Immunohistochemical Description and Dispensation of the Hypophyseal Cell Types in *Liza* Ramada

Original Article

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ABSTRACT

Introduction: The localization and characterization of the hypophyseal cell types in L. ramada is so far lacking.
Aim of the Work: The present study aimed to describe and determine the hypophyseal cells in L. ramada.
Material and Methods: Immunohistochemical localization of the hormone-secreting cells in the hypophysis of L. ramada was completed by antisera against both mammalian and piscine hormones.
Results: The hypophysis of L. ramada was comprised two main parts, the glandular part (adenohypophysis) and the nervous part (neurohypophysis). The adenohypophysis was divided into three subdivisions; rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Seven of hormone-secreting cells were distinguished in the glandular part according to their grouping, distribution, and immunoreactivity.
The acidophilic prolactin (PRL) cells occupied the bulk of the RPD and showed positive and specific immunoreaction with salmon PRL antiserum. Lead hematoxylin-positive (PbH+) cells, which were bordered the neurohypophysis in the RPD, gave positive immunoreactivity with human adrenocorticotrophic (ACTH) antiserum.
The acidophilic cells of the PPD immunoreacted positively and strongly with antiserum against salmon growth hormone (GH). In addition, the central region of PPD comprised basophilic cells which were showed specific and strong immunoreaction with antisera against salmon gonadotropin (GTH) subunits; Iβ and IIβ.
The presumptive thyrotropin (TSH) secreting cells were distinguished in the neurohypophysis between the RPD and the PPD, and they were negative immunoreacted with antiserum against rat thyrotropin (rBetaTSH).
Two cell types were found in the PI; the Periodic Acid-Schiff-positive (PAS+) cells which had positive immunoreaction with anti-salmon somatolactin (SL) and the Lead hematoxylin-positive (PbH+) cells which immunostained with anti-alpha-melanin-stimulating hormone (α-MSH).
Conclusion: The localization of hypophyseal cells of L. ramada, consider as basis for understanding and tracking hormonal changes during its reproductive cycle in captivity.

Received: 16 June 2021, **Accepted:** 23 July 2021

Key Words: Immunohistochemistry, liza ramada (Teleostei), pituitary gland.

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ISSN: 1110-0559, Vol. 45, No. 4

INTRODUCTION

The gonads and the hypophysis are considering the control system for fish propagation. Both histochemistry and physiology were used to identify the hormone adenohypophyseal producing cells of teleosts[1-3]. Furthermore, the hypophyseal hormone-producing cells were identified by immunohistochemical methods using both mammalian and piscine hormones antisera[4-6]. Seven different hormone-producing cells were characterized in most teleosts. They are belonging to three major origins: (i) GH/PRL group, containing PRL, GH and SL producing cells; (ii) glycopeptides hormones including GTHs and TSH; and (iii) proopio melanocortin (POMC) derived hormones such as ACTH and MSH[7-11].

Immunohistochemical characterization of the hypophysis during propagation of teleosts, in different environments, revealed that hypophyseal hormones play a main role in propagation in teleosts and may be regulated the related activities that directly or indirectly affect reproductive case[8,9,12].

In Egypt, it is well established that thin-lipped mullet, L. ramada play an important role in polyculture of different salinity. The importance of this species is related to its fast growing rate and worldwide distribution. The culture of mullet is closely dependent on the fry collection from nature, which it does not satisfy the increasing demand for the juveniles of L. ramada. To substitute the fry collection from wild stocks, it is necessary to develop and establish practical techniques for artificial propagation of mullet[13]. The resources of mature female breeders are vitally important, particularly when linked to mass propagation of juveniles in the hatcheries[12,14].

 DOI: 10.21608/ejh.2021.80941.1508

Regardless of of the great interest of L. ramada, some information on the subject of its reproductive endocrinology is available. Nevertheless, the characterization of the hypophyseal cell types in this fish not done yet. This work was designed to describe and identify the hypophyseal cell types by utilizing both histochemistry and immunohistochemistry, to gain insight on the reproductive glands of L. ramada and to provide basic information necessary for its successful propagation.

MATERIAL AND METHODS

Fish collecting

From saline water ponds (captivity), at El-Matareyya Research Station - National Institute of Oceanography and Fisheries - Egypt, 50 of adults L. ramada breeders, with a mean length of 35 cm. and a mean weight of 600 gm. were collected alive during their maturation months (November and December).

Histology and histochemistry

Before anatomy the alive fishes were narcotized in clove oil (Sigma) solution at a dose of 40 mg/l. As soon as possible after the anatomy, the hypophysis, united to the brain, was fixed in Bouin's fixative for 72 hour at 4 oC. The fixed brain united with hypophysis was thereafter histologically prepared as previously illustrated[15]. Successive sagittal sections of the hypophysis were cutted at a thickness of 4 µm. Selected sections from each hypophysis were stained with the following procedures.

1. Harris's alum hematoxylin and counter stain of aqueous eosin (1%)[16].
2. Combined stains of Periodic Acid-Schiff and Lead hematoxylin (PAS-PbH)[17,18]
3. Performic acid-Alcian blue (PFAAB) - Orange G (OG) stain[19].

Immunohistochemical procedures

Antibodies: Antisera used in the present study were mentioned in previous study[7] and summarized in (Table 1).

