on the right are in close association with nuclear envelope while MOs on the left are totally immersed (Scale bar = 0.5 μm. For abbreviations see Fig. 2).*

nucleoplasm is reduced to a narrow translucent space between the chromatin and the nuclear envelope. Most of the mitochondria form a layer at the anterior side of the nucleus and all MOs are positioned posteriorly just behind this mitochondria region.

FBs are clearly visible between mitochondria and nucleus in the older spermatids (Fig. 6B). These FBs are closely packed parallel filaments arranged into elongated cylinders radially oriented around the anterior part of the nucleus (Fig. 6B, C). The radial outfoldings of the nuclear envelope penetrate between FBs and overlying mitochondria (Fig. 6B, C). Occasionally, FBs and mitochondria also occur amongst the MOs in the posterior part of spermatids. A thin dense filamentous layer envelopes the compact complex of internal organelles, which separates these organelles from the external cytoplasm (Fig. 6B).

In young spermatids, the part of the cytoplasm that contains only cisternae of the RER is often segregated into large protuberances that disappear later in the

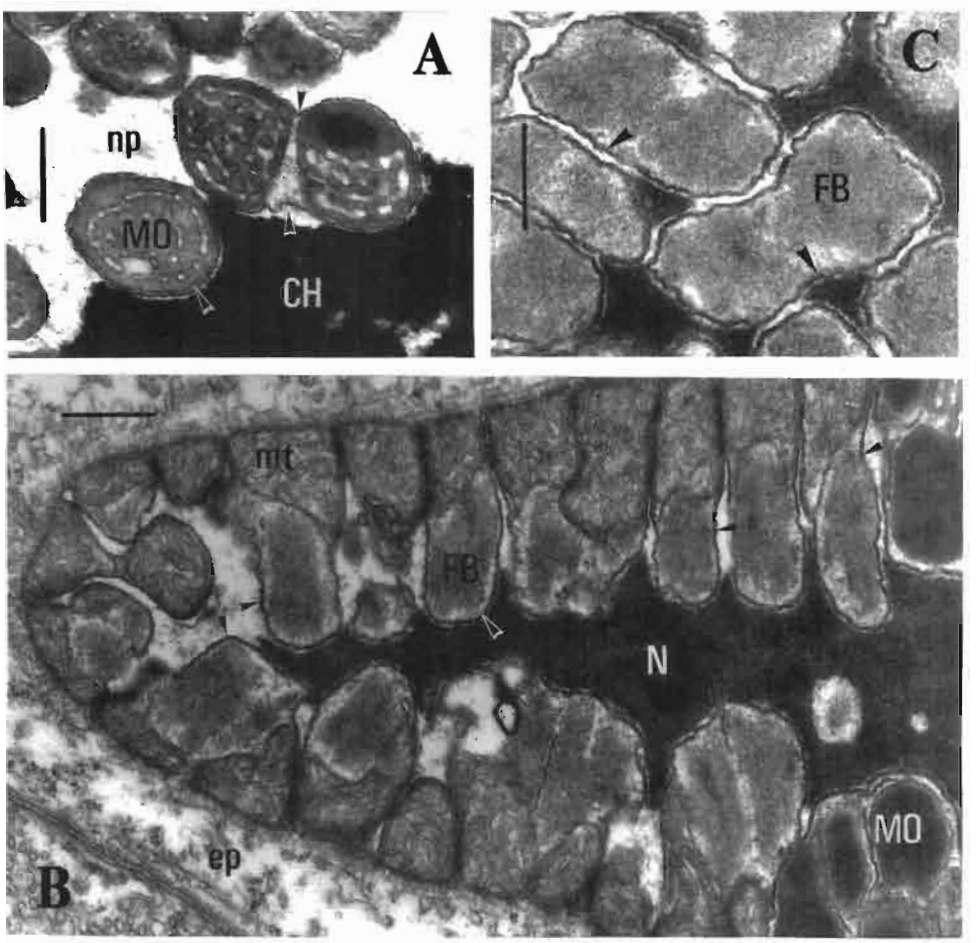

Fig. 6. *Spermatids. A: MOs surrounded by nuclear envelope (arrowheads) and associated with nuclear chromatin; B: anterior part of older spermatid (left to right = anterior to posterior; arrowheads indicate nuclear envelope); C: Transverse section through the FBs surrounded by the nuclear envelope (arrowheads). (Scale bars: A, C = 0.5 μm; B = 1 μm. For abbreviations see Fig. 2).*

