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# HISTOCHEMICAL STUDIES, CELL TYPE DISTRIBUTION AND SEASONAL VARIATION OF GONADOTROPIN CELLS IN PITUITARYGLAND OF FEMALE *DICENTRARCHUS LABRAX* IN RELATION TO MATURATION OF GONADS

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***Key words:*** *Pituitary gland, histochemistry, Dicentrarchus labrax.*

## ABSTRACT

*Dicentrarchus labrax* adenohypophysis is subdivided into three distinct zones innervated by the process of neurohypophysis. Six cell types were identified in the adenohypophysis using histochemical methods. The basophilic prolactin (PRL) and adrenocorticotropic hormone secreting cell (ACTH) were found in the rostral pars distalis region (RPD). The PRL cells showed strong affinity to Azan stain and gave red colour. The ACTH cells showed strong tinctorial response to Lead Hematoxylin and gave dark gray colour. The ACTH cell gave brown colour when stained with Azan technique. These cells were generally interlocated between neurohypophysis and PRL cells, forming a defined cell population clearly distinct from PRL cells which were restricted to RPD region. The third and fourth basophilic melanocyte stimulating hormone secreting cells (MSH) and somatotrops cells (STH) were observed in the proximal pars distalis region (PPD). They were mostly rounded in shape with indented nucleus in case of MSH, while the nucleus of STH was irregular in shape. The MSH cells stained light red with Azan. The STH cells gave yellow colour with Periodic acid-Orange G technique (PAS-OG). All acidophilic cell types did not change their activity throughout the year. The basophilic gonadotropic cells (GTH) and thyrotropic cells (TSH) react positively with PAS. One type of GTH cells were detected, Herlant technique gave blue colour for GTH cells , whereas Camerone and Steel technique gave green colour. The GTH cells exhibited both quantitative and qualitative variations during the ovarian cycle. During resting period (immature and mature) and pre-spawning period, the GTH cells were characterized by gradual accumulation of granules and reached to maximum diameters at the pre-spawning period. The activity of GTH cells was reflected by staining affinity and vacuolization of the cells. During ripening and spawning period (from December to March), slight decrease in the GTH diameters and staining affinity were recorded. During the spent stage (from April to June), the GTH cells stained weakly with presence of vacuolated and atretic cells. The neurohypophysis in *Dicentrarchus labrax* consisted of nerve fibers which extended below the pituitary stalk.

## INTRODUCTION

Sea bass belong to family Serranidae. In Egypt *Dicentrarchus labrax* is considered one of the most important commercial fish, which is found all-over the Mediterranean Sea and other waters. Sea bass is cultured in many fish farms (FAO, 1984). The identification and distribution of the cell types in the pituitary gland of several teleosts has attracted attention of some investigators from the histochemical, ultrastructural and immuno-cytochemical points of view as indicated by Marayan *et al.* 1985; Pectoff *et al*. 1994; Zaki *et al*. 1996; Mousa & Mousa 1997 a&b; Assem and El-Boray 2001; Ali 2003 and Al-Absawy 2004. They pointed that

in those fishes the identification and distribution of the different cell types indicate that the adenohypophysis consisted mainly of two groups: basophilic or PAS positive and acidophilic or PAS negative. The secretory cells of the pituitary gland show a vigorous distribution in the three well defined zones of the adenohypophysis. The PRL and ACTH cells are located in the RPD region, while the remaining cells are found in the PPD and PI regions. However, some differences have been noticed from species to another.

One gonadotropin- secreting cells type has been observed in some fish species (Toubeau *et al.* 1991; Assem 1995, El-Greisy 2000; Gaber 2000; Assem & El-Boray 2001 and Al-Absawy 2004. However, two GTH cell types were also described in other teleost species such as *Mugil cephalus* by Mousa& Mousa 1997 a &b.

