BIOCELL
2003, 27(3): 319-328

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Morphological study of the spermatogenesis in the teleost *Piaractus mesopotamicus*

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Keywords: *Piaractus mesopotamicus*, spermatogenesis, spermatogonia, spermiogenesis, Teleostei

ABSTRACT: The spermatogenesis of *Piaractus mesopotamicus* was investigated under light and transmission electron microscopy. The specimens were captured from their natural environment (Rio Miranda and Rio Aquidauana, Pantanal Matogrossense, Brazil) during April and September. The results were compared with the spermatogenic data of specimens under captivity condition. In both conditions, *P. mesopotamicus* presented the typical spermatogenesis pattern of the teleost fishes, showing no significative differences. The spermatozoon was classified as type I, which has a globular head without acrosome, a short middle piece and a long tail constituted only by the flagellum. This type of spermatozoon is considered the basic type in fishes.

Introduction

The sexual maturation of the gonads present characteristic patterns for each animal group and some particularities for each species. Hence, the study of the gametogenesis contributes for the differentiation of a species or groups of species, helping in phylogenetic classification and, forming a general panorama, creates possibilities for the management and preservation of the species (Bacetti, 1970; Billard, 1970; 1983; Frázen, 1970, 1977; Afzelius, 1978, 1979; Mattei and Mattei, 1978; Azevedo and Corral, 1983; Lahnsteiner *et al.*, 1991; Romagosa *et al.*, 2000).

Piaractus mesopotamicus (locally known as Pacu) is a teleost fish from the Pantanal Matogrossense's rivers, and constitutes an autochthonous species of the Prata Basin. This fish has seasonal reproductive cycle. The spawning is of the split less type (Romagosa, 1991; Bernardino *et al.*, 1986) and occurs mainly on November (Ferraz de Lima *et al.*, 1984), when the fishes present fully gonadal development. The spawning is done at the rivers margins, very far from the site where the adult fishes live and their reproduction does not occur under artificial conditions, except when hormonally induced.

The testes of *P. mesopotamicus* follow the Teleostei pattern, being constituted by a pair of elongated organs of tubular type. The seminiferous tubules anastomose and in the terminal portion form a single spermatic duct that opens into the genital papilla. This fish does not present accessory glands, or seminal vesicle (Romagosa, 1991).

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Received on February 5, 2003. Accepted on June 13, 2003

Contrary to the oogenesis, the developing processes of the male gonads in Brazilian native fishes have been poorly investigated (Cruz-Landim and Cruz-Höfling, 1986/87; Matos *et al.*, 1993, 1995; Matos and Azevedo, 1989; Romagosa *et al.*, 1993, 2000), and do not devoted to the spermatogenesis of individuals captured in their natural environment. In this sense, a histological and ultrastructural study of the spermatogenesis in *P. mesopotamicus* under natural condition was done, trying to contribute to the knowledge of the reproductive cycle in this species, comparing the present results with those obtained with fishes of the same species under captivity condition (Romagosa, 1991; Romagosa *et al.*, 1993).

Materials and Methods

Male specimens of *Piaractus mesopotamicus* (Teleostei) were captured at the Pantanal Matogrossense (Mato Grosso do Sul State, Brazil), in feeding and breeding sites at the Aquidauana and Miranda rivers during the months of April and September. Testis fragments were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, and post-fixed in 1% osmium tetroxide in the same buffer, after rinse in the buffer. The testis fragments were dehydrated in a acetone series (30 to 100%) and embedded in Epon 812. Semithin sections were used for light microscope studies after being stained with methylene blue and azur II. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined and photographed under a Zeiss EM9S2 transmission electron microscope (TEM).

