J. Product. & Dev., 22(3): 499 - 510(2017)

INDUCTION OF SPAWNING IN AFRICAN CATFISH (*Clarias gariepinus)* BY USING HUMAN CHORIONIC GONADOTROPIN

Fatma Mahsoub*; M. N. El Gaafary*; A. A. Rashwan*and A. M. Aker**.
** Department of Animal & Poultry Production, Faculty of Technology and Development, Zagazig University, Zagazig Egypt.*
*** Department of Fish Hatching and Reproductive Physiology, Central Laboratory for Aquaculture Research (CLAR), Abbassa Abo- Hammad, Sharkia, Egypt .*

ABSTRACT

This work was designed to study the effect of Human chorionic gonadotropin (HCG) on induction of spawning of African catfish (Clarias gariepinus). In this experiment a total number of 90 catfish (745.8±9.2g body weight) were divided into three experimental groups (30 each) .The first group was considered as a control group and injected intramusculary with carp pituitary extract (0.3ml/kg/ b.w.).The second group was injected with HCG (1200 IU / kg body weight). The third group was injected with both carp pituitary extract and HCG (0.15 ml/kg and 600 IU/kg body weight, respectively.

The results of the present experiment showed significant differences in survival rate , fertilization rate (%), hatching rate (%), latency period, egg weight (g), egg diameter (mm) and total protein (g/dl). However, insignificant differences were detected in initial weight (g), condition factor and specific growth rate, body weight before spawning, body weight after spawning, egg number/g, gonadosomatic index, hepatosomatic index, cortisol (ug/dl) and estradiol17β (pg/m). Females treated with HCG had the highest values of fertilization rate, hatching rate, latency time and survival rate.

***Conclusively,** the results of the present study indicated that at a level of 1200 IU from HCG was the most effective agent in inducing spawning of African catfish (Clarias gariepinus) females.*

Key words: Spawning, African catfish, *Clarias gariepinus*, HCG

INTRODUCTION

With increasing human populations all over the world and increasing food demands especially protein source, fish is considered to be an important and more valuable source of animal protein characterized by high nutritional value.

African catfish (*Clarias gariepinus*) is one of the most widely produced food fish in the world (Fasakin, *et al*., 2003; Al Dohail, 2005 and Sutriana, 2007) and it considered a native fish in all freshwater bodies of Egypt (Saleh, 2007). The attributes that make this species a farmers choice include faster growth rate and its bigger maturity size, easy to reproduce, accepts artificial feeds, tolerates to high stocking densities, adapting with poor water quality, high resistance to disease, lucrative in local regional and international markets, and its economical feasibility in pond culture systems, the most common culture system in East African Community (Teugels, 1986). However, lack of constantly available seed, low egg fertilization rate and low hatching rate are considered as the main obsticles(El– Sayed, 1999) . and are not yet be solved.

The present study was designed to investigate the effect of human chorionic gonadotropin (HCG) on induction of ovulation in female African catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

The present study was carried out in the Fish Hatchery belonging to the General Authority for Fish Resources Development, Abbassa, Abou-Hammad, Sharkia Governorate, Egypt. During year 2016.

In this experiment, a total number of 90 females of African catfish (745.8±9.2 initial body weight) was divided into three experimental groups (30 each). All fish were distributed in nine hapa (10 fish/hapa) and 27 females were used at the spawning season. The first group was considered as a control group and injected with carp pituitary extract at a level of 0.3ml/kg b.w. The second group was injected with HCG at a level of 1200 IU / kg body weight. The third group was injected with both carp pituitary extract (CPE) and HCG (0.15ml / kg plus 600 IU/kg body weight, respectively). Growth performance (condition factor, specific growth rate, and hepatosomatic index) and reproductive performance (latency time, ovulation rat, gonadosomatic index, egg parameters such as egg weight, egg number and egg diameter, fertilization rate, hatchability and survival rate) were determined according to the methods of Szabo *et al*.(2002); Adebayo (2006); Phelps *et al*., (2007) and El-Hawarry *et al*.(2016).

