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Comparative Study for Different Sources of Reproductive Stimulating Materials and Their Effects on the Reproductive Performance of African Catfish *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

African catfish, *Clarias gariepinus*, is a highly appreciated species for aquaculture. So, the present research was carried out to study the possibility of improving artificial reproduction of African catfish *C. gariepinus* brood stock by injection with some stimulating preparations. Females and males were divided into five treatments (T), each treatment had five females and two males, the females only were injected; T_2, was injected with imported Pregnyl®; T_3, was injected with imported Argent®; T_4, was injected with native carp pituitary gland and T_5, was injected with native catfish pituitary gland; but T_1, set as a control, without injection. The obtained results revealed that the ovary length was significantly ($p \leq 0.05$) longer in T_2, T_3 and T_4 than in T_1 and T_5. Also, T_3 gave the highest Absolute Fecundity (AF), being 119917 and T_2, T_4 and T_5 also represented higher AF, being 7.6, 7.8 and 3.9 times, respectively than T_1. Yet, T_3 gave the highest Relative Fecundity (RF), being 126.4 which was 8.5 times than T_1. There were significant differences among treatments for serum FSH, LH and progesterone (P4) but not for estrogen. Treatment No. 2 reflected the highest ($p \leq 0.05$) level of FSH and LH among all treatments but P4 level in each of T_2, T_3 and T_5 was significantly higher than T_1 and T_4. Histological examination of ovaries revealed normal structure of ovarian lamellae among all treatments and various development stages of oogenesis were observed. So, it could be concluded that T_3 followed by T_2 and T_5 were the best treatments for improving the reproductive performance of *C. gariepinus* concerning the significantly heaviest egg weight and highest AF, RF, serum FSH, LH and P4 than T_1 and T_4.

Key words: African catfish, reproductive performance, sexual hormones, fecundity

INTRODUCTION

African catfish *Clarias gariepinus* is a suitable alternative to tilapia in subsistence fish farming in Africa using low grade feed composed of some local agricultural and agro-industrial by-products, the yields of catfish from ponds could be as much as 2.5 times higher than those of tilapia (Verreth *et al*., 1993). In addition, this species is known with its high growth rate, resistance to handling and stress, relatively low requirements for water quality, amenability to high stocking densities, excellent meat quality and preference amongst consumers in many African countries (Hecht *et al*., 1996). Although, the potential use of *C. gariepinus* in Egyptian aquaculture is very high because of several factors related to the aquaculture system,

the species itself and existing market forces (Rezk, 2008). So, culturing of *C. gariepinus* is a growing interest, it once mass juvenile production becomes commercially and economically feasible. Furthermore, latest fish production statistics in Egypt revealed that African catfish production reached about 48750 tones (about 4.79% of the total aquaculture production in 2012 (GAFRD, 2012).

Reproductive problems are usually more serious in female brood stocks fish (Zohar and Mylonas, 2001). So, the high demand for fish fingerlings in the aquaculture industry has stimulated the need for artificial propagation of cultivable warm water fish (Nwokoye *et al*., 2007). Although, African catfish, *C. gariepinus*, is a highly appreciated species for aquaculture (De Graaf and Janssen, 1996). Yet, the seasonality of the reproductive cycle maintained catfishes in tropical fish ponds hampers the continuous production of fry and fingerlings for pond stocking (Nguenga *et al*., 2004), as well as catfish species are classified as single-time spawners (Tyler and Sumpter, 1996). Moreover, Dorman (2008) mentioned that, even with the genetic improvements in catfish brood stock, spawning success is often quite low, in average; only 50% of females actually spawn. Additionally, survival from the egg to fingerling stages, estimated at 60 to 70%. So, early success using injected extracts of fish pituitaries or human chorionic gonadotropin (hCG) has been exogenously administered to induce ovulation and spawning in females of captive brood stock (Nwokoye *et al*., 2007). Also, regulations of reproductive activity of fish by the brain-pituitary-gonad axis were reported by Yaron *et al*. (2003). In this manner, Carp Pituitary Extract (CPE) is the most predictable compound for the timing of ovulation (Liu *et al*., 1997), for induced spawn of female channel catfish (Lambert *et al*., 1999), as well as, catfish pituitary extract is as effective as CPE or luteinizing hormone-releasing hormone analogue (LHRHa) for inducing ovulation in females' channel catfish (Bosworth, 2005).

The need for high quality fish seeds has necessitated researchers to look for various ways of enhancing fertility to meet the growing demand (Dada *et al*., 2010). So, hormonal therapies have an important role in brood stock fish management (Mylonas and Zohar, 2001). Hence, various exogenous hormones have successfully been used to induce maturation and ovulation of postvitellogenic oocytes in fish (Richter and Van Der Hurk, 1982), including injection with salmon-GnRH and chicken-GnRH-II for spawning induction of *C. gariepinus* (Szabo *et al*., 2007); recently, Sharaf (2012) reported that gonadotropin-releasing hormone analog (GnRHa) is effective for induced spawning in *C. gariepinus*.

In Egypt, production of *C. gariepinus*, so far, is mainly from fisheries capture. Use of *C. gariepinus* for farming has encountered a number of obstacles including problems mainly related with reproduction and fry maintenance (Rezk, 2008). With regard to *C. gariepinus*, although artificial hatchery technology is now available to produce seeds, a few hatcheries have worked with *C. gariepinus* (Krouma, 2011). Therefore, the present research was designed as a comparative study to evaluate the possibility of improving artificial propagation of African catfish *C. gariepinus* using the intramuscular injection with some commercial imported and native preparations of reproductive hormones, by measuring different physiological, biochemical and histological parameters.

MATERIALS AND METHODS

Brood stock selection: This study was carried out at the fish farm belonging to the Egyptian Aquaculture Centre, Kafr El-Sheikh Governorate, Egypt. Brood stock African catfish in an earthen pond (8×20 m) were fed one time a day, 6 days a week for 1 month by demand feeder a tilapia

commercial floating feed (manufactured by Hendrix, Egypt factory, contained 32% crude protein, 4.51% crude fat, 4.48% crude fiber and 4160 kcal kg^{-1} gross energy and consisted of fish meal 72%, soybean meal 44%, wheat bran, corn gluten 60%, monocalcium phosphate, lime stone, soy oil, L-lysine and vitamins and minerals mixture). This period was to make brood stock ready for spawning. Rearing water quality parameters were measured weekly at 06.0 am and 06.0 pm to determine the values of pH (using Jenway Ltd, model 350-pH meter), dissolved oxygen concentration (using an oxygen meter model d-5509) and temperature degrees centigrade (using a thermometer). The values for water temperature ranged between 28 and 30°C, pH values 7.8-8.6 and dissolved oxygen 3.9-7.1 mg L^{-1}, these water parameters are within the acceptable ranges recommended for rearing brood stock catfish according to Boyd (1990). After this 1 month, twenty five females and ten males from brood stock in earthen pond were chosen to be equal in weight (within each sex), activity and ovary appropriate (eggs have a bright green color) approximately.

