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Research Article

Control of Reproduction by Using Hormonal Injection in Grass Carp (*Ctenopharyngodon Idella*) Reared In the Floating Cages in River Nile, Egypt

Abd-Elhakim E. El-Gamal[1], Samah T. Darwish[2] and Mohamed A. Sheha

[1]National Institute of Oceanography and Fisheries, Egypt
[2]Zoology Department, Faculty of Science, Al Arish University, Egypt

*Corresponding Author
Dr. Samah T. Darwish

Abstract: The experimental application of induced spawning on grass carp (*Ctenopharyngodon idella*) were carried out during the period of spawning extended from April to May 2016. The different doses level of human chorionic gonadotropin (HCG) and carp pituitary homogenate (CPH) were used separately or mixed together. The present results indicated that the best dose level that give higher number of fertilized and hatching eggs were 5mg/kg of body weight of CPH, following 9hours with 1500IU HCG per kg of body weight. No significance differences occurred (P> 0.05) in fertilized and hatching rates between HCG and CPH of treatment groups. In all experiment, the initial egg diameter before the initial injection was 600 micron in diameter. In mature stage, the ripe eggs were recorded to be more than 1 mm in diameters after injection with hormonal treatment.

Keywords: human chorionic gonadotropin (HCG), carp pituitary homogenate (CPH), grass carp, *Ctenopharyngodon idella* (Teleostei).

INTRODUCTION

The grass carp is one of the largest members of the family Cyprinidae, and is the only member of the genus Ctenopharyngodon as stated by Shireman and Smith (1983); Chilton and Muoneke (1992). Grass carp is a sub-tropical to temperate species, native in large rivers and lakes in eastern Asia. Grass carp, *Ctenopharyngodon idella* farmed throughout the world for two reasons, it considered as a rich of source of protein production especially in developing countries and also used as a biological control for aquatic weeds.

The main problem of grass carp farming in Egypt was the failure in natural spawning, since the female reached to mature state but the ovulation failed and atretic action accelerated, then oocyte absorbed again.

Several factors inhibit the reproductive process in teleosts, among these factors pollutants, salinity and water quality. In this respect, Singh *et al*., (2010) used several techniques for induction of fish breeding and stimulate ripe fish to breed in captive condition. However, in Egypt, there is no enough data on the suitable of different methods of spawning for this economic fish. So the present work planned to investigate the human chorionic gonadotropin (HCG) and carp pituitary homogenate (CPH) separately or mixed together on oocyte ripening, ovulation and spawning of mature female.

MATERIAL AND METHODS:

Fish Collection

The fish samples collected from floating cages in River Nile during prespawning season (April-May, 2016). The age of selected brooders ranged from 3-4 years in female with an average weight 4.925 kg for hormone injection trials and 4.430 kg was also used for extract of pituitary gland in injection trials. The age of selected males ranged from 2-3 years with an average weight 3kg for hormonal injection.

Selection of Brooders

Sexual state of male and females of grass carp under the present investigation showed ripping signs during experiment, since in the female, the pectoral fin

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was soft and the abdomen was rounded, while the males characterized by having a rough and large pectoral fin Fig. (1) as mentioned by Rashid *et al.*, (2014).

Fig. (1): A photo macrography picked up by using a camera showed the deciduous tubercles (DT) (pearl organs) on the dorsal and medial surfaces of the pectoral fins (PF)

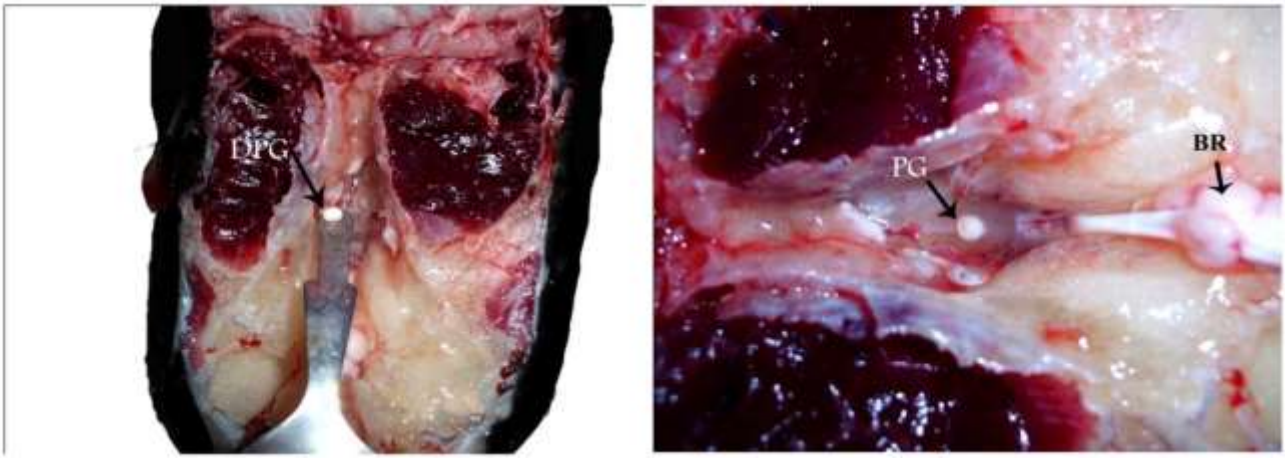

Fig. (2): A photo macrography picked up by using a camera showing dissected specimen of female grass carp, *Ctenopharyngodon idella* showing separated pituitary gland (SPG) from ventral surface of brain (BR).