Blood samples (5ml) were collected from tail vein at spawning. Samples were centrifuged immediately at 3000 r.p.m. for 15 minutes .The serum was collected and stored at -20C^{o} until analyzed for cortisol and stradiol17β concentrations by using Radioimmuniassay (Diagnostic

products corporation kits) as described by Eckert *et al.*,(2001) and Gore-langton and Armstrong(1988). Total protein was also determined as described by Henry, (1974), Histpathological examinations of both liver and kidney of the experimental animals were also studied .

Data were subjected to analysis of variance according to Snedecor and Cochran (1982). General linear model procedure of the statistical analysis system (SPSS, 2004) was used . Reproductive traits were analysed by the log linear model for the analysis of contingency tables.

Duncans New Multiple Range Test was used for multiple comparisons (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance traits:

The Initial weight (g), condition factor (K_{factor}), specific growth rate (SGR), gonadosomatic index and hepatosomatic index showed insignificant variation among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic gonadotropin (HCG) and their combination (Table1). These results are in agreement with those reported by Eman (2008) who found no significant variation in K_{factor} of common carp fish as a result of hormonal treatment, however, Jayaprakas and Sambthu (1995) reported that fish treated with gonadotropic hormones showed an increase in the growth parameters over the control. The same authors also found that human chorionic gonadotropin promoted better growth rate.

The results presented in Table (2) indicated that the differences in survival rate (SR%) among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic gonadotropin (HCG) and CPE plus HCG were significant ($P<0.05$). Females treated with HCG gave higher survival rate than those treated with either pituitary gland of common carp (CPE) CPE plus HCG. However, the differences in body weight before and after spawning (g) were not significant. These results agreed with those reported by EL-Hawary *et al.*, (2016) and Mylonas *et al.*, (1992). They reported an increase in survival rate when HCG was used. However, Ndimele and Owodeinde (2012) found that less survival rate was obtained following using ovaprim as compared with pituitary extract.

Table (1): Means and standard error of initial weight (g), condition factor, specific growth rate, gonadosomatic index%, hepatosomatic index% of African catfish females injected with PG extract of common carp, HCG and their combination.

Treatment groups	Initial weight (g)	Condition factor	Specific growth rate	Gonadoso-matic Index, %	Hepatoso-matic Index %
Control(CPE)	759.11 ±9.99	0.92 ±0.03	4.81 ±0.01	14.79 ±0.48	0.69 ±0.03
HCG	741.11 ±9.75	1.00 ±0.05	4.79 ±0.01	14.9 2±0.57	0.70 ±0.03
HCG+CPE	737.22 ±7.78	0.91 ±0.03	4.79 ±0.01	14.69 ±0.56	0.72 ±0.56
Significance	**NS**	**NS**	**NS**	**NS**	**NS**

NS= Not significant.

Table (2): Means and standard error of survival rate (SR%) ,body weight before and after spawning (g)of African catfish females after injection with CPE extract of common carp, HCG and their combination.

Treatment groups	Survival rate (%)	Body weight before spawning (g)	Body weight after spawning (g)
Control (CPE)	74.00^{b}±2.23	776.67±9.89	706.22±9.63
HCG	81.78^{a}±1.38	762.33±7.61	712.78±780
HCG + CPE	77.11^{ab}±2.00	755.89±7.08	689.67±6.68
Significance	*	**NS**	**NS**

-Means in the same column within the same classification having different litter differ significantly ($P < 0.05$). *= $P < 0.05$, NS= Not significant

2. Reproductive traits:

The differences in fertilization rate, hatching rate and latency time (Table3) among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic gonadotropin (HCG) and CPE plus HCG were significant ($P<.0.05$ or $P<0.01$) . Females treated with HCG had the highest values of fertilization rate, hatching rate and latency period than those treated with PG of common carp (CPE) and their combination. These

Table (3). Means and standard error of fertilization rate, hatching rate and latency time of African catfish females injected with PG extract of common carp, HCG and their combination.

