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Improvement of Sperm Quality Indices of Benni Fish (*Barbus sharpeyi*) by Application of LHRHA2 and Metoclopramide

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ABSTRACT

The main objective of this study was assessment of hormonal treatments effects on sperm quality improvement in *Barbus sharpeyi*. Results showed differences in effects of treatments with LHRHa2, LHRHa2 combined with a dopamine antagonist (MET), and CPE (Carp Pituitary Extract) on parameters of sperm volume, motility, spermatocrit, biochemical indices of seminal plasma and sperm efficiency on artificial propagation, incubation survival, and deformities. Biochemical analysis of seminal plasma showed that the majority of parameters were affected by different hormonal treatments. In conclusion, the highest sperm quality of Benni fish could be achieved by application of LHRHA2 (10 µg kg^{-1}+MET), LHRHA2 (5 µg kg^{-1}+MET) and LHRHA2 (5 µg kg^{-1}) at 8, 12, and 16 hours PHS, respectively.

Keywords: Hormone treatment, Dopamine antagonist, Carp pituitary extract (CPE), Sperm quality.

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INTRODUCTION

Barbus sharpeyi is one of the most important and economically valuable fish in Tigris and Euphrates wetlands (Alavi *et al.,* 2010; Kahkesh *et al.,* 2010). During the past 10 years, natural reserves of *B. sharpeyi* declined sharply and the best way to obtaining fingerlings for reconstructing natural stocks is successful artificial reproduction through injection of carp pituitary extract (Al Mukhtar *et al.,* 2009). Despite propagation of *B. sharpeyi* in Iran, the fertilization rate and efficiency of reproduction have been reported unsatisfactory (Pyka *et al.,* 2001). In the artificial rearing condition, few species are able to reach the final stages of maturation and reproduction, but in the other species, mature oocytes and sperm can only be obtained by hormone injection and quality of gamete would be different based on stimulation methods (Mabudi *et al.,* 2011).

Development of different carp species reproduction in the world, lead to more use of the carp pituitary gland. On the other hand, we should ensure that sufficient amount of GTH are available for successful induction of sexual maturation in the pituitary gland. This restriction has caused expansion and invention of various forms of LHRH hormone and its analogues (Arabaci *et al*., 2001).

Since the availability of sperm with high quality is essential for achieving high fertilization rate and larval quality (Verma *et al*., 2009; Bozkurt *et al.,* 2011), the present study can be worthwhile for completing basic information of gamete biology and increasing reproduction efficiency in *B. sharpeyi*. Different types of hormones such as GnRH and its analogues, Ovaprim (sGnRHa+Domperidone), Ovaplant

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(sGnRHa), CPE (carp pituitary extract), and HCG were used as effective tools for induction of fish spermiation and ovulation (Cejko *et al.,* 2011). But, in teleosts, GnRH (LHRH) has an important role in reproduction, regulation, and release of pituitary hormones (Li *et al.,* 2002). Also, GTH and GnRH are keys to regulation of fish reproduction (Albert, 2008). Earlier reports on hormonal treatment of *B. sharpeyi* revealed that application of Ovaprim (sGnRHa+Domperidone) had advantage in fertilization rate in comparison to CPE (Hashim *et al.,* 2006). Several studies indicate sperm volume, percentage of motile spermatozoa (Cejko *et al.,* 2011), sperm density (Cejko *et al.,* 2010), osmotic pressure of seminal plasma, velocity, motility, and fertilization capacity of spermatozoa are influenced by application of different hormonal treatments (Lin *et al.,* 1996) and stimulation methods (Mylonas *et al.,* 2010; Hajirezaee *et al.,* 2010). Caille *et al*. (2006) found the strongest stimulation of spermiation of *Tinca tinca* with LHRHa in dosage of 20 and 40 µg kg^{-1}. Also, Cejko *et al*. (2012) reported that sperm quality parameters of *Barbus barbus* declined with expanding time of hormonal treatment.

The main purposes of this study were to evaluate effects of LHRHA2 with and without Anti-Dopamine metoclopramide in comparison to CPE on fertilization parameters, sperm quality indices, and composition of seminal plasma in *B. sharpeyi* at 8, 12, and 16 hours following hormonal stimulation.

MATERIALS AND METHODS

This experiment was carried out at the Center of Endemic Fish Propagation, Khuzestan province, south of Iran, from beginning of March 2010 till the end of reproduction season of *Barbus sharpeyi* in May. Seventy-two males of *B. sharpeyi* (2^{+} years old, total average length and weight 41.56±1.43 cm and 797.86±55.60 g, respectively) were used as sperm donors. The broodstocks were caught from earth ponds using pond trap and held in raceway at 23°C.

Hormonal Treatments

Maturities of the males were checked by abdominal massaging and only males without sperm were selected. After that the males were weighed, tagged, and divided into 24 experimental groups (n= 3). LHRHA2 (Vehicle of 100 µg, made in China) was diluted in physiological solution (0.9% of NaCl) according to Table 1. Total volume of injection in each treatment was 1 ml kg^{-1} body weight and in each treatment three fish were injected.

Also, 15 females with average length and weight of 43.60±5.30 cm and 1117.70±372.77 g, respectively, received 4 mg kg^{-1} CPE homogenized in physiological solution. Injections of females were performed in 2 stages. In the first and second stage, 10 and 90% of the total CPE was injected, respectively. Ovulation occurred 12 hours after the second injection. Finally, all fish were anaesthetized and sperm samples were collected individually.

Assessment of Sperm Motility

Duration of sperm motility in each individual broodstock sperm samples was measured according to the method described by Alavi *et al.* (2009). Sperm motility was evaluated visually for total duration of motility (in seconds) after activation and was measured immediately after initiation of sperm activation until 95% of spermatozoa were immotile (Alavi *et al*., 2004). To induce the initiation of sperm motility, a 49 µl drop of the medium was placed on a glass slide and then a drop of 1 µl fresh sperm was diluted using a micro-sampler. All experiments were performed in triplicate at room temperature (17–20°C), using light microscopy (10X). To avoid subjective bias, all measurements were carried out by the same experimenter.

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Table 1. Summary of different hormonal treatments in male broodstocks of *Barbus sharpeyi*.

