

## COMPARATIVE EVALUATION OF SYNTHETIC OVAPRIM AND NATURAL CARP PITUITARY EXTRACT FOR INDUCED SPAWNING IN *CYPRINUS CARPIO* IN SYRIA

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### ABSTRACT

This study compared the effectiveness of synthetic hormone Ovaprim and natural Carp pituitary extract (CPE) in inducing spawning in common Carp. A total of 12 females and 24 males were divided into two groups. The first group received a single dose of Ovaprim (0.5 ml kg<sup>-1</sup> for females, 0.25 ml kg<sup>-1</sup> for males). The second group received CPE: females were given two injections (10% + 90%, total 4 mg kg<sup>-1</sup>), and males one dose (2 mg kg<sup>-1</sup>). Fertilization was done by dry method 8–10 hours after injection. Hatching occurred after 46 hours in Ovaprim group and 42 hours in CPE group. Statistical analysis showed significantly better results for Ovaprim in ovulation rate (100% vs. 83.33%), fertilization rate (96.5% vs. 76%), and hatching rate (97% vs. 63.21%). Latency period was longer with Ovaprim (12h 38m vs. 10h 21m).

**Keywords:** CPE, Fecundity, Hatching rate, Latency period, Ovulation rate.



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### INTRODUCTION

Aquaculture has become a major global food production sector, with freshwater aquaculture contributing significantly (51.3 million tons, 62.5%) to global output (Costello et al., 2020; FAO, 2024). Given the crucial role of *Cyprinus carpio* in freshwater aquaculture, alongside its role in aquatic ecosystems. Additionally, breeding techniques have been developed to suit various climatic conditions (Lukistyowati and Putra, 2023; Xue et al., 2023). It is currently farmed in over 100 countries (Shahi et al., 2022). The first recorded introduction of this species in Syria was by Beckman in 1962 (Beckman, 1962), later confirmed through data collected in 1998 (MNHN: Museum National de Histoire Naturelle, Paris, France; MSL: Materialien zum Sumerischen Lexikon). Native to the Caspian Sea region in Eurasia, genetic studies have identified two subspecies: *Cyprinus carpio carpio* from Europe and *Cyprinus carpio hematopterus* from Asia (Al-Hilli, 2009; Azawy and Issa, 2019). According to

Çiçek et al., (2023), *Cyprinus carpio* classified as a commercially important species. Its introduction was primarily for aquaculture and fisheries enhancement purposes (Çiçek et al., 2022). However, it faces threats due to water extraction, climate change, and rising temperatures (IUCN, 2023). *Cyprinus carpio* demonstrates high adaptability to intensive rearing conditions when adequate nutrition and aeration are provided (Fahad and Shuhaib, 2021; Jelena, 2024). It has played a pivotal role in the global blue revolution: the expansion of aquatic animal and plant farming (Garlock et al., 2020; Karnai and Szucs, 2018). Fish must evolve to meet growing demand through modern techniques such as hormonal-induced artificial breeding (Paul et al., 2022). Yaron et al., (2009) discussed the hormonal regulation of reproduction in female fish, highlighting two main methods of hormonal induction. The first is hypophysation, involving pituitary extracts such as carp pituitary extract (CPE) and salmon pituitary extract (SPE), commonly used in commercial