Treatment groups	Latency time (hrs)	Fertilization rate (%)	Hatching rate (%)
Control (CPE)	11.78^{a} ±.43	84.56^{b} ±1.34	74.78^{b} ±1.99
HCG	9.22^{b} ±.32	92.11^{a} ±2.38	86.11^{a} ±.69
HCG+CPE	11.11^{a} ±.56	87.89^{ab} ±2.56	79.11^{b} ±2.03
Significance	**	*	**

Means in the same column within the same classification having different litter differ significantly ($P < 0.05$).
*= $P < 0.05$, ** =$P < 0.01$.

results are in agreement with those reported by Haniffa *et al.* (2000); Haniffa and Sridhar (2002) and Leonardo *et al.* (2004). The same authors showed that the highest fertilization rate was obtained when HCG was used. Salami *et al.* (2006); Haniffa *et al.* (2000) reported that hatching rate was increased after injection with HCG in spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*). EL-Hawary *et al.*(2016) stated that the use of HCG produced higher latency period and less hatching rate. Ndimele and Owodeinde (2012) reported that induction of spawning in catfish (*Clarias gariepinus*) with synthetic hormone (ovaprim) produced offspring with better qualities than those induced with pituitary extract. Oyeleye *et al* (2016) found that the application of ovaprim in inducing spawning produced higher fertilization rate and hatching rate as compared with pituitary gland extract injection. However Zairin *et al.*, (1992); Brzuska, (2003) and (2004), Akar, (2006) and Akar and Ali, (2006) and Eman (2008) found that the mean fertilization and hatching percentage were considerably higher after injection with HCG combined with CPE of common carp. In contrary EL-Hawary *et al.*(2016) stated that the use of HCG produced less hatching rate.

The results of the present study (Table 4) showed that the difference in weight of eggs (g), and diameter of eggs (mm**)** among the experimental groups injected with pituitary gland of common carp (CPE), human chorionic

Table (4): Means ± SE of egg weight (g), number of eggs /g and diameter of eggs (mm**)** of African catfish females injected with CPE extract of common carp, HCG and their combination .

Treatment groups	Weight of egg (g)	Number of egg/ g	Diameter of egg (mm)
Control (PG)	70.56[a] ±2.83	730.00 ±22.04	1.46[a] ±.11
HCG	58.33[c] ±.94	782.78 ±13.25	0.94[b] ±.04
HCG+CPE	64.56[b] ±1.41	770.00 ±13.94	1.07[b] ±.06
Significance	**	NS	**

Means in the same column within the same classification having different litter differ significantly (P < 0.05).
-** =P < 0.01, NS= Not significant.

gonadotropin (HCG) and CPE plus HCG were significant (P<0.01). However, the differences in the number of eggs/g were not significant. Females treated with CPE only produced better weight of egg (g), and diameter of egg (mm**)** than CPE plus HCG and HCG. These results agreed with those reported by Brzuska,(2002& 2003); Ndimele and Owodeinnde, (2012); Oloniyi and Akinbola (2013); Oyeleye *et al*., (2016) and El-Hawary *et al* ., (2016). They also found higher quantity of egg were obtained after the application of synthetic ovulation stimulator than after the treatment with stimulator of natural origin such as carp pituitary or human chorionic gonadotropin. However, Zairin *et al* (1992) found that the injection of human chorionic gonadotropin in *Clarias lazera* stimulates ovarian development. It induces increases of weight and size of eggs (Haniffa and Sridhar, 2002). Salami *et al.* (1994) reported similar increase in egg weight and big sized eggs in *Clarias gariepinus.* Eman (2008) found that the mean total weight of eggs were considerably higher after injection with HCG combined with CPE of common carp. Oyeleye *et al*. (2016) and Bruzuska (2004) found that the application of ovaprim resulted in a higher weight of egg / kg body weight and higher quality eggs in comparison with pituitary extract. The same author stated that treatment of female's African catfish (*Clarias gariepinus*) with two doses or one dose of ovopel as ovulation stimulator had insignificant effects in either weight of eggs or eggs quality.

