FOLLICULAR ATRESIA IN CURIMATÁ-PIOA *PROCHILODUS AFFINIS* REINHARDT, 1874 (PISCES, CHARACIFORMES)

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(With 2 figures)

RESUMO

Atresia Folicular em Curimatá-pioa *Prochilodus affinis* Reinhardt, 1874 (Pisces, Characiformes)

Os eventos morfológicos da atresia folicular foram descritos em folículos pré-vitelogênicos e vitelogênicos de *Prochilodus affinis* criados em cativeiro na Estação de Hidrobiologia e Piscicultura de Três Marias, Minas Gerais, Brasil. Fêmeas não desovadas de curimatá-pioa apresentaram ovários atrésicos durante 5 meses, de março a julho/1986/87. O processo de regressão ovariana foi caracterizado em fase inicial, avançada e final. Atresia folicular foi observada principalmente os ovócitos vitelogênicos. Folículos pré-vitelogênicos em degeneração foram observados em algumas fêmeas capturadas em julho, após reabsorção da maior parte dos folículos vitelogênicos. Este longo período de regressão pode afetar a reprodução artificial da espécie, uma vez que processos degenerativos nos ovários reduzem a taxa de fertilização.

Palavras-chave: *Prochilodus affinis*, curimatá-pioa, teleósteo, atresia folicular, cativeiro.

ABSTRACT

The morphological events of follicular atresia were described in the previtellogenic and vitellogenic atretic follicles of *Prochilodus affinis* raised in captivity at the Três Marias Fishery Station, Minas Gerais, Brazil. Unspawned females of curimatá-pioa showed atretic ovaries during 5 months, from March to July/1986/87. The process of ovarian regression was characterized in initial, advanced and late phases. Follicular atresia was observed mainly in vitellogenic oocytes. Previtellogenic follicles in degeneration were observed in some females captured in July, after reabsorption of most vitellogenic follicles. This long regression period can affect the artificial reproduction of the species since degenerative process in ovaries reduce the rate of fertilization.

Key words: *Prochilodus affinis*, curimatá-pioa, teleost, follicular atresia, captivity.

INTRODUCTION

Follicular atresia is a degenerative process which occurs in vertebrate ovaries under natural and experimental conditions, leading to the reab-

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sorption of the oocyte (Saidapur, 1978). In teleosts, it is common during prespawning, spawning and postspawning (Guraya, 1986). Several factors such as stress, starvation, biocides agents, light, temperature, inadequate hormonal levels and/or blood supply induce atresia (Nagahama, 1983; Guraya, 1986). Fishes kept in captivity show more atretic follicles in their ovaries than those obtained from a natural environment (Guraya *et al.*, 1975). The morphological aspects of follicular atresia were described in some teleost species (Rai, 1966; Rastogi, 1969; Srivastava, 1969; Babu and Nair, 1983). Studies with Brazilian native species kept in confinement are scarce (Romagosa *et al.*, 1982, 1988; Lima *et al.*, 1991).

Prochilodus affinis is a major species captured by professional fishers in São Francisco river basin. It is a "piracema" (reophilic) fish (Britski *et al.*, 1988), that has free eggs and total spawning (Sato and Godinho, 1987). It is induced to reproduce at the Três Marias Fishery Station (Sato and Cardoso, 1988) from December to February in order to produce alevins for repopulation of the Três Marias reservoir. Recently, Rizzo and Bazzoli (1993) described the oocyte development, the postovulatory follicles, the micropylar apparatus and the oocyte surface of *P. affinis* raised in captivity and induced to spawn. The present paper describes the reabsorption of the previtellogenic and vitellogenic atretic follicles of unspawned *P. affinis*. The knowledge of the morphological events of the follicular atresia can be useful for improve fish artificial propagation methods.

MATERIAL AND METHODS

Females of curimatá-pioa *Prochilodus affinis* in advanced maturation (15-38 cm standard length and 2-3 years old), born from artificial propagation at the Três Marias Fishery Station in the São Francisco river, Minas Gerais, Brazil, were selectioned and kept in tanks of the station. Ovary fragments from 40 unspawned females were collected monthly in 1986/87. The specimens were fixed in Bouin's fluid, embedded in paraplast or glycol metacrylate plastic (JB-4, Polysciences), sectioned at 3 to 6 μm thickness and stained with haematoxylin/eosin, Gomori's trichrome, toluidine blue-sodium borate, periodic acid-Schiff (PAS)/haematoxylin and Dominici stain.

