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Cyclic Changes of Gonadotropin-Secreting Cells During Ovarian Cycle of *Mugil cephalus*

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ABSTRACT

Fish spawning strategies can be developed by taking into account the activity of pituitary gland gonadotropin secretion during gonad maturation. The goal of the current work was to study the immunocytochemistry of *M. cephalus* gonadotrophs during ovary development in natural habitat, utilizing an antibody raised against the Coho salmon gonadotropin II (GTHII) ß subunit. The cycle of oocyte development typically go through six stages: previtellogenic primary oocytes, oocytes at the beginning of yolk vesicles deposition, and the three yolky oocytes; primary, secondary, and tertiary yolk oocytes. In addition, the ripe oocytes were observed by hormonal induction. Based on the seasonal variations observed in the histology and gonadosomatic index, five phases were identified during ovaries maturation, namely peri-vitellogenic ovary, early-, mid-, and late-vitellogenic ovaries, and prespawning ovary. Furthermore, the ripe ovary was experimentally obtained by hormonal stimulation in saline water. The gonadotropin-secreting (GTH) cells made up the larger part of the proximal pars distalis (PPD). The anti-chum salmon GTH IIß subunit exhibited a strong and specific binding to the many secretory granules found in these cells. The size, number and intensity of immunoreactive granules of GTH cells represented certain seasonal changes, occurring concurrently with ovarian development. As the ovary developed and reached reproductive maturity, the activity of of the pituitary gland to produce the gonadotropin hormone generally increased noticeably. During the development of *M. cephalus* ovaries, the gonadotrophs indicated a rise in the immunostaining density, granulation, size and number during ovarian maturation. Furthermore, granulation disappeared, and secretory vacuoles, along with weak immunoreaction of GTH-secreting cells, were observed during induced ripening. The spawned stage was achieved through hormonal injection

INTRODUCTION

The principle that gonadotropins produced by the pituitary gland regulate the development, and the maintenance of gonadal function in vertebrates is no exception for teleosts **(Van Oordt & Peute, 1983)**. Gonadotropic cells and/or GTHs in two distinct forms are observed in some fish species **(Nozaki *et al.*, 1990; Mousa, 2002; Mousa *et al.*, 2021; Mousa *et al.*, 2024)**. Immunocytochemical investigations of the hypophysis cell type localization in mullets; *L. ramada M. cephalus* indicated the presence of only

one type of gonadotrophs **(Mousa *et al*., 2021; Mousa *et al*., 2024)** which secrets GTHIIß. Coincident with gonadal development, a notable alterations for the amount of gonadotropin in *M. cephalus* serum occurred with the maturity of gonads **(Mousa, 1994)**. According to **Marques *et al*. (2000)**, the pituitary produces and releases two types of gonadotropin hormones (GTHs) into blood: the hormones LH or luteinizing and FSH or follicle-stimulating. The hypothalamus produces gonadotropin-releasing hormone (GnRH), which triggers the production of both LH and FSH. Sex hormone synthesis in *M. cephalus* occurs in the follicle layers of the developed oocytes; theca, and granulosa, according to histochemical and ultrastructural observations. During oogenesis, follicular layers have shown concurrent changes both qualitative and quantitative **(Mousa *et al*., 2023)**. The primary way that follicular cells react to FSH is by inducing steroidogenesis, which results in the liver production of yolk vitellogenins and their absorption and processing into globules of yolk in the oocyte **(Lubzens *et al*., 2010; Reading *et al*., 2017)**. Conversely, LH is crucial to controlling oogenesis final phases, such as oocyte maturation as well as ovulation **(Lubzens *et al*., 2010; Mousa, 2010; Mousa *et al*., 2018)**. LH and FSH treatment in female *M. cephalus* induced vitellogenin production until the development of oocyte complete, which ultimately resulted in viable eggs. **(Ramos-Júdez *et al*., 2022a; Ramos-Júdez *et al*., 2022b)**.

The resources of mature breeders are crucial in an effort to extend *M. cephalus* breeding season and consistently produce fertile eggs especially if associated with the widespread production of young in hatcheries. Even with studies on artificially reproducing mullet in captivity, the farming method still depends on catching young fish from the nature **(Lee & Tamaru, 1988)**. The majority of investigators have focused on inducing spawning under natural circumstances during the time of year when they usually breed **(Lee *et al*., 1987; Mousa, 2010)**. The reproductive physiology of this species, including its propagation in captivity or under hormonal therapy, additionally to the arrangements and variations of hypophysial hormones throughout the annual cycle, is, however, poorly understood at this time. Comparative data collection is critical in the teleost reproduction investigation because different species exhibit various physiological and behavioral strategies **(Royan *et al*., 2021)**. The current study is an examination for GTHIIß in hypophysis of *M. cephalus* females during natural ovarian cycle to gain insight mullet's endocrinology and give the fundamental details required for its effective reproduction.

