MORPHOLOGICAL CHANGES OF SERTOLI CELLS DURING THE MALE REPRODUCTIVE CYCLE OF THE TELEOST *Piaractus mesopotamicus* (HOLMBERG, 1887)

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ABSTRACT

An investigation of the histological and ultrastructural changes of Sertoli cells during the male reproductive cycle in *Piaractus mesopotamicus* was made. The results showed that the Sertoli cell development is closely related with germ cell maturation. Therefore, these cells may have some role in germ cell maturation during the reproductive cycle of this species, whether in forming a tissue framework for the developing spermatogenic cysts, aiding in testes reorganization for a new reproductive cycle, in addition to other possible functions discussed in the text.

Key words: spermatogenesis, testes, cyst cells, reproduction.

RESUMO

Mudanças morfológicas nas células de Sertoli durante o ciclo reprodutivo de machos do teleósteo *Piaractus mesopotamicus* (Holmberg, 1887)

Realizou-se uma investigação das mudanças histológicas e ultra-estruturais das células de Sertoli durante o ciclo reprodutivo de machos de *Piaractus mesopotamicus*. Os resultados mostraram que o desenvolvimento das células de Sertoli está estritamente relacionado à maturação das células gaméticas. Portanto, as células de Sertoli têm algum papel na maturação das células germinativas durante o ciclo reprodutivo dessa espécie, talvez formando um tecido de sustentação para os cistos espermatogênicos em desenvolvimento, ajudando a reorganização testicular para um novo ciclo reprodutivo, além de outras possíveis funções discutidas no texto.

Palavras-chave: espermatogênese, testículos, células do cisto, reprodução.

INTRODUCTION

In teleosts, the testicular structure varies from species to species, although two basic types, lobular and tubular, may be identified according to differentiation of the germinal tissue (Billard, 1986, 1990; Grier, 1981; Grier *et al.*, 1980; Nakagama, 1983). According to Grier (1993), in lower teleost fish the germinal compartment is organized into anastomosing tubules, while that of higher fish is organized into branching lobules. The lobular-type testis, which is presented also by *Piaractus mesopotamicus*, is composed of numerous tubules separated from each other by a thin layer of fibrous or interstitial tissue having several types of cells and blood vessels (Cauty & Loir, 1995).

Within the lobules, primary spermatogonia undergo numerous mitotic divisions to produce groups or cysts containing differentiating spermatogonial cells, all of which are in the same stage of development in a given cyst (Billard *et al.*, 1982; Pudney, 1995). A layer of epithelial cells and

basal lamina separates the cysts from each other. Therefore, the lobules of teleost testis contain two cell types: somatic epithelial cells lining the periphery of the lobules and cysts of germ cells within them.

All the cysts are enveloped by differentiated cells, known as "cystic cells", "lobule boundary cells", or "Sertoli cells" (Cruz-Höfling & Cruz-Landim, 1984; Romagosa *et al.*, 2000). The terminology for these cells has long been confused. The term "lobule boundary cells" was first introduced by Marshall & Lofts (1956), followed by O'Halloran & Idler (1970), who considered these cells homologous with the mammalian Leydig cells. However, the lobule boundary cells seem to be more accurately homologous to Sertoli cells, since they are separated from the interlobular space by a basal lamina (Billard *et al.*, 1972, 1982; Grier, 1975; Grier & Linton, 1977; Mattei *et al.*, 1982, 1993; Nakagama, 1983; Nicholls & Graham, 1972) and, as in mammals, present follicle-stimulating hormone (FSH) receptors (Schulz *et al.*, 2001; Weltzien *et al.*, 2002).

