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Ultrastructure features of spermiogenesis and spermatozoa in *Diplodus vulgaris* (Geoffroy saint-hilaire, 1817) 'Egypt'.

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Abstract

The ultrastructure of spermatogenesis in *Diplodus vulgaris* is described by using light and transmission electron microscopes. The testis is lobular in shape and spermatogensis is of unrestricted type. Spermatogonia occur isolated or in clusters within the seminiferous lobules. The germ cells are found in cysts formed by sertoli cell processes. Cells within cysts are found in the same developmental stage. Spermiogenesis is characterized by chromatin condensation, movement of the centrioles, flagellum development, nuclear rotation, nuclear indentation and nuclear fossa formation, reduction of the cytoplasm and differentiation of the flagellar complex. The spermatozoon has no acrosome and has an oval heterogeneously electron dense nucleus with a deep axial nuclear fossa and a nuclear notch. The nuclear fossa contains the centriolar complex and part of the basal body of the axoneme. Two fibrous bodies are attaching the proximal centriole to the nucleus. The proximal and distal centrioles are perpendicular to each other and lie at right angle to the base of the head. The short midpiece contains one large mitochondrial ring. The flagellum reveals a typical axonemal configuration with two single central and nine double peripheral microtubules. Ultrastructure of spermatozoa has most recently served as a criterion for taxonomic and phylogenetic classification between different species. The ultrastructural features of spermiogenesis and spermatozoa of *D. vulgaris* are discussed and comparisons are made between these features and those present in the available literature.

Keywords: Ultrastructure, Spermatogenesis, Spermiogenesis, Spermatozoa, D. vulgaris.

1. Introduction

Family Sparidae contains a number of economically important species throughout the world (Nelson, 1976). They are considering valued and popular species for both aquaculture and fisheries. Twelve species of this family are frequent in the landed catch from Alexandria waters (Ibrahim & Soliman, 1996).

Despite of the immense ultrastructure studies on fish spermiogensis the fine structure of many marine fishes still lack detailed investigation. By using both scanning and transmission electron microscopy; some important specific morphological differences can be found among wide spectrum of teleost spermatozoa which can be used for taxonomic purposes (Jamieson, 1991; Mattei, 1991; Mansour *et al*., 2002; Ferreira & Dolder, 2003; Lahnsteiner, 2003 and Lee *et al*., 2006).

The objectives of this study are to focus on the histological and ultrastructure of spermiogenesis and spermatozoa of *D. vulgaris* to improve the understanding of the reproduction of this important species and to compare the distinguished features with those available on other fish groups to provide useful systematic characteristics.

2. Materials and Methods

Samples of *D. vulgaris* were collected during the breeding season 'From November to February, 2008' (Mahmoud *et al*., 2010) from Abu Qir Bay.

Mature males of *D. vulgaris* (Geoffroy saint-hilaire, 1817) varying in length between 14 and 20 cm were collected for clarifying their ultrastructure during spermatogenesis. Immediately after dissection of the fish, the testis was cut into small pieces; those parts were fixed overnight at 4°C in 4% buffered glutaraldehyde made in 0.12 M cacodylate buffer (pH 7.3) for 2.5 h. Specimens were washed in 5% sucrose in 0.05 M cacodylate buffer (pH 7.3) three times, each one 15 min. postfixed in 1% osmium tetroxide in 0.2 M cacodylate buffer (pH 7.3), for 2 hr. at 4°C. Samples were then washed 3 times and dehydrated through graded alcohol series and acetone and embedded in Spurr's resin (Spurr, 1969). Afterward, thick plastic sections were cut using a LKB ultramicrotome with a glass knife and stained with toluidine blue. Sections were examined and photographed in JEOL JEM-100CX II TEM.

Semithin sections (1µm) were cut using a LKB ultramicrotome with a glass knife and stained with

toluidine blue. When appropriate regions were found, ultrathin sections were subsequently made and stained with drops of 2% uranyl acetate followed by lead citrate for 30 min. Then, these sections were examined and photographed with an Olympus CX41 digital camera.

3. Results

The testis of *D. vulgaris* is lobular in shape and the germ cells are arranged in cysts or clusters within the seminiferous lobules of the unrestricted spermatogonial type (Figure 1). Spermatogenesis occurs in several places along the length of each lobule and spermatogonia undergo numerous mitotic divisions producing cysts containing different developmental spermatogenic stages.

During the spawning period, all phases of spermatogenesis are observed. Depending upon the morphology and size of the nucleus, presence of organelles and centriolar morphology, particularly the pericentriolar structures, the developing stages of spermatogenesis could be described.

3.1. Spermatogonia

Spermatogonia are comparatively large cells. Their nucleus contains irregular batches of granular chromatin. There are numerous micronucleoli distributed close to the nuclear membrane (Figure 2A). When this material detached from the nuclear envelope, it forms dense granules that are either free (nuage) or associated with mitochondria (cement) as shown in Figure (2A). Mitochondria are unevenly distributed throughout the cytoplasm and the endoplasmic reticulum is concentrically organized in the peripheral cytoplasm.