MATERIALS AND METHODS

Fish collection

From the Mediterranean Sea near the shore of Damietta, mature *M. cephalus* females, having total length about 30cm and total weight about 800gm, were observed between January 1, 2023 and December 30, 2024, during the ovarian cycle. Throughout the year, live fish were harvested at intervals of one month. To make sure that all phases

of gonad maturation were covered, mature females were gathered twice every month during the months of spawning migration from September to October.

As indicators of maturity, oocyte diameter, histological appearance, and gonadosomatic index (GSI) were employed. GSI was calculated as follows:

GSI = (Weight of ovary / Weight of gutted fish) x 100.

Five periods of ovarian development were recognized. The fish in the ripening stage was not visible. However, hormone induction was used to reach this stage with ripe ovaries according to **Mousa *et al.* (2018)**. In brief, mature females with prespawning ovaries were acclimated to seawater (35‰) and injected with two injections of hormones. The two injections were: a prime stimulating injection of common carp pituitary glands (20mg/ kg of body weight; BW), and after 24h, a resolving injection of gonadotropin-releasing hormone (4µg buserelin acetate/ kg BW) in combination with dopamine antagonist (5mg Metoclopramide/kg BW)

Histological appearances

Following fish decapitation, the pituitary gland and brain were removed and preserved for twenty-four hours in Bouin's fixative. After being dehydrated by a series of ascending ethanol concentrations and clearing in xylene, the fixed pituitary was embedded in paraffin wax with melting point between 56 & 58^{o}C. The pituitary gland was cut into consecutively sagittal sections, each with a thickness of 5µm. Similarly to the pituitaries, the ovaries were taken out, weighed, and processed for histological analysis. Gonadal sections were stained using Heidenhain's iron hematoxylin and eosin in an aqueous solution as a counterstain **(Conn, 1953)**.

Immunocytochemistry

According to **Mousa *et al*. (2024)**, a Vector Laboratories Vectastain Avidin-biotin peroxidase complex (ABC) Kit was typically used for the immunocytochemical staining of the hypophysis sections. The sections underwent a series of procedures, including xylene deparaffinization, graded ethanol rehydration, and two 10-minute rinses in phosphate-buffered saline (PBS; pH 7.4). The incubation was conducted at lab temperature, and each step was washed with PBS. The chum salmon GTH IIß subunit antibody was used to immunoreact with the sections, diluted 1:5000, for 12-18hr. Dr. H. Kawauchi (School of Fisheries Science, Japan) gave the antibody against the GTH IIß subunit of chum salmon. Then the sections of pituitary were reacted with the biotinylated secondary antibody for one hour and with avidin-biotin-conjugated peroxidase for forty-five minutes. After washing, sections were colored for five minutes using 3-,3`-diaminobenzidine tetrahydrochloride (DAB). Finally, the immunostained slides were dehydrated in ethanol, cleared in xylene and mounted in DPX.

Statistical analysis

The statistical software used for data processing was the SPSS (Statistical Package for Social Sciences). The "t" test for paired samples was used to compare the means. Moreover, the value of $P<0.05$ was considered statistically significant.

RESULTS

Ovarian development

There are six distinct stages in the female *M. cephalus* ovarian cycle based on seasonal variations observed in the histomorphology and GSI (Table 1). Fish in stage I had previtellogenic ovaries with GSI % of 0.55±0.12 and the diameter of largest oocytes was 75±10µm. The majority of the ovarian components in this stage are primary oocytes (Fig. 1a). Early vitellogenic ovaries in stage II females had a GSI of 0.78±0.24. It was observed that the majority of oocytes in these ovaries belonged to vesicle stage, with a 155±25µm mean diameter, and primary oocytes (Fig. 1d). Females in stage III had ovaries at mid stage of vitellogenesis with a mean GSI of 3.25±0.85 and oocyte diameter of 320±45µm. Most oocytes were in the primary and secondary yolk stages (Fig. 2a). The deposition of yolk globules is the characteristic of these oocytes. Ovaries in stage IV fish were at late vitellogenesis, with a GSI of 10.0±1.55. According to Fig. (2d), most of the oocytes were in the stage of secondary yolk formation, with a mean diameter of 430±35µm. Stage V female ovaries were at prespawning stage having 22.5±4.55 GSI %. In the prespawning ovaries, the majority of the oocytes were tertiary yolk ones (Fig. 3a) with 630±25 µm in diameter. The GSI of the ripe females was 28.0±6.5. From the obtained ripe ovaries, the majority of the oocytes are primarily ripe oocytes (Fig. 3d) with a mean diameter of 700±50µm.

Cyclic variation in the immunoreactivity of GTH cells

The majority of the PPD was made up of GTH cells, which had many secretory granules that bonded firmly and precisely to the antibody against ß subunit of chum salmon GTH II (Figs. 1, 2 and 3), but they were negatively stained with GTH Iß antiserum of chum salmon, and GTH Iß and GTH IIß of both coho salmon and killifish. Concurrent with ovarian development, the quantity, size, and intensity of GTH cell immunoreactive granules indicated specific seasonal variations (Figs. 1, 2 and 3).

