Tissue & Cell, 1999 **31** (5) 480–488

Article no. tice.1999.0045

Tissue&Cell

Ovarian follicular atresia in two teleost species: a histological and ultrastructural study

A. C. L. Miranda[1], N. Bazzoli[1], E. Rizzo[1], Y. Sato[2]

Abstract. Follicular atresia is a common phenomenon in vertebrate ovaries involving the oocyte and the follicular wall degeneration. Female *Astyanax bimaculatus lacustris* and *Leporinus reinhardti* were kept in aquaculture cages inside tanks from February 1994 to January 1995 for the study of the characteristics of different stages of follicular atresia. Histological and ultrastructural analysis demonstrated similarities in the degenerative events and in the resorption of oocytes in both species. Degradation of organelles, such as mitochondria, cortical alveoli, and annulate lamellae occurred in the peripheral ooplasm during the initial stage of the process. Follicle cells showed marked phagocytic activity with digestive vacuoles, myelin figures, and lipofuscin granules during the intermediate and advanced stages of follicular atresia. Granulocytes were in activity during the final stage of follicle resorption. The duration of follicular atresia was 4 months in *Leporinus reinhardti* and 7 months in *Astyanax bimaculatus lacustris*. When submitted to induced reproduction in December 1995, 50–60% of the females of both species responded to induced spawning, indicating the recovery of gonadal activity. It is suggested that, in captive conditions, follicular atresia is shorter in total-spawning fishes when compared to those showing partial spawning, and that it has no apparent deleterious effects on induced reproduction in the subsequent cycle. © 1999 Harcourt Publishers Ltd.

Keywords: follicular atresia, teleost, ovarian regression, ultrastructure, reproduction, follicle cells

Introduction

Despite the extensive literature on oogenesis in teleosts (Guraya, 1986; Selman & Wallace, 1989), little is known about the cellular and molecular aspects of follicular atresia involving the degenerative processes of the oocyte and the follicular wall (Lang, 1981; Besseau & Faliex, 1994; Jans & Van Der Kraak, 1997). Follicular atresia is a common phenomenon in vertebrate ovaries under both natural and experimental conditions (Saidapur, 1978) and can be induced by factors such as stress, fasting, biocidal agents, light, temperature, confinement and inadequate hormone levels (Nagahama, 1983). When reared in captivity, several fish species show changes in their gonadal cycles completing oocyte development; however, the spawning only occurs when artificially induced (Mylonas et al., 1997).

Studies on follicular atresia in confined fish are of vital importance for fish breeding, since the degenerative processes occurring in the ovary affect fertility rates (Lam, 1983; Fenerich-Verani et al., 1984; Rizzo & Bazzoli, 1995). The present study is a histological and ultrastructural analysis of the stages of resorption of the atretic follicles of *Astyanax bimaculatus lacustris* and *Leporinus reinhardti* maintained in cages within aquaculture tanks. The objectives are to establish the duration, possible causes as well as the functions of follicular atresia in teleosts. The species were selected based of their different reproductive strategies in natural conditions: *A. bimaculatus lacustris* has a long reproductive period characterized by partial spawning (Pelizaro et al., 1981) while *L. reinhardti* has a short reproductive period characterized by

[1]Laboratory of Ictiohistology, Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, CEP 30.161–970, CP 486, Brazil [2]Três Marias Hydrobiological and Fishculture Station (CODEVASF-CEMIG), Três Marias, Minas Gerais, Brazil

Received 6 April 1999
Accepted 30 July 1999

Correspondence to: Dr Nilo Bazzoli. Tel.: +55 31 499 2785; Fax: +55 31 499 2772; E-mail: ictio@mono.icb.ufmg.br

Table 1 Morphological characteristics of the atretic follicles in *A. bimaculatus lacustris* and *L. reinhardti*

	Morphological characteristics
Initial atresia stage (Fig. 1A, B; Fig.2A)	Rupture of the nuclear envelope and dispersion of chromatin in the ooplasm. Beginning of the disintegration of the organelles in the peripheral ooplasm: mitochondria, annulate lamellae, cortical alveoli and yolk globules. Tears in the zona pellucida. Hypertrophied follicle cells. Normal basal membrane and theca.
Intermediate atresia stage (Fig. 1C; Fig. 2B)	Liquefaction of the yolk globules. Disintegration and fragmentation of the zona pellucida. Follicle cells with phagocytic characteristics by engulf of the yolk. Theca showing little vascularization. Slightly convoluted basal membrane.
Advanced atresia stage (Fig. 1D, E; Fig. 3A)	Yolk almost completely phagocytated by the follicle cells. Numerous myelin figures in the cytoplasm of follicle cells. Theca richly vascularized. Thickened and highly convoluted basal membrane.
Final atresia stage (Fig. 2F; Fig. 3C)	Yolk completely reabsorbed. Reduction in the numbers of follicle and thecal cells. Accumulation of yellow-brownish pigments (lipofuscin granules). Connective tissue richly vascularized surrounds the remaining of the follicle. Convoluted and fragmented basal membrane persists. Granulocytes close to the atretic follicles.

total spawning (Rizzo et al., 1996). In tanks, *A. bimaculatus lacustris* spawns spontaneously (Andrade et al., 1985) while *L. reinhardti*, although in ovarian advanced maturation, requires artificial induction to initiate spawning.

