

ULTRASTRUCTURAL CHANGES IN FEMALE HEPATOCYTES DURING OVARIAN MATURATION OF *Steindachnerina insculpta* (PISCES: CURIMATIDAE)

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(With 4 figures)

ABSTRACT

During gonadal maturation the females of *Steindachnerina insculpta*, a teleost of the Furnas reservoir, Minas Gerais, Brazil, show low hepatosomatic and high gonadosomatic indexes, suggesting possible transference of hepatic substances to the ovaries. In comparison to the resting phase, in the maturation/mature one the hepatocytes show greater development of both smooth and rough endoplasmic reticula, increased electron density in the mitochondria, and glycogen scattered throughout the cytoplasm. In both resting and maturation/mature phases, the hepatic parenchyma contains melanomacrophagic centers that consist of degenerating cellular debris and are surrounded by capsules formed by cells, resembling fibroblasts, joined by desmosomes. No physiological relation of these centers with ovarian maturation was found.

Keywords: teleost fish, ovarian maturation, hepatocytes.

RESUMO

Mudanças ultra-estruturais nos hepatócitos de fêmeas de *Steindachnerina insculpta* (Pisces: Curimatidae) durante a maturação ovariana

Fêmeas de *Steindachnerina insculpta*, um teleósteo do reservatório de Furnas, Minas Gerais, Brasil, apresentaram, durante a maturação gonadal, um baixo índice hepatossomático e um alto índice gonadosomático, sugerindo que substâncias hepáticas são provavelmente transferidas para os ovários. Comparando-se fêmeas no estágio de repouso com aquelas no estágio maturação/maduro, observaram-se nos hepatócitos de fêmeas no estágio maturação/maduro maior desenvolvimento do retículo endoplasmático rugoso e liso, mitocôndrias com densidade eletrônica aumentada e glicogênio espalhado por todo citoplasma. No estágio de repouso, assim como no estágio de maturação/maduro, observaram-se, no parênquima hepático, centros melanomacrofágicos constituídos de restos celulares em degeneração delimitados por cápsula contendo células semelhantes a fibroblastos unidas por desmosomas. Não foram observadas relações fisiológicas entre os centros melanomacrofágicos e a maturação dos ovários.

Palavras-chave: peixe-teleósteo, maturação ovariana, hepatócitos.

INTRODUCTION

In non-mammalian vertebrates, the vitellogenesis stage is important because in it oocytes accumulate yolk in the form of globules. These contain vitellogenin, a phospholipo-glyco-protein secreted by the hepatocytes, which is then

incorporated into the oocytes by micropinocytosis, via the blood stream (Selman & Wallace, 1989). In recent years, data has increased substantially on vitellogenesis regulation and mechanisms, *i.e.* multiplicity of vitellogenin (Vg) genes, the oocyte Vg receptor, Vg processing enzymes and their

specific yolk products, and synthesis of various vitelline envelope (VE) precursor proteins in the liver and ovary (Patiño & Sullivan, 2002).

Vitellogenesis is regulated by estrogens produced by theca and stromal cells in response to gonadotrophins released by the pituitary gland (Van de Hurk & Peute, 1979). During the later stages of vitellogenesis, large amounts of yolk material, requiring fat and protein from dietary and somatic sources, are absorbed by the oocytes (Bohemen *et al.*, 1981). Many fish stop feeding during the gonadal maturation period that precedes spawning and leads to a loss in total body and hepatic weights (Tveranger, 1985). Therefore, gonadosomatic (GSI) and hepatossomatic (HSI) indexes are accurate parameters for determining reproductive periodicity (Maddock & Burton, 1999). In addition, differences in the distribution and degree of proliferation of rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), and mitochondria in the hepocytes may be related to oogenesis during ovarian maturation (Peute *et al.*, 1978; Bohemen *et al.*, 1981; Gillis *et al.*, 1990).

