SPERMATOGENESIS IN THE YELLOWTAIL TETRA *Astyanax altiparanae:* A HISTOLOGICAL ANALYSES WITH EMPHASIS TO SPERMATOGONIAL AND SPERMATID TYPES*

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ABSTRACT

This study aimed to detailed description of the characteristics of the different germ cell types found in *Astyanax altiparanae* during spermatogenesis. In this purpose, testes from 25 adult male's specimens of *A. altiparanae* were sampled and submitted to the usual techniques for light microscopy. Based on nuclear shape, chromatin condensation, nucleoli quantity and cell size were identified four spermatogonial types: type A undifferentiated ($A_{und.}$*); type A undifferentiated ($A_{und.}$); type A differentiated ($A_{dif.}$); and type B spermatogonia. Spermatocytes were observed in different phases of meiosis (leptotene/zygotene/pachytene and diplotene), metaphase I and II and secondary spermatocytes, being distinguish mainly by their chromosomal organization inside the nucleus. Were also identified three different types of spermatids, which were named as initial, intermediate and final, which can be differentiated by increase in nuclear condensation and spacing among cells inside the cysts, possibly by flagella arise and reduction in nuclear diameter . Thus, this study contribute to a better understand of spermatogenesis in this and other fish species.

Keywords: fish reproduction; germ cells; spermiogenesis; testis morphology

ESPERMATOGÊNESE NO LAMBARI-DO-RABO-AMARELO *Astyanax altiparanae:* UMA ANÁLISE HISTOLÓGICA COM ÊNFASE AOS TIPOS ESPERMATOGONIAIS E ESPERMÁTICO

RESUMO

O presente estudo teve como objetivo a descrição detalhada dos diferentes tipos de células germinativas encontradas em *Astyanax altiparanae* durante a espermatogênese. Deste modo, os testículos de 25 espécimes machos e adultos de *A. altiparanae* foram coletados e submetidos às técnicas usuais para microscopia de luz. Baseado no formato nuclear, condensação da cromatina, quantidade de nucléolos e tamanho celular foram identificados quatro tipos de espermatogônias: indiferenciadas do tipo A ($A_{und.}$*); indiferenciadas do tipo A ($A_{und.}$); diferenciadas do tipo A ($A_{dif.}$); e espermatogônia do tipo B. Os espermatócitos foram observados em diferentes fases da meiose (leptóteno/zigóteno, paquíteno e diplóteno), metáfase I e II e espermatócitos secundários, sendo distinguidos principalmente pela organização dos cromossomos nos núcleos. Também foram identificados três diferentes tipos de espermátides, que foram nomeadas como iniciais, intermediárias e finais, que se diferenciaram pelo aumento da compactação nuclear e espaçamento entre as células no cisto, pelo possível surgimento do flagelo, e pelo diâmetro nuclear. Assim, este estudo contribui para um melhor entendimento da espermatogênese nesta e nas demais espécies de peixes.

Palavras chave: células germinativas; espermiogênese; morfologia testicular; reprodução em peixes

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INTRODUCTION

Teleost represent the most diversify and abundant group among vertebrates, being formed for about 96% from all fish species. The success of this group is assigned to a series of adaptation, reflecting the huge diversity of morphology, life cycle pattern, but mainly in the reproduction type, characterized by varied reproductive strategy such as spawning rhythm, fecundity, maturity age, annual reproductive cycle, etc. (ALEXANDRINO *et al*., 1985; VAZZOLER, 1996; REIS *et al*., 2003; NÓBREGA *et al*., 2006; GRIER and ARANZÁBAL, 2009).

Reproductive strategies reflect directly in gonadal morphology. In teleost with external fertilization, for example, testes are commonly elongated paired organs located in coelomic cavity (GRIER and ARANZÁBAL, 2009; SIQUEIRA-SILVA *et al*., 2013). In Siluriformes, some species present testes with digitiform projections or fringes with varied aspects (LOPES *et al*., 2004; MAZZOLDI *et al*., 2007). In *Synbranchus marmoratus* the secondary males may show only one testis (LO NOSTRO *et al*., 2003).

The main testicular function is to keep spermatogenesis, one complex and extremely organized process, in which one initial diploid cells, named spermatogonia, undergo proliferation and differentiation to produce spermatozoa, which are haploid cells (GRIER *et al*., 1980; NÓBREGA *et al*., 2009; SCHULZ *et al*., 2010).