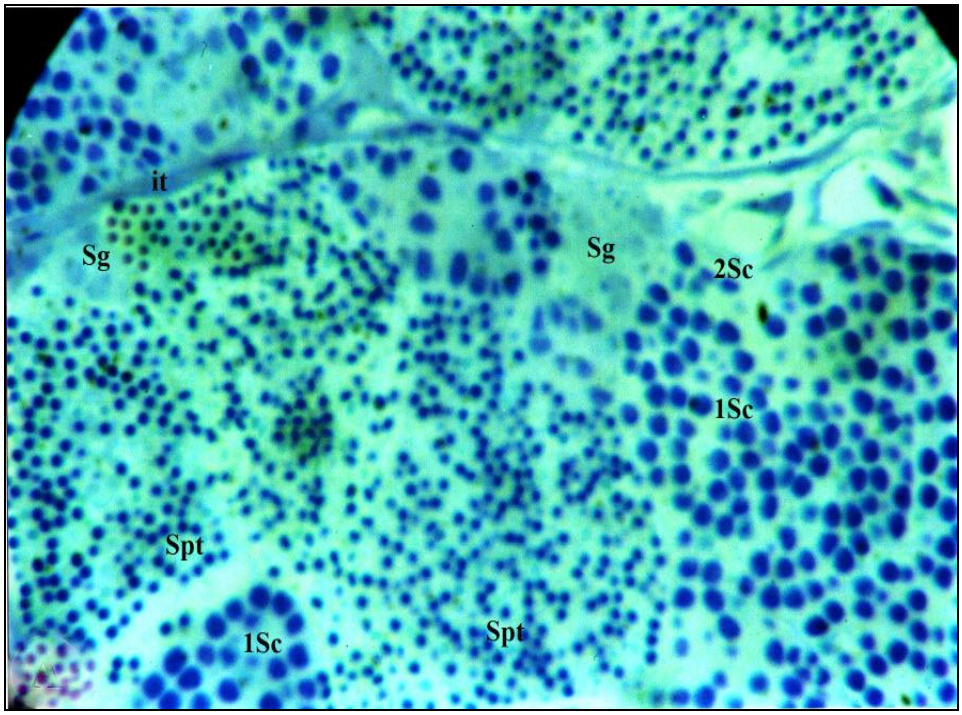


Figure 1: Light microscope micrographs of the testis of ripe male of *D. vulgaris* showing: Seminiferous lobules separated by interstitial tissue (it), groups of spermatogonia (Sg), primary spermatocytes (1Sc), secondary spermatocytes (2Sc) and spermatids (Spt) (X100).

3.2. Spermatocytes

Spermatocytes are found in cysts surrounded by sertoli cell processes. Primary spermatocytes are the largest spermatogenic cells; they are distinguished by having pale cytoplasm, large nucleus with dense granular chromatin. The nucleus appears clumped or slightly mottled in shape and the cytoplasm connected with the nucleus through the nuclear pores (np) as shown in Figure (2B). Mitochondria are distributed among the cytoplasm which is filled by free ribosomes and cisternae of endoplasmic reticulum.

Secondary spermatocytes are comparatively smaller in size with relatively smaller nucleus. The cytoplasm forms a narrow strand irregular in shape. The centriolar complex appears close to the plasma membrane to form the flagellum, mitochondria cells appeared to be distributed among the cytoplasm (Figure 3A). The heterochromatin is more condensed at places where the nuclear envelopes tend to bulge out, where the nuclear division precedes the cytoplasmic division, the nucleolus is not recognized (Figure 3A).

Sertoli cells have oval and large nucleus which contain marginal heterochromatin blocks and the

cytoplasm of these cells contain endoplasmic reticulum, ribosomes, Golgi complex, bundles of microfilaments and microtubules and mitochondria with dense matrix (Figure 3B).

