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Effect of different doses of human chorionic gonadotropin (HCG) hormone on stripping response and reproductive performance of the African catfish (*Clarias gariepinus*).

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ABSTRACT

This study was conducted to investigate the effect of different doses injection of human chorionic gonadotropin (HCG) hormone on stripping response and reproductive performance of African catfish (*Clarias gariepinus*). African catfish spawners were intramuscularly injected with different doses of HCG (500, 1500, 3000, 6000 IU/kg female); males were injected at half the female dose. The results showed that fish group injected by 6000 IU/ kg female had the highest ovaries weight and gonadsomatic index, but, recorded the lowest value from ovulated egg diameter. The lower latency period was recorded with 6000 IU/ kg female (12 h). While, the highest latency period were in 500, 1500 IU/ kg (28 h) and 3000 IU/ kg (22 h). The number of fertilized eggs/ female and fertilization rate (%) were significantly different (P£0.05) among the experimental treatments, with the highest number of fertilized eggs/ female and fertilization rate were observed with 6000 IU/ kg female (60848 fertilized eggs/ female with 84.45%) and the lowest number of fertilized eggs/ female and fertilization rate were presented with 500 IU/ kg female (3372 fertilized eggs/ female with 10.15%). The number of larvae/ female and hatching rate showed significant differences (P£0.05) among different level of HCG hormone with the highest number of larvae and hatching rate with 6000 IU/ kg female (49657 larvae/ female with 81.45%) followed by those 3000 IU/ kg female (43177 larvae/ female with 73.65%), 1500 IU/ kg female (12099 larvae/ female with 57.9 %), while the incubation eggs of group injected with 500 IU/ kg female don't show any hatching larvae. 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.

INTRODUCTION

Egyptian aquaculture has recently achieved a great successful story setting Egypt on the sixth rank of the world aquaculture (1561.5 thousand tonnes, live weight with 1.90 % of world total aquaculture) and first among African countries (FAO, 2020). Egyptian aquaculture was depended on little number species, Tilapia, Carp and Mullet which

accounting about 95% from the production, this led to a lack of diversity in aquaculture. Fortunately, African catfish farming has witness an increased production and gained a substantial importance in the aquaculture sectornewly in Egypt.

The African catfish (*Clarias gariepinus*) is one of the most important freshwater fish in Africa that has a widely distribution among African countries and has been introduced and cultured in many countries in Asia, Europe and South America (Marimuthu, 2019), due to its many advantages, high growth rate, low production cost, cultured under several systems, extremely resistant to diseases and stress. Catfish (*C. gariepinus*) is a strong fish, where it has tolerance with hard environmental conditions (e.g muddy, high stocking densities and decreasilly in water quality) (Mehrim *et al.*, 2014; Tyor and Pahwa, 2017). All of this advantages, turned African catfish from mere an undesirable species in tilapia ponds or a 'police-fish' to control overbreeding in mixed-sex tilapia culture to an important and potentially species for aquaculture.

With farmers increase more interested in this species, the demand for seed increases, in contrast, the scarceness of natural spawning in captivity. In addition the many problems of collecting fry from nature resources, which are represented in: seasonality, limited, time-wasting, stressful, its productivity is difficult to predict and uneconomic, since the seed availability is the chokepoint for successful culture of any fish species. Accordingly, spawning induction of captive African catfish becomes the best method to conquer this problems, improving in fertilization, hatching, survival rate, possibility to produce fry round all year, through injection of one of several hormones including; fish pituitary extracts, HCG hormone, gonadotropin hormone (GTH), luteinizing hormone-releasing hormone (LHRH) and LHRH agonists (LHRHa), gonadotropin-releasing hormone (GnRH) and GnRHa, Ovatide, Dagin, Ovaryprim, Ovaprim, Ovopel, Ovupin-L, Ovulin and Aquaspawn (Rutaisire and Booth, 2004; Ngueku, 2015; Mamndeyati *et al.*, 2018; Sukendi *et al.*, 2019).

Hormone-induced spawning of fish has been used for almost 60 years in fish hatcheries for production of fry or fingerlings which contributes significantly to the overall aquaculture production (Rahman *et al.*, 2011). It has opened the door of a new era throughout the world for high quality and high quantity of fish production (DOF, 2014**)**. In Africa induced breeding started after the Second World War, which the first successful production of fingerlings was that of *Clarias garepinus* in Egypt (Aliwa, 1982). Surprisingly, the same procedures, with only minor modifications, have been used to spawn an entire range of fishes from the ancient sturgeon and paddlefish to carp, catfish, salmon, sea bass, sea bream and mullet. In addition to breeding other desirable fish species, Induction of spawning using hormones provides a direct control over the final stages of the reproduction cycle in teleosts (Rottman *et al.*, 1991a).

