Structure and ultrastructure of spermatozoa of Chrysomya megacephala (Diptera: Calliphoridae)

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Abstract

The spermatozoa of Chrysomya megacephala are similar to those described for other Brachycera. In this species, the spermatozoa are long and thin, measuring about 590 µm in length, of which the head region measures approximately 60 µm. The head includes a monolayered acrosome with electron-lucid material, and the shape of the nucleus, in cross-sections, varies from circular to oval with completely condensed chromatin. The centriole was observed in the zone of flagellar implantation, below the "peg" region. In the region of overlap, the followings structures are observed: nucleus, centriolar adjunct, mitochondrial derivatives and axoneme. The two mitochondrial derivatives are of different lengths but similar diameter. The axoneme is of a conventional insectan type with a 9 + 9 + 2 microtubular arrangement, with accessory tubules flanked by the electron-dense intertubular material. The male internal reproductive tract consists of testis, vas deferens, seminal vesicle, accessory glands and ejaculatory duct.

Keywords: Entomology; Germ cells; Insect sperm; Microscopy; Reproductive system

1. Introduction

Approximately 150,000 species of flies are described in the world and more than 24,000 in the Neotropical region (Amorim et al., 2002). More than 1000 species of Calliphoridae are hitherto known, organized in 150 genera and 5 subfamilies: Calliphorinae, Mesembrinellinae, Chrysomyinae, Toxotarsinae and Rhiniinae (James, 1970 and Mello, 2003). In the neotropics about 130 species of blow flies are known (Carvalho and De Mello-Patiu, 2008). Calliphoridae (blow flies) present a great ecological diversity, occupying several habitats–from organic matter to animal tissues in decomposition, and are distributed worldwide. They cause medical problems and important losses to the animal industry (Zumpt, 1965, Ghandour, 1988 and David et al., 2008).

Chrysomya megacephala, the Oriental latrine fly, has been present in the dipteran fauna of Brasil since the mid 1975s, when it is believed to have been introduced, probably from Africa (Guimarães et al., 1978 and Guimarães et al., 1979). Greenberg, 1971 and Greenberg, 1973 reported that this species is among the most dangerous dipteran vectors of

enteric pathogens such as viruses, bacteria and helminthes. Nevertheless, in many regions of the world this species is an important pollinator (Anderson et al., 1982 and Castañeda-Vildózola et al., 1999).

Recently, studies have demonstrated that the structure and ultrastructure of internal reproductive organs and spermatozoa in insects (Jamieson et al., 1999) provide additional characters for taxonomic analysis and so can contribute to our understanding of relationships (Dallai et al., 1993, Dallai and Afzelius, 1995 and Carcupino et al., 1995).

The diversity in sperm structure in Diptera is greater than in all other insect groups taken together. Whereas the sperm structure of nematoceran Diptera is relatively well known, in the brachyceran group such studies are scarce. In the family Calliphoridae, only Calliphora vomitoria have been briefly described (Dallai and Afzelius, 1990). All the investigated species of brachyceran flies share a common spermatozoal model. There is an apical monolayered acrosome, a compact nucleus, fully crystallized mitochondrial derivatives and a 9 + 9 + 2 axoneme (Jamieson, 1987 and Jamieson et al., 1999).

The aim of this study is, therefore, to describe the ultrastructure of spermatozoa of Chrysomya megacephala and, in futures studies, make the comparison with others species from the family Calliphoridae.

2. Materials and methods

Chrysomya megacephala adult specimens were collected at the campus of the University of Brasilia (UnB), Brazil, to start a colony and begin laboratory analyses. The flies were attracted by fish carcasses and bovine minced meat, and collected with entomological nets.

In the laboratory, the flies were fed with a honey and water solution (1:1) and water ad libitum for 1 week and then provided with a 100 g bovine minced meat as an oviposition medium. Numerous eggs were found in the underside of the meat after 1 day and were placed 20 to a cup in tapered plastic, each containing 20 g of fresh bovine minced meat, until the pupariation and complete development (adult) stages.

2.1. Light microscopy

The flies were briefly submitted to cold-induced dormancy and the reproductive system of males was removed in its entirety by dissection of the animal in saline solution of

NaCl 0.9%. After dissection, these structures were placed on a slide for examination. Observations and image acquisitions were made using a Zeiss (SPEMI 2000C) stereomicroscope.

Testes used for transmission electron microscopy were also sectioned for light microscopy. Semi-thin sections (4 µm) were stained with 0.25% toluidine blue, pH 11, observed and photographed with an Axiophot Zeiss® Microscope equipped with a Zeiss® Axiocam MRc digital camera and Axiovision 4.5 software.

