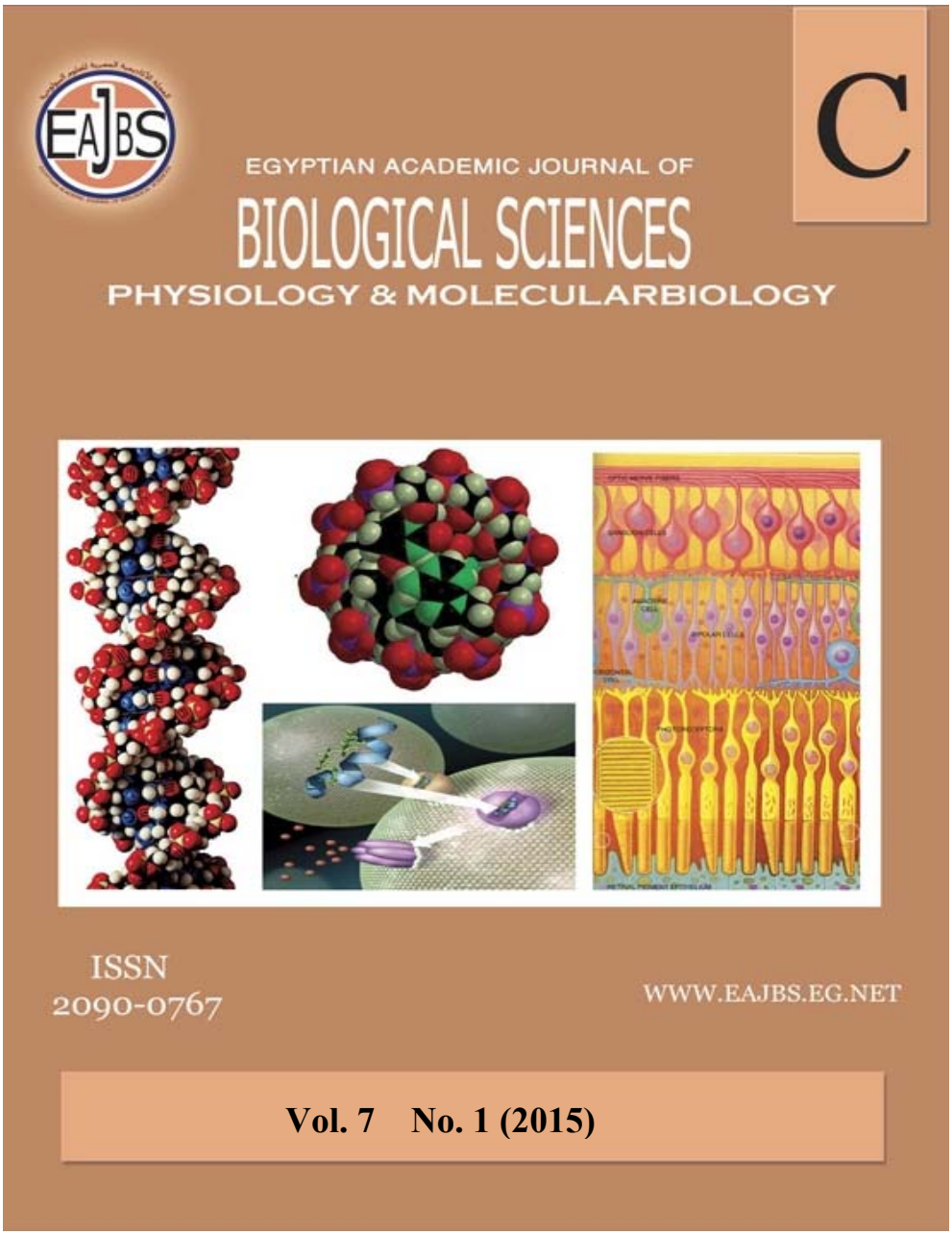

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Effect of Follicular Stimulating Hormone and Leutinizing Hormone on Reproduction, Physiological and Biochemical Changes of *Oreochromis niloticus*

