HISTOLOGY OF THE GONADS OF THE HYBRID *Pseudoplatystoma punctifer* X *Leiarius marmoratus*

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ABSTRACT

Considering the rapid expansion of fish farming in intensive systems and the use of hybrids in Brazil, the study regarding the breeding capacity of the most recent hybrid is of ecological importance. If these animals are fertile, they may breed with the parental species in the wild and can negatively affect the genetic variability of the population (parental species in the wild). Fragments of the gonads were collected and submitted to histological evaluation. Histological cuts were stained with eosin/hematoxilin and toluidine, and slides were randomly selected for observation of three fields each animal, through light microscopy. Gonads of all fishes were paired structures of elongated shape in the abdominal cavity, covered by an albuginea tunic. Male juveniles presented primary spermatocytes while juvenile females presented chromatine nucleolus oocytes. Adult females presented chromatin-nucleolus oocytes, perinuclear, cortical alveoli, and vitellogenic oocytes visible in various sizes. The presence of oocytes in different stages and primary spermatocytes indicate that these fish may be fertile. Fish hybridization represents a threat to the conservation of native species.

Keywords: pisciculture; germinative cells; spermatocytes; ovary

HISTOLOGIA DAS GÔNADAS DO HÍBRIDO *Pseudoplatystoma punctifer* X *Leiarius marmoratus*

RESUMO

Considerando a rápida expansão da piscicultura em sistemas intensivos e do uso de híbridos no Brasil, o estudo sobre a capacidade de reprodução do híbrido é de importância ecológica. Caso estes animais sejam férteis, podem afetar negativamente a variabilidade genética da população (espécies parentais em estado selvagem). Fragmentos das gônadas foram coletados e submetidos à avaliação histológica. Os cortes histológicos foram corados com eosina/hematoxilina e toluidina, e selecionados aleatoriamente para observação de três campos de cada animal, através de microscopia de luz. Gônadas de todos os peixes apresentaram-se como estruturas emparelhadas de forma alongada na cavidade abdominal, cobertas por uma túnica albugínea. Juvenis do sexo masculino apresentaram espermatócitos primários, enquanto as fêmeas juvenis apresentaram ovócitos cromatina nucléolo nos ovários. Fêmeas adultas apresentaram ovócitos cromatina-nucléolo nos ovários, alvéolo cortical, perinuclear e vitelogênico, visíveis em vários tamanhos. A presença de ovócitos em diferentes fases e espermatócitos primários indicam que estes peixes podem ser férteis. A hibridação de peixes pode representar uma ameaça à conservação das espécies nativas.

Palavras chave: piscicultura; células germinativas; espermatócitos; ovário

Artigo Científico: Recebido em 16/05/2014 – Aprovado em 13/05/2015

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INTRODUCTION

The use of hybrid fish has received special attention in Brazil in recent years as a way to gather favorable characteristics of the parental species in order to improve production yields. Similarly to agriculture, in which hybridization aims to increase production, to become more competitive and to improve customer acceptance to the final products (BOTERO *et al*., 2004), most fish producers seek for the same alternatives.

The production of fish hybrids resulting from hybridization programs produced such as, *Piaractus mesopotamicus* x *Colossoma macropomum*; *Piaractus brachypomus* x *C. macropomum*; *Leporinus macrocephalus* x *Leporinus enlongatus*; *Pseudoplatystoma reticulatum* x *Phractocephalus hemeliopterus*; *P. reticulatum* x *Pseudoplatystoma corruscans* are also currently in production in Brazil (PORTO-FORESTI *et al*., 2008; HASHIMOTO *et al*., 2009; 2010; 2012).

BARTLEY *et al*. (2001), argue that the use of hybridization programs in fish farming has been performed in numerous species of fish in order to produce sterile and animals that have better performance than the parental species (hybrid vigor). Therefore, studies describing the reproductive biology of hybrid fish, as well as, their potential risk to the environment are needed, before these fish are raised commercially in large scale (FAUSTINO *et al*., 2007).

