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HISTOCHEMICAL AND FINE STRUCTUR STUDIES OF CELL TYPE IDENTIFICATION, LOCALIZATION AND SEASONAL VARIATION IN PITUITARY GLAND IN RELATION TO GONADAL MATURATION OF FEMALE *OBLADA MELANURA*

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ABSTRACT

Oblada melanura adenohypophysis is subdivided into three distinct zones innervated by the process of neurohypophysis. Six cell types were identified and localized in the adenohypophysis using histochemical and electron microscopy methods. The acidophilic prolactin (**PRL**) and adrenocorticotropic hormone secreting cell (**ACTH**) were found in the rostral pars distails 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 the fourth acidophilic melanocyte stimulating hormone secreting cells (**MSH**) and somatotrops cells (**STH**) were observed in the proximal pars distails region (PPD). They were mostly rounded with indented nucleus in case of MSH, while the nucleus of STH was irregular in shape. The MSH cells stained light red with Azan. All acidophilic cell types did not change their activity throughout the year. The basophilic gonadotrophic cells (**GTH**) and thyrotrophic cells (**TSH**) react positively with PAS. One type of GTH cells were detected, Herlant technique gave blue colour for GTH cells. 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 as reflected by electron microscope. The activity of GTH cells was reflected by staining affinity and vacuolization of the cells. During ripening and spawning period (from May to August), slight decrease in the GTH diameters and staining affinity were recorded. During the spent stage (from September to November), the GTH cells was stained weakly with presence of vacuolated and atretic cells. The neurohypophysis in *Oblada melanura* consisted of nerve fibers which extended below the pituitary stalk.

1. INTRODUCTION

Porgies belong to family Sparidae. In Egypt *Oblada melanura* is found all-over the Mediterranean Sea and the Mediterranean coast of Alexandria as well as other waters (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 and Mousa, 1997 a & b; Assem and EL-Boray, 2001; Ali, 2003 and AL-Absawy, 2004; Assem, 2004; Fahmy, 2006 and Cinquetti and Dramis, 2006. 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 secretion cells of the pituitary gland show a vigorous distribution in the three 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 cell type has been observed in some fish species Toubeau *et al*.1991; Assem, 1995; El-Greisy, 2000; Gaber, 2000; Assem and EL-Boray, 2001; Assem, 2004; AL-Absawy, 2004 and Fahmy, 2006. Zaki *et al*. 1996 studied the cyclic changes in the pituitary 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 gonadotrophic cells in the female mullet, *Mugil cephalus* (teleost fish) by using immunocytochemical method. Assem and EL-Boray 2001 studied the cell type distribution in the pituitary gland of female *Rhabdosargus haffara*. Assem 2004 studied cell type distribution and seasonal variation of gonadotropic cells and their relation to maturation of gonads in female *Dicentrarchus labrax*.

The aim of the present work is to identify and localize the different cell types in the pituitary gland of *Oblada melanura* by histological and histochemical methods. Studying the GTH cells of the female at all maturity stages in relation to oocyte maturation and gonadosomatic indices were applied to provide basic information on the reproductive biology for this species. Electron microscopy was also used to provide a basis of future studies on the endocrine control of oogenesis and spawning in this fish.

2. MATERIALS AND METHODS

2.1. Fish sampling

Oblada melanura fish were collected throughout the period from January to December 2005 from El- Maadia coast at intervals of 2 weeks. The 180 specimens collected ranged in size from 15 to 25 cm and body weight between 180 to 340 gm. Sex and sexual maturity stages were identified by three methods as follows:

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

2.2. Histological and histochemical methods

After dissection of the fish, the pituitary gland with a piece of the brain 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).

2.3. Electron microscope technique

Five pituitary glands were immersed-fixed at 4ºC in 4 % glutaraldehyde and then in 1% osmium tetroxide for one hour at room temperature , rinsed twice in Cacodhlate buffer, dehydrated through a graded ethanol series, cleared in propylene acid and embedded in polarbed 812 (polaron) epoxy resin. Ultra thin sections were prepared by

using glass and diamond knives and stained with uranyl acetate and lead citrate. Sections were viewed on transmission electron microscopy.

2.4. Statistical analysis

Microsoft windows (2003), Excel program were used for analysis of correlation coefficient.

3. RESULTS AND DISCUSSION

3.1. Ovarian cycle

3.1.1. Maturity stages

On the basis of seasonal changes encountered in the histomorphology (oocyte diameter) and gonadosomatic index, (table 2) indicates that the ovarian cycle of female *Oblada melanura* 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 fellows:-

I- Immature stage:

Ovaries are thin almost cylindrical with two tapering ends. The average of GSI values was about 0.52 ± 0.24 and the mean oocyte diameters 27 µm ± 9.95 (table 2).

II- Mature stage:

Ovaries are enlarged in size, pinkish-red in colour and occupy about half of the body cavity. The average GSI values were about 0.667± 0.31 and oocyte diameters 88 µm ± 14.42.

III- Nearly ripe stage:

Ovaries are yellowish in colour, size reaches two third of body cavity, and eggs are distinguishable with the naked eye. This stage is detected in March and April with average GSI values of 1.19 ± 0.21 and oocyte diameters 127 µm ± 34.31.

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. This stage is detected from May to July, with average GSI values of 5.2 ± 2.22 and oocyte diameters 500 µm ± 89.

V- Spawning stage (partly spent):

The ovaries show slight shrinkage due to the discharge of a considerable amount of ripe oocyte during the course of spawning. This stage is detected in August, with average GSI values of 2.43 ± 1.21 and oocyte diameters 450 µm ± 14.14.

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. This stage is detected at December and January with average GSI values of 0.397± 0.2 and oocyte diameters 73 µm ± 10.42.

