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COMPARATIVE STUDIES OF SOLUBLE PROTEIN USING ISOELECTRIC FOCUSING (IEF) IN GONADS AND PITUITARY GLANDS OF FEMALE MUGIL CEPHALUS IN NATURAL HABITAT AND CAPTIVITY DURING REPRODUCTIVE CYCLE

By

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Key words: Biochemistry, Endocrinology, Grey Mullet.

ABSTRACT

*Soluble protein of gonads and pituitary glands of female **Mugil cephalus** in different stages of maturity in both natural habitat (saline water) and captivity (freshwater), was investigated using isoelectric focusing. The electrophoretic patterns of ovary protein showed differences between the two localities in terms of maturation stages. The pituitary gland protein for both saline and freshwater showed dimorphism in terms of isoelectric focusing. The stages of maturity differed in their protein pattern from one to another in the arrangement and number of intensively colored band. There are specific bands in each stage of maturity for ovaries and pituitary glands.*

INTRODUCTION

One of the most common fish species in the Mediterranean Sea is the black mullet "Grey Mullet" or "Jumping Mullet"; ***Mugil cephalus*** (L) is an euryhaline fish cultured in fresh, brackish and seawater. In nature ***Mugil cephalus*** matures and spawns during the autumn months in freshwater ponds and lakes.

The vitellogenesis of ovarian oocytes in captive females have been designated only up to the tertiary yolk globule stage (Mousa, 1994). Shireman (1975) marked gonadal atresia in Mugil cephalus in both male and female shortly before ripening in freshwater lakes. Teleostei are no exception to the rule that: vertebrate gonadal function is developed and maintained by gonadotropic hormones produced by the pituitary gland (Van Oordt and Peute 1983). Assem (1992) determined the variation in the gonads and pituitary gland protein during maturation stages in female and male ***Oblada melanura*** by isoelectric focusing, and found great variation in electrophoretic patterns with characteristic and specific bands for each stages of maturation.

A marked changes for serum gonadotrophic level in ***Mugil cephalus*** occurs with sexual maturity (Zaki ***et al.***, 1995). Mousa and Mousa (1997) investigated gonadotrophic cells in female ***Mugil cephalus***. during ovarian cycle in both fish reared in natural habitat and captivity using Immuno-cytochemistry.

Isoelectric focusing technique was used as a tool for the biochemical analysis of protein in different fish (EL-Gharabawy (1995); EL-Gharabawy and Zaki 1990 a & b; EL-Gharabawy ***et al*** 1995; Zaki ***et al.*** 1996 and 1997).

Assem (1995) studied comparative analysis of plasma protein using electrophoresis and isoelectric focusing in female and male ***Solea vulgaris*** and ***Solea aegyptiaca*** at various stages of maturation.

Assem (1998) studied the changes in protein patterns of gonad and pituitary gland during maturation stages of ***Solea aegyptiaca*** of the Egyptian Mediterranean waters.

Abdo (1996) found a great variation in electrophoretic patterns with characteristic and specific bands for each stages of maturation in female and male gonads and pituitary gland of ***Dicentrarchus labrax***.

The present study was undertaken to identify and localize the sexual variations in the soluble proteins of ovaries and pituitary gland during different maturation stages in both natural habitat and captivity to provide basic information necessary for its successful propagation.

MTERIAL AND METHODS

Fish collection:

Mature females of ***Mugil cephalus*** (with standard length ranged from 28 to 43cm and body weight from 700 to1500gm) were collected from El-Bardawill lagoon (natural marine habitat) and El-Manzalah freshwater (captivity fish farm) at intervals of about one month throughout the period from April 1997 to May 1998. The maturity stages were identified by three methods as follows:

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

Sample Preparation:

For each stage of maturity, pooled sample of five ovaries "0.1g. from each" was homogenized with 5ml of cold tris Hcl (PH. 8.0) whereas a pool of five pituitaries was homogenized with 0.5 ml of tris Hcl (PH 8.0). The homogenates were centrifuged at 6000 rpm for 10 minutes (at room temperature). The clear supernatant was pipetted into vials and stored at (-20°C) until use. Microsoft Windows (95), Excel program was used for statistical analysis (correlation coefficient and significant test).

