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Common Forms of Atresia in the Ovary of Some Red Sea Fishes During Reproductive Cycle

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Abstract: Four economic fish species were collected monthly from commercial catch land at El-Attaka fish landing (*Saurida undosquamis*, *Rhabdosargus haffara*, *Nemipterus japonicus* and *Liza carinata*). Histological examination of ovaries of these species indicated the presence of atretic oocytes (oocyte retention) as a natural phenomenon in fishes. Depending on the histological descriptions atretic oocytes may be classified into two main types: a, bursting b, non bursting. In the present study, frequency of degenerating oocytes were affected by the gonad maturation. In the early stages, the frequency is very low. It increased gradually reaching its maximum in spent stage about 50%. There is a significant difference between the atretic oocytes and gonad maturation ($p < 0.05$). Atresia is also affected by the length of the spawning season (Long or short). All examined ovaries of multiple spawners (*S. undosquamis*) should atretic oocytes. But in (*N. japonicus*, *R. haffara* and *L. carinata*) about 30 to 35% of the ova show atresia. These species have limited spawning season.

Key words: Atresia, spawning, maturity stages.

INTRODUCTION

Atresia is a natural phenomenon and is very common feature of the teleostean ovary. It may be caused by overproduction of oocytes in batch spawners as a result of environmental condition stress (Leino and Maccormik, 1997). Atretic oocytes may be observed in the ovaries amongst the normal oocytes at any stage of development (Simonsen and Gundersen, 2005). The present study aims to describe the different types and common atretic oocytes in the ovary of some red sea fishes during their reproductive cycles and correlate this phenomenon of atresia to gonadal activity of fishes.

MATERIALS AND METHODS

Sample collection: Samples of four Red Sea fish species (*S. undosquamis*, *N. japonicus*, *R. haffara* and *L. carinata*) were collected from commercial catch at EL-Attaka fish landing. The collection was carried out from September 2003 to May 2004.

Study of reproduction: Samples were dissected and gonads (ovaries) were removed, then weighed. Visual assessment of their maturity stages was made according to Ramadan (2003).

Histological examination: It was carried out by taking a small piece (0.5 cm) from ovary, fixed in Bouin's solution and transferred to 70% alcohol. The specimens then were dehydrated, cleared and embedded in paraffin wax. Tissue

was sectioned by microtome (7 μ) and stained with a heamatoxyline and eosin stains. Microscopic examination of sections was carried out by using a Nikon Microscope.

Statistical analysis: Data were analyzed by using ANOVA, test according to Duncan (1955).

RESULTS

Histological analysis revealed that most of the females of *S. undosquamis*, *N. japonicus*, *R. haffara* and *L. carinata* were undergoing mass atresia in all developmental stages are evident in oocyte sheath and content. On the basis of histological differences, in Red Sea fishes; atretic follicles were mainly of two types; non-bursting and bursting atresia according to Gupta and Matti (1986).

Non-bursting atresia: This type of atresia is very common in the early oocytes. It is characterized by the non ruptured follicular wall. This type of atresia was further classified into three types:

- Capsulated atresia, which is characterized by a drastic reduction in the size of ooplasm which appeared as a dark stain mass (Fig. 1A).
- Lipoidal atresia, in which the follicular wall looked crumpled and nearly thick. The ooplasm loaded with vacuoles which may be lipid materials (Fig. 2). In late lipoidal atresia the oocyte membrane wrinkled and become thick (Fig. 1B).

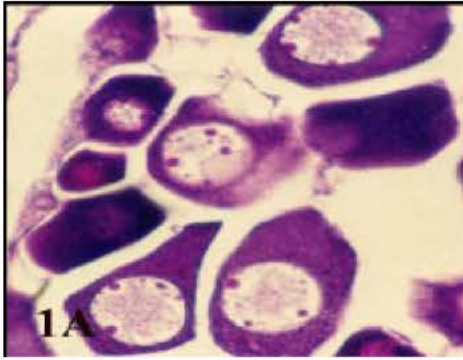


Fig. 1a: Photomicrograph of cross section in ovary of *Saurida undosquamis* showing a non bursting atresia (I-capsulate atresia) appear as a dark stain (Heamatoxylen and Eosin stain X 100)