GTH cells were consistently found in the pituitaries of ripe and vitellogenic fish as well as previtellogenic fish. In the previtellogenic fish, the GTH cells had small sizes and moderate immunostaining (Fig. 1b, c). Granulated, degranulated and vacuolated cells were present. Once vitellogenesis has begun (early and mid-vitellogenesis; yolk deposition), the size and quantity of the GTH positive cells increased, and their immunoreactivity varied (Figs. 1e, 1f, 2b and 2c). Most of the GTH cells had a revival of immunoreactive granules, and few cells exhibited a vacuolated appearance. In the late-vitellogenic females, the immunoreaction of the gonadotrophs was augmented due to the presence of immunoreactive granules in large amount, as well as the presence of degranulated GTH cells (Fig. 2e and f). Thereafter, in fish with prespawning ovaries, the GTH cells exhibited both hypertrophy and hyperplasia. The majority of the GTH cells in this stage showed strong immunoreactivity and seemed to be extremely granulated (Fig. 3b and c). Nevertheless, because of the significant loss of secretory granules, the cells secreting gonadotropin in hormonal induced ripe females appeared vacuolated and

degranulated with weak to moderate immunoreactivity due to significant granules secretion (Fig. 3e, f), and few of the GTH-secreting cells showed a large number of immunoreactive granules.

Table 1. Gonadosomatic index (GSI) and oocyte diameter (Mean±SD) of female *Mugil cephalus*, at different stages of maturation, obtained from natural habitat.

Ovary stage	Number of fish	Gonadosomatic index (%)	Largest oocyte diameter (µm)
Previtellogenesis (I)	20	0.55±0.12	75±10
Early previtellogenesis (II)	20	0.78±0.24	155±25
Mid previtellogenesis (III)	20	3.25±0.85	320±45
Late previtellogenesis (IV)	20	10.0±1.55	430±35
Prespawning (V)	20	22.5±4.55	630±25
Spawning (ripe) (VI)	20	30.0±3.5	700±50