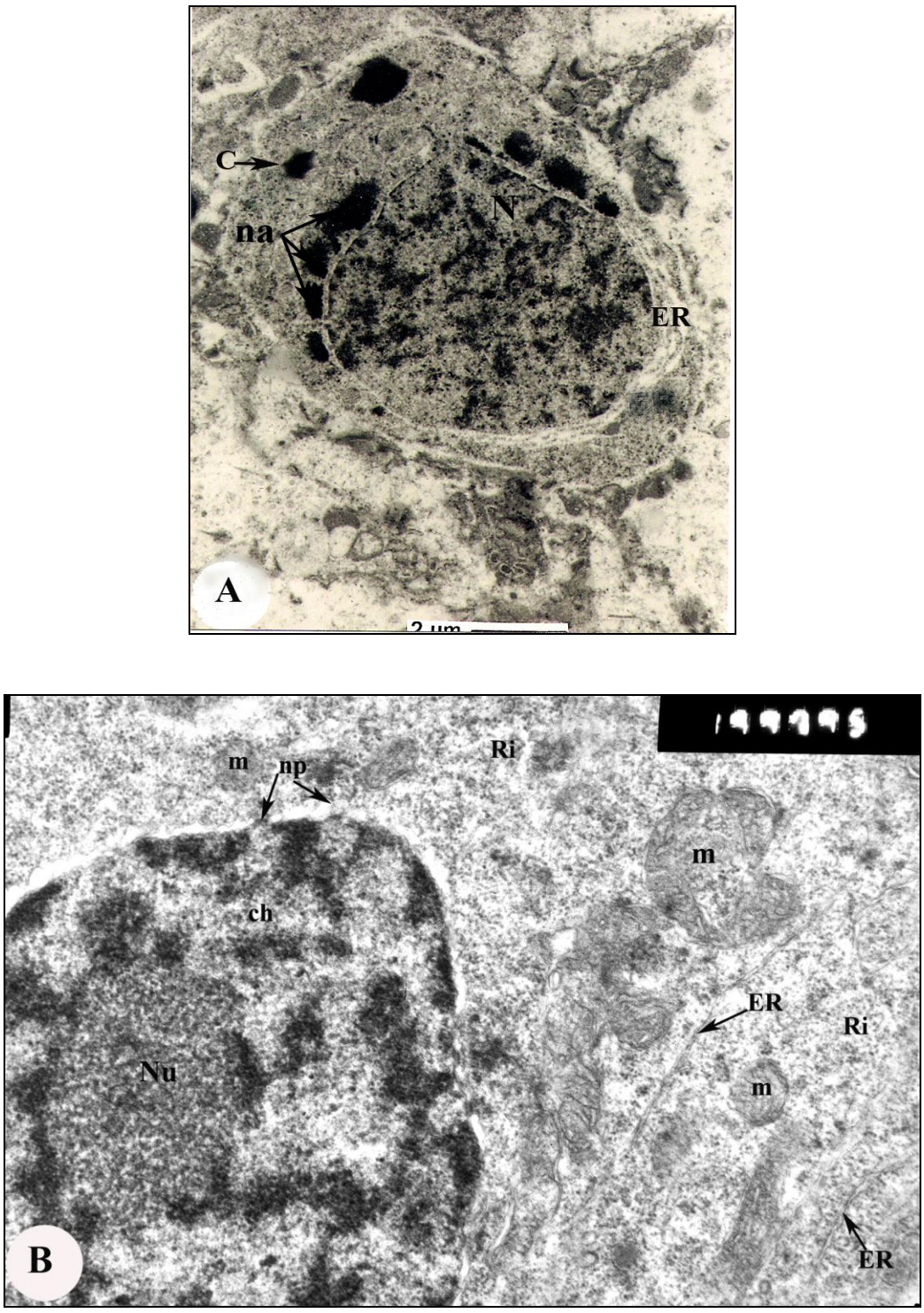


Figure 2: Transmission electron micrographs (TEM) showing: **A.** Spermatogonia of *D. vulgaris* with numerous micronucleoli (nuage 'na') distributed close to the nuclear membrane, cement (C) and Endoplasmic reticulum (ER) are noticed. **B.** The primary spermatocyte with large nucleus which contains accentric nucleolus (Nu) and chromatin blocks (ch). Note: nuclear pores (np), mitochondria (m), ribosomes (Ri) and endoplasmic reticulum (ER) (X13000).

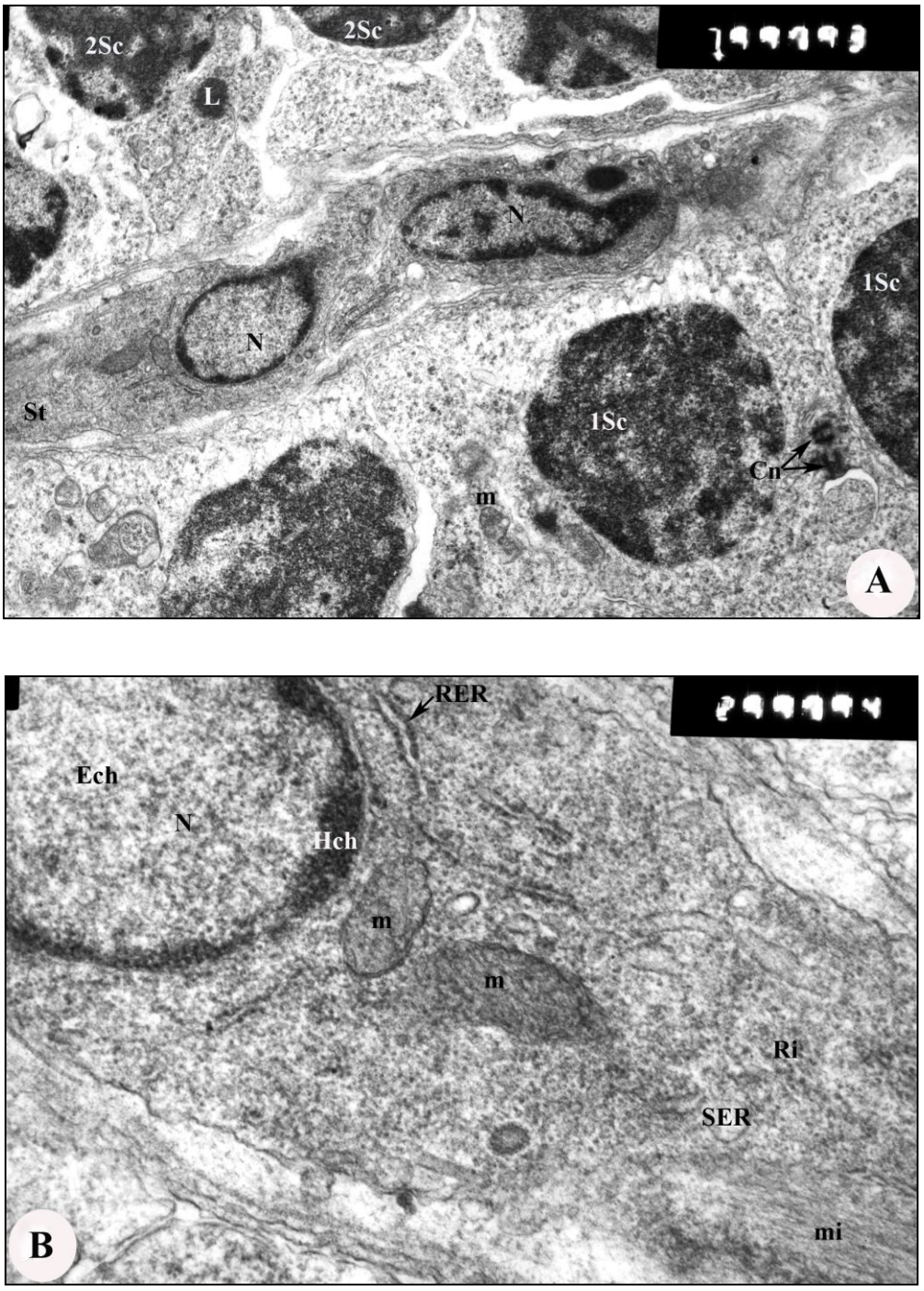


Figure 3: Transmission electron micrographs (TEM) of ripe male of *D. vulgaris* showing: **A.** Cyst of primary Spermatocytes (1Sc) and secondary Spermatocytes (2Sc) surrounded by Sertoli cell (St). Note, mitochondria (m) and lysosome (L) appear in the cytoplasm (X7500). **B.** The nucleus (N) of the Sertoli cell with heterochromatin (Hch) and Euchromatin (Ech) and surrounded by cytoplasm with ribosomes (Ri), smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), mitochondria (m), bundles of microfilament and microtubules (mi) (X25000).

