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Effects of Ovaprim, Pituitary Gland Extract and Human Chorionic Gonadotropin on Testosterone, 11-Ketotestosterone, and 17β-Estradiol Hormones of African Catfish *(Clarias gariepinus)*

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ABSTRACT

This study closely inspected the effects of ovaprim, pituitary gland extract, and human chorionic gonadotropin on 11-ketotestosterone, and 17β-estradiol hormones. Females and males (T1, T2, and T3) were injected with Ovaprim as 0.5 ml/kg of fish, pituitary gland extract of catfish, and Human Chorionic Gonadotropin (HCG), respectively. Results showed that female gonadosomatic index of the spring season (10.45 ±0.35%) were significantly increased compared to other seasons: autumn followed by summer and winter seasons (0.82 ±0.06%). Results showed that T1 was better than T2, but T2 was better than T3 in all reproductive performance parameters. Plasma 17β-estradiol in female catfish throughout 12 hours after injection of stimulating hormones ranged from (2.15 ng/ml) in T1 to (1.65 ng/ml) in T3 whereas testosterone in female of catfish throughout 12 hours were ranged from (0.031 ng/ml) in T1 to (0.036 ng/ml) in T3. Also, plasma 11-ketotestosterone in male of catfish was recorded in T1 (0.154 ng/ml) followed by T2 (0.107 ng/ml) and lowest level in T3 (0.083 ng/ml) but plasma testosterone in male was recorded in T1 (0.225 ng/ml) followed by T2 (0.189 ng/ml) and lowest level in T3 (0.143 ng/ml).

Key words: Catfish; *Clarias gariepinus*, Reproductive performance, Eggs number, Fertilization, 11-ketotestosterone and 17β-estradiol.

INTRODUCTION

Use of piscine pituitary hormone treatment from fish offal, particularly an extract from African catfish, is economically and

environmentally preferable in the artificial propagation of African catfish (**Natea *et al.*, 2017)**. **Tiogué *et al.*, (2018)** concluded that the hormone Ovaprim expresses the best performances at the beginning of reproduction. But this advantage is offset by poor survival rate, which is better with the pituitary gland. Therefore, it is clear that the use of the synthetic hormone is not economical for optimal production of *C. gariepinus* fry. In the same way a mastery of the use of the raffia fibers will improve the results and consequently will decrease the production costs. African catfish (*Clarias gariepinus*) garnered interest as a prospect for fish culture in Egypt due to its exponential growth, bulky weight, protein-rich content, and tasty boneless flesh. Hypophysation had been used by various researchers for instigating ovulation in female catfish (**Brzuska, 2001**; **Akar and Ali, 2006**). In fish breeding, Ovaprim is utilized as a spawning hormone (**Marte *et al.*, 1987**). Catfishes are a favored delicacy in Southeast Asia and India. The mainly cultivated species are *Clarias gariepinus*, *Clarias batrachus*, and *Clarias macrocephalus*; the first species exist in Africa while the second and third are in Asia. Even though *Clarias batrachus* naturally reproduces in ponds, the efficiency and catfish spawning induction with ovaprim has been assessed to be less than 50% (**Ngamvongchon *et al.*, 1988**), quite low in comparison to that of carps. African catfish have increased production in Egypt and turned it from tilapia ponds in mixed-sex tilapia in earthen ponds. Carp, tilapia, and mullet are stables of the Egyptian outcome and make up for 95% of fish outcome; this indicates the lack of diversity in Egyptian fish production (**El-Hawarry *et al.*, 2016**; **Rothuis *et al.*, 2013**). The African catfish was important in Egypt due to its growth rate, protein-rich content, and nutritious tasty flesh (**Wajahat Ameer, 2019**). Diverse strategies have been used for rearing under controlled conditions. Where few strategies require rudimentary introduced variations into ecological parameters, sexual isolation of developed examples or substrates for connection of eggs remains among other strategies. The optimal modern and efficient system was the utilization of hormones to actuate production (**Conte, 1988**). Gonadosomatic Index (GSI) is an imperative device in setting up the reproducing time frame in creatures and fish (**Singh and Srivastava, 2017**). At the point when coordinated assessment of gonadal development is not accessible, regenerative examinations are as often as possible dependent on lists of quantitative nature, as Gonad Somatic Index (GSI) and condition factor (K) facilitating the process which characterize conceptive cycles and conceivable variety in species' physiological states during their life expectancy (**Kreiner *et al.*, 2001**). Gonad Somatic Index (GSI), egg