Spermatogenesis in fish and other anaminote develop inside spermatocysts, which are formed by germ cells in synchronic development surrounded by cytoplasmic process of Sertoli cells, which are responsible for germ cells mechanical and nutritional. Didactically, spermatogenesis may be divided in three phases: spermatogonial or proliferative phase in which spermatogonia undergo successive mitotic divisions. This phase is specie-specific; spermatocitary or meiotic phase, in which the genetic material is duplicated, recombined and segregated; and spermiogenic phase, characterized by morphological and physiological changes of spermatids, with variation according the species, to originate the spermatozoa (GRIER *et al*., 1980; RUSSELL *et al*., 1990; VERÍSSIMO-SILVEIRA *et al*., 2006; NÓBREGA *et al*., 2009; SCHULZ *et al*., 2010; SIQUEIRA-SILVA *et al*.; 2012).

Despite the advantages provided by histological studies in attempt to better understand fish spermatogenesis, few data considering the basic aspect of this process and both a detailed morphological description and behavior from germ cells during this process are available (JAMIESON, 1991; LEAL *et al*., 2009).

Thus, the aim of the present study was the morphological description of germ cells characteristics in *Astyanax altiparanae* differentiating them according to the subdivisions in spermatogonial, spermatocytary and spermiogenic lineage during spermatogenic cycle. Popularly known as yellowtail tetra, this is a small fish (reaching a maximum of 20 cm), distributed in High Paraná, Paranapanema, Tibagi and Iguaçu basin (Brazil) in both lotic and lentic environments, being a substantial part of bigger fish diet, besides to be appreciated by human (GARUTTI and BRITISKI, 2000; ORSI *et al*., 2002; DE BEM, 2009). The choice of species was motivated by its good livestock characteristics, such as ease of handling, small size, early sexual maturity at 4 months of age, and intertidal spawning, which enable it as an appropriate model for investigating the biology of teleost and for the development of distinct studies in laboratory conditions (CASTILHO-ALMEIDA, 2007; YASUI *et al*., 2014).

MATERIAL AND METHODS

Animals

Twenty-five sexually mature male specimens of *A. altiparanae* of standard size ranging from 12 to 15 cm were selected from excavated tanks at Hydrobiology Engineer Souza Dias Station (CESP). Sexually mature males are easily distinguished from females by presenting hooks in anal fin.

Experimental procedures were carried out in strict accordance with the guide for care and use of laboratory animals of the University Estadual Paulista (UNESP). The research ethics committee of UNESP approved the protocols (Permit Nº: 006/2012/CEUA). All surgical procedures were performed under anesthesia with 0.5 g of

benzocaine in 5 mL of absolute ethanol, and all efforts were made to minimize fish suffering. Fish were identified and a group of voucher individuals was deposited in the collection of fishes of DZSJRP Ichthyology Laboratory, Department of Zoology and Botanic IBILCE/UNESP under the register number DZSJRP008999.

Sampling and analyses

Testes were dissected, cuted into transverse and longitudinal sections, fixed in 4% paraformaldehyde with 2% glutaraldehyde solution in Sorensen phosphate buffer, pH 7.4, for a minimum period of 24 hours. Then, the samples were dehydrated in ethanolic solutions at increasing concentrations and were impregnated in glycol methacrylate (Technovit 7100/historesin; HeraeusKulzer, Wehrheim, Germany). Finally, the samples were sectioned to a thickness of 3.0 µm using a microtome (LEICA RM 2145; Leica Instruments GmbH, Heidelberg Nussloch, Germany) equipped with a glass blade and stained with Haematoxylin and Eosin. Photo-processing and histological analyses were completed with a Zeiss optical microscopy equipped with an AXIOCAM-MRc5 camera (Carl Zeiss Microimaging GmbH, Göttingen, Germany). Measure from cell nuclei diameter was performed using Motic Images Plus 2.0 program. One hundred nuclei were measured for each spermatogonial type and spermatozoon and 4500 nuclei for each spermatid type.

RESULTS

Spermatogenesis in *A. altiparanae* was subdivided according the stage of germ cells inside germinal epithelium. Thus, morphological characteristics of germ cells allowed identification of four spermatogonial types and three different types of spermatids, besides spermatocytary stage commonly found during reproductive cycle in fish.

Spermatogonial types in mitotic or proliferative phases

Spermatogonial types were differentiated according to nuclear shape, chromatin condensation, nucleoli quantity and cell size, being divided in:

- Type A undifferentiated spermatogonia ($A_{und.}$*): these cells are isolated and dispersed throughout the germinal epithelium (Figure 1). Their nucleus show an irregular and elongated shape, being a little basophilic and heterochromatic; the average diameter was 7.67 ± 0.63 µm. More than one well-evidenced nucleolus is observed (Figure 2A).