Males and females were dissected and their testes and spermathecae were smeared on clean glass microscope slides to release the sperm within them, and fixed in a solution of 4% (wt/vol) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. After drying at room temperature, the preparations were observed in a photomicroscope (Zeiss® Axiocam MRc digital camera and Axiovision 4.5 software) equipped with differential interference and contrast phases.

For measurement of sperm nucleus length, some of these preparations were stained for 15 min with 0.2 µg/ml 4,6-diamino-2-phenylindole (DAPI) in PBS, washed and mounted with a solution of N-Propil-Galato. They were then examined by Axiophot Zeiss® and Confocal Leica SP5 microscopes equipped with epifluorescence and with 405 nm excitation filters, respectively.

2.2. Transmission electron microscopy

The testes and spermathecae were fixed for 4 h in a solution containing 2.5% glutaraldehyde, 4% paraformaldehyde, 5 mM CaCl2 and 3% sucrose, buffered in 0.1 M sodium cacodylate, at pH 7.2. After fixation, the specimens were rinsed in the same buffer, and post-fixed in 1% osmium tetroxide, 0.8% potassium ferricyanide, and 5 mM CaCl2 in 0.1 M sodium cacodylate buffer. In some cases the specimens were fixed in a mixture of 2.5% glutaraldehyde, 1% tannic acid in 0.1 M phosphate buffer, at pH 7.2, followed by block-staining in 1% uranyl acetate in distilled water (Afzelius, 1988). The material was dehydrated in an ascending acetone series (30–100%) and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed using a Jeol® 1011 transmission electron microscope operating at 80 kV.

For detection of basic proteins, the ethanolic-phosphotungstic acid method (E-PTA), modified from Bloom and Aghajanian (1968), was applied. Testes were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 4 h at 4 °C. After washing in phosphate buffer and dehydrating in alcohol, the material was treated “en bloc” with a solution of 2% de

PTA in absolute ethanol for 24 h at 4 °C, washed in absolute ethanol and embedded in Spurr's resin. Some ultrathin sections were observed, unstained and partly stained with uranyl acetate.

3. Results

In sexually mature males of Chrysomya megacephala, the spermatozoa are stored in the testes and seminal vesicle. In the sexually mature females, these cells are stored in the spermathecae after copulation.

The male internal reproductive system in Chrysomya megacephala comprises the following organs: two testes, paired vasa deferentia and accessory glands, and one seminal vesicle and ejaculatory duct. Typically, each of the testes opens into the vasa deferentia, which then unites to connect with the seminal vesicle. The accessory glands are directly connected to the seminal vesicle and the ejaculatory duct is located at the end portion of this organ (Fig. 1A). The testes in this species have an intense and brilliant reddish-brown color. The color is light when the male fly emerges from the puparium and normal intensity is reached after sexual maturation. The arrangement of germ cells in the testes shows that this structure is formed by a unique and long follicle with several cysts (Fig. 1B). The testicle is made up of an external wall, which surrounds the germinative cells. The testicular wall is formed by a peritoneal sheath, a muscular layer, a basement membrane, a follicular epithelium, tracheoles, and an epithelium at the base of the follicle. The cytoplasm of peritoneal sheath is rich in rounded grains containing reddish-brown pigments, which gives the organ its characteristic color. When observed in transmission electron microscopy, these grains show different sizes and electron-density (Fig. 1C).

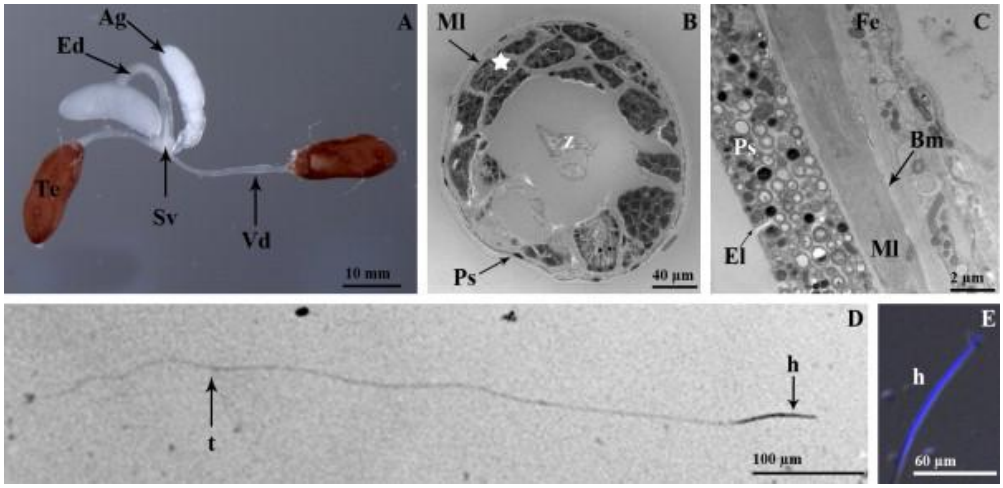

Fig. 1. (A and B) Light micrograph of male internal reproductive organs and structure of the testis in Chrysomya megacephala. (A) Male internal reproductive organs: testes (Te), vas deferens (Vd), seminal

