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The correlation between the hypophysial gonadal axis with special reference to the r ole of fatty acids and isoenzyme during the ovarian maturation in female grass carp, *Ctenopharyngodon idella*

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ABSTRACT

The present study was conducted to investigate the relationship between the gonad maturation of grass carp, Ctenopharyngodon idella and the changes of pituitary gland cells types coincides with oogonial development and their changes of fatty acid profile and isoenzyme electrophoresis. Five categories of oogenesis are detected such as immature, maturing, ripe spawning and follicular atresia. The pituitary gland has possessed six different kinds of cells. In the rostral pars distalis (RPD), two acidophilic cells types are distinguished; adrenocorticotropic hormone secreting cells (ACTH) and prolactin hormone secreting cells (PRL). The proximal pars distalis (PPD) exhibited two basophilic cell types include gonadotropic hormone secreting cells (GTH) and the thyrotrophic hormone secreting cells (TSH). During ripe stage, the gonadotrophic cells attained the highest changes of the GTH cells explained by increase of cytoplasmic secretory granules. The saturated fatty acids contents reached the maximum peak value and ranged from 38.45% to 39.19%. While unsaturated fatty acids (UFA) ranged from17.09.1% to17.49% and polyunsaturated fatty acids (PUFA) become 43.54% to 44.29%. The most abundant fatty acids were palmitic acid (C16:0), stearic acid (C18:0) in case of SFA, oleic acid (C18:1 n-9) in case of MUFA, linoleic (C18:2 n-6) in case of omega6 and docosahexaenoic (DHA) and acid (C22:6 n-3) Eicosapentaenoic (20:5n-3) (EPA) in case of omega3. The assayed isoenzymes electrophoresis was altered during the ovarian development. The authors finally concluded that there is a gradual change in the pituitary of endocrine cells parallel with changes of follicular development and assessed by alterations of isoenzyme electrophoresis and fatty acids content.

INTRODUCTION

Grass carp is economically important for Egyptian community because of its nutritional value, tasty food and its biological control in aquatic vegetation. In Egypt grass carp, *Ctenopharyngodon idella* failed to spawn, so the present work was carried out to put answers for solving the problem of production offspring. The gonad maturation in teleosts is a complicated process, mainly controlled by the hypothalamic–pituitary-gonadal–liver axis. The changes of pituitary cells types during annual cycle facilitated its influences on gonadal growth and maturatio (Swanson, 1991, Nagahama, 2000 and Arukwe and Goksøyr, 2003).

Many authors studied the influences of pituitary glands on gonadal development of teleost fishes such as *Solea impar* (AIi, 2003), *Trachinotus ovatus* (AI-Absawy, 2004), *Calotes versicolor* (Malashetty *et al*., 2009), *Xiphias gladius* (Minniti *et al*., 2009), *Ctenopharyngodon idella* (Grandi *et al*., 2014) and *Pagellus erythrinus L* (Balci and İkiz., 2017). The basophilic GTH exhibited marked variations during the ovarian cycle which explained in female *Oblada melanura* (AI-Absawy, 2004), *Pagellus erythrinus* (Balci and İkiz., 2017) and seabream (*Diplodus sargus*) (Ismail and Negm., 2018). On the other hand, Ctenopharyngodon idella somatolactin cells (SL) and GTH II cells are also, involved in gonadal differentiation (Grandi *et al*., 2014).

Fatty acid is a vital source of energy utilized during spawning season, sperm maturation and vitellogenesis and steroid hormone synthesis (Mazorra *et al*., 2003 and Jerez *et al*., 2006). Eicosapentaenoic acid (EPA, C20:5 n-3) and DHA are important for human especially for prevention of the cardiovascular diseases and managing the development and function of the nervous system, photoreception, and the reproductive system (Sidhu, 2003).The fatty acid profile of teleost fishes varied between species, environment and geographical distribution (Kacar *et al*., 2015 and Kayhan *et al*., 2015).

The Alkaline and acid phosphatases are important for egg maturation. Acid phosphatase play a great role in transfer phosphate to fish tissues required for development, growth and maturation (Blum, 1970; Hurkadli *et al*., 1985 and Gadekar and Baile, 2015).

Alkaline phosphatase (ALP) activities have been associated with the thyroid hormone level changes and estradiol driven hepatic vitellogenesis (Eales *et al.*, 1991; Crim *et al.*, 1992 and Johnston *et al*., 1992,1994). The present study assessed the correlations between oogonial development coincides with differentiation of pituitary cells and their enzyme activities and fatty acids accumulation.

MATERIALS AND METHODS

The studied teleost fish was collected from floating cages on River Nile during January to December (2016). Their average body weight ranged from 2552 to 4500 gm and age from 3-5 years.

According to Yoneda *et al*. (2001) and El-Gamal (2001), Gonadosomatic Index (GSI) was calculated using the following equation:

$$GSI = \frac{\text{Gonad weight (gm)}}{\text{Gutted weight (gm)}} \times 100$$

Histological techniques

The fishes were sacrificed, dissected and their ovaries and pituitary glands were separated. The specimens fixed immediately in bouin,s fluid for 24 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and embedded in molten paraplast 58°-62c°. Six µm thick histological sections were cut and stained with haematoxylin and eosin and Mallory triple stain for ovary. in case of pituitary gland, the paraffin sections were stained with Priodic acid–-orange G (PAS-OG), periodic acid Schiff's (PAS) reaction and AL-PAS- Orange G technique (Van Overbeekc and McBride, 1967 and Humason, 1972). The cell diameters of the GTH were measured by using ocular micrometer.

Fatty acid profile

Whole ovaries of captured fishes were assayed for the determination of fatty acids. BioAssay System method provides a sample, one-step and high – through put assay for measuring fatty acids. In this assay, free fatty acids are enzymatically converted to acyl-CoA and subsequently to H_2O_2 reacts with a specific dye to form a pink colored product. The optical density at 570nm or fluorescence intensity (530/385 nm) is directly proportional to the free fatty acid concentration in the sample (Enzy Chrom Free Fatty Acid Assay Kit, BioAssay System, USA) (Ackman and Sipos, 1964).

