

HISTOLOGICAL AND ENZYME HISTOCHEMICAL STUDIES ON THE TRANSITIONAL

GONADS OF GIANT GROUPER *E. tauvina* (PERCIFORMS: SERRANIDAE) FROM THE

ARABIAN GULF COAST OF SAUDI ARABIA.

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Abstract

The present work was carried out using twenty mature *Epinephelus tauvina* (Perciforms: Serranidae) collected from Arabian Gulf coast at Dammam City. Fishes of this species are known to undergo sex change during certain stage of their life cycle. Histological and enzyme histochemical studies were performed on gonads of the collected fishes. The examination of the gonads of *E. tauvina* revealed the presence of three developmental phases during the sex change process. These were: Female, early transition phase and late transition phase each one may subdivide into two stages. In female phase several developmental stages of the oocytes were recognized. At the beginning of early transition phase perinucleolar mature oocytes began degeneration while late transition phase characterized by rapid proliferation of spermatogenic cells. The histochemical studies demonstrated that alkaline phosphatase enzyme gave intense reaction in granulosa cells of mature oocytes while acid phosphatase gave an intense reaction in the interstitial cells and atretic follicles but $\Delta 5 - 3\beta$ hydroxysteroid dehydrogenase enzyme gave intense reaction to special cells in capsule of gonads.

Introduction:

Tropical fishes show an exceptional range of reproductive strategies with regards to the expression of their sexuality (Devlin and Nagahama, 2002). Along with this diversity many different physiological regulations of gonadal sex differentiation or sex change have been demonstrated or suggested, including for instance the participation of the brain in a hermaphrodite's sex change (Grober and Sunobe, 1996; Black *et al.*, 2004), and of external factors like temperature in species having Environmental Sex Determination (ESD) (Baroiller and D'Cotta, 2001). Despite this diversity of sex determination and sex differentiation processes, there is at least one well conserved factor common to nearly all teleost fish in the control of ovarian differentiation, which is the implication of estrogens and the enzyme

needed for their synthesis. (Guiguen *et al.*, 2010).

Groupers are widely distributed throughout the tropical and subtropical waters of the world and are regarded as a favourite marine food fish. However, being protogynous hermaphrodites, groupers have been considered as study model for development and reproduction, especially for sex determination or sex differentiation, owing to the advantage that grouper gonad development undergoes transition from ovary to intersexual gonad and then to testis (Shapiro, 1987; Zhou and Gui, 2008).

In such marine hermaphrodite fishes, sex changes are accomplished by complete alteration of gonadal anatomy and function as well as changes in behaviour (Tang *et al.*

1975; Reinboth, 1979 and Shapiro, 1987). It is still unknown when exactly, and what triggers their sex change from female to functional males. In contrast to numerous studies on reproductive cycles of protogynous hermaphrodite fishes (**Cardwell and Liley, 1991; Johnson et al. 1998; Lee et al., 2002; Mackie, 2006 and Liu & de Mitcheson, 2009**), very few studies have pointed out the endocrine changes in fish that undergo female to male sex changes (**Tan and Tan, 1974; Chen et al. 1980; Reinboth, 1979 & 1988; Nakamura et al. 1989; Siau, 1994 and Kobayashi et al., 2010**).

The different stages of oocyte maturation as well as the existence of protogyny in *E. tauvina* were previously reported by **Abu Hakima (1987)**. The present study aims to give some details on histological and histochemical changes on gonads during its sex change process.

Material and Methods:

Twenty sexually mature *E. tauvina* fishes were collected from the coast of Arabian Gulf at Dammam City. (The collected fishes ranged in total length between 30.5 and 95 cm and in weight between 345 and 14500 grams). The specimens were collected over one year period. For the identification of developmental stages and different phases, fresh gonads were fixed in 10 % neutral formalin sectioned at 5 µm thick and stained with haematoxylin and Eosin (**Bancroft and Gamble, 2008**).

For histochemical studies the frozen unfixed sections of gonads were cut on cryostat at - 10 °C at 10 µm thickness for studying enzymes (acid phosphatase enzyme by the Gomori lead method (**Bitensky, 1963**) and alkaline phosphatase enzyme by direct lead method (**El-Aser and Hassanein, 1975**) and Δ5-3β hydroxysteroid dehydrogenase (modified by **Nagahama et al., 1976**).

Result:

The gonads of *E. tauvina* are divided into two lobes located on either side of the

intestine attached to the peritoneal wall. On basis of histological changes, entire process of sex changes was assigned into three developmental phases: female, early transition and late transition phase. Each phase may sub-divide into two stages early and late.

In the female phase the ovarian tissue contains small oocytes in early development stage, it was arranged in lamellae, which extended into ventral, membrane bound ovarian cavity or lumen and also the presence of atretic follicles, Fig. (1).

The last phase characterized by presence of numerous yolk globules (circular in shape and acidophilic) in-between yolk vesicles. The nucleus increase in size with irregular contours. The oocyte surrounded by acidophilic zona radiata and granulosa and thecal cells, Fig. (2). During the same stage several oocytes appear at perinucleolar stage in which the oocyte increased in size with large nucleus contain many nucleoli arranged in the inner contour of the nucleus and the yolk globules varies in density of eosinophilic yolk from light to dark, Fig. (3).

Several individual of gonads of *E. tauvina* shows early transition phase shows many small oocytes with large nucleus contain 1-3 nucleolus surrounded by dark basophilic cytoplasm all these at chromatin nucleolus stage, Fig. (4). The last phase characterized by degeneration of primary oocytes and simultaneous spermatogonial proliferation in the germinal epithelium lining ovarian lumen.