Many fish producers justify the use of hybrids based on the shortage in the production cycle, possibility to obtain monosex populations without the use of hormones and the production of more docile fishes that are easier to handle. Approximately 20 types of fish hybrids have been produced in Brazil (PORTO-FORESTI *et al*., 2010). The great interest in raising the hybrid *Pseudoplatystoma punctifer* x *Leiarius marmoratus* in Brazil highlights the need for further investigation regarding its ability to breed. The determination of the female breeding stage based on the ovarian development, and a male readiness to spawn, based on the modification in the germ cells, are important information of the fish reproductive biology. Thus, the objective of the present study was to describe the histological characteristics of the hybrid *P. punctifer* x *L. marmoratus*, in order to support its culture.

MATERIAL AND METHODS

Thirty-one hybrid *P. punctifer* (female) x *L. marmoratus* (male) individuals, obtained at the São Paulo Fish Farm, State of Tocantins, Brazil, of different ages were evaluated in the present study: five 7-day old; five 14-day old; ten 9-month old; five 16-month old; and seven 36-month old fish.

The animals about were euthanized by using benzocaine (0.5 mL benzocaine L^{-1} water) and submitted to ventral longitudinal section for the following gonad morphological observation: anatomical position to the right (R) and left sides (L), location in the abdominal cavity and coloration. The gonads were dissected and measured by using a paquimeter, determining length in the longitudinal axis (mm) and width in the transversal axis (mm); weighted individually (g) and determining the GSI (Gonadossomatic Index).

Fragments of testicle and ovary were drawn and processed for light microscopy analysis by fixation in Bouin solution for 24 hours. After dehydrated, diafanized and embedded in paraffin, tissue samples were sliced in 4 μm and stained with hematoxilin-eosine and toluidine.

Histological analysis was done by randomly observing three slides of each animal. For each slide, three fields were chosen for light microscopy examination, in which the existing cells and the presence of germ cells were observed. The gamete counting was carried out by randomly observing of 10 fields and all sections examined were photomicrographed.

RESULTS

The hybrids *P. punctifer* x *L. marmoratus* presented no sexual dimorphism. Histologically, 7 and 14-day old fish presented no developed reproductive cells, presenting only somatic cells, volumous and primordial germ cells, which do not allow sexual differentiation, that can be done by the observation of germ cells that initiated the gametogenesis.

The cell differentiation analysis indicated 6 females and 4 males among the 9-month old fish while all 16 and 36-month old fish were females. Adult females were found having the size equivalent to the first gonadal maturation. Fish and gonad length and weight are presented in Table 1.

Table 1. Fish and gonad mean (± SD) weight and length of hybrid *Pseudoplatystoma punctifer* (female) x *Leiarius marmoratus* (male).

	Age					
	7 days	**14 days**	**9 months**		**16 months**	**36 months**
Sex	ND	ND	Female	Males	Female	Female
Body weight (g)	1.3 ± 0.9	20.3 ± 1.0	1,760 ± 0.5	1,870 ± 0.6	2,950 ± 1.2	4,610 ± 0.9
Body length (cm)	5.2 ± 1.2	15.3 ± 0.7	57.5 ± 1.1	60.5 ± 0.9	61.5 ± 1.4	68.8 ± 1.2
Gonad weight (g)	^{R} 0.036 ± 0.5	^{R} 0.386 ± 0.7	^{R} 0.388 ± 0.27	^{R} 0.743 ± 0.7	^{R} 4.75 ± 0.12	^{R} 9.21 ± 1.5
	^{L} 0.029 ± 0.5	^{L} 0.340 ± 0,7	^{L} 0.335 ± 0.10	^{L} 0.482 ± 0.5	^{L} 4.35 ± 0.9	^{L} 9.10 ± 0.9
Gonad length (cm)	^{R} 0.40 ± 0.47	^{R} 0.68 ± 0.6	^{R} 3.15 ± 1.2	^{R} 3.75 ± 1.3	^{R} 4.75 ± 2.0	^{R} 5.9 ± 2.4
	^{L} 0.30 ± 0.44	^{L} 0.60 ± 0.5	^{L} 3.20 ± 1.3	^{L} 3.40 ± 1.3	^{L} 4.30 ± 2.3	^{L} 6.20 ± 2.5

ND = Sex not determined; R = Right; L = Left.

