OVULATION INDUCTION IN AFRICAN CATFISH *(CLARIAS GARIEPINUS)* USING CARP AND CATFISH PITUITARY EXTRACT AND OVAPRIM

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Abstract

The effects of Ovaprim (O), Carp pituitary extract (CPE), Catfish pituitary extract (Cat PE) and combination of them for induction of spawning in African catfish (*Clarias gariepinus*) were studied. A total number of 80 African Catfish (40 female & 40 male) were used in two experiments 40 for each and in each experiment the brooders were divided into 4 equal groups. In the first experiment all fish were injected one dose only. The first group was injected with Carp pituitary extract (CPE). The second group was injected with Catfish pituitary extract (Cat PE). The third group was injected with Carp & Cat pituitary extract (C Cat PE).The fourth group was injected with Ovaprim (O). The results revealed that the group four which injected by Ovaprim were responded and give eggs but the first, the second and the third groups not respond. In the second experiment the brooders were injected two doses, the first group was injected with Carp pituitary extract (CPE). The second group was injected with dose Catfish pituitary extract (Cat PE). The third group was injected with Carp & Cat pituitary extract (C Cat PE).While the fourth group was injected a single Ovaprim (O). Weight of eggs produced and hatching percentages were recorded. The results revealed that the mass of eggs and mass of eggs as percentage of female body weight (egg weight index) were higher ($p<0.05$) in first group than in fourth group and in second group. The mean fertilization percentage and the percentage of live embryos after 24 hours were not significantly ($p<0.05$) different amongst the four groups.

INTRODUCTION

The African Catfish (*Clarias gariepinus*) is characterized by its rapid growth, large body weight and a high content of protein in the tasty boneless flesh. This fish has attracted interest as a potential species for fish culture in Egypt and other countries and may be propagated in natural conditions in small ponds, in semi-natural conditions using hypophysation and artificial nests installed in spawning grounds (Mis 1977) or under controlled conditions. Owing to the economic value of this species, the latter method of Catfish reproduction has become increasing popular in several different European countries since the mid. 1970s (Kouril *et al* 1996 and Linhart *et al* 2003). Several authors have used hypophysation for inducing ovulation in African Catfish females (Adamek1995; Brzuska *et al* 2000; Brzuska 2002 and Akar and Ali (2006).

For many years, fish farmers have been using hormone preparations for the artificial propagation of many cultured fish. In practice, acetone- dried pituitary has been the most commonly used agent to induce ovulation (Sharma and Singh 2002 and Akar 2005). A recent development in the technology of induced spawning is the stimulation of endogenous gonadotropin (Gth) release from the pituitary of the treated fish using a synthetic analogue of gonadotropin releasing hormone (GnRHa) (Szabo *et al.* 2002; Brzuska 2003). Because certain analogues are inexpensive and effective, this method is gaining acceptance throughout the world. GnRHa treatments have been found to induce and synchronize ovulation in several commercially important fresh water and marine species (Zohar and Mylonas 2001 and Szabo, 2003).

Satisfactory results have been obtained using Ovaprim as an ovulation stimulant in Carp (Brzuska and Adamek 1997), European catfish females (Brzuska and Adamek 1999), Nase (Szabo *et al.* 2002), Indian major carps (Sharma and Singh 2002) and in Northern pike (Szabo, 2003)

The aim of the present study was to determine the efficacy of the mammalian GnRH analogue applied with ovaprim (salmon GnRH) in comparison with hypophysation in the induction of ovulation in African catfish.

MATERIAL AND METHODS

The investigation was conducted in the fish hatchery of the Central Laboratory for Aquaculture Research at Abbassa in June 2007 which cones ides with peak period of, the natural spawning period of African catfish (*Clarias gariepinus*).

A total number of 40 (Exp.1) and 40(Exp.2) apparently healthy African catfish were divided into four equal groups (10 fish of each).

The experimental fish were selected from over wintered African catfish maintained in 6.5 faddan earthen pond with 1.5 meter depth, with stocking density 100 fish / feddan.

On the last days of April, the spawners, were caught, after selection and sexing transferred to a quarter faddan pond with 1meter depth.

At the spawning season, African catfish females showing spawning signs were randomly divided and distributed in four tanks of 3.5cubic meter volume. Each tank contained five individual. After acclimatization (24 hrs), the water quality parameter were measured and recorded (Dewis and Freilas, 1970) twice daily (Table 1).

Table. 1. Physico- chemical characteristics of the pond water where of African catfish was kept during the experimental periods.

