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Full Length Research Paper

Effects of exogenous hormones on plasma cortisol, sex steroid hormone and glucose levels in male and female grass carp, Ctenopharyngodon idellus, during the spawning induction

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Hormonal manipulation is a common spawning technique in aquaculture. In the present study, 15 male grass carp (*Ctenopharyngodon idellus*) with mean weight of 5 kg and 30 females with mean weight of 8 kg were used. Broodstock were injected with ovaprim (salmon gonadotropin- releasing hormone analogue in combination with domperidone) and human chronic gonadotropin (HCG) with a dosage of 4 mg for female/kg BW and 2 mg for male/kg BW in 2 injections at 24 h interval. At zero time, blood samples were taken from male and females and testosterone, estradiol, cortisol and glucose levels were measured and used as control values. Administration of hormones accelerated the final oocyte maturation and induced ovulation. Plasma levels of estradiol (E2) in female grass carp increased sharply at 6 h after the first injection and decreased after the second injection. Serum testosterone (T) in males increased parallel to the plasma estradiol in females. Also, the results show significant increase in the serum cortisol and glucose values during the artificial spawning period (P<0.05).

Key words: GnRHa, human chronic gonadotropin (HCG), sex steroid, cortisol, glucose, induced spawning, grass carp.

INTRODUCTION

For sustainable cyprinidae fish production, both from the point of view of conservation programmes (Kaminski et al., 2004; Yousefian, 2011) and aquaculture production (Mikolajczyk et al., 2004; Yousefian et al., 2011), the basic requirement is to successfully manage all phases of artificial reproduction by providing a sufficient amount of fry. Many fish species reared in captivity exhibit some form of reproductive dysfunction (Peter et al., 1988; Podhorec and Kouril, 2009). In addition, some species of fish will not readily breed in captivity due to environmental or culture conditions which may cause stress or may not provide the required conditions needed to complete the reproductive process. Hormone injections are used to induce spawning in numerous fish species in aquaculture (Mylonas and Zohar, 2001; Yousefian and Mousavi, 2011). Therefore, in the initial development stages of some species with aquaculture potential, eggs are usually obtained by the capture and hormonal induction of ovulation/spermiation of broodstock. This approach commonly involves the capture and transportation of fish to holding facilities, with fish being treated with exogenous hormones sometime after capture. Few studies have investigated the effects of hormonal treatment on subsequent endocrine responses. This is likely to be a particular problem in species that are particularly sensitive to stress, where plasma levels of estradiol (E2) and serum testosterone (T) are rapidly depressed within 1 h of capture (Carragher and Pankhurst, 1991; Haddy and Pankhurst, 1999). In teleosts, cortisol is a major 'stress-related' hormone and its plasma level increases in

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response to stress (Billard and Gillet, 1981; Sumpter et al., 1986). In the field of endocrinology, working with fish and taking sample of blood to determine the biochemical composition is very important because any handling or even using anesthesia induce stress in fish and affect hormonal level in blood.

Several methods used to induce spawning and improve spermatogenesis in cultured fish are based on the injection of GTH-II from different sources, including crude extract of carp pituitary gland (CPE), partially purified fish GTH-II and mammalian GTH, especially human chorionic hormone (HCG) and a series of treatments with ovaprim, ovaplant and combination of cPG with ovaprim or HCG, were used with increased gonado-somatic index (GSI) in both male and female fish (Crim and Bettles, 1997; Zohar and Mylones, 2001; Abol-Munafi et al., 2006; Yousefian and Mousavi, 2011). Among these hormones, HCG is routinely used and is effective in cyprinid species, such as grass carp and black carp (Lin and Peter, 1996; Yousefian and Mousavi, 2011). Additionally, to facilitate the GtH releasing activity of GnRHa, especially in cyprinids, it is necessary to combine it with a dopamine receptor antagonist such as domperidone, pimozide or metoclopramide (Peter et al., 1988).

Recently, GnRH analogue and in combination with dopamine were used. Carp species with the use of GnRH analogues, together with antidopaminergic drugs (pimozide, domperidone, etc.), has been successful in previous researches (Drori et al., 1994; Sukumasavin et al., 2000; Glasser et al., 2004) and there is success in the use of GnRHa alone or in combination with dopamine antagonist in spawning induction of various fish (Peter et al., 1988; Yaron, 1995; Zohar and Mylonas, 2001; Szabo et al., 2002).

