



## Biochemical evaluation of the carp hypophyseal homogenate as spawning stimulator in teleosts

Mohamed F. Kora<sup>1</sup>, Mostafa A. Mousa<sup>1\*</sup> and Noha A. Khalil<sup>2</sup>

1. Fish Reproduction Laboratory, National Institute of Oceanography and Fisheries, Alexandria.
2. Fish Reproduction Laboratory, National Institute of Oceanography and Fisheries, Qanater, Egypt.

\*Corresponding Author: [mostafa\\_mousa2002@yahoo.com](mailto:mostafa_mousa2002@yahoo.com)

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### ABSTRACT

Hypophyseal homogenate of common carp is considered one of the different hormonal sources for spawning induction in the teleosts. The present work aimed to measure the hormonal concentration of the hypophysis in different carp species, which can be used as a source of hormones for spawning induction in teleosts. The hypophysis of different mature carp species (both males and females); namely, common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) were evaluated biochemically to discuss their hormonal source. Five hormones in the pituitary gland, including: adrenocorticotropin hormone (ACTH), prolactin hormone (PRL), growth hormone (GH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were analyzed using electrochemiluminescence immunoassay (ECLIA). Compared to female carps, the hypophysis homogenate of male carps recorded lower levels of the measured hormones. The biochemical results showed that the hypophysis homogenate of *H. molitrix* showed higher levels of 426±13, 118±6, 2355±47, and 1005±35 ng/mg for PRL, GH, LH, and FSH, respectively. Whereas, lower level of ACTH (5±0.5 ng/mg) was recorded compared to those obtained in *C. carpio* and *C. idella*. However, the concentrations of those hormones recorded the lowest values in male carpfish, while the concentration of ACTH hormone was higher in male common carp, recording 13±1.2 ng/mg. The present biochemical observations revealed the suitability and effectiveness of the cheap silver carp hypophysis as a spawning inducer in fishes.

### INTRODUCTION

Reproduction in teleosts, as in higher animals, is normally controlled by the reproductive hormones secreted from the brain and the hypophysis, which secrete gonadotropins (GTHs) to manage the development of gonads in both sexes. Reproductive hormones such as fish hypophysis homogenate, human gonadotropin and gonadotropin releasing hormone are usually used for induction of gonadal development, maturation, and spawning in various fishes (Austriano *et al.*, 2006; Mousa, 2010; Mehdi & Ehsan, 2011; Mousa *et al.*, 2018a).

Recently, with the increase in the prices of synthetic hormones, the tendency to use the pituitary gland extract has witnessed a great increase as a stimulator for spawning in fish (Mehdi & Ehsan, 2011; Mousa *et al.*, 2018a). However, disadvantages have been detected with respect to this method, such as the lack of knowledge about the contents of different hormones extracted from the pituitary gland, which depend on the stage of fish maturity from which the gland is extracted (Mousa *et al.*, 2018a).

Two types of gonadotropins, GTH I and GTH II, have been observed and identified in teleosts (Suzuki *et al.*, 1988). GTH I and GTH II are similar to follicular stimulating hormone (FSH) and luteinizing hormone (LH), respectively (Swanson *et al.*, 1991). It is known that in teleosts, the GTH I and GTH II play similar roles to those found in higher animals for FSH and LH, respectively; as observed by the plasma hormonal profiles in rainbow trout females for FSH and LH (Prat *et al.*, 1996), which imitate the oestrus cycle in mammals.

Hormones of ACTH, GH, and PRL have a key role in the adaptation of fish to different salinities (Mousa *et al.*, 2015). In addition, ACTH plays a major role in the stress response in spawned fish (Pickering, 1981; Leloup-Hatey, 1985; Balm *et al.*, 1994; Mousa *et al.*, 2015; Mousa *et al.*, 2018a). Notedly, the gonadotropins (I, II), similar to the hormone (LH, FSH) in mammals, are the basis for stimulating the gonads in the secretion of sex hormones for the development and maturation of the gonads (Swanson, 1991; Prat *et al.*, 1996). Thus, it was necessary to define the concentration of those hormones in different carp fish to find out the most appropriate types of carp in terms of hormone amounts needed for maturation and ovulation processes, and evaluate them economically with regard to their cost. Most of the previous studies used the hypophysis of common carp and avoided other carp species. In our recent work, we used the hypophysis of silver carp which gave higher rate of ovulation in *L. ramada* compared to those of common carp hypophysis or human chorionic gonadotropin (Mousa *et al.*, 2018a).

