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Research Article

Effects of human chorionic gonadotropin and ovaprim on the reproductive performance of tench (*Tinca tinca*) broodstock during semi-artificial reproduction

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Abstract

This study investigated the effects of Ovaprim (sGnRHa + domperidone) and human chorionic gonadotropin (hCG) combined with metoclopramide on the reproductive performance of *Tinca tinca* broodstock under semi-artificial reproduction. A total of 42 broodfish (21 females and 21 males), weighing 804.2 ± 20.62 and 503.53 ± 15.31 g, respectively, were selected from the western basin of Anzali Lagoon, transferred to the Shahid Ansari Center, and maintained at 25.1±0.3°C. The experimental groups (n=14 for each treatment, including 7 males and 7 females) included: the control group (0.9% NaCl solution), the hCG group (a dose of 400 IU/kg of fish body weight), and the Ovaprim group (a dose of 0.5 mL/kg of fish body weight). Metoclopramide was injected as an antidopamine agent in combination with hCG (10 mg metoclopramide + 5000 IU hCG). Females received two injections (20% initial + 80% after 10 h), while males received one injection. The results indicated that the highest response to the hormone for spawning maturation (100%) and the highest egg fertilization rate (85.7%) were observed in the Ovaprim group compared to the hCG group (45.3%) ($p<0.05$). Moreover, the latency time in the Ovaprim group (12 hours) was significantly shorter than in the hCG group (20 hours) ($p<0.05$). Furthermore, no ovulation was observed in the control group ($p>0.05$). In conclusion, administration of Ovaprim at a dosage of 0.5 mL/kg body weight under controlled conditions significantly enhanced the reproductive performance of tench, indicating its potential as an effective hormonal agent for semi-artificial reproduction in aquaculture practices.

Article info

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Introduction

The tench (*Tinca tinca*) is an omnivorous freshwater fish species belonging to the family Cyprinidae, with a distribution range extending across various regions of Europe and parts of Asia, including northern Iran (particularly the basins of the Caspian Sea). Owing to its desirable growth performance, high-quality flesh, and ability to reproduce under captive conditions, the commercial production and conservation-based rehabilitation of this species have been expanding in several countries (Kottelat and Freyhof, 2007; Kujawa *et al*., 2011; Kucharczyk *et al*., 2024). With the increasing demand for tench production, the establishment of scientifically sound and efficient reproductive technologies and culture practices is becoming increasingly important.

In many species, wild-caught or captive-bred spawners fail to reproduce due to inadequate conditions (Zohar *et al*., 2010; Zupa *et al*., 2017). This failure to reproduce is often due to poor nutrition, stress in captivity, and lack of a suitable environment for spawning. This can lead to anovulation in females, or a lack of spermatogenesis, and poor sperm quality in males, especially in stress-sensitive species (Kumar *et al*., 2022; Bera *et al*., 2025).

To enhance the efficiency of artificial reproduction and ensure synchronized reproduction, hormonal treatments and appropriate methods can significantly promote this industry (Akbari Nargesi *et al*., 2022; Honji *et al*., 2025). Various hormones, such as synthetic gonadotropin-releasing hormone analogues (GnRHa) and specific gonadotropins, have caused successful ovulation and spermiation in many freshwater and marine species (Mylonas *et al*., 2010; Trudeau, 2022; Mechaly *et al*., 2024; Akbari Nargesi *et al*., 2025a). Despite the favorable effect of GnRH in stimulating reproduction in many vertebrates, the presence of dopamine as a factor inhibiting the action of gonadotropins in some teleosts has made the function of this hormone in artificial reproduction unsuccessful. Therefore, the use of dopamine antagonists along with this hormone is necessary to induce reproduction in such species (Peter *et al*., 1988). This hormonal manipulation treatment, which is currently known as the Linpe technique, has been extensively applied in various teleost species. It helps overcome dopaminergic suppression and elevates reproductive success, especially in cyprinids (Vazirzadeh *et al*., 2016; Trudeau, 2022; Hu *et al*., 2021; Mechaly *et al*., 2024; Nguyễn *et al*., 2025).

