ISSN 1110-0354
Bull. Nat. Inst. Of Oceanogr. & Fish., A.R.E., 1999. Vol. (25): 331 - 355

HORMONAL INDUCTION OF OOCYTE FINAL MATURATION AND OVULATION IN THIN-LIPPED GREY MULLET, LIZA RAMADA (Risso)

By

MOSTAFA A. MOUSA*

**National Institute of Oceanography and Fisheries, Alexandria.*

Key words: Oocyte, maturation, ovulation, immunocytochemistry, pituitary gland, *Liza ramada* (Teleostei).

ABSTRACT

*The application of a high potency synthetic analogue of luteinizing hormone-releasing hormone (LHRH-a) in combination with human chorionic gonadotropin (HCG) for inducing the final stages of maturation and ovulation in the grey mullet **Liza ramada** was investigated in prespawning females, with average egg diameters of 600 µm or more, by three hormonal injection strategies. The average GSI and diameter of ovarian oocytes increased gradually during induction of final oocyte maturation (FOM). Mature and transparent oocytes (mean diameter was 900 ± 50 µm) were observed in about 85% of the stimulated females; however, ovulation occurred only in 35% of injected females.*

Final oocyte maturation (FOM) of fish treated with LHRH-a in combination with HCG showed two phases: early phase associated with lipid-droplet coalescence and germinal vesicle (GV) migration, and late phase included germinal vesicle breakdown (GVBD) and yolk-globule coalescence. Translucent ovulated eggs had completely coalesced lipid and yolk masses. The changes in the activity of gonadotropin (GTH), somatolactin (SL), adrenocorticotropin (ACTH) and melanin-stimulating hormone (MSH)-secreting cells associated with hormonal-induced FOM were studied immunocytochemically. Early FOM was associated with high synthetic activity of the GTH,

ACTH and MSH. During late FOM, the secretory activity of GTH, ACTH and MSH cells was increased as indicated by degranulation and vacuolization of these cells. At ovulation further increase in the secretory activity of the GTH and MSH was obtained, while the synthetic activity of the ACTH cells increased again. In addition, SL showed gradual release during induction of FOM and ovulation as reflected by degranulation and decrease in size of SL-immunoreactive cells. The results of this study point to the possible role of SL, ACTH and MSH, in addition to GTH, in the regulation of FOM and ovulation during spawning of ***L. ramada.***

INTRODUCTION

The thin-lipped grey mullet ***Liza ramada*** (Risso) is an important and attractive species for farming in sea, brackish and fresh water. In Egypt, fingerlings of ***Liza ramada*** are reared successfully in freshwater-polyculture ponds together with common carp, grass carp, silver carp and tilapia. However, the culture of mullet is closely dependent on the availability of their fry. The present fry collection stations from nature can not satisfy the increasing demand for the juveniles of ***Liza ramada***. Consequently, man must help the nature in such respects through developing and creating other efficient means. In other words, it is necessary to develop and stablish practical techniques for artificial propagation of mullet in order to substitute the fry collection from wild stocks.

Like females of many commercially important fishes, grey mullets fail to complete ovarian development and do not undergo final maturation (FOM), ovulation or spawning when reared in captivity (Mousa, 1994; Mousa and Mousa, 1997; Mousa and El-Gamal, 1999). To facilitate a steady supply of seeds, oocyte maturation and ovulation need to be induced. The failure of captive fish to undergo FOM is thought to be caused by the shortage of gonadotropin synthesis (Mousa and Mousa, 1997) and/or the lack of pituitary GTH release (Zohar, 1989) at the end of vitellogenesis process. The use of luteinizing hormone-releasing hormone (LHRH) or gonadotropin-releasing hormone (GnRH) and their analogs for induced pituitary GTH release and the initiation of FOM has gained considerable favor (Zohar, 1988; Breton ***et al.***, 1990; Mylonas ***et al.***, 1998). Even so, the response of fish to these peptides are not yet fully characterized and appear to be species specific (Peter, 1986).

Further research is needed, in particular, optimal application protocols need to be determined for each species individually.

Thus, the present work was planned to investigate the effect of human chorionic gonadotropin (HCG) and luteinizing hormone-releasing hormone analog (LHRH-a) on oocyte final maturation and ovulation in prespawning females of ***L. ramada*** acclimated to seawater (32 ‰). Also, to clarify the changes of the gonads and functional state of the secretory cells of the pituitary gland in the grey mullet (***L. ramada***) females during and after hormonal injections.

MATERIAL AND METHODS

Experimental fish and treatments:

The present experiments were carried out, during the natural spawning season of ***L. ramada***, in December 1998. The fish used in these experiments were obtained from Damietta seawater fish farm (semi-natural habitat). Mature females of ***Liza ramada*** with at least two-years-old (290-550 gm body weight) were selected on the basis of the presence of a soft, swollen abdomen and a protruding genital papillae. Selected females were maintained in 15,000-liter butyl rubber-lined ponds (15 fish/pond) equipped with seawater and constant aeration. Fish were anesthetized in a solution (100 mg/l) of tricaine methane sulfonate (MS-222, Sandoz) befor handling. The maturity and the oocyte diameters of the females were staged by obtaining *in vivo* biopsy of the ovary using a polyethelene cannula (Shehadeh ***et al.***, 1973). For the measurement of oocyte diameter, the oocytes were preserved in a solution of 1% formalin in 0.9 NaCl. All of the females used possessed oocytes whose diameters were greater than 600 µm (Table I). Females that were found to have the appropriate egg diameters were then removed to 1000-liter cement ponds equipped with constant running seawater (32‰) and aeration. Water temperature ranged from 16 to 18°C.

The experimental design and the protocol of hormonal injection are presented in table (I). Human chorionic gonadotropin (HCG) "pregnyl" (Nile Co. for Pharmaceuticals, Cairo, A.R.E.) was injected into the dorsal musculature of each fish adjacent to the dorsal fin (priming injection). Twenty-four hours later, second "resolving" injection of LHRH-a was administered.

Table I

The experimental design, the protocol of hormonal injection and the response of female *L. ramada* to hormonal treatment during the induction of final maturation and ovulation in saline water (32‰).