Spawning induction of *C. gariepinus* has gained a significant international interest by many authors (Brzuska *et al.*, 1999; Brzuska, 2003; Brzuska, 2004; Adebayo and popoola 2008; Maradun *et al.* 2018); recently in Egypt (Saadony *et al.*, 2014; El-Hawarry

et al., 2016; Shourbela *et al.,* 2020). In the other hand, the use of HCG is the popular protocol to induce spawning in many fish species such as Sea bream, *Sparus aurata* (Badran *et al*., 2019); male Japanese eel, *Anguilla japonica* (Tanaka *et al*., 2003); Benni, *Barbuss sharpeyi* (Kahkesh *et al.*, 2010); Pigfish, *Orthopristis chrysoptera* (Di Maggio *et al.*, 2014). The stimulation of final oocyte maturation, ovulation and spawning of African catfish by using of human chorionic gonadotropin (HCG) was presented by many authors (Saadony *et al.,* 2014; Mehrim *et al.,* 2014; El-Hawarry *et al.,* 2016; Ahmed, 2018).

The objective of the present study was, to evaluate the overall effects of different doses injection of human chorionic gonadotropin (HCG) hormone on stripping response and reproductive performance of African catfish (*Clarias gariepinus* Burchell, 1822).

MATERIALS 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. This study was conducted to investigate the effect of different doses injection of human chorionic gonadotropin (HCG) hormone for improving artificial propagation of African catfish (*Clarias gariepinus* Burchell, 1822).

Broodstock- rearing conditions.

African catfish (*Clarias gariepinus*) broodstock (420-670 g/ fish, body weight) used in this study were purchased alive and in good condition from private fish farm, El-Fayoum Governorate, Egypt, then transported to the area where the study was occurred. 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. Fish were held under natural photoperiod condition throughout the experimental period. The average water quality criteria in broodstock tanks are presented in Table (1).

Selection of broodfish.

Twenty (20) ripe females and Twenty (20) ripe 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. For proper selection of the female broodfish, a catheter was used to get a small egg sample from their ovaries. The egg diameter of more than 90 % of the ripe ovaries was bigger than 900 μm when examined under a calibrated ocular micrometer. The reddening of the genital papilla was used as an indicator of ripeness the males (Ngueku, 2015; Gadisa, 2017; Shourbela *et al*., 2020). No feed were offered for fish before hormonal injection for three days. The total length (cm) of the brooders was measured with a fish Measuring Board and weight in grams (g) was determined with an electronic scale of all brooders prior to hormone administration.

Table (1). Water quality criteria of broodstock tanks.

Parameters	Measurement for broodstock tank
Temperature, °C	29.5
pH	8.15
Dissolved oxygen, mg/l	6.37
EC*, mS/cm **	3.14
Carbonate (CO_3), mg/l	32.55
Bicarbonate (HCO_3), mg/l	206.35
Total alkalinity, mg/l	238.9
Calcium (Ca), mg/l	37.45
Magnesium (Mg), mg/l	55.35
Hardness (Ca+Mg), mg/l	92.8

* Electrical conductivity ** mS/cm, millisiemens/centimeter

Experimental design and hormonal injection.

The male and female brooders were grouped into four treatments with five replicates each. The treatments with female to male ratios were (1:1 male ♂: female ♀). African catfish (*Clarias garipains*) spawners were intermuscularly injected by human chorionic gonadotropin (HCG) hormone, the commercial name is (choriomon®). This hormone is one of the most hormone used in human medicine as a stimulator to ovary, the HCG hormone was produced by a commercial pharmaceutical company (Sumach: Infar, India) and HCG solution was prepared according to the prescriptions of brochure supplied by the manufacturing company, it is freeze-dried powder corresponding to 5000 IU HCG per vial, the HCG hormone extracted from human urine; the origin of the urine; people's republic of china.

The experimental fish (female and male) were injected after weighted it to determine the dose of hormone according to dose of each group (Table 2). Then they intramuscularly injected at angle 30- 45 degrees by insulin syringes into the dorsal muscle above the lateral line toward the tail (El-Hawarry *et al.*, 2016). 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.

Table (2). Details of the experimental treatments (fish injection by HCG).