Sample application and silver staining technique:

Technical procedure for sample application and determination of protein isoelectric points (PI's) were performed as described by pharmacia LKB, 1987 (calibrator Kit no. 11-B-045-02). Silver staining technique as derived from the method of Heukshoven and Dernik (1985) was applied, the PI values of protein under investigation is determined by comparing its electrophoretic mobility with that of protein standards of a known PI values. The average sensitivity limit of this technique was estimated to be 1 to 5 ng proteins per band for isoelectric focusing and is approximately 20 times sensitive than coomassie staining method. Gels were dried and scanned using hoefer GS 300 catalogue number 10663/5-14-92. Microsoft windows (GS 365 W) catalogue no. 24512 was used for analysis.

RESULTS

Ovarian cycle:

A. In natural habitat "saline water"

The ovarian cycle of female ***M. cephalus*** can be classified into six stages:

Stage I: Consisted of fish with pre-vitellogenic ovaries, which had a GSI of 0.45±0.18 and Oocyte diameter of 0.095±0.02 mm. At this stage, the primary Oocyte dominates the ovarian components.

Stage II: The fish at stage II had early vitellogenic ovaries with GSI of 0.7±0.25, most of the oocytes at this stage were belong to the vesicle stages with a mean diameter of o.16±0.035mm.

Stage III: "Mid vitellogenic" ovaries which had GSI of 2.4±1.2 and oocyte diameter of 0.3±0.05mm. Most of the oocyte were in the primary and secondary yolk stages. These oocytes are characterized by yolk globules deposition.

Stage IV: This stage consisted of fish had late vitellogenic ovaries with GSI of 9±2.5. Most of the oocytes were noticed to belong to the secondary yolk stage with a mean diameter of 0.42±0.045 mm.

Stage V: Consisted of fish with pre-spawning ovaries, which had a GSI of 25.51±7.0. Most of the Oocyte in the pre-spawning ovaries belong mainly to the tertiary yolk stage with a mean diameter of 0.61±0.05 mm.

Stage VI: This stage had post-spawning "spent" ovaries, which had a GSI of 1.35 ± 0.45. The ovaries at this stage were characterized by a great number of relatively deformed Oocyte. The Oocyte diameter of 0.1± 0.01.

B-In captivity fish farm (freshwater):

In freshwater ponds, the growth and differentiation of pre-vitellogenic follicles was delayed.

Stage I: "pre-vitellogenic ovaries" Consisted of fish with GSI values of 0.7± 0.1 and Oocyte diameter of 0.13±0.02 mm. At this stage, the primary Oocyte dominates the ovarian components.

Stage II: "early vitellogenic ovaries" Consisted of fish with GSI of 1.85±0.6, and mean Oocyte diameter of 0.29±0.015 mm.

Stage III: "Mid vitellogenic ovaries" the fish had GSI values of 6.0±2.3 and oocyte diameter of 0.41±0.5 mm.

Stage IV: "Late vitellogenic ovaries" Consisted of fish with GSI values of 15.0±4.0. The mean Oocyte diameter of 0.55± 0.06 mm. At this stage, certain degenerative changes (atresia) were taken place in oocytes.

Stage V: "Post-spawning or spent ovaries" Consisted of fish, which had a GSI of 1.25 ± 0.9. The mean Oocyte diameter values of 0.11± 0.015.

I. Gonad protein in saline and freshwater fish in relation to stages of maturity:

For all stages of maturity the protein patterns of ovaries of both fresh and saline water were given in figure 1A and 1B (PH gradient from 3-9).

In female ***Mugil cephalus*** reared in saline water the ovaries protein were identified by three common protein fractions separated at PI's value of 4.9, 5.9 and 9.3 with different percentages of concentration for each stage of maturity. Stage I, of maturity was characterized by one specific bands separated at PI's value of 7.6. Two specific bands were separated from stage II of maturity at PI's values of 4.65 and 7.5.