RESULTS AND DISCUSSION

Females of *P. affinis* kept in confinement were prepared to reproduction from December to February. However, they are not induced to reproduction, thus showing atretic ovaries during the period from March to July. The process of ovarian regression last 5 months in the *P. affinis*, 5-6 months in the *Piaractus mesopotanicus* (Romagosa *et al.*, 1988; Lima *et al.*, 1991) and 2 months in the *Prochilodus scrofa* (Romagosa *et al.*, 1982), indicating that the period of follicular atresia can to be variable among species. Long regression period can affect the artificial reproduction procedures since degenerative processes in the ovaries reduce the rate of fertilization. On the other hand, ovaries of starved Northern anchovies kept in laboratory regressed rapidly and no yolk remained in the ovaries at 23 days after onset of starvation (Hunter and Macewicz, 1985) suggesting that the period of ovarian regression can to be reduced.

In the present study, the process of follicular atresia was subdivided into 5 stages in order to in-

Figs. 1-9 — Morphological events of the reabsorption of previtellogenic atretic follicles of *P. affinis*. Specimens embedded in glycol methacrylate plastic. Fig. 1 — Stage 1: vacuolated areas (*) close the nuclear envelope, shrinkage of the ooplasm leading to separation of the zona pellucida. Dominici stain – × 180. Fig. 2 — Stage 2: fractured zona pellucida (arrow), flocculent ooplasm (OP), scattered nuclear material (N). Dominici stain – × 150. Fig. 3 — Detail of Stage 2: ooplasmic material outside the zona pellucida (arrow), hypertrophied follicular epithelium (FE). Dominici stain – × 930. Fig. 4 — Stage 3: highly columnar follicular epithelium (FE), folded zona pellucida (arrow), vacuolated ooplasm (OP). Toluidine blue-sodium borate – × 180. Fig. 5 — Detail of Stage 3: highly columnar follicular epithelium (FE) with basophilic granules inside of phagocytic aspect, theca richly vascularized (V). Toluidine blue-sodium borate – × 1200. Fig. 6 — Stage 4: remains of the zona pellucida (arrow) and invading cells into ooplasm, vacuolated areas close the follicular epithelium (FE) and the invading cells. Periodic acid-Schiff (PAS) and haematoxilin – × 180. Fig. 7 — Stage 4: oocyte material almost completely reabsorbed, degeneration of the folicular epithelium (FE) and the invading cells (arrowhead). Toluidine blue-sodium borate – × 440. Fig. 8 — Stage 5: atretic follicle compaction and increase of cellular degeneration (arrowhead). Toluidine blue-sodium borate – × 450. Fig. 9 — Stage 5: group of pigment cells at the end of follicular atresia persisting in the ovarian stroma. Periodic acid-Schiff (PAS)/haematoxilin – × 410.

