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The anatomic structure and immunoreaction in cells with anti-hFSH and anti-hLH of the pituitary gland in Lake Van fish (*Alburnus tarichi* Güldenstädt, 1814)

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Abstract. The histology of the pituitary gland was investigated in the Lake Van fish (*Alburnus tarichi*). The adenohypophysis (AH) is composed of the rostral pars distalis (RPD), proximal pars distalis (PPD), and pars intermedia (PI). Seven different cell types were identified in the AH. Most of the cells in the RPD were periodic acid-Schiff (PAS)-negative. In the PPD, polygonal- and ovoid-shaped cells were distinguished, and they were PAS-positive. Polygonal cells were determined to be localized in the dorsal and ventral region of the PPD and around the boundaries of the PI and RPD. These cells showed a strong reaction with anti-human follicle-stimulating hormone (hFSH) and human luteinizing hormone (hLH). Ovoid-shaped cells were observed in the middle region of the PPD, and they showed a weak reaction with anti-hFSH and hLH. The cells localized in the PI were PAS-negative; however, some cells in the follicle surrounding the PI showed a cross-reaction with anti-hFSH and anti-hLH. These cells were more strongly stained with anti-hLH than with anti-hFSH. According to the results, we can conclude that glycoprotein secreting cells are present in all regions of the pituitary, but they are more intensive in the PPD than in the RPD and PI.

Keywords: *Alburnus tarichi*, pituitary, histology, histochemistry, Lake Van fish.

Introduction

The pituitary gland in vertebrates comprises two basic divisions, the adenohypophysis (AH) and the neurohypophysis (NH). In contrast to other vertebrates, the fish AH is divided into sub-divisions, namely the rostral pars distalis (RPD), proximal pars distalis (PPD), and pars intermedia (PI), whereas the entire NH penetrates the AH via numerous branches (Takashima & Hibiya 1995, Pandolfi et al. 2006, Camacho et al. 2020) The morphology of the teleostean pituitary varies considerably from one teleostean order to other; even within a given order variability is remarkable (Farrell 2011). In many teleosts, different cell types and their distributions have been identified histochemically and immunohistochemically (Cambre et al. 1986, Siegmund et al. 1987, Quesada et al. 1988, Shimizu et al. 2008, Nóbrega et al. 2017, De Jesus et al. 2014, 2017, Camacho et al. 2020). Two types of gonadotrophs (GTHs) were first isolated in teleosts, in chum salmon: FSH and LH (Suzuki et al. 1988a). These are chemically homologous to tetrapod follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Suzuki et al. 1988a, Ayala et al. 2003).

GTHs are known as regulators of reproductive functions. The FSH level increases during vitellogenesis, whereas LH increases during the final maturation and ovulation stages (Suzuki et al. 1988a). GTHs consist of a common α-subunit and β-subunit peculiar to the hormone (Suzuki et al. 1988b, Gen et al. 2000, Ohta et al. 2008).

Lake Van fish is the only fish species living in the lake of Van, the largest soda lake in the world. This species is an anadromous cyprinid fish adapted to extreme conditions (salinity 22 ‰, pH 9.8, and alkalinity 153 mEq/L) in Lake Van in eastern Turkey. Extreme conditions of the lake limit the living life to a great extent (Danulat & Selcuk 1992, Oğuz 2015). This fish is an important source of protein for the people of the region. It is only endemic to Lake Van, and it is hunted for 10.000 tons per year. Despite the importance of this species, information on growth, reproduction, and endocrine control of adaptation to different environments is insufficient. No study has been found about the pituitary gland of the Lake Van fish, even if it is of great importance in endocrine control. This study aimed to investigate the pituitary gland of Lake Van fish; therefore, this is a pioneering study both histologically and immunohistochemically.

Materials and Methods

All experimental animal procedures were carried out following animal study protocols approved by the Animal Researchers Local Ethics Committee of Van Yüzüncü Yıl University (protocol no: 2020/12).

