

## Research Article



# Comparative effects of Ovaprim<sup>TM</sup> and Ovapass<sup>TM</sup> hormones on some reproductive characteristics of *Schizothorax zarudnyi*

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### Abstract

This study was conducted to compare the effectiveness of Ovaprim<sup>TM</sup> and Ovapass<sup>TM</sup> hormones to induce spawning in *Schizothorax zarudnyi*. The female (n=48; 1535.12±180.09 g) and male broodstocks (n=53; 752.00±48.30 g) were randomly allocated in 4 experimental groups including T1: females which received 0.2, 0.5 and 0.5 mL/kg body weight (BW) Ovaprim with 24-h time intervals, T2: females which treated with 0.5 and 0.5 mL/kg BW Ovapass with 12-h time intervals, T3: females were injected with 0.2, 0.5 and 0.5 mL/kg BW Ovapass with 24-h time intervals, and T4: females were injected with a combination of Ovaprim (0.2 mL/kg) and Ovapass (0.5 and 0.5 mL/kg BW) with 24-h time intervals. The amount of injection in the first stage was 10%, the second stage was 90%, and the male fish were simultaneously injected with the second stage of the females at 0.3 mL/kg. The results showed that the highest rate of fertilization was detected in treatments 2 and 4 (93.33% and 92.33%, respectively), which had a significant difference with other groups ( $p<0.05$ ). No significant differences were observed between the treatments in the indices of relative and working fecundity and dry egg diameter ( $p>0.05$ ). The lowest and highest latency period was observed in treatments 2 (27.33±0.66 h) and 1 (36.20±3.77 h), respectively ( $p<0.05$ ). The results of this study clearly suggested that the Iranian Ovapass hormone is highly effective for *S. zarudnyi* reproduction and might be successfully replace with Ovaprim.

**Keywords:** *Schizothorax zarudnyi*, Artificial reproduction, Ovaprim, Ovapass, Fertilization

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## Introduction

The Hamun mahi, *Schizothorax zarudnyi*, is native to the Sistan basin that is located in the east of Iran (Oveisi and Kavosh 2021) and the species exists only in this region in Iran. In fact, this fish belongs to the Hamun international wetland, which is shared between Iran and Afghanistan (Vekerdy *et al.*, 2006). Several factors such as drought, man-made actions and the introduction of non-native species caused a decline in the population of this fish and it was in danger of extinction (Gharaei *et al.*, 2019). In addition to the biological and ecological importance of *S. zarudnyi*, it is delicious and in high demand in the seafood domestic market (Rahdari *et al.*, 2013). Therefore, the species could be a potential candidate in the aquaculture industry (Gharaei *et al.*, 2010). The introduction of new species for aquaculture is rapidly expanding due to the development of new technologies for fish breeding. On the other hand, the number of threatened or endangered species is increasing (Arthington *et al.*, 2016). There are many ways to protection of native species. Although artificial breeding is not the best solution, there is no other solution under the current conditions when other solutions such as restoring natural spawning grounds or removing non-native fish are not possible (Azari Takami, 2014). In sustainable aquaculture, artificial propagation is the most promising and reliable method to produce high egg quality throughout the year. It involves the use of natural (hypophysation) or synthetic hormones

to induce ovulation and spawning in farmed fish (Apollos, 2020; Asadi Eidivand *et al.*, 2022). In captivity, many commercial fish of aquaculture industry exhibit reproductive dysfunctions (Zohar and Mylonas, 2001). So, in these fish hormone therapy is the only way to achieve controlled and reliable reproduction, and not only spawning should be induced in this way, but it is necessary for having good quality gametes and followed having good larvae and fry (Podhorec and Kouril, 2009; Kerdgari *et al.*, 2022). The reason for the lack of spawning in these conditions is the cessation of the release of LH (luteinizing hormone) from the pituitary (Mylonas *et al.*, 1998). The use of hormone therapy causes the release of LH stored in the pituitary and, as a result, advances the final maturation of the oocyte and ovulation (Zohar and Mylonas, 2001). The use of GnRH analogs has been used for hormone therapy of many fish. Of course, in some fish such as carp, catfish, salmon, and some perciforms, there is a strong dopamine system that stops ovulation and sperm emission in captivity (Cabrita *et al.*, 2008).

