

EFFECT OF SOME OVULATION STIMULANTS ON REPRODUCTIVE EFFICIENCY OF COMMON CARP (*CYPRINUS CARPIO*)

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Abstract

The research was carried out during seasons 2004, 2005 on broodstock of common carp *Cyprinus carpio* L. at fish hatchery of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou- Hammad, Sharkia.

The studies were included five treatments (each treatment had 7 females) to improve reproductive performance of common carp by using injection of saline solution as a (Control), extracted pituitary gland of tilapia, extracted pituitary gland of carp, human chorionic gonadotropin (HCG) and pituitary gland extract (first dosage) in combination with human chorionic gonadotropin (HCG) in the second dosage (PG of carp then HCG). The injection was divided into two separate dosages and were injected twice (double injection). The first injection was usually 10 % of total dose, 90% of total dose was in the second injection. Males received only one injection at the second injection (3 mg/kg of male weight always one gland pituitary of common carp for each male).

The results of this study recorded an increase in number and weight of eggs which were produced from females of common carp during seasons 2004 and 2005. In the same time, increasing in fertilization and hatching rate when common carp (*Cyprinus carpio* L.) females injected with pituitary gland extract of carp, human chorionic gonadotropin (HCG) and pituitary gland extract in combination with human chorionic gonadotropin (HCG).

Generally, there were marked increases in the ovulation, fertilization and hatching rate of common carp females (*Cyprinus carpio* L.) which were injected with pituitary gland extract of common carp, HCG and pituitary gland extract of common carp in combination with HCG. On the other hand, injection with

pituitary gland extract of tilapia and saline solution didn't give any effect on ovulation of common carp females.

It could be concluded that the injection of pituitary gland extract (PGE) of common carp combined with HCG was the most effective and economic for the artificial spawning of common carp (*Cyprinus carpio L.*).

INTRODUCTION

The reproduction in fish is regulated by external environmental factors that trigger internal mechanisms into action and by either the fish's internal regulating factors by injected hormones or other substance. Hormone induced spawning is the only reliable method to induce reproduction in fish (Rottmann *et al.*, 1992).

Artificial reproduction including hormonal induction of oocyte maturation and/or ovulation followed by stripping of females and sometimes also permeation and stripping of males is a wide scale practice in fish farming. Obtaining eggs from fish and fertilizing them is known as spawning. The two basic procedures utilized for spawning fish commonly are referred to the natural and artificial methods. The fractional injections of hypophysis were found to be the most effective method in accelerating maturation (Konradt, 1968). Fresh pituitary extract had better inducing effects than the gonadotropin preparations (Carreon *et al.*, 1973).

Gonadotropin releasing hormone (GnRH) is a principal stimulator of follicle stimulating hormone (FSH=GTH I) and Luteinizing hormone (LH = GTH II) release in teleost fish (Van Der Kraak *et al.*, 1998).

Hodson and Sullivan (1993) Reported that the increase in ovulation may be due to that the HCG is necessary to accelerate the maturation of the eggs in fish.

HCG was also used in experiments on fish reproduction. It was shown that this hormone stimulated germinal vesicle break-down in oocytes of several fish species (Degani and Boker, 1992 and York *et al.*, 1993) and steroid production in vitellogenic and full-grown ovarian follicles (Srivastava and Van Der Kraak, 1994), which HCG proved to stimulate maturation of the gonads of several fish species (Scott and Canario, 1990 and Kagawa *et al.*, 1994).

HCG however is not effective on all species. HCG has been used in combination with common carp pituitary extract; for some species, the combination has shown to have improved potency than either preparation used alone. The two hormones can be prepared and injected separately, or the HCG solution can be used when mixing the pituitary extract (Rottmann *et al.*, 1992).

A high efficiency of ovulation with HCG was reported in the eastern region (Ngamvongchon *et al.*, 1988) and in the united state (Kozlowski, 1994 and Adamek,1995) where HCG was used in combination with carp pituitary suspension, but this application are not yet applied in Egypt (Akar, 2006). More recently, the reproduction utilizing different ovulation stimulates using various preparation of both natural and synthetic origin where carried out (Brzuska, 2003 & 2004). Application of HCG for stimulation showed good results in reproduction in *Cyprinus carpio L.*, (Rottmann *et al.*, 1992 and Brzuska and Bialowas, 2002). The aim of this study is to enhance the reproductive performance of the common carp (*Cyprinus carpio L.*) affected with pituitary gland extract (PGE) of tilapia and common carp, human chorionic gonadotropin (HCG) hormone and pituitary gland extract in combination with human chorionic gonadotropin (HCG).

