

Some Biochemical Changes Associated with Injection of Grass Carp (*Ctenopharyngodon idellus*) with Oviaprim and Pregnyl for Induction of Artificial Spawning

M.A.A. Metwally and I.M. Fouad

Department of Reproductive Physiology, Central Laboratory for Aquaculture Research,
Agriculture Research Centers, Abbassa, Egypt

Abstract: Hormonal injection is a common spawning technique in aquaculture season 18 males of grass carp (*Ctenopharyngodon idellus*) with mean weight of 5 kg and 36 females with mean weight of 8 kg were used. The fish are injected with Ovaprim (Salmon gonadotropin releasing hormone analog, dopamine antagonist and domperidone) and Pregnyl (Human chronic gonadotropin, HCG) (4 mg for female and 2 mg for male /kg BW), 2 injections were given 24hrs a part, either first or second injection. At zero time blood samples were taken from male and females before injection and some biochemical parameters were measured and used as control values. Administration of hormones accelerated the final Oocyte maturation and percent of ovulation. Plasma levels of Estradiol (E2) in female grass carp increased sharply at 6 hrs after the first injection of the two hormones, and it decreased after the second injection. Plasma Testosterone (T) in males increased parallel to the plasma Estradiol in females. Also the results showed significant increase in serum Cortisol, glucose and total protein values during the time of artificial spawning.

Key words: Grass carp • Spewing hormone • Oviaprim • Pregnyl

INTRODUCTION

Some species of fish will not readily bred 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 [1]. To improve the spermatogenesis a series of treatments with single hormone of Ovaprim, Ovaplant, human chronic gonadotropin (hCG), Carp pituitary (cPG) and combination of cPG with Ovaprim or hCG, were used with increased gonado somatic index (GSI) in both male and female fishes [2]. Spawning of *Channa punctatus* was observed at 0.3 and 0.5 ml kg⁻¹ body mass for Ovaprim and at 2000 and 3000 IU/kg for hCG [3], but for *Heteropneustes fossilis* successful spawning was observed at 0.3, 0.5 and 0.7 ml kg⁻¹ body mass 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 injected twice intraperitoneal with a total extract of Carp hypophysis (0, 6 mg kg⁻¹ Bw at Jo and 5, 4 mg kg⁻¹ BW at J1) are

compared to a lot receiving only saline solution. No ovulation is detected in the last group (receiving saline solution), but Estradiol level increases with exogenous c-GTH, and levels are low at the end of vitellogenesis [4]. Yu *et al.* [5] successful ovulation in the common carp can be achieved by a single administration of 10 µg/kg sGnRH_a, combined with 20 mg kg⁻¹ of the water-soluble dopamine receptor antagonist, metoclopramide (GnRH+MET). Single injections of luteinizing-releasing hormone analog (LHRH-a; 25 µg kg⁻¹). Females successfully ovulated; mean egg fertilization varied between 12.5 and 37.5%, hatching ranged from 8.7 to 46.2% [6]. European silver eels were stimulation by GnRH_a (Gonadotropin-Releasing Hormone agonist), females and males showed a two- to three-folds higher LHβ (luteinising hormone β subunit) [7].

This investigation aimed to study hormonal and some biochemical changes during artificial spawning in grass carp (*Ctenopharyngodon idellus*) by using pregnyl (Human chronic gonadotropin, HCG) and Ovaprim (salmon gonadotropin releasing hormone analog, dopamine antagonist, and domperidone).

MATERIALS AND METHODS

Hormones:

- Pregnyl chronic gonadotropin, HCG, produced by the Nile Co. for Pharmaceuticals and Chemicals Industries, Cairo, Egypt. Under License of Organon. Oss Holland.
- Ovaprim (Salmon gonadotropin releasing hormone analog, dopamine antagonist, and domperidone), obtained from Syndel, Laboratories, Ltd., Vancouver, British Columbia.