vesicle (Sv), accessory glands (Ag) and ejaculatory duct (Ed). (B) Cross-section shows the morphology of testis wall, the internal organization of the cyst and the spermatozoa. Peritoneal sheath (Ps), layer of muscles (Ml), cyst in distinct stages of differentiation (star), spermatozoa (Z). (C) Transmission electron micrograph (TEM) of a cross-section of the testis wall. In the strata, forming the testis wall, it is possible to see: external layer (El); peritoneal sheath (Ps) with pigmented grains; layer of muscle (Ml) delimited by basement membrane (Bm) and follicular epithelium (Fe). (D and E) Interferential differential contrast micrograph of a spermatozoon and the DAPI-stained head region, respectively. Head (h) and tail (t).

The differentiation of the spermatids of Chrysomya megacephala occurs within cysts. Inside each cyst, the spermatic cells are perfectly aligned and in the same stage of maturation. The number of spermatozoa per bundle is variable, but approximately 128 cells are very commonly found in each cyst (Fig. 2A).

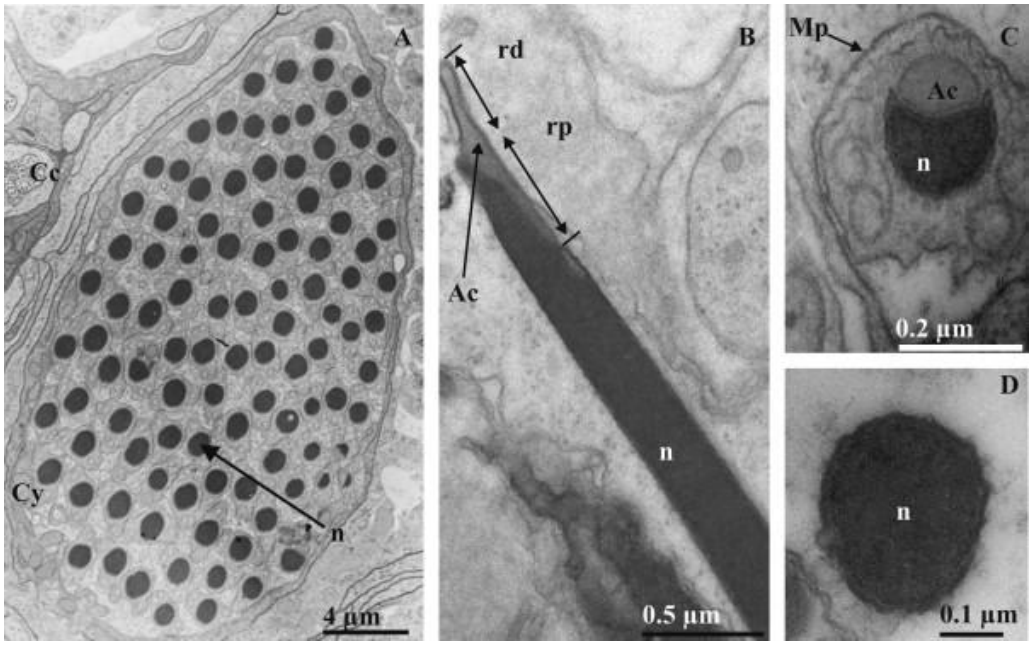

Fig. 2. (A–D) TEM of head region. (A) Cyst (Cy) showing spermatozoa head region. Cystic cells (Cc); nucleus (n). (B) Longitudinal view of acrosome (Ac) showing its two regions, distal (Rd) and proximal (Rp) and the nucleus (n). (C) In the proximal region of acrosome (Ac), the surface of this organelle is in contact with the nucleus (n). Plasmatic membrane (Mp). (D) The nuclear chromatin appears completely condensed. Nucleus (n).

The spermatozoon of the examined species (Fig. 1 and Fig. 5) is very long and filiform, measuring approximately 590 µm in total length, including the head and tail regions. The region of the head is about 60 µm in total length (Fig. 1E).

The head consists of an acrosome and nucleus. The acrosome is conical, monolayered and consists of a moderately electron-dense material. In longitudinal section, it fits closely into an indentation on the surface of the nucleus, and in cross-section it displays an elliptic substructure (Fig. 2 and Fig. 5).

The structure of the acrosome is divided into two regions: distal and proximal. The distal region (rd), comprising about 45% of the total length of the acrosome, is anterior to the nucleus, and the proximal region (pr) is closely associated with the nucleus (Fig. 2 and Fig. 5).