Males and females fishes, of all ages, presented similar long shaped paired gonads, in the right and left sides of the abdominal cavity. The testis of the 4 four males were tubular shape, pale, characterizing immature gonads, presenting little blood irrigation without fringes, which is commonly found in the Siluriforms. Histologically, the testis was covered by the albuginea tunic and presented seminiferous tubules with closed lumen, with ridges forming the spermatic duct connected to the urogenital papilla. The observation of the germ cells indicated the presence of primary spermatogonia and spermatocytes (Figure 1). No sperm was found, in this hybrid, that presented the initial stage of testicle formation, probably due to their young age (9 months old), but there were evidences of cells in preparation for its formation.

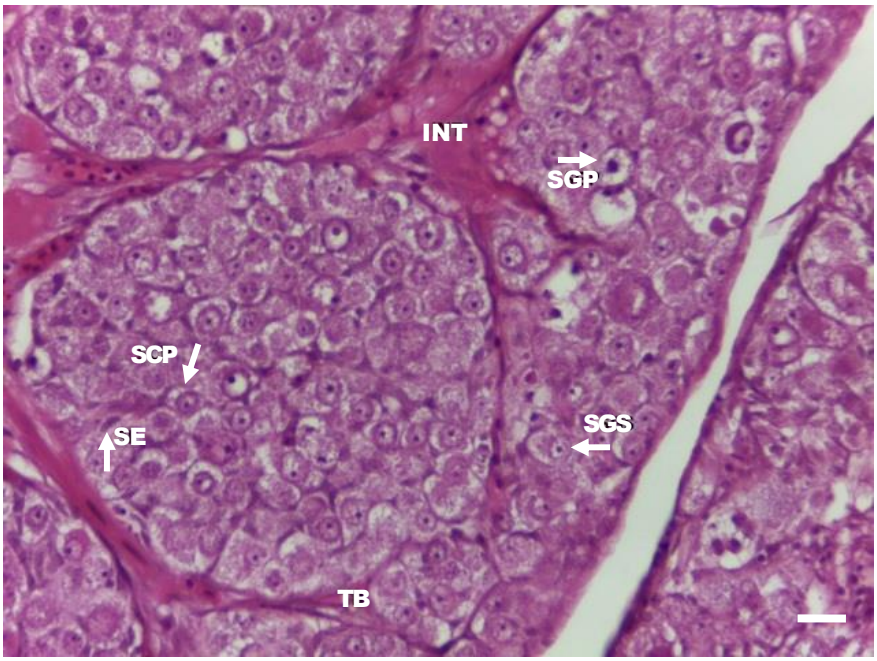

Figure 1. Testis of 9 months old hybrid *Pseudoplatystoma punctifer* (female) x *Leiarius marmoratus* (male). Primary spermatogonies (SGP); secondary spermatogonies (SGS); primary spermatocytes (SCP); Sertoli cells (SE); circulated seminiferous tubule (TB); interstitial space (INT). Hematoxiline/eosine stain; bar = 5 µm.

The ovaries of fish with different ages (9, 16 and 36 months) presented similar characteristics, except for size differentiation. Nine and sixteen months old fish ovaries presented translucid reddish color indicating the initial maturation stage. The 36 months old fish presented thicken

ovaries with lighted color, indicating the presence of oocytes.

Histologically, ovaries are covered by albuginea tunic formed by connective tissue, smooth muscular fibers and blood vessels. This tunic projects ridges to the strome, forming ovigerous lamellae where oocytes of different stages are attached. Ovaries are cistovarian type considering the connection of the lumen to the oviduct or ovarian duct through which the oocytes reach the urogenital papilla and the external environment.

Juvenile females presented chromatine nucleolus oocytes, but perinuclear and cortical alveoli oocytes in small quantity (Figures 2, 3 and 4). Adult females showed chromatin-nucleolus oocytes, perinuclear, cortical alveoli, vitellogenic and mature oocytes. Also, micropyle was observed in several mature oocytes (Figure 5).

