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The Effect of Different Hormonal Treatments on Semen Quality Parameters in Cultured and Wild Carp

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Abstract

The objective of this study was to assay the effectiveness injection of different hormones such as ovaprim, human chorionic gonadotropin (hCG) and carp pituitary gland (cPG) on spermatological parameters and seminal plasma composition of cultured and wild carp. At the dosage tested, motility duration, percentage of motile spermatozoa, spermatocrit and sperm volume were significant changes ($P<0.01$) in both stimulated groups and treatments. Composition of seminal plasma (Na^+, K^+, Cl^-, glucose, cholesterol and protein) were significant changes in cultured and wild carp, but values of Ca^{+2} and Mg^{+2}did not show significant difference ($P<0.01$) in treatments. In general, significant changes in all treatments for spermatological properties and composition of seminal plasma were observed. The results showed that ovaprim and carp pituitary gland hormones had more effective than human chorionic gonadotropin (hCG) in cultured and wild carp.

Keywords: Hormone treatment, Spermatological parameters, Seminal plasma, Cultured and Wild Carp

Doğal ve Kültürü Yapılan Sazan Balığının Sperm Kalitesi Üzerine Farklı Hormonal Uygulamaların Etkileri

Özet

Bu çalışmanın amacı, ovaprim, insan koryonik gonadotropini (hCG) ve sazan hipofiz bezi (cPG) gibi farklı hormon uygulamalarının kültüre ortamında ve doğal sazan balıklarının spermatolojik parametrelerine ve seminal plazma kompozisyonuna etkilerini araştırmaktır. Dozaj testinde, motolite süresi, hareket edebilen spermatozoa yüzdesi, spermotokrit ve sperm hacmi hem uyarılmış hem de muamele görmüş gruplarda önemli ($P<0,01$) değişimler göstermiştir. Seminal plazma kompozisyonu (Na^+, K^+, Cl^-,glukoz, kolesterol ve protein) kültüre alınan ve doğadan gelen sazanlarda önemli değişiklikler göstermiştir fakat Ca^{+2} ve Mg^{+2} muamelelerde önemli ($P<0,01$) fark göstermemiştir. Genelde, tüm muamele gruplarındaki spermotolojik özellikler ve seminal plazma kompozisyonundaki önemli değişimler incelenmiştir. Sonuçlar kültüre alınan ve doğadan gelen sazanlarda ovaprim ve sazan hipofiz bezi hormonlarının insan koryonik gonadotropininden (hCG) daha etkili olduğunu göstermiştir.

Anahtar Kelimeler: Hormon muamelesi, spermatolojik parametreler, seminal plazma, kültüre alınan ve doğadan gelen sazan.

Introduction

The common carp (*Cyprinus carpio* L. 1758**)** has been cultivated for several thousand years and nowadays it is abundantly cultured throughout of the world. Reproduction in fish is regulated by external environmental factors that trigger internal mechanisms into action (Rottmann *et al*., 1991). The reproductive cycle can be controlled by either placing the fish in an appropriate environment or by changing the fish internal regulating factors with injected hormones or other substances. Hormone induced spawning of fish has been used for almost 60 years (Rottmann *et al*., 1991). Sperm management by hormone treatment can become a vital tool for increasing artificial reproduction success in farms. Reproductive hormones have been used to stimulate reproductive processes and induce spermiation and spawning. Among various reproductive hormones, pituitary gland (PG), human chorionic gonadotropin (hCG) and GnRHa are commonly used for inducing or maintaining spermatogenesis in many fish species. The pituitary gland produces and stores gonadotropin hormones (GTH), which play a decisive role in ovulation and spermiation. Human Chorionic Gonadotropin (hCG) is purified gonadotropin hormone used for induced spawning (Rottmann *et al*., 1991). Over recent years, hCG has been increasingly

