# ON THE REPRODUCTION OF BRAZILIAN FISHES.IX.SPERMATOGENESIS OF THE SURUBIM *Pseudoplatystoma corruscans* AGASSIZ 1829 (PISCES, PIMELODIDAE)

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## SUMMARY

The authors studied morphologically seven types of germ cells during the process of spermatogenesis of the catfish (surubim) *Pseudoplatystoma corruscans*. Resting germ cells, primary spermatogonia, secondary spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa have been recognized.

**Key words:** Reproduction, fish, spermatogenesis, *Pseudoplatystoma corruscans*.

## RESUMO

**Sobre a reprodução de peixes brasileiros. IX. Espermatogênese do surubim *Pseudoplatystoma corruscans* Agassiz 1829 (Pisces, Pimelodidae)**

Os autores estudaram morfologicamente sete tipos de células germinativas, durante o processo de espermatogênese do surubim *Pseudoplatystoma corruscans*. Foram reconhecidas: células germinativas em repouso, espermatogônias primárias, espermatogônias secundárias, espermatócitos primários, espermatócitos secundários, espermátides e espermatozóides.

**Unitermos:** Reprodução, peixe, espermatogênese, *Pseudoplatystoma corruscans*.

## INTRODUCTION

The testicular changes during the reproductive cycle were studied macro and microscopically in a few teleostean species (see HOAR, 1957, 1969; DODD, 1960; LOFTS, 1968; DE VLAMING, 1974 for reviews). Gonads of Brazilian catfishes of Pimelodidae family were studied in bagre *Rhamdia quelen* and in *Pimelodella lateristriga* by IHERING & AZEVEDO (1936); in mandi *Pimelodus maculatus* by GODINHO et alii (1974, 1977) and by BASILE-MARTINS et alii (1975); in bagre *Rhamdia hilarii* by LEME DOS SANTOS et alii (1986b); in jurupensén *Sorubim lima* by LOPES et alii (1986a) and by WATANABE et alii (1986); in pintado-cachara *Pseudoplatystoma fasciatus* by LEME DOS SANTOS et alii (1986a), and in barbado *Pimelodus pirinampus* by LOPES et alii (1986b). In the present study, spermatogenesis is described in a fresh water catfish, *Pseudoplatystoma corruscans*, with special emphasis on the cytological changes occuring in the germ cells under microscopic observation.

## MATERIAL AND METHODS

A total of 48 males *Pseudoplatystoma corruscans* (common names: bagre-rajado, caçonete, caparari, loango, pintado, piracajiara, sorubim, surubi-de-cama, and surubim-pintado) were studied during 1984. Specimes were collected from Paraguai River (Corumbá-MS). The fish were vivisected without anesthesia. The testicles were carefully extirpated, fixed in Bouin fixative for 24 hours, embedded in paraffin, and cut in 7 µm- thick sections. Staining was done with hematoxylin-eosin and Masson trichrome.

## RESULTS

The testes of the adult *Pseudo*

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*platystoma corruscans* are soft, creamy white, paired elongated structures, each situated on either side, just ventral to the kidney in the posterior region of the abdominal cavity, and their ducts join posteriorly to open into the urogenital papilla. They remain attached to the body wall and the air bladder by means of mesorchia.

Histologically, the tests of surubim displays typical organization of seminiferous lobules of connective tissue fibres (Figure 1A) consisting of spermatogenic cysts. Each cysts is an autonomous unit with germ cells in the same stage of maturity (Figure 1A). In between the seminiferous lobules islets of in**terstitial cells** (Leydig cells) can be observed. The nuclei of interstitial cells possess one or two nucleoli, a little amount of cytoplasm with well defined cell boundary and show comparatively strong affinity to hematoxylin.

The recognized and described stages are the following: resting germ cells, primary spermatogonia, secondary spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, and spermatozoa. The terms used are those suggested by WILSON (1925).

**1. Resting germ cells:** They are the largest cells and when very active are known as the sperm mother cells. The sperm mother cells are large spherical structures with a large clear nucleus surrounded by cytoplasmic relatively large mass. The cytoplasm is clear and the cell membrane is usually distinct though weak. The nucleus contains nucleolus eccentrically placed and several chromatin threads stained with hematoxylin (Figure 1B). The sperm mother cells multiply and soon give origin to a large number of cells. Some of these cells grow in size and give rise to the primary spermatogonia.

**2. Primary spermatogonia:** They are smaller than resting germ cells. The cytoplasm is clear and somehow variable in quantity. The cell membrane is indistinct. The nucleoplasm is hyaline with a thin, delicate network of chromatin radiating from the prominent nucleolus (Figure 1A and 1B).