MATERIALS AND METHODS

The present study was carried out in Fish hatchery belongs to Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou- Hammad, Sharkia Governorate, Egypt. The broodstock of common carp fish (*Cyprinus carpio*) were carefully selected during the season of 2004 and 2005.

Thirty five females and thirty five females of the common carp (*Cyprinus carpio L.*). The broodfishes were divided into five groups each group contained 7 females.

The experimental studies included five treatments; by using injection of saline solution as a control, pituitary gland extract of tilapia, pituitary gland extract of carp, human chorionic gonadotropin (HCG) and pituitary gland extract (first

dosage) in combination with human chorionic gonadotropin (HCG) in the second dosage (PG of carp then HCG).

The doses of pituitary gland extract (3 mg pituitary gland /Kg of female), Human chorionic gonadotropin (HCG) is available in drug stores under the commercial name Pregnyl (dose 1200 IU / Kg of female). The dose was divided into two separate dosages and injected twice (double injection), and the first dose contain usually 10 % of the total dose while the second dose constituted 90 % of the total dose. Males always receive only one injection at the second injection (1 ml for each Male).

Preparation of injection

To prepare the dried glands, first the required amount of **PG** based on the total body weight of the broodfish was calculated. Second, the **PG was** put into a mortar and grind thoroughly into a fine powder and then mixed with 0.65 % saline solution (3 mg pituitary gland/ kg of female) which equal to one pituitary gland of carp or equal to 4 of pituitary gland of tilapia. The solution was centrifuged for 15 min at 3000 r.p.m Each 1ml of the resulted supernatant always contain the extract of one gland of common carp and four glands of tilapia. (Adebayo and Fagbenro, 2004).

Stripping and fertilization.

The stripping process began after (8-10) hours after the second injection. Stripped eggs were immediately weighed in a plastic bowl (which has previously been weighed empty). Milt was collected from anaesthetized males and mixed with the eggs of female. The eggs and sperms were left for 5 minutes then added the fertilization solution (4 gm of sodium chloride (salt) and 3 gm of urea) in 1 liter of water. The fertilization solution has been added to the eggs. It was very important to stir the mixture continuously for (1- 1.5) hours. (Làszlò *et al.*, 2002).

Fertilized eggs of common carp (*Cyprinus carpio L.*) were treated with tannin solution to remove any final traces of stickness (5 gm of tannin were added to 10

liter of water) for a few seconds then washed several times with fresh water and the eggs transferred into the zuger jars (Làszlò *et al.*, 2002). The average of water temperature was 25.5 ± 1.5 °C; pH was 7.8 ± 0.5 and dissolved concentration oxygen 4.6 ± 0.5 mg /L.

The condition factor (K_{factor}) was determined for the individual fish from the formula recommended by Bishai (1976):

$$K_{\text{factor}} = W / (L)^3 * 100$$

Where, W = Weight of fish (g)

L = Length (cm)

The percentage of fertilization and hatching were estimated according to Gheyas *et al.*, (2001) as follows:

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

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

Statistical analysis

The data were analyzed by analysis of variance. Duncan's Multiple Range test was applied to condition factors, egg weight, absolute fecundity, relative fecundity (number of fry/female and number of fry/gm of female body weight) all statistics were carried out using Statistical analysis systems (SAS, 2004).

RESULTS

The condition factors data cited in Table (1) showed that there were insignificant differences among all treatments during season 2004 and 2005, where common carp (*Cyprinus carpio L.*) females were injected with saline solution as a (Control), pituitary gland extract of tilapia, pituitary gland extract of carp, human chorionic gonadotropin (HCG) and pituitary gland extract in combination with human chorionic gonadotropin (HCG).