The nucleus of Chrysomya megacephala is long, measuring about 60 µm when stained for DAPI and examined by epifluorescence microscopy (Fig. 1E). It is fusiform and filled with condensed and uniform chromatin (Fig. 2B). When submitted to E-PTA methodology, the nucleus shows high concentrations of basic proteins (Fig. 4E). In cross-sections, the nucleus shape varies from circular to oval (Fig. 2, Fig. 3 and Fig. 5). During the last stages of spermiogenesis in Chrysomya megacephala, the nucleus shows a completely condensed chromatin, with many microtubules appearing around and it is possible to see the accessory membranes, in the cytoplasm of the cells (Fig. 3A). Since the initial stages of nuclear condensation, until the complete maturity of the cell, cross-sections reveal the presence of an accessory membrane adjacent to the nuclear envelope. There are two accessory membranes per nucleus, each on opposite sides of this organelle.

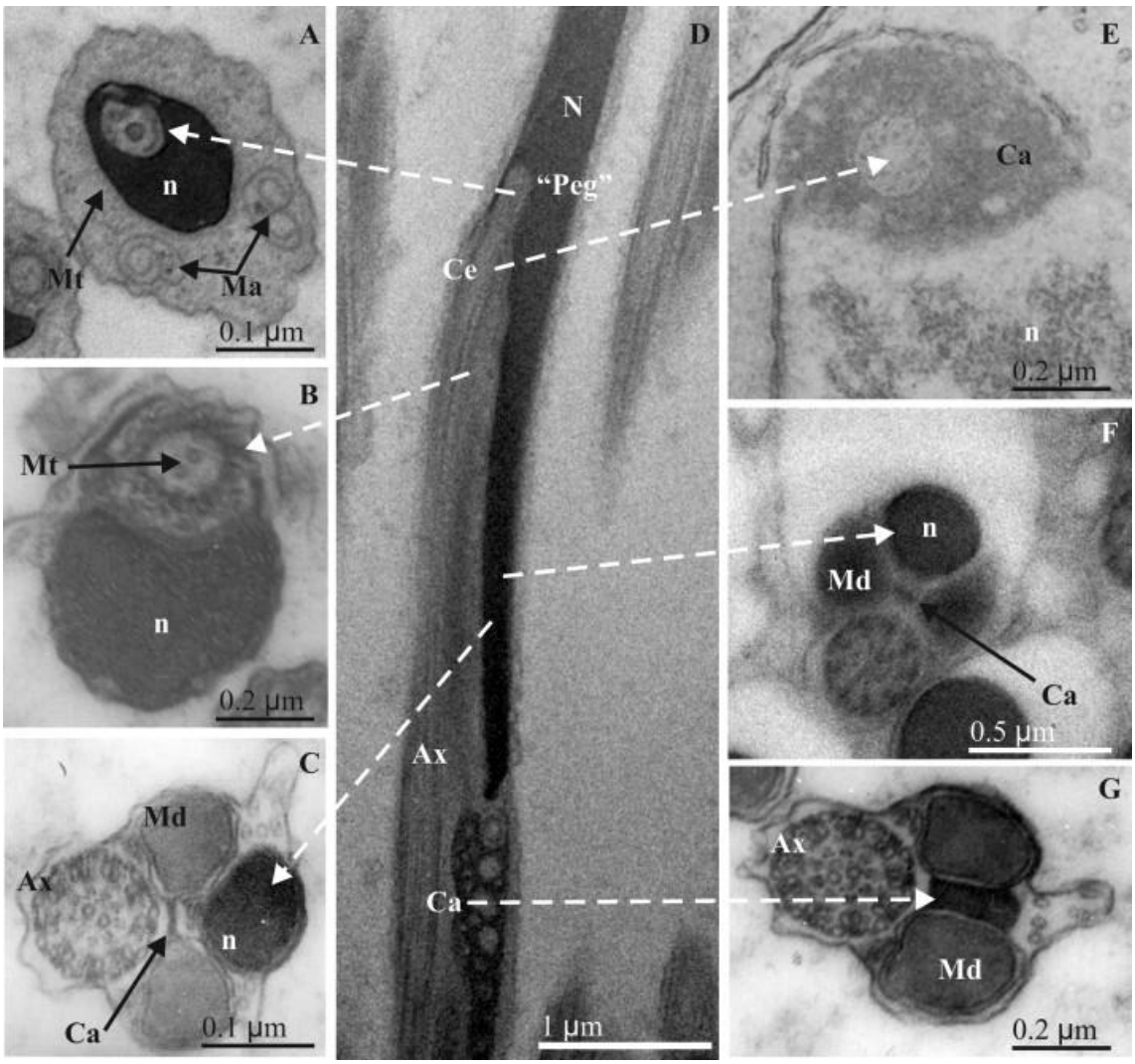

Fig. 3. (A–G) TEM of overlap region. (A) Region 1. In cross-sections, the cytoplasmic "Peg" the upper right portion of nucleus (n) can be seen. The accessory membranes (Ma) and microtubules (Mt) are visible in the cytoplasm. (B) Region 2. Showing the central pair of microtubules (Mt). Nucleus (n); axoneme (Ax). (C) Region 3. This region shows the complete axoneme (Ax), the two mitochondrial derivatives (Md), the nucleus (n) and the emergence of the centriolar adjunct (Ca). (D) Longitudinal view of overlap zone, showing emergence of axoneme (Ax) from small indentation of nucleus "Peg". Note the

notched appearance of nucleus (n). Centriolar adjunct (Ca) may be seen at the basis of the nucleus. (E) The centriole (Ce) is seen posteriorly to the nucleus (n). (F) Region 4. Centriolar adjunct (Ca) surrounded by nucleus (n), mitochondrial derivatives (Md) and axoneme (Ax). (G) The centriolar adjunct (Ca) can be seen between the mitochondrial derivatives (Md), also in the tail region.

The nucleus and the components of the tail, in the zone of overlap, could be seen in the same sections and consist of four different regions. The first region is recognizable in cross and longitudinal sections by the notched appearance of the nucleus, marked by the origin and emergence of the tail components. The tail begins in a small peg-like structure, where a “peg” of cytoplasm is completely surrounded by the nucleus (Fig. 3 and Fig. 5).