As far as the use of GnRHa or dopamine antagonists are concerned, several combinations are currently available on the market for example, Canadian preparation ovaprim (sGnRH + domperidone). In this respect, spawning of Channapunctatus was observed at 0.3 and 0.5 ml/kg body mass for ovaprim and at 2000 and 3000 IU/kg for HCG (Haniffa and Sridhar, 2002), but for *Heteropneustes fossilis*, successful spawning was observed at 0.3, 0.5 and 0.7 ml/kg body weight for ovaprim and at 1000, 2000 and 3000 IU/kg for HCG.

Plasma levels of carp gonadotropin (c-GTH) and estradiol-17 beta (E2) when fish were injected twice intraperitoneally with a total extract of carp hypophysis (0 and 6 mg/kg BW at J0 and 5 and 4 mg/kg BW at J1) are compared to those that received only saline solution. No ovulation is detected in the last group (receiving saline solution) but estradiol level has been increased respectively with exogenous c-GTH and levels are low at the end of vitellogenesis (Breton et al., 1983). Moreover, Yu et al. (1995) has shown that successful ovulation in the common carp can be achieved by a single administration of 10 µg/kg sGnRHa, combined with 20 mg/kg of the water-soluble dopamine receptor antagonist, metoclopramide (GnRH plus MET). With single injections of luteinizing-releasing hormone analogue (LHRH-a, 25 µg/kg BW), females successfully ovulated, mean egg fertilization varied between 12.5 and 37.5% and hatching ranged from 8.7 to 46.2% (Fatih et al., 2008).

In the present study, plasma levels of cortisol, sex steroids and glucose levels were measured in the grass carp (Ctenopharyngodon idellus) by using HCG and ovaprim during the hormonal spawning induction.

MATERIALS AND METHODS

The experiment was carried out at a private carp cultivation center, Sari, Iran, during the spawning season of grass carp. Sexually matured 2 to 3 years old fish were randomly selected and males (mean weight of 5 ± 0.3 kg, n = 15) and females (mean weight of 8 ± 0.5 kg, n = 30) of grass carp (*C. idellus*) were used. The fish are injected with ovaprim (Syndel Laboratories Ltd., India; 20 µg [D-Arg6,Pro9- Net] sGnRHa plus 10 mg domperidone per ml propylene glycol) and human chronic gonadotropin (HCG) (4 mg/kg BW for female and 2 mg/kg BW for male). The hormones were given at 24 h interval on two injections. Blood samples were taken from male and female fishes before injection for the determination of cortisol, estradiol (E2), testosterone (T) and glucose levels. Spawning experiments were conducted on three adult female grass carp groups. The first group served as the control group, the second group was treated with human chronic gonadotropin (HCG), while the third group was treated with ovaprim. All fish were disease-free, and sexually ripe. Brood fish were collected from spawning ground ponds in May 2011 (22 to 26°C). The fish was ripe enough for hormonal treatment. The abdomen of females was rounded and soft. Their genital opening and anus were swollen, protruding and reddish. In males, sperm were extracted just by gentle pressing at both side of male genital at the abdomen. The fish were then transported into the hatchery and placed in two polypropylene tanks, with running water of 22°C and oxygen level of 7 ± 0.8 mg/L.

The HCG and ovaprim were applied in two injection with 24 h apart. Control males and females were injected with saline. HCG was diluted in physiological saline (0.7% saline), and were prepared just before the injections. The hormones were injected, in two dosages, intraperitoneally through the ventral (bottom) part of the fish behind the pelvic fin. The preparatory dose brings the fish to the brink of spawning and the decisive dose induces ovulation. The preparatory dose was about 50% of the total dose for grass carp. Prior to injections or blood sampling, the fish were anesthetized and placed in tricaine methane-sulfonate (MS-222) bath at a concentration of 50 to 100 mg/l (Stanley, 1976).

Blood sampling

Blood samples were taken by lateral caudal vein using pre-heparinized 5 ml syringe from males and females before hormone treatments as control values (0 h) and after hormonal injection at 2, 4, 6, 8, 10 h and also after egg laying of broodstocks. Collected blood was left in order to clot at 37°C for 1 h, then centrifuged at 3000 rpm for 20 min. Serum was used for the determination of blood glucose, testosterone activity, estradiol activity and cortisol levels.

Radioimmunoassays

Concentrations of cortisol and sex steroids in the plasma samples

were determined by radioimmunoassays as described by Takahashi et al. (1985) for cortisol, Lou et al. (1985) and Shimizu et al. (1985) for testosterone (T) and estradiol-17β (E2), respectively. All samples were measured in duplicate determinations.