The present value in GSI and egg diameter are in agreement with Assem 2004 who studied cell type distribution and seasonal variation of gonadotropic cells as well as their relation to maturation of gonads in female *Dicentrarchus labrax*.

3.2. The pituitary gland

The fish pituitary gland made the subject of studies given by various authors for many years, without their anatomy and cell types are known in detail, for few teleost species. The identification and distribution of the various adenohypophysial cell types have been studied in few species (Cinquitti and Dramis, 2006).

3.2.1. Morphologically

The pituitary gland of *Oblada melanura* 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. The pituitary gland of *Oblada melanura* as in most teleost fish is of the leptobasic type i.e. the neurohypophysis has well a developed infundibular stalk and the adeohypophysis is cone shaped. The

neurohypophysis in *Oblada melanura* 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*; AI-Absawy, 2004 for *Trachinotus ovatus*; Fahmy, 2006 for *Chrysichthus rueppelli* and Cinquetti and Dramis, 2006 for *Padogobius martensi*).

Table (1): Location and staining properties of acidophilic and basophilic cell types in the pituitary gland of Oblada melanura (Culling, 1978 &Humason, 1979).

Types of staining technique	Types of the cells and Pituitary regions					
	Acidophilic cells				Basophilic cells	
	PRL	ACTH	STH	MSH	GTH	TSH
	RPD	RPD	PPD	PPD	PPD & PI	PPD & PI
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
(PAS-OG-LG)	yellow	yellow	yellow	yellow	green	green
(PAS-H-AF)	-ve	-ve	Light blue	Blue	Red	Red

PRL Prolactin cells,
ACTH Adrenocorticotropin hormone secreting cells.
STH Somatotropin hormone secreting cells.
MSH Melanocyte 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.

Table (2): Gonadosomatic index (GSI) and oocyte diameters of female *Oblada melanura* at different maturity stages.

Stages of maturity	No. of fish	Oocyte diameter (µm)			Gonadosomatic index Mean % ± SD
		Min.	Max.	Mean± SD	
Immature	28	22	42	27 ± 9.95	0.523 ± 0.24
Mature	20	78	96	88 ±14.42	0.667 ± 0.31
Nearly ripe	16	98	160	127 ±34.31	1.190 ± 0.21
Ripe	31	430	570	500 ± 89.81	5.200 ± 2.22
Spawning	25	410	530	450 ±14.14	2.430 ± 1.21
Spent	30	66	82	73 ±10.4	0.397 ± 0.20

3.2.2. 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 of three regions: the rostral pars distails (RPD), proximal pars distails (PPD) and pars intermediata (PI) as shown in figure 1A. No sharp line separation could be detected between these zones. Two major groups of cells were recognized according to their affinity to 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). Periodic acid Orange G- light Green stain gave green colour for basophilic cell types and yellow colour for acidophilic cell types as shown in figure 2A&b. PAS-H-AF stain gave red colour for basophilic cell types and gave light blue colour for acidophilic cell types (Fig. 5A).

Adenohypophysis· is subdivided into three distinct zones innervated by the neurohypophysis. Six cell types were identified in the adenohypophysis using the histochemical methods in the present study according to these results 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); *Solea impare* (Ali, 2003); *Dicentrarchus labrax* (Assem, 2004) and *Chrysichthus rueppelli* (Fahmy, 2006).

3.2.3. Cell type distribution in the pituitary gland of Oblada melanura

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

3.2.3.1. Prolactin hormone secreting 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. They are stained yellow with PAS Orange G-light Green (Fig.3A). However, they also form a compact mass and are stained red with Azocarmine in Azan technique (Fig.3B). These cells were inactively responsive to the annual cycle. These results are in agreement with Zaki *et al*. 1996 and Assem & El-Boray 2001 and Assem 2004.

3.2.3.2. Adrenocorticotropin hormone secreting cells (ACTH)

ACTH cells appear like cords bordering PRL cells or like islets 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 they are stained yellow with PAS Orange G-light Green (Fig.3A &B). ACTH cells have strong affinity to Azan stain to give brown colour (Fig.3C). These cells have affinity to Periodic acid-Schiff reagent lead Hematoxylin (PAS-PbH) and give dark gray colour (Fig.3D). In *Oblada melanura* the particular location of ACTH cells in the RPD region is observed in many teleosts (Toubeau *et al*., 1991; Yan and Thomas, 1991; Mousa and Mousa, 1997a; and Assem 2004).

3.2.3.3. Melanocyte hormone secreting 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.3E). MSH cells did not change their activity throughout the year.

3.2.3.4. Somatotropin hormone secreting cells (STH)

The fourth acidophilic cell type was present in the PPD region. They are 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 which have eccentric location. These acidophilic cells type are stained yellow red (orange) colour with Azocarmine in Azan technique (Fig.3E). The distribution and identification of STH and MSH cells in the present study agree with Zaki *et al*., 1996 on *Siganus rivulatus* as well as Assem, 2004 on *Dicentrarchus labrax*.

3.2.3.5. Gonadotrophic hormone secreting cells (GTH)

GTH occupied most of the PPD region. Aggregations of GTH cells were also observed in the PI region. These basophilic cells had variable shapes and sizes and withspherical nuclei that were eccentric in location. Herlant gave blue colour for GTH and TSH cells as shown in figure 4A. While, they are stained light blue colour with Azocarmine in Azan technique (Fig.3E). The basophilic granules in the GTH cells varied in number, size and intensity with the season. 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*; Naito *et al*. (1995) for *Oncorhynchus mykiss*; Ali (2003) for *Solea impar*; AI-Absawy (2004) for *Trachinotus ovatus* and Assem (2004) for *Dicentrarchus labrax*.