In breeding sectors, aquaculture species are artificially propagated using different hormones, which are produced by synthetic and recombinant methods. There are several reasons for the production of recombinant hormones such as the small number of desired peptides in the production tissues available, and relationship between the structure and function of the hormones is dedicated to removing or replacing one

or more amino acids (Yeganeh *et al.*, 2022). For artificial reproduction, synthetic hormones are cheaper and more stable than carp pituitary extract (CPE), which has been used in the past for the reproduction of carp, and therefore have a longer shelf life. Also, these hormones are mostly available in the market in pure form without any reproductive inhibiting agents (Yousefian and Mousavi, 2011). Ovaprim contains combinations of salmon gonadotropin-releasing hormone (sGnRH) and domperidone. The Ovaprim hormone has been used for the artificial breeding of many fish species, such as Indian carps (Cyprinidae) (Mijkherjee *et al.*, 2002; Sharma and Singh, 2002; Sarkar *et al.*, 2004; Naeem *et al.*, 2005 a, b), pike, *Esox lucius* (Khaval *et al.*, 2015) and Longspine scraper, *Capoeta trutta* (Zadmajid, 2016). In Danube bleak (*Alburnus chalcoides*), higher efficiency of Ovaprim than pituitary extract and combination of HCG (Human chorionic gonadotropin) with metoclopramide has been observed (Nosrati *et al.*, 2019). Induction of ovulation with Ovaprim in catfish, *Clarias gariepinus* species has been successfully performed (Musa

Ahmed and Hagar Talib, 2018). However, there is currently no published data on the use of two types of recombinant hormones (Iranian-made hormone: Ovapass with foreign-made hormone: Ovaprim) in the artificial reproduction of *S. zarudnyi*, but total reliance on Ovaprim hormone means that reproduction of this fish will be disrupted if unavailable. Therefore, the aim of this study was to compare the effect of the hormones Ovapass<sup>TM</sup> and Ovaprim<sup>TM</sup> on spawning induction in *S. zarudnyi*. So far, no study has been published about the effects of this hormone on fish.

## Materials and methods

### Broodstock selection and maintenance

In this experiment, the fish specimens were obtained from healthy broodstocks of Zahak Aquatic Restoration and Genetic Conservation Center (Sistan and Baluchestan province, Iran, 89°30'N, 67°61'E). Before the experiment, 48 female broodstocks (1535.12±180.09 g) and 60 male broods (752.00±48.30 g) were placed separately in rectangular-shaped concrete tanks of running water (3 m<sup>3</sup> volume) of 15-17°C for 22-24 h (Table 1).

**Table 1: Weights and lengths of injected groups (mean ± SD).**

Parameter		Groups			
		1	2	3	4
Female	Weight (g)	1601.33 ± 315.15	1483.33 ± 56.86	1550.00 ± 245.37	1505.83 ± 102.97
	Length (cm)	52.20 ± 0.97	52.60 ± 1.16	49.40 ± 1.24	49.60 ± 1.58
Male	Weight (g)	761.12 ± 29.41	783.59 ± 60.11	725.17 ± 67.12	738.12 ± 36.57
	Length (cm)	24.85 ± 0.63	27.86 ± 1.29	23.12 ± 0.81	24.35 ± 0.57

Females were characterized with soft, swollen belly and pink-red genital. Also, males were characterized by releasing

milt upon slight pressure to the abdomen were selected. Prior to injections, the fish were anesthetized with a clove

solution bath at 0.05-0.07 mg/L for 2-3 min (Rahdari *et al.*, 2018). The weighing (g), labeling, length measurement (cm), and hormone injection were performed while the fish were unconscious. Following the hormone injection, the specimens (12 females and 15 males) were kept together in circular concrete tanks with a flow-through circuit. Tanks were filled with filtered freshwater and 50% daily water exchanged. During the experiment, the water temperature was  $16.4 \pm 1.1^\circ\text{C}$  and values for dissolved  $\text{O}_2$  and pH were  $6.9 \pm 0.3$  mg/L and  $7.5 \pm 0.4$ , respectively. The photoperiod was kept natural (10 h light and 14 h darkness).