At the late phase of transition also appears few semineferous cysts contain spermatocytes and spermatids which appears in the interstitial connective tissue in between oocytes in the early stage, Fig. (5).

The gonads in the later stage surrounded by thick capsule consisting of numerous connective tissue fibers enclosing in between steroidogenic cells containing centrally located nucleoli, Fig. (6).

The histochemical examination of *E. tauvina* gonads revealed that in female stage the ovary surrounded by capsule containing cells aggregates, which gave moderate reaction to alkaline phosphatase enzyme. In addition, the ovary of *E. tauvina* filled with numerous oocyte which show an intense reaction to alkaline phosphatase in granulosa cells while the cytoplasm of oocyte gave reaction varies from week to moderate to same enzyme, Fig.(7).

By magnification of the later stages showed an intense reaction in granulosa cells while zona radiata gave no reaction to some enzyme. Few atretic follicles gave also an intense reaction to alkaline phosphatase enzyme, also the cytoplasm around the nucleus before its disappearance gave an intense reaction to the same enzyme, Fig. (8).

In early stages of transmission the gonads filled with mature oocytes which more crowded and gave an intense reaction of alkaline phosphatase in the outer contours of oocytes, Fig. (9). Also, few atretic follicles appeared with aggregation of interstitial spermatogenic cells in-

between mature oocytes and the both gave an intense reaction to alkaline phosphatase, and there were some clusters of cells in capsule gave an intense reaction to alkaline phosphatase, Fig. (10).

Concerning the reaction of acid phosphatase in the ovary of *E. tauvina*, it was noticed that the intense reaction of this enzyme were concerned to connective tissue in between mature oocyte while the granulosa cells which surround the oocyte gave moderate reaction, Fig. (11). The gonads in early stages of transition in addition to interstitial connective tissue there were aggregation of testicular tissue both gave moderate to intense reaction to acid phosphatase, Fig (12).

The study of $\Delta 5-3\beta$ hydroxy steroid dehydrogenase reaction in the ovary of *E. tauvina* showed intense reaction in some granulosa cells around mature oocytes, Fig.(13), while in gonads in early stages of transition also appeared groups of cells in connective tissue capsule which gave an intense reaction, Fig.(14). Meanwhile, in many situations some aggregation of interstitial connective tissue cells gave moderate reaction to the same enzyme.

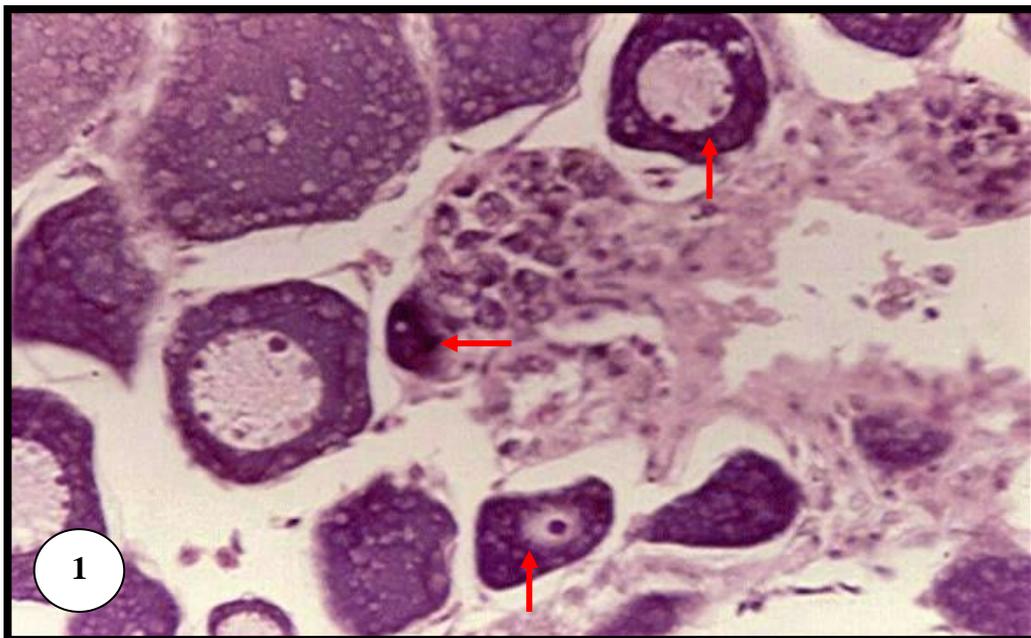


Fig. (1): Light micrograph of gonad of *E. tauvina* fish showing the ovarian tissues contains oocytes in early stage were arranged into lamellae and presence of atretic follicle (arrow). H&E stain (4 ×10).