In *Oblada melanura* the GTH cells stained blue colour with Herlant technique, while, PAS – OG – LG technique gave green colour for GTH cells, also GTH showed pronounced seasonal changes. Similar results were reported by Zaki *et al*., 1996; Gaber, 2000; Assem, 2004 and Fahmy, 2006.

3.2.3.6. Thyrotropin hormone secreting 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 dark blue colour with Azocarmine in Azan technique (Fig.3E). Herlant gave blue colour for GTH and TSH cells as shown in figure 4A. These cells appeared with probably the highest nucleocytoplasmic ratio among all adenohypophysis.

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 and Mousa 1997 a&b); *Bagrus bayad* & *B. docmac* (Gaber, 2000); *Solea impar* (Ali, 2003); *Trachinotus ovatus* (AI-Absawy, 2004); *Chrysichthus rueppelli* (Fahmy, 2006) and *Padogobius martensi* (Cinquetti and Dramis, 2006).

In *Oblada melanura*, the TSH cells did not change their activity in relation to seasonal variation and stained positively having blue colour with Azan technique. Similar results who reported by Zaki *et al*. 1996 for *Siganus rivulatus*, Assem 2004 for *Dicentrarchus labrax* and Fahmy, 2006 for *Chrysichthus rueppelli*.

3.2.4. Histochemical and fine structure of gonadotrophin cells during annual reproductive cycle in relation to oocyte maturation

Annual histological and ultrastructural changes of GTH cell diameters and their cytoplasm granulation in the pituitary gland of *Oblada melanura* were studied in relation to average percent of different oocyte developmental stages as indicated in table (3). In the present study, the GTH cells showed pronounced changes in correlation with the gonadal cycle. Moreover, the attribution of gonadotrophin hormone secretion with GTH cells or classical basophils was detected by immunocytochemical studies in *Micropogonias undulatlls*, *Cynoscion nebulosus* and *Sciaenops ocellatus* by Yan and Thomas, 1991; *Mugil cephalus* by Mousa and Mousa, 1997 a&b and *Padogobius martensi* by Cinquetti and Dramis, 2006. On the basis of histochemistry, basophiles 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; Ali, 2003 and Fahmy, 2006.

3.2.4.1. Resting stage (immature and maturing)

This stage extended from December, January to March. 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; while 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 vacant areas were detected between GTH cells. The average cell diameter of GTH was 5.5 ± 0.5 µm. similar results were recorded by Assem and EL-Boray 2001 and Assem 2004 who studied the cell type distribution seasonal changes in the pituitary gland of female in relation to oocyte developmental stages.

3.2.4.2. Pre-spawning stage

During this period the average cell diameter of GTH reaches it maximum value of 11.3 ± 0.11 µm as indicated in table 3. This stage extended from March to April. Vacant areas between cells decreased. Slight increase in the staining affinity of GTH cells was detected at this stage (Fig. 5A & B). These cells were situated mainly in the PPD region, while small number was detected in PI region. GTH cell in *Oblada melanura* 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; AIi, 2003; AI-Absawy, 2004; Assem, 2004 and Fahmy, 2006. They stated that through the prespawning 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.

Ultrastructure of GTH cells indicates their activity at this stage. Increased cytoplasmic granulation and nuclear chromatin density of GTH cells was detected (Fig. 6A & B). During this period ovaries contained mainly vacuolized oocytes (33.01 %).

3.2.4.3. Ripening and spawning stage

During the breeding period, which extended from May to August slight decrease in the average diameters of GTH cells was noticed (10.8 ± 1.9 µm) as indicated in table 3. The GTH cells increased in number and staining intensity as an indication of cell granulation (Fig. 6C). The GTH cells were widely distributed in all regions and vacant

areas between the cells disappeared. *Oblada melanura* GTH cells were continued in activity and granulation through ripening and spawning period, these results was in agreement with Garcia-Ayala *et al*., 2003 and Onuma *et al*. 2003.

At the spawning period the GTH ultrastructural secreting activity indicated by the slight decrease in nuclear density and cytoplasmic granulation. Also, this is indicated by presence of endoplasmic reticulum and the increased number of mitochondria in cytoplasm of GTH cells (Fig. 6D). All oocytes stages were detected as shown in table 2.

The Ultrastructure characteristics of different cell type of pituitary gland for several teleosts were studied by several authors, Batten *et al*., 1999 and Ishwar *et al*., 2005. In the present results, the gonadotropin cells were characterized by highly electron dense secretory vesicles. Also, these cells were recognized with well developed endoplasmic reticulum with parallel cisterna, while the Golgi apparatus was poorly developed and mostly consisted of small vesicles. Similar results were reported by Sharp-Baker *et al*., 1995 in African catfish *Clarias gariepinus*; Naito *et al*., 1995 in rainbow trout *Oncorhynchis mykiss* and Fahmy, 2006 in *Chrysichthus rueppelli*.

3.2.4.4. Spent stage

During the spent stage (from September to November), immature and maturing oocytes (51.67 and 40.33%) and small percentage of resorbed vacuolized and ripe oocytes (8%) were detected (table 2). The GTH cells stained very weakly indicating the presence of few granules (low activity). Few number of GTH cells were granulated, average diameters was about 5.1 ± 0.9 µm. most of the GTH cells became vacuolated and atretic (Fig. 7A). In present study, the GTH cells were inactive with decrease in average number and diameters during the spent period. These results are in accordance with the observation of Krishnan and Diwan, 1990; Assem & EI-Boray 2001; Ali, 2003; AI-Absawy, 2004 and Fahmy, 2006. Krishnan and Diwan, 1990 stated that during the resting period of *Etroplus suratensis* the gonadal steroidogenesis is much reduced, gametogenesis remains absent, the gonadotrops in the pituitary gland became smaller and showed a sign of internal breakdown and the pituitary content was at the lowest level in this period.

