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Atresia and Apoptosis in Pre- and Postovulatory Follicles of Sharptooth Catfish (*Clarias gariepinus*, Burchell, 1822)

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ABSTRACT

Ovarian follicular atresia and postovulatory regression in mammals is mediated by apoptosis, which is a natural occurring cause of cellular death. However, its function in fish is largely unknown. In order to discuss the possible role of apoptosis in fish ovarian follicular atresia and postovulatory regression, the pre and postovulatory follicles (POF) of the freshwater teleost, *Clarias gariepinus* were observed by light microscopy. The germinal vesicle and the cytoplasmic organelles of the oocyte were disintegrated. The theca of atretic mature oocytes was hypertrophied and persisted to form the interstitial cells, whereas the granulosa cells were regressed and disappeared. Erythrocytes and leukocytes were also detected at the advance stage of atresia. Apoptosis in the granulosa cells were clearly detected. The chromatin condensation against the nuclear envelope, cell shrinkage, surface blebbing and generation of apoptotic bodies by cell fragmentation were evidence of apoptosis. In pre and postovulatory follicles, karyorhexis and the budding phenomena were clearly detected. Karyorhexis and budding phenomena were also the sing of apoptosis. Atresia was also clearly detected in pre and postovulatory follicles. The detection of karyorhexis, characteristic of apoptotic cells may provide a better understanding in atresia mechanism. It seems that apoptosis has a major role in the elimination of POF and atretic oocytes in the ovaries of *C. gariepinus*.

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SC performed the histological work of the study and also wrote the manuscript. Experimental fish was provided and taking care of by EY.

Key words
Atresia, Apoptosis, *Clarias gariepinus*, Histology.

INTRODUCTION

Follicular atresia is a widespread degenerative process in the vertebrate ovary, by which the majority of oocytes at varying stages in their development are lost other than ovulation (Guraya and Greenwald, 1964; Wood and van der Kraak, 2001; Vieyra *et al*., 2008; Valdebnito *et al*., 2011; Morais *et al*., 2012; Wildner *et al*., 2013; Privalikhin *et al*., 2015; Senerat *et al*., 2015; Hannon and Flaws, 2015). Numerous studies suggest that for the removal of somatic and germ cells in the ovaries of mammals and birds, apoptosis is the mean molecular mechanism (Kumar and Joy, 2015; Chatterjee and Bhattacharjee, 2016). Apoptosis produces cell fragments called apoptotic bodies that phagocytic cells are able to engulf and quickly remove before the contents of the cell can spill out onto surrounding cells and cause damage and it often provides beneficial effects to the organism (Celestino *et al*., 2009; Chatterjee and Bhattacharjee, 2016). It has been described as programmed cell death (Drummond *et al*., 2000; Yu *et al*., 2004; Santos *et al*., 2008; Üçüncü and Çakıcı, 2009; Morais *et al*., 2012; Melo *et al*., 2015). Meanwhile atresia has been defined as a form of apoptosis (Hannon and Flaws, 2015). It occurs in two forms; pre-ovulatory and post-ovulatory atresia. The first form of atresia involves the breakdown of a follicular complex that includes an oocyte. This form of atresia can occur at all stages of oogenesis. It can be induced by factors such as stress, nutrition, biocides agents, captivity, light, temperature, inadequate hormone levels, salinity fluctuations (Guraya and Greenwald, 1964; Miranda *et al*., 1999; Leonardo *et al*., 2006; Morais *et al*., 2012; Harlıoglu, 2017). Accordingly, most studies to date on female teleost have mainly investigated the effects of these conditions on the reproductive success in terms of spawning performance (*e.g.,* fecundity, egg quality and survival of larva).

Abbreviations

POF, postovulatory follicles; Ly, liquefaction of yolk granules; Da, degeneration of cortical alveoli; T, theca; G, granulosa; AtZr, atretic zona radiata; P, pyknosis; K, karyorhexis; Ap, apoptotic body; Y, yolk; V, vacuole; Zr, zona radiata; A, atrium; YB, yellow bodies; O, oogonia; DPB, dark pigmented bodies; HGC, hypertrophied granulosa cells; PFC, phagocytic follicular cells; Lv, large vacuole; Ic, interstitial cells; Bm, basement membrane; MC, micropylar cell; AC, apoptotic cell; BV, blood vessels.

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In the second form of atresia, only the oocyte is released into the ovarian lumen, leaving behind the granulosa and empty follicular envelops. These are termed as post-ovulatory follicles (POF). The post–ovulatory follicle loses its shape because of its collapse as the oocyte is released. Degeneration of the POF is proposed to be due to cell death, termed apoptosis (Morais *et al*., 2012; Melo *et al*., 2015).

C. gariepinus is a resilient bottom-dwelling indigenous African teleost species, whose ovary development is well described (Çek and Yılmaz, 2007). The development pattern of its ovaries was categorized as group-synchrous type (Çek and Yılmaz, 2007). Since this species do not spawn spontaneously in captivity, a widespread process of follicular regression occurs in its ovaries during and particularly after reproductive period.

In mammals and birds, the role of apoptosis in atretic ovaries has been intensively studied. However, considering that morphological studies on apoptosis and atresia in pre and post ovulatory follicles in fish are scarce, the present investigation analyzes histological aspects of their regression in *C. gariepinus* in order to elucidate the mechanism responsible for atresia and apoptosis during ovarian development and recovery at the post-spawning period. It focuses on aspects related to follicular atresia and apoptosis process during different follicular stages.