Items		Items	
Oxygen (mg/l)	8.0	Nitrate (mg/l)	0.14
PH	8.5	Amonia (mg/l)	0.01
Temperature (c)	28	Salinity (mg/l)	0.46
Nitrite (mg/l)	0.01	Transparency	15

Salinity was calculated by relation (1000 micromhos =0.7g salinity according to Dewis and Freila, 1970). The experimental fish were injected by different substance as ovulation stimulant each group (Exp1and Exp2).

Table 2. Substances used as ovulation stimulant and doses.

Group	Substance	Dose*
1	CP	3 mg/ kg
2	Cat P	3 mg/ kg
3	CP& Cat P	Half CP & half Cat P
4	Ovaprim	0.5 ml/kg

*Dose per 1kg body weight: i.p. intraperitoneally, carp (CP) and catfish pituitary (CatP) and ovaprim (O).

Where in the first experiment all brooders were injected one dose.

Table 3. Substances used as ovulation stimulant and doses.

Group	Substance	Dose 1*	Dose 2* after eight hours
1	CP	3 mg/fish	3 mg / kg
2	CatP	3 mg /fish	3 mg / kg
3	CP & CatP	3 mg/ fish	Half CP & half Cat P
4	Ovaprim	0.5 ml/kg	0.0

*Dose per 1kg body weight: i.p. intraperitoneally, carp (CP) and catfish pituitary (CatP) and ovaprim (O) were applied at the same time in dose 1 injection (Szabo 2003)

After artificial fertilization, eggs were incubated in Zogar glass jars with continuous water at temperature 28°c.

Ovulation rate and PGSI were estimated according to Szabo (2003)

Ovulation ratio = number of ovulated females /number injected.

PGSI = weight of stripped egg mass/BW before stripping) *100

After 6 hrs of incubation, the percentages of fertilization and hatching were estimated according to Gheyas, *et al* (2001) and Linhart *et al* (2003) as follows:

Fertilization rate = Number of fertilized eggs / Total number of eggs * 100

Hatching rate = Number of hatched eggs (fry) /Total number of eggs *100

Then after 24 hrs of incubation the number living embryos were calculated. Statistical analysis of data carried out by applying the computer program SAS (1996). Differences among means were tested for significance according to Duncan (1955).

RESULTS AND DISCUSSION

In the first experiment the females responded to the injection with Ovaprim after 20 hrs but in the other 3 groups no response for the injection and the abdomen of females were distended (Table 4), also in the two experiment the time between the injection and ovulation was 20 hrs in group 4 where 60 % of females ovulated, while the time between the injection and ovulation was 9 hrs in group 1 and 3 where 75 and 70 % of females ovulated respectively. On the other hand was 11 hrs in group 2 where 50 % of females (Table4).Adamek (1999) and Brzuska (2003) showed that the ovulation time in European catfish females was 30 hrs in ovaprim treated group and 19 hrs in carp pituitary treated group.

The ovulation time was similar in the ovaprim, carp pituitary and carp& catfish pituitary treated groups .The ovaprim treatment does not surpass carp pituitary treatment in African catfish .This is confirmed by field trails in which 75% of African catfish females injected with carp pituitary ovulated in contrast to the ovaprim treatment with ovulation ratio of only 60% .These results were in agreement with the findings of Szabo *et al* (2002) and Szabo (2003).

The results of (Table 5 and Fig 1) showed that the mass of eggs and PGSI was significantly higher in group 1 than 2 and 4 groups while the differences between 1 and 3 were not significant in the same trait. These results were in agreement with findings Brzuska (1999) in grass carp and silver carp, Brzuska and Adamek (1999) in European catfish, Brzuska and Grzywanczewski (1999) in common carp and Szabo (2003) in northern pike.

Table 5 and Fig 1 showed that the mean fertilization percentages and the sac larvae after 24 hrs in group 1 was higher than group 2, 3, and 4 but all difference

were insignificant. Unfortunately, ovaprim treatment could not improve fertilization rate and thus eliminate the main drawback of induced spawning of catfish. The low and variable fertilization rate which probably depends on the quality of the ovulated egg had always been limiting factors in large scale production of catfish fry which in agreement with Szabo 2001.

A part from the positive properties of ovaprim mentioned above, a drawback of it's application is that not all the treated females yielded eggs at the same time in the conditions of the present experiment. This fact has to be taken into consideration when ovaprim is used as ovulation stimulant .In this case, the spawning requires attention for a longer time and fish are exposed to a greater stress being repeatedly caught for ovulation control (Kozlowski 1994 and Brzuska and Adamek 1999).

A high positive value of correlation was found between weight of female before, after injection, mass of eggs, percentage of fertilization and percentage of live embryos after 24 h .While the negative value of correlation was found between percentage of fertilization, percentage of live embryos after 24 h and mass of eggs, weight female before and after injection (Table 6).