Table 1. The Condition factor (K_{factor}) of common carp females (*Cyprinus carpio L.*) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

Seasons	2004			2005		
parameters	Wt. of. fish (gm)	Length (cm)	K_{factor}	Wt. of. fish (gm)	Length (cm)	K_{factor}
Treatments						
Control	2560 ± 0.06	55.43 ± 0.72	1.51 ± 0.05 ^a	2500 ± 0.012	54 ± 0.37	1.62 ± 0.03 ^a
PG of tilapia	2790 ± 0.08	56 ± 0.49	1.59 ± 0.016 ^a	2810 ± 0.09	56.29 ± 0.42	1.57 ± 0.03 ^a
PG of carp	2690 ± 0.06	55.43 ± 0.37	1.58 ± 0.009 ^a	2730 ± 0.04	55.86 ± 0.37	1.56 ± 0.008 ^a
HCG	2490 ± 0.04	54.43 ± 0.53	1.54 ± 0.02 ^a	2490 ± 0.04	54.43 ± 0.53	1.54 ± 0.02 ^a
PG of carp then HCG	2530 ± 0.05	54.14 ± 0.86	1.6 ± 0.05 ^a	2730 ± 0.07	55.86 ± 0.4	1.56 ± 0.015 ^a

The same letter in the same column is not significantly different at $P < 0.05$

The total weight of eggs during season 2004 showed that significant increase (258.5 ± 10.62 , 260.0 ± 8.078 and 261.43 ± 6.84 g) respectively at ($P < 0.05$) after injecting common carp (*Cyprinus carpio L.*) females with the pituitary gland extract of carp, HCG and combination, respectively when compared to the control group (injected with saline solution) and pituitary gland extract of tilapia

which didn't give any eggs, (0 ± 0). At the same time the total weight of eggs were insignificant between the three groups as presented in Table (2).

During season 2005 this value increased significantly at ($P < 0.05$) to 249.29 ± 5.92 , 251.43 ± 6.05 and 269.85 ± 8.74 g, after injecting fish with the pituitary gland extract of carp, HCG and the combination of them, respectively. The least total weight of eggs exhibited insignificant difference in fish group injected with pituitary gland extract of carp and HCG (249.29 ± 0.92 and 251.43 ± 6.05 g), respectively, while the highest one was observed after injection with combination of Pituitary carp then HCG with (269.85 ± 8.74 g).

Table 2. The total weight (T. wt.) of eggs of common carp females (*Cyprinus carpio* L.) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

Seasons	2004		2005	
parameters	Wt. of fish (gm)	T. wt. of eggs (gm)	Wt. of fish (gm)	T. wt. of eggs (gm)
Treatments				
Control	2560 ± 0.006	0 ± 0^b	2500 ± 0.012	0 ± 0^c
PG of tilapia	2790 ± 0.08	0 ± 0^b	2810 ± 0.09	0 ± 0^c
PG of carp	2690 ± 0.06	258.5 ± 10.62^a	2730 ± 0.04	249.29 ± 0.92^b
HCG	2490 ± 0.04	260.0 ± 8.078^a	2490 ± 0.04	251.43 ± 6.05^b
PG of carp then HCG	2530 ± 0.05	261.43 ± 6.84^a	2730 ± 0.07	269.85 ± 8.74^a

The same letter in the same column is not significantly different at $P < 0.05$.

The values in Table (3) considering that each number of the absolute fecundity was yield by dividing the natural number of the absolute fecundity by

(($10^3 \times \text{No}/\text{fish}$)). The absolute fecundity was increased significantly at ($P < 0.05$) 202.49 ± 10.12 , 192.42 ± 6.68 and 199.92 ± 5.91 after injecting the common carp females with pituitary gland extract of carp, HCG hormone and combination between them, respectively at season 2004. On the other hand, no effect is observed after the injection with saline solution (control) and pituitary gland extract of tilapia. Similarly in season 2005, the mean levels of absolute fecundity were significantly increased in all treatments as compared to the control group and pituitary gland extract of tilapia showed no effect. The maximum value of absolute fecundity was obtained at the combination group, $(209.36 \pm 8.126)10^3$ during season 2005. The minimum values were obtained from HCG group.