3.3. Spermiogenesis

The process of spermiogenesis is usually identified by polarization of spermatid cell, formation of the flagellum, rotation and condensation of the nucleus, the location of the centrosomes and the depletion of the cytoplasm.

Early spermatids are spherical with a small round nucleus with chromatin in small electron dense chromatin batches of heterogeneous density and cytoplasm filled by inconspicuous ribosomes (Figure 4A). They remain interconnected by cytoplasmic bridges.

In the middle stage of spermatid formation the centrioles migrate to the basal pole of the nucleus and some mitochondria of spherical or ovoid shape are located near the centrioles. Formation of the centrosome nucleus complex reveals obvious polarity of the spermatids (Figure 4B). The two centrioles are arranged at right angles to each other and appear to be interconnected by osmiophilic filaments. Both centrioles lie close to the plasma membrane and the distal centriole starts to form the flagellum (Figures 4B & 5A).

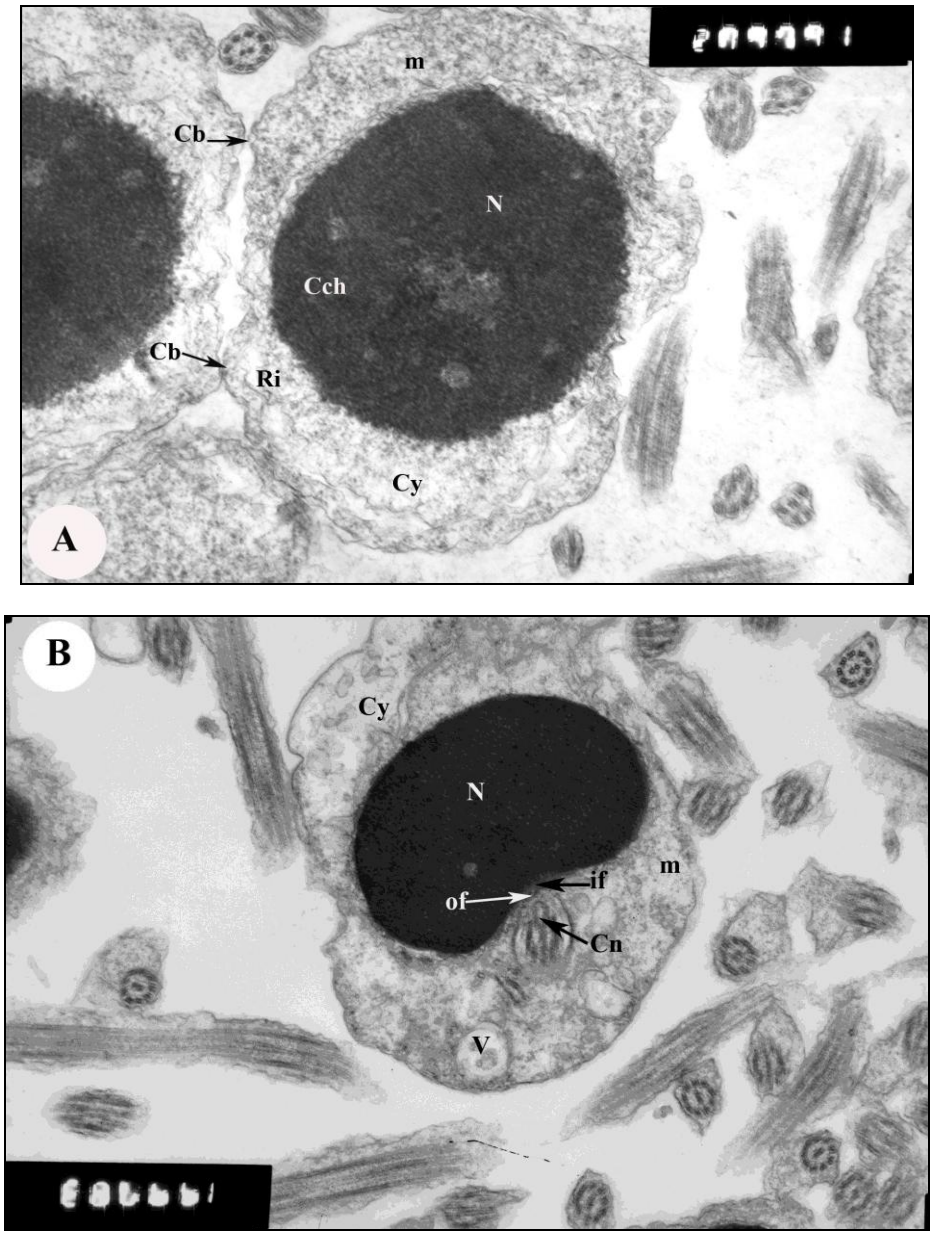
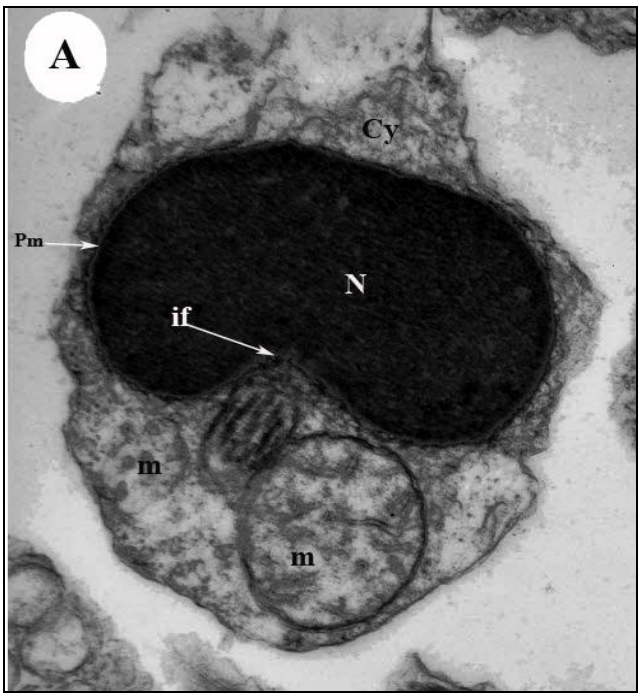


Figure 4: TEM showing **A.** The early stage of spermatide formation. Note: A small round nucleus (N) with condensed granular chromatin (Cch), cytoplasm (Cy) depletion which filled by inconspicuous ribosomes (Ri) and Cytoplasmic bridges (Cb) (X20000). **B.** Middle stage of spermatide formation the implantation fossa (if) starts to appear and the two centrioles (Cn) are fixed by an electron dense filament (of) and the distal centriole starts to form the flagellum. Mitochondria (m) and vacuoles (V) in spermatid's cytoplasm were noted (X15000).

