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ULTRASTRUCTURE OF MALE GERM CELLS AND CHARACTER OF SPERMATOZOA IN *BOOPS BOOPS* (FAMILY SPARIDAE) IN ALEXANDRIA COAST, EGYPT

ZAKI, M. I.[1*]; NEGM, R.K.[2]; EL-AGAMY, A.[3]; AWAD, G. S.[4]

[1-] National Institute of Oceanography and Fisheries, Alexandria.
[2-] National Institute of Oceanography and Fisheries,Alexandria.
[3-] Faculty of Science, Zagazig University.
[4-] Faculty of Science, Zagazig University.

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ABSTRACT

Ultrastructural studies of the spermatogonia showed masses of electron-dense opaque substances, outside the nuclear membrane that were named the perinuclear "nuage". Both primary and secondary spermatocytes are always grouped into cysts and appear to be connected by intercellular bridges. The initial phase of the spermatid development and its metamorphosis into sperm is the appearance of electron-lucent vesicles in the cytoplasm. The middle phase is the formation of the implantation fossa while the advanced phase is the development of the flagellum.

The sperm of *Boops boops* is morphologically simple. It appeared by the electron microscopy consisting of 3 regions, a round head, a short middle piece and a single very long flagellum (characteristic to marine species), that penetrates the posterior region of the sperm body through a relatively short cytoplasmic canal. No accessory structures were apparent on the flagellum.

INTRODUCTION

Studies of fish reproduction usually require knowledge of the stage of gonad development in individual fish. Such knowledge is often based on visual inspection of the external appearance of the gonad. This is probably the most rapid but least certain technique. A more precise and detailed analysis requires the use of histological methods (Wallace and Selman, 1981; West 1990 and Garcia-Diaz *et al*., 1997), but it is also the most time consuming one. The reproduction of many species has only been studied in females, since the small sized sex cells in males render ultrastructural analysis virtually necessary. Identification of cellular changes is difficult by light microscopy alone. Ultrastructural studies using scanning and transmission electron microscopy can resolve some of these difficulties, as well as provide additional valuable information to fish biologists, (Selman and Wallace, 1986).

Structure of fish spermatozoa is extremely diverse (Jamieson, 1991). This diversity can be explained at least partly by the diversity of modes of reproduction in fishes. Most externally fertilized teleosts have a simple type of the spermatozoon, called aquasperm, characterized by a rounded head and a short neck region with few mitochondria (Jamieson, 1991 and Mattei, 1991). In contrast, spermatozoa of many internally fertilized teleosts have an elongated head and other derived features. This was shown for example for groups, which contain both oviparous and viviparous species like in family Poeciliidae, and Zenarchopteridae (Jamieson and Grier, 1993).

The evolution of special structural features of spermatozoa of internally

*Corresponding author
E-mail: DrMagdaZaki@hotmail.com

fertilized fishes must have involved modification of the process of spermiogenesis. Compared with the wealth of information on spermatozoon structure, there are relatively few studies on fish spermiogenesis. Most of these studies described either spermiogenesis in fishes with simple spermatozoa or in viviparous fishes belonging to advanced fishes of the group Acanthopterygii (Billard 1970, 1983a, 1983b and 1986; Mattei 1970; Mattei and Mattei 1974; Grier 1975a; Bruslé 1981 and Gwo and Gwo 1993).

Ultrastructure of spermatozoa has been recently served as a criterion for taxonomic and phylogenetic classification of over 200 fish species (Jamieson, 1991 and Mattei, 1991). The fine structure of many marine fish spermatozoa still lacks detailed investigation.

The aim of the present study is to describe the changes in the male sexual cells of *Boops boops* to illustrate the sequential steps of spermatogenesis by using transmission and scanning microscopes.

MATERIAL AND METHODS

Fish samples

The samples used in the present work were obtained alive by commercial fishermen, from the Mediterranean coast near Kayet Bey castle at Anfoushy region, Alexandria. The fish were transported to the laboratory in aerated aquarium, dissected to determine sex and maturity stage. Then the gonads were cut into very minute pieces and immediately fixed in the universal E.M. fixative {4% glutraldhyde in 0.1 Cacodylate buffer (pH 7.2-7.4)} at 4^{o}c till processing.

The sample washing was done in 5% sucrose in 0.05M Cacodylate buffer overnight. Post fixation in 1% Osmium tetroxide in 0.2M Cacodylate buffer. Rinsing and washing in buffer at pH 7.2-7.4. Dehydration, embedding, sectioning and staining with uranel acetate (saturated in 70% alcohol). The examination was done by using Jeol CX 100 electron microscope and Zeiss 109 transmission electron microscope.

For scanning electron microscope, the fixation of the sample was applied as previous in transmission electron microscope on the sperm semen, then drying with critical point drier Samdri-PVT-3B and Coating with JEC-1100E ion Sputtering device.

RESULTS

In *Boops boops* as most teleosts fish, the testes morphologically changes in shape, colour and increasing magnitude towards spawning. In the present study the male fish less than 11cm were immature and larger than 12 cm were sexually mature.

The testes of *Boops boops* are characterized by the presence of branched seminiferous lobules composed of germ cells which were designated to different stages as the following: Spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa.

1-Spermatogonia

Spermatogonial cells are rounded or slightly oval cells with big eccentric ovoid nuclei, which are surrounded by thin layers of cytoplasm, indicating that the nucleus-cytoplasmic ratio is large. The homogenous nucleus contains small clumps of chromatin material consisting of chromatin granules and chromatin threads with one or two nucleoli that appeared in the central zone.

The nuclear membrane, posses nuclear pores, which are not uniformly distributed, but they are observed in that region of nuclear envelope which faces some cytoplasmic organelles.

Masses of electron-dense opaque substances are present outside the nuclear membrane and have been named the perinuclear “nuage” in spermatogonia. These masses are present, but only in small amounts, suggesting nuclear-cytoplasm exchange (fig. 1).

The spermatogonia are characterized by their faintly stained cytoplasm. They are also distinguished by their conspicuous boundary and their distinguishable nuclear membrane.

Granular round mitochondria are observed in the cytoplasm, they are numerous but small in size at this stage of development. They may be irregular or grouped by cementum. Free ribosomes and Golgi apparatus are also observed in the cytoplasm. Blood vessels and putative pericytes with endothelial cells are found between the spermatogonial cells.

Late stage of development of spermatogonia doesn't differ much from early stage except that the cell decrease in diameter and the chromatin material become much more condensed (fig. 2). The spermatogonia divide by mitosis to give primary spermatocyte.

