Structural and ultrastructural characteristics of the spermatogenesis of the grey armored catfish *Liposarcus anisitsi* (Holmberg, 1893) (Teleostei, Siluriformes)

Claudinei da CRUZ[1];
Antônio Marcos ORSI[2];
Karina SIMÕES[3];
Carlos Alberto VICENTINI[4]

1- Laboratório de Ecotoxicologia do Departamento de Fitossanidade da UNESP e CAUNESP, Jaboticabal - SP
2- Departamento de Anatomia da UNESP, Botucatu - SP
3- Departamento de Anatomia da UNICAMP, Campinas - SP
4- Departamento de Ciências Biológicas da UNESP, Bauru e CAUNESP, Jaboticabal - SP

Correspondence to:
ANTÔNIO MARCOS ORSI
Departamento de Anatomia
Universidade Estadual Paulista
Distrito de Rubião Júnior, s/n
Caixa Postal 510
18618-000 - Botucatu - SP
amorsi@ibb.unesp.br

Received: 08/08/2003
Accepted: 22/06/2004

Abstract

Liposarcus anisitsi is a freshwater teleost from Siluriform order with external fertilization that shows cystic spermatogenesis process with spermatogonial unrestricted lobular structure of the testes. In this species, isolated or intracystic primary spermatogonia were noted as the ¨stem cells¨ of the spermatogenetic lineage. Thus, as consequence of the cystic spermatogenesis process of *Liposarcus anisitsi* all the three germinative cells are observed inside specific spermatogenetic cysts, since the primary and secondary spermatogonias until the spermatocytes and early and late spermatids. Spermatogonia show spherical shape with central nuclei and great amount of round mitochondria and ER profiles mainly of rough type in their cytoplasm. Primary spermatocytes have inter-relations through cytoplasmic bridges and are clearly identified by presence of synaptonemic complexes inside the nuclei. Early spermatids beginning maturational phases, as well as the late spermatids, tend to exhibit spherical nuclei, variable chromatin pattern with progressive condensation during the end steps of the spermiogenesis. In this end steps, the late spermatids show the nuclear fossa formed to the fixation of the flagella. The spermatozoon of *Liposarcus anisitsi* shows round head, round nuclei, very dense chromatin, lack of acrosoma, short middle piece and long tail (principal and end pieces of the flagellum).

Key-words:
Spermatogenesis.
Ultrastructure.
Siluriforme.
Liposarcus anisitsi.

Introduction

In teleost fish, the description of the ultrastructure of spermatogenic cells during the pre-meiotic and meiotic phases of the kinetics of spermatogenesis has revealed some common nuclear and cytoplasmic features[1,2,3,4,5]. So, marked modifications have been observed in the nucleus and cytoplasm of spermatids during the process of germinative cell differentiation, which is mainly intracystic in most teleosts[2,4,5,6,7,8,9,10,11,12], including centriole migration, flagellum formation, chromatin condensation, formation of the nuclear fossa, migration of the mitochondria to the middle piece, and elimination of excess cytoplasm[2,12].

Some studies have been reported in the literature regarding the spermiogenesis of fish from temperate or subtropical regions[1,2,3,10,13,14] and concerning spermatozoon morphology[10,15] under these climatic conditions. Subcellular characteristics of the spermiogenic process and of its final product - the spermatozoon - under humid tropical (neotropical) and even

equatorial conditions have also been described for the Brazilian ichthyofauna[1,4,5,6,16,17,18,19,20,21], providing a comparative and adequate basis for the present study conducted on the grey armored catfish.

Concerning to the evolution of spermatozoa of teleost fish, some authors have reported structural transformations in the flagellar apparatus[22,23,24]. However, according to Lou and Takahashi[17], significant variations exist in the morphology and morphogenetic pattern of teleost spermatozoa, which might be related to the mode of reproduction of this phylum such as those resulting from external (most species) and internal fertilization processes.[1,5]

Few studies are available concerning the systematics, evolution and morphology of Siluriformes in South America, an order consisting of 14 families including the species *Liposarcus anisitsi*, although there are species with a zootechnical potential that can be reared in captivity[25]. In Brazil, fish of the order Siluriformes, including the grey armored catfish, are considered to be of economical value[26], representing a good option for aquaculture, due to their rusticity, a medium length which can reach up to 50 cm, and a medium weight of up to 900 g.