Glucose measurement

The glucose concentration was measured by using a kit, Glucose C2-test Wako based on an enzyme method by mutarotase and glucose oxidase (Wako Pure Chemical Ind. Ltd., Osaka) according to the instructions of the manufacturer. The linear range of standard glucose was 0.5 to 4 mg/ml.

Statistical analysis

The data were statistically analyzed using one way analysis of variance (ANOVA) (Snedecor and Cochran, 1969). Duncan's multiple range tests was used for comparing the different mean values (Duncan, 1955). Statistical significance was accepted at $P<0.05$. All data were expressed as mean ± standard error of the mean (SEM).

RESULTS

Testosterone level

Changes in serum testosterone level in male grass carp induced by ovaprim and HCG are given in Table 1. The level of testosterone in male grass carp during 24 h of the artificial spawning showed significant changes ($P<0.01$). The highest testosterone level induced by HCG was 17.3 ± 0.6 ng/ml obtained 8 h after the first injection but high level induction by ovaprim was 18.7±1.4 ng/ml obtained 10 h after the first injection.

Estradiol level

Changes in the serum estradiol level in female grass carp during 24 h of artificial spawning induced by ovaprim and HCG are shown in Table 1. The level of estradiol in female grass carp during 24 h of the artificial spawning showed significant changes ($P<0.01$). The high estradiol concentration induced by HCG was 856.5 ± 4.8 pg/ml obtained 10 h after the first injection, but high estradiole concentration induced by ovaprim was 894.4 ± 2.9 pg/ml obtained 12 h after the first injection.

Cortisol level

The changes in serum cortisol level of both male and female grass carp induced by injection of ovaprim and HCG are given in Tables 1 and 2. The highest cortisol level obtained for male grass carp after the first injection with HCG at 8 h was 12.6 ± 0.9 ng/ml and with ovaprim at 10 h was 13.2 ± 0.9 ng/ml. Also, the serum cortisol level recorded for female grass carp through hormonal induction period showed highly significant changes ($P < 0.05$) as compared to the control and the high concentration in female at 10 h was 13.2 ± 0.8 ng/ml for HCG treated group and 12.7 ± 0.5 ng/ml for ovaprim treated group.

Blood serum glucose

Changes in the serum blood glucose of male and female grass carp during 24 h of artificial spawning induced by injection of ovaprim and HCG are illustrated in Tables 1 and 2. The serum blood glucose concentration increased in male grass carp 6 h after the first injection by HCG which was 221.5 ± 0.7 mg/dl and 8 h after ovaprim was 242.2 ± 0.8 mg/dl. The highest serum blood glucose concentration was obtained for female grass carp before the second injection by HCG at 10 h (164.3 ± 1.4 mg/dl), whereas the highest concentration was obtained for ovaprim group at 10 h (170.6 ± 0.9 mg/dl).

DISCUSSION

In fish, the co-ordination between environmental stimuli and gonadotropins stimulate the secretion of FSH and LH, which regulate hormonal responses that are important for successful reproduction. These two factors are most important, because they can act, directly or through sense organs, on the glands that produce hormones, which in turn produce the appropriate physiological or behavioral responses that ultimately control the timing of spawning in fish. Therefore, understanding the manipulation of this reproductive status is an integral aspect of sustainable fisheries management. These parameters are important for an accurate evaluation of the effects of different treatments on sexual maturation in fish farming. Here in, we reported the effects of human chronic gonadotropin (HCG) and ovaprim (trade mark, salmon gonadotropin releasing hormone analogue, domperidone) on spawning profile and described changes in cortisol levels, glucose levels and sexual steroids in relation to gonadal maturation and development in male/female grass carp (*C. idellus*) during induced spawning. Among the most significant advancements in the field of aquaculture during recent decades, is the development of techniques to induce reproduction in fish using hormonal stimulation.

Gonadal development and spawning are regulated through the hypothalamus–pituitary–gonadal and hepatic axis, and thus leading to successful reproduction (Zohar et al., 2010). In addition, teleost gonadal steroids are known to modulate both the synthesis and release of GtHs (these were recently designated FSH and LH) by the pituitary and influence several brain functions that are apparently responsible for gender-specific differences in the regulation of hypothalamus–pituitary–gonadal (HPG) axis. Thus, gametogenesis in fish is regulated by the

Table 1. Testosterone, glucose and cortisol changes in male grass carp in 24-h artificial spermiation induced by using ovaprim and HCG hormones (Mean±S.E.M).

