Dynamics and cytochemistry of oogenesis in *Leporinus striatus* Kner (Teleostei, Characiformes, Anostomidae) from the Rio Sapucaí, Minas Gerais State, Brazil

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ABSTRACT. Oogenesis involves a sequense of transformations which are undergone by female germ cells. These cells change into oogonias and then into mature oocytes. Sexually mature females were collected monthly, during one year, from the Rio Sapucaí, tributary of the Rio Grande, which is part of the Furnas Reservoir system in the state of Minas Gerais. The observed material showed that oogonias were small spherical cells, had a big spherical nucleus, with a single nucleolus, and weakly stained cytoplasm with eosinophilic granules (FG stained), which indicate their protein content. The primary oocytes showed a big basophilic nucleus, with a large peripheral nucleolus, and several smaller nucleoli. They show a reduced cytoplasmic content. The previtellogenic oocytes presented voluminous cytoplasm and nucleus with several small peripheral nucleoli. The oocytes underwent vitellogenesis with the development of the zona radiata and the follicle cells. The zona radiata had two layers, the outer and the inner, which showed its protein content when stained with CM and FG techniques. TB pH 2.5 and pH 4.0 staining showed that oocytes undergoing vitellogenesis presented weakly stained cytoplasm and peripheral cytoplasmic vesicles. The follicle cells that were squamous became cuboidal. In mature oocytes, the yolk granules that filled the cytoplasm became green and blue when stained with FG and CM techniques, indicating their protein content. The perivitelline region showed rosy stained vesicles (TB pH 2.5 and pH 4.0) spread among the weakly stained peripheral vesicles, which seemed to be the cortical alveoli. The zona radiata cells, CM and FG stained, still showed two layers like the oocytes from the previous stage, but thicker.

KEY WORDS. Teleostei, Anostomidae, *Leporinus striatus*, oogenesis, cytochemical

Anostomidae are herbivorous fishes, of small and medium size, very appreciated as food and in game fishing (BRITSKI 1972), and usually found in neotropical rivers. Small size species, such as *Leporinus striatus* Kner, 1859, are also found in reservoirs, where they form the basis of the food chain (NOMURA 1984).

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Fish reproduction is generally cyclic, seasonal and influenced by environmental conditions (NAGAHAMA 1983). The female gametogenesis, due to the different morphological characteristics shown by the germ cells, from oogonias to mature oocytes, is used as a parameter to determine the time of the year in which they reproduce (WEST 1990). Therefore, describing cell types and, mainly, the chemical and morphological characteristics of oocyte structures, such as cortical alveoli, yolk globule and zona radiata, as well as the characteristics of follicle cells, is the starting point in the study of the reproductive biology of fish (GURAYA 1986).

Within this context, the purpose of the present work was to study the dynamics and cytochemistry of oogenesis in *Leporinus striatus* Kner, 1859, at the Rio Sapucaí, Furnas Reservoir, Minas Gerais State, Brazil.

MATERIAL AND METHODS

Leporinu striatus specimens were collected monthly during one year, from August 1998 to July 1999, in the Rio Sapucaí, a tributary of the Rio Grande, Furnas Reservoir, Alfenas, Minas Gerais, Brazil (latitude, 21°25'45"; longitude 45°56'50"; average altitude, 849,2 m). A set of gill nets (10 meters long, 1.5 m high, 1.5 cm mesh size) were exposed for a 10-15 hours period.

A total of 72 sexually mature females, removed alive from the nets, were taken to the laboratory, where they had their cellomic cavity opened and gonads removed, sectioned and fixed overnight in KARNOVSKY (1964).

The fixed material was embedded in historesin, and cut on a microtome equipped with glass knife. The 3-µm sections were differently stained with: hematoxylin-eosin (HE), for general morphological analysis; Toluidin blue pH 4.0 (TB pH 4.0), for the detection of acid polysaccharides and phosphated cellular components (DNA and RNA); Toluidin blue pH 2.5 (TB pH 2.5), for the detection of sulphated acid polysaccharides; Coello's Method (CM) (COELLO 1989), for the detection of neutral polysaccharides and proteins; and Fast Green pH 2.5 (FG), for the detection of total proteins. The slides thus prepared were observed, analyzed and photographed under an Olympus BX 50 photomicroscope.

