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Sharma R.K
Department of Zoology, Kurukshetra University, Kurukshetra - 136119, India

Bhat R.A.
Department of Zoology, Kurukshetra University, Kurukshetra - 136119, India

Correspondence:
Sharma R.K
Reproductive Physiology Laboratory, Department of Zoology Kurukshetra University, Kurukshetra-136119

Histomorphology of atretic follicles in rainbow trout (*Oncorhynchus mykiss*) from Kashmir

Sharma R.K. and Bhat R.A.

ABSTRACT
Follicular atresia is a prevalent degenerative phenomenon in vertebrate ovaries which involves the oocyte as well as follicular components. The present study was aimed to demonstrate the occurrence of follicular atresia and to determine the different histological characteristic features of the Rainbow trout ovaries at different stages of the development. The distinct features of atretic follicles in rainbow trout ovary in previtellogenic stages included the appearance of gap between zona pellucida and ooplasmic contents. Vitellogenic stage was characterized by the presence of pycnotic nuclei with hyaline cytoplasm, hypotrophy and hyperplasia in granulosa cells, disruption in thecal as well as granulosa cell layers, folding of zona pellucida upon itself, formation of loops in advanced stages of atresia and fully degenerated vitellus which got absorbed by the surrounding follicle cells.

Keywords: Follicular atresia, granulosa cells, hyperplasia, hypotrophy and Rainbow trout.

1. Introduction

Follicular atresia is a degenerative process by which oocytes in different developmental stages and growth are lost from the ovary, being the major cause of reduced fertility in any species [1]. Follicular atresia has been extensively studied in cyclostomes, teleosts and elasmobranchs [2, 3, 4]. For Salmonids, atresia has been reported in Atlantic salmon, *Salmo salar L.* [5, 6], Brook trout, *Salvelinus fontinalis* (Mitchill) [7, 8] and Rainbow trout, *Oncorhynchus mykiss* (Walbaum) [9, 8]. Follicular atresia in the fish ovary generally occurs during pre-spawning, spawning and post spawning periods. Despite the vast literature on oogenesis in teleost fish [10, 11], little is known about the cellular as well as molecular aspects of follicular atresia involving the degenerative processes of oocyte and follicular wall [12, 13]. Follicular atresia is considered to be a very common phenomenon in vertebrate ovaries under both natural as well as experimental conditions [3] and can be induced by external factors such as stress, fasting, biocidal agents, light, temperature, confinement and inadequate hormone levels [14]. The most important significance of follicular atresia during the normal course of reproduction is to limit the number of eggs that could be supported for vitellogenesis, maturation and ovulation by the female fish. Studies on follicular atresia in fish are of vital importance for fish breeding, since the degenerative processes occurring in the ovary affect fertility rates [15, 16, 17]. Ovarian atresia in non-mammalian vertebrates has received a very less attention than that of mammals, but in the recent past, morphological characteristics and the functions of atresia in the teleost ovaries are widely being discussed [18, 19, 20, 21, 22].

In the present study, rainbow trout ovaries from prespawning to spend season were analyzed histologically to determine the prevalence of atresia.

2. Materials and Methods

For the present study, mature Rainbow trout (*Oncorhynchus mykiss*) were procured from different hatcheries especially from Verinag (33.55̊ N and 75.25̊ E) and Kokernag (33.69̊ N and 75.22̊ E) hatcheries of Kashmir valley (Jammu and Kashmir) during February, 2013 to December, 2013. Soon after capture, the fish were sacrificed and ovaries were dissected out. For histological studies, the tissue was fixed in Bouin's fixative (picric acid, formalin and acetic acid in the ratio 75:20:5) for 24 h then transferred to 70% ethanol. These samples were processed through an ethanol grades for dehydration, cleared in xylene and embedded in wax for sectioning. Sections of about 5 μm thickness were cut and stained with haematoxylin and eosin. The stained slides were examined under light microscope.