Experiment (Exp.)	No. of samples	Body weight (Kg) (Min-Max) Mean±SD	Initial oocyte diameter (μm) (Min-Max) Mean±SD	Dosage/Kg body weight			Duration from the day 0	Response		Characters of oocytes and remarks
				1st injection Day 0	2nd injection Day 1	3rd injection Day 2		GSI (%)	Egg diameter (μm)	
Exp. I (10/12)										
Control	4	(390-540) 470±62	(600-625) 606±11	Saline	Saline	-	36h	(17.3-19.4) 18.5±0.86	(600-625) 606±11	At prespawning stage (no response).
Injected Fish (Day 0 = Dec. 10)	2	382-456	600	15,000 IU HCG	20,000 IU HCG +	-	24h	22.5-22.7	650	More than ten oil droplets.
	5	(374-528) 455±70	(600-625) 610±14	//	200 μg LHRHa	-	24-36h	(24.8-27.9) 26.1±1.6	(700-850) 760±65	Migratory nucleus stage with less than ten oil droplets.
	4	(360-460) 390±41	(600-625) 619±11	//	//	-	32-36h	(24.5-27.9) 27±1.4	(825-925) 869±44.6	One oil droplets.
	2	450-530	625-650	//	//	-	34-36h	25-27	900-950	One oil droplet and yolk homogenization.
Exp. II (16/12)										
Control	4	(395-540) 456±55	(625-650) 638±13	Saline	Saline	-	60h	(18.9-21.2) 20±0.9	(625-650) 638±13	At prespawning stage (no response)
Injected Fish (Day 0 = Dec. 16)	3	(350-460) 420±50	(600-625) 617±12	15,000 IU HCG	30,000 IU HCG +	-	40-50h	(28.8-33.5) 30.4±2.2	(850-900) 883±24	One oil droplet and yolk homogenization.
	5	(330-530) 461±76	(600-650) 635±22	//	200 μg LHRHa	-	40-59h	(27.8-35) 31.7±2.9	(925-950) 940±14	Mature and transparent oocytes.
	2	380-540	625	//	//	-	52-59h	38.4-46.5	1000	Ovulated, stripped and the eggs fertilized.
Exp. III (22/12)										
Control	4	(370-520) 429±59	(600-650) 631±21	Saline	Saline	Saline	60h	(17.3-21) 19±1.4	(600-650) 631±21	At prespawning stage (no response)
Injected Fish (Day 0 = Dec. 22)	3	(290-550) 423±106	(600-650) 625±20	15,000 IU HCG	20,000 IU HCG +	15,000 IU HCG	40-52h	(29-35) 31±2.6	950	Mature and transparent oocytes.
	4	(310-480) 386±61	(625-650) 631±11	//	200 μg LHRHa	//	52-58h	(33.4-45) 40.1±4.7	1000	Ovulation was noticed.

The LHRH-a (desGly^{10}[D-Ala^{6}]LHRH ethylamide) was purchased from Sigma Chemical Co., St. Louis, MO. Three experiments were carried out. In the first experiment, pregnyl (HCG) was used as a priming injection at a dose of 15,000 IU/ kg body weight. This injection was followed, 24 hs later, by resolving injection of 20,000 IU HCG in combination with 200 µg LHRH-a /kg body weight. The second experiment was carried out as the first one except the resolving injection was 30,000 IU HCG in combination with 200 µg LHRH-a / kg body weight. In the third experiment, the priming injection was 15,000 IU HCG and the resolving one was 20,000 IU HCG in combination with 200 µg LHRH-a followed, 24 hs later, by third injection of 15,000 IU HCG / kg body weight. Females were transferred just after second injection to 150-liter spawning aquaria equipped with constant running seawater and aeration (Temp. 16-18 °C). An individual female was placed with two ripe males in each aquarium. Controls were treated with 0.5 ml saline /Kg B. wt. All injections were administered between 0800 and 1000h. At 24h, 36h and 48h five females were dissected out for histological and immunocytochemical examination. The gonadosomatic index (GSI%) was calculated and the ovarian stage was distinguished.

Histological examination:

The gonadal biopsies were examined immediately after collection. Oocyte diameter and morphology were examined microscopically. The diameter of at least 25 of the largest oocytes was recorded from each fish, and the position of the germinal vesicle (GV) was determined after clearing the cytoplasm for 10min with a 1:1:1 v/v methanol: ethanol:acetic acid solution (Crim and Glebe, 1990). Immediately after the dissection, the pituitary gland, attached to the brain, was fixed in Bouin's fluid for 48 h at 4 °C.The fixed brain and pituitaries were thereafter dehydrated through graded ethanol solution, cleared and embedded in paraplast (M.P.: 56-58 °C). Consecutive median sagittal sections of the pituitary gland were made at 5 µm thickness. Gonads were also removed and prepared for histological examination using the same method as for the pituitaries. Sections of gonads were stained with Harris`s alum hematoxylin (Conn,1953) and aqueous solution of eosin as a counterstain.

Immunocytochemical procedures:

Antibodies:

Rabbit antiserum directed against human adrenocorticotropic hormone (ACTH) was obtained from National Institute of Health. Anti-ovine luteinizing

hormone (o-LH) was obtained from Dr. K. Wakabayashi (Gunma University, Japan). Antiserum to chum salmon (***Oncorhynchus keta***) hormones, chum salmon GTH Iβ subunit (Lot No.8707), chum salmon GTH IIβ subunit (Lot No.8506) and chum salmon somatolactin (SL) (Lot No. 8906) were obtained from Dr. H. Kawauchi (School of Fisheries Science, Kitasato University, Iwate, Japan). Antiserum to coho salmon (***Oncorhynchus kisutch***) hormones; coho salmon GTH Iβ subunit (Lot No.8622) and coho salmon GTH IIβ subunit (Lot No.37.3.92) were gifts from Dr. P. Swanson (University of Washington, Seattle). Dr. B. Colman (Whitney Lab., University of Florida, Florida, USA) provided the antiserum to killifish (***Fundulus heroclitus***) hormones; GTH Iβ subunit (Lot No.M87014) and GTH IIβ subunit (Lot No.M87015).

Immunocytochemical reactions:

Immunocytochemical staining for the sections of the pituitary gland was generally performed with a vectastain ABC (Avidin-biotin peroxidase complex) Kit (Vector Laboratories) as described previously (Mousa and Mousa, 1999 a, b).

RESULTS

Histological examination of post-vitellogenic oocytes:

All females prior to hormonal injection contained vitellogenic oocytes varying in diameter from 600 to 650 µm. Vitellogenic oocytes had a centrally located germinal vesicle (GV), and their cytoplasm was filled mostly with yolk globules and lesser number of unstained lipid droplets scattered throughout the cytoplasm (Fig. 1a). No cortical alveoli were visible at this stage. Three layers; theca, granulosa and zona radiata (Fig. 1b) cover the vitellogenic oocyte. The zona radiata was a very prominent feature. At higher magnification, the striated nature of the zona radiata was clearly visible (Fig. 1b).

Final oocyte maturation of hormonal-injected fish:

In experiment (I), none of injected females reached final maturation, however 50% of females exhibited migratory nucleus stage (Table I). A high rate (85%) of final oocyte maturation (FOM) was achieved in both experiment (II) and (III). FOM was associated with gradual increase in oocyte diameter and GSI (Table I). In experiment (II), ovulation occurred only in 20% of the stimulated females, however in experiment (III) ovulation occurred in 57% of injected females (Table I). Based on the morphological and histological changes FOM was identified to two stages: early FOM included lipid-droplet

coalescence and germinal vesicle migration, and late FOM included germinal vesicle breakdown (GVBD) and yolk-globule coalescence.

Early final oocyte maturation:

The first morphological change after hormonal induction was the fusion and coalescence of the lipid droplets. At this stage the GV was located between the center and the periphery of the oocyte, and was almost always in association with the largest lipid mass (Figs. 1c and 1d). Lipid-droplet coalescence usually began in the center of the oocyte (Fig. 1c), and continued until a single lipid mass was formed in the center of the oocyte during late FOM (Fig. 2a). The germinal vesicle was still intact at the end of the early FOM stage and was localized adjacent to the lipid mass in the peripheral cytoplasm (Fig. 1d). The yolk globules were displaced to the periphery, but the yolk did not appear to undergo any morphological changes since the yolk globules maintained their size, structure and staining properties (Fig. 1d). The cortical alveoli were visible at this stage (Figs. 1e and 1f). At higher magnification, the striated nature of the zona radiata was still visible (Fig. 1e). Also prominent at this stage was the micropylar cell covering and projecting into the micropyle which was located above an area of the oocyte occupied mostly with cytoplasm and only few yolk globules, and adjacent to the germinal vesicle (Fig. 1f). Development to this stage (early FOM) from the time of hormonal treatment (Exp. I) of post-vitellogenic oocytes required 24 to 36 hs.