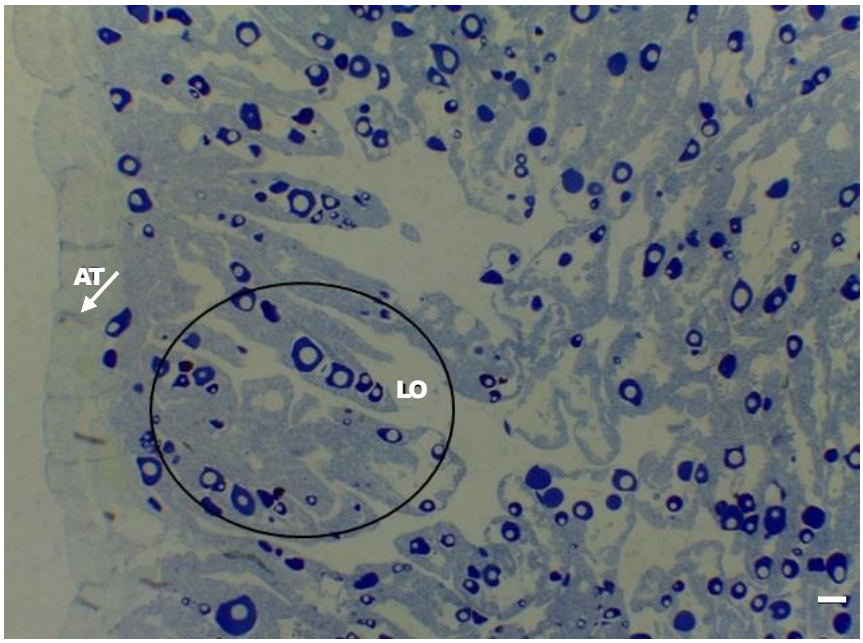

Figure 2. Ovary (9 months) of fingerling hybrid *Pseudoplatystoma punctifer* (female) x *Leiarius marmoratus* (male): general view. Albugine tunic (AT); circulated ovigerous lamellae (LO). Toluidine blue stain; bar = 10 µm.

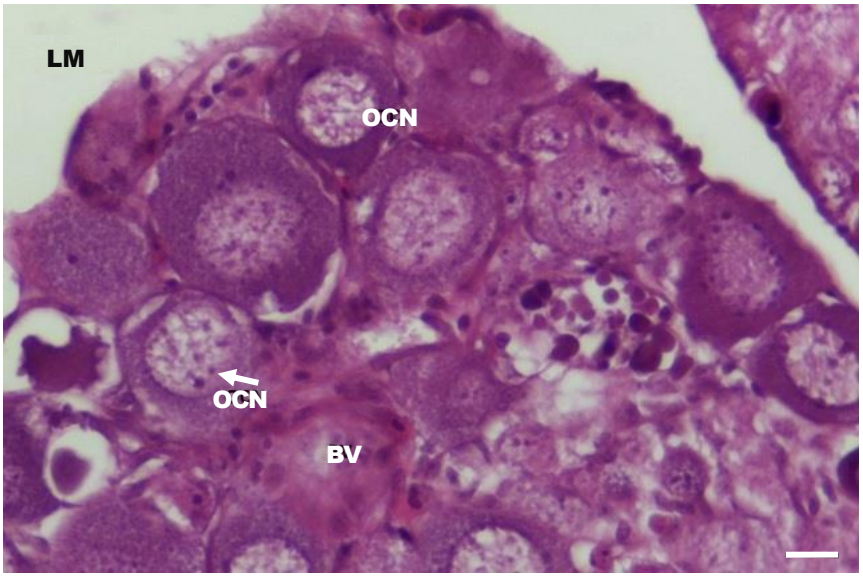

Figure 3. Ovary of 9 months old hybrid *Pseudoplatystoma punctifer* (female) x *Leiarius marmoratus* (male). Chromatine nucleole oocyte (OCN); lumen (LM); blood vessels (BV). Hematoxiline/eosine stain; bar = 20 µm.