Treatments	Female		Male	
	Number	Dose	Number	Dose
Treatment 1	5	500 IU/Kg	5	250 IU/Kg
Treatment 2	5	1500 IU/Kg	5	750 IU/Kg
Treatment 3	5	3000 IU/Kg	5	1500 IU/Kg
Treatment 4	5	6000 IU/Kg	5	3000 IU/Kg

Checking for ovulation

Female fish which were injected with different doses of HCG hormone were checked after ten (10) hrs, for ovulation and continued at one-hour intervals (Brzucka, 2004). Hence females were tested for ovulation by applying slight pressure on the abdomen towards the genital opening (eggs ooze out easily when the abdomen is gently pressed), hence females produced green-brown eggs were rated them as ovulated (El-Hawarry *et al.,* 2016). Directly latency period of each group and ovulation rate were determined.

Stripping process and egg fertilization

The stripping process occurs to collect the eggs oozed with slight pressure on abdomen in direction tail by thumb to collect the eggs into the pre-weighted plastic bowl. At the same, milt was collected from male broodfish by opening the abdomen, removed the testes and was dissected into small pieces to obtain the sperm. Milt was put on the stripped eggs, fresh water was added to activation the semen, mixed them by gently shaking of the bowl, and then eggs were rinsed in fertilizing solution (4 gram of sodium chloride and 3 gram Urea/ L water) (Saadony *et al*., 2014). Before this, about one gram of stripped egg from each female was put in the formalin solution 5% and storage them until determine the egg diameter and number of stripped eggs for each female. After two minutes of fertilization, eggs were washed with water and additional fresh water was added. Thereafter left the mix for 2-5 min before transport them into the incubation aquaria, after 12 hrs from incubation the sample of eggs were taken from the central part of the incubator of each group, and then examined under microscope to determine the percentage of fertilized eggs. And then the hatching rate was determined after 24-36 hrs from incubation of egg. Fertilized eggs were incubated in aquariums with dimensions of 80×50×50 cm supplied with dechlorinated tap water. Aquarium were continuously supplied with oxygen through oxygen pumps. The fertilized eggs were placed on nylon nets suspended in the water of the aquariums. The average water quality criteria in eggs incubators are presented in Table (3).

Table (3). Water quality criteria of eggs incubators.

Parameters	**Measurement for eggs incubators**
Temperature, °C	28
pH	7.35
Dissolved oxygen, mg/l	7.24
EC*, mS/cm **	0.83
Carbonate (CO_3), mg/l	21.45
Bicarbonate (HCO_3), mg/l	171.45
Total alkalinity, mg/l	192.9
Calcium (Ca), mg/l	35.4
Magnesium (Mg), mg/l	26.85
Hardness (Ca+Mg), mg/l	62.25

* Electrical conductivity ** mS/cm, millisiemens/centimeter

Measurement Parameters:

1- The morphometric measurements.

The morphometric measurements were carried out for each fish: body weight (g), total length (cm), standard length (cm), weight of gonad (g). The condition of the brooders was determined using the Fulton's condition factor (K, g/cm^3) from the relationship: Condition factor (g/cm^3) = (wet weight)/ (total length^3) ×100 (Htun-Han, 1978).

2- Ovulation rate.

Ovulation rate = number of ovulated females/ number of injected females x 100 (Szabo *et al.,* 2002).

3- Latency period.

The period from injection until the start of ovulation (hrs).

4- The calculation of gonadosomatic index (GSI).

The gonadsomatic 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 (Tseng and Chan, 1982).

5- The calculation of working fecundity.

Working fecundity = (the number of stripped eggs/ g fish body weight) (Kahkesh *et al.,* 2010).

6- Egg diameter.

One gram eggs were taken from each female and fixed in 5% formalin to determine egg diameter 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.

7- Fertilization rate.

Fertilization rate (FR)= (Number of fertilized eggs/ Total number of incubated eggs) x 100.

8- Hatching rate.

Hatching rate = (Number of hatched eggs (larvae)/ Total number of fertilized eggs) x 100.

Water quality.

Water temperature, dissolved oxygen, pH, electrical conductivity (EC) EC, Carbonate, Bicarbonate, Calcium (Ca), Magnesium (Mg) and total hardness were measured in broodstock tanks and eggs incubators.

Temperature, dissolved oxygen, pH and EC were measured daily at 1 pm, by centigrade thermometer, oxygen meter (Cole Parmer model 5946), Orion digital pH meter model 201 and Conductivity meter model (YSI.SCT-33), respectively. Carbonate, Bicarbonate, Calcium (Ca), Magnesium (Mg) and total hardness were measured using the methods described by Centre Laboratory for Soil, Water and Plant Analysis, Faculty of Agriculture, Fayoum University (Egypt).

Statistical analysis.

Morphometric parameters, stripping response and reproductive performance parameters 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 **(**SPSS, 2015**)** 23, released version.