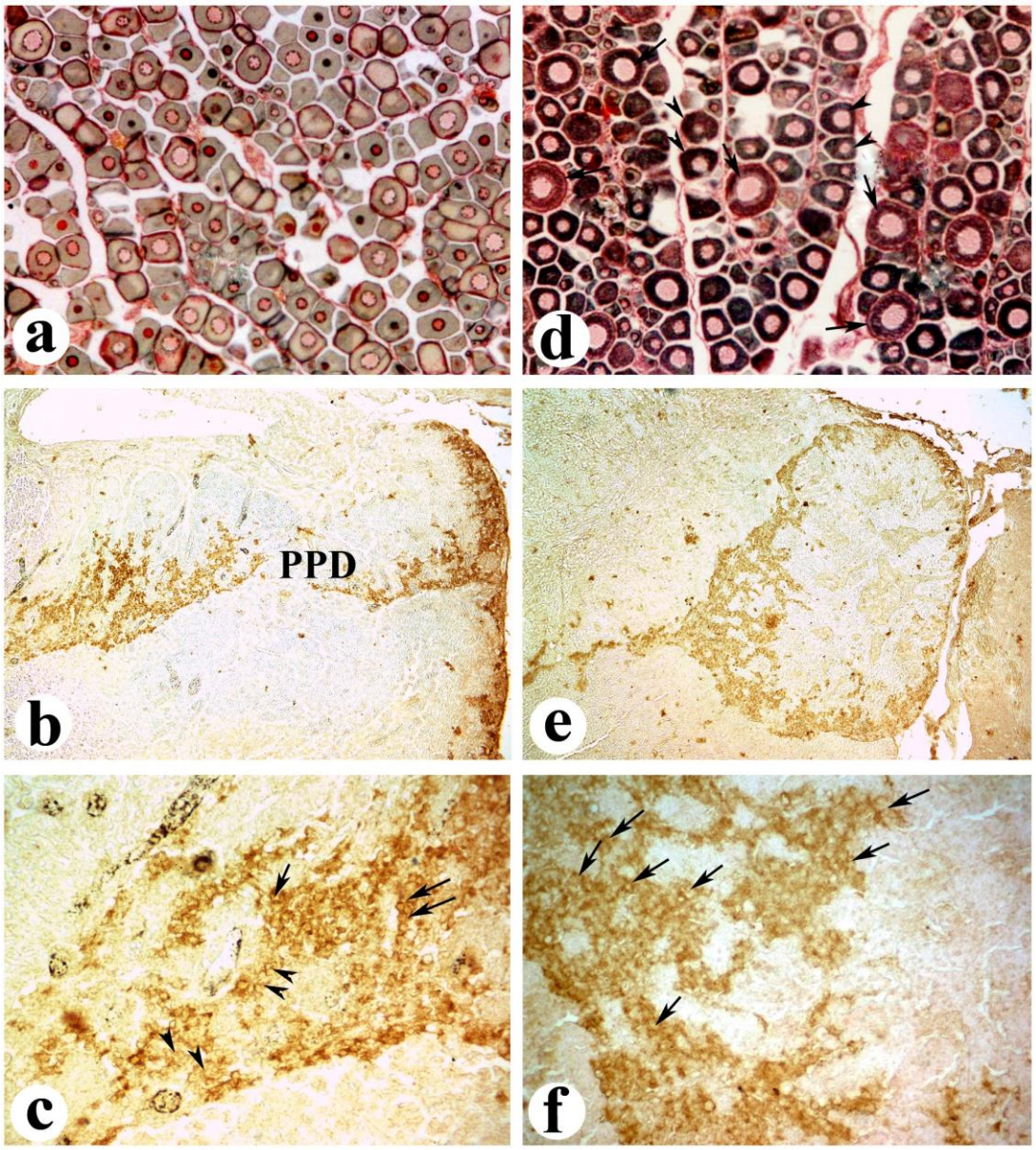

Fig. 1. Sections of *M. cephalus* ovary in different stages of development, stained with iron hematoxylin and eosin (**a** and **d**). X100. **a)** Only the primary oocytes are present in the previtellogenic ovary. Immunostaining of the GTH cells in the pituitary gland of female *M. cephalus* stained with anti-chum salmon GTH IIß subunit (**b, c, e** and **f**); **b)** Previtellogenic female pituitary with small size and moderate-immunoreactive GTH cells located in the PPD. X100. **c)** Enlarged GTH cells of females with previtellogenic ovary; both granulated (arrows) and degranulated (arrowheads) cells were present. X400. **d)** Early-vitellogenic ovary containing the vesicles oocytes (arrows) and primary oocytes (arrowheads). **e)** GTH cells of females with early-vitellogenic ovary moderately immunostained. X100. **f)** Magnified portion of **(e)** showing the revival of immunoreactive granules in most of the GTH cells (arrows). X400