In the present experiment the best results were obtained with ovaprim treatment. After the injection of this substance, spawning was induced in a high percentage of females, the yield of eggs was sufficiently high both in grams and in percentage of females body weight, and the best quality of eggs was observed out of the four (Table 4) investigated variants. The quality of eggs was demonstrated by the percentage of live embryos after 24 hrs incubation. The investigation was carried out in one spawning season and with a small number of fish: nevertheless, the preliminary results suggested that ovaprim might be recommended as a good ovulation stimulant for African catfish. In hatchery conditions, an important trait of ovaprim is its easy application to fish in form of one injection but the dose at the drug needs more investigation yield the best mass of eggs.

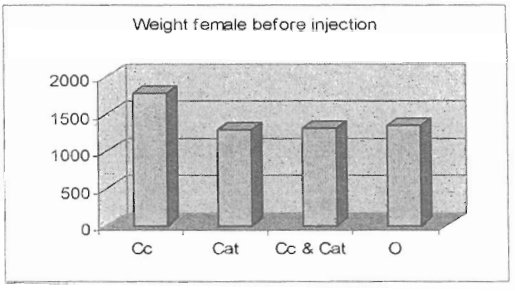
Table 4. Statistical characteristics of the investigated traits

Investigation traits	group 1	group 2	group 3	group 4
Weight female before injection	1430.0± 211	1260.0± 312	1310.0±251	1310.0±179
Weight female after injection	1460.0 ±264	1280.0 ±331	1350.0 ± 214	1160.0±126
Mass of eggs	0.0	0.0	0.0	150.0 ±12.2
Mass of eggs as percentage of female body weight	0.0	0.0	0.0	11.45 ±2.1
Percentage of fertilization	0.0	0.0	0.0	91.0±4.4
Percentage of live embryos after 24 hours	0.0	0.0	0.0	81.0 ±2.5

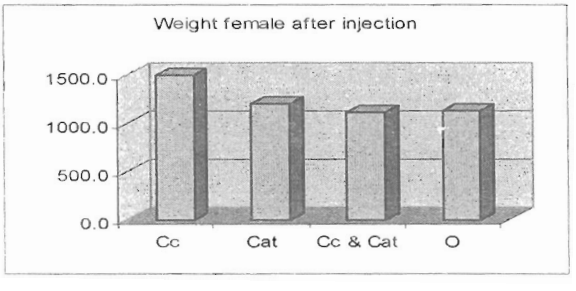
Table 5 . Statistical characteristics of the investigated traits.

Investigation traits	group1	group2	group3	group4
Weight female before injection	1770.0 ± 288.3 a	1280.0 ±321.1a	1300.0 +189.0a	1340.0+243.2a
Weight female after ovulation	1490.0 ±274.0a	1200.0 ±299.4a	1110.0 +171.7a	1126.0+226.9a
Mass of eggs	260.0±53.3a	80.0 ±33.9b	184.0 +18.8ab	94.0 +16.9b
Mass of eggs as percentage of female body weight (PGSI)	14.6 +1.1a	6.25 +0.4b	14.1 +1.2ab	7.0 +0.2b
Percentage of fertilization	91.4 ±1.5a	70.0 ±17.2a	88.0 +2.5a	90.0+1.2a
Percentage of live embryos after 24 hours	81.0 ±1.0a	64.0 ±16.0a	81.0+1.8a	82.0 +1.2a

Values in the same row having the same latter are not significantly different ($p<0.05$).



A



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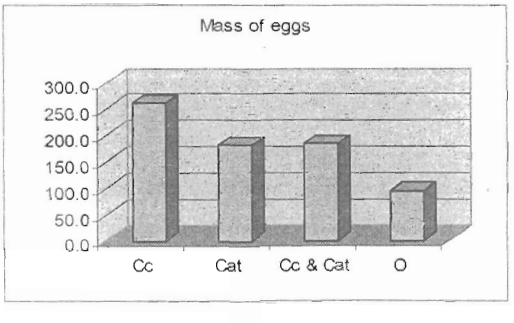
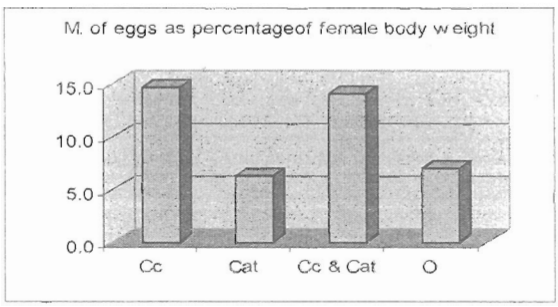