Alkaline and acid phosphatases isoenzymes electrophoresis

Ovary ALP is separated by electrophoresis through alkaline buffered (pH 9.1) according Sundblad *et al*. (1972). Electrophoresis was performed at a constant current at 2.5 mA per gel column at 18-20 to Co. Incubation for alkaline phosphatase (E.C.3.1.3.1) was carried out using Naphthol-AS-MX-phosphateas substrate and fast red violet LB salt as the coupler. Incubations of acid phosphatase (E.C.3.1.3.2) was undertaken at pH 5.0 using alpha-naphthyl phosphate as substrate and hexazotized pararosanilin as coupled agent, run in electrophoresis and processed for visualization and photographed (Frazen and Hasselgren, 1978).

RESULTS

1. Morphological, histological observations on the ovary of female grass carp

According to the morphological and histological investigations of ovaries, the oogonia were arranged into five stages Yamamoto *et al.,* (1956) in addition to some modifications.

1.1.Immature stage

During this stage the GSI ranged from 3.38 to 3.6%. Morphologically, the ovary was a colourless strand of tissue, attached to the upper abdominal wall of the abdomen and located above the kidney (Fig. 1A). Histologically, the oocyte nuclei have a characteristic chromatin nucleus stage, early & late perinucleolus stage (Fig. 1B).

1.2.Maturing stage

During which the GSI reached 4.68%. The ovaries matured and appeared opaque, red in colour and their eggs were identified macroscopically. The blood capillaries were distributed along the ovarian wall. Histologically, there was a detected late perinucleolus and early and mid-yolk vesicle accumulation (Figs. 1C, D).

1.3.Ripe stage

At this stage, the GSI ranged from 14.75 to 14.93% (Table, 1 and Fig. 4). Morphologically, the ripe stage was an opaque organ and appeared yellow in color and loaded with yolk. Macroscopically, the ovaries become granulated. The blood capillaries were distinguished and distributed as a network on the ovarian surfaces. Histologically, ripe stage is characterized by the presence of ova loading with yolk granules (Figs.1 E&F).

1.4.Spawning stage

No spawning was observed in this stage.

1.5.Atretic stage

During this stage, there was a sharp depletion of GSI which ranged from 2.98 to 3.25%. Morphologically, the ovary becomes flaccid, flabby and yellowish in colour. Some translucent and opaque residual eggs were detected macroscopically. Histologically, the granulosa cells become hypertrophied and degenerated coincides with liquification of the atretic oocytes and yolk granules (Figs.1 G&H).

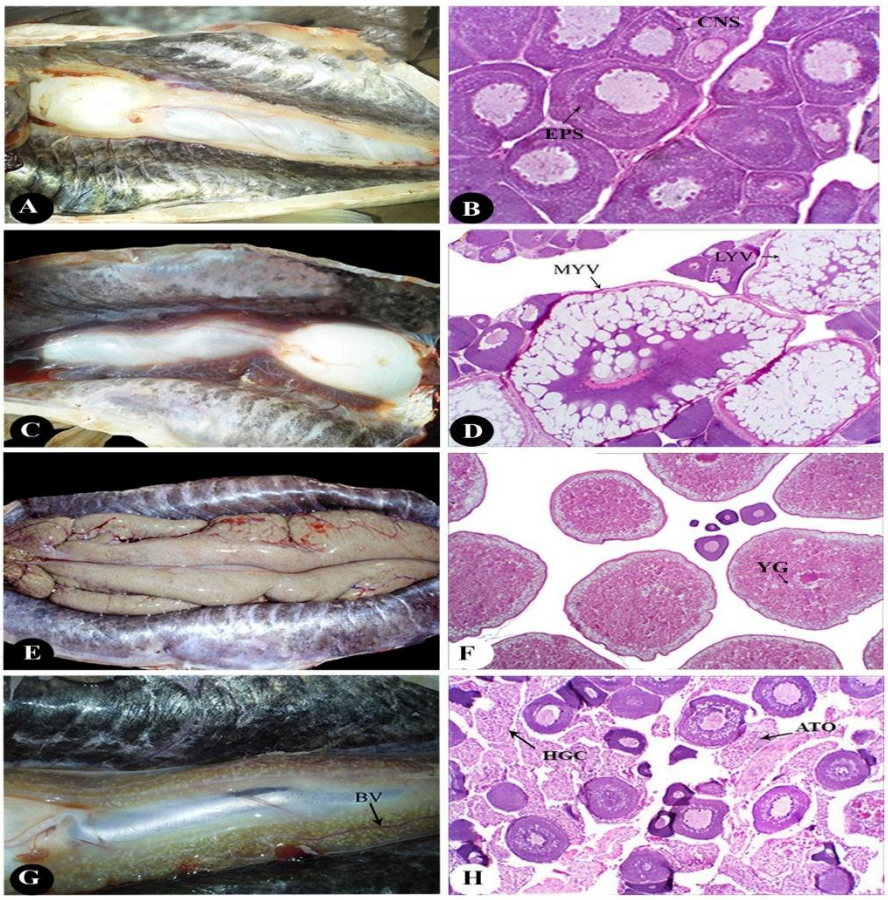

Fig. (1). Photomacrograph of gonads (A,C,E,G) and histological structure of the ovary of female grass carp. Dissected specimen in the ovary of female grass carp, showing different morphological stages of ovary; (A) Immature stage of the ovary appears as colourless organ (5x); (C) Maturing stage, of the

ovary appears orange in colour, elongated in shape. (5x); (E) Ripe stage, the ovary appears yellow in colour (5x); (G). Atretic stage, the ovary is reached with blood vessel (BV) (5x); Histological sections in different stages of maturation; (B) Immature ovary contain chromatin nucleus stage (CNS), early perinucleolus stage (EPS). H&E, X400; (D) Maturing stage contain different stages of midyolk vesicle (MYV) and late yolk vesicle (LYV) H&E, X200; (F) Ripe stage characterized with the presence of yolk granule stages (YG) H&E, X100; (H) Atretic stage showed presence hypertrophied granulose cell (HGC) and atretic oocytes (ATO) H&E, X400.

