Use of Ovaprim Hormone to Induce Ovulation and Hatching in Common Carp (*Cyprinus carpio*) Farmed in Syria

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□ ABSTRACT □

This study represents the first investigation conducted in Syria to evaluate the effectiveness of the Ovaprim hormone in inducing hormonal ovulation and hatching in common carp *Cyprinus carpio*. The research was carried out from July 6 to July 10, 2024, at a private fish hatchery located in the Kazo area of Hama Governorate. The primary objectives of this study were to estimate the ovulation rate, fertilization rate, hatching rate, latency period, and absolute fecundity.

A total of six sexually mature females, weighing between 3200 and 3500 grams with an average weight of 3250 grams, and twelve males (two males per female) were used in the experiment. Females were injected with a single dose of 0.5 ml/kg of body weight of Ovaprim, while males received 0.25 ml/kg of body weight.

The latency period averaged 12 hours and 38 minutes under temperatures ranging from 25 to 27 °C, at which point all females were ready for spawning. The absolute fecundity averaged 298,868 eggs, and the ovulation rate reached 100%, while the average fertilization rate was 96.67%. The average hatching rate was 92.0%.

Key words: Absolute fecundity, *Cyprinus carpio*, Ovaprim, hatching rate, latency period, ovulation rate.

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استخدام هرمون Ovaprim لتحفيز الإباضة والفقس في أسماك الكارب العام *Cyprinus carpio* المستزرع في سوريا

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□ ملخّص □

تمثل هذه الدراسة أول دراسة تجري في سورية لتقييم فعالية هرمون Ovaprim في التحفيز الهرموني للإباضة والفقس عند أسماك الكارب الشائع *Cyprinus carpio*. تمَّ إجراء البحث من 6 يوليو إلى 10 يوليو 2024 في مفقس أسماك خاص تقع في منطقة كازو بمحافظة حماة. كانت الأهداف الأساسية لهذه الدراسة تقدير معدل الإباضة ومعدل الإخصاب ومعدل الفقس وفترة الكمون والخصوبة المطلقة.

تمَّ استخدام 6 إناث ناضجة جنسياً بوزن يتراوح بين 3200 و3500غ بمتوسط وزن 3250غ و12 ذكر (ذكران لكل أنثى) في التجربة. وحُقنت الإناث بجرعة واحدة من الأوفابريم 0.5 مل/كغ من وزن الجسم، بينما أُعطيت الذكور 0.25 مل/كغ من وزن الجسم.

بلغ متوسط فترة الكمون 12 ساعة و38 دقيقة في درجات حرارة تراوحت بين 25 إلى 27 درجة مئوية، وعند هذه الدرجة أصبحت جميع الإناث جاهزة للتكاثر. بلغ متوسط الخصوبة المطلقة 298868 بيضة، وبلغ معدل الإباضة 100%، بينما بلغ متوسط معدل الإخصاب 96.67%. بلغ متوسط معدل الفقس 92.0%.

الكلمات المفتاحية: Ovaprim، *Cyprinus carpio*، فترة الكمون، معدل الإباضة، الخصوبة المطلقة، معدل الفقس.

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Introduction:

Given the crucial role of *Cyprinus carpio* in freshwater aquaculture, extensive research has been conducted in recent decades on various aspects of its physiology, genetics, nutrition, and diseases., alongside its role in aquatic ecosystems. Additionally, breeding techniques have been developed to suit various climatic conditions (Xue *et al*., 2023; Lukistyowati and Putra, 2023).

In Syria, the farming of common carp *Cyprinus carpio* has gained considerable importance in the aquaculture sector, attributed to several advantages such as high growth rates, low production costs, and ease of spawning, as they produce a large number of eggs. They can be cultured in both flowing and stagnant waters, in cages and lakes, and in temperate as well as tropical regions. However, they are highly sensitive to increases in salinity, with an optimal temperature for growth and reproduction of 24 °C. Common carp are also characterized by high resistance to diseases, stress, and changes in handling, transportation, temperature, and oxygen levels (Hamawi and Al-Samman, 2017).