Materials and methods

Females *A. bimaculatus lacustris* and *L. reinhardti*, in advanced maturation condition, were stocked in aquaculture cages. Each species was kept isolated within separated cages inside a tank at the Três Marias Hydrobiological and Fishculture Station, (CODEVASF-CEMIG) at Três Marias, Brazil (Lat. 18°11′S, Long. 45°13′W), during the period of February 1994 to January 1995. Commercial feed was used throughout the experiment. Water temperature of the tank changed from 22 to 29°C during the present study. Five to eight fishes of each species were collected monthly for the study on ovarian regression. Fragments of the ovaries were fixed in Bouin's fluid for 4 h, and prepared using routine techniques for histology: embedding in paraffin and/or glycol metacrylate plastic resin, sectioning under 2–5μm thickness, and staining with haematoxilin-eosin or toluidine blue-sodium borate. Furthermore the specimens were also fixed in 2.5% glutaraldehyde/phosphate buffer 0.1 M, pH 7.2, for 6 h at 4°C, post-fixed in 1% osmium tetroxide with 1.5% potassium ferrocyanide for 2 h at room temperature, dehydrated and embedded in Epon plastic resin for ultrastructural analyses. The ultrathin sections were cut with a diamond blade, contrasted with uranyl acetate and lead citrate, and examined under a Zeiss EM-10 transmission electron microscope. The diameters of the normal and atretic vitellogenic follicles were determined using an eyeglass micrometer attached to light microscope. The major and minor diameter of 50 follicles were measured in each stage of the follicular atresia in 30 randomly-chosen slides.

For macroscopic analysis of ovarian regression, the fish were dissected to examine the size and vascularization of the gonads, and the degree of opacity of the oocytes. A gonadosomatic index (GSI) was calculated for each ovarian regression phase, based on the relation: GSI = (gonad weight/body weight) x 100.

In order to examine the efficiency of induced reproduction in the fish stocked for 1 year in the aquaculture cages, female *L. reinhardti* and *A. bimaculatus lacustris* were hypophyzed according to the method of Ihering (1937), with injections of crude carp pituitary extract (CCPE) in the celomic cavity. Semen was obtained from males stocked in the tanks of the station. Female *L. reinhardti* received two doses of CCPE and female *A. bimaculatus lacustris* received a single dose. Males of both species received one dose of CCPE. Estimation of the time for egg stripping and hatching (in degree–h) and rate of egg fertilization (in %) was performed according to Woynarovich and Horváth (1980). The 'dry' method was used to fertilize the eggs. The fertilized eggs were placed in a funnel-type fiberglass incubator with 60 l capacity.

Results

Female *A. bimaculatus lacustris* and *L. reinhardti* in advanced stage of the oocyte development did not spawn spontaneously while stocked in the aquaculture cages, and their oocytes became atretic leading to macro- and microscopic changes in their ovaries. Atresia process was seen to be most frequent in