Brood stock spawning: Brood stock African catfish females (average weight 812±78.46 g $fish^{-1}$ and body length 46.5±1.16 cm) and males (average weight 1065±81.36 g $fish^{-1}$ and body length 53.6±1.24 cm) were taken from this earthen pond to the farm laboratory and were received in five glass tanks (50×40×50 cm) each contained 30 L fresh water mixed with 15 mL formalin as external disinfectant. After this treatment, these fishes were divided into five treatments each treatment has 5 females and 2 males. Brood stock fish were separated into 6 tanks (one tank for each treatment of females and one tank for all males). During this time, no feed was offered. The flow rate of water was 2 L^{-1}. Extracted pituitary gland from sacrificed males was kept for female's injection. Nine hours post treatment (only females' injection), females became ready for spawning and striping. Details of the experimental treatments and the preparation of the injected materials were illustrated in Table 1.

Pituitary keeping method: After the evisceration of pituitary from under brain of males, it was transferred into test tube with 1 mL of acetone for 5 min acetone was changed after this period and repeated after 15 min, 8 h and 24 h from starting this method according to Kumar (1992). During this time, the test tube was kept at room temperature. Next these 24 h, the pituitary was dried on candidacy paper until the complete dryness and kept in a dried bottle in a refrigerator (4°C).

Egg fertilization: Eggs were collected from three females per treatment and another two females were used for blood sampling. Males were killed to collect the milt from testis to fertilize eggs of each treatment. Milt was put on eggs and mixed with about 200 mL fresh water to stimulate the fertilization. After mixing eggs with the milt in a plastic plate for 2 min to ensure that all eggs were fertilized, then these fertilized eggs were distributed on a substrate which was made of a wood frame (2×0.5×0.08 m) with American fiber net (1 cm^2 contained 6 gaps, each of 20 μ). On which eggs did not accumulate over others. Each treatment was separated in a concrete pond (5 treatments/5 ponds, each of 8×3×1.25 m, whereas the water column was 60 cm), where eggs need 30 cm water depth to keep temperature between 25-28°C, tropic day. Fish were killed at the end of the experiment and soon the abdominal cavity was opened to remove gonads which were weighed individually and their length as well as size and density were measured and calculated. Gonado-Somatic Index (GSI) was calculated as:

$$GSI = \frac{\text{Gonads weight}}{\text{Fish weight}} \times 100 \quad \text{(Tseng and Chan, 1982)}$$

The brood stock fish measurements were taken for the nearest 0.1 g and 0.01 cm to calculate the condition (K) factor. The male's weight in average was 1065±81.36 g fish^{-1}, body length 53.6±1.24 cm, testis weight 12.3±1.62 g fish^{-1}, testis length 6.61±0.49 cm, k-factor 0.69±0.02 and GSI 1.14±0.11. Total egg weight and number per female as well as individual egg weight and diameter were measured too. Eggs number was counted using 1 mm insulin syringe, then related to ovary weight and body weight of fish. Absolute fecundity (AF, number of eggs/female) and relative fecundity (RF, number of eggs/g female weight) were calculated according to Bhujel (2000).

Serum hormones assay: At the end of the experiment, blood samples were collected from the remaining two females to obtain blood serum by centrifugation for 20 min at 3500 rpm. Serum Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), as well as the sex steroids hormones, Progesterone (P4) and Estrogen (E2) were quantitatively analyzed by a calibrated DPC IMMULITE® 1000 chemiluminescent immunoassay system (DPC, Los Angeles, CA, USA), accordingly to the manufacturer's instructions by test kits catalogs No. (LKFS1), (LKLH1), (LKPG1) and (LKE21), respectively.

Table 1: Details of the experimental treatments and the preparation of the injected materials

Treatments	Details	No. of fish	Preparations
T_1	Control	5♀ and 2♂*	Fish without injection
T_2	Injected with Pregnyl®	5♀ and 2 ♀*	Injection with imported Pregnyl® (hCG hormone, it is an injectable, highly purified preparation of human chorionic gonadotropin derived from the urine of pregnant women. It mimics the normal surge of Luteinizing Hormone (LH) and causes ovulation to begin): This hormone is one of the most hormones used in human medicine as a stimulator to ovary. One ampoule (has 5000 IU in 1 mL inj., from Organon, Netherland) was diluted to 5 mL distilled water, so each 1 mL contained 1000 IU. The intramuscular injection dose was 1.7 mL (=1700 IU kg^{-1}) of female into the dorsal muscle
T_3	Injected with Argent®, carp pituitary gland	5 ♀ and 2♂*	Injection with imported Argent® (from Argent Laboratories 870215 2nd Ave. N.E., Redmond WA 98052 USA), one carp pituitary gland per kilogram of female, each gland was extracted with 2 mL saline solution (sodium chloride 0.9%) in small grinder and injected into fish dorsal muscle
T_4	Injected with native carp pituitary gland	5 ♀ and 2 ♂*	Injection with one native carp pituitary gland per kg of female and used with the same method in T_3
T_5	Injected with native catfish pituitary gland	5 ♀ and 2 ♂*	Injection with one native catfish pituitary gland per kilogram of female and used with the same method in T_3

*Males without injection in all treatments, but females only injected with the tested agents

Histological examination: At the end of the experiment, fish were sacrificed and the target organs (female gonads) were sampled. Samples were fixed in 10% neutralized formalin solution followed by washing with tab water, then dehydrated by different grades of alcohol (70, 85, 96 and 99%). Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were sectioned to six micron. The sections were stained by Hematoxyline (H) and Eosin (E) and then subjected to a histological examination according to Roberts (2001).

