www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 17:1025-1030(2017) DOI: 10.4194/1303-2712-v17_5_18

RESEARCH PAPER

Effects of Ovaprim Administration on Reproductive Parameters of Shirbot, *Barbus grypus*, Cyprinidae

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Received 01 January 2017
Accepted 06 March 2017

Abstract

In the present study, we have investigated the *inducing effect* of ovaprim on reproductive parameters of Shirbot, *Barbus grypus* including: spawning rate, working fecundity, egg weight/g.bw, egg hatching percent and *larval survival* rate in order to found an alternative for carp *pituitary extract*. For this purpose, 105 female fish were divided in 6 experimental treatments and injected *intraperitoneally* in two stages (first stage by *10% hormone* and after 12 *hours* the second stage with *90% hormone*) with different doses of ovaprim including: T_1: 0.25 ml/kg.bw, T_2: 0.5 ml/kg.bw, T_3: 0.75 ml/kg.bw, T_4: 1 ml/kg.bw, T_5: 1.25 ml/kg.bw, T_6: 1. 5 ml/kg.bw. Also, one group was considered as control and its fish were injected by 3 mg/kg.bw *pituitary extract. To obtain spermatozoa, 3 maturing male fish were injected intraperitoneally* by 3/kg.bw *pituitary extract in one stage. According to our results*, fish of T_1, T_2 and T_6 did not respond to injection and thus spawning did not occur. The highest values of spawning rate, egg weight/g.bw and working fecundity were observed in T_4 and control group. The lower values of spawning rate, egg weight/g.bw and working fecundity were obtained in T_5 compared to other experimental groups. As well as, there were no significant differences between experimental groups in terms of fertilization percent, hatching percent and *larval survival* rate. The results of this study suggest that a dose of 1 ml/kg.bw ovaprim and 3 mg/kg.bw *pituitary extract have better and similar effects on some* reproductive parameters of Shirbot such as spawning rate, egg weight/g.bw and working fecundity. Thus, use of traditional method i.e. *pituitary extract could be useful yet to induce the reproduction in Shirbot.*

Keywords: Ovaprim, reproductive parameters, fecundity, fertilization, *pituitary extract*, Shirbot, *Barbus grypus.*

Introduction

The Shirbot, *Barbus grypus* is one of the important freshwater fish species in Forat and Dejles river basins. This species is nutritionally omnivorous and ecologically euryhaline and eurytherme and widely distributed in Iran, Turkey, Syria and Iraq (Nikpei, 1996; Abdoli, 2000). Shirbot is favorable among indigenous residents of south western provinces of Iran, especially Khuzestan Province (Banaee & Naderi, 2014). During last decade, the artificial reproduction of Shirbot has been conducted to restock its depleting populations in nature and also to meet market demands (Ghafleh Marammazi, 2000; Marammazi & Kahkesh 2011; Freyhof, 2014). At present, carp pituitary extract is used in the induced breeding of Shirbot, however, this method is costly and less efficient in induction of final maturation due to the existence of some inhibitory hormones in the extract such as dopamine (reviewed by Yousefian & Mousavi, 2011). So, trends in use of synthetic hormones are increasing due to their advantages. Synthetic hormones are much less expensive and they are more stable and thus have a longer shelf life. Also, these hormones are more available in market in a purified form without any reproductive inhibitory component (reviewed byYousefian & Mousavi, 2011). Ovaprim (sGnRHa+Domperidone) is a synthetic hormone which used widely for spawning induction of cyprinidae and other commercial fish species. Seifi, Imanpoor, Jafari and Makhdomi (2011) demonstrated that artificial induction of maturation by ovaprim enhances milt quality parameters in common carp, *Cyprinus carpio*. More, Bhandare, Shinde, Pathan, & Sonawane (2010) have investigated the ovaprim-induced maturation of three Indian carps, *Catla, Rohu* and *Mrigal*. These authors showed that a single intra-muscular injection of ovaprim (0.5-0.2 ml/kg.bw) can induces final maturation. Also, more percentage of fertilization (75- 90%) was found with ovaprim compared to pituitary extract (65- 80%). In the Asp, A*spius aspius*, the highest percentage of ovulation (100%) and embryo-survival to the eyed-egg-stage (81.3%) was recorded after the application of a combination of ovopel and ovaprim in compared to other groups. As well as, fish from the control