Using of the immunocytochemistry with piscine and mammalian hormones antisera could be used to characterize and identify the different hypophyseal hormones in many teleosts (García-Hernández *et al.*, 2002; Mousa, 2002; García Ayala *et al.*, 2003; Mousa & Mousa, 2006; Mousa *et al.*, 2006; Shimizu *et al.*, 2008; Ohkubo *et al.*, 2010). We have previously conducted immunocytochemical studies of the hypophysis on different carp fish available in Egypt (common, grass and silver carps). It was concluded that, the silver carps are the most appropriate fish from the anatomical point of view, and they contain hormones suitable for sexual maturity and induction of spawning in fishes. It is also less expensive than other carp species (Mousa *et al.*, 2018b).

To obtain the suitability of using hypophysis for other carp species, the hormone types and quantities in the hypophysis of the different species of carp (*C. carpio*, *C. idella*, and *H. molitrix*) should be evaluated. Therefore, this study was designed to measure the content of ACTH, GH, PRL, LH, and FSH in the hypophysis of different species of carp (*C. carpio*, *C. idella* and *H. molitrix*) to obtain the most appropriate species from a biochemical point of view and in terms of the cost of pituitary extract with biochemical methods using antibodies against human hormones.

## MATERIALS AND METHODS

### Pituitary gland sampling

Both mature 2-year-old males and females of common, grass and silver carp were collected, from the research farm at El-Serw, during the various stages of maturity. At the research station of El-Matareyya, the pituitary glands necessary for biochemical analysis were taken at different stages of maturation, prepared, and stored at  $-70^{\circ}\text{C}$  until use. At the end of all samples collection, biochemical analysis was estimated.

Before the dissection of samples, carp fishes were narcotized in clove oil at a dose of 40 mg/l of water (Mousa, 2004), and then the hypophysis was quickly segregated and dried in acetone. The acetone dried carp pituitary powder was weighed, homogenized in 1.0 ml of 0.08 M barbital buffer pH 8.6 (Crim *et al.*, 1981) and stored at  $-70^{\circ}\text{C}$  until biochemical analyses.

### Immunoassay procedure

The concentrations of the ACTH, GH, PRL, LH, and FSH were determined by electrochemiluminescence (Elecsys 2010; Roche Diagnostics, Mannheim, Germany) using the Elecsys Kits. The assay of hormones was obtained by microplate reader set to 450 nm.

### Hormonal ELISA Kits

Different types of kits were used for the determination of hormones as follows:

- 1- Elecsys ACTH (Catalog Number: 07026684190).
- 2- Elecsys prolactin (Catalog Number: 03203093190).
- 3- Elecsys growth hormone (GH) (Catalog Number: 5390125).
- 4- Elecsys 11732234122 LH
- 5- Elecsys 11775863122 FSH

### Statistical analysis

Differences between treatments were tested by one-way ANOVA using the treatment as factor of variance (Bailey, 1981). Tukey's test was used to identify the significance of different groups. Statistical significance was accepted at  $P < 0.05$ .

## RESULTS

### Hormonal levels in the hypophysis of carp

Table (1) and Figs. (1, 2) reveal that, the hormonal levels in the hypophysis of males of common, grass and silver carps were relatively lower than those in the females of these fishes.

#### ACTH levels

ACTH levels in males of silver, grass, and common carps recorded the values of  $3 \pm 0.4$ ,  $9 \pm 0.5$ , and  $13 \pm 1.2$  ng/mg, respectively (Table 1 & Fig. 1). High levels of ACTH were obtained in both common ( $17 \pm 1.5$  ng/mg) and grass carp females ( $12 \pm 0.3$  ng/mg), whereas low concentration ( $5 \pm 0.5$  ng/mg) was determined in females of silver carp.