MATERIALS AND METHODS

Experimental fish and system

The present study was conducted at the Aquarium research unites of Iskenderun Technical University Turkey. Female brood stocks were caught alive from the Asi River and brought to the laboratory. Twenty-five female fish in average weight of 113.19 g and 25.07 cm in total length were assigned to five aquariums, with five fish in each for 35 days. The water temperature was maintained at 27±1ºC, pH was measured at 6.9. Each 80-litter aquarium was continuously aerated using a pump. The aquariums were housed inside an experimental room with a natural photoperiod (12 h dark, 12 h light). A static water system was used, and 80% of the water in each aquarium was changed daily, before the morning feed. During the experiment, brood stock were fed with trout feed pellets (IDL ALFA, 2.2 mm; Inve, Aquamaks, Turkey) tree times a day. Fish experiments were approved by the Mustafa Kemal University in Turkey and were conducted in agreement with the guidelines of Republic of Turkey University of Mustafa Kemal laboratory animal ethics committee.

Histological procedure

Fish were anaesthetized in 0.04%, 2-phenoxethanol (Sigma Chem. Dorset, UK). The gonads were dissected from five sacrificed females for each week, were directly fixed in 10% neutral buffered formalin (prepared in neutral buffered saline modified for use with teleost tissue, 4g NaH_2PO_4, 6.5g $NaHPO_4$, 100ml formaldehyde and 900ml distilled water). A cross section from the center of each ovary was fixed in formalin, dehydrated in graded ethanol, embedded in paraffin, sectioned at 5µm thickness and stained with haematoxylin and eosin (MERCK) for histological examination (Çek *et al*., 2001; Çek and Yılmaz, 2009). After histological work, all slides were examined under a light microscope (CH-2 Olympus-Japan). Photomicrographs were taken to illustrate atresia and apoptosis in the ovary of *C. gariepinus*.

RESULTS

Atresia and apoptosis in pre-ovulatory follicles

Based on the morphological changes in the ooplasm and surrounding follicular layers, tree main phases of atretic oocytes (Atresia in the Primary growth phase, atresia in the secondary growth phase and atresia in the maturation and hydration phase) were identified. Atresia in the Primary growth phase further divided into atresia at the chromatin nucleolar and perinucleolar stages. The non-ruptured follicular wall characterized atresia in these stages. The ooplasm size was drastically reduced and appeared as a dark stain mass (Fig. 1A). Further the oocytes at the peinucleolar stage, shrinkaged and was closely accompanied by the development of clear spaces in the peripheral ooplasm (Fig. 1B). The nucleoli that were generally arranged to form a regular layer in the nuclear envelope of normal grooving previtellogenic oocytes were distributed irregularly in the nucleoplasm (Fig. 1B). Atretic oocytes at the secondary growth phase were divided into 3 stages, namely: atretic oocytes at stage three (atretic oocytes at cortical alveoli stage, early phase of vacuole formation were detected; Fig. 1C), atretic oocytes at stage 4 (atretic oocytes at early vitellogenesis stage; Fig. 1D) and atretic oocytes at stage 5 (atretic oocytes at vitellogenesis stage; Fig. 1E). The main characteristics of atresia of cortical alveoli, early vitellogenesis oocytes and vitellogenesis oocytes were the formation of peculiar marks in the cytoplasm and the thickening of the zona pellucida. In stage tree atretic oocytes, the ooplasm loaded with mass vacuoles, which may be lipoid materials (Fig. 1C). The oocytes membrane wrinkled and thickened (Fig. 1D). Yellowish yolk vesicles were detected at atresia in the early vitellogenesis phase. Hyperplasia and hypertrophy in granulosa cells were also clearly noted. Zona radiate were irregular in appearance, theca cells were slightly enlarged and basal membrane disintegrated (Fig. 1D).