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ABSTRACT

The fish were divided into four groups, control group and three groups injected intramuscularly with follicular stimulating hormone and leutinizing hormone (FSH and LH) 10, 20 and 40 µg kg− 1 body weight dissolved in 0.6% NaCl under a natural temperature regime. The fish were held in large tanks contained water from their farm. The fish were fed with 30 % protein diet during experiment. Females and males *Oreochromis niloticus* 150 - 160 g body weight from Abbasa farm were injected with a doses of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 µg kg− 1 body weight) three times a week, The result showed multiple ovulations and spawns within a period of approximately 30 days. Plasma levels of 17β-estradiol (E2), follicular stimulating hormone (FSH) and leutinizing hormone (LH) were elevated at 30 days post-treatment, preceding the spawns with the highest fecundity, concomitant with a decrease in the levels of plasma testosterone (T). However, follicular stimulating hormone and leutinizing hormone (10, 20 and 40 µg kg− 1 body weight) treatment in males increased plasma levels of testesterone and 11-ketotestosterone (11-KT) during injection. These results show that administration of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 µg kg− 1 body weight) induces multiple spawns in *Oreochromis niloticus.* where, approximately 100% increase in red blood cells (RBCs), white blood cells (WBCs), hematocrit value (Hc) and hemoglobin content (Hb) respectively. Also, there are increased in serum glucose level, total protein, aspartate and alanine amino transferase activities (AST and ALT) and increased serum creatinine and uric acid concentration after administration of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 µg kg− 1 body weight) respectively. The results indicate that the fish that administration of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 µg kg− 1 body weight) increase blood constituents, serum glucose level, serum total protein and serum AST, ALT activities, creatinine and uric acid level. These data suggest that fish respond to administration of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 µg kg− 1 body weight) by increasing their WBCs, enzymes of liver functions and kidney functions.

INTRODUCTION

In Teleosteii, the growth of ovaries can be subdivided into 6 stages: oogenesis, primary ooctyte growth, corticular alveolar stage, vitellogenesis, maturation and ovulation (Tyler & Sumpter 1996). At ovulation, the mature oocyte is released from its follicle wall enclosure in the ovary. Ovulation is the release of a mature oocyte from its follicle wall enclosure in the ovary.

The oocyte separates from the granulosa layer and the follicle wall ruptures to expulse the oocyte. Meiosis is completed at fertilisation with the penetration of the egg by sperm. Distinctive features of *O. tilapia* ovaries at different stages of maturation have been described (Babiker 1986; Tacon, Ndiaye, Cauty, Le Menn & Jalabert 1996). However, the most 16 accurate description is that of Tilapia zillii (Coward & Bromage 1998). Oocyte development can generally be called synchronous or asynchronous (Scott 1987). Furthermore, it is also not clear from which oocyte 17 growth stage the next oocytes are recruited for developing into mature oocytes (Jalabert & Zohar 1982). In *Oreochromis niloticus* and Tilapia zillii it was shown that each new group of maturing oocytes arose from a pool of pre-vitellogenetic eggs (Babiker 1986; Coward & Bromage 1998). In the tropics, *O. niloticus and O. aureus* are known to have a continuous sperm production all year round (Lowe-McConnell 1959; Hyder 1972). Hormonal regulation Two distinct gonadotropins have been found in a number of teleost fish and in tilapia (Suzuki, *et al* 1988; Bogomolnaya, *et al* 1989; Rosenfeld *et al* 2001). These are homologous to the mammalian follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These gonadotropins contain an α-subunit which is common to both FSH and LH and a β-subunit which is unique to each gonatropin (Rosenfeld *et al.* 2001). In tilapia, the presence of LHβ and FSHβ have been demonstrated (Rosenfeld *et al.,* 1997). However, in fish more is known on LH than on FSH (Van *et al*. 1998).The ease in cultivation, a rapid growth, a very palatable flesh and high resistance to disease, favors the farming of Nile tilapia. As a consequence research with this species is intensifying. In order to perform selective breeding, gynogenesis, hybridisation or sex inversion, large amounts of ripe unfertilised eggs are necessary. Due to aseasonality and an individual development of the ovaries, it is difficult to harvest adequate quantities of Nile tilapia eggs. The aquaculture of the warm water tilapia is also gaining in importance. Recently the world output of tilapia overtook that of salmonids in terms of quantity. Of all several tilapia species which are cultivated, the Nile tilapia (*O. niloticus*) is the most economically important. Furthermore no method has been developed yet which satisfyingly helps in controlling spawning in Nile tilapia for the purpose of egg production. The sexual differentiation of Tilapia gonads occurs around 15 to 30 days after fecundation (Nakamura & Takahashi 1973; Yoshikawa & Oguri 1978). Depending on environmental conditions, sexual maturity can already be reached after 2-3 months. The blood glucose levels have long been used as indicators of stress in fish as recorded by (Hattingh, 1976; Donaldson, 1981; Wedemeyer & Mcleay 1981). Hyperglycemia may provide additional energy during times of high metabolic need such as" fight or flight" response. Abdelmegid *et al*. (2002) reported that all mean difference total lipids, cholesterol, glucose and creatinine were significant increase and showed hyperglycemia and lipidimia and elevated levels of creatinine in serum as compared to the control.