#### PRL levels

PRL levels recorded the following values:  $419 \pm 15$ ,  $375 \pm 12$ , and  $350 \pm 48$  ng/mg in males of silver, grass and common carps, respectively (Table 1 & Fig. 1). Low levels of PRL were obtained in both common ( $360 \pm 36$  ng/mg) and grass carp females ( $382 \pm 8$

ng/mg), whereas high concentration ( $426 \pm 16$  ng/mg) was determined in female of silver carp.

#### **GH levels**

GH levels recorded the values of  $105 \pm 5$ ,  $83 \pm 4$ , and  $75 \pm 3.9$  ng/mg in males of silver, grass and common carps, respectively (Table 1 & Fig. 1). Low levels of GH were obtained in both common ( $80 \pm 4.2$  ng/mg) and grass carp females ( $92 \pm 6$  ng/mg), whereas a high concentration ( $118 \pm 6$  ng/mg) was determined in silver carp female.

#### **LH levels**

LH levels recorded the values:  $610 \pm 29$ ,  $530 \pm 36$ , and  $450 \pm 35$  ng/mg in males of silver, grass and common carps, respectively (Table 1 & Fig. 2). Low levels of LH were obtained in both common ( $1995 \pm 45$  ng/mg) and grass carp females ( $2090 \pm 55$  ng/mg), whereas high concentration ( $2355 \pm 47$  ng/mg) was determined in silver carp female.

#### **FSH levels**

FSH levels recorded the successive values:  $405 \pm 19$ ,  $310 \pm 26$ , and  $250 \pm 28$  ng/mg in males of silver, grass and common carps, respectively (Table 1 & Fig. 2). Low levels of FSH were obtained in both common ( $890 \pm 37$  ng/mg) and grass carp females ( $980 \pm 29$  ng/mg), whereas high concentration ( $1005 \pm 35$  ng/mg) was determined in silver carp female.

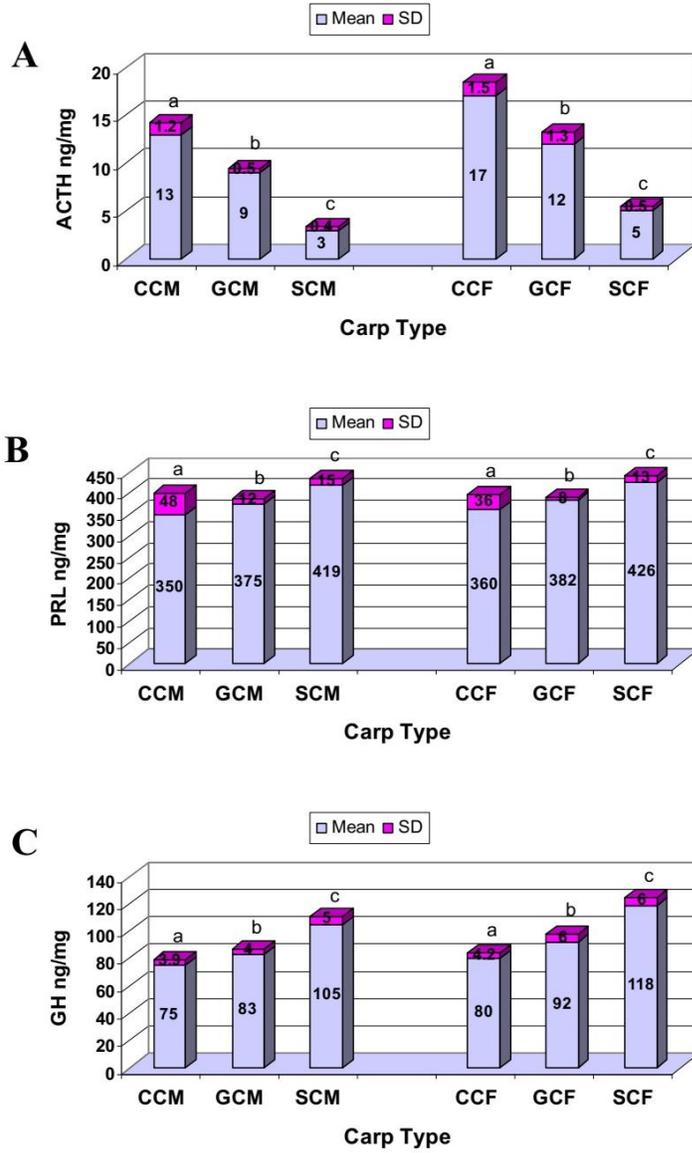
For the economic value of the gland of silver carp as a source of hormones, according to the prices of carp in Egypt, it is considered less expensive than those extracted from other types of carp.