employed in spawning induction trials of many fish species. A further of advantage of hCG is that it acts directly at the level of the gonad (Zohar and Mylonas, 2001). PG and hCG has been used to induce spermiation in Japanese eel (*Anguilla japonica*) (Miura *et al*., 1991), mullet (*Mugil cephalus)* (Shehadeh *et al*., 1973), bream (*Abramis brama*) (Kucharczyk *et al*., 1997), Pangasiid cat fish (Cacot *et al*., 2003) and European eel (*Anguilla anguilla*) (Perez *et al*., 2000; Austriano *et al*., 2006). Gonadotropin releasing hormone (GnRHa) is one of the most commonly used hormones which stimulates the pituitary and released GTH, thus subsequently milt production. Also GnRHa have been used to induce spermiation in European cat fish (*Silurus glanis*) (Linhart and Billard, 1994), Atlantic halibut (*Hippoglossus hippoglossus*) (Vermierssen *et al*., 2000), Green back flounder (*Rhombosolea tapirina)* (Lim *et al*., 2004) and Deccan mahseer (*Tor khudree*) (Basavaraj and Hedge, 2005).Treatment with GnRHa has also proven effective in enhancing milt production in fish (Zohar and Mylonas, 2001). The use of hormone treatments influences ovulation synchronization variously in different fish species. The reproduction of many fish species in hatcheries is impossible without the application of hormonal preparations. This refers to spawners originating from wild fish populations (Kucharczyk *et al*., 1997; Szabo *et al*., 2002), as well as from those reared pond conditions (Brzuska, 2003; Brzuska and Adamek, 2008). To evaluate the sperm quality several parameters have been documented including motility, spermatocrit, fertilizing capacity, osmolality and pH of seminal plasma, chemical composition of seminal plasma and several others (Rurangwa *et al*., 2004). Numerous factors can affect the quality of semen and seminal plasma composition. This factor includes season, temperature, nutrition, stress, hormonal stimulation, milt contamination and short-term storage (Ciereszko, 2008). Composition of seminal plasma are included inorganic constituent (Na^+, K^+, Ca^{2+} and Mg^{2+}) involved in the process of inhibition or activation of sperm motility (Morisawa, 1985). Also, organic compounds such as glucose, cholesterol and protein are found in seminal plasma (Lahnsteiner *et al*., 1994). To have controlled and successful production in fish farming, it is important to have adequate knowledge of sperm characteristics and seminal plasma composition of cultured and wild fish. The objective of the current study was to compare controlled cultured and wild carp reproduction when applying three different hormonal preparations under hatchery conditions.

Materials and Methods

Broodstock

The experiment was conducted at the Shahid Rajaee breeding center Sari, Mazandaran, Iran. The wild carp broodstocks were caught in the Caspian Sea during spawning season, May-June 2009 (water temperature 17–20°C) and then transferred to Shahid Rajaee breeding center for acclimination (7 days). Farmed carps used in this study were reared in earthen ponds of 0.5 ha of the farm Shahid Rajaee breeding center during two years. The experiments included 40 males of the wild carp and cultured carp average body weight 1500±110 and 1600±116 g respectively and marked by placing visible tags on the dorsal fin and randomly were divided to treatment groups. In both groups, four treatments with 5 fish per each treatment were tested. Saline solution (0.7% NaCl) was used as a control. Treatments injected intraperitoneally as follows: 3 mg/kg b.w. (cPG), 0.5 ml/kg b.w. ovaprim (sGnRHa+dompridon), and 1500 IU/kg b.w hCG. For the study effect of different hormonal treatments on semen quality parameters in Cultured and wild Carp, after injection of 24 h ovaprim (Salmon GnRH + domperidone, Syndel Laboratories, Canada), carp pituitary gland (cPG), Saline solution (0.7% NaCl) and 12 h of hCG, semen was collected.

Sperm Collection

Fish were anaesthetized in clove powder (50 ppm) before sampling. Sperm samples were collected with a syringe fitted with a plastic needle. We attempted to collect all the sperm at each stripping and to avoid its contamination with urine, mucus or blood; to provide enough oxygenation to the sperm by maintained enough head space in syringe. A syringe of sperm from each male was placed on ice (4°C) and immediately transferred to the laboratory for analyses.