distance across, fruitfulness and excessive weight were essentially ($p<0.05$) which were diminished in the entire distinctive feed angle when contrasted and that of the control (**Ekpo *et al.*, 2018**). Captive fish's spawning induction is presented as the optimal method as a solution to the problems at hand throughout the injection of one of several extraneous hormones such as the following: ovaprim, fish pituitary extracts, and Human Chorionic Gonadotropin (HCG) (**Hossain *et al.*, 2012; Dhara and Saha 2013**).

Aim of study is the enhancement of catfish spawning by improving reproductive hormones

MATERIAL AND METHODS

This work was carried out at the earthen ponds (hapas), Central Laboratory for Aquaculture Research, Agriculture Research Center in Egypt. This experiment was devoted to study the effects of stimulating hormones and seasonal changes on sex steroid hormones and the reproductive performance of catfish *(Clarias gariepinus)*. The experiment included three treatments: In the first treatment (T1), females and males were injected with Ova Prim as 0.5 ml/kg of fish. While in the second treatment (T2), females and males were injected with pituitary gland extract of catfish (*Clarias gariepinus*) as 4 mg/kg of fish whereas third treatment (T3), females and males were injected with Human Chorionic Gonadotropin (HCG) as 3000 IU/kg of fish during spawning season (spring). All treatments were put in hapas inside earthen ponds until injection then transferred to a fiberglass tank and eggs were collected in funnel spawning. In a parallel direction, the gonadosomatic index was seasonally calculated through 1 year. In the middle of each season, fish were randomly picked up at the same time anesthetized with tricaine methane sulfonate (MS-222) before they were killed by transection of the spinal cord. Fish body, testis, and ovary weights were recorded to calculate the gonadosomatic index.

The male was relinquished to get the gonads which house the milt. At the end of the latency time, the females were painstakingly evacuated, sulked with towels, and physically hand stripped for eggs. Slight pressure was applied on the midriff of the female introvert, and this prompted the ovulated eggs to overflow out effectively from the genital opening. Egg weight was assessed by **Szabó *et al.*, (2003).** The level of the prepared eggs was as follows: 100 eggs were taken from every female (**El-Hawarry *et al.*, 2012**) and then set in a petri dish and analyzed under a binocular magnifying instrument three times. Dull unfertilized eggs were

isolated from living ones while checking the number of prepared ones (**Ayinla, 1988**). This was done 12 hours post-fertilization.

Three subsamples of one gram of eggs from every female were taken and settled in 5% formalin for further checking totally the quantity of eggs to each gram of egg mass (**Phelps *et al.*, 2007**) before bringing forth subtests of eggs (1gm), kept on independent dishes and inspecting to survey the ripeness and hatchability rates (in percentages) as decided from tallying the quantity of dynamic hatchlings. Survivability assessment was watched for a time of around three days after hatching. The post-incubating survivability was assessed and recorded by **Bagenal (1978)**.

Fertilization Rate = Number of fertilized eggs x 100
Total number of eggs

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

Survival rate= Final number of surviving larvae x100
Initial number of fish embryo (**El-Ashram *et al.*, 1997**).