To date, various hormonal treatments have been employed to induce synchronized ovulation and facilitate out-of-season reproduction in different fish species. Ovaprim is a commercial liquid hormone formulation containing a salmon gonadotropin-releasing hormone analogue (sGnRHa) and the dopamine antagonist domperidone. It can be administered through intramuscular or intraperitoneal injection and acts directly on the pituitary gland to stimulate gonadotropin release. In recent years, Ovaprim has been successfully applied for reproductive induction in numerous fish species (Kucharczyk *et al*., 2024; Akbari Nargesi *et al*., 2025b). Human chorionic gonadotropin (hCG) is also one of the most popular and effective solutions used to induce the secretion of luteinizing hormone in humans, and there are successful experiences in inducing reproduction in various fish species (Kucharczyk *et al*., 2020; Falahatkar *et al*., 2022; Gorouhi *et al*., 2024).

Although several studies have investigated the artificial reproduction of wild tench using spawning-inducing agents (Kujawa *et al*., 2011; Targońska *et al*., 2012; Kucharczyk *et al*., 2024), research on the controlled reproduction of this species under captive conditions remains limited in Iran. In particular, no studies have yet evaluated the reproductive performance of tench broodstock under controlled breeding conditions in Iran. Therefore, adapting reproductive protocols to the specific environmental and climatic conditions of each region is essential for achieving optimal outcomes in hatchery operations. Accordingly, the present study aimed to compare the effects of hCG and Ovaprim on the reproductive performance of tench broodstock and to establish an effective protocol for semi-artificial reproduction of this valuable species.

Materials and methods

Collection and keeping of broodstock

The wild tench broodstock (n=50) used in this study were caught from the western basin of Bandar Anzali Lagoon using double-sided cone traps and then transported to the Shahid Ansari Teleost Fish Restocking and Genetic Conservation Center (Guilan, Iran) using special fish-carrying vehicles (plastic bags and oxygen capsules, with oxygen supplied continuously during transport). First, the broodstock were anesthetized using clove powder extract (200 mg/L) and examined for their apparent health. Then, healthy male and female broodstock were kept separately in two fiberglass tanks (2.0 × 2.0 × 0.5 m each) under the environmental conditions of the hatchery for two weeks before checking their readiness for reproduction. After the end of the adaptation period, the sexual maturity of female broodstock was assessed by examination of their appearance, the softness of their abdominal surface, and the genital opening. Moreover, the degree of sexual maturity of male broodstock was determined by examining the genital opening and applying gentle pressure on the abdominal area. The water physicochemical factors during the experiment period, including temperature, dissolved oxygen, and pH, were 25.1±0.3 °C, 7.8±0.6 mg/L, and 7.1-7.4, respectively. Natural photoperiod was considered during the experiment (D10:L14).

Experimental design and semi-artificial reproduction

After the adaptation period, the fish were divided into three experimental groups (Table 1). The experimental groups included: 1) the control group (0.5 mL of 0.9% saline solution per kg of fish body weight), 2) the hCG group (400 IU per kg of fish body weight), and 3) the Ovaprim (containing 20 µg/mL sGnRHa and 10 mg/mL domperidone) group (0.5 mL per kg of fish body weight). In the commercial hormone Ovaprim, a combination of hormone and antidopamine is present. For the hCG treatment, metoclopramide was added as an antidopamine agent to the hCG vial (10 mg for 5000 IU of hCG).

Table 1: Hormone doses administered during priming and resolving injections across different treatment methods in different genders of the tench (*Tinca tinca*).