group did not ovulate. The latency time was shorter in the groups where ovopel and ovopel with ovaprim was applied (40 h) than in ovaprim group (42–44 h) (Targo´nska, Kucharczyk, Zarski, Mamcarz, & Falahatkar, 2011). Naeem, Salam, and AJafar (2005) suggested that a single dose of ovaprim (0.7 mg/ kg.bw) successfully induces reproduction in *Catla catla*. In *Barbus sharpeyi*, intramuscular injection of LHRHa+CPE (Carp Pituitary) had more efficiency on reproductive parameters than ovaprim (Kahkesh, Yooneszadeh Feshalami, Amiri, & Nickpey, 2010). Gharaei, Abdolali, and Mostafa (2011) concluded that ovaprim is the most suitable for inducing spawning in Snow trout, *Schizothorax zarudnyi* when they observed more working fecundity and percentage of eyed egg to larvae in ovaprim treated group. Mabudi, Savari, and Javadzadeh (2013) demonstrated that 1.25 ml/kg.bw ovaprim improves spawning and fertilization rate in *Barbus xanthopterus*. To our knowledge, there are no studies regarding the inducing impacts of ovaprim on reproductive parameters of Shirbot, *Barbus grypus*. Thus,

In the present study, we examine the effects of various doses of ovaprim on reproductive properties of Shirbot. Such study can help to aquaculture enhancing of Shirbot.

Material and Methods

Experimental Design

The experiment was conducted at developmental center for endemic fishes, Susangerd, Ahvaz, Iran. Broodstocks of Shirbot (n=105 females, (total weight=1600±100 g); n=3 males (total weight=4260±450 g)) were captured from *tributaries* of Karkheh and Karun Rivers. The Broodstocks were transferred to hatchery by 1000 l tank supplied with *oxygen*. Then, females and males were separated and stocked in different ponds to prevent natural spawning. Female fish were identified though morphological characteristics such as pink to red and protruding genital papilla and distended abdomen. In matured males, sperm was released from genital papilla with slight pressure on the belly. In the present study, the cultured males with same size and age were considered for experiment. After 2 days acclimation to hatchery condition, female fish were injected intraperitoneally in two stages *(*first stage by 10% hormone and after 12 hours the second stage with 90% hormone*)* with various doses of ovaprim *(*20 µg GnRH+ 10 mg Domperidone, Asia-Company, Iran*)* depending on experimental treatments. In this regards 6 experimental treatments including*:* T_1: 0.25 ml/kg.bw, T_2: 0. 5 ml/kg.bw, T_3: 0.75 ml/kg.bw, T_4: 1 ml/kg.bw, T_5: 1.25 ml/kg.bw, T_6: 1.5 ml/kg.bw were designed. Also, one group was considered as control and its fish were injected intraperitoneally by 3/kg.bw pituitary extract. Also, male fish were injected intraperitoneally by 3/kg.bw pituitary extract in one stage when females received their last injection. Before hormone injection, the fish were anaesthetized by ethylene glycol monophenyl ether (30 ml/100 l water) (Gyes weii, 1992) and then hormones injected by 3 ml syringe. After final injection, fish were checked every 6 h interval up to ovulation. During the course of the experiment, the water condition (temperature, dissolved oxygen and pH) of each pond was checked and maintained in normal range. After 12 h, the ovulation occurred and egg collection carried out by hand-stripping. Before egg collection, fish were anaesthetized by ethylene glycol monophenyl ether (200 ppm) (Sarker & Satoh, 2007). The eggs were fertilized according to Billard, Cosson, Perchec, and Linhart (1995) by pooled milt samples of three males and then incubated separately in Veis incubators (250 g egg per Veis incubator) until hatching i.e. 3-4 days after fertilization.

Reproductive Parameters

The reproductive indices were spawning rate (%), egg weight/g.bw, working fecundity, fertilization percent (%), hatching percent (%) and larvae survival percent (%). These indices were measured as follow formula:

Spawning rate=The number of ovulated fish/total number of injected fish)×100

Fertilization rate=Number of fertilized egg/ total eggs×100.

Fertilization rate was determined under a dissecting loop 8h after fertilization, when were at the stage of gastrulation (Brommage & Cumalantunga, 1998).

Hatching percent=Number of viable embryos/total number of eggs)×100 (23) (Hanjavanit, Kitancharoen, & Rakmanee, 2008)

Working fecundity = The number of collected eggs/Kg.bw)

Egg weight/g.bw= The number of collected eggs (g)/total body weight (g)

Statistical Analysis

SPSS software (Ver.16) was used for data analysis. Data normality was investigated by *Kolmogorov–Smirnov* test. Differences between means were analyzed by one way analysis of variance (ANOVA) followed by Duncan's new Multiple Range test at minimum significant of $P<0.05$. Data measured in percentage scale were converted by angular transformation (arcsin $\sqrt{p}$) prior to analysis. All results are presented as means±standard error of the mean.