RESULTS AND DISCUSSION

Germ cells at different stages of development were observed in the ovaries of the sexually mature specimens of *L. striatus*.

Their oogonias, similarly to teleosts in general (DE VLAMING 1983; VAZZO-LER 1996; SILVA *et al.* 1998), were found grouped in nests and inserted in ovarian lamellae. These were small cells with a big, round and basophilic nucleus (Fig. 1) that underwent meiosis and thus originated the primary oocytes, which were enveloped by a layer of follicle cells forming the follicles. These primary oocytes left the nests and started to develop (Fig. 1).

The oocyte maturation in fishes is a continuous process. However, it can be classified in stages that vary according to the criteria used. BAZZOLI & GODINHO (1995) and VAZZOLER (1996) described six stages in this process, which can also be applied to *L. striatus*.
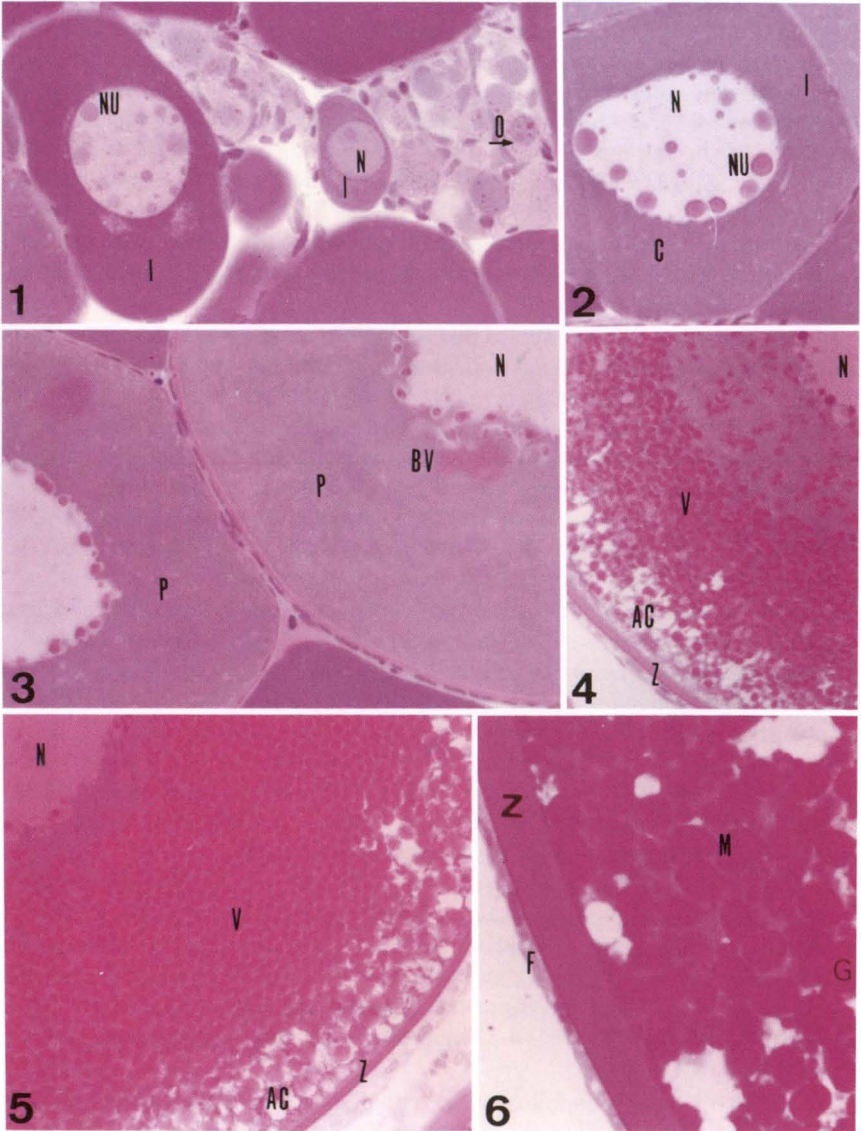
Figs 1-6. (1) oogonia (O), primary oocyte (I), nucleoli (NU), HE; (2) primary oocyte (I), cytoplasm (C), nucleus (N), nucleoli (NU), HE; (3) previtellogenic oocyte (P).nucleus (N), Balbiani's vitelline body (BV) HE; (4) vitellogenic oocyte (V), nucleus (N), cortical alveoli (AC), zona radiata (Z), HE; (5) vitellogenic oocyte (V),nucleus (N), cortical alveoli (AC), zona radiata (Z), HE; (6) mature oocyte (M), yolk globules (G), zona radiata (Z), follicle cells (F), HE.

