Songklanakarin J. Sci. Technol.
37 (5), 569-573, Sep. - Oct. 2015

http://www.sjst.psu.ac.th

Original Article

Classification stages of novel atretic structure in short mackerel *Rastrelliger brachysoma* (Bleeker, 1851) from the Upper Gulf of Thailand

Sinlapachai Senarat[1], Jes Kettratad[1], and Wannee Jiraungkoorskul[2]*

[1] *Department of Marine Science, Faculty of Science, Chulalongkorn University, Pathum Wan, Bangkok, 10330 Thailand.*

[2] *Department of Pathobiology, Faculty of Science, Mahidol University, Ratchathewi, Bangkok, 10400 Thailand.*

Received: 5 February 2015; Accepted: 14 June 2015

Abstract

An understanding of atretic follicles in wild population is required before investigating the reproductive cycle and spawning season but these follicles have never been reported on adult short mackerel *Rastrelliger brachysoma* (Bleeker, 1851). Histology and histochemistry were used to classify the stage of atretic follicles in *R. brachysoma*, obtained from the Upper Gulf of Thailand. Microscopically, it was clear that under atretic processing this species could be successively divided into two phases: atretic follicle during previtellogenic and vitellogenic stages, in which the latter was also classified into five steps (I, II, III, IV, and V). Histochemically the cortical alveoli, yolk granules and basement membrane were observed and discussed in this study.

Keywords: atretic follicles, histology, *Rastrelliger brachysoma*, short mackerel, fish, Thailand

1. Introduction

One of the major part in the oogenic processing in teleost fish and other vertebrates is called atretic follicles (Genten *et al.,* 2009). In terms of these follicles being referred to as unovulated oocytes are obviously observed under the degeneration and resorption of oogenic cells (Kennedy, 2002; Santos *et al*., 2008) and the physiological processes as well as homeostasis of the follicles (Hussein, 2005). Variability degrees of this follicle are dependent upon seasons, temperature, light, food (Saidapur, 1978) and chemical compounds, particularly endocrine disrupting chemicals (Johnson *et al.,* 2009). Therefore, investigations of follicles have been reported in several fish species and discussed in many aspects s including morphology and biochemistry as well as structural functions (Kennedy, 2002; Wood and van der Kraak, 2002, 2003; Santos *et al*., 2008). As to the present objectives, the classification of follicles can be useful for the estimation of oocytes and the prediction of the spawning frequency in the fish population as well as reproductive health status (Hunter and Glodberg, 1980; Hunter and Macewicz, 1985; Hunter and Lo, 1997; Blazer, 2002; Ganias *et al*., 2003; Johnson *et al*., 2009).

An economic important marine fish, *Rastrelliger brachysoma* is a very popular fish due to its low price and its consideration as a source of cheap protein for Thai consumers. The catch of *R. brachysoma* for the year 2009 (115,400 tons) was significantly less than the annual catches of approximately 143,500 tons in 2005 to 2008 (Department of Fisheries, 2009). The reduction of the wild population of *R. brachysoma* may due to overfishing and/or the deterioration of their natural habitats. If the decrease of the *R. brachysoma* population continues at this rate, this fish might become extinct in the Gulf of Thailand and food insecurity

* Corresponding author.
Email address: wannee.jir@mahidol.ac.th

could become a major issue in the near future. Up until now, it has also been exclusively considered as a good candidate fish for aquaculture in Thailand. An understanding of the gonadal structure in the wild population is required before investigating the reproductive cycle and spawning seasons. Although the gonadal structure and gametogenesis in this species living in the Upper Gulf of Thailand has been primarily reported using histological and histochemical investigation (Senarat *et al*. *in process*), it was not histologically defined as to the classification schemes in the atretic follicles.