Common carp reach sexual maturity at 18 to 24 months, with males mature about two months earlier than females and reach a smaller size. The breeding season extends from late January to March and from July to August. Under optimal conditions, Females are ready to spawn 42 to 60 days after their prior spawning (Parameswaran *et al*., 1972).

The significance of hormones in fish breeding has emerged with the use of hormonal induction techniques to enhance production, especially when one sex exhibits greater growth potential compared to the other, and to induce sex reversal. Hormonal treatments also aim to improve fecundity, synchronize ovulation timing among breeding groups, and increase fertilization and hatching rates (Elakkanai *et al*., 2015; El-Hawarry *et al*., 2016). Artificial reproduction techniques have been employed for various fish species, including common carp (Brzuska, 2004), utilizing numerous ovulation stimulants. The selection of hormonal stimulants depends not only on the fish species but also on their cost. Furthermore, hormones must be handled with great care to ensure biological, environmental, and food safety (Hakuc-Blazowska *et al*., 2009).

Most studies indicate that hormone treatment in fish yields results only if the females are at an appropriate stage of sexual maturity. Hormonal treatment is a continuation of certain physiological developments that culminate in ovulation. Age is a critical determinant of sexual maturity, with variations in the age at which fish reach maturity. For instance, common carp typically attain sexual maturity at about two years of age in the Middle East, while in Europe, this can extend up to four years (Tessema *et al*., 2020). It is essential to assess the diameter of the eggs prior to hormonal injection, as fish in their first year of maturity typically produce eggs that are not viable for fertilization.

The choice of hormone for injection depends on several factors, including the type of fish to be stimulated, the availability of the hormone, and its cost. The efficacy of the hormone in inducing ovulation in fish is influenced by factors related to the fish itself, such as sexual maturity stage and size, as well as environmental conditions like water temperature and breeding season.

Artificial insemination complements the natural maturation of fish, as hormonal injections stimulate the completion of oocyte development within the ovaries and induce spawning, leading to the production of gametes. To increase common carp production, sufficient quantities of fry are required, which is challenging to achieve through natural hatching, thus necessitating hormonal injections.

The primary sexual hormones utilized in fish farming are androgens and estrogens, which may be derived from natural sources (found in nature) or synthetic (chemically manufactured or produced in laboratories) (Hoga *et al*., 2018). Among studies focused on artificial spawning using Ovaprim for the first time in Basra, the findings of Al-Mukhtar *et al*. (2004) demonstrated the superiority of Ovaprim over other hormones, including CPH, in terms of egg weight relative to body weight, fertilization rates, and hatching success.
In a study by Brzuska (2021), a combination of natural and synthetic hormones was used, achieving the highest reproductive effectiveness, measured by the greatest number of live larvae and the highest egg weight, via the application of Ovaprim, CPH, and HCG + CPH.
Research conducted in India indicated a slight increase in fertilization rates and gonadosomatic index (GSI) when using CPH compared to Ovaprim (Pawar *et al*., 2020). Another study observed the ovulation response to Ovaprim compared to pituitary extracts in Indian major carp, reporting fertilization rates of 88.11% to 97.94% and hatching rates of 74.70% to 95.92% with Ovaprim, in contrast to fertilization rates of 53.19% to 85.48% and hatching rates of 60% to 58.82% with pituitary extracts (More *et al*., 2010).
Additionally, a study conducted in Basra tested Ovaprim and CPE, revealing that only four fish responded to pituitary hormone injections and produced gametes, while all fish in the second trial responded to Ovaprim injections and produced gametes without manual stripping (Jaber *et al*., 2010).
Therefore, the aim of our current study was to utilize and evaluate a new type of hormone for hormonal induction, testing its efficacy in the breeding of common carp *Cyprinus carpio* under the climatic conditions and aquaculture practices in Syria.