Fig. 2: Photomicrograph of cross section in ovary of *Nimeptrus japonicus* showing a non bursting atresia (II- Lipoidal atresia a) in which the ooplasm loaded with vacuoles (Heamatoxylen and Eosin stain X 50)

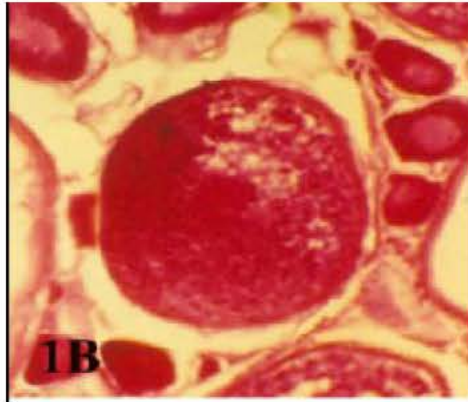


Fig. 1b: Photomicrograph of cross section in ovary of *Liza carinata* showing a non bursting atresia (I-capsulate atresia) appear as a dark stain (Heamatoxylen and Eosin stain X 100)



Fig. 3: Photomicrograph of cross section in ovary of *Rhabdosargus haffara* showing a non bursting atresia (III- Cystic) with reduction in size leaving a wide clear pervitelline space. (FE = Follicular Epithelium, ZR = Zona Radiata, OC = Oocyte Content) (Heamatoxylen and Eosin stain X 50)

- Cystic atresia, in which the oocyte lost its normal identity and reduced in size leaving a wide clear pervitelline space between the ooplasm and the oocyte membrane (Fig. 3).

Bursting atresia: This type was observed in the large developmental oocytes. There were several types of bursting atresia where they identified on the basis of their histological characters.

Multiple bursts: Atretic follicles of these types protrude at several regions of follicles. The wall of oocyte was thicker than normal one (Fig. 4A and B).

Single bursts: In these atretic oocytes the bursting site was single and the contents of the follicle extruded into the stroma. The wall appeared thick (Fig. 5A and B).

Liquified bursts: Atretic follicles of this type were contained large vacuoles in the ooplasm (liquefied) and the oocyte wall was thick and wrinkled in the late stages (Fig. 6A and B and C).

Phagocytic bursts: In this type the follicle cells transformed into phagocytic cells that invade the ooplasm through the ruptured parts of follicular wall. They showed reduction in size and gradual degeneration (Fig. 7).

Finally, all forms of atretic oocytes degenerated and disappeared in the stroma.

Relation between atretic oocytes and ovarian developmental stages of the four red sea fishes: The gonadal status of four fishes (*N. japonicus*,

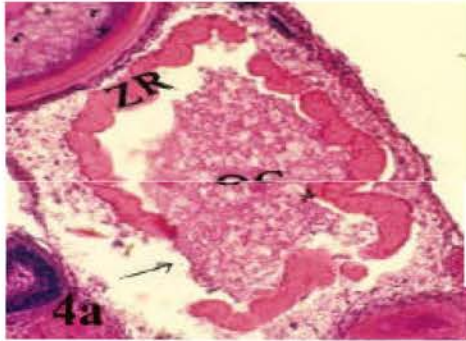


Fig. 4a: Photomicrograph of cross section in ovary of *Saurida undosquamis* showing a bursting atresia (I- multiple bursts) in which the ooplasm loaded with vacuoles (ZR = Zona Radiata, OC = Oocyte Content) (Heamatoxylen and Eosin stain X 50)

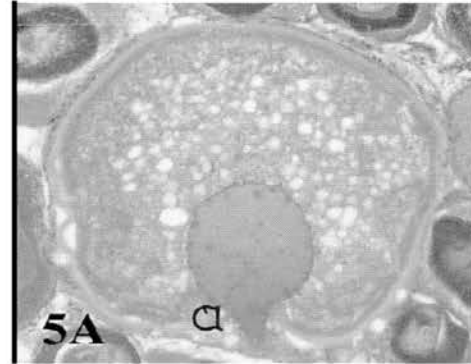


Fig. 5a: Photomicrograph of cross section in ovary of *Saurida undosquamis* showing a bursting atresia (II- single burst a) the content of the oocytes extruded into the stroma (Heamatoxylen and Eosin stain X 50)

