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Comparative Efficacy of Different Doses of Ovaprim (Synthetic Salmon Gonadotropin-Releasing Hormone and Domperidone) in Induced Breeding of Common Carp (*Cyprinus Carpio*)

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Abstract
Having no previous attempt in Common carp in Pakistan, we intended to assess the impact of different doses of Ovaprim on various reproductive parameters of Common carp in a semiartificial condition. A complete randomized design with one factor, four treatments, and three replications was used in experimentation. Ripe brood stock weighing 2 kg on average (48 in number) were placed in spawning tanks with a male-to-female ratio of 2:1. The treatments involved injecting different doses of Ovaprim: T_0 (control group), T_1 (0.3ml/kg to females_0.1 ml/kg to males), T_2 (0.5ml/kg to females_0.2 ml/kg to males), and T_3 (0.7ml/kg to females_0.2 ml/kg to males). With water quality parameters within the recommended ranges throughout the study period, the standard dose of Ovaprim (0.5ml/kg for females_0.3ml/kg for males) yielded optimal results in terms of latency time (5 hours), fecundity (272000 eggs/kg of gonads), fertility (81.76%), hatching (83%) and survival rate (91.98%). While deviations led to decreased outcomes. The recommended dose of Ovaprim affected all stages of reproduction from ovulation till survival and led to higher yield in Common carp in comparison to lower or higher than recommended dose. This is indicating the activation of cascade hormonal system at optimum level than the other doses with no specific effect due to species selection.

Keywords: Induced Breeding, Common Carp, Ovaprim, *Cyprinus Carpio,* Gonadotropin Releasing Hormone, Domperidone

Introduction

Ovaprim is gonadotropin-releasing hormone (GnRH) analogue with dopamine receptor antagonist that potentiate the receptor response to the GnRH (Zohar et al., 2001). The GnRH is secreted from the hypothalamus of the fore brain and activates the pituitary gland for secretion of gonadotropin hormones (especially Gonadotropin-II), resulting in stimulation of ovaries and tests to produce steroids and prostaglandins (Evans and Claiborne, 2006). These hormones act directly on the gonads to cause final maturation and release of oocytes (eggs) in females (ovulation), and the release of sperm in males (spermiation) (Evans and Claiborne, 2006). The use of GnRH has been reported to be used for induced breeding purposes in other species of fishes and brought positive outcomes (Nazir, 2023), but in Common carp to the best of our knowledge no such attempts have been made in Pakistan (Harvey et al., 1979; Malik et al., 2014). This was since Common carp normally breed in captive conditions inside standing water unlike other running water fishes such as silver carp, grass carp, mori, telia and rohu that cannot breed in captive condition (Mylonas & Zohar, 2007; Schreck et al., 2001). Hence, it is extracted that there is no need for artificial reproduction. So, an inverted idea would be to have research on Common carp breeding, putting it on top priority to study various breeding behaviors of Common carp in dynamic ways. Ovaprim is GnRH analogue with dopamine receptor antagonist that potentiates the receptor response to the GnRH (Zohar et al., 2001) used either alone or with profasi (human chorionic gonadotropin). The liquid form (1.0 ml) of it contains 20 μg of Salmon GnRH (d-Arg, TRP-7, LEU-8, PRO-9, NET) and 10 mg of domperidone dissolve in prophylene glycol (Brzuska et al., 1999; Hill et al., 2009). Ovaprim is used as a spawning aid to induce ovulation (release of mature oocytes/eggs) in female fish and spermiation (release of milt/sperm) in male mature brood-fish (Ali et al, 2015). Domperidone, the other active component of Ovaprim, helps block the inhibitory effects of dopamine (Yanong et al., 2009). This substance has been applied under controlled conditions in the culture of carps (Brzuska and Adamek, 1997), as well as other culturable species such as pike, *Esox lucius* (Szabo, 2003). With no previous straight attempts to see the effects of Ovaprim in induced breeding of Common carp in Pakistan, this study aims to determine the effect of Ovaprim use in Common Carp and

to check the most effective dose of Ovaprim for Common carp propagation in a semiartificial environment.