In region 2, an expansion of the “peg” of cytoplasm can be observed, showing a small and eccentrically located indentation of the nucleus. Its region is also characterized by the presence of microtubules, but they are not organized in either a centriole or an axoneme (Fig. 3 and Fig. 5).

In the initial stages of spermiogenesis, the centriole can be seen in cross-section as being located in the posterior head region in an indentation of the nucleus and surrounded by dense material, the centriolar adjunct (Fig. 3E). In the spermatozoon, this centriole is situated in the apical region of the axoneme, above the peg-like region (between the regions 2 and 3). The centriole in this species consists of nine triplets (Fig. 3D and E).

Region 3 is characterized by the emergence of the complete axoneme (with the 9 + 9 + 2 configuration of microtubules) and the two mitochondrial derivatives, associated with the nucleus. The mitochondrial derivatives are positioned side-by-side near the midline and ventral to the axoneme (Fig. 3 and Fig. 5).

In region 4 it is possible to see all the structures found in the overlap zone: the nucleus, axoneme, mitochondrial derivatives and centriolar adjunct. This structure is interpreted as being a support organelle, centrally located between the other organelles in the overlap zone in cross-section (Fig. 3 and Fig. 5). In this region, the nucleus appears in direct contact with the mitochondrial derivatives and the centriolar adjunct. The axoneme is always found at the opposite side of the nucleus (Fig. 3 and Fig. 5).

At the end of the overlap zone, and initial region of the flagellum, as the nucleus decreases progressively in size, the centriolar adjunct increases in proportion and eventually replaces the nucleus completely (Fig. 3 and Fig. 5). In the tail region, the major portion of the centriolar adjunct is seen under the nucleus (longitudinal section) and appears compact and very electron-dense (Fig. 3 and Fig. 5).

The mitochondrial derivatives are positioned side-by-side near the midline and ventral to the axoneme. Paracrystalline material occupies the entire interior of the derivatives (Fig. 4A and B), resembling a honeycomb structure in cross-section (Fig. 4B). These organelles follow

the axoneme along their length and one of them extends for almost the entire length of the spermatozoon, while the other is smaller, finishing early (Fig. 4 and Fig. 5). They differ in length, but not in diameter (Fig. 4 and Fig. 5).