Broodstock: During spawning season at May 20 males (Mean weight of 5 kg) and 10 female (Mean weight of 8 kg) of grass carp (*Ctenopharyngodon idellus*) were used. The fish are injected with Ovaprim and Pregnyl (4 mg kg⁻¹ BW for female and 2 mg kg⁻¹ BW for male), the hormones were given through 24hrs apart on two injections. Blood samples were taken from male and female fishes before injection (Zero time) for determination of some biochemical constituents.

Spawning experiments were conducted on three adult female grass carp groups (each contained 12fish with average body weight of 8 kg); the first group served as the control group, the second group treated by Pregnyl, while the third group treated with Ovaprim. Males with in an average body weight 5 kg (N=15) were used. All fish were disease free, and sexually ripe. This experiment takes places in artificial fish hatchery near, Central Laboratory for Aquaculture Research. Brood fish were collected from spawning ground ponds in May 2005 (22-26°C). There are several indicators of ripeness: In females; the abdomen is rounded and soft. The genital opening is swollen, protruding, and reddish. The anus is often also swollen and reddish. Secondary sexual characteristics are evident [8]. In males; Milt is released when the abdomen is pressed gently. Secondary sexual characteristics are evident [9]. After capture, fish were transported into the hatchery and placed in two polypropylene tanks, with running water, water quality parameters, such as temperature and oxygen kept at optimal levels. The fish were allowed to acclimation for period of one week.

Injecting the Fish: The injections of pregnyl and Ovaprim (4 mg kg⁻¹ Bw for female and 2 mg kg⁻¹ BW for male) were given through 24hrs apart on two injections. Control males and females were injected with saline. The hormones were injected into a fish; intraperitoneal through the ventral (bottom) part of the fish behind either

the pelvic or pectoral fin. Two dosage levels are commonly used: a preparatory dose and a decisive, or final, dose with a time gap generally of 12 to 24 hours between the two injections. The preparatory dose brings the fish to the brink of spawning and the decisive dose induces ovulation. The preparatory dose was about 50 percent of the total dose for grass carp. The fish were anesthetized and placed in MS-222 at a concentration of 50-100 mg l⁻¹ [10].

Biochemical Analysis: Blood samples were collected directly from the lateral caudal vein from males and females before hormone (pregnyl and Ovaprim) injection as control values(0 hrs) and after hormone injection at 2, 4, 6, 8, 10 hours and also after second injection 2, 4, 6, 8, 10 hours and after egg lying. Collected blood left to clot at 37°C for 1 hour, centrifuged at 3000 r. p. m for 20 minutes serum samples were used for determination of blood glucose, total protein, testosterone activity and estradiol activity.

Blood glucose was estimated by using glucose oxidase method [11]. Total protein in serum was determined colormetrically [12].

Testosterone Level: The testosterone level in serum was determined using kit from Bio- Merieux (VIDAS) which is an automated quantitative test for use on the VIDAS analyzer for the quantitative measurement of testosterone in serum using the ELFA technique.

Estradiol II (E211): The 17 β Estradiol concentration in serum was determined using Bio- Merieux kit using the ELFA technique.

Statistical Analysis of the Results: The obtained data were statistically analyzed using one way analysis of variance (ANOVA) [13]. Duncan's multiple range tests was used for comparing the different mean values [14].

RESULTS

Testosterone level: Changes in serum testosterone level in male grass carp during 24 hrs of artificial spawning induced by Ovaprim and Pregnyl are shown in Table 1. the level in male grass carp during the artificial spawning showed significant (P=0.01) changes. The highest testosterone level induced by Pregnyl was 17.33±0.65 ng ml⁻¹, obtained 8hrs after the first injection but high level induced by Ovaprim was 18.87±1.25 ng ml⁻¹ obtained 10 hrs after the first injection.