The function of Sertoli cells in fish is not well established, but the ultrastructural morphology demonstrates the presence of spherical mitochondria with parallel crystae and lipid deposits in the cytoplasm (Billard *et al.*, 1972; Cruz-Höfling & Cruz-Landim, 1984; Grier, 1975; Mattei *et al.*, 1982), which are characteristics of steroid-producing cells, suggesting a possible role in steroid synthesis, or at least locations where these hormones are stocked (Grier & Linton, 1977; Cruz-Höfling & Cruz-Landim, 1984; Mattei *et al.*, 1982). However, knowledge about endocrine control of spermatogenesis in teleost fish has mostly been drawn from measurements of hormone levels in the peripheral blood, injection of pituitary extracts, gonadotropins, and steroids into either intact or hypophysectomized specimens (Billard *et al.*, 1972; Fostier *et al.*, 1983; Schulz *et al.*, 2001; Weltzien *et al.*, 2002), in such a way the exactly local of control of the hormone-producing is unknown.

In most teleost fish studied, testis growth and development coincide with increased plasma levels of 11-ketotestosterone (11-KT) and to a lesser extent, testosterone (T) (Borg, 1994; Methven *et al.*, 1992; Norberg *et al.*, 2001; Scott *et al.*, 1980; Weltzien *et al.*, 2002). *In vivo* or *in vitro* studies show that 11-KT is most effective as a direct stimulator for spermatogenesis (Cavaco *et al.*, 1998), while T is most effective as a stimulator of hypothalamic (Amano *et al.*, 1994) and pituitary (Montero *et al.*, 1995) activity, leading to further activation of the testis. The sex steroid levels in fish are also influenced by behavior, e.g., social modulation (Oliveira *et al.*, 2002). The presence of cholesterol-positive lipids in Sertoli cell homologues seems be an insufficient criterion by which to identify them as steroid-producing cells (Cruz-Höfling & Cruz-Landim, 1984).

Although the lobule boundary cells, according to Marshall & Lofts (1956), often occur in testes of fishes not having typical interstitial cells (Leydig cells), ultrastructural observations clearly indicate that there are some species whose testes appear to have both interstitial and lobule boundary cells (Guraya, 1976; Nakagama *et al.*, 1982).

These cells seem to participate as well as in spermatozoa phagocytosis, mainly during the regression stage of testis development, but while this process is sometimes mentioned, its details have not yet been presented (Mattei *et al.*, 1993; Romagosa *et al.*, 2000; Weltzien *et al.*, 2002).

A histological and ultrastructural investigation of Sertoli cell development in the testes of *P. mesopotamicus* was made for the purpose of contributing the clarification of the role of these cells in teleost fishes with a seasonal reproductive cycle. This species presents local ecological importance and, through artificial breeding, may prove to be significant economically for riverine people. For both of these reasons, the reproductive mechanism and system of *P. mesopotamicus* should be understood.

MATERIAL AND METHODS

Male specimens of *Piaractus mesopotamicus* (Teleostei) were captured at the Pantanal Matogrossense (Mato Grosso do Sul State, Brazil) at breeding and feeding sites on the rivers Aquidauana and Miranda, during April and September. Testes fragments were fixed in 2.5% gluraldehyde in 0.1 M sodium cacodylate buffer, in which buffer they were then rinsed, and then post-fixed in 1% osmium tetroxide in that same buffer. They were dehydrated in an 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 uranile acetate and lead citrate prior to being examined and photographed under a Zeiss EM9S2 transmission electron microscope.

RESULTS

The testes of fishes with a seasonal reproductive cycle, as is the case of this species, show along the year different levels of gonadal development, which in males may be divided in four stages, according to the period of the year in which each one appears, and also to morphological and ultrastructural changes in testis tissues and germ cell line maturation. Morphological changes of the cystic cells were observed along these four stages of testis development in *P. mesopotamicus*.

The studied fishes were adult males that had already gone through previous reproductive cycles; therefore, at the beginning of the new seasonal cycle, remaining spermatozoa were sometimes found in the cysts (Fig. 1B), but most of the testes lobules were completely empty and lined only by the Sertoli cells that form an epithelial inner cover-like boundary at the beginning of the reproductive cycle (Fig. 1A).