RESULTS

Morphometric parameters of African catfish (*Clarias gariepinus)* male.

The characteristics of male broodstock used for artificial reproduction are shown in table (4). From the table and statistical analysis indicated significant differences ($P \leq 0.05$) between treatments in body weight, total length, and standard length and condition factor. The total body weight of male broodstock range from 420–670 gram, T_2 had the biggest male mean body weight (660±10 g) while the least mean body weight was recorded in T_4 (431.25±11.25 g). The total length of male brood stock used in artificial reproduction was in the range of (42.5–51 cm). Males in T_1 recorded the highest mean total length (49.37±1.63 cm) while, males in T_4 recorded the lowest mean total length (43±0.5 cm). Also in table (4) showed that the highest condition value for males that used in artificial reproduction were observed in T_3 and T_2 (0.58±0.025, 0.57±0.018 g/cm^3), respectively without significant differences between them, while, the lowest condition value for males was recorded in T_1 (0.45±0.029 g/cm^3).

Also in table (4) 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 in T_4 (0.93±0.03 %) followed by T_3 and T_1 (0.90±0.07, 0.86±0.01%), respectively, while T_2 recorded the lowest level from GSI, % (0.73±0.02%).

Table (4). Morphometric parameters of African catfish (*Clarias gariepinus*) male.

Morphometric parameters	Treatments			
	T_1	**T_2**	**T_3**	**T_4**
Body weight, g/ male	546.25±18.75^{b}	660±10^{a}	551.25±23.75^{b}	431.25±11.25^{c}
Total length, cm/ male	49.37± 1.63^{a}	48.75± 0.75^{ab}	45.5± 0.0^{bc}	43±0.5^{c}
Standard length, cm/ male	42.75± 1.75^{a}	42.5± 0.5^{a}	40± 0.0^{ab}	36.5± 0.5^{b}
Condition factor, g/cm^3	0.45± 0.029^{b}	0.57± 0.018^{a}	0.58± 0.025^{a}	0.54± 0.033^{ab}
GSI, %	0.86± 0.01^{ab}	0.73± 0.02^{b}	0.90± 0.07^{ab}	0.93± 0.03^{a}

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

T_1: male treat with 250 IU HCG/ kg body weight, T_2: male treat with 750 IU HCG/ kg body weight, T_3: male treat with 1500 IU HCG/ kg body weight, T_4: male treat with 3000 IU HCG/ kg body weight).

GSI: gonad somatic index

Morphometric parameters of African catfish (*Clarias gariepinus*) female.

The characteristics of female broodstock are shown in table (5). From the table no significant differences (P>0.05) between treatments in body weight, total length, standard length and condition factor, which the body weight of female broodstock range from 505–615 gram, but T_4 had the biggest female mean body weight (570±45 g), while the least mean body weight was recorded in T_2 (515±10 g). The total length of female brood stock used in artificial reproduction were in the range of 41.5–49.5 cm, but, T_4 had the longest female broodstock mean total length (46.75±2.75 cm), while, T_2 had the shortest female broodstock mean total length (42.75±1.25 cm).

Table (5). Morphometric parameters of *Clarias gariepinus* female.

Morphometric parameters	Treatments			
	T_1	**T_2**	**T_3**	**T_4**
Body weight, g/ female	530±10	515±10	532.75±19.75	570±45
Total length, cm/ female	43.25±0.75	42.75±1.25	45±0.00	46.75±2.75
Standard length, cm/ female	38.25±0.75	38±1	39.25±0.25	40.5±2.50
Condition factor, g/cm^3	0.65±0.02	0.66±0.04	0.58±0.02	0.56±0.05

T_1: female 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.

Stripping response of the African catfish (*Clarias gariepinus*).

The effect of different doses of HCG hormone on latency period (hrs), ovulated female (%), weighted of stripped eggs g/ female, egg diameter (mm), working fecundity (strip eggs/ g female) were summarized in table (6). The results showed that hormonal injection by different doses of HCG had significant effect (P≤0.05) on latency period and egg diameter. While no significant different were recorded among different doses of HCG hormone on ovulated female, %, weighted of stripped eggs and working fecundity.

From the results in table (6) latency period ranged from 12 to 28 h for the ovulated four experimental treatments. The lower latency period was recorded in T_4 (12 h) with a significant differences from all other treatments. In contrary, the highest latency period were in T_1, T_2 (28 h) and T_3 (22 h). In all treatments the ovulated female was 100%. No significant differences between treatments in weight of stripped eggs g/ female, but the highest value was showed in T_3 (50.95 g/ female) and the lowest value was observed in T_2 (24.6 g/ female).