**3. Secondary spermatogonia:** These are smaller than primary spermatogonia. Their cell boundaries are distinct and cytoplasmic area is thin and hyaline (Figure 1A and 1B). The amount of cytoplasm is usually much less than in the primary spermatogonia. Nuclei are well marked and contain centrally placed prominent nucleolus stained deeply with hematoxylin.

**4. Primary spermatocytes:** The next spermatogenetic stage is the primary spermatocyte. They arise by repeated mitotic division from secondary spermatogonia and is distinguishable from the later by a number of characteristics. This involves polarisation of chromatin material into one pole within the nucleus and the remaining parts of the chromosomes extend towards the centre of the nuclei formig a "bouquet". This stage is called synizesis stage. The nucleus is slightly smaller than in secondary spermatogonia. There is no distinct nucleolus (Figure 1A and 1B).

**5. Secondary spermatocytes:** These are again smaller than the primary spermatocytes, and they are formed by the first maturation division of primary spermatocytes. The nuclear wall becomes very distinct and possesses deeply stained thick cluster of chromatin material giving crystalline appearance. Secondary spermatocytes have a short duration and soon they get divided to form spermatids (Figure 1A and 1B).

**6. Spermatids:** They are formed by second maturation division. Like the secondary spermatocytes, spermatids have dense chromatin in their nuclei. The chromatin is uniformily condensed deeply stained with hematoxylin. At this stage cyst wall bursts librating the content in the lumen of the lobules (Figure 1A and 1B).

**7. Spermatozoa:** Spermatids undergo the final transformation into motile spermatozoa. They are the smallest among the germ cells. They possess a distinct rounded head stained deeply with hematoxylin and a tail stained with eosin.

## DISCUSSION

In *Pseudoplatystoma corruscans*

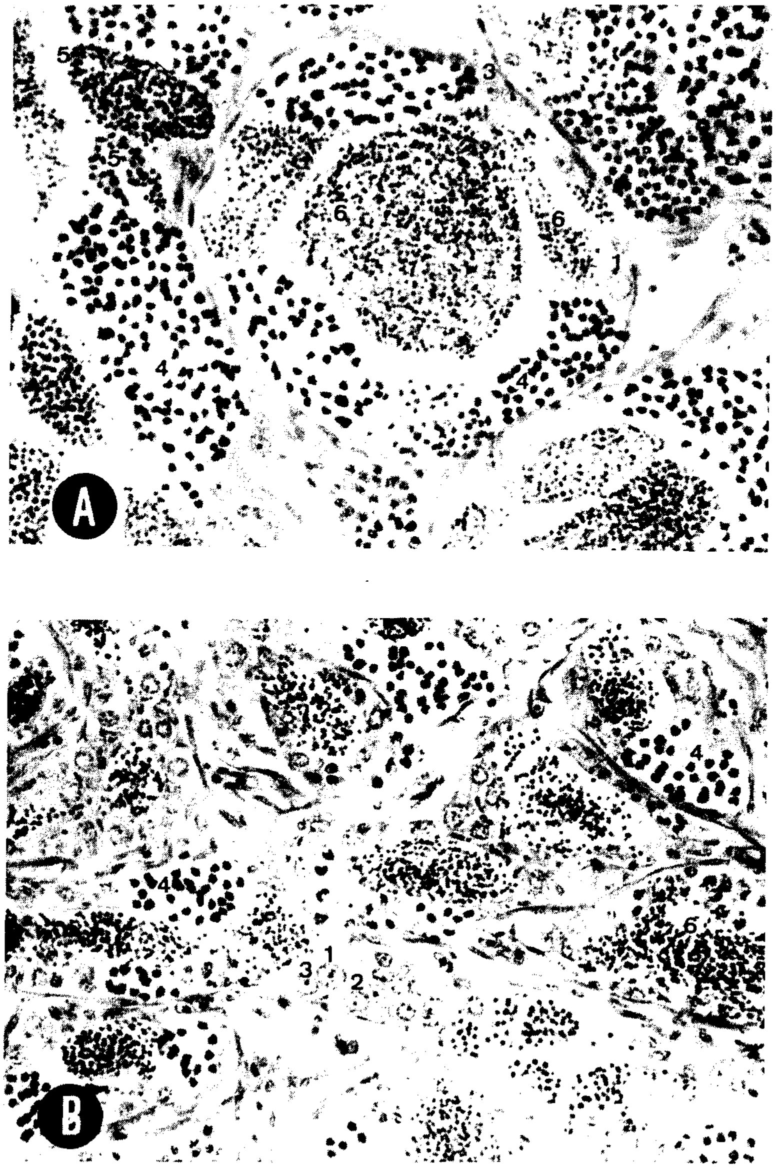


FIGURE 1 - Histological picture of the *Pseudoplatystoma corruscans*, showing germ cells in the following spermatogenic stages: resting germ cells (1), primary spermatogonia (2), secondary spermatogonia (3), primary spermatocytes (4), secondary spermatocytes (5) and spermatids (6).
Hematoxylin-Eosin (50x).