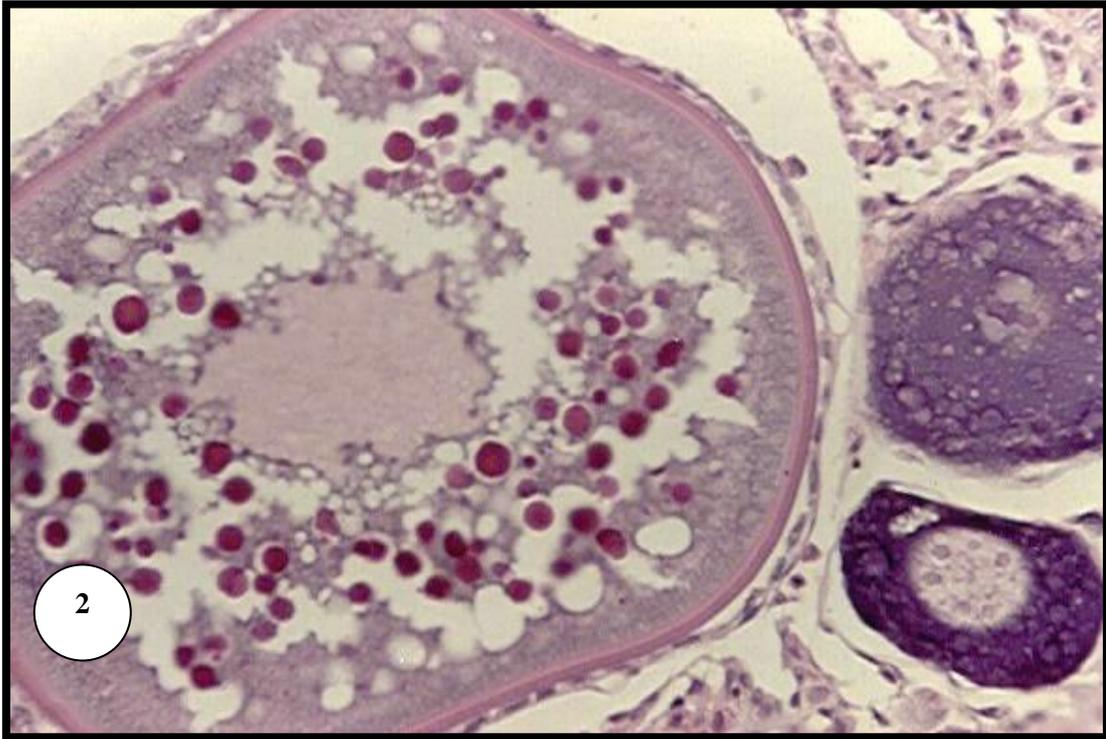


Fig (2): Light micrograph of gonad of E.tauvina fish showing female phase characterized by presence of circular acidophilic yolk globules (arrow). H&E stain (40 × 10).

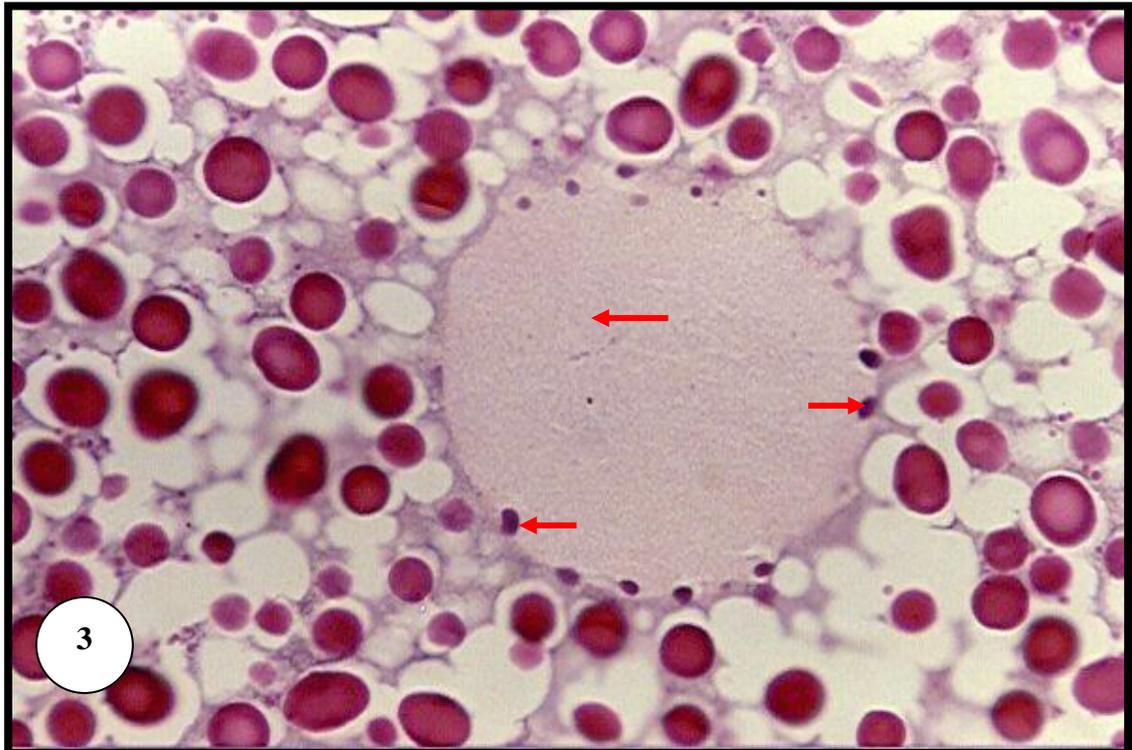


Fig. (3): Light micrograph of gonad of E. tauvina fish showing: perinucleolar stage showing numerous nucleoli arranged in the inner contour of nucleus (arrows). H&E stain, (40 × 10).



Fig.(4): light micrograph of gonad of *E. tauvina* fish showing : early transition phase showing degeneration of primary oocytes and appearance of testicular tissue (arrows) . H&E stain (40 × 10).