The beginning of the reproductive cycle (Stage I) is marked by proliferation of spermatogonia, which aggregate in groups close to the inner seminiferous tubule wall. In this stage, the Sertoli cells are clearly seen lining the entire extension of the inner seminiferous tubule wall (Fig. 1A) and associated with the spermatogonia that in this stage are already enveloped by them (Figs. 1A, B, C). Sertoli cells at this stage show an elongated nucleus, with an irregular contour, and with chromatin condensed only near the nuclear envelope (Figs. 1B, C). The scarce cytoplasm is poor in organelles, with some small and spherical mitochondria occurring as well as many large lipid droplets, which fill the cytoplasm. The smooth endoplasmic reticule (SER) is not very developed. The plasmic membrane emits short, thin projections into the lumen of the seminiferous tubule (Fig. 1B), which also envelop the spermatogonia (Figs. 1B, C) and, sometimes, the spermatozoa remaining in the cyst lumen from the previous cycle. The projections of two different Sertoli cells are linked through desmosomes, forming a closed comportment. The Sertoli cells may be in direct contact with the spermatogonia, but are not with the interstitial tissue forming the seminiferous tubule walls, due to the electron-lucid basal lamina separating them (Fig. 1B).

In a more advanced stage of testis maturation (Stage II), many cysts of the germ line in various maturation phases, e.g., spermatocytes, spermatids, and even spermatozoa can be observed in the seminiferous tubules. In this maturation stage, the amount of lipidic deposits in the Sertoli cells has increased, and the droplets are larger and more electrondense (compare Fig. 1A with 2A). Projections of the cell plasmic membrane become longer and more numerous, and are full of lipid droplets (Fig. 2B). In this stage, the Sertoli cells with their projections can be clearly observed enveloping each cyst, and forming a framework among them, i.e., sustentation tissue, for the developing cysts of the seminiferous tubule (Figs. 2A, B).

When the males are able to reproduce, they present fully developed or mature testes, which are those in Stage III. The seminiferous tubules are now replete with differentiating spermatides, with some regions predominantly presenting mature spermatozoa (Figs. 3A, B). The mature spermatozoa are released in the seminiferous tubule lumen by the rupture of the Sertoli cell barrier. In this stage, the Sertoli "epithelium" presents a decreased number and/or volume of cells (compare Fig. 2A with 3A). Cell extensions become filamentous and the lipid droplets concentrate in the "cell body", rather than in the cytoplasmic extensions (Fig. 3B). At this point, some spermatozoa seem to be reabsorbed by the Sertoli cells, which present residue of spermatozoa nuclei in the cytoplasm (Fig. 3B).

After the male reproductive period, most of the mature spermatozoa have already been released from the testis by the spawning. The testis begins a regression process signaling the end of the reproductive cycle (Stage IV), and the gonad enters a nonreproductive season, during which the new reproductive cycle is being prepared by reabsorption of the remaining spermatozoa in the seminiferous tubule, and restructuring of all the testis tissue. In this stage, the Sertoli cells present a disrupted luminal surface (Figs. 4A, B, C). The elongated

nucleus exhibits a regular shape due to the decreasing of lipid droplets (Fig. 4A) that filled the cytoplasm in the previous stages. The cytoplasm is poor in organelles, shows some SER, and seems to be undergoing reabsorption and regression marked by the presence of myelinic bodies and great vacuolization, respectively (Figs. 4A, C). In addition, spermatozoa reabsorption activity by the Sertoli cells increases (Figs. 4B, D).

DISCUSSION

In some species of fish, spermiogenesis, or part of the process, occurs outside the cysts, but in *Ophidion* sp., the mature germ cells complete spermiogenesis individually, and are not linked through cytoplasm bridges to the testes lumen (Mattei *et al.*, 1993). According to this author, this semi-cystic type of spermatogenesis has also been observed in *Neoceratis spinifer*, *Lepadogaster lepadogaster*, and various species of Blenniidae. In *P. mesopotamicus*, spermatogenesis is of the cystic type, since it occurs entirely within the cysts.