Late final oocyte maturation:

Once lipid-droplet coalescence was completed, germinal vesicle breakdown took place soon afterwards (Fig. 2a). By this time, a process began which is reffered to as 'clearing', since the oocytes appear progressively more translucent. By the time the oocytes were ovulated, their content was almost totally transparent (Fig.2d) and have a mean diameter of 900∀50 μm. Histological examination indicated that the 'clearing' effect was the result of the coalescence of the yolk globules into, eventually, a single mass (Fig. 2b). During yolk-globule coalescence, inclusions appeared inside the enlarging yolk mass (Fig. 2b). Later, these inclusions disappeared (Fig. 2c) and at the time of ovulation the oocyte contained a fully fused, uniform yolk mass surrounding the lipid mass (Fig. 2d). Late FOM lasted < 24 hs.

Hypophysial cell activity during induction of FOM:

The synthetic and secretory activities of different cell types of the pituitary gland of ***L. ramada*** showed differences in the immunoreactivity during the hormonal-induction of FOM and ovulation (Exp. II and III).

Gonadotrops (GTH cells):

Antiserum to chum salmon GTH IIβ bound strongly and specifically to the GTH cells (Figs. 3a-3d), but antiserum to GTH Iβ failed to bind the GTH cells. The GTH cells were also bound specifically by antiserum to the ovine luteinizing hormone (o-LH). In addition, the GTH cells showed negative immunoreactivity to antiserum to GTH Iβ and GTH IIβ subunits of **both** ***Oncorhynchus kisutch*** and ***Fundulus heteroclitus***. Both granulated and vacuolated GTH cells were found in post-vitellogenic females before induction of FOM (Fig. 3a). The synthetic activity of the GTH cells was increased during early FOM as reflected by devacuolization, granulation and the strong immunoreaction of the GTH cells (Fig. 3b). The secretory activity of the GTH cells was increased during late FOM as indicated by degranulation and vacuolization (Fig. 3c). At ovulation further increase in the secretory activity of the GTH cells was obtained, since the degranulated and vacuolated appearance of them (Fig. 3d).

Somatolactin (SL) cells:

The SL cells showed strong immunoreactivity to anti-chum salmon SL (Figs. 1e-1h). SL-immunoreactive cells of post-vitellogenic females were hypertrophied, intensely granulated and showed strong immunoreaction (Fig. 3e). SL showed gradual release during induction of FOM and ovulation (Figs. 3e-3h). The secretory activity of SL-immunoreactive cells was further increased during late FOM and ovulation as reflected by their degranulation and decrease in size (Figs. 3g and 3h).

Adrenocorticotrops (ACTH cells):

Antiserum to human ACTH bound strongly to the ACTH cells (Figs. 4a-4d). Most of the ACTH cells of post-vitellogenic females were degranulated (Fig. 4a). Early FOM was associated with high synthetic activity of the ACTH, since the intensely granulation and strong immunoreactivity of the ACTH cells (Fig. 4b). During late FOM, some of the ACTH became degranulated on account of the discharge of their secretory contents (Fig. 4c). Thereafter, the synthetic

activity of the ACTH cells increased again as indicated by hypertrophy, intensely granulation and strong immunoreaction of them. (Fig. 4d).

Melanotrops (MSH cells):

The melanin-stimulating hormone (MSH) secreting cells showed cross-reaction with anti-human ACTH (Figs. 4e-4h). Most of the MSH cells of post-vitellogenic females were degranulated (Fig. 4e). The synthetic activity of MSH was augmented during early FOM, as reflected by hypertrophy, accumulation of a large quantity of immunoreactive granules and strong immunoreactivity of MSH cells (Fig. 4f). Thereafter, MSH showed gradual release during late FOM and ovulation (Figs. 4g and 4h). The secretory activity of MSH cells was further increased during ovulation, since the degranulated appearance of them. (Fig. 4h).

DISCUSSION

Regarding oocyte cytology, thin-lipped grey mullet ***Liza ramada*** is similar its relatives the striped grey mullet ***M. cephalus*** (Mousa, 1994), the European sea bass ***Dicentrarchus labrax*** (Fausto ***et al.***, 1994), the white perch ***Morone americana*** (Jackson and Sullivan, 1995) and the white bass ***Morone chrysops*** (Mylonas ***et al.***, 1997a). At the beginning of the spawning season, ***Liza ramada*** ovary contained fully vitellogenic oocytes varying in diameter from 600 to 650 µm, a characteristic in agreement with its one-batch, synchronous spawning strategy.

A high rate (70%) of final oocyte maturation (FOM), was achieved utilizing the pregnyl (HCG) as a priming injection at a dose of 15,000 IU/kg body weight followed, 24 hs later, by resolving injection of 30,000 IU HCG in combination with 200 µg LHRH-a/kg body weight. A variety of hormonal treatments was used for inducing final maturation and changeover of ***M. cephalus*** female with vitelline oocytes (tertiary yolk stage) to spawning condition with much higher doses: 50,000-80,000 IU HCG/kg body weight (Kuo ***et al.***, 1973), 28-48 mg of fresh mullet pituitaries plus 10,000-80,000 IU chorionic gonadotropin/kg of body weight (Kulikova and Gnatchenko, 1987) and 20 mg carp pituitary homogenate/kg body weight of fish, followed by 200 µg LHRH-a/kg of fish (Lee ***et al.***, 1987; Suzuki ***et al.***, 1991).

The absence of an increase in oocyte diameter and GSI of untreated fish during the experiment, suggesting that no further vitellogenic growth or maturation can take place at this time, without hormonal stimulation. On the contrary, the process of FOM was associated with significant and continuous increases in oocyte diameter (about 50% of the initial diameter) and GSI (50-100% of the initial GSI). Similar increase in oocyte size during FOM has been observed for many fishes, and has been attributed primarily to water uptake (Wallace and Selman, 1981). With the onset of FOM, a Na^+, K^+-ATPase-driven increase in the concentration of inorganic ions inside the oocyte begins, resulting in the extensive water uptake (Greeley ***et al.***, 1991; LaFleur and Thomas, 1991). In addition, proteolytic cleavage of the yolk proteins increases the concentration of free amino acids (Wallace and Selman, 1985; Greeley ***et al.***, 1986; Matsubara ***et al.***, 1995), causing hydration of the maturing oocyte by increasing the intracellular osmotic pressure (Thorsen and Fyhn, 1991).

Early FOM began with lipid droplet coalescence, which occurred concomitantly with GV migration. The GV is always attached to the largest lipid droplet and appears to be pushed to the periphery by the mere enlarging of the coalesced lipid-mass, a characteristic similar to that of ***Dicentrarchus labrax***, ***M. cephalus*** and ***Morone saxatilis*** (Alvarino ***et al.***, 1992; Mousa, 1994; Mylonas ***et al.***, 1997b).

