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ARTICLE

Sex Hormones Changes in Blood and Their Effect on Fecundity of African Catfish (*Clarias gariepinus* Burchell, 1822) after Being Injected with Different Doses of Human Chorionic Gonadotropin (HCG) Hormone

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ABSTRACT

This study was conducted to investigate the effect of different doses injection of human chorionic gonadotropin (HCG) hormone on fecundity and serum sex hormones (FSH, LH, estrogen (E_2), progesterone (P_4), testosterone (T)) of African catfish (*Clarias gariepinus*). African catfish spawners were intermuscularly injected with different doses of HCG (500, 1500, 3000, 6000 IU/kg female), and group is not injected as a control; males were injected at half the female dose. The results showed that, fish group injected by 6000 IU HCG/ kg female had the highest gonadsomatic index, absolute fecundity and relative fecundity, while, the lowest value of absolute fecundity and relative fecundity were recorded with 500 IU HCG/ kg female. The group injected with the highest amount of HCG (6000 IU/ kg female) recorded the lowest value from egg diameter, while the highest egg diameter was observed in 500 IU HCG/ kg female. In females, the group injected with 6000 IU HCG/ kg female reflected the lowest level of FSH and the highest level of LH and the highest level of P_4 compared to other treatments. Level of T recorded the highest level with 1500 IU HCG/ kg female. The control group reflected the highest level of FSH and E_2, while the control group reflected the lowest level of T and P_4 level. In males, serum FSH, LH, P_4 and E_2 in male groups injected with HCG were relatively higher than those recorded in the control group. The highest level of T was recorded in treatment injected with the highest dose of HCG and decreased in other treatments until recorded the lowest level of T in the control group. It was observed, HCG hormone has successfully and accelerate induced spawning in African catfish (*Clarias gariepinus*) and increased in reproductive performance with the increase in HCG dosage and as compared to group not injected.

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1. Introduction

Reproduction in fishes is regulated by both internal mechanisms within the fish and external environmental factors. The environmental factors such as (water temperature, photoperiod etc.) trigger the internal mechanisms into action. The internal mechanism that controls the process of reproduction in fish is the brain-hypothalamus-pituitary gonad chain [1]. Environmental signals are transduced into an endocrine signal through activation of the hypothalamic-pituitary-gonadal (HPG) axis, initially through synthesis and release of gonadotropin-releasing hormone (GnRH), a decapeptide, from the hypothalamus [2]. GnRH stimulation of the pituitary results in the synthesis and release of the protein hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH) [3, 4]. FSH is involved in stimulating the early stages of gametogenesis, whereas LH modulates later stages of ovarian and testicular maturation, with both acting thought membrane-bound G protein-coupled receptors [3].

Most fish species used in research and aquaculture are gonochoristic. Therefore, a sexually mature fish would have either a functional testis (male) or a functional ovary (female). In most gonochoristic fish, FSH and LH stimulate the synthesis of three key sex steroids: E_2 serves as the principal estrogen and stimulates germ-cell proliferation and growth and vitellogenesis; 11 ketotestosterone (11-KT) serves as the central androgen that regulates spermatogenesis and spermiogenesis; 17α, 20βdihydroxy-4-pregnen-3-one (DHP) regulates final oocyte maturation and ovulation in females as well as spermatozoa maturation and spermiation in males [5]. Steroid hormones are the final endocrine effectors of gonadal development, in coordination with the pituitary GTHs steroidogenesis takes place in the somatic cells of the gonad, the granulosa and theca cells in the ovary and the interstitial Leydig cells and Sertoli cells in the testes. The major steroid hormones in the regulation of fish gametogenesis are the estrogen (E_2) in females and the androgen (11KT) in males [6-9].

Presently, hormonal inducement and artificial fertilization are attracting much attention in terms of research and its applicability in farm situations as they allow for greater control over various steps in the fish reproductive cycle. Many techniques using hormonal-induced ovulation and artificial insemination to ensure spawning and fertilization of the African catfish in captivity have been perfected [10]. Human chorionic gonadotropin (HCG) was used in experiments on fish reproduction. It was shown that this hormone stimulated Germinal Vesicle Breakdown (GVBD) in oocytes of several fish species [11, 12] and steroid production in vitellogenic and full-grown ovarian follicles [13]. Over recent years, HCG has been increasingly employed in spawning induction trials of many fish species.

In commercial breeding of African catfish the hormonal manipulation are very useful method to ensure high percentage of spawning and possibility to produce fish seed round all year. HCG is the common application to induce spawning in African catfish (*Clarias gariepinus*). When Ahmed [14] had been used different doses from HCG (1000, 2000 and 3000 IU/ Kg body weight of female) to induced spawning in catfish (*Clarias lazera*), there was significant between treatments in latency period (10, 9 and 6 hours), respectively. Eggs weight (g) was best when induced spawning with (3000 IU/ Kg which was, 63g), followed by 2000 IU/ Kg (25.67g) and finally 1000 IU/ Kg (9.67g), but there was no significant between treatments in the fertilization rate %, hatchability rate and survival rate.

The objective of the present study was, to evaluate the overall effects of different doses injection of human chorionic gonadotropin (HCG) hormone on ovaries weight, gonadsomatic index, absolute fecundity and relative fecundity and serum sex hormones (FSH, LH, estradiol, progesterone, testosterone) of African catfish (*Clarias gariepinus* Burchell, 1822).

2. Material and Methods

The present study was carried out at the Fish Farm in Agricultural Consulting Center, Faculty of Agriculture, El-Fayoum University, Egypt, in August 2019.