In this respect, studies on the spermatogenic process and on spermatozoon evolution in species of this order, as carried out here, are important to broaden the knowledge about the reproduction and reproducibility of neotropical teleost fish held in captivity. Thus, the aim of the present study was to describe aspects of the spermatogenesis and the morphological and cytophysiological characteristics of spermatogenic cells of the grey armored catfish *Liposarcus anisitsi*, a siluriform species widely distributed within Brazilian freshwater systems.

Material and Methods

Twenty-two adult grey armored catfish with a mean body weight of 550 g, provenient from Aquaculture Center of the UNESP (CAUNESP) were studied. Testicular tissue samples were immersed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.3, for 4 hours and postfixed in 1% osmium tetroxide in the same buffer (0.1 M) for 3 hours. After fixation of the tissue samples and dehydration in a growing acetone series, the material was embedded in Araldite (Merck®, USA).

Semi-thin sections (0.5 μm) were stained with Azur II and methylene blue for selection of adequate fields under a light microscope. Ultrathin sections (60 to 80 nm) of the selected areas were counterstained with uranyl acetate and lead citrate[27], analyzed and photodocumented with a Philips EM–301 transmission electron microscope (Philips®, Netherlands).

Spermatozoa were analyzed by scanning electron microscopy (SEM). Seminal fluid was manually collected from four fish and fixed in a glutaraldehyde and osmium tetroxide solution, brought to the critical point and sputtered with a colloidal mixture of palladium gold. The samples were then analyzed and photodocumented with a Philips® scanning electron microscope at the UNESP Electron Microscopy Center, Botucatu Campus, SP, Brazil.

For previous histological analysis, fragments of the testis were fixed in McDowell solution[28] for 24 h and embedded in Historesinâ (Leica-Germany). Sections of 3 and 5 μm were stained with Hematoxylin - Phloxin B.

Results

In the grey armored catfish, development of the spermatogenic cells occurred mainly within germinative cysts formed by cytoplasmic prolongations of supporting epitheliocytes (Sertoli cells), characterizing a process of spermatogenesis of the cystic type (Figure 1A). However, the primary spermatogonia were found isolated or in pairs during the different stages of gonadal development, when they were directly attached to the seminiferous lobular

wall, confirming a pattern of unrestricted spermatogonial distribution (Figure 2), as discussed below.

Ultrastructurally, the primary spermatogonia (Figure 1B) appeared as irregularly shaped, spherical and voluminous cells with a large nucleus located in a central position relative to the thin cytoplasm. The nuclear membrane showed irregular contours with fluting and intimate punctual contacts with the endomembrane of the endoplasmic reticulum. The nuclear chromatin was euchromatic, predominantly granular and diffusely dispersed within the nucleoplasm. A single large, spheroidal, highly electron-dense nucleolus of excentric intranuclear position was frequently observed. The cytoplasm surrounded the large nucleus as a narrow band, presenting a large reticulation produced by the lamellae and vesicles of the predominantly granular endoplasmic reticulum (Figure 1B). Free ribosomes and polysomes, round mitochondria adjacent to the lamellae of the endoplasmic reticulum and some electron-dense bodies were also observed. The plasma membrane was clearly visible and attached to the plasma membrane of Sertoli cells, forming a cystic wall (Figure 1A), and punctual reinforcements formed by desmosomes were also noted.

The secondary spermatogonia showed structural characteristics similar to those of the primary spermatogonia, but were never found isolated in the lobular wall and the several nucleolus was more prominent than that of primary spermatogonia. Hence, secondary spermatogonia (Figure 2) were exclusively visualized inside the germinative cysts, a finding differentiating them from primary spermatogonia.