Fig. (3): A photo macrography picked up by using a camera showed the injection dose in female at the base of a pelvic fin.

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Preparation of Pituitary Homogenate and Hormone Solution

Preparation of acetone-dried pituitary gland prepared according to Woynarovich and Horvath (1980). Since the pituitary gland, separated with remove the top of the skull with a saw or knife then brain is removed from the skull, the gland is left behind on the base of the skull. Carefully, pituitary gland was excised as showed in Fig. (2). Dried pituitary glands pulverized with mortar and pestle then the homogenate tissue mixed with sterile saline solution (0.7% NaCl) solution. This mixture was mixed thoroughly to produce a homogenate suspension.

The quantity of dried pituitary glands to be weighed is determined by multiplying the recommended dose by the total weight of brood fish. The volume of saline solution that must be mixed to obtain the desired volume and the recommended dose for injection calculated according to Rottmann *et al*., (1991). The recommended dose injected with needle at the base of a pelvic fin, where there are no scales, as shown in Fig. (3).

Human chorionic gonadotropin (HCG) as Pregnyl 5000 I.U. powder produced by the Nile Company for pharmaceuticals Cairo was also used.

Experimental Studies

Three experiments were carried out at same time during the experimental study period of study. Each experiment contained twenty four males and twelve females. The water temperature was recorded during the period of experiment and the other characteristic of water parameters was also measured.

Breeding and Spawning

Both males and females were put in the separate ponds before one month of spawning. The fish feed twice a day with food contains 25% protein and aquatic plants. After priming dose of injection of both male and female, they were put in the ratio 2:1 (male: female) i.e. one female needs two males. Then after acclimatization about 4-5 hours with create water waves as occurs in the natural habitat. After about 7-8 hours from the second injection of decisive dose, the males started to chase females and stimulate it to release eggs. The spawning took place after 15 to 18 hours of injection. The water temperature ranged from 24-26°C. The ovulated eggs of grass carp were whitish demersal in nature, then eggs absorbed water and became semi-pelagic and the brooders were transported from the ponds. The ovulated eggs for each trials group collected with drag net. The collected eggs are rinsed several times and transferred into the Zuger jar. After hatching, the fry transported to rearing ponds that must be covered, since the newly hatching fry is more sensitive to light. The fertilized and hatched fry were determined by counting the fertilized eggs and hatched fry and determined by the following equations:
Fertilization percentage % = Total No. of fertilized egg /Total No. egg x100.

Hatching percentage %= Total No. of hatching egg/Total No. of fertilized egg x 100.

Induced Spawning

The spawning occurred artificially using the following protocol of hormonal injection as represented in table (1). On 21 May 2016, thirty six females were used for experimentation, their average of total weight ranged from 53.33kg to 59.7kg. The average in diameter of oocyte was greater than 600 micron. These oocytes were taken by using polyethylene cannula inserted in the genital opening. Three experiments were carried out. During the period of spawning season the first experiment, dried pituitary extract of common carp CPH was used in the first and second dose.

The primary injection was 3mg of CPH/kg of body weight, following by the resolving doses of 5mg of CPH/kg of body weight. The interval time between the first and the second injection was 16 hours Table (1).

In the second experiment, Human chorionic gonadotropin (HCG) pregnyl was used. The first injection was 750I.U. HCG per kg of body weight, followed by dose levels 1500HCG/ kg of body weight. The interval time between the first and second injection was 24 hours Table (1).

In the third experiment, 3-5mg with suspension of pituitary extract CPH in the first and in

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the second injection the dose of HCG ranged from 750-1500mg/kg of body weight.

The males were only injected either with 5mg/kg of suspension of pituitary extracts (CPH) or 750 I.U.HCG per kg of body weight. The injection was used in the time of final decisive injection of females.

STATISTICAL ANALYSES:

Data were statistically analyzed using one-way ANOVA, followed by TUKEY multiple-range test for multiple comparisons ($P<0.05$). Statistical analysis was performed with SPSS software program.

RESULTS AND DISCUSSION

In the present investigation, the main problem of grass carp, *Ctenopharyngodon idella* as an exotic fish in Egyptian fauna was the failure of natural spawning, so the artificial spawning became the key of solving reproduction problem. The hormonal treatment achieved with two successive injections. In case of carp pituitary extract (CPE), the priming injection basically results a slight increase in oocyte size, while nucleus of oocytes remains in a central position. The final oocyte maturation and then ovulation is triggered by the final of decisive injection as shown in Table (1).