settings. CPE is suitable for warm-water fish, while SPE is preferred for cold-water species (Yaron et al., 2002). However, challenges such as variability in extract purity, dosage estimation, collection and storage methods, and genetic distance between donor and recipient fish limit its effectiveness. Additionally, the presence of multiple hormones in varying concentrations may antagonize gonadotropins (Hamwi and Al-Samman, 2017). Therefore, there was an urgent need to discover alternatives to it that did not have a negative impact on production characteristics, were less expensive than pituitary hormone, and were easy to handle and prepare (Al-Hilli and Salih, 2013; Asal and Saleh, 2015). In response, alternative methods emerged in the 1980s, notably Linpe's method, which uses hypothalamic peptides like GnRH or its analogs (GnRH<sub>a</sub>) combined with dopamine antagonists (DA) to inhibit endogenous dopamine, a suppressor of gonadotropin synthesis (Weil et al., 1986; Naeem et al., 2011). Several commercial products have since been developed based on this principle, including Ovaprim (a combination of sGnRH-A and domperidone) which was tested against CPE in this study (Hossain et al., 2013). Hamwi et al. (2025) previously demonstrated its efficacy under Syrian climatic and aquaculture conditions. Unfortunately, despite the existence of the general authority for Fisheries and its branches in Syria, knowledge regarding advanced hormonal breeding techniques remains limited, and many still rely on CPE despite its drawbacks. Furthermore, modern hatcheries are scarce, therefore, this study aimed to evaluate the use of Ovaprim compared to CPE based on key reproductive indicators: latency period, ovulation rate, absolute fecundity, fertilization rate, hatching rate, and time to hatching.

## **MATERIALS AND METHODS**

### **Study Site and Experimental Period:**

The experiment was conducted in a private fish hatchery located in Kazw village, Hama Governorate, Syria. The site is approximately 5.648 km from the city center (Al-Asi Square) and sits at an elevation of 287 meters above

sea level. The study was carried out over a period spanning from February to July 2024.

### **Hormones Used for Induced Spawning:**

**Ovaprim Hormone:** Ovaprim is a ready-to-use solution developed by Duopharma (Syndel Laboratories Ltd., BC, Canada, V9K 1V5). It contains synthetic salmon gonadotropin-releasing hormone analog (sGnRH<sub>a</sub>) at a concentration of 20 µg/ml and domperidone (a dopamine antagonist) at 10 mg/ml, with the following composition: ([D-Arg<sup>6</sup>, Pro<sup>9</sup>, Leu<sup>8</sup>, Trp<sup>7</sup>-NET]-LHRH + domperidone). A single intraperitoneal dose was administered to both sexes, with females receiving 0.5 ml kg<sup>-1</sup> and males 0.25 ml kg<sup>-1</sup>. This was done in parallel with the second CPE injection given to females. The injection was performed at a 45° angle beneath the pectoral fin. To prevent thermal shock, the temperature of the solution was adjusted to match that of the holding tank water (Dariusz et al., 2007). Its low viscosity allows easy administration (Syndel, 2004). The first successful application of Ovaprim using a single-dose protocol was reported in India by Nandeasha et al., (1990).

### **Carp Pituitary Extract (CPE):**

In March 2024, pituitaries were collected from 75 sexually mature common carp (*Cyprinus carpio*) weighing between 2.5–2.8 kg. The glands were immediately preserved in acetone for 2, 12 and 24 hours consecutively, to remove fat and moisture. The average dry weight of each gland was approximately 2.5 mg. The required amount of pituitary tissue was calculated based on female body weight (4 mg kg<sup>-1</sup>) and male body weight (2 mg kg<sup>-1</sup>), half an hour before injection. Glands were gently removed from storage using forceps, dried on filter paper for 2–3 minutes, and weighed using a sensitive scale. The glands were then ground into a fine powder using a porcelain mortar and suspended in physiological saline (0.9% NaCl). The mixture was centrifuged at 3000 rpm for 5 minutes to separate the supernatant, which was drawn into 5 ml syringes for injection. Females were injected intraperitoneally with CPE at a total dose of 4 mg kg<sup>-1</sup>, divided into two injections: a priming dose of 10% followed 12 hours later by a resolving dose of 90%. Males received a

single dose of 2 mg kg<sup>-1</sup> concurrently with the second injection for females.

#### **Experimental Fish:**

Broodstock aged 2–3 years were selected based on good morphological and health characteristics. Females had swollen, soft and flexible abdomens. Gentle pressure on the abdomen caused a small quantity of eggs to be released through the genital opening, which appeared reddish or pinkish with a red papilla shaped like an inverted "U". The anal opening was also red and slightly swollen. Males had smaller, firmer abdomens. Slight abdominal pressure released white, milky milt through a small, conical, whitish genital opening shaped like a "V". Females and males were separated in February 2024, two months prior to the natural spawning season, to avoid spontaneous spawning.