Zaki *et al.* 1996 studied the cyclic changes in the pituitary gland and gonads of *Siganus rivulatus* from the Red Sea (Hurgada area). Mousa and Mousa 1997 a&b identified the cell type distribution and the activity of the gonadotropic cell in the female mullet, *Mugil cephalus* (teleost fish) by using immunocytochemical method. Assem and El-Boray 2001 studied the cell type distribution and comparing between gonadotropin cells in the pituitary gland of female *Rhabdosargus haffara* reared in saline and brackish water.

The aim of this work is to identify and localize by both histological and histochemical methods the different cell types in the pituitary gland of *Dicentrarchus labrax* which seems to be suitable for aquaculture but, as with many species does not breed in captivity. The examination of GTH cells of the female at all stages of maturity in relation to oocyte maturation and gonadosomatic indices aims to gaining insight endocrinology of the species and providing basic information necessary for its successful propagation.

## MATERIALS AND METHODS

**Fish sampling***::*

*Dicentrarchus labrax* fish were collected throughout the period from January to December 2003 from EL-Maadia coast at intervals of 2 weeks.The 210 specimens collected ranged in size from 25 to 74 cm and body weight varied between 700 to 1800gm. Sex and maturity stages were identified by three methods as follows:

1- Gonadosomatic index (GSI= gonad weight / gutted body weight X 100) .
2- Oocyte diameters.
3- Histological appearance.

***Histological and histochemical methods:***

After dissection of the fish , the pituitary gland with a piece of brain and a piece of gonads were fixed in 10% buffered formalin. After fixation, dehydration and embedding, consecutive median sagittal sections of the brain and pituitary were made at 5 µ thickness. For each specimen selected sections were stained according to Culling, 1978 and Humason, 1979 as indicated in (Table 1) to determine the location of acidophilic and basophilic cells according to staining affinity and coloration of each type of cells. Hypertrophy and granulation of GTH cells were quantitatively determined by measuring the variation in nuclear density (the number of nuclei per unit area).

***Statistical analysis:***

Microsoft windows (2000), Excel program were used for analysis of correlation coefficient.

Table 1: Location and staining properties of acidophilic and basophilic cell types in the pituitary gland of *Dicentrarchus labrax*.

| Types of staining technique | Types of the cells and Pituitary regions | | | | | |
|---|---|---|---|---|---|---|
| | Acidophilic cell | | | | Basophilic cells | |
| | PRL | ACTH | STH | MSH | GTH | TSH |
| | RPD | RPD | PPD | PPD | PPD&PI | PPD&PI |
| PAS | – ve | – ve | – ve | – ve | Pink | Pink |
| PAS-OG | – ve | – ve | Yellow | – ve | Red | Magenta Red |
| PAS-PbH | – ve | Dark Gray | – ve | Gray | Pink | Pink |
| Azan | Light-Red | Brown | Yellow-Red | Light-Red | Light-Blue | Blue |
| Herlant (Ox-Ab-PAS-OG) | Pink | Pink | Orange | Pink | Blue | Dark- blue |
| Camerone & Steele | – ve | – ve | – ve | – ve | Green | Orange- purple |
| PAS-H-AF | – ve | – ve | Light Blue | Blue | Red | Red |
| (PAS-)G-LG | Yellow | Yellow | Yellow | Yellow | Green | Green |

PRL Prolactin cells,
ACTH Adrenocorticotropin hormone secreting cells.
STH Somatotropin hormone secreting cells.
MSH Melanocyte hormone secreting cells.
GTH Gonadotropin hormone secreting cells.
TSH Thyrotropin hormone secreting cells.
RPD Rostral pars distalis region.
PPD Proximal pars distalis region.
PI Pars intermediata region.
PAS Periodic acid –Schiff reagent.
OG Orange-G.
PbH Lead Hematoxylin.
OX-AB Oxidation and Alcian blue.
AF Acid Fuchsin.
H Hematoxylin.
LG Light green.