Data of the ovulated eggs diameter showed that the highest eggs diameter was showed in T_1 (the lowest dose from HCG hormone) (1.44 mm) followed by T_3 and T_2 with (1.33 and 1.31 mm) for eggs diameter, while the lowest eggs diameter was showed in T_4 (the highest dose from HCG hormone) (1.17 mm). The working fecundity in the broodstock was close to the range of 84-150 stipped eggs/g female. The recorded working fecundity (149 eggs/ g female) in treatment injected with (3000 IU HCG/ kg female) was higher than those recorded in all other treatments followed by the treatment

injected with the highest dose of HCG with (126 eggs/ g female). While the lowest value (84 and 87 eggs/ g female) was observed in T_2 and T_1 groups injected by (1500 and 500 IU/ kg female), respectively. In figures (1 and 2) 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.

Table (6). Effect of different doses of HCG on stripping response of the African catfish (*Clarias gariepinus*).

Parameters	Treatments			
	T_1	**T_2**	**T_3**	**T_4**
Latency period, hrs	28± 2[a]	28± 2[a]	22± 2[a]	12± 1[b]
Ovulated females, %	100	100	100	100
Weight of stripped egg, g/ female	27.7± 6.8	24.6± 5.6	50.95± 10.35	43± 2.8
Egg diameter, mm	1.44± 0.00[a]	1.31± 0.01[ab]	1.335± 0.05[ab]	1.175± 0.10[b]
Working fecundity, strip eggs/ g female	87.15 ±25.6	84.34 ±17.32	149.3 ±26	126.7 ±5.76

- (a, b, c ..) Average in the same row having different superscripts are differ significantly (P≤0.05).
T_1: female 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.

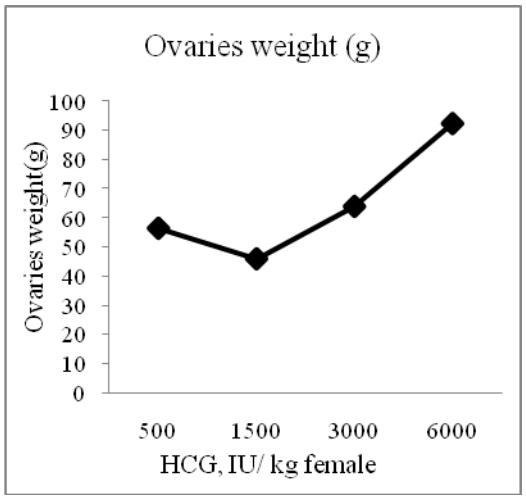

Fig 1. Changes of ovaries weight (g).

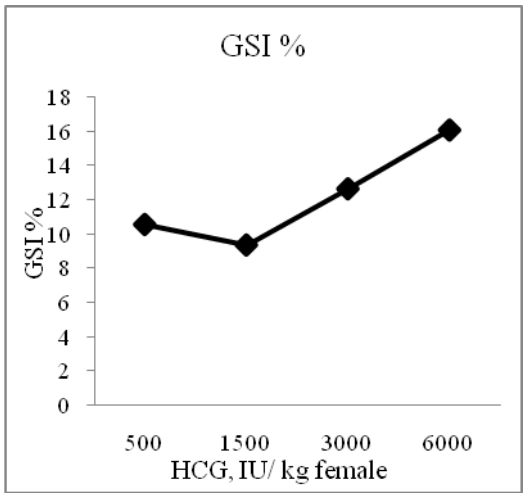

Fig 2. Changes of gonadosomatic index

Reproductive performance parameters.

Reproductive performance parameters of African catfish (*C. gariepinus*) under effect of injection of different doses of HCG are shown in table (7). The results showed that the induced spawning by different doses of HCG for African catfish had significant

effects (P≤0.05) on the reproductive performance parameters (number of fertilized egg/ female, fertilization rate, number of larvae/ female, hatching rate). While, number of stripped egg/ female showed insignificant differences between treatments.

Table (7). Effect of different doses of HCG on reproductive performance of African catfish (*Clarias gariepinus*).

Parameters	Treatments			
	T_1	**T_2**	**T_3**	**T_4**
No. of stripped egg/ female	45940.5± 12709.5	43264± 8076	80054± 16800	71962.5± 2416.5
No. of fertilized egg/ female	3372.5± 3372^b	20993± 2520^b	59077.5± 9107.5^a	60848± 4308^a
Fertilization rate, %	10.15± 10.15^c	49.15± 3.35^b	74.7± 4.3^a	84.45± 3.15^a
No. of larvae/ female	0.0^c	12099± 997^b	43177.5± 4551.5^a	49657± 4878^a
Hatching rate, %	0.0^c	57.9± 2.2^b	73.65± 3.65^a	81.45± 2.25^a

- (a, b, c ..) Average in the same row having different superscripts are differ significantly (P≤0.05).