Table 1: Immunohistochemical staining of the hypophysis of L. ramada

Antiserum to	Dilution	RPD		PPD		PI	
		P	C	S	G	PAS^+	PbH^+
Chum salmon PRL	1:5000	++	-	-	-	-	-
Human ACTH	1:500	-	++	-	-	-	+
Chum salmon GH	1:5000	-	-	++	-	-	-
Chum salmon GTH Iβ	1:1000	-	-	-	++	-	-
Chum salmon GTH IIβ	1:5000	-	-	-	++	-	-
Rat βTSH	1:500	-	-	-	-	-	-
Chum salmon SL	1:5000	-	-	-	-	++	-
α-MSH	1:1000	-	-	-	-	-	++

Note. RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia; P, PRL cells; C, ACTH cells; T, thyrotrops; S, somatotrops; G, gonadotrops; PAS+, periodic acid-Schiff reaction positive cell; PbH+, lead hematoxylin positive cell; PRL, prolactin; ACTH, adrenocorticotropin; TSH, thyrotropin; GH, growth hormone; GTH, gonadotropin; SL, somatolactin; -, +, ++, negative, weak and strong immunostaining responses, respectively

Immunohistochemical reactions: Immunohistochemical technique for the hypophyseal sections was preceded with Avidin-biotin complex (ABC) Kit as represented previously[7,15]. Briefly, hydrated sections were washed two times in phosphate-buffered saline (PBS; pH 7.4) for 10 min each. All staining steps were completed at ambient temperature. After each step, washing of slides was done in PBS. Incubation of sections with the primary antibodies was continued for overnight. The working dilutions of the primary antibodies were obtained experimentally (see table 1). Then, the incubation of sections with the secondary antibody for 1h., and with AB- conjugated peroxidase for 45 min. Afterwards, the immunoreaction was shown with 3, 3`-diaminobenzidine tetrahydrochloride (DAB). Finally, the immunostained slides were prepared as previously described and mounting in DPX[15].

To emphasize the used antibodies specificity, control hypophyseal sections were stained in the absent of the hormone antisera or using bovine serum as substitute for primary antiserum. No positive reaction obtained in the sections.

RESULTS

The hypophysis of L. ramada was comprised two main parts, the glandular part (adenohypophysis) and the nervous part (neurohypophysis). The adenohypophysis was divided into three subdivision; rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) as represented in figures (1) and (2). Furthermore, the adenohypophysis was innervated with neurohypophyseal branches (Figures 1,2). As a result of the hypophysis histochemistry and immunohistochemistry, seven cell types were distinguished in the adenohypophysis of L. ramada. Two types of hormone-producing cells were identified in the RPD; PRL-producing cells and human adrenocorticotrophic (ACTH) -producing cells. Both growth hormone (GH) -producing cells and gonadotropin (GTH) subunits producing cells (GTH Iβ and GTH IIβ) were localized in the PPD. However, the presumptive thyrotropin (TSH)-producing cells have been characterized in the intermediate area between PPD and RPD. Two of hormone-producing cells were localized in the PI; the melanin-stimulating hormone (MSH) -producing cells and the somatolactin (SL) -producing cells.

PRL-producing cells

The acidophilic prolactin (PRL) -producing cells occupied the bulk of the RPD (Figures 1,2). These cells have small sizes, irregular shapes and round nuclei with a definite nucleolus (Figure 3). The acidophilic PRL-producing cells stained with acidic stains; eosin and orange G (Figures 3,4), so correspondingly named "orangeophilous acidophils". The PRL-producing cells were immunoreacted positively with salmon PRL antiserum (Table 1 and Figures 5,6).

ACTH-producing cells

The ACTH-producing cells were found as threads separating the PRL-producing cells and the neurohypophysis

(Figures 7,8). They have small sizes, various shapes and eccentric small nuclei (Figure 3). The ACTH-producing cells stained specifically with lead hematoxylin-positive (PbH+) (Figure 9). They gave strong immunoreaction with human ACTH antiserum (Figures 7,8), which cross reacted with the PI PbH+ cells (Figure 10 and Table 1).

GH-producing cells

The GH-producing cells were located in the PPD (Figures 11-13). They are sorted in threads limiting the neurohypophysis and are also dispersed among the basophilic cells of the PPD. These cells have densely granulated cytoplasm and oval or spherical nuclei (Figure 11). The acidophilic GH-producing cells were stained with orange G (Figure 12). They immunostained strongly and specifically with salmon GH antiserum (Figures 13,14).

GTHs- (Iβ and IIβ) and TSH-producing cells

The GTHs-producing cells are occupying the central portion of the PPD (Figures 15-17). The basophilic GTHs-producing cells have various shapes, sizes and with rounded nuclei (Figure 15). They stained with alcian blue and PAS (Figures 12,16). The GTHs-producing cells gave specific and strong immunoreaction with antisera against salmon GTH subunits; Iβ and IIβ. (Figures 17-20).

Groups of smaller sized basophiles (thyrotrops) were demonstrated in the region of neurohypophysis, being located between the RPD and the PPD, (Figures 2,12,21). They reacted positively with alcian blue (Figure 12). These cells have elongated or angular shapes with a small cytoplasmic rim encompassing a small nucleus (Figure 21). TSH-producing cells gave a negative immunoreaction with antiserum to rat TSHβ subunit (Table 1).

SL- and α-MSH-producing cells

Two hormone-secreting cells were identified in the PI; the Periodic Acid-Schiff-positive (PAS+) cells and the Lead hematoxylin-positive (PbH+) cells (Figures 16,22). The PI PAS+ cells exhibited various shapes and sizes and were located singly or in aggregations (Figures 16,22). These cells immunoreacted specifically with salmon SL antiserum (Figures 23,24).

The PbH+ cells of the PI were immunostained with α-MSH antiserum (Table 1,Figure 25) and cross reacted with hACTH antiserum (Figs. 7 and 10). The α-MSH-producing cells have various sizes and shapes, and surrounded the neurohypophyseal processes, mixed with the SL-producing cells (Figures 16,22).