2-Primary and secondary spermatocytes

Both primary and secondary spermatocytes are always grouped into cysts (figs. 3 and 5) and appear to be connected by intercellular bridges. Cell divisions are frequent within each cyst (figs. 4 and 7). Primary spermatocyte divide by meiosis (first meiotic division) to give secondary spermatocyte (fig. 4) while secondary spermatocytes divide also by meiosis (second meiotic division) to give spermatid (fig. 7) which metamorphoses into the sperm cell. Accordingly primary spermatocytes have complete number of chromosomes as any somatic cell while the secondary spermatocytes have half the number of chromosomes that any somatic cell has in its nucleus.

Another difference between the primary and secondary spermatocytes is that they differ in size and nuclear morphology.

The spermatocyte stage is characterized by more complicated nuclear structures. These cells (primary and secondary spermatocyte) are oval in shape with a voluminous nucleus that has clumps of chromatin distributed centrally (as irregular strands) and at the nuclear periphery (figs. 3 and 5).

Numerous mitochondria were usually grouped "concentrated" at one pole of the cell, some being elongated and others are circular (fig. 3). Generally the mitochondria are bigger in size in secondary spermatocyte than in primary spermatocyte. The mitochondrial matrix is relatively electron-dense particularly during cell division. Individual cells often have many intercellular cytoplasmic bridges, some of which are considerably extended (figs. 3 and 5).

Microtubules were observed on the outside of the nuclear envelope (fig. 7). Synaptonemal complexes, diplosomes and Golgi apparatus were also observed. Apart from these structures, the spermatocytes had little endoplasmic reticulum, but the cytoplasm is densely occupied by ribosomes often in the form of polyribosomes (figs. 3 and 5).

In the primary spermatocyte, before the first meiotic division, the condensing chromosomes with the nucleolus will become attached to the nuclear envelope, which has many pores that -in contrast to early stages- are now distributed among the entire circumference of the nuclear envelope (fig. 3). The electron dense material "nuage" is subjacent to the nuclear pores and is seen among the mitochondria that increase in size than the previous stage and has now several layers of parallel cristernae.

During the division of the cells to produce secondary spermatocytes, the cells become elongated and the nucleus becomes surrounded by a broad band of cytoplasm. The heterochromatin is more condensed at places where the nuclear envelopes tend to bulge out, where the nuclear division proceeds the cytoplasmic division (fig. 4).

The secondary spermatocytes are clustered together within cysts, they are contiguous and synchronously dividing cells. They were found to have condensed granular chromatin at one pole of the nucleus "moon shaped chromatin material", that lie in the

center of the cell in the early reproductive season, when not in state of division (fig. 6).

The nucleolus seems to fade away and is never seen again in the subsequent stages. The cytoplasm is scarce, clear, finely granular that make it highly electron-dense and with few organelles.

3-Spermatids

During spermiogenesis, remarkable nuclear and cytoplasmic changes take place. Spermatids have a large rounded nucleus that contains electron-dense and granular chromatin.

During differentiation (metamorphosis), the nucleus had thick clumped chromatin, which gradually condensed and become homogeneous. The nuclear fossa appeared as a depression in the nucleus, where the centriolar complex will reside later (fig. 8).

As observed with electron microscopy, this complex consists of proximal and distal centrioles. The proximal centriole is in close contact with the nuclear membrane while the distal centriole is bounded to the plasma membrane in young spermatids (fig. 9). Each of these centrioles has nine pairs of peripheral microtubules but lacks central microtubules.

Concomitant with nuclear condensation, the flagellum is formed from the distal centriole (fig. 11). The flagellum reveals a typical axonemal configuration with nine pairs of peripheral and two central microtubules.

Cytoplasm is abundant at this stage of development in the spermatid and has a moderate electron density. It contains irregularly distributed mitochondria, endoplasmic reticulum and vesicles of smooth endoplasmic reticulum. Towards the completion of spermiogenesis, excess cytoplasm appears to be sloughed off the spermatid into the cyst lumen (Garcia-Diaz *et al,* 2002).

At the beginning of spermiogenesis, the nucleus migrates “it was central in position in young spermatid” and becomes eccentrically placed in an area that corresponds to the anterior region of the spermatid. At that time, a thin layer of cytoplasm separates the nucleus from the cytoplasmic membrane (fig. 8). The basal part of the nucleus forms the nuclear fossa, to which the proximal centriole migrates.

Due to the bond between the distal centriole and the plasma membrane, a cytoplasmic canal is formed. The flagellum is situated within this relatively short cytoplasmic canal. As the nucleus of the spermatid elongates, the cytoplasmic canal elongates.

Vesicles of pinocytosis appear in large numbers in the posterior region of the spermatid. These vesicles are found either at the surface of the cell or at the level of the plasma membrane that limits the cytoplasmic canal (fig. 9).

These vesicles are covered by a network of clathrines and their contents are electron dense at the beginning of their formation, vesicles of pinocytosis merge with each other and with vesicles of larger size. The coating of clathrines disappears only when those vesicles totally fuse to form large vesicles whose contents are electron-lucent.

At this stage (figs. 10 and 11), the spermatid is completely metamorphosed into a spermatozoa and shows a clear bilateral symmetry. Its long and short axis is defined not only by the shape of the nucleus but also by the position of the proximal centriole which is situated on the apical side of the spermatid and by the position of the mitochondria on the opposite side.

Most of the cytoplasm is concentrated on the mitochondrial posterior pole of the spermatid and on the flagellar side.

So we can say that the initial phase of the spermatid development and metamorphosis into sperm is the appearance of electron-lucent vesicles in the cytoplasm. The middle phase of development is the formation of the implantation fossa while the advanced phase is that the flagellum begins to develop.

4- **Spermatozoa**

The sperm of *Boops boops* is morphologically simple (figs. 14 and 16). It

appeared by the electron microscopy consisting of 3 regions, a round head, a short midpiece and a single very long flagellum (tail) that penetrates the posterior region of the sperm body through a relatively short cytoplasmic canal. No accessory structures were apparent on the flagellum.

The head (fig.15) of the mature sperm is circular in shape and contained a depression in the form of a pit shaped called hilus. Within the hilus, the proximal centriole is located.

The head consists mostly of the 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. Undulating cell and nuclear membranes run close together along the anterior side of the nucleus in the vicinity of which no acrosome is evident.

The midpiece (figs. 10, 11 and 16) contains eight large spherical mitochondria that surround the basal portion of the flagellum and the centrioles. Their matrix is electron-lucent, and their laminar cristae are irregularly arranged. In the longitudinal sections, two large mitochondria can be seen arranged in the midpiece, one to the left and the other to the right of the flagellum (figs. 10 and 11), Thus the mitochondria surround the flagellum at the midpiece region.