3. Results

During the present investigation, follicular atresia was frequently observed in vitellogenic stage. However previtellogenic phase was also affected. During atresia the oocyte were observed to shrink, and gradually undergo various stages of degeneration and resorption. The growth of Rainbow trout oocytes was divisible in seven stages, based on morphological basis. Stage I- Chromatin-nucleolus stage, Stage II– Perinucleolus stage, Stage III- Cortical alveolus stage, Stage IV- Primary vitellogenic Stage (Figure 1), Stage V- Secondary vitellogenic stage (Figure 2), Stage VI- Maturation stage and Stage VII- Spent stage. These types of oocytes were characterized by features like cortical alveoli, yolk globules, zona radiata, zona interna, zona externa, granulosa cells, thecal cells.

The atretic follicles at an early stage of atresia were characterized by the gap between zona pellucida and ooplasmic contents and decrease in follicular diameter (Figure 3). On advancement of the follicles in atresia stage i.e., in vitellogenic stage there was disorganization of the nucleus and cytoplasm of the oocyte and folding of the oocyte wall (Figure 4). The folding, fracturing and dissolution of zona pellucida in vitellogenic stage (Figure 5). The presence of pycnotic nuclei and undulation of the zona pellucida was also observed during vitellogenic stage. Hypotrophy was observed in some areas of granulosa cells and hyperplasia in localized areas (Figure 6). Some morphological differences were observed in granulosa cells, some of the cells were cuboidal and some of the enlarged granulosa cells were irregular in shape (Figure 7). The other morphological observations were folding of zona pellucida upon itself and formation of hairpin loop at certain places because of shrinkage of ooplasmic contents (figure 8); fusion and liquefaction of the yolk globules and enlargement of the thecal cells (Figure 9); formation of gap between theca and granulosa cells and hypotrophy in thecal cells [10].

Fig 1: Microphotograph showing previtellogenic oocytes with well-developed nucleus (N). Hematoxylin & Eosin (100X).

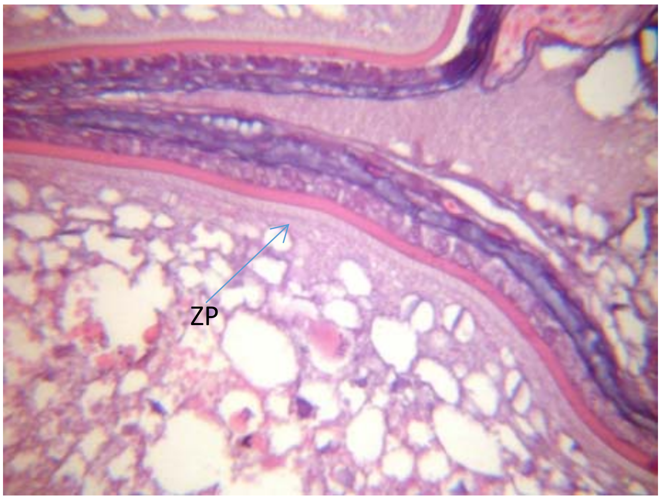

Fig 2: Microphotograph vitellogenic oocyte with well characterized zona pellucida (ZP).Hematoxylin & Eosin (400X).