The late FOM phase consisted of GVBD and yolk-globule coalescence. Once lipid-droplet coalescence was compeleted, GV breakdown (GVBD) took place soon afterwards. By this time, a process began which is reffered to as "clearing", since the oocytes appear progressively more translucent. The process of "clearing" was identified histologically to be the result of yolk globule coalescence into, eventually, a single mass. Similar observations were recorded for ***M. cephalus*** (Mousa, 1994). Fusion of yolk globules is common in many fishes, in most species it happens during FOM, however in some it occurs during vitellogenesis (Wallace and Selman, 1981). The staining properties of the yolk changed at the beginning of its coalescence and crystalline granules appeared in the yolk globules. We observed these changes in morphology and staining were brought about by proteolysis of the yolk proteins in ***M. cephalus*** (Mousa, 1994). This process is very common in fish, especially in marine species that spawn pelagic eggs (Wallac and Selman, 1985; Greeley ***et al.***, 1986; Matsubara ***et al.***, 1995).

Immunocytochemically the synthetic and secretory activities of the GTH in the pituitary gland of post-vitellogenic females of ***Liza ramada*** were not enough to produce GTH elevation. In the absence of a GTH elevation, the shift in steroidogenic production from estradiol-17β (E_2) to testosterone (T) and then to maturation inducing steroid (MIS), which takes place during FOM (Nagahama ***et al.***, 1994), did not occur and the fish failed to undergo FOM. By exogenous hormone (HCG) treatment, in ***Liza ramada*** as in other species (Patino and Thomas, 1990), GTH can induce GV migration and lipid coalescence (early FOM), as well as increase in the synthesis of GTH as reflected by devacuolization and granulation of GTH cells. However, endogenous GTH IIβ released by LHRHa treatment, in combination with exogenous GTH (HCG), can induce late FOM and ovulation. Degranulation and vacuoliztion of GTH cells reflect the releasing of GTH from pituitary during late FOM and ovulation. Similar immunocytochemical observation was obtained in ***M. cephalus*** (Mousa and Mousa, 1997). In addition, sustained elevations of GTH in response to GnRHa treatments have been documented in ***Oncorhynchus mykiss*** (Breton ***et al.***, 1990), ***Morone chrysops*** (Mylonas ***et al.***, 1997a) and ***Morone saxatilis*** (Mylonas ***et al.***, 1998).

In post-vitellogenic females, SL cells were enlarged and frequently more granulated, suggesting high synthetic activity of SL. During induction of FOM and ovulation, SL showed gradual release as reflected by degranulation and decrease in size of SL-immunoreactive cells. This may indicate the involvement of SL in energy mobilization related to reproduction, since the biological events during spawning (FOM and ovulation) require a great deal of energy. Activated SL cells identified immunocytochemically, were seen also in spawning ***Oncorhynchus nerka***, ***O. keta*** and ***O. tshawytscha*** (Olivereau and Rand-Weaver, 1994a,b), ***Oreochromis niloticus*** (Mousa and Mousa, 1999a) and ***M. cephalus*** (Mousa and Mousa, 1999b). In addition, the present immunocytochemical results received a good support from biochemical studies, which showed that SL levels have been shown to increase during spawning migration of ***Oncorhynchus keta*** (Kakizawa ***et al.***, 1995b) and ***O. kisutch*** (Rand-Weaver ***et al.***, 1992; Rand-Weaver and Swanson, 1993), and in stressed and exercised ***O. mykiss*** (Kakizawa ***et al.***, 1995a).

The ACTH secreted by the RPD controls interrenal synthesis and release of cortisol (Henderson and Garland, 1980) and activated by several factors such as stress, temperature, pollution, etc. (Leloup-Hatey, 1985; Balm ***et al.***, 1994). In

the present study, handling and stress induced by hormonal injection caused an activation of the ACTH cells as indicated by the strong immunoreactivity of them. This may be caused an increase in the release of cortisol. Jalabert and Fostier (1984) reported that high cortisol levels enhance the GTH stimulation of 17α,20β-Dihydroxyprogesterone secretion from mature oocyte follicles of ***Oncorhynchus mykiss***, this being in line with earlier *in vitro* studies suggesting stimulation by cortisol of oocyte maturation of ***O. rhodurus*** (Young ***et al.***, 1982). Also, an activation of MSH cells was obtained during induction of FOM and ovulation in ***Liza ramada***. This may be due to the increased release of SL and/or changes in background (Zhu and Thomas, 1996). The MSH cells are responsible for the colour background adaptation (Van Eys, 1980; Zhu and Thomas, 1996).

Thus the present results indicated that the use of pregnyl (HCG) as a priming injection at a dose of 15,000 IU/kg body weight followed, 24 hs later by resolving injection of 30,000 IU HCG in combination with 200 µg LHRHa/kg body weight, proved to be effective in inducing final oocyte maturation and ovulation in ***L. ramada*** at a time of 50 to 60 hs after hormonal injection. The data obtained on alteration in the functional state of the secretory cells of the pituitary gland in ***L. ramada*** before and after hormonal injections presuppose a connection between these changes and the nature of the response of females to the stimulation of spawning when other conditions are conductive to maturation and the possibility of effecting economy in the use of hormones for injections by a combination of the ecological stimulation of maturation.

Besides, the present results have urged and stimulated the need for further studies to know the possible reasones for fish death during ovulation and before oviposition, and optimizing the induced spawning technique of ***L. ramada***.

EXPLANATION OF FIGURES

Fig.(1): Transverse sections of ovaries of ***L. ramada*** stained with Harris's hematoxylin and eosin:

a- Ovary of post-vitellogenic female before injection of hormones, showing tertiary yolk oocyte which has central-located nucleus (N), ooplasm impregnated with yolk globules (Y) and lesser number of lipid droplets (L) distributed in the ooplasm. X100.

b- A magnified portion of (a) showing the wall of the tertiary yolk (post-vitellogenic) oocyte; theca layer (T), granulosa layer (G) and zona radiata (Z). Cortical ooplasm (OP) and yolk globules (Y) are also designated. X1000.

c- Ovary of injected female, undergoing early final oocyte maturation (FOM), showing the beginning of lipid droplets (L) coalescence and migration of germinal vesicle (GV) to the peripheral cytoplasm. X100.

d- Ovary of injected female, 24 hs from the priming injection, showing the advanced lipid-droplets (L) coalescence and the peripheral position of the GV. X100.

e- A magnified portion of (d) showing the wall of the periphery GV oocyte (pGV); theca (T), granulosa (G) and zona radiata (Z). The cortical alveoli (C) are apparent for the first time as they line against the cortical ooplasm (OP). X1000.

f- A section through a pGV oocyte, at high magnification, showing the zona radiata (Z) at the site where the micropylar cell (MC) penetrates the micropyle. The cytoplasm underneath is relatively void of yolk-globules. Cortical alveoli (C) and germinal vesicle (GV) are also illustrated. X400.

Fig. (2): Histological sections (a-c) of oocytes from injected female of ***L. ramada***, at various stages of late FOM, stained with Harris's hematoxylin and eosin.

a- Beginning of late FOM, with lipid droplets (L) completely coalesced, yolk globules displaced to the peripheral cytoplasm and breakdown of GV. X100.

b- A magnified portion of oocyte undergoing late FOM, showing advanced yolk-globules (Y) coalescence. Also, lipid mass (L) is represented. X400.

c- A magnified portion of oocytes immediately prior to ovulation with follicle (F) still intact. The yolk globules are almost completely coalesced. X400.

d- Translucent ovulated egg with completely coalesced lipid (L) and yolk (Y) masses. X100.