Methods and Materials:

Study Site and Experimental Period:

The research was carried out at a privately owned fish hatchery located in the Kazo area of Hama Governorate, approximately 5.648 kilometers from the city center (Al-Assi Square). The site is situated at an elevation of 287 meters above sea level, during the period from July 6 to July 10, 2024 (Fig. 1).

Figure (1) An aerial image showing the coordinates of the study area (36°42'14"E 35°09'28"N).

Hormones Used for Hormonal Induction:

Ovaprim Hormone:

Ovaprim is a ready-to-use solution that should be stored at temperatures below 25 °C, away from sunlight and heat sources. The Ovaprim (10 ml, Indian origin), developed by Duopharma in collaboration with Syndel Laboratories Ltd, Canada, contains a gonadotropin-releasing hormone analog for salmon (sGnRHa) at a concentration of 20 micrograms/ml, along with domperidone (a dopamine receptor antagonist) at a concentration of 10 mg/ml (Fig. 2). The dosage administered was 0.5 ml/kg for females and 0.25 ml/kg for males.

Figure (2) The Ovaprim hormone used in the study.

Selection of Experimental Fish:

Breeding stock (males and females) was selected based on their well-proportioned bodies, recent age, and absence of wounds, sores, bruises, parasites, and deformities in their reproductive organs. Females were separated from males in February 2024, two months prior to the breeding season, to prevent natural spawning.

Experimental Tanks:

Females and males were kept in two separate tanks (1500 m^3), containing clean, renewed water free from waste accumulation or excess feed. These conditions were suitable for growth and maturation, with continuous disinfection to maintain fish health (fish were disinfected in a 0.5 % saline solution for 5 minutes before being placed in the tanks). Oxygen pumps were used, and water temperature was monitored to provide optimal conditions for common carp growth, maintained at approximately 25 ± 1 °C (Guderley and Blier, 1988). Fish were fed a high-protein diet containing 35 % crude protein at a rate of 1-3 % of their body weight daily, depending on their acceptance and appetite (Table 1).

A total of six sexually mature females weighing between 3200 and 3500 grams (average 3250 ± 225.8 grams) and twelve males (at a ratio of two males per female) were selected to minimize the risk of fertilization failure due to the presence of an infertile male and to ensure the highest fertilization rates.

Table (1) Composition of Feed Pellets Manufactured by the General Organization for Fish

Primary Feed Material	Percentage %
Soybean Meal	44
Cottonseed Meal (Decorticated or Partially Decorticated)	12
Poultry Byproduct Meal	23
Wheat Bran	11
Yellow Corn	5
Dicalcium Phosphate	2
Choline Chloride	0.5
Methionine	0.5
Lysine	0.5
Minerals	0.5
Vitamins	0.5
Table Salt	0.5
Total	100

Additives

- **Antioxidants**
- **Antifungals**

Acclimatization of Experimental Fish:

After selecting the mature males and females from the breeding tanks, they were transferred to rectangular concrete tanks measuring 7 m × 3 m at the bottom and 9 m × 4 m at the top, with a height of 1.5 m. The fish were kept in these tanks for two days prior to hormonal injection to acclimatize and alleviate the stress associated with collection and transportation. No feeding was provided during the acclimatization period.

Anesthesia (Sedation) and Hormonal Injection:

The fish were anesthetized using a water bath with clove oil at a concentration of 80 mg/L, which has previously shown good results in common carp (Hamwi *et al*., 2021). In this study, sedation was applied without reaching a complete anesthetic state, which could inhibit artificial insemination in some species. The fish were weighed using an electronic scale and injected with a single dose of Ovaprim at 0.5 ml/kg of body weight, administered at the base of the pectoral fin. After injection, since female common carp release eggs immediately upon ovulation, the genital opening was sutured with a specialized needle and thread in a cross pattern over the genital opening, resembling an "X" shape, according to Hussain (1982).