One specific band was separated from stage III, IV and V of maturity at PI's values of 4.5, 4.4 and 5.6 respectively.

At stage VI of maturity, there were two specific bands separated from ovary protein in saline water at PI's value of 4.1 and 4.7 as shown in table 1.

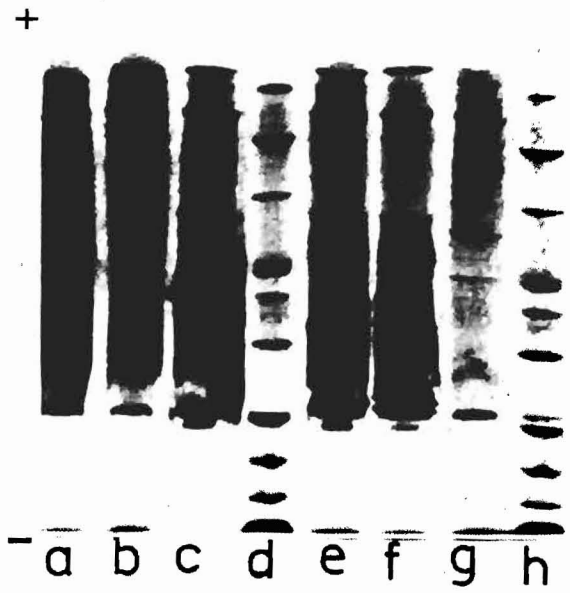


Fig. (1A): Isoelectric focusing of the ovaries proteins at all stages of maturity in ***Mugil cephalus*** reared in natural habitat (saline water) on polyacrylamid gel IEF (3-9). a-Previtellogenic; b- Early-vitellogenic; c-Mid-vitellogenic; d & h- Standard protein calibration; e-Late vitellogenic; f-Pre-spawning and g- Spent ovaries.

Fig. (1B): Isoelectric focusing of the ovaries proteins at all stages of maturity in ***Mugil cephalus*** reared in captivity (freshwater) on polyacrylamid gel IEF (3-9). a-Pre-vitellogenic; b- Early-vitellogenic; c-Mid-vitellogenic; d & g- Standard protein calibration; e-Late vitellogenic and f- Spent ovaries.

Table (1): The isoelectric point (PI's) values and the corresponding percentage (%) of Quantitative values of scanning pattern in gonad of female ***Mugil cephalus*** reared in saline water.

PI Values	Percentage of concentrations for stages of maturity					
	I	II	III	IV	V	VI
3.40	---	---	2.90	3.10	9.10	6.10
3.50	1.40	4.60	1.70	---	---	---
3.80	2.40	3.40	1.20	12.5	---	---
4.10	---	---	---	---	---	*4.30
4.20	5.20	7.30	7.30	---	---	---
4.40	---	---	---	*3.10	---	---
4.50	---	---	*3.90	---	---	---
4.60	4.80	7.00	---	4.90	4.50	---
4.65	---	*2.50	---	---	---	---
4.70	---	---	---	---	---	*16.8
4.90	# 5.40	3.00	3.70	4.00	1.20	4.40
5.00	3.30	---	3.30	2.50	1.80	9.50
5.20	2.50	5.00	1.90	---	1.20	---
5.30	4.40	2.50	3.50	1.50	---	4.10
5.40	---	5.10	2.00	1.60	1.60	4.30
5.50	3.00	4.00	---	1.60	1.00	---
5.60	---	---	---	---	*0.80	---
5.70	2.90	1.50	---	---	---	---
5.80	4.20	1.50	---	---	---	---
5.90	# 3.00	1.00	6.60	1.10	6.10	3.40
6.00	3.50	2.00	---	---	---	6.00
6.10	---	1.30	3.00	6.70	8.30	---
6.20	2.70	3.60	3.30	---	5.50	---
6.40	3.90	2.50	3.20	5.90	---	2.70
6.50	3.10	2.30	7.10	3.50	---	1.50
6.60	2.60	1.00	4.70	6.10	---	---
6.70	2.50	3.90	---	3.80	23.1	6.80
6.80	---	---	6.00	---	4.60	---
6.90	2.60	---	---	---	---	4.60
7.00	9.50	12.2	---	---	---	---
7.30	8.50	---	3.00	18.5	---	---
7.40	11.4	---	---	---	---	5.00
7.50	---	*7.00	---	---	---	---
7.60	*3.00	---	---	---	---	---
7.70	---	13.1	11.8	---	6.40	---
7.80	---	---	10.0	6.00	12.3	7.40
8.10	---	---	---	4.30	5.20	---
8.30	2.30	1.30	5.10	5.50	4.60	---
8.40	---	---	3.60	1.80	1.20	8.20
9.30	# 1.90	1.40	1.20	2.00	1.50	4.90