# RESULTS

## 1- Ovarian cycle:

***Maturity stages:***

On the basis of seasonal changes encountered in the histomorphology and gonadosomatic index Table 2 indicates that the ovarian cycle of female *Dicentrarchus labrax* can be classified into six stages, a scale for the maturity stages was adopted taking into account the two generalized scales: (Zaki *et al.*, 1996 and Assem, 2000) as follows:-

***I- Immature stage:***

Ovaries are almost cylindrical with two tapering ends. This stage consists of ovaries with chromatin nucleolus cells and early perinucleolus cells. The average GSI values was about 0.69% ± 0.13 and oocyte diameters 88 µm ± 36 (Table 2).

***II- Maturing stage:***

Ovaries are enlarged in size, pinkish-red in colour and occupy nearly half of the body cavity. Histologically most of the oocytes are detected in the early and late perinucleolus, with small number of ovae are in the vacuolized stage. The average GSI values was about 0.82% ± 0.14 and oocyte diameters 146 µm ± 60.

***III- Nearly ripe stage:***

Ovaries are yellowish in colour, size reaches about two-third of the body cavity,

and eggs are distinguishable with the naked eye. Histologically, most of the oocytes are in the vacuolized stage and few number at primary yolk stage oocytes. This stage is detected in October and November with average GSI values of 5.94% ± 0.19 and oocyte diameters 354 µm ± 112.

***IV- Ripe stage:***

At this stage ovaries show maximum development in thickness and width and occupy the entire length of the body cavity. Ovaries are stretched typically, and are orange yellow in colour. The belly of the female seems swollen, eggs can be evacuated by a slight press on the belly. Histologically most of the oocytes are in the primary and secondary yolk stage. This stage is detected from December to March , with average GSI values of 16.2% ± 1.9 and oocyte diameters 751 µm ± 96.

***V- Spawning stage (partly spent):***

The ovaries show slight shrinkage and decrease in weight due to the discharge of a considerable amount of sexual products during the course of spawning. Histologically most of the oocytes are in secondary, tertiary and liquefied yolk stage. This stage is detected in January, February and March with average GSI values of 14.6% ± 2.5 and oocyte diameters 812 µm ± 87.

***VI- Spent stage:***

Ovaries are severely shrunken, flaccid, collapsed and highly vascularized and reddish-purple in colour. They also have large number of blood vessels. Histologically most of the oocytes are detected in the early and late perinucleolus, This stage is detected from April to June with average GSI values of 0.76%±0.14 and oocyte diameters 113 µm ±40.

**Table2:** Gonadosomatic index (GSI) and oocyte diameters of female *Dicentrarchus labrax* at different stages of maturity.

| Stages of maturity | No. Of Fish | Oocyte diameters (µm) Mean ± SD | Gonadosomatic index Mean (%) ± SD |
|---|---|---|---|
| Immature | 25 | 88 ± 36 | 0.69 ± 0.13 |
| Maturing | 23 | 146 ± 60 | 0.82 ± 0.14 |
| Nearly-ripe | 28 | 354 ± 112 | 5.94 ± 0.19 |
| Ripe | 22 | 751 ± 96 | 16.2 ± 1.9 ** |
| Spawning | 27 | 812 ± 87 | 14.6 ± 2.5** |
| Spent | 28 | 113 ± 40 | 0.76 ± 0.14 |

## 2- The pituitary gland:

***I – Morphologically:***

The pituitary gland of *Dicentrarchus labrax* is a whitish cone-shaped structure, suspended ventrally from the floor of the diencephalons of the brain. It is attached to the brain by a stalk.