In the present work, we investigated the effectiveness of administration of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 μg kg^{-1} body weight) intramuscular injection in inducing ovulation and spermiation in *O. niloticus*. The changes in plasma levels of sexual steroids in response to administration of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 μg kg^{-1} body weight) treatments, in both males and females *O. niloticus*, were also

determined. Also, administration of follicular stimulating hormone and leutinizing hormone (10, 20 and 40 μg kg− 1 body weight) by increasing their WBCs, enzymes of liver functions and kidney functions in *O. niloticus*.

MATERIALS AND METHODS

O. niloticus 150 - 160 g body weight was obtained from Abbasa farm. The fish were held in large tanks contained water from their farm. The fish were fed with 30 % protein diet during experiment. The fish were divided into four groups, control group and three groups injected intramuscularly with follicular stimulating hormone (FSH) and leutinizing hormone (LH) 10, 20 and 40 μg kg^{-1} body weight dissolved in 0.6% NaCl under a natural temperature regime. The FSH and LH used was (Bachem Bioscience, King of Prussia, PA). hormonal treatments were given in duplicate tanks (10 males and females in each tank). The photoperiod was maintained at 13L: 11D, and the female: male sex ratio in each tank was approximately1:1.Throughout the experiments, fish were fed with the 30% protein diet. Control fish were treated with 0.6% NaCl. During experiment fish were injected 3 times per week with follicular stimulating hormone and leutinizing hormone (10, 20 and 40 μg kg^{-1} body weight) for 30 days. Each group consists of two-glass aquaria 30 x 60 x 40 cm^3. Each glass aquarium contains 10 fish. The blood samples were taken from fish after 30 dayes from arterial caudal with heparinzed syringes from control group and injected groups, respectively according to the methods of Soivio *et al*. (1972). The blood was centrifuged at 4000 rpm for 15 minutes to separate the serum for measuring Plasma levels of 17β-estradiol (E2), follicular stimulating hormone (FSH), leutinizing hormone (LH), testosterone (T) and 11-ketotestosterone. The last blood samples were taken from control fish group and injected fish with 1, 5 μg kg^{-1} body weight (BW) for measuring Plasma levels of 17β-estradiol (E2), follicular stimulating hormone (FSH) and leutinizing hormone (LH), testosterone (T) and 11-ketotestosterone.Also, measuring RBCs, WBCs, HB, HC, enzymes of liver functions and kidney functions in *Oreochromis niloticus*.

Egg collection and evaluation of gamete quality

Eggs were collected after injection three times a day using net collectors located at the outflow of the tanks. Buoyant eggs were separated from non-buoyant eggs, as a measure of egg quality, in a calibrated cylinder for about 1 h. The eggs were immediately incubated in different tanks at 19 °C for determination of hatching success. Fertilization was assessed in a fraction of 100 buoyant eggs under a stereomicroscope. Fecundity was expressed as total egg produce or relative fecundity (eggs kg^{-1} female).During the follicular stimulating hormone and leutinizing hormone (10, 20 and 40 μg kg^{-1} body weight) injection trials, spermiating males were identified by the release of sperm after application of gentle abdominal pressure approximately every week. When milt could be obtained, and was free of urine, the total volume of sperm was recorded by using a micropipette. Sperm density (SD) was determined in 1: 100 or 1: 200 diluted sperm using a haemocytometer and a Leica DMLB light microscope. Also when sperm was obtained clean, the spermatocrit (Sct) was determined using a capillary pipette that was centrifuged at 13,000 rpm for 10 min. To measure sperm motility, milt samples were diluted 100-fold in filtered water being rapidly placed on microscope slides and immediately viewed at ×200 magnifications under the microscope. The sperm motility duration (SMD) was defined as the time from the beginning of activation to the cessation of all

spermatozoa movement (Lim *et al*., 2004).

