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Comparative Study Showing the Effect of Injection With Different Doses of Sex Steroid Hormones on Reproductive Performance of Common Carp (*Cyprinus carpio* L.) and Grass Carp (*Ctenopharyngodon idella*)

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ABSTRACT

The present study was conducted to evaluate the effects of human chorionic gonadotropin (HCG), carp pituitary extract (CPE) and combination of them, one some reproductive performance for both, Common carp (*Cyprinus Carpio* L.) and Grass carp (*Ctenopharyngodon idella*) , studied in a total number of 40 carp that were divided into eight groups. Latency time, mass of eggs, egg weight index percentage (EWIP), ovulation rate, fertilization rate, hatching rate, cost of injection, return efficiency and economic efficiency were recorded. The results revealed that latency times in common carp injected by Gm1 and Gm4 were 10 hours, wherever Gm2 and Gm3 were 18 hours. On the other hand, the spawning percentage was 80, 75, 65 and 90% of females in groups Gm1, Gm2, Gm3 & Gm4, respectively. While, the mass of eggs and EWIP was significantly higher in Gm4 and Gm1 then decreased in Gm2 and Gm3 ,respectively. The mean fertilization percentage and hatching rate was higher insignificantly in Gm2 than in all groups. The return was higher significantly ($P<0.05$) in Gm4 and Gm1 then decreased in Gm2 and Gm3, respectively. While, the economic efficiency was higher significantly in Gm4 with slight decrease in Gm3 and Gm2, respectively) than in Gm1. The results of latency time in grass carp in both Gs1 and Gs4 was 8 hours, while, in Gs2 and Gs3 was 15 hours. On the other hand, the spawning percentages were 80, 70, 30 and 90% in Gs1, Gs2, Gs3 and Gs4, respectively. While, the mass of eggs were significantly higher in Gs1 and Gs4 then decrease in both Gs2 and Gs 3, respectively. Also, EWIP was significantly higher in Gs1 then decrease in Gs 4, Gs2 and Gs3, respectively. The fertilization percentage and hatching rate was higher significantly in both Gs4 and Gs1 than Gs 2, respectively. The return were higher significantly ($P<0.05$) in both Gs1 and Gs4 with

slight decrease in Gs2 and Gs3, respectively, but the difference was non significantly between Gs1 and Gs4. Present study concluded that Gs4 was the most effective treatment not only in the artificial spawning of both common carp and grass carp ,but also in economic efficiency.

Keywords: Reproductive performances; Carp pituitary extract; human chorionic gonadotropin, Common carp and grass carp.

INTRODUCTION

Fish farming expansion of the far eastern carp has been possible as a result of the development and improvement of propagation methods of these species under controlled conditions (Brzuska, 1999 & 2004 and Akar 2008) after introducing of these fishes in the countries of the temperate zone, controlled propagation was mainly conducted using hypophysation of spawners (Brzuska and Adamek, 1987 ; Essa, 1987 and Brzuska 1999). Also a high efficiency of ovulation with (HCG) was reported in the eastern regions (Ngamvongchon *et al.*, 1988) and in the United States (Rottmann and Shireman, 1979 and Adamek, 1995) where (HCG) was used in combination with carp pituitary suspension. Reproduction after utilizing different ovulation stimulates using various preparations of both natural and synthetic origin were carried out (Brzuska, 2000 & 2004). Application of (HCG) for stimulation showed good results in reproduction in *Cyprinus carpio* L., *(*Brzuska and Bialowas, 2002), in Silver carp (Haque *et al.*, 1995). Human chorionic gonadotropin (HCG) is the most common purified gonadotropic hormone used for induced spawning. The fish injection gonadotropin hormone, pituitary extracts and purified hormones such as (HCG) plus, mention , acting directly on the ovaries and testes. However, the comparatively large dose and frequent injection were required. The hormones last longer in the fishes system and have potent stimulatory effects on their ovulation and spermatism (Rottmann *et al.*, 1992). The beneficial traits of (HCG) gave the possibility of precise dosing without the necessity of weighing the preparation, a very simple method of preparing and storing injections and the elimination of an additional injection of the dopamine receptor blocker (Adamek, 1995).