T_1: female 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.

The results showed that the highest number of stripped egg was presented in T_3 (80054 eggs/ female) followed by T_4 (71962 eggs/ female), T_1 (45940 eggs/ female) and the lowest number was observed in T_2 (43264 eggs/ female).

From the result in table (7) the number of fertilized eggs/ female and fertilization rate (%) were significantly different (P≤0.05) among the experimental treatments, with the highest number of fertilized eggs/ female and fertilization rate were observed in T_4 (60848 fertilized eggs/ female with 84.45%) and the lowest number of fertilized eggs/ female and fertilization rate were presented in T_1 (3372 fertilized eggs/ female with 10.15%). The number of larvae/ female and hatching rate showed significant differences (P≤0.05) among different level of HCG hormone with the highest number of larvae and hatching rate in T_4 (49657 larvae/ female with 81.45%) followed by those T_3 (43177 larvae/ female with 73.65%), T_2 (12099 larvae/ female with 57.9 %), while the incubation egg in T_1 don't showed any hatching larvae.

DISCUSSION

Reproduction in fishes is a cyclic phenomenon and is regulated by environmental factors (exogenous) that induce internal mechanisms (endogenous) into action. In nature the fish spawning take places under good environmental conditions that are appropriate to

the survival of the fry. Final oocyte maturation, ovulation in female and spermation in males can be controlled by both environmental and hormonal manipulation this protocol (mainly hormonal induction) have become of practical importance in aquaculture for many reasons e.g in the current fish type (African catfish), improving fecundity, synchrony the time of spawning in the most broodstock and increasing the fertilization and hatching rate **(**Elakkanai *et al*., 2015; Hawarry *et al.,* 2016**)**. The advantageous traits of HCG gave the possibility of accurate dosing without the need of preparation and weighing of dose, preparing and storing injections of HCG hormone is a very simple method and don't need to be injected with dopamine antagonists (Adamek, 1995).

The most popular purified gonadotropin hormone utilized to induce spawning in fish is 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 (Rottmann *et al.,* 1991b). 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.

During the present study minimum latency period with mean value of (12 h) was recorded in T_4 (fish inject with 6000 IU HCG/ kg female) as compare to all other treatments. This similar with that obtained by El-Hawarry *et al.* (2016) reported that using of 4000 IU/ kg from HCG showed latency period (14.3 h) in African catfish. While, the results in the current study disagree with that obtained by Ahmed (2018) reported that using of different doses from HCG (3000, 2000, 1000 IU/ kg) showed short spawning time (6.33, 9, 10.33 h), respectively in *Clarias lazera*.

In other hormones, Shourbela *et al.* (2014) reported that latency period varied from (10.33–15 h) when females treated with different doses from GnRHa combined with or without pimozide or dompridone in African catfish. Mamndeyati *et al.* (2018) indicated that using of different doses from Ovulin (0.10, 0.30, 0.50 ml/ kg) to induce spawning of African catfish showed no significant differences between treatments in latency period with (10 h).

The present study showed that the suboptimal doses of 500, 1500 IU HCG/ kg injected in females was not appropriate for complete ovulation for which stripping was not easy and these showed in long latency period for which the latency period in was (28 h) when using this treatments. The higher of latency period might due to insufficient of plasma gonadotropin which this is necessary to final maturation and ovulation (Billard *et al.,* 1984; Sahoo *et al*., 2005).

The differences occurred in latency period may be due to the kind of hormones, the doses of hormonal injection that used, water parameter specially temperature, time of injection and fish species.

The results of ovulated females (%) recorded in the present study were greater than those reported for *Barbuss harpeyi* (Kahkesh *et al.,* 2010); *C. gariepinus* (Saadony *et al.,* 2014) who reported that the ovulation index (%) was ranged between 38 to 66 % for which the ovulation index was (70.25 and 66.56 %) in the fish groups injected with (1000, 3000 IU HCG/ kg female), respectively.

Meanwhile, the ovulation percentage in the present study was one hundred percent in all treatments except the fish in control group who failed to ovulate with zero % ovulation and this result may be due to lessen in plasma gonadotropin (GTH) level in those females where, GTH is necessary to start the final proceedings egg maturation and ovulation.

Sahoo *et al.* (2008) demonstrated that the good stripping response with the highest egg weight was present in *C. batrachus* when the hormonal injection was (3000-4000 IU HCG/ kg female) combination with latency period (14-23 h). While, Akar *et al.* (2010) reported that the ovulation index was 90% in common carp that injected with two doses from HCG hormone (the first and second doses were similar 1000 IU/ kg female).

