Al-Azhar Bull. Sci. Vol. 22, No. 1 (June.): pp. 1-12, 2011.

REPORT ON STATE OF ATRESIA IN THE OVARIES OF SOME MEDITERRANEAN SEA FISHES FROM THE EGYPTIAN COAST

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Abstract

Some common forms of atresia were observed in the ovaries of striped sea bream, *Lithognathus mormyrus*, spiny cheeked grunter, *Terapon puta* and bogue, *Boops boops* obtained from Abu Qir Bay, Alexandria. The ovarian atresia was recognized by batches of clear brownish fluid beneath the ovarian epithelium and the oocytes appear reddish in colour. The present study showed that, the ovarian atresia increases with the increasing the maturity stages and length of spawning season. The percentages of ovarian atresia was marked higher in *L. mormyrus* and middle in *T. puta* which characterized by long spawning season. The percentage of ovarian atresia was lower in *B. boops* which characterized by short spawning season. Histological examination of ovaries of *L. mormyrus*, *T. puta* and *B. boops* indicated that, the presence of atretic oocytes (oocyte retention) as a non natural phenomenon in the first maturity stages and natural in the last maturity one. Atretic oocytes may be classified into two main types: nonbursting and bursting. Capsulated atresia, lipoidal atresia and cystic atresia are belonging to the first type. Multiple bursts, single bursts and liquified bursts are of common phases in the second one. Finally, all forms of atretic oocytes degenerated and disappeared in the stroma.

Introduction

The reproductive capacity and factors affecting reproduction in wild fish populations is of great significance in the management of fisheries. The egg production by a population of fishes is a function of the abundance of mature females, their fecundity and the proportion of females that release ova at spawning (Trippel and Harvey, 1990 and Alne-Na-E and Radi, 1998).

Atresia is a natural phenomenon and is very common feature of the teleostean ovary. Atretic oocytes may be observed in the ovaries amongst the normal oocytes at any stage of development (Simonsen and Gundersen, 2005). One of its possible causes is the over production of oocytes in batch spawners as a result of environmental conditions stress (Leino and Maccormik, 1997).

Several studies have noted detrimental effects (atresia, failure to spawn, decreased fecundity and decreased hatchability of eggs) of environmental stressors on gonadal function in teleosts. Such stressors include disease (Munkittrick and Leatherland, 1984); increase in salinity (Al-Thani *et al*., 1996a&b and Moharram, 2000) changes in environmental quality and exposure to environmental toxicants such as organochlorines and heavy metals (Monsson *et al*., 1994; Chevreuil *et al*., 1995; Wiklund *et al*., 1996; Alne-Na-Ei and Rady, 1998 and Kristensen *et al.*, 2007).

The purpose of the present study is to document the different types and common atretic oocytes in the ovaries of some Mediterranean Sea fishes during the reproductive cycles and the correlation between atresia and maturity stages and period of spawning season.

Materials and methods

1-Specimens collection:

A total of 1076 specimens; 331 of *Lithognathus mormyrus*, 472 of *Terapon puta* and 273 of *Boops boops* were obtained monthly from the commercial catch of Abu Qir Bay, Alexandria during the period from January, 2010 to December, 2010 formed the materials for the present study. Trammel net, bottom trawls, purse seines and beach seines were the main fishing methods used to collect the fish. Wherever possible fishes were examined fresh or preserved in 10% formalin solution for latter examination. In the laboratory, fishes were identified and the standard length, total length and weight of the fish were recorded.

2-Study of atresia:

Fishes were dissected, ovaries were removed and examined. Maturity stages of ovary were recorded according to Khalaf Allah (2009). On the basis of the histological differences, the atretic oocytes may be classified according to Cupta and Matti (1986).

3- Histological studies:

To study histologial and histochemical features of the ovaries, small pieces of the ovaries (5 mm) were removed from the dissected specimens, and immediately fixed in alcoholic Bouin`s solution for at least 48 hours and transferred to 70% alcohol. Tissues were dehydrated, cleared and embedded in paraffin wax. Transverse sections were cut at 4 to 6 um and at least 8 slides from each region were prepared. They were stained with Harri`s haematoxylin, counter stained with eosin and examined under microscope.