The midpiece encircles the flagellum and is completely separated from it by the cytoplasmic channel (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 centriolar complex is formed of distal and proximal centrioles, both of which are similar in structure to centrioles of the spermatid (fig. 9). The centrioles have doublet of (9+2) arrangement of the microtubules. The distal centriole is parallel to the flagellum where as the proximal is oriented perpendicular or by a small acute angle to the distal centriole.

The flagellum is very long and thin (fig. 14), which is a character to the marine species. The insertion of the flagellum into the head is symmetric as indicated by the orientation of the centrioles and the nucleus. The flagella have a typical axonemal configuration of nine peripheral doublets and two central microtubules (9+2). The axoneme is covered by plasma membrane.

The spermatozoa that are free in the lumen of seminiferous lobules flow the deferent duct where they will by expelled (fig. 13).

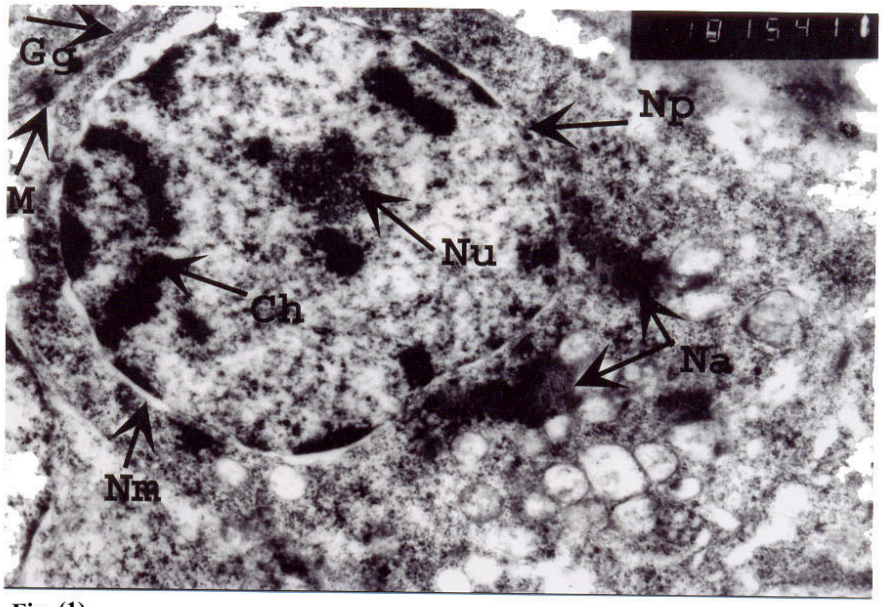
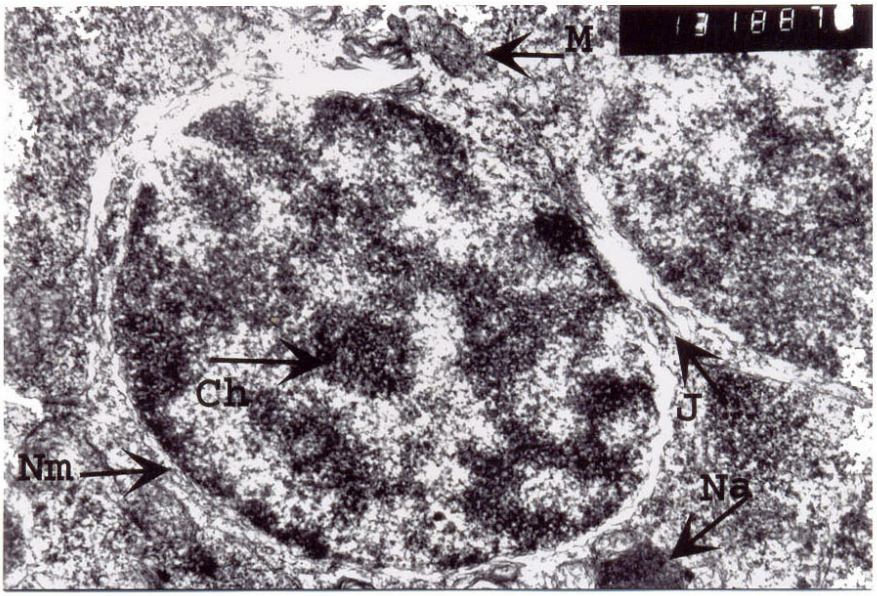


Fig. (1) X 10.000



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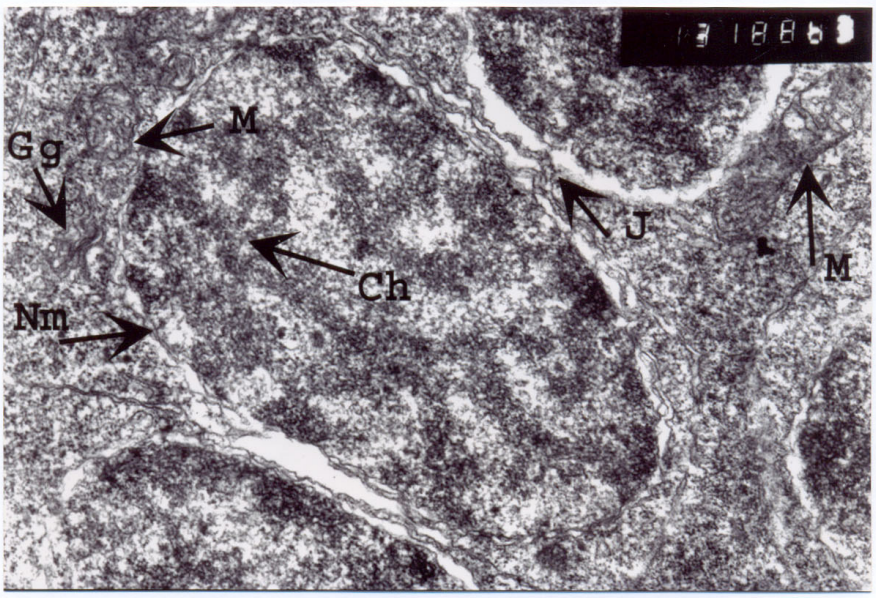

