



## INDUCED SPAWNING OF AFRICAN CATFISH, *CLARIAS GARIEPINUS* USING (GnRH<sub>a</sub>) COMBINED WITH DOPAMINE ANTAGONISTS.

Ramy, M. Shourbela<sup>1</sup>; Ashraf, M. Abd El-latif<sup>2</sup> and Abd-El-Azem .M.A<sup>3</sup>.

<sup>1</sup>Dept. of Animal Husbandry and Animal Wealth Development, (Fish breeding and production) Fac. of Vet. Med. Alex. Univ. Egypt. Ramy\_aqua@yahoo.com. <sup>2</sup> Dept. of Fish Diseases and Management, Fac. of Vet. Med., Benha Univ., Egypt. ashraf.mohamed@fvtm.bu.edu.eg. <sup>3</sup> Dept. of Pharmacology, Fac. of Vet. Med. Benha Univ. Egypt.

### ABSTRACT

A study was conducted to evaluate the effects of Gonadotropin releasing hormone analogue (GnRH<sub>a</sub>) alone or with dopamine antagonists (pimozide or domperidone) on induced spawning of African catfish (*Clarias gariepinus*). Fifty four female *C. gariepinus* were divided into 9 groups. Latency period, mass of eggs, ovulation rate, fertilization %, hatching rate, % of deformity, survival rate and estradiol (E2) were estimated. Results of the current study indicated successful spawning induction of African catfish using (GnRH<sub>a</sub>) with dopamine antagonists. The highest ovulation rate (100 %) was observed in groups 6, 8 and 9 which were injected with GnRH<sub>a</sub> 40 ug/kg.b.w plus 16mg pimozid, 10 and 20mg domperidone /kg.b.w respectively. Meanwhile, the lowest ovulation rate (16.67 %) was observed in group 3 which was injected only with GnRH<sub>a</sub> 80 ug/kg.b.w and group 4 which was injected with GnRH<sub>a</sub> 40 ug plus pimozid 4 mg/kg.b.w. The egg mass produced per female showed significant difference ( $P < 0.05$ ) among different groups with the highest values in group 6, while the lowest value was observed in group 4. There was significant highest percentage of deformed larvae (11.2 %) in group 4 and the lowest (4.17 %) was in group 5. Data showed that the level of 17 $\beta$ -estradiol (E2) increased at 6 h in all treated groups with a significant difference ( $P < 0.05$ ). The peak level of E2 was observed at 12 hr post injection in group 9. In contrast, there is a significant decrease in E2 at 24 hr post injection in all ovulated groups. In conclusion, the experiment clearly indicated that the use of GnRH<sub>a</sub> combined with dopamine antagonists was more effective in induction of spawning compared with the use of GnRH<sub>a</sub> alone in African catfish.

**KeyWords:** *Clarias gariepinus*, induced spawning, Gonadotropin releasing hormone analogue (GnRH<sub>a</sub>), dopamine antagonists, estradiol (E2).

(BVMJ-27(1):25-35, 2014)

### 1. INTRODUCTION

The African catfish (*Clarias gariepinus*) has attracted interest as a potential species for fish culture in Egypt due to its rapid growth, large body weight and a high content of protein in the tasty boneless flesh. Several authors have used hypophysation for inducing ovulation in catfish females (Adamek 1995; Brzuska 2001 and Akar and Ali 2006). In order to ensure a reliable supply of quality fish seed, various techniques have been developed for breeding pond fish under controlled

conditions. Where some methods require only elementary changes in environmental conditions, others require sexual segregation of mature specimens or substrates for attachment of eggs. The most sophisticated and efficient technique until date is the use of hormones to induce spawning (Staff, 1983; Richardson, 1988). Induced breeding by hypophysation was developed in India for catfishes such as *Heteropneustes fossilis* and *Clarias batrachus* (Ramaswamy and Sunderaraj

1956; Khan and Mukhopadhyay, 1976; Dehadrai *et al.*, 1985), the doses of pituitary extract required for singhi was found to be very high (30 mg/kg). In recent years, a combination of dopamine antagonists and LHRH analogue (LHRA $\alpha$ ) has been found successful in ovulation and induced breeding in some teleosts (De Leeuw *et al.*, 1985; Fremin, 1991). Carp pituitary extract (CPE) and luteinizing hormone-releasing hormone analogue (LHRH $\alpha$ ) are two well-known hormones for controlling ovulation in channel catfish (Fobes 2013). Since the 1990s, a drug known as ovaprim has been commonly used as a spawning hormone in fish breeding (Marte *et al.*, 1987). Ovaprim, which is a combination of salmon GnRH analog combined with a dopamine agonist Domperidone, has proved to be extremely successful in breeding of carps with a spawning rate of about 100%. In comparison, the success rate of spawning using GnRH analogs in other fishes are 100% in Grouper (*Epinephelus salmoides*) (Kungvankij *et al.*, 1986), and 99% in Milkfish (*Channos chanos*) (Kelley and Lee, 1986). Catfishes are a favorite food in India and Southeast Asia. Of the three species that are chiefly cultivated viz. *Clarias batrachus*, *C. gariepinus* and *C. macrocephalus*, the first and third are extensively cultured in Asia. Although *C. batrachus* breeds naturally in ponds, the efficiency and rate of induced spawning of catfish with ovaprim has been found to be less than 50% (Thalathiah *et al.*, 1988; Ngamvongchon *et al.*, 1988), which is very low compared to that of carps. Sex steroids in female fish perform major roles in oocyte maturation, ovulation and spawning. Synthesis of vitellogenin and increase in ovarian size during final oocyte maturation is controlled by 17 $\beta$ -estradiol. 17 $\beta$ -estradiol is directly related to gonadosomatic index (Tyler *et al.*, 1991; Sabet *et al.*, 2009; Coccia *et al.*, 2010).