Results

Data in Table (1) showed that, the spawning season of *Lithognathus mormyrus* and *Terapon puta* is extending from May to October (6 months). The percentages of atresia are 58.30% and 32.2% for *Lithognathus mormyrus* and *Terapon puta* respectively. These fishes are bottom feeders. While, the spawning season of *Boops*

boops is extending from February to April (3 months). The percentage of atresia is 8.05%. This fish is a semi-pelagic feeder.

The ovarian atresia was recognized by batches of clear brownish fluid beneath the ovarian epithelium and the oocytes appear reddish in colour. Females have external abnormalities such as skin ulcers and discoloration. Histological examination revealed that, the atretic oocytes are derived from either vitellogenic or mature oocytes that didnot develop further but degenerated and reabsorbed in the three species examined (Plate, 1). However, follicular atresia was noticed to takes place in the following way: the follicular epithelium is converted from squamous to the columnar pattern with no distinct boundaries between cells and their nuclei become rounded. The striation of the zona radiata of the oocyte had disappeared. Isolated ruptures appeared in it initially and then all of it soon breaks into isolated pieces. The cells of the follicular epithelium are apically emarginated with the oocyte and evidently function in the case as phagocytes. The follicular phagocytic cells invade the yolky materials of the oocytes. The yolky materials exhibit clear signs of deformation and the nuclei of the oocytes disappear. The phagocytic cells when invaded the oocyte had attacked the proteins at first, while the carbohydrates and lipids were still within the theca which still remained surrounding the oocytes till the completion of its reabsorption (Plate, 1).

On the basis of the histological differences, the atretic oocytes may be classified into two main types: nonbursting and bursting atresia. Nonbursting atresia:

This type of atresia is very common in the early oocytes. It is characterized by the non ruptured follicular wall and can be classified into three types:

1- Capsulated atresia characterized by a drastic reduction in the size of ooplasm which appeared as a dark stain mass. As the process of atresia proceeds, the cells of the stratified epithelium increase in number and invade the underlying liquefied yolk. In more advanced stage of atresia, the whole yolky mass becomes loose and intermixes with invading follicular cells. Moreover, the theca folliculi becomes highly vascular and increase markedly in the thickness. In late period of atresia, the atretic follicle loses its vascularity and appear as an empty sac which is resorbed through ovarian stroma (Plate, 1A&B).

2- Lipoidal atresia, in which the follicular wall looked crumpled and nearly thick. The ooplasm loaded with vacuoles which may be lipoid materials. In late lipoidal atresia the oocyte membrane wrinkled and become thick (Plate, 1C).

3-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 (Plate, 1D).

Bursting atresia:

This type of atresia was observed in the late developmental oocytes. It is characterized by the ruptured follicular wall and can be classified into three types:

1- Multiple bursts: atretic follicles are protruding at several regions of follicles. The wall of oocyte was thicker than the normal one (Plate, 1E&F).

2- Single bursts: in the atretic oocytes the bursting site is single and the contents of the follicle extruded into the stroma. The wall of atretic oocyte appeared thick (Plate, 1G).

3- Liquified bursts: atretic follicles of this type are contained large vacuoles in the ooplasm and the wall of oocyte was thick and wrinkled in the late stages (Plate, 1H). Finally, all forms of atretic oocytes degenerated and disappeared in the stroma.

Relation between atresia and the spawning season:

Results in Table (1) showed that, the percentage of ovarian atresia was marked higher in *Lithognathus mormyrus* (58.30%) and middle in *Terapon puta* (32.2%) which characterized by long spawning season. The percentage of ovarian atresia was marked lower in *Boops boops* (8.05%) which characterized by short spawning season.