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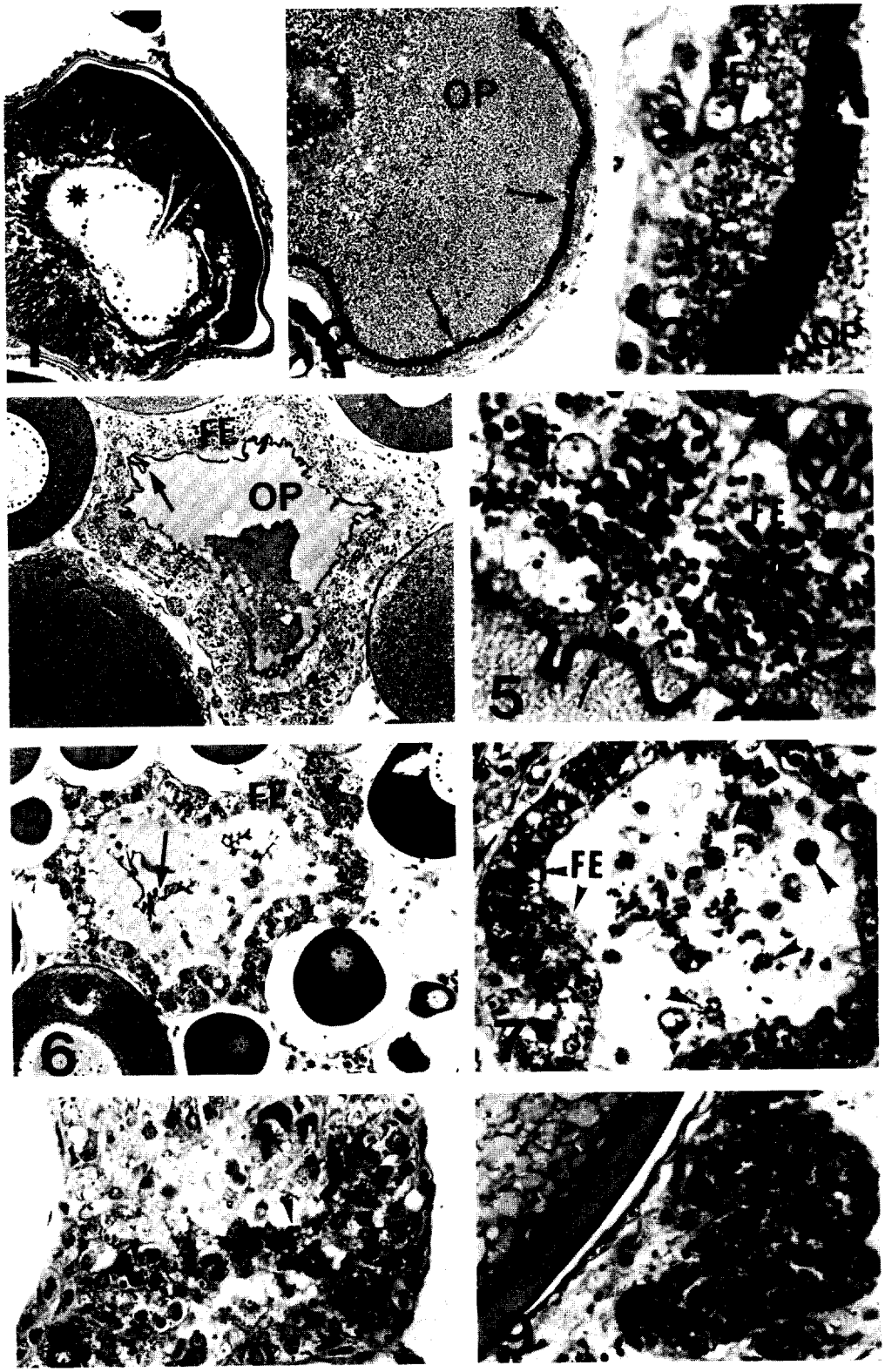

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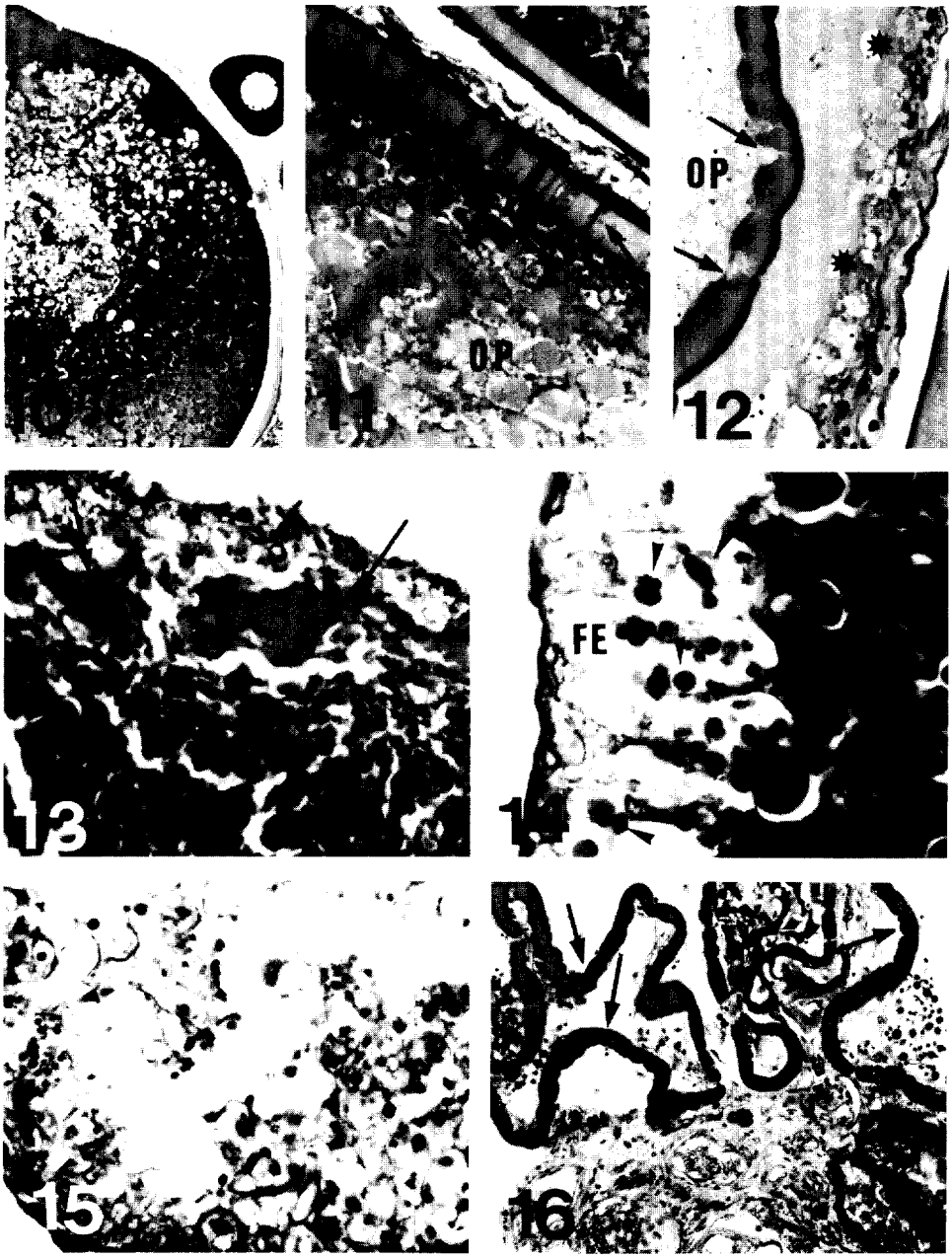