\# Prominent bands
* Specific bands

Table (2): The isoelectric point (PI's) values and the corresponding percentage (%) of quantitative values of scanning pattern in gonads of female ***Mugil cephalus*** reared in fresh water.

PI Values	Percentage of concentrations for stages of maturity				
	I	II	III	IV	V
3.35	---	---	---	---	*2.80
3.40	---	---	---	*3.80	---
3.50	#6.50	4.40	6.40	3.00	4.90
3.55	---	---	---	*3.80	---
3.70	#0.90	2.30	3.00	0.50	2.60
3.80	---	---	*3.00	---	---
3.90	0.90	2.00	---	---	2.70
4.00	3.00	---	---	---	2.10
4.10	4.50	1.90	---	---	---
4.40	---	---	3.90	3.80	---
4.50	2.00	5.00	---	2.70	6.50
4.55	*3.90	---	---	---	---
4.60	---	---	---	*6.50	---
4.80	5.00	---	7.00	4.40	---
5.00	---	---	---	---	*4.40
5.20	---	8.90	4.80	3.70	7.90
5.40	*6.70	---	---	---	---
5.50	---	3.80	---	2.70	3.90
5.60	---	4.60	1.50	2.00	5.20
5.70	*6.00	---	---	---	---
5.80	0.90	4.00	---	1.60	4.30
6.00	5.00	2.30	4.00	---	2.40
6.20	---	---	---	*2.00	---
6.30	7.40	5.00	7.50	6.40	---
6.40	3.00	---	2.50	2.30	17.6
6.50	4.90	2.10	---	2.50	3.50
6.60	---	---	*6.60	---	---
6.70	---	9.80	7.00	6.00	9.80
7.00	10.1	8.00	3.70	13.5	---
7.10	*3.90	---	---	---	---
7.20	4.00	2.70	3.50	6.20	---
7.30	#5.40	7.20	5.30	8.00	9.10
7.40	---	---	*10.7	---	---
7.50	5.50	11.0	11.0	---	6.00
7.55	#9.00	4.50	4.00	7.00	1.50
7.80	---	---	---	*5.10	---
8.10	---	*6.30	---	---	---
9.30	#1.50	4.20	4.60	2.50	2.80

Prominent bands
* Specific bands

Gradual decrease was recorded in total number of bands (25, 25, 23, 21, 19, 17) for ovaries in female fish reared in natural habitat with correlation coefficient (r= -0.98) in relation to stages of maturity.

Insignificant correlation between the total number of fraction in ovaries of female fish reared in saline water and specific number of fractions (r =0.16).

The protein patterns in ovaries of freshwater fish were identified by five common protein fractions separated at PI's value of 3.5, 3.7, 7.3, 7.55 and 9.3 as shown in table 2.

There were four specific bands in ovaries of freshwater fish at stage I of maturity separated at PI's value of 4.55, 5.4, 5.7 and 7.1. Whereas stages II of maturity was characterize by one specific fraction separated at PI's value of 8.1.

Stage III of maturity was characterized by three specific fractions separated at PI's value of 3.8, 6.6 and 7.4.

Five and two specific fractions were characterized the ovaries at stage IV and V of maturity and separated at PI values of (3.4, 3.55, 4.6, 6.2 and 7.8) for stage IV and (3.35 and 5.0) for stage V as shown in table 2.