The Sertoli cells are closely relation with germ cell maturation, since they are more developed when the testes are in maximum spermiogenic activity, or Stage II. In that phase, these cells enclose the already developing cysts and also fill the spaces among them, thus giving rise to the framework tissue that sustains them in the seminiferous tubule. When the testes have developed completely (Stage III) and are filled with mature spermatozoa, the Sertoli cells begin their reabsorption activity, which is more evident in the regression phase (Stage IV). This phagocytosis or reabsorptive activity of spermatozoa, and even of earlier maturing phases of germ cells, by the Sertoli cells is very poorly studied in fishes, but it has already been briefly described for some species or at least mentioned (Mattei *et al.*, 1993; Romagosa *et al.*, 2000; Weltzien *et al.*, 2002).

Nakaghi *et al.* (2003) reported that the Sertoli cells of *Colossoma macropomum* (tambaqui) were found at the periphery of the cysts of germinative lineage cells and the nuclei were shown to be smaller as these cells develop, bigger in the resting period, and smaller and flat in the maturation period due to the lipids droplets that fill the Sertoli cell cytoplasm. These results, which are in accordance with the present data, also suggest that these cells have some role in germ cell maturation in teleosts.

The developmental cycle of Sertoli cells in *P. mesopotamicus* coincides with the increased 11-KT plasma levels in teleost fish (Borg, 1994; Gazola & Borella, 1997; Methven *et al.*, 1992; Norberg *et al.*, 2001; Scott *et al.*, 1980; Weltzien *et al.*, 2002), and are more highly developed in the maximum testis development stage, when the titer of sex steroids is higher, suggesting that, directly or not, the developing germ cells may use Sertoli lipid deposits for their development.

In mammals, Sertoli cells present FSH receptors and constitute a framework to support developing germ cells; they also have at least four important functions in testis development. During embryogenesis, they produce the anti-Müllerian hormone, which suppresses the female reproductive tract, and stimulates interstitial tissue development and replication of Leydig cells, which produce the testosterone hormone responsible for inducing the development of masculine secondary sex characteristics. In adults, they activate spermatogonia development and stimulate maturation of the germ cell line (Alberts *et al.*, 2002).

In fishes, the sex hormone regulation of the testis is not yet completely understood. Autoradiographic studies have shown evidence of two types of receptors present in Coho salmon testis (Miwa & Swanson, 1994). The type RI receptors, found in Sertoli cells, interact with both FSH and LH, whereas receptor type RII, found in Leydig cells, interacts specifically with LH. The morphology and receptor-activity similarity between the lobule boundary cells and the mammalian Sertoli cells leads the authors to propose the term "Sertoli cell" for the lobule boundary cells in fish.

This study has provided some clues to understand the Sertoli cell function in teleost fishes. As in mammals, these cells may fashion a framework to support cysts in the seminiferous tubules, and perhaps create a microenvironment for developing germ cells within the cysts. Later on, during the interseasonal regression these cells participate in ridding the testes of spawning waste by reabsorbing the remaining spermatozoa, or even earlier, nonsynchronous germ cell lines. Thus, testes are prepared for the next reproductive season. In addition, the Sertoli cells may protect or support the early stages of spermatogonia proliferation development.