during the process of spermatogenesis a progressive cytological change occur followed by a gradual decrease in the size of germ cells. Seven types of germ cells have been identified in the process of maturation. The resting germ cell is characterised by its large size and distinct cytoplasmic and nuclear material. In *P. corruscans* sperm mother cells are present throughout the whole year. During the breeding period, however, their number is very much reduced, and they are seen in dormant condition along the lobule wall. Different names have been given to these solitary sperm mother cells by different investigators. HANN (1927) named them **dormant germ cells**; SUSUKI (1939) called the **reserve germ cells**, and WEISEL (1943) and JONES (1940) referred to them as **resting germ cells**. Some workers believe that after spermiation the testes is reconstituted by the migration of primary germ cells in it from an extra-testicular source (TURNER, 1919; GEISER, 1922; CRAIG-BENNETT, 1931; LOFTS & MARSHALL, 1957). In agreement with the findings of VAN OORDT (1925), HANN (1927), MATTHEWS (1938), BULLOUGH (1939) and BARR (1963), the observation of *P. corruscans* suggests that new generations of sex cells are produced by the mitotic division of the resting sperm mother cells present in the testis throughout the year.

The resting germ cell (or resting sperm mother cell) divides to form primary and secondary spermatogonia. These stages of germ cells are characterised by a gradual decrease in their size showing prochromosomal and leptotene stages of spermatogonia. The next stage is primary spermatocytes which passes through various stages of division and finally reduces in size. They are characterised by the formation of synzesis knot. The primary spermatocytes undergo first maturation division to give rise to the reduced number of chromosomes of the secondary spermatocytes. Secondary spermatocytes is a comparatively transient phase in the spermatogenetic cycle. Several other workers also agreed with this view (FOLEY, 1926; VAUPEL, 1929; BULLOUGH, 1939; WEISEL, 1943; AHSAN, 1966; SRIVASTAVA, 1984). The spermatids are formed by the second maturation division of secondary spermatocytes which finally transform into motile spermatozoa, and at the spermatid stage the cyst wall break open to release them into lobule lumen. Similar observations have been made by other workers (CRAIG-BENNETT, 1931; BULLOUGH, 1939; AHSAN, 1966; HYDER, 1969; SWARUP & SRIVASTAVA, 1978, 1979; SRIVASTAVA, 1984).

Similarly to the *Notropis bifrenatus* (HARRINGTON, 1957), *Couesius plumbeus* (AHSAN, 1966) and *Channa striatus* (SRIVASTAVA, 1984), in *P. corruscans* spermatogenesis involves two types of multiplication process: 1) Mitotic phase - this is very slow and the nuclear division is mitotic up to primary spermatocyte, and 2) Meiotic phase - this is rapid and of short duration in which secondary spermatocytes and spermatids are formed and finally transform into spermatozoa.

The occurense of interstitial cells and their role in teleosts presents a controversial picture:

a) CRAIG-BENNETT (1931) has correlated the seasonal cycle in the interstitial cells of the testis with the secondary sexual characters of *Gasterosteus aculeatus*.

b) MARSHALL & LOFTS (1965) have described typical interstitial Leydig cells in the testes of some teleosts, while lobule boundary cells which are considered as being modified connective tissue, and are Leydig-cell homologues, have been described by the authors in other species.

c) LOFTS & MARSHALL (1957) have shown that lobule boundary cells in *Esox lucius* possess cholesterol-positive lipids known to be the precursors of the male hormone, during prespawning period.

d) GOKHALE (1957) has assigned nutritive function to interstitial and lobule boundary cells in the testes of *Gadus*.

e) ESSENBERG (1933), VAN OORDT (1925), MATTHEWS (1938), RASQUIM & HAFTER (1951), and BARR (1963) could not find any evidence of glandular activity in these cells.

After spermiation, new spermatogenetic stages start forming, and new cysts of early germ cells begin to appear.