The results presented in Table 6 showed that the difference in total protein, among the experimental groups injected with pituitary gland of

Table (5). Means and standard errors of total protein (g/dl), cortisol (ug/dl) and estradiol (pg/ml) concentration of African catfish females after injection with PG extract of common carp, HCG and their combination.

Treatment groups	Total protein (g/dl)	Cortisol (ug/dl)	Estradiol (pg/ml)
Control (CPE)	6.29[a]	23.86	229.00
	±.11	±2.46	±44.80
HCG	5.84[b]	19.69	220.67
	±.07	±2.95	±39.02
HCG+ CPE	5.86[b]	23.74	254.75
	±.13	±1.68	±44.29
Significance	**	NS	NS

Means in the same column within the same classification having different litter differ significantly (P < 0.05). ** =P < 0.01, NS= Not significant-

common carp (CPE), human chorionic gonadotropin (HCG) and CPE plus HCG were significant (P<.0.01). However, the differences in cortisol and estradiol concentration were not significant in all hormonal treated groups. Females in the control group which was injected with CPE gave higher total protein concentration than those treated with either HCG or CPE plus HCG respectively. However, Eman (2008) found that a very highly significant decrease (P<0.001) in total protein was recorded in fish injected with combination of CPE of carp and HCG.

Histopatholoical results:

Histological examination indicated that both liver and ovary of catfish treated with human chorionic gonadotropin, common carp pituitary gland extract and their combinations showed normal histo-morphological structures (Figures 1 and 2). No differences were noticed between the treated groups and the control.

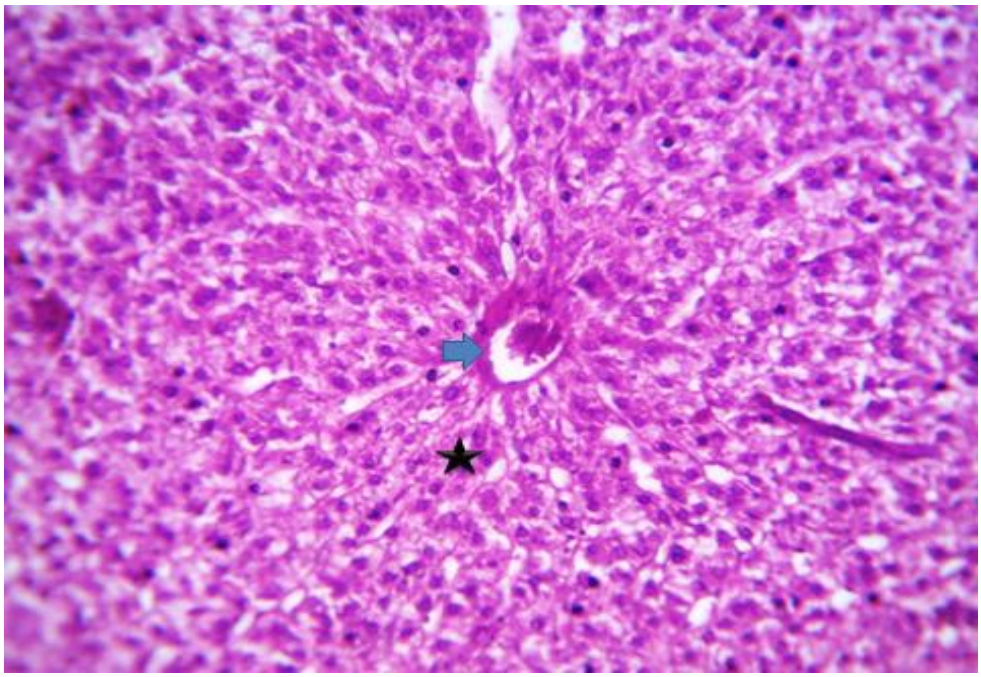
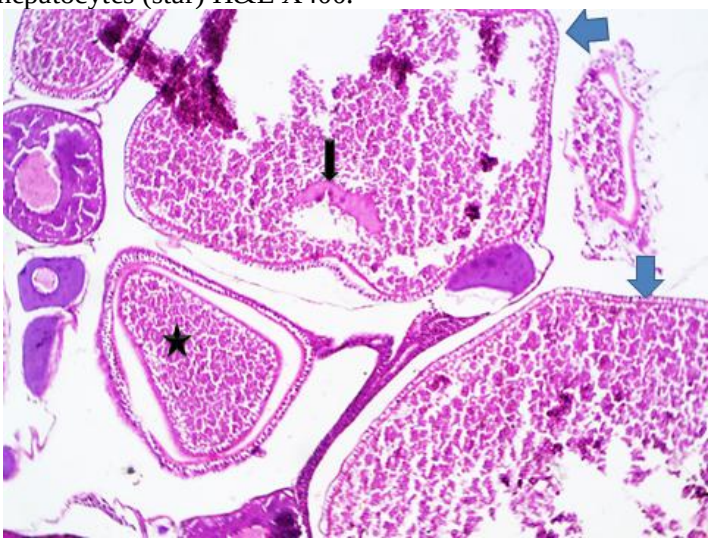