Sperm Analysis

An activating solution of 0.3% NaCl was used for estimating motility. For the evaluation of motility, about 1 µl of semen was placed on a test tube and 1,000 µl of activation solution was added and thoroughly mixed with the tip of a pipette, about 10 µl of semen diluted placed on a glass microscope slide and motility was recorded using a camera (Panasonic wv.cp240 Japan) mounted on a phase contrast microscope (Leica USA). Each motility determination was performed in triplicate for each semen sample. The duration of sperm motility was measured immediately after initiation of sperm activation until 100% spermatozoa were immotile and expressed as sperm movement duration. The Percentages of motile spermatozoa was defined as the percentage of progressively motile spermatozoa within each activated sample. Progressively motile spermatozoa were defined as actively swimming in a forward motion. Only forward moving sperm was judged motile and sperm cells that vibrated in place were not considered to be motile. Observations were made within two hours of semen collection. Semen was drawn into glass microhaematocrit capillary tubes (75

mm length, 1-1; 1-2 mm internal diameter) until 60–80% of the tube volume were occupied by semen. One end of the tube was then sealed with clay and the tubes were centrifuged for 8 min at 3,000 g (Sigma, 13 USA). Spermatocrit was defined as the ratio the total volume of white package material to the total volume of semen ×100 (Rurangwa *et al*., 2004). Measurements were taken in triplicate for each sample, and the average of the three measurements was used for the results. Sperm volume was measured in scaled vials and expressed as ml.

Seminal Plasma Characteristics

The pH of seminal plasma was immediately determined using a laboratory pH meter (pH meter, Iran 762). Sperm samples were centrifuged at 3000 rpm at 8 min (Eppendorf AG, Hamburg, Germany) and then seminal plasma (supernatant) was collected. Plasma was centrifuged twice to avoid possible contamination with spermatozoa. Organic and inorganic content of seminal plasma were analyzed. The minerals (Ca^{+2}, Mg^{+2} and Cl^{-}) and biochemical parameters (total protein, glucose and cholesterol) were measured spectrophotometrically (S2000-UV/VIS England). The concentrations of Na^{+} and K^{+} were determined with flame photometer (Jenway PFP, England) (Standard kits from Parsazmoon, Tehran, Iran).

Statistical Analysis

Motility duration, percentage of motile spermatozoa, spermatocrit and Sperm volume were calculated by mean and standard deviation (SD). Before analysis, data were tested for normality and homogeneity of variance. Mean were tested for significant differences by one-way ANOVA followed by Duncan's multiple range at 0.01 significant levels. Statistical analysis was performed with the SPSS software.

Results

The spermatological parameters of the semen are presented in Figures 1-4. Sperm motility duration ranged from 57.20 to 74.80 and 42.00 to 56.40 (sec) in cultured and wild carp respectively. The higher values obtained in carp pituitary gland (cPG) and ovaprim treatments of cultured carp (Figure 1). Stimulation of males by ovaprim and (cPG) changed significantly percentage of motile spermatozoa in cultured and wild carp compared to control, and highest values of percentage of motile spermatozoa

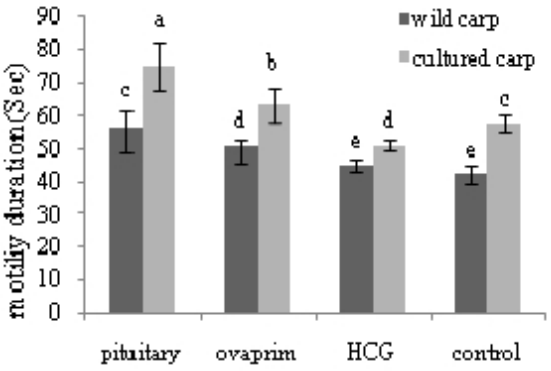

Figure 1. Changes in sperm motility duration treated with different hormones in cultured and wild carp (different letters correspond to significantly different results P<0.01) (n= 5 males).

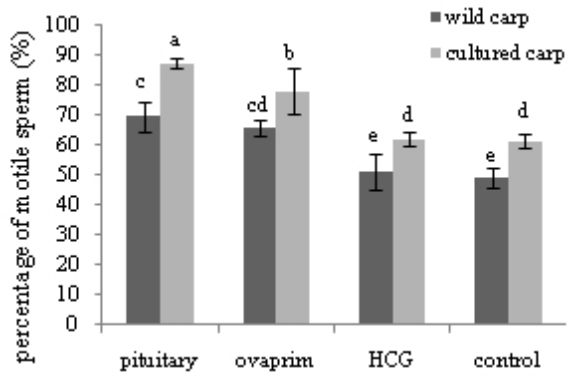

Figure 2. Changes in percentage of motile spermatozoa treated with different hormones in cultured and wild carp (different letters correspond to significantly different results P<0.01) (n= 5 males).