Materials and Methods

Experimental Design

This study was conducted between the mid of October and end of November 2022, in line with the breading season of the Common carp (Weimin, 2005). The study was conducted in the laboratory of Sher Abad Carp Hatchery and Training center (CH&TC), Mathra, Peshawar, Khyber Pakhtunkhwa (KP), Pakistan. The present experiment followed complete randomized design (CRD) with one factor, four treatments, and three replications. Synthetic hormones viz, Ovaprim (Synthetic Salmon Gonadotropin-Releasing Hormone and Domperidone) (Syndel laboratories, Nanaimo Canada) at different doses were injected i,e: T_0 (control group with no inducement), T_1 (0.3 ml/kg to females and 0.1 ml/kg to males), T_2 (0.5 ml/kg to females and 0.2 ml/kg to males), and T_3 (0.7 ml/kg to females and 0.2 ml/kg to males). We had followed the dosage recommendation (0.5 ml/kg for female fishes while 0.2 ml/kg for male fishes) of Ovaprim suggested by Nandeesha, et al., 1990 for *Aristichthys nobilis* and *Catla catla* and its manipulation. Details are presented in Table1.

Brooders Selection and Maintenance

The fish were reared in the hatchery brood pond (1 hec), maintained and fed with soft aquatic weeds i,e Azolla, lemna and supplementary feed of wheat bran and rice bran at 2:1 ratio. Growth of the brooders and water quality of the pond were monitored daily, weekly and summarized into monthly intervals. A total of 48 brooder of Common carp fishes (males and females) at the ratio of 2:1 was used in this experiment. The average body weight of the brooders was determined by the digital balance (Salter, model 235, Bromwich, England). Healthy brooders were selected randomly based on their overall external appearance having average body weight of 2 kg, as their normal weight ranges from 0.5 to 4 kg (Tomelleri and Eberle, 1990). These brooders were caught from the brooders stock ponds (1 hec) of hatchery by using dragging net and transferred to holding tanks. Both male and female brooders were acclimatized in separate holding tanks of 9.5 × 6.5 × 4 feet for 6 to 12 hours. Growth of the brooders and water quality of each holding tank were monitored at interval of 7 days.

Treatments

Ovaprim was injected in a single dose to randomly selected Common carps in evening time (7:00 pm). Brooders were injected Ovaprim through intramuscular routes into dorsolateral region of the fishes in a single dose (Haniffa et al., 2002) by using hypodermic syringe after cleaning the area with cotton swab. At the inner side of the basal part of the pectoral fin where it was scaleless, the needle was injected gently towards the head at an angle of 45° to longitudinal axis of the body to the depth of about 1.5 cm. The dosage of the inducing agent was calculated by the following formula:

$$Weight\ of\ fish\ \times\ Dosage = Quantity\ to\ inject$$

Male brooders of T_1, T_2 and T_3 were injected 0.1 ml/kg, 0.2 ml/kg and 0.3 ml/kg respectively, and females were injected 0.3 ml/kg, 0.5 ml/kg and 0.7 ml/kg respectively (Table 1). As we selected brooders of average weight 2 kg; so, the total quantity of Ovaprim injected to the male brooders of T_1, T_2, T_3 were 2 × 0.1 = 0.2 ml, 2 × 0.2 = 0.4 ml and 2 × 0.3 = 0.6 ml respectively, and to the female brooders of T_1, T_2, T_3 were 2 × 0.3 = 0.6 ml, 2 × 0.5 = 1ml and 2 × 0.7 = 1.4

ml respectively. The treatment and their injection quantity are summarized in table 2.2 (Table 1).

Spawning and Fertilization

A gentle water was supplied to the holding tanks from the biofiltered recirculating water system after each 12 hours at the rate of 30 liter per minute. Fishes were observed for its matting behavioral signs in holding tanks after dose administrations. Male stimulated female estrus− which was characterized by restlessness of females and its tail and abdomen became extremely constricted. While noting the matting behavior at the bottom of holding tank, male showed chasing behavior and touching the females aggressively which lasted for 30 to 60 minutes. Active male finally chased the female and continuously hit the vent and head of the females (Haniffa et al., 2007). Their stripping did not occur, rather spawned naturally after injection in tanks (semiartificial condition). At the time of breeding, males were associated at one side of the females and shed sperm on releasing eggs from females. The adhesive eggs ovulated from females− were fertilized outside the body of females by male and stick to the weeds in holding tank. After that fishes were netted out because of cannibalistic behavior of Common carp. The process of fertilization took 30 to 60 minutes. The non-adhesive eggs were carried out with water flow from the holding tanks and collected in separate containers. The fertilized eggs were observed in holding tanks. The fertilized eggs were removed from holding tanks and were suspended in separate standing water of hatching tank for further embryonic stages. The fertilized eggs absorbed water and attained the size of 1 to 1.4 mm in diameter and hatching was expected to start in 24 hours. Eggs were collected from holding tanks and transferred into hatching tanks of the same size. Eggs were spread inside each hatching tank.