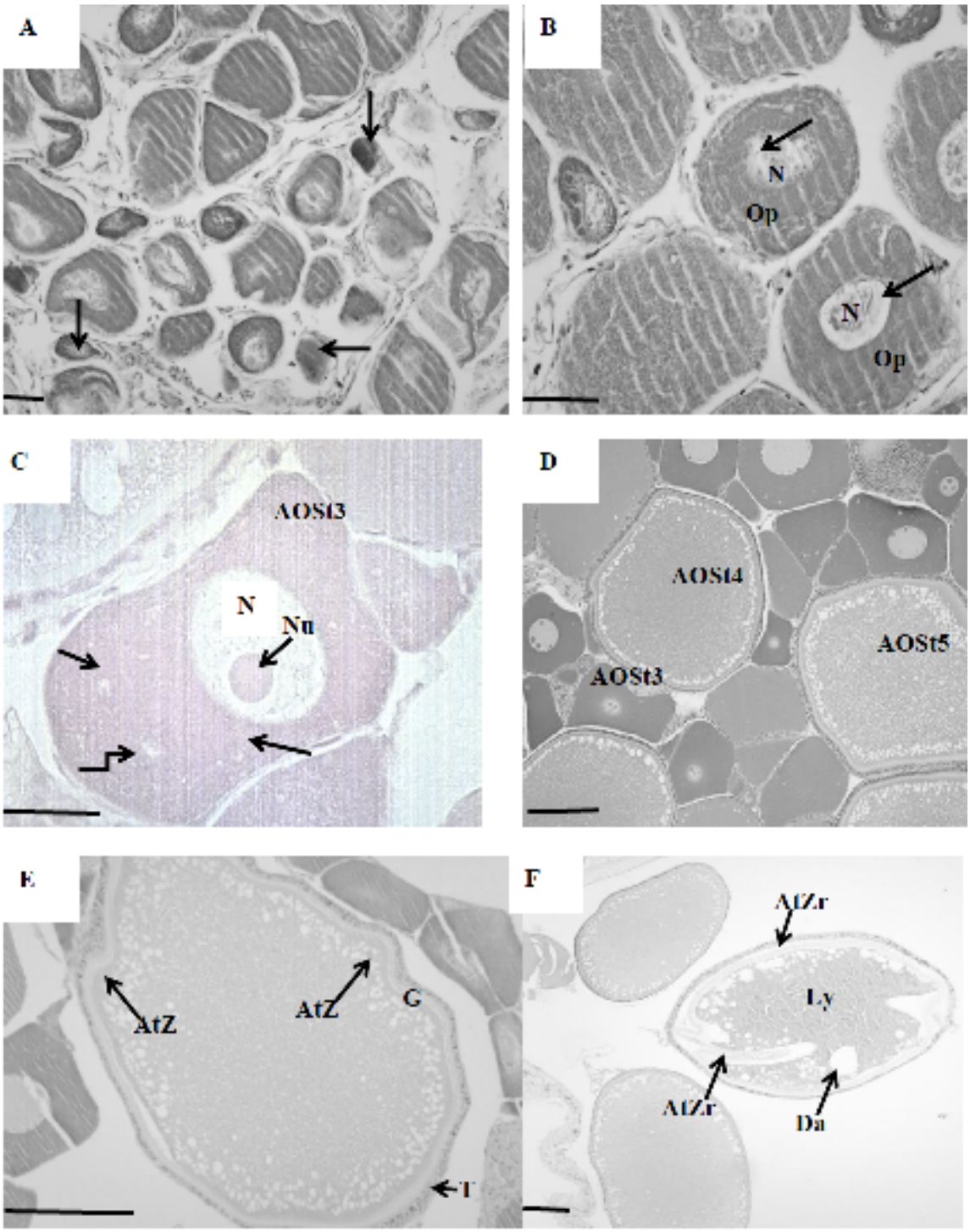

Fig. 1. Pre-ovulatory atretic ovarian follicles. A, arrows show atretic oocytes as dark stain mass in the chromatin nucleolar stage; **B**, atretic oocytes at the perinocleolar stage, arrows show the development of clear spaces in the peripheral ooplasm; **C**, arrows show ealy vacuole formation; **D**, early phase of vascuolization, oocytes resorption; **E**, early degeneration of zona radiata in vitellogenic oocyte; **F**, fregmentation of zona radiata, follicular cells with protrusion extending into the oocyte cytoplasm. Ly, liquefaction of yolk granules; Da, degeneration of cortical alveoli; T, theca; G, granulosa; AtZr, atretic zona radiata. Scale bars: A, 30µm; B-F, 60 µm. Stain: hematoxylin & eosin.

The first visible sing of atresia in vitellogenic oocytes (stage 5 atretic oocytes), was the thickening of the zona radiata. In advance stage five atretic oocytes, yolk liquefaction, zona radiata breakdown, yolk releasing and hypertrophy of the granulosa were observed (Fig. 1E, F). Degeneration of cortial alveoli were also clearly detected (Fig. 1F). During maturation and hydration phase, highly columnar granulosa cells exhibiting intense phagocytic activity ingested most of the yolk and zona radiata remains. Moreover, granulosa layer was completely bursting into pieces due to vesicles formation (Fig. 1F).

The results of the present study identified various morphological changes that occur during apoptosis in pre-ovulatory follicles. During the early process of apoptosis, cell shrinkage and pyknosis were visible by light microscopy (Fig. 2A). With cell shrinkage, the oocytes were smaller; the cytoplasm was dens (Fig. 2A). Pyknosis was the result of chromatin condensation and margination (the chromatin packed into smooth masses applied against the nuclear membrane). Apoptotic sings of blebbing membrane were observed. The nucleus of oocyte brooked up (karyorhexis) and the budding phenomenon were clearly detected in pre-ovulatory oocytes of *C. gariepinus* and this was the most important characteristic feature of apoptosis (Fig. 2B). Chromatin aggregation, condensed nucleus and condensed cytoplasm as the sings of apoptosis were also recorded (Fig. 2C). Apoptotic bodies and large vacuoles between hypertrophic granulosa cells were clearly detected (Fig. 2D). Remarkable enlarged granulosa cells were markedly showed the characteristics of phagocytic cells that devoured vitellus. Some of their nuclei were pyknotic: a crescent-shaped chromatin and cytoplasm material was distinguished as being of a characteristic feature of apoptosis (Fig. 2A, C).