Following this, the fish were revived by being placed in a well-aerated tank for 10 minutes and then returned to the prepared experimental tanks. They were monitored for 10 hours post-injection, with observations made hourly to note the hormonal stimulation response through the characteristic abdominal swelling of the females due to oocyte maturation in the ovaries, and to accurately determine the latency period for inducing ovulation.

Fertilization Process (Egg and Sperm Collection):

When final maturation signs were observed in the females, such as abdominal swelling and circular swimming movements, the collection process was conducted with two individuals present to minimize injury and stress to the females. The first person held the mature female by the tail and supported the pectoral fins with one hand, positioning the fish

slightly inclined with the tail down and directing the genital opening toward a collection container. The suture closing the genital opening was then removed, while the second person held the fish at the dorsal fin with the left hand and began to express the eggs with the right hand by applying pressure with the index finger and thumb, starting from the area near the ventral fins and moving backward toward the genital opening. A third person held a previously weighed egg collection container. The sperm was extracted from the mature male using the same technique. The eggs and sperm were mixed in a plastic container to prevent the eggs from sticking to the sides after the sperm was placed directly on top of the eggs.

Egg Fertilization Process:

The eggs collected from each female were weighed individually, and a sample weighing 1 gram was taken to count the number of eggs present, with the average value multiplied by the total quantity of eggs from each female. Subsequently, the sperm was added to the eggs at a ratio of 1 ml for every 100,000 eggs and mixed gently with a soft brush for two minutes (Fig. 3). After a short period, a small amount of water was added to the mixture to facilitate the fertilization process, ensuring the temperature was appropriate. The mixture was then washed with a fertilization solution instead of water; this solution was prepared by dissolving 0.3% urea and 0.4% salt in 10 liters of pure water, and the washing was conducted for a quarter of an hour, repeating the process 3 to 4 times to remove the high viscosity of the eggs and increase the egg diameter after water absorption, enhancing the fertilizing capacity of the sperm. The viability and motility of the sperm are highest in the ovarian fluids that accompany the eggs, where they remain alive for about 3.5 to 4 minutes, while sperm can stay active in water for approximately 30 seconds. Afterward, the eggs were washed with a tannin solution at a concentration of 0.5 g per 1 liter of pure water for 10 seconds, quickly removed, and then the eggs were washed several times with clean water (Fig. 4).

Figure (3) The fertilization process of the eggs.

Figure (4) The process of washing the fertilized eggs.

Estimation of Fertilization Rate:

The fertilization rate was determined after placing the fertilized eggs in incubators for about 6 to 10 hours, by which time the eggs had reached the gastrulation stage. Six samples were taken from each incubator as one sample, with 100 eggs counted randomly in each sample, excluding unfertilized, white, and opaque eggs, while fertilized eggs were transparent with a noticeable black dot indicating the beginning of eye formation. The diameter of the fertilized eggs ranged from 1.11 to 1.38 mm, with an average of 1.24 ± 0.09 mm.

Incubation and Hatching of Eggs:

After washing, the fertilized eggs from each female were transferred to six conical incubators, each with a capacity of 248 liters, ensuring oxygen levels of at least 4-5 mg/L. The water flow rate within the incubators was initially set at 18 liters per minute, gradually increasing to 20 liters per minute, before being reduced to 8 liters per minute by the end of incubation.

Estimation of Hatching Rate:

The hatching period was recorded after 46 hours of fertilization at temperatures ranging from 27 to 29 °C. After hatching, a sample was taken using a 1000 ml container from each incubator, and the number of hatched larvae was counted, multiplying the count by 248 (the capacity of one circular incubator) to obtain the total number of hatched larvae in each incubator. The time required for hatching was calculated as the interval between fertilizing the eggs and the completion of their hatching.