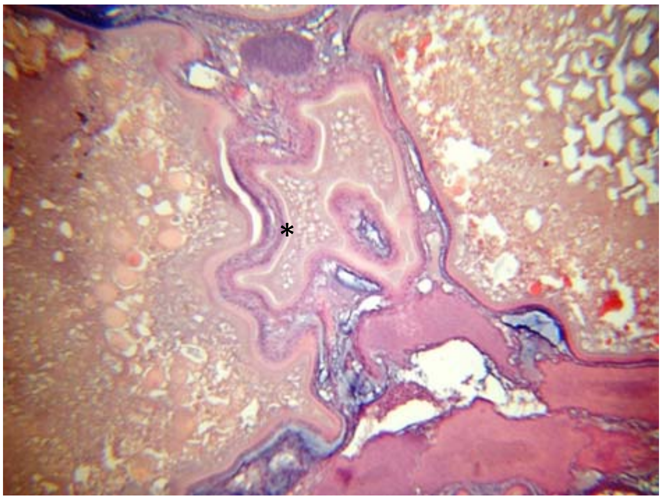

Fig 3: Microphotograph of oocyte shows gap (*) between zona pellucida and ooplasmic contents in previtellogenic follicles.Hematoxylin & Eosin (100X)

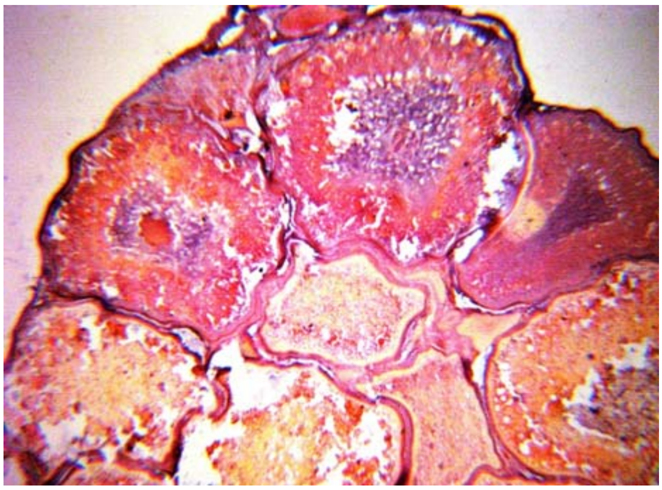

Fig 4: Microphotograph showing disorganization of the nucleus, cytoplasm of the oocyte and folded oocyte wall.Hematoxylin & Eosin (100X)

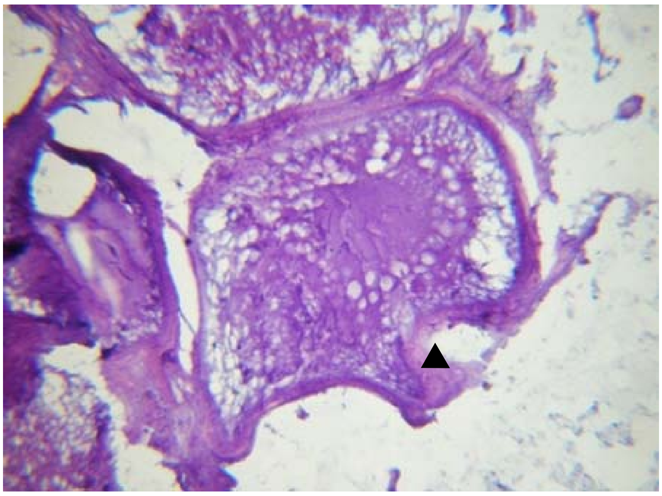

Fig 5: Microphotograph showing folding, fracturing and dissolution of zona pellucida (Arrow). Hematoxylin & Eosin (100X)

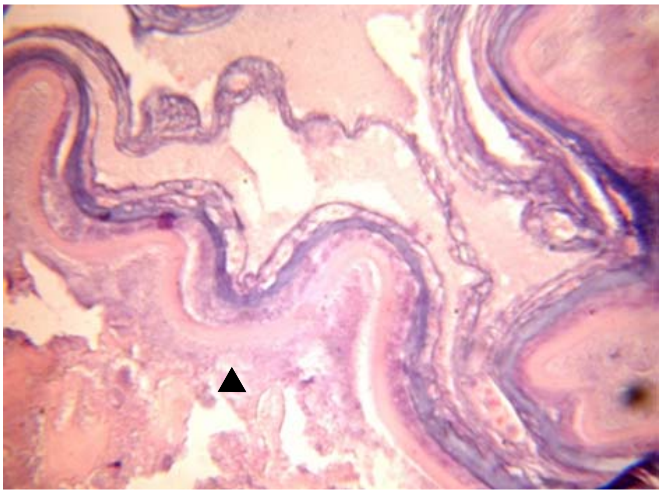

Fig 6: High power view showing the presence of pycnotic nuclei with hyaline cytoplasm, granulosa cell diffusion and undulation of zona pellucida (Arrow). Hematoxylin & Eosin (400X)