This work studies the morphological changes in hepatocytes during the resting and maturation/mature phases of the reproductive cycle of *Steindachnerina insculpta*, a small detritivorous fish species from Furnas Reservoir, the Grande River, Minas Gerais, Brazil.

MATERIAL AND METHODS

Specimens

For biometric analyses, two hundred and seventy-two adult females (ninety-one in the resting phase and one hundred eighty-one in the maturation/mature phase) were captured in the Furnas Reservoir (20° 40' S and 46° 19' W) between September 1992 and August 1994. All specimens, which were captured using gill nets, were fixed in 10% formaldehyde.

Gonadosomatic and hepatossomatic indexes

After dissection, gender identification, macroscopic determination of the reproductive cycle phase, the following data were recorded for each specimen: standard length (SL), body weight (BW), and that of the gonads (GW) and liver (LW). The biometric data obtained were used for calculating

the gonadosomatic ($GSI = GW/BW \times 100$) and hepatossomatic ($HSI = LW/BW \times 100$) indexes.

Light microscopy

For histological analysis of morphological changes in the hepatocytes, liver fragments of ten adult females in the resting phase and ten females in the maturation/mature one were collected. Specimens were fixed in Bouin's fluid for 12 h, embedded in glycol metacrylate plastic resin, sectioned (3-5 μ m), and stained with hematoxylin-eosin or toluidin blue-sodium borate. To determine the content of hepatocyte glycogen and lipids, the periodic -acid Schiff (PAS) and Sudan black techniques were used, respectively. Reproductive-cycle phases were confirmed histologically.

Transmission electron microscopy

The liver fragments were placed Karnovsky fixative with 0.1 M cacodylate buffer, pH 7.2-7.4, at 4 °C, for approximately 12 h; post-fixed in 1% osmium tetroxide in the same buffer for 2 h; dehydrated; and embedded in Epon 812. Ultra-thin sections were stained with 1% uranyl acetate and lead citrate, and observed in an electron microscope.

Statistical analysis

A student t test was performed to detect variations in the mean values of the gonadosomatic and hepatossomatic indexes calculated for resting and maturation/mature reproductive cycle phases.

RESULTS

Gonadosomatic and hepatossomatic indexes

Mean GSI increased from the resting (3.37 ± 2.80) to maturation/mature (13.17 ± 5.09) phase, while the mean HSI for females decreased from the resting (0.54 ± 0.32) to maturation/mature phase (0.37 ± 0.24). The GSI and HSI mean values were statistically different ($p < 0.05$) between resting and mature phases.

Histological analysis of hepatocytes

The liver of *S. insculpta* shows hepatocytes arranged in rows intercalated with sinusoidal capillaries. Each hepatocyte contains a large, round, centrally situated nucleus with a prominent nucleolus. The hepatic parenchyma of females in the resting and maturation/mature phases showed circular structures consisting of amorphous

material, surrounded by a thin connective capsule - the melanomacrophagic centers.

Ultrastructural analysis of hepatocytes

Female hepatocytes in the resting and maturation/mature phases showed morphological

variations in rough and smooth endoplasmic reticula, mitochondria, and glycogen (Table 1, Figs. 1-2). Degenerating cellular debris surrounded by fibroblast-like cells joined by desmosomes were seen in the melanomacrophagic centers (Figs. 3-4).

TABLE 1
Main ultrastructural differences between hepatocytes during the resting and maturation/mature phases of *S. insculpta* females captured in Furnas Reservoir.

Organelles/inclusions	Ultrastructural Characteristics of Hepatocytes	
	Resting	Maturation/ Mature
RER	Scarce, dispersed cisterns	Abundant, parallel cisterns
SER	Poorly developed	Abundant anastomosed networks of short tubules
Glycogen	Accumulated in peripheral areas of the cytoplasm	Scattered throughout the cytoplasm
Mitochondria	Abundant	Abundant, with increased electron density

RER = rough endoplasmic reticulum; and SER = smooth endoplasmic reticulum.