***II- Histochemically :***

In the sagittal section, the pituitary gland consisted of two components as follows:

a- Neurohypophysis: Consisted of nerve fibers which extended below the pituitary stalk (Fig.1A). The neurohypophysis penetrated deeply and ramified in the adenohypophysis.

b- Adenohypophysis: Consisted of epithelial tissue, which in turn comprised three regions: the rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermediata (PI) as shown in figure 1A. Although there was no sharp line separation between these zones, they were recognized after the use of different stains. Acidophilic cell types were characterized by their negative reaction to PAS. Herlant stain gave blue colour for basophilic cell types and pink colour for acidophilic cell types (Fig.1B). PAS-H-AF stain gave red colour for basophilic cell types and gave light blue colour for acidophilic cell types (Fig. 1C). Periodic acid Orange G-Light Green stain gave green colour for basophilic cell types and yellow colour for acidophilic cell type as shown in Figure 1D.

***III- Cell type distribution in the pituitary gland of Dicentrarchus labrax:***

Six different cell types were identified in the pituitary gland of *Dicentrarchus labrax*. These cells include prolactin cells (PRL) and adrenocorticotropic hormone secreting cells (ACTH) in the RPD. Somatotrops (STH), melanocytes stimulating hormone secreting cells (MSH) gonadotrops (GTH) and thyrotrops (TSH) were present in the PPD and PI regions.

***1-Prolactin cells (PRL):***

The prolactin cells occupy the major part of the RPD. These cells are small in size with irregular shape and have rounded nuclei (Fig. 2A). They also form a compact mass and are stained red with Azocarmin in Azan technique. However, they are negative to PAS and considered as acidophilic cell type.

***2- Adrenocorticotrops cell (ACTH) :***

ACTH cells appear like cords bordering PRL cells or like isles located among PRL cells and the neurohypophysis. They are small in size, spherical or oval in shape, with small eccentric nuclei. These cells have no affinity for PAS (acidophilic cell type), but ACTH cells have strong affinity to Azan stain to gave brown colour (Fig. 2B). These cels have affinity to Periodic acid-Schiff reagent Lead Hematoxylin (PAS-PbH) and give dark gray colour (Fig.2C).

***3- Melanocyte stimulating hormone cells (MSH):***

MSH cells were generally round in shape and ranged in diameter from 6 µm to 7 µm. They have rounded or indented nucleus which was centrally located in the cells. Although these cells were typically acidophilic and occupy the PPD region. MSH cells stained light red with Azocarmine (Fig. 2D). MSH cells did not change their activity throughout the year.

***4- Somatotrops cell (STH) :***

The fourth acidophilic cell type was present in the PPD region. They found intermingled with GTH cells. STH cells could not be detected in the RPD or PI regions. These cells were mostly rounded or ellipsoid, with oval or irregular nuclei and have eccentric location and ranged from 5 µm to 6.5 µm. These acidophilic cells type are stained yellow red colour with Azocarmine in Azan technique (Fig.2D). STH cells had strong affinity towards Orange-G to gave yellow colour with PAS-OG (Fig.3A).

***5-Gonadotrops cell (GTH):***

GTH occupied most of the PPD region. Aggregations of GTH cells were also

observed in the PI region. These basophilic cells manifested variable shapes and sizes and were exhibited spherical nuclei with eccentric location. The GTH cells contained numerous basophilic granules stained red colour with PAS-OG (Fig.3A). Cameron and steel gave green colour for GTH cells (Fig. 3B), while Herlant gave blue colour as shown in Figure 3C. The basophilic granules in the GTH cells varied in number, size and intensity of those granulations with the season.

***6-Thyrotrops cell (TSH):***

TSH cells were present between RPD and PPD. These cells appeared either angular or elongated in shape. These basophilic cells did not vary with the season. TSH cells reacted faintly with PAS and stained magenta red colour with PAS-OG (Fig.3A). Cameron and steel gave orange purple colour for TSH cells (Fig.3B). Herlant gave dark blue colour for TSH cells (Fig.3C). These cells appeared with probably the highest nucleocytoplasmic ratio among all adenohypophysis.

***IV- Gonadotropic cells during annual reproductive cycle in relation to oocyte maturation:***

Annual histological changes in diameters of GTH cells in the pituitary gland of *Dicentrarchus labrax* were studied in relation to average percent of different oocyte developmental stages as indicated in table 3.