Broodstock gender	Hormonal treatment	Overal dose/kg	Priming dose (%)	Time interval (h)	Resolving dose (%)	Number of injections
Female	Control [(0.9% saline, (mL/kg)]	0.5	20	10	80	2
	hCG (IU/kg) + Metoclopramide*	400	20	10	80	2
	Ovaprim (mL/kg)	0.5	20	10	80	2
Male	Control [(0.9% saline, (mL/kg)]	0.5	-	-	100	1
	hCG (IU/kg) + Metoclopramide*	400	-	-	100	1
	Ovaprim (mL/kg)	0.5	-	-	100	1

* Metoclopramide was injected as an antidopamine agent in combination with hCG (10 mg metoclopramide + 5000 IU hCG).

In the present study, the injected dose of hormones in different treatments was selected based on previous studies on other fish species (Falahatkar *et al*., 2019, 2022; Gorouhi *et al*., 2024). The average weight for male and female fish was 503.53±15.31 and 804.2 ± 20.62 g, respectively. Before injection, two broodstocks from each treatment were dissected to examine the condition of the ovaries and estimate related indices, including gonad weight and gonadosomatic index. Characteristics of the tench female broodstock used for induced spawning under controlled conditions are shown in Table 2. In all treatments, the injection was performed intraperitoneally under the ventral fin using a 1 mL syringe (Fig. 1).

Table 2: Characteristics of the tench (*Tinca tinca*) female broodstock used for induced spawning under controlled conditions.

Index	Treatments		
	Control	hCG	Ovaprim
Number of fish	7	7	7
Broodstock weight (g)	789.4±142.4	822.3±131.7	809.3±142.7
Broodstock length (cm)	35.7±1.5	36.1±1.6	35.8±1.8
Gonad weight	120.45±20.15	119.1±16.3	123.5±11.2
Gonadosomatic Index (%)	14.9±0.14	15.8±0.23	14.7±0.71
Survival rate (%)	100 ± 0.0	100 ± 0.0	100 ±0.0

Data expressed as mean ± standard deviation.

In all groups, male and female fish were kept in a 1:1 ratio after injection and were maintained in a 2.0-meter-long, 0.7-meter-wide hapa within the earthen pond. The interior of the hapa was covered with artificial nests made of nylon rope spawning mops.

Gamete's collection

During the post-injection period, the fish were monitored for possible spawning at two-hour intervals. If spawning was observed for any fish, the exact time of spawning was recorded. Then, the eggs were slowly added to the bucket of water using a sieve and weighed using a scale with an accuracy of 0.01 g.

Figure 1: The process of selecting and inducting tench (*Tinca tinca*) broodstock. A: Ventral view of a female fish; B: Ventral view of a male fish; C: Anesthesia process; D: Injection process.

Reproductive parameters analysis

The average number of water-hardened eggs per gram (n=3 for each treatment) was estimated by weighing 1 g of water-hardened eggs (in triplicate for each sample) with a scale of 0.001 g accuracy and counting the number of eggs. The latency time, response rate, fertilization rate, mean weight of released eggs, and relative fecundity were calculated using the following formulas (Abdissa *et al*., 2020; Akbari Nargesi *et al*., 2023):

Response rate (%) = (number of ovulated females / number of injected females) × 100

Latency time = number of hours until ovulation

Relative fecundity = number of eggs per fish / kg of fish body weight

Fertilization rate (%) = [(number of incubated eggs - number of dead opaque eggs) / total number of incubated eggs] × 100

Statistical analysis

The data of the present study were expressed as mean ± standard deviation. SPSS version 22 (IBM, Armonk, NY, USA) was used for statistical calculations in this study. One-way analysis of variance was performed to examine the difference between the mean variables within treatments. After observing a significant difference at the 0.05 level in the ANOVA test, Tukey's post hoc test was used for multiple comparisons.