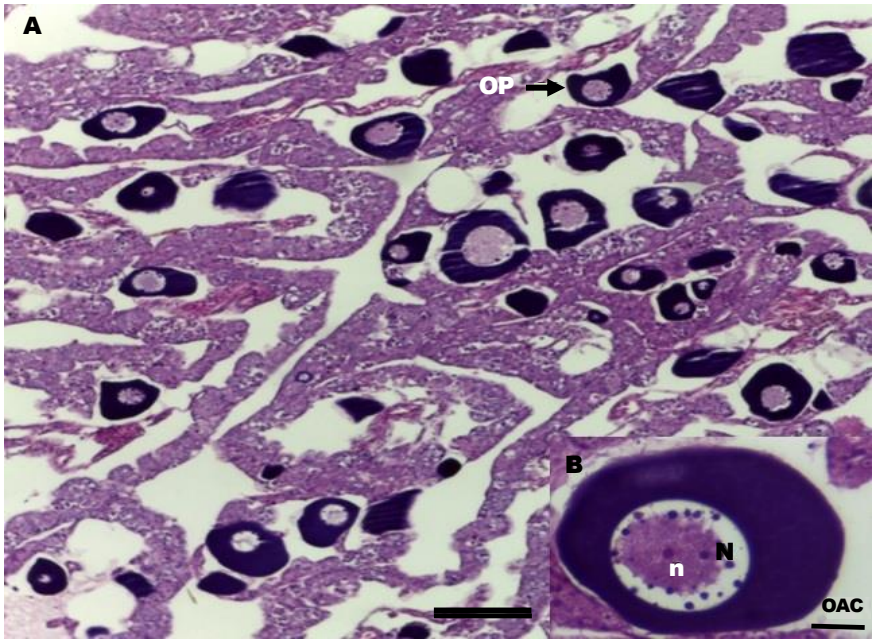

Figure 4. Ovary of 16 months old hybrid *Pseudoplatystoma punctifer* (female) x *Leiarius marmoratus* (male). (A) perinuclear oocytes (OP); bar = 40 µm. (B) showing cortical alveoli oocyte in initial stage (OAC), n=nucleus, N= nucleole. Hematoxiline/eosine stain; bar = 20 µm.

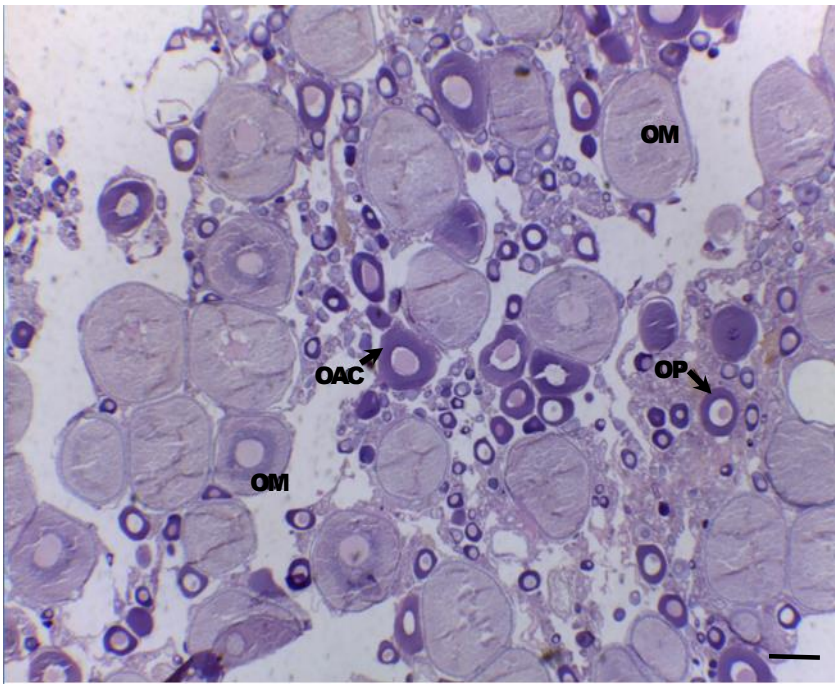

Figure 5. Ovary of 36 months old hybrid *Pseudoplatystoma punctifer* (female) x *Leiarius marmoratus* (male): general view. Perinuclear oocytes (OP); cortical alveole oocyte (OAC); mature oocyte (OM). Hematoxiline/eosine stain; bar = 20 µm.

It was observed a variation in the number of different cell types during observation of the germ cells, as presented in Table 2. The GSI presented low index in all stages of development and did not differ among sex or ages (Table 3).

Table 2. Oocytes based on the occurrence of different development stages of hybrid *Pseudoplatystoma punctifer* (female) *x Leiarius marmoratus* (male).