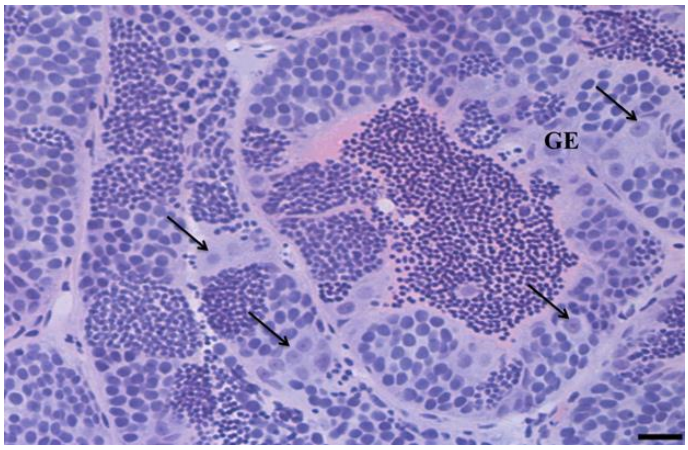

Figure 1. *Astyanax altiparanae* testes**.** Distribution of spermatogonia (arrow) through the germinal epithelium (GE). Scale bar: 20 µm.

- Type A undifferentiated spermatogonia ($A_{und.}$): are also isolated and dispersed throughout the germinal epithelium, however, are smaller than the previous one. These cells show an elongated and more basophilic nucleus with average diameter of the 6.12 ± 1.16 µm. In this spermatogonial type nucleoli are not well evidenced (Figure 2B).

- Type A differentiated spermatogonia ($A_{dif.}$): differently from the previous ones, type A differentiated spermatogonia are grouped in two or more cells in a cyst. Their nucleus is round/oval, presenting little condensed chromatin and only one evidenced nucleolus. The nucleus average diameter of this spermatogonia type was 4.55 ± 2.16 µm (Figure 2C).

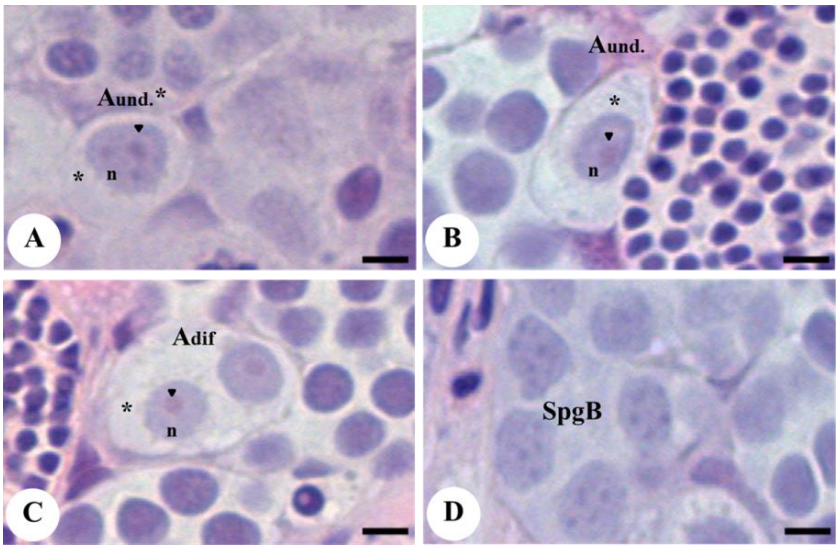

Figure 2. Spermatogonial type *of Astyanax altiparanae.* **A**: Type-A undifferentiated spermatogonia ($A_{und.}$*); **B**: Type-A undifferentiated spermatogonia ($A_{und.}$); **C**: Type-A undifferentiated spermatogonia ($A_{dif.}$); **D**: Type-B spermatogonia cyst (SpgB). n = nucleus; arrowhead= nucleolus; *= cytoplasm. Scale bars: 10 µm.

- Type B spermatogonia (SpgB): these cells are also grouped in cysts and the last germ cells to undergo mitotic divisions during spermatogenesis. Their nucleus is rounder, showing an increase in the amount of spread heterochromatic material. Nuclear average diameter of this spermatogonial type was 4.83 ± 0.54 µm and the nucleoli are little evidenced (Figure 2D). Meiotic phase, which is also named spermatocytary, starts after differentiation of spermatogonia B in primary spermatocyte.