The primary spermatocytes observed within the respective germinative cysts were round cells containing common organelles in their cytoplasm, with an apparent predominance of round mitochondria (Figure 1C). These cells were interconnected by cytoplasmic bridges and contained clearly visible pores in their nuclear membrane, which were found to be connected to the lamellae and cisternae of the predominantly granular endoplasmic reticulum. In addition, some lamellae of the endoplasmic reticulum extended from the nuclear membrane to the plasma membrane, thus confirming the connection between the plasma and nuclear membranes through a complex of cytoplasmic endomembranes (Figure 1C).

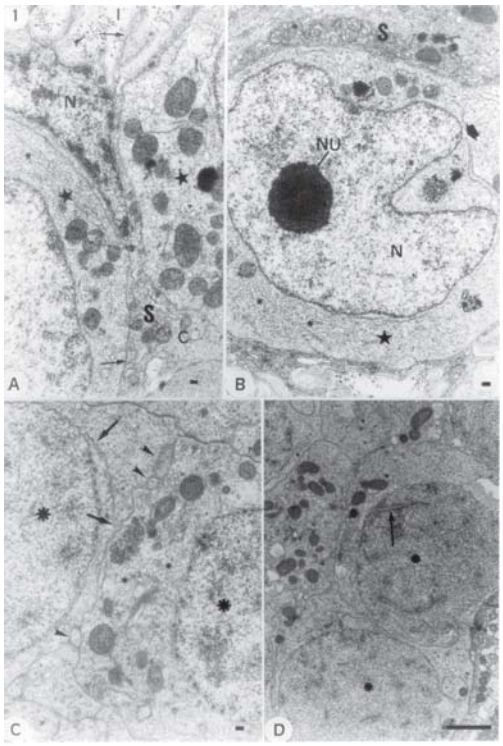

Figure 1 - Ultrastructure of spermatogenetic cells of the *L. anisitsi* showing primary spermatogonia in A and B and primary spermatocytes in C and D and their relations with Sertoli cells at the level of spermatogenetic cysts. The subcellular structures indicated are: cytoplasm (**C**) and nucleus (**N**) of Sertoli cell (**S**), cell prolongations (thin arrows), interstitium (**I**) between two cysts with collagen fibers (arrow head), cytoplasms of primary spermatogonias (stars) with large amount of ER (*) in A; the same structures of the primary spermatogonia are indicated with emphasis to the nucleolus (NU) and interrelation of nuclear envelope and profile of ER (large arrow) in B. Nuclei of primary spermatocytes (*), cytoplasmic bridges (large arrow heads) between two adjacent cells, interrelation between nuclear envelope and plasmic membrane by profile of ER (middle arrows) and large amount of lamellar-vesicular ER (*) in C; synaptonemic complexes (long arrow) in the nuclei of primary spermatocytes (*) and common organelles are seen in the cytoplasm of this cells in D. Scale bar = 1 mm.

The nuclear chromatin of the primary spermatocytes seemed relatively more dense than that of the spermatogonia, showing nuclear synaptonemal complexes. These complexes were formed from the inner surface of the nuclear membrane at a time when the chromosomes were still not condensed (Figure 1D). The junctions between the plasmatic membranes of the primary spermatocytes themselves and between them and the membranes delimiting the prolongations of Sertoli cells were clearly visible (Figure 1C). The secondary spermatocytes, which were also intracystic, were hardly visible since they rapidly underwent the second mitotic division.

The spermatids, also intracystic, were found to be connected by cytoplasmic bridges during the initial phase of cell differentiation (spermiogenesis). They contained a spherical nucleus, with a predominance of heterochromatin while sparse areas of less condensed chromatin were still noted (Figures 3A, 3B). No acrosome was observed apically since this structure is not formed in this species. Large areas of non-condensed chromatin were visible for some time mainly in the central portion of the nucleus (Figure 3B).

During the final phase of spermiogenesis, when the final differentiation of the elongated (late) spermatids is completed, the chromatin appeared strongly and homogenously heterochromatic, forming caudally the nuclear fossa. This fossa consisted of a depression in the nuclear envelope where the proximal and distal centrioles reside, which then migrate from the supranuclear part of the cytoplasm to form the flagellum. The two centrioles were connected by dense anchor fibers (Figure 3C).