Parameter	0 h (Time of first injection)	2 h	4 h	6 h	8 h	10 h
Testosterone (ng/ml) in HCG treated group	5.9±1.6^{e}	8.7±1.3^{d}	11.6±0.8^{c}	14.7±0.6^{b}	17.3±0.6^{a}	16.3±1.5^{a}
Testosterone(ng/ml) in ovaprim treated group	5.9±1.5^{f}	7.8±1.7^{e}	9.5±0.4^{d}	12.7±1^{d}	16.3±0.5^{b}	18.7±1.4^{a}
Glucose (mg/dl) in HCG treated group	95.3±0.5^{l}	130.3±1.3^{i}	176.3±0.9^{f}	221.5±0.7^{a}	217.6±0.8^{b}	205.5±0.5^{c}
Glucose (mg/dl) in ovaprim treated group	94.8±0.9^{l}	140.±1.7^{i}	178.6±1^{e}	237.3±1^{b}	242.2±0.8^{a}	218.7±0.4^{c}
Cortisol (ng/ml) in HCG treated group	5.7±0.7^{c}	6.9±0.8^{c}	7.9±1.3^{b}	9.3±0.9^{b}	12.6±0.9^{a}	9.6±0.8^{b}
Cortisol (ng/ml) in ovaprim treated group	5.3±0.8^{d}	5.9±0.5^{d}	7.3±0.3^{c}	8.8±0.8^{b}	10.3±1^{b}	13.2±0.9^{a}
Parameter	**12 h (Time of second injection)**	**14 h**	**16 h**	**18 h**	**20 h**	**24 h (Time of ovulation)**
Testosterone(ng/ml) in HCG treated group	16.2±1^{a}	14.6±2.2^{b}	14.1±1.3^{b}	13.6±0.8^{b}	9.8±1.3^{c}	7.4±1.5^{d}
Testosterone(ng/ml) in ovaprim treated group	18.5±0.6^{a}	16.8±1.3^{b}	14.8±1.4^{c}	14.8±0.4^{c}	11.9±1.5^{c}	9.7±1.1^{d}
Glucose (mg/dl) in HCG treated group	191±0.9^{d}	182.4±1.2^{e}	172±1.2^{g}	157.5±1^{h}	129±1.2^{j}	112.5±0.7^{k}
Glucose (mg/dl) in ovaprim treated group	198.5±0.4^{d}	175±1.4^{f}	169.5±1.6^{g}	148.4±0.9^{h}	132.7±1.5^{j}	121.7±0.9^{k}
Cortisol (ng/ml) in HCG treated group	9.1±0.7^{b}	8.7±0.7^{b}	7.8±1.2^{b}	6.9±0.9^{c}	6.6±1.2^{c}	6.2±0.8^{c}
Cortisol (ng/ml) in ovaprim treated group	12.8±0.8^{a}	10.4±0.6^{b}	9.6±0.9^{b}	7.4±0.9^{c}	6.9±0.9^{c}	5.9±1.4^{d}

Mean with the same letter for each parameter are not significantly different, highly significant differences between groups ($p < 0.01$). One-way ANOVA followed by Duncan´s post-hoc test was used.

pituitary FSH and LH and by sex steroids (Yaron et al., 2003). Based on established theories, environmental factors (example photoperiod and water temperature) provide necessary cues that are perceived by the central nervous system (CNS) that initiate the oocyte developmental processes. In response, gonadotropin-releasing hormone (GnRH) is secreted from hypothalamus, which in turn stimulates the release of FSH and LH from the pituitary. During the breeding season, a strong increase in the blood levels of several different hormones can be seen due to the activities of gonadotropic hormones, especially the LH (Schulz and Miura, 2002).While FSH is mainly involved in the vitellogenic process, LH plays a role in final oocyte maturation and ovulation (Nagahama, 1994; Pham et al., 2010). In accordance, plasma levels of FSH show an extended increase during vitellogenesis, while plasma levels of LH remain low throughout vitellogenesis and are elevated dramatically during spawning (Nagahama, 1994; Pham et al., 2010). The endocrine control of oocyte maturation has also been thoroughly studied in several teleost

Table 2. Some serum biochemical changes of female grass carp in 24-h artificial hatchery induced by using ovaprim and HCG hormones (Mean ± S.E.M).