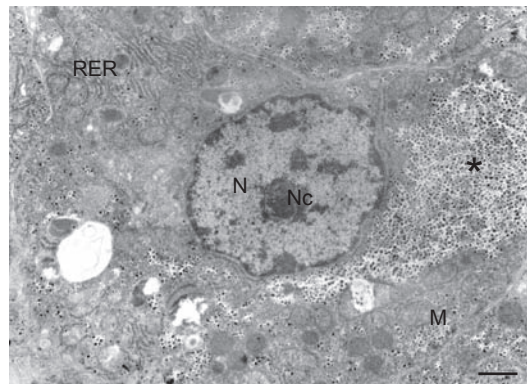


Fig. 1 — Hepatocyte of female in resting phase: rough endoplasmic reticulum (RER) with scarce, dispersed cisterns, accumulated glycogen (asterisk) in peripheral regions of the cytoplasm, and abundant mitochondria (M). N = nucleus; Nc = nucleolus. Bar = 1.0 μ m.

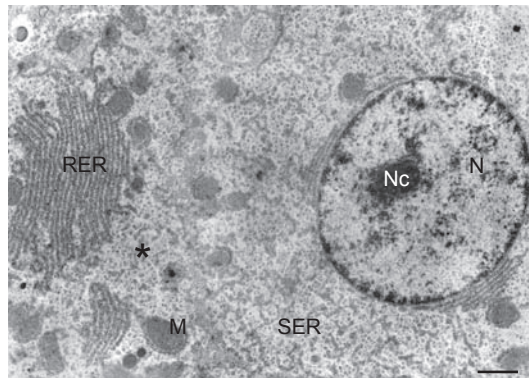


Fig. 2 — Hepatocyte of female in maturation/mature phase: rough endoplasmic reticulum (RER) with parallel cisterns, smooth endoplasmic reticulum (SER) constituted of anastomosed network of short tubules, glycogen (asterisk) dispersed throughout the cytoplasm, and mitochondria (M) with increased electron density. N = nucleus; Nc = nucleolus. Bar = 1.0 μ m.

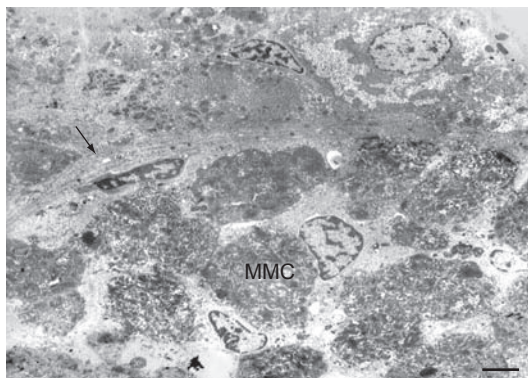


Fig. 3 — Melanomacrophagic center (MMC) containing amorphous, heterogeneous, electron dense bodies and nuclei of cells. Arrow = capsule. Bar = 3.0 μm .

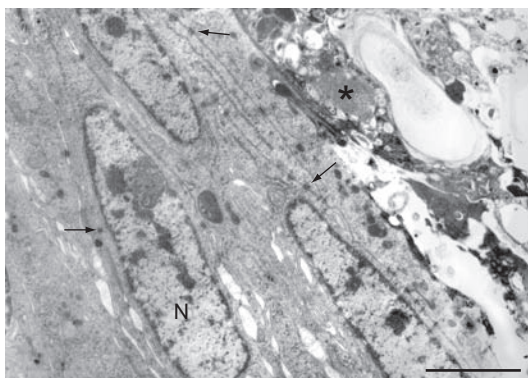


Fig. 4 — Detail of capsule of the melanomacrophagic center, with cells, resembling fibroblasts, joined by desmosomes (arrow). Nucleus (N); amorphous material (asterisk). Bar = 1.0 μm .