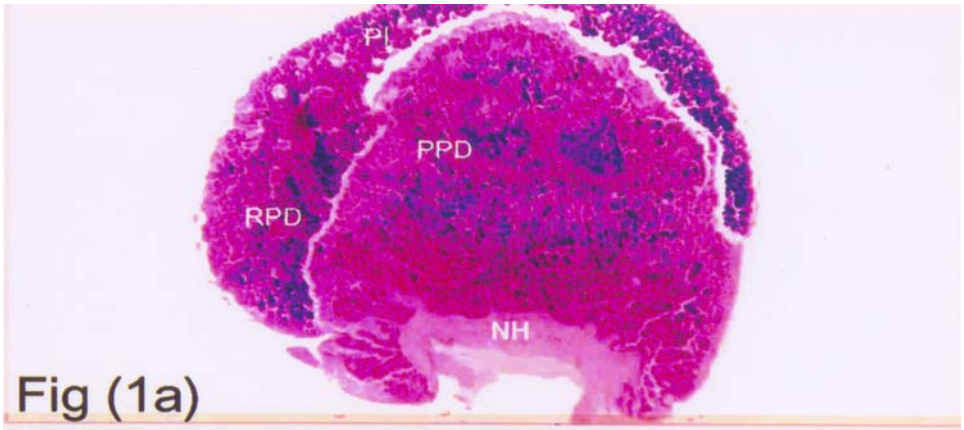
Ultrastructure of GTH cells indicated by decreased cytoplasmic granulation and nuclear chromatin density (Fig. 7 B).

Table (3): Average percent of histological oocyte maturation in relation to GTH cell diameters in female Oblada melanura through the period from January to December 2005.

Months	Stages of maturity	Average (%) of oocyte stages					GTH cell diameter (µm)		
		I	II	III	IV	V	Max	Min	Average ± SD
December & June-March	Resting (Immature and Mature)	48.17	40.83	10.67	0.00	0.00	6	5	5.5 ± 0.51
March-April	Prespawning	15.17	27.04	33.01	23.33	1.05	12	10	11.3 ± 0.11**
May-August	Ripe and Spawning	8.83	2.33	25.17	36.17	26.83	12	9	10.8 ± 1.9**
September-November	Spent	51.67	40.33	2.33	5.67	0.00	6	4	5.1± 0.9

I Immature oocyte
II Mature oocyte
III Vacuolized oocyte
IV Oocyte at primary yolk stages
V Secondary and tertiary Yolk stages

** Highly significant correlation at P< 0.001



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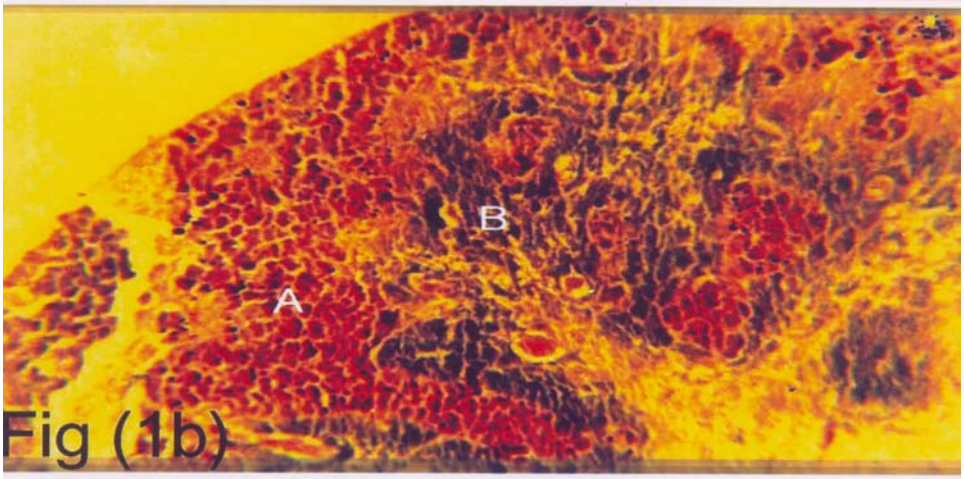

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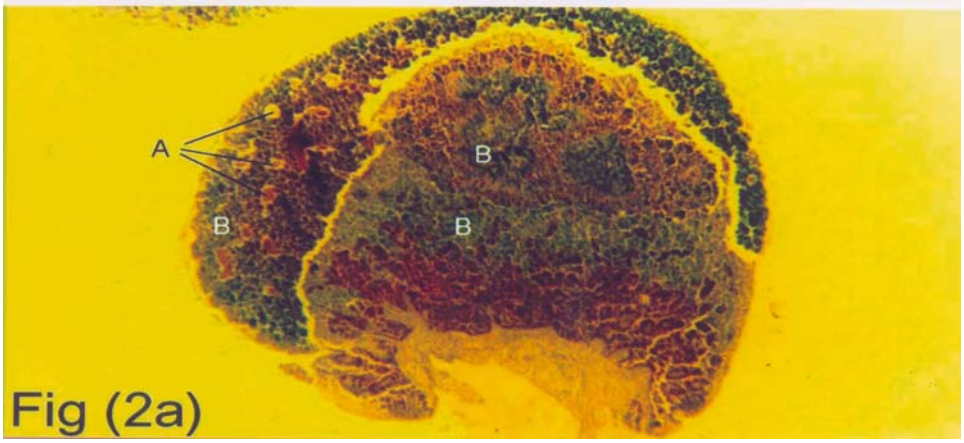