Table 1: Some serum biochemical changes in male grass carp through 24-hrs of artificial hatchery induced by using of Ovaprim and Pregnyl hormones (Mean±SE)

| Parameters | 0 h (before 1 st | 2h | 4h | 6h | 8h | 10 h (before 2 nd |
|--|-----------------------------|--------------|--------------|--------------|--------------|------------------------------|
| | (injection) | | | | | (injection) |
| Testosterone (ng ml ⁻¹) in Pregnyl treated group | 5.95±1.56e | 8.69±1.27d | 11.58±0.85c | 14.66±0.58b | 17.33±0.65a | 16.27±1.52a |
| Testosterone (ng ml ⁻¹) in Ovaprim treated group | 5.95±1.56f | 7.95±1.88e | 9.48±0.55d | 12.76±0.83d | 16.38±0.43b | 18.87±1.25a |
| Cortisol (ng ml ⁻¹) in Pregnyl treated group | 5.72±0.69c | 6.82±0.87c | 7.93±1.29b | 9.23±0.83b | 12.52±0.86a | 9.58±0.74b |
| Cortisol (ng ml ⁻¹) in Ovaprim treated group | 5.29±0.75d | 5.95±0.49d | 7.24±0.33c | 8.83±0.77b | 10.35±0.99b | 13.16±0.89a |
| Total protein (gm/dl) in Pregnyl treated group | 3.66±0.55d | 5.87±0.86b | 7.32±0.96a | 8.64±0.43a | 7.83±0.39a | 5.97±0.74b |
| Total protein (gm/dl) in Ovaprim treated group | 3.83±0.47c | 5.42±0.69b | 7.80±0.43a | 8.25±0.72a | 8.74±0.56a | 7.33±0.59a |
| Blood sugar (mg dl ⁻¹) in Pregnyl treated group | 95.22±0.56l | 130.15±1.24i | 175.32±0.95f | 220.54±0.78a | 218.64±0.69b | 205.43±0.56c |
| Blood sugar (mg dl ⁻¹) in Ovaprim treated group | 94.89±0.78l | 140.00±1.65i | 178.67±0.88e | 238.26±0.93b | 241.24±0.83a | 218.65±0.37c |
| Parameters | 12 h (after 2 nd | 14h | 16h | 18h | 20h | 24h after |
| | (injection) | | | | | ovulation |
| Testosterone (ng ml ⁻¹) in Pregnyl treated group | 16.11±0.99a | 14.54±2.16b | 14.12±1.24b | 13.56±0.76b | 9.76f±1.22c | 7.41±1.44d |
| Testosterone (ng ml ⁻¹) in Ovaprim treated group | 18.45±0.63a | 16.81±1.25b | 14.82±1.35c | 14.77±0.36c | 11.86f±1.52c | 9.65±1.12d |
| Cortisol (ng ml ⁻¹) in Pregnyl treated group | 9.04±0.69b | 8.66±0.73b | 7.79±1.22b | 6.83±0.94c | 6.64±1.18c | 6.11±0.88c |
| Cortisol (ng ml ⁻¹) in Ovaprim treated group | 12.86±0.79a | 10.44±0.55b | 9.55±0.98b | 7.33±0.92c | 6.96±0.88c | 5.88±0.97d |
| Total protein (gm/dl) in Pregnyl treated group | 5.83±1.23b | 4.96±0.79c | 4.78±0.83c | 4.37±0.45c | 4.19±0.85c | 3.98±1.31 d |
| Total protein (gm/dl) in Ovaprim treated group | 6.75±0.84a | 4.44±0.93b | 4.12±0.94b | 4.00±0.59b | 3.98±0.73c | 3.75±0.75c |
| Blood sugar (mg dl ⁻¹) in Pregnyl treated group | 190.96±0.89d | 182.34±1.10e | 171.99±1.14g | 157.56±0.96h | 128.96±1.22j | 112.47±0.66k |
| Blood sugar (mg dl ⁻¹) in Ovaprim treated group | 198.45±0.38d | 174.68±1.42f | 169.54±1.65g | 148.39±0.84h | 132.67±1.43j | 121.74±0.89k |

Mean with the same letter for each parameter are not significantly different, highly significant differences between groups (p < 0.01)

Estradiol Level: Changes in the serum Estradiol level in female grass carp during 24 hrs of artificial spawning induced by Ovaprim and Pregnyl are shown in Table 1. The level in female grass carp during the artificial spawning showed significant (P=0.01) changes. The high Estradiol concentration induced by Pregnyl was 855.54±5.4 pg ml⁻¹ obtained 10 hrs after the first injection, but high Estradiole concentration induced by Ovaprim was 894.42±2.9 pg ml⁻¹ obtained 12 hrs after the first injection.

Cortisol level: The changes in serum cortisol level of both male and female grass carp during 24hrs of artificial spawning induced by injection of Ovaprim and Pregnyl are shown in Tables 1and, 2.

The highest cortisol level obtained for male grass carp after the first injection with pregnyl at 8 hrs was 12.52±0.86 ng ml⁻¹. whereas; highest cortisol level obtained for male grass carp after the first injection with Ovaprim at 10 hrs was 13.16±0.89 ng ml⁻¹. Also the serum cortisol level recorded for female grass carp through

Table 2: Some serum biochemical changes of female grass carp through 24-hrs of artificial hatchery induced by using of Ovaprim and Pregnyl hormone (Mean + SE)

| Parameters | 0 h (before 1 st | 2h | 4h | 6h | 8h | 10 h (before 2 nd |
|---|-----------------------------|--------------|--------------|--------------|--------------|------------------------------|
| | (injection) | | | | | (injection) |
| Estradiol (pg ml ⁻¹) in Pregnyl treated group | 489.42±6.1j | 514.33±3.4i | 600.17±6.5f | 716.72±5.7c | 800.27±8.2b | 855.54±5.4a |
| Estradiol (pg ml ⁻¹) in Ovaprim treated group | 467.89±8.2i | 545.24±3.9g | 588.95±6.8f | 710.47±7.7d | 776.34±5.5c | 886.27±6.4b |
| Cortisol (ng ml ⁻¹) in Pregnyl treated group | 5.44±1.25d | 8.20±0.76c | 9.14±0.79c | 10.15±1.37b | 12.43±0.77a | 13.16±0.82a |
| Cortisol (ng ml ⁻¹) in Ovaprim treated group | 5.86±0.58g | 7.48±0.95f | 8.79±0.86d | 10.10±0.85c | 11.98±0.59b | 12.79±0.43a |
| Serum total protein (gm/dl) in Pregnyl treated group | 3.50±0.92b | 3.89±0.95b | 5.35±0.89a | 6.10±0.35a | 6.97±0.72a | 7.38±0.56a |
| Serum total protein (gm/dl) in Ovaprim treated group | 3.22±0.88e | 3.95±0.76d | 4.96±0.79c | 5.81±0.66c | 6.58±0.93b | 7.83±0.77a |
| Serum blood sugar (mg dl ⁻¹) in Pregnyl treated group | 96.97±1.66k | 114.81±1.22j | 121.36±0.72i | 135.81±0.74f | 153.37±0.55c | 165.32±1.38a |
| Serum blood sugar (mg dl ⁻¹) in Ovaprim treated group | 91.99±1.42j | 110.54±1.55i | 132.12±0.88f | 143.43±0.81e | 162.41±0.93b | 170.66±0.89a |
| Parameters | 12 h (after 2 nd | 14h | 16h | 18h | 20h | 24h after |
| (injection) | (injection) | | | | | ovulation |
| Estradiol (pg ml ⁻¹) in Pregnyl treated group | 798.53±4.4b | 723.75±7.6c | 687.45±8.4d | 610.35±4.5e | 585.34±5.3g | 523.51±4.4h |
| Estradiol (pg ml ⁻¹) in Ovaprim treated group | 894.42±2.9a | 777.77±8.1c | 721.37±7.7d | 658.26±8.3e | 591.72±9.1f | 534.77±6.4h |
| Cortisol (ng ml ⁻¹) in Pregnyl treated group | 12.92±1.56a | 12.48±1.74a | 12.10±0.88a | 11.16±0.94b | 8.75±1.55c | 7.38±0.88c |
| Cortisol (ng ml ⁻¹) in Ovaprim treated group | 13.98±0.89a | 12.77±0.44a | 12.00±0.49b | 11.82±0.48b | 9.55±0.42c | 8.22±0.86e |
| Serum total protein (gm/dl) in Pregnyl treated group | 6.80±0.28a | 6.23±0.96a | 6.18±0.58a | 5.75±0.23a | 4.52±0.35b | 4.29±0.42b |
| Serum total protein (gm/dl) in Ovaprim treated group | 7.98±0.55a | 6.83±0.75b | 6.36±0.44b | 5.40±0.66c | 4.68±0.42c | 3.98±0.83d |
| Serum blood sugar (mg dl ⁻¹) in Pregnyl treated group | 159.92±1.62b | 149.75±2.16d | 144.85±1.73e | 133.75±1.61g | 126.54±0.34h | 116.83±0.25j |
| Serum blood sugar (mg dl ⁻¹) in Ovaprim treated group | 169.62±0.99a | 160.23±1.45b | 158.92±1.24c | 146.89±1.14d | 129.67±0.74g | 120.14±0.81h |

Mean with the same letter for each parameter are not significantly different, highly significant differences between groups (p < 0.01).

artificial hatchery showed highly significant (P=0.01) changes compared to control and the high concentration in female at 10hrs was 13.16±0.82 ng ml⁻¹.

Total Protein: The changes in serum total protein of male and female grass carp during 24 hrs of artificial spawning induced by injection of Ovaprim and Pregnyl are illustrated in Table 1 and 2. The total proteins showed significant (P=0.01) increase in male grass carp after

injection with Ovaprim and Pregnyl. The highest concentration was observed 8 and 6 hrs after the first injection (8.74±0.56 and 8.64±0.43 g dl⁻¹, respectively).

In female, serum total protein concentration showed significant (P<0.01) changes in Ovaprim and Pregnyl groups as compared to the control group. The highest concentration was observed in fish injected by Ovaprim after 12hrs 7.98±0.55 g dl⁻¹ and for Pregnyl after 10hrs 7.38±0.56 g dl⁻¹.

Blood Serum Glucose: Changes in the serum blood glucose of male and female grass carp during 24 hrs of artificial spawning induced by injection of Ovaprim and Pregnyl are illustrated in Table 1 and 2.

The serum blood glucose concentration increased in male grass carp 6 hrs after the first injection by Pregnyl 220.54 ± 0.78 mg dl⁻¹ and 12 hrs after Ovaprim 241.24 ± 0.83 mg dl⁻¹.

The highest serum blood glucose concentration was obtained for female grass carp before the second injection by Pregnyl at 10hrs (165.32 ± 1.38 mg dl⁻¹). Whereas the highest concentration was obtained for Ovaprim group at 10 hrs (170.66 ± 0.89 mg dl⁻¹).

DISCUSSION

This study was designed to investigate the effects of Pregnyl (Human chorionic gonadotropin, HCG) and Ovaprim (Salmon gonadotropin releasing hormone analog, dopamine antagonist, and domperidone) on some biochemical changes during artificial spawning in grass carp (*Ctenopharyngodon idellus*).

The highest testosterone level induced in male grass carp by Pregnyl and Ovaprim were obtained 8-10 hrs after the first injection. These results were in line with other studies indicated improving the spermatogenesis with a series of treatment hormone of Ovaprim, Ovaplant, HCG, cPG and combination of cPG with Ovaprim or HCG [2, 5] in white silver carp (*Hypophthalmichthys molitrix*). On the other hand, Zvi [15] stimulation of the sperm duct by gonadotropin, or by 17, 20 -dihydroxy-4-pregnen-3-one (17,20-P) in common carp, in white bass (*Morone chrysops*) were exposed to an increase in temperature and treated with a gonadotropin-releasing hormone antagonist (GnRHa) enhancing milt production in white bass [16] and on bass (*Dicentrarchus labrax* L.) [17].

In female fish, the high Estradiol level induced by Pregnyl and Ovaprim obtained 10-12 hrs after the first injection. Similar results were obtained in female Korean spotted sea bass (*Lateolabrax maculatus*) by Francisco *et al.* [18]. Administration of GnRHa alone or combined with PIM accelerated the final oocyte maturation (FOM) and induced spawning. Plasma levels of 17-estradiol (E₂) increased sharply at 12 hrs after the first injection of GnRHa alone or combined with PIM, but it decreased after the second injection of GnRHa alone [19]. Steroid levels in the hormone treated grass carp groups with HCG was significantly increased to 6 hrs after treatment, followed by a rapid decline at 12 hrs. Pituitary extracts were the most potent steroid induces ovulation in grass carp. Also, Tamás *et al.* [20] found that female (*Chondrostoma nasus*,

Cyprinidae) receiving pituitary extract injection at lower doses of 3 mg kg⁻¹ BW ovulated partially. spotted sea bass (*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) [21]. In the present study, Pregnyl and Ovaprim were the most potent steroid induces ovulation in grass carp.

The highest Cortisol level obtained for male grass carp after the first injection with Pregnyl at 8hrs was 12.52 ± 0.86 ng ml⁻¹. Whereas highest Cortisol level obtained for male grass carp after the first injection with Ovaprim at 10 hrs was 13.16 ± 0.89 ng ml⁻¹. Also Cortisol level obtained for female grass carp induced by injection of Pregnyl showed highly significant changes compared to control, and the high level in female at 10hrs was 13.16 ± 0.82 ng ml⁻¹. Whereas the serum Cortisol level obtained for female grass carp through artificial hatchery induced by injection of Ovaprim showed highly significant changes compared to control, and the high level in female at 12 hrs was 13.98 ± 0.89 ng ml⁻¹. Cortisol increase during many metabolic processes like reproduction, this results are in agreement with [22] sockeye salmon (*Onchorynchus nerka*), plasma level of cortisol, is the main stress hormone in fish, increases during the spawning period and Cortisol levels were higher in females than in males [23]. In common carp (*Cyprinus carpio* L.) using androgenetic progeny groups were stimulate plasma cortisol, and lactate concentrations [24]. Plasma Cortisol of *Cyprinus carpio*, during ovulation induced by carp pituitary extract. Cortisol appeared to be stimulated by the priming dose of pituitary extract and fell rapidly after ovulation.

The total proteins were significantly different in male grass carp after injection with Ovaprim and Pregnyl compared to control fish, the high concentration showed after the first injection at 8hrs 8.74 ± 0.56 g dl⁻¹ and at 6 hrs, 8.64 ± 0.43 g dl⁻¹, respectively. But serum total protein concentration obtained for female grass carp through artificial hatchery induced by injection of Ovaprim and Pregnyl showed highly significant changes compared to control, and the high concentration in fish injected by Ovaprim noticed at 12hrs 7.98 ± 0.55 g dl⁻¹. Whereas, the total protein in fish injected by Pregnyl showed after 10 hrs was 7.38 ± 0.56 g dl⁻¹, respectively, these results are in agreements with [25] seasonal changes in the mRNA levels of glycoprotein alpha, gonadotropin (GTH) and thyrotropin (thyroid-stimulating hormone (TSH)) subunits in the pituitary of goldfish during spawning [26]. In goldfish, plasma gonadotropin levels increase during spawning in both males and females (GTH surge). From

the obtained results, it can be concluded that, injection of Ovaprim and Pregnyl during artificial spawning showed biochemical and physiological changes.

The serum glucose concentration increased for male grass carp after the first injection by Pregnyl at 6 hrs (220.54 ± 0.78 mg dl⁻¹), but the concentration induced by Ovaprim (241.24 ± 0.83 mg dl⁻¹) at 12hrs. Also the highest serum blood glucose concentration obtained for female grass carp before the second injection by pregnyl at 10 hrs was 165.32 ± 1.38 mg dl⁻¹. Whereas, the highest serum blood glucose concentration obtained for female grass carp injected by Ovaprim at 10hrs was 170.66 ± 0.89 mg dl⁻¹. This result is in agreement with Kime [24] who reported increased plasma glucocorticoides of *Cyprinus carpio*, during ovulation induced by carp pituitary extract, stimulate an increase cortisol and glucose levels showed large variations. Levels of glucose fell rapidly after ovulation *Anguilla japonica*. In common carp (*Cyprinus carpio* L.) [23]. Sockeye salmon (*Onchorynchus nerka*) [22], this study concluded that Ovaprim and Pregnyl induced spawning and some biochemical responses in males and females grass carp in artificial hatchery.

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