During the breeding season, histological and histochemical approaches were used to investigate the histological stage of atretic follicles in *R. brachysoma*, as obtained from the Upper Gulf of Thailand. Surely, this result will enrich the understanding regarding the gonadal histological evidences of this species. Moreover, the atresia has not yet been applied to the natural habitats of this fish so it can be used to establish the preparation for the criteria in both estimating and quantifying during the spawning season, fish health as well as sustainable management.

2. Materials and Methods

2.1 Sample collection and study site

Sexual mature female, *R. brachysoma* with a weight of 90-120 g and total length of 16.5-20.0 cm were caught during the breeding season (January 2013 to February 2014; n=10) with bamboo strake trap from Samut Songkram province (13°16'18.4" N, 100°02'13.4" E), which were lived in the Upper Gulf of Thailand. The species identification was according to the identification key of FAO (FAO, 2010). The experimental protocol was approved by the Animal Care and Use Committee of Faculty of Science in accordance with the guide for the care and use of laboratory animal prepared by Chulalongkorn University (Protocol Review No.1423003).

2.2 Histological and histochemical observations

In the laboratory, all fish were euthanized by rapidly cooling shock (Wilson *et al*., 2009). Afterwards, ovaries were dissected out and immediately fixed in Davidson's fixative (about 36 hrs.) for further histological analysis. Ovarian tissues were primary dehydrated, cleared and embedded in paraffin. Cross and longitudinal sections were cut at a thickness of 6-7 µm and then, they were stained by hematoxylin and eosin (H&E). Other sections were histochemically stained by Masson's trichrome (MT), aniline blue pH 2.5 (AB) periodic acid-schiff (PAS) and reticulin method (RT) (modied from Puchtler and Waldrop, 1978; Humason, 1979; Vidal, 1988; Bancroft and Gamble, 2007). Microscopically, it was examined to assess the histological stage of the atretic follicles according to the guidelines of Ganias *et al*. (2003).

3. Results and Discussion

The gross anatomy of ovaries in the *R. brachysoma* during the breeding season had paired and elongated structures showing yellow color (before fixation) and white color (after fixation) within the peritoneal cavity (Figure 1A and 1B). At the light microscopic level, the analysis of ovaries of *R. brachysoma* obviously exhibited during breeding season as containing the differential stages of oocyte because it was considered as asynchronous developmental oocytes according to MT staining (Figure 1C to 1D). In this study, it could be classified into two phases based on size, histological structure and staining properties as follows:

3.1 Phase I the classification of the atretic follicle during previtellogenic stage

The characterization of atretic follicle in the previtellogenic stage was similarly seen with normal stage, but the ooplasm shown as basophilic cytoplasm with a surrounding thin follicular layer (PAS staining) (Figure 2A and 2B).

3.2 Phase II the classification stage of the atretic follicles during vitellogenic stage

In general, the characterization of normal vitellogenic stage based on histology and histochemistry was 250-300 µm in diameter with numerous small yolk granules. Among its yolk granules, the oil droplets and cortical alveoli were distinctly detected. Also, this stage was surrounded by

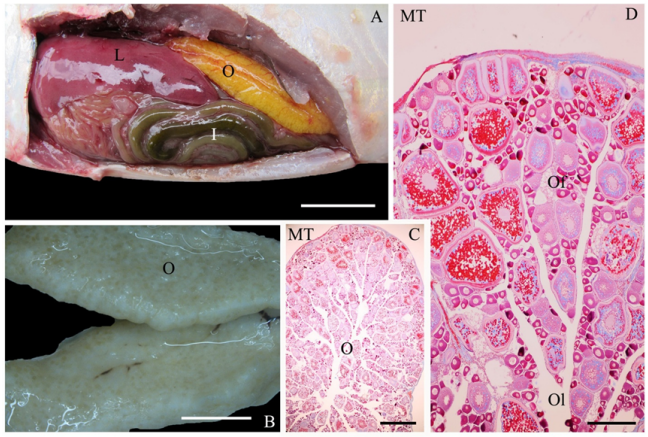

Figure 1. Micrograph of gross anatomy and histology of ovaries in *Rastrelliger brachysoma* during breeding season; (A) ovarian morphology (pre-fixation) = 1 cm; (B) ovarian morphology (post-fixation) = 1 cm; (C-D) ovarian histology = 200 µm. *I = intestine, L = liver, O = ovarain tissue; Of = ovigerous fold; Ol = ovarian lumen. (MT = Masson's trichrome stain).*