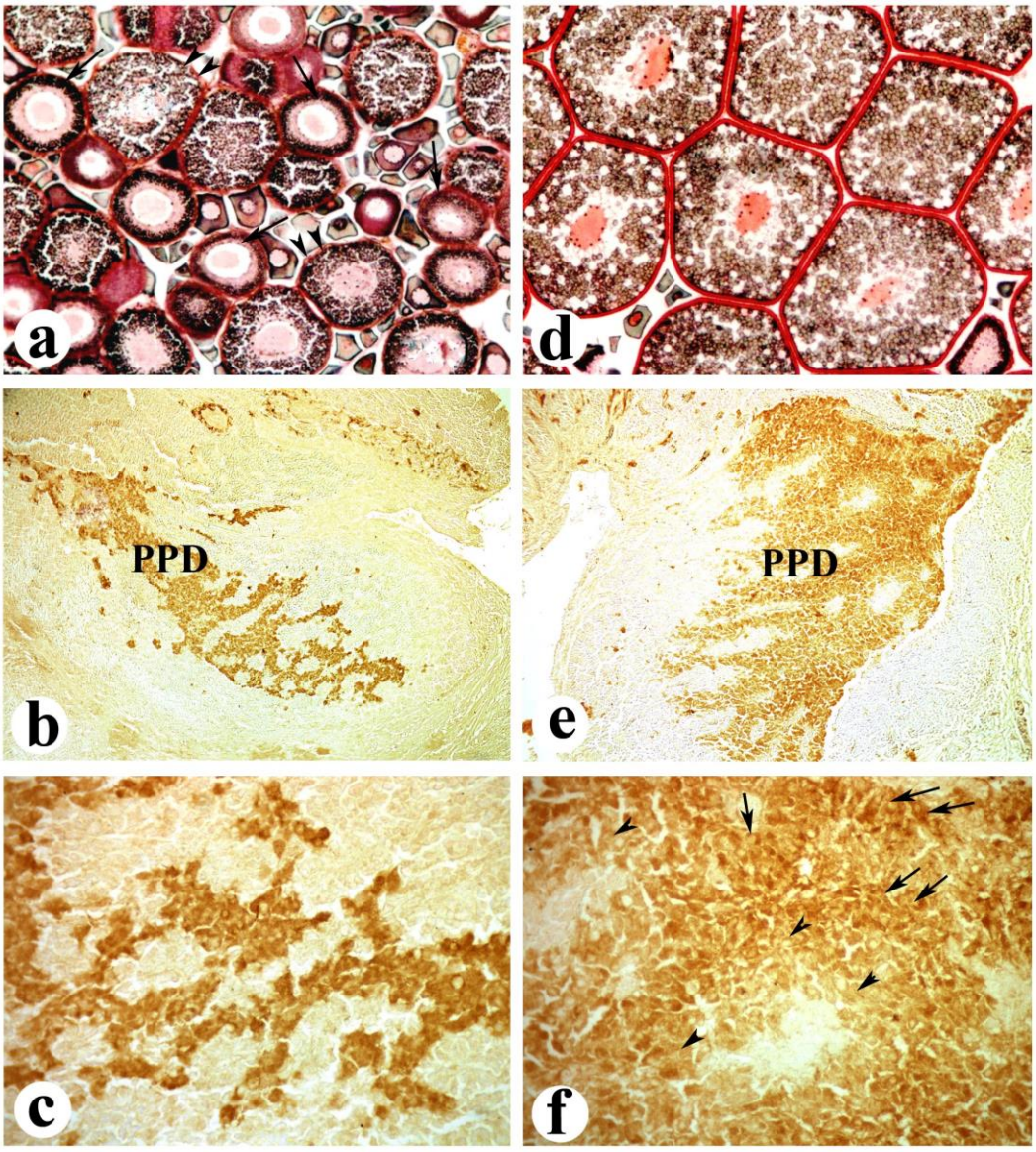

Fig. 2. *M. cephalus* ovary sections at mid-vitellogenesis **(a)** and late-vitellogenesis **(d)** stained with iron hematoxylin. X100. **a)** Mid-vitellogenic ovary, showing the protied yolk granules stained black with iron hematoxylin- in the primary (arrows) and secondary yolk oocytes (arrowheads). **b)** Immunoreactive GTH cells in the PPD of Mid-vitellogenic female. X100. **c)** Enlarged area of the GTH cells in mid-vitellogenesis female which are increased in number and size and with variable immunoreactivity. Note the revival of immunoreactive granules in most of the GTH cells. X400. **d)** Ovary that is late-vitellogenic and contains secondary yolk stage oocytes. **e)** Immunostained GTH cells of female with late-vitellogenic ovary occupied most of the PPD. X100. **f)** Enlarged portion of **(e)** showing granulated GTH cells (arrows) with intensely immunoreactive granules and some degranulated GTH cells (arrowheads). X400