Group number	Treatment	Time after injection (Hours)
A	LHRHA2 (2.5 µg kg^{-1}) LHRHA2 (5 µg kg^{-1}) LHRHA2 (10 µg kg^{-1}) LHRHA2 (2.5 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) LHRHA2 (5 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) LHRHA2 (10 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) Positive Control: Carp Pituitary Extract (2 mg kg^{-1}) Negative Control: physiological solution (0.9% of NaCl)	8
B	LHRHA2 (2.5 µg kg^{-1}) LHRHA2 (5 µg kg^{-1}) LHRHA2 (10 µg kg^{-1}) LHRHA2 (2.5 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) LHRHA2 (5 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) LHRHA2 (10 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) Positive Control: Carp Pituitary Extract (2 mg kg^{-1}) Negative Control: physiological solution (0.9% of NaCl)	12
C	LHRHA2 (2.5 µg kg^{-1}) LHRHA2 (5 µg kg^{-1}) LHRHA2 (10 µg kg^{-1}) LHRHA2 (2.5 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) LHRHA2 (5 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) LHRHA2 (10 µg kg^{-1})+ Metoclopramide (2.5 mg kg^{-1}) Positive Control: Carp Pituitary Extract (2 mg kg^{-1}) Negative Control: physiological solution (0.9% of NaCl)	16

Spermatocrit and Sperm Volume

Micro-haematocrit capillary tubes (75 mm length, 1.1-1.2 mm inner diameter) were filled with individual sperm samples and one end of each tube was sealed with clay for microhaematocrit centrifuge (Behdad HAEMATOKRIT) and the tubes were centrifuged for 5 min at 10,000 g and spermatocrit were calculated (Agarwal and Raghuvanshi, 2009). Total volume of sperm value was measured during the collection of sperm in triplicate (Cejko *et al.,* 2011).

Biochemical Composition of Seminal Plasma

Assessment of Organic and Inorganic Composition of Seminal Plasma

Individual sperm samples were centrifuged (Spectrafuge 16 M Labnet) at 3,000 rpm for 3 min, followed by a 10-minute centrifuge at 10,000 rpm and the supernatant was isolated and stored frozen at -20°C for analysis (Alavi *et al.,* 2010). Levels of Ca^{2+} and biochemical parameters of seminal plasma (glucose, triglyceride, and alkaline phosphatase) were assessed by using an autoanalyzer (standard analysis kits from Parsazmoon, Tehran, Iran) (Golpour and Imanpoor, 2010). Amounts of Na^{+} and K^{+} in seminal plasma were measured using the flame photometric method (NAK SEACK, ITALY) (Bozkurt *et al.,* 2011; Alavi *et al.,* 2010).

Seminal Plasma Osmolality

The osmolality of samples were measured by using an osmometer (Gonotec-Osmomat 030, Momatso, Japan) and distilled water was used as the control solution (Wilson-leedy *et al.,* 2009).

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Fertilization Protocols

The total obtained ova were mixed together and were used for all treatments. In each of 3 replicates, 10 ml pooled fish oocytes (from 15 female oocytes pools) were added and fertilized by mixing with 10 micro liters of fresh sperm (from individually collected sperm) and 1 ml hatchery water. Adhesiveness of the eggs was removed with continuous stirring for half an hour in the hatchery tap water, then, the eggs were rinsed twice with a 5% solution of tannic acid for 20 seconds for final elimination of stickiness (Horváth, *et al.,* 2007).

Incubation Survival, Hatching, and Deformity

Twelve hours following fertilization, the embryonic development stage of eggs was calculated. For this purpose, about 500 eggs in each replication were taken out of the incubator with siphoning. Then, eggs with a nervous belt were calculated as a fertilized egg (Linhart *et al.,* 2008).

After hatching, the numbers of obtained larvae in each treatment were counted for calculation of hatching (Ottesen and Babiak, 2007) and deformity rate (Krejai and Palikova, 2006).

Statistical Analysis

Normality of variance was tested by the Kolmogorov–Smirnov and all data were reported as Mean±Standard Deviation (SD). Two-way ANOVA was employed for analysis of data. Means were separated by Duncan's New Multiple Range Test, and considered significant at $P< 0.05$. Statistical analysis was conducted using SPSS software version 16.0.

RESULTS

Results from Two-way ANOVA showed that interactions of PHS time and hormonal treatments were significant ($P< 0.05$) and as follows:

Sperm Motility

Application of LHRHA2+MET 10 µg kg^{-1} had the highest duration of sperm motility in comparison to the other treatments at 8 hours PHS ($P< 0.05$, Figure 2-a).

The highest and lowest duration of sperm motility were achieved by application of LHRHA2 in dosages of 10 and 2.5 µg kg^{-1} at 12 hours PHS, respectively ($P< 0.05$, Figure 2-a).

Injection of 5 µg kg^{-1} LHRHA2 had the highest duration of sperm motility at 16 hours PHS, whereas application of CPE showed the lowest rate (47.66±2.90 second) among treatments at the same time ($P< 0.05$, Figure 2-a).

Spermatocrit and Sperm Volume

All males in the negative control groups that were injected by physiological solution (0.9% of NaCl) in different PHS times had no spermiation at all. However, the highest spermatocrit and sperm volume were achieved by injection of CPE and LHRHA2+MET (10 µg kg^{-1}) at 8 hours PHS, respectively ($P< 0.05$, Figures 2-b and -c).

The highest spermatocrit value was measured by application of LHRHA2+MET (10 µg kg^{-1}), at 12 hours PHS, whereas the highest sperm volume was obtained by injection of LHRHA2 (10 µg kg^{-1}) and LHRHA2+MET (5 µg kg^{-1}) at the same time ($P> 0.05$, Figures 2-b and -c).

The highest spermatocrit and sperm volume were achieved by injection of LHRHA2 (10 µg kg^{-1}) and LHRHA2+MET (2.5 µg kg^{-1}) at 16 hours PHS, respectively ($P< 0.05$, Figures 2-b and -c).

Composition of Seminal Plasma

Results of hormonal treatments at different time PHS on organic (Na^{+}, K^{+}, Ca^{+2}) and inorganic (glucose, triglyceride and alkaline