Meiotic or spermatocytary phases

In this phase, spermatocytes were found in different phases from meiosis and were mainly distinguished by their nuclear characteristics, such as size, condensation and chromosome arrangement:

- Leptotene/Zygotene (L/Z), pachytene and diplotene primary spermatocytes and secondary spermatocyte: Leptotene/Zygotene spermatocytes show rounded nucleus with apparently clearest chromatin and some heterochromatic points that are subjacent to the nuclear envelope (Figure 3A);

Cells in pachytene (P) are apparently smaller than the previous one, show a denser nucleus with more condensed chromosomes (Figure 3B);

Spermatocyte in diplotene phase (D) are found in the same cyst with that ones in metaphases I (M). In this cell phase, chromosome reach their maximum nuclear condensation (Figure 3C).

At the end of first meiotic division, secondary spermatocytes (Sc2) are found. In *A. altiparanae* they were characterized by their round nucleus and dense chromatin (Figure 3D).

Spermiogenesis and sperm generation

After secondary spermatocyte undergo the second meiotic division, spermatids are originated giving start to spermiogenic phase, also called differentiation phase, which in this species can be observed in three different stages of development, according to the increase in nuclear compression and spacing among them inside the cysts, probably due to the appearance of flagellum and elimination of cytoplasmic reside. The spermatid types in this species were named initial, intermediate and final spermatids.

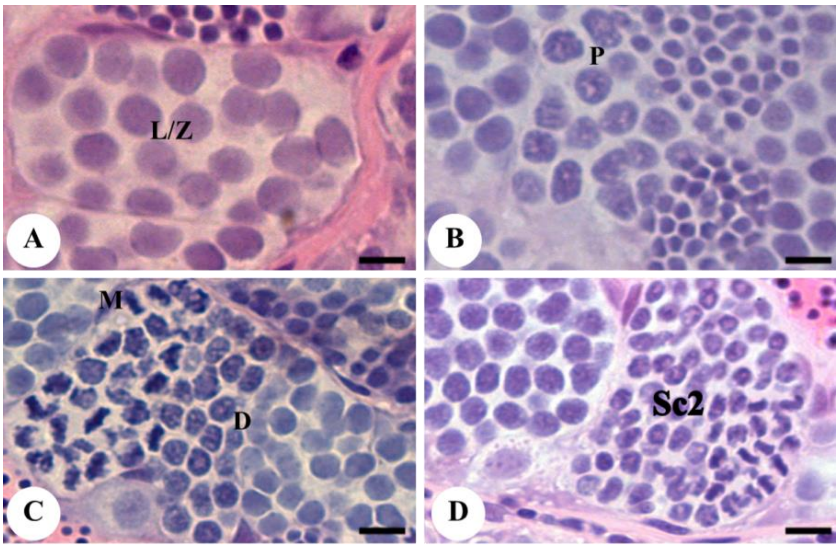

Figure 3. Meiotic phase in *Astyanax altiparanae*. **A**: cyst of leptotene/zygotene spermatocytes (L/Z); **B**: cyst of pachytene spermatocytes (P); **C**: cyst of diplotene spermatocytes (D) and metaphases I (M); **D**: cyst of secondary spermatocytes (Sc2). Scale bars: 10 µm.

Initial spermatids (St1) are smaller than both spermatogonial and spermatocyte cells and are very compressed inside the cysts. It shows a rounder and more condensed nucleus in comparison to cells from previous phases. Their nucleus showed a great reduction in average diameter measuring 2.54 ± 0.42 µm (Figure 4A).

Intermediate spermatids (St2) are smaller than previous ones. The nucleus is more compressed and rounder and the cytoplasmic volume is visibly smaller. Nucleus average diameter of this spermatid type is 1.38 ± 0.53 µm (Figure 4B).