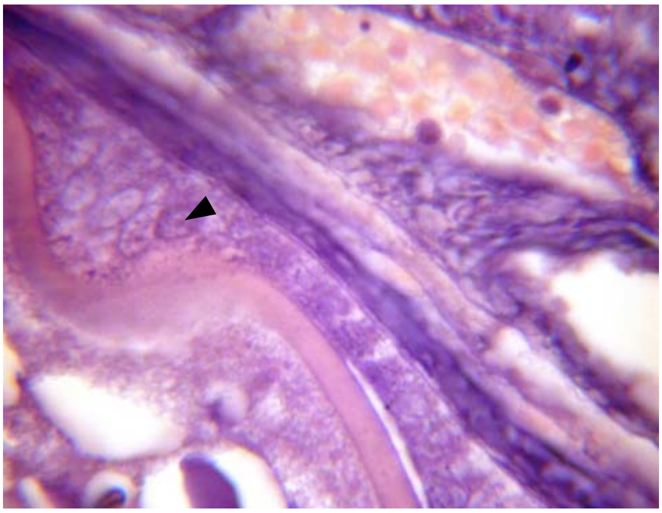

Fig 7: Microphotograph showing granulosa cells (Arrow) of different morphological shapes and sizes. Hematoxylin & Eosin (400X).

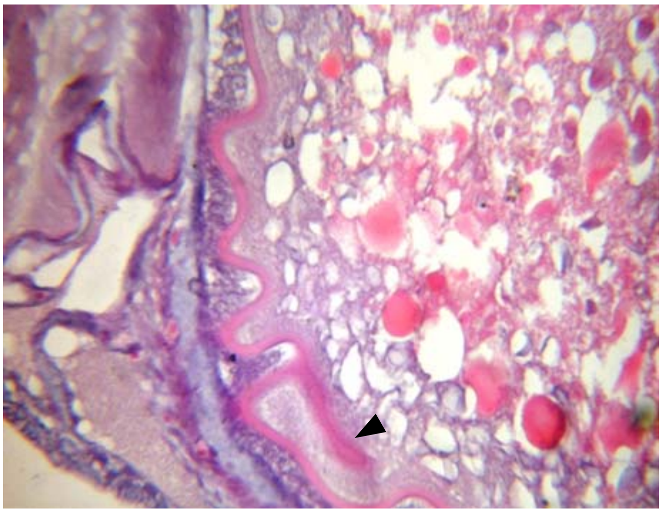

Fig 8: Microphotograph showing folding of zona pellucida upon itself and forms hair loop structure (Arrow) and thecal cell hypotrophy. Hematoxylin & Eosin (400X)

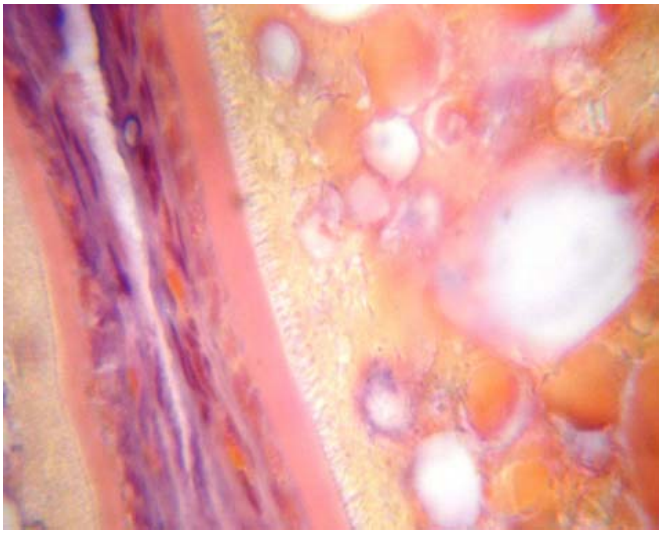

Fig 9: Microphotograph showing fusion and liquefaction of the yolk globules. Hematoxylin & Eosin (1000X)

