INDUCED SPAWNING OF AFRICAN CATFISH, *CLARIAS GARIEPINUS* USING (GnRHa) COMBINED WITH DOPAMINE ANTAGONISTS.

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A B S T R A C T

A study was conducted to evaluate the effects of Gonadotropin relasing hormone analogue (GnRHa) 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 (GnRHa) with dopamine antagonists. The highest ovulation rate (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 domperidone /kg.b.w respectively. Meanwhile, the lowest ovulation rate (16.67 %) was observed in group 3 which was injected only with GnRHa80 ug/kg.b.w and group 4 which was injected with GnRHa 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 17β-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 GnRHa combined with dopamine antagonists was more effective in induction of spawning compared with the use of GnRHa alone in African catfish.

KeyWords: *Clarias gariepinus*, induced spawning, Gonadotropin releasing hormone analogue (GnRHa), dopamine antagonists, estradiol (E2).

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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 (LHRAa) 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 (LHRHa) 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 17b-estradiol. 17b-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 ± 1.05 ℃, pH was 8.57 ± 0.19 and Dissolved oxygen (DO) was 6.57 ± 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 GnRHa and dopamine antagonists:

2.2.1. Gonadotropin releasing hormone analogue GnRHa: Cystorelin® (vial 20 ml) each 1 ml contains 50 µg 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® manifactured 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® manifactured by EIPICO Campany, 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 + Pimozide	40 µg + 4mg
G5		40 µg + 8mg
G6		40 µg + 16mg
G7	GnRHa + Dompridone	40 µg + 5mg
G8		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 ovulatation after injecting physiological saline. In the same respect group (2) received GnRHa 40ug/kg.b.w showed no ovulatation. While group (3) injected with GnRHa 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 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.

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, pimozide 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^{a}±12.57	0	0	0	0	0	0	0
G2 (Gn 40ug)	620.10^{a}±16.87	0	0	0	0	0	0	0
G3 (Gn 80 ug)	578.45^{a} ±2012	16.67^{d}	33.4^{d} ± 0.00	15.0^{a} ± 0.00	62.27^{b}±3.22	70.02^{b}±3.74	9.12^{b}±2.11	71.55^{b} ± 18.1
G4 (Gn 40 ug +P 4mg)	586.83^{a}±12.93	16.67^{d}	30.4^{d} ± 0.00	13.0^{b} ± 0.00	63.33^{b} ± 3.25	73.22^{b}±2.56	11.2^{a}±3.11	75.45^{b} ± 20.1
G5 (Gn 40 ug +P 8mg)	628.17^{a}±19.83	83.33^{b}	55.94^{b} ± 6.34	11.8^{c} ±0.42	79.87^{ab}±4.21	86.67^{a}±2.17	4.17^{c}±1.08	85.12^{a} ± 10.54
G6(Gn 40 ug + P 16mg)	628.30^{a}±16.55	100^{a}	67.27^{a} ± 3.69	11.33^{c}±0.42	65.17^{b}±4.36	75.24^{b}±0.99	10.05^{a}±1.06	80.68^{a} ± 11.54
G7 (Gn 40 ug +D 5mg)	625.50^{a}±18.67	50^{c}	40.18^{c} ± 5.16	12.33^{bc}±0.96	68.44^{b}±5.62	76.33^{b}±1.94	5.17^{c}±1.17	76.04^{a} ± 6.11
G8 (Gn40 ug + D 10 mg)	548.17^{a}±17.47	100^{a}	57.42^{b} ± 7.33	11.5^{c}±0.34	83.17^{a}±3.75	89.17^{a}±2.27	4.51^{c}±0.76	89.50^{a} ± 4.2
G9 (Gn 40ug + D 20 mg)	546.18^{a}±12.45	100^{a}	54.82^{b} ± 6.34	10.33^{d}±0.42	67.17^{b}±6.24	72.66^{b}±5.39	8.04^{b}±1.13	81.33^{a} ± 8.33

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

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

Gn=GnRha P=Pimozide 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 17β-estradiol (E2) concentrations measured at zero, 6, 12 and 24 h post-injection. After 6 h, there was an increase in the level of 17β-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 ovulatation after injecting physiological saline. In the same respect group (2) received GnRHa 40ug/kg.b.w showed no ovulatation. 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 Ghezel, 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 *(Hetropneustes 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 17β-estradiol (E2) concentrations measured at zero, 6, 12 and 24 h post-injection. After 6 h, there was an increase in the level of 17β-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 GnRHa combined by dopamine antagonist (Pimozide or Dompridone) might be considered an effective procedure for induction of spawning in *Clarias gariepinus*. Also, administration of GnRHa 40ug plus domperidone 10mg/kg.b.w. gave the best spawning results.

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التفريخ الصناعي للقرموط الافريقي باستخدام GnRHa مع مثبطات الدوبامين

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الملخص العربي

استهدفت هذه الدراسة تقييم الحقن بهرمون سيستوريلين GnRHa وحده او مع مثبطات الدوبامين مثل دومبريدون وبيموزايد لإحداث التفريخ الصناعي لإناث القرموط الافريقي. واستخدم لهذا 54 سمكه (اناث) قسمت الى 9 مجموعات. وتم تسجيل الزمن بين الحقن والتبويض وقياس كميه البيض ونسبه التبويض والاخصاب ونسبه الفقس ونسبه التشوهات ونسبه الاعاشه كما تم قياس تركيز هرمون استراديول في السيرم. وأظهرت النتائج ما يلي: نجاح التبويض في سمكه القرموط الافريقي بمعدل يتراوح بين 100% في المجموعات(6و8و9) التي تم حقنها ب 40 ميكروجرام GnRHa مضافا اليه 16مجم بيوزايد، 10و20 مجم دومبريدون 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 بيوزايد لكل كجم من وزن الجسم. اظهرت النتائج ان كتله البيض المنتجة من المجموعة السادسة كانت هي الأعلى بينما كانت المجموعة الرابعة هي الاقل. كانت اعلي نسبه تشوهات في الزريعه 11.2% في المجموعه الرابعه بينما كانت الاقل 4.17% في المجموعة الخامسة. اظهرت النتائج زيادة تركيز هرمون استراديول في السيرم تدريجيا بعد 6ساعات من الحقن بالمقارنه بتركيزها عند الحقن ووصلت لاعلاها عند 12ساعه ثم بدات في التراجع عند 24 ساعة مع وجود اختلاف نوعي في المجموعات. اوضحت التجربة ان استخدام (GnRha) مع مثبطات الدوبامين مثل دومبريدون او بيموزايد أكثر تأثيرا في احداث التفريخ في سمكه القرموط الافريقي مقارنه باستخدام GnRha وحده.

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