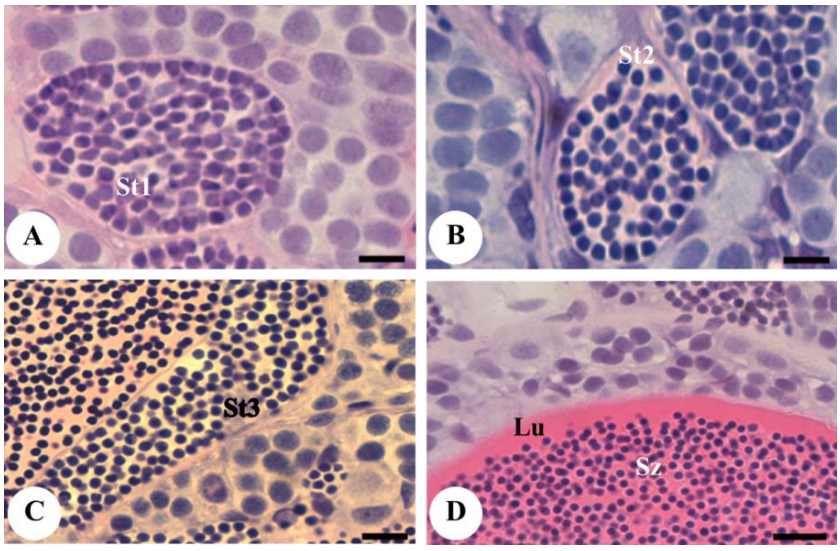

Figure 4. Spermiogenesis at *Astyanax altiparanae*. **A**: initial spermatid cyst (St1); **B**: intermediate spermatid cyst (St2); **C**: final spermatid cyst (St3); **D**: detail of spermatozoon (Sz) in seminiferous tubule lumen. Lu = lumen. Scale bar: 10 µm.

Final spermatids (St3) are the smallest cells from spermiogenic lineage and consequently from all spermatogenesis process before sperm generation. Due to flagellum generation and elimination of cytoplasmic resides there is an increase in the spacing among these cells inside

the cysts. Its nucleus is very condensed and surrounded by a narrow strip of cytoplasm. Nucleus average diameter of this spermatid type is 1.31 ± 0.19 µm (Figure 4C).

At the end of spermatogenesis the sperm are formed. It is smaller than final spermatids with nuclear average diameter 1.02 ± 0.16 µm. These cells are found only into tubular lumen (Figure 4D).

DISCUSSION

Morphological characteristics of spermatogenesis in teleost assist the comprehension of testicular functions, contributing to understand the numerous tactic and reproductive strategies developed by this group (VAZZOLER, 1996; NÓBREGA *et al*., 2009). Spermatogenic study in *A. altiparanae* enabled histological characterization of four spermatogonial and three spermatid types.

According to VILELA *et al.* (2003), there is a difference among spermatogonial types in fish, varying according both the nuclear chromatin arrangement and germ cell size, which is species-specific and can be genetically defined (ANDO *et al*., 2000). Differently from observations in *A. altiparanae,* five types of spermatogonia were described in zebrafish *(Danio rerio)* and they were classified as type-A undifferentiated ($A_{und.}$*), type-A undifferentiated ($A_{und.}$), type-A differentiated ($A_{dif.}$) and initial and final type-B spermatogonia (LEAL *et al*., 2009). However, in Nile tilapia (*Oreochromis niloticus)*, only three types of spermatogonia were differentiated, being named type-Aor immature spermatogonia, type-A or mature differentiated spermatogonia and type-B spermatogonia (VILELA, *et al*., 2003; SCHULZ *et al*., 2005) and for the South American species, *Gymnotus carapo,* only two types of spermatogonia were observed, defined as type-A spermatogonia (SPGA) and type-B spermatogonia (SPGB) (VERGÍLIO *et al*., 2012).

Spermatogonia are fundamental to spermatogenesis, since they can develop distinct functions. $A_{und.}$* spermatogonia, also named isolated spermatogonia, for example, is the biggest germ cell inside the epithelium, being frequently named as stem cell, due to its renewal potential in each reproductive cycle and for giving origin to differentiated daughter cells, which will contribute to tissue functions. Spermatogonial cells show fundamental role in the transmission of inherited characters, since spermatogonia compromised with sperm generation, named type-A undifferentiated spermatogonia, carry all parental genetic material (MORENA *et al*., 1996; De ROOIJ and RUSSELL, 2000; NÓBREGA *et al*., 2010; SCHULZ *et al*., 2010; VERGÍLIO *et al*., 2012). Type-A differentiated spermatogonia are originated from previous ones. It show cytoplasmic bridges among them, due to incomplete cytokinesis during mitosis process (LEAL *et al*., 2009; SIQUEIRA-SILVA *et al*., 2012), and can be found in cysts with two to eight germ cells according to the species (SCHULZ *et al*., 2010).