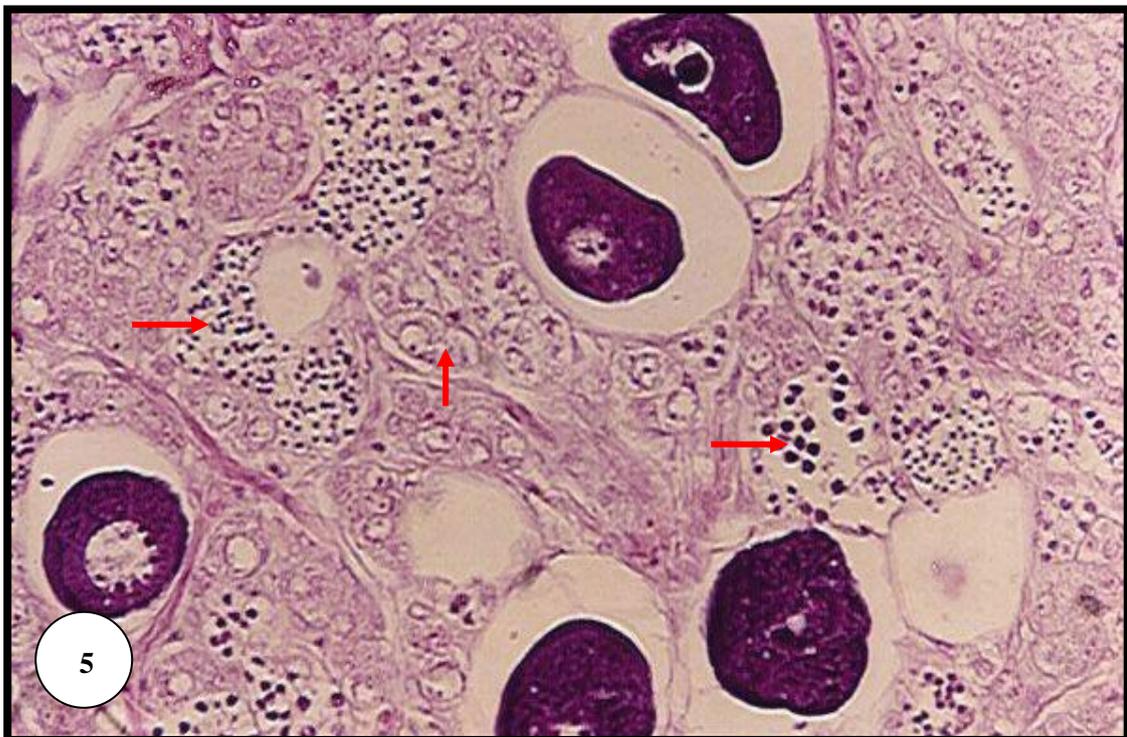


Fig. (5): light micrograph of gonad of *E. tauvina* fish showing: oocytes in early stages and increase number of cysts which filled with different stages of spermatogenesis from spermatogonia to spermatids (arrows). H&E stain (40 ×10).

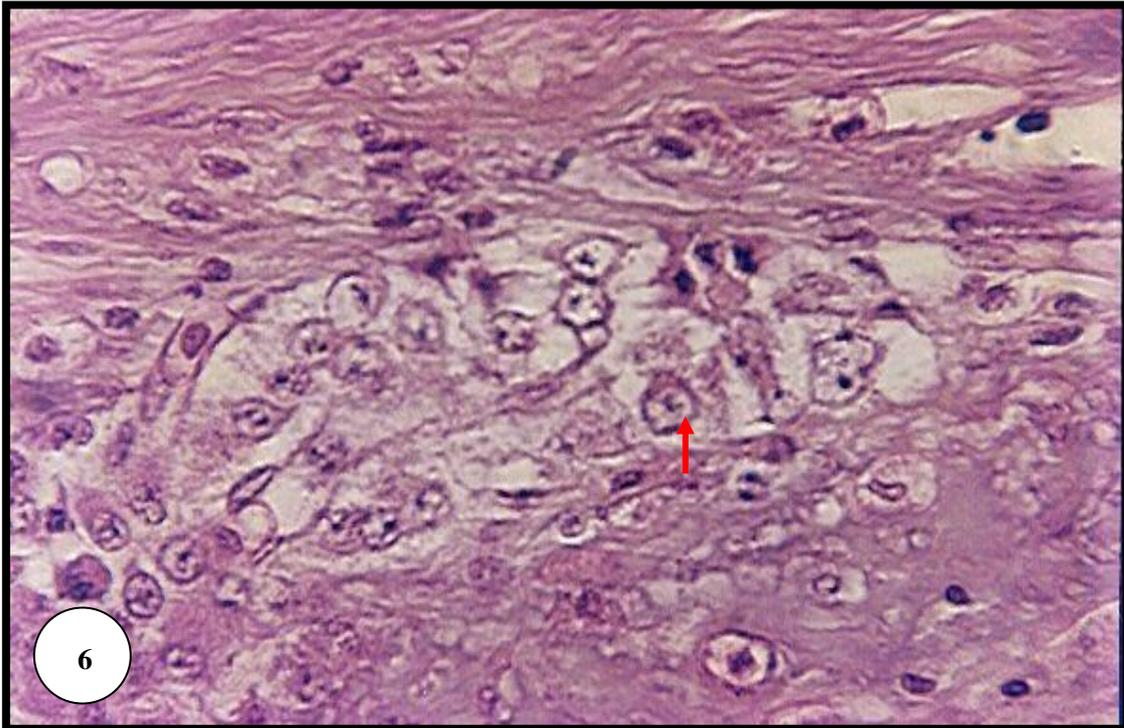


Fig. (6): light micrograph of gonad of E. tauvina fish showing: capsules consist of connective tissue fibers enclosing aggregations of steroidogenic cells highly vacuolated with centrally located nuclei. (arrow). H&E stain (10 × 100).