**Table 1.** Hormonal levels in different carp types; common carp, grass carp and silver carp

Type of hormone		Carp Type		
		Common carp	Grass carp	Silver carp
ACTH ng/mg of pituitary	Male	$13 \pm 1.2$	$9 \pm 0.5^a$	$3 \pm 0.4^{a,b}$
	Female	$17 \pm 1.5$	$12 \pm 1.3^a$	$5 \pm 0.5^{a,b}$
PRL ng/mg of pituitary	Male	$350 \pm 48$	$375 \pm 12^a$	$419 \pm 15^{a,b}$
	Female	$360 \pm 36$	$382 \pm 8^a$	$426 \pm 13^{a,b}$
GH ng/mg of pituitary	Male	$75 \pm 3.9$	$83 \pm 4^a$	$105 \pm 5^{a,b}$
	Female	$80 \pm 4.2$	$92 \pm 6^a$	$118 \pm 6^{a,b}$
LH ng/mg of pituitary	Male	$450 \pm 35$	$530 \pm 36^a$	$610 \pm 29^{a,b}$
	Female	$1995 \pm 45$	$2090 \pm 55^a$	$2355 \pm 47^{a,b}$
FSH ng/mg of pituitary	Male	$250 \pm 28$	$310 \pm 26^a$	$405 \pm 19^{a,b}$
	Female	<b><math>890 \pm 37</math></b>	<b><math>980 \pm 29^a</math></b>	<b><math>1005 \pm 35^{a,b}</math></b>

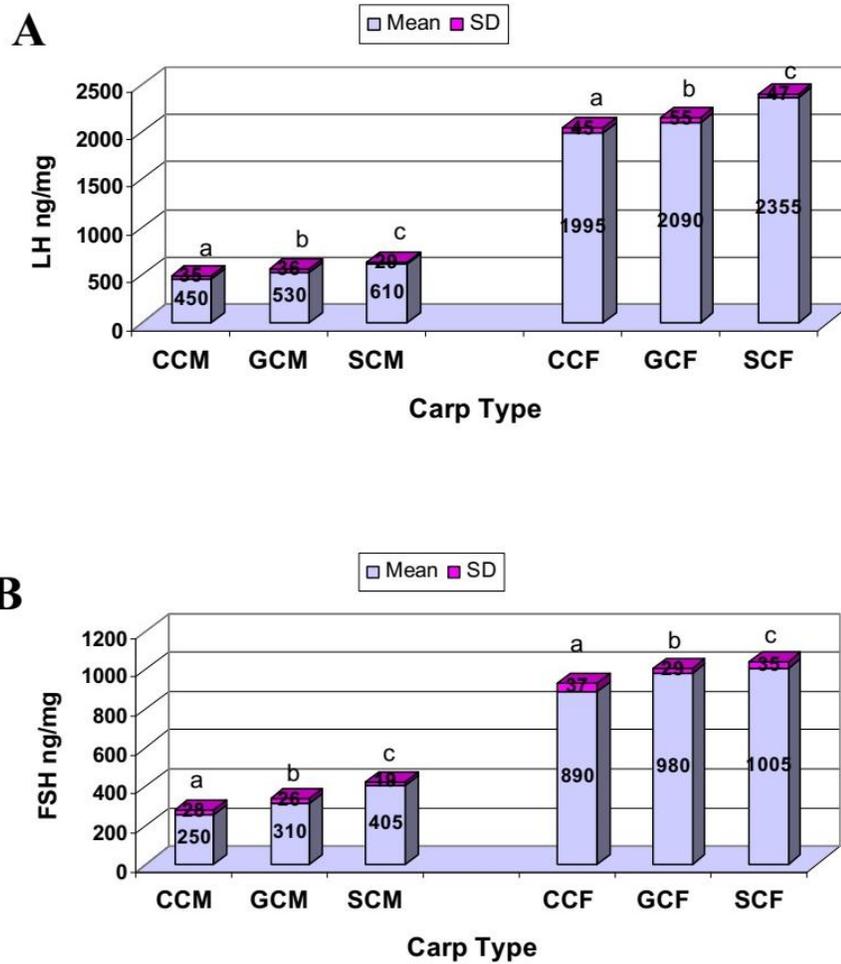
<sup>a</sup>: Significant differences when compared to common carp ( $P < 0.05$ ).