There were a significant correlation between the total number of fraction (r = 0.78) in ovaries of freshwater fish and specific number of bands. No trend was recorded between total number of band for ovaries in freshwater and natural habitat (r = 0.125).

Insignificant correlation between total number of band for freshwater fish ovaries (specific and non-specific number 22, 20, 19, 23, 19) in relation to stages of maturity (r = -0.26).

There were highly significant correlation between the total number of fraction in ovaries in natural habitat (r= 0.99) in relation to number of non specific bands (24, 23, 22, 20, 18, 15). At the same time this relation in freshwater are weak (r =0.51). (Table 1 and 2).

Gradual significant decrease relationship were recorded between non-specific number of bands in ovaries of fish reared in natural habitat (r = -0.97) with maturation.

No obvious trend were recorded between non-specific number of bands in ovaries of freshwater fish (18, 19, 16, 18, 17) with relation to stages of maturity (r= -0.42).

II Pituitary gland protein in saline and freshwater fish in relation to stages of maturity:

For all stages of maturity the protein patterns of pituitary gland of both saline and freshwater fish were given in Fig 2A and 2B (PH gradient from 3-9).

The pituitary gland protein of female ***Mugil cephalus*** reared in saline water was characterized by four common bands separated at PI values of 3.3, 3.8, 6.4 and 7.8 with different percentages of concentration for each stage of maturity.

Stage I, III and V of maturity were characterized by two specific fractions separated at PI values of (3.4and 6.8); (4.5 and 6.6) and (3.5 and 6.1) respectively as shown in table 3.

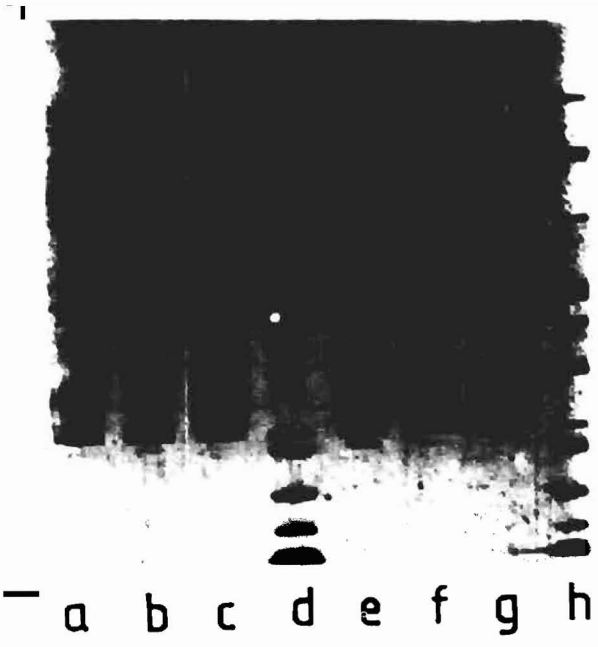
Three specific fractions were separated from pituitary gland protein in natural habitat from stage II of maturity at PI values of 5.0, 6.7 and 6.9.

One specific fraction was separated from pituitary gland at stage IV of maturity at PI's value of 7.1 and 3.6 respectively.

There were insignificant decreases in specific number of fraction in pituitary gland of natural habitat (2, 3, 2, 1, 2 and 1) in relation to stages of maturity (r =-0.64), whereas no trend were recorded in freshwater pituitary (r = -0.14) in number of specific fraction (4, 1, 3, 2 and 3) with maturation.

Significant correlation between total number of fraction (r = 0.71) and specific number in natural habitat was recorded, whereas insignificant correlation was found in freshwater fish (r = 0.28).

The pituitary gland protein in freshwater ***Mugil cephalus*** was characterized by four common bands separated at PI values of 3.5, 6.6, 7.3 and 7.8 with different percentages of concentration for each stage of maturity.