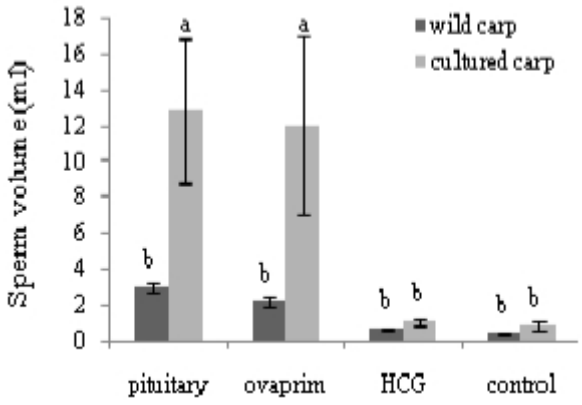

Figure 3. Significant changes in sperm volume treated with different hormones in cultured and wild carp (different letters correspond to significantly different results P<0.01) (n= 5 males).

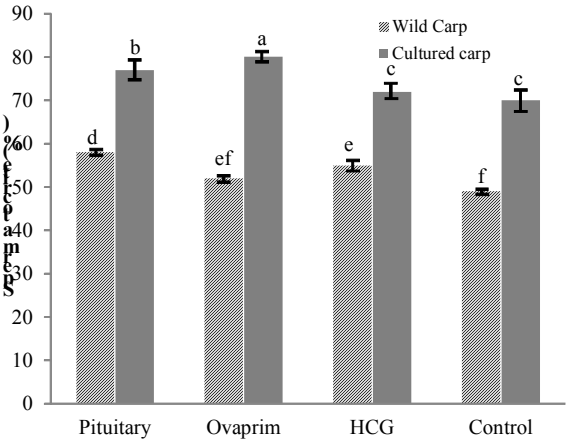

Figure 4. Changes of spermatocrit at different hormonal treatments in cultured and wild carp (different letters correspond to significantly different results P<0.01) (n= 5 males).

were observed in (cPG) treatment of cultured carp (Figure 2). A similar tendency was observed in sperm volume. The males stimulated by ovaprim and (cPG) hormones observed changed significantly sperm volume in cultured carp than wild carp and treatments (Figure 3). Spermatocrit demonstrated a clear increase in all treatments hormones of cultured carp than wild carp treatments. The males of cultured carp injected with ovaprim hormone showed increase in value of spermatocrit than other treatments (Figure 4). Compositions of seminal plasma in cultured and wild carp are shown in Table 1. As seen Table 1, the value of the pH of seminal plasma treated with different hormones was significant difference (P<0.01) in both stimulated groups and higher value of pH was recorded in (cPG) and ovaprim treatments of cultured carp. Also, values of Na^+, K^+ and Cl^- were significant changes among treatments and higher contents of Na^+, and K^+ were observed in treatment of wild carp control than other treatments. The Cl^- ion concentration was higher in (cPG) and hCG of cultured carp treatments compared to wild carp treatments. The Ca^{2+} and Mg^{2+} ion concentrations were significant changes in both stimulated groups and higher content of Ca^{2+} and Mg^{2+} ions were recorded in (cPG) and ovaprim treatments of wild carp. Seminal plasma of metabolites (glucose, cholesterol and protein) was changed between stimulated groups. The higher value of glucose of seminal plasma was observed in control treatments of cultured and wild carp in comparison with other treatments. The cholesterol content was lower in hCG treatment than other treatments in cultured and wild carp.

Discussion

Hormonal stimulation is a common practice in aquaculture to stimulate milt availability in teleost (Zohar and Mylonas, 2001). In Iran, carp pituitary gland (cPG) is a traditional agent for ovulation induction in cyprinid fish. In recent years, (cPG) has become expensive and not readily available largely for use in hatcheries. To reduce hormone costs, we selected two hormones; ovaprim (Salmon GnRH + domperidone) and human chorionic gonadotropin (hCG) compared to carp pituitary gland (cPG) to stimulate milt production in cultured and wild carp.