The aim of this study is to investigate the effects of using GnRH analogues with or without dopamine antagonists (pimozide or domperidone) on the spawning

performance parameters of African catfish and to record the progress in the circulating levels of ovarian hormone: oestradiol, representing the main steroid involved in vitellogenesis.

## 2. MATERIAL AND METHODS

### 2.1. Fish and experimental design:

A total number of Fifty-four apparently healthy female and one hundred and sixty two male African catfish (*Clarias gariepinus*) were obtained from private fish farm, Alexandria governorate, Egypt in august 2013. The fish were transported to Lab of department of animal husbandry and animal wealth development, (fish breeding and production) at Fac. of Vet. Med, Edfina, Alexandria University and maintained in fiber glass tanks, supplied with oxygenated dechlorinated tap water for 15 days for acclimation. The fish were fed twice daily with pelleted food (30 % crude protein) in a rate of 3% of their body weight. Water parameters were monitored, temperature was  $28.6 \pm 1.05$  °C, pH was  $8.57 \pm 0.19$  and Dissolved oxygen (DO) was  $6.57 \pm 0.19$  mg/L. Female catfish, which showed spawning signs, were randomly divided into 9 groups with two replicates, each contained three females as shown in table (1).

### 2.2. Preparation of GnRH $\alpha$ and dopamine antagonists:

2.2.1. Gonadotropin releasing hormone analogue GnRH $\alpha$ : Cystorelin® (vial 20 ml) each 1 ml contains 50  $\mu$ g synthetic Gonadorelin (GnRh) diacetate tetrahydrate manufactured by CEVA SANTLE ANIMAL –FARANCE.

2.2.2. Pimozide tablets, each tablet contains 4mg from the active principle (under trade name Orap Forte® manufactured by Cilag, Belgium Campany). Pimozide tablets were powdered and then dissolved in dimethyl sulfoxide (Omeljaniuk *et al.*, 1987).

2.2.3. Domperidone tablets: each tablet contains 10 mg from the active principle (under trade name Gastromotil® manufactured by EIPICO Company, Cairo, A. R. E.) were powdered and then resuspended in physiological saline 0.9 % when

needed (Alok *et al* 1997). The volume of injection of different doses of Pimozide or dompridone were adjusted at 2ml /Kg.B.w and injected intramuscularly combined with GnRHa.

Table (1) Showing GnRHa and dopamine antagonists doses used in different groups

Groups(each group 6 female fish)	Drug	Dose/ kg / B.wt.
G1 ( control)	Saline	1ml
G2	GnRHa	40 µg
G3	GnRHa	80 µg
G4	GnRHa +	40 µg + 4mg
G5	Pimozide	40 µg + 8mg
G6		40 µg + 16mg
G7	GnRHa +	40 µg + 5mg
G8	Dompridone	40 µg + 10mg
G9		40 µg + 20mg

**N.B.** The volume of injection of different doses of Pimozide or dompridone were adjusted not exceed 2ml /Kg. B.w and injected intramuscularly combined with GnRHa.

### 2.3. Stripping and fertilization of eggs:

Female fish, which were injected by GnRHa alone or in combination with dopamine antagonists, were checked for ovulation after six hrs. post injection and continued at one-hour intervals. The fish that yielded stream of transparent green-brown eggs were rated as ovulated. The weight of total egg mass from each female was recorded for total egg production. Eggs obtained from each female were fertilized by mixing with milt obtained from macerated testes of three killed males according to Rothbard (1981). The fertilized eggs were spread into plastic bowl containing 4 L of water (dechlorinated tap water) for incubation. Dull, unfertilized dead eggs were separated from transparent, living ones. After hatching, the percentage of normal and deformed larvae was calculated. Fertilization success was determined under a dissecting microscope

24 h after fertilization, when eggs were at the stage of gastrulation (Razavi, 1984). Survival rate was determined according to (El-Ashram, 1997).

### 2.4. Assessment of results:

2.4.1- *Ovulation ratio* = number of ovulated females / number of injected females x 100 Szabo *et al.* (2002)

#### 2.4.2- *Egg weight*:

Egg weight were estimated according to Szabo *et al.* (2002)

#### 2.4.3- *Percentage of fertilized eggs*:

A sample of 100 eggs was taken from the central part of the plastic bowel of each female (Brzuska, 2004) placed in Petri dish and examined under binocular microscope 3x. Dull unfertilized eggs were separated from transparent living ones and counting the number of fertilized one (Ayinla, 1988). This was done after 12 hrs post fertilization.