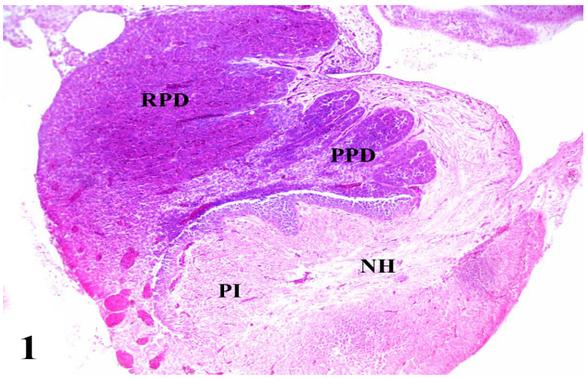

Fig. 1: Sagittal section in the hypophysis of L. ramada stained with hematoxylin and eosin, showing the glandular part including RPD, PPD and PI and the neurohypophysis (NH).X40.

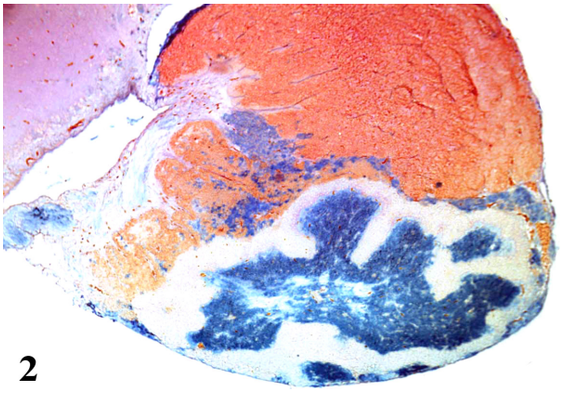

Fig. 2: Sagittal section in the hypophysis of L. ramada stained with AB-PAS-OG, displaying the adenohypophyseal cells; acidophilic or orangeophilic cells and basophilic cells stained with alcian blue. X40.

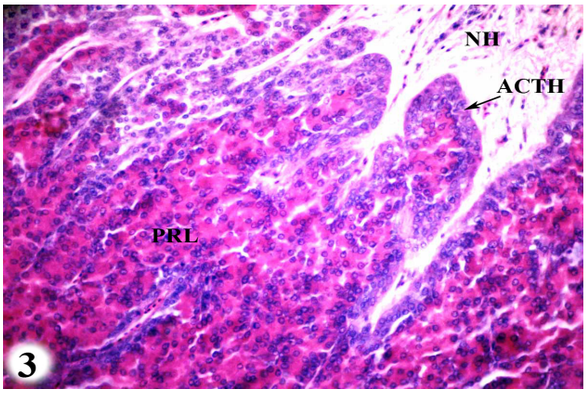

Fig. 3: Enlarge portion of the section in figure (1) displaying the PRL cells have various shapes and round nuclei with prominent nucleolus. Also, note the ACTH cells and neurohypophysis (NH). X400.

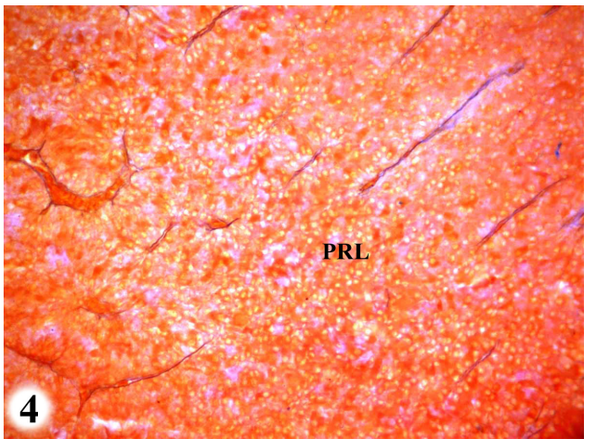

Fig. 4: A magnified portion of the section in figure (2) displaying the orangeophilic PRL cells. X400.

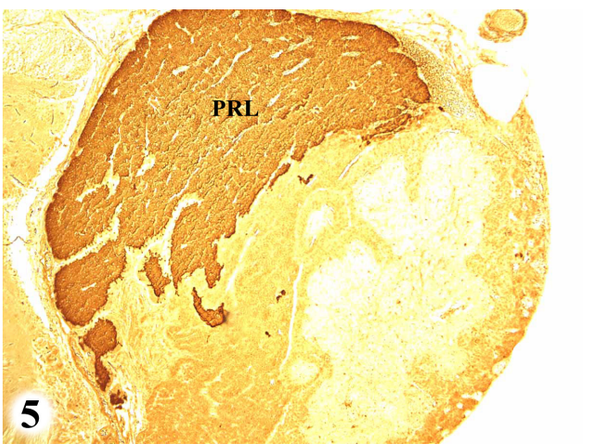

Fig. 5: Sagittal section of the hypophysis of L. ramada immunoreacted with anti- salmon PRL antiserum. PRL cells are located in the RPD. X40.

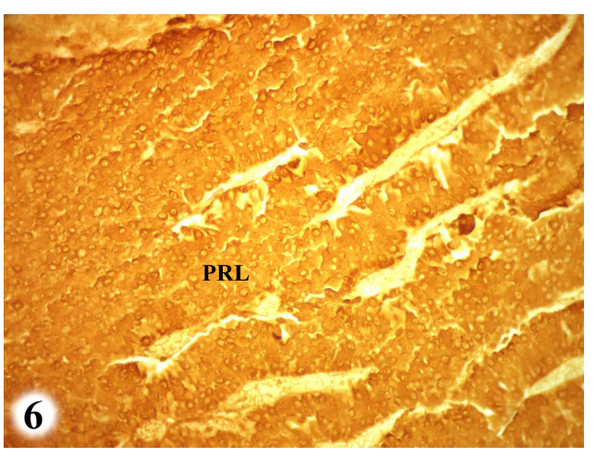

Fig. 6: A magnified portion of the section in figure (5) showing strong immunoreaction of PRL cells. X400.