Concomitantly with the appearance of the flagellum in the maturing spermatids, large part of the cytoplasmic mass of these haploid cells was displaced caudally to the region of the nuclear fossa (Figure 3C), and later absorbed by the cytoplasm of the sustentacular cells (Sertoli cells). Finally, during spermiation the spermatozoon cysts ruptured, releasing

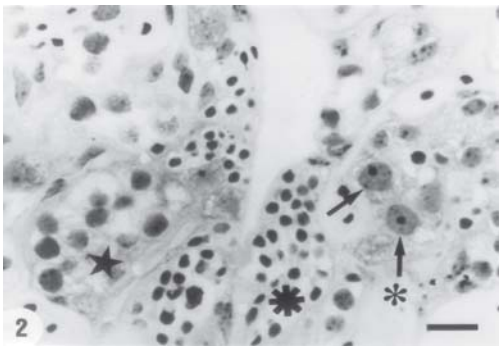

Figura 2 - Photomicrography op the testis structure the *L. anisitsi* showing a spermatogonial cyst (H), a spermatid cyst (*) and two isolated primary spermatogonia (arrows) close related to lobular wall (\). Scale bar = 2,5 mm

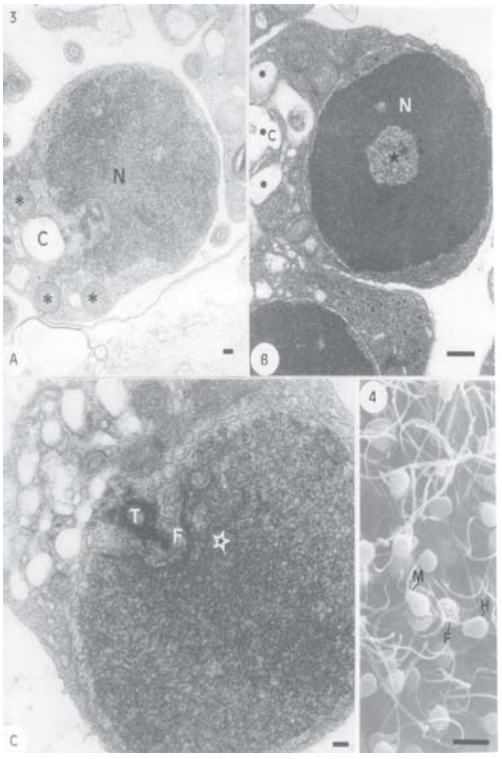

Figura 3 - Ultrastructure of spermatids in different stages of the maturation phase inside spermatids cysts. It is showed rounded nucleus (**N**), scarce cytoplasm (**C**) with inferior distribution and mitochondria in this position (*) in A; differentiation with centralized euchromatin (H), light areas of probably cytoplasmic resorption (*) in B; nuclear fossa (**F**) and centrioles (**T**) in the inferior extremity of spermatid with dense nuclear heterochromatin in C (white star). Scale bar = 1 mm

Figura 4 -Scanning electron microscopy of spermatozoa showing a head (**H**), the middle piece (**M**) and the flagellum (**F**). Scale bar = 81,4 mm

spermatozoa into the lobular lumen from where they are expelled during ejaculation. The spermatozoa consisted of a round head, short middle piece and long flagellum (Figure 4).

Discussion

Srivastava and Sing[29] found the spermatogenic cells of *Channa punctatus* which are in a resting state to be the largest cells of the testicular germinative lineage, with a large and pale nucleus and an excentric nucleolus. However, in the present study on *Liposarcus anisitsi* the largest spermatogenic cell identified was the primary spermatogonium, which is apparently a stem cell of the testicular germinative lineage based on its mitotic rate of cell division or proliferation in fish[14], as well as compared to mammals in which it also represents a spermatogenic stem cell[30,31,32].