After mitotic divisions, secondary or type-B spermatogonia are originated. They are the last spermatogonial generations and can form cysts with 16 or more cells according to the species, as in *D. rerio*, whose total number of spermatogonia B per cyst was about 208 cells (LEAL *et al*., 2009; SCHULZ *et al*., 2010). In *A. altiparanae* only one type of spermatogonia B was identified. However, in *D. rerio* these cells were divided between initial and final spermatogonia B (LEAL *et al*., 2009), corroborating De ROOIJ and RUSSELL (2000) idea who admit that spermatogonial terminology is confuse, due to the complexity of spermatogonial kinetics. Type-B spermatogonia differentiated in primary spermatocytes, giving origin to spermatocytary phases in which meiosis occur (GRIER *et al*., 1980; SILVA, 1987; VERÍSSIMO-SILVEIRA *et al*., 2006; NÓBREGA *et al*., 2008; SCHULZ *et al*., 2010).

In this phase, spermatocytes can be distinguished by their size and chromosome condensation, but mainly by "meiotic figure" or arrangement of the chromosomes into the nucleus (LEAL *et al*., 2009). Morphological characteristics of *A. altiparanae* spermatocytes are similar to observations already described to other teleost, as for the species *G. carapo* (VERGÍLIO *et al*., 2012) highlighting that this phase might be highly preserved in this group.

After secondary spermatocytes finish the second meiotic division, the spermatids are originated. These cells are committed to undergo events of cell differentiation, characterized by two main morphological phenomena: intracellular

movements and structural changes. Together, both of these phenomena are named spermiogenesis, which can also vary among species (ROOSE-RUNGE, 1962; BRUSLÉ, 1981; BILLARD, 1984; SILVA, 1987; LO NOSTRO *et al*., 2003). As can be notice by difference in spermatid types, since other Neotropical fish such as *Cyphocharax modestus*, *Potamorhina altamazonica* and *Steindachnerina insculpta* presented only two types of spermatids, named early and late spermatid (QUAGIO-GRASSIOTTO *et al*., 2003). However, LEAL *et al*. (2009) also estimated three spermatid types to *D. rerio* species, which separated from the studied species around 153 million years ago. Thus, the number of spermatids does not seem to follow a set pattern among Teleostei fish.

CONCLUSION

Considering these aspects, it can be concluded that this study provides important tools for better understanding of spermatogenesis in teleosts. Moreover, understanding of the morphological events of spermatogenesis in this species, enable it as an appropriate model for the development of methodologies such as germ cell transplantation involving other Neotropical fish and Characiform species.

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REFERENCES

ANDO, N.; MIURA, T.; NADER, M.R.; MIURA, C.; YAMAUCHI, K. 2000 A method for estimating the number of mitotic divisions in fish testes. *Fisheries Science*, *66*(2): 299-303.

ALEXANDRINO, A.C.; PHAN, M.T.; PINHEIRO, E.F.G. 1985 Caracterização macroscópica e microscópica das gônadas do curimbatá, *Prochilodus scrofa* (Steindachner, 1881), durante o ciclo reprodutivo. *Boletim de Zoologia*, *9*: 159-175.

BILLARD, R. 1984 Ultrastructural changes in the spermatogonia and spermatocytes of *Poecilia reticulata* during spermatogenesis. *Cell Tissue Research*, 237(2): 219-226.

BRUSLÉ, S. 1981 Ultrastructure of spermiogenesis in *Liza aurata* Risso, 1810 (Teleostei, Mugilidae). *Cell Tissue Research*, *217*(2): 415-424.

CASTILHO-ALMEIDA, R.B. 2007 ***Astyanax altiparanae*** *(Pisces, Characiformes) como modelo biológico de espécie de peixe para exploração zootécnica e biomanipulação*. Botucatu. 119p. (Doctoral Thesis. Universidade Estadual Paulista - UNESP). Available at: <http://www.ibb.unesp.br/posgrad/teses/zoologia_do_2007_rodrigo_almeida.pdf> Access on: 20 Nov. 2014.

DE BEM, J.C. 2009 *Desenvolvimento gonadal inicial e reversão sexual em* ***Astyanax altiparanae*** *(Teleostei, Characidae)*. Rio Claro. 96p. (Masters Dissertation. Universidade Estadual Paulista - UNESP). Available at: <http://repositorio.unesp.br/bitstream/handle/11449/87742/bem_jc_me_rcla.pdf?sequence=1&isAllowed=y> Access on: 20 Nov. 2014.

DE ROOIJ, D.G. and RUSSELL, L.D. 2000 All you wanted to know about spermatogonia but were afraid to ask. *Journal of Andrology*, *21*(6): 776-798.