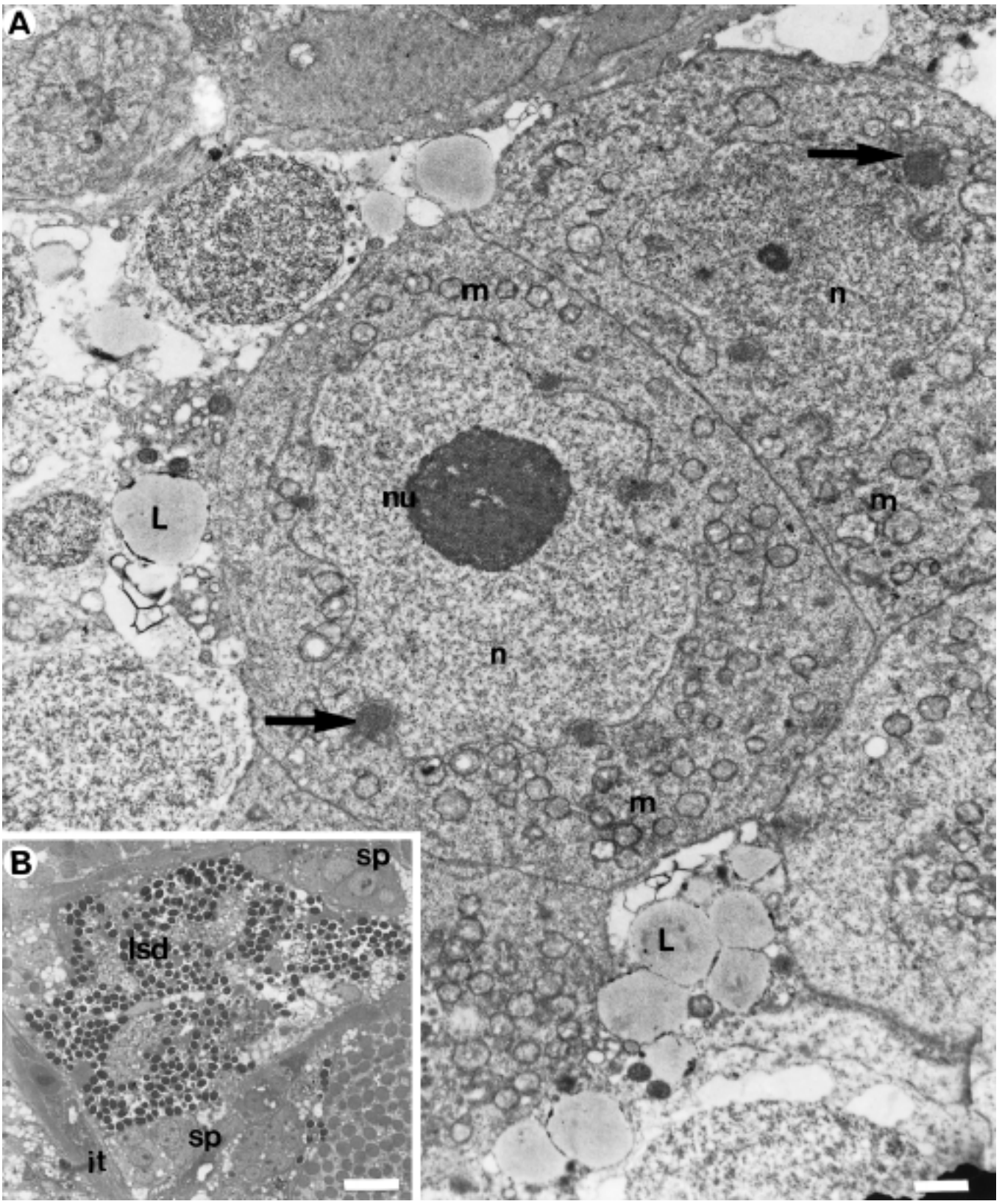
Results

During the annual reproductive cycle, the testes of *P. mesopotamicus* pass by four stages of development: stage I (or resting); stage II (or maturation); stage III (or maturated) and stage IV (or regression). Each stage shows characteristic phases of gamete development in the testicular tubules.

During the stage I, the fishes are entering in maturity, the testes show a pink-translucid coloration and present an increase number of spermatogonia in the seminiferous tubules. Therefore, in this stage, new spermatogonia arise by mitotic division of the quiescent primary spermatogonia, forming groups of secondary spermatogonia close to the testis tubular wall (Figs. 1A,B). However, if it is a resting inter-cycle stage, besides spermatogonial proliferation, spermatozoa in reabsorption will be also present (Fig. 2C).