The primary spermatogonia, similar to our earlier considerations, have also been characterized as the largest spermatogenic cells in fish of temperate or subtropical climates.[2,12] In the grey armored catfish, primary spermatogonia were observed separated or in pairs and located close to the inner surface of the seminiferous lobular wall, a finding characterizing the testicular type of this species as lobular, cystic with unrestricted distribution of the primary spermatogonia throughout the lobular wall.[5,7]

In addition, primary spermatogonia were also visualized inside the germinative cysts specific of primary spermatogonia, as also reported for *Brycon orbignyanus*.[1] This pattern of predominantly intracystic cellular development observed here seems to be frequent in teleost testes[1,2,4,5,6,10,11,12,13,21]. Thus, spermatocytogenesis and spermiogenesis predominantly occur within germinative cysts specific for the different spermatogenic cells[21]. On the other hand, the concomitant presence of "free" primary spermatogonia on the internal lobular wall or in an encysted form might correspond to a semicystic spermatogenic pattern[9] as discussed for the spermatogenesis of *Brycon orbignyanus*[1].

In general, primary and secondary spermatogonia showed structural aspects similar to those observed for *Sorubim lima*[4], with emphasis on the presence of a voluminous nucleus, predominance of nuclear euchromatin and free ribosomes or ribosomes aggregated with polysomes, and round mitochondria, with the organelles being randomly distributed within the perinuclear cytoplasm. However, these ultrastructural characteristics, which confirm the presence of intense cell proliferation activity peculiar to stem cells of the mammalian spermatogenic lineage[30,31,32], were more marked in intracystic primary spermatogonia than in secondary spermatogonia, in agreement with the observations on *Brycon orbignyanus* testis[1].

The junctions observed here between the plasma membranes of intracystic primary and secondary spermatogonia and the plasma membrane of the pericystic projections of supporting epitheliocytes[33], or Sertoli cells[34,35], including the presence of desmosome between membranes, is a peculiar characteristic of teleosts with intracystic spermatogenesis[1,5]. In mammals, these spermatogenic-Sertoli cell junctions are aimed at providing structural stability to the seminiferous tubular epithelium[33,34,35,36], preventing, for example, early spermiation during spermiogenesis[33,34,35]. Based on our observations of the structure of the cysts in the lobular wall of the grey armored catfish testis, we may hypothesize that the presence of spermatogenic-Sertoli cell junctions, as well as the presence of cytoplasmic bridges observed between intracystic primary spermatocytes, are aimed at providing stability to the cystic wall similar to the findings reported for mammals, thus permitting the synchrony between intracystic cell differentiation and development and, consequently, stabilization of the structure of the seminiferous lobular wall.

The peculiar distribution of nuclear chromatin observed in the primary spermatocytes of *L. anisitsi*, which was relatively more heterochromatic than that of

the spermatogonia, as well as the presence of intranuclear synaptonemal complexes, has also been reported for *Sorubim lima*[4]. The process of intimate pairing of homologous chromosomes observed in these cells during the prophase of the first meiotic division explains the presence of these intranuclear synaptonemal complexes[30,36].

With respect to the observation of perinuclear cisternae in the predominantly granular endoplasmic reticulum of the primary spermatocytes of the grey armored catfish, and considering the fact that some lamellae are apparently interconnected with the plasma and nuclear membranes, similar to the findings reported for these cells in the mammalian seminiferous epithelium[36,37,38], two cytophysiological hypotheses can be raised. First, the perinuclear cisternae of the endoplasmic reticulum seem to originate from the nuclear membrane to which they partially adhere and which they also surround[37]. Moreover, these cisternae might act as favored sites of storage and translocation of proteins from the nucleus to the cytoplasm or vice-versa[36,37,38]. The observation of these apparently complex functions in fish, even in evolutive terms, cannot be ruled out. Fish represent the "first" vertebrate phylum. Initial and complex biological processes differing from the mechanisms of life maintenance and prolongation of Chordata are observed in this phylum, which, in our understanding and based on the extensive literature cited here, show a sufficiently rich complexity and diversity.