Figure 1: Fish liver (car pituitary extract ,HCG and their combination treated groups) showed normal hepatic vein (arrow), cords, sinusoids and hepatocytes (star) H&E X400.



Figure,2: Fish Ovary (car pituitary extract ,HCG and their combination treated groups) showed normal mature follicles (thick arrows) which contains yolk globules (star) and oocytes (thin arrow) H&E X400.

Conclusively, the results of the present study indicated that at a level of 1200 IU from HCG was the most effective agent in inducing spawning of African catfish (*Clarias gariepinus*) females.

REFERENCES

Adebayo, O.T. (2006). Reproductive performance of African Clariid Catfish *Clarias gariepinus* broodstock on varying maternal stress. *J. Fish Inter*, 1:17-20.

Akar, A. M. (2006). Biologic and economic efficiency of human chorionic gonadotropin and carp pituitary extract for spawning induction in silver carp. *Egypt. J. Exp Biol. (Zool*), 1: 73-76

Akar, A. M. and Ali, M. A. (2006). Artificial spawning of African catfish *(Clarias gariepinus)* after stimulation of ovulation with carp pituitary extracts ,human chorionic gonadotrophin and mixed injection. *Egypt. J. of Appl. Sci.,* 21 (2): 8- 16.

Al-Dohail, M. A. S. (2005). The effect of mixed feeding schedules on growth performance and diurnal digestives protease activities of African Catfish *Clarias gariepinus*. M.Sc. Thesis, University Sains Malaysia, Malaysia.

Brzuska, E. (2002). Artificial spawning of African catfish (*Clarias gariepinus*) stimulation of ovulation using carp pituitary or ovopel. *J. of Appl. Aquac.* , 12 (4): 13- 22.

Brzuska, E. (2003): Artificial propagation of the carp *(Cyprinus carpio L.)* two year reproduction results of females of Hungarian line W and Polish Line 6 after ovulation stimulation with carp pituitary or mGnRH-a and dopaminergic inhibitor. *Czech J. Anim. Sci*., 48: 139-151.

Brzuska, E. (2004). Artifical propagation of African catfish(*Clarias gariepinus*): The application of a single dose of pellets containing D-Ala6, Pro 9NEt-mGnRH and dopamine inhibitor metoclopramide *Czech . J. Anim. Sci., 49, (7): 289–296*.

Duncan, D.B.(1955). Multiple range and Multiple F test. Biometrics, 11:1-42.

Eckert, S.M.; Yada , T . and Shepherd ,B. S. (2001) . Hormonal control of osmoregulation in the channel catfish Ictalurus punctatus. Cen. Comp. *Endocrinol*. , 122: 270-286.

El-Hawarry N. W. , Soliman H. A. , Ramy M. S (2016) Breeding response and larval quality of African catfish (*Clarias gariepinus*, Burchell

1822) using different hormones/hormonal analogues with dopamine antagonist . *The Egyptian J. Aqu . Res*., 42(2): 231-239**.**

EL-Sayed, E. A. (1999). Some studies on artificial propagation of tilapia. Ph.D. Thesis, Faculty of Vet. Med., Zagazig. Univ**.** Zagazig Egypt.