The primary spermatogonium was the largest cell of the germ line and showed a very large and spherical nucleus with central position, with irregular contour and de-condensed chromatin (Fig. 1A). Very defined central nucleoli may be observed (Fig. 1A). The cytoplasm (Fig. 1A) was not abundant, containing many spherical mitochondria, developed smooth endoplasmic reticule (SER), free ribosomes, rarely Golgi complexes and rough endoplasmic reticule (RER). In this stage, electrondense material in close apposition to the nuclear envelope on the cytoplasm side and in association with two or three mitochondria was observed lodged into nuclear pits, and dispersed in the cytoplasm (Fig. 1A). This electrondense material has been named “nuage” (Clérot, 1979).

FIGURE 1. **A.** Transmission electron microscopy (TEM) of a secondary spermatogonia group after mitotic division. Notice nuage lodged in pits of the nuclear envelope and dispersed in the cytoplasm (arrows), associated with two or three mitochondria (m). n, nucleus; nu, nucleoli; Scale Bar = 0.7 μm. **B.** Light Micrograph (LM) of a seminiferous tubule in a stage II testis. Notice primary spermatogonia (sp) in close association with the seminiferous tubule walls and cyst of late spermatids (lsd). it, interstitial tissue; Scale Bar = 100 μm.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Each secondary spermatogonium become wrapped by a thin layer of flattened cells, known as cyst cells. Into the cyst cell envelope, the spermatogonia proliferate by mitosis and, in a later stage, undergo meiosis and spermatozoa differentiation. The secondary spermatogonia were smaller than the primary ones, once they underwent successive mitotic divisions, without much growth between them. The chromatin may or not be more decondensed and the nucleolus is usually evident. The same intracytoplasmatic organelles observed in primary spermatogonia were observed in the secondary ones, but there was an increasing of the "nuage" lodged in nuclear envelope pits and dispersed in the cytoplasm. This material may be distributed all around the extension of the nuclear envelope (Fig. 1A). The stage I, therefore, is characterized as the stage of mitotic proliferation of the secondary spermatogonia and cyst organization, and occurred in fishes captured at the feeding place.

In the next stage or stage II the testes present whitish coloration, being this stage correspondent to the spermatogenic period of the testis development, therefore, in this stage intense spermatogenic activity occurs in the seminiferous tubules, where spermatogonia, spermatocytes, early and late spermatids may be observed (Fig. 2A,B).

Primary spermatocytes are cells in prophase of the first meiotic division, and are characterized by synaptonemal complexes (Fig. 2A, B) present in zygotene and pachytene nuclei. The first meiotic division originates the secondary spermatocytes.