Determination of Breeding Performance

For recording the breeding performance of the fishes, the following mentioned direct and indirect parameters were observed and determined respectively, which may have potentially affected the breeding performance of the fishes.

Water Quality Parameters

The standard methods of American public health association (APHA, 1995) were used as standard for analyzing some important physicochemical water parameters like water temperature (°C), pH, dissolved O_2 (mgl^{-1}), free CO_2 (ppm), hardness of water (mgl^{-1}), acidity (mIL^{-1}), alkalinity (g L^{-1}), chlorine and nitrogen concentration (mgL^{-1}). The water quality parameters of all holding tanks were measured from 20 October to 27 November at intervals of seven days. The temperature was checked through digital thermometer (Hanna instruments HI-8634, Woonsocket, United states) by dipping the sensor in water for about three minutes and then the given values were noted. With the help of digital pH meter (Jenway 370-pH meter, London, Essex, E2 9DU, United Kingdom), pH of water was determined. The other chemical parameters of i,e dissolved O_2, free CO_2, hardness of water, total chlorine and total nitrogen were determined by taking water sample from holding tanks in clean plastic bottles and analyzed by digital multidirect machine (Tintometer ATES0205200003, Amesbury, United Kingdom). All these water-related analyses were carried out in the laboratory of CH & TC Peshawar.

The breeding performance of the fish were determined by observing and recording the latency period, fecundity rate, fertilization rate, hatchability rate and survival rate (Haniffa et al., 2007; Saha et al., 2017). Latency period (in hours) was calculated for the time between injection of Ovaprim up to ovulation of eggs. Hence, the difference between the time of injection and the time of ovulation which is expressed in hours.

Each female effective fecundity rate (in percentage) was determined through random sampling of released eggs. First the sample of fish was taken from each holding tank of different treatments and the ripen ovary of the fishes was weighted. After determining the weight of the ovary, 3 samples of 100 mg were taken from the anterior, posterior and middle part of ovary. We counted the number of eggs in each sample and the fecundity rate was determined by following formula.

$$\textbf{Fecundity} = \frac{\text{Average number of ova obtained from 3 samples of ovary} \times \text{total weight of ovary}}{100}$$

Fertilization of the eggs ovulated from the females occurred outside the body and was completed in the time range of 30 to 60 minutes after spawning while 3 to 4 hours after fertilization blastomeres were formed inside fertilized eggs. The formation of blastomeres were confirmed by appearance of clear yellowish eyespot and distinguished from the unfertilized eggs which was appearing whitish and cloudy, and some became ruptured. When fertilization was completed, then the total number of fertilized eggs were determined in holding tanks by the help of volumetric method, in which number of eggs per ml was counted, and that number was multiplied with the total volume occupied by total eggs (Muir and Robert, 1985):

$$Fertility\ rate = \frac{no.of\ fertilized\ eggs}{total\ no.of\ eggs} \times 100$$

The fertilized eggs were incubated in the same tank. A steady flow of fresh water was provided to keep eggs in motion and oxygenated. After 19 to 24 hours hatching occurred from the fertilized eggs at water temperature of 20 to 24.5 °C and special rectangular tank was used to keep the hatching for three days until yolk was absorbed. Hatchability rate was determined by the following formula adopted from Lambert (2008):

$$Percentage\ hatchability\ = \frac{no.of\ eggs\ hatched}{total\ no.of\ fertilized\ eggs} \times 100$$