Statistical analysis: The obtained numerical data were statistically analyzed using SAS software package (SAS, 2001) for one-way analysis of variance (ANOVA). Ratio and percent data were arcsine-transformed prior to statistical analyses and evaluated by using the following model:

$$Y_{ij} = \mu + A_i + e_{ij}$$

where, Y_{ij} is an observation of body weight, length and K-factor, ovaries weight, ovaries size, ovary density, ovary length and GSI, total egg weight, egg diameter, individual egg weight, AF and RF and blood serum FSH, LH, P4 and E2; μ is least square mean; A_i is the fixed effect of treatments (T_1-T_5) and e_{ij} is the random error. Statistical significant ($p \geq 0.05$) differences between mean were compared by using Duncan's multiple ranges test (Duncan, 1955), which was described by Bailey (1995).

RESULTS

Brood stock characteristics: In respect to the female brood stock measurements (body length and K-factor) in Table 2, there were no significant ($p \geq 0.05$) differences among treatments; yet, T_4 was heavier ($p \leq 0.05$) in body weight than T_1 and T_3. Table 3 illustrates mean±standard error

Table 2: Body weight, length and K-factor data of brood stock female African catfish as affected by the experimental treatments (Mean±SE)

	Treatments					
Traits	T_1	T_2	T_3	T_4	T_5	p-value
Body weight (g)	708.0±49.23^{b}	796.0±31.87^{ab}	752.0±47.58^{b}	956.0±68.82^{a}	848.0±82.85^{ab}	0.04
Body length (cm)	46.60±2.68^{A}	47.20±0.37^{A}	45.00±1.05^{A}	47.80±1.28^{A}	46.20±1.20^{A}	0.74
K-factor	0.72±0.09^{A}	0.76±0.03^{A}	0.82±0.04^{A}	0.87±0.06^{A}	0.85±0.05^{A}	0.35

Mean having different small letters are significantly different ($p \leq 0.05$), but mean having capital letter are not significantly different ($p \geq 0.05$)

Table 3: Ovarian measurements of African catfish as affected by the experimental treatments (Mean±SE)

	Treatments					
Traits	T_1	T_2	T_3	T_4	T_5	p-value
Ovaries weight (g)	151.3±3.10^{A}	175.8±6.35^{A}	205.0±33.7^{A}	189.1±47.9^{A}	143.2±21.8^{A}	0.55
Ovaries size (cm^3)	57.5±17.5^{A}	87.5±7.50^{A}	100.0±5.00^{A}	87.5±17.5^{A}	82.5±7.50^{A}	0.29
Ovary density (g cm^{-1})	2.88±0.98^{A}	1.95±0.25^{A}	2.00±0.20^{A}	2.11±0.10^{A}	1.70±0.10^{A}	0.51
Ovary length (cm)	10.0±0.00^{b}	13.4±0.35^{a}	12.8±0.75^{a}	12.3±0.70^{a}	9.50±0.50^{b}	0.01
GSI (%)	23.65±1.25^{A}	20.75±1.95^{A}	23.85±3.95^{A}	20.35±0.75^{A}	17.40±0.10^{A}	0.39

Mean having different small letters are significantly different ($p \leq 0.05$), but mean having capital letter are not significantly different ($p \geq 0.05$)

(SE) of the ovarian traits including ovaries weight, size and density (specific gravity), as well as the GSI which were all not significantly ($p \geq 0.05$) different among treatments, except the ovary length which was significantly ($p \leq 0.05$) longer in T_2, T_3 and T_4 than in T_1 and T_5.

Egg quality and fish fecundity: Brood stock *C. gariepinus* egg quality measurements are presented in Table 4, whereas no significant ($p \geq 0.05$) differences were found in egg diameter (mm) and egg weight (mg egg^{-1}) among all treatments. However, total egg weight (g female^{-1}) was significantly ($p \leq 0.05$) heavier in T_3 (152.2±14.3 g female^{-1}) and T_2 (145.6±33.6 g female^{-1}) than in T_1 (24.2±17.9 g female^{-1}) by 6.29 and 6.0 times, respectively. Data of AF and RF are presented also in Table 4, which revealed significant ($p \leq 0.05$) differences among treatments in both AF and RF. Fish injected with imported Argent® (T_3) gave the highest egg number per female (i.e., AF, being 119917 eggs female^{-1}) which was 9.75 times that of the control (T_1). The other treatments (T_2, T_4 and T_5) also represented higher total egg number female^{-1}, being 7.6, 7.8 and 3.9 times, respectively than the control. The RF took the same trend, since T_3 gave the highest egg number g^{-1} female body weight (i.e., RF, being 126.4 eggs g^{-1} female weight) which was 8.5 times that of the control (T_1). The other treatments (T_2, T_4 and T_5) also represented higher RF, being 8.5, 6.6 and 3.2 times, respectively than the control. However, there were no significantly ($p \geq 0.05$) differences between treatments T_2 and T_5 and between T_1 and T_5 in AF.

Blood serum hormones: Figure 1 presents the data of blood serum hormones (FSH, LH, P4 and E2) determinations. It is clear that there were significant ($p \leq 0.05$) differences among treatments for all tested hormones, except for E2. Treatment No. 2 (Pregnyl®) reflected the highest level of FSH (2.17±0.18 mIU mL^{-1}) and LH (26.67±7.05 mIU mL^{-1}) but P4 level was the highest in each of T_2 (Pregnyl®), T_3 (Argent®) and T_5 (native catfish pituitary) without significant ($p \geq 0.05$) differences among these treatments which were significantly ($p \leq 0.05$) higher than the control (T_1) and T_4 (native carp pituitary).

Histology of the ovaries: Histological examination of ovaries of experimental African catfish brood stock revealed the presence of different stages of oocytes, which developed at various stages of oogenesis in ovaries of fish injected with experimental materials compared with fish's ovaries without injection (T_1, control), as illustrated in Fig. 2a-h.