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## REFERENCES

AHSAN, S.N. Cyclical changes in the testicular activity of the lake chub, *Couesius plumbeus* (Agassiz). *Can.J. Zool.*, *44*:149-159, 1966.

BARR, W.A. The endocrine control of the sexual cycle in the plaice, *Pleuronectes platessa* (L.). III. The endocrine control of spermatogenesis. *Gen. Comp. Endocrin.*, *3*:205-215, 1963.

BASILE-MARTINS, M.A.; GODINHO, H. M.; FENERICH, N.A.; BRAMLEY BARKER, J.M. Influência de fatores abiõticos sobre a maturação dos ovãrios de *Pimelodus maculatus* LAC. 1803 (Pisces, Siluroidei). *Bol. Inst. Pesca, Santos*, *4*:1-28, 1975.

CRAIG-BENNETT, A. The reproductive cycle of three spined sticleback, *Gastrosteus aculeatus* Linn. *Phil. Trans. Roy. Soc.* (London), Series B, *219*:197-279, 1931.

DE VLAMING, V.L. Environmental and endocrine control of teleost reproduction. In:SCHRECK, C.B. ed. *Control of Sex in Fishes.* Blacksburg, *Sea Grant and Virginia Polytechinique Institute and State University Press*, 1974. p.13-83.

DODD, J.M. Gonadal and gonadotrophic hormones in lower vertebrates. In: PARKES, A.S. ed. *MARSHALL'S Physiology of Reproduction.* New York, *Longmans Green*, 1960. Vol. I, 417-582.

ESSENBERG, J.M. Sex differentiation in the viviparous teleost, *Xiphophorus helleri*. *Biol.Bull.*, *45*:46-97, 1923.

FOLEY, J.G. The spermatogenesis of *Umbralimi* with special reference to behaviour of the spermatogonial chromosomes in the first maturation division. *Biol. Bull.*, *50*:117-140, 1926.

GODINHO, H.M.; BASILE-MARTINS, M. A.; FENERICH, N.A.; NARAHARA, N. Y. Fecundidade e tipo de desova do mandi *Pimelodus maculatus* Lacepẽrẽ, 1803 (Pisces, Siluroidei). *Rev. Brasil Biol.*, *37*:737-744, 1977.

GODINHO, H.M.; FERRI, S.; MEDEIROS, L.O.; BARKER, J.M.B. Morphological changes in the ovary of *Pimelodus maculatus* Lacepẽrẽ, 1803 (Pisces, Siluroidei) related to the reproductive cycle. *Rev. Brasil. Biol.*, *34*:581-588, 1974.

GOKHALE, S.V. Seasonal histological changes in the gonads of whiting (*Gadus merlangus*) and the Norway pout (*G. esmarkii*). *Indian J. Fish.*, *4*:92-112, 1957.

HANN, H.W. The history of germ-cells of *Cottus bairdii*. *J. Morph.*, *43*:427-497, 1927.

HARRINGTON, R.W. Sexual photoperiodicity of the cyprinid fish, *Natropis bifrenatus* (Cope) in relation to the phases of its annual reproductive cycle. *J. Exp. Zool.*, *135*:529-556, 1957.

HOAR, W.S. The gonads and reproduction. In: BROWN, M.E. ed. *The physiology of Fishes.* New York, London, *Academic Press.* 1957. Vol. I, 287-321.

HOAR, W.S. Reproduction. In:HOAR, W.S. & RANDALL, D.I. eds. *Fish Physiology.* New York,London, *Academic Press*, 1969. Vol.III, 1-72.

HYDER, M. Histological studies on the testis of *Tilapia leucosticta* and other species of the genus *Tilapia* (Pisces: Teleostei). *Trans. Amer. Microsc. Soc.*, *88*:211-231, 1969.

IHERING, R. von; AZEVEDO, P. A desova e a hypophysação dos peixes. Evolução de dois nematognathas. *Arq. Inst. Biol.* São Paulo, 7:108-117, 1936.

JONES, J. W. Histological changes in the testis in the sexual cycle of male salmon parr (*Salmo salar* L. Juv.). *Proc. roy. Soc.*, *B128*:499-509, 1940.

LEME DOS SANTOS, H.S.; LOPES, R.A.; PAULA, C.V.; PAULA-LOPES, O. V.; NUTI-SOBRINHO, A. Sobre a reprodução de peixes brasileiros. V. Espermatogenẽse do pintado-cachara *Pseudoplatystoma fasciatus* Linnaeus 1766 (Pisces, Pimelodidae). In: ENCONTRO DE PESQUISAS VETERINÃRIAS, 11º, Jaboticabal, 6 e 7/novembro/1986a.