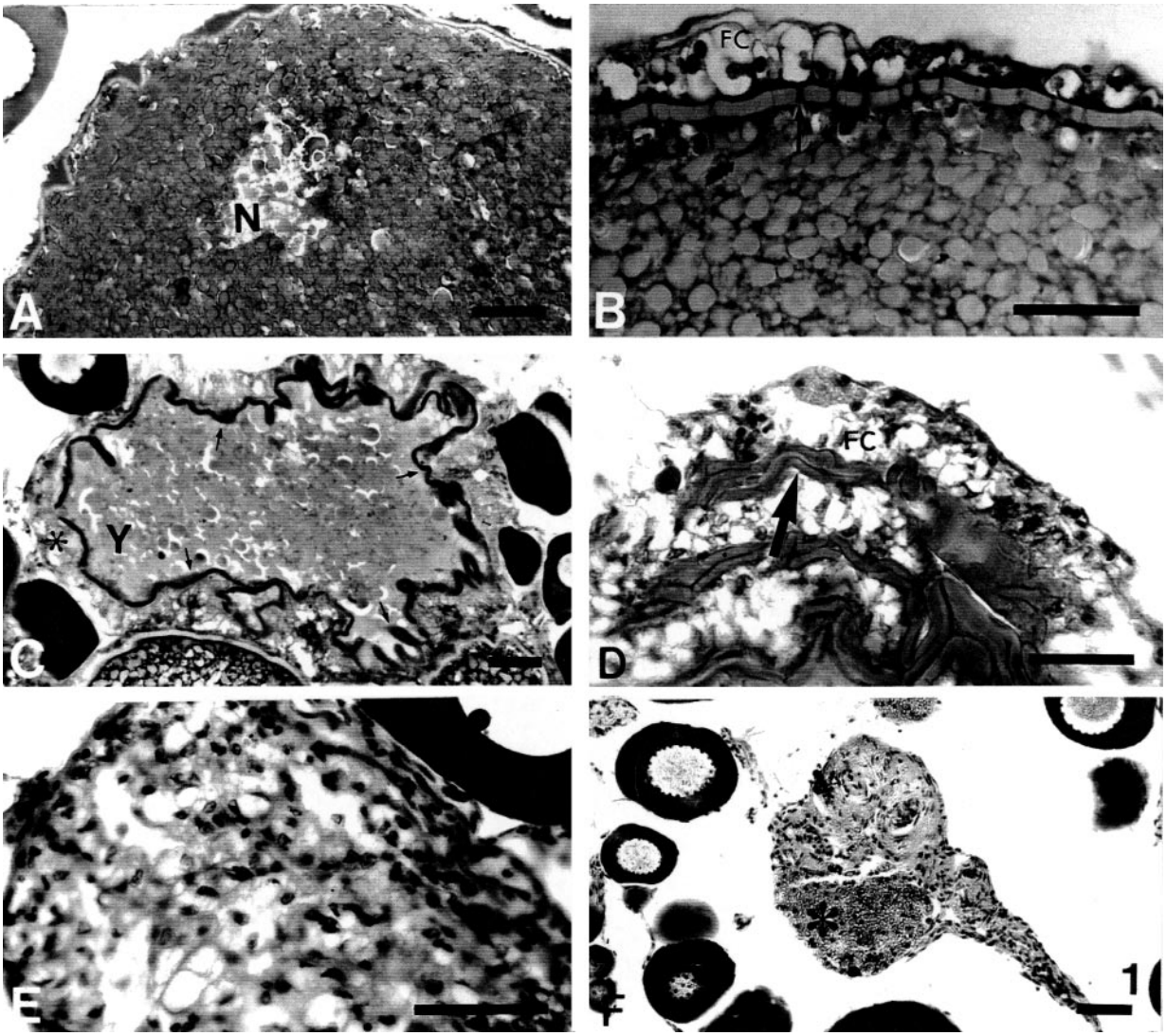

Fig. 1 Histological sections of follicular atresia at vitellogenic oocytes: **A** Initial atresia stage in *L. reinhardti*, showing nuclear degradation (N), with chromatin dispersed in the ooplasm. HE. **B** Tears in the zona pellucida (arrow) and hypertrophied follicle cells (FC) with a vacuolated appearance containing phagocyted material during initial atresia in *L. reinhardti*. Toluidine blue. **C** Intermediate atresia stage in *L. reinhardti* with vacuolated areas in the ooplasm, liquefied yolk globules (Y), convoluted zona pellucida (arrows) and hypertrophied follicle cells (asterisk). Toluidine blue. **D** Advanced atresia stage in *A. bimaculatus lacustris* with the yolk almost completely phagocyted, the zona pellucida still remaining (arrow), follicle cells with vacuolated cytoplasm. HE. **E** Advanced atresia stage in *A. bimaculatus lacustris* with the yolk and zona pellucida completely reabsorbed, and vacuolated follicle cells. HE. **F** Final atresia stage in *L. reinhardti* showing yellow-brownish bodies (asterisk), enclosed by connective tissue richly vascularized. HE. Scale bars: 50 μm.

vitellogenic follicles, and of rare occurrence in previtellogenic follicles. During atresia, the oocytes shrank, gradually undergoing the various stages of degeneration and resorption. For better understanding of the dynamics of follicular atresia, the process was divided into four stages: initial, intermediate, advanced, and final (see Table 1). The morphological alterations arising during atresia were compared with the features of normal vitellogenic follicles: central or eccentric nucleus showing various nucleoli; ooplasm with mitochondria, rough endoplasmic reticulum (RER), annulate lamellae, cortical alveoli and yolk globules; zona pellucida in two layers; squamous follicle cells, with vesiculous nucleus and evident nucleoli, well-developed RER, Golgi complex, and mitochondria with parallel cristae; moderately dense basal membrane and theca containing cells similar to fibroblasts.

The histological and ultrastructural characteristics of follicular atresia were similar in both *A. bimaculatus lacustris* and *L. reinhardti* females. A sequence of events characterized by 1) disorganization of the nucleus and cytoplasm of the oocyte; 2) distortion of the follicle; 3) folding, fracturing and dissolution of zona pellucida; 4) fusion and liquefaction of the yolk globules, following hypertrophy of the follicle cells which acquired phagocytic features for ingestion and digestion of the yolk was observed. In the final stages of this involutive process, the ooplasm was invaded by the follicular and thecal cells forming a yellow-brownish pigmented cell