2.1 Broodstock- rearing conditions

African catfish (*Clarias gariepinus*) broodstock used in this study were purchased alive and in good condition from private fish farm, El-Fayoum Governorate, Egypt. The females were ranged from 505-615 g/fish in body weight and 41.5-49.5 cm/fish in body length, while the males were ranged from 420-670 g/fish in body weight and 42.5-51 cm/fish in body length. The brood fish were disinfected with formaldehyde (0.15 ml/ 10 L of water, i.e. 15 ppm) for 6 hours, and then stock and maintained the female fish separated from the male fish in rectangular tanks (3×2×1.2 m^3), supplied with aerated water, where tanks water was continually replaced for 14 days for fish acclimatization to farm water conditions. Water temperature around 29.5 °C, pH around 8.15 and dissolved oxygen concentration 6.37 mg/l approximately during the experimental period. Fish were held under natural photoperiod condition throughout the experimental period.

2.2 Experimental Design and Hormonal Injection

Twenty five (25) ripe females and Twenty five (25) ripe

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males with sex ratio (1:1 male ♂: female ♀) were selected for the breeding experiment. Ripeness of females was determined by external morphological characteristics the females had a soft, distended abdomen and round swollen genital papilla and readiness to spawn.

The male and female brooders were grouped into four treatments with five replicates each. African catfish (*Clarias garipains*) spawners were intermuscularly injected with different doses of human chorionic gonadotropin (HCG) hormone, the commercial name is (choriomon®). The doses were 500 (T_1), 1500 (T_2), 3000 (T_3) and 6000 (T_4) IU/Kg body weight of female and 250 (T_1), 750 (T_2), 1500 (T_3) and 3000 (T_4) IU/Kg body weight of male and group is not injected as a control. The injection was made in the evening between 5 pm and 6 pm, and after that, the injected females were returned into the containers until the checking for ovulation.

2.3 Egg Diameter and Fish Fecundity

One gram eggs were taken from each female and fixed in 5% formalin to determine egg diameter, fish fecundity (Absolute fecundity (AF) and relative fecundity (RF)), then, they were taken on a slide, egg diameter was determined by using an eye-piece micrometer in the binuclear at a power magnification of 10 X and then the measurement were converted into mm.

The most accurate method of enumeration of fish eggs that is fecundity is probably by actual count. This method of direct counting was found to be much time consuming and rather impossible in case of fishes which are highly fecund. When the actual counting of eggs is impracticable, approximate fecundity may be obtained by gravimetric methods, which has been successfully used by Doha and Hye [15]; Shafi *et al.* [16]; Dewan and Doha [17]; Das *et al.* [18]. The egg in the sample was counted, number of egg of the sample multiplied by the total weight of both the ovaries which gave the total number of eggs of a particular fish. In this way fecundity average fish were obtained by using following equation:

AF = (N × Gonad weight (g)) /Sample weight (g); Where N = Number of egg in the sample. RF= The number of eggs (AF)/ g [19]

2.4 The Calculation of Gonadosomatic Index (GSI)

The gonad somatic index (GSI) values were measured by recording of gonad weight and body weight of male and female separately on an electronicbalance throughout the study period. Following equation was used to determine GSI:

GSI = gonads weight/ fish weight × 100 [20].

2.5 Blood samples and Serum Hormones Assay

Blood samples were collected from the caudal vein (each female and male) at 12 hrs post-injection without anticoagulant then transferred to Wasserman tubes. Blood was allowed to clot at room temperature for 45 min then centrifuged at 3500 rpm for 20 minute to obtain serum sample [21]. The Serum samples were pipetted into Eppendorf tube, labeled and stored in deep freeze at -20°C till assayed.

Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH), as well as the sex steroids hormones, Progesterone (P_4) and Testosterone were quantitatively analyzed by i-CHROMA™ Reader System, estradiol was determined by Enzyme Immunoassay using standard estradiol (0, 20, 100, 300, 800 and 3200 pg/ml) (biocheck, Inc. Foster City, CA 94404 U.S.A.).

2.6 Statistical Analysis

Gonad somatic index, absolute fecundity, relative fecundity, egg diameter, and serum sex hormones were analyzed as mean ± standard error of the mean (S.E.M). The obtained data were subjected to one-way ANOVA. Differences between means were tested at the 5% probability level using Waller Duncan's test. All the statistical analyses were done using Statistical Package for Social Sciences program (SPSS) for Windows 23, released version [22].

3. Results

3.1 Ovarian Measurements and Fecundity of African Catfish (*Clarias gariepinus*)

Under artificial reproduction the effect of different doses of HCG on ovarian measurements and fecundity of African catfish (*Clarias gariepinus*) are tabulated in table (1). The results cleared that the injection by different doses of HCG had significant effects ($P \leq 0.05$) on the fecundity (Absolute fecundity (AF), Relative fecundity (RF)) and egg diameter. While, ovaries weight (g) and gonadosomatic index (GSI, %) showed insignificant differences between treatments, but, the highest values were observed in treatment injected by 6000 IU/ kg female.

The results also showed that, the highest absolute fecundity (192404 eggs/ female) was observed in treatment injected by 6000 IU/ kg female, thereafter, treatment injected by 3000 IU/ kg female, control, and 1500 IU/ kg female, while the lowest value of absolute fecundity was observed in treatment injected by 500 IU/ kg female. The treatment of catfish injected by (6000 IU/ kg female) had a significantly the highest values of RF (339.75 eggs/ g female) and significantly differences than all other treatments followed by T_3 (3000 IU/ kg female) with (133.41

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eggs/ g female), while the T_1 (500 IU/ kg female) had the lowest value of RF (102.99 eggs/ g female).

The results indicated that, the highest egg diameter (1.31 mm) was observed in treatment injected by 3000 IU/ kg female, while the lowest egg diameter (1.11 mm) was observed in treatment injected by 6000 IU/ kg female.

3.2 Gonadosomatic index for males

Figure (1) showed that the hormonal injection by differences doses of HCG had significant effects ($p \leq 0.05$) on males gonadsomatic index (GSI, %) between treatments, the highest level of GSI was observed with 3000 IU HCG/ kg male (0.93±0.03 %) followed by 1500 IU HCG and 250 IU HCG (0.90±0.07, 0.86±0.01%), respectively, while 750 IU HCG recorded the lowest level from GSI (0.73±0.02%).