2. Histological changes of pituitary gland

The pituitary gland appeared oval shaped, compressed dorso-ventrally and attached to the ventral region of the hypothalamus. The gland is located behind the optic chiasma and protected within a bony chamber of the para-sphenoid bone (Figs. 2A&B). Histologically, the gland was composed of two main parts; the neural part called neurohypophysis (NH) and is formed of nerve fibers and and penetrating the glandular part (Fig. 2C), While the glandular part called the adenohypophysis. Three regions are distinguished; rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI (Fig. 2C). The RPD is composed of a thin strip of cells and aggregated in mass forming cords around the branches of nerves of the neurohypophysis. It is characterized by the presence of two types of hormonal secreting cells; acidophilic cells (adrenocorticotropic hormone secreting cells (ACTH) and prolactin hormone secreting cells (PRL)) closely packed with a few basophilic thyrotrophic hormonal secreting cells (TSH) (Fig. 2D).

The prolactin hormonal secreting cells (PRL) are abundant and arranged in cord-shapes around the nerves branching of NH (Fig. 2D). Following periodic-acid Schiffs' (PAS) Orange- G reaction, these cells appeared acidophilic having a higher affinity with orange G and take yellow color. The cells irregularly shaped and varying in size and some of them become spindle-shaped. Their nuclei were centrally located. There is no detected changes of cell structure throughout the maturity stage of gonadal cycle (Fig. 2D).

The Adrenocorticotropic hormone secreting cells (ACTH) are characterized by their oval or polygonal shape with eccentric nuclei enclosed in a thin cytoplasmic sheath (Fig. 2D). It is aggregated in between cells of the PRL. It showed negatively affinity with orange G following periodic- acid schiffs' (PAS) Orange- G technique (Fig. 2D).

2.1.Proximal pars distalis (PPD)

The PPD is located behind the RPD and anterior to the pars intermedia (PI). Gonadotropic (GTH) and thyrotrophic basophilic secreting cells (TSH)) interspersed with the acidophilic somatotrophic hormonal secreting cells (STH) (Fig. 2C).

The somatotrophic hormonal secreting cells (STH) showed acidophilic affinity with PAS-Orange G and appeared as polyhedral cells stained with yellow color (Figs. 2E&F). Also STH showed negatively staining affinity to PAS stain and viewed as unstained cell in between two basophilic cell GTH &TSH (Fig. 2H). The histological structures of these cells were not changed following the cyclic change throughout the gonadal maturation.