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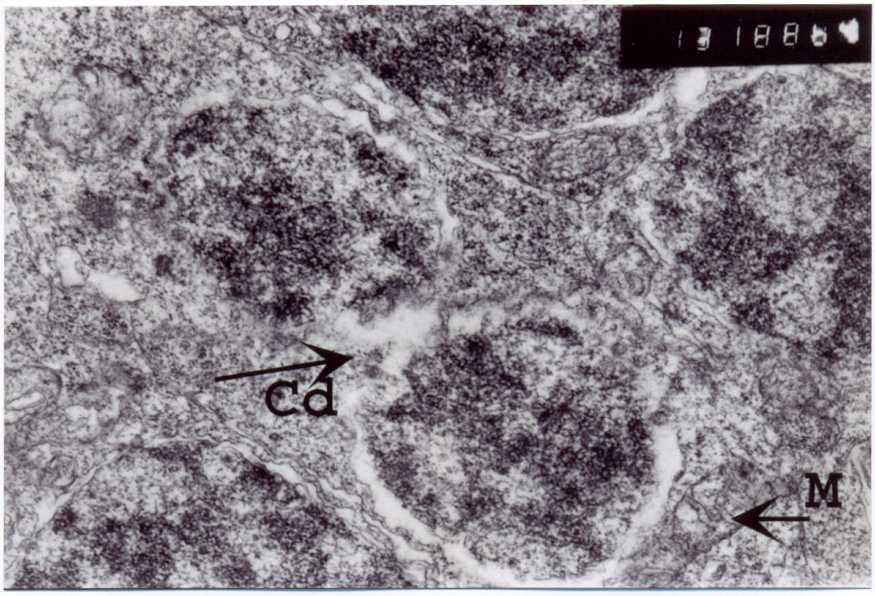

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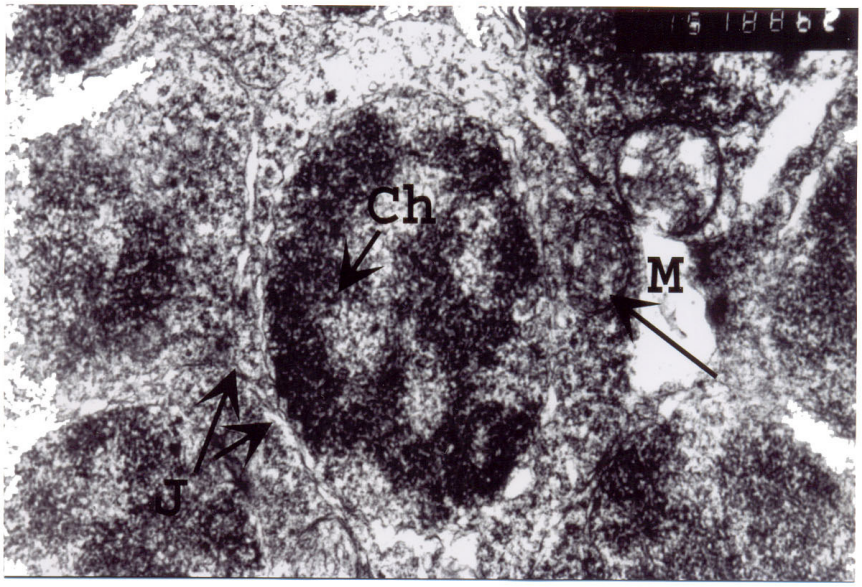

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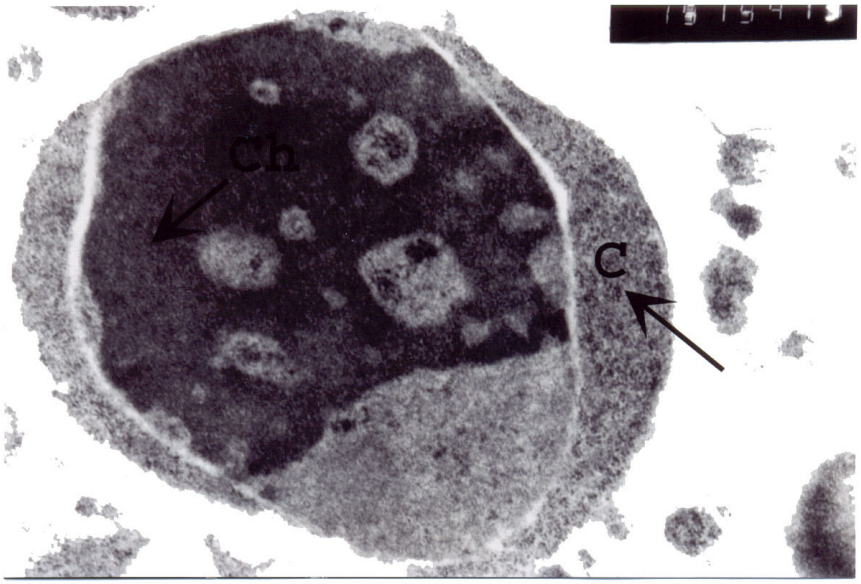


Fig. (6) X 15.000

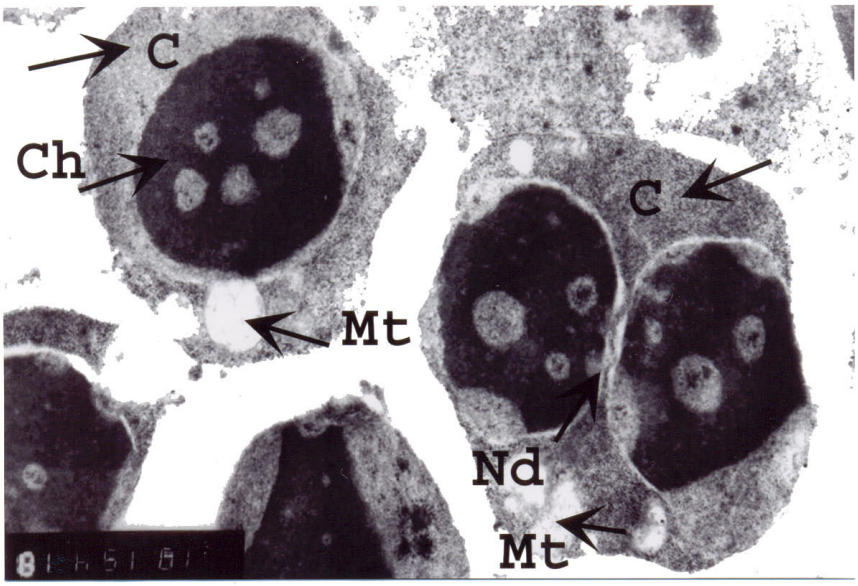
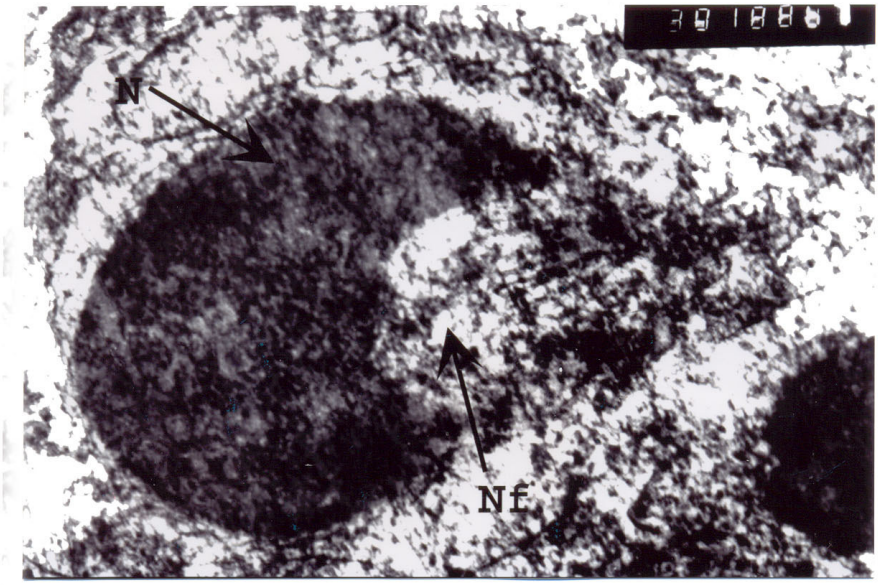