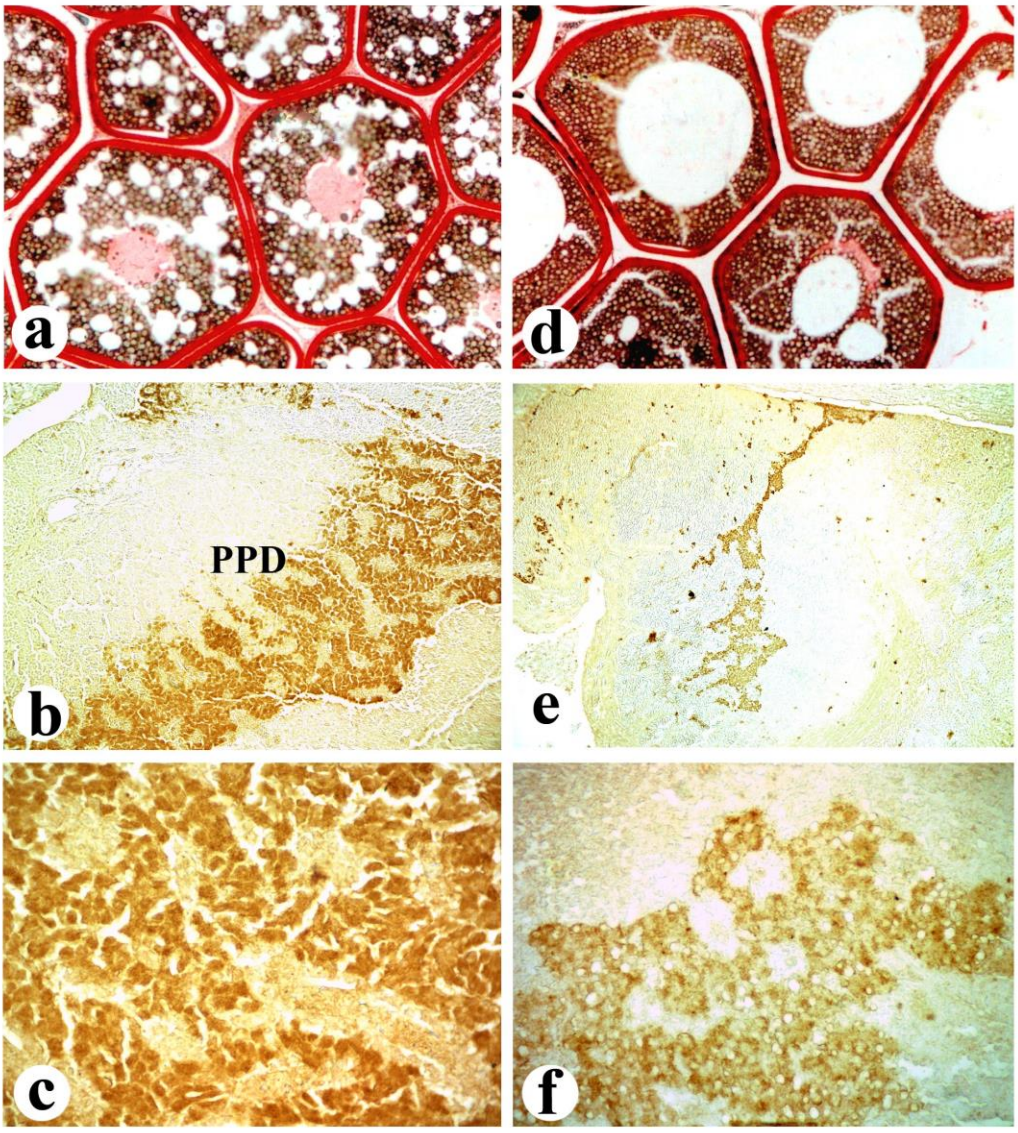

Fig. 3: Iron hematoxylin-stained ovaries of *M. cephalus* prespawning female **(a)** and spawning (ripe) female, induced experimentally by injection of hormones **(d)**. X100. **a)** The tertiary yolk oocytes are found in the prespawning ovary. **b)** The GTH cells of prespawning female are increased in number and located in the largest portion of PPD with strong immunoreactivity. X100. **c)** Higher magnification of the GTH cell area in prespawning female indicated the accumulation of large coarse immunoreactive granules in all of the GTH cells. X400. **d)** Hormonal-induced spawning ovary, having the ripe oocytes with central located one large lipid vesicle. **e)** GTH cells showing weak immunoreactivity in the hypophysis of induced ripe female. X100. **f)** The PPD magnification of spawned female appeared the degranulation and vacuolization of in most of the GTH cells. X400

DISCUSSION

In teleost species, two distinct GTHs (GTH I and GTH II) were recorded **(Nozaki *et al.*, 1990; Naito *et al.*, 1991; Mitparian *et al.*, 2023)**. In the previous paper, we reported that the pituitary gland of *M. cephalus* contains a single type of GTH-secreting cells **(Mousa *et al.*, 2024)** which secrets both GTHI and GTHII. The current investigation confirms this observation in every phase of ovarian growth that we examined. Similarly, one cell type in the pituitary gland of *L. niloticus*, *S. solea*, and *L. ramada* showed colocalization of chum salmon βGTHI and βGTHII immunoreactivities **(Mousa, 2002; Mousa & Khalil, 2004; Mousa *et al.*, 2021)**. GTH cell distribution in *M. cephalus* is similar to that which can be discovered in other telesosts **(Toubeau *et al.*, 1991; Mousa, 2002; Mousa & Khalil, 2004; Mousa *et al.*, 2021)**. In particular, gonadotropin, which is generated by gonadotrops, is intimately linked to reproduction since it promotes the production of steroids, vitellogenin absorption, ovulation, maturation of eggs, and sperms release **(Wallace & Selman, 1981; Goetz, 1983; Ramos-Júdez *et al.*, 2022b)**. Gonadotropin-induced elevation of plasma estradiol-17β (E1) levels in female teleosts drives sexual maturation **(Nagahama, 1987; Fakriadis *et al.*, 2024)**. Gonadotropin (s) promotes the oocyte's uptake of vitellogenin, whereas E1 activates the liver's vitellogenin (VTG) synthesis and secretion, a precursor to the protein in the yolk **(Mommsen & Walsh, 1988; Ferdinand *et al.*, 2023)**.

Throughout the ovarian cycle, the GTH-immunoreactive (-ir) cells showed changes in both quality and quantity. In females with previtellogenic ovaries, and at the beginning of yolk deposition (early-vitellogenic and mid-vitellogenic ovaries), the cells secreting gonadotropins are characterized by a gradual formation of GTH-immunostained granules and are also featured by a decrease in secretory activity, being evident from the presence of vacuoles and lack of granulation in some cells, releasing a small amount of the hormone which is just enough to initiate vitellogenesis. In the stage of late-vitellogenic ovaries, the immunoreactivity of the GTH-ir cells is augmented owing to the cumulation of numerous immunostained granules. Countless GTH cells appear to empty their secretory contents to release the hormone essential for the oocyte's yolk formation. Later, on approach to the prespawning stage, when the entire ooplasm becomes heavily impregnated with yolk globules, the GTH cells reach the peak of their activity showing tremendous changes represented in the form of hyperplasia, hypertrophy and accumulation of large amounts of GTH-ir granules in the oocytes. The present observations are consistent with the immunocytochemical findings reported in *Salmo gairdneri irideus* **(Nozaki *et al.*, 1990)**. Additionally, the current findings align with the histological findings in numerous fish, including: *Heteropneustes fossilis* **(Sundararaj, 1959)**, *Oncorhynchus nerka* **(Van Overbeek & McBride, 1967)**, *Chanos chanos* **(Tan, 1985)**, *Etroplus suratensis* **(Krishnan & Diwan, 1990)**, and *M. cephalus* **(Mousa, 1994)**. Furthermore, the present immunocytochemical observations in female *M. cephalus* are