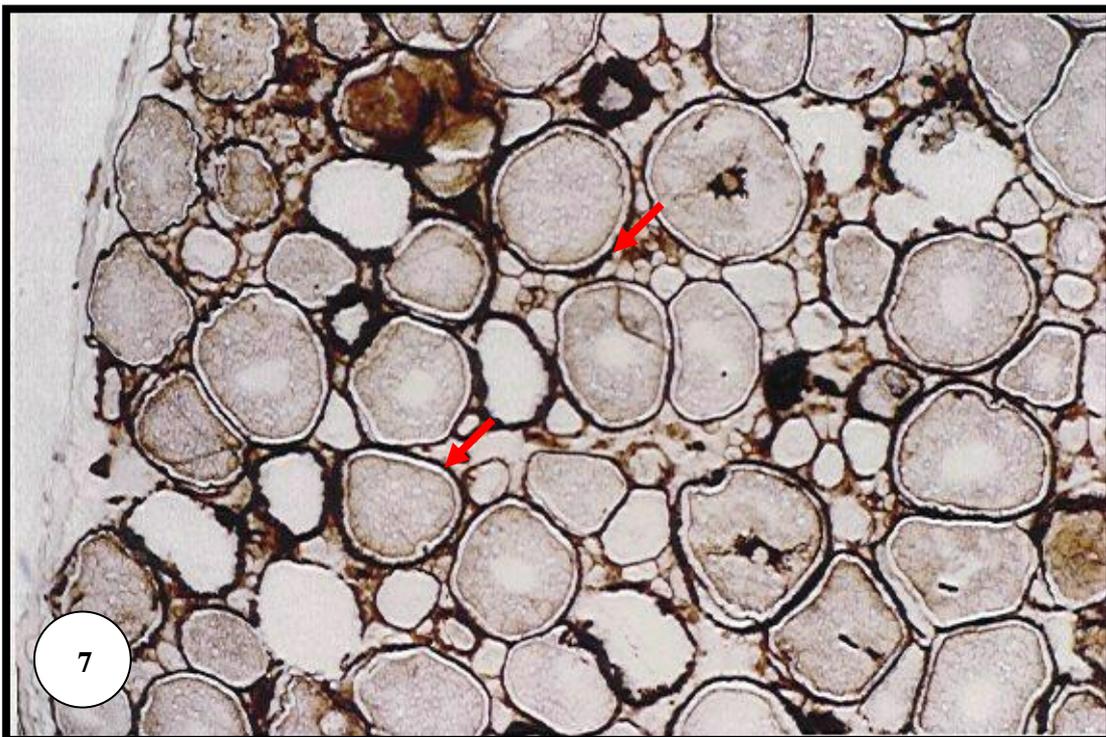


Fig. (7): light micrograph of gonad of E. tauvina fish showing: an intense reaction of alkaline phosphatase appeared in granulosa cells which surrounded the mature oocyte (arrows), moderate reaction of same enzyme in cytoplasm of oocyte .Gomori reaction (10×4).



Fig. (8): light micrograph of gonad of E. tauvina fish showing: an intense reaction around mature oocyte and around the wall of atretic follicle, also in yolk around nucleus of mature oocyte (arrows). Gomori reaction (10×10).

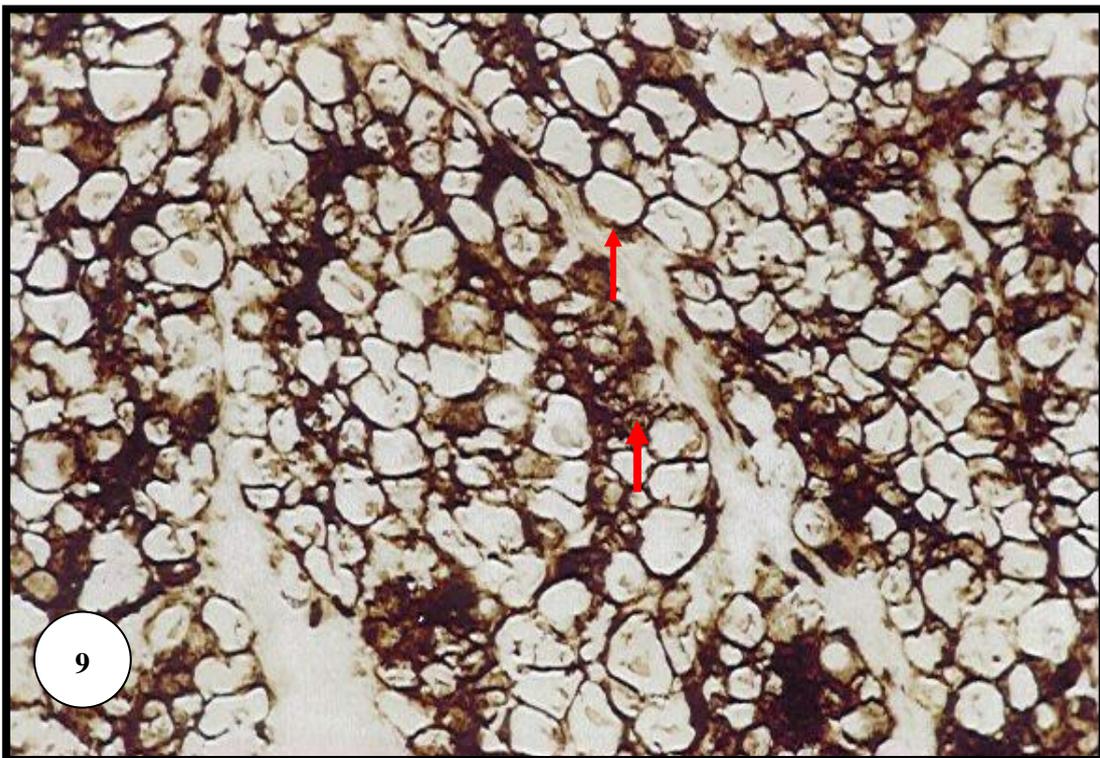


Fig. (9): light micrograph of gonad of E. tauvina fish showing: mature stages shows numerous mature oocytes counter membrane and some atretic follicles and few interstitial spermatogenic cells reacted positively with alkaline phosphatase (arrows), and negative reaction in cytoplasm of mature oocytes . Gomori reaction (10×10).