Blood Sampling Procedure

Blood samples were collected from fish at different time intervals (0, 6 and 12 h) after hormone administration during spawning season (spring). Blood (3ml) was collected from the caudal vasculature using a heparinized needle. The samples were stored in Eppendorf tubes and kept in ice after centrifugation at 6000 rpm for 2 min to separate the blood plasma. The sex steroid hormones (testosterone, 11-ketotestosterone, and 17β-estradiol) were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA). This was done by using commercially available enzyme-linked immunosorbent assay kits from Cayman Chemical Company, USA. The sex steroids hormones were evaluated following the assay kit procedures and methods described by **Cuisset *et al.*, (1994)** and **Nash *et al.*, (2000).**

Measurable Analysis

The acquired information was exposed to ANOVA one-path investigation of change to weigh the impact of different stimulating hormones injections on reproductive performance and steroid hormones simultaneously (**Duncan, 1955**). Duncan's multiple range tests were utilized as a posthoc test to assess means at $P \leq 0.05$. The product SPSS,

form 20 (SPSS, Richmond, VA, USA), was utilized as portrayed by **Dytham, (1999).**

RESULTS

The obtained results in table (1) showed that the average ovary weights were recorded in spring as 52.25±3.64g, autumn 18.45±1.43g, summer 9.1±0.56g, and winter 4.1±0.3g with significant differences. The female gonadosomatic index was significantly increased in winter in a gradual pace of 0.82±0.06%, summer 1.82±0.8%, autumn 3.69±0.76%, and in spring 10.45±0.35%. The gonadosomatic indices showed significant differences between four seasons.

Table (1): Body weight (g), ovary weight (g), and gonadosomatic index (GSI) (as average ± SD) of female catfish (*Clarias gariepinus*) in different seasons during one year.

Items \ Season	Spring	Summer	Autumn	Winter
Average Body Weight (g)	500 ±13^{a}	500 ±13^{a}	500 ±13^{a}	500 ±13^{a}
Average Ovary Weight (g)	52.25 ±3.65^{a}	9.1 ±0.56^{c}	18.45 ±1.43^{b}	4.1 ±0.3^{d}
G.S.I (%)	10.45 ±0.35^{a}	1.82±0.8^{c}	3.69 ±0.76^{b}	0.82 ±0.06^{d}

Means that have the same letter in the same row are not significant (P>0.05).

In table (2), the average testis weights were recorded in spring (7.98=0.43g), followed by autumn (4.76±0.22g), summer (3.99±0.43g), and last in winter (2.35±0.35g). Male gonadosomatic indexes were recorded in winter (0.46±0.02%), summer (0.78±0.02%) and autumn 0.93=0.03%, but gonadosomatic index was significantly increased in spring to be 1.56±0.22%. Gonadosomatic index showed significant differences between the four seasons.

Table (2): Body weight (g), testis weight (g), and gonadosomatic index (as average ± SD) of male catfish (*Clarias gariepinus*) in three treatments during different seasons.

Items \ Season	Spring	Summer	Autumn	Winter
Average Male Weight (g)	512 ±17^{a}	512 ±17^{a}	512 ±17^{a}	512 ±17^{a}
Average Testis Weight (g)	7.98 ±0.43^{a}	3.99 ±0.43^{c}	4.76 ±0.22^{b}	2.35 ±0.35^{d}
Average G.S.I (%)	1.56 ±0.22^{a}	0.78 ±0.02^{c}	0.93 ±0.03^{b}	0.46 ±0.02^{d}

Means that have the same letter in the same row are not significant (P>0.05).

Table (3) showed that the recorded results of the reproductive performance parameters (testis, ovary, and eggs weights and eggs and larvae numbers) in the three treatments during breeding season per one female or in relation to one kilogram of female catfish (*Clarias gariepinus*). These results detected that the testis weight per kilogram of male had significant differences between T1, T2, and T3 (15.96±0.12g, 15.27±0.32 g, and 15.44±0.21g), simultaneously. Also, female ovary weight per kilogram weight had significant differences between the three treatments as following: 106.2±0.42g, 104.5±0.74g, and 102.1±0.51g in T1, T2, and T3, respectively. Also, eggs weight and number per kilogram of female weight were significantly increased in T1, T2, and T3 (82.32±1.1g, 78.61±1.2g, and 64.21±1.2g), but egg number per kilogram of female was recorded as 52140±1180, 48251± 1180, and 37520±1520 in T1, T2, and T3. Larvae number/kg of females at three days was recorded in T1, T2, and T3 as 44921±1243, 40253±1321, and 22370±1121, respectively with significant differences between different treatments. Eggs weights per female (g) were illustrated in table (3) as follows: 40.8± 0.9g, 38.7±0.7g, and 32.2±1.2g in T1, T2, and T3 with significant differences. Eggs number per female were illustrated in table (3) as follows: 26070± 679, 24115±867, and 18752±573 in T1, T2, and T3 with significant differences. Eggs numbers per gram of egg weight are illustrated in table (3) as follows: 658±9, 611±8, and 586±9 in T1, T2, and T3, respectively. Larvae number/female at three days are illustrated in table (3) as follows: 22289±1020, 19540±619, and 11183±518 in T1, T2, and T3, respectively but fertilization and hatching rates were detected while considering significant differences between T1, T2, and T3.