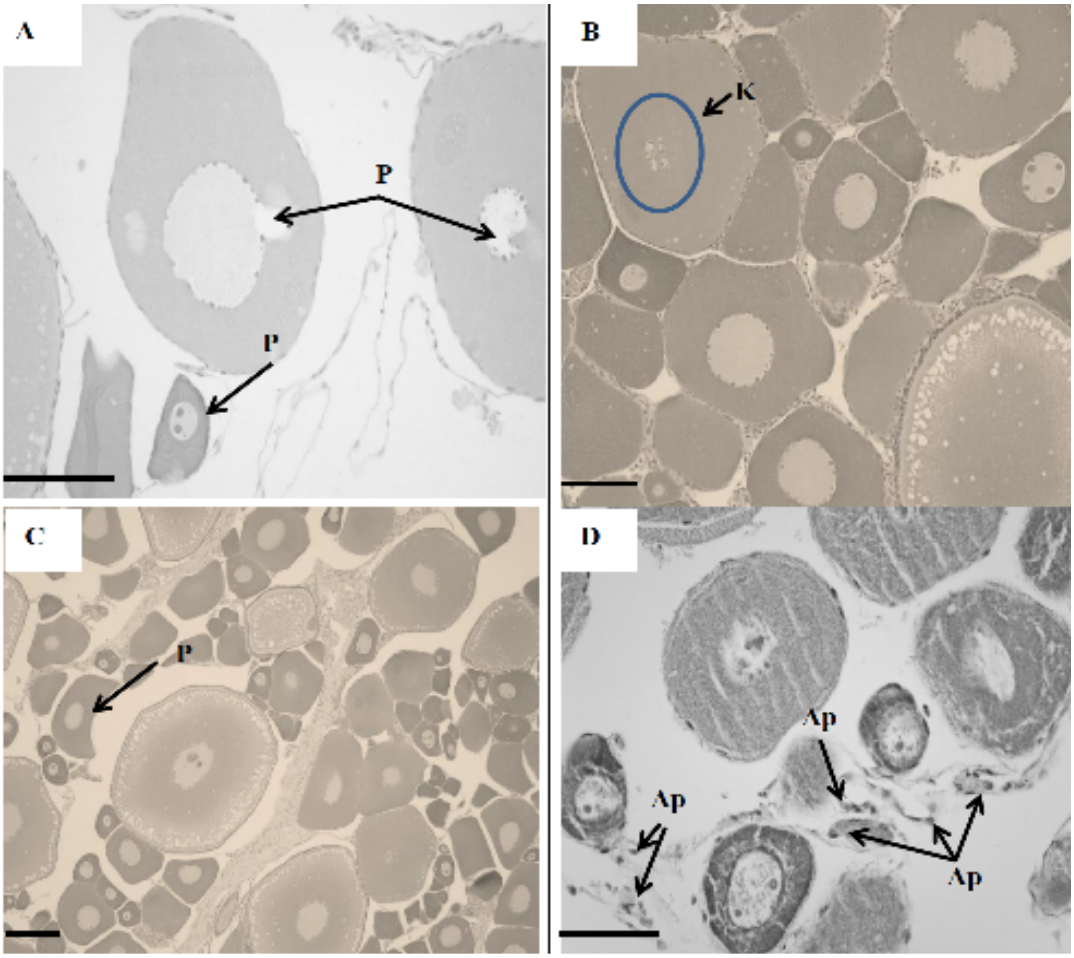

Fig. 2. Apoptosis in pre-ovulatory follicles of *C. gariepinus*. **A and C**, arrow shows pyknosis, irreversible condensation of chromatin in the nucleus and condensation of cytoplasm of oocytes undergoing apoptosis; **B**, pyknosis followed by the fragmentation of the nucleus. (karyorhexis) and the budding phenomenon detected in pre-ovulatory oocytes of *C. gariepinus*. Arrow shows the budding phenomenon. P, pyknosis; K, karyorhexis; Ap, apoptotic body. Scale bar, 200µm. Stain: hematoxylin & eosin.

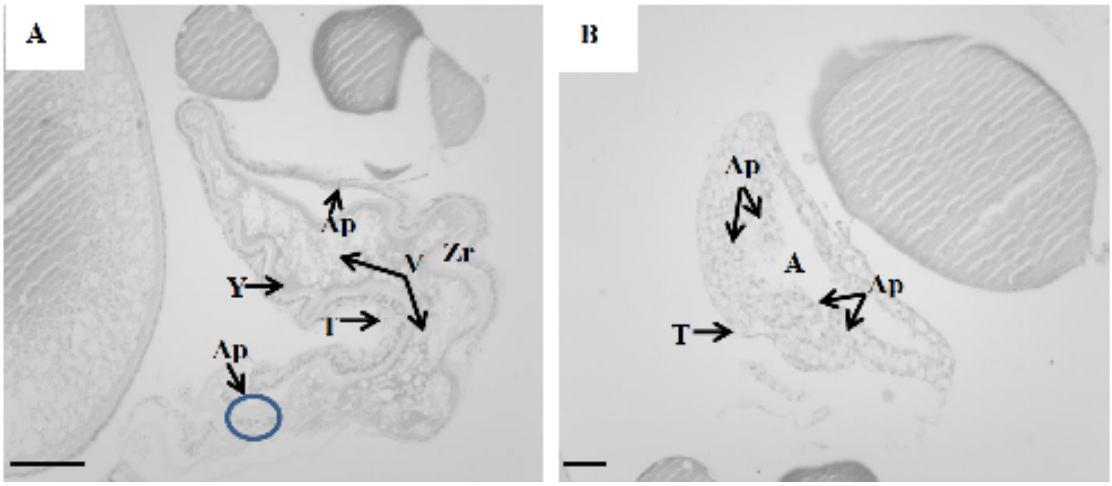

Fig. 3. Apoptosis in pre-ovulatory follicles of *C. gariepinus*. **A**, engulfed yolk, disintegrated zona ratiata, hypertrophic theca cells, invading granulosa cells; **B**, apoptotic body formation is shown. Ap, apoptotic body; Y, yolk; V, vacuole; T, theca; Zr, zona radiata; A, atrium. Scale bar, 200µm. Stain: hematoxylin & eosin.