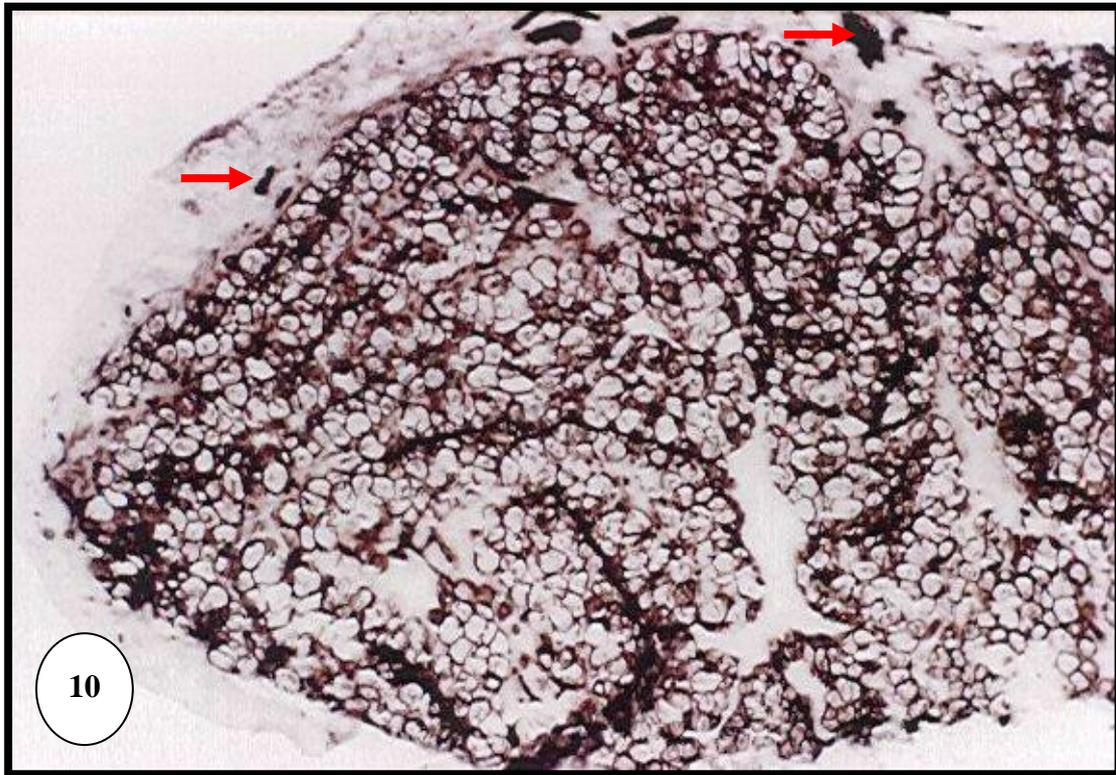


Fig. (10): light micrograph of gonad of *E. tauvina* fish showing: an intense reaction as mentioned in Fig. (9) In addition to some aggregation of steroidogenic cells in the capsule gave an intense reaction to alkaline phosphatase (arrows). Gomori reaction (10×4).

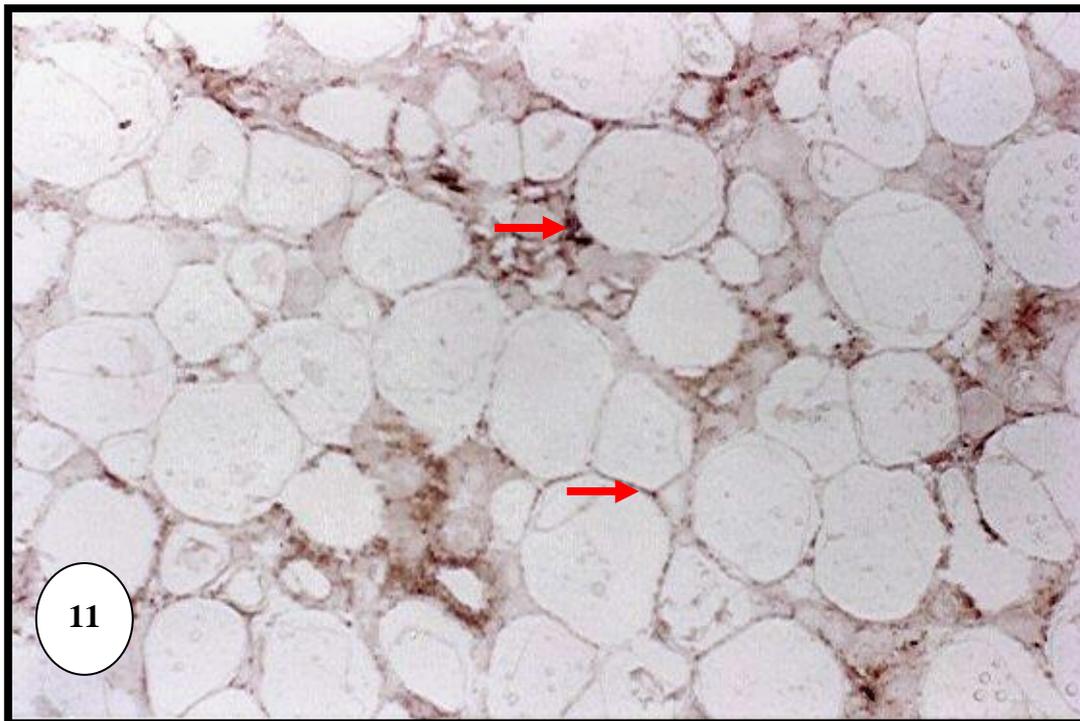


Fig. (11): light micrograph of gonad of *E. tauvina* fish showing: an intense reaction of acid phosphatase in the connective tissue in-between mature oocytes and moderate reaction in granulosa cells surrounded mature oocytes. Gomori reaction (10×4).