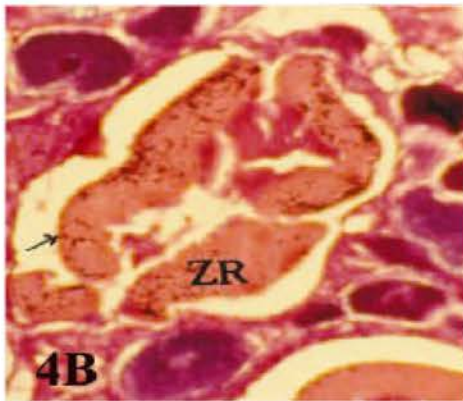


Fig. 4b: Photomicrograph of cross section in ovary of *Rhabdosargus haffara* showing a bursting atresia (I- multiple bursts) in which the ooplasm loaded with vacuoles (ZR = Zona Radiata) (Heamatoxylen and Eosin stain X 50)

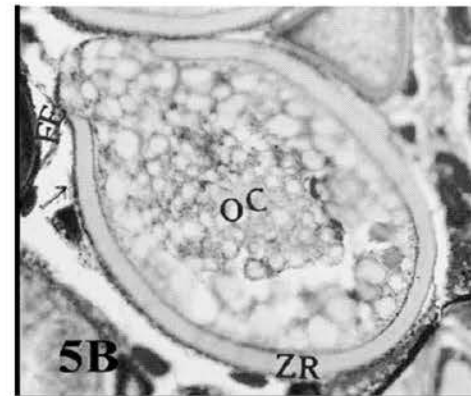


Fig. 5b: Photomicrograph of cross section in ovary of *Nimeptrus japonicus* showing a bursting atresia (II- single burst) the content of the oocytes extruded into the stroma (FE = Follicular Epithelium, ZR = Zona Radiata, OC = Oocyte Content) (Heamatoxylen and Eosin stain X 50)

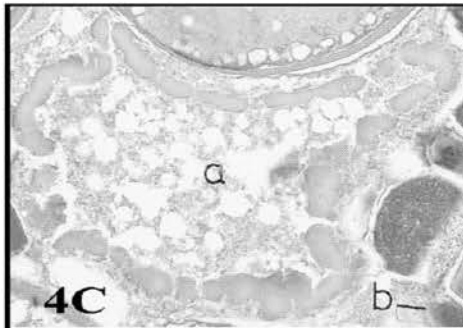


Fig. 4c: Photomicrograph of cross section in ovary of *N. japonicus* showing a bursting atresia (I- multiple bursts) in which the ooplasm loaded with vacuoles (a = oocyte content, b = small cystic type) (Heamatoxylen and Eosin stain X 50).

S. undosquamis, *R. haffara* and *L. carinata*) were assessed through the histological examination and the ovarian developmental stages classified, into seven stages, according to Ramadan (2003) as follows, stage I (immature), stage II (developing), stage III (developed), stage IV (fully developed), stage V (gravid), stage VI (spawning) and stage VII (spent) (Fig. 8).

Examination of gonads revealed that, fishes were in immature phase (stage I) no atretic oocytes were found. However, in stage II according the above scheme contained a very small percent of atretic oocytes to normal ones (1 and 2%, respectively).