***1- Resting stage (immature & maturing):***

This stages extended mainly from July to September. Throughout the resting period the average percentage of immature, maturing and vacuolized oocytes are shown in Table 2. Pituitaries of resting females (Fig.4A&B) contained GTH cells situated mainly in the PPD, they were few in number in the RPD. The diameter averaged 6.4 ± 0.9 µm. They were also degranulated and not present in the PI region. In resting stage various marked vacuoles were detected between GTH cells. The average nuclear density of GTH cells varied between 20 and 22 nuclei per 0.2 $mm^2$.

***2- Pre-spawning stage:***

During this period ovaries contained mainly vacuolized oocytes (60.76%). This stage extended from October to end of November. Vacuoles between cells decreased in size in the pituitaries of the nearly-ripe females with average GTH cells were recorded (12.3 ± 0.22 µm) as indicated in Table 3. These cells were situated mainly in the PPD region, while small number was detected in PI region. The average nuclear density of the GTH cells was about 38 nuclei per 0.2 $mm^2$. Slight increase in the staining affinity of GTH cells was detected at this stage (Fig.4C).

**3- *Ripening and spawning stage:***

During this breeding period, which extended from December to March, all oocytes stages were detected. During ripening period slight decrease in the average diameters of GTH cells was noticed (11.1 ± 2.1µm) as indicated in table 3. The GTH cells increased in number and staining intensity as an indication of cell granulation. The average nuclear density of GTH cells was about 30 nuclei per 0.2 $mm^2$. The GTH cells were widely distributed in all regions (Fig. 5 A&B), vacuoles between the cells disappeared. At the spawning period the average nuclear density of GTH cells was about 25 nuclei per 0.2 $mm^2$. Vacuoles between GTH cells began to appear (Fig.5C&D).

***4- Spent stage:***

During the spent stage (from April to June), immature and maturing oocytes (50.6% and 42.19%) and small percentage of resorbed oocytes (7.21%) were detected (table 2). The GTH stained very weakly indicating the presence of few granules (low activity). Few number of GTH cells were granulated, average diameters was about 5.8 ± 1.2 µm. Most of the GTH cells became vacuolated and atretic (Fig. 6A&B).

**Table 3:** Average percent of histological oocyte maturation in relation to GTH cell diameters in female *Dicentrarchus labrax* throughout the period from January o December 2003.

| Months | Stages Of Maturity | Average (%) of oocyte stages | | | | | GTH cell diameter (µm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | Max. | Min. | Average ± SD |
| July-Sept. | Resting (Immature & mature) | 43.1 | 55.61 | 1.39 | --- | --- | 7 | 5 | 6.4 ± 0.9 |
| Oct.- Nov | Pre-spawning | 10.2 | 20.2 | 60.76 | 8.94 | --- | 13 | 11 | ** 12.3 ± 0.22 |
| Dec.- March | Ripe & spawning | 5.1 | 13.12 | 19.68 | 37.29 | 24.81 | 13 | 9 | ** 11.1 ± 2.1 |
| April-June | Spent | 50.6 | 42.19 | --- | 7.21 | --- | 7 | 4 | 5.8 ± 1.2 |

** Highly significant correlation at P< 0.001.
I Immature oocytes.
II Maturing oocytes.
III Vacuolized oocytes.
IV Ripe oocytes at primary yolk stages.
V Secondary and tertiary yolk stage oocytes.

## DISCUSSION

The pituitary gland of *Dicentrarchus labrax* as in most teleost fishes is of the leptobasic type i.e. the neurohypophysis has well developed infundibular stalk and the adeohypophysis is cone shaped. The neurohypophysis in *Dicentrarchus labrax* consisted of nerve fibers which extended below the pituitary stalk. Similar results were obtained in a number of teleost species (Assem, 1995 for *Solea vulgaris* and *Solea aegyptiaca* ; Zaki *et al*., 1996 for *Siganus rivulatus* and Al-Absawy, 2004 for *Trachinotus ovatus*).