Results

According to our results, fish of T_1, T_2 and T_6 did not respond to injection and thus spawning did not occur. There were significant differences between experimental groups in terms of spawning rate (Figure 1), working fecundity (Figure 2) and egg weight/g.bw (Figure 3) (P<0.05). In this regard, the highest values of spawning rate were observed in T_4 and control group (Figure 1, P<0.05). Also, there were no significant differences between T_4 and control group (Figure 1, P>0.05). Fish of T_5 showed the lowest spawning rate among all experimental groups (Figure 1, P<0.05). The highest values of egg weight/g.bw obtained in fish administrated with ovaprim (1 ml/kg.bw: T_4) and with pituitary extract (3 mg/kg.bw: control) compared to other experimental groups (Figure 3, P<0.05). The lower values of egg weight/g.bw yielded in T_5 compared to other experimental groups (Figure 3, P<0.05). Similar to spawning rate and egg weight/g.bw, more working fecundity was found in T_4 and control group (Figure 2, P<0.05). Also, the lowest working fecundity was observed in T_5 (Figure 2, P<0.05). As well as, there were no significant differences between experimental groups in terms of fertilization percent (Figure 4), hatching percent (Figure 5) and *larval survival* rate (Figure 6) (P>0.05).

Discussion

In the present study, a combination of *GnRH* and Domperidone (as anti-dopamine) was applied for first time for inducing the spawning in Shirbot. Since the inhibitory action of dopamine on LH (luteinizing hormone) secretion is very strong in cyprinids, the use of dopamine antagonists combined with GnRH agonists is necessary to induce the ovulation in a sufficient percentage of fish brooders (De Leeuw, Reasink, Rooyakkers, & Goos, 1985; Mikolajczyk, Chyb, Sokolowska, Mikolajczyk, Enright, Epler, Filipiak, & Breton, 2003). The use of various dopamine antgonists to induce ovulation in cyprinids fish is a well-known method in aquaculture (Peter,

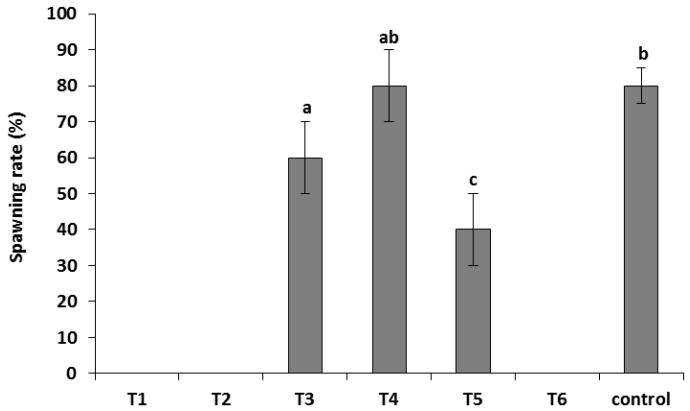

Figure 1. Comparison of spawning rate (%) of Shirbot between experimental groups. T_1: fish administrated with 0.25 ml/kg.bw ovaprim, T_2: fish administrated with 0. 5 ml/kg.bw, T_3: fish administrated with 0.75 ml/kg.bw, T_4: fish administrated with 1 ml/kg.bw, T_5: fish administrated with 1.25 ml/kg.bw, T_6: fish administrated with 1. 5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences (P<0.05).

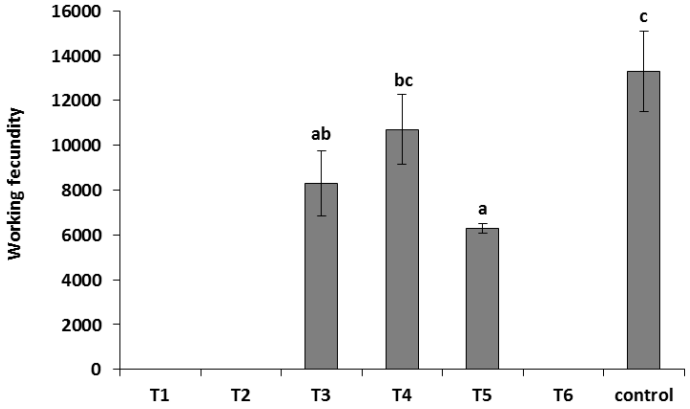

Figure 2. Comparison of working fecundity of Shirbot between experimental groups. T_1: fish administrated with 0.25 ml/kg.bw ovaprim, T_2: fish administrated with 0. 5 ml/kg.bw, T_3: fish administrated with 0.75 ml/kg.bw, T_4: fish administrated with 1 ml/kg.bw, T_5: fish administrated with 1.25 ml/kg.bw, T_6: fish administrated with 1. 5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences (P<0.05).