Haque *et al.* (1995), Akar,(2006) and Akar and Ali (2006) injected the silver carp and the bighead carp with (HCG) and pituitary gland carp, during the breeding season, and found that a mixture of (HCG) and pituitary gland was better than either (HCG) or pituitary gland separately in induce spawning of females.

The present study aimed to evaluate the effects of (HCG), carp pituitary gland or their combination on some reproductive performance parameters of common carp and grass carp.

MATERIALS AND METHODS

The present study was conducted at Abbassa Fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Sharkia,Egypt. Forty fish (twenty common carp and twenty grass carp) apparently healthy were divided into eghit equal randomly groups, each of group five fish. The fish were selected from over-wintered carp maintained in 6.5 feddan(4200m) earthen ponds that has 1.5 meter depth, with stocking density of 200 fish/ feddan. In the late days of April, the spawners were caught, selected , sexed and transferred to a quarter feddan pond with 1 meter depth. At the spawning season, female carp which showed spawning signs were randomly divided and distributed in eight tanks of 3.5 m volume filled with filtered Nile water supplied with aerator. Each tank contained five spawners.

After acclimatization of fish for 24 hrs the water quality was measured (Dewis and Freiles 1970) twice daily (Table1).

The fish were subjected to the following treatments. First group (G1) was injected with carp pituitary extract (CPE) double doses (first dose 3mg / fish and dose secondary 3mg / kg fish). Secondary group(G2) was injected with single dose carp pituitary extract (CPE) (3mg / kg fish). Third dose (G3) was injected with human chorionic gonadotropin (HCG) (first dose 1000 Iu / fish and second dose 1000 Iu / kg) and fourth group G4 was injected with CPE & HCG (first dose 3mg / fish and second dose 1.5 mg and 500 Iu / kg). In general, injection was intra-peritoneal and the second dose injected after 8 hours

Table1: ***Physico-chemical characteristics of earthen water ponds of carp during climatic period before the experimental.***

Items	Mean	Items	Mean
Temperature(c)	23-25 ^{o}C	Nitrate (mg/l)	0.01
PH	8.7	Nitrite (mg/l)	0.02
Oxygen (mg/l)	8.1	Salinity (mg/l)	0.3

Salinity was calculated by relation (1000 micromos = 0.7g salinity according to *Dewis and Freila (1970).*

from first dose . After treatments, fertilized eggs were separately incubated in zogar glass jars supplied with continuous water at temperature of 23-25 ^{o}C.

Ovulation rate and egg weight index percentage (EWIP) were estimated according to **Szabo *et al.* (2002)**

Ovulation rate = number of ovulated females / number of injected females x 100

Egg weight index percentage (EWIP) = (weight of stripped egg (g) / body weight of females before stripping (g)) x 100

In all fertilized eggs treatments, the percentage of fertilization was estimated after 6 hrs from incubation according to Gheyas, *et al.* (2001) as follows:

Fertilization rate = (Number of fertilized eggs / Total number of eggs) x100

Hatching rate = (Number of hatched eggs (larvae) /Total number of eggs) x100

Then after 48 hrs in common carp and 24 hrs in grass carp incubation period hatching percentage was estimated.

Economic efficience was estimated according to (Kazlowski 1994) as follows:

Injection cost = injection cost/ kg x weight of fish.

Fry production = Weight of eggs x percentage of fertilization x 700.000 (common carp) or 450.000(grass carp) /1000 x 100.

Return = Fry production – injection cost.

Economic efficiency = (Many return/ Injection cost) x 100

Statistical analysis were carried out using statistical analysis systems (SAS, 2004).