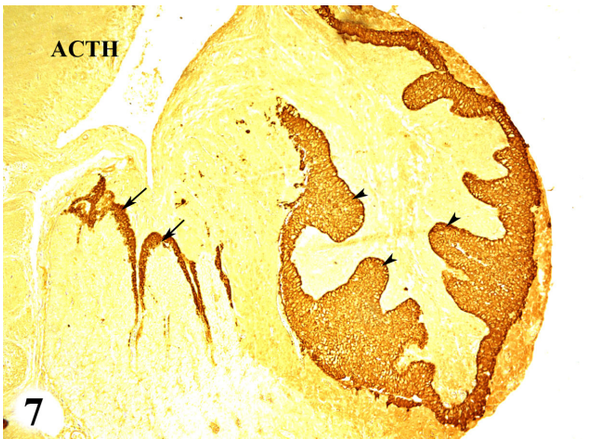

Fig. 7: Sagittal section of the pituitary gland of L. ramada immunostained with anti-hACTH. Showing the immunoreactive ACTH cells (arrows) and cross-reactive PbH+ cells in the PI (arrowheads). X40.

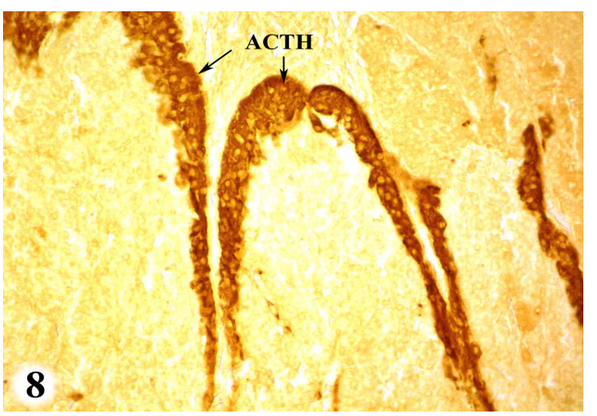

Fig. 8: A magnified portion of figure (7) showing strong immunoreaction of ACTH cells. X400.

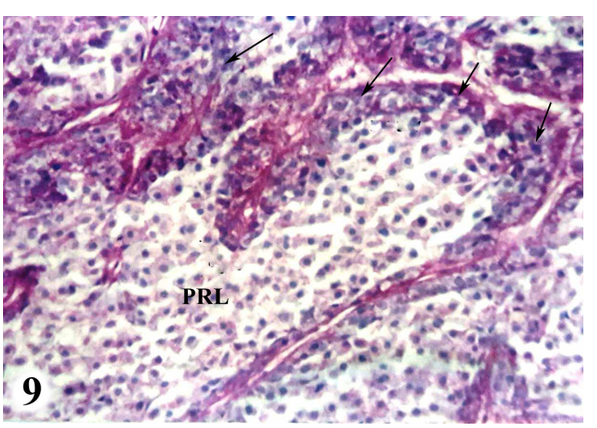

Fig. 9: Sagittal section of the pituitary gland of L. ramada stained with PAS and PbH stains, displaying the ACTH-producing cells stained with PbH (arrows). X400.

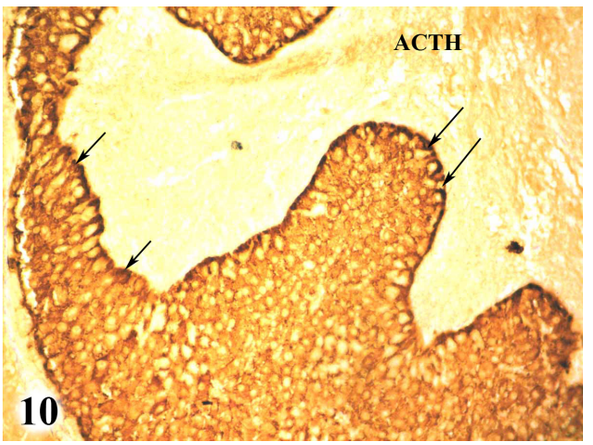

Fig. 10: A magnified portion of the section in figure (7) showing the PbH+ cells (arrows), in the PI, displaying cross immunoreaction with anti-human ACTH antiserum. X400.

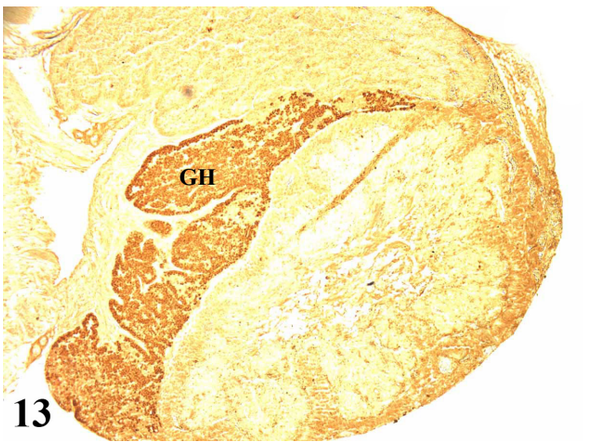

Fig. 13: Sagittal section of the pituitary gland of L. ramada immunoreacted with salmon GH antiserum. The GH-positive cells are located in the PPD. X40.

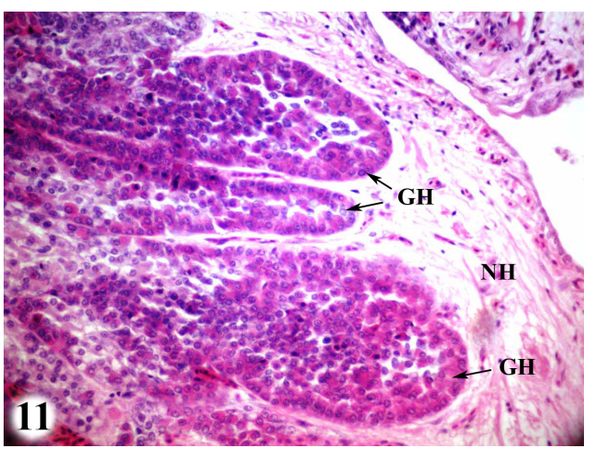

Fig. 11: A magnified portion of the section in figure (1) showing the GH cells distributed throughout the PPD and NH. X400.