Table 4: Egg measurements of African catfish as affected by the experimental treatments (Mean±SE)

	Treatments					
Traits	T_1	T_2	T_3	T_4	T_5	p-value
Total egg weight (g female^{-1})	24.2±17.9^{b}	145.6±33.6^{a}	152.2±14.3^{a}	126.4±44.0^{ab}	68.5±53.4^{ab}	0.03
Egg diameter (mm)	0.96±0.088^{A}	0.87±0.033^{A}	1.0±0.153^{A}	1.0±0.058^{A}	0.86±0.033^{A}	0.64
Individual egg weight (mg egg^{-1})	0.64±0.091^{A}	0.66±0.056^{A}	0.76±0.085^{A}	0.73±0.014^{A}	0.82±0.081^{A}	0.12
Total egg number female^{-1} (AF)	12288±7656^{b}	93583±14543^{a}	119917±8890.88^{a}	93130±39434^{a}	47828±34359^{ab}	0.03
Relative fecundity (RF)	14.7±7.95^{d}	124.9±23.8^{a}	126.4±23.3^{a}	97.9±35.5^{b}	47.8±29.2^{c}	0.02

Mean having different small letters are significantly different ($p \leq 0.05$), but mean having capital letter are not significantly different ($p \geq 0.05$)

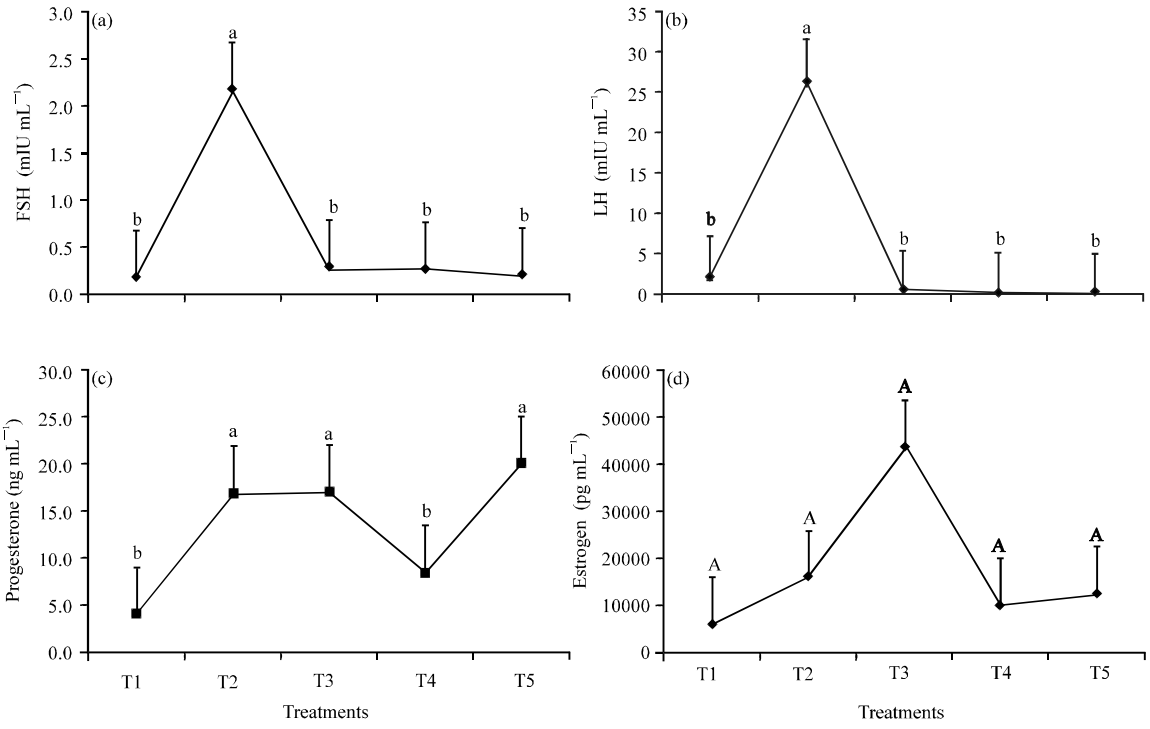

Fig. 1(a-d): Data of serum hormones of the experimental African catfish. Mean having different small letters are significantly different ($p \leq 0.05$) but mean having capital letter are not significantly different ($p \geq 0.05$)

DISCUSSION

In the present study, body measurements of female brood stock *C. gariepinus* revealed the heaviest ($p \leq 0.05$) body weight of T_4 compared with T_1 and T_3 and the longest ovary length ($p \leq 0.05$) of T_2, T_3 and T_4 compared with in T_1 and T_5 but there were no significant ($p \geq 0.05$) differences among treatments concerning body length, K-factor, ovaries weight, size and density and GSI. The non-significant differences may be attributed to the fact that the ovarian weight is usually a negligible fraction of the somatic (body) weight. De Graaf *et al.* (1995) found similar findings for *C. gariepinus*, using artificial propagation techniques. Similarly to the present findings, Sharaf (2005) noted no significant differences in GSI between *C. gariepinus* females as affected with single dose of synthetic GnRHs and hCG. Yet, earlier established linear relationship was found between fecundity, ovarian weight and length, GSI and somatic weight of *C. gariepinus* (Eyo and Mgbenka, 1992). This relationship is important in estimating fecundity from ovarian weight and length, GSI and somatic weight. In this respect, Nguenga *et al.* (2004) found that oocyte diameter and absolute fecundity increased with increasing the weight of *Heterobranchus longifilis*.

Egg quality measurement means specific parameters of egg, such as egg diameter, egg weight, total egg weight and total egg number per female (AF) or egg number per g female body weight (RF) to measure the quality of egg represented by fish treated with the tested materials, where egg quality is an important parameter for commercial fish hatcheries, as well as the quality of fry produced from a brood stock (Babin *et al.*, 2007). The present findings concerning egg quality and fecundity showed significantly ($p \leq 0.05$) heavier total egg weight (g female^{-1}) in T_3 and T_2 than in