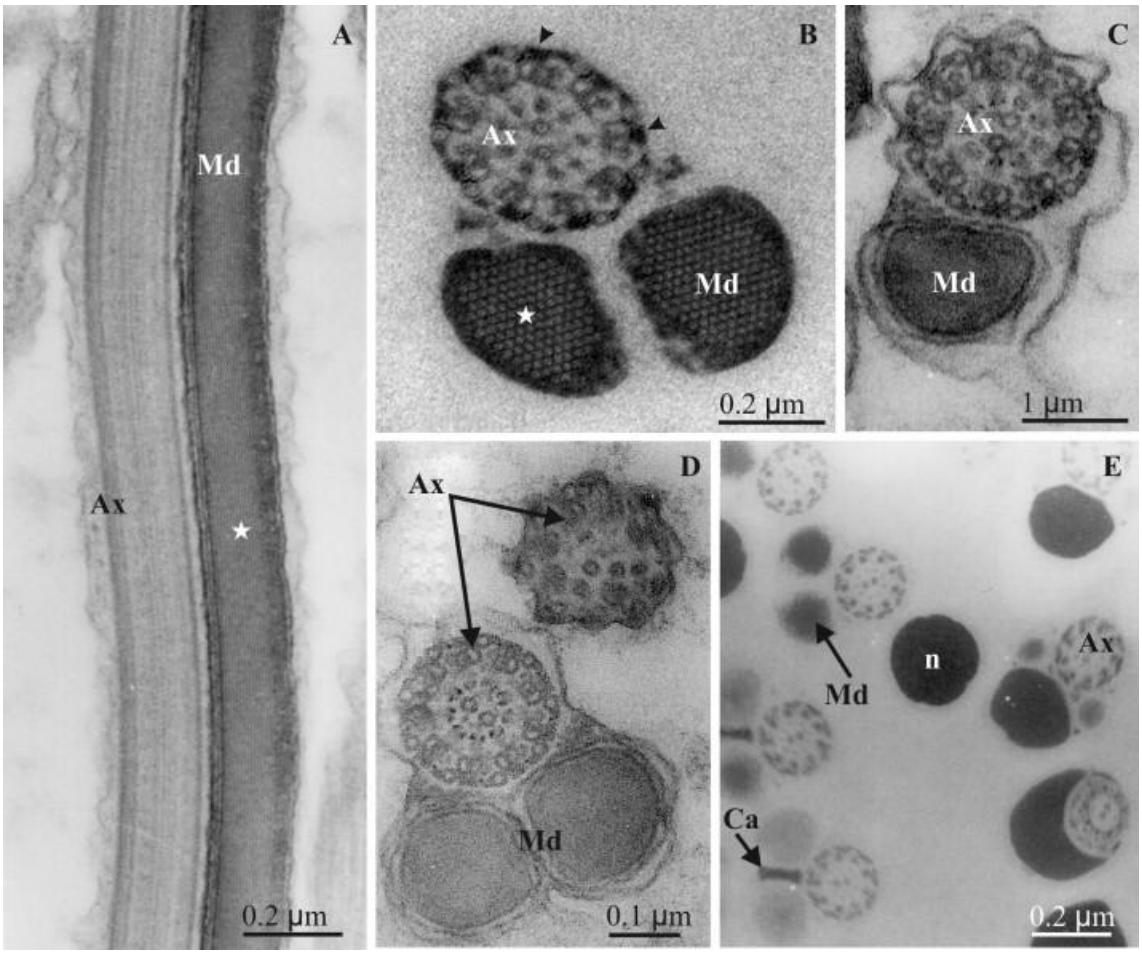

Fig. 4. (A–E) TEM of the tail region. (A) Mitochondrial derivatives (Md), filled with paracrystalline material (star), were seen in longitudinal section. Axoneme (Ax). (B) Spermatozoon obtained from spermatheca and fixed with tannic acid. The axoneme (Ax) is made up of nine accessory microtubules, nine doublets and a central pair. Dense fibers (arrows head) are seen between the accessory microtubules. Mitochondrial derivatives (Md). (C) In the posterior tip, it was possible to see one mitochondrial derivative (Md). Axoneme (Ax). (D) Final portion of flagellum showing the gradual disorganization of the axoneme (Ax). Note the accessory microtubules as the last to be lost at the axoneme tip. (E) Cross-section of spermatozoa treated with E-PTA. Nucleus (n), centriolar adjunct (Ca) and microtubules of axoneme (Ax) are E-PTA positive and the mitochondrial derivatives (Md) are sometimes positive.

Fig. 5. Schematic representation of the Chrysomya megacephala spermatozoon: (i) acrosome; (ii) nucleus; (iii) emergence of flagellum; (iv) overlap zone; (v) flagellar region; (vi and vii) endpiece of flagellum.

The axoneme displays the typical insectan pattern of 9 + 9 + 2 microtubules, with electron-dense material between the accessory tubules. The nine single accessory microtubules are the most external, followed internally by the nine doublets and a central pair (Fig. 4 and Fig. 5). The endpiece is characterized by a progressively reduced number of microtubular elements. In this species, the nine doublets finish first, followed by the two central microtubules and, finally by accessory ones (Fig. 4D).

The centriolar adjunct and the axoneme with all their structures are E-PTA positive. However, the mitochondrial derivatives only sometimes appear as E-PTA positive (Fig. 4E).

4. Discussion

The male internal reproductive organs in Chrysomya megacephala (Calliphoridae) comprise: testes, vas deferens, accessory glands, seminal vesicle, ejaculatory duct and resemble the pattern structure observed in other Diptera (Joly et al., 2003 and Sinclair et al.,

2007). Such reproductive organs in Diptera are poorly known, with few detailed comparative studies (Sinclair et al., 2007).