We captured 20 adult Lake Van fish (*Alburnus tarichi*) (weight 98.6–116.8 g; length 18–20.6 cm) from Lake Van and the Karasu River, which pours into the lake, in April and May (in the maturation stage). The fish, aged 3+ years, were brought to the laboratory alive and anesthetized using MS 222 (Sigma Chemical Co., St. Louis, MO). Fish were sacrificed by decapitation after anesthetic. For routine histology, the pituitaries were removed and immediately fixed in Bouin solution for 24h at room temperature. The samples were dehydrated in increasing ethanol concentrations (70, 80, 90, 95, and 100 %), cleared in xylene (two changes, 20 min each), and embedded in Paraplast (Leica Microsystems, Wetzlar, Germany). Sections (5 µm) were cut from the paraffin blocks using a microtome (HM 325 Manual Microtome, MICROM International GmbH, Waldorf, Germany). They were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), periodic acid Schiff Orang G (PAS-OG), Orang G-acid fuchsin (OFG), Masson's tricrom (T) (Bancroft & Gamble 2002).

Pituitaries were fixed in 4% paraformaldehyde at 4 °C. Tissues were dehydrated by graded ethanol series (70, 80, 90, 95, and 100%), cleared in xylene, embedded in paraffin wax, and sectioned at 5µm with a microtome. Immunohistochemical staining for the sections of the pituitary gland was generally performed using an ABC (streptavidin-biotin peroxidase complex) Kit (Dako lsab 2). The sections were deparaffinized in xylene, rehydrated through graded ethanol, and washed in phosphate-buffered saline (PBS; pH 7.4) three times, for 10 min each time. All incubations were carried out at 4 °C, and PBS was used for washing after each step. The sections were

incubated with rabbit anti-hFSH and hLH (1:100 dilution, Abcam, Cambridge, UK) overnight at 4 °C. After that, the sections were incubated with a biotinylated secondary antibody for 1 h and PAP complex for 30 min. The sections were washed and stained with 3-amino 9-ethyl/carbazole in N, N-dimethylformamide (AEC). Finally, the sections were counterstained with Mayer's hematoxylin. The slides were mounted with coverslips and viewed using a digital camera (DFC 490, Leica, Wetzlar, Germany) attached to a microscope (DMI 6000B, Leica, Wetzlar, Germany).

Results

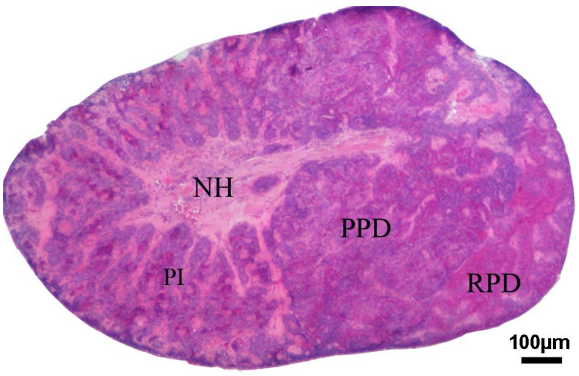

Figure 1. The longitudinal section of the pituitary gland in Lake Van fish. Neurohypophysis (NH); Pars intermedia (PI); Rostral pars distalis (RPD); Proximal pars distalis (PPD). Scale bar = 100 µm.

General morphology

The pituitary gland of the Lake Van fish is ovoid, with two regions of approximately equal lengths that could be distinguished morphologically (Figure 1). The anterior region connected to the brain is opaque, yet the posterior part is quite transparent.

Histology

The adenohypophysis (AH) is divided into the rostral pars distalis (RPD), proximal pars distalis (PPD), and pars intermedia (PI). While the anterior region includes the RPD and PPD, the posterior part has only the PI (Figure 1). The neurohypophysis (NH) is interdigitated with all regions of the AH. The interdigitation of the NH with the PI is very deep and elaborate.

The acidophilic and basophilic cells could easily be distinguished in the pituitary using the PAS, OFG, PAS-OG, and Mallory's trichrome staining methods. The acidophilic cells and nuclei were generally round, while the basophilic cells were ovoid or polygonal with a spherical nucleus.

Rostral pars distalis

The RPD was located in the anterior part of the pituitary and was scarcely occupied by the neurohypophysis processes. Two endocrine cell types were apparent with the PAS and trichrome staining. Most of the cells were PAS-negative and stained in brick red with trichrome (Figure 2a). Most of these cells were round, although some were polygonal. The PAS-positive cells in this region were located along the border with NH branches (Figure 2b), and they were stained blue with trichrome (Figure 2a). These cells were oval or polygonal in shape (12.25–9.25 µm in diameter). In this region, the PAS-positive cells were immunoreactive to anti-hFSH and hLH (Figure 3a, 3b). The number of immunoreactive cells with anti-hFSH was higher than that of the immunoreactive cells with anti-hLH; they were strongly immunoreactive (Table 1).