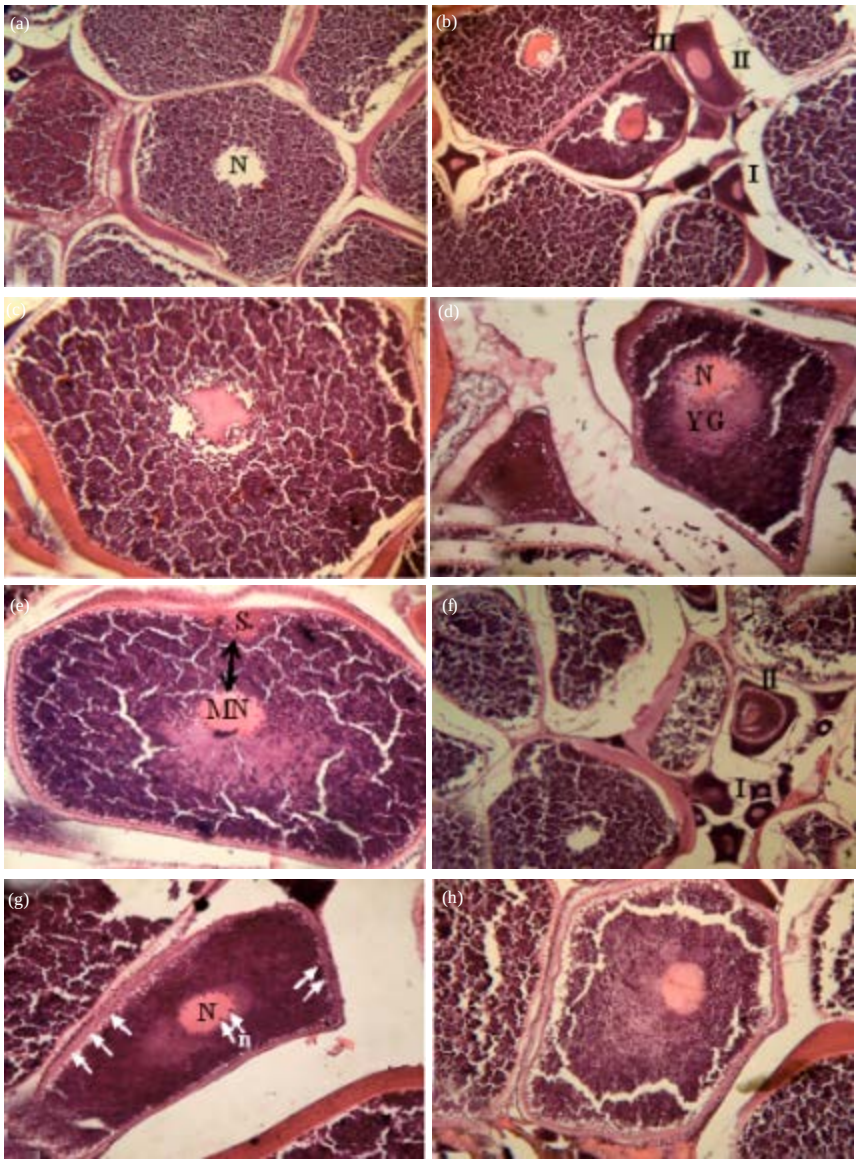

Fig. 2(a-h): Transverse section in ovary of African catfish brood stock (stained with H and E),(a) T_1 (control), showing primary yolk stage, N: nucleus, (b and c) Fish injected with Pregnyl® (T_2) showing normal structure of ovarian lamellae, which contains oocytes at various stages of oogenesis, (c) Showing oocytes in mature stage, (d and e) Fish injected with carp pituitary (Argent, T_3) showing oocytes in yolk globules (YG) stage, (e) Showing migratory nucleus (MN) stage, S: Spore, (f and g) Fish injected with carp pituitary (native,T_4) showing normal structure of ovarian lamellae, which contains oocytes at various stages of oogenesis, (g) Showing oocytes in early yolk vesicles (EYV, arrows), stage, n: nucleolus and (h) Fish injected with catfish pituitary (native, T_5) showing oocytes in late vitellogenic stage. Magnification, a, b and f (and 60); c, d, e, g and h (×120)

T_1. However, no significant ($p \geq 0.05$) differences in individual egg diameter and egg weight (mg egg^{-1}) among all treatments were found. Data of both AF and RF revealed significant ($p \leq 0.05$) differences among treatments, whereas fish injected with experimental materials (T_2, T_3, T_4 and T_5) gave the highest AF and RF compared with the control group (T_1). Similarly, in *Heterobranchus bidorsalis* no significant difference ($p \geq 0.05$) of egg diameter among all treatments was found (Nwokoye *et al*., 2007). So, Carillo *et al*. (1995) opined that egg diameter is not a good indicator of egg and larval quality. Increasing fecundity of *C. gariepinus* in this study could be attributed to the injection with native or commercial hormonal materials, which are capable of increasing the production of FSH, LH, E2 and P4, the key hormones involved in the production and maturation of eggs in the ovary. Appropriate hormonal therapies do not usually have a negative effect on egg quality (Haffray *et al*., 2005), whereas at times they can even improve fish fecundity compared to spontaneously maturing populations (Mikolajczyk *et al*., 2004).

Hormonal therapies have an important role in brood stock fish management and will continue to be a necessary tool even after fish become properly (Mylonas and Zohar, 2001). Hence, many studies confirmed the important role of gonadotropins hormone (GtHs), hCG or fish pituitary extracts injection to improve the reproductive performance of catfish sp., such as *C. gariepinus* due to hCG administration than CPE (Salami *et al*., 1994). Artificial spawning and high hatching rates (≥84%) were induced in *Heterobranchus bidorsalis* by single hormone injections of Carp Pituitary Suspensions (CPS), Homoplastic Pituitary Suspension (HPS) or hCG over 3 month (Adebayo and Fagbenro, 2004), as well as, they noted that using of HPS would save operational costs in fish hatchery management in African countries. Also, the highest fertilization (75-89%) and hatching (66-78%) could be obtained from the females of *C. batrachus* injected with at 3000-5000 IU doses of hCG with 14-17 h post- injection (Sahoo *et al*., 2007). Additionally, Sadek (2009) concluded that injection of Pregnyl® (hCG) have higher potent than Receptal® (GnRH) in the artificial propagation of *C. gariepinus*.

In contrary of the present findings, Brazilian catfish *Pseudoplatystoma fasciatum* treated with combined CPE/hCG was not influenced (Leonardo *et al*., 2004), as well as in yaqui catfish *Ictalurus pricei*, hCG, catfish pituitary extract and combined sGnRHa/DA treatments were ineffective (Mylonas and Zohar, 2001). Accordingly of these results, the efficacy of combinations of GtHs together with GnRHa, do not seem to be better compared to treatments using only one of the hormones for the induction of spawning in fish (Wen and Lin, 2004). Meanwhile, LH or hCG may be more appropriate in inducing oocyte maturation, ovulation and spawning (Garcia *et al*., 2001).

