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# Light and electron microscopic examination of the pituitary gland of common Pandora (*Pagellus erythrinus* L., 1758)

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**Abstract**
The purpose of this study was to identify cell types of the pituitary gland of Common Pandora using histochemical methods. Infundibulum region of the organ was cleared through the anterior and posterior lobes. Regional and cellular differences were detected in the anterior lobe using different staining properties. Very dense acidophilic cells were especially located in the rostral pars distalis and it constituted a dark-painted region. There were many basophilic cells in the proximal pars distalis. Pars intermedia boundaries were not very clear, but distinguished by the presence of colloid. Stained in different ways, small acidophils and large basophils reflected differences in hormone contents in granules. Electron microscopic examination of pituitary revealed that cells showed similarities to the features of endocrine secreting proteins and to the fine structure of gonadotrophin in adenohypophysis. Necessary for the hormone synthesis, a well-developed Golgi complex and membrane-located near, there were more intense mid-secretory vesicles a large number of round or oval. There were oval euchromatic nucleus.

**Keywords:** *Pagellus erythrinus*, pituitary gland, light and electron microscopic, histology

## 1. Introduction
A member of the Sparidae family, Common Pandora (*Pagellus erythrinus* L., 1758) has been considered one of the alternative species of high economic value in the aquaculture sector especially in recent years [1]. *P. erythrinus* is among the most captured species for the small-scale fishing fleet in many Mediterranean countries, playing an important role in the local micro economy by the volume of catches and by its high price [2, 3, 4, 5]. It was reported that the common pandora is a suitable species for aquaculture in the Mediterranean and that the correct determination of the species spawning period is also very important [6]. Spawning period, sex-ratio, GSI, length at first maturity and length weight relationship were studied in different regions such as, Agean sea [4, 5, 7], southern Portugal [5, 8], the western Mediterranean [6], Tyrrhenian sea [9], in the Gulf of Tunis [10, 11], and in the Bay of Monastir [5, 12], in Agadir's Bay of Morocco [5, 13]. However, there have been very few studies conducted on the culture of Common Pandora even though it is regarded as an alternative species as well as the other members of the Sparidae family [14]. One of the main problems encountered in the contribution of alternative species to intensive aquaculture is lack of information regarding their biotic and abiotic requirements. In addition, gathering information on morphology and development of organs of those species has also been one of the most discussed topics in recent years. However, a detailed description of the cell types in the pituitary of Common Pandora has not yet been done. The aim of this study was to determine in terms of the place of secretion, structural and functional characteristics the different pituitary cell types using light and electron microscopy histology. The identification of pituitary cell types will provide useful information for future physiological studies on pituitary gland function in *P. erythrinus.*

## 2. Materials and Methods
### 2.1 Hyphophysectomy
Hyphophysectomy refers to the process in which pituitary gland is removed from fish through cranial incision method. Captured fish were brought to the laboratory for hyphophysectomy, connective tissue around the brain was cleaned, the ends of the optic nerves were cut, and the

brain was held with the help of nippers and lifted from front to back. Pituitary gland could be removed together with the brain or could sometimes remain in its own depression in the parasphenoid bone. In such a case, it was removed with the help of fine-tipped nippers [15, 16]. (Figure 1-2).