#### **Acclimation and Injection Procedure:**

Mature broodstock were kept in tanks two days prior to hormone injection to allow acclimation and reduce stress. No food was provided 24 hours before injection to empty the digestive tract and facilitate ovulation. Each female was anesthetized using clove oil at a concentration of 80 mg L<sup>-1</sup> (Hamwi et al., 2021), gently wrapped in a cloth, and injected at the base of the pectoral fin. The genital opening was then sutured in an X-shaped pattern to prevent premature egg release.

#### **Reproductive Parameters Studied:**

##### **Latency Period:**

Defined as the time interval between hormone injection and the start of egg collection from females. Ovulation Rate (%): Calculated as (Number of ovulated females / Total number of hormonally treated females) × 100. Absolute Fecundity (Total Egg Count per Female): Each female's egg batch was weighed separately, and a 1-gram subsample was counted to estimate the total number of eggs. This value was then multiplied by the total eggs weight to calculate absolute fecundity: Eggs per female = Eggs per gram × Total egg weight, Fertilization Rate: *Cyprinus carpio* eggs are highly viscous, making fertilization difficult. To address this, using a solution containing 30 g urea and 40 g salt per 10 L of distilled water. A small amount of this solution was initially added to help the eggs

absorb water through the micropyle, increasing their size and facilitating fertilization. The process was repeated 3–4 times to remove excess stickiness, enhance swelling, and improve sperm penetration. This method was later refined by Woynarovich and Woynarovich (1980) using a solution of 200 g urea and 40 g salt per 10 L of water, which reduced fertilization time. Fertilization rate was determined microscopically after incubation for 6–10 hours. Random samples were taken from each incubation tray: unfertilized eggs appeared opaque and white, while fertilized eggs were transparent. The fertilization rate was calculated using the following formula: Fertilization Rate (%) = (Number of fertilized eggs / Total number of eggs in sample) × 100, Hatching Rate: -treated ones, at water temperatures ranging between 27–29°C. Upon completion of hatching, a random sample of 1000 ml was taken from each Fertilized eggs were placed in incubators with continuous water flow and controlled temperature. Hatching rate was calculated using the following formula: Hatching Rate (%) = (Total number of hatched larvae / Total number of fertilized eggs) × 100 Hatching occurred 46 hours post-fertilization for Ovaprim-treated females and 42 hours for CPE incubator to count the hatched larvae. The hatching duration was recorded as the time elapsed between fertilization and full hatching.

##### **Statistical analysis:**

Statistical analysis was carried out using Microsoft Excel and SPSS software (version 30). Data were subjected to one-way analysis of variance (ANOVA) to compare means across multiple groups. For comparisons between two groups, Student's t-test was applied. Differences were considered statistically significant at  $P \leq 0.05$ .

#### **RESULTS AND DISCUSSION**

Statistical analysis using one-way ANOVA revealed no significant differences in the initial body weights of females between the two treatment groups before hormonal induction ( $P > 0.05$ ). The average weight of Ovaprim-treated females was 3250 g, while that of CPE-treated females was 2820 g. However, a noticeable difference was observed in post-treatment egg weight: Ovaprim-treated

females produced significantly heavier eggs (517.50 g) compared to those treated with CPE (357 g). Similarly, the ratio of egg weight to body weight was higher in the Ovaprim group (15.92 %) than in the CPE group (12.67 %). These differences were statistically significant ( $P \leq 0.05$ ), indicating a stronger hormonal response in the Ovaprim group. This statistical significance reflects the greater effectiveness of Ovaprim in stimulating increased egg production and mass. A higher egg-to-body weight ratio is generally considered an indicator of better reproductive performance

suggesting that females responded more favorably to Ovaprim, resulting in higher quality and quantity of eggs relative to their body size. The latency period (the time from hormone injection to ovulation) ranged from 12 hours and 17 minutes to 12 hours and 58 minutes (mean: 12h 38m) for Ovaprim-treated fish, and from 10h to 10h 42m (mean: 10h 21m) for CPE-treated fish, at water temperatures ranged between 25–27°C. The shorter latency period observed in the CPE group was statistically significant ( $P \leq 0.05$ ) (Table 1).