Other significant sings of apoptosis in pre-ovulatory oocytes were theca and granulosa disruption and vesicle formation. Cytoplasm containing yolk was almost completely engulfed and the vitellus was detected as a partly fractured mass (Fig. 3A). Presence of apoptotic bodies among scattered clusters of granulusa cells indicates that apoptotic process was not fully completed. At the end of the process, vitellus was completely absorbed and left a large cavity behind which termed as atrium (Fig. 3B).

Atresia and apoptosis in postovulatory follicles

After ovulation, the granulosa and theca of the follicle remained within the ovary, which subsequently formed the post ovulatory follicle (POF). They were hollow collapsed structures with a hypertrophied granulosa in the space previously occupied by the oocyte (Fig. 4A). Some of the post-ovulatory follicles had an opening to the peritoneal cavity through which oocytes had been released (Fig. 4A). POF gradually collapsed and showed progressive occupation of the lumen while granulosa decreased in size and theca thickened (Fig. 4B, C). The theca of atretic follicles hypertrophied and persisted to form the interstitial cells (Fig. 4B). Hypertrophy of granulosa cells were clearly detected (Fig. 4C). Apoptotic figures, large vacuoles, interstitial cells were recorded. Connective tissue strands were folded and clearly detected among post-ovulated ovaries. The theca of post-ovulatory follicles was continuous with the epithelial cell layer lining the ovigerous lamellae. The basement membrane of the hypertrophied granulosa was of variable thickness and in some places; the granulosa was separated from the theca at the basement membrane. Many phagocytic follicular cells were detected (Fig. 4D). Occasionally oogonial nests were found amongst the connective tissue and beside the post-ovulatory follicles (Fig. 4E). Yellow bodies were detected at the terminal stages of POF and atretic oocytes. These were prominently represented in post-ovulated ovaries. They consisted of a loosely bound cell mass in which black pigmented bodies were embedded (Fig. 4F).

Apoptotic figures were frequently observed at the termination of ovulation phase. These figures were seen among scattered clusters of granulosa and blood cells (Fig. 5A). The blood cells increased in number whereas the granulosa cells regressed and disappeared. In advance stages of POF degeneration, granulosa cells formed an irregular mass attached to the basement membrane (Fig. 5B). Following irregular inflection of basement membrane, nuclei with condensed chromatin and showing pyknosis, were detected at the lumen of the follicle and at the follicular wall (Fig. 5C). The apoptotic cell appeared as a round or oval mass wıth dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments (Fig. 5C). Blood vessels were most commonly detected close to the connective tissue and follicular cells (Fig. 5D).

DISCUSSION

The developmental pattern of ovaries of *C. gariepinus* was categorized as the group-synchronous type (Çek and Yılmaz, 2007). Cumaranatunga (1985) and Tyler and Sumpter (1996), observed increased number of atresia in group-synchronous Rainbow trout ovaries at vitellogenic stage. The results of the present study were consistence with these findings.