Table 1. Changes of seminal plasma composition in cultured and wild carp stimulated with different hormones

		carp pituitary gland	ovaprim	hCG	control
Cultured carp	pH	8.36±0.15^{b}	8.20±0.12^{c}	7.94±0.05^{d}	8.04±0.11^{d}
	Na^{+} (m ML^{-1})	102.00±34.92^{c}	81.20±21.09^{c}	123.00±32.71^{c}	310.60±48.54^{b}
	K^{+} (m ML^{-1})	65.40±11.92^{e}	60.40±8.67^{e}	68.20±11.07^{e}	124.80±27.22^{d}
	Cl^{-} (mEq L^{-1})	102.97±2.85^{a}	94.86±7.31^{a}	100.94±3.27^{a}	73.42±14.83^{c}
	Ca^{2+} (m ML^{-1})	3.62±0.97^{b}	3.86±0.71^{b}	3.99±1.12^{b}	4.23±0.82^{b}
	Mg^{2+} (m ML^{-1})	1.54±0.27^{b}	1.75±0.44^{ab}	1.60±0.32^{ab}	1.77±0.58^{ab}
	Glucose (mgdL^{-1})	3.29±0.74^{d}	4.22±1.51^{d}	4.65±1.38^{d}	13.86±1.65^{a}
	Cholesterol (mgd L^{-1})	15.33±1.90^{d}	12.08±3.42^{e}	18.11±2.68^{d}	15.52±1.81^{d}
	Total protein (gd L^{-1})	0.65±0.13^{de}	0.63±0.10^{e}	0.74±0.09^{de}	1.04±0.14^{c}
Wild carp	pH	8.78±0.08^{a}	8.72±0.08^{a}	8.50±0.17^{b}	8.52±0.14^{b}
	Na^{+} (mML^{-1})	267.00±35.63^{b}	298.00±39.62^{b}	298.60±18.70^{b}	379.80±41.29^{a}
	K^{+} (mML^{-1})	219.00±23.82^{b}	165.60±15.77^{cd}	171.00±17.17^{c}	343.00±83.23^{a}
	Cl^{-} (mEqL^{-1})	82.33±2.42^{b}	26.93±1.41^{d}	24.12±1.41^{d}	19.33±1.11^{d}
	Ca^{2+} (mML^{-1})	5.85±1.11	5.59±0.77	5.86±0.96	5.46±0.55
	Mg^{2+} (mML^{-1})	2.07±0.26	2.10±0.28	2.10±0.20	2.09±0.35
	Glucose (mgdL^{-1})	11.21±2.69^{b}	8.15±1.49^{c}	11.43±1.29^{b}	11.71±1.14^{b}
	Cholesterol (mgdL^{-1})	32.48±3.61^{b}	31.02±2.16^{b}	20.32±1.71^{c}	47.77±2.07^{a}
	Total protein (gdL^{-1})	1.38±0.23^{b}	0.82±0.03^{d}	1.78±0.10^{a}	1.36±0.12^{b}

Gonadal maturation in teleost fish is primarily regulated by the brain–pituitary–gonadal axis. The GnRH stimulates the synthesis and release pituitary gonadotropin (GTH) (Melamed *et al*., 1996), and GTH stimulates the production of steroid hormones in the gonads (Van der Kraak *et al*., 1992). Manipulations of various environmental parameters, such as temperature, photoperiod and etc, can often improve the reliability of spawning (Zohar, 1989; Yaron, 1995). However, in some species, hormonal treatments are the only effective way for controlling reproduction. The reproduction in captivity of fish caught in the wild during the spawning season does not always produce the anticipated results. This is linked to both spawning phonology, when the males and females can appear at the spawning grounds at different times, as well as to the risk of obtaining an inadequate number of spawners at the appropriate moment (Szabo *et al*., 2002). Wild carp stimulation to produce of milt in captivity requires hormonal stimulation. Szabo *et al*. (2002) reported that positive reproduction results with nase (*Chondrostoma nasus*) can be obtained with the application of either carp pituitary extract (CPE) or an analogue of gonadotropin-releasing hormone (GnRH). In our study, we focused on a practical evaluation of three hormones to stimulation cultured and wild carp. In teleost fish, sperm motility is a one of biomarkers for assessment of sperm quality (Lahnsteiner *et al*., 1998). Krol *et al*. (2009) observed motility duration in European smelt (*Osmerus eperlanus*) treated with ovaprim + domperidone hormone was increased. In another study was reported that hCG injection in European eel does not affected on sperm motility (Austriano *et al*., 2006). Various marine and freshwater species have been examined successfully in treatment with GnRHa and have been shown as an effective method for improving sperm quantity and quality (Mylonas *et al*., 1997a; Clearwater and Crim, 1998; Zohar and Mylonas, 2001). GnRH acts at the hypothalamus–pituitary–gonad axis than gonadotropin. Subsequently GnRH can regulate stimulation of physiological events by directly or indirectly affecting the release of other hormones necessary for successful spermiation and milt production (Zohar and Mylonas, 2001). The our research demonstrated using (cPG) and ovaprim (GnRHa) treatments increased percentage of motile spermatozoa in cultured and wild carp compare to control. Several studies have been shown increase the percentage of motile spermatozoa, for example, in yellowtail flounder (*Limanda ferruginea*) treated with two different types of GnRHa-delivery systems increased the percentage of motile sperm from 20% to 40–90%, (Clearwater and Crim, 1998). Also, in Atlantic halibut (*Hippoglossus hippoglossus*) GnRHa implants increased sperm motility (Vermeirssen *et al*., 2004). An increase in percentage of motile sperm in European cat fish (*silurus glanis*) was reported by Linhart and Billard (1994). The similar results obtained from males of smelt (*Osmerus eperlanus*) stimulated by Ovaprim (Krol *et al*., 2009). Semen with an increased percentage of motile spermatozoa would have a better chance to successfully fertilize larger numbers of eggs. Sperm volume significantly increased in (cPG) and ovaprim (GnRHa) treatments of cultured carp than wild carp. Caille *et al*. (2006) observed increased in sperm volume of tench (*Tinca tinca*) after inducing with 2 mg /Kg b.w carp pituitary extract compare to control group injected with ringer soloution. Similar results have been observed in paddlefish (*Polyodon spatula*) (Linhart *et al*., 2000), European cat fish (*Silurus glanis*) (Linhart *et al*., 2004). In contrast, significant increase in sperm volume after GnRHa (ovaprim) injection compared to controls in European cat fish (*silurus glanis*) and