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

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

2.4.5-*latancy period*

Period from injection till the onset of ovulation (hrs).

2.4.6-*% of deformity* marked curved tail and shortened body. The percentage of undeveloped eggs, normal fry and deformed fry was noted in each case as suggested by De Leeuw *et al.* (1985)

2.4.7. *Survival rate*= (Final number of survival larvae / Initial number of fish embryo) x 100 (El-Ashram, 1997)

2.4.8- *Hormone determinations*

Blood samples were collected from the caudal vein at 0,6,12 and 24 hrs post-injection without anticoagulant then transferred to Wasserman tubes. Blood was allowed to clot at room temperature for 45 min then centrifuged at 3000 rpm for 15 minute to obtain serum sample (Bernet *et al.*, 2001). The Serum samples were pipetted into Eppendorf tube, labeled and stored in deep freeze at -20°C till assayed. Oestradiol was determined by Enzyme Immunoassay using standard oestradiol (0, 10, 30, 100, 300, and 1000 pg/ml) (biocheck, Inc. Foster City, CA 94404 U.S.A.),

2.4.8-*Statistical analysis:*

One-way analysis of variance (ANOVA) was applied used using (Statistical analysis System (SAS, 2004) software (SAS Institute Cary, North Carolina, USA, 2004.

### 3. RESULTS:

As presented in Table 2. The percentage of ovulation for the experimental groups was significantly different according to

treatment program. Control group showed no ovulation after injecting physiological saline. In the same respect group (2) received GnRH<sub>a</sub> 40ug/kg.b.w showed no ovulation. While group (3) injected with GnRH<sub>a</sub> 80 ug/kg.b.w showed low ovulation (16.67 %).

The highest percentage of ovulation(100 %) was observed in groups 6, 8 and 9 which were injected with GnRH<sub>a</sub> 40 ug/kg.b.w plus 16mg pimozid,10 and 20mg dompridone /kg.b.w respectively, followed by (83.33%) in group 5 injected with GnRH<sub>a</sub> 40 ug/kg.b.w plus 8mg pimozid. While in group 7 which were injected with GnRH<sub>a</sub> 40 ug/kg.b.w plus 5mg dompridone /kg. b.w was (50 %). In contrast, the lowest ovulation percentage (16.67 %) was observed in group 4 which were injected with GnRH<sub>a</sub> 40 ug/kg.b.w plus 4mg/kg.b.w pimozid.

The latency period ranged from 10.33 to 15 hrs for the seven ovulated groups (Table 2). Data demonstrated significant differences among these groups. The lowest latency period was (10.33 hrs) in group 9. In the contrary, the longest latency period was (15 hrs) in group 3. The weight of egg mass produced per female showed significant difference ( $P < 0.05$ ) among different groups with the lowest values (30.4 gm) in group 4 which was significantly differed from other treatments. While, the highest value (67.27 gm) was observed in group 9 (Table 2).

As shown in Table 2 the percentage of fertilization among the different experimental groups were significantly differed ( $p < 0.05$ ) with the lowest percentage of fertilization observed in group 4 (63.33 %) which was significantly differed ( $P < 0.05$ ) from all other treatments. On the other hand, the highest percentage of fertilization was found in group 8 (83.17 %) followed by group 5 (79.87 %).

Table (2) the effect of GnRHa, pimoziide and dompridone on spawning parameters in catfish (n = 6).

GROUPS	Fish weight (g)	Ovu. %	Egg weight (g)	Latency period (h)	Fertilization %	hatching rate %	% of deformity	Survival rate
G1 (control)	614.08 <sup>a</sup> ±1 2.57	0	0	0	0	0	0	0
G2 (Gn 40ug)	620.10 <sup>a</sup> ±1 6.87	0	0	0	0	0	0	0
G3 (Gn 80 ug)	578.45 <sup>a</sup> ±2012	16.6 7 <sup>d</sup>	33.4 <sup>d</sup> ± 0.00	15.0 <sup>a</sup> ± 0.00	62.27 <sup>b</sup> ±3. 22	70.02 <sup>b</sup> ±3.7 4	9.12 <sup>b</sup> ±2.1 1	71.55 <sup>b</sup> ± 18.1
G4 (Gn 40 ug +P 4mg)	586.83 <sup>a</sup> ±1 2.93	16.6 7 <sup>d</sup>	30.4 <sup>d</sup> ± 0.00	13.0 <sup>b</sup> ± 0.00	63.33 <sup>b</sup> ± 3.25	73.22 <sup>b</sup> ±2.5 6	11.2 <sup>a</sup> ±3.1 1	75.45 <sup>b</sup> ± 20.1
G5 (Gn 40 ug +P 8mg)	628.17 <sup>a</sup> ±1 9.83	83.3 3 <sup>b</sup>	55.94 <sup>b</sup> ± 6.34	11.8 <sup>c</sup> ±0.42	79.87 <sup>ab</sup> ±4 .21	86.67 <sup>a</sup> ±2.1 7	4.17 <sup>c</sup> ±1.0 8	85.12 <sup>a</sup> ± 10.54
G6(Gn 40 ug + P 16mg)	628.30 <sup>a</sup> ±1 6.55	100 <sup>a</sup>	67.27 <sup>a</sup> ± 3.69	11.33 <sup>c</sup> ±0. 42	65.17 <sup>b</sup> ±4. 36	75.24 <sup>b</sup> ±0.9 9	10.05 <sup>a</sup> ±1. 06	80.68 <sup>a</sup> ± 11.54
G7 (Gn 40 ug +D 5mg)	625.50 <sup>a</sup> ±1 8.67	50 <sup>c</sup>	40.18 <sup>c</sup> ± 5.16	12.33 <sup>bc</sup> ±0 .96	68.44 <sup>b</sup> ±5. 62	76.33 <sup>b</sup> ±1.9 4	5.17 <sup>c</sup> ±1.1 7	76.04 <sup>a</sup> ± 6.11
G8 (Gn40 ug + D 10 mg)	548.17 <sup>a</sup> ±1 7.47	100 <sup>a</sup>	57.42 <sup>b</sup> ± 7.33	11.5 <sup>c</sup> ±0.3 4	83.17 <sup>a</sup> ±3. 75	89.17 <sup>a</sup> ±2.2 7	4.51 <sup>c</sup> ±0.7 6	89.50 <sup>a</sup> ± 4.2
G9 (Gn 40ug + D 20 mg)	546.18 <sup>a</sup> ±1 2.45	100 <sup>a</sup>	54.82 <sup>b</sup> ± 6.34	10.33 <sup>d</sup> ±0. 42	67.17 <sup>b</sup> ±6. 24	72.66 <sup>b</sup> ±5.3 9	8.04 <sup>b</sup> ±1.1 3	81.33 <sup>a</sup> ± 8.33