The weight of stripped eggs in the present study was in the range between 24 to 50 g/ female, where, the highest value of stripped eggs (50.95 g/ female) was observed in T_3 (3000 IU HCG/ kg female) followed by T_4 (6000 IU HCG/ kg female) with 43.28 g/ female. While, the minimum weights of stripped eggs were presented in T_1 and T_2 with (27.27 and 24.6 g/ female, respectively. This similar with that obtained by Ahmed (2018) who found that the egg weights that obtained in *C. gariepinus* by using (1000, 2000 and 3000 IU HCG/ kg fish) was (9.67, 25.67 and 63 g), respectively. In addition, the egg weights were ranged between (30 - 67 g) when females of African catfish stimulated with different doses from GnRHa combined with or without pimozide or dompridone with significant differences among treatments (Shourbela *et al.,* 2014). The egg weights in the current study was higher than those observed for *C. gariepinus* with Adebayo and popoola (2008) who reported that when stimulated spawning in females with weight in range between 530 to 540 g by using (Frog pituitary extract, 0.5 ml ovaprim/ kg fish and *C. gariepinus* pitiutary extract) the egg weights was (26.26, 18 and 21.28 g/ female), respectively.

According to the present results the working fecundity in the broodstock was the highest value (149.3 egg/ g female) was recorded in the fish injected with 3000 IU HCG/ g female) followed by 6000 IU HCG/ g female (126.7 egg/ g female) with insignificant different between treatments. These results were higher than those obtained in Benni fish (*Barbus sharpeyi*) by Kahkesh *et al.* (2010) who reported that working fecundity was (33, 56, 34, and 33 stripped eggs/ g female) when *Barbus sharpeyi* females induction to spawning by different matrials (not inject, 10 µ LHRHa/ kg, 10 µ LHRHa/ kg plus 2 mg/

Carp pitiutary extract/ kg female, 0.5 ml Ovatide/ kg female and 0.5 ml Ovaprim/ kg female), respectively.

In the present study, the results showed that the fertilization rate was significantly different ($P \leq 0.05$) among the experimental treatments, which the fish group injected by 6000 IU/ kg female had the highest fertilization rate (84.5%), while the lowest fertilization rate was recorded in T_1 (the fish group inject by 500 IU/ kg female (10% fertilization rate). These results agree with results of Ahmed and Manofal (2017) who found that when the females of *C. gariepinus* injected by different doses from HCG (500, 1500, 2250, 2500 IU/ kg fish) found that the increase in the hormonal dose that used from HCG led to an increase in the fertilization rate (0, 65, 60, 75%), respectively. Also, Maradun *et al.* (2018) Who reported that the dosage of the hormone administered influenced the fertilization rate as the increase in dosage resulted in more fertilization percentage and found that the fertilization percentage was higher (88.12%) in fish group injected with higher dose of Ovulin (0.7 ml/ kg fish) than those injected with lower dose from Ovulin (0.3 and 0.5 ml/ kg fish) with (72.73 and 80.62 %), respectively when induced spawning in *C. gariepinus*.

Similarly several studies carried out for induction spawning in African catfish (*C. gariepinus*). Saadony *et al.* (2014) found that the fertilization rate was (40, 72.5 %) when females of African catfish injected by (1000, 3000 IU HCG/ kg fish). And El-Hawarry *et al.* (2016) indicted that the using of (4000 IU HCG/ kg fish, 4000 plus 10 mg Dopamine antagonist) in *C. gariepinus* showed fertilization rate (85.1, 82.2).

In the other hand, Ahmed (2018) found that no significant differences in fertilization rate, % (86, 85, 87%) when the females of *C. gariepinus* were stimulate to spawning by using (3000, 2000, 1000 IU HCG/ kg fish), respectively.

In the present study, the results indicated that the increasing in concentration of HCG doses that used to induce spawning caused the increasing in percentage of hatching. Meanwhile, the hatching rate in the current study showed significant differences among treatments with the highest value (81.45%) was recorded in T_4 (females injected with 6000 IU HCG/ kg) followed by T_3 and T_2 (3000, 1500 IU HCG/ kg) with (73.65, 57.9 %), respectively. This results are similar with Maradun *et al.* (2018) who found that the rates of (74.10, 79.84 and 82.07 %) were observed in the hatching rate when the African catfish groups injected by increasingly doses from Ovulin (0.3, 0.5, and 0.7 ml/ kg female weight), respectively.