The mitochondria aggregate and their distribution are irregular on both sides of the midpiece around the base of the flagellum. The axoneme then detaches itself from the perinuclear region, bringing with it the plasma membrane (Figure 5A). Golgi apparatus appears as vesicular cisternae. The nucleus is electron dense with prominent batches of fine and coarse granular heterochromatin (Figure 5B).

The cell outline appears very irregular in shape and cytoplasmic bridges become narrower than in previous stages (Figure 5B). The cells display a finely granular appearance because of the homogeneity of chromatin. The nucleus becomes indented and a nuclear fossa is formed as a depression in the nucleus. Due to the rotation of the nucleus (90°) the diplosome flagellar axis becomes perpendicular to the base of the nucleus (Figure 5B).



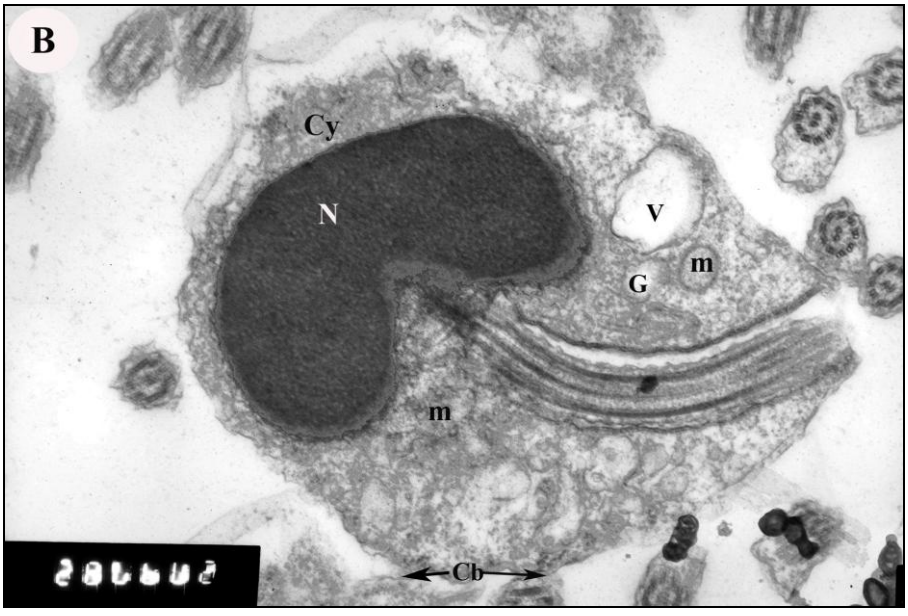


Figure 5: TEM showing polarity of spermatid**. A.** The mitochondria (m) in the middle stage of spermatide formation are aggregate on both sides of the midpiece around the base of the flagellum. The implantation fossa (if) and the plasma membrane (Pm) are appear clearly (20000). **B.**The nucleus (N) of late stage of spermatide formation is electron dense with finely granular appearance duo to the homogeneity of chromatin. Flagellum starts to be elongated. Golgi apparatus (G), mitochondria and vacuoles in depleted cytoplasm and narrow cytoplasmic bridges (Cb) are noticed (X20000).

In late stage of spermatid formation the proximal centriole is located within the nuclear fossa. A nuclear notch appears in the region between the proximal and distal centriols (Figure 6A). In addition, two fibrous bodies appear perpendicular to each other above the proximal centriole within the nuclear fossa (Figures 6A & 7B). The two bodies are interconnected with osmiophilic filaments and the upper one attaches to the nucleus with two bands of osmiophilic filaments; similarly the lower body connects with the proximal centriole. Moreover, a basal foot appears laterally in the basal part of the distal centriole and anchors it to the nucleus (Figures 6A & 7B).