When the larvae were fully mature and shifted to the stocking pond then the reading was recorded again. The total number of survived larvae were determined by same volumetric method. Survival rate was determined by the following formula:

$$Survival\ rate\ (\%)\ = \frac{no.of\ fishes\ survived\ upto\ fry\ stage}{total\ eggs\ hatched} \times 100$$

Results

Water Quality Assessment

The observed mean water temperature for T_0, T_1, T_2 and T_3 were "19.6 °C, 21.2 °C, 22.8 °C and 20.4 °C respectively (Table 2). The mean dissolved oxygen for T_0, T_1, T_2 and T_3 were "3.18 mgl^{-1}", 3.58 mgl^{-1}", "3.72 mgl^{-1}" and "3.72 mgl^{-1}" respectively. The average free CO_2 level for T_0, T_1, T_2 and T_3 were "11.2 ppm", "11.2 ppm", "11.2" and "11.2 ppm" respectively. Water hardness for T_0, T_1, T_2 and T_3 averaged at "25 mgL^{-1}", "8.2 mgL^{-1}" "8.4 mgL^{-1}" and "8.0 mgL^{-1}" respectively. The acidity of water for T_0, T_1, T_2 and T_3 was observed to be "0.50 mlL^{-1}" "0.46 mlL^{-1}" "0.42 mlL^{-1}" and "0.42 mlL^{-1}" respectively. Alkalinity values for T_0, T_1, T_2 and T_3 were recorded as "0.124 gL^{-1}", "0.174 gL^{-1}", "0.176 gL^{-1}" and "0.174 gL^{-1}" respectively. Chlorine concentration for water of T_0, T_1, T_2 and T_3 was observed to be "0.25 mgL^{-1}", "0.26 mgL^{-1}" "0.26 mgL^{-1}" and "0.28 mgL^{-1}" respectively. Nitrogen concentration of water for T_0, T_1, T_2 and

T_3 was recorded as "0.8 mgL^{-1}", "0.7 mgL^{-1}", "0.7 mgL^{-1}" and 0.7 mgL^{-1} respectively (Table 2).

Latency Period and Spawning

There was no latency time observed for T_0 because fishes of control group were not injected with Ovaprim but, natural spawning of the control group was observed one day later from other treatment (24 hours) groups. The latency time of T_1, T_2 were "8:00 hrs, 5:30 hrs" respectively (Table 3). In T_1 fishes spawned partially while in T_2 complete spawning was observed. Latency time for T3 was not noted because spawning was not observed in current and one day after injection of Ovaprim.

Fecundity Rate

The various fecundity rates calculated for T_0 were as follows (Table 3). The observed average absolute fecundity (Total ripen eggs in ovary) per female was 48000 eggs and average total absolute fecundity was 192000 eggs. Relative fecundity (Eggs per kg of ovary) per female of the T_0 was observed as 24000 eggs and average total relative fecundity was 96000 eggs. For T_1 the absolute fecundity (total ripen eggs in ovary) per female averaged at 29892 eggs and total absolute fecundity was 119568 eggs, while average relative fecundity (eggs per kg of ovary) per female was 14946 eggs and total relative fecundity was 59784 eggs. The absolute fecundity per female for T_2 group was observed 68000 eggs and average total absolute fecundity was 272000 eggs. The average relative fecundity (eggs per kg of ovary) per female of the T_2 was 34000 eggs and average total relative fecundity was 136000 eggs. The fecundity rate observed for T_3 was zero. It was observed that females in T_3 did not spawn in holding tank. The highest fecundity rate was observed in T_2 than in comparison to all other treatments and control groups (Table 3).

Fertilization Rate

The observed fertilization rate for T_0 was 57% where 109440 eggs out of 192000 total eggs for all females were fertilized (Table 3). However, the observed fertilization rate for all females of T_1 was 17936 eggs out of total 119568 eggs with percentage fertility of 15%. In T_2, it was observed that 55597 eggs were fertilized out of 68000 per female. For total females of T_2 it was observed that 2222388 eggs out of total 272000 eggs were fertilized with percentage fertility of 81.76%. Among all groups the observed fertilization rate for T_0 was 42.00%; greater than T_1 while observed fertilization rate in T_2 was higher than all other treatments, T_2 fertilization rate was 24.76% greater than T_0 and 66.76% than T_1.