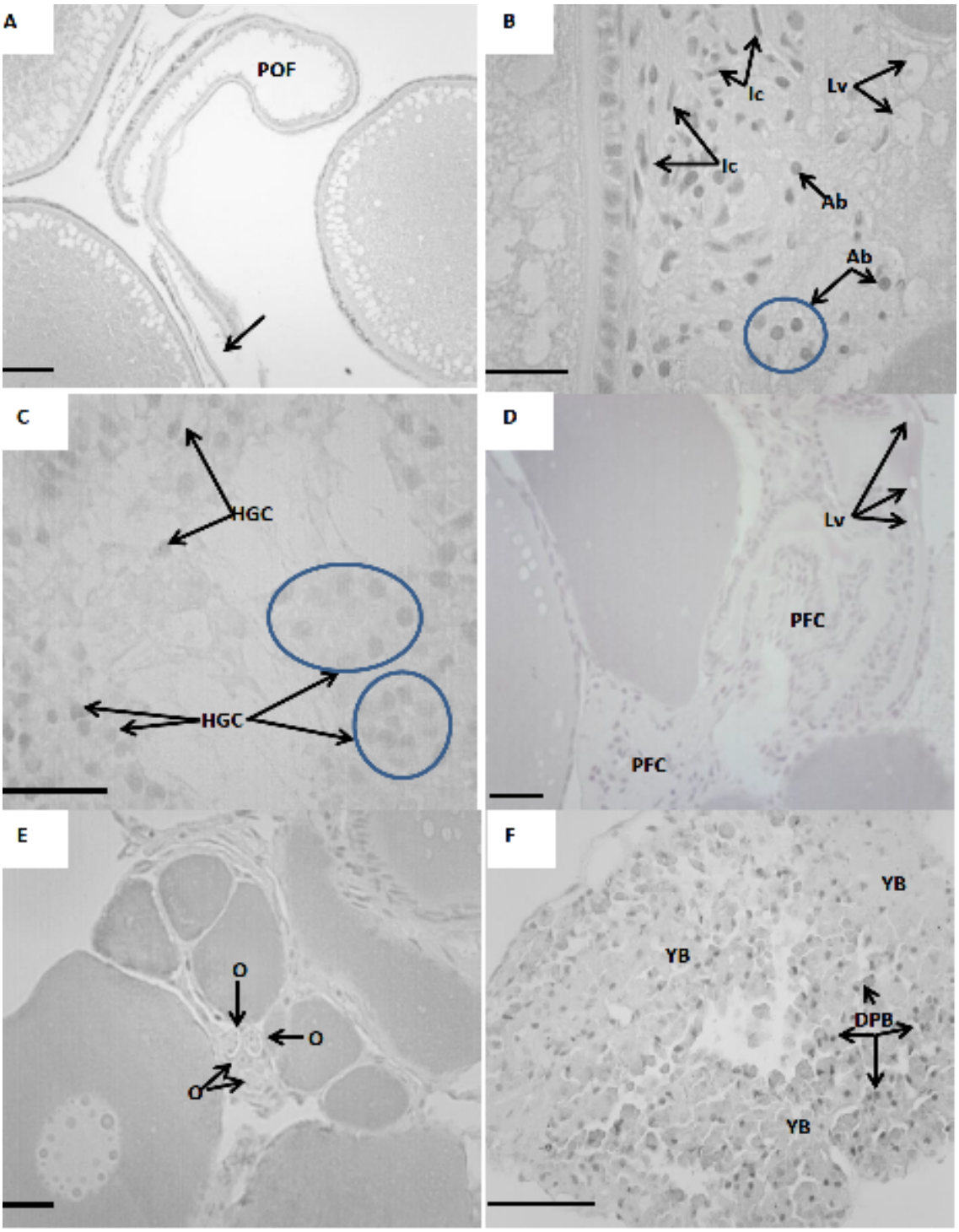

Fig. 4. Atresia at post ovulatory follicle of *C. Gariepinus*. **A**, arrow shows evidence of the rupture of the follicular wall after ovulation; **B**, formation of interstitial cells; **C**, hypertrophy of granulosa cells are shown; **D**, phagocytıc follicular cells are shown; **E**, Arrow shows oogonia which were occasionaly detected amongst the connective tissue; **F**, arrows show yellow bodies which prominently represented in post ovulated ovaries. Dark pigmented bodies are also shown. POF, post ovulatory follicle; YB, Yellow bodies; Ab, apoptotic bodies; O, oogonia; DPB, dark pigmented bodies; HGC, hypertrophied granulosa cells; PFC, phagocytic follicular cells; Lv, large vacuole; Ic, interstitial cells. Scale bar, 200µm. Stain: hematoxylin & eosin.