development (Fig. 7A). This part of the cytoplasm is not a residual body because it does not detach and the remnants of RER are removed from the older spermatid with the true residual body. Initially, the residual cytoplasm is segregated at the periphery of the cell and surrounds the central complex of organelles (Figs 6B; 9D). Then, the residual cytoplasm with ruptured cisternae of the RER, large Golgi bodies, ribosomes, and numerous vesicles move to the anterior pole of the spermatid, where the residual body arises and finally detaches (Fig. 7B). Only a thin ectoplasm and a dense fibrous layer enclose the central complex of organelles in the newly formed spermatozoon.

Immature spermatozoon

The immature spermatozoa fill the dilated proximal regions (seminal vesicles) of both testes. The sperm cells are 25 μm long and conical in shape with a widened (up to 12 μm) anterior region. The centre of the sperm cell is occupied by a strongly condensed and usually elongate nucleus with a nuclear envelope, which is separated from the chromatin by a narrow electron-transparent space (Figs 7C; 9E). Numerous outfoldings of the nuclear envelope are interspersed with surrounding organelles.

The anterior part of the nucleus is surrounded by a layer of radially oriented FBs that are in contact with the external layer of mitochondria (Figs 7C; 9E; 10F). Sperm cells in the proximal part of the seminal vesicle have elongated and randomly distributed FBs (Fig. 8). The region of mitochondria and FBs terminates approximately at the middle of the nucleus. A mass of MOs surrounds the rest of the nucleus and fills the prominent posterior part of the spermatozoon. The outer bipolar MOs form a clearly arranged