In most species of Diptera studied, the testis is considered to be a subdivided sac-like organ equivalent to tubular follicles observed in the testes of other insect orders (Williamson, 1989). In Drosophila (Drosophilidae), the most widely studied group in Diptera (Kiefer, 1966, Bairatti, 1967, Tokuyasu et al., 1972a, Tokuyasu et al., 1972b and Hicks et al., 1999), as well as in Ceratitis capitata (Tephritidae) (Guillén, 1983 and Báo and Dolder, 1991) and in Chrysomya megacephala, the spermatozoa derive from primordial germ cells located at the apical end of the testes, and these stem cells go through a synchronous processes of mitosis without cellular division to produce the spermatocytes. In contrast, patterns of cellular development are quite different in non-feeding oestrid flies. In the testis of the third instars of Hypoderma (Oestridae), an apical zone with a mass of cells was observed and the spermatogonial cysts are arranged around it basally (Boullard, 1967).

In C. capitata and Anastrepha ludens (Tephritidae) as well as in Chrysomya megacephala, the testes are made up of an external and pigmented wall which surrounds the germ cells. The testicular wall in both species consists of a peritoneal sheath and a muscular layer. In the peritoneal sheath it is possible to see the presence of a great number of vesicles containing pigment, which gives the organ its characteristic color. According to Báo and Dolder (1991), the pigmented epithelium is responsible for forming an outer physical barrier, protecting the testis and, for Valdez (2001), these vesicles could store nutrients temporally, en route from the blood to the transforming germ cells. The muscular layer is presumably associated with the peristaltic contractions, and possibly contributes to the displacement of the spermatid bundle and frees spermatozoa into the testes and toward the posterior duct, leading out of the testis. According to Chapman (1998), although the muscle layer is very common in some Diptera, it is absent in most insects.

The spermatids found in the testes, at the distal and medial regions of this organ, are encapsulated by a somatic cell, forming the cyst, where they completes the differentiation process. In the proximal region of the testes, near to the insertion of the seminal vesicle, these cells are free. These aspects of testicular organization were also described in some Tephritidae (Báo and Dolder, 1991 and Valdez, 2001), in Drosophila melanogaster (Kiefer, 1966, Bairatti, 1967, Tokuyasu et al., 1972a and Tokuyasu et al., 1972b), in Sarcophaga bullata (Sarcophagidae) (Warner, 1971) and in a large number of other insect orders (Phillips, 1970).

In Chrysomya megacephala, as in most insects, the development of the germinative cells takes place within such cysts (Phillips, 1970). According to Virkki (1969), archaic orders of

insects have a greater number of sperm per bundle than recent orders, in other words, the most recent or specialized groups tend to have a smaller number of sperm per bundle.

In most insects it was observed that the number of spermatozoa per bundle is variable. Research shows that for Diptera, the number of spermatids per bundle varies among different species depending on the number of spermatogonial premeiotic divisions (Oguma et al., 1987, Quagio-Grassiotto and Lello, 1996 and Cruz-Landim, 2001). For Aedes aegypti (Culicidae), it was suggested by Owusu-Daaku et al. (2007), that the maximum number of spermatozoa per cyst for this species is probably 512, being the result of nine divisions from the original spermatocyst mother cell (stem cell), according to what has been proposed by Virkki (1969). In the RA mutant of C. capitata the number of germ line cells observed was constant, approximately 250 per cyst (Báo and Dolder, 1990), the result of eight divisions. In Chrysomya megacephala, the number of sperm per cyst varies, but it is common to find 128 sperm per cyst. A similar feature was observed in S. bullata (seven divisions) (Warner, 1971). When compared with other groups, this feature is consistent with the current taxonomic and phylogenetic position of this species within Diptera.

The structure of spermatozoa in Chrysomya megacephala is similar to the general description for insect sperm (Phillips, 1970 and Baccetti, 1972; see also reviews by Jamieson, 1987 and Jamieson et al., 1999). They are filiform as in the majority of the Diptera and do not show great variations in length, as found in the spermatozoa of D. melanogaster (Joly et al., 1991, Pitnick et al., 1995 and Jamieson et al., 1999).

The monolayered condition of the acrosome observed in the spermatozoa of Chrysomya megacephala is apparently shared by all brachyceran spermatozoa already examined. According to Dallai et al. (1984), the acrosome in Diptera has different forms. In some families it appears small and devoid of perforatorium and extra-acrosomal layers and in other families it is an elongated organelle, partially lateral to the nucleus and including an internal crystalline fiber. In the studied species, the acrosome possesses the latter aspect, but the crystalline fiber was not observed. In cross-sections, this structure is elliptical, becoming more circular as the section nears the tip. The striated filaments or crystalline fibers occurring in the acrosome were observed in C. capitata (Báo et al., 1989) and S. bullata (Warner, 1971).