Eman, M. Zaki (2008). Physiological studies on reproductive performance of carp fishes MSc., Faculty of Science Zagazig University, Zagazig, Egypt.

Fasakin, E.A.; Balogun, A.M. and Ajayi, O.O (2003). Evaluation of full fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. *Aquaculture Research*, 34:733-738.

Gore-Langton, R.E. and Armstrong, D.T. (1988) *Follicular Steroidogenesis and Its Control.* In Knobil, E. and Neill, J. (eds), *The Physiology of Reproduction.* Raven Press, New York, pp. 331–385.

Haniffa, M. A.; Merlin Rose, T. and Francis, T. (2000) : Induced spawning of the striped murrel *Channa striatus* using pituitary extracts, human Chorionic gonadotropin, Luteinizing hormone releasing hormone analogue and ovaprim. *Acta Icht. Piscat*., 30: 53- 60.

Haniffa, M. A. and Sridhar, S. (2002). Induced spawning of spotted murrel *Channa punctatus* and catfish *Heteropneustes fossilis* using hormone (ovaprim). Veterinarski arhtv, 72 (1): 51- 56.

Henry, R. J. (1974): *Clinical Chemistry Principles and Techniques.* 2nd edition, Harper and Row publ., New York, PP 525.

Jayaprakas, V. and Sambthu, C. (1995): Effect of dietary hormones on food utilization, growth and body composition of the pearlspot; *Etroplus suratensis* (Bloch) in a brakish water pond. *Indian J. of Marine Sciences,* 24 (1): 32– 36.

Leonardo, A. F. G.; Romagosa, E.; Borella, M. I. and Batlouni, S. R. (2004). Induced spawning of hatchery– raised Brazilian catfish, cachara *Pseudoplatystoma fasciatum* (Linnaeus, 1766). *Aquaculture*, 240: 451– 461.

Mylonas, C. C.; Hinshaw, J. M. and Sullivan, C.V. (1992). GnRH-a induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. Aquaculture, 106: 379- 392.

Ndimele P.E. and Owodeinde F.G. (2012). Comparative reproductive and growth performance of *Clarias gariepinus* (Burchell, 1822) and its hybrid induced with synthetic hormone and pituitary gland of *Clarias gariepinus*. *Turk. J. Fish. Aquat. Sci*., 12 (3): 616-626 .

Oyeleye, O. O. ; Ola S. I. and Omitogun O. G. (2016) Ovulation induced in African catfish (Clarias gariepinus, Burchell 1822) by hormones produced in the primary culture of pituitary cells .*Inter. J .Fish. & Aquac.* ,8(7), 67-73.

Olaniyi, C.O and Akinbola, D.O (2013) Comparative studies on the hatchability, performance and survival rate of african catfish (*Clarias gariepinus*) larval produced: Using ovaprim and catfish pituitary extract hormones. *J. Biol., Agric . & Health*., 3(9): 2224-3208.

Phelps R.P., Hastey R., Pendetar A., Linley L., Papanikos N. and Dunham R.A. (2007) Effects of temperature on the induced spawning of channel catfish and the production of channel blue catfish hybrid fry. *Aquaculture*, 273 (1): 80–86

Salami, A. A.; Fagbenro, O. A.; Edibit, L. and Fagbemiro, S. O (1994): Induced spawning of the african catfish *Clarias gariepinus* using non-piscine pituitary extracts. *World Aquaculture Sci*, 25: 166- 168.

Salami A. A; Fagbenro O. A; Balogun A. M; Atoyebi O. and Olowoyeye M. F. (2006). Effective dose of amphibian pituitary extracts for the induced spawning of the Clariid Catfish, *Clarias gariepinus* (Burchell 1822). *J. Aquac. Tropics*, 11:9-12

Saleh, M.A. (2007). Freshwater fish seed resources in Egypt, In: M.G. Bondad- Reantaso (ed.). Assessment of Freshwater Fish Seed Resources for Sustainaable Aquaculture. FAO Fisheries Technical Paper. No.501.Rome, FAO, 241-255**.**

Snedecor , G. W. and Cochran , W.C. (1982) . *Statistical Methods*. 8th Edition , Iowa State University Press, Ames, Iowa, U.S.A.