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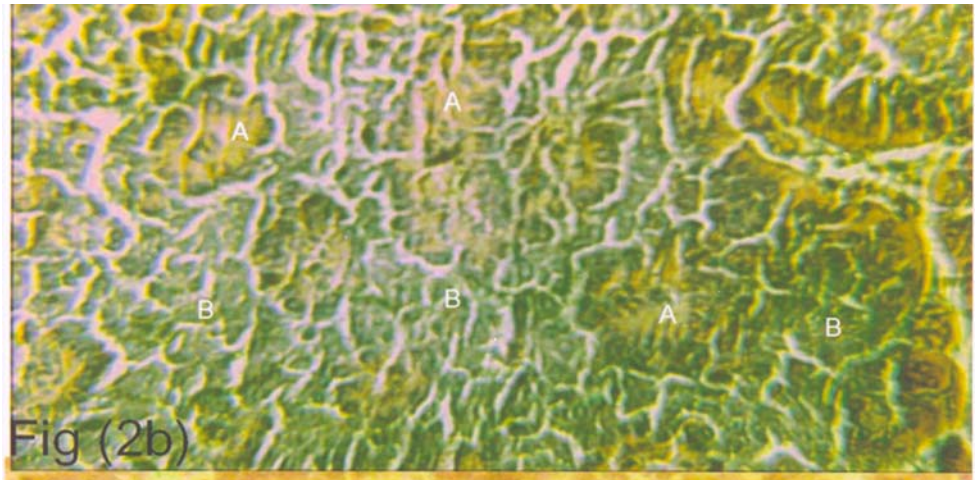
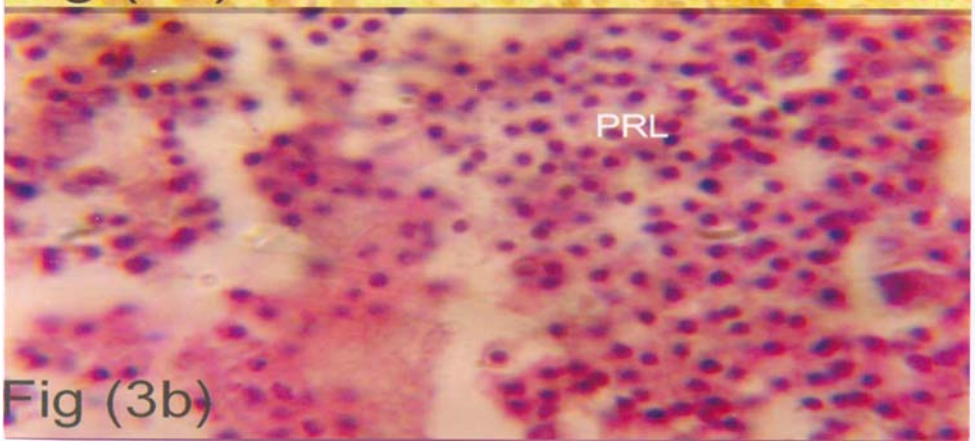


Fig (2b)

Fig (3a)



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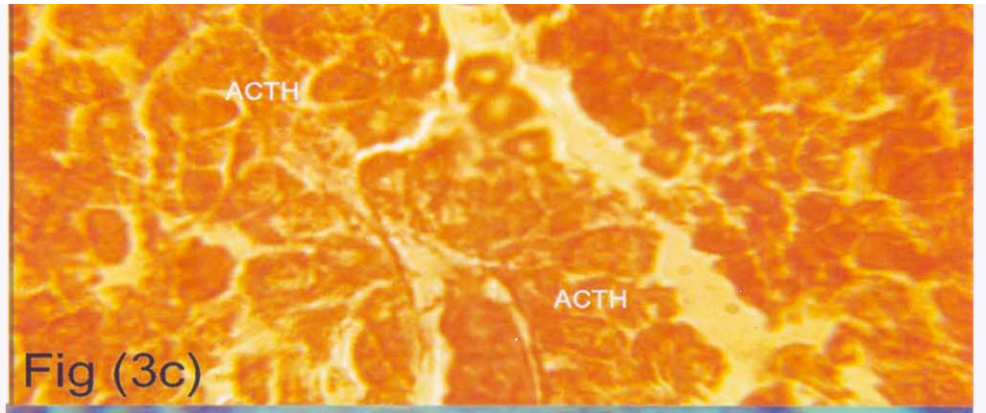

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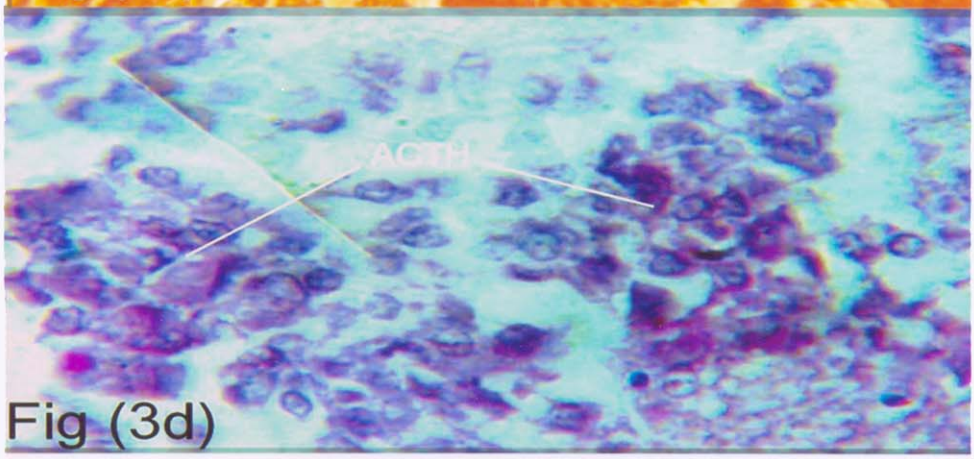