Analytical techniques

Blood samples were drawn from arterial caudalis with heparinized syringes. Plasma levels of 17β-estradiol (E2), follicular stimulating hormone (FSH) and leutinizing hormone (LH), testosterone (T) and 11-ketotestosterone. Plasma levels of free 17β-estradiol (E_2; measured only in females), testosterone (T) and 11-ketotestosterone (11-KT; measured only in males) were quantified by enzyme-linked immunosorbent assay (ELISA) following the method described by Rodríguez et al. (2000). Blood samples were drawn from arterial caudalis with heparinized syringes. Red blood cells and white blood cells were determined by hemocytometres improved neubaure, hawksley, Germany. Hematocrite value was determined by using a microcapillary tubes where, these tubes filled with blood immediately after blood collection and centrifuged in a microcapillary centrifuge. The amount of red blood cells was determined as a percentage of total blood (hematocrit) by the use of microcapillry reader. Hemoglobin content measured according to Dacie and Lewis (1975). The remaining blood samples were centrifuged for determination of concentration of serum glucose, total protein, the activities of AST, ALT activities, creatinine and uric acid. The glucose was determined by enzymatic colorimetric method according to Trinder (1969). The total protein was determined by rapid colorimetric method according to Peter (1968). The activities of AST and ALT were determined by colorimetric method according to Reitman and Frankel (1957). The creatinine and uric acid levels were determined by clorimetric methods according to Henry (1974).

STATISTICAL ANALYSIS

Data were reported as means ± S. E. (n). Significant differences ($p<0.05$) within each group were tested with Student's two-tailed t-test and one-way ANOVA by SPSS for windows XP2 2002. Comparisons between groups were tested by Student's two-tailed t-test unpaired design, ($p<0.05$).

RESULTS

I- Reproductive parameters:

Oreochromis niloticus males and females were initially stimulated by injection of follicular stimulating hormone (FSH) and leutinizing hormone (LH) 10, 20 and 40 μg kg^{-1} body weight (Table 1). Control fish did not show any spontaneous spawning. In contrast, injection with 10 or 20 μg FSH and LH kg^{-1} induced spawning 3–4 days after the treatment. Females injected with 10 μg FSH and LH kg^{-1} produced 7,000 eggs with a relative fecundity of 4.564 eggs kg^{-1}, whereas the dose of 40 μg FSH and LH kg^{-1} induced more ovulations per female with a total production of 10,000 eggs and a relative fecundity of 8.921 eggs kg^{-1}.

Table 1: Effect of FSH and LH injection for a month on the spawning performance of females *Oreochromis niloticus.*

Treatment (μg FSH &LH	N	Mean weight (g)	Spawnin gs (n)	Total eggs	Relative fecundity (eggs kg− 1)	Egg buoyancya (%)
Saline	10	150 ± 10	0	–	–	–
10 μg FSH kg^{-1}	10	150 ± 10	3	7,000	4.564	30.1 ± 14.1^{ab}
20 μg FSH kg^{-1}	10	150 ± 10	5	8,000	5.832	38.5 ± 17.1^{ab}
40 μg FSH kg^{-1}	10	150 ± 10	8	10.000	8.921	43.7 ± 11.1^{ab}
10 μg LH kg^{-1}	10	150 ± 10	2	3.000	2.322	22.4 ± 16.1^{ab}
20 μg LH kg^{-1}	10	150 ± 10	6	7565	6.684	33.7 ± 1.23^{b}
40 μg L kg^{-1}	10	150 ± 10	7	8,456	7.228	53.7 ± 0.8^{a}

Significant at $p<0.05$, ** highly significant at $p< 0.01$, *** very highly significant at $p < 0.001$.

In both cases, the percentage of buoyant eggs was similar (30.1% and 38.5 ± 43.7%). However, none of the eggs produced were fertilized.

As indicated for FSH and LH which injected 3 times weekly for a period of 30 days, these treatments resulted in an increased number of spawns per female with respect to that observed in the preliminary trial, the number of spawns being higher in females injected with 40μg FSH and LH kg− 1after 30 days (16 spawns) than in those treated with 10 and 20 μg FSH and LH kg− 1after 30 days (32 spawns), while control fish did not spawn (Table 1). Both the number of eggs and relative fecundity after FSH and LH treatments were higher with respect to the preliminary trial, and these increased according with the number of FSH and LH injections applied. Thus, 7000 eggs and 8000 eggs kg− 1; and 10000 eggs and 3000, 7565 and 8456 eggs kg− 1, were obtained from females injected 3 times weekly treated with 10 and 20 μg FSH and LH kg− 1after 30 days (Table 1). However, although the percentage of buoyant eggs was similar between treatments (43.7 ± 1.3% and 53.7 ± 0.8%) (Table 1).

In males, saline- and FSH and LH - treated groups showed approximately the same number of spermiating fish, which produced small amounts of sperm after gentle pressure of the abdomen (Table 2). In addition, no statistical differences were detected between FSH and LH - treated and untreated fish in sperm volume, Sct, SD or SMD recorded during the treatment period (Table 2).