Table 1. Effect of different doses of HCG on ovarian measurements and fecundity of African catfish (*Clarias gariepinus*).

Items	Dose of HCG, IU/ kg body weight				
	Control	T_1	T_2	T_3	T_4
Ovaries weight, g	30±5	56.5± 36.5	46±5	64±9	92.5± 5.5
Gonadosomatic index, %	5.82± 1.19	10.53± 7.17	9.32± 0.7	12.63± 1.96	16.09± 0.16
Absolute fecundity, eggs/ female	67324.5± 8599.5^{b}	54864.5± 15604.5^{b}	63713.5± 5493.5^{b}	71213± 6313^{b}	192404± 656^{a}
Relative fecundity, eggs/ g female	129.10± 12.81^{b}	102.99± 27.49^{b}	123.55± 8.26^{b}	133.41± 6.90^{b}	339.75± 27.97^{a}
Egg diameter, mm	1.12± 0.07^{abc}	1.171± 0.01^{bc}	1.26± 0.03^{ab}	1.31± 0.03^{a}	1.11± 0.0^{c}

Notes: (a, b, c ..) Average in the same row having different superscripts are differ significantly ($P \leq 0.05$).

Control: without hormonal injection, T_1: female brood stock treat with 500 IU HCG/ kg body weight, T_2: female treat with 1500 IU HCG/ kg body weight, T_3: female treat with 3000 IU HCG/ kg body weight, T_4: female treat with 6000 IU HCG/ kg body weight.

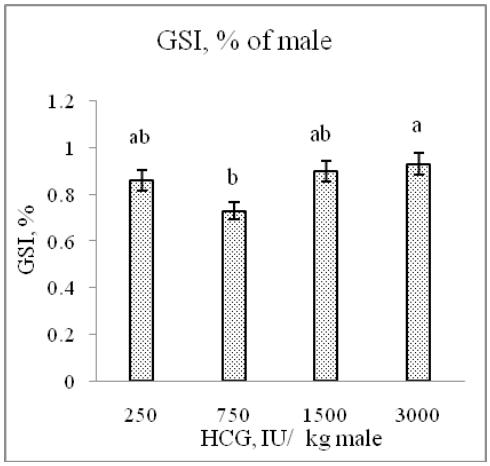

Figure 1. Gonadsomatic index of males (a, b, c ..) Average in having different superscripts are differ significantly ($P \leq 0.05$)

3.3 Latency Period, Fertilization and Hatching Rate

From the results in Figure (2) latency period (the period from injection until the start of ovulation, hrs) ranged from 12 to 28 h for the ovulated four experimental treatments. The lower latency period was recorded with 6000 IU HCG/ kg female (12 h) with a significant differences from all other treatments. In contrary, the highest latency period were in 500 IU HCG and 1500 IU HCG (28 h) and 3000 IU HCG (22 h).

From the result in figure (3) the highest fertilization rate were observed with 6000 IU HCG/ kg female (84.45%) and the lowest fertilization rate were presented in 500 IU HCG/ kg female (10.15%). The highest hatching rate with 6000 IU HCG/ kg female (81.45%) followed by those 3000 IU HCG/ kg female (73.65%), 1500 IU HCG/ kg female (57.9 %), while the incubation egg in 500 IU HCG/ kg female don't showed any hatching larvae.

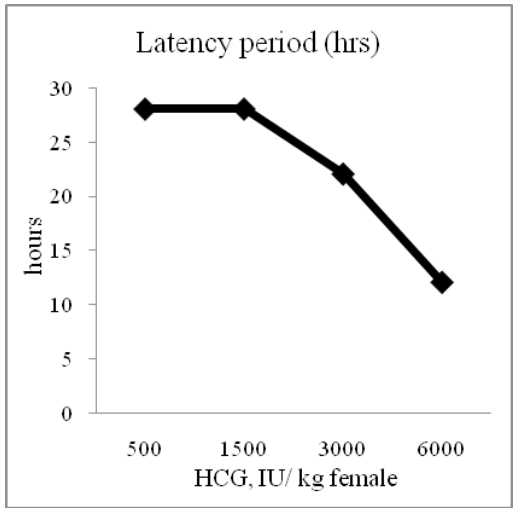

Figure 2. Latency period

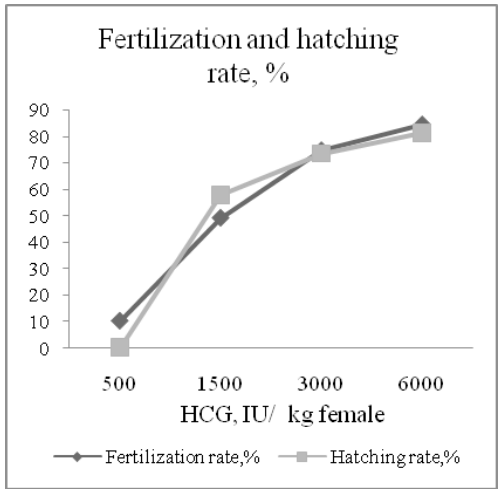

Figure 3. Fertilization and hatching rate

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3.4 Blood Serum Hormone for Female

The results of blood hormones for female *C. gariepinus* broodstock treat with different level from HCG hormone are shown in figures (4-8). The results showed that significant differences (P≤0.05) were obtained among treatments for all tested hormones.

Control (not inject) reflected the highest level of FSH (0.54±0.01 mIU/ ml) and estrogen (E_2) (5297±7.57 pg/ ml), while the control group reflected the lowest level of testosterone (2.86±0.06 ng/ ml) and progesterone (P_4) level (1.5±0.12 ng/ ml). The results of FSH level showed that was significant differences between control and all other treatments, but were insignificant differences between treatments (figure 4).

From figure (5) LH levels was the highest in each of 1500, 3000 and 6000 IU HCG/ kg female (2.7±0.15, 2.8±0.32, 2.9±0.11 mIU/ ml), respectively, without significant differences among these treatments with significantly (P≤0.05) higher than the control and 500 IU HCG/ kg female (1.2±0.11, 1.6±0.11 mIU/ ml).