Table 3 - The effect of GnRHa , pimoziide and dompridone on serum estradiol levels in catfish ( n = 3).

Groups	At injection	6 h.	12 h.	24 h
G1- (control)	0.34 <sup>a</sup> ±0.05	0.42 <sup>c</sup> ±0.17	0.37 <sup>c</sup> ±0.09	0.51 <sup>c</sup> ±0.10
G2 (Gn 40ug)	0.36 <sup>a</sup> ±0.04	0.50 <sup>bc</sup> ±0.09	0.66 <sup>d</sup> ±0.09	0.78 <sup>b</sup> ±0.08
G3 (Gn 80 ug)	0.35 <sup>a</sup> ±0.07	0.56 <sup>bc</sup> ±0.10	0.98 <sup>d</sup> ±0.12	1.05 <sup>b</sup> ±0.09
G4 (Gn 40 ug +P 4mg)	0.33 <sup>a</sup> ±0.02	0.60 <sup>b</sup> ±0.11	1.17 <sup>d</sup> ±0.13	1.25 <sup>a</sup> ±0.17
G5 (Gn 40 ug +P 8mg)	0.35 <sup>a</sup> ±0.08	0.84 <sup>ab</sup> ±0.07	1.67 <sup>b</sup> ±0.13	0.90 <sup>b</sup> ±0.09
G6(Gn 40 ug + P 16mg)	0.33 <sup>a</sup> ±0.05	0.93 <sup>a</sup> ±0.12	1.98 <sup>a</sup> ±0.15	0.56 <sup>c</sup> ±0.17
G7 (Gn 40 ug +D 5mg)	0.34 <sup>a</sup> ±0.03	0.60 <sup>b</sup> ±0.11	1.37 <sup>c</sup> ±0.13	0.97 <sup>b</sup> ±0.010
G8 (Gn40 ug + D 10 mg)	0.35 <sup>a</sup> ±0.07	0.83 <sup>ab</sup> ±0.12	1.88 <sup>b</sup> ±0.17	1.05 <sup>b</sup> ±0.12
G9 (Gn 40 ug+ D 20 mg)	0.35 <sup>a</sup> ±0.07	0.94 <sup>a</sup> ±0.12	2.21 <sup>a</sup> ±0.19	0.93 <sup>b</sup> ±0.12

Gn=GnRha    P=Pimoziide    D = Dompridone    Ovu. % = Ovulation %  
Means within the same column carrying different letters are significantly different at (P < 0.05).

Percentage of hatchability showed significant differences ( $P < 0.05$ ) among the groups. The highest significant hatchability percentage observed in group 8 (89.17%) followed by group 5 (86.67%). The lowest significant hatchability percentage observed in group 3 (70.02 %).

Percentage of deformed larvae for the ovulated groups were significantly differ from each other ( $P < 0.05$ ) and the lowest percentage was reported in group 5 (4.17 %) followed by group 8 (4.51%) (Table 2). There was significant higher percentage of deformed larvae for group 4 (11.2 %) followed by group 6 (10.05%) and group 3 (9.12%).

Survival rate for the ovulated groups were significantly differ from each other ( $P < 0.05$ ) and significant lowest percentage was reported for group 3 ( $71.55^b \pm 18.1$ ). There was significant highest percentage of Survival rate for group 8 ( $89.50^a \pm 4.2$ ) compared to other groups.