DISCUSSION

The GSI and HSI of *S. insculpta* showed an inverse relation throughout the reproductive cycle, similarly to what was reported by Yoneda *et al.* (1998). The HSI reached higher values in the resting phase and decreased abruptly during the maturation/mature one, at which time the GSI became higher, indicating decreased hepatic reserve reserves during ovarian maturation. This is known to occur as a result of depleted storage of hepatocyte glycogen and lipids (Svedäng & Wickström, 1997), and also because of vitellogenin synthesis and transfer to the oocytes (Luzzana *et al.*, 1996). The HSI may also vary between species of teleosts, due to the differences in energy storage capacity, physiological condition, feeding habits, and nutrient availability (Svedäng & Wickström, 1997). In the mature stage, female hepatocytes of *S. insculpta* showed RERs with proliferated

cisterns arranged in parallel stacks, observed also by Bohemen *et al.* (1981) and Gillis *et al.* (1990). According to Peute *et al.* (1978), rapid proliferation of rough endoplasmic reticula results from increasing vitellogenin production. Bohemen *et al.* (1981), however, detected no vitellogenin in the liver, even in the presence of high HSI values, suggesting that this protein is released into the blood stream immediately after its synthesis by the hepatocytes. Therefore, increasing liver weight may be related to the storage of non-proteic substances and/or to the development of the organelles in these cells during vitellogenesis (Bohemen *et al.*, 1981). The smooth endoplasmic reticula of the hepatocytes in the mature phase was proliferated, forming an extensive, anastomosed network of short tubules, which according to Leatherland & Sonstegard (1988), is because the teleost liver is involved in metabolizing and converting sex hormones. Kime (1978) and Schulz (1986) showed

teleost liver to be capable of converting cortisol into androgens, possibly signifying a role of the liver in normal steroidogenesis of gonadal hormones. In this study, similarly to what was reported by Peute *et al.* (1978), glycogen granules were grouped in peripheral cytoplasm of the hepatocytes during the resting phase, and dispersed throughout the cytoplasm during the maturation/mature phase.

Hepatocytes of *S. insculpta* females in the mature phase showed mitochondria a highly electron-dense matrix, as described by Peute *et al.* (1978) for *Brachydanio rerio*. The authors suggest that density of the mitochondria matrix during this stage results from increasing energy demand and metabolic rate related to the use of triglyceride-derived free fatty acids as the oxidative substrate.

In teleosts, melanomacrophagic centers varying in number, size, and content are found in the spleen, kidneys, and liver (Herraez & Zapata, 1991). Vogelbein *et al.* (1987) described these centers as a special type of granuloma formed by an accumulation of monocytes drawn from peripheral circulation. Meseguer *et al.* (1994) observed macrophage mobility in the hepatic parenchyma and involvement of these cells in melanomacrophagic center formation. The presence of these centers in hemocytopoietic tissue is related to health, nutritional state, age, and stress, accounting for the suggested use of these structures as environmental-quality bio-indicators (Agius & Roberts, 1981). In this study, melanomacrophagic centers located near blood vessels and containing heterogeneous pigments, similar to those observed in *Carassius auratus* by Herraez & Zapata (1991), were seen in the hepatic parenchyma during both resting and maturation/mature phases. As reported by Gonzalez *et al.* (1993) and observed in the present work, those centers did not affect the hepatic physiology related to vitellogenin synthesis, since ovarian maturation occurred normally. In *S. insculpta*, the melanomacrophagic centers evolved to granulomas, a finding corroborated by the findings of Vogelbein *et al.* (1987) and Meseguer *et al.* (1994).

The first account of hepatocyte involvement in vitellogenesis, as shown by ultrastructural features of a neotropical teleost species, was carried out in the present work. However, further molecular studies must be conducted to quantify vitellogenin content throughout the reproductive cycle of Brazilian teleosts.

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