#### *Hormones and experimental design*

Ovapass™ was purchased from Protein Array Saman (PAS) Company (Tehran,

Iran). Its composition is declared similar to Ovaprim™. Ovaprim (each milliliter contains 20  $\mu\text{g}$  Salmon gonadotropin-releasing hormone analog (GnRH) and 10 mg domperidone), purchased from Syndel Laboratories, Ltd., Vancouver, Canada.

Females ( $n=48$ ) were allocated into four experimental groups. 24 h after adaptation at  $15-17^\circ\text{C}$ , four treated groups received hormones via intraperitoneal injections at the base of the pectoral fins as shown in Table 2. The males ( $n=60$ ) were allocated into four groups and received hormones synchronized to the 2<sup>nd</sup> female's injection for inducing spermiation (Table 3).

**Table 2: The doses of hormones used to induce spawning in female *S. zarudnyi*.**

		Injections dosage/kg BW					
Treatment		Fish No.	First	Second	Third	Time interval	Reference
T1	Ovaprim (mL/kg)	12	0.2	0.5	0.5	24h	Rahdari <i>et al.</i> , 2013
T2	Ovapass (mL/kg)	12	0.5	0.5	-	12h	Recommended by the manufacturer
T3	Ovapass (mL/kg)	12	0.2	0.5	0.5	24h	
T4	Ovaprim (mL/kg) + Ovapass (mL/kg)	12	0.2 Ovaprim	0.5 Ovapass	0.5 Ovapass	24h	

**Table 3: The doses of hormones used to induce spawning in male *S. zarudnyi*.**

Groups	Treatment	Fish No.	Injection's dosage/kg BW.
1	Ovaprim (mL/kg)	15	0.3
2	Ovapass (mL/kg)	15	0.3
3	Ovapass (mL/kg)	15	0.3
4	Ovaprim (mL/kg) + Ovapass (mL/kg)	15	0.3

To avoid repeated anesthesia and stress to the brood stocks, an arrangement was made to inject the first stage of the

female broodstocks immediately after labeling and weighing. The injections were done at the base of the pectoral fin

(Rahdari *et al.*, 2013). To minimize stress in the broodstocks, insulin syringes were used for injection. The male and female brood stocks, which were kept separately in rectangular ponds before the injection, were transferred together to the round pond in the indoor facility, after the injection, for further stimulation. After the second

injection, the females were checked every 6 h. Therefore, the fish were anesthetized and the eggs were stripped manually, when ovulation was observed. The spawning rate index (%) was calculated with the following relationship (Billard, 1990):

$$\text{Spawning rate} = (\text{Number of ovulated fish} / \text{Total number of injected fish}) \times 100$$

#### *Collection of Gametes, fertilization, and incubation*

During the examination, ripe gamete donors were anaesthetized in a solution containing 0.05-0.07 mg/L clove powder. The females were checked each 8 to 10 h after the second injection. The milt was collected from the male donors by stripping. The ovum was collected from individual female donor by abdominal gently stripped method. Eggs were stripped into a plastic vessel and were fertilized using a "dry method" (Kucharczyk *et al.*, 1997). The eggs were placed on trays and the milt and eggs were mixed slowly with a turkey feather for one min. In general, the ratio

of males to females was 3:1 (Gharaei *et al.*, 2019). All spawners were kept one week after gamete collection and all fish were closely monitored to record survival rate.

The fecundity rate was calculated using volumetric technique. This technique uses simple proportionality to estimate total fecundity from a given number of eggs in a known subsample volume and total sample volume value, and then calculates the total number of eggs in the ovary. To determine the relative fecundity, the ratio of the number of extracted eggs to the weight of the fish was calculated (Billard, 1990):

Working fecundity = The total volume of stripped eggs  $\times$  The average number of eggs in the samples

Relative fecundity = Working fecundity / total body weight (kg)

For fertilization, a very small amount of water was added so that the sperms become active and fertilization takes place. Then, the adhesion was removed gradually with the water of the hatchery facility. Egg diameter (mm) was

measured from 10 eggs from each fish, using an eyepiece micrometer fitted to a dissection microscope. The water was changed over time and after half an hour at most, the adhesion of the eggs was completely removed then left to water

harden for 30 min. Fertilized eggs were incubated in jar incubators (Vase) to start the incubation stage. To determine the percentage of fertilization about 24 h after transferring the eggs to jar incubators, about 100 eggs of each brooder were sampled and after clarification in the clarification solution

Fertilization rate (FR) (%) = Number of fertilized egg / total eggs × 100

Two types of incubators, jar Vase and troughs California, were used for the incubation stages of *S. zarudnyi* eggs. In this way, from the beginning to the egg-hatching stage, a jar Vase was used, and from hatching to post-hatching, and after that, California incubators were used. In each jar incubator, 300 mL of water-

Hatching rate (%) = (Number of viable larvae / total number of eyed eggs) × 100

Larvae Samples were fixed in formaldehyde and external condition were checked by a loop.