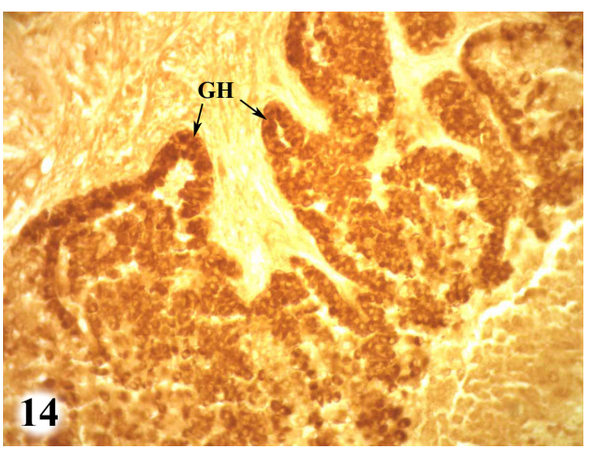

Fig. 14: A magnified portion of figure (13) showing the GH cells immunostained strongly with anti-chum salmon GH antiserum. X400.

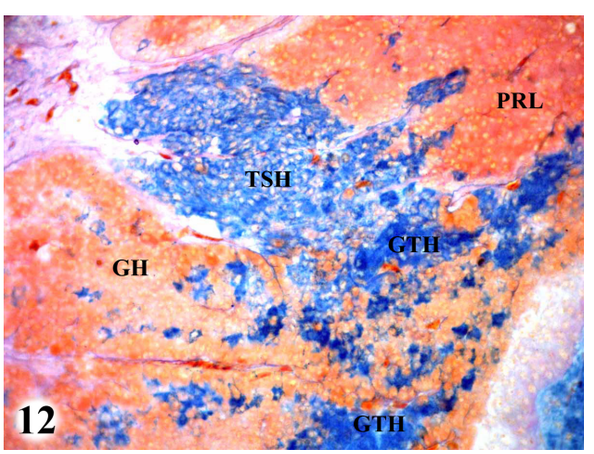

Fig. 12: Enlarged portion of section in figure (2) showing the orangeophilic PRL-producing cells and GH-producing cells, beside the basophilic GTH cells and TSH cells stained with alcian blue. X400.

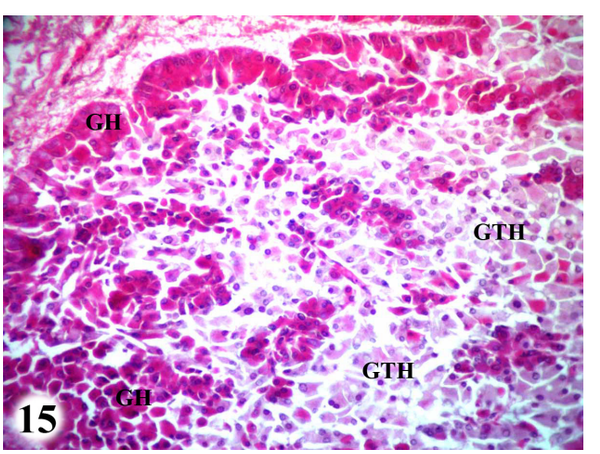

Fig. 15: Sagittal section of the pituitary gland of L. ramada stained with hematoxylin and eosin, displaying the GTH cells have different sizes and shapes, beside GH cells. X400.

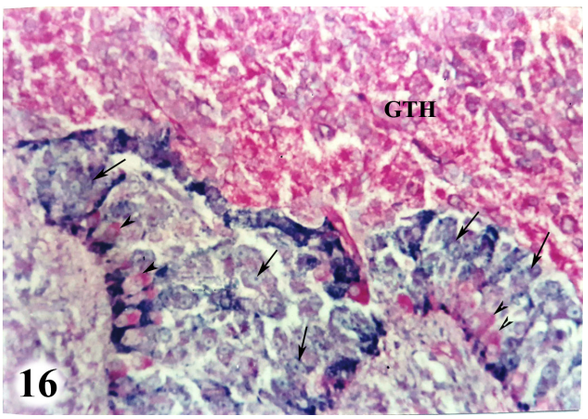

Fig. 16: Sagittal section of the pituitary gland of L. ramada stained with PAS and PbH stains, illustrating the GTH-PAS+ cells, beside the PI cells; the PAS-positive cells (arrowheads) and PbH-positive cells (arrows). X400.

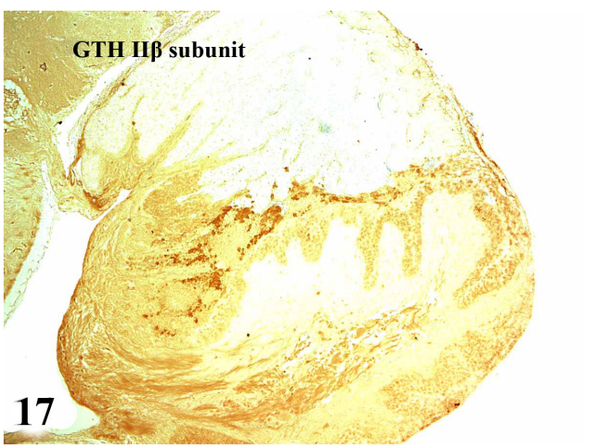

Fig. 17: Sagittal section of the pituitary gland of L. ramada immunoreacted with salmon GTH IIβ antiserum. The GTH IIβ producing cells are located in the PPD. X40.