Table (3) showed the serum  $17\beta$ -estradiol (E2) concentrations measured at zero, 6, 12 and 24 h post-injection. After 6 h, there was an increase in the level of  $17\beta$ -estradiol in all groups and a significant difference ( $p < 0.05$ ) was observed. Peak level of serum E2 was observed at 12 h post injection for group 9 followed by group 6. In the present study treatment with GnRHa with Pimozide or domperidone could increase the amount of sex steroids and subsequent ovarian development in *Clarias gariepinus*.

#### 4. DISCUSSION

Concerning to the results illustrated in Table 2. The percentage of ovulation for the experimental groups was significantly different according to treatment program. Control group showed no ovulation after injecting physiological saline. In the same respect group (2) received GnRHa 40ug/kg.b.w showed no ovulation. While group (3) injected with GnRHa 80 ug/kg.b.w showed low ovulation (16.67 %). These findings was closely similar to those described by (Richter et al 1987) in their

work in *C. macrocephalus*, as in *C. gariepinus*. The highest percentage of ovulation(100 %) was observed in groups 6, 8 and 9 which were injected with GnRHa 40 ug/kg.b.w plus 16mg pimozid, 10 and 20mg dompridone /kg.b.w respectively, followed by (83.33%) in group 5 injected with GnRHa 40 ug/kg.b.w plus 8mg pimozid. While in group 7 which were injected with GnRHa 40 ug/kg.b.w plus 5mg dompridone /kg.b.w was (50 %). In contrast, the lowest ovulation percentage (16.67 %) was observed in group 4 which were injected with GnRHa 40 ug/kg.b.w plus 4mg/kg.b.w pimozid. These results are nearly agreed with the results obtained by Brzuska, 2003 reported that combination of GnRHa with dompridone showed high ovulation rate (87.5) in African catfish .Also Nayak et al (2001) demonstrated that administration of LHRHa combined with pimozid caused high rate of ovulation in Indian catfish (*Hetropneustes fossilis*).On the other hand, Dorafshan *et al* (2003) and Ghezal, 1993 stated that combination of GnRHa with a dopamine receptor antagonist such as domperidone is necessary for spawning induction in cyprinid fish such as common carp. Aizen *et al.* (2005) reported that, the addition of some additives to HCG or CPE as a dopamine antagonist causes the stimulator was more potent in inducing ovulation compared to GnRHa alone and this attributed to Dopaminergic inhibition is a major barrier along the reproductive axis that arrests spontaneous spawning. The latency period ranged from 10.33 to 15 hrs for the seven ovulated groups (Table 2). Data demonstrated significant differences among these groups. The lowest latency period was (10.33 hrs) in group 9. In the contrary, the longest latency period was (15 hrs) in group 3. Our results were nearly similar with that obtained by Brzuska, 2003 reported that combination of GnRHa with dompridone showed latency time 13 h in African catfish (*Clarias gariepinus*) and De Leeuw et al. (1985) reported that the latency time was 12.3 h in African catfish (*Clarias gariepinus*) injected with LHRHa and

pimozide. On the other hand, there were long argument around the latency time obtained by other several studies [Akar. 2006 and El-Hawarry *et al* 2012] in silver carp, Basavaraja *et al.*, 2007 in Indian carp and [Brzuska, 2006 and Vazirzadeh *et al.*, 2011] in common carp]. This difference may due to change in hormone used or difference in water parameters as temperature. Also species of fish, time of injection and injection regime for each special treatments. The weight of egg mass produced per female showed significant difference ( $P < 0.05$ ) among different groups with the lowest values (30.4 gm) in group 4 which was significantly differed from other treatments. While, the highest value (67.27 gm) was observed in group 9 (Table 2). This variation may be attributed to different doses of dopamine antagonists. The percentage of fertilization among the different experimental groups were significantly differed ( $p < 0.05$ ) with the lowest percentage of fertilization observed in group 4 (63.33 %) which was significantly differed ( $P < 0.05$ ) from all other treatments. On the other hand, the highest percentage of fertilization was found in group 8 (83.17 %) followed by group 5 (79.87 %). Similar results were obtained by Nayak *et al* (2001) who demonstrated that administration of LHRHa combined with pimozid caused high rate of fertilization in Indian catfish (*Hetroptneustes fossilis*). Percentage of hatchability showed significant differences ( $P < 0.05$ ) among the groups. The highest significant hatchability percentage observed in group 8 (89.17%) followed by group 5 (86.67%). The lowest significant hatchability percentage observed in group 3 (70.02 %). This lowest rate may be attributed to the poor quality eggs in this group. Percentage of deformed larvae for the ovulated groups were significantly differ from each other ( $P < 0.05$ ) and the lowest percentage was reported in group 5 (4.17 %) followed by group 8 (4.51) (Table 2). There was significant higher percentage of deformed larvae for group 4 (11.2 %)

followed by group 6 (10.05%) and group 3 (9.12%). This Significant increase in the percentage of deformed larvae in fish of these groups may be attributed to the low egg quality that led to higher larval deformities. This high deformity may be attributed to the fertilization of unripe ova, which led to abnormal embryonic development (Azuadi *et al.*, 2011). Survival rate for the ovulated groups were significantly differ from each other ( $P < 0.05$ ) and significant lowest percentage was reported for group 3 ( $71.55^b \pm 18.1$ ). There was significant highest percentage of Survival rate for group 8 ( $89.50^a \pm 4.2$ ) compared to other groups.