Results

According to the results, only two females ovulated in the hCG-injected group after 20 hours of injection (Table 3). The abdomen size of the other five broodstock was only visibly larger than in the control treatment, but no signs of egg release were observed. According to Table 3, the fertilization rate in this group was 48.3%, and the average weight of the released eggs per fish was 91.8 g. All the Ovaprim-injected females ovulated approximately 12 hours after injection, and the eggs were fertilized. According to Table 3, the fertilization rate in this group was 85.7%, and the average weight of the released eggs was 104.6 g. However, in the control group, which was only treated with physiological serum, no ovulation was observed, and no changes were detected in the fish of this group, either in terms of appearance. The results of the present study showed that the response rate, relative fertility, and fertilization rate in the Ovaprim-injected broodstock increased significantly compared to other groups ($p<0.05$). According to the results, the response rate of this hormone in this fish species was 100%.

Table 3: Comparison of reproductive parameters of semi-artificially propagated tench (*Tinca tinca*) broodstock after induction with hCG and Ovaprim (mean ± standard deviation) (n=7 for each treatment).

Index	Treatments		
	Control	hCG	Ovaprim
Latency time (hrs)	nd	20±4.2	12±2.8
Number of oocytes per gram	1995.1±45.1	1969.0±46.9	1981.4±42.8
Number of water-hardened eggs per gram	-	857.6±15.0	848.3±12.7
Response rate (%)	0^c	28.6^b	100^a
Mean weight of released eggs (g)/fish	0^b	$91.8±4.7^a$	$104.6±13.1^a$
Relative fecundity (eggs/kg body weight)	nd	$197020.8±13644.6^a$	$258405.8±33158.3^a$
Fertilization rate (%)	nd	$48.3±7.9^b$	$85.7±4.3^a$

Different letters in each row indicate significant differences ($p<0.05$).

Discussion

It has been well established that the use of hormonal induction in aquaculture programs can be a practical method for controlling the reproduction process and increasing reproductive efficiency in captive broodstock. Given the significance of the wild tench population, this study examined the effects of administering different hormonal agents (hCG+metoclopramide and Ovaprim) on the semi-artificial reproduction and reproductive performance of this valuable species. In addition, the present study is among the first to provide evidence comparing the effects of hCG combined with metoclopramide and the synthetic hormone Ovaprim in fish.

According to our results, the latency time documented in this study was lower in fish treated with Ovaprim than in those treated with hCG. Various factors affect the latency time in fish reproduction, including the dose of the hormonal agent, the type of hormone, and the fish species (Kujawa *et al*., 2022). The reason for this difference in the present study may be attributed to the better effectiveness of Ovaprim and its compounds for the tench. In a similar study, Akbari Nargesi *et al*. (2022) administered Ovaprim, Ovopel, and their combinations to stimulate female Caspian roach (*Rutilus caspicus*) and found that the shortest latency period of 20 hours occurred after injecting 0.1 and 0.5 mL/kg body weight of Ovaprim.

The fertilization rate reported in this study was higher in fish treated with Ovaprim than in those treated with hCG. It is likely that hormonal stimulation by Ovaprim, in addition to affecting the dynamics of female maturation, had a significant effect on ovulation. Injecting Ovaprim into male fish also enhanced hatching success and improved embryo survival rates. Recent studies on various fish species, including blackfin sea bream (*Acanthopagrus berda*) (Abbas *et al*., 2019), Caspian Kutum (*Rutilus frisii*) (Akbari Nargesi *et al*., 2024), and common bream (*Abramis brama*) (Akbari Nargesi *et al*., 2025b), have shown that administering an appropriate dose of Ovaprim can result in a high fertilization percentage, which is consistent with the results observed in the present study.