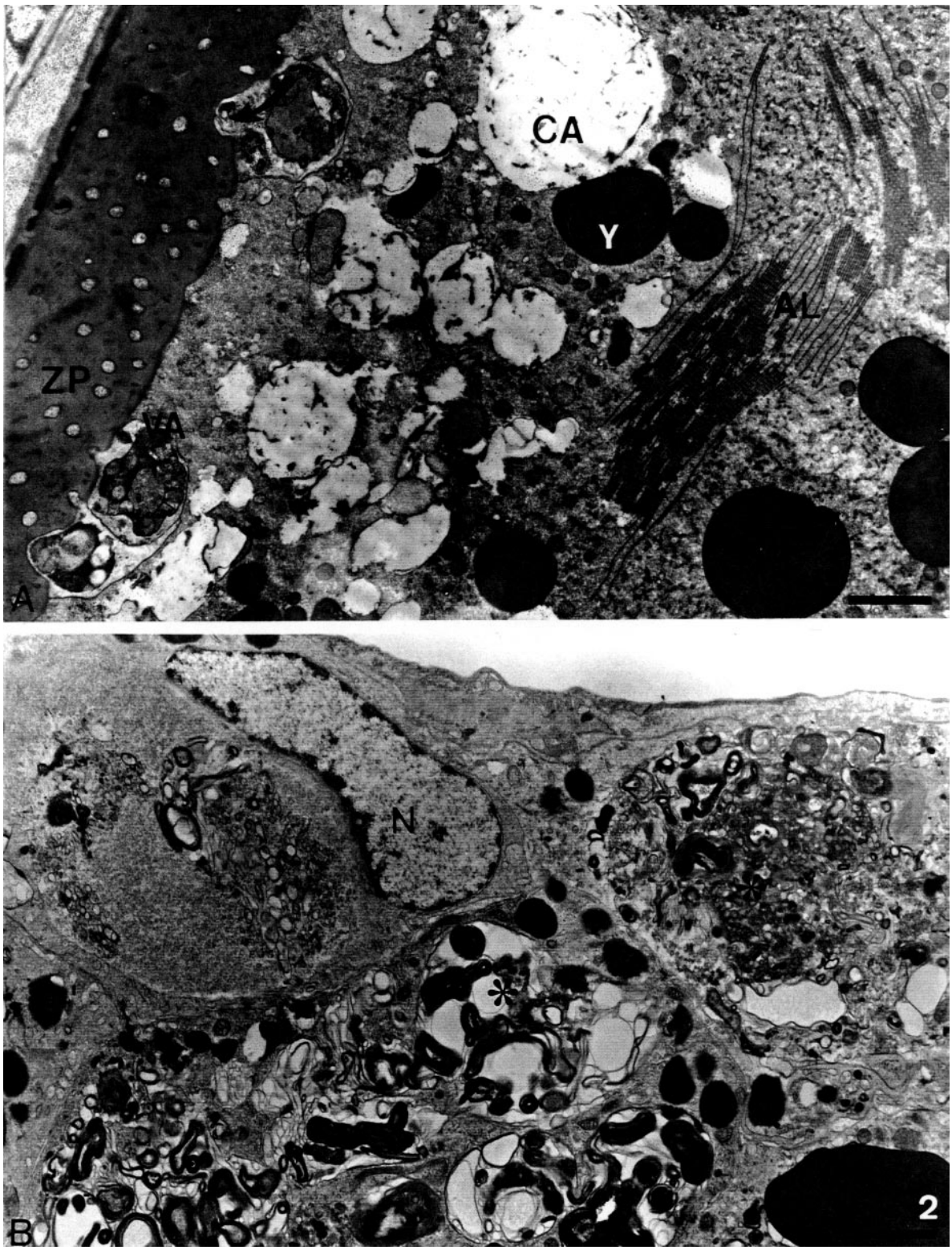

Fig. 2 **A** Ultrastructural characteristics of initial atresia in vitellogenic follicle of *L. reinhardti*: morphological changes in the peripheral ooplasm with vacuoles (VA), beginning of the formation of tears (arrow) in the zona pellucida (ZP), annulate lamellae starting to disintegrate (AL), flocculent cortical alveoli (CA) and yolk globules (Y). Scale bar: 2 μm. **B** Ultrastructural characteristics of the intermediate atresia stage in vitellogenic follicles of *A. bimaculatus lacustris*: follicle cells with a vesiculous nucleus (N) and cytoplasm replete with vacuoles containing membranous structures in degeneration (asterisk). Scale bars: 2 μm.

Table 2 Diameters (μm) of normal vitellogenic follicles and those in different stages of follicular atresia in *A. bimaculatus lacustris* and *L. reinhardti* maintained in aquaculture cages

	A. bimaculatus lacustris	*L. reinhardti*
Vitelogenic follicles	486.42 ± 51.36	606.94 ± 55.39
Inicial atresia	358.72 ± 63.47	503.77 ± 99.15
Intermediate atresia	248.43 ± 57.38	286.73 ± 91.51
Advanced atresia	187.89 ± 54.61	142.43 ± 37.26
Final atresia	103.57 ± 29.93	85.41 ± 34.90

The values are means and ± SD of 50 follicles

mass surrounded by richly vascularized connective tissue. Granulocytes were also observed in activity close to the atretic follicles at the final stage of resorption.

During atresia, follicular diameter diminished gradually by 10–30% in the initial stage, 30–70% in the intermediate stage, 70–80% in the advanced stage, and compacting was above 80% of the follicle initial size at the final stage (Table 2).

In both *A. bimaculatus lacustris* and *L. reinhardti*, ovarian regression was divided into three stages based on macro- and microscopic changes: initial, intermediate and final (see Table 3), all of which involved a gradual reduction in the values of the gonadosomatic index (Table 4). The dynamics of the ovarian regression lasts 7 months in *A. bimaculatus lacustris* (from December to June), and 4 months in *L. reinhardti* (from March to June).

Three of the five female *L. reinhardti* and five of the 10 female *A. bimaculatus lacustris* stocked in the aquaculture cages spawned following hormone induction. The histological analysis of the ovaries of these females demonstrated the presence of numerous post-ovulatory follicles and few atretic vitellogenic oocytes. The ovaries of *L. reinhardti* and *A. bimaculatus lacustris* which did not respond to hormone induction, showed a number of atretic vitellogenic follicles and oocytes in all stages of development. Oocyte extrusion occurred 211–216 degree–h after the second hormone injection in *L. reinhardti* and 305–312 degree–h in *A. bimaculatus lacustris* at 23 to 24°C. The average fertility for *L. reinhardti* from the cage was approximately 80% at 15 h postspawning. It was not possible to collect the semen for fertilization of the eggs of *A. bimaculatus lacustris*.

Discussion

Follicular atresia is an involutive process widespread in ovaries of fishes and other vertebrates, and observed in all stages of the reproductive cycle, although most frequently during postspawing period (Guraya, 1994). In *A. bimaculatus lacustris* stocked in tanks inside cages of the Três Marias Hydrobiological and Fishculture Station, we observed atretic follicles during the prespawning, spawning and postspawning periods (Miranda, 1996).