Regarding to the results in Table (3), the serum  $17\beta$ -estradiol (E2) concentrations measured at zero, 6, 12 and 24 h post-injection. After 6 h, there was an increase in the level of  $17\beta$ -estradiol in all groups and a significant difference ( $p < 0.05$ ) was observed. Peak level of serum E2 was observed at 12 h post injection for group 9 followed by group 6. In the present study treatment with GnRHa with Pimozide or domperidone could increase the amount of sex steroids and subsequent ovarian development in *Clarias gariepinus*. These results agree with these previously reported for sea bass *Dicentrarchus labrax* by Prat *et al.*, 2001 where the treatment of GnRHa and pimozide induced an increase of plasma sex steroids. The sharp increase in plasma E2 could occur because of a high aromatase activity in the ovary at the moment of GnRHa administration. Also our results were nearly similar to that obtained by Sigal-Drori *et al* (1993) reported that The rise in circulating Gonadotrophin Hormones following GnRH+Metoclopramide was gradual and reached its peak 14 h after treatment of common carp. The failure of fish to ovulate after treatment with GnRHa and the control vehicles may suggest that plasma gonadotropin (Gth) levels of those fish had remained low. A surge in Gth can initiate final events of oocyte maturation and

ovulation in *C. macrocephalus*, as in *C. gariepinus* (Richter et al 1987).

**CONCLUSION:** The results demonstrated that treatment with GnRH<sub>a</sub> combined by dopamine antagonist (Pimozide or Domperidone) might be considered an effective procedure for induction of spawning in *Clarias gariepinus*. Also, administration of GnRH<sub>a</sub> 40ug plus domperidone 10mg/kg.b.w. gave the best spawning results.

## 5. REFERENCES

- Adamek, J. (1995). Rozrodi podchow wyegen surnaa fry kanskiego (*Clarias gariepinus*). Przege. Ryb, 1:36-42.
- Aizen J, Meiri, I., Tzchori, I., Levavi-Sivan, B., and Rosenfeld, H. (2005). Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. General comparative endocrinology, 142 (1-2): 212-217.
- Akar. M.A. (2006). Biologic and economic efficiency of human chorionic gonadotropin and carp pituitary extract for spawning induction in silver carp (*Hypophthalmichthys molitrix*). Egypt. J. Ex Biol. (zool).1:73-76.
- Akar. M.A. and Ali, M.A. (2006). Artificial spawning of African catfish (*Clarias gariepinus*). After stimulation of ovulation with carp pituitary extract, human chorionic gonadotropin and mixed injection. Egypt. J. of Appl. Sci., 21(2):8-16.
- Alok, D., Deyika, P. and Lalit, G. (1997): Effect of d-Lys6 salmon sGnRH alone and in combination with domperidone on the spawning of common carp during the late spawning season. Aquaculture International. 5(4): 369-374
- Ayinla, O.A. (1988): Nutritive and reproductive performance of *Clarias gariepinus* (Burchell 1822). Unpublished Ph. D Thesis, University of Ibadan, Nigeria. pp. 433.
- Azuadi, N.M., Siraj, S.S., Daud, S.K. Christianus, A., Harmin, S.A., Sungan, S. and Britin, R. (2011): Enhancing ovulation of Malaysian Mahseer (*Tor tambroides*) in captivity removal of dopaminergic inhibition. J. Fish. Aquatic Sci., 6: 740 - 750.
- Basavaraja, N., Nandeesh, M.C., Akash, N., Rangaswamy, B.L., Mahesh, T. and Murthy, C.K. (2007). Successful induced spawning of Indian major carps following injection of buserelin and domperidone. Asian Fisheries Science, 2: 287-297.
- Bernet, D., Schmidt, H., Wahli, T. and Burkhardt-Holm, P. (2001): Effluent from a sewage treatment works causes changes in serum biochemistry of brown trout (*Salmo trutta L.*). Ecotoxicol. Environ. Saf. 48:140-147.
- Brzuska, E. (2001): Artificial spawning of European catfish, differences between propagation results after stimulation of ovulation with carp pituitary and ovopel. Aquaculture Research 32: 11-19.
- BRZUSKA, E. (2003): Artificial propagation of African catfish (*Clarias gariepinus*): differences between reproduction effects after stimulation of ovulation with carp pituitary homogenate or GnRH-a and dopaminergic inhibitor. Czech J. Anim. Sci., 48(5): 181-190
- Brzuska, E. (2004): Artificial spawning of carp (*Cyprinus carpio L.*); differences between the effects of reproduction in females of Hungarian, Polish and French origin treated with carp pituitary homogenate or [DTel6, ProNHEt9] GnRH (Lecirelin). Aquaculture research, 35:1318- 327.
- Brzuska, E. (2006). Artificial spawning of female Lithuanian strain B carp (*Cyprinus carpio L.*) after treatment with carp pituitary homogenate,