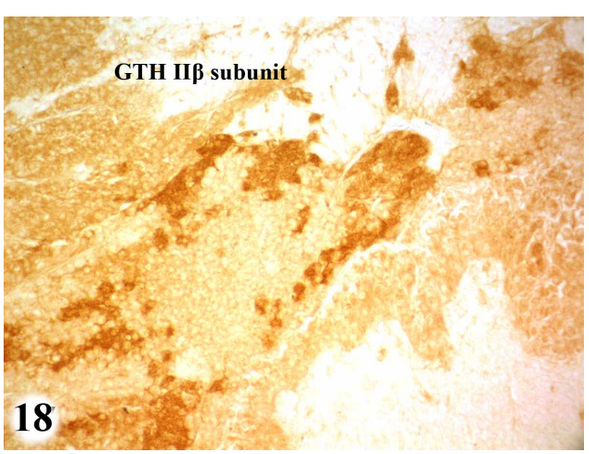

Fig. 18: A magnified portion of the section in figure (16) showing the GTH IIβ cells immunostained strongly with salmon GTH IIβ subunit antiserum. X400.

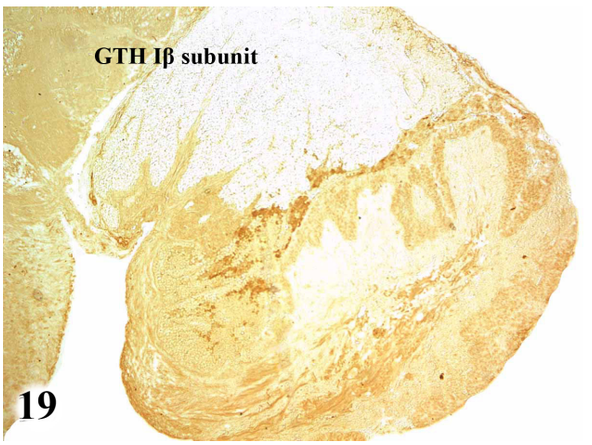

Fig. 19: Sagittal section of the pituitary gland of L. ramada immunoreacted with salmon GTH Iβ antiserum. GTH Iβ immunoreaction was obtained in the same region immunostained with salmon GTH IIβ subunit antiserum. X40.

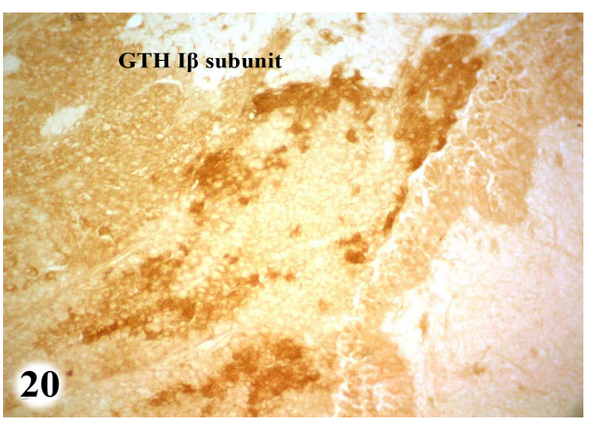

Fig. 20: A magnified portion of the section in figure (16) showing the GTH Iβ cells immunostained strongly with salmon GTH Iβ subunit antiserum. X400.

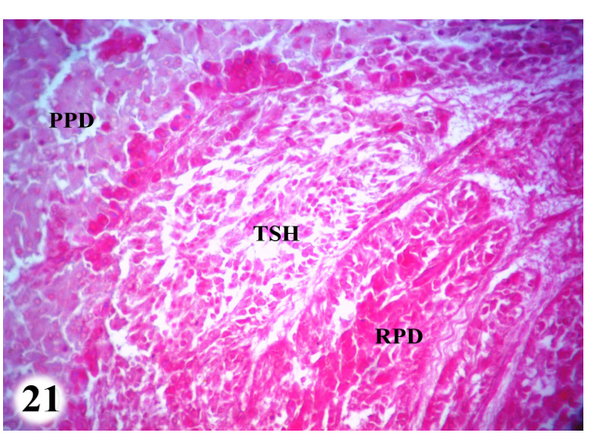

Fig. 21: Sagittal section of the pituitary gland of L. ramada stained with hematoxylin and eosin, illustrating groups of TSH cells exhibited small sizes and present in the neurohypophysis between the PPD and the RPD. X400.

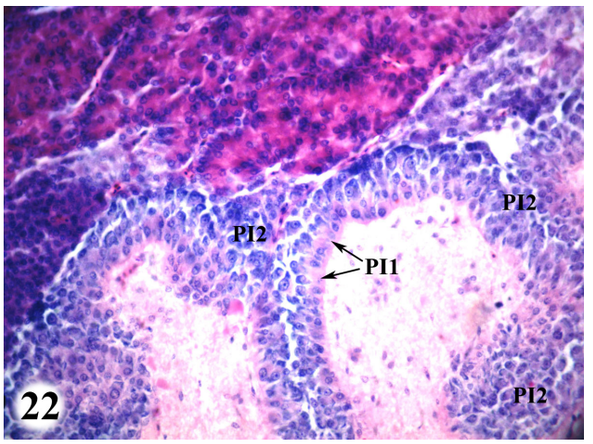

Fig. 22: Sagittal section of the pituitary gland of L. ramada stained with hematoxylin and eosin, displaying the PI cell types; the eosin-stained PI1 cells and the haematoxylin-stained PI2 cells. X400.