follicular complex which was clearly divided into three well-developed layers: (i) zona pellucida composing of two layers; inner and outer zona pellucida, (ii) granulosa and (iii) theca cells, respectively. Among normal vitellogenic stage, histological details of several atretic follicles during vitellogenic stage in this species were detected. It could be completely divided into four steps according to shape, characterizations of nucleus and follicle complex (Figure 2D to 2I).

Stage I, the histological appearance in this stage was quite similar to that of normal vitellogenic stage, but some microstructures were initially seen including nucleic disintegration with irregular shapes. Some areas of yolk granules were digested, especially in the periphery of the ooplasm. Irregularity in shape and degeneration with separation between inner and outer layers of the zonapellucida were initially and continuously detected. Additionally, hypertro-

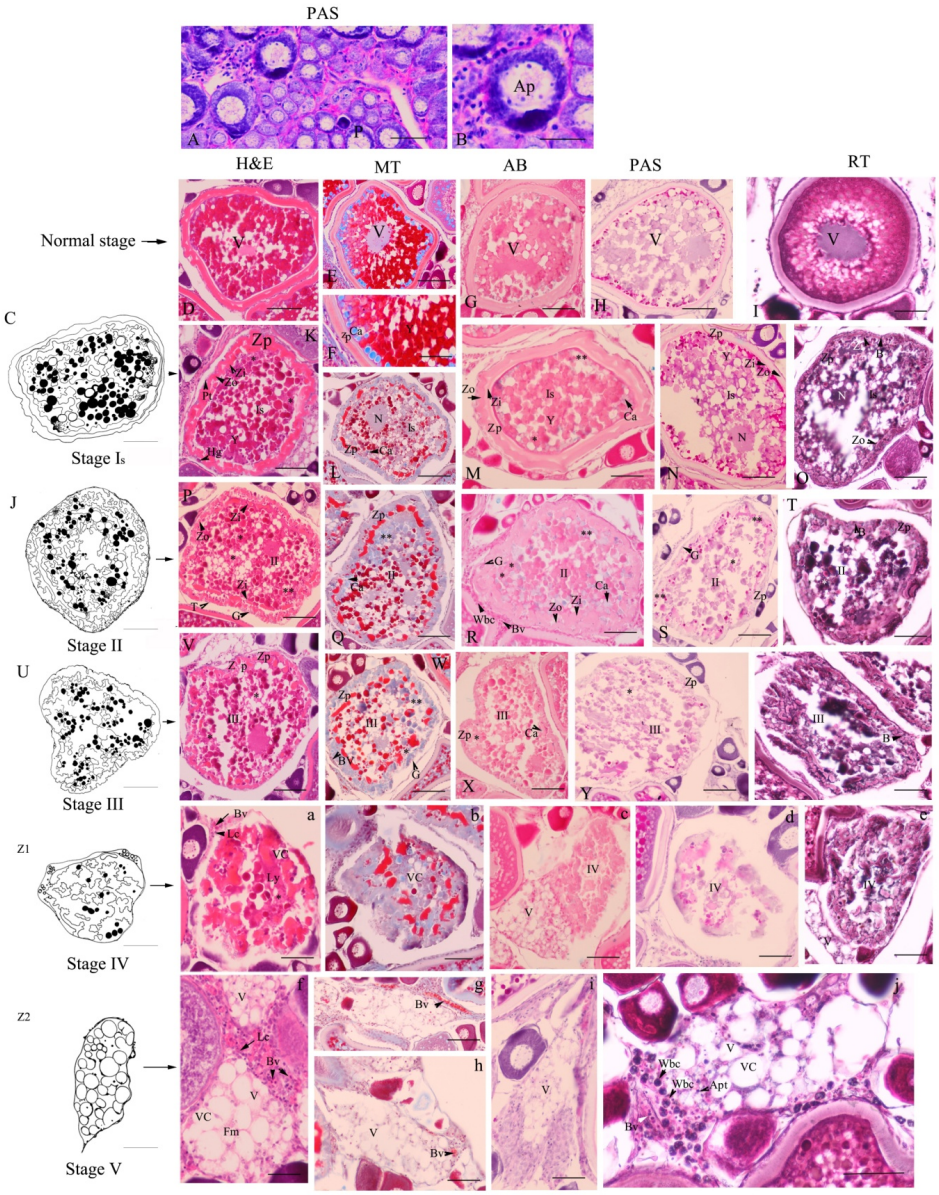

Figure 2. Schematic diagram, micrograph of histology and histochemistry of atretic follicles in *Rastrelliger brachysoma*; (A-B) atretic follicle of previtellogenic stage (Ap), A = 100 µm, B = 50 µm; (D-I) Normal vitellogenic stage (V) = 70 µm; (C, K-O) Stage Is = 50 µm; (J, P-T) Stage II = 50 µm; (U-Z) Stage III = 50 µm; (Z1, a-e) Stage IV = 50 µm; (Z2, f-j) Stage V = 50 µm; *Apt = apoptosis, B = basement membrane, Bv = blood vessel, Ca = Cortical alveoli, G = granulosa cell, Hg = hypertrophy of granulosa cell, Lc = lymphocytes, Ly = liquefaction of yolk granules, N = nucleus, P = previtellogenic stage, Pt = pynotic nuclei, T = theca cell, VC = vacuole, Wbc = white blood cells, Y = yolk granules, Zi = inner layer of zonapellucida, Zo = outer layer of zonapellucida, Zp = zonapellucida, * = degeneration of yolk granule, ** = degeneration of cortical alveoli.(MT = Masson's trichrome, PAS = periodic acid-schiff, AB = aniline blue, Rt = reticulin method).*