<sup>b</sup>: Significant differences when compared to grass carp ( $P < 0.05$ ).



**Fig. 1.** Concentration of hormones in different mature carp pituitary homogenate; common carp male (CCM), grass carp male (GCM), silver carp male (SCM), common carp female (CCF), grass carp female (GCF) and silver carp female (SCF): **(A)** ACTH; **(B)** PRL and **(C)** GH.

- Data are reported as means  $\pm$  SD. Significantly different means ( $P < 0.05$ ) are indicated by different letters (Tukey's test).



**Fig. 2.** Concentration of hormones in different mature carp pituitary homogenate; common carp male (CCM), grass carp male (GCM), silver carp male (SCM), common carp female (CCF), grass carp female (GCF) and silver carp female (SCF): **(A)** LH and **(B)** FSH. Data are reported as means  $\pm$  SD. Significantly different means ( $P < 0.05$ ) are indicated by different letters (Tukey's test).

## DISCUSSION

There are several hormones used to stimulate spawning of marine fish comprising hypophyseal homogenate of common carp, human chorionic gonadotropin and gonadotropin releasing hormones (Austriano *et al.*, 2006; Mousa, 2010; Mehdi & Ehsan, 2011; Mousa *et al.*, 2018a). The pituitary gland is commonly used from common carp without using other types of carp. Recently, the use of silver carp hypophyseal homogenate increased the ovulation rate in *L. ramada* than those of human chorionic gonadotropin and common carp (Mousa *et al.*, 2018a). Furthermore, the hypophysis of silver carp gave strong immunoreactions for somatolactin (SL) and gonadotropin (GTH II $\beta$ ) secreting cells, which increased in size and number compared to those of grass carp and common carp. In addition, the immunoreactivity of stress response hormones (ACTH

and CRF) in silver carp was lower than those of grass carp and common carp; since small size and lower number of ACTH immunoreactive cells were observed (**Mousa et al., 2018b**).

The present biochemical results indicate that the hypophysis of silver carp female had higher levels of GTHs;  $2355\pm 47$  and  $1005\pm 35$  ng/mg for LH and FSH, respectively, in comparison with those of common carp and grass carp. Additionally, the immunohistochemistry revealed that, the hypophysis of silver carp showed strong immunoreaction for gonadotropin hormone than those of grass carp and common carp (**Mousa et al., 2018b**). This demonstrates the superiority of the pituitary gland of silver carp over that of both grass carp and common carp for induction of ovulation in thin-lipped mullet (**Mousa et al., 2018a**). In teleosts, the hypophysis secretes the gonadotropins, which are responsible for sex steroids production, gonad development, maturation and spawning (**Weltzien et al., 2004; Kamei et al., 2005**). So, the high concentration of gonadotropin in donor hypophysis can increase its efficiency during the spawning induction in teleosts.