Hatchability Rate

The hatchability rates for T_0, T_1 and T_2 were "19, 19, 22, hours" respectively. In T_0, 43776 hatchlings were hatched out of a total of 109440 fertilized eggs with percentage hatchability of 40% (Table 3). The percentage hatchability rate for T_1 was 40% where 7175 hatchlings were hatched out of total 17936 fertilized eggs. Out of a total of 222388 fertilized eggs, 184582 were hatched out in T_2 with the percentage hatchability of 83%. In T_3, there were no hatchlings observed. The percentage hatchability was observed same in T_0, T_1 while highest percentage hatchability was observed in T_2.

Survival Rate

Out of the total 43776 hatchlings of T_0, 26266 survived up to fry stage with percentage survival rate of 60.00% (Table 3). In T_1, 2851 hatchlings were observed to survive up to fry stage in total 7175 hatchlings with percentage survival rate of 39.93%. While; the percentage survival rate was highest for the T_2, which was 91.98% where 169778 hatchings were survived out of total

184582 hatchlings. In T_3, 0% survival rate was observed. The observed percentage survival rate of T_0 was 20.26% greater than T_1. While the survival rate of T_2 was greater than all other treatments (Table 3).

Discussion

This research was conducted with the aim to know the most effective dose of Ovaprim for the induced breeding of Common carp (tried for the first time in Pakistan) in semiartificial conditions. Among all the experimental and control groups, experimental group T_2, was the best in term of latency period (5.30 hours), total fecundity (272000 eggs/kg of gonads), fertilization rate (81.76%), hatching rate (83%), and survival rate (91.98%).

Productivity and sustainability of aquaculture depend upon quality seed in adequate quantity of commercially important fish species, which could be possible by induce spawning and nursing from small to large scale at hatcheries. It depends on several factors such as brood-stock management, breeding techniques, nursing methodologies, farming and marketing. The use of synthetic hormone Ovaprim in artificial ovulation of carp fishes is well accepted (Sarkar et al., 2004; Mujkherjee et al., 2002; Pandey and Singh 1997; Sharma and Singh, 2002; Szabó, 2003). However, it does depend on the response of fish species. The use of Ovaprim was tried on many other resembling species, however, it was never tried on Common carp in Pakistan. We tried for the first time to get the best of our knowledge. In our study we observed that the latency period decreased with the increase in Ovaprim dose, and a huge difference was observed not only between control and experimental groups (standard, lower and higher doses), but also within the experimental groups. This effect was obviously due to the combination of sGnRH and domperidon in Ovaprim solution at standard doses of 0.5 ml/kg, which stimulated gonadotropin (GTH II or LH) secretion in the pituitary gland of the brood fish to an optimum extent without causing adverse effect and then induced maturation and ovulation (Nuraini et al., 2017). The influence of sGnRH and domperidon at the standard dosage most likely inhibited secretion of dopamine and stimulated the secretion of pituitary gland (Nuraini et al., 2017). Pituitary gland secrets GTH. The GTH in this case, GTH II, would stimulate the theca cells which secrete hormone 17-alpha hydroxyprogesterone, which would then be converted into maturation-inducing steroid by the enzyme 20-beta-dihydroxy steroids, stimulating follicles to burst as the oocytes hydrate. (Goetz et al., 1997; Patiño et al., 2002). The higher levels of gonadotropin lead to inducement of ovulation faster than the normal (Lam et al., *1985*). However according to Ithisom et al,. (2008), the hormone works optimally at a certain dose and any change in the dose will reduce effectiveness.

These findings are in consonance with the studies of Usman et al. (2015), Arabaci et al., (2001), and Drori et al., (1994). Usman et al., (2015) tried Ovaprim induction in *Cyprinus carpio* in Nigeria, while Arabaci et al,. (2001) had tried induced spawning in *Cyprinus carpio* in Tukey. Drori et al., (1994) had performed the induced spawning in *Cyprinus carpio* in Israel. Apart from species, there were other factors which may have influenced the differences in results to other studies. These findings possibly could have been affected by the physicochemical parameters of the water. Growth and persistence of fish depends on the quality of water including physical properties such as color, air temperature and water temperature in addition to the chemical properties such as dissolved oxygen, alkalinity, total dissolved solid, pH and electric conductivity (Bolorundaro and Abdullah, 1996). Among all these parameters the water temperature has a determining effect on breeding fishes affecting the fish maturation, fish eggs development, hatching rate, larval behavior and even certain morphological features (Karadede and Unlu, 2007; Gardeur, 2007). Therefore, the determination of the optimal temperature for Common carp brood stock is necessary to maximize the fish seed production (El-Gamal et al.,

2009). The normal range of temperature for Indian major carps is 13 to 32 °C. In our study the water temperature we had observed was 21 °C, in line with the previous studies for better growth, development and reproductive outcomes (Weimin, 2005).