Based on the classical staining and distribution, adenohypophysis is subdivided into three distinct zones innervated by the neurohypophysis process. Six cell types were identified in the adenohypophysis using the histochemical methods. Five to nine different cell types in pituitary gland have been described in other teleost as *Barbus barbus* (Toubeau *et al*., 1991); *Solea vulgaris* and *Solea aegyptiaca* (Assem, 1995); *Siganus rivulatus* (Zaki *et al*., 1996) *Rhabdosargus haffara* (Assem & El-Boray, 2001) and *Solea impar* (Ali, 2003).

In present study the basophilic PRL and ACTH cells were found in the RPD region. These cells were inactively responsive to the annual cycle. The PRL cells showed strong affinity to Azan to give red colour. The ACTH cells showed strong tinctorial response to Lead Hematoxylin. In agreement with these results Zaki *et al*. 1996. and Assem & El-Boray 2001 indicated that ACTH cells were generally interlocated between neurohypophysis and PRL cells. In *Dicentrarchus labrax* the particular location of ACTH cells in the RPD region is observed in many teleosts ( Toubeau *et al*., 1991; Yan and Thomas, 1991; Zaki *et al*., 1996 and Mousa & Mousa, 1997a).

The acidophilic somatotrops cells (STH) and melanocytes (MSH) were observed in the PPD region. STH have the ability to be yellow under the effect of PAS-OG technique. MSH stained light red with

Azan. The distribution and identification of STH and MSH cells in the present study is in agreement with previous studies on *Siganus rivulatus* (Zaki *et al*., 1996) .

On the basis of histochemistry, basophils which lie in the adenohypophysis are the GTH and TSH cells. In these basophilic cells types the PAS converts the glycol group to aldehyd one that able to react with Schiff reagent. The basophilic cells were scattered and reacted similar to other teleosts as indicated by Assem, 1995; Gaber, 2000 and Ali, 2003.

In the present work one type of GTH cells was detected as reported by many authors: Yan and Thomas, (1991) for *Micropogonias undulatus*, *Cynoscion nebulosus* and *Sciaenops ocellatus* and Naito *et al.*, (1995) for *Oncorhynchus mykiss*.

The present results indicate that the TSH cells were intermingled between GTH cells. These cells appeared to be situated in approximately similar zone in *Mugil cephalus* (Mousa, 1994); *Bagrus bayad & B. docmac* (Gaber, 2000); *Solea impar* (Ali, 2003) and *Trachinotus ovatus* (Al-Absawy, 2004).

In *Dicentrarchus labrax*, the TSH cells did not change their activity in relation to seasonal variation and stained positively having orange purple colour with Cameron and steel technique. Similar results reported by Zaki *et al.*1996 for *Siganus rivulatus*.

In present study, the GTH cells showed pronounced change in correlation with the gonadal cycle. Moreover, the attribution of gonadotropin hormone secretion with GTH cells or classical basophils was detected by immunocytochemical studies in *Micropogonias undulatus*, *Cynoscion nebulosus* and *Sciaenops ocellatus* by Yan and Thomas, (1991) and *Mugil cephalus* by Mousa & Mousa 1997 a&b.

In *Dicentrarchus labrax*, the GTH cells stained blue colour with Herlant technique, whereas Cameron and Steel technique gave green colour for GTH cells. Similar results were reported by Zaki *et al*., 1996 and Gaber, 2000.

GTH cell in *Dicentrarchus labrax* were characterized by a gradual accumulation of granules in relation to maturation stages of the ovaries. This activity was reflected by staining affinity and vacuolization of the cells. This is in conformity with Krishnan and Diwan (1990) *for Etroplus suratensis* ; Gaber, 2000; Ali, 2003 and Al-Absawy, 2004. They stated that through the pre-spawning period the GTH cells showed a sign of activity, these cells increased in number, size and occupied a considerable area in the PPD region and they started to invade the PI region. These cells were continued in activity and granulation through ripening and spawning period.