Table (3): Reproductive performance parameters (average ± SD) results per one female and relative to one kilogram of female weight of catfish *(Clarias gariepinus)* in three treatments during breeding season

Items \ Treatment		T1	T2	T3
Testis Weight per kg of Male	**(g)**	15.96±0.12[a]	15.44±0.11[b]	15.17±0.08[c]
Ovary Weight per kg of Female	**(g)**	106.2±0.22[a]	104.5±0.34[b]	102.1±0.51[c]
Eggs Weight per kg of Female	**(g)**	82.32±1.1[a]	78.61±1.2[b]	64.21±1.2[c]
Egg Number per kg of Female		52140±1180[a]	48251± 1180[b]	37520±1520[c]
Larvae Number/kg of Female in Three Days		44921±1243[a]	40253±1321[b]	22370±1121[c]
Eggs Weight per Female	**(g)**	40.8± 0.9[a]	38.7±0.7[b]	32.2±1.2[c]
Eggs Number per Female		26070± 679[a]	24115±867[b]	18752±573[c]
Eggs Number per Gram of Eggs Weight		658± 9[a]	611±8[b]	586± 9[c]
Larvae Number/ Female at Three Days		22289±1020[a]	19540±619[b]	11183±518[c]
Fertilization Rate	**(%)**	90.5± 1.3[a]	86.4± 1.2[b]	71.3± 1.7[c]
Hatching Rate	**(%)**	95.2± 3.2[a]	94.1± 3.2[a]	84.4± 3.1[b]

Means that have the same letter in the same row are not significant (P>0.05).

Plasma testosterone (ng/ml) in catfish females throughout 0, 6 and12 hours after injection of stimulating hormones was illustrated in figure (1). After 12 hours from injection, the highest level was recorded in T3 which was 0.036 ng/ml, succeeded by T2 and T1 which were 0.034 and 0.031 ng/ml. After six hours from injection, the highest level was recorded in T3 (0.031 ng/ml) followed by T2 (0.029 ng/ml), and the lowest in T3 (0.027 ng/ml). Plasma testosterone (ng/ml) in catfish males throughout 0, 6 and12 hours after injection of stimulating hormones was illustrated in figure (2). After 12 hours from injection, the highest level was recorded in T1 (0.225 ng/ml) followed by T2 (0.189 ng/ml), and the lowest in T3 (0.143 ng/ml). After six hours from injection, the highest level was recorded in T1 (0.172 ng/ml) followed by T2 (0.107 ng/ml), and the lowest in T3 (0.095 ng/ml) as illustrated in figure 2. In figure (3), plasma 17β-estradiol (ng/ml) in catfish females throughout 0, 6 and 12

hours after injection of stimulating hormones was illustrated in figure (3), showing its levels increasing with passage of time. After 12 hours from injection, the highest level was recorded in T1 (2.15 ng/ml) followed by T2 (1.87 ng/ml), and the lowest in T3 (1.65 ng/ml). After six hours from injection, the highest level was recorded in T1 (1.75 ng/ml) followed by T2 (1.41ng/ml), and the lowest in T3 (1.34 ng/ml). Plasma 11-ketotestosterone (ng/ml) in catfish males throughout 0, 6 and12 hours after injection of stimulating hormones was illustrated in figure (4). After 12 hours from injection, the highest level was recorded in T1 (0.154 ng/ml) followed by T2 (0.107 ng/ml), and the lowest in T3 (0.083 ng/ml). After six hours from injection, the highest level was recorded in T1 (0.075 ng/ml) followed by T2 (0.065 ng/ml), and lowest in T3 (0.054 ng/ml).