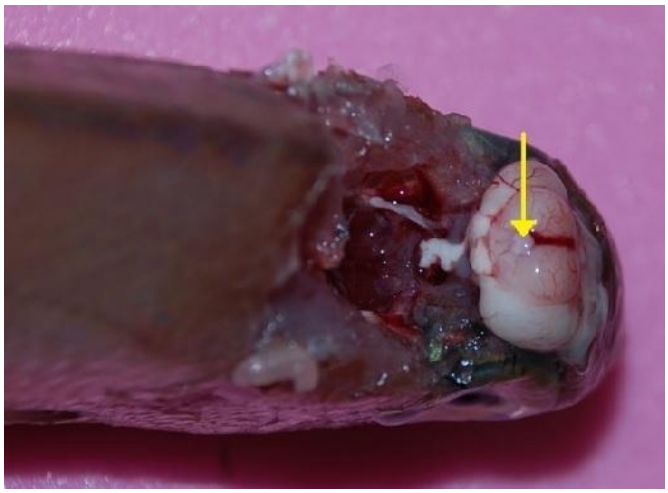

**Fig 1:** Pituitary gland is removed from fish through cranial incision method.

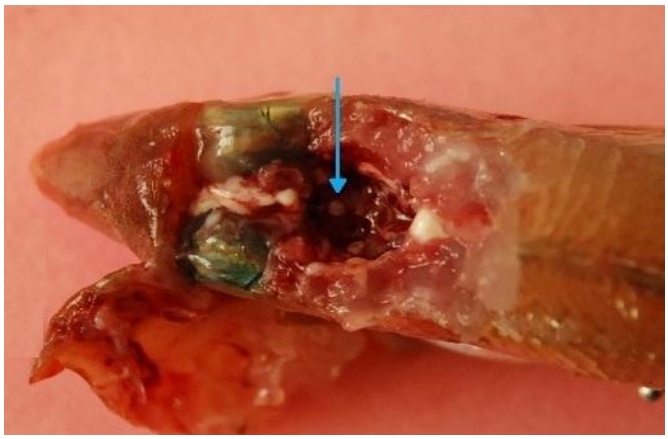

**Fig 2:** Pituitary gland (own depression in the parasphenoid bone)

### 2.2 Light Microscopic Studies

Depending on the histochemical techniques to be applied, pituitaries removed from the fish were kept in a Bouin's and 10% neutral formaldehyde solution for at least 24-48 hours. The tissues were detected in the neutral formaldehyde. They were washed for one hour in order to eliminate the effect of the detection fluid. The tissues kept in different concentrations of alcohol for a certain period after the detection process were subjected to xylol and paraffin baths and blocked and made ready for cutting with microtome. Sections of 5 µ thickness cut from the tissues blocked for normal histology were kept in an oven at 40 °C overnight. They were stained according to the Harris's hematoxylin-eosin and Periodic Acid Schiff (PAS) staining procedures for the general histological diagnosis. The slides stained and mounted in Canada balsam were examined under a binocular research microscope (Olympus CX21) and their microphotographs were taken for evaluation in order to identify the histological details [17- 20].

### 2.3 Electron Microscopic Studies

Tissues were kept in 4% glutaraldehyde for the preliminary determination process and then were kept in 1% osmium tetroxide for the secondary determination process and then subjected to dehydration in order to determine the pituitary samples used in the electron microscopic examination. After the necessary procedures, semi-thin (1-2 µm) and then ultra-thin (100-200 A°) sections were taken using ultramicrotomy and the samples were prepared for the analysis [21]. Semi-thin sections (1-2 µm) obtained by Ultratom for electron microscope were stained toluidine blue and all stained sections were examined using a light microscope (Olympus BH 2; Japan) after they were covered with entellan. In addition, 100-200 A° sections were stained with uranyl acetate and lead citrate and the results were evaluated using Leo Zeiss-906 Transmission Electron Microscopy (TEM) and then photographed [21].

## 3. Results

### 3.1 Light Microscopic

The opening of the frontal part of the skull of the fish through Hyphophysectomy operation revealed that the brain was surrounded by a protective connective tissue. The pituitary gland, which was creamy white in color, was embedded in its own depression referred to as "sella turcica" in the parasphenoid bone of the skull. Infundibulum region referred to as pituitary stalk had a fine and thin structure; and therefore could be broken very quickly during Hyphophysectomy and its inner cavity could not extend inwards. Sometimes, however, it was removed together with the brain. It was also observed that the pituitary gland had a smooth surface. Histological examinations revealed that the pituitary gland had a smooth circular rounded shape. The infundibulum region, the anterior and posterior lobes of the organ were quite evident. The overall image of the $III^{+}$ age Common Pandora samples showed that the pituitary gland was connected to the brain by the thin and short infundibulum (Figure 3.A-3.B). As in other vertebrates in general, the anterior pituitary of the fish consisted of two main parts, namely, the anterior lobe which was stained dark (Adenohypophysis) and the posterior lobe which was stained pale (Neurohypophysis). Pars intermedia (intermediate) lobe contained cleft remnant. The anterior and the posterior lobes had a very rich vascular network while pars intermedia did not. It was observed that neurohypophysis region extended to the pars intermedia through finger-like projections (Figure 3.C-3.D). It was observed that, during breeding time, the size of the adenohypophyses grew more than the neurohypophysis. The samples of the beginning of April when breeding started showed larger adenohypopheses in size than neurohypophysis (Figure 3.B). The samples of June when the breeding ended showed smaller adenohypopheses in the sagittal section (Figure 3.C). Neurohypophysis was quite compact. It was distinguished as the region where neurohypophysis axons branch out and spread and these axons extended from the paraventricular and supraoptic nuclei of the hypothalamus (Figure 3.C). There were no differences in the neurohypophysis in the reproductive stages except for spatial changes. Specialized glial cells referred to as pituicyte were identified in the neurohypophysis (Figure 3.E). Regional and cellular differentiation in the anterior lobe was observed in the form of different staining properties. Adenohypophysis showed a typical feature of the endocrine glands. The Pars distal of the adenohypophysis was composed of acidophilus, basophil and chromophobe cell lines which form anastomosis of strands with each other (Figure 3.F). In particular, acidophilus cells in the rostral pars were quite densely located