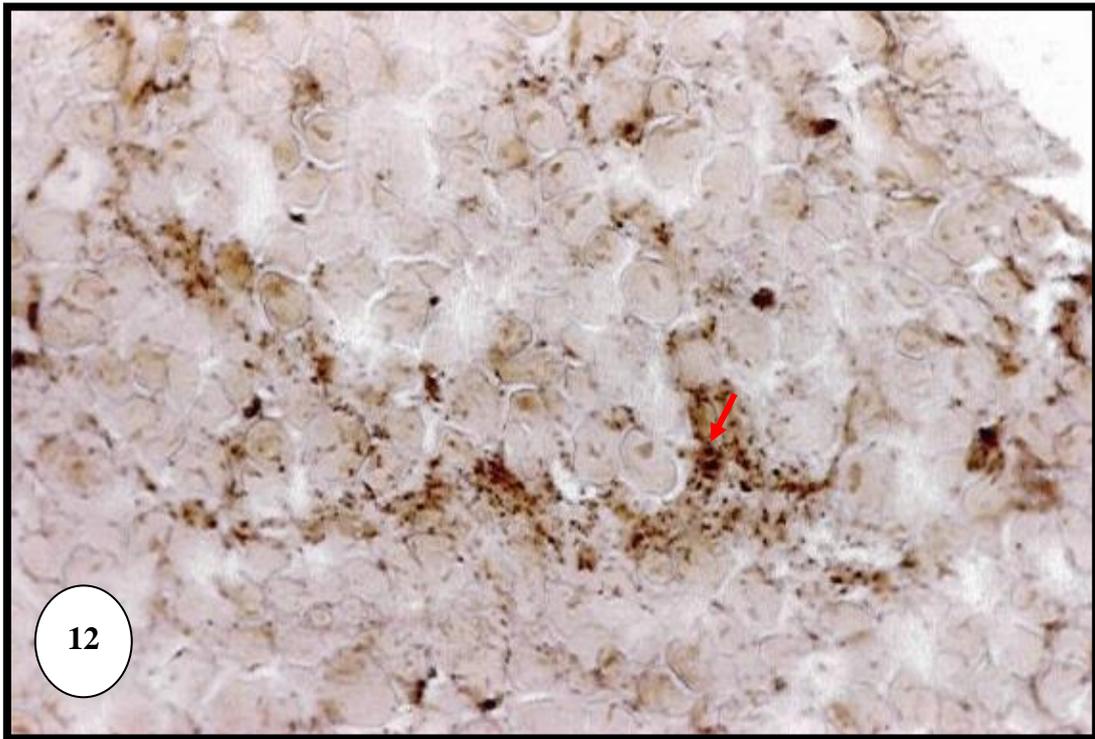


Fig. (12): light micrograph of gonad of *E. tauvina* fish showing: an interstitial connective tissue and aggregations of testicular tissues gave reaction to acid phosphatase varies from moderate to intense reaction (arrows). . Gomori reaction (10×10).

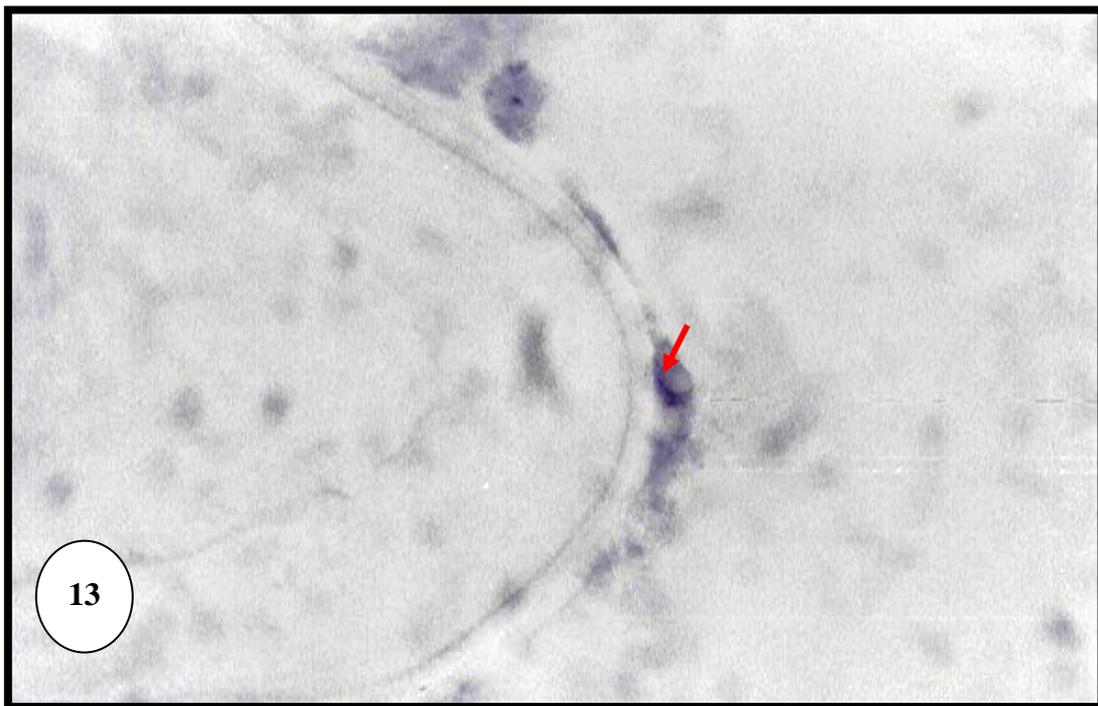


Fig. (13): light micrograph of gonad of *E. tauvina* fish showing: intense reaction of enzyme $\Delta 5 - 3 \beta$ HSD in some granulosa cells around mature oocytes. (10×100).