Fig. (3): Sagittal sections of the pituitary gland of *L. ramada*, during induction of FOM and ovulation, immunostained with (a-d) antiserum to chum salmon gonadotropin (GTH IIβ), and (e-h) antiserum to chum salmon somatolactin (SL). X1000.

a- Immunostained GTH cells of post-vitellogenic female before induction of FOM. Note the presence of both granulated (arrowheads) and vacuolated (arrows) cells.

b- Immunostained GTH cells of injected female, undergoing early FOM, became devacuolated & granulated, and having strong immunoreactivity.

c- GTH cells of injected female, undergoing late FOM, exhibiting secretory vacuoles (V). Also, granulated (arrowheads) and degranulated (arrows) cells are illustrated.

d- Immunostained GTH cells of ovulated female having many large secretory vacuoles (V) and less immunoreactive granules.

e- Immunostained somatolactin (SL) cells of post-vitellogenic female, before induction of FOM, exhibiting large sizes and heavily impregnated with immunoreactive granules.

f- SL-immunoreactive cells of injected female, undergoing early FOM, having different sizes. Note the degranulated appearance of some SL cells (arrows).

g- Immunostained SL cells of injected female, undergoing late FOM. The SL cells are small in size and number, and degranulated. Note some of the SL cells with moderately immunoreaction.

h- SL-immunoreactive cells of ovulated female. Note the increase of secretory activity as reflected by degranulation of most of SL cells (arrows).

Fig. (4): Sagittal sections of the pituitary gland of ***L. ramada***, during induction of FOM and ovulation, immunostained with (a-d) antiserum to human ACTH, & (e-h) cross reacted with antiserum to human ACTH. X1000.

a- Immunostained ACTH cells of post-vitellogenic female, before induction of FOM. Note the degranulated appearance of most ACTH cells (arrows).

ii- ACTH-immunoreactive cells of injected female, undergoing early FOM, became granulated and having strong immunoreactivity.

c- Immunostained ACTH cells of injected female, undergoing late FOM. Note the degranulated appearance of some ACTH cells (arrows).

d- ACTH- immunoreactive cells of ovulated female, exhibiting large sizes and heavily impregnated with immunoreactive granules.

e- MSH cells of post-vitellogenic female, before induction of FOM, having degranulated appearance and with moderately immunoreaction.

f- MSH cells of injected female, undergoing early FOM, became granulated and having strong immunoreactivity.

g- MSH cells of injected female, undergoing late FOM. Note the release of MSH as reflected by degranulation.

h- MSH cells of ovulated female. Note the increase of secretory activity as reflected by degranulation of most of MSH cells (arrows).