Table 3 Macro- and microscopic aspects of normal ovaries in advanced maturation and during ovarian regression in *A. bimaculatus lacustris* and *L. reinhardti* maintained in aquaculture cages

Stage	Macroscopic aspects	Microscopic aspects
Initial regression	Voluminous ovaries. Numerous normal and some opaque oocytes visible to the naked eye. Abundant vascularization.	Normal young (O1), previtellogenic (O2), with cortical alveoli follicle (O3) and numerous vitellogenic follicle (O4). Some follicles in initial atresia stage.
Intermediate regression	Gelatinous, hemorrhagic, and reduced ovaries. Numerous opaque oocytes, few normal oocytes visible to the naked eye.	Numerous follicles in intermediate and advanced atresia stage. Few normal O1, O2, O3, O4.
Final regression	Reduced, thin and whitish ovaries. Little vascularization.	Numerous follicles in advanced and final atresia stage. Presence of O1 and O2.

Table 4 Gonadosomatic index (GSI) during ovarian regression in *A. bimaculatus lacustris* and *L. reinhardti* maintained in aquaculture cages

	A. bimaculatus lacustris		*L. reinhardti*	
Stage	Occurrence	GSI	Occurrence	GSI
Initial regression	December January	14.46 ± 4.54	March April	11.53 ± 1.20
Intermediate regression	February March April	12.15 ± 6.31	May	5.1 ± 4.36
Final regression	May June	1.48 ± 1.46	June	0.88 ± 4.36

The values are means and ± SD.

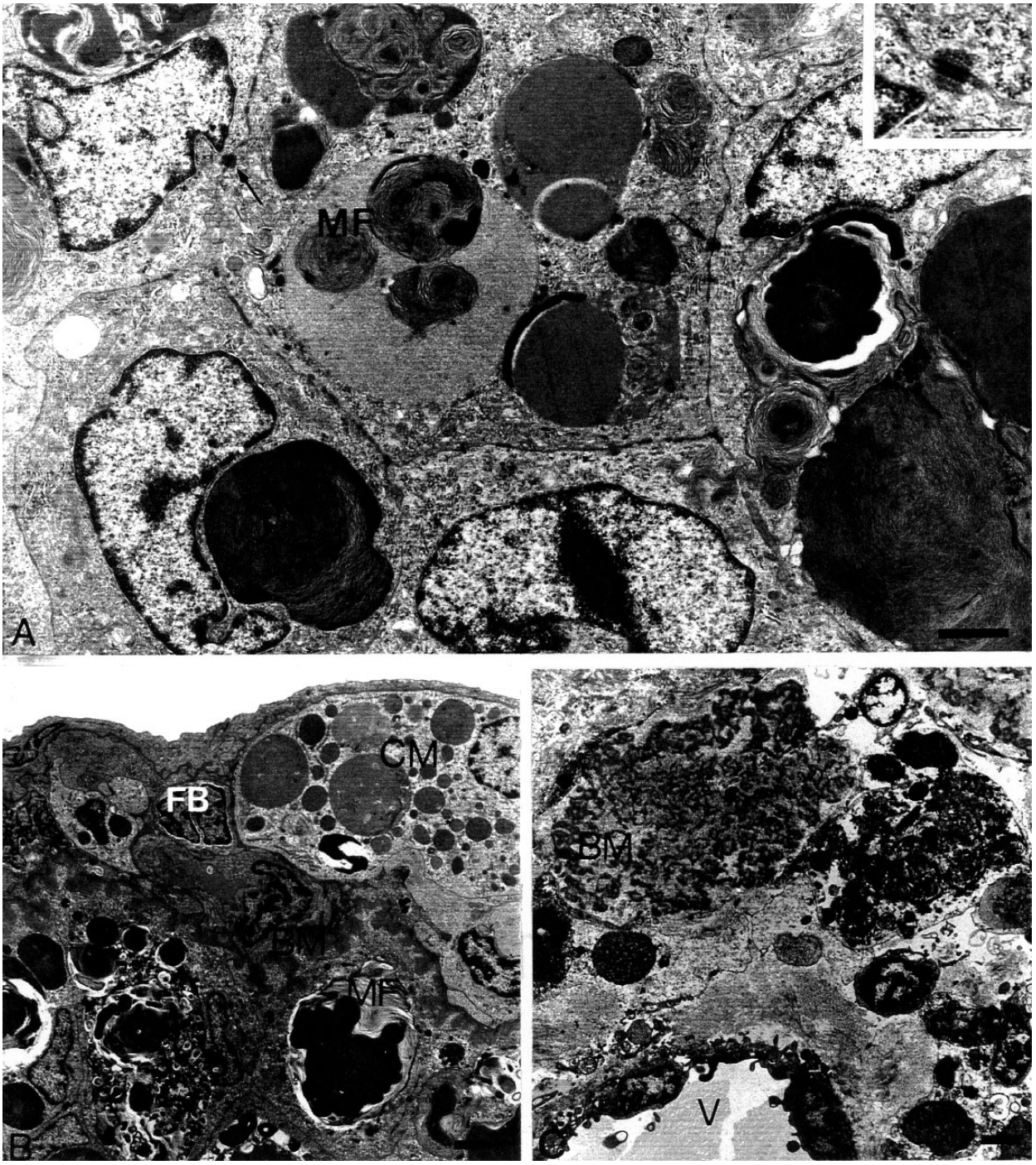

Fig. 3 Ultrastructural characteristics of the advanced atresia stage in vitellogenic follicles of *L. reinhardti*: **A** Follicle cells connected by desmosomes (arrows and inset) with myelin figures in the cytoplasm, characteristic of cells in degeneration. Scale bar 1 μm. Inset: 0,5 μm. **B** Thecal layer showing cells with macrophagic characteristics (CM) and cells similar to fibroblasts, highly convoluted and basal membrane (MB). Numerous electron-dense structures in the form of myelin figures (MF) in the cytoplasm of the follicle cells. Scale bar 2 μm. **C** Ultrastructural characteristics of final atresia in vitellogenic follicles of *L. reinhardti*: the fragmented and convoluted basal membrane (MB) still persists close to connective tissue, blood vessels (V) and lipofuscin granules. Scale bars: 2 μm.