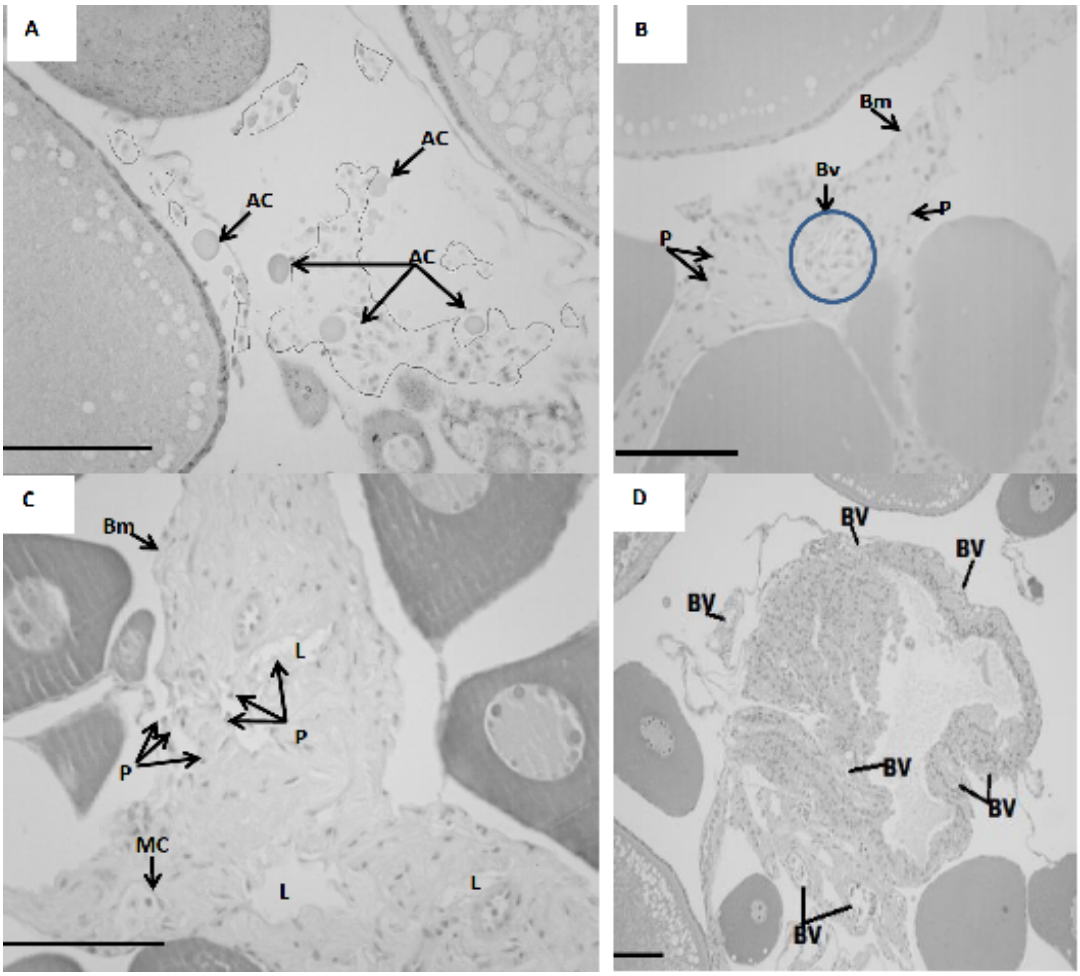

Fig. 5. Apoptosis in POF. **A**, all irregular circulus show various type of blood cells. Arrows show apoptotic cells; **B**, arrow shows blood vessels. Piknosis were also detected; **C**, following inflection nuclei with condensed chromatin and showing pyknosis, were detected at the lumen of the follicle and at the follicular wall (arrows); **D**, blood vessels close to the follicular cells. Bm, basement membrane, MC, micropylar cell, AC, apoptotic cell; BV, blood vessels; P, pyknosis. Scale bars, 200µm. Stain: hematoxylin & eosin.

In *C. gariepinus*, we observed atresia in pre-ovulatory, post ovulatory follicles and in all developmental stages of oocytes. In our study, the process of ovarian follicle atresia and resorption was preceded by marked morphological changes in both the oocyte and follicular cells, such as the disintegration of the oocyte germinal vesicle and of other cytoplasmic changes, the fragmentation of the zona radiate and the hypertrophy of the granulosa cells. These cells become phagocytic with digestive vacuoles, incorporate, and digest the oocytes yolk as well as other oocyte components and organelles. Guraya (1986) suggested that these cells might also secrete enzymes, which digest the yolk. Theca and granulosa cells were also defined as invasive cells by Guraya (1986), Miranda *et al*. (1999), Privalikhin *et al*. (2015) and Senarat *et al*. (2015).

Atresia in pre-ovulatory follicles of *C. gariepinus* was mainly divided into three stages; atresia in primary growth phase, atresia in the secondary growth phase and atresia in the maturation and hydration phase. The non-ruptured follicular wall characterized atresia in primary growth phase. The ooplasm size was drastically reduced and appeared as a dark stain mass. The nucleoli that were generally arranged to form a regular layer in the nuclear envelope of normal grooving previtellogenic oocytes were distributed irregularly in the nucleoplasm. Atretic oocytes at the secondary growth phase were divided into 3 stages, namely: atretic oocytes at stage three, four and five. During maturation and hydration phase (atresia in the maturation and hydration phase), highly columnar granulosa cells exhibiting intense phagocytic activity

ingested most of the yolk and zona radiata remains. Moreover, granulosa layer was completely bursting into pieces due to vesicles formation. Similar classification of atresia in the pre-ovulatory follicles of rainbow trout was previously made by Cumaranatunga (1985). In our study, the yolk at pre-ovulatory stage and zona pellucida after post-ovulation degenerated, which were engulfed by hypertrophied follicular cells as has also been reported for other several species (Tyler and Sumpter, 1996; Thome *et al*., 2009; Üçüncü and Çakıcı, 2009). In the pre-ovulatory phase, germinal vesicle migration towards the micropyle was detected in most oocytes. This result was interpreted as an important event of the final oocytes maturation.

In the present study, granulosa cells become highly columnar during the onset of postovulatory regression. POF gradually collapsed and showed progressive occupation of the lumen while granulosa decreased in size and theca thickened. Hypertrophy of granulosa cells was clearly detected. The theca of post-ovulatory follicles was continuous with the epithelial cell layer lining the ovigerous lamellae. Similar results were also detected in other fish species (Santos *et al*., 2005, 2008; Celestino *et al*., 2009; Thomé *et al*., 2009; Üçüncü and Çakıcı, 2009). At advanced atresia and in POF regression, we often observed the presence of blood cells such as erythrocytes and leukocytes, possibly, derived from the ovarian stroma and/or the theca, which invaded the degenerating oocyte. The presence of granulocytes (polymorphonuclear leukocytes) in atretic follicles is reported in other fish species (Santos *et al*., 2005; Tingaud-Sequeira *et al*., 2009; Kumar and Joy, 2015; Privalikhin *et al*., 2015) and suggested a relationship between follicular regression and immune cells. The specific function of immune cells (eosinophilic granulocytes and macrophages) during follicular atresia in fish is not well known, although it has been proposed that they may act synergistically with follicular cells in the resorption of the oocyte by releasing their granules containing lytic enzymes (Besseau and Faliex, 1994). Lang (1981) did not revealed any effects of immune cells in the resorption of the oocytes from the ovary. According to Besseau and Faliex (1994), follicle and immune cells invade the degenerating oocyte and leading to the formation of yellow brown bodies. In our study, we detected yellow-brown bodies at the terminal stages of POF and atretic oocytes. These were prominently represented in post-ovulated ovaries. They consisted of a loosely bound cell mass in which black pigmented bodies were embedded. Kumar and Joy (2015) studied on the catfish, *Heteropneustes fossilis* and suggested melanin assay to be used as a follicular atresia biomarker. Since they indicated melanin in the yellow-brown bodies. In our study, yellow-brown bodies were most detected close to the blood vessels similar to those founding by Besseau and Faliex (1994). Privalikhin *et al*. (2015) have documented macrophage cell involvement in *Theragra chalcogramma*. They suggested two pathways regarding to involvement of immune cells in atresia; follicular and follicular histiocytic (mononuclear phagocyte system); degeneration of oocytes in early stages of development occurs with the participation of follicular cells, whilst late developmental stages characterized by both follicular and histiocyte cell involvement. The histiocytes are likely to be of hematogenous or connective tissue origins and they engaged in phagocytic functions (Privalikhin *et al*., 2015). In the present study, blood vessels were most detected close to the connective tissue and follicular cells similar to those founding by Besseau and Faliex (1994) and Privalikhin *et al*. (2015) (Fig. 5D). Therefore, our results support the involvement of both, theca and blood cells in digesting oocytes contents.