GARUTTI, V. and BRITSKI, H.A. 2000 Descrição de uma espécie nova de *Astyanax* (Teleostei: Characidae) da bacia do alto Rio Paraná e considerações gerais sobre as demais espécies do gênero na bacia. *Comunicações do Museu de Ciências e Tecnologia da PUCRS, Série Zoologia*, *13*: 65-88.

GRIER, H.J.; LINTON, J.R.; LEATHERLAND, J.F.; VLAMING, V.L. 1980 Structural evidence for two different testicular types in Teleost fishes. *The American Journal of Anatomy*, *159*(3): 331-345.

GRIER, H.J. and ARANZÁBAL, M.C.U. 2009 The testis and spermatogenesis in Teleosts. In: JAMIESON, B.G.M. *Reproductive Biology and Phylogeny of Fishes: Agnathans and Bony Fishes,* 8. St. Lucia: University of Queensland Press. p.119-142.

QUAGIO-GRASSIOTTO, I.; GAMIERO, M.C.; SCHNEIDER, T.; MALABARBA, L.R.; OLIVEIRA, C. 2003 Spermiogenesis and spermatozoa ultrastructure in five species of the Curimatidae with some considerations of spermatozoa ultrastructure in the Characiformes. *Neotropical Ichthyology*, *1*(1): 35-45.

JAMIESON, B.G.M. 1991 *Fish Evolution and Systematics: Evidence from spermatozoa*. Cambridge: Cambridge University Press. 319p.

LEAL, M.C.; CARDOSO, E.R.; NOBREGA, R.H.; BATLOUNI, S.R.; BOGERD, J.; FRANÇA, L.R.; SCHULZ, R.W. 2009 Histological and stereological evaluation of zebrafish (*Danio rerio)* spermatogenesis with an emphasis on spermatogonial generations. *Biology of Reproduction*, *81*(1): 77-187.

LO NOSTRO, F.; GRIER, H.; GUERRERO, G.A. 2003 Involvement of the gonadal germinal epithelium during sex reversal and seasonal testicular cycling in the protogynous swamp eel, *Synbranchus marmoratus* Bloch 1795 (Teleostei, Synbranchidae). *Journal of Morphology*, *257*(1): 107-126.

LOPES, D.C.J.R.; BAZZOLI, N.; BRITO, M.F.G.; MARIA, T.A. 2004 Male reproductive apparatus in the South American catfish *Conorhynchus conirostris. Journal of Fish Biology*, *64*(5): 1419-1424.

MAZZOLDI, C.; LORENZI, V.; RASOTTO, M.B. 2007 Variation of male reproductive apparatus in relation to fertilization modalities in the catfish families Auchenipteridae and Callichthyidae (Teleostei: Siluriformes). *Journal of Fish Biology*, *70*(1): 243-256.

MORENA, A.R.; BOITANI, C.; PESCE, M.; FELICI, M.; STEFANINI M. 1996 Isolation of Highly Purified Type A Spermatogonia From Prepubertal Rat Testis. *Journal of Andrology*, *17*(6): 708-717.

NÓBREGA, R.H.; LO NOSTRO, F.L.; GRIER, HARRY, J.; QUAGIO-GRASSIOTTO, I. 2006 Cellular proliferation in fish male germinal epithelium. In: INTERNATIONAL SYMPOSIUM ON ANIMAL BIOLOGY OF REPRODUCTION - From sex differentiation to reproductive biotechnology. *Animal Reproduction*, *3*: 199-199.

NÓBREGA, R.H.; LACERDA, S.M.N.S.; COSTA, G.M.J.; SILVA, R.C.; SCHULZ, R.W.; FRANÇA, L.R. 2008 Development of germ cell transplantation in zebrafish (*Danio rerio*): a means to study the spermatogonial stem cells in fish. In: INTERNATIONAL SYMPOSIUM ON ANIMAL BIOLOGY OF REPRODUCTION, 2., São Paulo, 19-22/Nov./2008. *Anais*… São Paulo: Brazilian College of Animal Reproduction.

NÓBREGA, R.H.; BATLOUNI, S.R.; FRANÇA, L.R. 2009 An overview of functional and stereological evaluation of spermatogenesis and germ cell transplantation in fish. *Fish Physiology Biochemistry, 35*(1): 197-206.