Fig. (14): light micrograph of gonad of *E. tauvina* fish showing: some aggregations of cells in capsule gave an intense reaction to $\Delta 5 - 3 \beta$ HSD enzyme. (10×10).

Discussion:

The groupers are wide spread in both tropical and temperate seas are commonly heavily fished. The brown spotted grouper, *Epinephelus tauvina*, is one of the most important food fishes in Saudi Arabia and in Kuwait, (Abu-Hakima, 1987). In our findings we classify the gonads of *E. tauvina* into three stages: female, early transition and late transition phase, which also subdivided into two stages. This most probably included in the seven stages recognized by Abu- Hakima (1987).

The *E. tauvina* shows protogynous hermaphroditism in accordance with (Bhandari *et al.*, 2003) our findings in *E. tauvina* not of that the oocytes showed several developmental stages which arranged into lamellae in accordance with (Bouain and Siau, 1983). There were several changes occurs during ripening of oocytes which easily recognized by presence of yolk in the ooplasm (Macer, 1974).

The present results revealed that at the beginning of early transition stage both

perinuclear and mature oocyte appeared and accompanied with few spermatogenic cells in accordance with (Bhandari *et al.*, 2003) who added that there were clear proliferation of spermatogonia towards the centre of lamellae.

In late transition phase stage we notice further degeneration of oocyte and rapid proliferation in addition to active spermatogenesis in accordance with (Abbasi *et al.*, 2007) who described the transitional gonads of the protogynous *Epinephelus coioides* were characterized by the concurrent degeneration of all oocytes and the proliferation of spermatocysts.

Foci of atresia and atretic bodies were routinely observed in histological sections of the gonads at different stages in *E. tauvina*. Atresia in the gonads of protogynous hermaphrodites is well documented by Smith (1965). In the present study, atretic follicles were most evident in the transforming gonads. Hastings (1981) has described three modes of resorption of ovarian tissue in the transforming gonads of *Hemanthias vivanus*. These include fragmentation of pre-vitellogenic oocytes, phagocytosis of

vitellogenic oocytes and invasion and breakdown of mature, unovulated eggs. In the present study, all three modes of breakdown were noted in the transforming gonads of *E. tauvina*. The invasion and breakdown of the mature eggs is similar to pattern typically associated with resorption of unshed eggs (**Saidapur, 1978**).

In an attempt to find a role between enzymatic activity and transition of female to male membrane in gonads of *E. tauvina*. Our result revealed an intense reaction to alkaline phosphatase in granulosa cells and atretic follicles indicating its importance in synthesis of yolk during maturation.

The present results showed moderate to intensive reaction of acid phosphatase in the granulosa cells which surround the oocytes and in connective tissue in between mature oocytes, also the same result was observed in the interstitial connective tissue and the aggregation of testicular tissue in the gonads of *E. tauvina* at the early transitional stage, which revealed the effective role of these cells in digestion and removing the yolk and cellular components of the oocytes in the ovary preparing it to the next stage (**Saidapur, 1978 and Alkaabi & Salim, 2002**).

Concerning $\Delta 5-3\beta$ hydroxysteroid dehydrogenase our result revealed that some granulosa cells around mature oocytes gave intense positive reaction while the gonads in transition stage some cells in capsule in addition to some interstitial connective tissue cells distributed all over the gonads gave positive reaction to same enzyme in accordance with (**Fedorov et al. 1990 and Alkaabi & Salim, 2002**), also with (**Alam et al., 2005**) who have observed clusters of strongly immunopositive cells in tissue near blood vessels in the tunica ovary surrounding the outer periphery in protogynous grouper *Epinephelus merra* these cells possibly play a physiological role in oocyte growth and gonadal restructuring during the sex change of individuals of this species, in contrast immunopositive cells were localized in the

theca layer surrounding the outer periphery of oocytes.

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دراسات نسيجية وكيميائية نسيجية إنزيمية على المناسل المتحولة للهامور العملاق
Epinephelus tauvina من ساحل الخليج العربي للمملكة العربية السعودية

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تمت الدراسة على مناسل أسماك الهامور العملاق *Epinephelus tauvina* بعدد 20 سمكة تم اصطيادها من ساحل الخليج العربي بمدينة الدمام . وقد خضعت المناسل للدراسة النسيجية حيث صنف نمو المناسل نسيجياً في هذه الأسماك إلى ثلاثة أطوار: طور الأنثى وطور التحول الجنسي المبكر وطور التحول الجنسي المتأخر وصنف كل طور إلى مرحلتين . ففي طور الأنثى شوهد العديد من مراحل نمو البويضات بينما في مستهل طور التحول الجنسي المبكر يبدأ تحلل البويضات الناضجة محيطية النواة فيما تميز طور التحول الجنسي المتأخر بتكاثر سريع للخلايا المنوية . ولقد أظهرت نتائج الدراسة الكيميائية نسيجية تفاعل كثيف لإنزيم الفوسفاتيز القلوي في خلايا الغشاء المحبب المحيط بالبويضات الناضجة بينما ظهر تفاعل كثيف لإنزيم الفوسفاتيز الحامضي في الخلايا اليبينية والجريبات الممتصة , وأيضاً ظهر تفاعل كثيف لإنزيم $\Delta 5-3\beta$ hydroxysteroid dehydrogenase في خلايا خاصة في محفظة المنسل.