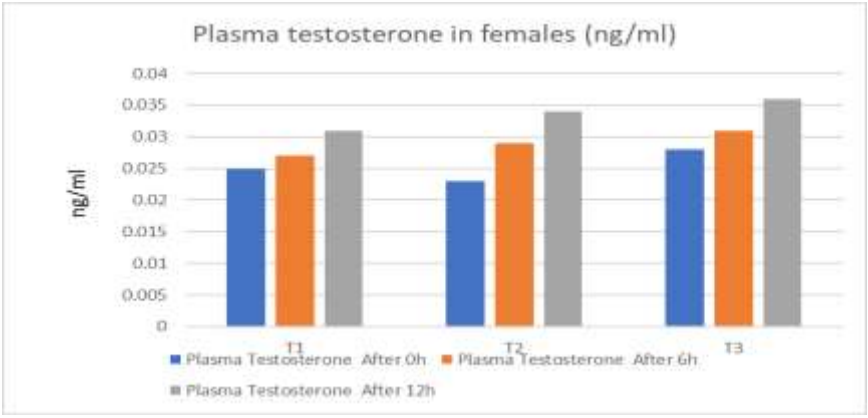

Figure (1): Plasma female testosterone ng/ml in African catfish during breeding season after injection of different stimulating hormones at zero, 6, and 12 hours after injection in different treatments.

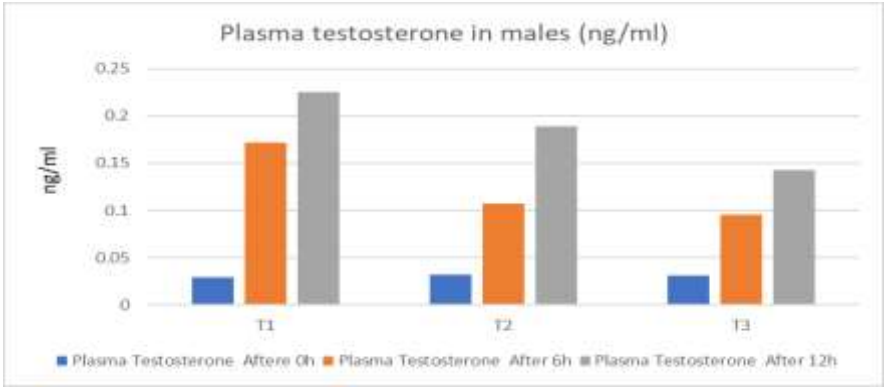

Figure (2): Plasma male testosterone ng/ml in African catfish during breeding season after injection of different stimulating hormones at zero, 6, and 12 hours after injection in different treatments**.**

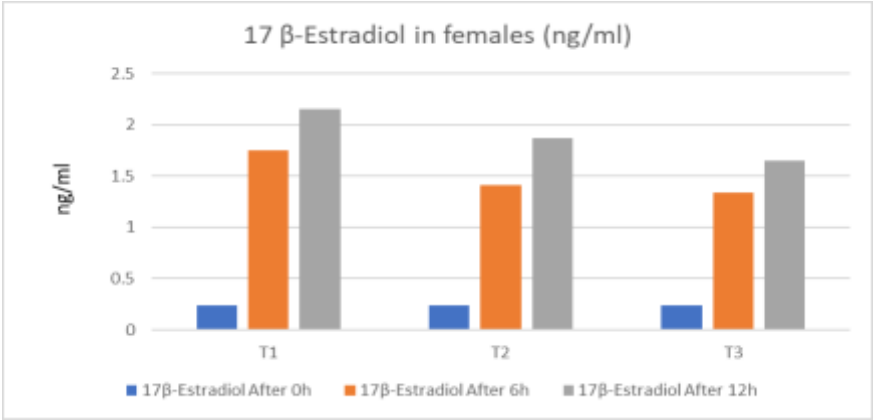

Figure (3): Plasma female 17β-estradiol (ng/ml) in African catfish during breeding season after injection of different stimulating hormones at zero, 6, and 12 hours after injection in different treatments.

