

Immunohistochemical study of gonadotropin-releasing hormone and somatolactin during induced spawning of *Liza ramada*

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Article

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ABSTRACT

Introduction: Gonadotropin releasing hormone (GnRH) is a crucial regulator of gonadal development and reproduction in fish. In addition, it is a possible secretagogue of somatolactin (SL) in teleosts. Little is known about the possible association of GnRH and SL during reproduction of *Liza ramada*.

Aim of the work: The present study was designed to investigate the GnRH and SL immunoreactivities during inducing the final stages of maturation, ovulation and spawning of *Liza ramada*; to assess the possible actions of these hormones on sexual maturation and spawning of teleosts.

Materials and Methods: Histological and immunohistochemical techniques were generally performed to describe the immunoreactivity of both GnRH and SL during ovarian maturation and spawning of *Liza ramada*.

Results: The mammalian GnRH (mGnRH) cell bodies were observed in the medulla oblongata. However, SL-immunoreactive (-IR) cells were detected in the pars intermedia (PI) of the pituitary gland. The GnRH-IR nerve terminals are in close contact with SL-IR pituitary cells. Importantly, the activity of both mGnRH-IR neurons and SL-IR pituitary cells were increased as reflected with the increased cell number and size during sexual maturation and spawning. The activity of these cells was more pronounced as indicated by the increased cells number and size with more immunoreactivity in pre-spawning female. During final maturation and spawning, mGnRH-IR neurons and SL cells showed an increase in the secretory activity as reflected by their small sizes, vacuolated appearance and weak immunoreactivity.

Conclusions: Taken together, these findings suggest that the close contact of mGnRH-IR terminals with SL-IR cells in the pituitary, together with the concomitant changes of GnRH and SL immunoreactivity in response to progress in the reproductive events suggest that GnRH may regulate SL release and the regulation of reproductive activity in *L. ramada*.

Key Words: Gonadotropin-releasing hormone, Immunocytochemistry, *Liza ramada*, maturation, ovulation, somatolactin, spawning.

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INTRODUCTION

Spawning induction of cultured fish by hormonal treatments is one of the ways to produce fry for stocking ponds. Similar to females of many commercially important fishes, mullet do not undergo final oocyte maturation (FOM), ovulation or spawning in captivity^[1-3]. Consistently, the ova will not advance to final maturation and ovulation without exogenous hormone stimulation, but will undergo atresia and degenerate. Therefore, it is necessary to induce maturation and ovulation with exogenous hormone stimulation. Indeed, the induced ovulation technique using acute injections of hormones including GnRH is an important step in the development of the mullet^[3, 4] or *Chondrostoma nasus*, Cyprinidae^[5] culture.

GnRH is a decapeptide neuroendocrine hormone that is considered to play important roles in the regulation

of teleosts reproduction, mainly by stimulation of gonadotropin release from the pituitary gland^[6-9]. Teleost fishes lack a functional-hypophyseal portal system, but GnRH nerve fibers terminate in the vicinity of the pituitary gonadotrophs^[10, 11]. Recently, a growing body of data suggests the involvement of GnRH in regulating the secretion of various pituitary hormones including SL-expressing cells were observed in *L. niloticus*^[11, 12], genus *Oncorhynchus*^[13-15], the steelhead trout and *O. mykiss*^[16]. SL is a pituitary hormone closely related to GH and is only found in fish. SL is involved in the regulation of different physiological processes, including reproduction^[17-22], acid-base and calcium regulation^[13, 23, 24], phosphate and fat metabolism^[25, 26], and background adaptation^[27-29]. SL has also been linked to fish reproduction. Indeed, SL cells in the PI are activated during the reproductive phase in the genus *Oncorhynchus*^[19, 30] and *Odontesthes bonariensis*^[31]. In addition, seasonal variations in the

number, size, and intensity of the immunoreaction of SL cells were observed concomitant with the development of the gonads and spawning in *O. niloticus* and *Mugil cephalus*^[17, 18]. Moreover, the close association of GnRH-IR fibers with various pituitary hormones secreting cells including SL-expressing cells; suggests that SL-producing cells are regulated by GnRH^[11, 32]. Both hormones regulate the reproductive activity in teleosts, however, little is known about the possible association of GnRH and SL during sexual maturation and spawning of thin-lipped grey mullet. Therefore, we aimed to investigate GnRH and SL immunoreactivities during stimulating oocyte final maturation, ovulation and spawning to assess the possible actions of these hormones on sexual maturation and spawning of thin-lipped grey mullet; *L. ramada*. Our results may provide information that could be critical for the development of reliable methods of spawning induction in this and other commercially important species.

PATIENTS AND METHODS

This study was carried out at El-Matareyya Research Station and Zoology Department, Faculty of Science-Zagazig University during the spawning season in the period from 1st November, 2014 and 31st. January 2015.

Broodstock collection

Mature breeders of thin-lipped grey mullet, at least two-years-old, with total weights ranged from 300 to 600 g with total lengths 30 - 40 cm, were collected alive, by draining water completely, during the spawning season (November to January) from freshwater culture ponds (at El-Serw Fish Research Station). Sexes were identified based on spermiation for males and slightly distended abdominal condition for females.