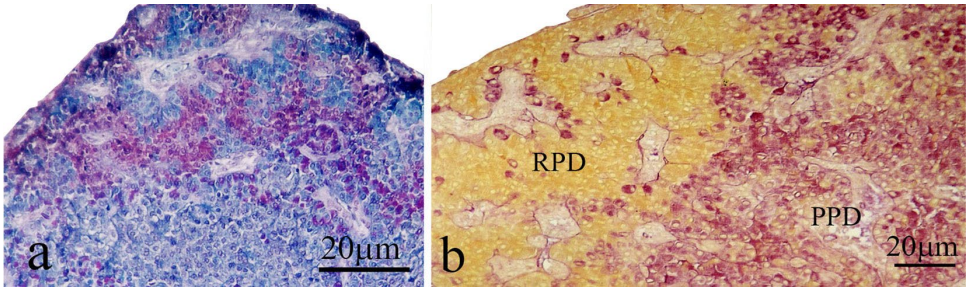

Figure 2. Sections of the RPD of the pituitary gland of Lake Van fish. a) Trichrome staining. b) PAS-positive cells stained with PAS-OG. Proximal pars distalis (PPD). Scale bars = 20 µm.

Proximal pars distalis

This region was situated between the RPD and the PI and showed no definite arrangement of cells. Three cell types were identified in this region. The prominent cells of this region were basophilic, which were PAS-positive and stained blue with trichrome (Figure 4a). The cells in the dorsal and ventral part of the PPD were polygonal or oval, often contained granules, and were relatively more extensive (11.75–9.25 µm in diameter) than the cells in the central region. The acidophilic cells, stained purple with trichrome, were localized around the NH branches. They were ovoid (13.25–7 µm in diameter) and contained an eccentric large irregular nucleus (Figure 3a). The cytoplasm of the PAS-positive cells in this region cross-reacted with the anti-hFSH and hLH (Figure 4b, 4c) (Table 1). However, the cells in the lower and upper extremities of the PPD, as well as the adjoining regions in the RPD and PI, were darkly immunostained with both anti-hFSH (Figure 4b) and hLH (Figure 4c, 4d), although the cells in the center were poorly stained.

Pars intermedia

The central part of the PI was occupied by a deep NH (Figure 1). All cells in this region were located along the border with NH branches. The cells were mostly round or elliptical and measured 10.3–10.0 µm in diameter. Two types of cells were identified, which were PAS-negative, although some of the cells in the central region were stained brownish purple with

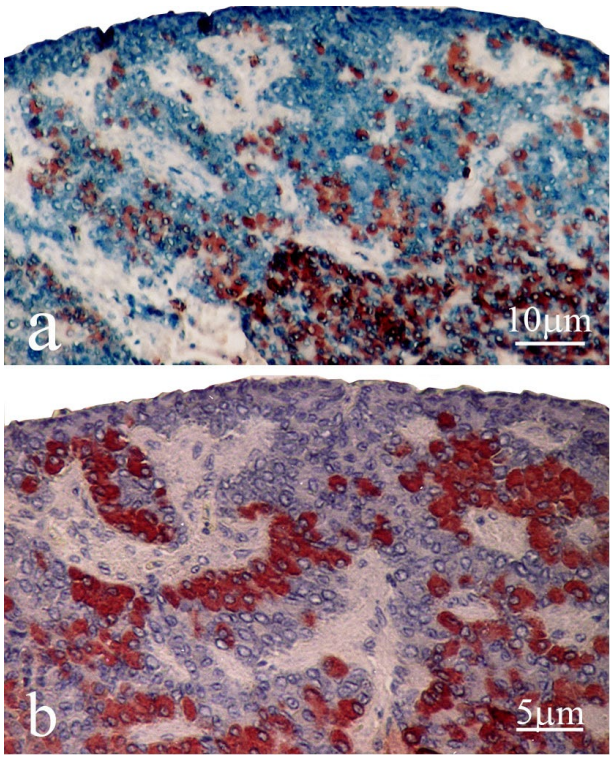

Figure 3. Sections of the RPD of the pituitary gland of Lake Van fish. a) Immunostaining with anti-hFSH. Scale bar = 10 µm.; b) Immunostaining with anti-hLH. Scale bar = 5 µm.