Fig. (7) X 10.000



X 30.000

Fig. (8)

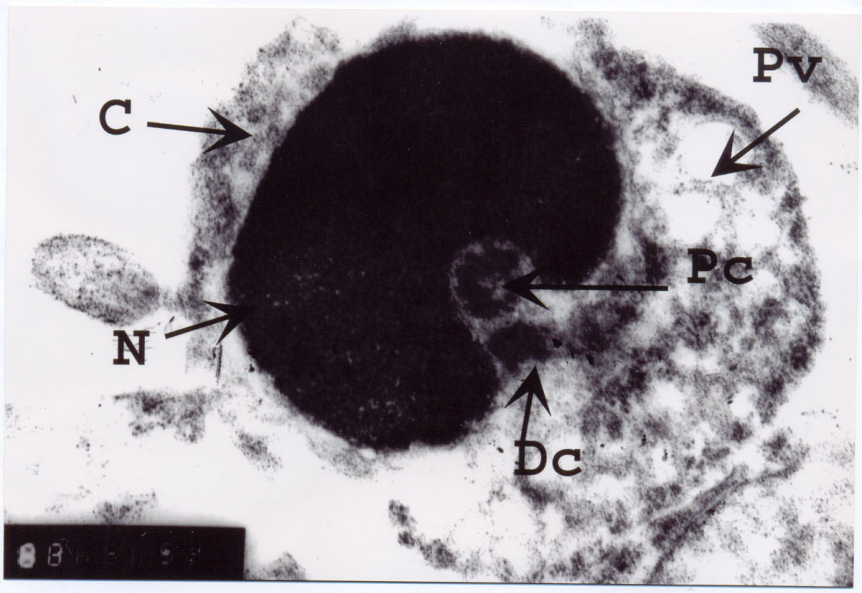
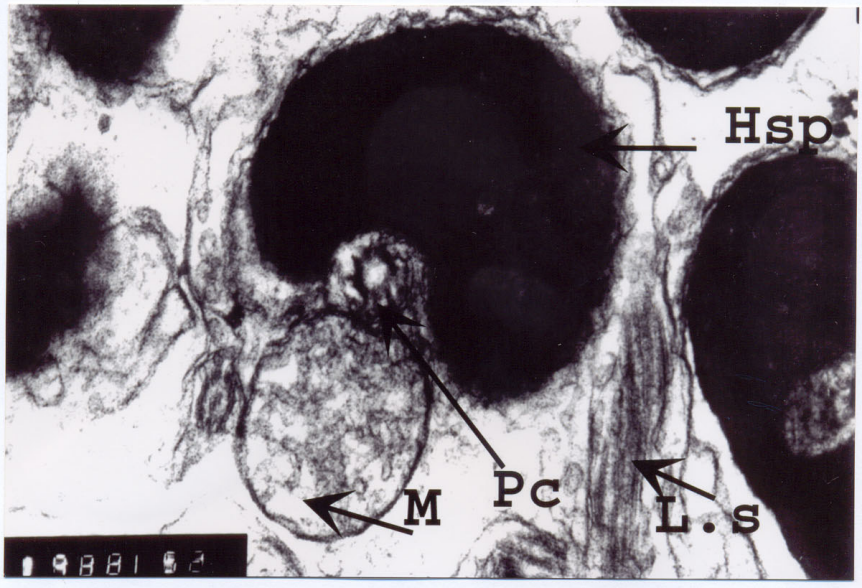


Fig. (9) X 25.000



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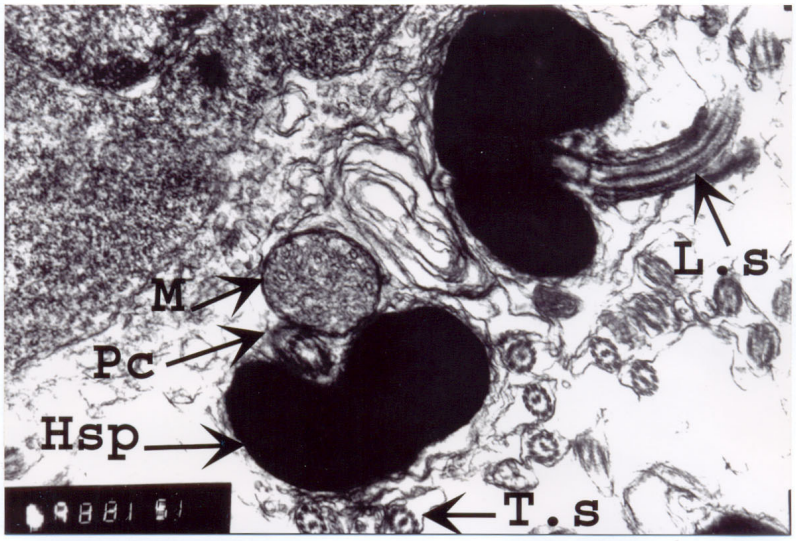
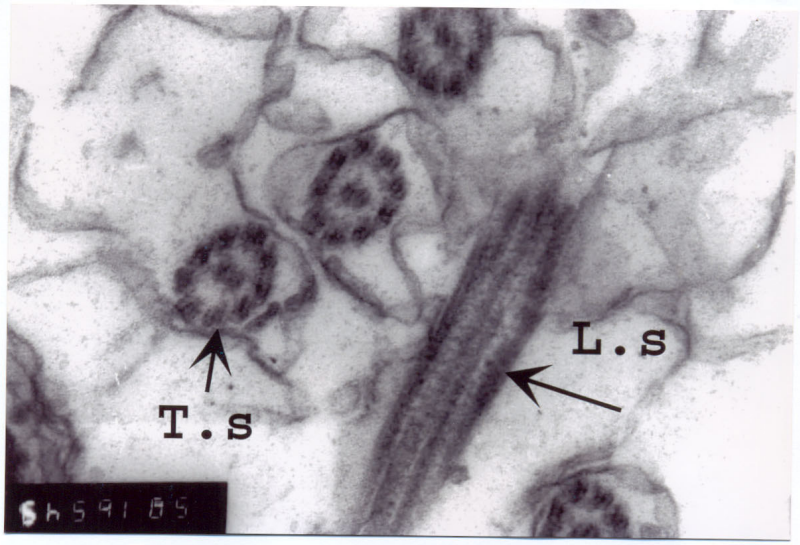