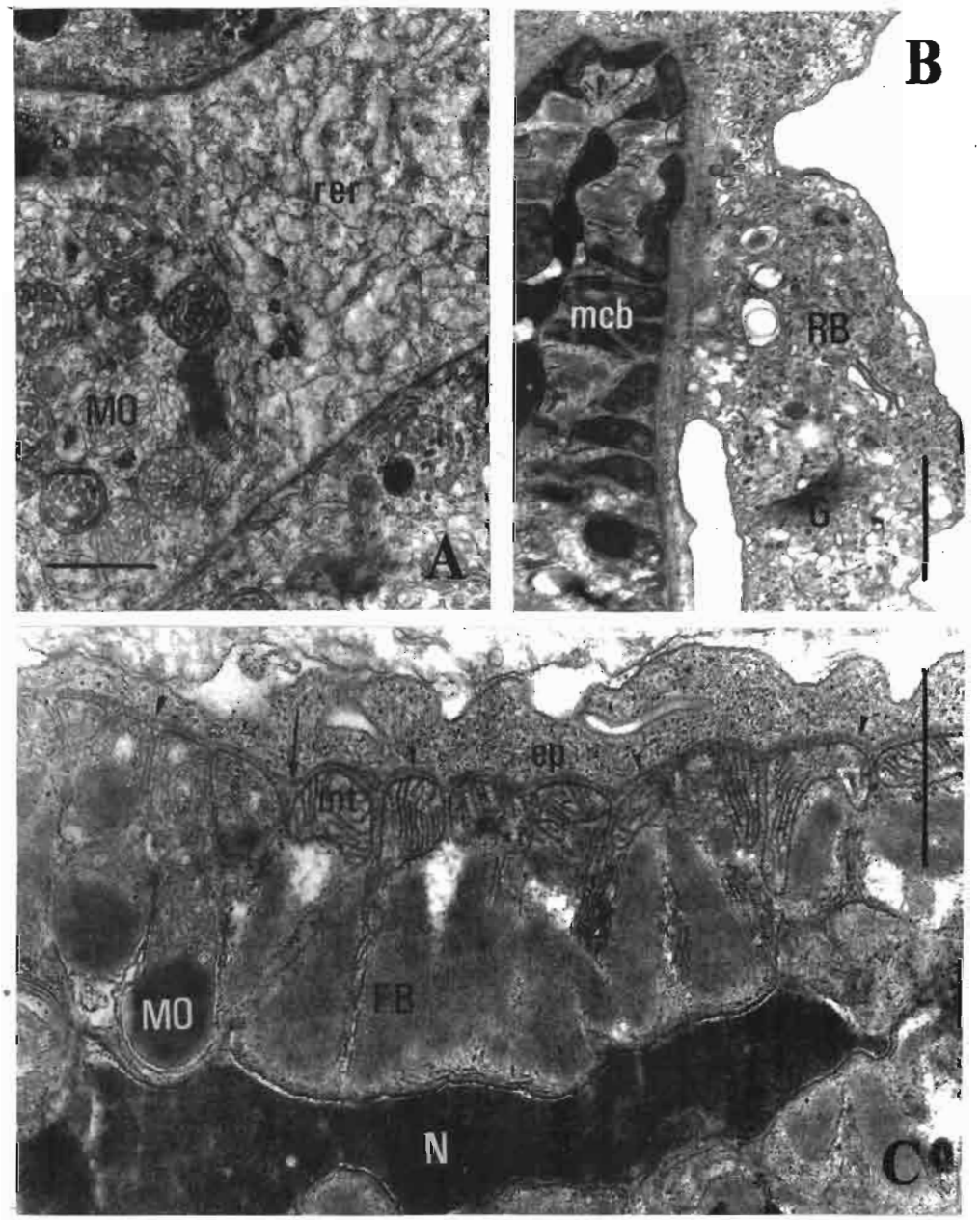

Fig. 7. *A: Part of young spermatid with cytoplasmic protuberance filled with cisternae of the RER; B: The residual body is forming at the anterior pole of the older spermatid; C: The anterior part of the immature spermatozoon (left to right = posterior to anterior); arrowheads indicate the fibrous layer separating internal organelles from the ectoplasm; arrow indicates the border between posterior mass of MOs and anterior mitochondria associated with underlying FBs. (Scale bar = 1 μm. For abbreviations see Fig. 2).*

layer where the MO poles with internal cisternae are oriented toward the surface of the cell. A few mitochondria and FBs occur between MOs in the posterior portion of the sperm cell.

The closely-packed cluster of sperm organelles is enveloped by a thin (50 nm) fibrous layer, which is contiguous with the ectoplasm (Figs 7C; 8; 9E; 10F). The latter is devoid of organelles and forms numerous