Figs. 10-16 — Morphological events of the reabsorption of vitellogenic atretic follicles of *P. affinis*. Specimens embedded in glycol metacrylate plastic (Figs. 10, 11, 12 and 16) and paraplast (Figs. 13, 14 and 15). Fig. 10 — Stage 1: nuclear material (N) scattered in the peripheral ooplasm, shrinkage of the ooplasm. Toluidine blue-sodium borate – × 90. Fig. 11 — Stage 2: slits in the zona pellucida (arrow), cuboidal follicular cells, yolk globules melting into ooplasm (OP). Toluidine blue-sodium borate – × 390. Fig. 12 — Stage 2: exit of ooplasmic material through the slits of the zona pellucida (arrow), vacuolated areas (*) close the folicular epithelium. Periodic acid-Schiff (PAS) and haematoxilin – × 250. Fig. 13 — Stage 3: desintegration and reabsorption of the zona pellucida (arrow). Haematoxilin/eosin – × 360. Fig. 14 — Stage 3: yolk reabsorption (arrowhead) by the follicular cells. Haematoxilin/eosin – × 980. Fig. 15 — Stage 4: oocyte cavity invaded by follicular cells which acquired a cord-like aspect. Haematoxilin-eosin – × 380. Fig. 16 — Stage 5: regression-resistant zona pellucida (arrow) persisting in the ovary associated with connective cells of the ovarian stroma. Dominici stain – × 140.

dicate the morphological events of the oocyte reabsorption in both previtellogenic (Figs. 1 to 9) and vitellogenic (Figs. 10 to 16) atretic follicles. The morphological characteristics of each stage were indicated in the Table I.

Follicular atresia in captive *P. affinis* was observed mainly in vitellogenic oocytes in accordance with other studies (Rastogi, 1969; Guraya *et al.*, 1975; Saidapur, 1978; Babu and Nair, 1983; Lima *et al.*, 1991). Some females captured in July presented very previtellogenic follicles undergoing degeneration, after reabsorption of the most vitellogenic oocytes. According to Guraya (1986) atresia of previtellogenic follicles is also of rare occurrence in teleosts at natural conditions. The main events of the reabsorption process appear to be similar in the previtellogenic and vitellogenic atretic follicles of *P. affinis*. However, there are

TABLE I

Main morphological characteristics of the 5 stages of follicular atresia in previtellogenic and vitellogenic atretic follicles of *P. affinis* (Pisces: Characiformes).