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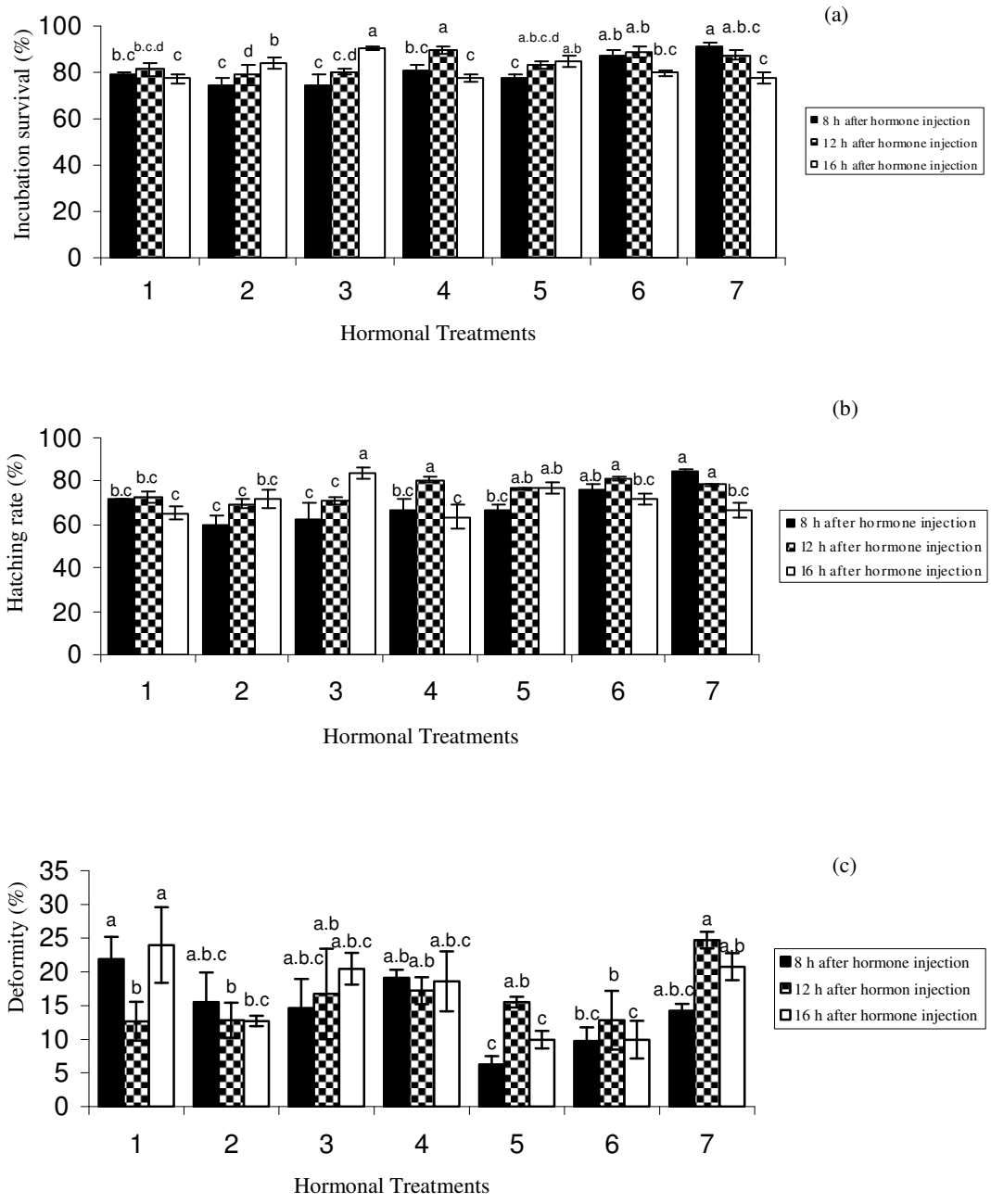

Figure 1. Results of incubation survival (a); hatching rate (b); and deformity percentage (c) in *Barbus sharpeyi*. Values are means of three replicates per treatment. Bars with different letters are significantly different ($P< 0.05$). Hormonal treatments included: (1) Carp pituitary extract (2 mg kg^{-1}); (2) LHRHA2 (2.5 µg kg^{-1}); (3) LHRHA2 (5 µg kg^{-1}); (4) LHRHA2 (10 µg kg^{-1}); (5) LHRHA2+MET (2.5 µg kg^{-1}); (6) LHRHA2+MET (5 µg kg^{-1}), (7) LHRHA2+MET (10 µg kg^{-1}).

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phosphatase) composition of seminal plasma are shown in Tables 3, 4.

Seminal Plasma Osmolality

Results of hormonal treatments at different times PHS on osmolality of seminal plasma is shown in Tables 2, 3, and 4. The highest osmotic pressure value in seminal plasma was recorded by injection of LHRHA2+MET (10 µg kg^{-1}), but the lowest value was achieved by application of LHRHA2 (2.5 µg kg^{-1}) ($P< 0.05$, Table 2).

Treatments injected by LHRHA2+MET (5 µg kg^{-1}) had the highest osmotic pressure of seminal plasma in comparison to other groups at 12 hours PHS ($P< 0.05$, Table 3).

The highest and lowest osmotic pressure of seminal plasma were measured after treatment by LHRHA2 (10 µg kg^{-1}) and CPE, respectively ($P< 0.05$, Table 4).

Incubation Survival, Hatching, and Deformity Rate

Results showed that the highest incubation survival rate at 8, 12, and 16 hours PHS were achieved by injection of LHRHA2+MET (10 µg kg^{-1}) and LHRHA2 (10 and 5 µg kg^{-1}), respectively ($P< 0.05$, Figure 1-a).

Also, the highest hatching rate was achieved by injection of LHRHA2+MET (10 µg kg^{-1}) at 8 hours PHS and statistically significant differences were found among the tested groups ($P< 0.05$, Figure 1-b).

Our results indicated that the highest hatching rate was recorded by injection of LHRHA2 (5 µg kg^{-1}) at 16 hours PHS ($P< 0.05$, Figure 1-b), while injection of CPE, LHRHA2+MET (5 µg kg^{-1}), and LHRHA2 (2.5 and 5 µg kg^{-1}) had the lowest result at the same PHS time, respectively ($P< 0.05$, Figure 1-b).

The highest percentages of deformity rate belonged to CPE, LHRHA2+MET (10 µg kg^{-1}) and CPE, at 8, 12, and 16 hours PHS, respectively ($P< 0.05$, Figure 1-c).

DISCUSSION

In this study, sperm volume, spermatocrit, and duration of spermatozoa motility in *B. sharpeyi* were affected by hormonal treatments, in accordance with the results of Cejko *et al*. (2011).

As in the other cyprinid fish, Na^+ and K^+ are the major ionic content of seminal plasma in *B. sharpeyi* and their levels are high enough to suppress the spermatozoa motility through the high level of osmotic pressure, in agreement with the results of Alavi *et al*. (2010) in *B. sharpeyi*.

For fishes injected by CPE, result of osmotic pressure value in the present study was similar to that reported by Alavi *et al*. (2010) on *B. sharpeyi*, but the higher value was recorded by application of LHRHA2 in comparison to CPE. The highest dose of LHRHA2 in combination with metoclopramide caused the highest osmotic pressure value which was correlated with Na^+ level in seminal plasma. Microscopic observations showed that head of some sperm cells were swollen after treatment of *B. sharpeyi* with higher dosage of LHRHA2. Probably, increasing of LHRHA2 dosage lead to bursting of some sperm cells, and consequently, their internal ionic was diffused to the seminal plasma. So this could lead to increasing of osmotic pressure of seminal plasma.

Morisawa (1985) reported that, in teleost fish, sodium and potassium levels of seminal plasma were 75-175 and 32-86 mM L^{-1}, respectively. In the present study, the highest (99 ± 2.08 mM L^{-1}) and lowest (65.33 ± 7.31 mM L^{-1}) Na^+ values were obtained by application of LHRHA2 (10 µg kg^{-1}) and CPE at 16 and 8 hours PHS, respectively, which was lower than perch (124 mM L^{-1}, Lahnsteiner *et al.,* 1995) and catfish (164 mM L^{-1}, Tan-Fermin *et al.,* 1999) and higher than trout (46.21 mM L^{-1}, Bozkurt *et al.,* 2011).