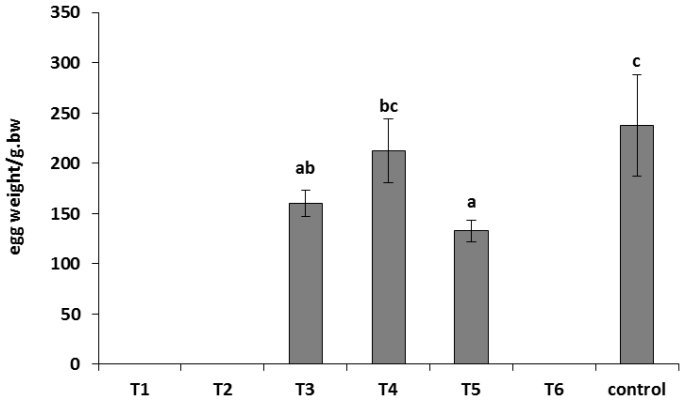

Figure 3. Comparison of egg weight/g.bw of Shirbot between experimental groups. T_1: fish administrated with 0.25 ml/kg.bw ovaprim, T_2: fish administrated with 0. 5 ml/kg.bw, T_3: fish administrated with 0.75 ml/kg.bw, T_4: fish administrated with 1 ml/kg.bw, T_5: fish administrated with 1.25 ml/kg.bw, T_6: fish administrated with 1. 5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences ($P<0.05$).

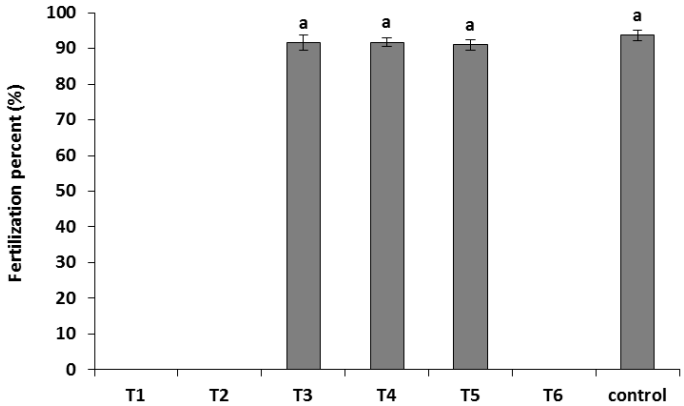

Figure 4. Comparison of fertilization percent of Shirbot between experimental groups. T_1: fish administrated with 0.25 ml/kg.bw ovaprim, T_2: fish administrated with 0. 5 ml/kg.bw, T_3: fish administrated with 0.75 ml/kg.bw, T_4: fish administrated with 1 ml/kg.bw, T_5: fish administrated with 1.25 ml/kg.bw, T_6: fish administrated with 1. 5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences ($P<0.05$).

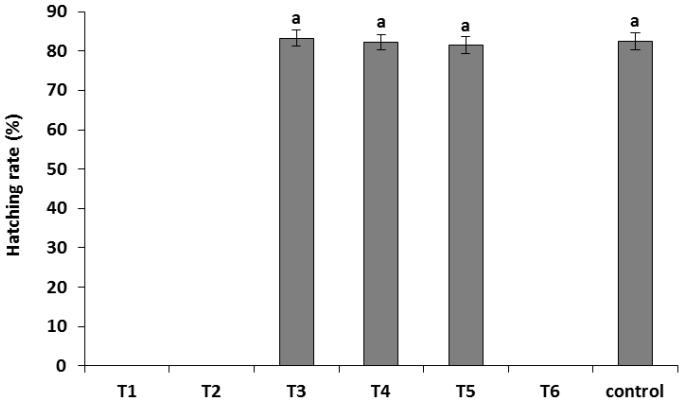

Figure 5. Comparison of hatching percent of Shirbot between experimental groups. T_1: fish administrated with 0.25 ml/kg.bw ovaprim, T_2: fish administrated with 0. 5 ml/kg.bw, T_3: fish administrated with 0.75 ml/kg.bw, T_4: fish administrated with 1 ml/kg.bw, T_5: fish administrated with 1.25 ml/kg.bw, T_6: fish administrated with 1. 5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences ($P<0.05$).