Seawater acclimation and induction of spawning

The present experiments were carried out, during the natural spawning season of thin-lipped grey mullet. The fish were anesthetized in a solution (40 mg/l) of clove oil (Sigma) before handling^[33]. Mature females of thin-lipped grey mullet were selected on the basis of the presence of a soft, swollen abdomen and protruding genital papillae. The maturity and the oocyte diameters of the females were staged by obtaining *in vivo* biopsy of the ovary using a polyethylene cannula^[34]. The females that were used possessed oocytes whose diameters were greater than 600 μ m. Ripe males in which milt could be easily extruded, by gentle pressure on their bellies, were used. Selected breeders were acclimated in 2000-litre circular fiberglass tanks (10 fish/tank). In brief, fish were transferred to water with 10% salinity (for 12 h) which gradually increased to 35% (for another 12 h).

Acclimated breeders were transferred to 500-litre fiberglass tanks equipped with constant running ozonated seawater (35%) and aeration (Female + 2 males/tank) for induction of spawning with human chorionic gonadotropin (HCG) "pregnyl" (Nile Co. for Pharmaceuticals, Cairo,

ARE) alone or in combination with LHRH-a. The protocol of hormonal injection was previously described^[31]. Water temperature and salinity were 19°C and 35‰, respectively.

Tissue processing and histology

Ten fish from each stage during induction of spawning; pre-spawning, mature and post-spawning females, were taken for histological and immunohistochemical investigations. The fishes were anesthetized in a solution (40 mg/L) of clove oil (Sigma) before handling^[33]. The gonadal biopsies were examined immediately after anesthesia. Oocyte diameter and morphology were examined microscopically. The diameter of at least 25 of the largest oocytes was recorded from each fish, and the position of the germinal vesicle (GV) was determined after clearing the cytoplasm for 10 min with a 1:1:1 v/v methanol: ethanol: acetic acid solution^[35]. The anesthetized fish perfused via the ascending aorta with 20 ml of normal saline, followed by 50 ml of Bouin's fluid at 4°C. Immediately after the dissection, the pituitary gland, attached to the brain, was post-fixed in Bouin's fluid for 24 h at 4°C. The gonads were removed and post fixed in Bouin's fluid for 24 h at 4°C. Thereafter, the fixed gonads, brain and pituitaries were dehydrated through graded ethanol solution, cleared and embedded in paraplast (M.P.: 56–58°C). Consecutive transverse sections were made at 4 μ m thickness. Sections of gonads were stained with Harris's alum hematoxylin^[36] and aqueous solution of eosin as a counter stain.

Immunohistochemical procedures

Antibodies: Rabbit antisera directed against GnRH antisera: mGnRH (83LRF) (G. Tramu, Avenue des Facultés, Talence, France), salmon GnRH (Lot No. 1668) (J.A. King, University of Cap Town, South Africa), cGnRH-II (Rüdiger W. Schulz, University of Utrecht, Faculty of Biology, The Netherlands), cGnRHIII (aCII6) (Koichi Okuzawa, National Research Institute of Aquaculture, Mie, Japan), Lamprey I GnRH (Lot 21134-), and Lamprey III GnRH (Lot 3952) (Stacia Sower, University of New Hampshire, USA). In addition, antiserum directed against chum salmon somatolactin (Lot No. 8906) was obtained from Dr. H. Kawauchi (School of Fisheries Science, Kitasato University, Iwate, Japan).

Immunohistochemistry

Immunocytochemical staining for the sections of the pituitary gland and brain was generally performed with a vectastain ABC (avidin–biotin peroxidase complex) Kit (Vector Laboratories) as described previously [17]. In brief, sections were deparaffinized in xylene, rehydrated through graded ethanol, washed in phosphate-buffered saline (PBS; pH 7.4) for two times 10 min each. All incubations were done at 4°C and PBS was used for washing after each step. Sections were incubated with the antisera to the various GnRHs overnight at 4°C (1:1000 for each of lGnRH-I, lGnRH-III, sGnRH (1668), cGnRH-II, and mGnRH (83LRF)). Thereafter, the sections were incubated with

the biotinylated secondary antibody (Vector Laboratories) for 1 h and with avidin–biotin-conjugated peroxidase for 45 min. Finally, the sections were washed and stained with 3', 3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma) including 0.01% H₂O₂ in 0.05M Tris-buffered saline (pH 7.6) for 3–5 min. After the enzyme reaction, the sections were washed in tap water, dehydrated in ethyl alcohol, cleared in xylene, and mounted in DPX (Din-butylPhthalate in Xylene; Fluka Chemie AG, Buchs, Switzerland).

Immunohistochemical double staining

To study co-localization of mGnRH and SL using double peroxidase immunohistochemistry with a vectastain ABC (Avidin-biotin peroxidase complex) Kit (Vector Laboratories) as described previously^[11]. The sections of the pituitary gland and brain were deparaffinized in xylene, rehydrated through graded ethanol and washed in phosphate-buffered saline (PBS). Then, the sections were treated with 0.6% H₂O₂ for 30 min to inactivate peroxidase in ABC and washed in several changes of PBS. Sections stained for mGnRH were then incubated with a second primary antibody against SL (Lot No. 8906, 1:1 000 dilution) (H. Kawauchi, School of Fisheries Science, Kita-sato University, Iwate, Japan). All incubations were done overnight at 4°C. Slides were then washed in PBS, exposed to the biotinylated secondary antibody (Vector Laboratories) for 1 h. and with avidin –biotin-conjugated peroxidase for 45 min. Finally, the sections were washed and stained with 3', 3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma) including 0.01 % H₂O₂ in 0.05 M Tris-buffered saline (pH 7.6) for 35- min. Sections stained with anti-GnRH antibody were washed in several changes of PBS. Sections were then incubated, overnight at 4°C, with a second primary antibody against chum salmon somatolactin, washed in PBS, exposed to the biotinylated secondary antibody (Vector Laboratories) for 1 h. and with avidin-biotin-conjugated peroxidase for 45 min. Finally, the sections were washed and stained with 3', 3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma) including 0.01 % H₂O₂ in 0.05 M Tris-buffered saline (pH 7.6) for 35- min. During staining with DAB, the nickel solution was used to differentiate between the double immunostaining. The chromogen DAB used for the first primary antiserum appeared black, whereas the one used for the second primary antiserum appeared brown. After the enzyme reaction, the sections were washed in tap water, dehydrated in alcohol, cleared in xylene and mounted in DPX.