The secretion of gonadotropin in both pituitary and gonads might have important roles on sex steroids synthesis, undifferentiated gonad and in turn on sex differentiation through brain-pituitary-gonad axis (Wu *et al*., 2009). Data of serum hormones (FSH, LH, P4 and E2) cleared that there were significant ($p \leq 0.05$) differences among treatments for all tested hormones, except for E2. Treatment No. 2 reflected the highest level of blood FSH and LH but P4 level was the highest in each of T_2, T_3 and T_5 without significant ($p \geq 0.05$) differences among these treatments which were significantly ($p \leq 0.05$) higher than T_1 (control) and T_4. In females, FSH is thought to be responsible for the regulation of vitellogenesis, while LH is responsible for oocyte maturation and ovulation (Yaron *et al*., 2003). Fish possess two GtHs similar to FSH and LH in other vertebrates. Furthermore, the presence of two distinct GtHRs in a single fish species was concermed by the molecular cloning of two different cDNAs in several fish species including different catfish sp. *C. gariepinus* (Vischer and Bogerd, 2003) and *Icralurus puncrarus* (Kumar *et al*., 2001). Based on

the other scientific point of view, the exception of salmonids, there are no assays available to measure FSH, due to difficulties in producing anti-FSH antibodies from the very small FSH amounts contained in fish pituitaries (Yaron *et al*., 2003). Also, they added that the role of FSH during vitellogenesis was based only on measurements of FSH β-mRNA levels in the pituitary and not of the released protein in the blood (Yaron *et al*., 2003). However, as in the present findings, plasma FSH and LH were measured in *C. gariepinus* by Sayed *et al.* (2012).

Pituitary gonadotropins FSH (GTH-I) and LH (GTH-II) are key reproductive hormones that are involved in controlling gonadal development steroidogenesis and ovulation (Yadetie and Male, 2002). So, different pattern was illustrated for LH in *C. gariepinus* (Schulz *et al*., 1997) and black carp (Gur *et al*., 2000). In these species, LH levels are already detectable in juveniles, steadily increasing concomitant with gonadal growth and maturation. Swanson *et al.* (1991) found that plasma FSH levels remain high throughout vitellogenesis but decline later in the Coho salmon, being low during oocyte maturation and ovulation without showing the "two-peak pattern" as in trout. In this manner, Daghash and Hussein (1999) reported that an intramuscular injected dose of 20 μg GnRH kg^{-1} BW increased the serum total cholesterol ($p \leq 0.05$) and progesterone ($p \leq 0.01$) levels of treated *C. gariepinus* females.

Generally, from the economic point of view, a complementary study (Abdelhamid *et al*., 2010) related to the present study was conducted, where the produced fry from all treatments were used to alleviate the cannibalism phenomena among the African catfish, *C. gariepinus* fry via periodical grading to eliminate the jumpers fry. Since, the cannibalism phenomena are a major problem in the African catfish farming, which led to negative economic effects in the commercial hatcheries and in the aquaculture of this species. From the other side, concerning the human public health, usage of hCG hormone is safety, where approximately 80% of its level is metabolized, predominantly in the kidneys. Intramuscular and subcutaneous administrations of hCG were found to be bioequivalent regarding the extent of absorption and the apparent elimination half-lives of approximately 33 h (Chen *et al*., 1993).

Fish sexual maturity and gonadal development is associated with increased circulating levels of gonadotropins and the gonadal steroids (Huggard-Nelson *et al*., 2002). The histological characteristics of ovaries of experimental *C. gariepinus* brood stock revealed the presence of different development stages of oocytes. These observations in the present study were accordingly with those reported by West (1990). Whereas, major developmental events can be divided into six phases: Oogenesis, primary oocyte growth, cortical alveolar stage, vitellogenesis, maturation and ovulation (Tyler and Sumpter, 1996). Similar structures were reported by Hussein (1984). More histological description of different stages of fish oocytes development was reported by Selman and Wallace (1989). So, in the present study, results of *C. gariepinus* injected with tested hormonal materials revealed the superiority in development stages of oocytes compared with the control treatment. This superiority of injected materials in the present study may be due to mechanism to regulate fish fecundity and the physiological role of these injection hormones or fish pituitary extracts, concerning the significantly ($p \leq 0.05$) highest egg weight, absolute fecundity and relative fecundity, as well as blood hormones than the control treatment. In this trend, Miwa *et al.* (2001) reported that hCG stimulated 17, 20 β-P and 20 β-S production, resulting in final maturation of most mature oocytes, synchronously, in *S. asotus*. Meanwhile, Chowdhury *et al.* (2010) indicated a decrease in the number of the immature ovarian follicles, thus elucidating the rationale behind partial success of Ovaprim® induced spawning in the *C. batrachus*, this decrease in spawning

efficiency may be attributed to a decrease in the expression of GnRH-receptor II in the catfish ovary, leading to a concomitant decrease in the development and maturation of primary follicles and subsequent ovulation.

CONCLUSION

From the aforementioned results, it could be concluded that T_3 (injected with imported Argent®, carp pituitary gland) followed by T_2 (injected with imported Pregnyl®) and T_5 (injected with native catfish pituitary gland) were the best treatments for improving the reproductive performance of brood stock African catfish *C. gariepinus* compared with T_1 (control, without injection) and T_4 (injected with native carp pituitary gland). So, we recommend the useful usage of these materials in African catfish hatcheries, as well as further studies are needed by these materials at the commercial scale, which may be lead to the positive economic effects in the commercial hatcheries and fish farms not only for African catfish but also for fresh or marine water fish species also.

REFERENCES

Abdelhamid, A.M., I.A. Radwan, A.I. Mehrim and A.F.B. Abdelhamid, 2010. Improving the survival rate of the African catfish, *Clarias gariepinus*. J. Anim. Poult. Prod. Mansoura Univ., 1: 409-414.

Adebayo, O.T. and O.A. Fagbenro, 2004. Induced ovulation and spawning of pond raised African giant catfish, *Heterobranchus bidorsalis* by exogenous hormones. Aquaculture, 242: 229-236.

Babin, P.J., J. Cerda and E. Lubzens, 2007. The Fish Oocyte: From basic Studies to Biotechnological Applications. Springer-Verlag, Dordrecht, The Netherlands, ISBN-13: 9781402062339, Pages: 510.

Bailey, N.T.J., 1995. Statistical Methods in Biology. 3rd Edn., Cambridge University Press, Cambridge, UK., Pages: 252.

Bhujel, R.C., 2000. A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) broodfish in seed production systems, especially hapa-based systems. Aquacult., 181: 37-59.