Table 2: Effects of FSH and LH injection for a month on sperm production and quality of males *Oreochromis niloticus*.

Treatment (μg FSH &LH	N	Mean weight (g)	Spermiating males (n)	Sperm volume (μl)	Sct1 (%)	SD2 (×1 09ml− 1)	SMD3(s)
Saline	10	150 ± 10	-	-	-	-	-
10 μg FSH kg^{-1}	10	150 ± 10	5	30 ± 5	21.2 ± 7.2	3.1 ± 1.0	132 ± 49
20 μg FSH kg^{-1}	10	150 ± 10	6	31 ± 6	23.2 ± 4.8	2.6 ± 1.0	96 ± 50
40 μg FSH kg^{-1}	10	150 ± 10	8	34 ± 6	25.4 ± 4.7	2.0 ± 1.0	60 ± 20
10 μg LH kg^{-1}	10	150 ± 10	4	27 ± 5	22.2 ± 7.2	3.1 ± 1.0	132 ± 49
20 μg LH kg^{-1}	10	150 ± 10	5	30 ± 6	23.2 ± 4.8	2.6 ± 1.0	96 ± 50
40 μg L kg^{-1}	10	150 ± 10	7	32 ± 6	25.4 ± 4.7	2.0 ± 1.0	60 ± 20

significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001,

1Stc, spermatocrit; 2SD, sperm density; 3SMD, spermatozoa motility duration. These parameters did not significantly affected by injection, and thus the mean ± S.E.M. of all sampling points is indicated.

Plasma levels of 17β-estradiol (E2), follicular stimulating hormone (FSH) and leutinizing hormone (LH), testosterone (T) and 11-ketotestosterone were determined. During injection plasma E2 levels in females increased at 30days after FSH and LH treatment (table. 3). the increase of plasma E2 levels was stronger in females injected with 40 μg FSH and LH kg− 1than in females treated 10 and 20 μg FSH and LH kg− 1. In both cases, plasma E2 increased after 30 dayed injections with FSH and LH than in controls. A similar effect on plasma FSH and LH levels was observed in FSH and LH injected females, but plasma levels of testosterone (T) and 11-ketotestosterone were much lower than those determined in control.

The highest levels of E2 were detected in some females injected with 10 and 20 μg FSH and LH kg− 1, although the values were not statistically significant with respect to those from

control fish. Females treated with 40µg FSH and LH kg− 1 did not show increased plasma E2 levels (Table 3). Plasma T decreased as E2 increased, thus showing an inverse relationship with E2 during the period of FSH and LH treatment. T levels were significantly lower in females treated with 40 µg FSH and LH kg^{-1} 3 time weekly form a period of 30 days. The females injected with 10 and 20 µg FSH and LH kg^{-1} showed the lowest plasma T levels (Table 3).

Table 3: Effects of FSH and LH injection for a month on the levels of 17β-estradiol (E2), FSH, LH, testosterone (T) and 11-ketotestosterone of *Oreochromis niloticus*.

Treatment (µg FSH &LH	n	Female			Male	
After 30 day		(E2) ng/ml	FSH mIU/ml	LH mIU/ml	T ng/ml	11-KT ng/ml
Saline	10	14.5±0. 58	12.2±0.45	4.4±0.14	0.58±0.55	40.2±0.45
10 µg FSH kg^{-1}	10	20.4±0.59**	14.6±0.27	6.1±0.20*	0. 33±0.018*	22.4±0. 5**
20 µg FSH kg^{-1}	10	50.5±005***	15.2±0. 71**	9.3±0. 12**	0.43±0.41**	25.4±0.42**
40 µg FSH kg^{-1}	10	65.9±0.431	18.9±0.642	11.975±0.90	0.48±0.25	34.2±0.16
10 µg LH kg^{-1}	10	20.8±0. 85**	12.8±0.55**	9.5±0. 61*	0.33±0.08*	10.9±0. 21**
20 µg LH kg^{-1}	10	27.9±0. 4***	20.7±0.43***	13.2±0. 3**	0.43±0.202**	16.8±0.64***
40 µg LH kg^{-1}	10	55.9±0.58***	25.7±0.83***	16.2±0.73**	0.463±0. 02**	18.8±0.88***

significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001,

FSH and LH treatment appeared to decrease the levels of both androgens, since males injected with 10, 20 and 40µg FSH and LH kg^{-1} showed significantly (P < 0.05) lower plasma T and 11-KT levels than those in control fish (Table 4).

[1]GSI, gonadosomatic index; [2]TSI, testis spermatozoa index. The TSI were significantly increase than control group after the injection of the fish with 10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days.