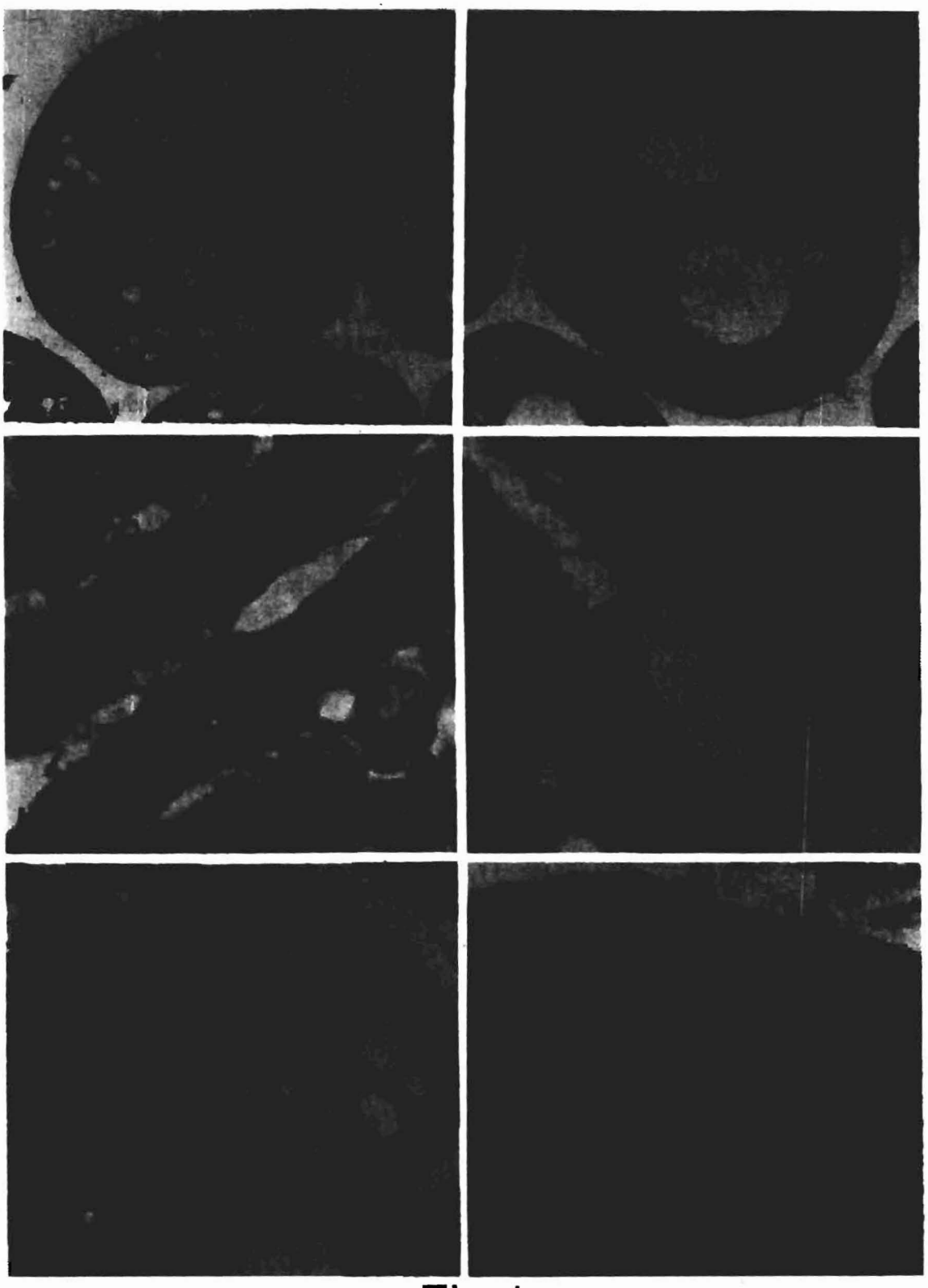


Fig.1

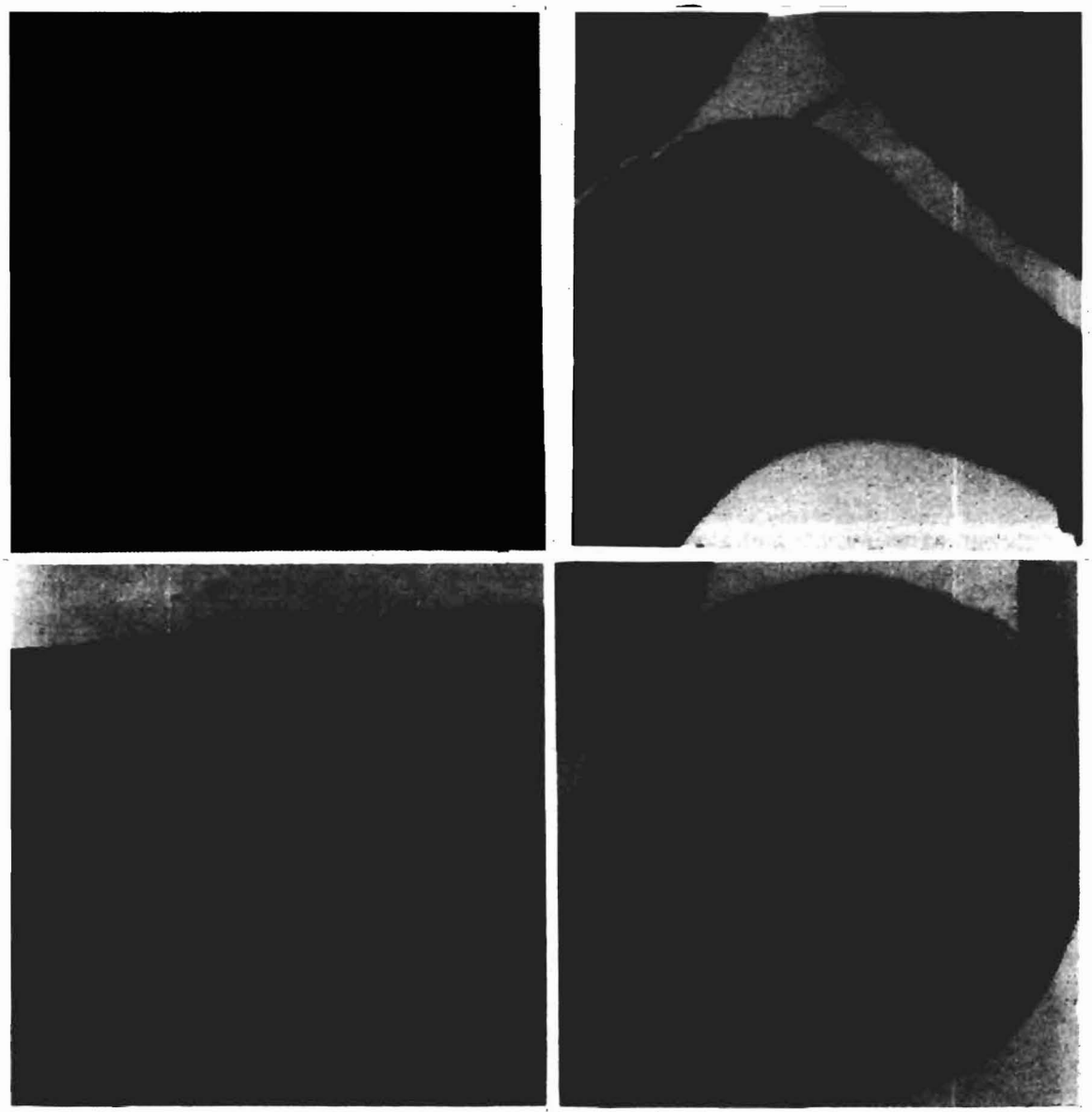


Fig.2

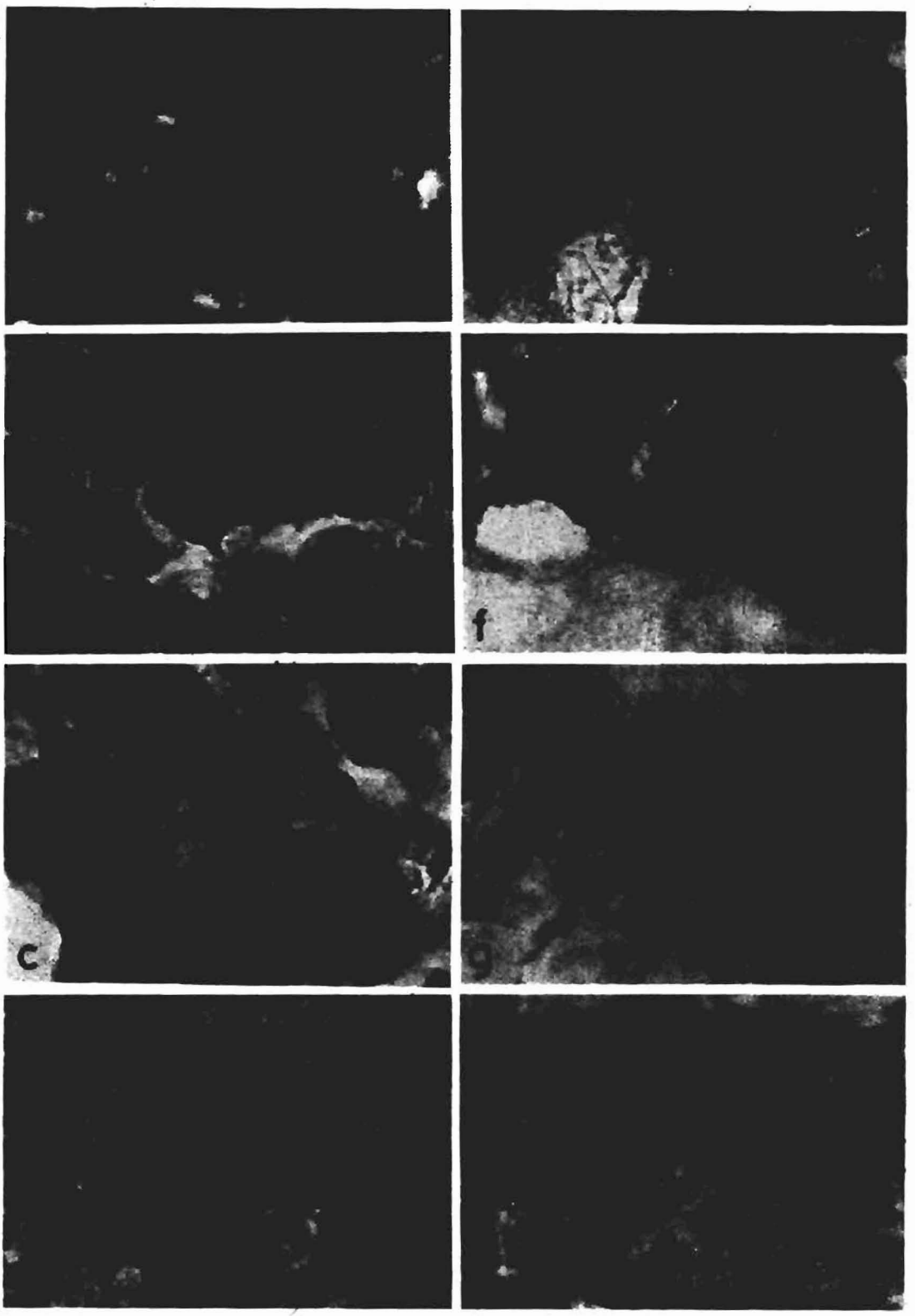
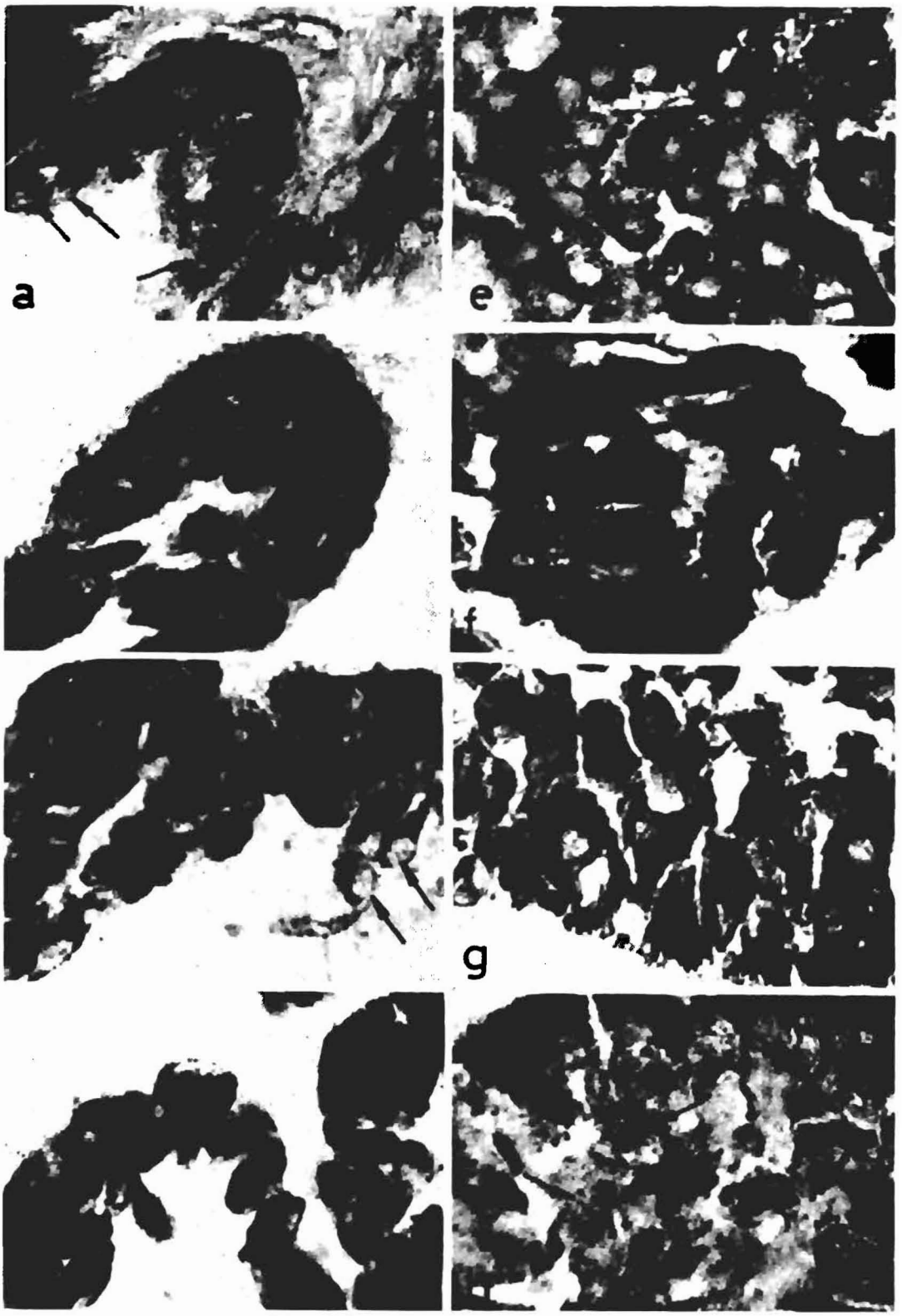