As observed in the majority of teleosts (VAZZOLER 1996), we found that, in *L. striatus*, the primary oocytes show reduced cytoplasm, a big, central and basophilic nucleus and a central nucleolus (Fig. 1). These oocytes grow, increase significantly in volume, and show a decreased nucleus-cytoplasm relationship. Within them, several small and big nucleoli appear and start to migrate to the periphery of the nucleus (Fig. 2). These findings agree with those reported by WALLACE & SELMAN (1981), NAGAHAMA (1983), DE VLAMING (1983), SELMAN & WALLACE (1989), BAZZOLI & RIZZO (1990) and RIZZO & BAZZOLI (1993) about this type of cell. The increased number of nucleoli found in primary oocytes is likeky to be related to the rise of RNA synthesis activity, necessary to their posterior maturation progress.

In our study, it was observed that previtellogenic oocytes had a nucleus with several small and peripherical nucleoli, basophilic cytoplasm and showed the formation of Balbiani's vitelline body (Fig. 3). According to DE VLAMING (1983), such structure is a complex composed of cytoplasmic organelles, including mitochondria, multivesicular bodies, endoplasmatic reticulum, Golgi apparatus and fibrogranular material. Similarly to what was observed in previtellogenic oocytes of other teleosts (YAMAMOTO & YOSHIOKA 1964; ANDERSON 1968; BEAMS & KESSEL *apud* WALLACE & SELMAN 1981; GURAYA 1986; TYLER & SUMPTER 1996; VAZZOLER 1996), this same structure is formed in the cytoplasm of *L. striatus* (Fig. 3).

We observed that *L. striatus* began vitellogenesis as clear vesicles appeared in the peripherical cytoplasm, the vitellogenic oocytes shown in figure 4. Generally, the endogen vitellogenesis preceed the exogen, with superposition only in some cases. During the endogen vitellogenesis the yolk vesicles are the first structure that appear in cytoplasm and then reunite on oocyte perifery to form the cortical alveoli (WALLACE & SELMAN 1981; HIBIYA 1983; N*AGAHAMA* 1983; DE VLAMING 1983; ZANUY 1987; SELMAN & WALLACE 1989; SUMPTER *et al.* 1984; TYLER & SUMPTER 1996). According to BAZZOLI & GODINHO (1995), the Balbiani's vitelline body takes part in the formation of the cortical alveoli in teleosts.

In *L. striatus*, the nucleus of oocytes in primary vitellogenesis showed irregular outline, and contained several peripherical and round nucleoli, making evident its intense activity of synthesis (Fig. 4). Still at this stage, we observed the development of zona radiata, a continuous acidophilic pellicle, with two acellular layers, attached to the oocyte plasmatic membrane. The concomitant onset of zona radiata and cortical alveoli (Figs 4-5) might be a characteristic of this stage of oocyte development (WEST 1990).