Relation between atresia and the ovarian maturity stages:

Data in Table (2) showed that, no atretic oocytes were found in pre-vitellogenesis of *Lithognathus mormyrus*. However, early-vitellogenesis contained small values of atretic oocytes in *Lithognathus mormyrus* and *Terapon puta*. In the mature phase (mid-vitellogenesis, late-vitellogenesis and pre-spawning), the values of ovarian atresia was marked higher in late-vitellogenesis, pre-spawning in *Lithognathus mormyrus* and in mid-vitellogenesis, pre-spawning in *Terapon puta*. In *Boops boops*, no atretic oocytes were found in pre-vitellogenesis and early-vitellogenesis. In mid-vitellogenesis, the value of ovarian atresia was very small and slightly increases in resting stages.

Discussion

Reproduction is the most critical function in aquatic animals affected by chronic toxicant stress (Birge *et al*., 1985). The ovaries of female fishes that fail to spawn become atretic shortly after the spawning period (Guraya, 1994). Ovarian atresia of female fishes has been suggested to associate with several factors, including overcrowding and heavy metal concentration (Levavi-sivan *et al*., 2004); higher temperature (Saxena and Sandhu, 1994 and Pankhurst *et al*., 1996); paucity of males (Tripple and Harvey, 1990): elevated pesticide (sukumar and Karpaganapathy, 1992); dietary deficiencies (Kjesbu *et al*., 1991 and Cerda *et al*., 1995); cyanide compounds (Alne-Na-Ei, 1997); confinement of fishes (Coward *et al*., 1998); sediment contamination (Alne-Na-El and Rady, 1998); spawning success (Levavi-sivan *et al*., 2004) and polluting chemicals or global warming (Unal *et al.*, 2007).

In the present study, atresia occurred in some ovaries of three species examined. It may be due to some factors such as heavy metals concentration, sediment contamination and changes in environmental quality. Abu-Qir Bay receives different pollutants contributing to various waste sources. The shore line configuration and coastal sedimentation have been modified by artificial structures such as jetties at the inlet of Edku lagoon and seawalls emplaced at the outer margin of Rosetta promontory (Firhy *et al.*, 1994). This of course affects the wild fish community as a result of reproductive disorder and leads to serious histopathological alternation of vital organs in fishes.

In the present study, the percentages of ovarian atresia was marked higher in *Lithognathus mormyrus* (58.30%) and middle in *Terapon puta* (32.2%) which characterized by long spawning season and bottom feeders. The percentage of ovarian atresia was marked lower in *Boops boops* (8.05%) which characterized by short spawning season and semi-pelagic feeder.

It may be emphasized that, the short of spawning season and feeding behavior of *Boops boops* may influence the low percentage of ovarian atresia i.e. the fish are mainly semi-pelagic feeder, not direct contact with sediment. The long of spawning season and feeding behavior of *L. mormyrus* and *T. puta* in Abu-Qir Bay may influence the highly percentages of ovarian atresia i.e. the fishes are mainly bottom feeder, the direct contact with sediment may cause ovarian atresia in various frequencies depending on the degree of sediment contamination. In agreement with these results, mature plaice had more ovarian lesions and ovarian cyclic changes because they live on the bottom of partially buried in the sediment highly contaminated with crude oil (Stott *et al*., 1983). Alne-Na-Ei and Rady (1998) mentioned that, the frequency of ovarian atresia of *Chrysichthys rueppelli* in Alrayah Almenoufi, due to bottom dewelling of the fish directly to sediment contamination.

Lithognathus mormyrus and *Terapon puta* were bottom feeders. The percentage of ovarian atresia was marked higher in *L. mormyrus* (58.30%) than in *T. puta* (32.2%) because, the former feed mainly on infouna, more direct contact with sediment may cause more of ovarian atresia.

Generally, the contaminated sediment has been suggested to cause potential adverse effects such as reduced growth, impaired reproductive success, backbone degradation, enlarged livers and tumor fin (Lesko *et al*., 1996). According to Munkittrick and Leatherland (1984), the heavy metals stress may cause a dysfunction of the hypothalamic-pituitary-gonad axis, which prevents the release of mature eggs, but not their development or maturation or ovarian atresia and spawning failure of the goldfish may be related to dysfunction in the timing of gonadotropin release or possibly related to the fact that steroid production, which is under gonadotropic regulation, is inappropriate for needs of normal gonad maturation, ovulation and spawning. The disturbance in liver functions of rainbow trout exposed to sediment extracts contaminated with heavy metals was documented by Gange *et al*. (1996) and Ghanem (2006) in *Mugil cephalus*.