In figure (6) the results showed that the highest level of progesterone were observed in 3000 and 6000 IU HCG/ kg female (4.3±0.03, 4.6±0.08 ng/ ml), respectively, without significant differences, while the lowest level of P_4 were presented in control (1.5±0.12 ng/ ml).

From figure (7) the results indicated that the highest level of testosterone was showed in 1500 IU HCG/ kg female (5.39±0.04 ng/ ml) followed by those 3000, 6000, 500 IU HCG/ kg female (5.12±0.01, 4.82±0.04, 3.28±0.11 ng/ ml), respectively, while the lowest level of testosterone was observed in control (not inject) (2.86±0.06 ng/ ml).

In figure (8) the highest level of E_2 was observed in control (5297±7.57 Pg/ ml) followed by those 500, 3000 and 6000 IU HCG/ kg female (4397±8.08, 3375±7.76, 3389±2.08 Pg/ ml), while the lowest level of E_2 was showed in 1500 IU HCG/ kg female (3330±5.29 Pg/ ml).

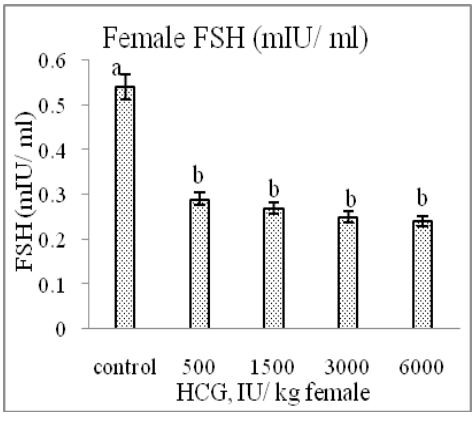

Figure 4. Changes of female FSH

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

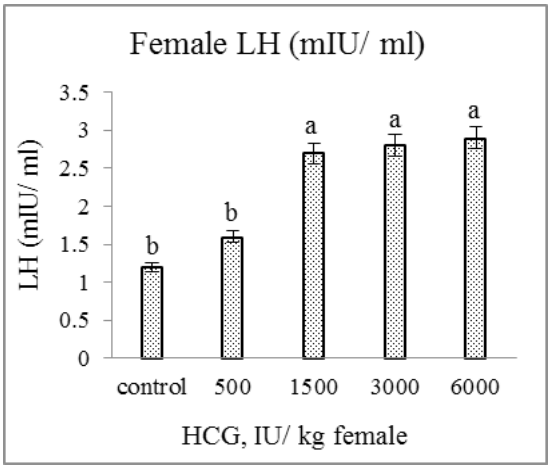

Figure 5. Changes of female LH

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

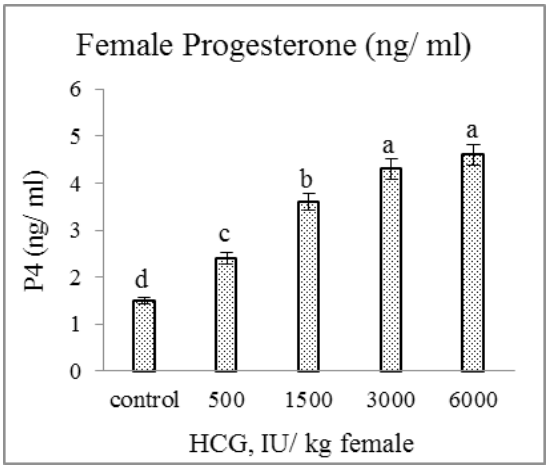

Figure 6. Changes of female progesterone

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

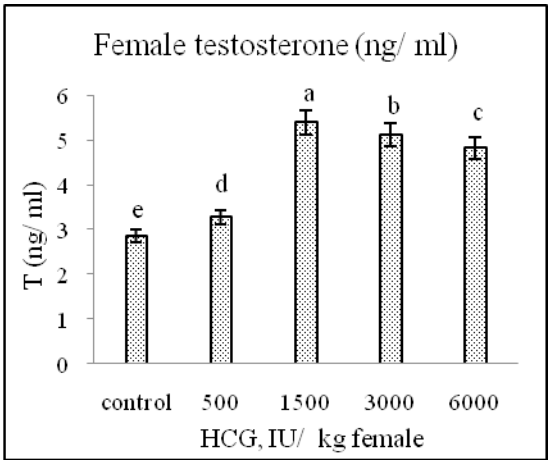

Figure 7. Changes of female testosterone

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

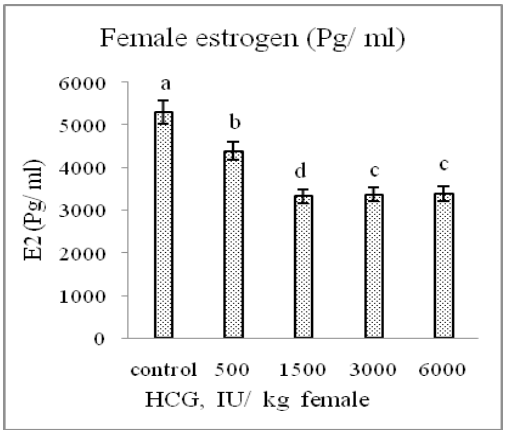

Figure 8. Changes of female estrogen

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

3.5 Blood Serum Hormone for Male

Figures (9-13) presents the data of male blood serum hormones (FSH, LH, progesterone (P_4), testosterone, estrogen (E_2) determinations. It is clear that these were significant differences (P≤0.05) among treatments for all male blood serum hormones were tested.

In figure (9) 750 IU HCG/ kg male reflected the highest level of FSH for male (0.49±0.01 mIU/ ml) followed by 1500, 3000 IU HCG/ kg male and control (0.43±0.00, 0.39±0.01, 0.31±0.01 mIU/ ml), while the lowest level of FSH was presented in 250 IU HCG/ kg male (0.26±0.01 mIU/ ml).