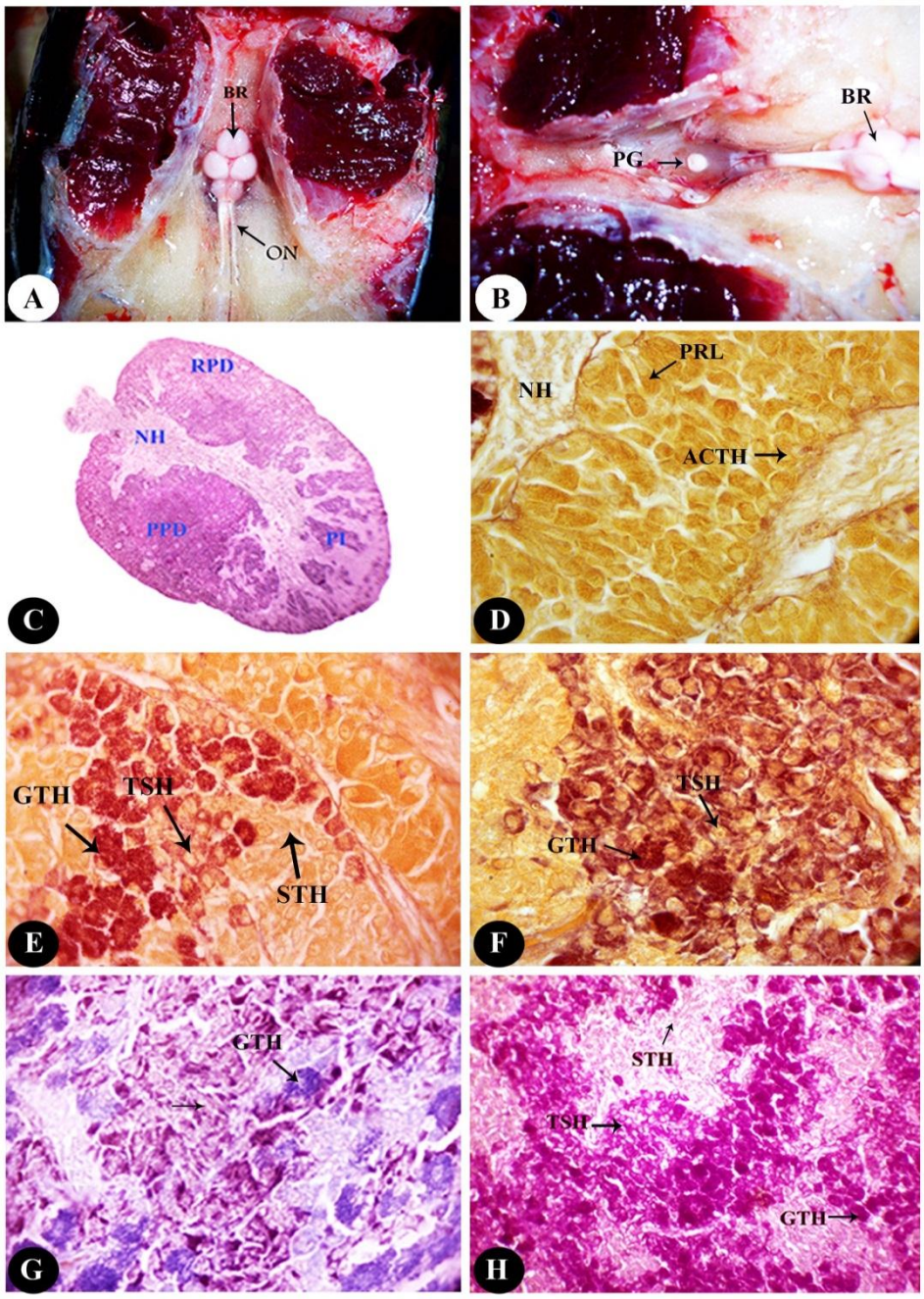

Fig. (2): Sagittal sections of pituitary gland of female grass carp showing, (A&B) Dissected specimen of female showing pituitary gland (PG) located down to ventral surface of brain (BR); (C) showing the extension neural fibers of neurohypophysis (NH), from anterior part of gland proximal pars distalis (PPD) and posterior part pars intermedia (PI) H&E, X400; (D) showing the PRL cell take elongated shape. Small and rounded ACTH cells interspread between PRL cells and bordering neurohypophysis (NH) branches in RPD (PAS) Orange- G1000x; (E) Showing acidophilic cells (PRL and ACTH) localized in the RPD and aggregation of STH localized in the PPD basophilic localized in the PPD, (GTH&TSH) cells stained red colour Orange- G1000x; (F) showing basophilic cells GTH stained with red color and acidophilic cell STH stained with pink located in PPD Orange- G1000x; (G) showing the basophilic cells stained with light blue and red colour AL-PAS1000x and (H) showing acidophilic affinity to cells of PI, when PAS technique is applied PAS. 400X.

Table (1): Fluctuation of gonadosomatic index (GSI %) of females grass carp, *Ctenopharyngodon idella* and GTH cell diameter during maturation cycle.

maturity Stage	No. of fish	Average GSI (%) ± SD			GTH cell diameter (in micron)		
		Max.	Min.	Average ± SD	Max.	Min.	Average± SD
Immature	5	3.6	3.38	3.5±0.057	5.77	5.4	5.52±16
Maturing	5	4.82	4.57	4.68±0.1053	11.66	10.98	11.2±0.22
Ripe	5	14.93	14.75	14.798±0.098	9.9	9.77	9.82±0.03
Atretic	5	3.255	2.98	3.097±0.1250	5.82	5.55	5.66±0.08

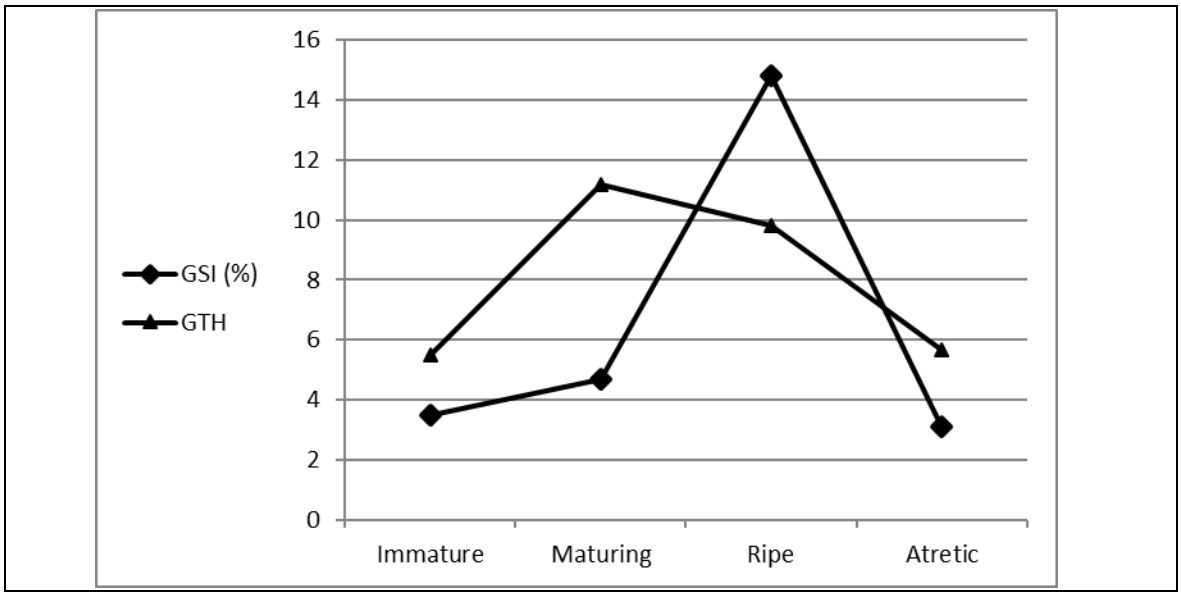

Fig. (3): Fluctuation of gonadosomatic index (GSI %) of females grass carp, *Ctenopharyngodon idella* and GTH cell diameter during maturation cycle.