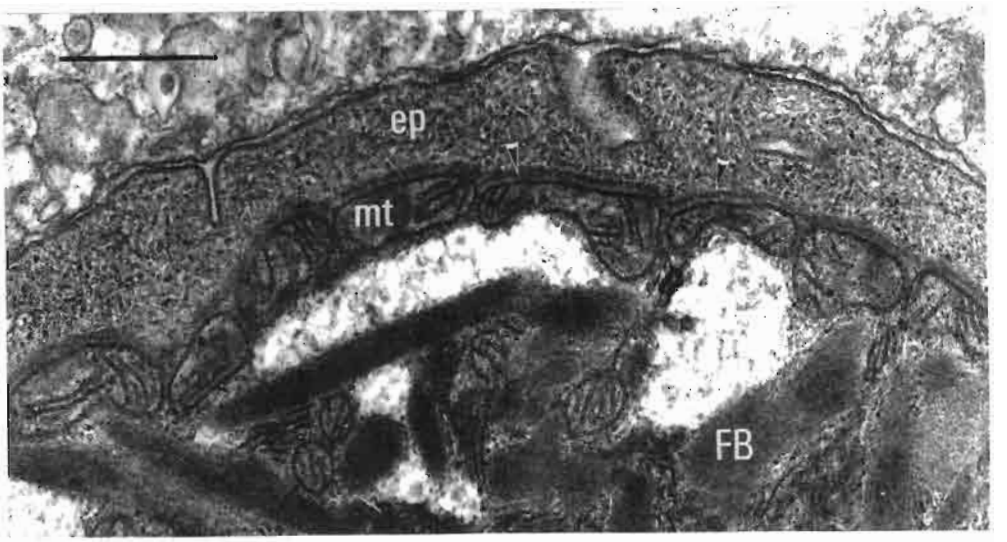

Fig. 8. *The anterior pole of the maturating spermatozoon from the distal part of the seminal vesicle, ectoplasm forms only short pseudopods filled with tube-like vesicles, note the disarrangement of FBs and mitochondria, arrowheads indicate fibrous layer. (Scale bar = 0.5 μm. For abbreviations see Fig. 2).*

short (0.3-0.5 μm) pseudopods all around the cell. The cytoplasm of these pseudopods is filled with numerous tube-like vesicles.

The immature spermatozoa described here seem to represent the final stage of sperm development in the testes. In the proximal region of the testis, the wall epithelium becomes distinctly glandular. There, the epithelial cells develop extensive RER, numerous Golgi bodies, and long protuberances filled with large secretory vesicles of filamentous content.

Discussion

The mature (*in utero*) spermatozoa of *E. anisospiculus* and *E. demani* were described previously as pseudopod-bearing cells with MCB containing nucleus, mitochondria, and MOs (Yushin & Malakhov, 1994). A very similar type of sperm also occurs in many orders of nematodes. However, the nuclear envelope is retained as a clear ancestral feature only in enoplids (Baccetti *et al.*, 1983; Yushin & Malakhov, 1994). Our ultrastructural data on spermatogenesis in *E. anisospiculus* show that the nuclear envelope is in fact reconstituted after the last meiotic division and the fate of the envelope may be traced throughout the stages of spermiogenesis. In the spermatids, the nuclear envelope comes into contact with mitochondria and captures the MOs to integrate future organelles of the sperm MCB. In the immature sperm, the nuclear envelope is also closely associated with radially arranged FBs. Thus, the nuclear envelope of enoplids is reconstituted to play the role of an integrating and anchoring structure for the organelles of future sperm.

The presence of the well developed pseudopod and typical MOs in mature spermatozoa of *Enoplus* spp. suggests that enoplids have to develop special MO-FB complexes in the cytoplasm of spermatocytes (Yushin & Malakhov, 1994). These complexes were described in many nematodes as a necessary component of the differentiating cytoplasm during spermatogenesis (Foor, 1983). As a rule, MO-FB complexes appear in the spermatocytes in the form of membranous vesicles and cisternae connected with growing paracrystalline FBs. In the spermatids, the MO-FB complexes dissociate into free FBs (later desegregated and transformed into the pseudopod cytoskeleton) and MOs, which attach to the plasma membrane and release their content via pores (Foor, 1983). This pattern of sperm development is usual for most of the species studied that belong to the subclass Rhabditia (McLaren, 1973; Shepherd & Clark, 1976; Wolf *et al.*, 1978; Ugwunna & Foor, 1982; Foor, 1983, and others) and for the only representative studied from the subclass Chromadoria (Noury-Srairi *et al.*, 1993).