Parameter	0 h (Time of first injection)	2 h	4 h	6 h	8 h	10 h
Estradiol(pg/ml) in HCG treated group	488.4±6.7^{j}	512.3±3.4^{i}	600.2±6.7^{f}	718.7±5.4^{c}	800.4±8.4^{b}	856.5±4.8^{a}
Estradiol (pg/ml) in ovaprim treated group	467.8±8.3^{i}	545.2±3.9^{g}	588.7±6.9^{f}	710.5±7.8^{d}	776.4±5.5^{c}	886.3±6.4^{b}
Glucose (mg/dl) in HCG treated group	96.9±1.6^{k}	114.8±1.3^{j}	121.4±0.7^{i}	135.8±0.7^{f}	153.4±0.6^{c}	164.3±1.4^{a}
Glucose (mg/dl) in ovaprim treated group	91.9±1.5^{j}	110.5±1.5^{i}	132.4±0.7^{f}	143.4±0.7^{e}	162.5±0.9^{b}	170.6±0.9^{a}
Cortisol (ng/ml) in HCG treated group	5.4±1.3^{d}	8.2±0.7^{c}	9.1±0.8^{c}	10.2±1.4^{b}	12.5±0.8^{a}	13.2±0.8^{a}
Cortisol (ng/ml) in ovaprim treated group	5.8±0.6^{g}	7.4±0.9^{f}	8.7±0.8^{d}	10.2±0.8^{c}	11.9±0.6^{b}	12.7±0.5^{a}
Parameter	**12 h (Time of second injection)**	**14 h**	**16 h**	**18 h**	**20 h**	**24 h (Tim of ovulation)**
Estradiol (pg/ml) in HCG treated group	798.6±4.5^{b}	723.7±7.6^{c}	687.5±8.4^{d}	610.3±4.5^{e}	585.3±5.3^{g}	523.6±4.3^{h}
Estradiol (pg/ml) in Ovaprim treated group	894.4±2.9^{a}	777.7±8.0^{c}	721.4±7.7^{d}	658.2±8.3^{e}	591.8±9.2^{f}	534.7±6.4^{h}
Glucose (mg/dl) in HCG treated group	159.9±1.6^{b}	149.7±2.2^{d}	144.9±1.8^{e}	133.8±1.7^{g}	126.6±0.4^{h}	116.8±0.3^{j}
Glucose (mg/dl) in Ovaprim treated group	169.6±1^{a}	160.2±1.5^{b}	159±1.3^{c}	146.9±1.2^{d}	129.7±0.8^{g}	120.1±0.8^{h}
Cortisol (ng/ml) in HCG treated group	12.9±1.6^{a}	12.5±1.8^{a}	12.1±0.9^{a}	11.2±1^{b}	8.8±1.6^{c}	7.3±0.9^{c}
Cortisol (ng/ml) in Ovaprim treated group	14±0.9^{a}	12.7±0.4^{a}	12.0±0.5^{b}	11.8±0.5^{b}	9.6±0.4^{c}	8.2±0.8^{e}

Mean with the same letter for each parameter are not significantly different, highly significant differences between groups ($p < 0.01$). One-way ANOVA followed by Duncan´s post-hoc test was used.

species where it has been established that oocyte maturation in fish is regulated by three main hormonal mediators, GtHs, maturation-inducing hormone (MIH) and maturation-promoting factor (MPF) (Nagahama, 1994; Nagahama, 1997). In accordance with the hormonal control of oocyte growth,GtH (probably LH) secreted from the pituitary stimulates the production of MIH in the granulosa cells. In the present study, we used

GnRHa and HCG to induce spawning and enhance reproductive performance in grass carp. An interesting observation was that these exo-genous gonadotrophs produced different effects on plasma sex steroid hormone levels, cortisol and blood glucose levels, furthermore, they accelerated spawning rate. Particularly, for sex steroid hormones, GtHs are known to have broad range activities that include regulation of ovarian steroid-genesis by follicular cells. In the present study, maximum effects were observed over time for plasma T and E2 levels, and they were differentially affected by the HCG and ovaprim (trade mark) treatment and these effects varied with time. The effects of GnRHa and HCG on gonadal steroid production is in accordance with the increasing evidence that GtHs may regulate several aspects of ovarian and testis development and function by several steroid-dependent and independent actions (Planas et al., 2000; Pham et al., 2010).