phies together with pyknosis of some granulosa and theca cells were detected (Figure 2C and 2K). Based on histochemistry, the cortical alveoli were still positive with MT as reddish and AB as bluish. Other characterizations, yolk granules with slight positively stain with PAS reaction, were specially surrounded by basement membrane (black line with RT) (Figure 2L to 2O).

Stage II, the early stage of stage II was irregular in shape. A sequence of event characterization as follows: the degradation and regression of yolk granules in some area located in the peripheral ooplasm were detected more than in the previous stage, as indicated by the fusing granules. According to H&E, the inner zona pellucida became broken down and fragmented from the degenerating of oocyte, whereas the outer zonapellucida increased highly, containing several fragments as well as islets greater than the inner zonapellucida. The hypertrophy of granulosa cells were also continuously proliferated, which each cell had a spherical nucleus with surrounding the eosinophilic cytoplasm. Externally, the thecal cell layer was located around few blood vessels (Figure 2J and 2P). AB, MT and PAS reactions conformed to previous stage but RT reaction distinctly showed the degeneration due to the fragment of basement membrane (Figure 2Q to 2T).

Stage III, the disorganizations both irregular in shape and shrinkage were obviously seen when compared with the prior stage. The nucleus in this stage was seen and slightly observed in some oocytes among the degeneration and digestion of yolk granules. The highly increasing fragmentations of zona pellucida gradually continued more than in the previous stage. Exclusively, it was also confirmed that inner layers of zonapellucida were first degenerated and continuously observed in its outer layer. The granulosa and theca were not separated and rarely seen. Surprisingly, in this stage, the leucocytes were mostly found and continued to be found near oocyte (Figure 2U and 2V). Histochemically, the cortical alveoli were slightly seen and began to degenerate (AB and MT stains) whereas RT reaction confirmed that no basement membrane was seen during this stage (Figure 2W to 2Z).