### Statistical Analysis

Data analysis was done with SPSS 16 software (Chicago, IL, USA). First, their normality was checked by the Kolmogorov-Smirnov test and the homogeneity of variances was checked by Levene's test. Then, differences among the experimental groups were analyzed using One-way variance followed by Duncan's test at a 95% confidence level. Data are represented as mean ± standard deviation.

### Results

According to our results, ovulation and spawning occurred between 85% and

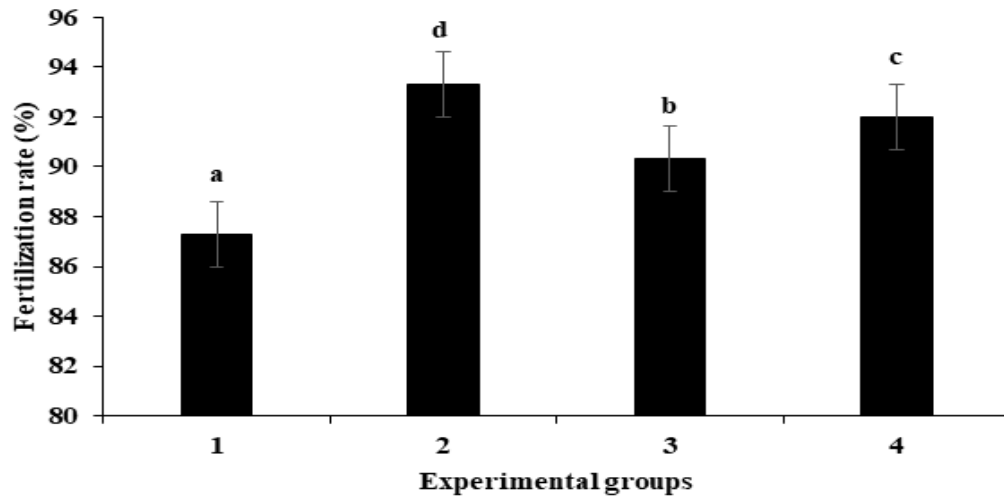
(1:1:1 methanol+acetic acid+distilled water (Zarei *et al.*, 2019)), the stages of embryo growth and development was observed under the loop. Samples with a neural streak were counted to determine the fertilization rate (Pankhurst *et al.*, 1996):

hardened eggs were poured and water flow was established. After all the eggs in each jar incubator reached the hatching time, they were transferred to the trough's Californian incubator. The percentage of egg-to-larvae conversion was calculated using the following relationships (Pankhurst *et al.*, 1996):

100% in all groups after hormone injection. There were significant differences between experimental groups in terms of fertilization rate (FR) (Fig. 1), the number of dry eggs per milliliter, spawning success (Table 4), latency period, eyed eggs, and hatching rate ( $p < 0.05$ ; Table 5). In this regard, the highest values of FR were observed in T2 ( $p < 0.05$ ). Fish of T2 showed the highest spawning rate among all experimental groups, also, there were no significant differences between T2 and T4 ( $p > 0.05$ ). As well as, there were no significant differences between experimental groups in terms of working fecundity and relative fecundity (Table 4), the diameter of dry egg and total hormone injected ( $p > 0.05$ ; Table 5). Also, the lowest working fecundity and

relative fecundity were observed in T1 ( $p>0.05$ ; Table 4). Also, the lowest and highest values of total hormone injected were observed in T2 and T1, respectively. The lowest and highest values of the latency period, which is the time interval between the first injection and the time of ovulation, were observed

in T1 and T2, respectively ( $p>0.05$ ; Table 5). The results of the incubation period showed that the time to reach the hatching stage and from hatching to post-hatching was influenced by the water temperature of the incubators.