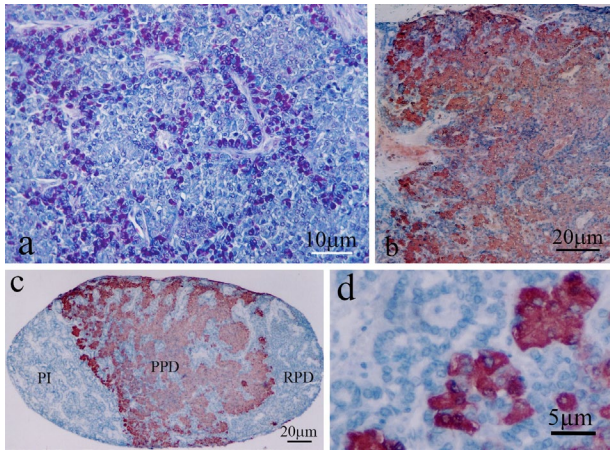

Figure 4. The sections of the PPD of the pituitary gland of Lake Van fish. a) Trichrome staining. Scale bar = 10 µm.; b) Immunostaining with anti-hFSH. Scale bar = 20 µm.; c) Immunostaining with anti-hLH. Scale bar = 20 µm.; d) High magnification of the immunoreactive LH cells. Scale bar = 5 µm.

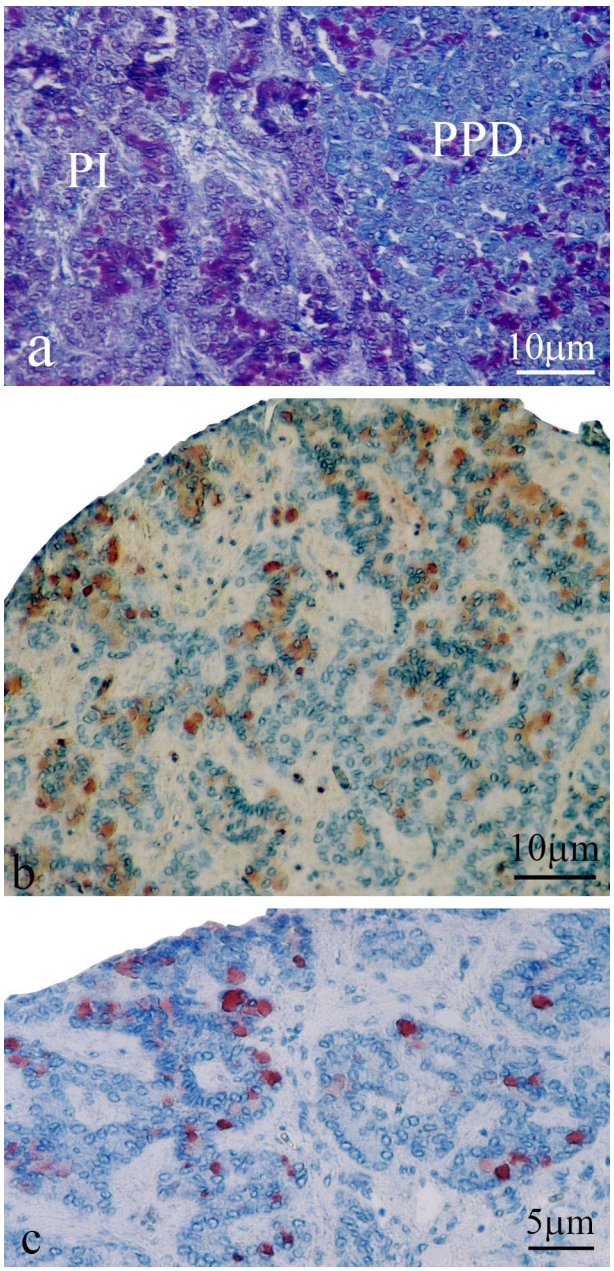

Figure 5. Sections of the PI of the pituitary gland of Lake Van fish. a) Trichrome staining; Scale bar = 10 µm. b) Immunostaining with anti-hFSH. Scale bar = 10 µm.; c) Immunostaining with anti-hLH. Scale bar = 5 µm.