- Ovopel or [D-Tle6, ProNHEt9] LH-RHa (Lecirelin). *Aquaculture research*, 37: 264-271.
- Coccia, E., De Lisa, E., Di Cristo, C., Di Cosmo, A., Paolucci, M. (2010). Effects of estradiol and progesterone on the reproduction of the freshwater crayfish *Cherax albidus*. *Biol Bull* 218:36-47
- De Leeuw, R., Th. Goos, H.J., Richter C.J. and Eding, E.H. (1985). Pimozide-LHRHa induced breeding of the African catfish, *Clarias gariepinus* (Bruchell). *Aquaculture*, 44:295-302.
- Dehadrai, P.V., Kamal, M.Y. and Das, K.K. (1985). Package of practices for increasing production of air-breathing fishes. In: *Aquaculture Extension Manual* 3:1-14. Central Inland Fisheries Research Institute, Barrack pore.
- Dorafshan S, Hossein Mostafavi and Bagher MojaziAmiri (2003). Induction of spawning in common carp *Cyprinus carpio*, using pituitary extract and GnRH analogue in combination with Domperidone. *Iranian journal of biotechnology*, 1(4).
- El-Ashram, A.M. (1997). Some reproductive studies on some freshwater fish with regard to some pathological and toxicological factors. Ph.D. Thesis, Zagazig univ., Egypt.
- El-Hawarry W.N, Nemaatallah, B.R. and Shinaway, A.M. (2012). Induced Spawning of Silver Carp, *Hypophthalmichthys molitrix* Using Hormones/Hormonal Analogue with Dopamine Antagonists. *Online J. Anim. Feed Res.*, 2(1): 58-63.
- Fermin, C.A. (1991). LHRHa and domperidone-induced oocyte maturation and ovulation in bighead carp, *Aristichthys nobilis* (Richardson). *Aquaculture*, 93:87-94.
- Fobes, M.C. (2013): Controlled Spawning of White Catfish, *Ictalurus catus*, and Brown Bullhead Catfish, *Ameiurus nebulosus*, Using Carp Pituitary Extract and LHRHa. Master of Science Thesis, Faculty of Auburn University.
- Ghezel, H.G (1993). Spawning induction in common carp, *C. carpio* using GnRH, HCG, PMSG, M. Sc thesis, Faculty of Veterinary Medicine, University of Tehran (in Persian) pp: 60-124.
- Khan, H.A. and S.K. Mukhopadhyay (1976). Production of stocking material of some air-breathing fishes by hypophysation. *J. Inland Fish. Soc. India*, 7:156-161.
- Kelley, C.D., & Lee, C.S. (1986). Artificial propagation. In: Lee C-S, Gordon and MS, Watanabe WO (Eds.), *Aquaculture of milkfish (Chanos chanos)*. State of the art. Oceanic Institute, Hawaii, 83-116.
- Kungvankij, P., Tiro, L., Pudadera, B.P., & Potestas, I.O. (1986). Induced spawning and larval rearing of grouper (*Epinephelus salmoides* Maxwell). In: J.L. Maclean, L.B. Dizon and L.V. Hosillos (Eds.), the First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.
- Marte, C.L., Sherwood, N.M., Crim, L.W., & Harvey, B. (1987). Induced spawning of maturing milkfish (*Chanos chanos forsskal*) with gonadotropin-releasing hormone (GnRH) analogues administered in various ways. *Aquaculture*, 60:303-310.
- Nayakp. K., Mishra, T.K., Singh, B.N., Pandey, A.K. and Das, R.C. (2001): Induced maturation and ovulation in *Heteropneustes fossilis* by using LHRHa, pimozide and ovaprim for production of quality eggs and larvae. *Indian J. Fish.*, 48(3): 269-275.
- Ngamvongchon, S., Pawaputanon, O., Leelapatra, W., and Johnson, W.E. (1988). Effectiveness of LHRH analogous for the induced spawning of carp and catfish in Northeast Thailand. *Aquaculture*, 74, 35 - 40.