Specificity controls

To demonstrate specificity of staining, the following controls were included as mentioned in details elsewhere^[11]: (1) Preabsorption of antibody against mGnRH and SL with a synthetic peptide for mGnRH (Sigma) and SL (H. Kawauchi) for 24 h at 4°C (2) omission of either the first or second primary antibody and either the first or second and secondary antibody.

Cell size measurements

Quantification of SL–immunoreactive cells in the PI was calculated from four sections of each individual animal (n = 8 in each maturity stage). The soma sizes of neurons immunoreactive to mGnRH in the NLTP as well as SL-ir pituitary cells were measured using computer-aided analysis (the Image-Pro Analysis package, Media Cybernetics) of digital images viewed via microscope (Axioskope, Zeiss, Oberkochen, Germany). A3CCD color video camera, (Sony) was used for a minimum of 50 mGnRH-ir neurons from each brain area under investigation (NL TP) per animal as well as 50 SL-ir cells from PI, the cross-sectional area was measured for neurons with the nucleus in the plane of section.

Statistical analysis

Differences between treatments were tested by one-way ANOVA using the treatment as factor of variance. Student-Newman-Keuls test or Holm-Sidak method were used to identify significant differences between all groups or compared to control, respectively. Statistical significance was accepted at P<0.05.

RESULTS

Induced spawning

The breeders were successfully acclimated to seawater (35%) prior to hormonal injection. High rate (100%) of ovulation and spawning was achieved in L. ramada females utilizing pregnyl (HCG) as priming and resolving injections. All the injected females were spawned at a time of 44 to 52 h after hormonal injection.

Histological characters of post-vitellogenic and mature oocytes

All females prior to hormonal injection contained vitellogenic oocytes varying in diameter from 600 to 650 µm. Vitellogenic (tertiary yolk) oocytes had a centrally located germinal vesicle (GV), and their cytoplasm was filled mostly with yolk globules and lesser number of unstained lipid droplets scattered throughout the cytoplasm (Fig. 1a).

Final oocyte maturation and spawning of hormonal-injected fish

Based on the morphological and histological changes; oocyte maturation was identified into two stages. Early maturation stage, which included lipid-droplet coalescence and germinal vesicle migration, and the late maturation stage included germinal vesicle breakdown (GVBD) and yolk-globule coalescence (Figs. 1a, 1b, 2a-2c).

The first morphological change after hormonal induction was the fusion and coalescence of the lipid droplets. At this stage the GV was located between the

center and the periphery of the oocyte, and was always in association with the largest lipid mass (Figs. 1b and 2b). The germinal vesicle was still intact at the end of the early maturation stage and was localized adjacent to the lipid mass in the peripheral cytoplasm (Figs. 1b and 2b). Development to this stage (early maturation) from the time of hormonal treatment of post-vitellogenic oocytes required 24 to 36 hs.

Once lipid-droplet coalescence was completed, germinal vesicle breakdown took place soon afterwards. By this time, the clearing process began in which the oocytes appear progressively more translucent (Fig. 3c). Late oocyte maturation lasted after 2430- hs. The ovulated eggs were free, pelagic and transparent and vary in diameter between 0.9 and 1.0 mm (Fig. 3c).

The post-spawning ovaries were characterized by a great number of relatively deformed oocytes and remains of empty follicles (Figs. 1c and 1d). In addition, the post-spawning ovaries of *L. ramada* contained primary oocytes (Figs. 1c and 1d).

Immunohistochemistry of GnRH during induction of spawning in female L. ramada

Different GnRH antisera were used in the present study. Only mGnRH was localized in neurons located in the medulla oblongata in the brain of *L. ramada* females (Figs. 3a-3f). During the pre-spawning stage of the ovaries, the synthetic activity of mGnRH neurons was high as reflected with strong immunoreactivity and increased sizes and numbers (Figs. 3a and 3b). At this stage mGnRH-IR neurons were impregnated with coarse immunoreactive granules (Figs. 3a and 3b). By approach the final maturation of the ovary, the immunostaining intensities and size of mGnRH neurons were markedly reduced compared to that of pre-spawning female (Figs. 3a - 3d). Most of mGnRH-IR neurons became elongated in shape and contained coarse immunoreactive granules (Figs. 3c and 3d). In the post-spawned fish with spent ovaries, most of mGnRH neurons appeared to empty their secretory contents as indicated by their small size and vacuolated cytoplasm (Figs. 3e and 3f).

Double immunostaining of mGnRH and SL in the pituitary gland during induction of spawning in female L. ramada

mGnRH-IR fibers appear in close contact with SL-IR cells in the PI of the pituitary gland of *L. ramada* females (Figs. 4a-4c). mGnRH-IR fibers exhibited high intensities and little decrease of mGnRH immunoreactivity during final ovarian maturation compared to that of pre-

spawning female (Fig. 4a and 4b). After spawning, the density and immunoreactivity of mGnRH-IR fibers were decreased (Fig. 4c).