Table 3. The absolute fecundity of common carp females (*Cyprinus carpio L.*) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

Seasons	2004		2005	
Parameters Treatments	Wt. of. fish (gm)	Absolute Fecundity ($10^3 \times \text{No}/\text{fish}$)	Wt. of. fish (gm)	Absolute Fecundity ($10^3 \times \text{No}/\text{fish}$)
Control	2560 ± 0.006	0.0 ± 0^b	2500 ± 0.012	0.0 ± 0^c
PG of tilapia	2790 ± 0.08	0.0 ± 0^b	2810 ± 0.09	0.0 ± 0^c
PG of carp	2690 ± 0.06	202.49 ± 10.12^a	2730 ± 0.04	189.28 ± 72.80^b
HCG	2490 ± 0.04	192.42 ± 6.68^a	2490 ± 0.04	186.78 ± 34.15^b
PG of carp then HCG	2530 ± 0.05	199.93 ± 5.91^a	2730 ± 0.07	209.36 ± 81.26^a

The same letter in the same column is not significantly different at $P < 0.05$.

The data presented in Table (4) the relative fecundity which was increased significantly at ($P < 0.05$) 757.86 ± 12.9 , 743.97 ± 6.34 and 755.0 ± 8.24 , after injecting the common carp females by pituitary gland extract of carp, HCG hormone and combination between them, respectively, during season 2004. On the other hand, no effects were observed after injection common carp females by saline solution (control) and pituitary gland extract of tilapia. During season 2005, the mean values of relative fecundity were increased significantly in all treatments after being compared to the control group but no effect was observed in case of pituitary of tilapia. The highest value was observed in the fish group injected with pituitary gland extract of carp in combination with HCG (775.0 ± 8.24). The minimum relative fecundity was obtained in fish injected with HCG hormone (743.97 ± 6.34).

Table 4. The relative fecundity of common carp females (*Cyprinus carpio L.*) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

Seasons	2004		2005	
Parameters	Wt. of. fish (gm)	Relative Fecundity	Wt. of. fish (gm)	Relative Fecundity
Treatments				
Control	2560 ± 0.006	0.0 ± 0^c	2500 ± 0.012	0.0 ± 0^c
PG of tilapia	2790 ± 0.08	0.0 ± 0^c	2810 ± 0.09	0.0 ± 0^c
PG of carp	2690 ± 0.06	782.14 ± 12.62^a	2730 ± 0.04	757.86 ± 12.9^{ab}
HCG	2490 ± 0.04	739.29 ± 5.28^b	2490 ± 0.04	743.57 ± 6.34^b
PG of carp then HCG	2530 ± 0.05	767.14 ± 8.37^a	2730 ± 0.07	775.0 ± 8.24^a

The same letter in the same column is not significantly different at $P < 0.05$.

Results of fertilization rate demonstrated in (Table 5 and Figure 1) recorded very high significant in the fertilization rate during season 2004 as 80.43 ± 0.95 , 85.14 ± 1.06 and 87.71 ± 1.19 , after injection of common carp females with pituitary gland extract of carp, HCG hormones and combination of them, respectively, on the other hand, there was no effect observed after injection with saline solution (control) and pituitary gland extract of tilapia on fertilization rate of female common carp *Cyprinus carpio* L. Similar to season 2005, results of fertilization rate were significantly increased when females injected with pituitary gland extract of carp in combination with HCG and other treatments (87.86 ± 0.77 , 89.36 ± 1.15 and 93.6 ± 1.17), respectively, but the females injected with saline solution (control) and pituitary gland extract of tilapia showed no response. The highest fertilization rate in season 2004 was recorded at the combination group (87.71 ± 1.19). Similar in season 2005 the highest level of these parameters was recorded in the same group (PG of carp then HCG) (93.6 ± 1.17).

Table 5. The fertilization rate of common carp females (*Cyprinus carpio* L.) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

Seasons	2004		2005	
Parameters	Wt. of. fish (gm)	Fertilization rate	Wt. of. fish (gm)	Fertilization rate
Treatments				
Control	2560 ± 0.006	0.0 ± 0^d	2500 ± 0.012	0.0 ± 0^c
PG of tilapia	2790 ± 0.08	0.0 ± 0^d	2810 ± 0.09	0.0 ± 0^c
PG of carp	2690 ± 0.06	80.43 ± 0.95^c	2730 ± 0.04	87.86 ± 0.77^b
HCG	2490 ± 0.04	85.14 ± 1.06^b	2490 ± 0.04	89.36 ± 1.15^b
PG of carp then HCG	2530 ± 0.05	87.71 ± 1.19^a	2730 ± 0.07	93.6 ± 1.17^a

The same letter in the same column is not significantly different at $P < 0.05$.