Table 1. Semiquantitative analysis of FSH and LH positive cells in the different pituitary regions in adult female Lake Van fish. (++) high, (+) low

Antibody	Rostral pars distalis	Proximal pars distalis	Pars intermedia
FSH	++	++	++
LH	+	+	+

trichrome (Figure 5a). Some of the cells appeared in the cross-reactions with both anti-hFSH and hLH. These cells were localized in a disorganized manner in the outer layers of the PI. The number of immunoreactive cells with anti-hFSH was higher than that of the immunoreactive cells with anti-hLH, but many of these showed poor reaction (Figure 5b) (Table 1). A small number of immunoreactive cells with anti-hLH were observed in the PI, and they were generally more strongly stained (Figure 5c).

Discussion

This study evaluated the histology of the pituitary and the immunoreactive cells with anti-hFSH and hLH in Lake Van fish. As has already been identified in other fish species

(Narayan et al. 1985, Farrell 2011), the pituitary of Lake Van fish also consists of three different regions, the RPD, PPD, and PI. The entities of various secretion cells and their localization in the pituitary of other fish species have been determined with histochemical and immunohistochemical methods (Narayan et al. 1985, Siegmund et al. 1987, Yan & Thomas 1991). Seven different secretion cell types (two in the RPD, three in the PPD, and two in the PI) were determined in Lake Van fish via histochemical methods. This is consistent with the number reported in most fish species (Follenius et al. 1978, Farrell 2011). However, it was reported to be different in *Mugil cephalus*, with six cell types (Narayan et al. 1985). All PAS-positive cells in the RPD of the pituitary and some cells stained blue with trichrome showed a cross-reaction with anti-hFSH and anti-hLH.

The gonadotropic cells in the pituitary of fish have been investigated more than the other cells, and the presence of two types of GTH, according to immunocytochemical and ultrastructural properties, have been determined (Olivereau & Nagahama 1983, Kagawa et al. 1998). Two different gonadotropins, determined as FSH and LH in the pituitary of salmon and Atlantic croaker, were isolated (Suzuki et al. 1988a). In some fish, thyroid-stimulating hormone (TSH) cells have been reported to show a reaction in some cases, even with very specific fish antisera, like anti-carp GTH and anti-salmon GTH (Lindahl 1980). Gonadotropins (I and II) and TSH are included in the family of adenohypophyseal glycoprotein hormones. These hormones have an identical α-subunit but different β-subunit (Pierce & Parsons 1981, Suzuki et al. 1988b, Copeland & Thomas 1993). Therefore, the use of specific antiserum against β-subunits of TSH and GTH is necessary for the specific immunocytochemical determination of TSH and GTH cells. Quesada et al. (1988) reported that some PAS-positive cells showed a cross-reaction with anti-hLH and that the other PAS-positive cells, which were supposed to be TSH cells, showed a slight reaction when the hLH was diluted to a point lower than 1:500. Therefore, some of the PAS-positive cells in the PPD, which showed a poor response with anti-hFSH and anti-hLH in Lake Van fish, may have been TSH synthesized cells.

These GTH cells showed positive immunoreactivity against the anti-human GTHs antiserum in some fish and exhibited cross-reactivity with immunohistochemical methods. It has been reported that GTH cells are localized only in the PPD in some fish (Olivereau & Nagahama 1983), whereas in some other fish, these cells are localized in both the PPD and PI (Siegmund et al. 1987, Quesada et al. 1988). Some studies also reported that these cells could also be localized in the PPD and RPD (Cambre et al. 1986). It was also reported that GTH cells could also be localized in the PPD and RPD in some fish (Dubourg et al. 1985, Kaneko et al. 1985, Cambre et al. 1986, Segura-Noguera et al. 2000, Laíz-Carrióon et al. 2003, Shimizu et al. 2003). We used anti-hFSH and anti-LH non-specific antibodies for the fish FSH and LH hormone. However, when assessing the results of the histochemical and immunohistochemical examinations, we can say that the GTH cells intensified in the PPD and were localized in both the RPD and PI. Further studies are required to determine the GTH cells in the pituitary of Lake Van fish.