Fig. (11) X 15.000



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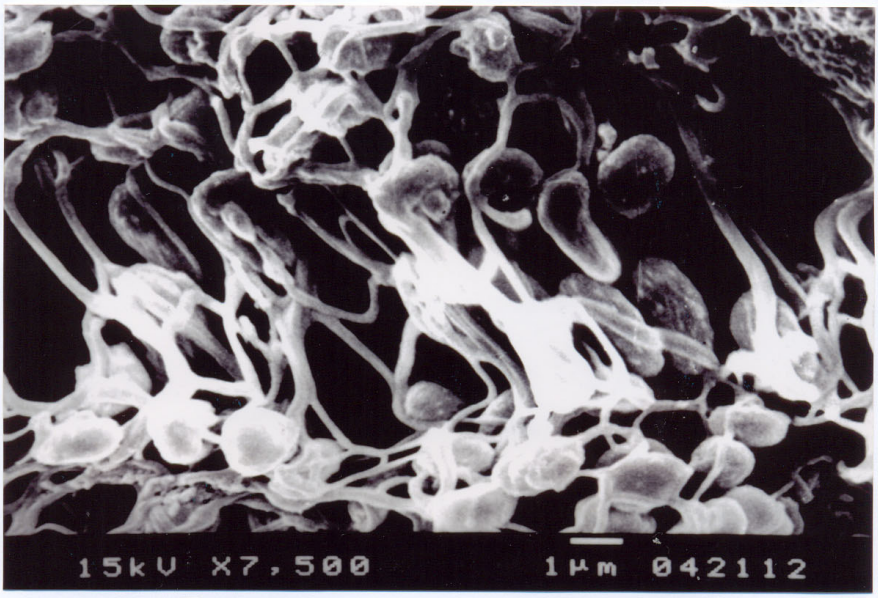

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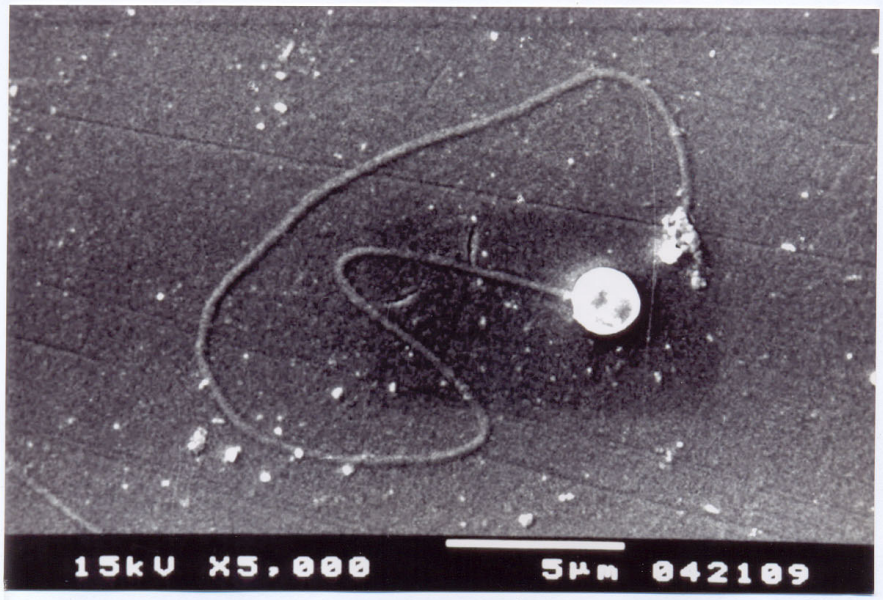