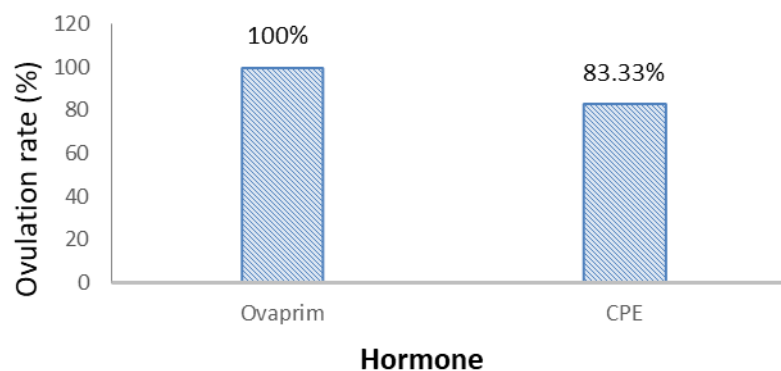
**Table 1. Latency period, female weight, egg weight, egg count per gram, and absolute fecundity of females induced to spawn using Ovaprim and carp pituitary extract (CPE)**

Hormone	Latency Period (min)	Female Weight (g)	Egg Weight (g)	Eggs per (g)	Absolute Fecundity
Ovaprim	742.5 ± 9.4	3250.0 ± 225.8	517.50 ± 38.31	577.0 ± 10.14	298.868.33 ± 26.668.02
CPE	613.0 ± 10.4	2820.0 ± 178.9	357.00 ± 17.18	476.0 ± 9.62	169.84 ± 6.250.04
Level of Signification	<0.001**	0.004*	<0.001**	0.032*	<0.001**

(\*\*): means high Significant and (\*): means Significant variation.

The variation in latency periods can be attributed to several factors, including differences in hormonal composition, dosage, frequency of injections, and the maturity level of the fish. Our results differ from some previous studies. For example, Solomon et al., (2015) reported a latency period of 9.30 hours following Ovaprim administration, whereas Yeasmin et al., (2013) observed spawning within 6 hours using both Ovaprim and CPE. In some studies, it has been suggested that pituitary extracts act directly on the gonads through the hypothalamic-pituitary-gonadal axis, leading to a rapid increase in

gonadotropin (GtH) levels in the bloodstream, which may explain the shorter latency times associated with CPE use (Brzuska and Białowas, 2002). Our study showed a 100% ovulation rate among Ovaprim-treated females (6 out of 6), with all individuals releasing eggs smoothly and without physical stress or damage to the eggs, which could otherwise reduce fertilization rates. In contrast, only 83.33% of CPE-treated females (5 out of 6) ovulated successfully, and the process was notably more difficult, affecting subsequent egg quality (Fig. 1).



**Figure 1. Relationship between hormone type and ovulation rate**

These findings are consistent with Yeasmin et al., (2013), where all fish responded well to Ovaprim. However, they differ from Brzuska (2021), where the ovulation rate was 80%. The superior performance of Ovaprim can be attributed to its dual mechanism: as a GnRH analog, it stimulates gonadotropin release, while domperidone (dopamine antagonist) prevents dopamine-induced suppression of GnRH, enhancing overall hormonal stimulation. This combination appears to be more effective than CPE, which contains multiple hormones at variable concentrations and lacks dopamine inhibition. Additionally, the females that ovulated successfully had eggs at advanced stages of maturation, with

nuclei having migrated beyond half the radius of the oocyte. The single-dose protocol used with Ovaprim also reduces the risk of over-ripening due to prolonged hormonal stimulation, unlike the double-injection method required for CPE. Fertilization rates in the Ovaprim group ranged from 95% to 98%, with an average of 96.67 %, and the number of fertilized eggs varied between 255.408 and 318.165 per female (average: 288,990). In comparison, CPE-treated females had fertilization rates between 72 and 80%, averaging 75.4 %, with fertilized egg counts ranged from 121.125 to 141.360 per female (average: 128.166.2) (Table 2).