Evidently, in the mature phase (from stage III to stage VII) the percentage was increase reaching its maximum

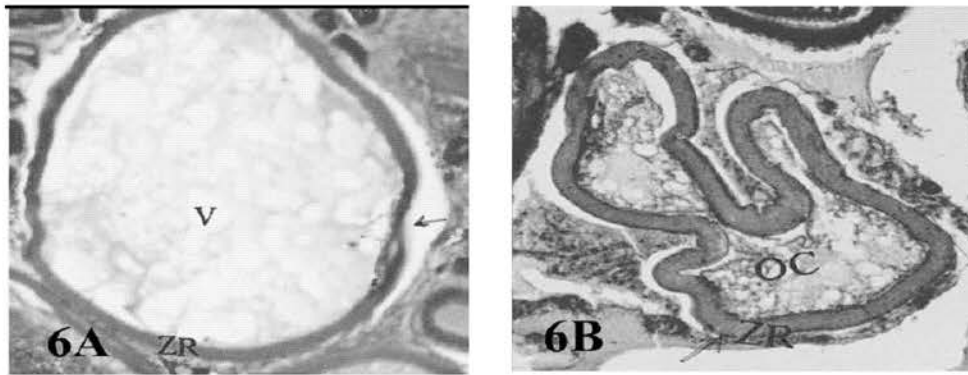


Fig. 6a: Photomicrograph of cross section in ovary of *Liza carinata* showing a bursting atresia (III-liquefied burst) (V = Vacuoles, ZR = Zona Radiate) (Heamatoxylen and Eosin stain X 50)

b: Photomicrograph of cross section in ovary of *Rhabdosargus haffara* with a bursting atresia showing late liquefied with wrinkled and thick oocyte membrane (ZR = Zona Radiata, OC = Oocyte Content) (Heamatoxylen and Eosin stain X 50)

Table 1: Frequency of ovarian atresia in four Red Sea fish species (September 2003 to May 2004)

Fish species	Total no.	No. of atretic female	No. of non-atretic female	Frequency of ovarian atresia (%)	Spawning season
<i>Saurida undosquamis</i>	380	180	0	100	All over the year ^a
<i>Nemipterus japonicus</i>	275	96	179	35	Extend from May to October ^b
<i>Rhabdosargus haffara</i>	117	41	76	35	Extend from December to March ^c
<i>Liza carinata</i>	120	36	84	30	Extend from November to January ^d

^aRammadan (1995), ^bEL-Halfawy (1995), ^cEL-Halfawy (2001) and ^dEL-Halfawy (2004)

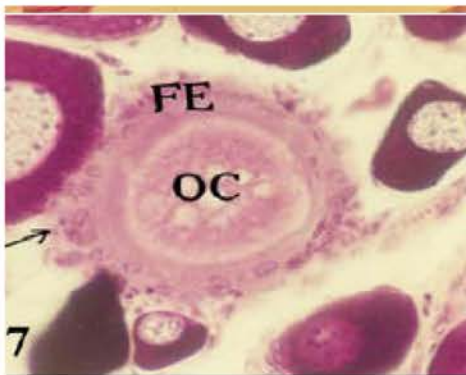


Fig. 7: Photomicrograph of cross section in ovary of *Saurida undosquamis* showing a bursting atresia (IV-phagocytic bursts) (FE = Follicular Epithelium, OC = Oocyte Content) (Heamatoxylen and Eosin stain X 50)

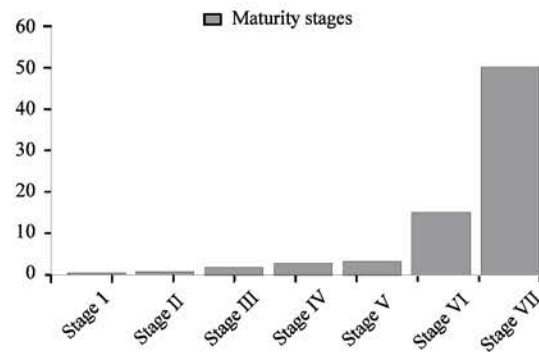


Fig. 8: Frequency of atretic oocytes in relation to ovarian developmental stages of some Red Sea fishes (2003)

values in the spawning(stage VI) and spent (stage VII) stages (15 and 50%, respectively).