Fig. (14) X 5.000

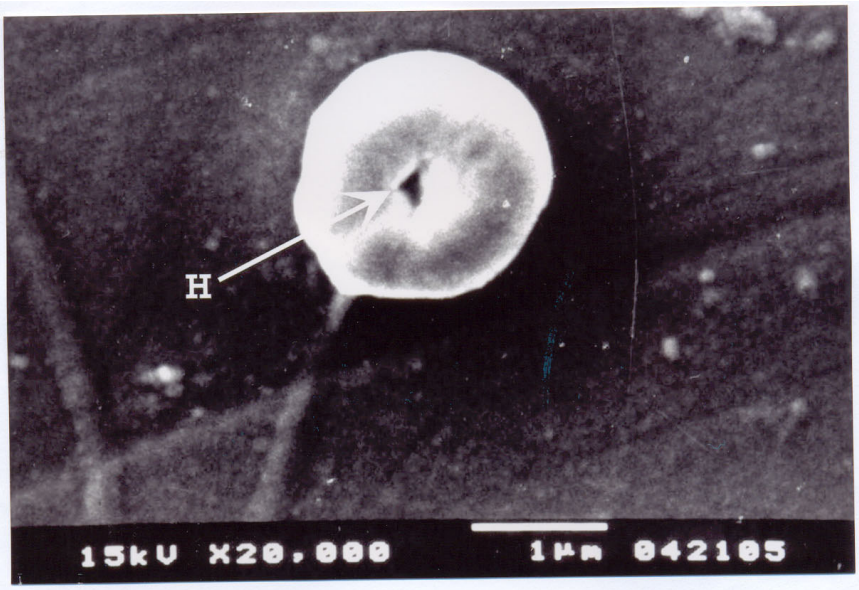

Fig. (15) X 20.000

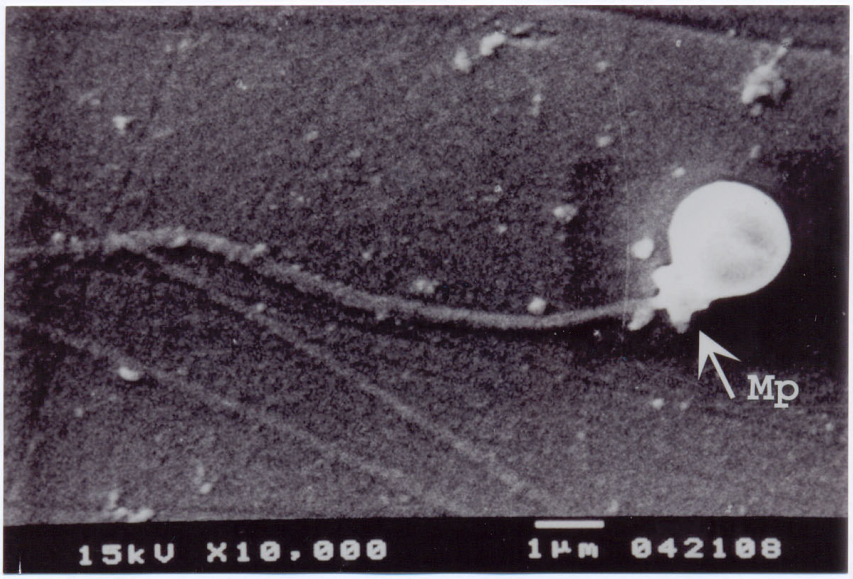

Fig. (16) X10.000

DISCUSSION

In the present study, by using the electron microscope, it was noticed that the mother spermatogonia of *Boops boops* is characterized by having big nucleus that divide mitotically into a number of daughter spermatogonia which are less in volume and their nucleui have more condensed chromatin material.

The spermatogonia showed round mitochondria, which are observed in the cytoplasm. These mitochondria may be irregular or grouped together by cementum, resembling those observed by Clerot (1979), who reported that this cementum is made up of ribosomic RNA of nuclear origin.

The present ultrastructural investigations for spermatogonia cells showed that their cytoplasm was faintly stained and that the nucleui were homogenous in appearance. Similar results were reported by Silveira *et al*. (1990) in *Blennius pholis*; Mousa (1994) in *Mugil cephalus* and Assem (1999) in *Caranx crysos*.

The distinctive changes that are observed in *Boops boops* spermatogonia to develop into spermatocytes are as follows: decreased size, increased nuclear-cytoplasmic ratio and a reduced number of mitochondria. These characteristics of germ cells are similar to findings in *Mugil* (*Liza*) *Auratus* (*Risso*) (Bruslé and Bruslé, 1978) and in *Acanthopagrus schlegeli* (Huang *et al*., 2002).

Perinuclear nuage is generally considered to be associated with germ cells (Hogan, 1978). In *Acanthogagrus schlegeli* (family Sparidae) Huang *et al*. (2002), found aggregates of masses of an electron-opaque substance being associated with or without mitochondria. He also reported that origin and function of the perinuclear nuage in male germ cells is not clear. The perinuclear nuage is also observed in *Boops boops* spermatogonia, usually not associated with mitochondria.

The synaptonemal complexes are formed, by pairing of the homologous chromosomes during the first meiotic division (Grier, 1975b; Cruz-Landim and Cruz-Höfling, 1986). The synaptonemal complexes are characterizing to the primary spermatocytes (Andrade *et al*., 2001 and Huang *et al*., 2002). These synaptonemal complexes were observed in *boops boops*. In mammals, basic proteins of the synaptonemal complexes combine with DNA and are involved in the chromosome structural organization that takes place in meiotic prophase (Offenberg *et al*., 1998 and Yuan *et al*., 1998).

Two types of nuclear chromatin, heterogeneous and homogenous, were found in the spermatocytes and spermatids (Huang *et al*, 2002). Spermatocytes having either heterogeneous or homogenous nuclear chromatin have been observed in our study. This may be related to the transformation of basic proteins in the nucleus (Grier, 1981). There were fewer spermatocytes with homogenous nuclear chromatin than with heterogeneous chromatin in *Boops boops*.

The nucleui in the spermatid cells of *Boops boops* had condensed chromatin material. Sprando *et al*. (1988) for bluegill *lepomis macrochirus* concluded that the condensation of chromatin in the nucleui of spermatids as they mature occur in a specific pattern that is often in the region adjacent to the developing flagellum.

Cytoplasmic bridges were observed in spermatocytes and spermatids but not in the spermatozoa. Spermiogenesis is achieved with the rupture of the cytoplasmic bridges (Bruslé, 1981).

The stages of the spermiogenesis in *Boops boops* were based on the modifications that occurred in the nucleus and spermatid cytoplasm before the spermatozoon is formed. The distribution and organization of the cytoplasmic organelles and implantation fossa of spermatids of *Boops boops* follow an identical pattern to that described by Thiaw *et al*. (1988). During spermatid differentiation,

pinocytotic vesicles uptake exogenous substances that are important to the process of cytoplasm elimination (Thiaw *et al*., 1988). This extruded material is phagocytosed by sertoli cells (Matos and Azevedo, 1989).

Movement of the cytoplasmic organelles and a decrease in the cytoplasmic volume were found in the stages of spermatocytes and spermiogenesis. Sprando and Russell (1988) indicated three possible ways to decrease cytoplasm in fish: 1) formation of residual bodies and phagocytization by sertoli cells. 2) Formation of tubular complexes and then disintegration. 3) Dehydration and chromatin condensation. In *Acanthopagrus schlegeli* (family Sparidae), the elimination of the cytoplasm occurred by the second method (Huang *et al*., 2002) while in *Boops boops*, the elimination of the cytoplasm is probably achieved by the first method.

Spermatozoa of teleosts present a great variety of shapes and structures. The only common feature is the absence of acrosomes and this characteristic may be related to the presence of a micropyle in the oocyte (Mattei, 1970 and Bromage and Roberts, 1994). However, vacuoles which are vestiges of acrosomes, can be found in some teleosts (Billard, 1983a). In *Boops boops* as in *Plagioscion squamosissimus* (Gusmáo *et al*., 1999) and *Bryconops affinis* (Andrade, 2001), no acrosomes or vestiges of these structures were observed.

Details of spermiogenesis may vary considerably even amongst closely related teleosts species (Grier, 1981). The morphology of spermatozoa has also become of interest from a taxonomic point of view in recent years (Jamieson, 1991). In particular the head morphology, nuclear invagination and number of mitochondria differ considerably among different teleosts (Lahnsteiner and Patzner, 1995 and 1996 and Yao *et al*., 1995).

The differences in the shape of spermatozoa, the number of mitochondria, the arrangement of centrioles as well as the occurrence of lateral flagellar ribbons among teleost species, may have consequences on the swimming behavior with respect to sperm velocity, swimming types and head detachment (Lahnsteiner and Patzner, 1995). However, knowledge of sperm morphology in relation to the motility of spermatozoa of marine fishes is very limited. Garcia-Diaz *et al*. (1999) described the spermatozoon morphology in Serranus species in the Canary Islands. They concluded that the spermatozoon size and morphology is related with distribution depth of the species.