C

Fig 1. Least- square means for the interaction of ovulation stimulator body weight, (A) weight female before injection, (B) weight female after injection, (C) mass of eggs,



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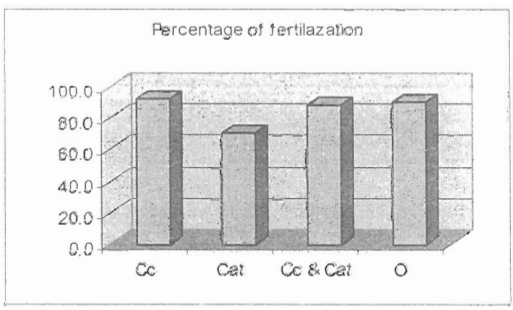

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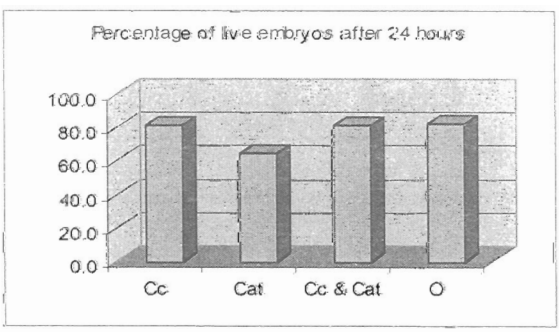

F

Fig 2. Least- square means for the interaction of ovulation stimulator body weight, (D) M. of eggs as percentage of female body weight, (E) percentage of fertilization, (F) percentage of live embryos after 24 h.

Table 6 . Pearson correlation coefficients between the traits of females of catfish.

	Before injection	After injection	M. of eggs	P. fertilization	P. live embryos
Before injection	1.000				
After injection	0.98**	1.00			
M. of eggs	0.55*	o.40	1.00		
P. fertilization	- 0.12	- 0.21	0.35	1.00	
P.live embryos	- 0.10	- 0.18	0.34	0.98**	1.00

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إحداث التبويض في القرموط الأفريقي باستخدام مستخلص الغدة النخامية للمبروك العادي والقراميط الأفريقية ومركب الأوفابريم

عادل محمد عبد الحميد عكر

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استهدف هذا البحث دراسة تأثير مركب االأوفابريم ومستخلص الغدة النخامية للمبروك والقراميط والخليط بينهما وتأثير ذلك على تفريخ القراميط الأفريقية.

حيث تم عمل تجربتين في التجربة الأولي تم حقن الأسماك في كل المجموعات مرة واحدة حيث تم تقسيمها إلي أربع مجموعات متساوية (١٠ سمكة لكل مجموعة) المجموعة الأولي تم حقنها بمستخلص الغدة النخامية للمبروك العادي- المجموعة الثانية تم حقنها بمستخلص الغدة النخامية للقراميط الأفريقية- المجموعة الثالثة تم حقنها بالخليط بينهما المجموعة الرابعة تم حقنها بمركب الأوفأبريم.

وكانت الاستجابة في المجموعة الرابعة بعد ٢٠ ساعة والتي تم حقنها بالاوفابريم أما المجموعات الثلاثة الاخري فلم تستجب للحقن وانتفخ بطنها. أما في التجربة الثانية والتي تم تقسيمها أيضا إلي أربع مجموعات متساوية (١٠ سمكة لكل مجموعة) المجموعة الأولي تم حقنها بمستخلص الغدة النخامية للمبروك العادي- المجموعة الثانية تم حقنها بمستخلص الغدة النخامية للقراميط الأفريقية- المجموعة الثالثة تم حقنها بالخليط بينهما المجموعة الرابعة تم حقنها بمركب الأوفابريم .

حدثت زيادة معنوية في كمية البيض وكمية البيض منسوبة لوزن الأمهات المنتج من الأمهات في المجموعة الأولي عن المجموعة الثانية والرابعة.

حدثت زيادة غير معنوية في كل من:

نسبة الإخصاب والإعاشة في المجموعة الأولي والرابعة عن المجموعة الثالثة والثانية

وجد ارتباط قوي بين وزن الأمهات قبل الحقن وبعد التبويض وبين الخصوبة والإعاشة .كما وجد ارتباط معنوي بين وزن الأمهات وكمية البيض .

مما سبق يتضح لنا أن استخدام مركب الأوفابريم أ حدث استجابة كبيرة في إنتاج البيض بحقنة كجرعة واحدة في كلتا التجربتين بينما الغدة النخامية(للمبروك العادي والقراميط والخليط بينهما) لم يحدث استجابة لها إلا بعد حقنها مرتين(جرعتين). هذا يتطلب منا إجراء بحوث عديدة آخري لتحديد الجرعة المناسبة لسهولة استخدامه .