**Figure 1:** Fertilization rate of different groups injected with Ovaprim and Ovapass hormones (T1: Fish that received 0.2, 0.5 and 0.5 mL/kg body Ovaprim with 24-h time intervals, T2: females treated with 0.5 and 0.5 mL/kg BW Ovapass with 12-h time intervals, T3: females were injected with 0.2, 0.5 and 0.5 mL/kg BW Ovapass with 24-h time intervals, and T4: females were injected with a combination of Ovaprim (0.2 mL/kg) and Ovapass (0.5 and 0.5 mL/kg BW) with 24-h time intervals).

**Table 4:** The effect of different doses of two types of hormones, Ovaprim and Ovapass, on different parameters of *S. zarudnyi* eggs and larvae (mean  $\pm$  standard deviation; n=12).

Group	Female brooder weight (g)	Spawning success (%)	Working fecundity	Relative fecundity	The volume of dry eggs per female fish (mL)	The number of dry eggs/mL
1	1601.33 $\pm$ 315.15	85 $\pm$ 00.00 <sup>a</sup>	42258.33 $\pm$ 10651.89	26504.79 $\pm$ 2821.39	153.67 $\pm$ 38.73 <sup>b</sup>	275.00 $\pm$ 1.00 <sup>a</sup>
2	1483.33 $\pm$ 56.86	100 $\pm$ 00.00 <sup>c</sup>	45070 $\pm$ 10203.03	30236.04 $\pm$ 5803.13	160.00 $\pm$ 36.06 <sup>a</sup>	281.67 $\pm$ 1.53 <sup>b</sup>
3	1550.00 $\pm$ 245.37	85 $\pm$ 00.00 <sup>a</sup>	52977.22 $\pm$ 6191.04	34522.62 $\pm$ 4946.76	191.67 $\pm$ 23.63 <sup>b</sup>	278.00 $\pm$ 1.00 <sup>a</sup>
4	1505.83 $\pm$ 102.97	90 $\pm$ 00.00 <sup>b</sup>	50746.67 $\pm$ 5306.65	33785.46 $\pm$ 3229.86	210.00 $\pm$ 55.68 <sup>a</sup>	273.00 $\pm$ 1.65 <sup>a</sup>

<sup>a,b</sup> Different superscripts within a column indicate significant differences ( $p<0.05$ ). (T1: Fish that received 0.2, 0.5 and 0.5 mL/kg body Ovaprim with 24-h time intervals, T2: females treated with 0.5 and 0.5 mL/kg BW Ovapass with 12-h time intervals, T3: females were injected with 0.2, 0.5 and 0.5 mL/kg BW Ovapass with 24-h time intervals, and T4: females were injected with a combination of Ovaprim (0.2 mL/kg) and Ovapass (0.5 and 0.5 mL/kg BW) with 24-h time intervals).

As the temperature increased, the duration of these periods became shorter and counter. The lowest values of completion of hatching (h) obtained in fish administrated with Ovapass (35±3.2 h; T2) compared to other experimental

groups ( $p<0.05$ ; Table 6). No abnormality was observed in the offsprings of the experimental groups after hormone therapy and spawning.

**Table 5: Effect of different doses of two types of hormones, Ovaprim and Ovapass, on different parameters of *S. zarudnyi* eggs and larvae (mean ± standard deviation; n=12).**

Group	Diameter of dry egg (mm)	Total hormone injected (mL)	Latency period (h)	Eyed egg (%)	Hatching rate (%)
1	1.67 ± 0.25	1.93 ± 0.36	36.1 ± 3.8 <sup>c</sup>	87.27 ± 0.23 <sup>a</sup>	80.67 ± 0.12 <sup>a</sup>
2	1.48 ± 0.012	1.52 ± 0.98	27.33 ± 0.66 <sup>a</sup>	93.33 ± 0.58 <sup>d</sup>	87.67 ± 4.93 <sup>b</sup>
3	1.61 ± 0.12	1.70 ± 0.19	32.38 ± 2.20 <sup>b</sup>	90.33 ± 0.58 <sup>b</sup>	82.00 ± 1.00 <sup>a</sup>
4	1.69 ± 0.03	1.80 ± 0.15	31.8 ± 1.30 <sup>b</sup>	91.33 ± 0.58 <sup>c</sup>	89.33 ± 0.58 <sup>b</sup>

<sup>a,b</sup> Different superscripts within a column indicate significant differences ( $p<0.05$ ). (T1: Fish that received 0.2, 0.5 and 0.5 mL/kg body Ovaprim with 24-h time intervals, T2: females treated with 0.5 and 0.5 mL/kg BW Ovapass with 12-h time intervals, T3: females were injected with 0.2, 0.5 and 0.5 mL/kg BW Ovapass with 24-h time intervals, and T4: females were injected with a combination of Ovaprim (0.2 mL/kg) and Ovapass (0.5 and 0.5 mL/kg BW) with 24-h time intervals).