However, the K^+ contents (ranged from 26.73 ± 5.42 to 33.76 ± 0.99 mM L^{-1}) in *B. sharpeyi* seminal plasma were similar to the

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Table 2. Seminal plasma indices of *Barbus sharpeyi,* 8 hours after different hormonal treatments**.**

Seminal plasma indices	Hormonal Treatments						
	Carp pituitary extract	LHRHA2			LHRHA2+MET (2.5 mg kg^{-1})		
	2 mg kg^{-1}	2.5 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}	2.5 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}
Na^{+} (mM L^{-1})	65.33±7.31 $^{c\,a}$	76.66±4.4 $^{b.c}$	73±2.51 $^{b.c}$	86±3.05 $^{a.b}$	78.33±3.33 $^{b.c}$	94±2.64 ^{a}	98.33±4.40 ^{a}
k^{+} (mM L^{-1})	32.9±1.05 ^{a}	30.53±0.12 ^{a}	31.40±0.30 ^{a}	31.73±0.14 ^{a}	30.06±1.15 ^{a}	30.06±0.25 ^{a}	31.50±0.65 ^{a}
Na^{+}/k^{+} (mM L^{-1})	1.98±0.21 ^{c}	2.51±0.15 ^{b}	2.32±0.09 $^{b.c}$	2.71±0.10 $^{a.b}$	2.61±0.13 ^{b}	3.07±0.11 ^{a}	3.12±0.13 ^{a}
Ca^{+2} (mg dl^{-1})	9.20±0.41 ^{a}	6.30±0.15 ^{d}	7.66±0.12 $^{b.c.d}$	8.23±0.87 $^{a.b.c}$	8.76±0.23 $^{a.b}$	7.06±0.26 $^{c.d}$	9.70±0.60 ^{a}
Alkaline phosphates (u L^{-1})	23.40±1.40 ^{c}	60±20.81 $^{b.c}$	43.20±15.97 $^{b.c}$	40.76±2.11 $^{b.c}$	43.43±15.29 $^{b.c}$	119.23±16.08 $^{a.b}$	170.40±56.05 ^{a}
Glucose (mg dl^{-1})	6.83±0.73 ^{a}	5.50±0.17 ^{a}	5.80±0.15 ^{a}	5.96±0.37 ^{a}	7.53±1.53 ^{a}	7.40±1.95 ^{a}	5.86±0.29 ^{a}
Triglyceride (mg dl^{-1})	66.83±0.52 ^{a}	68.63±0.52 ^{a}	62.83±1.56 ^{b}	65.66±0.88 $^{a.b}$	65.73±1.26 $^{a.b}$	68.30±1.76 ^{a}	68±0.64 ^{a}
Osmotic pressure (mOsm kg^{-1})	284.33±6.22 $^{c.d}$	281.33±4.91 ^{d}	284.33±4.48 $^{c.d}$	300.67±2.96 $^{b.c}$	291.33±3.84 $^{b.c.d}$	304.33±4.33 ^{b}	324.33±7.31 ^{a}

^{a} Values are presented as mean ±SD. Values in the same row having different letters are significantly different (P< 0.05).

Table 3. Seminal plasma indices of *Barbus sharpeyi,* 12 hours after hormonal treatments.

Seminal plasma indices	Carp pituitary extract	LHRHA2			LHRHA2+MET (2.5 mg kg^{-1})		
	2 mg kg^{-1}	2.5 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}	(2.5 mg kg^{-1})	5 µg kg^{-1}	10 µg kg^{-1}
Na^{+} (mM L^{-1})	70±2.88 $^{c\,a}$	87.66±4.33 $^{a.b}$	75.66±2.96 $^{b.c}$	90.33±6.06 $^{a.b}$	77.33±3.92 $^{b.c}$	98.33±4.40 ^{a}	81.66±7.26 $^{b.c}$
k^{+} (mM L^{-1})	93.30±0.47 $^{b.c}$	33.76±0.99 ^{a}	29.73±0.76 ^{c}	31.40±0.96 $^{b.c}$	31.23±0.53 $^{b.c}$	31.26±0.33 $^{b.c}$	32.10±0.43 $^{a.b}$
Na^{+}/k^{+} (mM L^{-1})	2.26±0.09 ^{c}	2.59±0.06 $^{b.c}$	2.54±0.12 $^{b.c}$	2.88±0.23 $^{a.b}$	2.47±0.09 $^{b.c}$	3.14±0.17 ^{a}	2.55±0.26 $^{b.c}$
Ca^{+2} (mg dl^{-1})	7.06±0.23 ^{b}	7.40±0.55 ^{b}	8.33±0.73 ^{b}	6.76±0.36 ^{b}	9.56±1.65 $^{a.b}$	7.66±0.42 ^{b}	11.96±1.22 ^{a}
Alkaline phosphates (u L^{-1})	55.96±11.22 ^{a}	72.70±12.67 ^{a}	67.63±11.09 ^{a}	61.70±11.99 ^{a}	78.33±12.01 ^{a}	77.30±4.56 ^{a}	20.83±8.63 ^{b}
Glucose (mg dl^{-1})	5.33±0.54 ^{b}	6.26±1.27 ^{b}	7.23±1.99 $^{a.b}$	6.03±0.31 ^{b}	10.76±0.62 ^{a}	6.26±0.06 ^{b}	11.03±2.50 ^{a}
Triglyceride (mg dl^{-1})	66.66±3.28 ^{a}	68.90±1.53 ^{a}	69.26±3.14 ^{a}	69.43±0.34 ^{a}	67.66±1.45 ^{a}	69.86±0.72 ^{a}	66.46±1.44 ^{a}
Osmotic pressure (mOsm kg^{-1})	275.33±5.20 ^{c}	309.33±7.88 $^{a.b}$	290±1.73 $^{b.c}$	304.33±8.66 $^{a.b}$	295.30±11.86 $^{a.b.c}$	318.67±9.61 ^{a}	308.33±3.92 $^{a.b}$

^{a} Values are presented as mean±SD. Values in the same row having different letters are significantly different (P< 0.05).

Table 4. Seminal plasma indices of *Barbus sharpeyi,* 16 hours after hormonal treatment.