Given the faster response to injection and 100% response of broodstock induced with Ovaprim, it can be concluded that this hormone is more suitable than hCG for inducing spawning of tench. The lack of spawning in the control group and in some fish in the hCG group may indicate a decrease in plasma concentrations of gonadotropin hormones in these fish; a vital factor that is considered essential for initiating the final stages of oocyte maturation and stimulating the spawning process (El-Hawarry *et al*., 2016; Akbari Nargesi *et al*., 2022). Our results highlight the vital role of stimulating hormones in coordinating reproductive processes, as well as the higher efficacy of Ovaprim due to its particular composition and multiple mechanisms compared to hCG in stimulating ovulation in this species. hCG performs as an analogue of luteinizing hormone (LH) and directly induces ovarian follicles to ovulate; nonetheless, it lacks a dopamine inhibitory mechanism (Mylonas *et al*., 2010). The simultaneous use of hCG and metoclopramide has a synergistic effect in inducing ovulation. Metoclopramide inhibits dopamine by blocking D_2 receptors and subsequently increases LH secretion, resulting in the final maturation of follicles and ovulation (Yaron and Levavi-Sivan, 2011; Trudeau, 2022). However, the inhibitory effect of metoclopramide on dopamine is probably less than that of domperidone in the tench, which is why a weaker response was observed in the hCG group than in the Ovaprim group. Moreover, hCG may have lower affinity and efficacy for LH receptors in this fish species.

Ovaprim is a strategic combination of a gonadotropin-releasing hormone analogue (GnRH-a) agonist and a dopamine receptor antagonist such as domperidone (20 µg and 10 mg per mL, respectively), which simultaneously activates two key pathways for ovulation induction. The mechanism of action of Ovaprim is that GnRH-a, by binding to pituitary receptors, elevates LH and FSH secretion, which helps the final egg maturation and ovulation, while dopamine inhibition avoids the negative response of this neurotransmitter on the secretion of GnRH and eventually gonadotropins (Zohar *et al*., 2010). Therefore, the difference in results obtained in the Ovaprim group compared to the hCG group in the present study may be related to the presence of synergistic effects of FSH through the increase in steroid precursors in the Ovaprim group. These precursors are crucial for the early maturation of follicles and induction of LH receptors. Furthermore, Ovaprim probably caused a complete and coordinated stimulation through natural stimulation of the hypothalamic-pituitary-gonadal axis, whereas hCG exerted a partial and potentially uncoordinated stimulation.

The high efficacy of Ovaprim in this study is consistent with previous investigations revealing that the utilization of a combination of GnRH-a and a dopamine inhibitor might lead to increased egg production and reduced induction periods (Peter *et al*., 1988; Levavi-Sivan *et al*., 2010). GnRHa analogs containing potent dopamine antagonists are usually very effective in stimulating both wild and farmed species. Similarly, the results of the current study are in agreement with the results reported for other cyprinids such as Catla, Rohu, and Mrigal (fertilization rates of 88–98% in Ovaprim group) (More *et al*., 2010), *Barbus grypus* (highest spawning rate and fecundity at 1 ml/kg Ovaprim) (Ghanemi and Khodadadi, 2017), *Leuciscus idus* (100% ovulation rate) (Kucharczyk *et al*., 2020), and *Labeobarbus brevicephalus* (high fecundity, fertilization, and hatching rates with Ovaprim) (Abdissa *et al*., 2020).

Conclusion

The findings of this investigation indicate that Ovaprim has a significant effect on the reproductive performance of tench under semi-artificial conditions. In the control group, there was no reproductive response (0%), while the hCG-treated group showed a response rate of only 48%. In contrast, the group treated with Ovaprim achieved a 100% success rate in inducing spawning. Additionally, the relative fecundity and fertilization percentage in the Ovaprim-treated group were significantly higher compared to the other groups. These results demonstrate that Ovaprim is an effective and reliable option for semi-artificial reproduction programs of tench, and could play a crucial role in enhancing reproductive efficiency in aquaculture programs of this valuable species.

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Declaration of AI Use

The authors used ChatGPT-4.5 for English language editing, grammar checks, and stylistic improvements. Following the use of this tool, they carefully reviewed and revised the content as necessary and took full responsibility for the final version of the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

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