**Table 6: Characteristics of water temperature and period of eyed and hatching eggs.**

	Group 1	Group 2	Group 3	Group 4
Water temperature until eyed egg period (°C)	16.0±1.3	16.0±1.1	16.8±1.2	17.2±1.0
Eyed egg period (h)	170.0±10.0 <sup>b</sup>	165.0±12.5 <sup>b</sup>	158.5±11.5 <sup>a</sup>	152.6±8.5 <sup>a</sup>
Water temperature from eyed egg stage to hatching (°C)	18.4±1.5	19.5±1.9	17.3±1.1	17.3±1.0
Completion of hatching (h)	48.0±3.5 <sup>b</sup>	35.0±3.2 <sup>a</sup>	52.0±4.3 <sup>b</sup>	52.0±4.1 <sup>b</sup>

<sup>a,b</sup> Different superscripts within a line indicate significant differences ( $p<0.05$ ).

## Discussion

In the present study, a combination of Ovaprim and Ovapass was applied for the first time for inducing spawning in *S. zarudnyi*. *S. zarudnyi* belongs to the Cyprinidae family that has a strong dopamine system (Peter *et al.*, 1986). One of the strong types of evidence for this claim is that the use of pituitary extract and HCG never induces ovulation in the female fish. Therefore, to weaken and eliminate the dopamine system, an anti-dopamine agent (e.g. domperidone, reserpine, metoclopramide or pimozide) must be used (Brzuska, 2021). So far, the

Ovaprim hormone has been used for the artificial breeding of this fish (Rahdari *et al.*, 2013; Gharaei *et al.*, 2019). However, pituitary extract has been effective for inducing spermatogenesis in male *S. zarudnyi*, and even some of its sperm cognitive parameters have been better than Ovaprim. For example, the highest levels of sperm volume, spermatocrit, and sperm density were observed in the treatment that used the pituitary extract (Arabnejad *et al.*, 2013).

The efficacy of two synthetic (Ovaprim and Ovatide) for the induced breeding of Indian major carps were compared. The results revealed that



Ovaprim is more effective than ovatide for the breeding of *Catla catla* (Dhawan and Kaur, 2004). Akbari Nargesi *et al.* (2022) showed that two injections of Ovaprim and/or a priming dose of Ovopel (mammalian GnRH analog+metoclopramide, at 18-20 µg and 9-10 mg, respectively) with a resolving dose of Ovaprim were suitable for the artificial reproduction of Rudd (*Scardinius erythrophthalmus*) female breeders, however two injections of Ovaprim recommended for Rudd spawning due to the proper effects and easy application.

The reason for the success of Ovaprim in inducing the spawning of *S. zarudnyi* should be found in the composition of this hormone, which contains dopamine antagonists. In other words, each milliliter contains 20 micrograms of sGnRH (Salmons GnRH) and 10 mg of the anti-dopamine domperidone (Ovaprim™ [www.syndel.com](http://www.syndel.com)). In this study, the effectiveness of Ovapass in inducing spawning of *S. zarudnyi* was compared to Ovaprim. The composition of both hormones is similar, but there are two non-structural differences between them. First of all, Ovapass is an available preparation for fish fry producers. Secondly, the protocol provided for the use of these two hormones by the manufacturing companies is different from each other. This difference becomes important when the duration of hormone therapy until spawning and the amount of hormone used decrease (T2). The frequency of injection is one of the parameters that is important in terms of time, cost and stress to the brooders. In