References

Ayala, A.G., Villaplana, M., Hernandez, M.P.G., Pozo, E.C., Agulleiro, B. (2003): FSH-, LH-, and TSH-expressing cells during development of *Sparus aurata* L. (Teleostei). An immunocytochemical study. General and Comparative Endocrinology (134): 72-79.

Bancroft, J.D., Gamble, M. (2008): Theory and practice of histological techniques. Elsevier Health Sciences.

Camacho, L.R., Pozzi, A.G., de Freitas, E.G., Shimizu, A., Pandolfi, M. (2020): Morphological and immunohistochemical comparison of the pituitary gland between a tropical *Paracheirodon axelrodi* and a subtropical *Aphyocharax anisitsi* characids (Characiformes: Characidae). Neotropical Ichthyology 18(1): e190092. DOI: 10.1590/1982-0224-2019-0092.

Cambre, M.L., Verdonc, W., Ollevier, F., Vandesande, F., Batten, T.F.C., Kühn, E.R. (1986): Immunocytochemical identification and localization of the different cell types in the pituitary of the Seabass (*Dicentrarchus labrax*). General and Comparative Endocrinology 61(3): 368-375.

Copeland, P.A., Thomas, P. (1993): Isolation of gonadotropin subunits and evidence for two distinct gonadotropins in Atlantic croaker (*Micropogonias undulatus*). General and Comparative Endocrinology (91): 115-125.

Danulat, E., Selcuk, B. (1992): Life history and environmental conditions of the anadromous *Chalcalburnus tarichi* (Cyprinidae) in the highly alkaline Lake Van, Eastern Anatolia, Turkey. Archiv fur Hydrobiologie 126(1): 105-125.

De Jesus, L.W.O., Bogerd, J., Vieceli, F.M., Branco, G.S., Camargo, M.P., Cassel, M., Moreira R.G., Yan, C.Y.I., Borella, M.I. (2017): Gonadotropin subunits of the characiform *Astyanax altiparanae*: Molecular characterization, spatiotemporal expression and their possible role on female reproductive dysfunction in captivity. General and Comparative Endocrinology 246: 150-163.

De Jesus, L.W.O., Chehade, C., Costa, F.G., Borella, M.I. (2014): Pituitary gland morphogenesis and ontogeny of adenohypophyseal cells of *Salminus brasiliensis* (Teleostei, Characiformes). Fish Physiology and Biochemistry 40(3): 897-909.

Dubourg, P., Burzawa-Gerard, E., Chambolle, P., Kah, O. (1985): Light and electron microscopic identification of gonadotrophic cells in the pituitary gland of the goldfish by means of immunocytochemistry. General and Comparative Endocrinology 59: 472-481.

Farrell, A.P. (2011): Encyclopedia of Fish Physiology: From Genome to Environment. Academic Press, San Diego, California.

Follenius, E., Doerr-Schott, J., Dubois, M.P. (1978): Immunocytology of pituitary cells from teleost fishes. International Review of Cytology 54: 193-223.

Gen, K., Okuzawa, K., Senthilkumaran, B., Tanaka, H., Moriyama, S., Kagawa, H. (2000): Unique Expression of Gonadotropin-I and II Subunits Genes in Male and Female Red Seabream (*Pagrus major*) During Sexual Maturation. Biological Reproduction 63: 308-319.

Kagawa, H., Kawazoe, I., Tanaka, H., Okuzawa, K. (1998): Immunocytochemical identification of two distinct gonadotropic cells (GTH I and GTH II) in the pituitary of Bluefin Tuna, *Thunnus thynnus*. General and Comparative Endocrinology 110: 11-18.

Kaneko, T., Kobayashi, M., Aida, K., Hanyu, I. (1985): Ultrastructural immunocytochemistry of gonadotrophs in the goldfish pituitary gland. Cell Tissue Research 239: 337-342.

Laíz-Carrióon, R., Segura-Noguera, M.M., Martin del Rio, MdP., Mancera, J.M. (2003): Ontogeni of adenohypophyseal cells in the pituitary of the American shad (*Alosa sapidissima*). General and Comparative Endocrinology 132: 454-464.

Lindahl, K. (1980): The gonadotropic cell in parr, precocious parr male and smolt of the Atlantic salmon, *Salmo salar*. An immunocytological, light-and electron microscopic study. Acta Zoologica 61(2): 117-125.