The variations on this scheme in Rhabditia have been described for tylenchids and oxyurids. In *Meloidogyne incognita* (Tylenchida), the FBs (also the precursors of pseudopod cytoskeleton) are formed freely in the cytoplasm of the spermatocytes and no MOs were observed (Shepherd & Clark, 1983). The spermatogenesis of the oxyurid nematode *Aspiculuris*

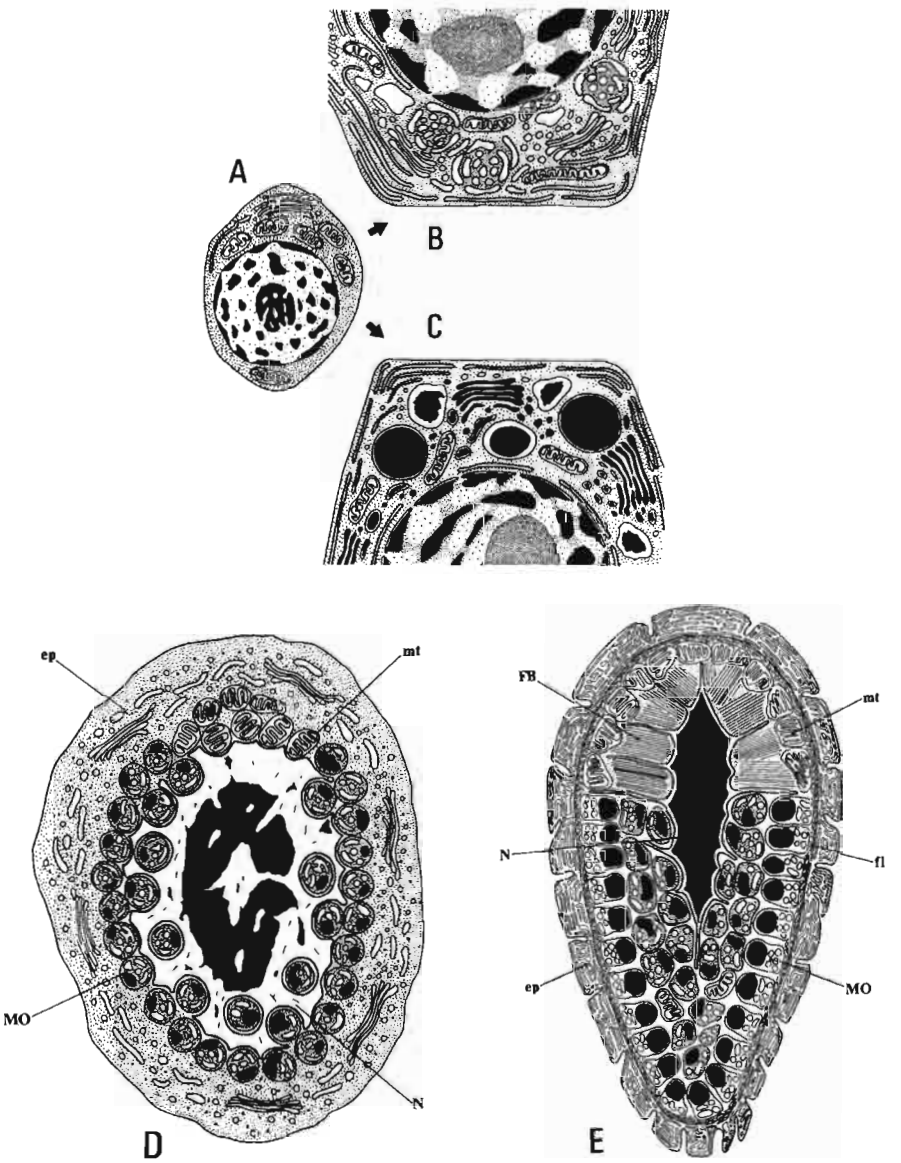

Fig. 9. *Schematic representation of the sperm development in the testis of* Enoplus anisospiculus*; A: Spermatogonia; B, C: Two types of young spermatocytes according to two lines of MO development (B: Type 1, MOs are formed initially as a spherical system of cisternae derived from Golgi bodies; C: Type 2, Golgi bodies form large electron-dense spheres surrounded by unit membrane); D: Young spermatid with condensing nucleus, anteriorly grouped mitochondria and MOs associated with shrunken nuclear envelope; E: Immature spermatozoon, anterior part of the nucleus is surrounded by radially oriented FBs and mitochondria, posterior part of the sperm is filled with MOs, thin fibrous layer delimits ectoplasm from the central cluster of organelles (For abbreviations see Fig. 2).*

tetraptera proceeds without MOs and FBs (Lee & Anya, 1967).