Seminal plasma indices	Carp pituitary extract	LHRHA2		LHRHA2+MET (2.5 mg kg^{-1})		
	2 mg kg^{-1}	2.5 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}	2.5 µg kg^{-1}	5 µg kg^{-1}	10 µg kg^{-1}
Na^{+} (mM L^{-1})	69.66±1.45 $^{c\,a}$	87±1.52 ^{b}	81.33±2.02 ^{b}	99±2.08 ^{a}	85±2.88 ^{b}	76.33±2.33 ^{b,c}	70±7.63 ^{c}
k^{+} (mM L^{-1})	33.23±0.98 ^{a}	30.63±0.27 ^{a}	30.10±0.80 ^{a}	33.56±0.71 ^{a}	30.23±0.93 ^{a}	26.73±5.42 ^{a}	30.70±0.96 ^{a}
Na^{+}/K^{+} (mM L^{-1})	2.10±0.10 ^{a}	2.84±0.07 ^{a}	2.70±0.09 ^{a}	2.95±0.12 ^{a}	2.82±0.18 ^{a}	3.22±0.90 ^{a}	2.27±0.22 ^{a}
Ca^{+2} (mg dl^{-1})	6.30±0.05 ^{c}	8.23±0.43 ^{b,c}	10.33±0.59 ^{a,b}	7.60±0.72 ^{c}	10.56±1.27 ^{a}	6.93±0.40 ^{c}	10.73±0.75 ^{a}
Alkaline phosphates (u L^{-1})	73.26±29.96 ^{a,b,c}	86.8±5.87 ^{a,b}	92.26±4.44 ^{a,b}	57.33±15.34 ^{b,c}	105.90±7.28 ^{a}	45.33±14.72 ^{b,c}	26.83±5.21 ^{c}
Glucose (mg dl^{-1})	6.90±0.23 ^{b}	6.46±0.48 ^{b}	6.50±0.95 ^{b}	6±0.10 ^{b}	11.43±2.24 ^{a}	5.23±0.18 ^{b}	5.70±0.10 ^{b}
Triglyceride (mg dl^{-1})	69.76±2.25 ^{a,b}	66.96±1.12 ^{b}	66±0.95 ^{b}	71.06±2.96 ^{a,b}	73.46±0.86 ^{a}	66.06±1.55 ^{b}	65.80±1.62 ^{b}
Osmotic pressure (mOsm kg^{-1})	276±0.57 ^{c}	299.33±2.02 ^{b}	302.67±0.33 ^{b}	320.67±2.96 ^{a}	304.67±3.84 ^{b}	279.33±3.92 ^{c}	294±9.16 ^{b}

^{a} Values are presented as mean ±SD. Values in the same row having different letters are significantly different (P< 0.05).

results reported by Alavi *et al.* (2010), which were higher than that in perch (10 mM L^{-1}, Lahnsteiner *et al.,* 1995), catfish (18 mM L^{-1}, Tan-Fermin *et al.,* 1999), and lower than that in trout (46 mM L^{-1}, Bozkurt *et al.,* 2011) and common carp (70 mM L^{-1}, Morisawa *et al.,* 1983).

The ratio of Na^{+}/K^{+} in seminal plasma is used as a tool for estimating the viability of spermatozoa (Hwang and Idler, 1969, Aramli *et al.,* 2013). As we showed, the higher Na^{+}/K^{+} ratio were achieved by injection of LHRHA2+MET (10 and 5 µg kg^{-1}) and LHRHA2+MET (5 µg kg^{-1}) at 8, 12, and 16 hours PHS, respectively.

The importance of glucose in seminal plasma is unclear but it has been correlated to the high energy requirement of the testes during spermatogenesis or to lipid synthesis of spermatozoa, also it is responsible for protection membrane of spermatozoa (Bozkurt *et al.,* 2006, Kalbassi *et al.* 2013).

The triglyceride level of seminal plasma determines energy of spermatozoa for motility activation, and its low level would cause inadequate supply of energy, decreased duration of spermatozoa motility, and lower ability of fertilization (Bozkurt *et al.,* 2009). However, findings from the present study revealed that the highest incubation survival and hatching rate had no correlation with the highest levels of glucose and triglyceride.

Activity of the alkaline phosphates over 10u/l seems to indicate feces contamination and, for this reason, alkaline phosphates activity can be used as an indicator of sperm purity (Ciereszko and Dabrowski, 1994; Lahnsteiner *et al.,* 1996, Aramli *et al.,* 2013). High levels of alkaline phosphates by injection of 10 µg LHRHA2+MET at 8 hours PHS, can be correlated to high contamination of sperm with feces or urine

Results of the present study showed that use of the higher doses of LHRHA2 could lead to the highest fertilization and hatching (Figures 1-a, -b, and -c) at earlier time following hormonal stimulation (8 hours). In this regards, use of metoclopramide improved the outputs of fertilization and

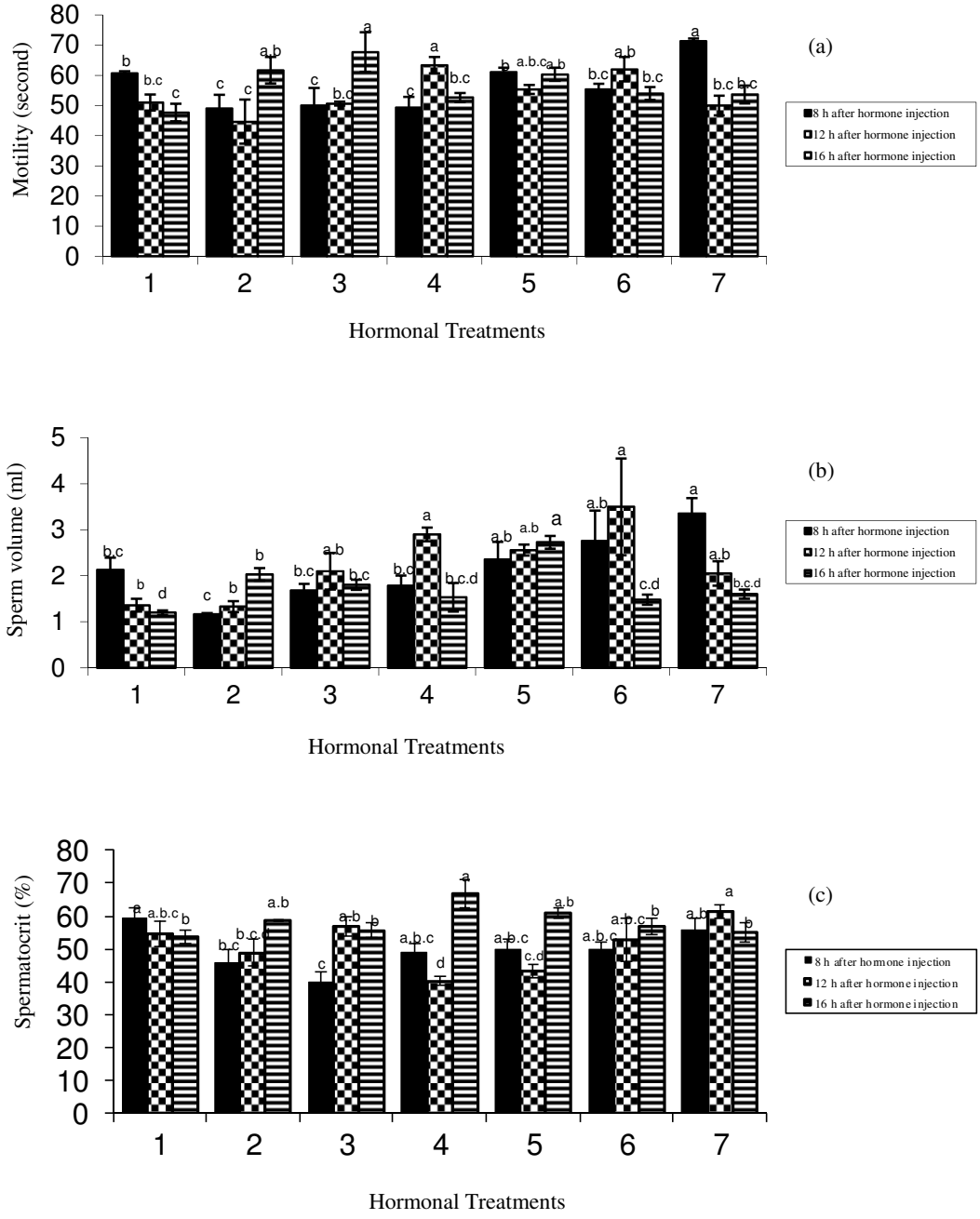

Figure 2. Results of motility (a); Sperm volume (b); and Spermatocrit (c) in *Barbus sharpeyi*. Values are means of three replicates per treatment. Bars with different letters are significantly different ($P<0.05$). Hormonal treatments as in Figure 1.