Narayan, A., George, K.C., Diwan, D. (1985): Histology of the pituitary gland of the grey mullet, *Mugil cephalus* L. Journal of Fish Biology 26: 381-390.

Nóbrega, R.H., De Jesus, L.W.O., Honji, R.M., Borella, M.I. (2017): Characterization of gonadotropic cells during continuous and seasonal spermatogenesis of two freshwater fish species: a histochemical and immunohistochemical study. Fish Physiology and Biochemistry 43(1): 51-63.

Oğuz, A.R. (2015): Histological changes in the gill epithelium of endemic Lake Van Fish (*Chalcalburnus tarichi*) during migration from alkaline water to freshwater. North-Western Journal of Zoology 11(1): 51-57.

Ohta, K., Mine, T., Yamaguchi, A., Matsuyama, M. (2008): Sexually dimorphic expression of pituitary glycoprotein hormones in a sex-changing fish (*Pseudolabrus sieboldi*). Journal of Experimental Zoology 309 (9): 534–541.

Olivereau, M., Nagahama, Y. (1983): Immunocytochemistry of gonadotropic cells in the pituitary of some teleost species. General and Comparative Endocrinology 50: 252-260.

Pandolfi, M., Nostro, F.L.L., Shimizu, A., Pozzi, A.G., Meijide, F.J., Vazquez, G.R., Maggese, M.C. (2006): Identification of immunoreactive FSH and LH cells in the cichlid fish *Cichlasoma dimerus* during the ontogeny and sexual

differentiation. Anatomy and Embryology 211(5): 355-365.

Pierce, J., Parsons, T.F. (1981): Glycoprotein hormones: Structure and function. Annual Review of Biochemistry 50: 465-495.

Quesada, J., Lozano, M.T., Ortega, A., Agulleiro, B. (1988): Immunocytochemical and ultrastructural characterization of the cell types in the adenopituitary of *Sparus aurata* L. (Teleost). General and Comparative Endocrinology 72: 209-225.

Segura-Noguera, M.M., Laiz-Carrion, R., Martin del Rio, M.P. Mancera J.M. (2000): An Immunocytochemical study of the pituitary gland of the hite seabream (*Diplodus sargus*). Histochemical Journal 32: 733-742.

Shimizu, A., Hamaguchi, M., Ito, H., Ohkubo, M., Udagawa, M., Fujii, K., Kobayashi, T., Nakamura, M. (2008): Appearances and chronological changes of mummichog *Fundulus heteroclitus* FSH cells and LH cells during ontogeny, sexual differentiation, and gonadal development. General and Comparative Endocrinology 156(2): 312-322.

Shimizu, A., Tanaka, H., Kagawa, H. (2003): Immunocytochemical applications of specific antisera raised against synthetic fragment peptides of mummichog GtH subunits: examining seasonal variations of gonadotrophs (FSH cells and LH cells) in the mummichog and applications to other acanthopterygian fishes. General and Comparative Endocrinology 132: 25-45.

Siegmund, I., Troncoso, S., Caorsi, C.E., Gonzalez, C.B. (1987): Identification and distribution of the cell types in the pituitary gland of *Austromenidia laticlavia* (Teleostei, Atherinidae). General and Comparative Endocrinology 67: 348-355.

Suzuki, K., Kawauchi, H., Nagahama, Y. (1988a): Isolation and characterization of two distinct gonadotropins from Chum Salmon pituitary glands. General and Comparative Endocrinology 71: 292-301.

Suzuki, K., Kawauchi, H., Nagahama, Y. (1988b): Isolation and characterization of subunits from two distinct Salmon gonadotropins. General and Comparative Endocrinology 71: 302-306.

Takashima, F., Hibiya, T. (1995): An Atlas of fish Histology. 2nd edition, Kodansa Ltd. Japan.

Yan, H.Y., Thomas, P. (1991): Histochemical and immunocytochemical identification of the pituitary cell types in three Sciaenid Fishes: Atlantic Croaker (*Micropogonias undulatus*), Spotted Seatrout (*Cynoscion nebulosus*), and Red Drum (*Sciaenops ocellatus*). General and Comparative Endocrinology 84: 389-400.