A number of factors has been described as causing follicular atresia in teleost fish. These include hypophysectomy, administration of steroid hormones, biocides, temperature change, starvation and confinement (Guraya, 1986). In captivity, atresia is more frequent in vitellogenic oocytes, although it can also be found in previtellogenic oocytes, as seen in this study as well as those of Guraya (1986) and Rizzo and Bazzoli (1995).

The first signs of atresia observed under the light microscope in both *A. bimaculatus lacustris* and *L. reinhardti* were the disintegration of the oocyte nucleus, followed by the fragmentation of the zona pellucida and hypertrophy of

the follicle cells, as also described by Babu and Nair (1983), Guraya (1986), Lima et al. (1991), Rizzo and Bazzoli (1995), Lowerre-Barbieri et al. (1996), and Mylonas et al. (1997). During the process of atresia in *Salmo gairdneri*, Schulz and Blüm (1983) described two types of oocyte nuclear disintegration. In the first type, described as intracellular nuclear disintegration and recorded for the majority of teleosts including those used in the present study, the nuclear content disappears rapidly in the ooplasm following lysis of the nuclear envelope. In the second type, the oocyte nucleus, together with part of the ooplasm, is eliminated through an opening in the follicle wall into the ovarian lumen, where it breaks up into various fragments. This type of nuclear disintegration was not observed in *A. bimaculatus lacustris* and *L. reinhardti*.

Except for Lang (1981) and Besseau and Faliex (1994), the literature contains no data on the ultrastructure of the atretic follicles in teleost fish. The ultrastructural analysis in the present study revealed changes in the ooplasm, including the disintegration of the cytoplasm organelles as also reported by authors above. The annulate lamellae lost their organization, thus showing a disintegration process in the atretic vitellogenic oocytes of *L. reinhardti*. As suggested by Lang (1981) and Besseau and Faliex (1994), the follicle cells ingest the yolk, indicating their active participation in the atretic process. The phagocytic nature of the hypertrophied follicle cells has also been reported in a histochemical study (Lambert, 1970) and by pinocytosis of vital stain (Bazzoli & Rizzo, 1995). According to Chieffi-Baccari et al. (1992), in electric ray, *Torpedo marmorata*, the phagocytic activity of the follicle cells involves incorporation and digestion not only of the yolk but also of oocyte components such as mitochondria and other organelles. Shrivastava (1969) reported that these cells also secrete enzymes which digest the yolk.

Several authors have mentioned the presence of blood cells derived from the theca in atretic fish oocytes which are involved in their resorption (Shrivastava, 1969; Lima et al., 1991; Ferreira, 1993). According to Guraya (1986) and Palmer et al. (1995), the cells derived from the ovarian stroma and/or the theca may act together with the follicle cells in the resorption of the atretic follicles, although Lang (1981) did not attribute any function to the immune cells in the digestion of oocyte material during atresia. Besseau and Faliex (1994) suggested a synergetic action of the somatic cells (follicle cells) and the immune cells (eosinophilic granulocytes and macrophages) in the resorption of gametes in *Lithognathus mormyrus*. According to the latter, these cells invade the degenerating oocyte, releasing their granules containing lytic enzymes, and degenerate at the end of the process leading to the formation of a deposit of yellow-brownish pigments, characterized as lipofuscins. In both species studied here, the advanced stage of follicular atresia was marked by the appearance of deposits of yellow-brownish pigments close to granulocytes and blood vessels. Our observations suggest that these residual bodies are slowly reabsorbed in the ovaries, as their presence throughout the reproductive cycle has been recorded in *A. bimaculatus lacustris* (Miranda, 1996). Ultrastructural analyses showed that these structures had no steroidogenic characteristics as referred earlier by Hoar (1983), Lambert (1970) and Babu and Nair (1983). Therefore, these deposits of pigments constitute only the result of the degeneration of the atretic follicle and correspond to the 'brown bodies' previously described in the literature.

Little information is available concerning the period it takes for the atretic vitellogenic follicles to be completely reabsorbed. In captive conditions, oocyte resorption lasts 2 months in *Prochilodus scrofa* (Talmelli et al., 1994), and 5 to 6 months in *Piaractus mesopotamicus* (Lima et al., 1991) and *Prochilodus affinis* (Rizzo & Bazzoli, 1995), thus indicating that the period of follicular atresia can be variable among species of the tropical fish. In this study, although the main morphological features of follicular atresia were similar in the two species studied, the period for complete resorption of the atretic follicles was clearly distinct: 4 months in *L. reinhardti* and 7 months in *A. bimaculatus lacustris*. Follicular atresia was probably shorter in *L. reinhardti* than in *A. bimaculatus lacustris* because of their different reproductive strategies. *L. reinhardti* shows total spawning, releasing all the vitellogenic oocytes during a short period of the year, and retaining only those which are young and previtellogenic (Rizzo et al., 1996). However, *A. bimaculatus lacustris* is a partial spawner, laying eggs for more prolonged periods, and having vitellogenic and atretic oocytes for the most part of the annual cycle (Andrade et al., 1985).