smelt (*Osmerus eperlanus*) was reported respectively (Linhart and Billard, 1994; Krol *et al*., 2009). Also, similar results from striped bass (*Morone saxatilis*) and winter flounder (*Pseudopleuronectes americanus)* were obtained (Pankhurst, 1994; Mylonas *et al*., 1997b) respectively. Furthermore, increase in the volume of stripes sperm compare to control group have been observed in with bass (*Morone chrysops*) (Mylonas *et al*., 1997a), Atlantic salmon (*Salmo salar*) (King and Young, 2001). In addition, hCG was shown significantly increase the sperm volume collected 6 h post-injection in gold fish (Zheng and Stacey, 1996). In this experiment, spermatocrit increased in cultured carp than wild carp using (cPG), ovaprim and hCG hormones. Ohta and Tanaka, (1996) observed that spermatocrit had periodical changes during each weekly injection of hCG. Several studies have documented spermatocrit after injection with GnRHa. Reduced spermatocrit following post-injection GnRHa have been observed in winter flounder (Shangguan and Crim, 1999), Green back flounder (Lim *et al*., 2004) and Deccan mahseer (Basavaraj and Hedge, 2005). In contrast, increase in sperm volume of winter flounder (Harmin and Crim, 1993), white bass (Mylonas *et al*., 1997a) and yellow tail flounder (*Pleuronectes ferrugineus*) were not accompanied by reductions in spermatocrit (Clearwater and Crim, 1998). It is not known whether the observed differences in spermatocrit were depending upon the mode of GnRHa treatment, spawning season and species specific characteristics. In this study, seminal plasma of pH changed after hormones injection in both simulated group and higher value of pH was measured in (cPG) and ovaprim treatments of cultured carp. An increase in seminal plasma pH has been observed in yellow tail flounder after injection with GnRHa (Clearwater and Crim, 1998). Linhart *et al*. (2003) reported stimulation of paddle fish (*Polyodon spatula*) with carp pituitary powder (CCP) and LHRHa resulted in differences in ionic composition of seminal plasma. In our study demonstrated that values of Na^+, K^+ glucose, cholesterol and protein of cultured and wild carp changed after injection with different hormones. In mrigal (*Cirrhinus mrigala*), seminal plasma parameters significantly influenced after injection with ovaprim (salmon GnRH + domperidone) (Verma *et al*., 2009). Our results showed that application ovaprim hormone is an effective method for improving spermiation and increasing sperm production in cultured and wild carp. Also, these results suggest that the administration of ovaprime can use instead on (cPG) in large hatcheries of Iran due to it is currently readily available largely and less expensive than (cPG).

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