consistent with the physiological observation presented by **Amano *et al*. (1992)** in female *Oncorhynchus masou*. They observed that changes in pituitary GTHIIß contents resembled those of GSI, and a rapid increase occured according to vitellogenesis and ovulation. Our results also support and explain seasonal variations in the gonadotropin level obtained during the ovary development of *M. cephalus* **(Mousa, 1994; Zaki *et al*., 1995)**. The previous authors postulated that serum gonadotropin increased during vitellogenesis and added that a dramatic hormone increase was observed during late-vitellogenesis (stage IV) after which it returned to low level at the end of vitellogenesis (prespawning stage), causing synthesis of gonadotropin in the pituitary gland by the mechanism of negative-feedback, attributed to the GTH cells strong immunoreactivity of the prespawning female in the present study. **Mousa (1994)** concluded that in the ripe *M. cephalus* female (ripe stage obtained with hormonal injection), a pronounced drop in serum estradiol-17ß and testosterone levels occurred during final maturation of oocyte (though before oviposition), removing the negative feedback and allowing the serum gonadotropin to rise. The releasing of gonadotropin from pituitary during final oocyte maturation and ovulation is reflected by degranulated and vacuolated appearance and weak staining of the GTH-ir cells in the hormone-induced ripe females.

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REFERENCES

Amano, M.; Aida, K.; Okumoto, N. and Hasegawa, Y. (1992). Changes in salmon GnRH and chicken GnRH-II contents in the brain and pituitary, and GTH contents in the oituitary in female masu salmon, *Oncorhynchus masou*, from hatching through ovulation. Zool. Science, 9: 375-386.

Con, H.J. (1953). "Biological stains" (Williams and Wilkins Company, Baltimore).

Fakriadis, I.; Meiri-Ashkenazi, I.; Bracha, C.; Rosenfeld, H.; Corriero, A.; Zupa, R.; Pousis, C.; Maria Papadaki, M. and Mylonas, C. C. (2024). Gonadotropin expression, pituitary and plasma levels in the reproductive cycle of wild and captive-reared greater amberjack (*Seriola dumerili*). General and Comparative Endocrinology, 350, 114465.

Ferdinand, B.R.; Venci Candida, X.; Suganthi, A.S. and Padua, J.C. (2023). Functional Aspects of Vitellogenin in Oogenesis and Its Regulatory Mechanism. In *Vitellogenin in Fishes-Diversification, Biological Properties, and Future Perspectives* (pp. 75-91). Singapore: Springer Nature Singapore.

Goetz, F.W. (1983). Hormone control of oocyte final maturation and ovulation in fishes. In "Fish Physiology" (W.S. Hoar, D.J. Randall, and E.M. Donaldson, Eds.), Vol. *9*, Part B, pp. 117-170. Academic Press. New York.

Krishnan, L. and Diwan, A.D. (1990). Seasonal changes in gonads and their relationship with gonadotrophs of the pituitary in *Etroplus suratensis* (Block). J. Mar. Biol. Assoc. India 32 (1-2): 5-9.

Lee, C.S. and Tamaru, C.S. (1988). Advances and future prospects of controlled maturation and spawning of grey mullet (*Mugil cephalus* L.) in captivity. Aquaculture 74: 63-73.

Lee, C.S.; Tamaru, C.S.; Miyamoto, G.T. and Kelley, C.D. (1987). Induced spawning of grey mullet (*Mugil cephalus*) by LHRH-a. Aquaculture 62: 327-336.

Lubzens, E.; Young, G. and Bobe, J. (2010). Oogenesis in teleosts: How eggs are formed. Gen. Comp. Endocrinol. 165: 367–389. doi:10.1016/j.ygcen.2009.05.022

Marques, P.; Skorupskaite, K.; George, J. T. and Anderson, R. A. (2000). "Physiology of GNRH and gonadotropin secretion," in Endotext [internet]. K. R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, W. W. de Herder, K. Dhatariya, et al. (South Dartmouth (MA).

Mitparian, T.; Kettratad, J.; Kangwanrangsan, N.; Ampawong, S.; Kaneko, G.; Iida, A.; Senarat, S. and Charoenphon, N. (2023). Brain structure and ultrastructure of gonadotropic cell in female grunting toadfish, *Allenbatrachus grunniens* (Linnaeus 1758). Agriculture and Natural Resources, 57 (2): 281-288.

Mommsen, T.P. and Walsh, P.J. (1988). Vitellogenesis and oocyte assembly. In: W.S. Hoar and D.J. Randall (Editors), Fish Physiology, Vol. XIA. Academic Press, Inc., New York, NY, pp. 347-406.

Mousa, M.A. (1994). Biological studies on the reproduction of mullet (*Mugil cephalus* L.) in Egypt. Ph.D. Thesis. Ain Shams University. PP278.

Mousa, M.A. (2002). Immunocytochemical identification and distribution of the cell types in the pituitary gland of the Nile perch, *Lates niloticus* (Teleostei, Centropomidae). Bull. Nat. Inst. of Oceanogr. & Fish., A.R.E., *28*: 157-178.

Mousa, M.A. (2010). Induced spawning and embryonic development of *Liza ramada* reared in freshwater ponds. Anim. Reprod. Sci., 119: 115-122.

Mousa, M.A. and Khalil, N.A. (2004). Immunocytochemical study of cell type distribution in the pituitary gland of the common sole, *Solea solea* (Linnaeus, 1758). J. Union Arab Biol. Cairo, 22 (A) Zool.:51-74.