Chang, Nahorniak, Omeljaniuk, Sokolowska, Shih, & Billard, 1986; Peter & Yu, 1997; Barth, Justice, & Ngai, 1997; Brzuska, 2000). Spawning rates, working fecundity and egg weight/g.bw in the present study were between 40-80%, 6200-13300, 130-240. The highest values of spawning rate, working fecundity and egg weight/g.bw were observed when fish administrated with 1 ml/kg.bw ovaprim and 3 mg/kg.bw *pituitary extract.* According to previous studies, the responses of cyprinid fishes to ovaprim

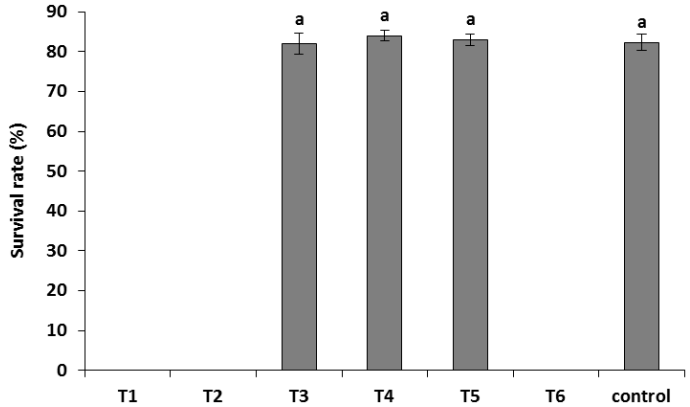

Figure 6. Comparison of larval survival rate (%) of Shirbot between experimental groups. T_1: fish administrated with 0.25 ml/kg.bw ovaprim, T_2: fish administrated with 0. 5 ml/kg.bw, T_3: fish administrated with 0.75 ml/kg.bw, T_4: fish administrated with 1 ml/kg.bw, T_5: fish administrated with 1.25 ml/kg.bw, T_6: fish administrated with 1. 5 ml/kg.bw, control: fish administrated with 3 mg/kg.bw pituitary extract. Different letters indicate significant differences ($P<0.05$).

are different. For example, Nandeesha, Rao, Jayanna, Parker, Varghese, Keshavanath, and Shetty (1990) obtained the best results in inducing reproduction of Indian carps at dose of 0.3-0.4 ml kg ovaprim. However, for the Snow trout a much higher dose of 1.5 ml/kg.bw made the best results (Gharaei *et al*., 2011). These results along with our results indicate that reproductive responses to ovaprim are dose-dependent and are different depending on kind of species. *In our study, the* spawning *did not occur in fish of administrated with* 0.25, 0.5 and 1.5 ml/kg.bw ovaprim. *In fact,* ovaprim both in low and high doses had no inducing effect on reproduction of Shirbot. Some studies demonstrated that induction of fish spawning by using hormonee therapy had not been successful when the hormones had been used in much low and high doses (*Rottmann, Shireman, & Chapman*, 1991). In low doses, a reproductive hormone cannot support all maturation events such as: germinal vesicle migration and breakdown and subsequently ovulation (*Rottmann et al*., 1991). Also, some authors have indicated that the reproduction is impaired due to the physiological negative feedbacks on pituitary gland when the high doses of reproductive hormones are used; as it was found in fish of T_6 (1.5 ml/kg.bw ovaprim) in our study. Therefore, a high dose of ovaprim in our study might affect the pituitary gland as a negative signal and suppress the secretion of gonadotropin. In the present study, since the highest values of spawning rate, working fecundity and egg weight/g.bw were obtained in fish administrated with 1 ml/kg.bw ovaprim, thus this dose is suggested for artificial induction of Shirbot. However, non-significant differences between T_4 and control group in terms of spawning rate, working fecundity and egg weight/g.bw suggest that the commonplace method i.e. use of pituitary extract could be a useful method yet for induction of spawning in Shirbot. In addition, this result shows that the inhibitory action of dopamine in Shirbot is probably weak compared to other cyprinid fishes. In our study, there were no significant differences between experimental groups in terms of fertilization percent, hatching percent and *larval survival* rate. This case suggests that the viability of eggs after ovulation is independent from dose of applied hormone. In conclusion, the results of the present study showed that ovaprim at dose of 1 ml/kg.bw improves more the reproductive parameters of Shirbot. As wells, the inhibitory action of dopamine is probably low in this species since the results in fish administrated with 1 ml/kg.bw ovaprim were similar to those administrated with 3 mg/kg.bw pituitary extract.

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