The data of hatching rate tabulated in (Table 6 and Figure 2) revealed no significant differences in hatching rate during season 2004 as 74 ± 0.93 , 75.57 ± 0.84 and 76 ± 1.11 , after injecting of the common carp with pituitary gland extract of carp, HCG hormone and combination between them, respectively, on the other hand, there was no effect observed after injecting with pituitary gland extract of tilapia and the control groups on hatching rate of female common carp *Cyprinus carpio* L. Similar, during season 2005 as 76 ± 0.93 , 80.29 ± 0.6 and 86.71 ± 0.75 after injecting the common carp by pituitary gland extract of carp, HCG hormone and combination between them, respectively. The mean values of hatching rate were significantly increased in all treatments, while no effect concerning pituitary gland extracts of tilapia and the control groups. The highest hatching rate in season 2004 was recorded at the combination group (76.0 ± 1.11). Similar in season 2005 the highest level was recorded in the same group (PG of carp then HCG) (86.71 ± 0.75).

Table 6. The hatching rate of common carp females (*Cyprinus carpio* L.) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

Seasons	2004		2005	
Parameters	Wt. of. fish (gm)	Hatching rate	Wt. of. fish (gm)	Hatching rate
Treatments				
Control	2560 ± 0.006	0.0 ± 0 ^b	2500 ± 0.012	0.0 ± 0 ^d
PG of tilapia	2790 ± 0.08	0.0 ± 0 ^b	2810 ± 0.09	0.0 ± 0 ^d
PG of carp	2690 ± 0.06	74.0 ± 0.93 ^a	2730 ± 0.04	76 ± 0.93 ^c
HCG	2490 ± 0.04	75.57 ± 0.84 ^a	2490 ± 0.04	80.29 ± 0.6 ^b
PG of carp then HCG	2530 ± 0.05	76.0 ± 1.11 ^a	2730 ± 0.07	86.71 ± 0.75 ^a

The same letter in the same column is not significantly different at P < 0.05.

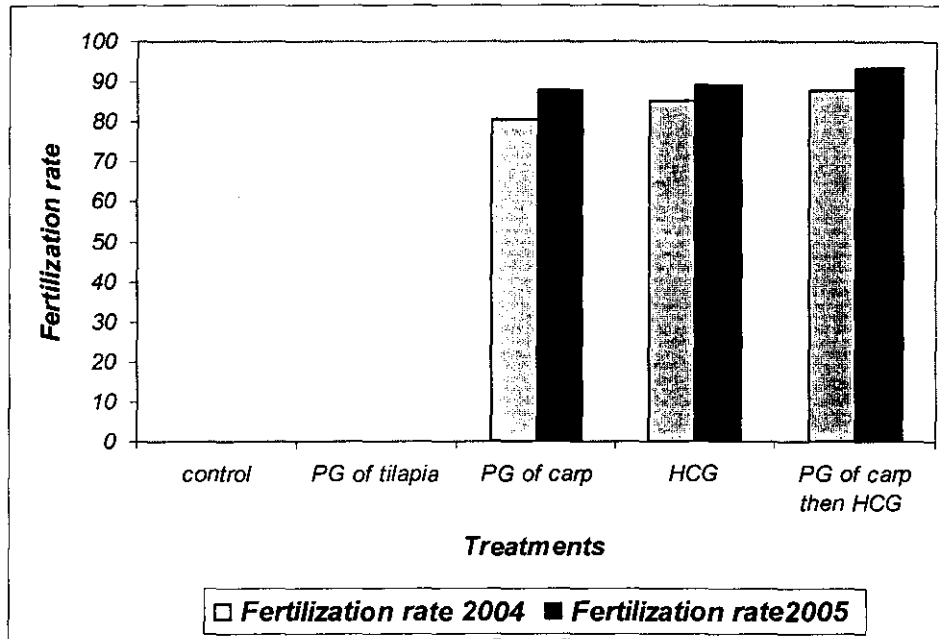


Figure 1. The fertilization rate of common carp females (*Cyprinus carpio L.*) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