Studied Reproductive Indicators:

1. Latency Period: The time interval between injection and the start of egg collection from females (El-Hawarry *et al*., 2016).
2. Reproduction Rate (RR):

RR = (Number of females ready to spawn\ Total number of hormonally stimulated females) x 100 (Kulikovsky *et al*, 1996)

3. Ovulation Rate (OR):

OR = (Number of egg-laying females\ Total number of hormonally stimulated females) x 100 (Szabo *et al*, 2002)

4. Absolute Fecundity (AB): The total number of mature vitellogenic oocytes taken from each female (AB = Weight of eggs taken from the female (g) × Number of eggs in the 1 g sample).
5. Fertilization Rate (FR):

FR = (Number of fertilized eggs\ Total number of eggs) x 100 (Okomoda *et al*, 2018).

6. Hatching Rate (HR):

HR = (Number of hatched larvae\ Total number of fertilized eggs) x 100 (Křišťan *et al*, 2013)

Results and Discussion:

The latency period from the time of injection until egg collection ranged from 12 hours and 17 minutes to 12 hours and 58 minutes, with an average of 12 hours and 38 minutes. This duration was recorded at water temperatures ranging from 25 to 27 °C (Table 2).

The variation in the latency period can be attributed to several factors, including water temperature, biological characteristics (species, age, weight), the type of commercial hormones and concentrations used for stimulation, the number of injections, timing of injections, and the maturity of the fish (Billard, 1990; Yaron, 1995). Therefore, our results differ from those of other studies, such as the one conducted by Solomon *et al*. (2015), which reported a latency period of (9.30 ± 0.27 hours). Our findings are consistent with the study by Jaber *et al*. (2010), where all experimental fish spawned after 12 hours at a temperature of 22 °C, likely due to a longer thermal acclimatization period of 25 days.

In the current study, the ovulation rate reached 100% (6 females out of 6), with all injected females releasing eggs in a rapid flow without difficulty. This was achieved by applying gentle pressure around the abdominal area through the genital opening without the expulsion of blood or fluids with the flowing eggs (Fig. 5). We attribute this success to the fact that Ovaprim is formulated to naturally stimulate gonadotropin production, meaning its action is not only to add stimulating hormones but also to enhance the secretion of these

hormones from the fish itself. Furthermore, using a single injection dose reduces the likelihood of the fish reaching a state of super-maturity, which can occur due to prolonged hormonal stimulation. This approach eliminates the need to extract eggs from females twice, as would be necessary with two injections, such as when using pituitary gland extracts (PG).

These results align with findings from experiments conducted by Jaber *et al*. (2010), Rottman *et al*. (1991), and Yeasmin *et al*. (2013), where all experimental fish responded to the Ovaprim treatment and released gametes without the need for manual stripping.

Figure (5) The flow and release of eggs from one of the females that was induced to spawn with Ovaprim hormone.

With the use of Ovaprim, ovulation typically occurs between 12- and 14-hours post-injection. Females were monitored 10 hours after injection, and signs of final maturity were observed in all hormonally treated females, resulting in a reproduction rate of 100% after 12 hours.

Table (2) The latency period, weight of females, weight of eggs, and total number of eggs for the fish females that were induced for artificial reproduction using Ovaprim solution.

No. of fish	Latency period (min.)	Female weight (g)	Eggs weight (g)	Absolute Fecundity
6	730-755	3200-3500	470-560	268370-328005
Average	742.5	3250	517.50	298868.33
STDEV	9.4	225.8	38.31	26668.02

The number of eggs collected from the females ranged from 268,370 to 328,005 eggs, with an average of (298,868.33 ± 26,668.02 eggs). Analyzing the correlation between female weight and absolute fecundity revealed a very strong positive correlation (r = 0.99) (Fig. 6) (Table 2).