In the present study, the highest testosterone level induced in male grass carp by HCG and ovaprim were obtained 8 to 10 h after the first injection. These results are in agreement with other studies, indicating improve-ment in the spermatogenesis with a series of treatment hormone of ovaprim, ovaplant, HCG, cPG and combination of cPG with ovaprim or HCG (Abol-Munafi et al., 2006; Yu et al., 2005) in white silver carp (*Hypophthalmichthys molitrix*). On the other hand, Yaron (1995) stimulated the sperm duct using gonadotropin, or 17,20-dihydroxy-4-pregnen-3-one (17,20-P) in white bass (*Morone chrysops*) exposed to an increase temperature and treated with a gonadotropin-releasing hormone antagonist (GnRHa) enhancing milt production in white bass (Mylonas et al., 1997) and in bass (*Dicentrarchuslabrax L.*) (Rodríguez et al., 2001). In female fish, the high estradiol level induced by HCG and ovaprim was obtained 10 to 12 h after the first injection. Similar results were obtained in female European sea bass (*Dicentrarchuslabrax* L.) by Prat et al. (2001). In contrast, both HCG and GnRHa were effective in elevating spermiated fish and this is similar to the response shown by greenback flounder (*Rhombosoleatapirina*) (Lim et al., 2004).

Administration of GnRHa alone or in combination with PIM accelerated the final oocyte maturation (FOM) and induced spawning. Plasma levels of 17-estradiol (E2) increased sharply at 12 h after the first injection of GnRHa alone or combined with PIM, but it decreased after the second injection of GnRHa alone (Miwa et al., 2001). Steroid levels treated grass carp groups with HCG was significantly increased to 6 h after treatment, followed by a rapid decline at 12 h. Plasma E2 levels increased significantly after 2 h, in response to GnRHa injection, and peaked after 12 h. A similar increase in E2 levels from post vitellogenic oocytes before final oocyte maturation was also reported in the carp (Levavi-Zermonsky and Yaron, 1986), long finned eels (*Anguilla dieffenbachia*) (Lokman et al., 2001) and gilthead seabream (Gothilf et al., 1997).

Pituitary extracts were the most potent steroid that induced ovulation in grass carp. Also, Szabo et al. (2002) found that female *Chondrostomanasus* (Cyprinidae) receiving pituitary extract injection at lower doses of 3 mg/kg BW ovulated partially. Spotted seabass (*Lateolabrax maculatus*) serum estradiol-17 (E2) level increased in September, reached their highest levels in October and early November, and then decreased in mid November and in late November ($P<0.01$) (Lee and Yang**,** 2002). In the present study, HCG and Ovaprim were the most potent steroid that induced ovulation in grass carp.

The cortisol level was increased in male and female grass carp after treatment with ovaprim and as compare to HCG which was markedly enhanced. There was significant change in cortisol level in both ovaprim and HCG as compared to the control ($P<0.05$). These results are in agreement with the work of Kubokawa et al. (1999) who studied sockeye salmon (*Onchorynchusnerka*). Tanck et al. (20l01) also stated that plasma cortisol levels is the main stress hormone in fish, it increases during the spawning period. In their study, the cortisol levels were higher in females than in males. In common carp (*Cyprinus carpio* L.) using androgenetic progeny groups stimulated plasma cortisol and lactate concentrations (Kime and Dolben, 1985). Plasma cortisol of *C. carpio*, during ovulation was induced by carp pituitary extract. Cortisol appeared to be stimulated by the priming dose of pituitary extract and fell rapidly after ovulation. In goldfish, plasma gonadotropin levels increase during spawning in both males and females (GTH surge) (Kobayashi et al., 1997). From the obtained results, it can be concluded that injection of ovaprim and HCG during artificial spaw-ning showed biochemical and physiological changes.

The increase of serum glucose concentration was higher in ovaprim than HCG and higher in male than female. The variation in increase cortisol and glucose levels was observed by Kime and Dolben (1985) who reported increased plasma glucocorticoides of *C. carpio*, during ovulation induced by carp pituitary extract. Levels of glucose fell rapidly after ovulation of *Anguilla japonica*. With common carp (*C. carpio* L.) (Tanck et al., 2001), sockeye salmon (Onchorynchus nerka) (Kubokawa et al., 1999), this study concluded that ovaprim and HCG induced spawning and some biochemical responses in males and females grass carp in artificial hatchery.

Conclusion

The present study shows that the injections of HCG and ovaprim to grass carp broodstock elevated levels of testosterone, estradiol, cortisol and glucose significantly ($P<0.05$) after 2 h of first injection and peaked after 10 to 12 h of first injections in both hormonal injections (HCG and ovaprim). Furthermore, sex steroids, cortisol and

blood glucose significantly declined after 12 h of second injection in both hormonal treatments. In conclusion, both hormonal treatments used in the present study were effective in spawning induction of male and female grass carp broodstocks.

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