Secondary spermatocytes were rarely observed in the testis of *L. anisitsi* due to the rapid occurrence of the second meiotic division in this species, similar to the findings of Romagosa et al.[11] in fish and also frequently observed for the testis of mammals[31,32].

The ultrastructural characteristics of the intracystic spermatids of *L. anisitsi* during the initial and final evolutive spermiogenic process observed here were similar to those described for *Plagioscion squamosissimus*[16]. The spermatids of the two species were found to be in the same stage of evolutive development in each cyst, including the well-known stages of nuclear chromatin condensation, appearance of the nuclear fossa for implantation of the flagellum, differentiation of the proximal and distal centrioles to form the flagellum, absence of an acrosome which is not formed in species with external fertilization, and absorption of excess spermatid cytoplasm by the cytoplasm of sustentacular cells[35].

Finally, in *L. anisitsi*, as a final step of the spermatocytogenic process, spermatozoa differentiate into forms with a round head and nucleus, a short flagellum and a highly marked middle piece and mitochondrial battery, as also observed for the spermatozoa of other teleosts that reproduce by external fertilization[1,5].

Características estruturais e ultraestruturais da espermatogênese do cascudo-cinza *Liposarcus anisitsi* (Holmberg, 1893) (Teleostei, Siluriformes)

Resumo

Liposarcus anisitsi é um teleósteo de água doce da ordem Siluriforme, com fertilização externa, possui um processo de espematogênese cística com estrutura espermatogonial lobular irrestrita dos testículos. Nesta espécie, a espermatogônia primária intracística ou isolada é considerada como a "stem cell" da linhagem espermatogenética. Então, como consequência do processo da espermatogênese cística de *Liposarcus anisitsi* todas as três células germinativas são observadas no interior de cistos espermatogenéticos específicos, desde as espermatogônias primárias e secundárias até os espermatócitos e espermátides jovens e maduras. A espermatogônia possui forma

Palavras-chave:
Espermatogênese.
Ultra-estrutura.
Siluriforme.
Liposarcus anisitsi .

esférica, com núcleo central e grande quantidade de mitocôndrias redondas e retículo endoplasmático, principalmente do tipo rugoso, em seu citoplasma. Os espermatócitos primários se inter-relacionam através de pontes citoplasmáticas e são claramente identificados pela presença de complexos sinaptonêmicos no interior de seus núcleos. Espermátides jovens no início da fase maturacional, tanto quanto as espermátides maduras tendem a exibir núcleos esféricos, um modelo variável de progressiva condensação cromatínica durante o estágio final da espermiogênese. Nestes estágios finais, as espermátides maduras mostra uma fossa nuclear formada para a fixação do flagelo. O espermatozóide de *Liposarcus anisitsi* possui cabeça redonda, núcleo redondo, cromatina densa, ausência de acrossoma, curta peça intermediária e longa cauda (peças principal e terminal do flagelo).

References

1 AIRES, E. D. **Características morfológicas e histofisiológicas de *Brycon orbignyanus* (Pisces: Teleostei)**. 1999. 69 p. Tese (Doutorado) – Instituto de Biociências, Universidade Estadual Paulista, Botucatu, 1999.

2 BILLARD, R Ultrastructural changes in the spermatogonia and spermatocytes of *Poecilia reticulata* during spermatogenesis. **Cell Tiss. Res.**, v. 237, p. 219-226, 1984.

3 MATTEI, X. et al. Peculiarities in the organization of testis of Ophidion sp (Pisces, Teleostei): evidence for two types of spermatogenesis in teleost fish. **J. Fish Biol**., v. 43, p. 931-937, 1993.

4 QUAGIO-GRASSIOTO, I.; CARVALHO, E. D. The ultrastructure of *Sorubim lima* (Teleostei, Siluriformes, Pimelodidae) spermatogenesis: premeiotic and meiotic periods. **Tiss. Cell**, v. 31, p. 561-567, 1999.

5 VICENTINI, C. A. **Estrutura comparativa da espermatogênese em *Prochilodus scrofa, Astyanax scabripinis, Phalloceros caudimaculatus* (Pisces, Teleostei)**. 2002. 102 p. Tese (Livre Docência) – Faculdade de Ciências, Universidade Estadual Paulista, Bauru, 2002.