The occurrence of atresia in the ovaries of fishes which did not spawn after hormonal inducing indicates that this degenerative process may be a factor in the failure of the method (Rizzo & Bazzoli, 1997; Mylonas et al., 1997). According to Kjesbu et al. (1991) and Palmer et al. (1995), follicular atresia can reduce the reproductive potential since it is observed when the gonadosomatic index is still high, and by the presence of atretic oocytes seem adjacent to normal oocytes at the ovulation. However, *L. reinhardti*, stocked in the aquaculture cages during the year and submitted to induced spawning, showed a fertilization rate of approximately 80%. At least in this species, the process of follicular atresia of one cycle does not appear to affect fertility rates of the next cycles which is in agreement with De Vlaming (1983), who considered atresia as not causing a drastic reduction of fecundity in fish.

The mechanisms which initiate and regulate oocyte atresia in teleost fish are poorly known, especially at the molecular level. In the initial stages of atresia in *A. bimaculatus lacustris* and *L. reinhardti*, we observed degeneration characterized by necrosis such as dissolution and disappearance of the nucleus and changes in the mitochondria and other organelles, suggesting that the death of the oocyte could be involved in this process. Some studies have shown that apoptosis, a type of physiological cell death, is connected with the process of follicular atresia in birds and mammals (Palumbo & Yeh, 1994; Hsueh et al., 1994). These studies provided evidence that apoptosis could be the

mechanism of cell death of the follicle and theca cells during the process of follicular atresia in physiological conditions and after induction in vitro. Drummond (1996) recorded a number of apoptotic figures in the ovaries of *A. bimaculatus lacustris* during the process of resorption of postovulatory follicles in females submitted to hypophysation, indicating that this phenomenon occur in fish ovaries. In present work, no apoptotic figures were clearly observed in the ultrastructure study, but in the histological analysis we reported apoptotic figures in the advanced atresia stage, indicating occurrence of apoptotic cell death during regression of the atretic follicles in teleost fish. Jans and Van Der Kraak (1997) also reported the occurrence of apoptotic cell death in ovarian follicles from teleost fish. Results suggest that apoptosis is involved in teleost ovarian development and the several of the hormonal factors acting as follicle survival factor in mammalian and avian ovaries may play a similar role in teleost ovarian follicles, adding to the increasing knowledge of the universality of this cell death process.

AL: annulate lamellae
BM: basal membrane
CA: cortical alveoli
CM: cell with macrophagic characteristics
FB: cell similar to fibroblast
FC: follicle cell
MF: myelin figure
N: nucleus
V: blood vessel
VA: vacuoles
Y: yolk globules
ZP: zona pellucida

ACKNOWLEDGEMENTS

The authors are grateful to the staff of Três Marias Hidrobiological and Fishculture Station (CODEVASF) for the assistance during the collection of the fishes, to the Electron Microscopy Centre CEMEL/UFMG and the Brazilian research foundations: PRPq-UFMG, CNPq, FAPEMIG for financial support.

REFERENCES

Andrade, D.R., Godinho, H.P., Ribeiro, S.P. and Castro, E.F.T. 1985. Ciclo reprodutivo anual de lambaris (*Astyanax bimaculatus* Linnaeus, 1758) em viveiros. Arq. Bras. Med. Vet. Zoot., 37, 435–447.

Babu, N. and Nair, N.B. 1983. Follicular atresia in *Amblypharyngodon chakaiensis*. Z. mikrosk. – anat. Forsch., 97, 499–504.

Bazzoli, N. and Rizzo, E. 1995. Reabsorção dos folículos atrésicos em *A. bimaculatus lacustris* (Pisces, Teleostei) mantidos em confinamento. Bios, 2, 37–41.

Besseau, L. and Faliex, E. 1994. Resorption of unemitted gametes in *Lithognathus mormyrus* (Sparidae, Teleostei): a possible synergic action of somatic and immune cells. Cell Tissue Res., 276, 123–132.

Chieffi-Baccari, G., Minucci, S., Di Matteo, L. and Chieffi, G. 1992. Ultrastructural investigation of the corpora atretica of the eletric ray *Torpedo marmorata*. Gen. Comp. Endocrinol., 96, 72–80.

De Vlaming, V. 1983. Oocyte development patterns and hormonal involvements among Teleosts. In: Rankin, J.C., Pitcher, T.J. and Duggan, R.T.N. (eds). Control Process in Fish Physiology Croom Helm, London, 176–199.

Drummond, C.D. 1996. Folículo pós-ovulatório de lambari *Astyanax bimaculatus lacustris* (Pisces, Characidae) submetido a desova induzida: estudo histológico e ultra-estrutural. Dissertation, Federal University of Minas Gerais, Brazil.

Fenerich-Verani, N., Godinho, H.M. and Narahara, M.Y. 1984. The size composition of the eggs of curimbatá *Prochilodus scrofa*, induced to spawn with human chorionic gonadotropin (HCG). Aquaculture, 24, 37–41.

Ferreira, B.P. 1993. Reproduction of the inshore coral trout *Plectropomus maculatus* (Perciformes: Serranidae) from the central Great Barrier Reef, Australia. J. Fish Biol., 42, 831–844.

Guraya, S.S. 1986. The Cell and Molecular Biology of Fish Oogenesis. Karger, Basel.

Guraya, S.S. 1994. Gonadal development and production of gametes in fish. Proc. Indian natn. Sci. Acad., 60, 15–32.

Hoar, W.S. 1983. Reproduction. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (eds) Fish Physiology. Academic Press, London, 1–72.