Table 4: Effects of FSH and LH injection for a month on gonado somatic index and testis spermatozoa index of *Oreochromis niloticus.*

Treatment (µg GnRHa kg− 1)	N	Mean weight (g)	GSI1	TSI2
Saline	10	150 ± 10	0.07 ± 0.01	267 ± 35
10 µg FSH kg^{-1}	10	150 ± 10	0.08 ± 0.01	296 ± 66*
20 µg FSH kg^{-1}	10	150 ± 10	0.09 ± 0.02*	310 ± 78*
40 µg FSH kg^{-1}	10	150 ± 10	0.10 ± 0.01*	342 ± 66*
10 µg LH kg^{-1}	10	150 ± 10	0.08 ± 0.02	226 ± 54*
20 µg LH kg^{-1}	10	150 ± 10	0.09 ± 0.02*	310 ± 71*
40 µg L kg^{-1}	10	150 ± 10	0.10 ± 0.01*	351 ± 33*

significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001,

II- Hematology Parameters

The red blood cells (RBCs) in the groups of the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days were significantly increased compared to control group as showed in Table (5). Table (5) showed a significant increase in the white blood cells (WBCs) in the groups of the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days respectively. Hemoglobin (Hb) was a significantly increase in the groups of the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days compared to control group. Hematocrit (Hct) was a significantly increased in the groups of the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days compared to control group as shown in Table (4).

Table 5: Effects of FSH and LH injection for a month on blood constituntes of *Oreochromis niloticus*.

parameters	RBCs x 106	WBCs x 103	Hb gm / 100ml	Hct %
Control	1333333.3 ± 57831	16866.6±866.6	7.11±0.19	22.25±0.57
10 µg FSH kg^{-1}	1596666.7±54873*	18933.3±762.3	8.99±0.17*	25.96±0.54*
20 µg FSH kg^{-1}	1713333.3±35276**	23566.6±1026.8*	9.36±0.12**	27.14±0.35**
40 µg FSH kg^{-1}	1806666.7±49328**	30416.6±1651.3**	9.68±0.09**	28.06±0.28**
10 µg LH kg^{-1}	1830000±55075**	76200±2193.1***	9.76±0.18**	28.3±0.55**
20 µg LH kg^{-1}	1880000±36055**	93733.3±3339.8**	9.92±0.12**	28.8±0.36**
40 µg LH kg^{-1}	2006667±90184**	133166.7±9116.3**	10.36±0.16**	30.1±0.55**

Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001,

III- Biochemical Parameters

Table (6) showed that the activity of aspartate amino transferase (AST) in the serum of fish that injected with10, 20 and 40 µg FSH and LH kg− 1 for a period of 30 days compared to control group. The serum activity of alanine amino transferase (ALT) of the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days respectively were very highly significant increased compared to control group. The serum uric acid leveled was significantly increased in the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days compared to control groups.

Table 6: Effects of FSH and LH injection for a month on liver functions enzymes and kidney functions of *Oreochromis niloticus*.

parameters	AST U/ L	ALT U/ L	Uric acid mg %	Creatinine mg %
Control	6.46±0.50	10.45±0.68	5.71±0.65	1.57±0.11
10 µg FSH kg^{-1}	11.3±0.75*	13.6±0.95	10.3±0.20*	1.80±0.05
20 µg FSH kg^{-1}	14.5±0.40*	15.73±0.90*	11.4±0.57*	1.86±0.03
40 µg FSH kg^{-1}	19.16±0.61**	20.8±0.88**	12.66±0.88**	1.87±0.03
10 µg LH kg^{-1}	19.36±0.52**	23.16±0.61**	12.06±0.14**	2.33±0.046
20 µg LH kg^{-1}	22.36±1.09**	25.86±0.80**	13.8±0.40**	2.47±0.0818
40 µg LH kg^{-1}	25.03±0.84***	27.06±0.46***	14.66±0.89***	2.59±0.097

Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001,

There were no significant increased in the serum creatinine level of the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days compared to control group. There was no significant increased in the serum glucose level of the fish that injected with10µg FSH and LH kg^{-1} for a period of 30 days compared to control group. But, the serum glucose level was significantly increased in the fish which injected with 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days in their serum glucose level compared to control group. The serum total protein of the fish that injected with10, 20 and 40 µg FSH and LH kg^{-1} for a period of 30 days were highly significant increased compared to control group as shown in Table (7).