In the pre-spawning females, SL-IR cells exhibited strong immunoreactivity, increased sizes and numbers (Fig. 4a). During the final maturation of the ovary, SL-IR cells appeared with reduced immunoreactivity in comparison to that of pre-spawning female (Figs. 4a and 4b). After spawning, SL-IR cells appeared to empty their secretory contents as indicated by their immunoreactivity and vacuolated cytoplasm (Fig. 4c).

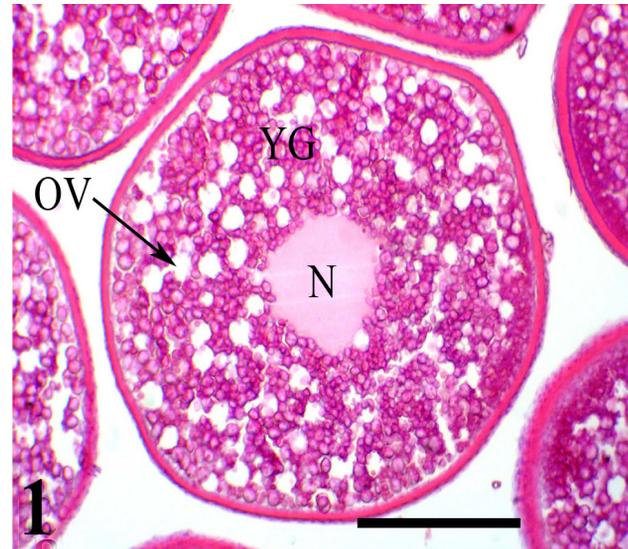


Fig. 1: Transverse section of ovary of post-vitellogenic *L. ramada* female before injection of hormones, stained with Harris's hematoxylin and eosin: showing tertiary yolk oocyte which has central-located nucleus (N), ooplasm impregnated with yolk globules (YG) and lesser number of oil vesicles (OV) distributed in the ooplasm. Scale bar = 250 μ m.



Fig. 2: Transverse section of ovary of injected *L. ramada* female, stained with Harris's hematoxylin and eosin: undergoing maturation, showing the oil vesicles (OV) coalescence, packed yolk globules (YG) and migration of germinal vesicle (GV) to the peripheral cytoplasm. Scale bar = 250 μ m.

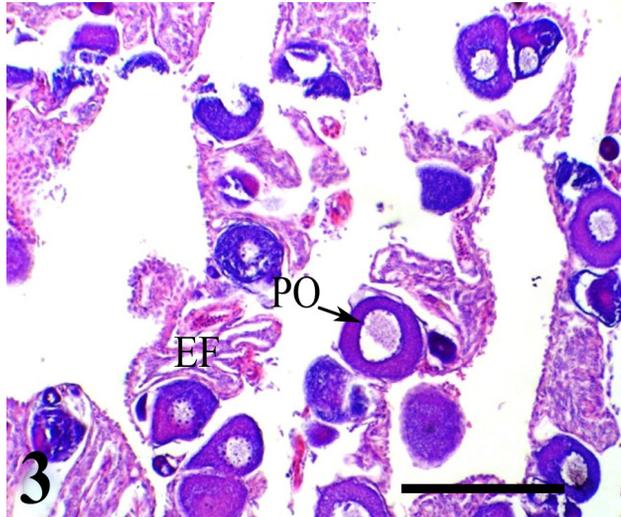


Fig. 3: Transverse sections of spent ovary of hormonal-induced spawning *L. ramada*, stained with Harris's hematoxylin and eosin: showing empty follicles (EF) and primary oocyte (PO). Scale bar = 250 μ m.

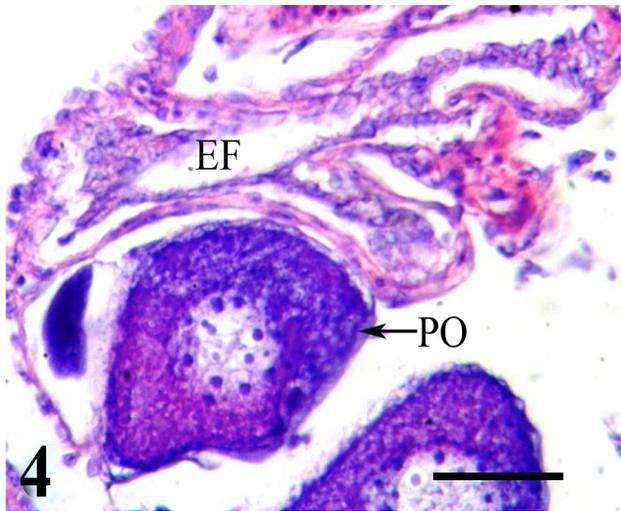


Fig. 4: A magnified portion of figure (3) showing empty follicles (EF) and primary oocyte (PO). Scale bar = 50 μ m.



Fig. 5: Macroscopic view of pre-spawning tertiary yolk oocytes which have central-located nucleus (N), ooplasm impregnated with yolk globules (YG). Scale bars = 400 μ m.



Fig. 6: Macroscopic view of mature oocytes, of injected *L. ramada* female, showing the oil vesicles (OV) coalescence and migration of germinal vesicle (GV) to the peripheral cytoplasm. Scale bars = 400 μ m.