6 CRUZ-LANDIM, C.; CRUZ-HOFFLING, M. A. Aspectos da espermatogênese de tucunaré *Cichla ocellaris* Schneider, 1801 (Teleostei, Cichlidae). **Acta Amaz.**, v. 16-17, p. 65-72, 1986-87.

7 GRIER, J. J.; PARENTI, L. R. Reproductive biology and systematics of phallostethid fishes as revealed by gonad structure. **Env. Biol. of Fishers,** v. 41, p. 287-299, 1994.

8 GRIER, H. J.; FITZSIMONS, J. M.; LINTON, J. R. Structure and ultrastructure of the testis and sperm formation in Goodeid teleost. **J. Morphol.**, v. 156, p. 419-438, 1978.

9 MATTEI, C.; MATTEI, X La spermiogenèse dun poisson Téléostéen (*Lepadogaster lepadogaster*): la spermatide. **Biol. Cell**, v. 32, p. 257-266, 1978.

10 MATTEI, X. Spermatozoon ultrastructure and its systematic implications in fishes. **Can. J. Zool**., v. 69, p. 3038-3055, 1991.

11 ROMAGOSA, E. et al. Ultrastructure of the germ cells in the testis of matrinxã, *Brycon cephalus* (Teleostei, Characidae). **Tiss. Cell**, v. 31, p. 540-544, 1999.

12 SELMAN, K.; WALLACE, R. A. Gametogenesis in Fundulus heteroclitus. **Am. J. Zool**., v. 26, p. 173-192, 1986.

13 ALEXANDRINO, A. C.; PHAN, M. T.; PINHEIRO, E. F. G. Caracterização macroscópica e microscópica das gônadas do curimbatá *Prochilodus scrofa*, durante o ciclo reprodutivo. **Bol. Zool. Univ. São Paulo**, v. 9, p. 159-175, 1985.

14 BILLARD, R. Quantitative analysis of spermatogenesis in the trout, *Salmo trutta fario*. **Cell Tiss. Res.,** v. 230, p. 493-502, 1983.

15 LAHNSTEINER, F.; PATZNER, R. A.; WEISSMANN, T. Testicular main ducts and spermatic ducts in some cyprinid fishes: morphology, fine structure and histochemistry. **J. Fish Biol**., v. 44, p. 937-951, 1994.

16 GUSMÃO, P.; FORESTI, F.; QUAGIO-GRASSIOTO, I. Ultrastructure of spermiogenesis in *Plagioscion squamosissimus* (Teleostei, Perciformes, Sciaenidae). **Tiss. Cell**, v. 31, p. 627-633, 1999.

17 LOU, H. Y.; TAKAHASHI, H. Spermiogenesis in the nile tilapia *Oreochomis niloticus* with notes on a unique pattern of nuclear chromatin condensation**. J. Morphol**.,

v. 200, p. 321-330, 1989.

18 MATOS, E. et al. Ultraestrutura do espermatozóide do pacu *Metynnis maculatus* Kner, 1860 (Pisces, Teleostei) do rio Amazonas. **Rev. Bras. Ciênc. Morfol.**, v. 10, p. 7-10, 1993.

19 MATOS, E. et al. Ultrastructural study of the spermatozoon of *Crenicichla satilis*, (Pisces, Teleostei) from the Amazon region. **Braz. J. Morphol. Sci**., v. 12, p. 109-114, 1995.

20 MATOS, E. et al. Aspectos morfológicos e ultraestruturais do espermatozóide de *Curumata inornata* (Pisces, Teleostei), do rio Amazonas. **Acta Amaz**., v. 28, p. 449- 453, 1998.

21 VICENTINI, C. A.; VICENTINI, I. B. F.; BENETTI, E.; ORSI, A. M. Testicular ultrastructure and morphology of the seminal pathway in *Prochylodus scrofa*. **J. Submicrosc. Cytol. Pathol**., v. 33, p. 357-362, 2001.