The yolk deposition followed the formation of zona radiata and the onset of cortical alveoli. The vitellus, as observed in *L. striatus*, initially appeared in the cortical cytoplasm, and moved centerwise causing an increase in the oocyte volume (Figs 4-5). According to BAZZOLI & RIZZO (1990), these oocytes are called vitellogenic. The mature oocytes of *L. striatus* showed a contracted nucleus with small (Fig. 7), round and peripherical nucleoli and loss of cytoplasmatic basophilia. They became significantly bigger due to the rapid increase of the number of acidophilic yolk globules (Fig. 6). The zona radiata became thicker and a funnel-like depression was formed, the micropyle (Fig. 12). The initially squamous follicle
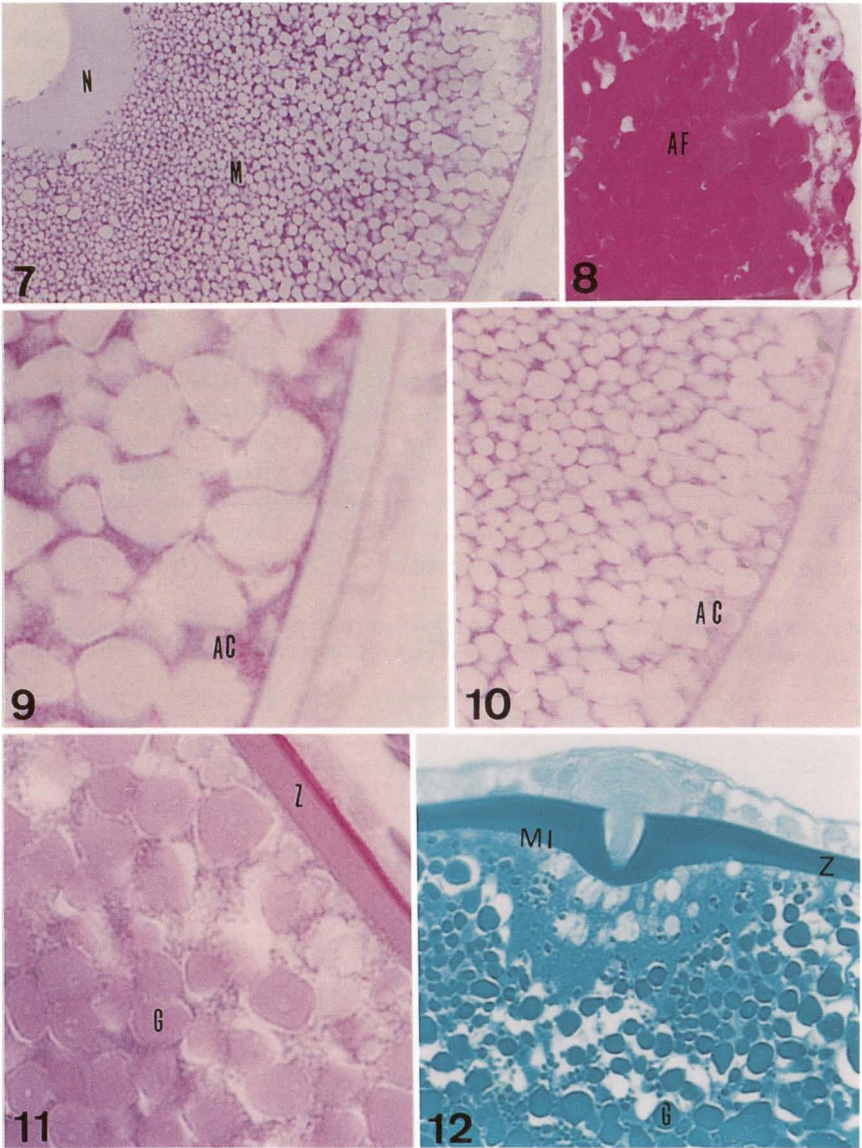
Figs 7-12. (7) mature oocyte (M), nucleus (N), AT 4.0; (8) atresic follicle (AF), HE; (9) cortical alveoli (AC), AT 4.0; (10) cortical alveoli (AC), AT 2.5; (11) yolk globules (G), zona radiata (Z), MC; (12) mycropile (MI), yolk globules (G), zona radiata (Z), FG.

cells, became cuboidal (Fig. 6). The follicle epithelium is also cuboidal in *Serrasalmus brandtii* Reinhardt, 1874, *Acestrorhynchus britiskii* Menezes, 1969 and *Schizodon knerii* (Steindachner, 1875), but it may be squamous in other species of fish (BAZZOLI & RIZZO 1990). In *Prochilodus affinis* Reinhardt, 1874, the squamous

follicle epithelium appears enveloped by a thin layer of conjunctive tissue, the theca (RIZZO & BAZZOLI 1993). According to VAZZOLER (1996), in freshwater fishes these oocytes are ready to be eliminated from the follicle.