Table 7: Effects of FSH and LH injection for a month on Glucose mg / dl and total protein g% of *Oreochromis niloticus*.

parameters	Glucose mg / dl	Total protein g%
Control	66.21±1.15	5.86±0.14
10 µg FSH kg^{-1}	73.33±1.66	6.56±0.18*
20 µg FSH kg^{-1}	77.33±1.45*	7.34±0.086**
40 µg FSH kg^{-1}	82.66±1,45**	8.5±0.17**
10 µg LH kg^{-1}	108.66±4.66**	8.5±0.17**
20 µg LH kg^{-1}	119.33±1.76**	8.46±0.14**
40 µg LH kg^{-1}	123±1.52**	8.76±0.088**

Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001

DISCUSSION

In the present work, untreated, control females did not undergo spontaneous ovulation during experiment. However, treatment of females with FSH and LH injection inhanced vitellogenesis, proved to be effective in inducing ovulation and spawning.

The *Oreochromis niloticus* shows a "group-synchronous" ovarian development in which successive batches of vitellogenic oocytes are recruited into FOM and ovulation during the spawning season (Wallace and Selman, 1981). This pattern of ovarian development, also observed in other flatfish species, often gives rise to multiple ovulations after GnRH treatment (Mylonas and Zohar, 2001). Consistent with these observations, repeated injections with FSH and LH were able to induce successive and even daily spawnings of *O. niloticus*.

The plasma levels of gonad steroids in females treated with FSH and LH showed that plasma E2 increased transiently within month after hormone treatment and decreased thereafter, while plasma levels of T decreased progressively. An initial increase of plasma E2 concentrations after natural or FSH and LH induced spawning has been observed in marine species with group-synchronous ovaries as Senegal sole, such as the North sea plaice (Scott *et al.,* 1999), yellowtail flounder (*P. ferrugineus*; Larsson *et al.,* 1997), European sea bass (Prat *et al.*, 2001), and greenback flounder (*Rhombosolea tapirina*; Poortenaar and Pankhurst, 2000). The increase of plasma FSH and LH and concomitant decrease of plasma T, preceded the spawns with the highest fecundity thus suggesting that FSH and LH possibly induced a massive recruitment of oocytes into vitellogenesis with increased aromatase activity converting T into E2. The subsequent decrease of E2 plasma levels could be caused by the shift of estrogen into progestogen production by the ovary related to oocyte maturation and ovulation processes. However, these conclusions must await the quantification of endogenous gonadotropin plasma levels after FSH and LH treatment.

In contrast to females, treatment of males with FSH and LH did not increase the production of spermatozoa, which could be related to the absence of egg fertilization observed in this work. The ineffectiveness of FSH and LH in inducing spermiation in *Oreochromis niloticus*, however, is not totally surprising, since similar results have been obtained in other flatfish species (Mylonas and Zohar, 2001; Berlinsky *et al.,* 1996 and Berlinsky *et al.*, 1997). A better situation was observed in Atlantic halibut producing low milt volume and poor sperm motility at the end of the spawning season, where treatment with FSH and LH containing EVAc implants resulted in an enhancement of milt volume and sperm motility, but also a concomitant decrease in spermatocrit (Vermeirssen *et al.,* 2000 and Vermeirssen *et al.,* 2004). Similarly, in the North Sea plaice (Vermeirssen *et al.,* 1998), starry flounder (*Platichthys stellatus*; Moon *et al.*, 2003) and greenback flounder (Lim *et al.,* 2004). The present study shows that plasma levels of androgens, T and 11-KT, were significantly decreased after FSH and LH injection. Treatment with FSH and LH implants is generally effective at increasing androgen plasma levels in a variety of teleosts including flatfish (Harmin and Crim, 1993 and Moon *et al.,* 2003), and thus it seems reasonable to find elevated plasma T and 11-KT levels in GnRH-treated Senegal sole males. However, large elevations of androgen plasma levels in flatfish are not always associated with enhanced milt volume, since in some

species GnRH is effective in stimulating milt production regardless of the levels of androgens in plasma (Clearwater and Crim, 1998, Vermeirssen *et al.*, 2000, Moon *et al*., 2003 and Lim *et al.*, 2004). These findings in flatfish would be consistent with the current accepted model for most teleosts in which spermatogenesis is regulated by androgens, while progestogens such as 17α,20β-dihydroxy-4-pregnen-3-one (17,20βP) have been suggested to be the main steroids involved in spermiation and milt hydration (e.g., Borg, 1994, Moon *et al*., 2003, *Vermeirssen et al.*, 1998 and Vermeirssen et al., 2004). In the present work, the plasma levels of progestogens were not determined, and therefore the effects of FSH and LH on progesterone synthesis and/or conversion in the testis that may explain the ineffectiveness of FSH and LH to improve milt production in *O. niloticus* remain to be investigated.