LH level was the highest in 3000 IU HCG/ kg male (1.95±0.02 mIU/ ml) followed by 1500, 750 and 250 IU HCG/ kg male (1.87±0.07, 1.83±0.03, 1.8±0.03 mIU/ ml), respectively, without significant differences between 750, 1500 IU HCG/ kg male, while the lowest level of LH was observed in the control (1.52±0.02 mIU/ ml) in figure (10).

From figure (11) P_4 level was the highest in each of 250 and 1500 IU HCG/ kg male (0.2 ng/ ml) without significant differences among these treatments which were significantly higher than the control, 750 and 3000 IU HCG/ kg male.

From figure (12) 3000 IU HCG/ kg male reflected the highest level of testosterone hormone (19.15±0.06 ng/ ml) followed by 1500, 750 and 250 IU HCG/ kg male respectively, while the control reflected the lowest level of testosterone (1.48±0.03 ng/ ml).

In figure (13) the results showed that the highest level of E_2 was observed in each of 250 and 750 IU HCG/ kg male (232.9±6.59, 221.5±3.59 Pg/ ml), respectively, without significant differences which were significantly differences with other treatments, while 3000 IU HCG/ kg male reflected the lowest level of E_2 (143.8±0.95 Pg/ ml).

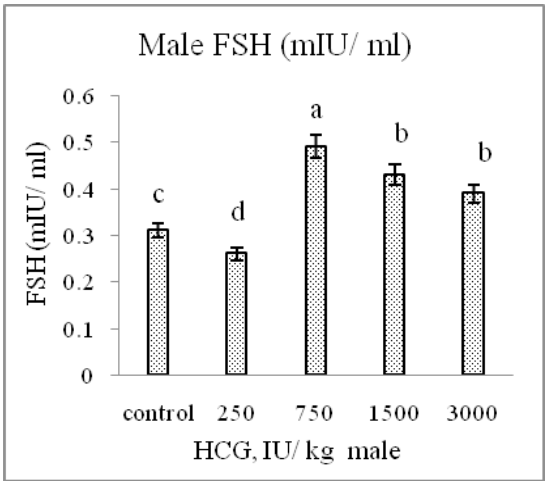

Figure 9. Changes of male FSH

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

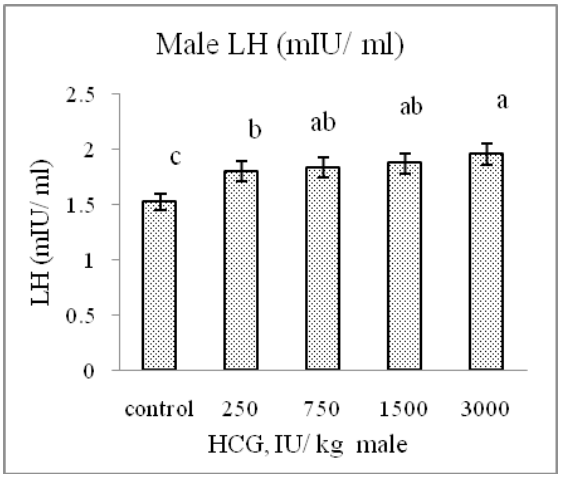

Figure 10. Changes of male LH

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

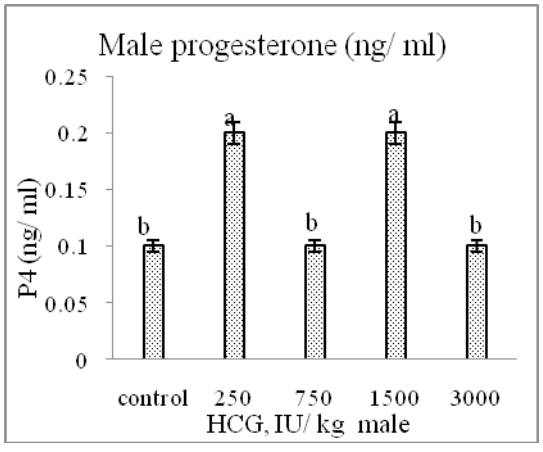

Figure 11. Changes of male progesterone

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

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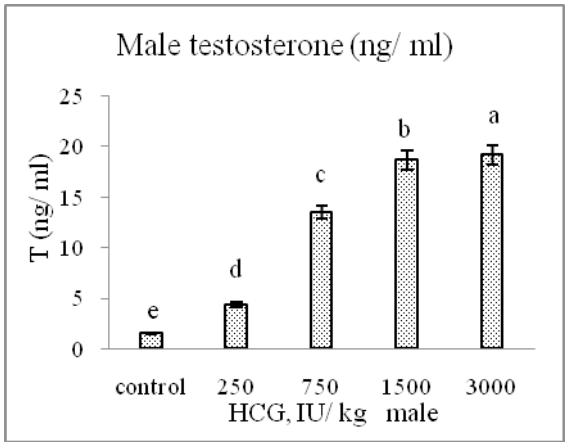

Figure 12. Changes of male testosterone

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

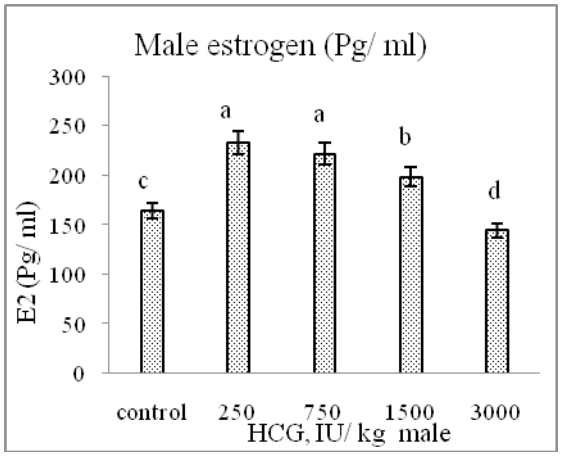

Figure 13. Changes of male estrogen

Note: (a, b, c ..) Average in having different superscripts are differ significantly (P≤0.05).