RESULTS AND DISCUSSION

Common carp (*Cyprinus carpio*)

The present results (Table 2) showed that, latency time (the time between the second injection and ovulation) of CPE double dose(Gm1) and CPE & HCG (Gm4) was 10 hours, wherever CPE single dose (Gm2) and HCG (Gm3)was 18 hours, In these groups 80, 75, 65 and 90% of females were spawned in groups Gm1, Gm2, Gm3 and Gm4, respectively. These results were in agreement with the findings of Brzuska (2002 & 2004). On the contrary , Mahmood (2006) showed that splitting of the CPE dose(double dose) did not affect the ovulation time and ovulation response

Table 2: ***Statistical characteristics of the investigation traits of common carp.***

Investigation	CPE double doses (G1)	CPE single dose (G2)	HCG (G3)	CPE with HCG (G4)
Latency time (hours)	**10**	**18**	**18**	**10**
Body weight before spawning (g)	**3390±572.5^{a}**	**2498±787.8^{a}**	**2402±794.8^{a}**	**3000±173.0^{ab}**
Body weight after spawning (g)	**3140±684.5^{a}**	**2260±679.5^{a}**	**2230±595.4^{a}**	**2710±201.2^{ab}**
Mass of eggs (g)	**250±64.8^{b}**	**238±91.5^{b}**	**172±63^{c}**	**362±91.2^{a}**
EWIP (%)	**10.2±2.7^{ab}**	**8.7±2.4^{b}**	**5.62±5.7^{c}**	**11.94±1.5^{a}**
Fertilization rate (%)	**76±6.5^{a}**	**78 2±0.7^{a}**	**76±5.5^{a}**	**74±4.2^{a}**
Hatching rate (%)	**73±5.7^{a}**	**74±4.1^{a}**	**73±5.7^{a}**	**69±4.2^{a}**
Cost (LE)	**30±4.2^{a}**	**16.8±2.7^{b}**	**7.7±0.6^{c}**	**24±6.0^{b}**
Return (LE)	**1142.1±197.3^{a}**	**878.9±143.4^{b}**	**408.8±82.3^{c}**	**1295±161.8^{a}**
Economic efficiency	**4013.9±464.3^{b}**	**4963.2± 559.5^{a}**	**5470.02± 984.3^{a}**	**5498.2±658.5^{a}**

Means with the same letter in the same row were not significantly different (P < 0.05)

in climbing perch (Anabas testudineus), also Kouril *et al.* (2003) showed that higher percentage of females tench *(Tinca tinca)* spawned after lecirelin treatment than after CPE (89 % and 23 % respectively). This might be due to species differences.

The results of Table 2 and Figs 2&3 showed that the mass of eggs and EWIP was significantly higher in group 4 (362±91.2 g &11.94±1.5 %) and group 1 (250±64.8 g & 10.2±2.7%) than in group 2 (238±91.5 g & 8.7±2.4%) and group 3 (172±63 g & 5.62±5.7), respectively. These results were in agreement with findings Brzuska (2002&2004), Akar (2006) and Akar and Ali (2006).

Table 3: ***Correlation between reproductive performances of common carp injected with pituitary extract; human chronic gonadotropin hormone and combination of.***

	WT BE	WET AF	egg	EWIP	F	H	CO	RE
WET AF	0.971							
Egg	0.486	0.37						
EWIP	0.342	0.264	0.735					
F	-0.093	-0.054	-0.223	-0.411				
H	-0.253	-0.171	-0.457	-0.368	0.842			
CO	0.662	0.648	0.498	0.529	-0.09	-0.22		
RE	0.367	0.336	0.694	0.847	-0.0119	-0.137	0.678	
EC	-0.197	-0.227	0.272	0.165	-0.293	-0.298	-0.421	-0.188

The mean fertilization percentage and hatching rate was higher insignificantly in group 2 than in other groups, but the fertilization rate in group 4 was the lowest . The present results are in agreement with Brzuska (2004) and Akar (2006) .