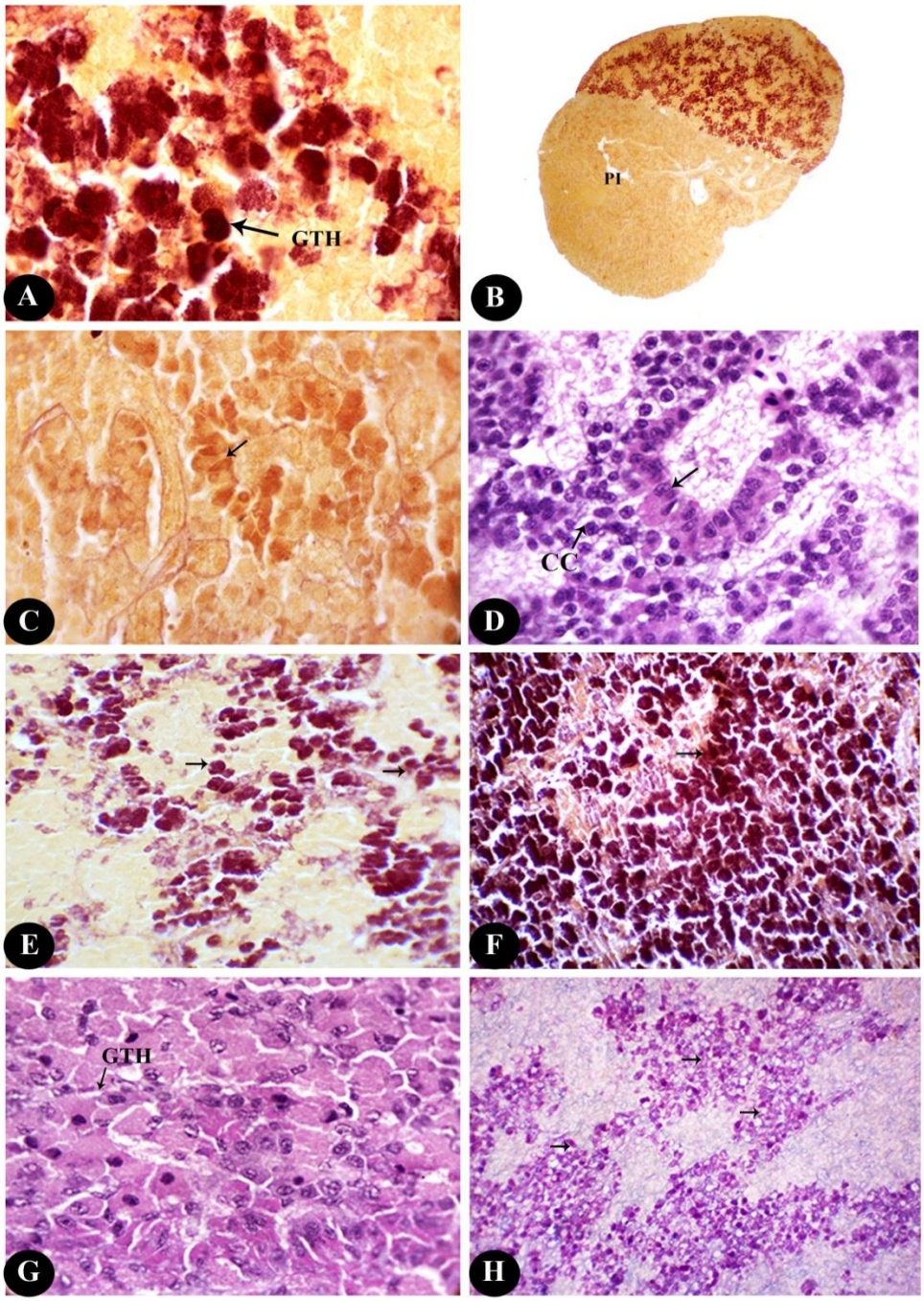

Fig. (4) Sagittal sections of pituitary glands showing the basophilic cell GTH during the different degree of maturation. (A) Showing GTH located in PPD with different degree of granulation (PAS Orange-G 400X); (B) pars intermedia (PI) occupy the most size of gland. (PAS Orange- G 50 X); (C) showing acidophilic affinity to cells of PI. (PAS Orange- G 400X); (D) unstained cytoplasm of Chromophilic cell (CC) and nucleolus dark stain with blue color. (H&E 800X); (E) showing starting of granulation of GTH cells during immature stage (PAS Orange- G 400X); (F) during ripe stage, the PPD compacted with basophilic cells GTH stained with dark magenta red color (PAS Orange- G 400X); (G) pituitary gland compacted with GTH during ripe stage (H&E 800X); (H) during atretic stage, vacuolization of basophilic GTH cell.

Thyrotrophic hormonal secreting cells (TSH) are restricted in contact area between the RPD and the dorsal area of PPD. TSH cells detected isolated or in small cell mass, appeared in peripheral zone of RPD. The TSH showed basophilic in affinity nature, since they were stained in pink red colour after applying PAS-Orange G technique (Figs. 2E, F). These cells appeared in small size in comparison to other basophilic cell GTH and stained with magenta red colour. The TSH cells were scattered among acidophilic ACTH and PRL cells in RPD) and among GTH cells in PPD (Fig. 2E, F).

The Gonadotropic hormone secreting cells (GTH) formed the majority of cells type in the PPD without any defined arrangement and stained with magenta red color (Fig. 2A). These cells are interspersed as single or small Island in the ventral zone of RPD. The GTH varied in shape from irregular to polygonal, and also were varied in size during changing the maturation state. The GTH diameter increased to the maximum value during maturing stage, then decline with degranulation of cytoplasm (Table1 and Fig. 3). The cytoplasmic vacuolization of GTH was concomitant with the maturation stage. The basophilic granules of the GTH stained with blue color when AL- (PAS) (Fig. 2G).and magenta color with (PAS) Orange- G technique was applied (Figs. 4A).

2.2. Pars intermedia (PI)

The pars intermedia (PI) formed the posterior part of the pituitary gland and characterized with branched NH (Fig. 2C,4B). The cells types of PI, showing acidophilic affinity to PAS Orange- G technique and stained with yellow color (Fig. 4B,C) and acidophilic character with haematoxylin and eosin stain (Fig. 4D). Also Chromophilic cell (4D) with unstained cytoplasm and dark stain nucleolus were detected. The PI cells showed an elongated shape and surrounded the neurohypophysis processes (Fig. 4C,D).