Bosworth, B.G., 2005. Comparison of carp pituitary extract, catfish pituitary extract, or LHRHa for induced spawning of channel catfish females to produce channel catfish x blue catfish hybrid fry. Proceedings of the Aquaculture America Conference, January 17-20, 2005, New Orleans, LA., pp: 386.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. 2nd Edn., Birmingham Publishing Co., Birmingham.

Carillo, M., S. Sanuy, F. Prat, J. Cerda, J. Ramos, E. Mananos and N. Bromage, 1995. Sea Bass (*Dicentrachus labrax* L). In: Broodstock Management and Egg and Larval Quality, Bromage, N. and J. Roberts (Eds.). Blackwell Science, Osmead, pp: 424.

Chen, R.J., S.C. Huang, S.N. Chow and C.Y. Hsieh, 1993. Human chorionic gonadotropin pattern in maternal circulation. Amniotic fluid and fetal circulation in late pregnancy. J. Reprod. Med., 38: 151-154.

Chowdhury, M., A. Chatterjee, A. Mondal and U. Chatterji, 2010. Ovaprim abrogates expression of GnRH Receptor-II in the Indian catfish. Int. J. Biol., 2: 189-198.

Dada, A.A., E.O. Adeparusi and O.V. Alale, 2010. Dietary dried *Kigelia Africana* fruits meal as fertility enhancer in female *Clarias gariepinus* (Burchell, 1822). Agric. Biol. J. N. Am., 1: 791-795

Daghash, H.A. and S.Y. Hussein, 1999. The role of gonadotropin releasing hormone (GnRH) injection on spawning, spermiation, some blood constituents and sex hormones in the African catfish, *Clarias gariepinus*. Proceedings of the Conference of Egyptian Society for Animal Reproduction and Fertility, January 26-28, 1999, Egypt, pp: 185-202.

De Graaf, G.J. and J.A.L. Janssen, 1996. Artificial reproduction and pond rearing of the African Catfish, *Clarias gariepinus* in Sub-Saharan Africa. FAO Fisheries Tech Paper No. 36, pp: 73.

De Graaf, G.J., F. Galemoni and B. Banzoussi, 1995. The artificial reproduction and fingerling production of the African catfish *Clarias gariepinus* (Burchell 1822) in protected and unprotected ponds. Aquacult. Res., 26: 233-242.

Dorman, L., 2008. Hatchery and brood fish management. University of Arkansas, Cooperative Extension Program, August 26, 2008. http://en.engormix.com/MA-aquaculture/news/hatcherybrood-fish-management-t13244/p0.htm.

Duncan, D.B., 1955. Multiple range and multiple *F* test. Biometrics, 11: 1-42.

Eyo, J.E. and B.O. Mgbenka, 1992. Aspect of the biology of *Clarias gariepinus* in Anambra river basin I: Oocyte diameter fecundity and sex ratio. J. Agric. Sci. Tech., 2: 47-51.

GAFRD, 2012. General Authority for Fish Resources Development: Fish Statistics Year Book. Ministry of Agriculture and Land Reclamation Publications, Egypt, Pages: 106.

Garcia, A., M.V. Diaz and B. Agulleiro, 2001. Hormonal induction of spawning and embryonic development of Mediterranean yellowtail (*Seriola dumerili*, Risso). Monografias Instituto Canario Ciencias Marinas, 4: 561-566 (In Spanish).

Gur, G., P. Melamed, A. Gissis and Z. Yaron, 2000. Changes along the pituitary-gonadal axis during maturation of the black carp, *Mylopharyngodon piceus*. J. Exp. Zool., 286: 405-413.

Haffray, P., W.J. Enright, M.A. Driancourt, T. Mikolajczyk, P. Rault and B. Breton, 2005. Optimization of breeding of salmonids: Gonazon™, the first officially approved inducer of ovulation in the EU. World Aquacult. Mag., 3: 52-56.

Hecht, T., L. Oellermann and L. Verheust, 1996. Perspectives on clariid catfishes culture in Africa. Aquat. Living Resour., 9: 197-206.

Huggard-Nelson, D.L., P.S. Nathwani, A. Kermouni and H.R. Habibi, 2002. Molecular characterization of LH-â and FSH-â subunits and their regulation by estrogen in the goldfish pituitary. Mol. Cell. Endocrinol., 188: 171-193.

Hussein, K.A., 1984. Gonadal maturation and fecundity of a fish with partial spawning (*Tilapia nilotica* L.). Arabian J. Sci. Res., 2: 683-699.

Krouma, I., 2011. Regional review on status and trends in aquaculture development in the near East and North Africa-2010. FAO Fisheries and Aquaculture Circular No. 1061/6, FAO, Rome, pp: 75.

Kumar, D., 1992. Fish culture in undrainable ponds. A manual of extension, FAO Fisheries Technical Paper No. 325. Rome.

Kumar, R.S., S. Ijiri and J.M. Trant, 2001. Molecular biology of the channel cat?sh gonadotropin receptors: 2. Complementary DNA cloning, functional expression and seasonal gene expression of the follicle-stimulating hormone receptor. Biol. Reprod., 65: 710-717.

Lambert, D.M., B.J. Argue, Z. Lmz and R.A. Dunham, 1999. Effects of seasonal variations, thyroid and steroid hormones and carp pituitary extract on the artificial production of channel catfish *Zctalurus punctatus* X blue catfish Z. furcatus Hybrids. J. World Aquac. Soc., 30: 80-89.

Leonardo, A.F.G., E. Romagosa, M.I. Borella and S.R. Batlouni, 2004. Induced spawning of hatchery-raised Brazilian catfish, cachara *Pseudoplatystoma fasciatum* (Linnaeus, 1766). Aquaculture, 240: 451-461.

Liu, Z.J., P. Li, B.J. Argue and R.A. Dunham, 1997. Gonadotropin á-subunit glycoprotein from channel catfish (*Icralurus puncrarus*) and its expression during hormone-induced ovulation. Mol. Mar. Biol. Biotechnol., 6: 217-227.

Mikolajczyk, T., J. Chyb, P. Szczerbik, M. Sokolowska-Mikolajczyk and P. Epler *et al*., 2004. Evaluation of the potency of azagly-nafarelin (GnRH analogue), administered in combination with different formulations of pimozide, on LH secretion, ovulation and egg quality in common carp (*Cyprinus carpio* L.) under laboratory, commercial hatchery and natural conditions. Aquaculture, 234: 447-460.