Hsueh, A.J.W., Billig, H. and Tsafriri, A. 1994. Ovarian follicle atresia: a hormonally controlled apoptotic process. Endocr. Rev., 15, 707–724.

Ihering, R von. 1937. A method for inducing fish to spawn. Prog. Fish Cult., 34, 15–16.

Jans, D.M. and Van Der Kraak, G. 1997. Suppression of apoptosis by gonadotropin, 17 β-estradiol, and epidermal growth factor in rainbow trout preovulatory ovaries follicles. Gen. Comp. Endocrinol., 105, 186–193.

Kjesbu, O.S., Kulngsoyr, J., Kryvi. H., Witthames, P.R. and Walker, M. 1991. Fecundity, atresia and egg size of captive atlantic cod (*Gobus morhua*) in relation to proximate body composition. Can. J. Fish Aquat. Sci., 48, 2333–2343.

Lam, T.J. 1983. Environmental influences on gonadal activity in fish. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (eds) Fish Physiology. Academic Press, London, 65–116.

Lambert, J.G.D. 1970. The ovary of the guppy, *Poecilia reticulata*. The atretic follicle, a corpus atreticum or a corpus luteum praeovulationis. Z. Zellforsch., 107, 54–67.

Lang, I. 1981. Electron microscopic and histochemical investigations of the atretic oocyte of *Perca fluviatilis* L. (Teleostei). Cell Tissue Res., 220, 201–212.

Lima, R.V.A., Bernardino, G., Val-Sella, M.V., Fava de Moraes, F., Schemy, R.A. and Borella, M.I. 1991. Tecido germinativo ovariano e ciclo reprodutivo de pacus (*Piaractus mesopotamicus* Holmberg, 1887) mantidos em cativeiro. Bol Téc. 4, 1–46.

Lowerre-Barbieri, S.K., Chittenden, M.E. and Barbieri, L.R. 1996. The multiple spawning pattern of weakfish in the Chesapeake Bay and Middle Atlantic Bight. J. Fish Biol., 48, 1139–1163.

Miranda, A.C.L. 1996. Reprodução de *Astyanax bimaculatus lacustris* em viveiros e Estudo histológico e ultta-estrutural da atresia folicular de *A. bimaculatus lacustris* e *Leporinus reinhardti* em gaiolas de acüicultura. Dissertation, Federal University of Minas Gerais, Brazil.

Mylonas, C.C., Woods III, L.C. and Zohar, Y. 1997. Cyto-histological examination of post-vitellogenesis and final oocyte maturation in captive-reared striped bass. J. Fish Biol., 1997, 50, 34–49.

Nagahama, Y. 1983. The functional morphology of teleost gonads. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (eds) Fish Physiology. Academic Press, London, 233–275.

Palmer, E.E., Sorensen, P.W. and Adelman, I.R. 1995. A histological study of seasonal ovarian development in freshwater drum in the Red Lakes, Minnesota. J. Fish Biol. 1995, 47, 199–210.

Palumbo, A. and Yeh, J. 1994. In situ localization of apoptosis in the rat ovary during follicular atresia. Biol. Reprod., 51, 888–895.

Pelizaro, M.G., Leme dos Santos, H.S., Lopes, R.A. and Castagnoli, N. 1981. Rhythm of development in the oocyte of the Tambiu *Astyanax bimaculatus* (Reinhardt, 1874) (Pisces: Characidae) a morphometric and histochemical study. Arch. Biol., 92, 415–431.

Rizzo, E. and Bazzoli, N. 1995. Follicular atresia in curimatá-pioa *Prochilodus affinis* Reinhardt, 1874 (Pisces, Characiformes). Rev. Brasil. Biol., 55, 697–703.

Rizzo, E. and Bazzoli, N. 1997. Atresia folicular em surubins *Pseudoplatystoma coruscans* submetidos à hipofisação. In: Miranda, M.O.T (ed). Surubim. Brasil, Série Estudos Pesca, 91–100.

Rizzo, E., Sato, Y., Ferreira, R.M.A., Chiarini-Garcia, H. and Bazzoli, N. 1996. Reproduction of *Leporinus reinhardti* Lütken, 1874 (Pisces: Anostomidae) from the Três Marias Reservoir, São Francisco river, Minas Gerais, Brazil. Ciênc. Cult., 48, 189–192.

Saidapur, S.K. 1978. Follicular atresia in the ovaries of non mammalian vertebrates. Int. Rev. Cytol., 54, 225–244.
Schulz, R. and Blüm, V. 1983. Elimination of the nucleus in preovulatory oocytes of the rainbow trout, *Salmo gairdneri* Richardson (Teleostei). Cell Tissue Res., 232, 685–689.
Selman, K. and Wallace, A.R. 1989. Cellular aspects of oocyte growth in teleosts. Zool. Sci., 6, 211–231.
Shrivastava, S.S. 1969. Formation of the corpora atretica in *Notopterus notopterus* (Pallas). Acta Zool., 50, 77–89.
Talmelli, E.F.A., Narahara, M.Y., Romagosa, E. and Vazzoler, A.E.A.M. 1994. Fases de degeneração ovocitária em curimbatá *Prochilodus scrofa* (Steindachner, 1881), mantido em confinamento. Rev. UNIMAR, 16, 83–96.
Woynarovich, E. and Horváth, L. 1980. The artificial propagation of warm-water finfishes – a manual for extension. FAO (Food and Agriculture Organization of the United Nations) Fisheries Biology Technical Paper.