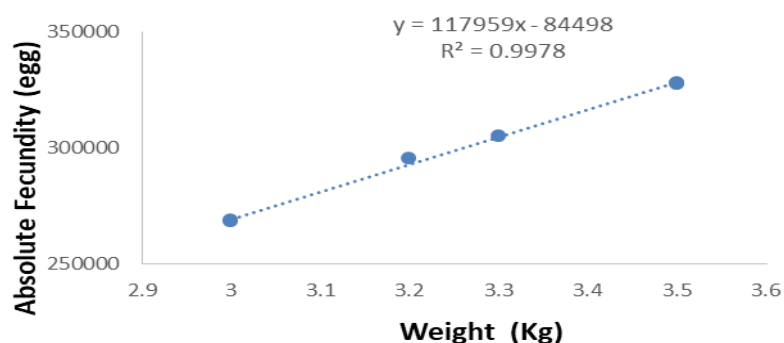
**Table 2. Fertilization rate, number of fertilized eggs, hatching rate, and number of hatched larvae in females induced with Ovaprim and CPE**

Hormone	Fertilization Rate (%)	Fertilized Eggs	Hatched Larvae	Hatching Rate (%)
Ovaprim	96.67 ± 1.03	288.990 ± 26.997	264.946.67 ± 12.949.94	92.01 ± 4.31
CPE	75.40 ± 3.21	128.166.2 ± 9.382.8	74.40 ± 1.960.61	58.26 ± 3.91
Level of Signification	<0.001**	<0.001**	<0.001**	<0.001**

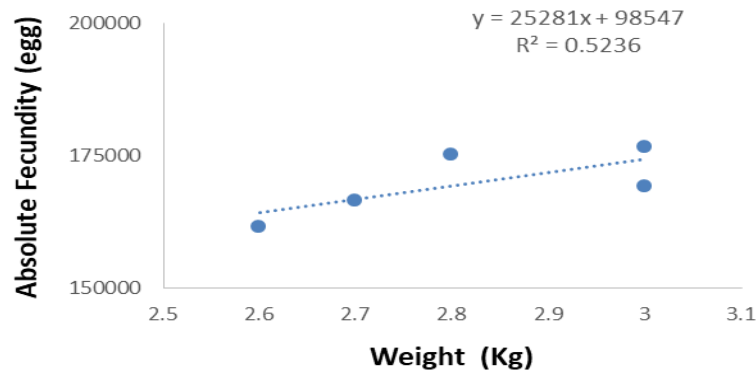
(\*\*): means significant variation.

Hatching rates followed a similar trend: Ovaprim-treated females achieved hatching rates between 86% and 97%, with an average of 92 ± 4.3%. In contrast, CPE-treated females showed hatching rates between 53.51% and 63.21%, averaging 58.26 ± 3.91% (Tab. 2). These values differ from those reported in other studies. For instance, More et al., (2010) found fertilization rates between 88.11 and 97.94% with Ovaprim, and between 53.19 and 85.48% with CPE, along with hatching rates of 74.70–95.92% and 58.82–60%, respectively. Similarly, Yeasmin et al., (2013) reported fertilization rates of 82.38% (Ovaprim) and

79.22% (CPE), with hatching rates of 71.48 and 69.55%, respectively. The total number of eggs collected from Ovaprim-treated females ranged from 268.370 to 328.005 with an average of 298.868.33 eggs per female (Table 1). Strong positive correlation was observed between female body weight and absolute fecundity ( $R^2 = 0.99$ ) (Fig. 2). In contrast, CPE-treated females yielded between 161,500 and 176,700 eggs, with an average of 169,840 eggs per female (Tab. 1), showing a moderate positive correlation with body weight ( $R^2 = 0.52$ ) (Fig. 3).