The silver carp female exhibited lower content of  $5\pm 0.5$  ng/mg for ACTH, in the present work, than those of grass carp and common carp. This, in turn, minimized stress during spawning induction in thin lipped mullet with the hypophysis of silver carp, which exhibited weak immunoreaction for the stress-response hormones; CRF and ACTH (**Mousa et al., 2018b**). In teleosts, the hypophysis secreting the ACTH controls the production of cortisol from the interrenal glands (**Henderson and Garland, 1980**). Several factors such as stress, temperature, and pollution activate the secretion of cortisol (**Donaldson, 1981; Pickering, 1981; Leloup-Hatey, 1985; Balm et al., 1994; Mousa & Mousa, 1999; Mousa et al., 2015**). Furthermore, an increase of ACTH-ir cells activity was detected in the hypophysis and CRF-ir in the brain and hypophysis during osmoregulation as well as the maturation of gonad and the induction of spawning. In addition, an increase of plasma cortisol was noted during spawning; all of which confirm the earlier findings (**Hirose & Ishida, 1974; Cook et al., 1980; Bry, 1985; Rivier & Rivest, 1991; Mousa & Mousa, 1999; Rotllant et al., 2000**), which reinforce the possible roles of those hormones on stress during reproduction and spawning in thin lipped mullet (**Mousa & Mousa, 2006**). However, the MSH-secreting cells regulate the color background adaptation (**Van Eys, 1980**). According to the previous events, we must be aware about the type of the hypophysis used for spawning induction in marine teleosts to reduce the stress as far as possible.

Furthermore, the high levels of PRL and GH ( $426\pm 13$  and  $118\pm 6$ , respectively) obtained in the present work for the females of silver carp, compared to those of grass carp and common carp, play a great part during the induction of spawning in thin lipped mullet using the hypophysis of silver carp, particularly in the osmoregulation (**Mousa et al., 2018a**). It is worth mentioning that, PRL and GH hormones have a key role in fish adaptation to different salinities (**Mclean & Donaldson, 1993; Mousa et al., 1999; Mousa et al., 2015**). The former hormone plays a principal role in the osmoregulation of fish, in low salinity habitats, particularly in euryhaline fish species (**Mancera et al., 1993; Mousa et al., 1999**). In fishes, GH plays a main role in growth (**Mclean & Donaldson, 1993**). In addition, GH participates in various physiological operations, such as immunity, metabolism and reproduction of teleosts (**Björnsson, 1997**).

## CONCLUSION

The present biochemical results of the silver carp hypophysis confirm its suitability as a cheap and appropriate source of hormones necessary for spawning induction in fishes.

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## REFERENCES

- Austriano, J.F.; Mrco-Jimenez, F.; Perez, L.; Balasch, S.; Garzon, D.L.; Penaranda, D.S.; Vicente, J.S.; Viudes-de-Castro, M.P. and Jover, M. (2006). Effect of HCG as spermiation inducer on European eel semen quality. *Theriogenology*, 66: 1012-1020.
- Bailey, N.T.J. (1981). *Statistical Methods in Biology*. 2nd Ed. Biological Science Texts., Pp: 216.
- Balm, P.; Pepels, P.; Helfrich, S.; Hovens, M.L. and Bonga, S.E. (1994). Adrenocorticotrophic hormone in relation to interrenal function during stress in tilapia (*Oreochromis mossambicus*). *Gen Comp Endocrinol*, 96: 347–360.
- Björnsson, B.Th. (1997). The biology of salmon growth hormone: from daylight to dominance. *Fish Physiol. Biochem.*, 17: 9-24.
- Bry, C. (1985). Plasma cortisol levels of female rainbow trout (*Salmo gairdneri*) at the end of the reproductive cycle: relationship with oocyte stages. *Gen. Comp. Endocrinol.*, 57: 47-52.
- Cook, A.F.; Stacey, N.E. and Peter, R.E. (1980). Periovulatory changes in serum cortisol levels in the goldfish, *Carassius auratus*. *Gen. Comp. Endocrinol.*, 40: 507-510.
- Crim, L.W.; Peter, R.E. and Billard, R. (1981). Onset of gonadotropic hormone accumulation in the immature trout pituitary gland in response to estrogen or aromatizable androgen steroid hormones. *Gen. Comp. Endocrinol.*, 44 (3): 374-381.
- Donaldson, E.M. (1981). The pituitary–interrenal axis as an indicator of stress in fish. In: Pickering, A.D. (Ed.), *Stress in Fish*. Academic Press, London (1981) pp. 11- 47.
- García-Ayala, A.; Villaplana, M.; García-Hernández, M.P.; Chaves Pozo, E. and Agulleiro, B. (2003). FSH-, LH-, and TSH-expressing cells during development of *Sparus aurata* L. (Teleostei). An immunocytochemical study. *Gen. Comp. Endocrinol.*, 134: 72-79.
- García-Hernández, M.P.; García-Ayala, A.; Zandbergen, M.A. and Agulleiro, B. (2002). Investigation into the duality of gonadotropic cells of Mediterranean yellowtail (*Seriola dumerilii*, Risso 1810): immunocytochemical and ultrastructural studies. *Gen. Comp. Endocrinol.*, 128: 25-35.
- Henderson, I.W. and Garland, H.O. (1980). The interrenal gland in pisces: part 2. Physiology. In: Chester-Jones, I., Henderson, I.W. (Eds.), *General, Comparative and Clinical Endocrinology of the Adrenal Cortex*, vol. 3. Academic Press, New York (1980) pp. 473–523.