Oocyte stage*	Age				
	7 days	14 days	9 months	16 months	36 months
1°	-	-	+	+	++
2°	-	-	+	+	+
3°	-	-	+	+	+
4°	-	-	-	-	+
5°	-	-	-	-	+
6°	-	-	-	-	-

** 1° = oogonium; 2° = chromatine nucleolus oocyte; 3° = perinuclear oocyte; 4° = cortical alveole oocyte; 5° = mature oocyte; 6° = atretic follicle. (-) absent; (+) present; (++) frequent; (+++) abundant.*

Table 3. Gonadossomatic index of hybrid *Pseudoplatystoma punctifer* (female) x *Leiarius marmoratus* (male).

	Age					
	7 days	14 days	9 months		6 months	36 months
Sex	ND	ND	Female	Males	Female	Female
GSI %	0.0014	0.041	0.039	0.064	0.135	0.345

ND = Sex not determined; GSI = Gonadossomatic index.

DISCUSSION

The absence of sexual dimorphism is common in fish. The external characteristics that may indicate sexual maturity or sex identification in fishes are frequently not evident, especially before the final maturation. Traditional sex identification and maturation is done by visual evaluation of the abdomen swelling and flaccidity and reddishness of the urogenital papilla (CREPALDI *et al*., 2006a).

Body weight and length may differentiate the fish on the first and second maturation and adult hybrid females present the same size as of female *P. punctifer* in the first maturation, according to LUCA (2010), which corroborate previous results found by RESENDE *et al*. (1995). On the contrary, ROMAGOSA *et al.* (2003a) found that farm raised *Pseudoplatystoma fasciatum* females reached first maturation at 37.65 cm in length. CREPALDI *et al*. (2006a) questions the use of these parameters because gonad maturation depends on other factors, such as food availability and environmental conditions in order to the fish to reach early or late maturation.

The hybrid presented similar characteristics of their parental species when comparing the results of anatomical disposition of the reproductive organs (CREPALDI et al., 2006b; CHAVES, 2011).

According to LUCA (2010), *P. punctifer* presents spermatocytes distributed along the tubular testis. Siluriforms testis also presents fringers throughout its length with different characteristics according to fish life cycle according to ANDRADE (2007) and BRITO (2003) during the analysis of *P. corruscans* and by LUCA (2010) in *P. punctifer*.

The ovaries examined in this study were similar to those observed in most teleosts. Histological analysis perfomed on the sample gonads allowed the clear identification of five developmental stages of the oocytes (young cells, chromatine nucleolus oocyte, perinuclear oocyte, cortical alveole oocyte, mature oocyte). Terminologies and features used to differentiate and identify the distinct stages of an oocyte formation may vary according to the authors and species studied (ABASCAL and MEDINA, 2005).

Average GSI varies from 15% to 19% according to the stage of maturation, especially in the more advanced stages as found in *P. fasciatum* (ROMAGOSA *et al*., 2003a). GSI also assist in determining gonadal stage of maturation and

higher values indicate high breeding activity (ROMAGOSA *et al*., 2003b). Mean values of the juveniles and adults of *P. punctifer* (female) x *L. marmoratus* (male) indicate that these fish were in breeding recess period. Human-mediated hybridization is a leading cause of biodiversity loss worldwide. How hybridization affects fitness and what level of hybridization is permissible pose difficult conservation questions with little empirical information to guide policy and management decisions (MUHLFELD *et al*., 2009). If used without proper monitoring and management, interspecific hybridization and introgression may impose serious threats to the aquaculture industry (HASHIMOTO *et al*., 2011).

CONCLUSIONS

Juvenile male and female of hybrid *P. punctifer* x *L. marmoratus* (9 and 16-month old) are in the initial stage of ovary and testis development and maturation, containing different types of germ cells.

Adult females (36-month old) presented cells of various stages, such as, chromatin-nucleolus oocytes, perinuclear oocytes, cortical alveole oocytes, vitelogenic oocytes and mature oocytes. The presence of female and male germ cells in different stages in the histological evaluation indicates that these fish are probably fertile.

New experimental studies must be carried to the knowledge of the reproductive biology of this hybrid to assess the potential environmental impact, because it can be fertile and thus can influence the genetic trait of the parental wild, where fertilization occurs.

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