**Figure 2. Relationship between female body weight and absolute fecundity in Ovaprim treated fish.**



**Figure 3. Relationship between female body weight and absolute fecundity in CPE treated fish.**

Interestingly, our absolute fecundity values were higher than those reported by Solomon et al., (2015), who found an average of 124.754 eggs per female using Ovaprim. This discrepancy may be due to differences in female body sizes across studies. The average number of eggs per gram was also higher in the Ovaprim group, ranging from 566 to 591 eggs per gram (average: 577), compared to the CPE group, which ranged from 465 to 490 eggs per gram (average: 476). This suggests that Ovaprim enhances egg maturation and ease of release, leading to higher yields. When eggs are released easily and uniformly, the full complement of mature eggs is typically expelled, resulting in a higher count per gram. Statistical analysis confirmed highly significant differences ( $P \leq 0.05$ ) between the two treatments in terms of fertilization rate, hatching rate, absolute fecundity, and egg count per gram - all favoring Ovaprim. These results clearly indicate that synthetic hormonal stimulation contributes not only to increased egg production but also to improved reproductive efficiency overall.

### CONCLUSION

This study is the first in Syria to compare the effectiveness of synthetic Ovaprim and natural CPE hormones in inducing spawning of *Cyprinus carpio* fishes. Results indicated that a single dose of 0.5 ml/kg Ovaprim significantly improved ovulation and hatching rates compared to CPE. The advantages of Ovaprim include simplified injection protocols, reduced stress on fish, and higher reproductive efficiency. Based on these

findings, we recommend adopting Ovaprim in artificial breeding programs for common carp in Syria for its practical and economic benefits.

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### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

### AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript were prepared by the authors. All procedures were conducted in accordance with international standards for the care of aquatic animals. We also hereby declare that this work is our original research, has not been previously published, and has not been submitted for publication elsewhere.

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### AUTHORS' CONTRIBUTION STATEMENT

F.D., conducted the practical experimental work, gathered relevant references, and wrote the initial draft of the manuscript.

N.H., conceived the research idea, oversaw its execution, and conducted the final review of the manuscript.

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## تقييم مقارن لهرموني Ovaprim الصناعي وCPH الطبيعي لتحفيز التكاثر في أسماك الكارب العام *Cyprinus carpio* في سوريا

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### المستخلص

قورنت فعالية الهرمون الصناعي Ovaprim ومستخلص الغدة النخامية (CPE) في تحفيز التكاثر عند أسماك الكارب العام. استخدمت 12 أنثى و24 ذكر سمكة وقسمت إلى مجموعتين. المجموعة الأولى حقنت بجرعة واحدة من Ovaprim (0.5 مل كغم<sup>-1</sup> للإناث، 0.25 مل كغم<sup>-1</sup> للذكور)، بينما المجموعة الثانية حصلت على جرعات من CPE (10% ثم 90% بعد 12 ساعة، بإجمالي 4 ملغم كغم<sup>-1</sup> للإناث، و2 ملغم كغم<sup>-1</sup> للذكور). تم الإخصاب بالطريقة الجافة بعد 8-10 ساعات. الفقس تم بعد 46 ساعة في مجموعة Ovaprim و42 ساعة في CPE أظهرت النتائج تفوقاً معنوياً لصالح Ovaprim في معدل الإباضة (100% مقابل 83.33%)، ومعدل الإخصاب (96.5% مقابل 76%)، ومعدل الفقس (97% مقابل 63.21%). كانت فترة الكمون أطول قليلاً مع Ovaprim (12 ساعة و38 دقيقة مقابل 10 ساعات و21 دقيقة).

الكلمات المفتاحية: CPE، الخصوبة، معدل الفقس، فترة الكمون، معدل الإباضة.