In present study, the GTH cells were inactive with decrease in average number and diameters through spent period. These results are confirmed with the observation of Krishnan and Diwan, 1990; Assem & El-Boray 2001 and Ali, 2003.

Krishnan and Diwan, 1990 stated that during the resting period of *Etroplus suratensis* the gonadal steroidogenesis was much reduced, gametogenesis remains absent, the gonadotrops in the pituitary gland become smaller and showed a sign of internal breackdown and the pituitary content was at the lowest level in this period.

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## EXPLANATION OF FIGURES

Fig.(1): Midsagittal section of the pituitary gland of *Dicentrarchus labrax* stained with: Fig.(1A) Herlant stain showing, rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermediata (PI) which comprise the adenohypophysis and neurohypophysis (NH). (X100).

Fig.(1B): Magnification (X250) (A) the acidophilic cell type (pink) and (B) basophilic cell (blue).

Fig.(1C): Periodic acid- Hematoxylin-Acid Fucksin stain (PAS-H-AF) gave light blue colour for acidophilic cells (A) and gave red colour for basophilic cells (B) (X250).

Fig.(1D): PAS-OG- Light Green stain gave yellow colour for acidophilic cells and gave green color for basophilic cells (B) (X 250).

Fig. (2): Midsagittal section of the pituitary gland of *Dicentrarchus labrax* stained with:

Fig.(2A):Azocarmin displaying the prolactin cells (PRL) stained red (x1000).
Fig.(2B): Azan stain gave brown colour for ACTH cells (X1000).
Fig.(2C): PAS-PbH gave gray colour for ACTH cells (X250).
Fig.(2D): Azan stain gave light red colour for somatotrops (STH) and dark red for melanocyte (MSH) (X 250).

Fig.(3): Midsagittal section of the pituitary gland of *Dicentrarchus labrax* stained with:
Fig.(3A): PAS-OG gave yellow colour for STH cells; red colour for GTH cells and magenta red colour for TSH cells(X400).
Fig.(3B): Cameron and Steel gave green colour for GTH cells and orange purple colour for TSH cells (X1000).
Fig.(3C): Herlant gave blue colour for GTH cells and dark blue colour for TSH cells (X250).

Fig.(4 A) Sagittal section of the pituitary gland of Dicenrarchus labrax at immature stage stained with Herlant stain showing, the small number of the blue basophilic cells (GTH&TSH) in the PPD with spaces between the cells (arrows) and neurohypophysis (NH) (X1000).

Fig.(4B): Sagittal section at maturing stage stained with Azan stain showing, the blue GTH cells and the dark blue TSH cells and the light red MSH cells, basophilic cells increased in number. Notice free spaces between the GTH cells (arrows) (X250).

Fig.(4C): Section at nearly ripe stage stained with periodic acid -Schiff reagent (PAS), the GTH cells appear pink with different diameters. There are small spaces between the cells (arrows) (X250).

Fig.(5A) Section at ripe stage stained with PAS-H-AF stain showing, aggregation and crowded of the red GTH cells. There are no spaces between the cells (X 400).

Fig. (5B) Magnification (X1000) showing, large diameter of GTH and aggregation with blood cells (BL).

Fig.(5C): Section at spawninge female stained with Camerone and Steel stain showing, the green GTH cells and orange purple TSH cells. Note some free spaces between the cells (arrows)(X 400).

Fig.(5D): Magnification (X1000). Note spaces between the cells (arrows).

Fig.(6A): Sagittal section at spent female stained with Cameron and Steel showing, deformated and abnormal GTH cells, free spaces between the cells (arrows) (X400).

Fig.(6B) Magnification (X1000) note deformed GTH cells and spaces between the cells (arrows).