4. Discussion

The most popular purified gonadotropin hormone utilized to induce spawning in fish is Human Chorionic Gonadotropin (HCG). The injected HCG in fish mimics the natural GtH synthesized and released by the fish's pituitary. Similarly just like the case with pituitary extracts, HCG bypass the brain-pituitary link, acts directly on gonads (ovaries and testes), so HCG may be more appropriate because it acts much faster, via direct induction of the gonad to induce synthesize and release sex steroid hormones which in turn act a key role in final oocyte maturation (FOM), spermiation and spawning [23]. It was observed in the current study that this specific hormone has successfully and accelerate induced spawning in *C. gariepinus* and increased in reproductive performance with the increase in HCG dosage.

In some fish species such as African catfish (*C. gariepinus*) the application of hormonal induction is the only way for controlling reproduction reliably. The usage of HCG to induce spawning has been tested successfully in various fish species and has been shown as an efficient strategy for improving fecundity, fertilization and hatching rate in females and improving quantity and quality of milt in males [14, 21, 24-27].

The fecundity is a necessary parameter for commercial finfish hatcheries, as well the quality and quantity of fry produced from a brood fish [28]. The physiological activity of gonads affect by increase or decrease of gonadsomatic index (GSI). Where, the increasing of GSI work as an indicator to start of the spawning season of fish [25].

In the present study, African catfish spawners were intermuscularly injected with different doses of HCG hormone (500, 1500, 3000, 6000 IU/kg female), and group is not injected as a control, males were injected at half the female dose. GSI for treatment injected by 6000 IU/ kg female weight had the highest GSI (16.09 %) followed by T_3 (3000 IU/kg female with GSI (12.63 %). While the lowest value of GSI (5.82 %) was observed in the control group without hormonal injection. This results similar to that reported by Saadony *et al.* [25] who described that GSI was (13.89 and 16.93 %) when *C. gariepinus* injected with (1000 and 3000 IU HCG/ kg female weight), respectively. Also, Sharaf [24] reported that GSI was 10.69 and 11.39 after 6 and 12 h, respectively after the females of *C. gariepinus* injected by 200 IU HCG/ kg female weight.

In the other hand, the results of GSI in the current study was lower than those described by Mehrim *et al.* [21] who reported that GSI was (20.75, 23.85, 20.35 and 17.40 %) in combination with body weight of *C. gariepinus* females ranged between (708 to 956 g) when these fish injected by (1700 IU HCG/ kg fish, import carp pituitary gland, carp pituitary gland and catfish pituitary gland), respectively.

The results of ovaries weight in this study were lower than those illustrated by Mehrim *et al.* [21] who found that ovaries weight was (175.8, 205, 189 and 143 g) when *C. gariepinus* induced to spawn by using 1700 IU HCG/ kg fish, import carp pituitary gland, carp pituitary gland or catfish pituitary gland), respectively. While in the present study the highest ovaries weight (92.5 g) was reported in T_4 (6000 IU HCG/ kg female weight) followed by 3000, 500 and 1500 IU HCG/ kg female with (64, 56 and 46 g), respectively; meanwhile the control group recorded the lowest ovaries weight with (30 g).

In the present study, fish group injected by 6000 IU HCG/ kg female had the highest absolute fecundity (192404 egg/female) which was high significantly

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differed from all other treatments.While, the lowest value of absolute fecundity was recorded in T_1 (500 IU HCG/ kg female) with (54864.5 egg/female). These results was higher than indicated by Ahmed [14] who found that egg number was (8410, 16443, 22312 egg/female) when females of *C. gariepinus* treated with (1000, 2000, 3000 IU HCG/ kg fish), respectively. While, the current results agree with Saadony *et al.* [25] who reported that absolute fecundity was (47 x 10^3 and 104 x 10^3) in African catfish females treated with (1000 and 3000 IU HCG/ kg fish) from this results can suggest that the fecundity may be as positive correlation in the increasing hormonal doses from HCG. El-Hawarry *et al.* [27] concluded that the egg number was higher (22339 egg/ female) in fish group injected with 4000 IU HCG/ kg fish than those injected with 4 mg carp pituitary extract (21132 egg/ female). In further studies, Sharaf [24] found that absolute fecundity was 65 x 10^3 and 56 x 10^3 after 6 and 12 hour, respectively, when the females of *C. gariepinus* injected with (200 IU HCG/ kg fish).

In the present study, showed that the dosage of the hormone applied influenced relative fecundity as the increase in dosage resulted in more egg number/ g female for which the highest relative fecundity (339.75 eggs/ g female) was recorded in T_4 (6000 IU HCG/ kg female) with significantly differences among other treatments. The fish group injected with (3000 IU HCG /kg female) recorded relative fecundity (133.41 eggs/ g female), While, the treat 1 (500 IU HCG/ kg female) had the lowest value (102.99 eggs/ g female). Saadony *et al.* [25] reported that when stimulated spawning in African catfish by using (1000 and 3000 IU HCG/ kg fish) relative fecundity was (104.26 and 142.25), respectively, in African catfish and these results agreement with the present study. Likewise, Mehrim *et al.* [21] found that the fish group treated with (1700 IU HCG/ kg fish) showed relative fecundity (124.9), furthermore, the relative fecundity obtained in females injected with (1700 IU hCG/ kg fish) was (124.9) better than those obtained in treatments treated with one carp pituitary gland/ kg fish or one native catfish pituitary gland/ kg fish.