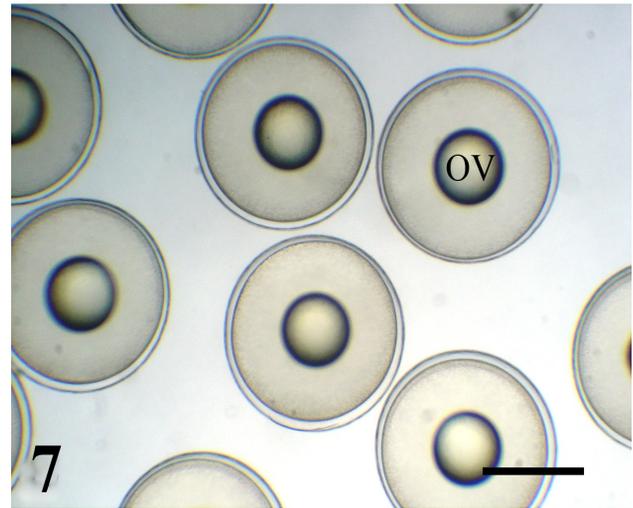


Fig. 7: Macroscopic view of ovulated eggs with one oil vesicle (OV). Scale bars = 500 μ m.

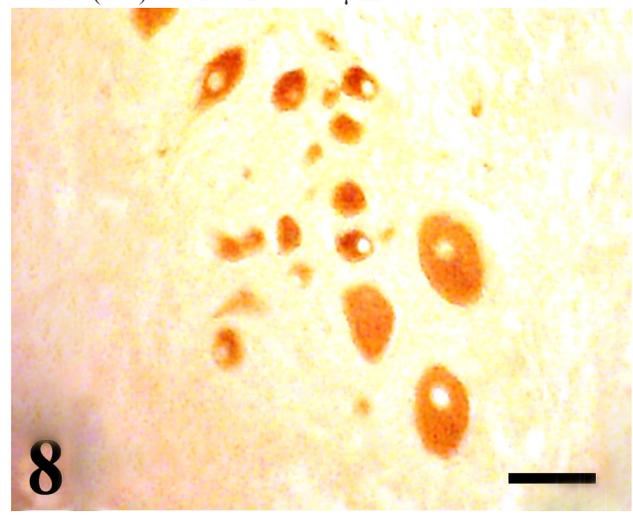


Fig. 8: Sagittal sections through the medulla oblongata in the brain of *L. ramada* female, showing mGnRH-IR cell bodies of pre-spawning female have different shapes and sizes, with strong immunoreaction. Scale bars = 20 μ m.

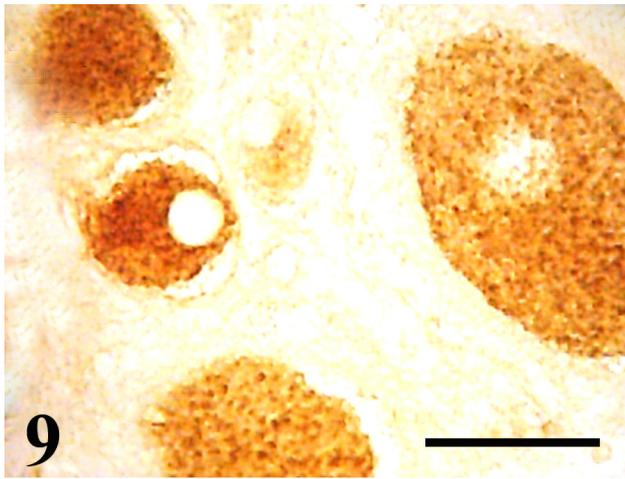


Fig. 9: Sagittal sections through the medulla oblongata in the brain of pre-spawning *L. ramada* female, showing mGnRH-IR cells impregnated with large (coarse) immunoreactive granules. Scale bars = 10 μ m.

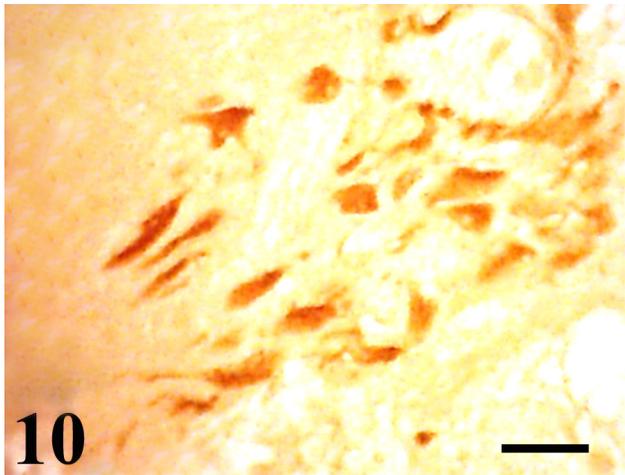


Fig. 10: Sagittal sections through the medulla oblongata in the brain of *L. ramada* female, during ovarian maturation and spawning, showing mGnRH-IR cell bodies of mature injected female, having small sizes, with moderate immunoreaction. Scale bars = 20 μ m.