Economic efficiency

Table 2 and Fig. 5 show that return was higher significantly ($P<0.05$) in groups 4 & 1 (1295±161.8 &1142.1±197.3 LE) than in groups 2 and 3 (878.9±143.4 & 408.8±82.3 LE), respectively. While, higher significantly in the economic efficiency of groups 2, 3&4 were 4963.2± 559.5, 5470.02± 984.3 & 5498.2±658.5, respectively.

The results showed that a high positive value of correlation (0.971) was found between body weight before spawning and body weight after spawning, also, between EWIP (%) and return (LE) was 0.847. While, high negative values of correlation were found between mass of eggs (g) and percentage of hatchability (%) was -0.457. Also, between EWIP (%) and percentage of fertilization was -0.411 as shown in table 3.

***Grass carp*(*Ctenopharyngodon idella*)**

The results revealed that, latency time in both Gs1 and Gs4 was 8 hours, while, in both Gs2 and Gs3 was 15 hours as shown in Table 4. On the other hand, the spawning percentages were 80, 70, 30 and 90% in groups Gs1, Gs2, Gs3 andGs4, respectively. These results were agreement with Brzuska (2002 & 2004) and Kouril *et al*. (2003) they showed that females of Tench (*Tinca tinca*) treatment by lecirelin higher percentage spawned than treatment by CPE (89 % and 23 % respectively). Also, Akar (2006) showed that synchronization of ovulation silver carp was observed in all the females after the injection by Aquaspawn.

The results in Table 4 and Fig 8 showed also that the mass of eggs was significantly higher in both Gs1 and Gs4 (530±88.7 & 440±119.4g) than in Gs2 and Gs3 (200±79.1 & 30±9.4 g), respectively. Also, EWIP was significantly higher in G1 (15.7±1.65) than in Gs4, Gs2

Table 4: ***Statistical characteristics of the investigation traits of grass carp.***

Investigation	CPE double doses (G1)	CPE single dose (G2)	HCG (G3)	CPE with HCG (G4)
Latency time (hours)	**8**	**15**	**15**	**8**
Body weight before spawning (g)	**3330±762.1[a]**	**2906±292[a]**	**2986±1205.4[a]**	**3560±746.1[a]**
Body weight after spawning (g)	**2800±629.5[a]**	**2500±583.1[a]**	**2946±1209.5[a]**	**2920±752.9[a]**
Mass of eggs (g)	**530±88.7[a]**	**200±79.1[b]**	**30±9.4[c]**	**440±119.4[a]**
EWIP (%)	**15.7±1.65[a]**	**6.5±1.4[c]**	**1.12±0.33[d]**	**10.6±3.1[b]**
Fertilization rate (%)	**72±4.4[a]**	**63±7.4[b]**	**20±5[c]**	**75±5 [a]**
Hatching rate (%)	**68±4.5[a]**	**60±17.3[a]**	**17±4.5[b]**	**70±5 [a]**
Cost (LE)	**28.8 ±5.02[a]**	**20.4±3.3[b]**	**12.4±4.5[c]**	**26.4±6.9[ab]**
Return (LE)	**3588.7±911[a]**	**1749.4±437.3[b]**	**162.9±67.3[c]**	**3733.7±823.6[a]**
Economic	**12452.2±903.1[b]**	**8232.4±1693.1[c]**	**1491.2±473.9[d]**	**14358.3±684.3[a]**

Means with the same letter in the same row were not significantly different (P < 0.05)

and Gs3 (10.6 ± 3.1, 6.5±1.4 and 1.12±0.33,10.6±3.1) ,respectively, Also Gs4 had significantly higher EWIP that of than Gs2 and Gs3.as shown in Table 4 and Fig 10. These results were in agreement with findings of Brzuska (2002 & 2004), Akar (2006) and Akar & Ali (2006). The increase in the ovulation activities the Common carp and Grass carp may be due to the Oogenesis is controlled by stimulating hormone (FSH) and Luitnasing hormone (LH) but need also participation of several paracrine autocrine mechanisms of regulation as reported by Kouril *et al,* (2003). Also the increase in ovulation activities may be due to HCG is increase the speed of eggs maturation in fish as reported by Hodson and Sulivan (1993).