- Hirose, K. and Ishida, R. (1974). Effects of cortisol and human chorionic gonadotropin (HCG) on ovulation in ayu, *Plecoglossus altivelis*. J. Fish. Biol., 6: 557-564.
- Kamei, H.; Kawazoe, I.; Kaneko, T. and Aida, K. (2005). Purification of follicle-stimulating hormone from immature Japanese eel, *Anguilla japonica*, and its biochemical properties and steroidogenic activities. Gen. Comp. Endocrinol., 143:257- 266.
- Leloup-Hatey, J. (1985). Environmental effects on the fish interrenal gland. In: Follett, B.K., Ishii, S., Chandola, A. (Eds.), "The Endocrine System and the Environment". Jpn Sci Soc Press, Tokyo, (1985) pp. 13–21.
- Mancera, J.M.; Fernandez-Liebrez, P.; Grondona, J.M. and Perez-Figares, J.M. (1993). Influence of the environmental salinity on prolactin and corticotropic cells in the euryhaline gilthead sea bream (*Sparus aurata* L.). Gen. Comp. Endocrinol., 90: 220 - 231.
- Mclean, E. and Donaldson, E.M. (1993). The role of growth hormone in growth of poikilotherms. In: Schreibman, M. P.; Scanes, C. G. and Pang, P. K. T., eds. The Endocrinology of Growth, Development and Metabolism in Vertebrates. New York: Academic Press (1993) pp. 43-71.
- Mehdi, Y. and Ehsan, M.S. (2011). A review of the control of reproduction and hormonal manipulations in finfish species. Afr. J. Agricul. Res., 6 (7): 1643-1650.
- Mousa, M.A. (2002). Immunocytochemical and histochemical study on oogenesis in thin-lipped grey mullet, *Liza ramada*. J. Egypt. Ger. Soc. Zool., 39 (C): 549-567.
- Mousa, M.A. (2004). The efficacy of clove oil as an anaesthetic during the induction of spawning of thin-lipped grey mullet, *Liza ramada* (Risso). J. Egypt. Ger. Soc. Zool., 45 (A): 515-535.
- Mousa, M.A. (2010). Induced spawning and embryonic development of *Liza ramada* reared in freshwater ponds. Anim. Reprod. Sci., 119: 115-122.
- Mousa, M.A. and Mousa, S.A. (1999). 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. (2006). Involvement of corticotropin releasing factor and adrenocorticotrophic hormone in the ovarian maturation, seawater acclimation and induced-spawning of *Liza ramada*. Gen. Comp. Endocrinol., 146: 167-179.
- Mousa, M.A.; El-Shebly, A.A. and Khalil, M-B.A. (1999). Effect of salinity on prolactin and growth hormone cell activity in *Mugil cephalus*. Egypt. J. Aquat. Biol. & Fish., 3 (2): 85-101.
- Mousa, M.A.; Khalil, N.A. and Gaber, S.A. (2006). Distribution of immunoreactivities for adeno-hypophysial hormones in the pituitary gland of the Nile mormyrid, *Mormyrus kannume* (Teleostei, Mormyridae). J. Egypt. Ger. Soc. Zool., 51 (C): 33-56.
- Mousa, M.A.; Ibrahim, A.A.E.; Hashem, A.M. and Khalil, N.A. (2015). The effect of water quality on the immunoreactivity of stress-response cells and gonadotropin-secreting cells in the pituitary gland of Nile tilapia, *Oreochromis niloticus*. J. Exp. Zool., 323A: 146-159.