The decreased latency period with increase in Ovaprim does not go upto a level was not generalized, where a contrast to our results to previous findings from other authors was observed (Nuraini *et al.,* 2017; Achionye-Nzeh *et al*., 2012; Sahoo *et al.*, (2007). These differences may be attributed to the selection of species, sample size, dosage of Ovaprim and different climatic conditions of the study area, because the species along with the locality used by Nuraini *et al.,* (2017), Achionye-Nzeh *et al*., (2012) and Sahoo *et al.,* (2007) were Siben fish in Indonesia, African mudfish (*Larias gariepinus)* in Nigeria and *Clarias batrachus* in India respectively. The Ovaprim dosage used by them were 0.7 ml/kg, 1.5 ml/kg and o.5 ml/kg respectively. According to previous studies, the responses of fishes to Ovaprim are different. For example, Nandeesha et al., (1990) got the best results in inducing reproduction of Indian carps at dose of 0.3-0.4 ml kg Ovaprim. However, for the Snow trout the best result obtained at much higher dose of Ovaprim at 1.5 ml/kg of body weight (Gharaei et al., 2011). These results in combination with our results indicate that reproductive responses to Ovaprim are dose dependent and are different depending on species kind. Consequently, and evidently; in our study, we observed the highest fecundity rate for T_2 (standard dose) followed by T_0 (control group) and T_1 (high dose). The fecundity rate of T_2 was higher than all treatments and control groups. These findings agree with earlier research on Common carp by Yeasmin et al., (2013). Yeasmin et al carried out their study in Bangladesh on Common carp and reported 63149 eggs/kg from Common carp at dosage of 0.5 ml Ovaprim. However, some contrasting literature also exists to our findings like studies of Bakkara et al., (2015), Nuraini et al., (2017) and Naeem et al., (2008). Naeem et al., (2013), Malik et al., (2014) and Bakkara et al., (2015) performed experimental study on lelan fish (*Osteochilus pleurotaenia*) in Indonesia and got 26,826, 30,024, 23,350 eggs/g of gonads by injecting Ovaprim of various doses of 0.5 ml/kg, 0.6 ml/kg and 0.7 ml/kg respectively. Nuraini et al., (2017) studied Siban fish (*Cyclocheilichthys apogon)* in Indonesia and obtained 5.5 ± 0.71, 10.67 ± 6.03, 11.00 ± 0.00 and 19.00 ± 11.53 (eggs g^{-1} of BW) eggs by injecting 4 doses of Ovaprim (0.25 ml/kg, 0.3 ml/kg, 0.5 ml/kg, 0.7 ml/kg) respectively. Naeem et al., (2008) conducted experiment on Grass carp (C*tenophyrngoden idella*) in Multan, Pakistan and reported 62532 kg^{-1} eggs by using single dose of Ovaprim of 0.4 ml/kg. Naeem et al,. (2013) carried out their experiment on *Labeo rohita* and obtained 63574 eggs / kg.

Malik et al., (2014) conducted experiment on Koi carp in Sindh, Pakistan and obtained 9930 eggs/kg by injection of single dose of Ovaprim at 0.5 ml/kg. The results of which are lower than the present finding of T_2 indicating differences in species selection in all these studies. Different fish species having genetic diversity exhibit unique reproductive behaviors and physiological characteristics in addition to the physicochemical parameters of water body, that can affect their response to hormonal induction. While hormonal protocols itself and health of the fish may also influence the reproductive outcomes of the fishes. For instance, the reproductive cycle and hormone receptors in Common carp might not respond to Ovaprim in the same way as other species studied in different locations (Sahoo and Dash, 2012). Fish with different genetic backgrounds might have varying sensitivities to hormone doses and exhibit different reproductive behaviors (Pšenička and Gela, 2007). For instance, Bakkara et al., (2015) found the best result for *Osteochilus pleurotaenia* by applying 0.6 ml/kg of Ovaprim while Yeasmin et al., (2013) found best result for *Cyprinus carpio* by applying 0.5 ml/kg of Ovaprim dose. Water quality, temperature, and photo period are critical environmental factors influencing fish reproduction. Variations in these factors between study locations can impact the success of