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دراسات مقارنة لمعرفة تأثير الحقن بجرعات من هرمونات التناسل علي الكفاءة التناسلية للمبروك العادى والحشائش

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المعمل المركزي لبحوث الثروة السمكية بالعباسة

استهدف هذا البحث دراسة تأثير الحقن بمستخلص الغدة النخامية(كجرعه واحدة أو جرعتين) وهرمون الجونادوتروبين البشري والخليط بينهماعلي الكفاءة التناسلية للمبروك العادى ومبروك الحشائش . واستخدم لذلك البحث والذى جرى فى المعمل المركزى لبحوث الاسماك بالعباسة - ابوحماد - شرقية ٢٠ سمكة مبروك عادى ١٠ ذكور و ١٠ إناث و٢٠ سمكة مبروك حشائش ١٠ ذكور و١٠ اناث وقسمت هذه الاسماك الى ٨ مجموعات الاربعة مجموعات الاولى مبروك عادى والاربعة مجموعات الثانيه مبروك حشائش والمجوعة الاولى تم حقنها بالغدة النخامية للمبروك علي جرعتين والمجموعة الثانية جرعة واحدة من الغدة النخاميه والمجموعة الثالثة بهرمون الجونادوتروبين البشري والمجموعة الرابعه خليط بينهما فى هذا البحث تمت دراسة الزمن بين الحقنة الأولي والتبويض وكمية البيض ودليل المناسل ونسبة الإخصاب ونسبة الفقس والعائد والكفاءة الاقتصادية في المبروك العادي والحشائش.

وكانت النتائج كما يلي: :

اولا : المبروك العادى:

الزمن بين الحقنة الأولي والتبويض كان أسرع وأفضل في المجموعة الآولي والرابعة (٨ ساعات) عن المجموعة الثانية والثالثة (١٠ ساعات) ونسبة التبويض فى المجموعه الاولى ٨٠% والثانية ٧٥% والثالثة ٦٥ والرابعة ٩٠١% و حدثت زيادة معنوية لكمية البيض ودليل المناسل في المجموعة الأولي والرابعة عن

باقي المجاميع بينما لم تكن معنوية في نسبة الإخصاب ونسبة الفقس في المجموعة الأولي والرابعة وحدثت زيادة معنوية في العائد والكفاءة الاقتصادية في المجموعة الرابعة عن باقي المجاميع.

ثانيا: مبروك الحشائش:

الزمن بين الحقنة الأولي والتبويض كان أسرع وأفضل في المجموعة الآولي والرابعة (٨ ساعات) عن المجموعة الثانية والثالثة (١٥ ساعه) ونسبة التبويض فى المجموعه الاولى ٨٠% والثانية ٧٠% والثالثة ٣٠ والرابعة ٩٠% وحدثت زيادة معنوية لكمية البيض في المجموعة الأولي والرابعة وقلت فى المجموعة الثانية والثالثة وزياده فى دليل المناسل فى المجموعة الاولى وقل فى باقى المجموعات وحدثت زيادة معنوية في نسبة الإخصاب ونسبة الفقس في المجموعة الأولي والرابعة وحدثت زيادة معنوية في العائد والكفاءة الاقتصادية في المجموعة الرابعة عن باقي المجاميع .

مما سبق نوصي باستخدام خليط من مستخلص الغدة النخامية وهرمون الجونادوتروبين البشري عند تفريخ المبروك العادي والحشائش.