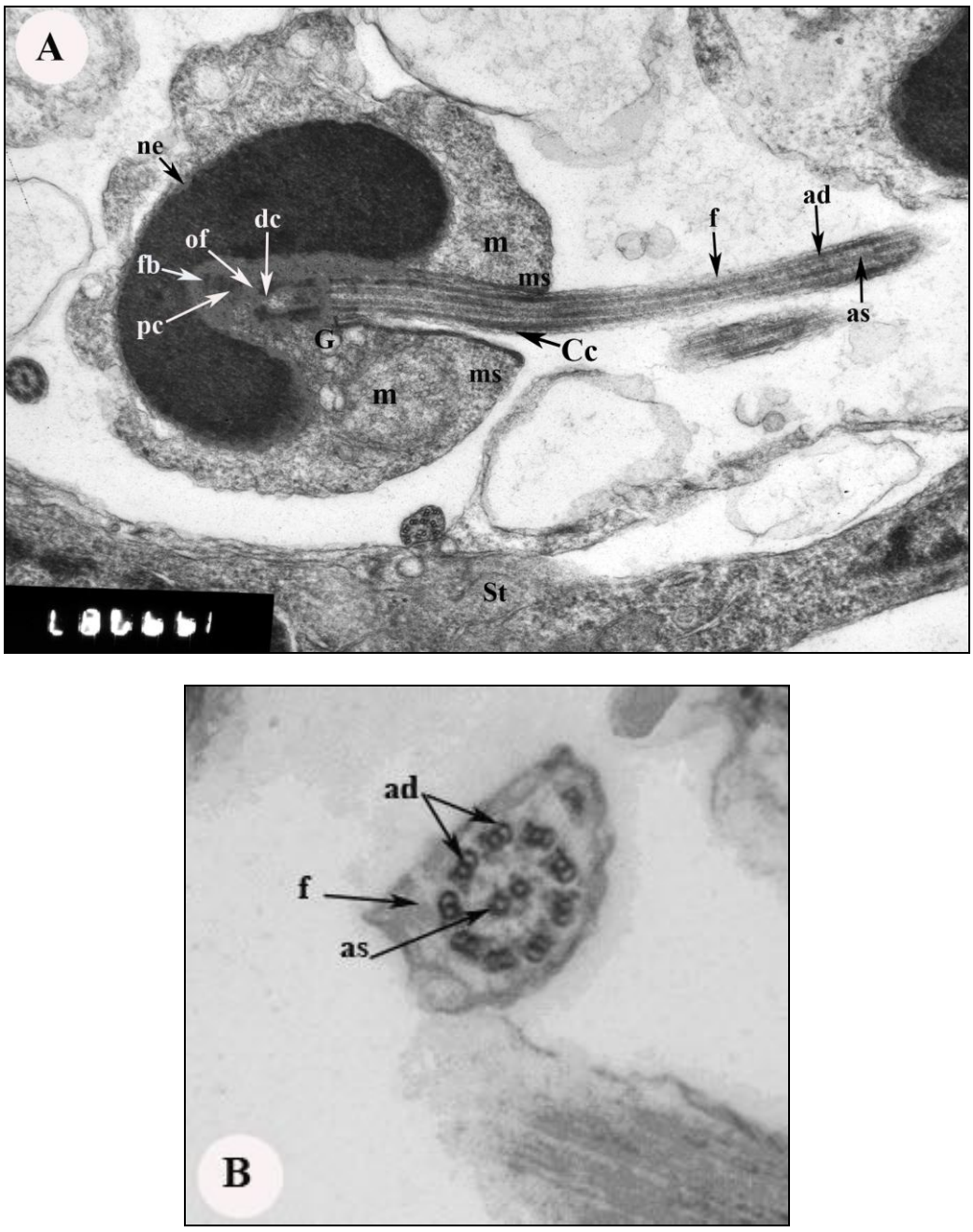


Figure 6: TEM in late spermatide stage shows: **A.** The proximal centriole (pc) in the nuclear fossa. The distal centriole (dc) connected with the proximal one by electron dense filaments (of). Two fibrous bodies (fb) appear above the proximal centriole within the nuclear fossa. The nucleus appears with condensed chromatin structure and irregular nuclear envelope (ne). The Mitochondria (m) are strongly aggregate in one side of mitochondria sleeve (ms) and less abundant in the other one. Flagellum (f) structure is clear (axonemal singlet microtubules (as) and axonemal doublet microtubules (ad)). Sertoli cells (St) appear surrounded the spermatide (X15000). **B.** A transverse section of flagellum (f) with a typical two centeral axonemal singlet microtubules (as) and nine peripheral axonemal doublet microtubules (ad) (X30000).

The axoneme appears surrounded by mitochondria, which are separated from the flagellum by the cytoplasmic canal (Figure 6A). Most of the cytoplasm is concentrated on the mitochondrial posterior pole of the spermatid and on the flagellar side. As development proceeds, the size of the spermatids decreases and the intercellular spaces enlarge to form a lumen in the cyst.

The nucleus becomes more compact with increasing dense and thick chromatin filaments. The nuclear fossa is further expanded into the nucleus and completely surrounds the centriolar complex. As the nuclear fossa develops, the nucleus condenses and a small nuclear notch appears at the level of the proximal centriole, which gradually increases and becomes filled with electron dense material (Figure 6A).

Figure (6B) shows a transverse section of flagellum with a typical two central axonemal singlet and nine peripheral axonemal doublet microtubules.

The cell begins to discard most of its cytoplasm to form a mature spermatozoon, leaving the residual bodies of various sizes and shapes in the intercellular spaces (Figure 7A). The proximal and distal centrioles occupy only the distal part of the nuclear fossa and are perpendicular to each other and lie at right angle to the base of the head (Figure 7B).