Miwa, T., G. Yoshizaki, H. Naka, M. Nakatani, K. Sakai, M. Kobayashi and T. Takeuchi, 2001. Ovarian steroid synthesis during oocyte maturation and ovulation in Japanese catfish (*Silurus asotus*). Aquaculture, 198: 179-191.

Mylonas, C.C. and Y. Zohar, 2001. Use of GnRHa-delivery systems for the control of reproduction in fish. Rev. Fish Biol. Fish., 10: 463-491.

Nguenga, D., G.G. Teugels, M. Legendre and F. Ollevier, 2004. Influence of tropical seasonal changes on oocyte diameter, responses to hormonal induction and hatching quality in two strains of African catfish, *Heterobranchus longfilis* (Siluriformes, Clariidae). Aquacult Res., 35: 1349-1357.

Nwokoye, C.O., L.A. Nwuba and J.E. Eyo, 2007. Induced propagation of African clariid catfish, *Heterobranchus bidorsalis* (Geoffrey St Hillarie, 1809) using synthetic and homoplastic hormones. Afr. J. Biotechnol., 6: 2687-2693.

Rezk, M.A., 2008. Genetic improvement of *Clarias gariepinus* at the WorldFish center, Abbassa, Egypt. Proceedings of the Workshop on the Development of a Genetic Improvement Program for African Catfish *Clarias Gariepinus*, November 5-9, 2007, Accra, Ghana, pp: 96-103.

Richter, C.J.J. and A.J. Van Der Hurk, 1982. Effects of II-esoxcorticosterone-acetate and carp pituitary suspension on follicle maturation in the ovaries of African catfish *Clarias lazera* (C and V). Aquaculture, 29: 53-66.

Roberts, J.R., 2001. Fish Pathology. 3rd Edn., Churchill Livingstone, UK., ISBN-10: 0702025631, pp: 380-470.

Sadek, A.S.M., 2009. Nutritional and physiological studies on African catfish (*Clarias gariepinus*). Ph.D. Thesis, Faculty of Agriculture, University of Kafr El-Sheikh, Egypt.

Sahoo, S.K., S.S. Giri, S. Chandra and A.K. Sahu, 2007. Spawning performance and egg quality of Asian catfish *Clarias batrachus* at various does of HCG injection and latency periods during spawing induction. Aquaculture, 266: 289-292.

Salami, A.A., O.A. Fagbenro, L. Edibite and S.O. Fagbemiro, 1994. Induced spawning of the African catfish, *Clarias gariepinus* using non-piscine pituitary extracts. J. World Aquacult. Soc., 25: 166-168.

SAS, 2001. Statistical Guide for Personal Computer. SAS Institute Inc., Carey, NC., USA.

Sayed, A.E., U.M. Mahmoud and I.A.A. Mekkawy, 2012. Reproductive biomarkers to identify endocrine disruption in *Clarias gariepinus* exposed to 4-nonylphenol. Ecotoxicol. Environ. Saf., 78: 310-319.

Schulz, R.W., M.A. Zandbergen, J. Peute, J. Bogerd, W. van Dijk and H.J.T. Goos, 1997. Pituitary gonadotrophs are strongly activated at the beginning of spermatogenesis in African catfish, *Clarias gariepinus*. Biol. Reprod., 57: 139-147.

Selman, K. and R.A. Wallace, 1989. Cellular aspects of oocyte growth in teleost. Zool. Sci., 6: 211-231.

Sharaf, S.M., 2005. Effect of gonadotropin-releasing gormone (GnRHs) and Human Chronic Gonadotropin (HCG) on ovarian development and plasma levels of sex steroids in African catfish *Clarias gariepinus*. Egypt J. Applied Sci., 20: 23-37.

Sharaf, S.M., 2012. Effect of GnRHa, pimozide and Ovaprim on ovulation and plasma sex steroid hormones in African catfish *Clarias gariepinus*. Theriogenology, 77: 1709-1716.

Swanson, P., K. Suzuki, H. Kawauchi and W.W. Dickhoff, 1991. Isolation and characterization of two *Coho salmon* gonadotropins, Gth-I and Gth-II. Biol Reprod., 44: 29-38.

Szabo, T., F. Radics, T. Barth and L. Horvath, 2007. *In vivo* activity of native GnRHs and their analogues on ovulation in the African catfish, *Clarias gariepinus* (Burchell). Aquacult. Res., 38: 140-146.

Tseng, W.Y. and K.L. Chan, 1982. The productive biology of the rabbit fish in Hong Kong. J. World Maricul. Soc., 13: 313-321.

Tyler, C.R. and J.P. Sumpter, 1996. Oocyte growth and development in teleosts. Rev. Fish Biol. Fish., 6: 287-318.

Verreth, J., E.H. Eding, G.R.M. Rao, F. Huskens and H. Segner, 1993. A review of feeding practices, growth and nutritional physiology in larvae of the catfishes *Clarias gariepinus* and *Clarias batrachus*. J. World Aquac. Soc., 24: 135-144.

Vischer, H.F. and J. Bogerd, 2003. Cloning and functional characterization of a gonadal luteinizing hormone receptor complementary DNA from the African cat?sh (*Clarias gariepinus*). Biol. Reprod., 68: 262-271.

Wen, H.S. and H.R. Lin, 2004. Effects of exogenous neurohormone, gonadotropin (GtH) and dopaminergic drugs on the serum GtH content and ovulatory responsiveness of wild catfish, *Silurus asotus* (Linnaeus, 1758). Aquacult. Res., 35: 204-212.

West, G., 1990. Methods of assessing ovarian development in fishes: A review. Aust. J. Mar. Freshwater Res., 41: 199-222.

Wu, F., X. Zhang, W. Zhang, B. Huang, Z. Liu, C. Hu and D. Wang, 2009. Expression of three gonadotropin subunits in Southern cat?sh gonad and their possible roles during early gonadal development. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 153: 44-48.

Yadetie, F. and R. Male, 2002. Effects of 4-nonylphenol on gene expression of pituitary hormones in juvenile Atlantic salmon (*Salmo salar*). Aquat. Toxicol., 58: 113-129.

Yaron, Z., G. Gur, P. Melamed, H. Rosenfeld, A. Elizur and B. Levavi-Sivan, 2003. Regulation of fish gonadotropins. Int. Rev. Cytol., 225: 131-185.

Zohar, Y. and C.C. Mylonas, 2001. Endocrine manipulations of spawning in culture fish: From hormones to genes Aquaculture, 197: 99-136.