Also sperm morphology reflects to large degree the reproductive patterns. For example, nuclear elongation possibly coupled with elongation of the mid-piece, occurs in teleosts with internal fertilization (Grier, 1981), and is therefore deduced to give some advantage to the spermatozoon internal environment within the female (Jamieson, 1991). Internal fertilization can therefore be related to long middle piece and external fertilization to a short one. Among the teleosts, most externally fertilizing species produce typical "aquasperm" described by Jamieson (1991) as having a spherical nucleui, where as internally fertilized species often have modified spermatozoa with elongated nuclei (Jamieson, 1991; Mattei, 1991).

However, in family Zoarcidae (Jamieson, 1991) and the only member of family Atherinidae *Labidesthes sicculus* (Grier *et al*., 1990), are known to be internally fertilized, and the females are inseminated with typical aquasperm. Thus, it is clear that elongation of the sperm nucleus is not essential for internal fertilization (Burns *et al*., 1995). Energy required by the spermatozoon in internal fertilization is supplied by mitochondria, which breathe and consume the endogenous substrate of the middle piece (Baccetti and Afzelius, 1976).

Boops boops is characterized by having anacrosomal aquasperm as defined by Jamieson and Leung (1991), which is of typical external fertilization, i.e. short round head, few mitochondria (eight) and a barely differentiated middle piece.

Two types of spermiogenesis have been described in teleosts (Mattei, 1970): in type I, rotation of the nucleus occurs, the diplosome enters the nuclear fossa, and the flagellum is symmetrically located. Rotation of the nucleus during spermiogenesis has been found in several unrelated teleosts, for example in *Lepomis macrochirus* (family Centrarchidae) (Sprando *et al*., 1988) and *Acanthopagrus schlegeli* (family Sparidae)(Gwo and Gwo, 1993) and (Huang *et al*., 2002), even in species with elongated spermatozoon head like in family Poeciliidae and family Zenarchopteridae. In this type I, the long axis of the sperm head is continuous with the flagellar axis, thus the sperm is symmetrical (Jamieson and Grier, 1993). The spermatozoon of *Boops boops* belongs to type I due to the formation of cytoplasmic canal, diplosome-flagellum and nuclear rotation as several species of Balistidae, Soleidae, Monacanthidae, Diodontidae and Scorpaenidae (Jamieson, 1991) Mullidae (Biossin *et al*., 1969; Mattei, 1970; Lahnsteiner and Patzner, 1995) and Zoarcidae (Yao *et al*., 1995).

In type II of (Mattei, 1970), there is no nuclear rotation, the diplosome remains outside the fossa and the flagellum is asymmetrically located. This type of spermiogenesis has been found for example in *Liza aurata* (Mugilidae) (Bruslé, 1981).

Spermatozoa of most teleost species are monoflagellate (Jamieson, 1991) while biflagellate spermatozoa occur, as far as it is known, in *Porichthys notatus* (Stanley, 1965) Gobiescoidae (*Lepadogaster lepadogaster*) (Mattei & Mattei, 1978) also in Batrachoidae (*Opsanus tau*) (Casas *et al*., 1981). The reason for the co-occurrence of monoflagellate and biflagellate spermatozoa remains uncertain. Biflagellate spermatozoa could have higher swimming velocities or specific motility patterns in comparison to the monoflagellate ones. For biflagellate spermatozoa, however, the entrance into the narrow micropylar channel could be more complicated than for the monoflagellate ones as the two flagella could entangle with and the spermatozoa become "stuck" in the micropylar channel. Also the centriolar complex is very specific as the basal body is fastened at the nucleus while the proximal centriole is completely reduced. The reason for this structure in relation to sperm functionality is unknown.

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FIGURE LEGENDS

Figure 1. Electromicrograph of T.S. in testes of *Boops boops* showing an early stage of development of spermatogonia.

Figure 2. Electromicrograph of T.S. in testes of *Boops boops* showing late stage of development of spermatogonia.

Figure 3. Electromicrograph of T.S. in testes of *Boops boops* showing a nest of primary spermatocyte.

Figure 4. Electromicrograph of T.S. in testes of *Boops boops* showing the first meiotic division of primary spermatocyte to give secondary spermatocyte. Notice that the nuclear division precedes the cytoplasmic division.

Figure 5. Electromicrograph of T.S. in testes of *Boops boops* showing a cyst of secondary spermatocyte.

Figure 6. Electromicrograph of T.S. in testes of *Boops boops* showing a secondary spermatocyte cell.

Figure 7. Electromicrograph of T.S. in testes of *Boops boops* showing the second meiotic division.

Figure 8. Electromicrograph of T.S. in testes of *Boops boops* showing the spermatid.

Figure 9. Electromicrograph of T.S. in testes of *Boops boops* showing the metamorphosis of the spermatid.

Figure 10. Electromicrograph of T.S. in testes of *Boops boops* showing the structure of the spermatozoa.

Figure 11. Electromicrograph showing L.S. in the midpiece region and the flagellum. Notice that the midpiece encircles the flagellum and is completely separated from it by the cytoplasmic canal.

Figure 12. Electromicrograph showing L.S. and T.S. in the tail region, notice that the axoneme is covered by plasma membrane.

Figure 13. Group of spermatozoa with circular head and long flagellum released into the lumen of the seminiferous lobules by scanning electron microscope.

Figure 14. scanning electron microscope showing the simple morphology of a single sperm to illustrate its round head and very long flagellum.

Figure 15. Magnification to the round head by scanning electron microscope to show the depression in the form of a pit shaped (hilus).

Figure 16. Magnification to the head of the spermatozoa to show the short midpiece region by scanning electron microscope.

Abbreviations

1. C = cytoplasm.
2. Cd = cytoplasmic division.
3. Ch = chromatin material.
4. Dc = distal centriole.
5. Gg = golgi apparatus.
6. H = hilus.
7. Hsp = head of the sperm.
8. J = junction.
9. L.S = longitudinal section in the tail region of sperm.
10. M = mitochondria.
11. Mp = middle piece.
12. Mt = microtubules.
13. N = nucleus.
14. Na = nuage.
15. Nd = nuclear division.
16. Nf= nuclear fossa.
17. Nm = nuclear membrane.
18. Np = nuclear pores.
19. Nu = nucleolus.
20. Pc = proximal centriole.
21. Pv = vesicles of pinocytosis.
22. T.S = cross section in the tail region of the sperm.