The egg diameter in the present study had significantly differences among treatments for which the highest egg diameter (1.31 mm) was observed in T_3 (3000 IU HCG/ kg female weight) followed by T_2 (1500 IU), T_1 (500 IU) and the control group (1.26, 1.17 and 1.12 mm), respectively. Whereas, the group injected with the highest amount of HCG hormone (6000 IU/ kg female) recorded the lowest value from egg diameter (1.11 mm). The egg diameter recorded in the current study was higher than those explained for African catfish by Mehrim *et al.* [21] who found that egg diameter was (0.87, 1.0 and 0.86 mm) when females injected with 1700 IU HCG/kg fish, one carp pituitary gland/ kg fish or one native catfish pituitary gland/ kg fish, respectively with no significant differences among treatments. In addition, these results are similar with Saadony *et al.* [25] who demonstrated that using of different doses from HCG hormone (1000 and 3000 IU/ kg female weight) caused more increasing in egg diameter (1.2 and 1.43 mm), respectively.

In the other hand, the egg diameter had the positive correlation with the increasing in broodstock weight. Bichi *et al.* [29] who found that the egg diameter increasing from 1.1 to 1.6 mm when the weight of broodstock increasing from 800 to 1500 g.

One of the methods that used for assess stage of reproductive maturation enclose the determination of sex steroid hormones or plasma levels of vitellogenin in only females, where these change dramatically and dependably during the different stages of gonad development and maturation. Most aquaculture fish are gonochoristic, African catfish is gonochoristic and in most gonochoristic fish, the gonadotropin that released from pituitary gland into the blood circulation (FSH and LH) acts as the key regulators of gonadal development and spawning, where, FSH and LH acts on gonads to stimulate the synthesis of three main sex steroids: estrogen E_2; (11-KT) 11 ketotestosterone; DHP [30].

In female fish, FSH acts on ovary to produce estrogen hormone where, E_2 serves as the major estrogen and induces germ-cell proliferation, gonadal estrogens induce the synthesis and release of vitellogenin (VTG), the primary storage protein in fish oocytes by the female liver, during the first phase from reproductive cycle in fish. In addition, Androgens and E_2 (under FSH) are involved in the appearance of lipid droplets in previtellogenic oocyes [31]. Where in fish like in other vertebrate there are two major phase in the reproductive cycle of fish the first phase: the proliferation, growth and differentiation of the gametes (spermatogenesis in male and vitellogenesis in female), while the second phase: final maturation and preparation of the spermatozoa and oocytes for release and insemination (spermiation and final oocyte maturation and ovulation [32].

During the reproductive cycle, levels of FSH increase in circulation during the gonadal development step (first phase) and then the levels of FSH decrease during final oocyte maturation and ovulation (second phase) in contrary to levels of LH. In the second phase from reproductive cycle, LH acts on the ovarian follicle to produce DHP, the maturation-inducing hormone, (MIH) in most fishes. Meanwhile, DHP regulates final oocyte

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maturation and ovulation in females. In addition, DHP is involved in the meiosis onset of ogonia in the ovary [30].

In the present study after 12 h from hormonal injection, the group injected with the highest dose from HCG hormone (6000 IU HCG/ kg female) reflected the lowest level of serum FSH (0.24 mIU/ ml) and the highest level of serum LH (2.9 mIU/ ml) and the highest level of serum progesterone (4.6 ng/ ml) compared to other treatments. It suggest that the highest doses from hormonal therapy, that tested to induced spawning of African catfish in the current study caused speed up in ovarian development and ovulation by increasingly stimulated the ovary to produce the steroid hormone that involved in spawning of fish. Because of this process, the latency period (the period from injection until the start of ovulation, hrs) recorded the lowest time in T_4 (6000 IU HCG/ kg female) and leaded to accelerated hormonal stimulation, caused in increasing in fecundity but this leaded to decrease in egg diameter. The control group (not inject) reflected the highest level of FSH (0.54±0.01 mIU/ml) and gradually decrease toward the increasing in doses of hormonal injection. While, the fish group injected with the highest dose from HCG (6000 IU/ kg female) reflected the lowest level of FSH hormone.

Similar results were obtained by Sharaf [33] found that induced spawning by usage GnRHa with or without pimozide (PIM) increased levels of sex steroids in consequence increasing in ovarian development of African catfish, the same reported by Shourbela *et al.* [34].

In the present study, level of testosterone hormone recorded the highest level in T_2 (1500 IU HCG/ kg female) with (5.39 ng/ml) and had significantly differences with other treatments. While testosterone level recorded in T_4 (6000 IU HCG/ kg female) was lower than observed in T_2 and T_3 (1500 and 3000 IU HCG/ kg female).

Reading and Sullivan [35] reported that increased level of testosterone can be affected by FSH hormone which acts on the ovary (theca cell layer) and then it thought that hormonal therapy stimulate the ovary (in granulosa cells) to convert testosterone (T) to estradiol (E_2) (under FSH) this process lead to an increase in the level of estrogen hormone in blood circulation which in turn stimulate the liver to synthesize vitellogenin during vitellogenisis stage. Kim *et al.* [36] report that after injection of female eels (*Anguilla japonica*) with HCG hormone the plasma levels of T and E_2 increased slightly during vitellogenesis and decreased afterward. While, plasma levels of T and E_2 in the control female group were not changed during the study period.

In the present study, T_4 (injected with the highest dose 6000 IU HCG/ kg female) recorded the lowest egg diameter (1.17 mm) and the lowest level of FSH hormone. While, the highest egg diameter (1.44 mm) was observed in T_1 (500 IU HCG/ kg female) and level of serum FSH in T_1 was higher than other treatments. These results agree with Achionye-Nzeh and Obaroh [37] who reported that increased egg diameter is thought to occur because the FSH content increases so that the follicle develops and egg diameter increases. Mehrim *et al.* [21] reported that an intramuscular injected dose of 1700 IU HCG/ kg female weight increased the serum FSH level and LH level of treated African catfish females.

In the present study, the control (not inject) reflected the highest level of FSH (0.54±0.01 mIU/ ml) and estrogen (E_2) (5297±7.57 pg/ ml), while the control group reflected the lowest level of testosterone (2.86±0.06 ng/ ml) and progesterone (P_4) level (1.5±0.12 ng/ ml). The failure of fertilized eggs to hatch in fish group injected with 500 IU HCG/ kg female weight may suggest that insufficient hormonal dose used to reach the full maturity of eggs in this group.