hatching rate (Figures 1-a and -b). Perhaps, combination of the higher dosage of LHRHA2 with metoclopramide induced more regular hormonal cycles of *B. sharpeyi* in earlier time following hormonal stimulation. It seems that longer times post hormonal treatments lead to aging of spermatozoa and decrease of sperm quality.

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Our study indicated that using lower dosages of LHRHA2 can lead to higher incubation survival rate and lower deformity rate in comparison to higher hormonal treatments with expanding of time PHS (16 hours). Probably, production of self antidopamine by male of *B. sharpeyi* was related to decrease of deformity and increase in incubation survival rate.

Larval deformity can be induced by several parameters such as hormonal treatments (Bonnet *et al.,* 2007), direct impact of contamination (Von Westernhagen *et al.,* 1988), parental factors and condition of artificial spawning (Jezierska, *et al.,* 2000), and environmental factors (Mis *et al.,* 1995). In our study, injection of high dosages of LHRHA2 increased larval deformity (Figure 1-c). Also, the highest number of deformed larva belonged to the fish treated by CPE at 8 and16 hours PHS (Figure 1-c). Perhaps, injecting male of *B. sharpeyi* with higher dosage of LHRHA2 caused anomalies in some spermatozoa cells, which could increase larval deformity. Also, probably, CPE did not have sufficient amount of GTH and, afterward, process of spermatogenesis was not completed.

As a final conclusion, the present study was an attempt to provide preliminary data on the effects of LHRHA2 and its combination with metoclopramide on *Barbus sharpeyi* propagation. Our results showed strong stimulation effect on spermiation after 8, 12, and 16 hours PHS by LHRHA2 injection. Also, the highest incubation survival and hatching rate were achieved in comparison to the control males that were induced by CPE.

The shorter time needed to obtain the highest levels of sperm volume at higher dosages of LHRHA2 with combination of metoclopramide and sperm volume and spermatocrit values were reduced with the expanding of time after treatments. Maybe uses of metoclopramide accelerate process of spermatogenesis processes and afterward, stimulate secretion of high amount of sperm volume in comparison to groups that have not metoclopramide. Perhaps, longer times post hormonal stimulation lead to reuptake of seminal plasma and then decline the amount of sperm volume via decrease of spermatogenesis processes.

It seems more time is needed after hormone injection for stronger stimulation of *B. sharpeyi* males with lower dosages of LHRHA2 without metoclopramide. Perhaps, longer times post hormonal treatment compensate absence of metoclopramide. Also, use of metoclopramide was not essential for stimulation of spermiation of *B. sharpeyi*, by injection of LHRHA2 at the higher times PHS (16 hours), but injection of LHRHA2 alone (without metoclopramide) can lead to spermiation and males of *B. sharpeyi* can produce the required anti-dopamine. It can be due to modulation of spermatogenesis processes and production of self antidopamine by male of *B. sharpeyi* with longer times following hormonal treatments.

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REFERENCES

1. Agarwal, N. K. and Raghuvanshi, S. K. 2009. Spermatocrit and Sperm Density in Snowtrout (*Schizothorax richardsonii*): Correlation and Variation during the Breeding Season. *Aquacult.*, **291**: 61-64.
2. Al Mukhtar, M. A., Saleh, J. H., Jaber, A. A. and Hatam, A. 2009. Artificial Propagation and Fingerlings Production of *Barbus sharpeyi* (Gunther 1874) in Basrah during the Spring of 2006. *Iraqi J. Agric.,* **14**: 87-193.
3. Alavi, S. M. H., Cosson, J., Karami, M., Mojazi Amiri, B. and Akhoundzadeh, M. A. 2004. Spermatozoa motility in the Persian Sturgeon, Acipenser persicus: effects of pH, dilution rate, ions and osmolality. *Reproduction*, **128**: 819-828.

[DOR: 20.1001.1.16807073.2014.16.1.14.8]