In this work, atresic follicles were occasionally observed in the ovaries of *L. striatus*. These structures resulted from non-ovulated mature oocytes, in which there was loss of cellular turgescence and rupture of zona radiata. Yolk globules, when present, lost their individuality becoming amorphous masses of acidophilic substances with disintegrated nuclei (Fig. 8). According to NAGAHAMA (1983), DE VLAMING (1983) and TYLER & SUMPTER (1996), numerous follicle atresia may arise from environmental stress.

The cytochemical analysis (Tab. I) of *L. striatus* oocytes revealed positive response to TB pH 4.0 and TB pH 2.5 in cortical alveoli, indicating the presence of sulphated acid polysaccharides (Figs 9-10). In specimens of *Hemmigrammus marginatus* Ellis, 1911, *Moenkhausia costae* (Steindachner, 1907) and *Roeboides xenodon* (Reinhardt, 1851), BAZZOLI *et al.* (1996) observed the presence of neutral glycoproteins in these structures. The cortical alveoli also contain neutral glycoproteins in *Lophiosilurus alexandri* Steindachner, 1876, and carboxylated acid glycoconjugates in *Pseudoplatystoma coruscans* (Spix & Agassiz, 1829) (BAZZOLI & GODINHO 1997).

Table I. Cytochemical reactions in cortical alveoli, yolk globules and zona radiata in *Leporinus striatus*, collected in Rio Sapucaí, tributary of the Rio Grande, Furnas Reservoir, Mina Gerais, Brazil.

Cytochemical reactions	Cortical alveoli	Yolk globules	Zona radiata	
			OL	IL
Coello's method	–	Purplish blue	Magenta	Rosy
Fast Green PH 2.5	–	Green	Green	Green
Toluidin Blue PH 4.0	Rosy	–	–	–
Toluidin Blue pH 2.5	Rosy	–	–	–

(OL) Outer layer, (IL) inner layer, (–) no response.

In *Oligosarcus argenteus* Günther, 1864, NEVES *et al.* (1995), using TB pH 3.0 and 4.0, observed metachromasia in TB pH 3.0 indicating the presence of sulphated and non-sulphated acid polysaccharides in cortical alveoli. In specimens of *Leporinus* Agassiz, 1829, BAZZOLI & GODINHO (1994) observed that these structures contained carboxylated acid glycoconjugates in some species and neutral glycoproteins in others. Cortical alveoli were shown to be sudanophobic in *Leporinus* sp., using Sudan Black B, Sudan III, Oil Red O and Nile blue sulphate (BAZZOLI & GODINHO 1994).

In *Serrasalmus brandtii*, these structures are positive to AB pH 2.5 and AB pH 0.5 indicating the presence of carboxylated and sulphated acid polysaccharides or sulphated groups only. In these species, the cortical alveoli respond negatively to the lipid detection techniques (BAZZOLI & RIZZO 1990).

The yolk globules of vitellogenic oocytes of *L. striatus* respond positively in blue to CM, and also to FG, indicating their protein content (Figs 11-12). BAZZOLI *et al.* (1996) observed the presence of neutral glycoproteins and neutral lipids in the

yolk globule of *H. marginatus*, *M. costae* and *R. xenodon*. In *L. alexandri* and *P. coruscans* the yolk globules, besides containing neutral glycoproteins, are positive to Sudan Black B, Sudan III, Oil Red O and Nile blue sulphate techniques, indicating the presence of neutral lipids (BAZZOLI & GODINHO 1997).

In *L. striatus*, the zona radiata showed 2 layers that reacted differently to the staining used. With CM the outer layer stained magenta and the inner layer stained rosy; with FG, both responded in green, thus indicating their glycoproteinic content (Figs 11-12). BAZZOLI *et al.* (1996) detected neutral glycoproteins in the zona radiata of *H. marginatus*, *M. costae* and *R. xenodon*. In the outer layer of the zona radiata of *M. costae*, they have also detected carboxylated acid glycoconjugates, and in *H. marginatus* glycoproteins rich in sialic acid. The same authors have shown that in *L. alexandri* and *P. coruscans*, the zona radiata is composed by only one layer containing neutral glycoproteins.

The chemical composition of the different oocytes structures and the oogenesis dinamics in *Leporinus striatus* is similar to the ones observed in other freshwater teleosts with external fertilization, according to the idea that the female gametogenesis in Teleostean fishes is linked to the reproductive tactics of the species.

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