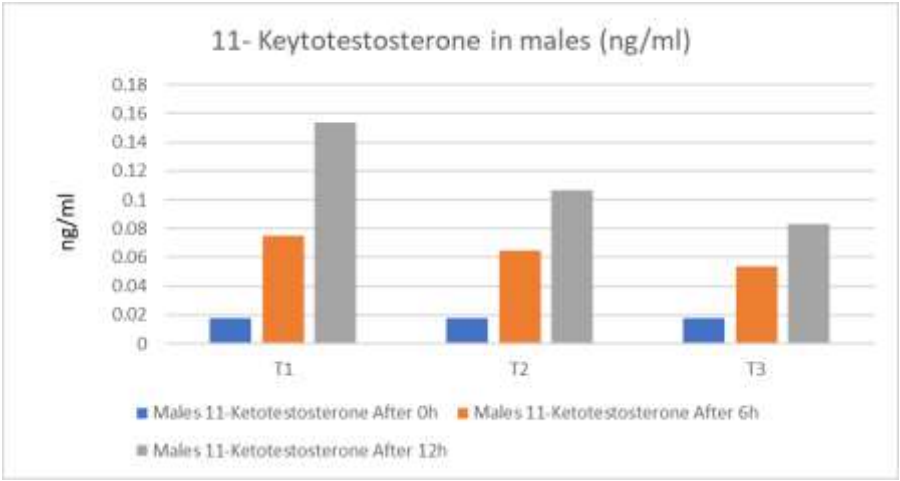

Figure (4): Plasma male 11-ketotestosterone (ng/ml) in African catfish during breeding season after injection of different stimulating hormones at zero, 6, and 12 hours after injection in different treatments

DISCUSSION

The weights of ovary and testis were increased in spring followed by autumn. Also, the gonadosomatic index ratio was changed throughout the year and it was found that it has a strong relationship with breeding season. These results were attributed with **Hunter and Macewicz (2003)** who found that of the parameters contributing to fish investigations, the Gonadosomatic Index (GSI) was one of them. GSI utilization took place

by identifying via dratted ovaries and thusly recognizing regenerative period from increments in weight. A few specialists have written about the GSI, fruitfulness, and Nigerian waters fish egg size, incorporating (**Anene and Okorie, 2008; Fawole and Arawomo, 2000**). Gonadosomatic record esteems (GSI) were examined, which give a proportion of gonad estimate with respect to body weight (**Wootton, 2012)**.

From the obtained results, reproductive performance of catfish (*Clarias gariepinus*) was significantly increased with ovaprim-inducing hormone followed by pituitary-extract hormone then human chronic gonadotropin hormone of mature females. In all reproductive performance parameters, egg weight and number and fertilization, hatching, and survival rates percentages were significantly increased in the same previous trend for all mature females of all treatments. This conclusion agrees with the findings quantity of eggs in 1 g acquired in this examination similar to the ones announced by **Viveen, *et al.*, (1985)** in *C. gariepinus.* The fruitful enlistment of bringing forth of *Clarias gariepinus* by utilizing both heteroplastic and homoplastic pituitary concentrate has been accounted for before (**Hecht, 1985**).

Production of seeds in catfish was controlled by stimulating hormones such as ovaprim, pituitary extract gland, and human chronic gonadotropin during breeding season. Stimulating hormones were increased sex steroid hormones in females and males such as plasma 17β-estradiol, testosterone, and 11-ketotestosterone. Sex steroids hormones in female fish crucially contribute to ovulation, oocyte maturation, and spawning.17β-estradiol is utilized to control the mechanisms that synthesize vitellogen in and surge ovarian size during the final oocyte maturation. 17β-estradiol is directly related to the gonadosomatic index (**Sabet *et al.*, 2009**; **Coccia *et al.*, 2010)**. Many hormones are involved in the control of maturation and spawning of fishes especially those produced by hypothalamus: the small peptide hormone (releasing hormone) and gonadotropin hormone which pass into the blood to reach the gonads (**Choi *et al.*, 2016**). Even though alternative synthetic hormone substances are trendy when it comes to inciting spawning in catfish alongside other cultured fishes, hypophysation remains the most common practice (**Van Oordt and Goos, 1987**) and is still vastly utilized especially for the fish which are economically essential but do not spawn in confined waters of aquaculture like African catfish and carps (**Hecht *et al.*, 1982**). This method is one of the first that demonstrated the effectiveness of crude pituitary extract for induced breeding. *Clarias gariepinus* has been considered as an ideal species for the development of