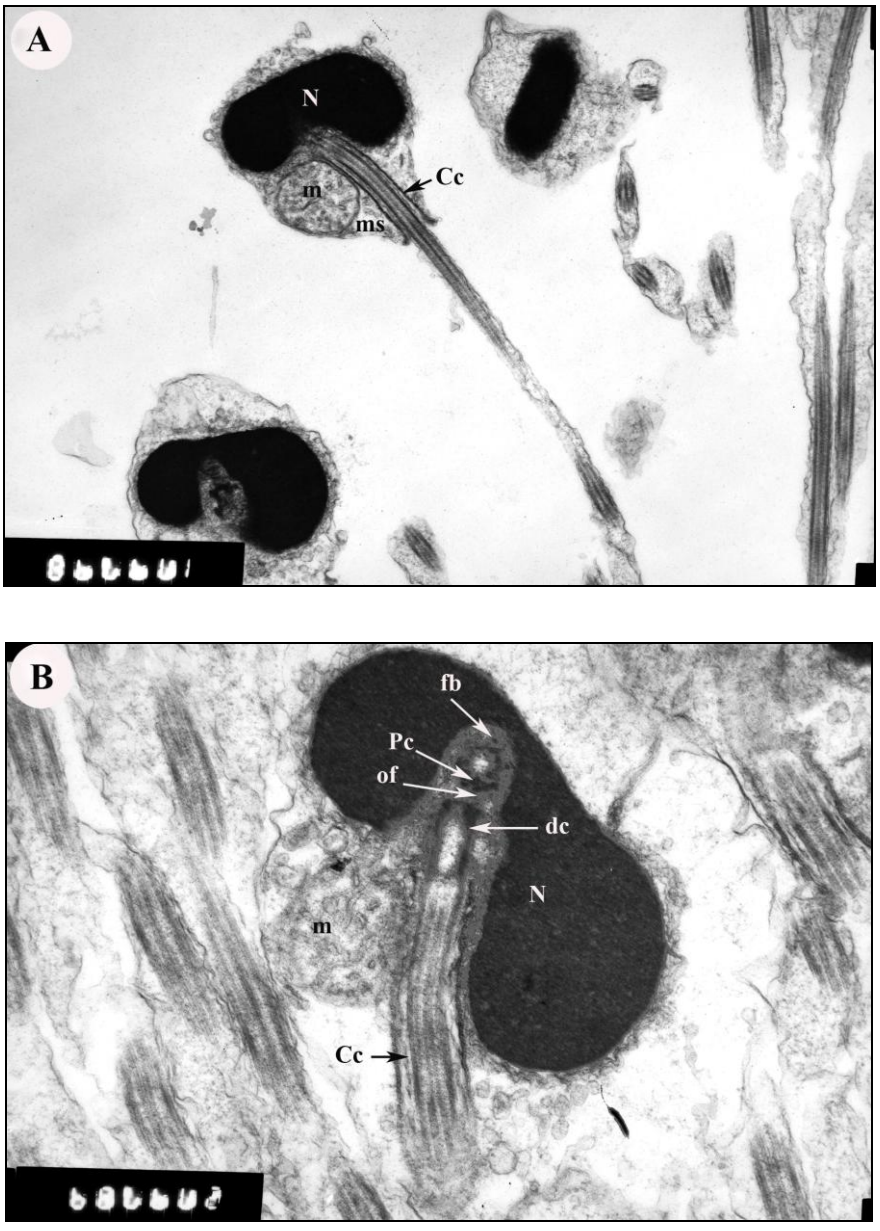


Figure 7: TEM in spermatozoa stage shows: **A.** Shows the mature spermatozoa with an oval nucleus (N) and condensed chromatin. One large ring of mitochondria (m) appears in one side lie within mitochondria sleeve (ms). The cytoplasmic canal (Cc) and long tail are noticed (X10000). **B.** The cell begins to discard most of its cytoplasm. The nuclear fossa is further expanded into the nucleus. The axoneme appears surrounded by mitochondria (m), which are separated from the flagellum by the cytoplasmic canal (Cc) (X20000).

The mature spermatozoon is a simple elongated cell composed of a head, a short mid piece and a relatively long tail or flagellum (Figure 7A). The head consists mostly of an oval nucleus, that has very dense, homogenous and osmophilic chromatin material. The sperm has no acrosome. The residual cytoplasm surrounding the nucleus has a granular appearance.

The midpiece encircles the flagellum and is completely separated from it by a cytoplasmic canal, that is an invagination of the plasmalemma, which runs longitudinally from the caudal to the cranial end of the midpiece and in which the flagella are located and emerged from the distal centriole. The centrosome deeply engulfed by the nucleus in the nuclear fossa (Figure 7B). The cytoplasm extends toward a very long and thin flagellum which is a character to the marine species and is surrounded by the flagellar plasma membrane. The insertion of the flagellum into the head is symmetric as indicated by the orientation of the centrioles and the nucleus. One large ring of mitochondria appears in one side lie within shallow depression of the nuclear caudal surface (mitochondria sleeve) (Figures 7 - A & B).

4. Discussion

According to the present study, Spermatogonia and Spermatocytes of *D. vulgaris* have limited fine structural variations with other teleosts species; however spermatids and spermatozoa have some peculiar structures different from some other fish species.

Spermatogonia occur singly or in small groups in all parts of the testis and each being almost completely surrounded by sertoli cells. These cells perform several functions for providing support and nutrition to the spermatogenic cells and in addition they phagocytize residual spermatozoa (Billard, 1970). They are connected together by intering digitations, desmosomes and tight junctions.

In *D. vulgaris*, mature spermatozoon has an oval nucleus, which appears to be similar to the nuclei described in some characids (Burns *et al*., 1998 and Mattei *et al*., 1995) and *C. auratus* (Shahin, 2007), while in some other teleosts species the nucleus is found to be spherical in shape (Gwo *et al*., 1993 and Gwo, 1995).

As mentioned in the present study rotation of the nucleus is 90°, the diplosome inters the nuclear fossa and the flagellum is medial in position. Similar findings have been recorded in *Acanthopagrus schlegeli* (Gwo *et al*., 1993) and *Alestes dentex* (Shahin, 2006).

On the contrary, rotation of the nucleus are not complete as in *Cyprinus carpio* and *Oreochromus niloticus* (Lou & Takahashi, 1989) or entirely does not occur as in *Liza aurata* (Brusle, 1981) and *Diapoma* sp. (Burns *et al*., 1998). In these cases, the diplosome remains outside the nuclear fossa and the flagellum inserts laterally to the head.

Grasssioto *et al*., 2001 indicated that, in the common teleost the nuclear fossa peneterates almost to the tip of the nucleus. The centriolar complex and the initial segment of the axoneme enter this fossa.

This is in accordance with the present observations in *D. vulgaris;* the nuclear fossa contains the centriolar complex and proximal portion of the flagellum. This type is common in the spermatozoa of many species as *O. niloticus* (Lou & Takahashi, 1989), *Plecoglossus altivelis* (Gwo *et al*., 1994), *Acanthopagrus latus* (Gwo, 1995), *Chanos chanos* (Gwo *et al*., 1995), *Pagellus Erythrinus* (Assem, 2003) and *C. auratus* (Shahin, 2007).