- Mousa, M.A.; Kora, M.F. and Khalil, N.A. (2018a). Evaluation of the effectiveness and cost of different hormones in stimulating the spawning of thin lipped grey mullet, *Liza ramada*. Egypt. J. Histol., 41 :( 3): 275-284.
- Mousa, M.A.; Khalil, N.A.; Kora, M.F. and El-Gohary, N.M. (2018b). Immunohistochemical evaluation of the pituitary gland of carp as a source of hormones needed to stimulate spawning in marine fish. Egypt. J. Histol., 41 (4): 419- 430.
- Ohkubo, M.; Katayama, S. and Shimizu, A. (2010). Molecular cloning and localization of the luteinizing hormone  $\beta$  subunit and glycoprotein hormone  $\alpha$  subunit from Japanese anchovy *Engraulis japonicus*. J. Fish. Biol., 77: 372-387.
- Pickering, A.D. (1981). The concept of biological stress. In: Pickering, A.D. (Ed.), Stress and Fish. Academic Press, London (1981) pp. 1–11.
- Prat, F.; Sumpter, J.P. and Tyle, C.R. (1996). Validation of radioimmunoassays for two salmon gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (*Oncorhynchus mykiss*). Biol. Reprod., 54: 1375-1382.
- Shimizu, A.; Hamaguchi, M.; Ito, H.; Ohkubo, M.; Udagawa, M.; Fujii, K.; Kobayashi, T. and Nakamura, M. (2008). Appearances and chronological changes of mummichog *Fundulus heteroclitus* FSH and LH cells during ontogeny, sexual differentiation, and gonadal development. Gen. Comp. Endocrinol., 56: 312-322.
- Suzuki, K.; Kawauchi, H. and Nagahama, Y. (1988). Isolation and characterization of two distinct gonadotropins from chum salmon pituitary glands. Gen. Comp. Endocrinol., 71 (2): 292-301.
- Swanson, P.; Suzuki, K.; Kawauchi, H. and Dickhoff, W.W. (1991). Isolation and characterization of two coho salmon gonadotropins, GTH I and GTH II. Biol. Reprod., 44: 29-38.
- Rivier, C. and Rivest, C. (1991). Effect of stress on the activity of the hypothalamic-gonadal axis: peripheral and central mechanisms. Biol. Reprod., 45: 523-532.
- Rotllant, J.; Balm, P.; Ruane, P.H.M.; Perez-Sanchez, N.M.; Wendelaar Bonga, J. and Tort, S.E.L. (2000). Pituitary proopiomelanocortin-derived peptides and hypothalamus–pituitary–interrenal axis activity in gilthead sea bream (*Sparus aurata*) during prolonged crowding stress: differential regulation of adrenocorticotropin hormone and  $\alpha$ -melanocyte-stimulating hormone release by corticotropin-releasing hormone and thyrotropin-releasing hormone. Gen. Comp. Endocrinol., 119: 152-163.
- 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: II. The PAS-positive cells. Cell Tissue Res., 210: 171-179.
- Weltzien, F.A.; Andersson, E.; Andersen, O.; Shalchian-Tabrizi, K. and Norberg, B. (2004). The brain-pituitary-gonad axis in male teleosts, with special emphasis on flatfish (*Pleuronectiformes*). Comp. Biochem. Physiol., Part A 137:447–477.