aquaculture in Africa (**Houssay, 1931**; **Dekempe and Micha, 1994**). Although HCG was effectively employed in spawning incitation of fishes including several catfish species, e.g., *Clarias gariepinus* (**Inyang and Hettiarachchi, 1994**) and *Heteropneustes fossilis*, (**Kather Haniffa and Sridhar, 2002**), stimulation with ovaprim hormone has been applied to a wide variety of families and species of fish to enhance ovulation and spermiation (**Haniffa *et al*., 2007**).

CONCLUSION

Artificial spawning of catfish was enhanced by using stimulating hormones, but ovaprim gave the best results followed by pituitary extract gland then human chorionic gonadotropin. These stimulating hormones were increased sex steroid hormones with time and reproductive performances.

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تأثير الأوفابريم، مستخلص الغدة النخامية والجونادوتروبين المشيمي البشري علي هرمونات التستوستيرون، ١١-كيتوتستوستيرون و١٧-بيتا إستراديول في سمكة القرموط الأفريقي.

الملخص العربى

تفقدت هذه الدراسة عن كثب تأثيرات الأوفابريم ، ومستخلص الغدة النخامية ، وهرمون الغدد التناسلية المشيمية البشرية علي كل من هرمونات التستوستيرون، ١١ كيتوتستوستيرون ، و ١٧-استراديول. تم حقن الإناث والذكورفي المعاملات الأولي والثانية والثالثة باستخدام الأوفابريم بمعدل ٠,٥ مل / كجم من الأسماك ، ومستخلص الغدة النخامية من سمك اللقرموط الأفريقي، ومستخلص الغدد التناسلية المشيمية البشرية الجونادوتروبين، على التوالي. أظهرت النتائج أن معامل المناسل للإناث لفصل الربيع (١٠,٤٥ ± ٠,٣٥٪) قد زاد معنوياً مقارنة بالمواسم الأخرى: الخريف يليه فصلي الصيف والشتاء (٠,٨٢ ± ٠,٠٦٪). أظهرت النتائج أن المعاملة الأولي كانت أفضل من الثانية تليهما المعاملة الثالثة في جميع متغيرات الأداء التناسلي. تراوحت البلازما ١٧بيتا-استراديول في إناث سمكة القرموط طوال ١٢ ساعة بعد حقن الهرمونات المحفزة من (٢,١٥ نانوغرام / مل) في المعاملة الأولي إلى (١,٦٥ نانوغرام / مل) في المعاملة الثالثة بينما تراوحت التستوستيرون في أنثى سمك القرموط طوال ١٢ ساعة من (٠,٠٣١ نانوغرام / مل) في المعاملة الأولي إلى (٠,٠٣٦ نانوغرام / مل) في المعاملة الثالثة، تم تسجيل البلازما ١١-كيتوتستوستيرون في ذكر سمك القرموط في المعاملة الأولي (٠,١٥٤ نانوغرام / مل) يليه المعاملة الثانية (٠,١٠٧ نانوغرام / مل) وأدنى مستوى في المعاملة الثالثة (٠,٠٨٣ نانوغرام / مل) ولكن هرمون التستوستيرون البلازمي في الذكور فقد سجل (٠,٢٢٥ نانوغرام / مل) للمعاملة الأولي يليه المعاملة الثانية (٠,١٨٩ نانوغرام / مل) وأدنى مستوى في المعامة الثالثة (٠,١٤٣ نانوغرام / مل).