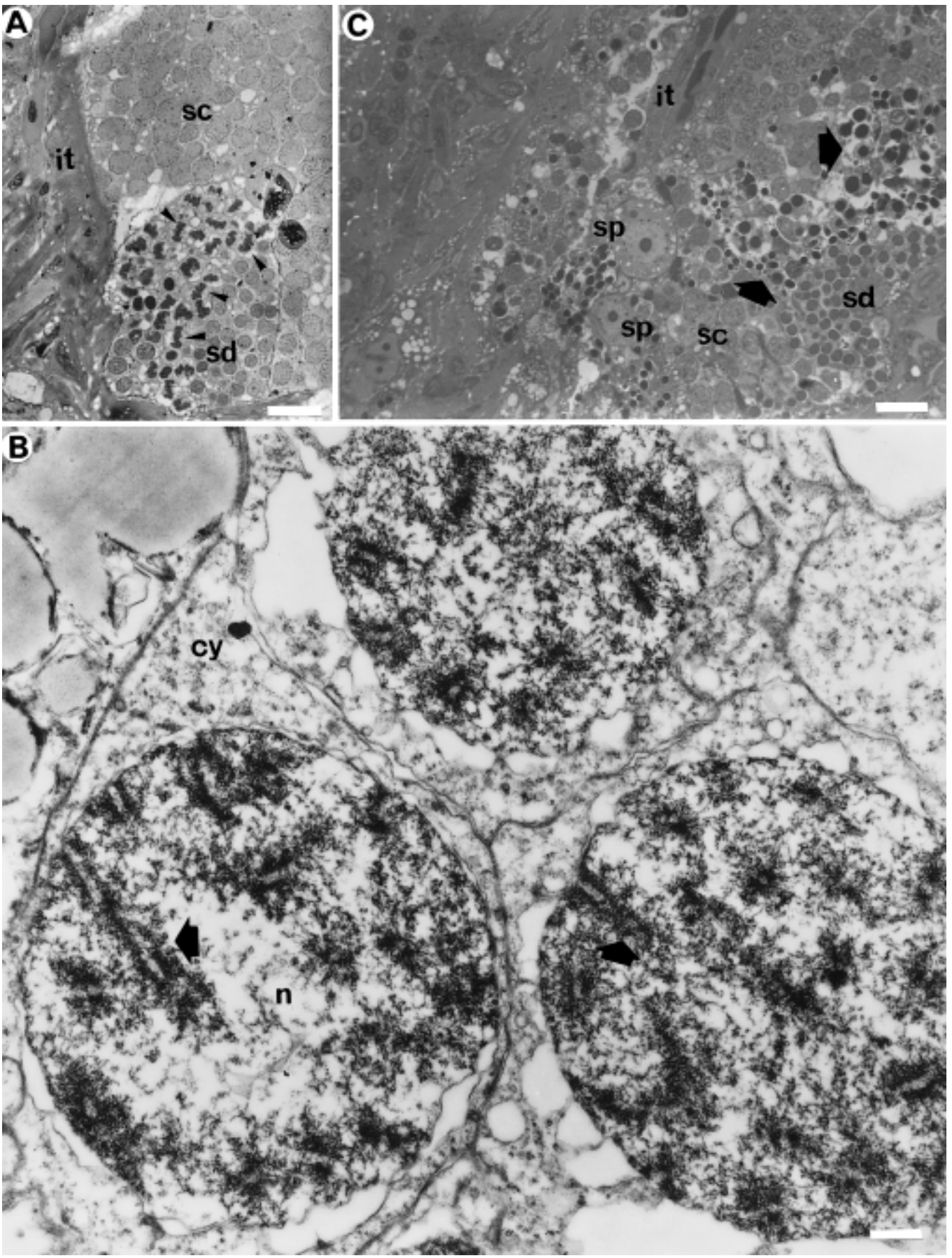
The secondary spermatocytes are smaller cells than the spermatogonia and the nucleolus is rarely visible.

The second division of meiosis gives rise to the spermatids that are smaller cells than the spermatocytes (Fig. 2A,C) and, inside the cysts, will transform in the spermatozoa by the process of spermiogenesis. The spermiogenesis is characterized by changes in the spermatid organelles, mainly in the nucleus featured by the condensation of the chromatin (Figs. 2A,C and 3A-F) and production of the flagellum by the centrioles. Firstly, the nuclei of the spermatids show granular chromatin distributed homogeneously (Fig. 3A,B). The spermatid nuclei present a subsequent increase of the chromatin condensation, which form larger electrondense granules and a decrease of the nuclear volume (Fig. 3A,C). Thereafter, large vacuoles of non-eliminated nucleoplasm appear in the nucleus (Fig. 3D). At this step of chromatin condensation, the centrioles are already located in a distal depression of the nucleus, the cytoplasm and mitochondria displaced from around the nucleus to near the centrioles, forming the middle piece, and the flagellum starts to elongate from the centrioles (Fig. 3E). The nucleus presents changes in its form, showing in the distal part a cavity, the future nuclear fossa (Figs. 3B,D-F). The cytoplasm, very reduced, almost disappears from around the nucleus being present only in the middle piece (Fig. 3E,F). At this stage, this cell is a late spermatid (Fig. 3E).

In all stages, the cells stay together through cytoplasm bridges, which are very evident in this stage (Fig. 3B) and permit the synchronic development of the germ cells inside the cysts. Fishes with testes in this stage were captured in the feeding place.