El-Hawarry *et al.* (2016) reported that the hatching percentage was (85.1, 83.7 %) when induced spawning in C. *gariepinus* by using (4000 IU HCG/ kg fish, 4000 plus 10 mg Dopamine antagonist), respectively. Furthermore the hatching rate that obtained by El-Hawarry *et al.* (2016) with using (4000 IU HCG/ kg fish) was higher than those obtained when using 40 µg GnRH-a with or without 10 mg Dopamine antagonist. with (81.9 and 75.2%), respectively. While, Shourbela *et al.* (2014) demonstrated that the hatching rate was ranged between (70.02 to 89.17 %) when African catfish females

injected by different doses from GnRH-a combined with or without pimozide or domperidone.

Sahoo *et al*. (2009) tested five HCG doses in combinations with five latency periods during stimulate spawning of catfish (*C. batrchus*) and reported that the HCG dosage 3000 IU/ kg in combined with latency period 14-23 h and 4000 IU/ kg in combined with latency period 14-17 h were better to reduce the deformed larvae among hatchling.

In the other hand, Ahmed (2018) reported that the hatching rate showed insignificant differences between treatments injected by (1000, 2000, 3000 IU HCG/ kg fish) with hatching rate in average 82 to 83 % in African catfish.

In the present study, 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.

CONCLUSION

From results of the present study the hormonal injection by 6000 IU HCG/ kg female was some advantages over the other treatments in terms of higher ovaries weight, gonadsomatic index, fertilization rate and hatching rate and the latency period recorded the lowest time in African catfish females. 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. Because of this process, the latency period recorded the lowest time with 6000 IU HCG/ kg female and leaded to accelerated hormonal stimulation, caused in increasing in gonadsomatic index 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.

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ARABIC SUMMARY

تاثير حقن أسماك القرموط الافريقى بجرعات مختلفة من هرمون الجونادوتروبين البشري (HCG) على الاستجابة للتجريد والأداء التناسلي.

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أجريت هذه الدراسة لتقييم تأثير حقن جرعات مختلفة من هرمون الجونادوتروبين البشري (HCG) على الاستجابة للتجريد والأداء التناسلي للقرموط الأفريقي. تم حقن الأسماك عن طريق الحقن العضلي بجرعات مختلفة من HCG (٥٠٠، ١٥٠٠، ٣٠٠٠، ٦٠٠٠ وحدة دولية/ كجم أنثى)، وتم حقن الذكور بنصف جرعة الإناث. أظهرت النتائج أن المجموعة السمكية التي تم حقنها بـ ٦٠٠٠ وحدة دولية/ كجم أنثى لها أعلى وزن للمبيض ومعامل الغدد التناسلية (GSI) لكنها سجلت أقل قيمه لقطر البيض الذي تم تجريده من الإناث. تم تسجيل أقصر فترة للاستجابة لتجريد البيض مع ٦٠٠٠ وحدة دولية/ كجم أنثى (١٢ ساعة) بينما كانت أطول فترة استجابة مع ٥٠٠، ١٥٠٠ وحدة دولية/ كجم أنثى (٢٨ ساعة) و٣٠٠٠ وحدة دولية/ كجم أنثى (٢٢ ساعة). لوحظ أكثر عدد من البيض المخصب/ أنثى ومعدل الأخصاب مع ٦٠٠٠ وحدة دولية/ كجم أنثى (٦٠٨٤٨ بيضة مخصبة/ أنثى بنسبة ٨٤,٤٥٪) وأقل عدد بيض مخصب/ أنثى ومعدل الأخصاب مع ٥٠٠ وحدة دولية/ كجم أنثى (٣٣٧٢ بيضة مخصبة/ أنثى بنسبة ١٠,١٥٪). سجل أكثر عدد يرقات ومعدل الفقس مع ٦٠٠٠ وحدة دولية/ كجم أنثى (٤٩٦٥٧ يرقة/ أنثى بنسبة ٨١,٤٥٪) يليها ٣٠٠٠ وحدة دولية/ كجم أنثى (٤٣١٧٧ يرقة/ أنثى بنسبة ٧٣,٦٥٪)، ١٥٠٠ وحدة دولية/ كجم أنثى (١٢٠٩٩ يرقة/ أنثى بنسبة ٥٧,٩٠٪). بينما البيض المحضن الناتج من المجموعة السمكية المحقونة بـ ٥٠٠ وحدة دولية/ كجم أنثى لم يظهر أي يرقات فاقسة. دلت النتائج أن هرمون HCG نجح في تسريع التبويض للقرموط الأفريقي وزاد الأداء التناسلي مع زيادة جرعة هرمون الجونادوتربين البشرى HCG.