and constituted the dark stained region. The number of basophilic cells in the proximal pars distalis was high. Pars intermedia boundaries were not very clear and could only be distinguished by the presence of colloids. The staining of the small acidophils and large basophiles in different colors reflected the difference in the hormone content of the granules. Sinusoidal capillaries (SC) were present between parenchymal cell stacks (Figure 3.F). The presence of a common vascular network was also observed. This network enabled the transfer of hormones secreted by the adenohypophysis cells to target organs and feed the cells in the anterior lobe of the adenohypophysis by carrying the stimulant and inhibitory factors which originated from the hypothalamic-hypophyseal portal system (Figure 3.F-3.G-3.H). The applied staining method indicated that basophilic gonadotropin cells of the pituitary gland of the male and female fish showed no vacuolation during the breeding season. Gonadotropin cells were shown in magenta color with PAS staining (Figure 3.G-3.H). It was observed that a vacuolar space was present in the cytoplasm of the proximal pars distalis region of the adenopituitary gland of the Common Pandora other than during spawning period while gonadotropin cells which contained secretion granules spread over a large area of the cell during spawning period (Figure 3.G-3.H).

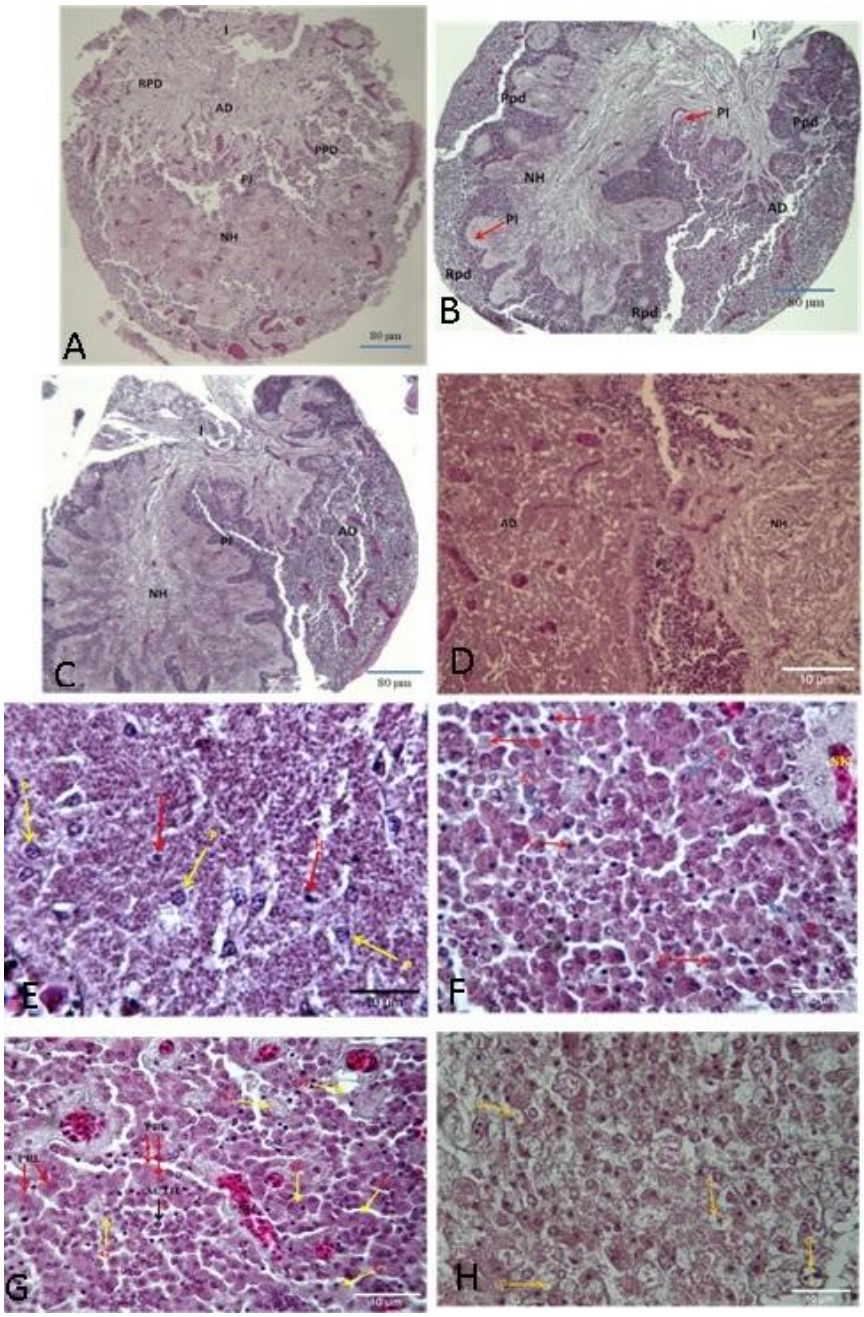


**Fig 3:** A) The general anatomy of the pituitary gland of Common Pandora belongs to the september (PAS) B) Sagittal section of the pituitary gland belongs to April (H+E) C) Sagittal section of the pituitary gland belongs to June (H+E) D) The pituitary gland belongs to July (H+E) E) The cells of Neurohypophysis (H+E) F) Adenohypophysial cell types in Common Pandora (PAS) G) The gonadotropin hormone cells during spawning of common pandora. (PAS) H) Gonadotrop hormone cells (vacuoles) done hormonal secretion after the reproduction period of common pandora (H+E). NH:Neurohypophysis PI:Pars İntermedia AD:Adenohypophysis PPD:Proximal pars distalis RPD:Rostral pars distalis I:İnfundibulum P:Pituicyte cells H:Herring bodies G:Gonadotropin hormone cells V:Vacuolar space A:Acidophilic cells B:Basophilic cells K:Chromophobic cells SK: Sinusoidal capillary G:Gonadotropin hormone cells PRL:Prolactin cells ACTH: Adrenocorticotropic hormone

### 3.2 Electron Microscopic

Thin structural properties of the gonadotropin cell in the adenopituitary gland showed similarities to the proteins-secreting endocrine cells. A well-developed Golgi complex which is essential for hormone synthesis and numerous round or oval secretory vesicles with dense midpoint located close to the membrane were present. Oval was a euchromatic core. February samples showed abundant secretory granules in the gonadotropin cells. Concurrent gonadal development corresponded to the stages of viteliogenesis and spermatogenesis in female and male fish, respectively (Figure 4.A). Very few and irregular secretory vesicles with low-contrast view were present in gonadotropin cells in old individuals and other than during spawning period. November samples showed that secretory vesicles in the gonadotropin cells decreased and vacuolation increased. Gonadotropin cells were observed to be small and scattered in the samples that was not reached sexual maturity (Figure 4.B). Electron microscopic examinations of the pituitary gland of the young individuals showed that there was a large number secretory vesicles in the gonadotropin cells and spread over a wide area during spawning period (Figure 4.C). Sinusoidal capillaries (SC) network was present between parenchymal cell stacks. Capillaries had electron density due to erythrocytes in them (Figure 4.D). In active cells, a prominent Golgi complex, a large number of mitochondria and granulated endoplasmic reticulum and dense secretory vesicles were found. Irregular shaped nucleus had one or more than one nucleolus (Figure 4.E-4.F).