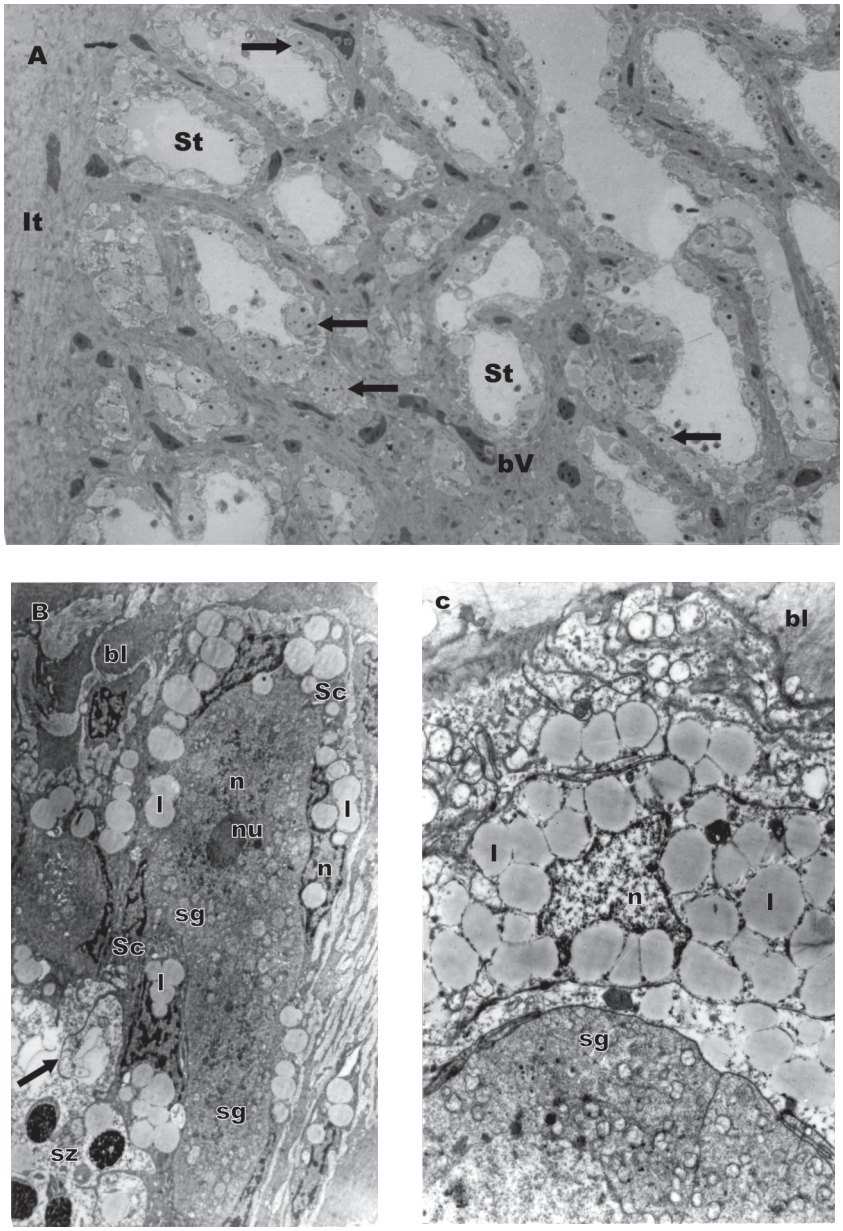

Fig. 1 — A. Light Micrograph (LM) of a *Piaractus mesopotamicus* nonspermatogenic testis, showing empty seminiferous tubules (st) lined by Sertoli cells. The arrows point to groups of spermatogonia, which are enveloped by Sertoli cells. bv, blood vessel; it, interstitial tissue. 500x. **B.** Transmission electron micrograph (TEM) of cyst walls, showing around the spermatogonia (sg), flat Sertoli cells (Sc), with irregular nuclei (n) and lipid droplets (l). The cells emit apical philopodia toward the testis lumen (arrow). bl, basal lamina, sz = spermatozoa. 7200x. **C.** TEM of a Sertoli cell around spermatogonia (sg), with cytoplasm filled with lipid (l). bl, basal lamina. 8400x.