(2A): Isoelectric focusing of the pituitary gland proteins at all stages of maturity in female ***Mugil cephalus*** reared in natural habitat (saline water) on polyacrylamid gel IEF (3-9). a-Pre-vitellogenic; b- Early-Vitellogenic; c-Mid-vitellogenic; d & h- Standard protein calibration; e-Late- vitellogenic; f-Pre-spawning and g- Spent ovaries.

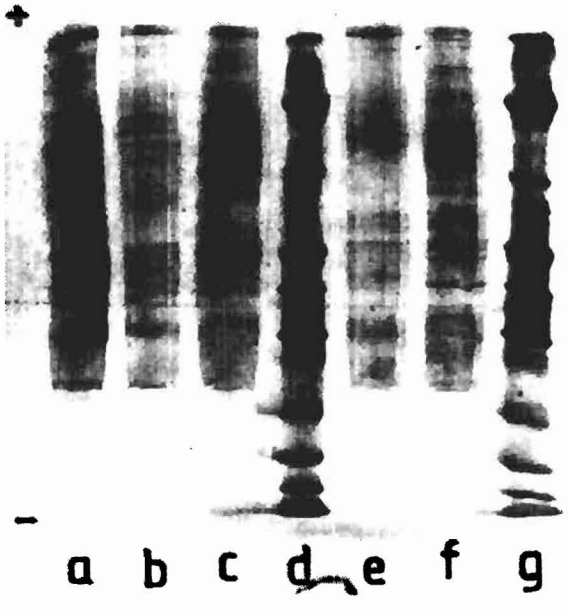


Fig. (2B): Isoelectric focusing of the pituitary gland proteins at all stages of maturity in female ***Mugil cephalus*** reared in captivity (freshwater) on polyacrylamid gel IEF (3-9). a-Pre-vitellogenic; b- Early-vitellogenic; c-Mid-vitellogenic; d & g- Standard protein calibration; e-Late vitellogenic; and f- Spent ovaries.

Table (3): The isoelectric point (PI's) values and the corresponding percentage (%) of quantitative values of scanning pattern in pituitary gland of female ***Mugil cephalus*** reared in saline water.

PI Values	Percentage of concentrations for stages of maturity					
	I	II	III	IV	V	VI
3.30	#8.20	14.0	2.60	6.50	2.90	7.40
3.40	*3.00	—	—	—	—	—
3.50	—	—	—	—	*10.0	—
3.60	—	—	—	—	—	*6.40
3.80	#6.80	11.9	5.50	12.1	9.20	4.50
4.30	6.00	—	—	—	—	5.70
4.50	—	—	*4.20	—	—	—
4.70	2.00	10.2	—	11.8	7.60	—
4.80	6.10	—	1.10	8.50	—	18.5
4.90	7.00	5.60	—	—	—	—
5.00	—	*4.70	—	—	—	—
5.20	7.30	—	—	—	—	6.90
5.90	9.60	1.00	2.00	1.00	—	—
6.10	—	—	—	—	*11.8	—
6.40	#4.60	11.7	8.80	4.40	4.50	14.1
6.60	—	—	*4.40	—	—	—
6.70	—	*4.80	—	—	—	—
6.80	*1.80	—	—	—	—	—
6.90	—	*5.90	—	—	—	—
7.00	1.00	—	5.90	2.00	6.00	—
7.10	—	—	—	*2.90	—	—
7.30	—	—	9.30	—	9.80	13.4
7.40	—	10.7	14.9	16.2	—	—
7.50	26.9	2.00	18.9	19.2	20.0	—
7.60	—	—	—	—	8.10	18.2
7.70	—	2.00	11.9	—	—	—
7.80	#9.70	15.0	10.5	13.7	10.1	4.90
7.90	—	0.50	—	1.70	—	—

\# Prominent bands
* Specific bands

Table (4): The isoelectric point (PI's) values and the corresponding percentage (%) of quantitative values of scanning pattern in pituitary gland of female ***Mugil cephalus*** reared in freshwater.