- Omeljaniuk, R.J., Shih, S.H., and Peter, R.E. (1987). In-vivo evaluation of dopamine receptor-mediated inhibition of gonadotrophin secretion from the pituitary gland of the goldfish, *Carassius auratus*. *J Endocrinol*; 114:449–58.
- Prat, F., Zanuy, S., and Carrillo, M. (2001): Effect of gonadotropin-releasing hormone analogue (GnRH $\alpha$ ) and pimozide on plasma levels of sex steroids and ovarian development in sea bass (*Dicentrarchus labrax* L.). *Aquaculture*, 198:325–38.
- Ramaswamy, L.S. and Sunderaraj, B.I. (1956). Induced spawning in the Indian catfish. *Science*, 123:1080
- Razavi Sayyad, B. and Sefid (1984). *Rutilus frisii* kutum (Kamenskii, 1901). Iranian Fisheries Research Organization, pp. 32
- Richardson, A. (1988). Hatchery Manual for the White Sturgeon *Acipenser transmontanus* with Application to Other North American Acipenseridae. Cooperative Extension, University of California, Division of Agriculture and Natural Resources.
- Richter, C.J.J., Eding, E.H. Goose, H.J.T., De Leeuw, R., Scott, A.P. and Van Oordt, P.G. (1987): The effect of pimozide/LHRH $\alpha$  and 17 alpha-hydroxyprogesterone on plasma steroid levels and ovulation in the African catfish, *Clarias garpienus*, *Aquaculture*; 63:157–168.
- Rothbard, S., (1981). Induced reproduction in cultivated cyprinids-the common carp and the group of Chinese carps. I. The technique of induction, spawning and hatching. *Bamidgeh*, 33: 103-121.
- Sabet, S.S., Reza, I.M., Bagher, A.F., Saeed, G. (2009). Study on sexual maturity and levels of gonad steroid hormones in female kutum *Rutilus frisii* kutum (Kamenskii, 1901) during spawning season from river Sefid-Rood of the Southern Caspian Sea. *J Cell Anim Biol* 3:208–215
- SAS (2004): SAS institute / STAT Guide for personal computers, 6<sup>th</sup> ed. Cary, NC.
- Sigal-Drori et al. (1993). Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH super active analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. *Aquaculture*, 119: 393-407.
- Staff, F. (1983). Alaska Department of Fish and Game, Division of Fisheries Rehabilitation, Enhancement and Development. *Fish Culture Manual*
- Szabo, T., Modgyasszay, C.S. and Horvath, L. (2002). Ovulation induction in nase (*Chondrostom anasus*). Using pituitary extract or GnRH analogue combined with domperidone. *Aquaculture* 203: 389-395.
- Thalathiah, S., Ahmad, A.O., and Zaini, M.S. (1988). Induced spawning techniques practiced at Batu Berendam, Malaka, Malaysia. *Aquaculture*, 74: 23-33.
- Tyler C.R., Sumpter J.P., Kawauchi H., and Swanson P. (1991) Involvement of gonadotropin uptake of vitellogenin into vitellogenic oocytes of the rainbow trout *Oncorhynchus mykiss*. *Gen Comp Endocrinol* 84:291–299
- Vazirzadeh A, Amiri, B.M., Yelghi, S., Hajimoradloo, A., Nematollahi, M.A. and Mylonas, C.C. (2011). Comparison of the effects of different methods of mammalian and salmon GnRH $\alpha$  administration on spawning performance in wild-caught female carp (*Cyprinus carpio*) from the Caspian Sea. *Aquaculture*, 320: 123-128



## التفريخ الصناعي للقرموط الأفريقي باستخدام GnRHa مع مثبطات الدوبامين

رامي محمود شرييله<sup>1</sup>، أشرف محمد عبد اللطيف<sup>2</sup>، عبد العظيم محمد عبد العظيم<sup>3</sup>  
<sup>1</sup> قسم رعاية الحيوان وتنمية الثروة الحيوانية (تربية وإنتاج الأسماك) كلية الطب البيطري -جامعة الاسكندرية  
<sup>2</sup> قسم امراض الاسماك ورعايتها. كلية الطب البيطري -جامعة بنها  
<sup>3</sup> قسم الفارماكولوجي -كلية الطب البيطري -جامعة بنها

### الملخص العربي

استهدفت هذه الدراسة تقييم الحقن بهرمون سيستوريلين GnRHa وحده او مع مثبطات الدوبامين مثل دوميريديون وبيموزايد لإحداث التفريخ الصناعي لإناث القرموط الأفريقي. واستخدم لهذا 54 سمكه (إناث) قسمت الى 9 مجموعات. وتم تسجيل الزمن بين الحقن والتبويض وقياس كمية البيض ونسبه التبويض والاختصاص ونسبه الفقس ونسبه التشوهات ونسبه الاعاشه كما تم قياس تركيز هرمون استراديول في السيرم. وأظهرت النتائج ما يلي: نجاح التبويض في سمكه القرموط الأفريقي بمعدل يتراوح بين 100% في المجموعات (6 و8 و9) التي تم حقنها ب 40 ميكروجرام GnRHa مضافا اليه 16مجم بيموزايد، 10 و20 مجم دوميريديون لكل كجم من وزن الجسم على التوالي. بينما لوحظ اقل نسبه تبويض (16.67%) في المجموعه الثالثه التي تم حقنها ب 80 ميكروجرام GnRHa والمجموعه الرابعه التي تم حقنها ب 40 ميكروجرام GnRHa مضافا اليه 4مجم بيموزايد لكل كجم من وزن الجسم. اظهرت النتائج ان كتله البيض المنتجه من المجموعه السادسه كانت هي الأعلى بينما كانت المجموعه الرابعه هي الاقل. كانت اعلي نسبه تشوهات في الزريعة 11.2% في المجموعه الرابعه بينما كانت الاقل 4.17% في المجموعه الخامسه. اظهرت النتائج زياده تركيز هرمون استراديول في السيرم تدريجيا بعد 6ساعات من الحقن بالمقارنه بتركيزها عند الحقن ووصلت لاعلاها عند 12ساعه ثم بدأت في التراجع عند 24 ساعة مع وجود اختلاف نوعي في المجموعات. اوضحت التجربة ان استخدام (GnRha) مع مثبطات الدوبامين مثل دوميريديون او بيموزايد أكثر تأثيرا في احداث التفريخ في سمكه القرموط الأفريقي مقارنة باستخدام GnRha وحده.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(1):25-35, سبتمبر 2014)