The Stage III corresponds to the testis mature for spawning. The testes are now white, the cysts have broken and the spermatozoa are free in the tubule lumen. Spermatozoa are the smallest testicular cells (Fig. 4A). In this species, spermatozoa are constituted by a globu-

FIGURE 2. A. LM of a seminiferous tubule portion undergoing spermatogenesis. Notice a cyst undergoing metaphase (arrowheads) surrounded by cysts containing spermatocytes I (sc) in pachytene and early spermatids (sd). it, interstitial tissue; Scale Bar = 30 μm. **B.** TEM detail of spermatocytes in pachytene, showing the synaptonemal complex (arrowheads). cy, cytoplasm; Scale Bar = 0.3 μm .**C.** LM of a seminiferous tubule in a testis in stage IV. Notice spermatogonia (sp) associated to the inner wall and remnant cysts under reabsorption (arrowheads). sc, spermatocyte; sd, spermatid. it, interstitial tissue; Scale Bar = 100 μm.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lar head without acrosome, a short middle piece and a tail, constituted only by the flagellum (Fig. 4B,C). The tail appears very weakly stained by stains used for light microscopy (Fig. 4A) and electronlucid under transmission electron microscopy (Fig. 4B). On the flagellum insertion region, the nucleus presents a nuclear fosse, which lodges the centriolar complex and the initial part of the flagellum (Figs. 3F, 4B). The distal centriole localizes below and perpendicularly to the proximal one and both have similar structure (Fig. 4B). The middle piece corresponds to about 2/3 of the head length and forms a sheath surrounding the initial part of the flagellum, that in the longitudinal sections seems like two symmetrical christae that become thinner in the distal portion (Fig. 4B). Small spherical mitochondria with few christae form a collar-like structure in the initial portion of the middle piece (Figs. 3F, 4B). The tail is long and starts from the initial part of the middle piece (Fig. 3F, 4B). The flagellum with 9+2 microtubule organization presents a thin cytoplasm cape that emits thin and regular membranous lateral expansions all along its extension (Fig. 4C). Fishes in this gonadal stage were found in the reproduction place.

The testis enters in regression or stage IV soon after the spawning, showing disorganization of the seminiferous tubule cysts although some late spermatids may still be present. The lumen presented remain spermatozoa being reabsorbed by the cyst cells. The spermatids remaining in the latecomer cyst were also reabsorbed by the cyst cells. After the reabsorption is over, the primary spermatogonia start dividing to restart the cycle (Fig.2C).