4. Alavi, S. M. H., Jorfi, E., Hatef, A. and Mortazavi, S. A. S. 2010. Sperm Motility and Seminal Plasma Ccharacteristics in *Barbus sharpeyi* (Gu¨nther, 1874). *Aquacult. Res.,* **41**: 688-694.
5. Alavi, S. M. H., Rodina, M., Viveiros, A. T. M., Cosson, J., Gela, D., Boryshpolets, S.and Linhart, O. 2009. Effects of Osmolality on Sperm Morphology, Motility and Flagellar Wave Parameters in Northern Pike (*Esox lucius L.*). *Theriogenol.,* **72**: 32-43.
6. Albert, J. B. 2008. An Overview of 30 Years of International Research in Some Selected Fields of the Reproductive Physiology of Fish. *Cybium. Suppl.,* **32**: 7-13.
7. Arabaci, M., Cagirgan, H. and Sari, M. 2001. Induction of Spawning in Common Carp (*Cyprinus carpio*) using LHRHa ([D-Ser (tBu)^6, Pro^9-NEt]-LHRH) combined with Haloperidol: Effects of Different Treatment Time and Determination of Latency Period Dependence on Temperature. *Turkish J. Fish. Aquat. Sci*., **1**: 1-5.
8. Aramli, M. S., Nazari R. M., Kalbassi M. R. and Aramli S. 2013. Effects of Short-term Storage on the Motility, Oxidative Stress, and ATP Content of Persian Sturgeon (*Acipenser persicus*) Sperm. *Anim. Reprod. Sci.* **143**(1-4):112-7.
9. Aramli, M. S., Kalbassi M. R. and Nazari R. M. 2013. Sex Steroid Levels of Persian Sturgeon, *Acipenser persicus*, Borodin, 1897, Males in Negative and Positive Responding to LH-RH-analogue. *J. Appl. Ichthyol.* DOI: 10.1111/jai.12347
10. Aramli, M. S., Kalbassi M. R. and Nazari R. M. 2013. Comparative Study of Sex Steroid Levels of Persian Sturgeon, *Acipenser persicus* Males in Responding Negative and Positive to LHRH-A2 Hormone. *J. Aquac. Res. Development.* **4:**177-182
11. Aramli, M. S., Kalbassi M. R. and Nazari R. M. 2013. Study of Sperm Concentration, Seminal Plasma Composition and their Physiological Correlation in the Persian Sturgeon, *Acipenser persicus*. *Reprod. Domest. Anim*. DOI: 10.1111/rda.12207
12. Bonnet, E., Fostier, A. and Bobe, J. 2007. Characterization of Rainbow Trout Egg Quality: A Case Study Using Four Different Breeding Protocols, with Emphasis on the Incidence of Embryonic Malformations. *Theriogenol.,* **67**: 786-794.
13. Bozkurt, Y., Ogrctmen, F., Secer, F. S. and Ercin, U. 2009. Relationship between Seminal Plasma Composition and Spermatological Parameters in Scaly Carp (*Cyprinus carpio*). *J. Anim. Vet. Adv.,* **8**: 2745-2749.
14. Bozkurt, Y., Ogretmen, F., Kokcu, O. and Ercin, U. 2011. Relationships between Seminal Plasma Composition and Sperm Quality Parameters of the *Salmo trutta macrostigma* (Dumeril, 1858) Semen: With Emphasis on Sperm Motility. *Czech J. Anim. Sci.,* **56**: 355–364.
15. Bozkurt, Y., Secer, S., Bukan, N., Akcay, E. and Tekin, N. 2006. Relationship between Body Condition, Sperm Quality Parameters and Fertilization Success in Rainbow Trout (*Salmo trutta fario*) Sperm. *Pak. J. Biol. Sci.,* **9**: 940-944.
16. Caille, N., Rodina, M., Kocour, M., Gela, D., Hans, F. M., and Linhart, O. 2006. Quantity, Motility and Fertility of Tench *Tinca tinca* (L.) Sperm in Relation to LHRH Analogue and Carp Pituitary Treatments. *Aquacult. Int.,* **14:** 75–87.
17. Cejko, B. I., Kowalski, R. K., Kucharczyk, D., Targońska, K., Krejszeff, S., Żarski, D. and Glogowski, J. 2010. Influence of the Length of Time after Hormonal Stimulation on Selected Parameters of Milt of Ide *Leuciscus idus L. Aquacult. Res.,* **41**: 804–813.
18. Cejko, B. I., Kowalski, R. K., Zarski, D., Targonska, K., Chwaluczyk, R., Kucharczyk, D., Glogowski, J. 2012. The Influence of the Length of Time after Hormonal Treatment with [(d-Ala6, Pro9 NEt)-mGnRH+Metoclopramide] i.e. Ovopel on Barbel *Barbus barbus* (L.) Milt Quality and Quantity Indicators. *J. Appl. Ichthyol.,* **28**: 249–253.
19. Cejko, B. I., Kucharczyk, D., Targonska, K., Kubiak, D., Sarosiek, B. and Glogowski, J. 2008. Quality Parameters and Selected Biochemical Markers of ASP, *Aspius Aspius* (L.), Semen Obtained after Hormonal Stimulation with Ovaprim or Ovopel. *Arch. Pol. Fish.,* **16**: 179-188.
20. Cejko, B., Kowalski, R. K., Kucharczyk, D., Zarski, D., Targonska, K. and Glogowski, J. 2011. Effect of Time after Hormonal Stimulation on Semen Quality Indicators of Common Carp, *Cyprinus carpio* (Actinopterygii: Cypriniformes:

[DOR: 20.1001.1.16807073.2014.16.1.14.8]

Cyprinidae). *ACTA Ichthyol. Pisc.,* **41**: 75-80.

21. Ciereszko, A., Dabrowski, K. 1994. Relationship between Biochemical Constituents of Fish Semen and Fertility: The Effect of Short-term Storage. *Fish Physiol. Biochem.,* **12**: 357-367.
22. Golpour, A. and Imanpoor, M. R. 2010. Relationships between Compositions of Seminal Plasma and Blood Plasma Parameters in Caspian Roach (*Rutilus rutilus caspicus*) during the Reproductive Season. *Res. J. Fish. Hydrobiol.,* **5**:105-110.
23. Hajirezaee, S., Mojazi Amiri, B. and Mirvaghefi, A. 2010. Fish Milt Quality and Major Factors Influencing the Milt Quality Parameters: A Review. *Afr. J. Biotechnol.,* **9**: 9148-9154.
24. Hashim, A. L., Hussain, A. N. A., Wazir, A. R., Hamid, W. A. and Balasem, A. 2006. The New Eden Master Plan for Integrated Water Resource Management in the Marshland Areas.–Agriculture: Overview of Present Conditions and Current Use of the Water in the Marshland Area. New Eden Group, Italy-Iraq, **4(1):** 256.
25. Horváth, A., Miskolczi, E., Mihalffy, S., Osz, K., Szabo, K. and Urbanyi, B. 2007. Cryopreservation of Common Carp *(Cyprinus carpio*) Sperm in 1.2 and 5 ml Straws and Occurrence of Haploids among Larvae Produced with Cryopreserved Sperm. *Cryobiol.,* **54**: 251–257.
26. Hwang, P. C. and Idler, D. R. 1969. A Study of Major Cations, Osmotic Pressure, and pH in Seminal Components of Atlantic Salmon. *J. Fish. Res. Bd . Can.,* **26**: 413-419.
27. Jezierska, B., Lugowska, K., Witeska, M. and Sarnowski, P. 2000. Malformation of Newly Hatched Common Carp Larvae. *Electronic Journal of Polish Agricultural Universities*. Available via http://www.ejpau.media.pl/volume3/issue2/fisheries/art-01.html.
28. Kahkesh, F. B., Yooneszadeh Feshalami, M., Amiri, F. and Nickpey, M. 2010. Effect of Ovaprim, Ovatide, HCG, LHRH-A2, LHRHA2+CPE and Carp Pituitary in Benni (*Barbus sharpeyi*) *Artificial Breed. Glob. Vet.,* **5**: 209-214.
29. Kalbassi M. R. and lorestani R. 2013. Analysis of Saline Activator Solution Effects on Sperm Quality Indices of *Barbus sharpeyi* by Image J Software. *Iran. J. Fish. Sci.* **12**: 357-377.
30. Krejai, R. and Palikova, M. 2006. Potassium Dichromate as a Reference Substance for Embryonic Tests of Toxicity in the Common Carp (*Cyprinus carpio L.*). *ACTA Vet. Brno.,* **75**: 259–263.
31. Lahnsteiner, F., Berger, B., Weismann, T. and Patzner, R. 1995. Fine Structure and Motility of Spermatozoa and Composition of the Seminal Plasma in the Perch. *J. Fish. Biol.,* **47**: 492-508.
32. Lahnsteiner, F., Berger, B., Weismann, T., Patzner, R. A. 1996. Motility of Spermatozoa of *Alburnus alburnus* (Cyprinidae) and Its Relationship to Seminal Plasma Composition and Sperm Metabolism. *Fish Physiol. Biochem.,* **15**:167-179.
33. Li, W. S., Lin, H. R. and Wong, A. O. 2002. Effects of Gonadotropin-Releasing Hormone on Growth Hormone Secretion and Gene Expression in Common Carp Pituitary. *Comp. Biochem. Physiol.,* **132**: 335–341.
34. Lin, F., Ciereszko, A. and Dabrowski, K. 1996. Sperm Production and Cryopreservation in Muskellunge after Carp Pituitary Extract and Human Chorionic Gonadotropin Injection. *Progressive Fish Cult.,* **58**: 32-37.
35. Linhart, O., Alavi, S. M. H., Rodina, M., Gela, D. and Cosson, J. 2008. Comparison of Sperm Velocity, Motility and Fertilizing Ability between Firstly and Secondly Activated Spermatozoa of Common Carp (*Cyprinus carpio*). *J. Appl. Ichthyol.,* **24**: 386-396.
36. Mabudi, H., Jamili, S., Majd, N. E., Vosoughi, G., Fatemi, M. R. and Rashed, S. 2011. The Effects of Ghrelin on Ovary Histology in *Barbus sharpeyi*. *J. Anim. Physiol. Anim. Nutr.,* **95**: 599-602.
37. Mis, J., Bieniarz, K., Epler, P., Sokolowska-Mikolajczyk, M. and Chyb, J. 1995. Incubation of Fertilized Common Carp (*Cyprinus carpio* L.) Eggs in Different Concentrations of Copper. *Pol. Arch. Hydrobiol.,* **42**: 269-276**.**
38. Morisawa, M. 1985. Initiation Mechanism of Sperm Motility at Spawning in Teleosts. *Zool. Sci.,* **2**: 605-615.
39. Morisawa, M., Suzuki, K. and Morisawa, S. 1983. Effects of Potassium and Osmolality on Spermatozoan Motility of Salmonid Fishes. *J. Exp. Biol.,* **107**: 105-113.
40. Mylonas, C. C., Fostier, A. and Zanuy, S. 2010. Broodstock Management and