According to Woynarovich and Horvath, 1980 and Cacot *et al*., 2002 who reported that the aim of the first injection (HCG) is to prepare the gonad for spawning, increasing oocyte sensitivity to the second stage of hormonal treatment. The second dose of injection includes the process of oocyte maturation since; the germinal vesicle migrated to the animal pole near to micropyle of the oocyte and then the germinal vesicle breakdown (GVBD). After that, the oocyte become mature ova and ready for releasing from the follicle and ovulated outside.

Table (1): Effect of injections HCG, CPH and mixtures HCG+CPH treatment on spawning of grass carp, *Ctenopharyngodon idella.*

Items	[1]CPH injection	[2]HCG injection	HCG+ CPH injection
No. of treated females	12	12	12
Total weight of females kg	53.23	59.7	55.33
Total no. of eggs	4342400	4828400	5126030
Average of total no. of eggs for one fish	361866.66	402366.66	427169
Total no. of ovulated eggs/kg	81578	80877	92644
Total no. of fertilized eggs	3502600	3966000	4363276
Overall fertilization percentage %	80.66	82.14	85.12
Total no. of fertilized eggs/kg	65801.23	66432.16	78859
No. fertilized egg for one fish	291883.33	330500	363606
Total no. of hatching	3042200	3264800	3824411
Overall hatching percentage %	86.85	82.31	87.65
Average no. of hatching eggs/kg	57151.98	54686.76	69120
No. hatching egg for one fish	253516.66	272066.66	318700
Dosage/kg of body weight	3-5mg/kg	750-1500 IU/kg	3-5mg/kg plus 750-1500 IU/kg
Time interval between 1st and 2nd injection	16	24	9

[1]CPH: Carp pituitary homogenate, [2]HCG: Human chorionic gonadotropin* $P < 0.05$ as determined by ANOVA data and Tukey test and multiple comparison test.

The Human chorionic gonadotropin (HCG) is used for induction of artificial spawning of fishes as in the present study, Since this hormone stimulated germinal vesicle breakdown (GVBD) in oocytes of several fish species as reported with several authors (Patino and Thomas 1990; Degani and Boker 1992; York *et al*., 1993) also HCG triggering ovulation in case of catfish as reported by Legendre *et al*., (2000), Adebayo and Fagbenro, (2004).

Application of HCG for stimulation the spawning showed good results in reproduction of *Cyprinus carpio* L., as reported by Brzuska and Bialowas, (2002) and silver carp Haque *et al*., (1995). In the present study, Human chorionic gonadotropin (HCG) as in the most common purified gonadotropic hormone used for induced spawning alone or combined with injection of CPE as shown in Table (1).

As reported in the other fishes HCG and CPE were injected in the silver carp and the bighead carp during the breeding season and the results indicated that a mixture of HCG and pituitary gland were found better than either HCG or pituitary gland separately in induce spawning of females (Haque *et al*., 1995 & Akar and Ali 2006). However Liao and Chen, (1984) reported that the use of HCG alone, was found to be successful in inducing ovulation/spawning in captive milkfish *Chanos chanos* in Taiwan.

The injection doses or recommended hormone doses vary considerably for different species of fish and even from different hatcheries spawning of the same species as observed by Rottmann *et al*., (1991). In the present study, the good results gave after injection dose 500IU/kg for HCG and 5mg/kg in case of CPH injection as shown in table (1), while Rashid *et al*., (2014) used injection of single dose of Ovatide 0.7 and

0.8-0.9ml/kg body weight for female grass carp and silver carp respectively. Also HCG was used for increasing spawning rates in Nile Tilapia with different dose and the results showed that the dosage ranged from 2000-3500IU of HCG/kg of fish were more effective than 500-3500IU of HCG/kg of fish as stated by Abiado *et al*., (1994).

In the present study, no significance differences (P<0.05) in fertilization and hatching rates recorded between HCG and CPH in the injection trials. As shown in table (1), since the fertilization rate recorded 80.66 % from ovulated eggs in case of CPH injection trials, while in case of HCG injection group recorded 82.13% from ovulated eggs. The hatching rates recoded in HCG and CPH injected trials 86.85% and 82.31% from the fertilized eggs in HCG and CPH respectively. Similar results were obtained with Naeem *et al*., (2011) in their studies on inducing the spawning of grass carp, *Ctenopharyngodon idella* with a single dose of ovaprim-C, since the ovulated eggs were 62532 kg-1, while overall fertilization and hatching rate was 80.36% and 79.49%, respectively. The results were also similar with Jamroz *et al*., (2008) on *Leuciscus Idus*.

CONCLUSION:

It can be concluded that the best results obtained by using the injection with CPH 3-5 mg/kg followed 9 hours by HCG for resolving injection. These results indicated that the injected of female gave a large number of fertilized eggs and hatched larvae. Further studies are needed to determine economic differences between the injection with HCG and CPH and in the future study, the effect the single dose of gonadotropin releasing hormone (GnRH) in combination with dopamine antagonist like ovaprim, is essential for improving the ovulation and hatching rates.

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