the case of *S. zarudnyi*, at least three consecutive injections are needed for ovulation to occur, while according to the brochure of the manufacturer of Ovaprim, in most carps, only one injection of Ovaprim at the rate of 0.5 mg/kg is enough for ovulation to occur ([www.syndel.com](http://www.syndel.com)). The issue of the necessity of multiple injections in white fish is true for both hormones, Ovaprim and Ovapass, with the difference that the interval between injections for Ovaprim was 24 h, but for Ovapass, it was 12 h, which reduces the time by half, at least in terms of time and cost. The need for one or more injections is related to the characteristics of the fish, such as, in contrast to *S. zarudnyi*, in *Capoeta razii*, the weight of its brooders was less than 100 g (Abdolahpour *et al.*, 2021) and/or in *Cyprinus rubrofasciatus* with an average weight of 536.7±6 g (Malik *et al.*, 2014), spawning occurred only with a single injection of 0.5 mL of Ovaprim per kilogram of body weight. Also, in Indian cyprinid, a single injection of 0.5-2.5 mg/kg of Ovaprim has caused the final treatment (More *et al.*, 2010). On the other hand, the total amount of hormone injected into the reproductive system until the induction of ovulation, in treatment 2 (Ovapass), although it did not have a statistically significant difference with other treatments, the amount was 0.4 mL less than Ovaprim, which is practically and economically very important.

Spawning ratio or Spawning success is one of the good indicators to evaluate hormonal effects on ovulation (Szabo *et al.*, 2002). In this research, fish of T2

showed the highest spawning rate (100 %) among all experimental groups, which is a very acceptable ratio in fish reproduction.

The observation of high FR in treatments 2 and 4 (93.33 and 90.33 percent) compared to the other two treatments cannot be attributed to the type of hormone, because the composition of Ovaprim and Ovapass is similar and probably other factors such as the type of brooders, sperm quality and inoculation operation have been involved. The fact that the percentage of fertilization did not decrease with Ovapass compared to Ovaprim, indicates the absence of a negative effect of Ovapass. In *Barbus grypus*, the percentage of fertilization in fish injected with Ovaprim was 75-90%, while it was 65-80% in pituitary extract treatment (Ghanemi and Khodadadi, 2017).

In the present study, there was no significant difference in some reproductive parameters, including total hormone injected, fecundity (working and relative), and egg diameter among different treatments, which indicates the comparable efficiency of Ovapass with Ovaprim. Egg quality is defined based on its ability to become an embryo (Bobe and Labbé, 2010). The substances used to induce spawning affect not only the time of spawning but also on the number of fish ready to spawn and the quantity and quality of the obtained gametes (Kucharczyk *et al.*, 2007). In perch, *Sander lucioperca*, the quality of eggs collected after induction of ovulation by GnRHs was better than in fish induced

with gonadotropic compounds (such as HCG) (Ljubobratović *et al.*, 2019). In the present study, Ovaprim and Ovapass were GnRH hormones, therefore it cannot be concluded that hormone therapy had an effect on egg quality indicators such as egg diameter.

The lowest latency period in the present study was related to T2 ( $27.33 \pm 0.66$ ), which used Ovapass. In fish, the latency period depends on various parameters such as water temperature, biological characteristics (species, age and weight), hormone type and injection frequency (Billard, 1990; Yaron, 1995). In grass carp *Ctenopharyngodon idella*, there was no significant difference in the latency period between the pituitary extract injected group and Ovaprim group (Khodabandeh Shelamani *et al.*, 2012). In *Aspius aspius*, the latency period in the group that received Ovopel (mammalian GnRH+anti-dopamine metoclopramide) or Ovopel and Ovaprim was 40 h, but in the group that received only Ovaprim it was longer (42-44 h) (Targońska *et al.*, 2011). In *Abramis brama*, the shortest latency period was also observed when the pituitary extract was used together with HCG hormone, and the duration of the period was increased with Ovopel (Kucharczyk *et al.*, 2007). Since in our study, all treatments were performed on the same species and weight, age and hormone composition were the same. So, the difference between the latency periods of the treatments may be related to the dose and frequency of hormone injections (Rahdari *et al.*, 2014).

In conclusion, the artificial spawning of *S. zarudnyi* was enhanced by using stimulating hormones, but Ovapass gave the best results followed by Ovaprim. These stimulating hormones have enhanced reproductive performances. The data of the present study showed that the effectiveness of Iranian Ovapass hormone for inducing spawning of *S. zarudnyi* for reasons such as good performance, cheapness, and easy availability is very suitable and it can be replaced Ovaprim hormone.

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