3. Annual variation of the GTH cells during the annual cycle

The GTH cells showed seasonally changes in size, number and the staining affinity during the annual cycle of fish female. According to the changes of ovarian maturation events and the changes of GTH cell numbers and size, the GTH state classified to main four stages resting, maturing, ripe and atretic stage.

3.1. Resting stage (immature and maturing)

The resting stage considered as dominant stage during the period of spawning season that extended from middle of March till the end of July. The size of pituitary gland was small and increase with maturation of gonad, since it became rich with GTH content. Histologically GTH cells localized mainly in the PPD; and distributed as separated island between acidophilic cell types (Figs. 4E). The GTH cells are small in size and measured 5.5µm with spherical shape. Both the type of acidophilic and basophilic cells were roughly equal in number

3.2.Maturing stage

The GTH was the dominant cell type detected in adenohypophysis. Number and size of GTH increased and recorded 11.2 µm especially in PPD and also interspersed in the ventral zone of RPD and bordering the PI near to branches of NH. The acidophilic cells undergo hyperplasia, while basophilic cells undergo both hyperplasia and hypertrophy. The staining affinity of GTH increase to the maximum level and cytoplasm became more granulated. At the end of this stage the pituitary gland compacted with GTH cell in PPD region and reached to the maximum size (Fig. 4F).

3.3.Ripe stage

During ripe stage the most conspicuous changes occur in the PPD, since the granulation decreased to some extent and the GTH cell size was recorded 9.82 µm in diameter and seems to be as compact of secretory granules (Fig. 4A). The vacant areas between the GTH cells decreased and seem to be a compacted by the cell mass (Fig. 4G).

3.4.Spawning stage

No spawning ovaries were observed during the spawning period.

3.5.Atretic stage

In present study, the GTH decreased in average number as showed in (Fig. 4H) and its diameters was recorded 5.66 µm during the atretic period. . Few number of GTH cells were still granulated and most of the GTH cells became vacuolated. The staining affinity of GTH cells for staining decreased (Fig. 4H)

4. Lipid content of ovarian development

From Table (2), USFA was detected as a dominant fatty acid (FA) with percent ranged from 60.81% in atretic stage to 61.55% in maturing stage, while SFA recorded 38.45% in maturing stage and to 39.19% in atretic stage. PUFA formed the major constitute of USFA with value ranged from 43.54% to 44.29%. While monounsaturated fatty Acids (MUFA) was ranged from17.09% to17.49%. The most abundant SFA was palmitic (16:0) which the maximum value attained in maturing ovary with 23.95% while; it recorded 24.39% in atretic stage. The second constitute of SFA was Stearic (18:0) with 14.18% to 14.65 % in immature and atretic respectively. MUFA constituted nearly 17% of the total fatty acids in fish gonads. The MUFA recorded 17.09% in immature stage the maximum percentage recorded in ripe ovary with 17.49%.The value of Oleic (18:1n-9) increase from16.07% in immature and to 16.28% in ripe stage.

Total omega-6 formed the major percentage of PUFA reaching nearly 38%, while total omega-3 attained 5%. Linoleic (18:2n-6) fatty acid formed the abundant fatty acid of Omega-6 reached approximately 25%. On the other hand, omega-3 eicosapentaenoic (20:5n-3) (EPA) formed the abundant constituent of fatty acid with percentage around 2.2%. EPA recorded 2.16% in immature ovary and increased to 2.25% in maturing stage and peaked in ripe stage with percentage 2.27%, then decline to 2.21% in atretic stage. The ARA formed 1.72 % from PUFA (Omega-6) group during immature stage then declined to 1.62 in maturing stage and reached to 1.97 in atretic stage of ovary.

5. Ovarian isoenzymes electrophoresis

Acid phosphatase (AcPs) isoenzyme was expressed in Fig. (5A). In immature stage, acid phosphatase isoenzyme electrophoresis is expressed in more dense two bands (I&II), the second band is duplicated. During mature stage, except isoenzyme fraction three which was missing, three isoenzyme bands were identified (I,II,IV). During ripe and atretic stage, three isoenzyme bands were observed. However, the isoenzyme fraction III was less dense in atretic stage compared to ripe one.

Alkaline phosphatase (ALP) isoenzymes isolated into two bands in all ovarian stages, except in ripe stage as showed in (Fig. 5B). During ripe stage, band (I) is duplicated into two bands, while band II missed in maturing and atretic stages. The band II of isoenzyme was denser in ripe stage. However in band III not detected in immature and ripe stages.

Table (2): Percentages of fatty acid profile during ovary maturation stages of female grass carp, Ctenopharyngodon idella.

	Immature	Maturing	Ripe	Atretic
A. Saturated Fatty Acids (SFA)				
Myristic (14:0)	0.15	0.17	0.15	0.15
Palmitic (16:0)	24.29	23.95	24.16	24.39
Stearic (18:0)	14.18	14.33	14.62	14.65
Total SFA	38.62	38.45	38.93	39.19
B. Unsaturated Fatty Acids USFA				
1. Mono unsaturated Fatty Acids (MUFA)				
Palmitoleic (16:1n-7)	1.02	1.11	1.21	1.22
Oleic (18:1n-9)	16.07	16.21	16.28	16.05
Total MUFA	17.09	17.32	17.49	17.27
2. polyunsaturated fatty acids (PUFA)				
2.1. Omega-6				
Linoleic (18:2n-6)	26.51	26.15	25.64	25.34
Gamma-linolenic (18:3n-6)	1.59	1.55	1.59	1.58
Eicosadienoic (20:2n-6)	1.91	1.82	1.91	1.89
Dihomogammalinolenic (20:3n-6)	1.95	1.88	1.98	1.92
Arachidonic (20:4n-6) (ARA)	1.72	1.62	1.96	1.97
Docosatetraenoic (22:4n-6)	2.41	2.37	2.33	2.51
Docosapentaenoic (22:5n-6)	3.28	3.41	2.95	3.17
Total omega-6	39.37	38.8	38.36	38.38
2.2. Omega-3				
Alpha-linolenic (18:3n-3)	1.25	1.33	1.31	1.34
Eicosapentaenoic (20:5n-3) EPA	2.16	2.25	2.27	2.21
Docosahexaenoic (22:6n-3) (DHA)	1.51	1.85	1.64	1.61
Total omega-3	4.92	5.43	5.22	5.16
Total PUFA	44.29	44.23	43.58	43.54
USFA/ SFA	1.58	1.6	1.56	1.55
EPA	2.16	2.25	2.27	2.21
ARA	1.72	1.62	1.96	1.97
EPA/ARA	1.25	1.38	1.16	1.12
(DHA)	1.51	1.85	1.64	1.61
DHA/ARA	0.88	1.14	0.83	0.82