Stage IV, the cell size was intermediately decreased when compared with former stage. Unlike the previous stage, the zona pellucida was greatly degenerated among liquefaction of yolk granules. During reabsorption, some area in the ooplasm was shown to contain the vacuoles, referred as empty space. Then, the follicular cells became phagocytize degenerating materials because several leucocytes were presented among a few blood vessels (Figure 2Z1 and 2a). No mucopolysaccharide was observed according to AB and MT, indicating the complete degeneration of cortical alveoli (Figure 2b and 2e).

Stage V follicle was an amoeboid-shape and decreased in size. The follicle (both yolk granules and zona pellucida) itself was completely digested. The large vacuole associated with yellow-brownish pigments within the ooplasm was accumulated (Figure 2Z2 and 2f). Some follicular cells were also presented. Moreover, this stage was surrounded by fibroblast-like cells, as strongly positive with MT and PAS stained (Figure 2g and 2h). It should be noted that this stage could potentially cause by the apoptosis under RT reaction (Figure 2j). The leucocytes were found around the apoptotic follicles (based on RT stained).

Until now, the characterization of atretic follicle has been basically reported in several fishes under regulation of reproductive hormone and reproductive physiology (Ganias *et al*., 2003; Nagahama, 1983). Also in this study, its feather has been applied to determine the fecundity and spawning season (Jans and van der Kraak, 1997) and reproductive health (Blazer, 2002). The previous research of the classification stages of atretic follicle has been exclusively investigated; especially Grier *et al*. (2009) which established and basically classified the atretic follicles during secondary growth stage into two successive events in teleost fish. In comparison, these characterizations of atretic follicles were also found in *R. brachysoma* too. The first process the degeneration and resorption of oocyte and zona pellucida together with hypertrophy of the granulosa cells which were found in stage I-V. Of additional importance, the second process is called as phagocytosis. The accumulations of yellow-brownish pigments, the greater degeneration and reabsorption of follicular cells occurred. This characterization was similar to stage V of this fish.

It can be concluded that the processing of ovarian follicles in this fish could be successively divided into two phases: atretic follicle during previtellogenicand vitellogenic stages (I, II, III, IV and V). Herein, the present results are the first report provided the basic information and the charges of chemical details during the ovarian follicle atretic denegation that could be applied for further studies including spawning and reproductive seasons in natural habitat and culture of *R. brachysoma*.

Acknowledgments

The authors are grateful to The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship for financial support. We are thankful to the members of the Fish Research Unit, Department of Pathobiology, Faculty of Science, Mahidol University, Thailand for their technical support in laboratory. Appreciations are also extended to Dr. P. Poolprasert and Dr. N. Kangwanrangsan for their informative discussions. We also specially thank Dr. D.V. Furman for critically reading the manuscript.

References

Bancroft, J.D. and Gamble, M. 2007. Theory and Practice of Histological Techniques. 6th edition. Churchill Livingstone, London, U.K., 744p.

Blazer, V.S. 2002. Histopathological assessment of gonadal tissue in wild fishes. Fish Physiology and Biochemistry. 26, 85-101.

Department of Fisheries. 2009. Fisheries statistics of Thailand. Available from: http://www.fisheries.go.th/itstat/yearbook/data_2552/Yearbook/Yearbook2009.pdf. [February 5, 2015].

FAO. 2010. Report of the First Workshop on the Assessment of Fishery Stock Status in South and Southeast Asia. Bangkok, 16-19 June 2009. FAO Fisheries and Agricultural Report No. 913, Rome, FAO, 30p. Available from: http://www.fao.org/docrep/012/i1555e/i1555e00.pdf [February 5, 2015].

Ganias, K., Somarakis, S., Koutsikopoulos, C., Machias, A. and Theodorou, A. 2003. Ovarian atresia in the Mediterranean sardine, *Sardinapilchrdussardina*. Journal of the Marine Biological Association of the United Kingdom. 83, 1327-1332.