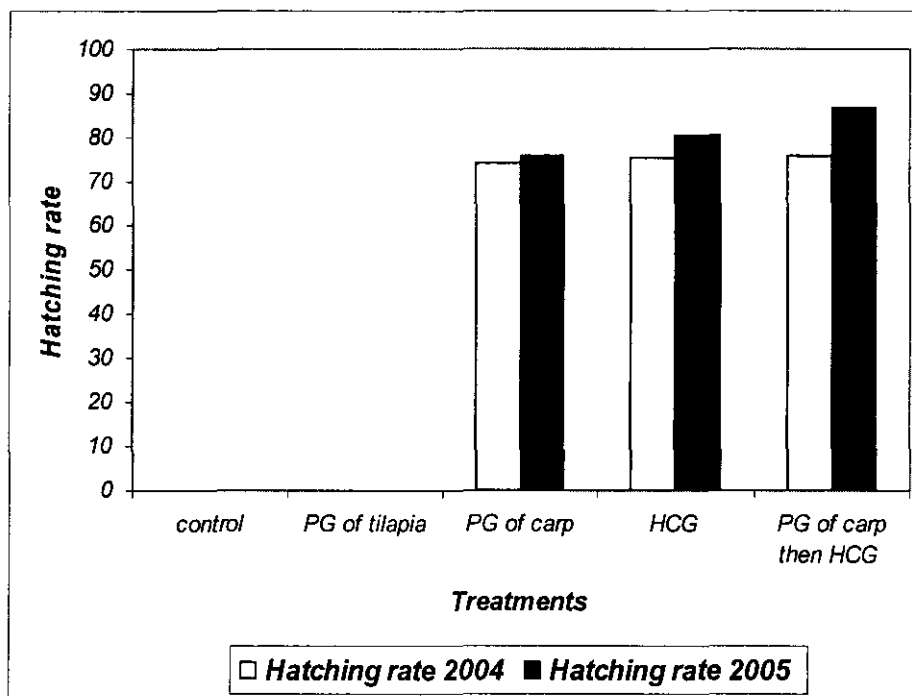


Figure 2. The hatching rate of common carp females (*Cyprinus carpio* L.) injected with saline solution (control), pituitary gland extract of tilapia, pituitary gland extract of carp, HCG and combination between the last two doses during seasons 2004 and 2005.

DISCUSSION

Reproductions in fish were regulated by internal mechanisms within the fish and external environmental factors that trigger internal mechanisms into action. The internal factors that control the process of reproduction in fish were the brain's hypothalamus-pituitary gland chain. Hormones inducing spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. Many variables impact the ability of injected hormones to induced spawning, including condition of the fish; stage of sexual maturity; size of the fish; previous spawning history; water temperature and season of the year (Rottmann *et al.*, 1992). Pituitary of carp play an important role

in a number of reproductive processes which was most effective in accelerating maturation. Ovulation has to be provoked by hormonal stimulation. The only successful method of induced spawning was injection of purified pituitary extract (Mikolajczyk *et al.*, 2003), in which gonadotropin releasing hormone (GnRH) was a principal stimulator of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Van Der Kraak *et al.*, 1998).

Human chorionic gonadotropin (HCG) was the most common purified hormone used for induced spawning, which acts directly on the ovaries and testes (Rottmann *et al.*, 1992). Jayaprakas and Sambthu (1995) reported that the fish injected with hormone showed an increase in the growth over control. Human chorionic gonadotropin promoted better growth, while Hodson and Sullivan (1993) found that the increase in ovulation may be due to that the HCG was necessary to speed the maturation of the eggs in fish.

On the other hand, El-Bolock *et al.* (1979) was in support of using HCG, they pointed out that using fish pituitary from mature fish either of the same species or from other donor such as carp was expensive and uneconomic but HCG was cheap and available as powder at drug-stores. Moreover, two of the advantages of using HCG were that the potency was standardized in international units and therefore results can be compared among users and its purity.

HCG has been used in combination with common carp pituitary extract; the combination has shown to have improved potency than either preparation used alone. The reproduction utilizing different ovulation stimulates using various preparation of both natural and synthetic origin (Brzuska, 2003 and 2004; Akar, 2006 and Akar and Ali, 2006).