Atresia and spawning season: From Table 1 frequency of ovarian atresia was marked higher in fishes which characterized by long spawning season like *S. undosquamis* than in short spawning season like others

species (*N. japonicus*, *R. haffara* and *L. carinata*). In *S. undosquamis* (amultiple spawner) in which spawning season extend all over the year (Ramadan, 1995) all the examined females show atretic follicles (100%). Meanwhile, in the partial spawning fishes in which spawning extends about 3 to 4 months in the year, the frequency of atretic females ranged from 30% in *L. carinata* to 35% in *N. japonicus* and *R. haffara*.

DISCUSSION

Several factors have been associated with egg retention by female fishes including overcrowding, environmental activity, elevated concentration of heavy metal and spawning success (Levavi-Sivan *et al.*, 2004). Degeneration of oocytes has been observed in the ovaries of all teleosts that have been carefully examined histologically (Tripple and Harvey, 1990). These atretic follicles may be a source of progesterone, they appear to function chiefly in the production of nutritive fluid (Wallace and Selman, 1981). Atresia occurs when oocytes abort to develop and fail to be spawned from the ovary and then are resorbed back into the gonad (Arocha, 2002).

Study of serial sections of the ovary of four Red Sea fish species (*S. undosquamis*, *N. japonicus*, *R. haffara* and *L. carinata*) revealed two main types of atresia i.e., nonbursting and bursting described as a common types. Ramadan *et al.* (1987) indicated that deformation of the wall of oocyte was considered as the first step of atresia and phagocytosis of oocytes. Kamel (1990) classified the atretic follicles according to the diameters, but in the present study classification depended on the histological descriptions. As in the finding of Ramadan *et al.* (1987), the atresia for both types nonbursting and bursting begin from 2 to 15% of oocytes fail to undergo maturation or ovulation in the peri and during spawning in stages (II to VI stages) and about 50% in the post spawning (in stage VII) spent stage.

There was a significant difference in maturity level with atretic oocytes ($p < 0.05$) and increased significantly in stage VII ($p < 0.02$). So, in the post spawning stage the atresia become most abundant immediately after egg laying. These findings were confirmed with Lowerre-Barbier *et al.* (1996) who reported that the atretic follicles occur at any stage of follicular development. Higher prevalence of atresia in the early vitellogenic phase has been described as a natural process regulating the surplus of oocytes in the early vitellogenic stage recruited into successive stage of development (Bromley *et al.*, 2000). However, Erpino (1973) and Assem and EL-Zaeem (2005) stated that atresia does not occur in the pre-vitellogenic developmental stages. Similar suggestion have been reported by Trippel and Harvey (1990). There was no evidence that atresia interfered with gamete development subsequently.

In the present study, the ratio of atresia increased by gonad maturation reaching its maximum values in the spent stage VII and this results agree with results obtained by Kurita *et al.* (2003). They stated that atresia has been noted to be wide spread in later phase of

maturation process and associated with the a valuable energy resources and environmental conditions. But this disagreement with Simonsen and Gundersen (2005) who reported that atresia was highest in early phases of maturation in Green land halibut but relatively high levels of atresia were also observed in fish in more advanced maturity phase.

However the investigators did not correlate atresia to spawning season and fish activity. Our study touch this correlation when investigate four species differ in the time and long of spawning season. As far as known, the multiple spawners as *S. undosquamis*, which have repetitive cycles of spawning when spawn of one brood the oocytes of the next brood are maturing. The atretic ovaries found allover the year and in all gonadal maturity by 100% of examined ovaries. This may be due to the higher activity of this species and the over production causes in failer of some oocytes to be spawned.

On the other hand, the other species which have a limited or short spawning season like *N. japonicus*, *R. haffara* and *L. carinata*, the atretic ovaries occurred in about 3 to 30% of examined females. This ratio concentrated after spawning season and few ratio in peri and during spawning season was observed. This also may be due to its limit activity in egg production. So, atresia may be important as it affects the estimated spawning potential of stock. Besides the previous study and earlier workers on atresia phenomenon, further investigation to understand the mechanism and significance of atresia to fish behavior.