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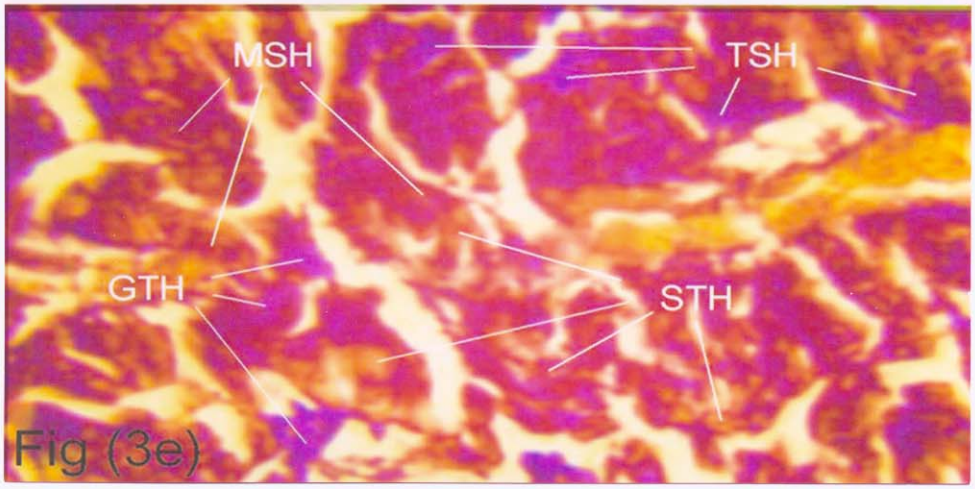

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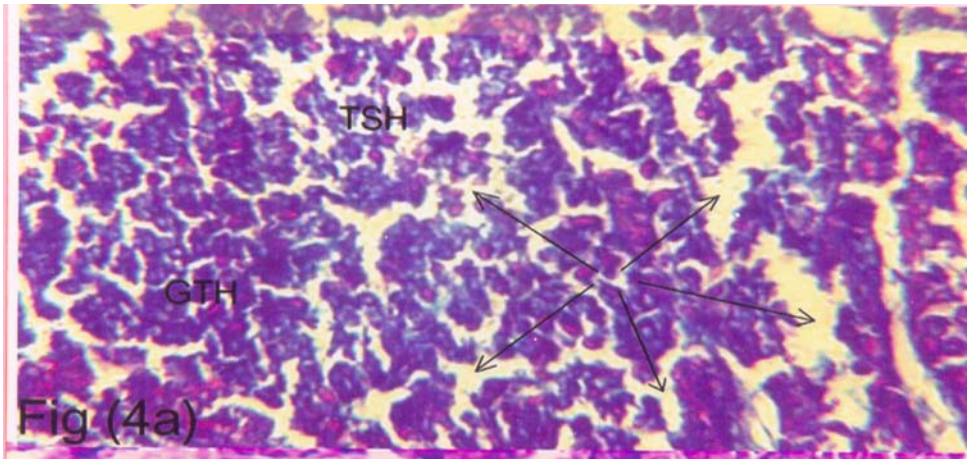

Fig (4a)

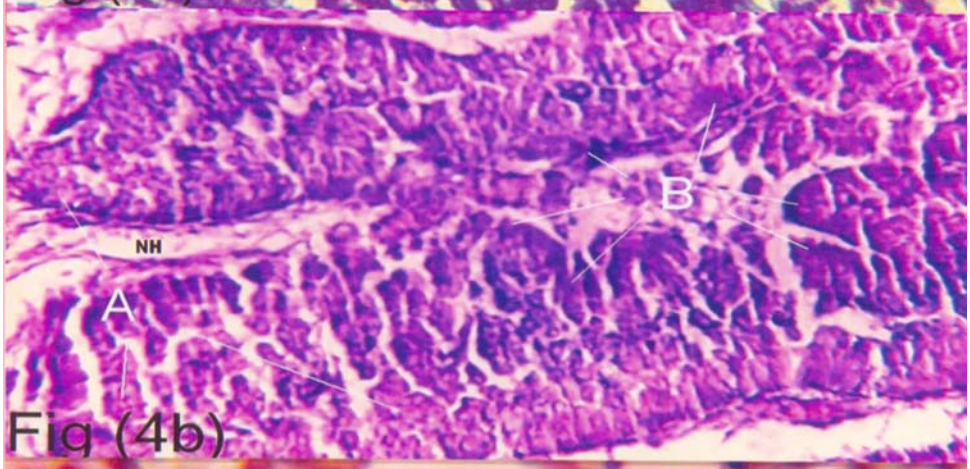

Fig (4b)

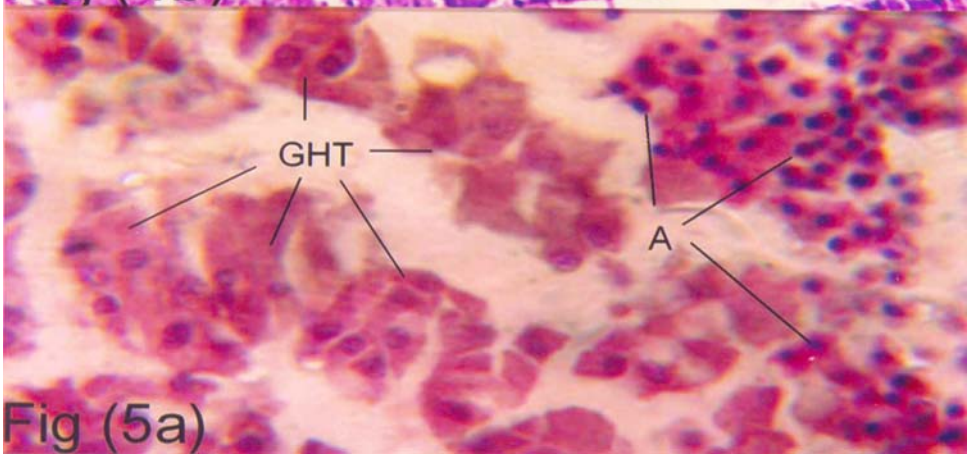

Fig (5a)