Fig.3


a
e
f
g

ACKNOWLEDGMENTS

The authors are extremely grateful to Drs. K.Wakabayashi, H.Hideshi Kawauchi and B. Colman for kindly donating the antisera used in this study.

REFERENCES

Alvarino, J.M.R.; Carrillo, M.; Zanuy, S.; Prat, F. and Mananos, E., 1992. Pattern of sea bass oocyte development after ovarian stimulation by LHRHa. J. Fish Biol. *41*: 965-970.

Balm, P. H. M., Pepels; P., Helfrich, S., Hovens, M. L. M. and Wendelaar Bonga, S.E., 1994. Adrenocorticotropic hormone in relation to interrenal function during stress in Tilapia (***Oreochromis mossambicus***). Gen. Comp. Endocrinol. *96*: 347-360.

Breton, B.; Weil, C.; Sambroni, E. and Zohar, Y., 1990. Effects of acute versus sustained administration of GnRHa on GtH release and ovulation in the rainbow trout, ***Oncorhynchus mykiss***. Aquaculture *91*: 371-383.

Conn, H. J., 1953. ABiological stains@ (Williams and Wilkins Company, Baltimore).

Crim, L.W. and Glebe, B.D., 1990. Reproduction. In Methods for Fish Biology (Schreck, C.B. and Moyle, P.B., eds), pp. 529-553. Bethesda, Maryland: American Fisheries Society.

Fausto, A.M.; Carcupino, M.; Scapigliati, G.; Taddei, A.R. and Mazzini, M., 1994. Fine structure of the chorion and micropyle of the sea bass egg ***Dicentrarchus labrax*** (Teleostea, Percichtydae). Bollettiuo di Zoologia *61*: 129-133.

Greeley, M.S., Jr; Calder, D.R. and Wallace, R.A., 1986. Changes in teleost yolk proteins during oocyte maturation: correlation of yolk proteolysis with oocyte hydration. Comp. Biochem. Physiol. *84B*: 1-9.

Greeley, M.S., Jr; Hols, H. and Wallace, R. A., 1991. Changes in size, hydration and low molecular weight osmotic effectors during meiotic maturation of *Fundulus* oocytes in vivo. Comp. Biochem. Physiol. *100A*: 639-647

Henderson, I. W. and Garland, H.O., 1980. The interrenal gland in pisces: Part 2.Physiology. In AGeneral, Comparative and Clinical Endocrinology of the Adrenal Cortex (I.Chester-Jones and I.W. Henderson, Eds.), Vol. *3*., pp. 473-523. Academic Press, New York.

Jackson, L.F. and Sullivan, C.V., 1995. Reproduction of white perch: the annual gametogenic cycle. Trans. Amer. Fish. Soc. *124*: 563-577.

Jalabert, B. and Fostier, A., 1984. The modulatory effect *in vitro* of estradiol-17*ß*, testosterone or cortisol on the output of 17*a*-hydroxy-20*ß*-dihydroxyprogesterone by rainbow trout (***Salmo gairdneri***) ovarian follicles stimulated by the maturational gonadotropin s-GTH. Reprod. Nutr. Dev. *24*: 127-136.

Kakizawa, S.; Kaneko, T.; Hasegawa, S. and Hirano, T., 1995a. Effects of feeding, fasting, background adaptation, acute stress, and exhaustive exercise on the plasma somatolactin concentrations in rainbow trout. Gen. Comp. Endocrinol. *98*: 137-146.

Kakizawa, S.;Kaneko,T.; Ogasawara, T. and Hirano, T., 1995b. Changes in plasma somatolactin levels during spawning migration of chum salmon (***Oncorhynchus keta***). Fish Physiol. Biochem. *14*: 93-191.

Kulikova, N.I. and Gnatchenko, L.G., 1987. Response of prespawning female Black Sea striped mullet, ***Mugil cephalus***, to chorionic gonadotropin. J. Ichthyology *27 (3)*: 44-53.

Kuo, C.M.; Nash, C.E. and Shehadeh, Z.H., 1973. Induced spawning of captive grey mullet (***Mugil cephalus*** L.) females by injection of human chorionic gonadotropin. Aquaculture *1*: 429-432.

LaFleur, G.J. and Thomas, P., 1991. Evidence for a role of Na^+, K^+-ATPase in the hydration of Atlantic croaker and spotted seatrout oocytes during final maturation. J. Exp. Zool. *258*: 126-136.