SPSS(2004). Statistical package for social science, Chicago, U.S.A.

Sutriana, A. (2007). The nutritional value of Cassava (Manihot esculenta Crantz)based diet for African Catfish *Clarias gariepinus* .M.Sc**.** Thesis, Universiti Sains Malaysia, Malaysia.

Szabo, T.; Modgyasszay, C. S. and Horvath, L. (2002). Ovulation induction innase *(Chondrostom anasus)* using pituitary extract or GnRH analogue combined with domperidone. *Aquaculture* , 203: 389-395.

Teugels GG (1986). Taxonomy, Phylogeny, biogeography of catfishes (Ontario physi, Siluroidei): *An Overview: Aquat. Living Resour*. ,9:34.

Zairin, M.; Asahina, K.; Furukawa, K. and Aida, K. (1992): Plasma steroid hormone profiles during HCG induced ovulation in female walking catfish, *Clarias batrachus*. *Zool. Sci.,* 9(3): 607- 617.

احداث التفريخ فى اسماك القرموط الافريقي باستخدام الهرمون الكريونى البشري

فاطمه محسوب* - محمد ناجى الجعفري* - على عبد العظيم رشوان* - عادل محمد عكر .**

*قسم الانتاج الحيوانى والداجني كليه التكنولوجيا والتنميه جامعه الزقازيق .

**قسم التفريخ وفسيولوجى التكاثر -المعمل المركزى لبحوث الاسماك -العباسه -شرقيه .

أجريت هذه الدراسة في مفرخ الأسماك التابع للهيئة العامة لتنمية الثروة السمكية،عباسه، أبو حماد، محافظة الشرقية، مصر.

صممت هذه التجربه لدراسة تأثير الهرمون الكريونى البشري على تفريخ اسماك القرموط الافريقى .. تم استخدام عدد 90من اناث القرموط الافريقى (وزن 745.8 ± 9.2) تم تقسيمهم الى ثلاث مجموعات تجريبية (30 سمكه/ مجموعه)، وتم اعتبار المجموعة الأولى مجموعة المقارنه وحقنت بمستخلص الغده النخاميه للمبروك (3. مل / كجم)، بينما وتم حقن المجموعة الثانية باستخدام الهرمون الكريونى البشري بمعدل 1200 وحدة دولية / كجم من وزن الجسم .تم حقن المجموعة الثالثة وحقنها بمستخلص الغده النخاميه للمبروك و الهرمون الكريونى البشري 15. مل / كغ و 600 وحدة دولية / كجم من وزن الجسم، على التوالي . وتم دراسه معدل اداء النمو و الاداء التناسلى .

وقد أظهرت نتائج التجربة الحالية وجود فروق معنوية في معدل البقاء على قيد الحياة ومعدل الاخصاب (٪) ومعدل الفقس (٪)ووزن البيض (g) وقطر البيض (مم) والبروتين الكلي بينما كانت، الاختلافات في الوزن الأولي ، معامل االحاله ومعدل النمو المحدد، ووزن الجسم قبل التفريخ، وزن الجسم بعد التفريخ، عدد البيض و دليل وزن الجسم للغدد الجنسيه و دليل وزن الكبد و الكورتيزول واستراديول 17 غير معنوي كما اظهرت النتائج ان معامله الإناث بالهرمون الكريونى البشري أعلى القيم في معدل الاخصاب، معدل الفقس، ومعدل البقاء على قيد الحياة .وأظهرت نتيجة الدراسة ايضا أن استخدام 1200 وحدة دولية من الهرمون الكريونى البشري كان فعالا في تفريخ اسماك القرموط الأفريقي .

التوصية: قد خلصت نتائج التجربه الي معامله الهرمون الكريونى البشرى بمعدل 1200وحده دوليه كان الاكثر فاعليه فى احداث التبويض في اسماك القرموط الافريقي .