REFERENCES

- Arocha, F., 2002. Oocyte development and maturity classification of swordfish from the north western Atlantic. *J. Fish Biol.*, 60: 13-27.
- Assem, S.S. and S.Y. EL-Zaeem, 2005. Application of biotechnology in fish breeding. II: Production of highly immune genetically modified Redbelly *Tilapia zilli*. *Afr. J. Biotechnol.*, 4: 449-459.
- Bromley, P.J., C. Ravier and P.R. Wilthames, 2000. The influence of feeding regime on sexual maturation, fecundity and atresia in first-time spawning turbot. *J. Fish Biol.*, 56: 264-278.
- Cupta, S.K. and R.R. Matti, 1986. Study of atresia in the ovary during the annual reproductive cycle and nesting cycle of the *Pied myna*. *J. Morphol.*, 190: 285-296.
- Duncan, D.B., 1955. Multiple-range and multiple F-test. *Biometric*, 11: 1-42.
- EL-Halfawy, M.M., 1995. Reproduction studies of *Nemipterus japonicus* in Gulf of Suez. M.Sc. Thesis, Faculty of Sci., Suez Canal University.

- EL-Halfawy, M.M., 2001. Effect of types of food on the development, growth and biochemical composition of the fish *Rhabdosargus haffara* (Forsskal, 1775), (Family: Sparidae) in the North Suez Gulf. Ph.D. Thesis, Faculty of Sci., Suez Canal University.
- EL-Halfawy, M.M., 2004. Reproductive biology of *M. seheli* (Family: Mugilidae) reared in fish farm. Egyptian J. Aquatic Res., 30: 234-240.
- Erpino, M.J., 1973. Histogenesis of atretic ovarian follicles in a seasonal breeding bird. J. Morphol., 139: 239-250.
- Kamel, S.A.M., 1990. Study of atresia in the ovary of the Nile bolty, *Oreochromis niloticus*, during its annual reproductive cycle. Proc. Zool. Soc. A.R.E., 18.
- Kurita, Y., S. Meier and O.S. Kjesbu, 2003. Oocyte growth and fecundity regulation by atresia of atlantic herring (*Clupea harengus*) in relation to body condition throughout the maturation cycle. J. Sea Res., 49: 203-219.
- Leino R.L. and J.H. McCormik, 1997. Reproductive characteristics of ruffe, *Gymnocephalus cernuus*, in the st. Louis river estuary on western lake superior: A histological examination of the ovaries over one annual cycle. Can. J. Fish. Aquat Sci., 54: 256-263.
- Levavi-Sivan, B., Viman, O. Sachs and A. Tzchori, 2004. Spawning induction and hormonal levels during final oocyte maturation in the silver perch. Aquacult. 229: 419-431.
- Lowerre-Barbieri, S.K., M.E. Chittnden, J.R. and L.R. BarBieri, 1996. The multiple spawning pattern of weak fish in the Chesapeake bay and Middle Atlantic Bight. J. Fish Biol., 48: 1139-1163.
- Ramadan, A.A., A.A. Ezzat, S.E.M. Khadre, N.A. Muguid and Aziz El-Sha, 1987. Seasonal histological changes in the ovary of *Sparus aurata*, a hermaphrodite teleost marine fish (Family: Sparidae). Folia Morph. 35: 251-264.
- Ramadan, A.M., 1995. Reproduction studies on Lizard fish, *Saurida undosquamis*. M.Sc., Thesis, Faculty of Science, Suez Canal University.
- Ramadan, A.M., 2003. Effect of artificial feeds on growth, gonad maturation and chemical composition of gilthead seabream, *Sparus aurata*. Ph.D. Thesis, Faculty of Science, Suez Canal University.
- Simonsen, C.S. and A.C. Gundersen, 2005. Ovary development in Green Land halibut, *Reinhardtius hippoglossoides*, in west Green land waters. J. Fish Biol., 67: 1299-1317.
- Tripple, E.A. and H.H. Harvey, 1990. Ovarian atresia and sex ratio imbalance in white sucker, *C. commersoni*. J. Fish. Biol., 36: 231-239.
- Wallace, R.A. and K. Selman, 1981. Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zoologist, 21: 325-343.