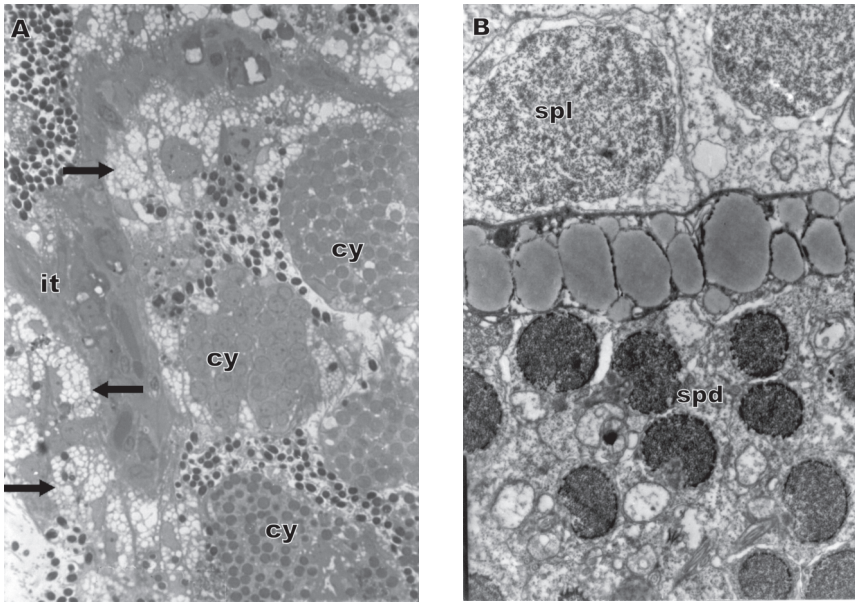

Fig. 2 — A. LM of a Stage II testis showing the Sertoli cells replete with lipid droplets (arrows) and several stages of spermatogenic cysts (cy). 200x. it, interstitial tissue. **B.** TEM showing a branch of a Sertoli cell dividing a cyst containing spermatocytes I (spI) from another containing late spermatids (spd). 15000x.

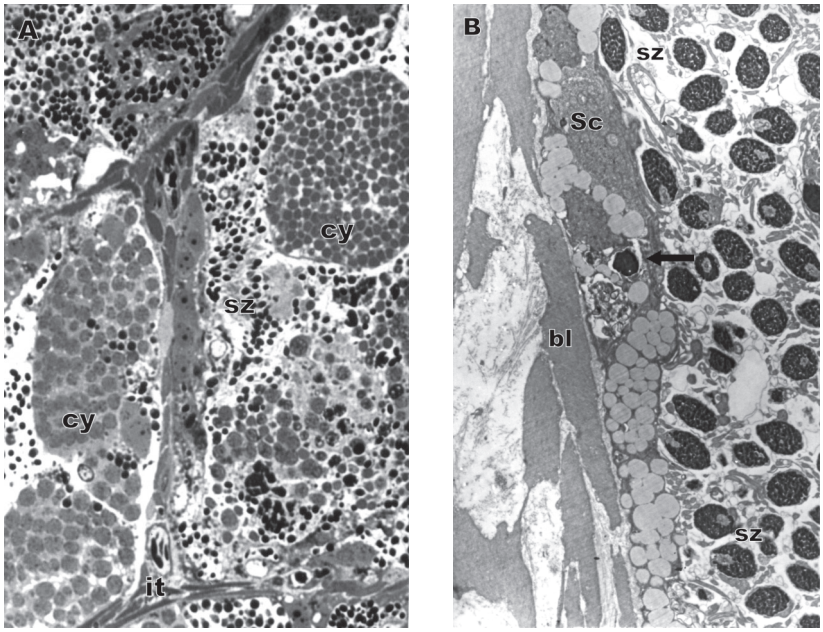

Fig. 3 — A. LM of a mature testis showing reduction of the lipid droplets from the Sertoli cells. cy, cysts; it, interstitial tissue; sz, spermatozoa. 200x. **B.** TEM of late spermatic cysts showing some degenerating sperm (arrow) in projections of flat Sertoli cells (Sc). bl, basal lamina; sz, spermatozoa. 7200x.