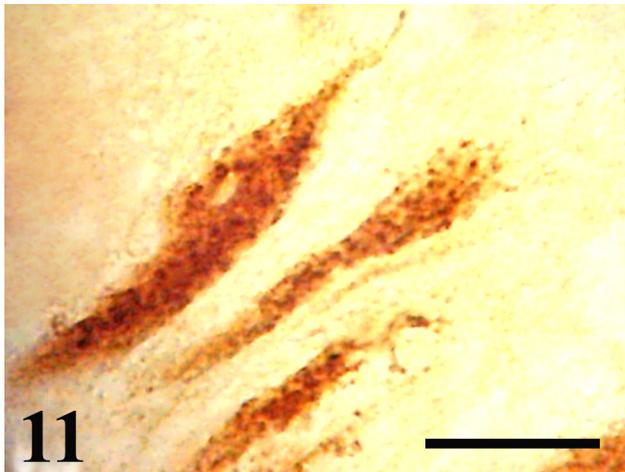


Fig. 11: Sagittal sections through the medulla oblongata in the brain of mature injected *L. ramada* female showing mGnRH-IR cells decreased in size and with large (coarse) immunoreactive granules. Scale bars = 10 μ m.

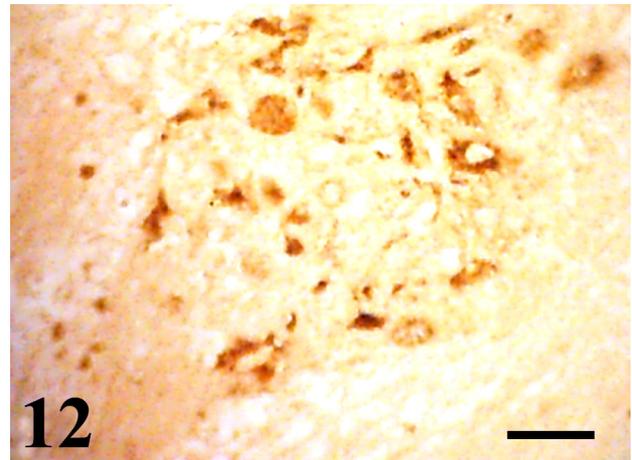


Fig. 12: Sagittal sections through the medulla oblongata in the brain of *L. ramada* female, during ovarian maturation and spawning, showing mGnRH-IR cell bodies of postspawning female, have small sizes and weak immunoreactivity. Scale bars = 20 μ m.

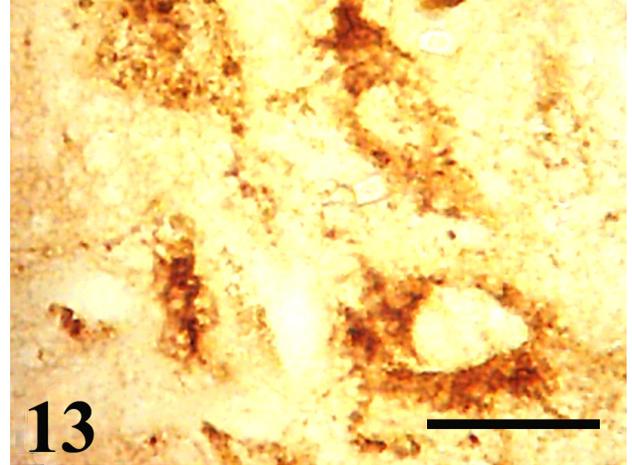


Fig. 13: Sagittal sections through the medulla oblongata in the brain of *L. ramada* postspawning female, showing mGnRH-IR cells have different sizes and with few immunoreactive granules. Scale bars = 10 μ m.

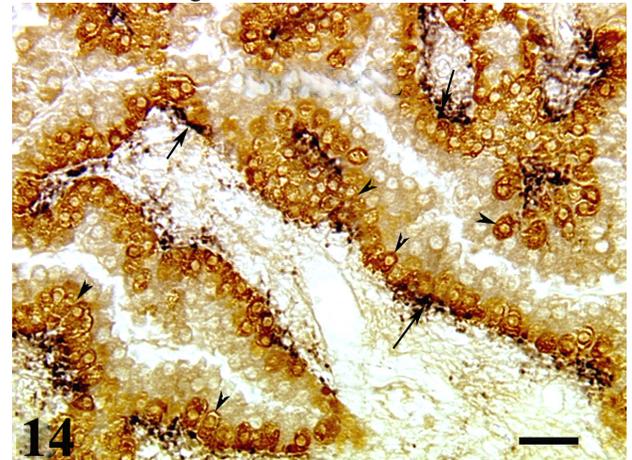


Fig. 14: Sagittal sections through the pituitary gland of *L. ramada* female, showing black mGnRH-IR fibers (arrows) in close contact with brown SL-IR cells (arrowheads) within the pituitary gland of pre-spawning female. Note the strong immunoreactivity of both mGnRH-IR fibers and SL-IR cells. Scale bars = 20 μ m.

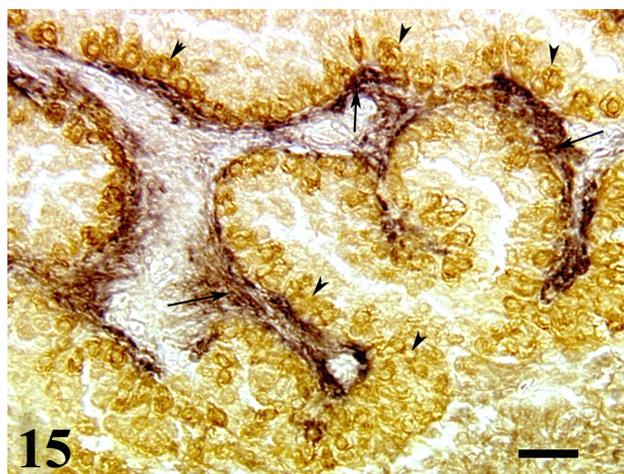


Fig. 15: Sagittal sections through the pituitary gland of *L. ramada* female, showing black mGnRH-IR fibers (arrows) in close contact with brown SL-IR cells (arrowheads) within the pituitary gland of mature injected female. Note the moderate immunoreactivity of both mGnRH-IR fibers and SL-IR cells. In addition, the density of mGnRH-IR fibers was higher than in pre-spawning female. Scale bars = 20 μ m.