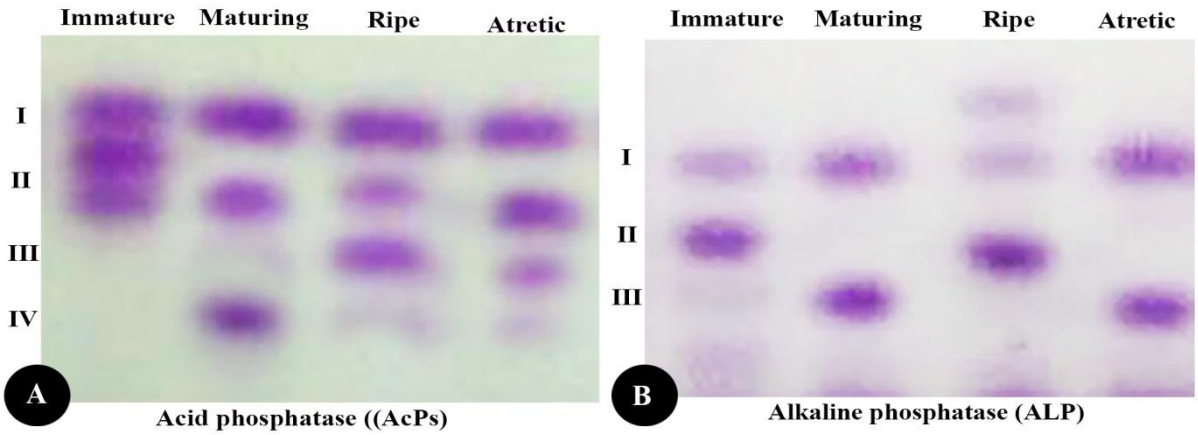

Fig. (5): Isoenzyme electrophoresis of (A) acid phosphatase ((AcPs)); (B) alkaline phosphatase (ALP).

DISCUSSIONS

The present work showed a strong close relationship between ovarian development and cytological changes of pituitary gland, especially basophilic cell type (GTH) which were abundant especially during maturation period. Annually, the ovary is undergoing morphological and histological alterations which become more prominent during spawning stage. The ovaries attained more increase of GSI, where the ova developed to a ripe ovary characterized with the presence of yolk granule stages. The results supported the work of Bose and Chakrabarti, (2014) in case of *Liza parsia*.

The present findings were confirmed by alterations of only one type of GTH in case of pituitary gland of grass carp. The present findings supported the work of AI-Absawy (2004) on *Oblada melanura*, and Balci and İkiz (2017) in case of *Pagellus erythrinus L*. However the present findings contradicted with the work of Grandi *et al*. (2014) in case, of *Ctenopharyngodon idella* and Minniti *et al*. (2009) in *Xiphias gladius* whom reported the presence of two types of gonadotrophic cells. In case *Ctenopharyngodon idella*, immunohistochemical technique was used to investigate the basophilic cell type GTH in the adenohypophysis and reported two types Also, Minniti *et al*. (2009) reported that two distinct GTH I and II are identified in *Xiphias gladius L*.

Histological changes of GTH during ovarian maturation have been studied in several species of teleosts since, during the prespawning stage the GTH cells increased in number, size and occupied a considerable area in the PPD (AIi, 2003; AI-Absawy, 2004; Fahmy, 2006 and Malashetty *et al*., 2009). In present study, GTH cells reached to the maximum degree of granulation during maturing stage since, cytoplasm was compacted with very fine granules that loaded with gonadotropin hormone. During the resting period of Etroplus suratensis the GTH became smaller in size and showed a sign of internal breakdown and the pituitary content was at the lowest level (Krishnan and Diwan, 1990). During ripe stage of grass carp, the cytoplasm degranulated after release the hormone these observations also confirmed by Yamamoto *et al*. (1956) in case of pituitary gland of *Liza parsia* since, degranulation and vacuolization processes of the Cyanophil-I cells appeared to be concomitant with vitellogenesis.

The basic knowledge about ovarian fatty acid composition of grass carp is an important tool to assist in broodstock diet requirements to insure successful spawning (Anido *et al*., 2015). Generally it is widely accepted that fatty acid composition of fish tissues reflects dietary fatty acid composition (Suloma and Ogata, 2011).

The obtained results of fatty acids composition of females grass carp was SFA 38.45% to 39.19%, MUFA17.09.1% to17.49 and PUFA=43.54% to 44.29%, while Anido *et al*. (2015) found that the main of fatty acids groups of wild female (Rhamdiaquelen) detected were: saturated SFA= 35.5%, MUFA= 28.1% and PUFA=33.5%. The SFA of grass carp and bighead carp were more than in case of *Siberian sturgeon* and wels catfish (Pyz-Aukasik and Kowalczyk-Pecka, 2017). While Ljubojevic *et al*. (2013) indicated a higher SFA content in bighead carp (32.82%) and wels catfish (30.22%) but lower in grass carp (28.72%) and recorded 25.99% in sturgeon (Badiani *et al*., 1996) and also, SFA in case of pike, zander, bream, tilapia, and pangasius, ranged from 36.28% to 42.18% (Łuczynska *et al*., 2014). The variation of constitute of fatty acid profile differed from species to another and also during the maturation process. The present results showed no significant difference observed in the total SFA with regard to the reproduction period and season. Several authors reported the important roles of DHA, EPA and ARA as constituents of neural tissues, as precursors of hormone-like molecules, and are involved in the immune system development of the embryo's and hatching ova (Wolfe and Horrocks, 1994 and Sorbera *et al*., 1998).