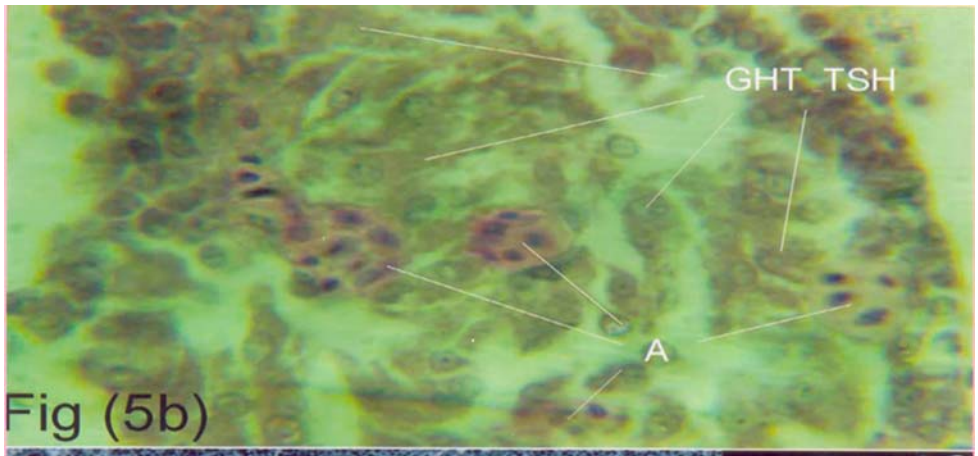

Fig (5b)

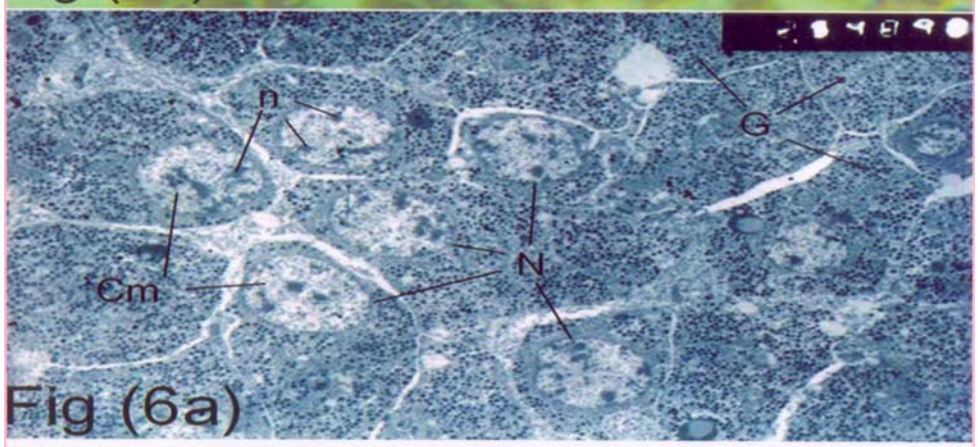

Fig (6a)

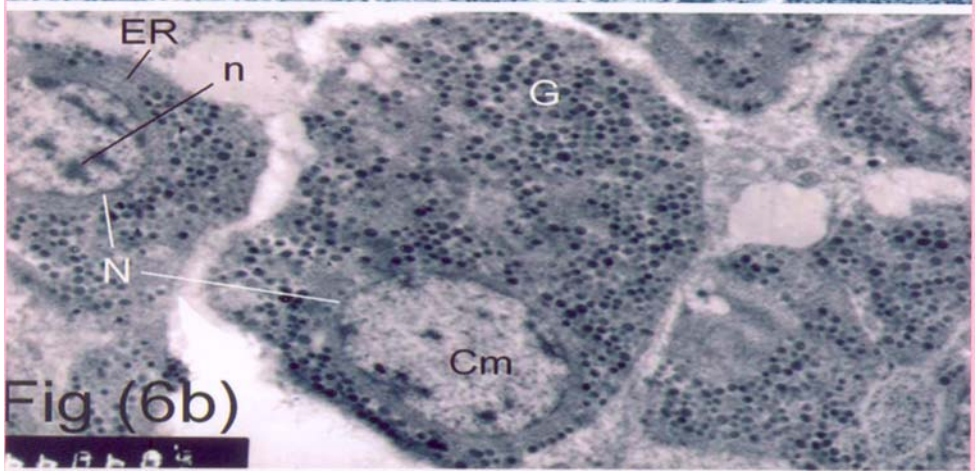

Fig (6b)

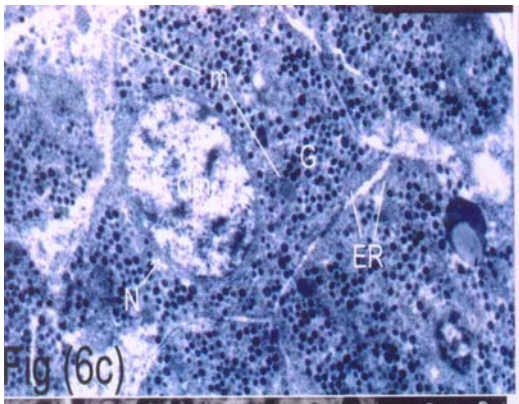

Fig (6c)