LEME DOS SANTOS, H.S.; ZUIM, S.M. F.; LOPES, R.A. Sobre a reprodução de peixes brasileiros. XVI. Estudo morfomẽtrico dos ovõcitos de *Rhamdia hilarii* Valenciennes 1840 (Pisces, Pimelodidae). *Ars Veterinãria*, *2*, Jaboticabal, 13-

26, 1986b.

LOFTS, B. Patterns of testicular activity. In: BARRINGTON, E.J.W. & JORGENSEN, C.B. eds. *Perspectives in Endocrinology*. New York, London. Academic Press, 1968, p.239-304.

LOFTS, B.; MARSHALL, A.J. Cyclical changes in the distribution of the testis lipids of a teleost fish *Esox lucius*. *Quart.J. Micr. Sci.*, *98*:79-88, 1957.

LOPES, R.A.; LEME DOS SANTOS, H.S.; NUTI-SOBRINHO, A.; WATANABE, I.; PAULA-LOPES, O.J.; PAULA,C.V. Sobre a reprodução de peixes brasileiros. VI. Estudo ao microscópio óptico do ritmo de desenvolvimento dos ovócitos do jurupensém *Sorubim lima* Schneider, 1801. (Pisces, Pimelodidae). In: ENCONTRO DE PESQUISAS VETERINÁRIAS, 11º, Jaboticabal, 6 e 7/novembro/ 1986a.

LOPES, R.A.; PAULA-LOPES, O.V.; PAULA, C.V.; LEME DOS SANTOS, H.S.; NUTI-SOBRINHO, A. Sobre a reprodução de peixes brasileiros. X. Ritmo de desenvolvimento dos ovócitos do barbado *Pimelodus pirinampus* Spix 1829 (Pisces, Pimelodidae). In: ENCONTRO DE PESQUISAS VETERINÁRIAS, 11º, Jaboticabal, 6 e 7/novembro/1986b.

MARSHALL, A.J.; LOFTS, B. The Leydig-cell homologue in certain teleost fishes. *Nature*, London, *177*:704-705, 1956.

MATTHEWS, S.A. Seasonal cycle in the gonads of *Fundulus*. *Biol. Bull.*, *75*:66-75, 1938.

RASQUIN, P.; HAFTER, E. Age changes in the testis of the teleost, *Astyanax mexicanus* *J. Morph.*, *89*: 397-407, 1951.

SRIVASTAVA, S. Spermatogenesis in a freshwater large murrel, *Channa striatus* (Bloch). *Bolm. Zool., Univ. S.Paulo*, *8*:107-114, 1984.

SUZUKI, K. Regeneration of the gonads in *Plecoglossus altivelis* after spawning season. *Cytologia*, *10*:113-126, 1939.

SWARUP, K.; SRIVASTAVA, S.T. Cyclic changes in the testicular activity of the freshwater large murrel, *Channa striatus* (Bloch). *Arch. de Biol.*, Bruxelles, *89*: 329-342, 1978.

SWARUP, K.; SRIVASTAVA, S.T. Cyclic changes in the testicular activity of the freshwater large murrel, *Channa marulius* (Ham). *Nat. Acad.Sci.Letters*, *2*:83-84, 1979.

TURNER, C.L. The seasonal cycle in the spermary of the perch. *J. Morph.*, *32*:681-711, 1919.

VAN OORDT, G.J. The relation between the development of the secondary sex characters and the structure of the testis in the teleost *Xiphophorus helleri*. *J. exp. Biol.*, *3*:43-59, 1925.

VAUPEL, J. The spermatogenesis of *Lebistes reticulatus*. *J. Morph. & Physiol.*, *47*:555-585, 1929.

WATANABE, I.; LOPES, R.A.; LEME DOS SANTOS, H.S.; NUTI-SOBRINHO, A.; PAULA, C.V.; PAULA-LOPES, O.V. Sobre a reprodução de peixes brasileiros. VII. Estudo ao microscópio eletrônico de varredura dos ovócitos do jurupensém *Sorubim lima* Schneider 1801 (Pisces, Pimelodidae). In:ENCONTRO DE PESQUISAS VETERINÁRIAS, 11º, Jaboticabal, 6 7/novembro/1986.

WEISEL, G.E. A histological study of the testes of the sokeyed salmon, *Oncorhynchus nerk*. *J.Morph.*, *73*:207-230, 1943.

WILSON, E.B. *The cell in development and heredity*. New York, Macamillan Co., 1925. 1,232p.