Mousa, M. A.; Kora, M. F. and Khalil, N. A. (2018). Evaluation of the effectiveness and cost of different hormones in stimulating the spawning of thin lipped grey mullet, *Liza ramada*. Egypt. J. Histol., 41(3): 275-284.

Mousa, M.A.; El-messady, F.A. and Khalil, N.A. (2021). Immunohistochemical description and dispensation of the hypophyseal cell types in *Liza ramada*.

Egypt.
J. Histol., 45 (4): 1068-1078.

Mousa, M. A.; El-Messady, F. A. and Khalil, N. A. (2023). Description of the sex hormone-secreting cells during oocyte development and atresia in flathead grey mullet *Mugil cephalus* (*Mugilidae*) in Al-Bardawil Lagoon, Egypt. Journal of Ichthyology, 63 (3): 513-523.

Mousa, M. A.; Khalil, N. A. and Kora, M. F. (2024). Hormone-secreting cells in the hypophysis of *Mugil cephalus*. Egyptian Journal of Aquatic Biology & Fisheries, 28 (4): 935 – 949.

Nagahama, Y. (1987). Gonadotropin action on gametogenesis and steroidogenesis in teleost gonads. Zoological Science, 4: 209- 222.

Naito, N.; Hyodo, S.; Okumoto, N.; Urano, A. and Nakai, Y. (1991). Differential production and regulation of gonadotropins (GTH I and GTH II) in the pituitary gland of rainbow trout *Oncorhynchus mykiss*, during ovarian development. Cell Tissue Res., 266: 457-467.

Nozaki, M.; Naito, N.; Swanson, P.; Dickhoff, W.W.; Nakai, Y.; Suzuki, K. and Kawauchi, H. (1990). Salmonid pituitary gonadotrophs. II. Ontogeny of GTH I and GTHII cells in the rainbow trout (*Salmo gairdneri irideus*).. Gen. Comp. Endocrin., 77: 358-367.

Ramos-Júdez, S.; Giménez, I.; Gumbau-Pous, J.; Arnold-Cruañes, L.S.; Estévez, A. and Duncan, N. (2022a). Recombinant FSH and LH therapy for spawning induction of previtellogenic and early spermatogenic arrested teleost, the flathead grey mullet (*Mugil cephalus*). Sci. Rep. 12: 6563. doi:10.1038/s41598-022-10371-0

Ramos-Júdez, S.; Danis, T.; Angelova, N.; Tsakogiannis, A.; Giménez, I.; Tsigenopoulos, C. S.; Duncan, N. and Manousaki, T. (2022b). Transcriptome analysis of flathead grey mullet (*Mugil cephalus*) ovarian development induced by recombinant gonadotropin hormones. Frontiers in Physiology, 13: 1033445.

Reading, B.; Sullivan, C. and Schilling, J. (2017). Vitellogenesis in fishes. doi:10. 1016/B978-0-12-809633-8.03076-4

Royan, M. R.; Siddique, K.; Csucs, G.; Puchades, M. A.; Nourizadeh-Lillabadi, R.; Bjaalie, J. G.; Henkel, C.V.; Weltzien, F. A. and Fontaine, R. (2021). 3D atlas
of the pituitary gland of the model fish medaka (*Oryzias latipes*). Frontiers in Endocrinology, 12, 719843.

Sundararaj, B.I. (1959). A study on the correlation between the structure of the pituitary gland of the indian catfish, *Heteropneustes fossilis*, and the seasonal changes in the ovary. Acta Anat. 37: 47-80.

Tan, J.D. (1985). A histological study of the hypophysial- gonadal system during sexual maturation and spawning in the milkfish, *Chanos chanos* (Forskal).. J. Fish Biol. 26: 657-668.

Toubeau, G.; Poilve,A.; Baras, E.; Nonclercq, D.; De Moor, S.; Beckers, J.F.; Dessy-Doize, C. and Heuson-Stiennon, J.A. (1991). Immunocytochemical study of cell types distribution in the pituitary of *Barbus barbus* (Teleostei, Cyprinidae). Gen. Comp. Endocrinol., 83: 35.

Wallace, R.A. and Selman, K. (1981). Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool., 21: 325-343.

Van Oordt, P.G.W.J. and Peute, J. (1983). The cellular origin of pituitary gonadotropins in teleosts.. In Fish Physiology. W.S. Hoar, D.J. Randall, and E.M. Donaldson, eds. (Academic, New York, London.), pp. Vol. IX, PP: 137-186.

Van Overbeeke, A.P. and McBride, J.R. (1967). The pituitary gland of the sockeye, *Oncorhynchus nerka*, during sexual maturation and spawning. J. Fish. Res. Bd. Can. *24*, 1781-1807.

Zaki, M.I.; El-Gharabawy, M.M., and Kamil, S.A. (1995). Seasonal changes in the gonadotropic and sex steroid hormones in the blood serum of the Gray mullet, *Mugil cephalus*, in the Sabkhet el Bardawil of the Mediterranean Sea. J. Ichthyology 35 (2): 1-7.