PI 's Values	Percentage of concentrations for stages of maturity				
	I	II	III	IV	V
3.50	#10.2	6.00	5.50	6.10	3.50
3.60	—	—	*3.20	—	—
3.70	—	—	—	*4.20	—
3.80	1.80	3.20	—	—	1.70
4.20	4.00	4.60	4.80	—	—
4.30	—	*6.00	—	—	—
4.70	16.7	—	10.3	4.50	3.00
4.80	—	12.9	3.00	6.20	14.6
4.90	—	—	—	8.40	2.00
5.00	—	—	13.7	14.0	—
5.10	—	5.30	—	—	10.4
5.20	*8.40	—	—	—	—
5.50	—	3.30	—	6.10	—
5.70	9.30	4.20	5.70	—	—
5.90	—	7.20	7.30	—	4.00
6.10	3.20	—	—	3.00	—
6.20	—	—	*2.80	—	—
6.30.	—	—	3.40	4.30	—
6.40	—	—	—	—	*5.70
6.50	*4.40	—	—	—	—
6.60	#5.40	3.50	4.10	3.70	5.20
6.70	—	—	—	*5.10	—
6.75	*3.90	—	—	—	—
6.80	2.50	5.00	—	—	12.4
6.90	1.00	3.20	—	—	—
7.00	5.60	6.50	—	—	—
7.30	#14.3	18.9	23.2	23.8	9.00
7.40	—	—	—	—	*2.00
7.70	3.50	9.50	6.00	—	—
7.80	#0.80	0.70	3.00	10.6	26.3
8.10	*5.00	—	—	—	—
9.20	—	—	*4.00	—	—
9.30	—	—	—	—	*0.20

\# Prominent bands

* Specific bands

Stage I of maturity in freshwater pituitary gland's protein was characterized by four specific bands separated at PI values of 5.2, 6.5, 6.75 and 8.1 with different percentage of concentrations. One specific band was separated from stage II of maturity at PI's value of 4.3.

Three specific bands were separated from pituitary gland protein in freshwater fish at stage III and V of maturity at PI values of (3.6, 6.2 and 9.2) for stage III whereas (6.4, 7.4 and 9.3) for stage V. Two specific bands were separated from stage IV of maturity at PI values 3.7 and 6.7 as shown in table 4.

There were gradual decreases in total number of bands (17, 16, 15, 13, 14) for pituitary gland in freshwater fish in relation to stages of maturity ($r = -0.9$).

In female pituitary gland of freshwater ***Mugil cephalus*** there were significant correlation between number of non-specific bands (13, 15, 12, 11, 11) in relation to total number of bands (17, 16, 15, 13 and 14) ($r = 0.76$).

DISCUSSION

Mugil cephalus belong to family Mugillidae, which spawn in the sea, but after enter the rivers and lakes for feeding where they can be well adapted to variations in temperature and salinity.

The Grey mullet, do not complete its reproductive cycle when kept in captivity. Gonadal atresia was marked by Shireman (1975) to occur in both male and female mullet shortly before ripening in freshwater lakes.

In the present study, the utilization of isoelectric focusing of soluble proteins has easily permitted the accurate variations in gonads and pituitary gland in relation to maturation stages.

The gonad and pituitary gland protein in female ***M. cephalus*** for both saline and freshwater showed dimorphism in terms of isoelectric focusing patterns.

The stages of maturity differed in their pattern from one to another by dramatic changes in morphology (the arrangement and number of intensively

colored band) and in chemical composition (relatively rapid and pronounced changes in amounts of specific proteins).

Gonad protein in female ***M. cephalus*** showed great differences corresponding to maturation stages and was characterized by a number of specific bands. These results supported by the work of Abdallah (1996) in ***Diplodus vulgaris***, Abdo (1996) in ***Dicentrarchus labrax***, Assem (1992) in ***Oblada melanura***, El- Gharabawy ***et al*** (1995) in ***Mugil seheli***, Zaki ***et al*** (1997) for ***Chrysichthys rueppelli*** & ***C.auratus*** and Assem (1998) for ***Solea aegyptiaca***.