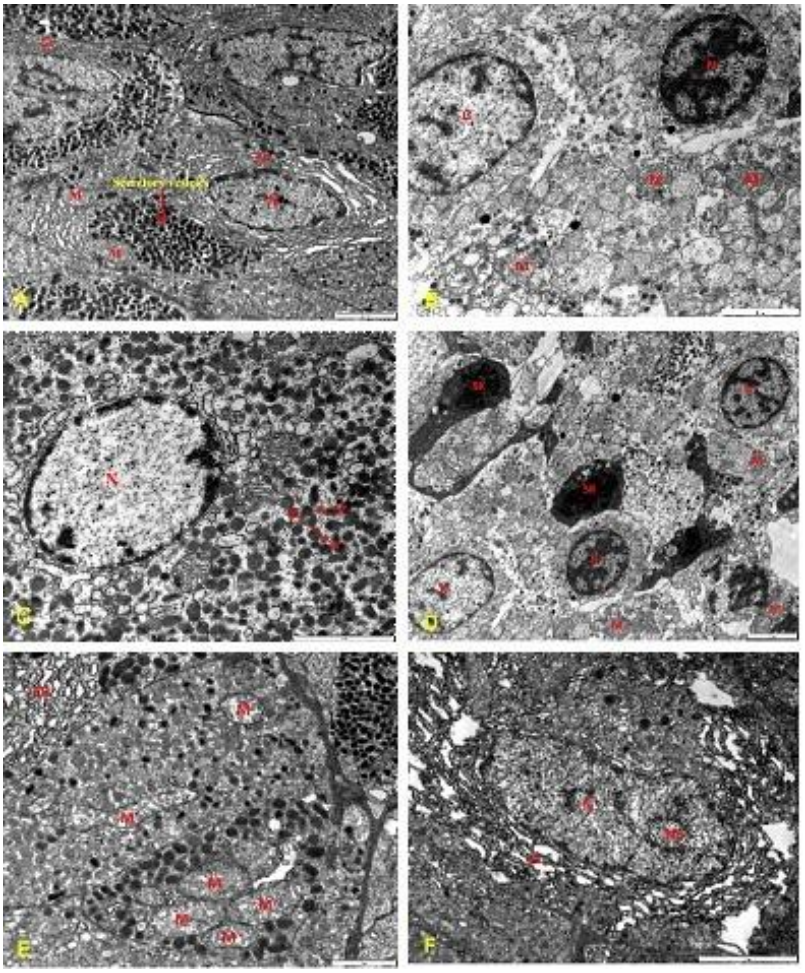

**Fig 4:** A) Electron microscopy image of Gonadotropin cell (Scale bar:2µm) B) Mitochondria and Gonadotropin cells in VI+ years old individuals (Scale bar:2µm) C) Secretory granules in the gonadotropin cell in the pituitary gland of II+ years old fish (Scale bar:2µm) D) Electron microscopy image of Adenohypophysis (Scale bar:2µm) E) Electron microscopy image of mitochondria in the cytoplasm of Gonadotropin cell of Adenohypophysis (Scale bar:1µm) F) Electron microscopy image of inactive Gonadotropin cell (Scale bar:2µm). ER:Endoplasmic Reticulum M:Mitochondria G:Gonadotropin hormone cells N:Nucleus Nu:Nucleolus Gc:Golgi complex g:Secretory granules SK:Sinusoidal capillaries GER:Granular Endoplasmic Reticulum

## 4. Discussion

This study investigated the light and electron microscopic level changes in the pituitary gland of the Common Pandora (*P. erythrinus* L., 1758) fish. it was pointed out that the pituitary gland was located right below the brain, embedded in its own depression called sella turcica in the parasphenoid bone of the skull of the fish as in other vertebrates [15, 16]. In this study, the samples indicated that the pituitary gland was buried in its own depression referred to as "sella turcica," which means Turkish Saddle, in the parasphenoid bone of the skull. Although birds and some mammals do not have pars intermedia region, it are found in fishes [15, 22]. However, it was observed that it was not as easily distinguished as pointed out by those studies. Neuropituitary gland was distinguished as the region composed of myelinated nerve fibers as defined in any other bony fish and scattered glial cells were observed [15]. It was pointed out that pituitary gland was in the shape of an acorn in common carps, round in vimba breams and cone-shaped in sander lucioperca [15]. Moreover, it was stated that pituitary gland attached to the brain through a short and thin