[DOR: 20.1001.1.16807073.2014.16.1.14.8]

Hormonal Manipulations of Fish Reproduction. *Gen. Comp. Endocrinol*., **165**: 516-534.
41. Ottesen, O. H. and Babiak, I. 2007. Parental Effects on Fertilization and Hatching Success and Development of *Atlantic Halibut* (*Hippoglossus hippoglossus L.*) Embryos and Larvae. *Theriogenol.,* **68**:1219–1227.
42. Pyka, J., Bartel, R., Szczerbowski, J. A. and Epler, P. 2001. Reproduction of Gattan (Barbus xanthopterus Heckel), Shabbout (*Barbus grypus* Heckel) and Bunni (*Barbus sharpeyi* Gunther) and Rearing Stocking Material of Theses Species. *Arch. Pol. Fish.,* **9**: 235-246.
43. Tan-Fermin, J. D., Miura, T., Adachi, S. and Yamauchi, K. 1999. Seminal Plasma Composition, Sperm Motility and Milt Dilution in the Asian Catfish *Clarias macrocephalus* (Gunther). *Aquacult.,* **171:** 323- 338.
44. Verma, D. K, Routray, P., Dash, C., Dasgupta S., Jena, J. K. 2009. Physical and Biochemical Characteristics of Semen and Ultrastructure of Spermatozoa in Six Carp Species. *Turk. J. Fish. Aquat. Sci.,* **9**: 67-76.
45. Von Westernhagen, H., Hoar, W. S. and Randall, D. J. 1988. Sublethal Effects of Pollutants on Fish Eggs and Larvae. *Fish Physiol.,* **11**: 253-346.
46. Wilson-Leedy, J. G., Kanuga, M. K. and Ingermann, R. L. 2009. Influence of Osmolality and Ions on the Activation and Characteristics of Zebrafish Sperm Motility. *Theriogenol.,* **71**:1054–1062.

بهبود فاکتورهای کیفی اسپرم ماهی بنی(*Barbus sharpeyi*) با بکارگیری LHRHA2 و متوکلوپرامید

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چکیده

در این تحقیق، تاثیر تیمارهای هورمونی بر کیفیت اسپرم در ماهی بنی بررسی شد. نتایج بدست آمده تفاوت در پارامترهای حجم اسپرم، تحرک، اسپرماتوکریت، پارامترهای بیوشیمیایی پلاسمای منی و کارایی اسپرم در تکثیر مصنوعی (بازماندگی انکوباسیون و بدشکلی) با تیمارهای متفاوت هورمونی LHRHA2، LHRHA2 در ترکیب با آنتی دوپامین (متوکلوپرامید) و عصاره غده هیپوفیز را نشان داد. بالاترین میزان بازماندگی انکوباسیون و دوره تحرک اسپرماتوزوآ با بکارگیری هورمونLHRHA2 (دوز 10 میکروگرم به ازاء کیلوگرم وزن بدن + متوکلوپرامید) و هورمونLHRHA2 (دوزهای 10 و 5 میکروگرم به ازاء کیلوگرم وزن بدن) به ترتیب در 8، 12 و 16 ساعت پس از هورمونوتراپی، بدست آمد. بالاترین حجم اسپرم به ترتیب در فاصله زمانی 8 و 12 ساعت پس از هورمونوتراپی با هورمونLHRHA2 (دوز 10 میکروگرم به ازاء کیلوگرم وزن بدن + متوکلوپرامید) و هورمونLHRHA2 (دوز 10 میکروگرم به ازاء کیلوگرم وزن بدن) بدست آمد، در حالیکه بکارگیری هورمونLHRHA2 (دوز 2/5 میکروگرم به ازاء کیلوگرم وزن بدن + متوکلوپرامید) بالاترین میزان حجم اسپرم را در 16 ساعت پس از القاء هورمون داشت. بالاترین درصد اسپرماتوکریت به ترتیب در 8، 12 و 16 ساعت پس از القاء هورمونی، با تزریق عصاره غده هیپوفیز،

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هورمونLHRHA2 (دوز 10 میکروگرم به ازاء کیلوگرم وزن بدن + متوکلوپرامید) و هورمونLHRHA2 (دوز 10 میکروگرم به ازاء کیلوگرم وزن بدن) ارزیابی شد. آنالیز بیوشیمیایی پلاسمای منی نشان داد که اکثر پارامترهای مورد مطالعه تحت تاثیر تیمارهای متفاوت هورمونی قرار گرفتند. بعنوان جمعبندی نهایی می توان عنوان نمود که بالاترین کیفیت اسپرم در ماهی بنی را می توان به ترتیب در 8، 12 و 16 ساعت پس از القاء هورمونی، با بکارگیری هورمونLHRHA2 (دوز 10 میکروگرم به ازاء کیلوگرم وزن بدن + متوکلوپرامید)، LHRHA2 (دوز 5 میکروگرم به ازاء کیلوگرم وزن بدن + متوکلوپرامید) وLHRHA2 (دوز 5 میکروگرم به ازاء کیلوگرم وزن بدن) استحصال نمود.

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