The usage different doses of HCG hormone have significant effect on gonadotropin and serum steroid hormones when hormonal injection applied in male African catfish as can be seen in the parameters of serum FSH and LH level and level of serum testosterone (T), estrogen (E_2) and progesterone (P_4). Saadony *et al.* [25] reported that the best hormone was HCG with the best dose (3000 IU/ kg fish) to hormonal stimulation for male African catfish.

In this study, serum FSH, LH, P_4 and E_2 in male groups injected with HCG hormone were relatively higher than those recorded in the control group. These results agree with Shoker [38] who showed that the level of serum FSH, T and E_2 were higher than those obtained in the control group when used GnRH to induce spawning of African catfish. And similar results were observed in grass carb by Mousavi and Yousefian [39].

Steroid hormones are synthesized and released of the testes by the somatic Leydig cells, under stimulation of GTH pituitary (FSH and LH) [40]. In the present study, the highest level of FSH was recorded in T_2 (750 IU HCG/ kg male), while, FSH level observed in T_4 (males injected with the highest dose) was lower than those obtained in T_2 and T_3. This was illustrated by Mylonas *et al.* [32] who showed that the levels of FSH hormone in males are high during early spermatogenesis, increase to extreme levels during the rapid testicular growth stage after that, the decline of FSH levels occur after spawning. On the other hand, levels of LH hormone are low during early spermatogenesis, increases during spermiation and peaks during the spawning season [41].

In the current study, hormonal injection with different

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doses of HCG hormone led to increase in level of serum LH and testosterone where, the highest level of serum LH and T were observed in treatment injected with the highest dose from HCG hormone. Miura and Miura [40] showed that the LH hormone is the mainly regulator to stimulate Leydig cells to produce androgens. Consequently, in the present study thought that hormonal stimulation with the high dose from HCG led to increase in level of serum T which in turn caused increased in GSI % where, the highest value of GSI was observed in treat 4 (males injected with the highest dose) while the lowest GSI was recorded in the control group.

In the present study, the results showed that the highest level of testosterone was recorded in treatment injected with the highest dose of HCG (T_4) and decreased in other treatments until recorded the lowest level of T in the control group. Miura and Miura [40] reported that the steroid hormones have vital and distinct roles in controlling spermatogenesis in males fish. In addition, Androgens (mainly 11 keto testosterone, 11KT) is the major regulator of spermatogenesis.

The HCG injection effectively induces spermiation and milt expression in silver perch *Leiopotherapon plumbeus* [26]. In the present study, when tested four HCG doses in male African catfish, GSI had significantly increased with the increase in hormonal dose and as compared to the control and the highest GSI in male was observed in treatment injected with the highest dose (3000 IU HCG/ kg male), and this agree with Saadony *et al.* [25] who reported that the best dose was 3000 IU HCG/ kg) to induce spermation in male African catfish. Furthermore, HCG hormone has successfully induced spermiation in several fish species such as pangasiid catfish *Pangasius bocourti* [42], European sea bass *Dicentrarchus labrax* [43], European eel *Anguilla anguilla* [44-46] and Japanese eel *Anguilla japonica* [47, 78].

Mousavi and Yousefian [39] reported that HCG or Ovaprim were efficient to induce spawning of male and female grass carp. Where showed that the injections of HCG and ovaprim led to increase in levels of testosterone in males, and estrogen in female significantly ($P<0.05$) after the start first injection with 2 h and peaked after 10 to 12 h of first injections in both hormonal injections (HCG and ovaprim), respectively. Moreover, levels of sex steroid hormones significantly decreased after 12 h of second hormonal injection.

Saadony *et al.* [25] found that the hormonal injection by different materials (HCG, Ovaprim and GnRHa) due to successfully induced spawning in African catfish with significantly increased in reproductive performance as compared to the control group. In addition, Mehrim *et al.* [21] showed that HCG injected in *C. gariepinus* was good hormone to improving in reproductive performance of African catfish and observed that injection by HCG hormone increased in levels of serum FSH, LH and progesterone than that observed in control group.

The efficiency of HCG to successfully induce spawning after a single injection is probably due to this GtH's relatively long retention time in blood circulation. This is not linked to the fact that it is a heterologous hormone for fish where, in humans it as well has a significantly longer half-life in blood circulation, contrasted with the gonadotropins of pituitary origin (FSH and LH) [49]. On the other side, usage of HCG hormone is safety in connection with the public health of humans, where approximately 80% of its level is metabolized chiefly in the kidneys. Intramuscular and subcutaneous administrations of HCG hormone were found to be bio equivalent regarding the extent of absorption and the apparent elimination half-lives of approximately 33 hours [50].

5. Conclusion

From this results the hormonal injection by 6000 IU HCG/ kg female was some advantages over the other treatments in terms of higher gonadsomatic index, fecundity and fertilization rate and hatching rate in African catfish females. Also, the best dose for males was (3000 IU HCG/ kg fish). It suggest that the highest doses from hormonal therapy, that tested to induced spawning of African catfish in the current study caused speed up in ovarian development and ovulation by increasingly stimulated the ovary to produce the steroid hormone that involved in spawning of fish. Because of this process, the latency period (the period from injection until the start of ovulation, hrs) recorded the lowest time with 6000 IU HCG/ kg female and leaded to accelerated hormonal stimulation, caused in increasing in fecundity but this leaded to decrease in egg diameter. The failure of fertilized eggs to hatch in fish group injected with 500 IU HCG/ kg female weight may suggest that insufficient hormonal dose used to reach the full maturity of eggs in this group. It was observed, HCG hormone has successfully and accelerate induced spawning in African catfish (*Clarias gariepinus*) and increased in reproductive performance with the increase in HCG dosage and as compared to group not injected.

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