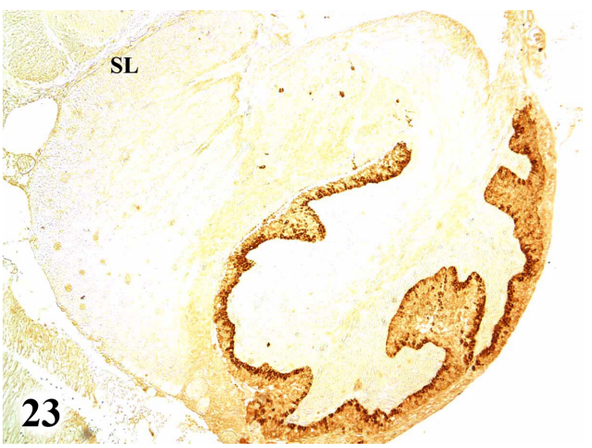

Fig. 23: Sagittal section of the pituitary gland of L. ramada immunoreacted with anti-salmon SL. Note the immunoreactive SL cells in PI. X40.

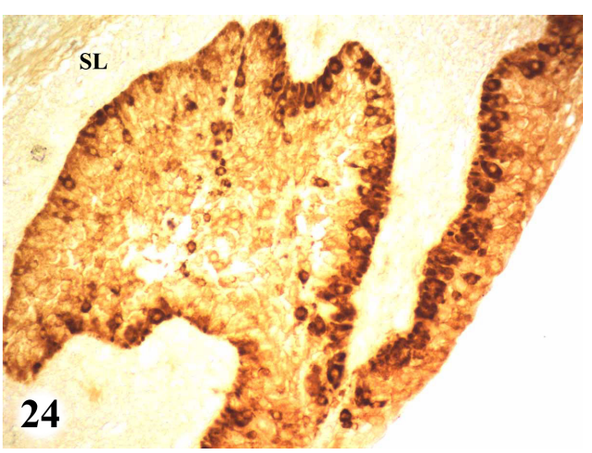

Fig. 24: A magnified portion of the section in figure (23), showing the positive immunostained SL cells in the PI. X400.

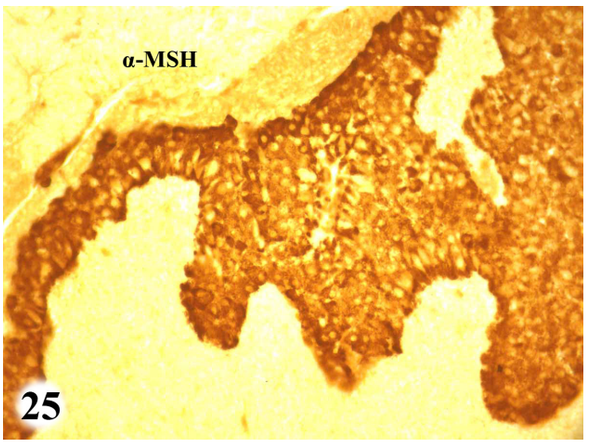

Fig. 25: Sagittal section of the pituitary gland of L. ramada immunostained with anti- α-MSH antiserum. Note, the intensely immunostained MSH (PbH+) cells in the PI. X40.

DISCUSSION

The hormone-producing cells in the hypophysis of L. ramada were stained by utilizing both histochemistry and immunocytochemistry. The present results indicated that the adenohypophysis of L. ramada containing seven of hormone-producing cells. Our findings concerning the allocation of cell types are in accordance to those observed in other fish species[7,20].

PRL-producing cells

The PRL-producing cells of L. ramada gave positive and specific immunoreaction with the PRL antiserum of salmon. Similar immunohistochemical results were obtained in other fish species[7,20,21]. However, the PRL antisera used to localize the PRL-producing cells in other fishes cross reacted with the GH-producing cells[22]. Similar to other fish species, the PRL-producing cells of L. ramada are mainly characterized in the RPD[6,23]. The obtained results indicated the principal role of PRL in osmoregulation of fish in low salinity habitats, particularly in euryhaline fish species[24,25].

GH-producing cells

The GH-producing cells of L. ramada were concentrated in the PPD, as observed in other fish species[4,7,23]. They were localized and immunostained with salmon GH antiserum, that used in the identification of the GH-producing cells of salmonid fish species[26] and non-salmonid species[7,20,21]. In fishes GH has been play a main role in growth[27]. In addition, GH has been participating in different physiological processes such as immunity, metabolism and reproduction of teleosts[28]. Furthermore, involvement of GH in osmoregulation has been observed in salmonid fish species and non-salmonid species[25,29].

SL-producing cells

Two cell types were located in the PI; the Periodic Acid-Schiff-positive (PAS+) cells and the Lead hematoxylin-positive (PbH+) cells. The immunohistochemistry revealed that the PbH+ cells were MSH-producing cells while the PAS+ cells were the SL-producing cells. The last hormone identified among the hypophyseal hormones, belonging to the group of GH/PRL, was the SL hormone[30]. Many investigations have been done on the molecular biology, immunocytochemistry and physiology of SL to determine its function. However, until now the SL physiological role is remaining undetermined[31]. In present work, we utilized the SL antiserum of salmon whish immunostained specifically the SL-producing cells. Similar allocation and characterization of SL-producing cells was obtained in other fish species[4,6,7,8,12,15,23].

In spite of its sharing in many of physiological processes such as sexual maturation, metabolism, mineral balance, stress and color adaptation, the function of SL hormone is remain undetermined[12,31]. The observed immunocytochemical changes in the SL-producing cells, in accompanied to reproductive activity, showing the potential role of SL in the reproductive activities of M. cephalus, O. niloticus, Oncorhynchus keta and O. nerka[12,15,32]. These immunocytochemical observations were in agreement with the biochemical findings obtained in Oncorhynchus kisutch that indicated the possible role of SL in sexual hormones synthesis[33]. In similar manner, a correlation between the SL level and sex steroids showed the possible role of SL in sexual maturation in O. kisutch[34].