In the present study, linoleic fatty acid formed the abundant fatty acid constituent of Omega-6, while ARA was not form more than 1.97% of total PUFA. On the other hands it forms 7.3 to 12% in case of *Rhamdiaquelen* (Anido *et al*., 2015), 10.1% in *Siganus virgantus*, 7.4% in *Lethrinus Atkinson* and 3.7% in *Lethrinus miniatus* (Suloma and Ogata., 2011). The role of ARA in fish reproduction was conducted with many of authors. The eicosanoids EPA generated from ARA that are responsible for regulating oocyte maturation, vitellogenesis and ovulation (Sorbera *et al*., 2001 and Bell and Sargent, 2003). Also Tocher (2010) reported that ARA is one of the major nutrients to ensure reproductive success in many fish species. ARA is the main precursor for the 2-series prostaglandins (PG-2), eicosanoids that stimulate steroid synthesis in the ovary, trigger oocyte maturation and affect the sexual behavior of females (Mercure and Van der Kraak, 1996 and Tocher, 2003).

So, the frailer of ovulation of matured ova may be due to the lowest content of ARA so the conversion to EPA ceased and the adequate synthesis of prostaglandins that considered the decisive factor of ovulation stopped. The hight concentrations of ARA in the final gonadal maturation stages may negatively affect egg quality (Furuita *et al*., 2007). Several studies with marine and fresh water species demonstrate that adequate ARA concentrations in the diet stimulate oocyte maturation (Mercure and VanderKraak, 1996; Pérez *et al*., 2007 and Norambuena *et al*., 2012).

In the present study, the value of EPA is more than ARA and more than DHA in all stages of grass carp ovaries, while in case of Tropical Coral Reef fish ARA level more than EPA level ranged from Ovarian DHA level higher than EPA (Suloma and Ogata, 2011). Also, in case of Epinephelus coioides, a relatively high ARA level recorded in ovarian tissue since, DHA >ARA >EPA with a DHA/EPA ratio of 6.8 and a DHA/ARA ratio of 2.5 in the ovarian total lipids. So the failure of ovulation in grass carp may be related to disorder of the percent between EPA, ARA and DHA (Alava *et al*., 2004).

It is important to add adequate ARA concentrations in the diet for brood stock of grass carp during oocyte maturation to stimulate success maturation and ovulation (Pérez *et al*., 2007 and Norambuena *et al*., 2012). The decrease of ARA in PUFA profile of grass carp may be due to the fish considered as exotic species, so cannot collect this type of PUFA from aquatic fauna or may be the fact of degree of the preferential retention of ARA in gonadal polar lipids appears larger in tropical species than in cold and temperate water species (Suloma and Ogata, 2011). Several authors related decline of ARA to Scarce of food chain that considered the main source of ARA and related the high ARA levels in coral reef fish, to existence of an ARA-rich food chain in coral reef area (Suloma and Ogata, 2011). EPA forms the major HUFA in tropical phytoplankton of the South China Sea (Shamsudin, 1998). Thus, ARA appears to be provided primarily from some organisms existing in/on benthic substrate and benthic detritus rather than pelagic organisms. Such speculation might be supported by the findings of Svetashev *et al*. (1991).

The results of the present investigation showed that acid phosphatase (AcPs) isolated in three isoenzyme bands during all maturation stages, while in case of *Puntius chilinoides* AcPs isolated to four bands at the spent stage, only two bands at the mature stage and three bands at the immature and maturing (Nuriyal and Singh, 1985). The depletion of isoenzyme band III during the maturation cycle may be explained with its role in maturation process, since the band denisity decrease toward final maturation then suddenly increase during atretic stage after failure of ovulation; and these findings also confirmed with Nuriyal and Singh (1985).

The ALP isolated into two isoenzymes bands detected in immature, maturing and atretic stages but two isoenzymes bands were detected in ripe stage. The fluctuation of band 2 and 3 showed a role in maturation, since the bands 2 and 3 were missed in immature and atretic stage. The decline of ALP during ripe stage can be explained with the active role of ALP in estradiol driven hepatic vitellogenesis as reported by (Eales *et al*., 1991; Johnston *et al*., 1992,1994 and Crim *et al*., 1992). Moreover, our result is consistent with Johnston *et al*. (1990,1992) findings in case of *Salmo salar*; as ALP activity increases during gonadal development and then decreases in the following spawning months. Furthermore, ALP recorded a higher activity in the ovaries of *Clarias batrachus* during maturation (Shaffi *et al*., 1974).

Conclusion

It can be concluded that, the annual fluctuation in the gonadotropic hormones secreting cells (GTH) in related to the ovarian maturation is outmost importance. In ripe stage, the GTH increased in activity and the ovary reached to its maximum size. However, in the immature and atretic stages, the GTH decreased in size and the ovary reached to the minimum size. The studies on the different fatty acids and their correlation with gonadal maturation are outmost importance in captivity. These results showed a disorder in the percent of fatty acids especially between EPA, ARA and DHA during ovarian maturation of grass carp. This process may be explained the failure of ovulation of grass carp. In the future study, it must be added an adequate quantity of fatty acid, especially ARA in diet and mixed them well before feeding process to stimulate the oocyte maturation, ovulation and successful propagation.

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