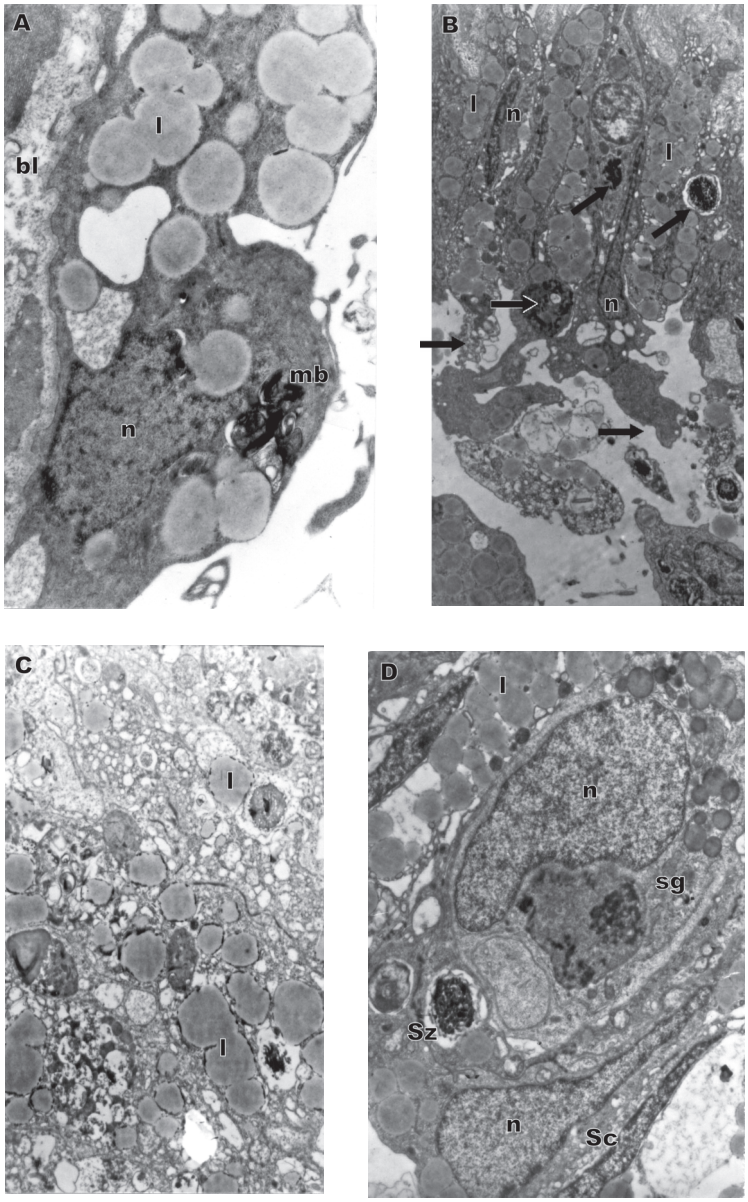

Fig. 4 — Testes in Stage IV. **A.** Myelinic bodies (mb) in the Sertoli cytoplasm, signalizing auto- or heterophagy. bl, basal lamina; l, lipid droplets; n, nucleus. 15000x. **B.** TEM showing all Sertoli cells in degeneration, with depleted lipidic deposits (l) and apical disrupted membrane (arrows). Notice the elongated nucleus (n) and some degenerating spermatozoa in their cytoplasm (arrowheads). 7200x. **C.** Disarranged cyst in a post- spawning testis, showing the disrupted Sertoli cell. l, lipid droplets. 7200x. **D.** Sertoli cell reabsorbing spermatozoa (sz), apparently surrounding degenerating spermatogonium (sg), in a post-spawning testis. l, lipid droplets; n, nucleus; Sc, Sertoli cells. 7200x.

As in other species of fishes, the Sertoli cell ultrastructure contains in the cytoplasm many lipid droplets and spherical mitochondria with parallel crystae. This morphology has led some authors to propose a possible role of these cells in steroid synthesis, or to suggest that they may be the storage place of these hormones, since the synthetic machinery of the cells does not appear to be very active (Grier & Linton, 1977; Cruz-Höfling & Cruz-Landim, 1984; Mattei *et al.*, 1982). The absence of a well-developed synthetic apparatus has also been noticed in this investigation, in which the cytoplasm of these cells was found to be poor in organelles, showing a smooth, poorly developed endoplasmic reticule.

Nevertheless, at least in this study, there was no evidence of exogenous substance uptake. The possibility that Sertoli cells accumulate non-hormonal lipids, as energy sources for the germ cell consumption during maturation, has not been raised here. But this could be the case. If so, these cells could have a role homologous to that of adipose tissue in mobilizing triglycerides.

Whatever the function of the Sertoli cells, the present morphological data show a close relationship between these cells and the spermatogenesis cycles.

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