The subclass Enoplia shows great variability in cytological events during spermatogenesis. In *Xiphinema theresiae* (Dorylaimida), MOs and FBs appear in spermatocytes as separate cytoplasmic components (Kruger, 1991). Then the MOs disappear, but the FBs form the peripheral cytoskeleton of the unpolarized sperm cell.

In *Gastromermis* sp. (Mermithida), MOs begin developing from membrane-bound pockets only in immature spermatozoa (Poinar & Hess-Poinar, 1993).

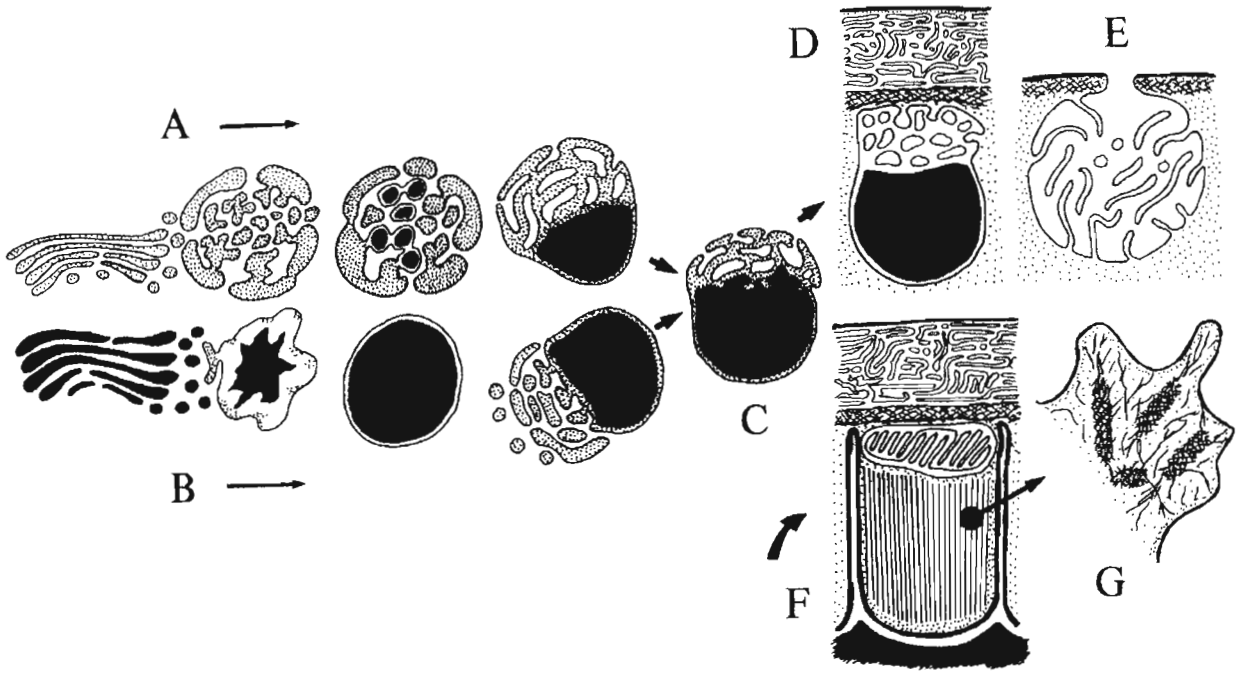

Fig. 10. *Schematic representation of the evolution of MOs and FBs during the sperm development in* Enoplus anisospiculus*; A, B: Two lines of MO formation in spermatocytes (A: Golgi bodies form the spherical system of cisternae, the content of* cisternae *condenses into several dense particles, which fuse to form the large eccentric electron-dense body; B: Golgi bodies form large vesicle with osmiophilic substance which condenses to form a large spherical body, then the system of the internal cisternae appears eccentrically inside the MO); C: Uniform bipolar MOs in older spermatocytes and spermatids; D: External MO in immature sperm, membranous part is opposed to the fibrous layer and ectoplasm; E: Emptied MO connected to plasma membrane of the mature (*in utero*) sperm; F: FBs (appearing initially only in older spermatid) are positioned in the immature sperm between the nucleus and the external layer of mitochondria; G: Large motile pseudopod of the mature sperm is filled with cytoskeleton proteins, possibly the derivatives of the FBs. (E and G are extrapolation after Yushin and Malakhov, 1994. For abbreviations see Fig. 2).*

No structures resembling FBs appear during spermatogenesis of *Gastromermis* sp., even though the mature sperm possesses a distinct pseudopod. This pseudopod is filled with microtubules that are unlike the filamentous content of the pseudopods described in other nematodes (Foor, 1983).

The spermatocytes of *Capillaria hepatica* (Trichurida) show significant metabolical activity, but neither MOs nor FBs were observed at this stage of spermatogenesis (Neill & Wright, 1973). In the immature sperm, the simple MOs appear as double membrane-bound vesicles formed by the fusion of small cisternae. In spite of the absence of FBs during sperm development, the spermatozoa of *C. hepatica* are bipolar, amoeboid, and possess an anterior pseudopod.