NÓBREGA, R.H.; GREEBE, C.; DE KANT, H.V.; BOGERD J.; FRANÇA, L.R.; SCHULZ, R.W. 2010 Spermatogonial stem cell niche and spermatogonial stem cell transplantation in zebrafish. *PlosOne*, *5*(9): e12808. [on line] doi:10.1371/journal.pone.0012808

ORSI, M.L.; SHIBATTA, O.A.; SILVA-SOUZA, A.T. 2002 Caracterização biológica de populações de peixes do rio Tibagi, localidade de Sertanópolis. In: MEDRI, M.E.; BIANCHINI, E.; SHIBATTA, O.A.; PIMENTA, J.A. *A bacia do rio Tibagi.* Londrina: Universidade Estadual de Londrina. p.425-432.

REIS, R.E.; KULLANDER, S.O.; FERRARIS JR, C.J. 2003 *Check list of the freshwater fishes of South and Central America.* Porto Alegre: PUCRS Press. 742p.

ROOSE-RUNGE, E.C. 1962 The process of spermatogenesis in mammals. *Biological Reviews*, *37*(3): 343-377.

RUSSELL, L.D.; ETTLIN, R.A.; SINHA HIKIM, A.P.; CLEGG, E.D. 1990 Mammalian spermatogenesis. *Histological and histopathological evaluation of the testis.* Clearwater: Cache River Press. 40p.

SCHULZ, R.W.; MENTING, S.; BOGERD, J.; FRANÇA, L.R.; VILELA, D.A.R; GODINHO, H.P. 2005 Sertoli cell proliferation in the adult testis: evidence from two fish species belonging to different orders. *Biology of Reproduction*, *73*(5): 891-898.

SCHULZ, R.W.; FRANÇA, L.R.; LAREYRE, J.J.; LEGAC, F.; CHIARINI-GARCIA, H.; NOBREGA, R.H.; MIURA, T. 2010 Spermatogenesis in fish. *General and Comparative Endocrinology*, *165*(3): 390-411.

SILVA, M. 1987 *Morfologia ultra-estrutural do testículo, cinética da espermatogênese e barreira hemotesticular da tilápia do Nilo,* ***Oreochromis niloticus****.* Belo Horizonte. 164p. (Doctoral Thesis. Universidade Federal de Minas Gerais - UFMG).

SIQUEIRA-SILVA, D.H.; DA-SILVA-COSTA, R.; NINHAUS-SILVEIRA, A.; VICENTINI, C.A.; VERÍSSIMO-SILVEIRA, R. 2012 Ultrastructural

analysis of spermiogenesis in the neotropical cichlid Kullander & Ferreira, 2006 (Perciformes: Cichlidae). *Journal of Applied Ichthyology*, *28*(6): 878-882.

SIQUEIRA-SILVA, D.H.D.; VICENTINI, C.A.; NINHAUS-SILVEIRA, A.; VERISSIMO-SILVEIRA, R. 2013 Reproductive cycle of the Neotropical cichlid yellow peacock bass *Cichla kelberi*: A novel pattern of testicular development. *Neotropical Ichthyology*, *11*(3): 587-596.

VAZZOLER, A.E.A.M. 1996 *Biologia da reprodução de peixes teleósteos: teoria e prática*. Maringá: Eduem. 169p.

VERÍSSIMO-SILVEIRA, R.; GUSMÃO-POMPIANI, P.; VICENTINI, C.A.; QUAGIO-GRASSIOTTO, I. 2006 Spermiogenesis and spermatozoa ultrastructure in *Salminus* and *Brycon*, two primitive genera in Characidae (Teleostei: Ostariophysi: Characiformes). *Acta Zoologica*, *87*(4): 305-313.

VILELA, D.A.R.; SILVA, S.G.B.; PEIXOTO, M.T.D.; GODINHO, H.P.; FRANÇA, L.R. 2003 Spermatogenesis in teleost: insights from the Nile tilapia (*Oreochromis niloticus)* model. *Fish Physiology and Biochemistry*, *28*(1): 187-190.

VERGÍLIO, C.S.; MOREIRA, R.V.; CARVALHO, C.E.V.; MELO, E.J.T. 2012 Characterization of mature testis and sperm morphology of *Gymnotus carapo* (Gymnotidae, Teleostei) from the southeast of Brazil. *Acta Zoologica, 0*: 1-7.

YASUI, G.S.; SANTOS, M.P.; NAKAGHI, L.S.O.; SENHORINI, J.A.; ARIAS-RODRIGUEZ, L.; FUJIMOTO, T.; SHIMODA, E.; SILVA, L.A. 2014 Improvement of gamete quality and its short-term storage: an approach for biotechnology in laboratory fish. *Animal*, *9*(3): 464-470.