Lee, C.S., Tamaru, C.S., Miyamoto, G.T., and Kelley, C.D., 1987. Induced spawning of grey mullet (***Mugil cephalus***) by LHRH-a. Aquaculture *62*: 327-336.

Leloup-Hatey, J., 1985. Environmental effects on the fish interrenal gland. In A The Endocrine System and the Environment@. (B. K. Follett; S. Ishii, and A.Chandola Eds), pp. 13-21. Jpn. Sci. Soc. Press, Tokyo / Springer-Verlag, Berlin.

Matsubara, T.; Adachi, S.; Ijiri, S. and Yamauchi, K., 1995. Changes of lipovitellin during *in vitro* oocyte maturation in Japanese flounder ***Paralichthys olivaceus***. Fisheries Science *61*: 478- 481.

Mousa, M.A., 1994. Biological studies on the reproduction of mullet (***Mugil cephalus*** L.) in Egypt. Ph.D. Thesis. Ain Shams University. pp278.

Mousa, M.A. and El-Gamal, A.E., 1999. Experimental study on the ovarian development and the gonadotropic cell activity in thin-lipped grey mullet, ***Liza ramada*** (Risso) in captivity. J.Egypt. Ger. Soc. Zool. *30* (c):51-65.

Mousa, M.A. and Mousa, S.A., 1999a. Immunocytochemical study on the localization and distribution of the somatolactin cells in the pituitary gland and the brain of ***Oreochromis niloticus*** (Teleostei, Cichlidae). Gen. Comp. Endocrinol. *113*: 197-211.

Mousa, M.A. and Mousa, S.A., 1999b. Possible involvement of somatolactin in the regulation of sexual maturation and spawning of ***Mugil cephalus***. Bull. Nat. Inst. Oceanogr. & Fish., ARE, 25: 289 – 309.

Mousa, S.A. and Mousa, M.A., 1997. Immunocytochmical studies of the gonadotropic cells in the pituitary gland of female mullet, ***Mugil cephalus*** during the annual reproductive cycle in both natural habitat and captivity. J. Egypt. Ger. Soc. Zool. *23* (c): 17-36.

Mylonas, C.C.; Magnus, Y.; Gissis, A.; Klebanov, Y. and Zohar, Y., 1997a. Reproductive biology and endocrine regulation of final oocyte maturation of captive white bass. J. Fish Biol. *51*: 234-250.

Mylonas, C.C.; Woods, L.C., III and Zohar, Y., 1997b. Cyto-histological examination of post-vitellogenesis and final oocyte maturation in captive-reared striped bass. J. Fish Biol. *50*: 34-49.

Mylonas, C.C.; Woods, L.C.,III; Thomas, P. and Zohar, Y., 1998. Endocrine profiles of female striped bass (***Morone saxatilis***) in captivity, during postvitellogenesis and induction of final oocyte maturation via controlled-release GnRHa-delivery systems. Gen. Comp. Endocrinol. *110*: 276-289.

Nagahama, Y.; Yoshikoni, M.; Yamashita, M. and Tanaka, M., 1994. Regulation of oocyte maturation in fish. In Fish Physiology, Volume XIII, Molecular Endocrinology of Fish (Farrell, A.P. and Randall, D.J., eds), pp. 393-439. San Diego, California: Academic Press.

Olivereau, M. and Rand-Weaver, M., 1994a. Immunocytochemical study of the somatolactin cells in the pituitary of pacific salmon, ***Oncorhynchus nerka***, and ***O. Keta*** at some stages of the reproductive cycle. Gen. Comp.Endocrinol. *93*: 28-35.

Olivereau, M. and Rand-Weaver, M., 1994b. Immunoreactive cells in the pituitary of young, migrating and spent chinook salmon (***Oncorhynchus tshawytscha***). Fish Physiol. Biochem. *13*: 141-151.

Patino, R. and Thomas, P., 1990. Effects of gonadotropin on ovarian intrafollicular processes during the development of oocyte maturational competence in a teleost, the Atlantic croaker: evidence for two distinct stages of gonadotropic control of final oocyte maturation. Biol. Reprod. *43*: 818-827.

Peter, R.E., 1986. Structure-activity studies on gonadotropin releasing hormone in teleosts, amphibians, reptiles, and mammals. In: Comparative Endocrinology: Developments and Directions (C. L. Ralph; Editor),. Alan R. Liss, New York, NY, pp. 75-93.

Rand-Weaver, M.; Swanson, P.; Kawauchi, H. and Dickhoff, W.W., 1992. somatolactin, a novel pituitary protein: purification and plasma level during reproductive maturation of coho salmon. J. Endocrinol. *133*: 393 - 403 .

Rand-Weaver, M. and Swanson, P., 1993. Plasma somatolactin levels in coho salmon (***Oncorhybchus Kisutch***) during smoltification and sexual maturation. Fish physiol. Biochem.*11*:175-182.

Shehadeh, Z.H.; Kuo, C.M. and Milisen, K. (1973): Validation of an *in vivo* method for monitoring ovarian development in the grey mullet (***Mugil cephalus***). J. Fish Biol. *5*: 489-496.

Suzuki, K., Ashina, K., Tamaru, C.S., Lee, C.S., and Inano, H., 1991. Biosynthesis of 17-*a*, 20*ß*- Dihydroxy-4-pregnen-3-one in the ovaries of grey mullet (***Mugil cephalus***) during induced ovulation by carp pituitary homogenates and an LHRH analogue. Gen. Comp. Endocrin. *84*: 215-221.

Thorsen, A. and Fyhn, H.J., 1991. Osmotic effectors during preovulatory swelling of marine fish eggs. In Proceedings of the Fourth International Symposium on Reproductive Physiology of Fish (Scott, A.P.; Sumpter, J.P.; Kime, D.E. and Rolfe, M.S., eds), pp. 312-314. Sheffield: Fish Symposium 1991.

Van Eys, G.J.J.M., 1980. Structural changes in the pars intermedia of the cichlid teleost ***Sarotherodon mossambicus*** as a result of background adaptation and illumination : I.The MSH-producing cells.Cell Tissue Res. *208*: 99-110.

Wallace, R.A. and Selman, K., 1981. Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist *21*: 325-343.

Wallace, R.A. and Selman, K., 1985. Major protein changes during vitellogenesis and maturation of *Fundulus* oocytes. Dev. Biol. *110*: 492-498.

Young, G.; Kagawa, H. and Nagahama, Y., 1982. Oocyte maturation in the amago salmon (***Oncorhynchus rhodurus***): *in vitro*, effects of salmon gonadotropin, steroids, and cytoketone (an inhibitor of 3β-hydroxy- steroid dehydrogenase). J. Exp. Zool. *224*: 265-275.

Zhu, Y. and Thomas, P., 1996. Elevations of somatolactin in plasma and pituitaries and increased alpha-MSH cell activity in red drum exposed to black background and decreased illumination. Gen. Comp. Endocrinol. *101*: 21-31.

Zohar, Y., 1988. Gonadotropin releasing hormone in spawning induction in teleosts: basic and applied considerations. In Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics (Zohar, Y. and Breton, B., eds), pp. 47-62. Paris: INRA Press.

Zohar, Y., 1989. Fish reproduction: its physiology and artificial manipulation. In Fish Culture in Warm Water Systems: Problem and Trends (Shilo, M. and Sarig, S., eds), pp. 65-119. Boca Raton: CRC Press.