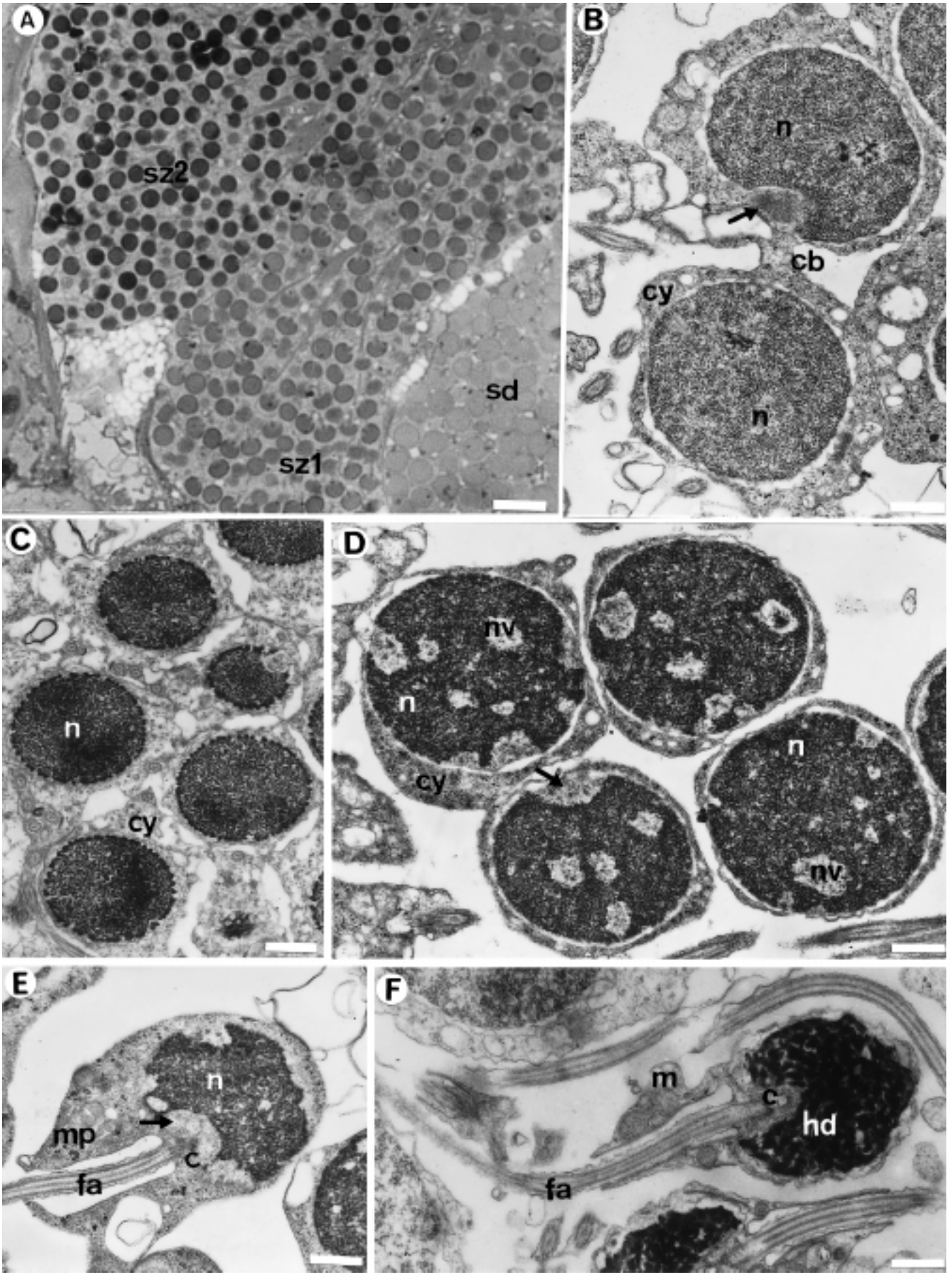
Discussion

Comparing the present results with those from Romagosa (1991) and Romagosa *et al.* (1993), the stages of spermatogenesis and testis development of *P. mesopotamicus* is very similar under natural and captivity conditions, being also similar to the other species of teleost fishes (Grier, 1981; Matos *et al.*, 1993, 1995; Cruz-Landim and Cruz-Höfling, 1986/87; Billard, 1970, 1983; Lahnsteiner *et al.*, 1991; Romagosa *et al.*, 2000). Therefore, although this fish reproduces only under natural conditions, the captivity seems do not to interfere in the gonadal development, at least in this specie. A question for a future study is about the behavior the gonads of fishes that were not induced to spawning through hormonal stimulation under artificial conditions at the end of the spermatogenic cycle. Will all spermatozoa be reabsorbed after spermatogenic regression?

As observed by Romagosa (1991) and Romagosa *et al.* (2000) the organization of the germ cells in the testis of *P. mesopotamicus* is of unrestricted type, which is characteristic of teleost fishes (Grier, 1981; Grie *et al.*, 1980) and means that the spermatogonia are localized in all extension of the inner seminiferous tubule wall, not restricted to a special portion.

The spermatozoa belong to the group III of Billard (1970) fish spermatozoon classification, in which the spermatozoa without acrosome is constituted by a globular head, a short middle piece organized as a collar of mitochondria surrounding the anterior extremity of the tail, and a tail, constituted only by the flagellum. The flagellum presents regular lateral membranous expan-