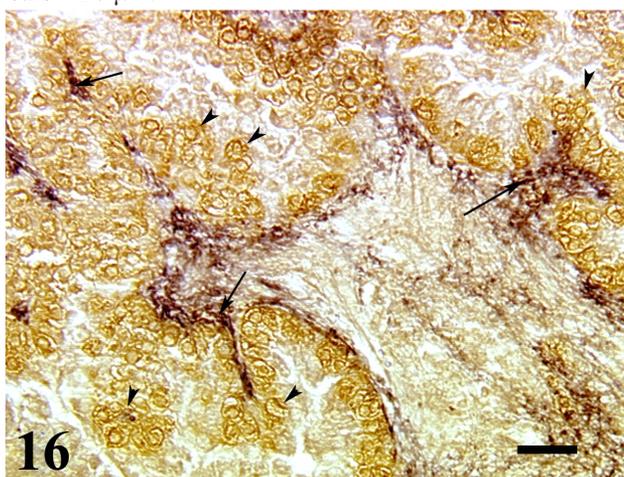


Fig. 16: Sagittal sections through the pituitary gland of *L. ramada* female, showing black mGnRH-IR fibers (arrows) in close contact with brown SL-IR cells (arrowheads) within the pituitary gland of postspawning female. Note the weak immunoreactivity of both mGnRH-IR fibers and SL-IR cells. Scale bars = 20 μ m.

DISCUSSION

The females of *L. ramada* did not complete final maturation in the captivity. Therefore, the completion of maturation and spawning of these fish is possible in the captivity by hormonal therapies in combination with environmental factors, particularly water temperature and salinity^[3, 37]. Our results revealed that the use of pregnyl (HCG) as a priming and resolving injections proved to be effective in inducing ovulation and spawning in *L. ramada*, at 44 to 52 h after hormonal injection. This may be due to the direct effect of HCG on the gonads. However, luteinizing hormone-releasing hormone analogue des-

Gly10 (D-Ala6) LHRH-Ethylamide (LHRHa) has been successfully used to induce final maturation and synchronize ovulation of some fish^[38-43]. The process of oocyte maturation was associated with significant and continuous increases in oocyte diameter. Similar increase in oocyte size during oocyte maturation has been observed for many fishes, and has been attributed primarily to water uptake^[44]. Oocyte maturation began with lipid droplet coalescence, which occurred concomitantly with GV migration. The GV is always attached to the largest lipid droplet and appears to be pushed to the periphery by the mere enlarging of the coalesced lipid-mass, a characteristic similar to that of *Dicentrarchus labrax*, *M. cephalus* and *Morone saxatilis*^[1, 45, 46]. Fusion of yolk globules is common in many fishes, in most species it happens during oocyte maturation, however in some it occurs during vitellogenesis^[44]. This process is very common in fish, especially in marine species that spawn pelagic eggs^[47-50]. The late oocyte maturation phase consisted of GVBD and yolk-globule coalescence in which clearing process began and consequently the oocytes appear progressively more translucent. Similar observations were recorded for *M. cephalus*^[1].

It is well established that GnRH, a decapeptide, represents one of the main stimulatory factors involved in the synthesis and releasing of GTHs in fish^[7, 8, 51]. In this research, we used specific antibodies for GnRH and chum salmon SL, immunohistochemistry to investigate the immunoreactivities of these hormones in the brain and pituitary gland of *L. ramada* during induced spawning. The mGnRH-IR cell bodies were observed in the medulla oblongata within the brain of *L. ramada*. However, the SL-IR cells were only detected in the PI of the examined individuals. Moreover, our double immunostaining revealed a close association between mGnRH-IR fibers and SL-IR cells in the PI area of pituitary gland. These findings are in agreement with previous study demonstrated mGnRH-IR fibers in close vicinity with SL-IR pituitary cells in the Nile perch^[11]. There is pharmacological and morphological evidence showing that GnRH could be involved in SL release. GnRH stimulated dopamine inhibited SL release in vitro^[13]. In addition, injection of GnRH stimulated the release of SL during the induction of maturation and ovulation in *Liza ramada*^[50]. The immunohistochemical results obtained in *O. mykiss*^[16], *O. bonariensis*^[32] and in *L. niloticus*^[11, 12] suggest that GnRH-IR fibers ending in contact with SL-IR cells can be the morphological substrate of GnRH action on SL release and this could be a common pattern in teleost fishes.

Interestingly, the present study demonstrated that the activity of the mGnRH-IR neurons in the medulla oblongata and mGnRH-IR fibers as well as SL-IR pituitary cells in the PI increased during spawning of

L. ramada. Similar immunohistochemical observations were obtained in *Oncorhynchus nerka* and *O. keta*^[19, 30], *O. niloticus*^[17], *M. cephalus*^[18], *O. bonariensis*^[31] and in *L. niloticus*^[12]. In addition, seasonal changes in GnRH concentrations were observed during the reproductive cycle of *Sebastes rastrelliger*^[52] and *Myxine glutinosa*^[53]. Also, mGnRH-ir fibers and SL-IR cells in the PI showed quantitative and qualitative seasonal changes during gonadal maturation and spawning in both sexes of *L. niloticus*^[12].