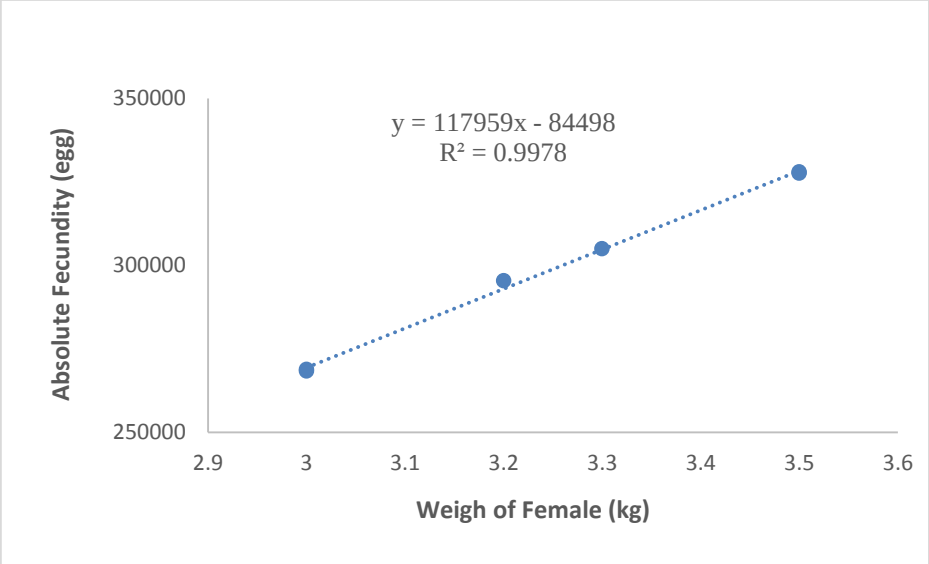

Figure (6) The correlation between the weight of females and absolute fecundity when injected with Ovaprim hormone.

The absolute fecundity values in this study were higher than those reported in some other studies. For instance, Solomon *et al*. (2015) found that the number of eggs stripped from common carp females with an average total weight of (1267.0 ± 318.0 g) was (124,754.0 ± 22,864.0 eggs). The difference in fertility may be attributed to the laboratory or field conditions of the experiments, in addition to the concentrations used for the hormonal doses and the condition of the fish used, such as specific weight and age.

The fertilization rate for females stimulated with Ovaprim ranged from 95% to 98%, with an average of (96.67 ± 1.0 %). The number of fertilized eggs varied from a minimum of 255,408 eggs to a maximum of 318,165 eggs, with an average of (288,990 ± 26,997 eggs) (Table 3). These fertilization rates differ from those recorded in several other studies; for instance, More *et al*. (2010) reported a high fertilization rate of 97.94%, while Yeasmin *et al*. (2013) recorded a lower rate of 82.38%. The difference in fertilization rates can also be attributed to the technical method employed in the fertilization process and the genetic makeup of the fish.

The hatching rate of the fertilized eggs ranged from a minimum of 86% to a maximum of 97%, with an average of (92.0 ± 4.3 %) (Table 3). The results of this experiment were lower than those reported by More *et al*. (2010), who found a hatching rate of 95.92%. However, our results were higher than those recorded by Yeasmin *et al*. (2013) and Solomon *et al*. (2015), which were 79.22% and 52.53%, respectively. As for the differences in hatching rates, they are attributed to the nature of the extracted eggs (whether they are fully matured or not, and if the nucleus has migrated towards the micropyle to be ready for fertilization, which would consequently reduce fertilization rates and, subsequently, hatching rates) as well as water temperature and oxygen levels.

Table (3) The number of fertilized eggs, fertilization rate, number of hatching larvae, and hatching rate for the fish females that were induced for artificial reproduction using Ovaprim solution.

No. of fish	Fertilization Rate (%)	Number of fertilized eggs	Number of hatched larvae	Hatching Rate (%)
6	95-98	255408-318165	248000-280240	86-97
Average	96.67	288990	264946.67	92
STDEV	1.03	26997	12949.94	4.3

Conclusion:

This study is the first in Syria to examine the efficacy of the Ovaprim hormone for inducing ovulation and hatching in common carp *Cyprinus carpio*. The results confirm its effectiveness in enhancing ovulation and hatching in farmed common carp in Syria, contributing to improved productivity and quality.

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