Results of the present study showed that, the ovarian atresia recognized by patches of clear brownish fluid beneath the ovarian epithelium and the oocytes appear with reddish colour. Females have external abnormalities such as skin ulcers and skin discoloration. This result was agreement with Alne-Na-E and Radi (1998).

In the present study, histological examination in three species examined showed that, the atretic oocytes may be classified into two main types: nonbursting and bursting atresia. Kamel (1990) classified the atretic follicles according to the diameters, but in the present study, classification depended on the histological descriptions. The same results obtained by Ramadan and El-Halfawy (2007). He indicated that, deformation of the wall of oocytes was considered as the first step of atresia and then phagocytosis of oocytes.

In the present study, the ratio of atresia increased by gonad maturation reaching its maximum values in more advanced maturity stages. This result agrees with results obtained by Kurita *et al*. (2003) and Ramadan and El-Halfawy (2007). They stated that, atresia has been noted to be wide spread in latter phases of maturation process and associated with 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.

Our study concluded that, Atresia needs further investigation to understand the mechanism and significance of atresia to fish behavior.

Table (1): Frequancy of ovarian atresia in some Mediterranean Sea fish species.

Species	No. of female	No. of atretic female	atresia %	Spawning season	Mode of feeding
Lithognathus mormyrus	331	193	58.30 %	May - October	Bottom feeder
Terapon puta	472	152	32.20 %	May - October	Bottom feeder
Boops boops	273	22	8.05 %	February - April	Semi-pelagic feeder
Total	1076	367	98.55	--	--

Table (2): Frequancy of atretic females in relation to ovarian stages of some Mediterranean Sea fish species.

Maturity stages	Atretic females			Total	%
	lithognathus	*Terapon*	*Boops*		
I	1	-	-	1	0.27
II	17	16	-	33	9
III	20	53	-	73	19.89
IV	15	22	2	39	10.63
V	61	40	5	106	28.88
VI	64	8	7	79	21.52
VII	15	13	8	36	9.81
Total	193	152	22	367	100

Explanation of PLATE

Plate 1(A): Part of T.S. in the ovary of *Lithognathus mormyrus*, showing the non bursting atresia (capsulate atresia) appear as a dark stain (H-E x 1200).

Plate 1(B): Magnified portion of T.S. in the ovary of *Lithognathus mormyrus*, showing the non bursting atresia (capsulate atresia) appear as a dark stain (H-E x 2000).

Plate 1(C): Magnified portion of T.S. in the ovary of *Terapon puta,* showing the non bursting atresia (lipoidal atresia) in which the ooplasm loaded with vacuoles (V) (H-E x 2000).