During post-ovulatory phase, ovarian regression, shrinkage and resorption of both postovulatory and atretic follicles occurred and the ovaries returned to the resting period to initiate another reproductive cycle. At this stage, ovaries of *C. gariepinus* showed growing follicle in all stages of development besides germ cells and remarkable number of oogonia along the germinal epithelium were detected. During the ovarian regression of *C. gariepinus* oogonia continued to proliferate and increased in number. In addition, many perinucleolar oocytes were detected. This finding was consistent with the results of Santos *et al*. (2008), Wildner *et al*. (2013) and Pan *et al*. (2016). However, it was contradictory to mammals where restricted number of oogonia presented in the gonads of adult females (Zuckerman, 1951; Hirshfield, 1991; Jalabert, 2005). In recent years, dogmatic view that oogonia and oocytes cannot be renewed in mammals after birth was challenged when Tilly and her colleagues (Tilly *et al*., 2009) and others (Woods *et al*., 2012; Pan *et al*., 2016) reported that the rate of oocyte loss through follicular atresia and ovulation was much higher than the net rate of oocyte decline.

Programmed cell death or apoptosis is a genetically regulated process, which plays a fundamental role during the developmental homeostasis of multicellular organisms (Jenkins *et al*., 2013). It is characterized by cell fragmentation forming apoptotic bodies, which are engulfed by phagocytes or neighboring cells, thus avoiding inflammatory reaction (Drummond *et al*., 2000). The presence of apoptotic bodies was observed only in atretic follicles, which was likely due to the absence of survival factors in oocyte that induce the activation of the endogenous apoptosis pathways (Quirck *et al*., 2004) leading to oocyte atresia. In our study, apoptosis

was detected in pre and post-ovulatory follicles. In pre-ovulatory follicles, cell shrinkage and pyknosis were visible by light microscopy. Pyknosis was the result of chromatin condensation and margination. Similar results were also detected by Santos *et al*. (2005, 2008) and Üçüncü and Çakıcı (2009) in different fish species. Karyorhexis and the budding phenomenon were detected in pre-ovulatory oocytes of *C. gariepinus* and this was the most important characteristic feature of apoptosis. Majno and Joris (1995) defined Karyorhexis and the budding phenomenon in detail. Remarkable enlarged granulosa cells were markedly showed the characteristics of phagocytic cells that devoured vitellus. Some of their nuclei were pyknotic. Other significant sings of apoptosis in pre-ovulatory oocytes were theca and granulosa disruption and vesicle formation. Presence of apoptotic bodies among scattered clusters of granulusa cells indicates that apoptotic process was not fully completed. At the end of the process, vitellus was completely absorbed and left a large cavity behind which termed as atrium. Üçüncü and Çakıcı (2009) detected this feature of apoptosis in zebra fish. Apoptotic bodies were observed in theca cells and follicular cells from follicles after ovulation. In the mammalian ovary, apoptosis is mainly involved in the removal of oocytes during fetal life and in the removal of granulose cells of the growing follicles during adult life. In teleost, apoptosis is important in the selection and recruitment of follicles for vitellogenesis and in ovarian regression after spawning (Santos *et al*. 2008).

According to Drummond *et al*. (2000), Santos *et al*. (2005) and Santos *et al*. (2008), the main morphological characteristics of the apoptosis in POF are: scattered single cells affected rather than clusters of continuous cells, absence of inflammation, aggregates of condensed chromatin as a crescent cap adjacent to the nuclear envelope, apoptotic body formation. These features were also detected in the present study. In addition to these features, karyorhexis and budding phenomena were also clearly observed in the pre-ovulatory follicles of *C. gariepinus*, suggesting that the apoptosis could be the major mechanism responsible for the elimination of follicular cells during inflection of the pre and post ovulatory follicles of *C. gariepinus*. Although, apoptosis may be a very important event during the ovarian cycle of *C. gariepinus*, the mechanism of the process were not explained in detail, in the present study. Janz and van der Kraak (1997) suggested that apoptosis might have an important role in the selection and recruitment of follicles into vitellogenesis in Rainbow trout. It is also possible that other types of cell death, aside from apoptosis will also be occurring in the POF of *C. gariepinus*.

CONCLUSION

Atresia in *C. gariepinus* occurred throughout all periods of oogenesis and it should be regarded as a normal reaction to stressful conditions, (nutrition, biocides agents, captivity, light, temperature, inadequate hormone levels, salinity fluctuations). The apoptosis may be the major mechanism responsible for the resorption of pre and POFs of *C. gariepinus* and accounts for deletion of the follicular cells. Although the morphological evidence of apoptosis in pre and POFs of *C. gariepinus* appears to be unequivocal, additional studies are necessary to evaluate the fragmentation of the DNA by using in situ labeling with TUNNEL technique.

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Statement of conflict of interest

The authors declare that they have no conflict of interest.

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