During the final maturation stage of the ovaries in *L. ramada*, mGnRH-IR neurons, mGnRH-IR fibers and SL-IR cells exhibited a marked degree of hypertrophy and showed strong immunoreaction. Finally, by the approach of postspawning phase of the ovary, the immunostaining intensities and size of mGnRH-IR neurons, mGnRH-IR fibers and SL-IR cells appeared greatly reduced, most of mGnRH-IR neurons and SL-IR cells appeared to empty their secretory contents as indicated by their small size and presence of secretory vacuoles. The active release of SL granules during spawning of *L. ramada* may indicate the involvement of SL not only in gonadal development but also in energy mobilization related to reproduction, since the biological events, during spawning process, concerned with reproduction require a great deal of energy. Activated SL cells identified immunohistochemically, were seen also in spawning of *Oncorhynchus nerka*, *O. keta* and *O. tshawytscha*^[19, 30], *M. cephalus*^[18] and in *L. niloticus*^[12]. Also, Somatolactin in the pituitary of blue gourami (*Trichogaster trichopterus*) was higher in females at low vitellogenesis compared to females with oocytes in maturation^[22]. Moreover, our immunohistochemical results are in agreement with the biochemical studies, which revealed increase of SL levels during the spawning migration of chum salmon *Oncorhynchus keta*^[54]. Furthermore, a hypercalcemic effect of SL was indicated in previous studies: SL cells are activated by low environmental Ca²⁺ levels and plasma SL levels are elevated in association with the increase in plasma Ca²⁺ levels in stressed and exercised fish^[24, 55, 56]. Although the activation of SL cells during ovarian maturation development suggests a hypercalcemic effect of SL, it is likely that other hormones, such as prolactin, stanniocalcin and calcitonin, are involved in calcium homeostasis in teleosts^[57-59]. It is clearly necessary to examine the role of SL in calcium homeostasis in the context of other calcemic hormones.

In summary, the dominance of mGnRH in the medulla oblongata and the pituitary gland and its immunoreactivity in relation to seasonal changes in reproductive condition establishes the concept that mGnRH is the functional form of GnRH with respect to SL release and the regulation of reproductive activity in *L. ramada*. Also, the gradual stimulation of

SL synthesis and release during spawning of *L. ramada* suggests that SL may be involved in the control of some biological events concerned with reproduction, such as calcium metabolism and energy mobilization.

CONFLICT OF INTEREST

The author declares there are no conflicts of interest.

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الملخص العربي

دراسة هستوكيميائية مناعية للهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين أثناء التفريخ المحفّز لأسماك الطوبار

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المقدمة: يعتبر الهرمون المحرر لهرمون الجونادوتروبين منظم فعال لتطور المناسل والتفريخ في الأسماك. بالإضافة إلى أنه يعتبر متحكماً في إفراز هرمون السوماتولاكتين في الأسماك. بالرغم من ذلك لا توجد معلومات متاحة توضح العلاقة بين الهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين أثناء تكاثر أسماك الطوبار.

الهدف من الدراسة: وقد صممت هذه الدراسة لفحص التفاعل المناعي لكل من الهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين أثناء تحفيز النضج النهائي، التبييض والتفريخ في أسماك الطوبار وذلك من أجل توضيح دور هذه الهرمونات في النضج الجنسي والتفريخ في الأسماك

المادة والطرق: تم استخدام الطرق الهستولوجية والهستوكيميائية المناعية لوصف التفاعل المناعي لكل من الهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين أثناء نضج المبيض والتفريخ لأسماك الطوبار.

النتائج: تم إكتشاف الخلايا العصبية المفرزة للهرمون المحرر لهرمون الجونادوتروبين الندي في منطقة النخاع المستطيل من المخ. بينما تم تحديد التفاعل المناعي للخلايا المفرزة لهرمون السوماتولاكتين في الجزء المتوسط من الغدة النخامية. وقد وجد إتصال وثيق بين النهايات العصبية والخلايا المفرزة لهرمون السوماتولاكتين. وأظهرت النتائج إزدياد نشاط كل من الهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين أثناء النضج الجنسي والتفريخ لأسماك الطوبار والذي تمثل في زيادة أعداد تلك الخلايا وحجمها. وقد إتضح إزدياد نشاط تلك الخلايا بزيادة حجمها وعددها وتفاعلها المناعي في مرحلة ما قبل الهجرة للإناث. بينما إزداد النشاط الإفرازي للخلايا العصبية المفرزة للهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين أثناء النضج النهائي والتفريخ كما إتضح من صغر حجمها وامتلاكها لفجوات إفرازية وتفاعل مناعي ضعيف.

الخلاصة: تشير النتائج الحالية إلى وجود إتصال وثيق بين النهايات العصبية المفرزة للهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين في الغدة النخامية وأيضاً وجود تغيرات متزامنة لكل من الهرمون المحرر لهرمون الجونادوتروبين وهرمون السوماتولاكتين يؤدي إلى الإفتراض بأن الهرمون المحرر لهرمون الجونادوتروبين ربما يكون مسؤولاً عن تنظيم إفراز هرمون السوماتولاكتين أثناء التكاثر لأسماك الطوبار