infundibulum, which this infundibulum part of the brain is macroscopically difficult to detect [15]. The fact that infundibulum is larger in *mycteroperca rubra* than in *epinephelus caninus* is accounted by as a species-specific difference [16]. Some differences regarding the morphology and the size of the pituitary gland of the teleost fish and the presence of infundibulum were observed. These differences were stated to depend on seasonal changes, age and sex even in the same conditions [15, 23]. The infundibulum region, anterior and posterior lobes of the organ were evident. Infundibulum region referred to as pituitary gland stalk was quite large and the interior space did not extend inward. The studied samples showed that the pituitary gland was connected to the brain through a short infundibulum. Regional and cellular differentiations in the anterior lobe was presented as different staining properties. The samples pointed to a circularly uniform-shaped pituitary gland. The pituitary glands of the mycteroperca rubra and epinephelus caninus are smooth round shaped as stated [16]. William *et al,* (2007) [24] stated that the histological differences between the pars distalis of the anterior lobe and the neuropituitary gland of the posterior lobe are more evident and mentions dark stained glandular epithelium feature due to a large number of firmly formed parenchymal cell populations in the structure of the anterior lobe. In addition, it is also stated that the posterior lobe consists of nerve tissues and therefore observed more clearly [24]. The study samples showed, in general, dark stained anterior lobe (Adenopituitary gland) and pale painted posterior lobe (Neuropituitary gland). Pars intermedia contained nerve cells between adenopituitary gland and neuropituitary gland regions, and the anterior lobe and posterior lobe had very rich vascular network while pars intermedia did not have this property. Neuropituitary gland and adenopituitary gland were observed to extend towards the pars intermedia through finger-shaped protrusions. The samples indicated that the pars distalis region of the adenopituitary gland constitutes 60% of the gland. Rostral pars distalis is the smallest part of this region and constitutes 15% of the gland in carps and this region located in posterio-dorsal is named anterior lobe, anterior glandular region, pro-adenopituitary gland and Hauptlappe [25]. During spawning season, adenopituitary gland was observed to grow more in size than neuropituitary gland. Neuropituitary gland could be distinguished as the region where axons branch out and spread [16]. The study indicated that neuropituitary gland had a compact structure and that the region was distinguished as the region where axons branched out and spread. Irregular shaped oval nucleated cells were reported in the gland [24]. Specialized glial cells referred to as pituicyte in the neuropituitary gland were observed. Chromophilic (acidophilic and basophilic feature-cells) and chromophobic (no stain receiving) cells found in the pars distalis of the adenopituitary gland in mammals were stated to be found in the histological sections of the adenopituitary gland [15, 26]. The pars distalis of the adenopituitary gland was observed to be composed of acidophilus, basophil and chromophobe cell lines which form anastomosis of strands with each other. In particular, acidophilus cells in the rostral pars were quite densely located and constitute the dark stained region. The number of basophilic cells in the proximal pars distalis was high. Pars intermedia boundaries were not very clear and could only be distinguished by the presence of colloids. The staining of the small acidophils and large basophiles in different colors reflects the difference in the hormone content of the granules. Sinusoidal capillaries were present between parenchymal cell stacks in this study. It was stated that gonadotropin cells in pars distalis are found only in adult fish and that they are large in the spring which is the maturation period of the gonads [27]. It was determined that a vacuolar space was present in the cytoplasm of the proximal pars distalis region of the adenopituitary gland of the Common Pandora other than during spawning period while gonadotropin cells which contain secretion granules spread over a large area of the cell during spawning period. The widespread presence of the pars distalis region in the pituitary gland could be viewed as the sample fish being in preparation for an intensive spawning period. This structural condition was consistent with the explanation of gonadotropin-secreting cells being basophilic and located in the proximal pars distal of the pituitary gland reported by Garcia-Hernandez, *et al,* (1996) [22] and Johnson, *et al,* (1998) [28]. Few small longish shaped, round nucleated basophilic gonadotropin cells found scattered in the rostral and pars distalis of the adenopituitary gland were stained ametist with histochemical stains (PAS, OFG) [15]. This method stained the basophilic gonadotropin cells in the pituitary gland of the male and female fish various colors in order to determine whether they exhibit vacuolation or not during spawning. Gonadotropin cells reported to produce FSH and LH hormones were shown in ametist through rust staining [29]. Massoud, *et al,* (1983) [27] reports that gonadotrops are observed in the proximal pars distalis region of fish that have reached sexual maturity. It is determined that mature gonadotrops form in spring and gonads develop. In this study, electron microscopic examination of the samples were compared the young and old individuals of the *P. erythrinus* and indicated that secretory vesicles in the pituitary gland of the old individuals decreased and vacuolation increased. Ultrastructural features of the gonadotropin cells are typical examples of protein-secreting endocrine cells. Many organelles which are essential for hormone synthesis such as Golgi complex and secretory vesicles near the cell membrane are present [24]. This study determined numerous round or oval dense secretory vesicles located near the cell membrane and an oval euchromatic core. Also, the presence of a well-developed Golgi complex is essential for hormone synthesis.

## 5. Conclusıon

According to histological and electron microscopic examinations of the gonadal development were determined that these fish showed reproductive activity from the end of April until the beginning of September.

When immunocytochemistry method was used in conjunction with electron microscopy, it was possible to distinguish between different types of secretory granules. Taking into account the immunocytochemistry staining together with the diameter, shape and staining characteristics of the secretory granules, fine structure cell definition of the granules will be possible.

## 6. Acknowledgements

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