Stage/ (Figures)	Morphological characteristics	Previtellogenic atretic follicle	Vitellogenic atretic follicle
Stage 1/	shrinkage of the ooplasm	Yes	Yes
(1 and 10)	detachment of the zona pellucida	Yes	Yes
	vacuolated areas appear close the nucleus	Yes	No
	intermixing of chromatin into ooplasm	Yes	Yes
	cortical vesicles are grouped	No	Yes
Stage 2/	zona pellucida is fractured	Yes	Yes
(2, 3, 11	ooplasmic material escapes to the extracellular space	Yes	Yes
and 12)	follicular cells hypertrophy becoming cuboidal	Yes	Yes
	thecal cells proliferate	Yes	Yes
	ooplasm show flocculent aspect	Yes	No
	yolk globules appear to melt	No	Yes
Stage 3/	ooplasm is partially destroyed and reabsorbed	Yes	Yes
(4, 5, 13	zona pellucida is fractured and folded	Yes	Yes
and 14)	follicular epithelium becomes highly columnar	Yes	Yes
	follicular cells show phagocytic granules	Yes	Yes
	theca is thick and richly vascularized	Yes	No
Stage 4/	ooplasm and zona pellucida were almost completely reabsorbed	Yes	Yes
(6, 7 and 15)	blood cells invade the oocyte cavity	Yes	No
	follicular cells invade the oocyte cavity	No	Yes
	remains of the oocyte appear to be reabsorbed by invading cells	Yes	Yes
	atretic follicle acquire a cord-like aspect	No	Yes
	follicular cells are in degeneration	Yes	No
Stage 5/	atretic follicle is in degeneration and compaction	Yes	Yes
(8, 9 and 16)	yellow-green pigments accumulate in some cells	Yes	Yes
	group of pigment cells can persist in the ovary	Yes	Yes
	sometimes, fractured zona pellucida can persist in the ovary	No	Yes

morphological differences in the follicular atresia depending on the species and stage of oocyte development (Guraya, 1986). Moreover, the reabsorption of the previtellogenic atretic follicles appeared to occur more rapidly, lasting less than a month in *P. affinis*. According to Rastogi (1969), the duration of atresia depends upon the size of the oocyte and the amount of yolk.

The phagocytic character of the hypertrophied follicular cells was confirmed by histochemical techniques (Lambert, 1970) and ultrastructural studies (Lang, 1981). The peripheral ooplasm of oocytes undergoing resorption could also be enzymatically active, suggesting that follicular cells could secrete enzymes during atresia (Livni, 1971). Blood cells derived from the theca and/or ovarian stroma were also involved in follicular atresia (Rai, 1966; Braeckevelt and McMillan, 1967; Srivastava, 1969; Rastogi, 1969) and they participated in phagocytosis of degenerating granulosa cells remains (Guraya, 1986).

Three distinct phases of the ovarian regression process in *P. affinis* were characterized as follows:

1. *Initial regression*

The ovary presented a great number of normal vitellogenic oocytes, some vitellogenic atretic follicles at stage 1, showing the first signs of atresia: shrinkage of the ooplasm, detachment of the zona pellucida, rupture of the nuclear envelope and intermixing of chromatin and the ooplasm. This phase was observed mainly in March and April.

2. *Advanced regression*

Vitellogenic atretic follicles in stage 2, 3 or 4 were frequent; young (01) and previtellogenic (02) oocytes were generally normal. This phase was most frequently observed in May and June.

3. *Late regression*

The ovary showed a great number of normal O1 and O2 oocytes; some vitellogenic atretic follicles at the end of the reabsorption process (Stage 5); pigment cell groups were seen close to the blood vessels and, sometimes, previtellogenic atretic follicles. This phase occurred mainly in July.

The fact that the glandular appearance of teleost atretic follicles is similar to mammalian corpus luteus lead some investigators to suggest a steroidogenic function for them (Ball, 1960; Browning, 1973). Histochemical studies refuted this possibility and the majority of the authors have considered the atretic follicles of fishes as structures in degeneration and reabsorption only (Lambert, 1970; Saidapur, 1978; Lang, 1981; Babu and Nair, 1983; Nagahama, 1983; Guraya, 1986). However, the thecal cells of previtellogenic atretic follicles appeared to give rise to the ovarian interstitial gland cells, which showed features of steroidogenic activity (Guraya, 1972; Saidapur and Nadkarni, 1976). Additional studies are necessary for better understanding of the functional significance of the follicular atresia and the role of interstitial cells in fish ovaries.

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