The results reported in the present study showed that: the protein patterns of ovaries in natural habitat "saline water" showed a gradual decrease in the number of bands with maturation reaching its minimum in the post-spawning ovaries (spent) (r =-0.98). These results are in agreement with Assem (1998) for ***Solea aegyptiaca***, which reported that the total protein fraction of the ovary decreased with maturation (r = -0.87).

No obvious trend was recorded for ovaries of female reared in captivity "freshwater" ***Mugil cephalus*** (r = 0.26). These results confirmed by the work of Mousa and Mousa (1997), who reported that: the immunocyto-chemistry of the gonadotropic cells in the pituitary gland of ***Mugil cephalus*** reared in captivity, appeared with low activity which causing a decline in the ovarian activity reflected in the form of low GSI and earlier resorption of the ovaries (atresia).

El-Halfawy (1993) identified ***Nemipterus*** species in Suez Gulf using (IEF) and also determined the variation, which occur in the gonad proteins during maturation stages. He found differences between species in number and concentration of bands.

El-Gharabawy ***et al***. (1995) reported that the number of protein fractions in ovaries of female ***Mugil seheli*** was decreased with advancing maturity.

In the present results the great differences between the number of bands at each stage of maturity in female gonad and pituitary gland may reflects an intimate relationship between cytoplasmic maturation and the change in electrophoretic distribution pattern of Oocyte proteins.

In agreement with our results Iwamatsu ***et al*** (1992) indicated that in ***Oryzias latipes*** some of the Oocyte proteins that become evident as maturation proceeds may be involved in the acquisition of developmental capacity by the Oocyte so that it can respond to the stimulus of sperm or pricking thus, these proteins seen to initiate development.

Thorsen and Fyhn (1991) found a significant decrease in protein content during hydration process in some marine eggs. They concluded that a large amount of free amino acid pool present in ripe pelagic eggs was created by hydrolysis of yolk protein.

Carnevali ***et al*** (1992) studied the changes in the electrophoretic pattern of yolk proteins during vitellogenesis in the gilthead bream, ***Sparus aurata***. They found major changes in the ovulated eggs, suggesting that they occur during the process of maturation. The largest components formed during vitellogenesis either disappeared or diminished. The explanation for yolk protein changes during Oocyte maturation in sea bream is consistent with the pre-existing yolk proteins in pre-maturational oocytes proteolytically altered during maturation.

Iwamatsu ***et al*** (1992) determined the changes in isoelectrophoretic patterns of Oocyte proteins during Oocyte maturation in ***Oryzias latipes***. They found that a few of the protein spots disappeared during the process of Oocyte maturation.

The results reported in the present study revealed that the protein patterns of female pituitary gland in natural habitat (saline water) showed a gradual decrease in the number of bands reaching its minimum in the spent stage ($r = -0.98$). The minimum number of bands in pituitary gland of captivity (freshwater) were recorded in late-vitellogenic stage and then increase in spent stage.

Van Oordt and Goos (1987) and Van Oordt ***et al*** (1987) reported that the pituitary gland of teleosts as well as in all vertebrates consists of two parts separate on the bases of embryology, structure and function. These are adenohypophysis and neurohypophysis Ball and Baker (1969). Devlaming (1974) reported that the central role of adenohypophysis is the regulation of gonadal function in teleost.

In the present results, in female ***Mugil cephalus*** reared in natural habitat the total protein fraction of pituitary gland was significantly decreased with maturation (r = -0.98).

Mousa and Mousa (1997) in his work on immunocyto chemical studies of the gonadotropic cells in the pituitary gland of female ***M. cephalus***, revealed that: the gonadotropic potency of the pituitary gland in general had undergone an obvious increase during ovarian development reaching a peak at the time of reproductive maturity. Degranulation, vacuolization and weak immunoreactivity of gonadotrophs cells have occurred during spawning in natural habitat.

In the present results, in fresh water ***M. cephalus*** gonads, there was insignificant correlation in total no. of bands (r = -0.26) in relation to stages of maturity. In agreement with our results Mousa and Mousa (1997) reported that, the immunocyto-chemistry of pituitary gland of female ***M. cephalus*** reared in captivity, appeared with low activity which causing a decline in the ovarian activity.

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