The mature sperm of *Dioctophyme renale* (Dioctophymida) have a small pseudopod, but no MOs and FBs were described in spermatocytes and spermatids (Foor, 1970, 1983).

Our data on spermatogenesis of *E. anisospiculus* provide no evidence of formation of MO-FB complexes at any stages of sperm development. The MOs appear in young spermatocytes in close contact with Golgi bodies and their development includes the accumulation of dense material, polarization, integration with nuclear envelope, and (after insemination) attachment to the plasma membrane (Figs 9, 10 of this paper; Yushin & Malakhov, 1994). The FBs appear only in older spermatids, but we failed to detect any involvement of MOs in the formation or release of the paracrystalline substance of FBs. In the immature spermatozoa of *E. anisospiculus,* radially arranged FBs occupy the anterior part of the cell and it is very likely that they have a storage function for the cytoskeletal proteins of the pseudopod of the mature sperm (Fig. 10F, G), as was demonstrated for many other nematodes (Foor, 1983). In any case, there are no FBs in the MCB of pseudopod-bearing spermatozoa

from the uterus of *E. anisospiculus* (Yushin & Malakhov, 1994).

Thus, the available data on spermatogenesis of representative members of Enoplia do not clarify the formation of the MO-FB complexes during sperm cell development because these specific structures were never observed in this subclass. Consequently, the MO-FB complexes found in the spermatocytes of Rhabditia and Chromadoria must be considered as a specific feature of these two subclasses. Additional observations on spermatogenesis of different representatives of Enoplia are necessary to elucidate the phylogenetic significance of different events in their sperm development.

Finally, we stress the ultrastructural peculiarities of the sperm development in *E. anisospiculus*: *i*) reconstitution of the nuclear envelope in the spermatid after the last meiotic division; *ii*) presence of a nuclear envelope functioning as an integrating structure for the organelles; *iii*) separate development of MOs and FBs without formation of MO-FB complexes; *iv*) presence of two types of MO development coinciding with two types of young spermatocytes; *v*) absence of FBs in spermatocytes and young spermatids and their appearance in older spermatids. These unique characters of spermatogenesis distinguish *E. anisospiculus* from other nematodes studied.

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