22 AFZELI1- AIRES, E. D. **Características morfológicas e histofisiológicas de *Brycon orbignyanus* (Pisces: Teleostei)**. 1999. 69 p. Tese (Doutorado) – Instituto de Biociências, Universidade Estadual Paulista, Botucatu, 1999.

23 AFZELIUS, B. A. The flagellar apparatus of marine spermatozoa: evolutionary and fuctional aspects. In: AMOS, W. B., DUCKETT, J. G. **Procaryotic and Eucaryotic Flagella**. London: Cambridge University Press, 1982. p. 495-519.

24 BACCETTI, B. The evolution of the sperm tail. In: AMOS, W. B.; DUCKETT, J. G. **Procaryotic and Eucaryotic Flagella**. London: Cambridge University Press, 1982. p. 521-532.

25 GOSLINE, W. The palatine-maxillary mechanism in catfishes, with comments on the evolution and zoogeography of modern siluroids. **Occean Pap. Calif. Acad. Sci.,** v. 120, p. 1-31, 1975.

26 ANTONIUTTI, D. M. et al. Relação peso total/ comprimento total, crescimento e idade do cascudo *Pleostomus albopunctatus*: Regan 1908 (Osteichithyes, Loricariidae), do rio Jaguari. **Bol. Inst. Pesca,** v. 12, p. 105-120, 1985.

27 REYNOLDS, E. S. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. **J. Cell Biol**., v. 17, p. 208-212, 1963.

28 MCDOWELL, E. M.; TRUMP, B. F. Histologic fixatives suitable for diagnostic light and electron microscopy. **Arch. Pathol. Lab. Med**., v. 100, n. 8, p. 405-414, 1976.

29 SRIVASTAVA, S. J.; SINGH, R. Seasonal changes in the testes of freshwater murrel, *Channa punctatus*. **Naturalia**, v. 19, p. 119-130, 1994.

30 ANDERSEN, K. Fine structure of spermatogonia and spermatocytes of the blue fox (*Alopex lagopus*). **Acta Ve.t. Scand.**, v. 19, p. 229-242, 1978.

31 CLERMONT, Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonia renewal. **Physiol. Rev.**, v. 52, p. 196-236, 1972.

32 ORSI, A. M.; FERREIRA, A. L. Re-exame do processo da espermatogênese em mamíferos, principalmente em rato e gambá. **Ciênc. Cult.**, v. 30, p. 65-718, 1978.

33 SINOWATZ F, A. M.; SELGUBER, W. Ultrastructure of sustentacular (Sertoli cells) in the bovine testis. **Acta Anat**., v. 133, p. 274-281, 1988.

34 RUSSEL, L. Spermatid-Sertoli tubulobulbar complexes as devices for elimination of cytoplasm from the head region of the late spermatids of the rat. **Anat. Rec**., v. 194, p. 233-246, 1979.

35 RUSSEL, L. et al. Sertoli ectoplasmic specializations during spermatogenesis. In: STEINBERGER, A.; STEINBERGER, E. **Testicular development, structure and function**. New York: Raven Press., 1980. p. 55-69.

36 ORSI, A. M.; VICENTINI, C. A.; VIOTTO, M. J. S. Características ultraestruturais de espermatogônias e espermatócitos primários do morcego hematófago *Desmodus rotundu,, G***. Naturalia**, v. 18, p. 15- 26, 1993a.

36 ORSI, A. M. et al. Características ultraestruturais das células de Sertoli do morcego hematófago (*Desmodus rotundus*, G.), **Rev. Bras. Biol**. , v. 53, p. 583-590, 1993b.

37 CHEMES, H. E.; FAWCETT, D. W.; DYM, M. Unusual features of the nuclear envelope in human spermatogenetic cells. **Anat. Rec.**, v. 192, p. 493- 512, 1978.

38 NISTAL, M.; PANIAGUA, R.; ESPONDA, P. Development of the endoplasmic reticulum during human spermatogenesis. **Acta Anat**., v. 108, p. 238-249, 1980.