ACTH- and α-MSH-producing cells

The ACTH-producing cells were appeared as threads separating the neurohypophysis and the PRL cells. The immunohistochemical observation revealed that human ACTH antiserum reacted positively and strongly with the ACTH-producing cells of L. ramada as obtained in other fishes[7,20,35]. The antiserum against human ACTH gave also cross reaction with the MSH-producing cells in L. ramada as appeared in other fish. There is a similarity between the ACTH precursor, POMC, and the α-MSH molecule[36]. This may explain the cross-immunoreaction of MSH-producing cells with anti-human ACTH obtained in L. ramada. In addition, the ACTH-producing cells and the MSH-producing cells stained specifically with lead hematoxylin (PbH+).

The α-MSH-producing cells in L. ramada surrounded the neurohypophyseal processes in the PI and mixed with the SL-producing cells. Similar location and arrangement of these cells were observed in other fish species[6,7,20,35]. The MSH-producing cells in L. ramada were specific immunostained with anti-α-MSH as reported in other teleosts[7,20].

It is known that the ACTH stimulates the inter-renal tissue of fish for production of cortisol[37], which participate in metabolism, osmoregulation and stress response[38]. But the MSH has a principal role in color adaptation and in stress response[38,39].

TSH- and GTH-producing cells

The group of glycopeptides hormones in the hypophysis was including GTHs and TSH. Anti-rat β-TSH showed negative immunoreaction with both TSH and GTH cells of L. ramada. However, this antiserum has been cross-reacted with the TSH-secreting cells of some fish species[20,40]. Furthermore, the GTH-producing cells gave a weak immunoreaction with rat β-TSH antiserum in other teleosts[41,42]. The immunohistochemical results indicated that the GTH-producing cells of L. ramada gave a specific and strong immunoreaction with salmon GTH subunits (Iβ and IIβ) antisera. Similarly, the co-localization of GTH subunits (Iβ and IIβ) was obtained in the same cells of S. dumerilii[43]. As in other fish species, the GTHs-producing cells in L. ramada were obtained in the PPD[7,11,20].

In teleosts GTH play main physiological roles related to their reproduction, including sex steroids production, development and maturation of gonads and spawning[10,44,45]. In female teleosts, sexual maturation is driven by a gonadotropin-induced increase in plasma estradiol-17β (E1) levels[46]. E1 stimulates the synthesis and secretion of vitellogenin (VTG), which is a yolk protein precursor, by the liver while gonadotropin (s) stimulates vitellogenin uptake by the oocyte[47,48].

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التعريف الهستوكيميائى المناعى وتوزيع أنواع الخلايا فى الغدة النخامية لسمكة الطوبار

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المقدمة: التعريف الهستوكيميائى المناعى للخلايا المفرزة فى الغدة النخامية لأسماك الطوبار غير موجود حتى الآن.
الهدف من البحث: يهدف هذا البحث إلى التعرف على ووصف الخلايا المفرزة للهرمونات في الغدة النخامية لأسماك الطوبار.
المادة والطرق: تم التعرف على أنواع الخلايا المفرزة للهرمونات فى الغدة النخامية لأسماك الطوبار باستخدام الطرق الهستوكيميائية المناعية والاستعانة بالأمصال المضادة لهرمونات الغدة النخامية للأسماك والثدييات؛ الإنسان والفئران.
النتائج: يتكون الجزء الغدى من ثلاثة أجزاء وهى: الجزء اللامركزى البعيد، الجزء اللامركزى القريب والجزء المتوسط. تتوزع سبعة أنواع من الخلايا المفرزة للهرمونات فى الجزء الغدى للغدة النخامية. تشغل الخلايا المفرزة لهرمون البرولاكتين معظم الجزء اللامركزى البعيد وهى خلايا محبة للأصباغ الحمضية. أما الخلايا المفرزة لهرمون الأدرينوكورتيكوتروبين، الموجبة للهيماتوكسيلين الرصاصى، فهى تكون شريط خلوى مجاور للنسيج العصبى على حافة الخلايا المفرزة لهرمون البرولاكتين أو كمجموعات بين خلايا البرولاكتين.
وجدت الخلايا المفرزة لهرمون النمو المحبة للصبغيات الحمضية فى الجزء اللامركزى القريب مرتبة فى أشرطة مجاورة لمنطقة النسيج العصبى. أما بالنسبة للخلايا المفرزة لهرمون الجونادوتروبين فهى تشغل وسط الجزء اللامركزى القريب. تفاعلت خلايا الجونادوتروبين إيجابيا مع الصبغيات القاعدية واتحدت بقوة وتميز مع كل من المصل المضاد لهرمون الجونادوتروبين ١- بيتا والمصل المضاد لهرمون الجونادوتروبين ٢- بيتا لأسماك السالمون. أما الخلايا المفرزة لهرمون الثيروتروبين فقد وجدت على شكل مجموعة من الخلايا فى المنطقة الواقعة بين الجزء اللامركزى البعيد والجزء اللامركزى القريب وقد أعطت تفاعلا مناعيا سلبيا.
وُجد نوعين من الخلايا فى الجزء المتوسط؛ الخلايا الموجبة التفاعل مع حمض البيرأيوديك شيف والتى تفاعلت موجبا مع المصل المضاد لهرمون السوماتولاكتين لأسماك السالمون والخلايا الموجبة التفاعل للهيماتوكسيلين الرصاصى والتى تفاعلت إيجابيا مع المصل المضاد لهرمون ألفا - ميلانوتروبين.
الخلاصة: يعتبر التعرف على الخلايا المفرزة للهرمونات فى الغدة النخامية لسمكة الطوبار أساسا لفهم وتتبع التغيرات الهرمونية أثناء دورة تكاثرها فى الأسر.