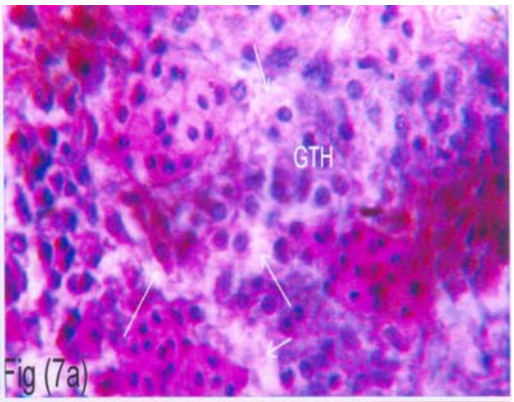

Fig (7a)

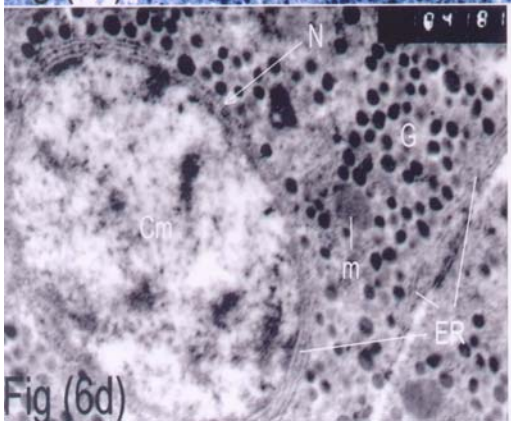

Fig (6d)

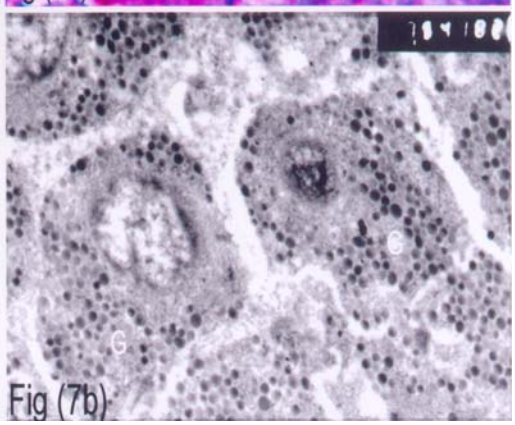

Fig (7b)

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EXPLANATION OF FIGURES

Midsagittal section of the pituitary gland of female Oblada melanura stained with:

Fig. (1A) Herlant technique showing rostral pars distails (RPD), proximal pars distails (PPD) and pars intermedita (PI) which comprise the adenohypophysis and neurohypophysis (NH) (X100).

Fig. (1B) magnification of (A) acidophilic cell type (pink) and (B) basophilic cell type (blue) Herlant technique (X250).

Fig. (2A) Periodic acid (PAS), orange G (OG) and Light green (LG) stains showing (A) acidophilic cell type stained yellow colour and (B) basophilic cell type stained green colour (X100).

Fig. (2B) magnification of (A) acidophilic cell type stained yellow colour and (B) basophilic cell type stained green colour with (PAS), (OG) and (LG) stains (X250).

Fig. (3A) (PAS), (OG) and (LG) stains showing the prolactin cells (PRL) and adrenocorticotropic cells (ACTH) stained yellow (X400).

Fig. (3B) Azan staining technique showing prolactin cells (PRL) stained light red colour (X1000).

Fig. (3C) Azan staining technique showing adrenocorticotropic cells (ACTH) stained brown colour (X1000).

Fig. (3D) (PAS) and lead hematoxylin (PbH) stains showing adrenocorticotropic cells (ACTH) stained gray colour (X1000).

Fig. (3E) Azan staining technique showing somatotrops (STH) stained yellow red, melanotrops (MSH) stained light red, gonadotrops (GTH) stained light blue and thyrotrops (TSH) stained blue (X1000).

Fig. (4A) Midsagittal section of the pituitary gland of female Oblada melanura at immature stage stained with Herlant technique showing: spaces (arrows) between the blue basophilic (GTH and TSH) in PPD region. (X250).

Fig. (4B) Magnification of midsagittal section of the pituitary gland at immature stage with Herlant technique stain showing acidophilic (pink) (A) basophilic (blue) (B) and neurohypophysis (NH) (X400).

Fig. (5A) Photomicrograph of midsagittal section of the pituitary gland at nearly ripe stage stained with PAS- hematoxylin (H) and acid Fuchsin (AF) showing aggregation and maximum diameter of GTH (red) and acidophilic cells (blue) (X1000).

Fig. (5B) Photomicrograph of midsagittal section of the pituitary gland at nearly ripe stage stained with PAS-OG-LG showing the maximum cell diameter of both GTH and TSH cells (green), also acidophilic cells (yellow) (X1000).

Fig. (6A) Photoelectrograph of the pituitary gland at prespawning stage stained with lead citrate and uranyl acetate showing maximum diameter of gonadotropic cells (GTH) their granulation (G), nucleus (N), nucleolus (n) and chromatin material (Cm) (X3000).

Fig. (6B) Magnification of (GTH) cells at prespawning stage showing granulation (G), endoplasmic reticulum (ER), nucleus (N), nucleolus (n) and chromatin material (Cm) (X5000).

Fig. (6C) Magnification of (GTH) cells at spawning stage showing granulation (G), mitochondria (m), endoplasmic reticulum (ER), nucleus (N) and chromatin material (Cm) (X4000).

Fig.(6D) Magnification of one (GTH) cell at spawning stage showing granulation (G), mitochondria (m), endoplasmic reticulum (ER), nucleus (N), chromatin material (Cm) (X5000).

Fig. (7A) Sagittal section of pituitary gland of female Oblada melanura at spent stage stained with PAS-H-AF stains showing deformed and abnormal GTH cells, also free spaces between cells (arrows) (X1000).

Fig. (7B) Magnification of (GTH) cells at spent stage showing their cytoplasm with reduced number of granulation (G) (X4000).