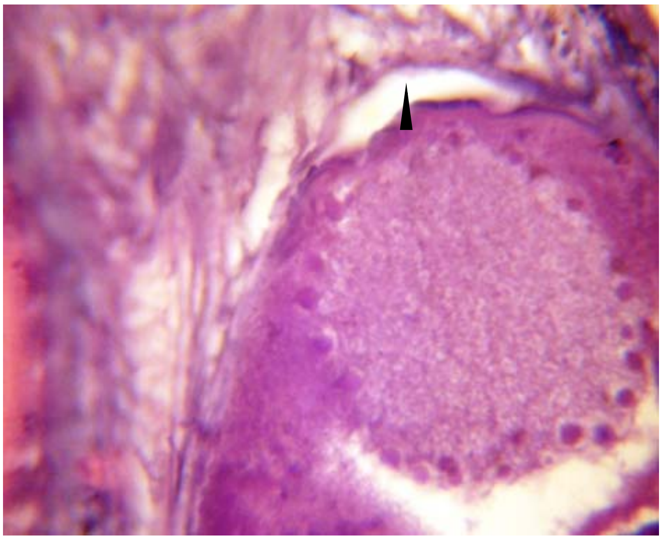

Fig 10: Microphotograph showing gap between theca and granulosa cell (Arrow) and hypotrophy in thecal cells. Hematoxylin & Eosin (400X)

4. Discussion
Atretic follicles are important indicators of environmental impact on fish ovaries. However, the mechanism of follicular atresia is not yet fully understood [22]. Rainbow trout is single spawning fish and most of its ovarian follicles are at synchronous stage of development. In previtellogenic follicles in fish, the first sign of atresia was the development of clear spaces in the peripheral ooplasm. The ooplasm got separated from the nucleus by a clear space as a result of shrinkage. Another characteristic feature of follicular atresia in this stage was the disorganization of ooplasmic and nuclear components. The disintegration of the oocyte nucleus was followed by the folding of the chorion and development of spaces in the peripheral ooplasm. Similar features of follicular atresia were observed in earlier studies in many fish species [23, 24, 25]. Follicular atresia was observed to be more frequent in vitellogenic oocytes in rainbow trout fish. Increased frequency of follicular atresia was also observed in vitellogenic oocytes in *Carassius auratus* [18], *Seriola dumerilii* [26] and *Brycon orthotaenia* [27]. Structural alterations in the present study endorse the earlier reports on vitellogenic follicles. The main histological features of Follicular atresia in vitellogenic stages were: Folding and dissolution of zona pellucida, disorganization and liquefaction of yolk and ooplasm, undulations and formation of the hair loop structure. The variation in frequency of atresia in pre vitellogenic and vitellogenic follicles is possibly due to varied hormone titer and availability of receptors [3].

5. Significance of follicular atresia
The most important significance of follicular atresia during the normal course of reproduction is to limit the number of eggs that could be supported for vitellogenesis, maturation and ovulation of the female fish. But there are some environmental factors that may increase follicular atresia affecting fecundity and adversely affecting the fish survival and reproduction. The mechanism that initiates and regulates follicular atresia is poorly understood at the molecular level. However, factors such as age, stage of the reproductive cycle, hormones, light, temperature, nutrition, irradiation and chemicals like biocides bear a significant impact on follicular atresia.

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