Nevertheless, the nuclear fossa is moderately developed in the curimatidae species (Grassiotto *et al*., 2003) and in the subfamilies Aphyocharacinae (Burns *et al*., 1998) while it is poorly developed in *Epinephelus malabaricus* and *Plectropomus leopardus* (Gwo *et al*., 1994). On the contrary, it is completely absent in most oviparous and some viviparous species as eels (Todd, 1976).

Moreover, it has been described that the position of centriolar complex is related to the shape of nuclear fossa, when the nuclear fossa is deep, the centriolar complex is located inside it. If the nuclear fossa is moderately deep, it may contain the entire centriolar complex or part of it, or only one of the centrioles, while the other one lies outside, but if it is completely absent, the centriolar complex usually lies close to the nucleus (Grassiotto *et al*., 2003).

The present study shows that, *D. vulgaris* has two fibrous bodies which occupy the upper part of the nuclear fossa and connect the proximal centriole with the nucleus and the basal foot, and alar sheets that attach the basal body to the nucleus and plasma membrane, respectively. They consist of osmophilic disks alternating with lighter material and lie perpendicularly above the proximal centriole in the upper part of nuclear fossa. These two dense bodies give rise to two short electron dense fibers, which connect them together with the proximal centriole and anchor the latter to the nucleus. Similar findings have been described in the salmon *O. m. formosanus* (Gwo *et al*., 1996) and in the gadiform *M. merluccius* (Medina *et al*., 2003).

Additionally, it has been pointed out that the position of the nuclear fossa and accordingly the attachment of the flagellum to the nucleus depend upon the rotation of the nucleus (Grassiotto *et al*., 2003). So, when the nuclear rotation is incomplete, the nuclear fossa is eccentric and so is the flagellum, which is perpendicular to the nucleus (Grassiotto *et al*., 2003; Jamieson, 1991and Burns *et al*., 1998). If the rotation is complete (90°) as in *D. vulgaris,* the nuclear fossa and the flagellum are medial and perpendicular to the nucleus. But when the nucleus does not rotate, the nuclear fossa is lateral in position or may be also absent

and thus the flagellum is parallel to the nucleus (Burns *et al*., 1998) or the nuclear fossa is medial and shallow and the initial segment of the flagellum arises directly in a perpendicular position to the basal pole of the nucleus (Shahin, 2006).

In *D. vulgaris*, the midpiece is short and contains a short cytoplasmic canal, similar cases have been recorded in the majority of Characiformes (Grassiotto *et al*., 2003) and in many teleosts (Mattei, 1991and Gwo, 1995). However, long midpiece and long cytoplasmic canal have been observed in some characids (Shahin, 2006).

Furthermore, the midpiece exhibits two situations among teleosts, one is at the posterior end of the nucleus as in *D. vulgaris*, many teleosts (Jamieson, 1991 and Shahin, 2006) and some Characiformes (Burns *et al*., 1998). In the other situation, the midpiece is located laterally to the nucleus as in some members of Characiformes (Burns *et al*., 1998).

Since mitochondria are energy producers for tail movement, they move toward the flagellum. In *D. vulgaris* the mitochondria are located adjacent to the caudal pole of the nucleus and surround the initial segment of the axoneme and are separated from it by a cytoplasmic canal (Jones and Butler, 1988 and Lahnsteiner & Patzner, 1990). Similar location of mitochondria has been also reported among some subfamilies as *Hyphessobrycon innesi* of the Tetragonopterinae (Jamieson, 1991) where they are grouped in the anterior third of the midpiece.

In species of the Glandulocaudinae, the elongate mitochondria are grouped and located close to the posterior end of the nucleus (Burns *et al*., 1998). The Acestrorhynchidae have few elongate mitochondria located around the nucleus and around the initial region of the axoneme. However, mitochondria are found in the nuclear indentation in many blenniid species (Silveira *et al*., 1990) and several eels (Todd, 1976). In the citharinid species, mitochondria are located close to the nucleus near the centriolar complex (Mattei *et al*., 1995).

The number and distribution of mitochondria are frequently variable among teleosts. In some species as *D. vulgaris* in the present study the mitochondria even become reduced to a single large mitochondrion (Todd, 1976). In some others, they might be several (up to ten) mitochondria. For example, one mitochondrion has been found lying either in a concavity in the anterior end of the nucleus as in *A. japonica* (Gwo *et al*., 1992), lateral to the flagellum as in *P. altivelis* (Gwo *et al*., 1994) or encircling the base of the flagellum as in *C. chanos* (Gwo *et al*., 1995), *Boops boops* (Zaki *et al*., 2005) and *O. m. formosanus* (Gwo *et al*., 1996). Nevertheless, it has been reported that there are three mitochondria in *A. latus* (Gwo, 1995), four in *A. schlegeli* (Gwo *et al*., 1993), five (rarely six) in *E. malabaricus* and *P. leopardus* (Gwo *et al*., 1994) and *C. auratus* (Shahin, 2007), eight in *Boops boops* (Zaki *et al*., 2005) and several, up to ten, mitochondria in *M. merluccius* (Medina *et al*., 2003), which surround the base of the flagellum.

The sperm of *D. vulgaris* in this study proved that, it has one tail (flagellum) like most of the other fish species. However, there are some species which have two tails (biflagellate spermatozoa) as cited by Yao *et al*. (1995). Biflagellate sperms are not common among teleosts but they have been previously reported in several species including *Polypterus senegalus* (Cladistia), *I. punctutas* (Siluroidei), *Lampanyctus sp*. (Myctophiformes), *L. lepadogaster* (Gobiesoci formes), and in *apogonid* (Perciformes) (Jamieson, 1991 and Yao *et al*., 1995).

Ultrastructure of spermatozoa has recently served as a criterion for taxonomic and phylogenetic classification between different species. The present study throws light on the ultrastructure features of spermiogenesis in *D. vulgaris* and gives a basis for comparing them with those of other species.

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