The chromatin of the nucleus is highly condensed in Chrysomya megacephala and this pattern is found in other Brachycera (Jamieson, 1987 and Jamieson et al., 1999). When treated with E-PTA, this organelle is homogeneous and completely positive. A peculiar characteristic, observed in the longitudinal and cross-sections of the nucleus, is the notched appearance of this structure, where the emergence of the tail components occurs. In the region know as overlap zone, basically all the organelles found in the tail (axoneme,

mitochondrial derivatives, centriolar adjunct) are seen in the same section. This characteristic was described in Megaselia scalaris (Phoridae) (Curtis et al., 1989) and is not very common in other families of insects.

The two accessory membranes observed laterally in the nucleus in cross-sections in Chrysomya megacephala, also appear in spermatids of Coelopa frigida (Coelopidae) (Schrankel and Schwalm, 1974) and named 'scroll-like structure'. These structures can be observed, in cross-sections of spermatids, as two membranes parallel two the nuclear envelope, extending along the nucleus. With increased condensation of the nucleus, the accessory membranes tend to disappear. According to Schrankel and Schwalm (1974), the function of these structures is unknown. The accessory membranes were described in C. capitata (Báo et al., 1989 and Báo and Dolder, 1990) and in S. bulatta (Warner, 1971), where the term 'whorl' has been used.

The centriole in the mature sperm was first proposed by Phillips (1970) as an organelle with nine microtubular triplets. The classical structure proposed by Phillips was found in D. melanogaster (Dallai and Afzelius, 1991), in C. capitata (Báo and Dolder, 1991) and is described here in Chrysomya megacephala. However, in some species this structure shows great variations. In Dacus oleae (Tephritidae), as in other Diptera an unusual configuration was observed for this structure: the presence of two central microtubules in the region of the centriole (Dallai and Afzelius, 1991).

In most insects, the nucleus is attached to the flagellum by a very electron-dense structure identified as the centriolar adjunct (Jamieson, 1987 and Jamieson et al., 1999). The centriolar adjunct in Chrysomya megacephala is relatively long, and was observed in the overlap region surrounded by the nucleus and flagellar structures (mitochondrial derivatives and axoneme) and extends into the region of the flagellum.

The mitochondrial derivatives in Chrysomya megacephala are symmetric in diameter, but asymmetric in length, while in Musca domestica (Muscidae) these structures are asymmetric in diameter and showing a considerable difference in length. In both species the derivative is completely filled with paracrystalline material (Gassner, 1970 and Gassner and Klemetson, 1981).

According to Dallai et al. (1993), the sperm structures of axoneme in Brachycera are relatively uniform, whereas in suborder Nematocera there is a considerable diversity, which is greater than that in any other insect order.

As expected for most Brachycera (Dallai et al., 1993 and Jamieson et al., 1999), Chrysomya megacephala have an axoneme with the 9 + 9 + 2 microtubules arranged parallel to

each other, and in fact, the pattern is similar to that described for most other insects (Dallai and Afzelius, 1990).

The end piece of flagellum in Chrysomya megacephala is characterized by a progressively reduced number of microtubular elements with the nine doublets finishing first, followed by the two central microtubules and with the accessory being the last ones to terminate. Unfortunately, the disorderly aspect of axoneme has not been taken into consideration in studies of Diptera spermatozoa. In some other groups, this parameter has been used as an important tool since it probably indicates a phylogenetic relationship (Zama et al., 2001 and Zama et al., 2005).

In the flagellar region of Chrysomya megacephala spermatozoon, the ethanolic-phosphotungstic method permitted the identification of different microtubule types in the axoneme, and in the centriolar adjunct structure. Basic proteins are associated with the central, peripheral and accessory microtubules and sometimes, to a very slight degree, with the mitochondrial derivatives. A similar aspect to the one described above for the tail region, was observed in Culex quinquefasciatus (Báo et al., 1992), where the centriolar adjunct, the mitochondrial derivatives and the microtubules are positive for the technique.

The similarity of sperm structure in Chrysomya megacephala to that of other brachycerans demonstrated here confirms the taxonomic and phylogenetic value of sperm ultrastructure.

Acknowledgements

K.P.O. Name would like to thank Professor J.A. Vexenat and J.S. Souza (UnB), for teaching me to capture and identify the Calliphoridae species. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Empreendimentos Científico e Tecnológico (FINATEC), Financiadora de Estudos e Projetos (FINEP) and Coordenação de Pessoal de Nível Superior (Capes).

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