FIGURE 3. Ultrastructural aspects of spermiogenesis in Stage II testes. **A.** LM of a seminiferous tubule presenting early spermatids (sd) and two stages in a more advance spermatid degree of maturation (zp1 and zp2) according to their nuclei condensation; Scale Bar = 30 μm . **B.** Initial step of chromatin condensation. Notice the primordial nuclear fossa (arrow) and a cytoplasmic bridge (cb) linking two spermatids. cy, cytoplasm; n, nucleus; Scale Bar = 0.5 μm. **C.** Spermatids in a more advanced stage of chromatin condensation. Notice the scarce cytoplasm (cy) around the nucleus (n); Scale Bar = 0.8 μm. **D.** Nuclear condensation originating vacuoles of nucleoplasm (nv). The cytoplasm persists as a halo surrounding the nucleus (n). arrow, nuclear fosse; Scale Bar = 0.5 μm. **E.** Late spermatids with condensed nucleus (n), with nuclear fossa (arrow), where the pair of centrioles (c) are lodged. Notice the formation of the middle piece (mp) and flagellum (fa); Scale Bar = 0.5 μm. **F.** Spermatozoa in a later step of maturation. Notice that the cytoplasm was almost eliminated and the head (hd), middle piece and flagellum (fa) were formed. c, centrioles; m, mitochondria; Scale Bar = 0.5 μm.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sions that in transversal sections present a wings-like aspect. These spermatozoa are considered less derived or the basal type (Billard, 1983; 1992; Grier, 1981; Cruz-Landim and Cruz-Höfling, 1986/87; Silva and Godinho, 1991; Matos *et al.*, 1993).

Some authors argue that the acrosome absence is not a basal condition, instead, it might be a derived condition, since in less derived fishes and lower phylogenetic groups the acrosome is present (Boisson *et al.*, 1967; Jespersen, 1971; Matos and Azevedo, 1989; Matos *et al.*, 1993). According to Afzelius (1979), along the evolution, some fishes lost the acrosome. The reason for this is still unknown (Matos *et al.*, 1993; Matos *et al.*, 1995). In the literature there are controversies about the reason why some fish's spermatozoa lack acrosome. The absence of the acrosome has been explained by the existence of micropyle in the eggs of the teleost fishes, which might facilitate their entrance through the corium (Pasteels, 1965; Nicander, 1970) and the consequent non-necessity of an acrosomal material.

The present authors think that the presence of micropyle in the eggs does not justify the absence of acrosome. In other groups of animals, as insects, the micropyle is present and their spermatozoa have acrosome. The function of acrosome is more than to permit the entrance of the spermatozoon in the egg. It promotes an acrosomal reaction with liberation of the content of the egg cortical granules that impedes other spermatozoa to enter the egg, hardening the vitellinic membrane, besides other effects. Therefore, we suggest that the absence of acrosome is a less derived condition and the polyspermy avoidance might be done by the cortical granules present in the eggs of this species.