In addition, plasma levels of E2 were increased at 30 days after injection with FSH and LH. In males, a similar situation apparently occurred, since both T and 11-KT plasma levels of fish treated with FSH and LH were lower than those of control. These observations may be related to physiological changes throughout the reproductive cycle in the sensitivity of the pituitary and/or gonads to FSH and LH gonadotropins, respectively. Although the causes of this differential responsiveness to FSH and LH are unknown, several potential mechanisms, such as gonadotropin receptor desensitization or negative feedback mechanisms on gonadotropin release by steroids, could be involved (Cerdà *et al.,* 1997, Schulz *et al.*, 2001 and Amsterdam *et al.,* 2002). In any event, the elucidation of the processes that may regulate pituitary and/or gonad responsiveness to GnRHa treatment during the reproductive cycle of Senegal sole requires further investigation.

This study indicated that there were an increase in the number of red blood cells and white blood cells. Also, an increased in the hematocrit value and hemoglobin content under the effect of aluminum chloride at the different periods. An increase in the number of red blood cells and hematocrit and hemoglobin contents could also have contributed the results that reported by Witter *et al.* (1987). The increase in the red blood cells, hematocrit and hemoglobin are agreement with (Goss and Wood 1988 and Miligan & Wood, 1982) who indicated the blood parameters were increased in rainbow trout. Total serum protein in *O. niloticus* was increased in this study and this may be due to stress of FS|H and LH on the fish. This increase was agreement with the results of Gross and Wood (1988) who reported the total serum protein was increased in the fish that exposed to stress. This study observed that the increase in the serum proteins are agreement with (Abdelmegid *et al*. 2002) that reported the liver protein was increased in Tilapia zilii under the effect stress. This study revealed that the increase in the serum AST and ALT activities, creatinine, uric acid and serum glucose level due to stress in the fish by affecting of FS|H and LH. This studies are agreement with the result of (Goss and Wood 1988) who reported the validity of cortisol and glucose as indicators of the stress in the fish. The blood glucose levels have long been used as indicators of stress in fish as recorded by (Hattingh, 1976; Donaldson, 1981; Wedemeyer & Mcleay 1981) who reported under condition of stress, hyperglycemia may provide additional energy during times of high metabolic need such as" fight or flight" response. This study observed that the increased in the serum creatinine and glucose level are agreement with Abdelmegid *et al*. (2002) who reported that all mean difference total lipids, cholesterol,

glucose and creatinine were significant increase and showed hyperglycemia and lipidimia and elevated levels of creatinine in serum as compared to the control.

CONCLUSION

Hormonal treatment with FS|H and LH both through repeated injection was able to induce ovulation and spawning of *Oreochromis niloticus* females, which usually fail to reproduce in captivity. However, FS|H and LH treatment of males was ineffective in stimulating reproductive processes (i.e., increasing spermiation or milt production), which may be related with the absence of egg fertilization in the spawnings produced by FS|H and LH treated females. Further studies are needed to identify the social, environmental and endocrine mech-anisms regulating male reproduction in Senegal sole, as well as the factors responsible for FS|H and LH failure in enhancing sperm production, contrary to the situation in most other studied teleosts.It can be concluded from this study that disturbance in the RBCs and WBCs, hematocrit value, hemoglobin content, serum total protein, serum glucose level, serum activities of (AST, ALT) and (creatinine and uric acid) concentrations as a result of stress of FS|H and LH injection on *O. niloticus* reflect the disturbance in all metabolic function.

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ARABIC SUMMARY

تأثير مسامي تحفيز الهرمونات و هرمون Leutinizing على التكاثر و التغيرات الفسيولوجية و البيوكيميائية للبلطى النيلى

السيد احمد محمد شكر
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اوضحت النتائج ان حقن البلطى النيلى بجرعات مختلفة من FSH and LH ١٠ و ٢٠ و ٤٠ ميكروجرام /كجم زيادة واضحة فى مكونات الدم.

ايضا وجد زيادة واضحة فى سكر الدم و كذلك فى وظائف الكبد و وظائف الكلى.

اوضحت النتائج ان الحقن بالهرمونين FSH and LH للبلطى ادى الى زيادة هرمونات التكاثر فى الذكور و الاناث.

يمكن القول بان الحقن بالهرمونين FSH and LH يؤدى الى زيادة التكاثر و الانتاج للبلطى النيلى.