Genten, F., Terwinghe, E. and Danguy, A. 2009. Atlas of Fish Histology, CRC Press, U.S.A., 223p.

Grier, J.H., Uribe, M.C. and Patiño, R. 2009. The ovary, folliculogenesis and oogenesis in teleosts.In Reproductive Biology and Phylogeny of Fishes (Agnathans and Bony Fishes, B.G.M. Jamieson, editor. Enfield, New Hampshire, Science Publishers, U.S.A., pp. 25-84.

Humason, G.L. 1979. Animal Tissue Techniques.4th edition. San Francisco, Freeman, U.S.A.

Hunter, J.R. and Goldberg, S.R. 1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulismordax*. Fishery Bulletin. 77, 641-652.

Hunter, J.R. and Lo, N.C.H. 1997. The daily egg production method of biomass estimation: some problems and potential improvements. Ozeanografika. 2, 41-69.

Hunter, J.R. and Macewicz, B. 1985.Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulismordax*.Fishery Bulletin. 83, 119-136.

Hussein, M.R. 2005. Apoptosis in the ovary: molecular mechanisms. Human Reproduction Update. 11, 162-178.

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. General and Comparative Endocrinology. 105, 186-193.

Johnson, R., Wolf, J. and Braunbeck, T. 2009. OECD guidance document for the diagnosis of endocrine-related histopathology of fish gonads. Available from: http://www.oecd.org/dataoecd/33/27/ 42140701.pdf [February 5, 2015]

Kennedy, A.M. 2002. Reproduction of striped bass *Moronesaxatilis*: a structural, biochemical and functional characterization of atresia. MSc Thesis, North Carolina State University.Available from: http://www.lib.ncsu.edu/theses/available/etd-06162002-210408/ [February 5, 2015]

Nagahama, Y. 1983. The function morphology of teleost gonads. In Fish Physiology: Volume IX: Reproduction, Part A: Endocrine Tissues and Hormones, W.S. Hoar, D.J. Randall, E.M. Donaldson, editors. No 9. Academic Press, New York, U.S.A., pp 223-275.

Puchtler, H. and Waldrop, F.W. 1978. Silver impregnation methods for reticulum bers and reticulin: A re-investigation of their origins and specicity. Histochemistry. 57, 177-187.

Saidapur, S.K. 1978. Follicular atresia in the ovaries of nonmammalian vertebrates. International Review of Cytology. 54, 225-244.

Santos, H.B., Sato, Y., Moro, L., Bazzoli, N. and Rizzo, E. 2008. Relationship among follicular apoptosis, integrin beta1 and collagen type IV during early ovarian regression in the teleost *Prochilodusargenteus* after induced spawning. Cell and Tissue Research. 332, 159-170.

Vidal, B.C. 1988. Histochemical and anisotropical properties characteristics of silver impregnation: the differentiation of reticulin bers from the other interstitial collagens. ZoologischeJahrbücher. 117, 485–494.

Wilson, J.M, Bunte, R.M. and Carty, A.J. 2009. Evaluation of rapid cooling and tricainemethanesulfonate (MS222) as methods of euthanasia in zebrafish (*Daniorerio*). American Association for Laboratory Animal Science. 48, 785-789.

Wood, A.W. and van der Kraak, G. 2002. Inhibition of apoptosis in vitellogenic ovarian follicles of rainbow trout (*Oncorhynchusmykiss*) by salmon gonadotrophin, epidermal growth factor and 17β-estradiol. Molecular Reproduction and Development. 61, 511-518.

Wood, A.W. and van Der Kraak, G. 2003. Yolk proteolysis in rainbow trout oocytes after serum-free culture: Evidence for a novel biochemical mechanism of atresia in oviparous vertebrates. Molecular Reproduction and Development. 65, 219-227.