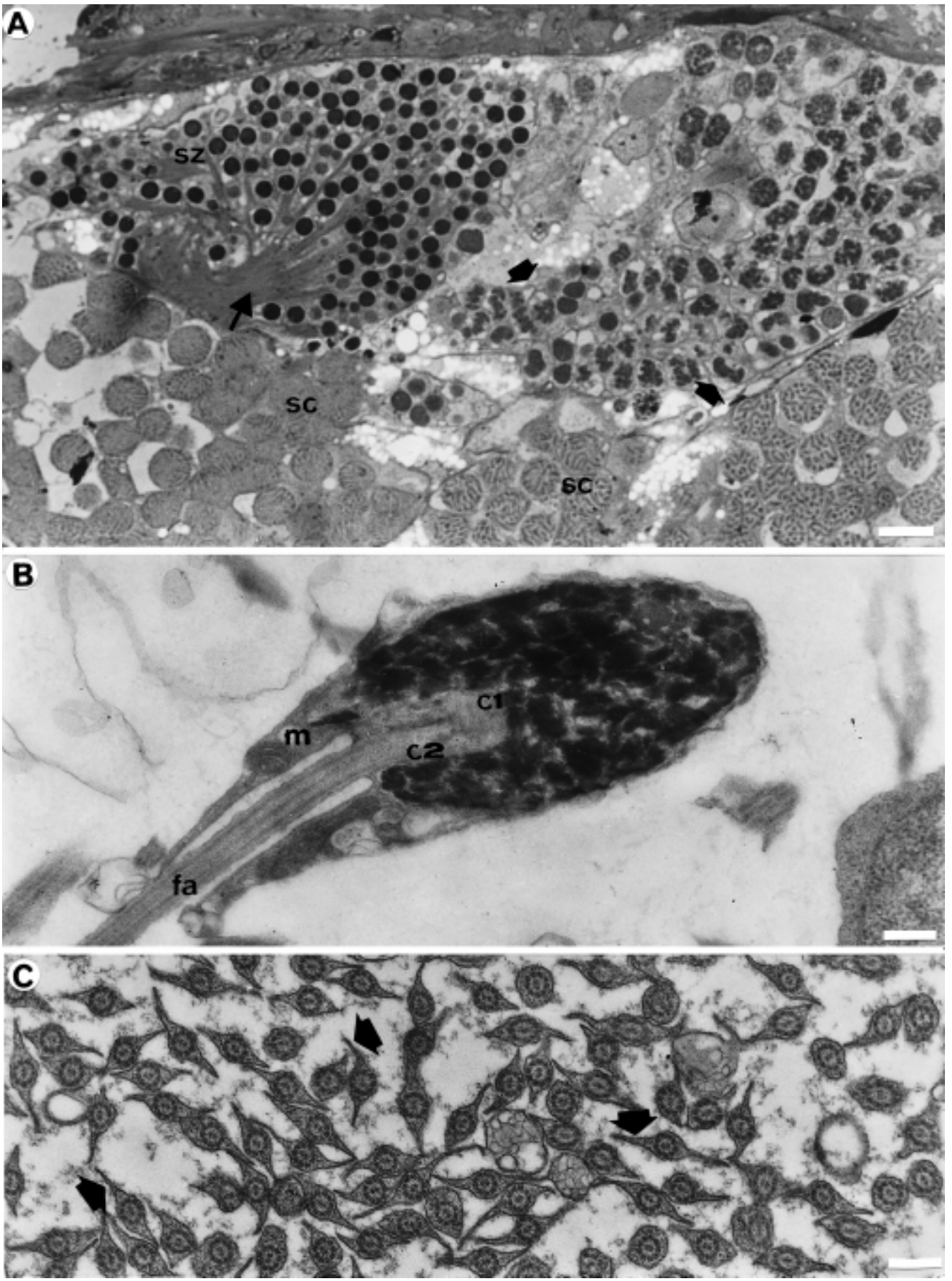
The long tail of the spermatozoa of *P. mesopotamicus* with regular lateral membranous extensions represents an adaptation for propulsion and orientation capacity of the spermatozoa, since this species of fish present external fertilization.

The function of the cyst cells is unknown, but by their localization were considered as homologue of the Sertoli cells of other vertebrates. Also unknown is the "nuage" function and its chemical nature, although in others species it was identified as ribonucleoproteins. In *P. mesopotamicus*, the "nuage" appear in spermatogonia, lodged in nuclear envelope pits or dispersed in the cytoplasm, always associated with mitocondria, being attributed to it, by Clérot (1979), function in mitochondrial replication.

Acknowledgments

Financial support given by FAPESP.

FIGURE 4. A. LM showing late spermatids (sz) still in the cyst. Notice sheaf of late spermatid long tails (arrow), side by side with cyst of larger spermatocytes II. Arrowhead, cells undergoing metaphase and spermatocytes I (sc) in pachytene; Scale Bar = 14 μm. **B.** Ultrastructural aspects of a spermatozoon in testis in stage III. Notice the condensed nucleus, forming the globular head, the nuclear fossa lodged the pair of centrioles (c1 and c2), which are in perpendicular disposition. The middle piece in longitudinal section forms two symmetrical crystae that become thinner in the distal extremity. A collar of mitochondria (m) is present in the initial part of the middle piece. The flagellum (fa) starts from the initial part of the middle piece and is very long; Scale Bar = 0.3 μm. **C.** Detail of the plasmic membrane extensions of the flagellum, forming wing-like projections (arrowheads). Notice the 9+2 microtubular organization of the flagellum; Scale Bar = 0.3 μm.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