induced breeding. For example, Usman et al., (2015) performed study on common carp in three successive months viz, January, February and March in Nigeria, where they found 16.55 ± 0.13, 13.00 ± 0.13 and 11.32 ± 0.07 latency time respectively. Differences in environmental conditions might lead to variations in egg production among Common carp populations (Nagahama, 1994). We conducted this study in the month of October where the photo period was 11 to 12 hours. The success of hormonal induction depends on factors such as dosage, timing, and administration of the hormone. Variations in these protocols can lead to different outcomes. Even slight differences in hormone dosages or timing can significantly affect the results of induced breeding (Bromage and Roberts, 1995). For instance, Nuraini et al., (2017) obtained 5.5 ± 0.71, 10.67 ± 6.03, 11.00 ± 0.00 and 19.00 ± 11.53 (eggs g-1 of BW) eggs by injecting 4 doses of Ovaprim (0.25 ml/kg, 0.3 ml/kg, 0.5 ml/kg, 0.7 ml/kg) respectively to Siban fish. The health and overall condition of the fish play a crucial role in their response to hormonal treatments. Fish that are stressed, malnourished, or diseased might not respond as effectively to hormone injections, leading to variations in egg production (Chatakondi and Mehta, 2007). A study by Smith et al., (2022) on rainbow trout (*Oncorhynchus mykiss*). They divided the fish population into two groups: one group consisting of healthy, well-nourished fish, and another group consisting of fish that were intentionally exposed to chronic stress by overcrowding and inadequate feeding. Both groups received the same hormone injection to stimulate egg production. The results revealed a clear difference in the response to the hormone treatment between the two groups. The healthy and well-nourished fish displayed a substantial increase in egg production, with nearly 90% of the treated fish releasing viable eggs. On the other hand, the stressed and malnourished fish exhibited a much lower response, with only 50% of the treated fish producing viable eggs. The stressed fish also showed delayed egg release and reduced egg quality. Apart from these factors the experimental design, including factors such as fish size, age, handling procedures, and study duration, can influence induced breeding outcomes (Bakkara et al., 2015; Yeasmin et al., 2013). Younger or smaller fish might respond differently than older or larger ones, and the duration of hormone exposure can impact the overall success of the process (Basavaraja and Bhatnagar, 2001).

After ovulation, the later reproductive events are equally sensitive to the prevailing conditions, may also vary among experimentation and similarly affected by the described conditions. In our results the Common carp showed the highest fertilization rate (82%) for T_2 to which standard Ovaprim dose was injected, followed by T_0 (57%) and T_1 (15%). The higher fertilization rate in T_2 was because of substantial and optimum induced ovulation resulting in the availability of a greater number of eggs to sperm to be fertilized. Against that the low dose of Ovaprim injected into fishes in T_1 could not fully initiate the cycle of ovulation; thus, had lower fecundity and lesser eggs available for fertilization. No fertilization rate was observed in T_3 because fishes in T_3 did not spawn due to overdosage of Ovaprim causing more negative feedback and thus suppress the hormone secretions of pituitary glands necessary for ovulation. The 82% fertilization rate obtained in our results for the standard dose of Ovaprim is in consonance with few studies Yaesmin et al., (2013) and Naeem et al., (2008) in spite that species were different, but in contrast to other studies Malik et al., (2013), Nuraini et al., (2017), Naeem et al., (2013) and Marimuthu et al., (2015). Yaesmin et al., (2013) and Naeem et al., (2008) reported similar fertilization rates of 82.38% and 80.36% respectively in Common carp and Grass carp by administration of 0.5 ml/kg of Ovaprim. Malik et al., (2013), Nuraini et al., (2017), