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ORIGINAL ARTICLE

Reproductive, hematological, and biochemical changes of wild female *Cyprinus carpio*, subjected to induced reproduction by Ovulin and/or pituitary extract

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Abstract
The present study aimed to determine the effects of pituitary extract or Ovulin (containing s-GnRH and anti-dopamine) injection on reproductive parameters, hematological, and serum biochemical characteristics of the female Sazan carp, *Cyprinus carpio*. The female broodstocks were caught from the Caspian Sea and transferred to the Sijeval Center of Fish Propagation and Reconstruction of Genetic Reserves of Bony Fish in Bandar-e-Turkmen, Iran. After acclimation, the fish were divided into two experimental groups: one injected with pituitary extract (PT) and the other with Ovulin (OV). Blood samples were taken from the fish before the injections and 12 hours after, when egg samples were also collected. The results showed that OV treatment significantly increased number of egg, egg dimeter, fecundity, fertilization percentage, hatching percentage and survival percentage compared to PT treatment. Serum cortisol significantly increased in OV treatment after the injection. Serum glucose and erythrocyte count significantly increased after the injections, whereas serum cholesterol and sodium concentrations decreased. In conclusion, OV is beneficial as an alternative to PT extract to stimulate final maturation in female Sazan without any health problems.

Keywords: Sazan, Reproduction, Hematological parameters, Biochemistry, Hormone

INTRODUCTION

Common carp, *Cyprinus carpio*, is found in various geographical regions, including the Caspian Sea (locally known as Sazan), and is considered an important food resource for local communities. However, its population has declined due to overfishing and degradation of its spawning grounds, particularly in the southern part of the Caspian Sea and its tributaries (Ghelichpour et al. 2013). Therefore, conservation efforts are necessary.

Currently, a stock rehabilitation program for Sazan is being implemented. This involves releasing artificially propagated fry into the rivers connected to the Caspian Sea. The process begins with collecting broodstocks from the Caspian Sea and then proceeding with artificial propagation (Hosseini & Hoseini 2012). Given the limited availability of Sazan broodstocks, it is crucial to achieve high success rates in propagation. To achieve this, the broodstocks are stimulated through exogenous hormonal induction to promote final maturation and maximize the release of sexual products.

The oldest stimulating hormone used in fish artificial induction is pituitary (PT) extract. This extract contains gonadotropins that act at the gonadal levels to promote final maturation (Mohammad Nejad et al. 2022). However, this product has certain disadvantages such as high price, low availability, not consistent hormonal composition, and presence of other materials not applicable in the reproduction processes (Mohammad Nejad et al. 2022). As a result, various synthetic materials were produced commercially as its alternative. These materials generally contain gonadotropin-releasing hormone analogues that act at the PT levels (Schneider et al. 2006). Also, they may contain other effective compounds such as anti-dopamine that are necessary in some fish species for successful reproduction. These synthetic products are cheap, available, and durable, and have consistent outputs. Ovulin (OV), ovaprim, ovatide, human chorionic gonadotropin (HCG), ovopel, dagin, and aquaspawn are examples of these materials that are used commercially (Zohar et al. 2022).

Along with promoting reproduction, hormone-therapy has diverse effects on fish. For example, immune inductions have been reported in gilthead seabream, *Sparus aurata*, administered with 17-beta estradiol or testosterone (Cuesta et al. 2007). Female bennei, *Barbus sharpeyi*, exhibited different cortisol responses to induction by carp PT extract alone, ovaprim, and gonadotropin-releasing hormone (GnRH) analogue + carp PT extract; the later induced lower cortisol responses (Mohammadian et al. 2015). Increase in doses of GnRH (Shokr 2020) or gonadotropins (Shokr 2015) induced hepatic and renal damages (characterized by elevation in blood transaminase enzymes, uric acid, and creatinine levels) and stress (characterized by cortisol/glucose elevations) in African catfish, *Clarias gariepinus*, and Nile tilapia, *Oreochromis niloticus*, respectively. Ovulin contains an analogue of salmon GnRH and a dopamine inhibitor, which promotes final maturation at 0.5 and 0.25mL/kg in females and males, respectively. Limited information on the efficacy of OV in Sazan broodstocks focuses on the effects of various doses on the fish (Mohammad Nejad et al. 2022). Also, hematological and serum biochemical responses to different doses of OV have been assessed in female Sazan (Mohammad Nejad et al. 2022). The present study aimed to assess the impact of the effective dose of OV (0.25 mL/kg body weight) on reproductive, hematological, and serum biochemical parameters in female Sazan. The results were also compared to the broodstocks stimulated by 2mg/kg PT extract.

MATERIALS AND METHODS

Experimental fish: The study was conducted in the spring of 2020 at the Sijeval Center of Fish Propagation and Reconstruction of Genetic Reserves of Bony Fish in Bandar-e-Turkmen, Golestan province, Iran. Approximately 24 Sazan carp broodstocks were collected from the southeastern Caspian Sea. They were then transported to the center using a vehicle equipped with oxygen capsules. After the arrival, the female fish were separated (8 fish) and their weight (1860-1950g) and length (56.2-58.7cm) were recorded. The fish scales were used for age determination (3-5 years). Then, the fish were acclimatized to the new conditions for two weeks, during which water temperature, dissolved oxygen, and pH were 21±2°C, 6.0±0.3mg/L, and 7.7±0.2, respectively.

Hormone injection: Common carp PT extract was obtained from the local market. OV was purchased from Ningbo Sansheng Pharmaceutical Company Ltd. (China). After the acclimation, the fish were divided into two groups held in two 2000-L tanks and kept in these tanks for one week, during which the fish were fed a commercial feed (Beyza 21 Co., Iran). Then, fish of one tank were injected with PT extract; whereas, fish of the other tank were injected with OV. For this, the fish were individually caught and placed in a 200-L tank, containing clove extract (0.2g/L). After anesthesia, the fish were injected peritoneally below the pectoral fin base at doses of 2mg/kg PT extract or 0.25mL/kg OV.

Blood samplings: The fish were sampled twice, one before the hormone injection, and the other 12 hours after the injection, when the fish completed ovulation. The fish were caught and anesthetized in a 200-L tank containing 2g/L clove extract (Hoseini & Ghelichpour 2012). Blood samples were taken by caudal puncture and collected in heparinized tubes. Then, the fish were marked by colored wool threads and returned to their corresponding tanks for the post-injection sampling and egg collection.

Egg collection and analysis: After the post-injection blood sampling, egg samples were taken by stripping the fish abdomen form the head to tail and collected in a syringe (Hajirezaee et al. 2010). The egg samples were collected in sterile tubes and used for reproduction efficiency indicators determination.

Blood and serum analysis: The blood erythrocytes (RBC) and leukocytes (WBC) were determined using the Dacie diluting solution, as suggested before (Yousefi et al. 2022a). Hemoglobin (Hb) levels were measured using a commercially available kit from

Table 1. Mean of number of egg, egg dimeter, fecundity, fertilization percentage, hatching percentage and survival percentage in the female Sazan treated with either PT or OV.

Treatment	Number of egg	Egg dimeter	Fecundity	Fertilization percentage	Hatching percentage	Survival percentage
PT	969±0.06	1.97±0.09	276593.30±14906.85	52.33±0.05	56.14±0.62	53.57±1.84
OV	1237.33±0.05	2.10±0.11	304220.70±14087.42	81.70±0.05	75.52±1.87	73.79±1.002

Zistchem Co. (Tehran, Iran) and a spectrophotometer (Yousefi et al. 2022a). Hematocrit (Hct) percentages were calculated by micro-centrifuging the blood samples (Abbasi et al. 2023). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formula developed by Blaxhall (1972). The differential WBC count was performed by preparing a blood smear and staining it with Giemsa, following the method described by Taheri Mirghaed et al. (2023).

Serum separation was done by centrifugation at 4°C (3000g; 7min) and the obtained materials were kept at -20°C until analysis (Rajabiesterabadi et al. 2020). Serum cortisol levels were assessed using a commercially available kit (Monobind Co., CA 92630 USA) at a wavelength of 450 nm and a temperature of 20 °C. This kit has been previously utilized and validated for cortisol analysis in various fish species (Mirzargar et al. 2022; Taheri Mirghaed et al. 2022; Yousefi et al. 2022b) and is based on the competitive ELISA method with a detection range of 0-500 ng/mL (sensitivity of 3.6ng/mL). Serum glucose, total protein, albumin, cholesterol, triglyceride, and calcium levels were measured using commercial biochemical kits (Zist Chem Co., Tehran, Iran), employing the glucose oxidase method (wavelength 546 nm; detection range 5-400mg/dL; sensitivity 5mg/dL), Biuret method (wavelength 546nm; detection range 0.5-6g/dL; sensitivity 0.005g/dL), bromocresol green method (wavelength 620nm; detection range 0.5-6g/dL; sensitivity 0.003mg/dL), colorimetric-enzymatic method (wavelength 505nm; detection range 15-1000 mg/dL; sensitivity 1.33mg/dL), colorimetric-enzymatic method (wavelength 505 nm; detection range 30-700mg/dL; sensitivity 5.6mg/dL), and arsenazo method (wavelength 660 nm; detection range 5-15mg/dL; sensitivity 0.14mg/dL), respectively (Hoseini et al. 2016; Taheri Mirghaed et al. 2018; Yousefi et al. 2018). Serum sodium and potassium concentrations were determined using a flame-photometer, as suggested by Mazandarani et al. (2017).

Statistical analysis: The blood and serum parameters were subjected to two-way repeated measure ANOVA to find the significant effects of sampling time (before-after injection) and hormone type (PT vs. OV). When there was an interaction effect of the sampling time and hormone type, pair comparisons were conducted by Duncan test, among the treatments. Data are expressed as mean±standard deviation and alpha was set at 0.05. The statistical analyses were performed in SPSS v. 26.

RESULTS

The results showed that the fish injected with OV had significantly higher number of egg, egg dimeter, fecundity, fertilization percentage, hatching percentage and survival percentage, compared to those injected with PT (Table 1).

Serum cholesterol significantly decreased after the hormone injections. However total protein, albumin and triglyceride significantly increased after the OV injections (Table 2). Serum potassium and calcium levels significantly increased after the OV injection. However, serum sodium significantly decreased after the OV injection (Table 3).

Injection with OV hormone had no significant effects on the blood WBC. However, granulocytes percentage significantly decreased after the OV injection (Table 4). MCH and MCHC significantly increased after the injection with OV, whereas, RBC, blood Hb, Hct and MCV non-significant decreased at this time. (Table 5). Serum cortisol and glucose levels significantly increased after OV injection (Table 6).

Table 2. Serum total protein, albumin, triglyceride, and cholesterol levels in the female Sazan treated with either PT or OV.

	Total protein (g/dL)	Albumin (g/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)
PT	3.55±0.05	1.49±0.03	130.5±1.5	193±1
OV	3.95±0.05	2.13±0.03	170±2	182.5±1.5

Table 3. Serum Na, K, and Ca levels in the female Sazan treated with either PT or OV.

	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mg/dL)
PT	148.50±1.5	0.35±0.05	12.41±0.28
OV	141±1	0.95±0.05	20.89±0.48

Table 4. Blood WBC and granulocytes in the female Sazan treated with either PT or OV.

	WBC (× 10^3 cell/µL)	Granulocytes (%)
PT	17.5±0.10	33.10±0.50
OV	17±0.20	27±0.50

Table 5. Blood RBC, Hb, Hct, MCV, MCH, and MCHC in the female Sazan treated with either PT or OV.

	RBC (× 10^6 cell/µL)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
PT	121.65±0.65	12.45±0.25	33.40±0.20	179±6.50	67.05±1.15	36.60±0.1
OV	117.55±0.25	12.60±0.20	32.85±0.25	181±1.2	71.65±0.45	45.50±0.1

Table 6. Serum cortisol and glucose levels in the female Sazan treated with either PT or OV.

	Cortisol	Glucose
PT	18.60±0. 5	74 ±1
OV	23.35±0.95	106±5

DISCUSSION

The present results suggest that OV is a superior inducing hormone than PT extract in Sazan, considering the improvements in the reproductive parameters. The duration of fecundity depends on several factors and prolonged periods increase the chance of successful fertilization (Lahnsteiner et al. 1999; Bastami et al. 2010; Mousavi-Sabet et al. 2012a; Mousavi-Sabet et al. 2012b; Mousavi-Sabet et al. 2012c; Mousavi-Sabet et al. 2013; Mousavi-Sabet et al. 2017; Merino et al. 2023; Yousefi et al. 2023; Yousefi et al. 2023). This study revealed the fish injected with OV had significantly higher number of egg, egg dimeter, fecundity, fertilization percentage, hatching percentage and survival percentage, compared to those injected with PT.

Cortisol is the main stress hormone that increases during energy-demanding events to stimulate catabolic process and supply required energy for fish (Barton 2002). Reproduction is an energy-demanding phenomenon and needs energy supply for fish (Mousavi-Sabet e al. 2011; Mousavi-Sabet et al. 2015a; Mousavi-Sabet et al. 2015b; Mousavi et al. 2019; Rahi et al. 2023). A common outcome of cortisol elevation is hyperglycemia and supplying glucose for target tissues to produce energy through the citric acid cycle and oxidative phosphorylation in the mitochondria (Birnie-Gauvin et al. 2023; Chen et al. 2024), which explains the results of serum glucose levels in the present study. Similar results were reported in the African catfish injected with GnRH (Shokr 2020), and grass carp, *Ctenopharyngodon idellus*, injected with either HCG, ovaprim, or pregnyl (Metwally & Fouad 2008; Mousavi & Yousefian 2012).

Increase in serum total protein level without a change in serum albumin is an indication of higher serum globulin levels. A consequence of elevation of energy expenditure is decrements in synthesis of new proteins and allocating the resources for more energy production (Jerez-Cepa et al. 2020). Thus, it can be stated that protein synthesis has been increased after the hormonal treatments to shift the energy resources toward gonads for final maturation. Shokr (2020) reported a decrease in serum total protein levels after African catfish injected with GnRH. Also, ovaprim or pregnyl injections significantly decreased serum total protein in the male grass carp (Metwally & Fouad 2008).

There was a decrease in serum sodium concentration after the injection. Sodium is the main cation of the fish blood that is tightly controlled. The exact mechanisms responsible for such a decrease are not clear, but the results are similar to Mohammad Nejad et al. (2022), who found such a decrement after OV injection to the male common carp. Nevertheless, such a small (although statistically significant) decrease in the serum sodium concentration may not be an indication of health deterioration in the fish, particularly when the other ions remained unchanged.

The decrease in serum cholesterol after the injection of the OV injection can be due to its utilization for steroid hormone synthesis (Schulz et al. 2010). Steroid hormone is synthesized from cholesterol, albeit through a multi-step process, and increases during the final maturation (Schulz et al. 2010). Thus, the decrease in the plasma cholesterol levels after the hormonal stimulations can be associated to a surge in production of steroid hormone. Similarly, injection of 0.2-0.35 mg/kg OV to the male wild carp resulted in a significant decrease in the serum cholesterol concentrations (Mohammad Nejad et al. 2022).

An increase in RBC count was observed after OV injection, which can be due to the action of reproduction. According on Shokr, 2020, Testosterone concentration increases after injecting of GnRH analogues in fish and this hormone has been shown to stimulate erythropoiesis in fish (Pottinger & Pickering 1987; Soldatov & Kukhareva 2015). Low Hb can be due to iron deficiency, and RBC with less Hb contents tend to be smaller, which explains the decreases in MCV and MCH values after the PT injection in the present study. Similar results were obtained in the male and female bighead carp, *Hypophthalmichthys nobilis*, injected with PT extract (Heydari et al. 2014). In contrast, the male African catfish had significantly higher RBC, Hct, Hb, MCV, MCH, and MCHC after GnRH injection (Shokr 2020). These contradictions raise the need for mechanistic studies on this topic.

In conclusion, OV improves reproductive parameters, compared to PT extract, which can increase fertilization success. Also, hematological and biochemical parameters showed no noticeable health problem in the fish treated by OV. Therefore, considering the lower price and higher availability of OV compared to PT extract, its application in Sazan reproduction and stock-rehabilitation program is recommended.

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مقاله کامل

تغییرات تولیدمثلی، هماتولوژیکی و بیوشیمیایی ماهی کپور ماده *Cyprinus carpio*، در معرض تولیدمثل القایی با هورمون اوولین و/یا عصاره هیپوفیز

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چکیده: مطالعه حاضر با هدف تعیین تأثیر تزریق عصاره هیپوفیز یا اوولین (حاوی s-GnRH و آنتی‌دوپامین) بر پارامترهای تولیدمثلی، هماتولوژیک و خصوصیات بیوشیمیایی سرمی ماهی کپور ماده *Cyprinus carpio* انجام شد. مولدهای ماده از دریای خزر صید و به مرکز تکثیر و بازسازی ذخایر ژنتیکی ماهیان استخوانی بندرترکمن منتقل شدند. پس از سازگاری، ماهیان به دو گروه آزمایشی تقسیم شدند: در یک گروه با عصاره هیپوفیز (PT) و دیگری با اوولین (OV) تزریق انجام شد. نمونه‌گیری از خون ماهی قبل از تزریق و ۱۲ ساعت پس از تزریق زمانی‌که که نمونه‌های تخم جمع‌آوری شد، گرفته شد. نتایج نشان داد که تیمار OV نسبت به تیمار PT باعث افزایش معنی‌دار در تعداد تخم، قطر تخم، باروری، درصد لقاح، درصد هچ و درصد بقا شده است. کورتیزول خون پس از تزریق در گروه تیمار شده با OV به‌طور قابل توجهی افزایش یافت. همچنین تعداد گلوکز و گلبول‌های قرمز سرم پس از تزریق به‌طور معنی‌داری افزایش یافت، در حالی‌که غلظت کلسترول و سدیم خون کاهش یافت. نتایج نشان داد که تزریق هورمونOV می‌تواند به‌عنوان یک جایگزین مفید نسبت به عصاره PT برای تحریک بلوغ نهایی در ماهی ماده کپور مورد استفاده قرار گیرد بدون اینکه تأثیر منفی بر سلامت ماهی داشته باشد.

کلمات‌کلیدی: ماهی کپور، تولیدمثل، پارامترهای خونی، بیوشیمی، هورمون