The obtained results indicated that the common carp females injected with pituitary gland extract of carp, HCG hormone and combination between them respectively were more effective than the control and pituitary gland extract of tilapia, so the pituitary gland was stimulated the reproductive performance in *Cyprinus carpio L.*; these results were in agreement with the findings of (Sharaf, 1992).

Srisakultiew and Wee (1988) and Zonneveld *et al.* (1988) recorded a successful inducing spawning when injecting intra muscular with pituitary gland extract of carp. Mikolajczyk *et al.* (2004) also reported that the ovulation was provoked by hormonal stimulation after injections of purified pituitary extract.

The results of the present study are in agreement with Rottmann *et al.* (1992); Hodson and Sullivan (1993) and Srivastava and Van Der Kraak (1994) they reported that the increase in ovulation may be due to the role of HCG which was necessary to accelerate the maturation of the egg in fish.

On the other hand, the mean fertilization and hatching percentage were higher after injection with PG of common carp in combination with HCG, in the same time increasing occurred in total weight of eggs, absolute and relative fecundity as compared with injection by HCG and PG of common carp separately, which considered as the economic and most effective in the artificial spawning of *Cyprinus carpio L.* and accompanied with significant increase on fertilization and hatching.

The results obtained showed that the successful use of inducing agents as PG of common carp, HCG and PG of common carp in combination with HCG, which were considered as the most important result presented in this work in induced ovulation of *Cyprinus carpio L.*

The present work indicated that the injection of PG of common carp combined with HCG was the most effective treatment, these results were in agreement with the findings of (Zairin *et al.*, 1992; Brzuska, 2003 and 2004; Akar, 2006 and Akar and Ali, 2006), which indicated that the use of pituitary gland of common carp in combination with HCG considered the economic and most effective in the artificial spawning of common carp (*Cyprinus carpio L.*).

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تأثير بعض المحفزات التبويضية على الكفاءة التناسلية لأسماك المبروك العادي.

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أجريت هذه الدراسة بالمفرخ السمكي بالمعمل المركزي لبحوث الثروة السمكية بالعباسة أبوحماد شرقية خلال موسمي التفريخ ٢٠٠٤ م ، ٢٠٠٥ م على أمهات أسماك المبروك العادي الدراسات التجريبية كانت تتضمن خمسة معاملات (كُلّ معاملة ٧ إناث) وذلك لتحسين أداء التناسل لأسماك المبروك العادي باستخدام الحقن بواسطة المحلول الملحي ، بمستخلص الغدة النخامية لأسماك البلطي ، بمستخلص الغدة النخامية لأسماك المبروك العادي ، هرمون الجونادوتروبين البشري ثم المعاملة الاخيرة تم حقنها بمستخلص الغدة النخامية للمبروك العادي في الجرعة الأولى والجرعة الثانية بهرمون الجونادوتروبين البشري ، والحقن كان على جرعتين الحقنة الأولى كانت ١٠ % من الجرعة الكلية و الحقنة الثانية ٩٠ % من الجرعة الكلية أما الذكور فكانت تعطى حقنة واحدة فقط (تنشيطية).

سجلت نتائج هذه الدراسة زيادة في أعداد وأوزان البيض المنتج من أمهات أسماك المبروك العادي خلال موسمي ٢٠٠٤ م ، ٢٠٠٥ في نفس الوقت، زيادة معدل الإخصاب و نسبة الفقس.

عموماً، كان هناك زيادة في التبويض ، معدل الإخصاب ونسبة الفقس لأسماك المبروك العادي عند الحقن بمستخلص الغدة النخامية لأسماك المبروك العادي ، هرمون الجونادوتروبين البشري ثم بمستخلص الغدة النخامية للمبروك العادي في الجرعة الأولى و الجرعة الثانية بهرمون الجونادوتروبين البشري. أما عند الحقن بمستخلص الغدة النخامية لأسماك البلطي و المحلول الملحي لم يعطي أي تأثير للتبويض.

أظهرت النتائج السابقة أن المعاملة التي تم حقنها بمستخلص الغدة النخامية للمبروك العادي في الجرعة الأولى ثم الحقن بهرمون الجونادوتروبين البشري أعطت أفضل النتائج عن المعاملات الاخرى. لذا يمكن التوصية باستخدام هذه المعاملة لأنها أكثر تأثيراً على التفريخ الصناعي لأسماك المبروك العادي.