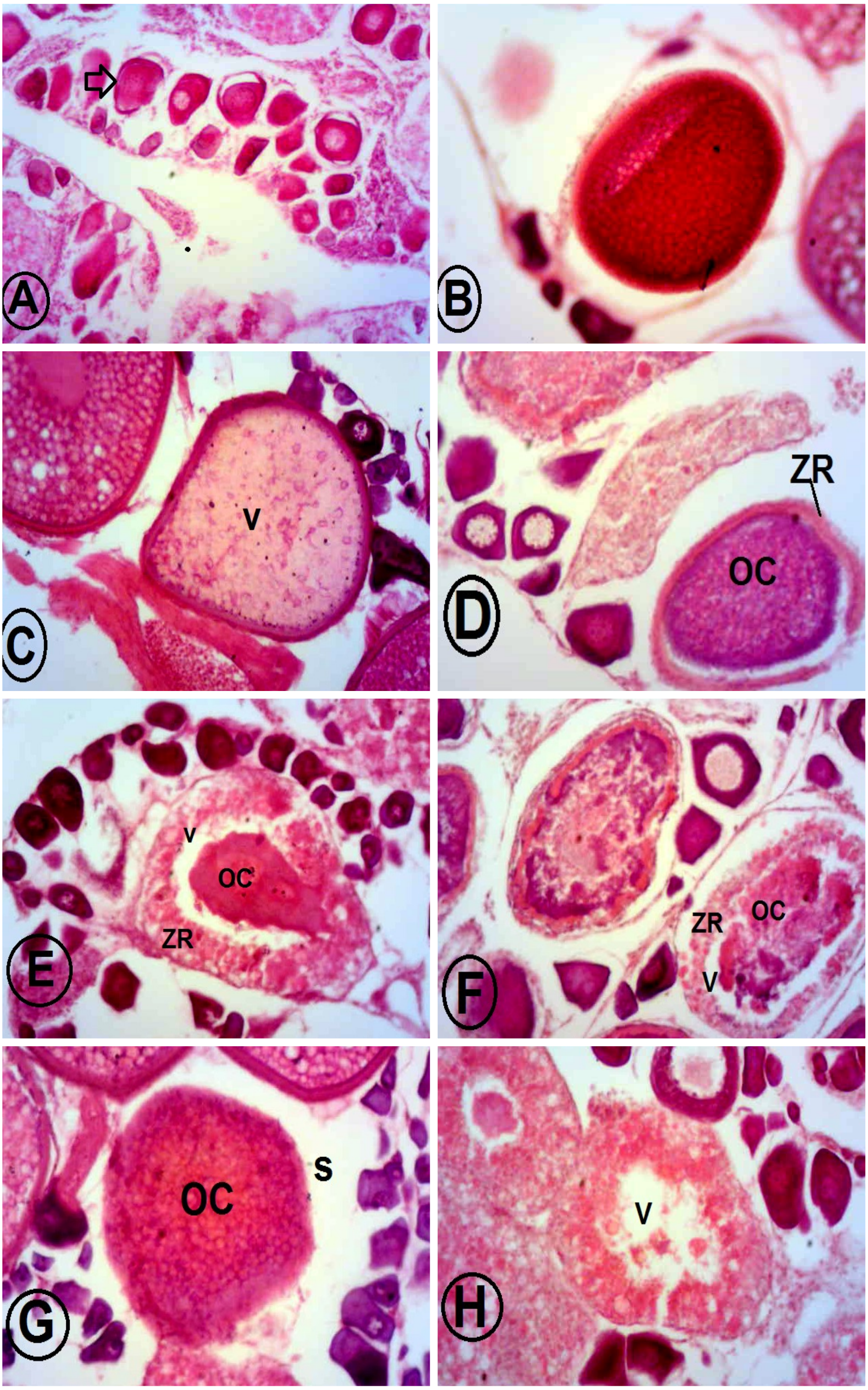
Plate 1(D): Magnified portion of T.S. in the ovary of *Lithognathus mormyrus*, showing the non bursting atresia (cystic atresia) with reduction in size and previtelline space between zona radiata (ZR) and oocyte content (OC) (H-E x 2000).

Plate 1(E): Magnified portion of T.S. in the ovary of *Lithognathus mormyrus*, showing the bursting atresia (multiple bursts) in which the ooplasm loaded with vacuoles (V) between zona radiate (ZR) and oocyte content (OC) (H-E x 2000).

Plate 1(F): Magnified portion of T.S. in the ovary of *Terapon puta*, showing the bursting atresia (multiple bursts) in which the ooplasm loaded with vacuoles (V) between zona radiate (ZR) and oocyte content (OC) (H-E x 2000).

Plate 1(G): Magnified portion of T.S. in the ovary of Boops boops, showing the bursting atresia (single bursts); the oocyte content (OC) extruded into the stroma (S) (H-E x 2000).

Plate 1(H): Magnified portion of T.S. in the ovary of Boops boops, showing the bursting atresia (liquified bursts); the ooplasm loaded with vacuoles (V) (H-E x 2000).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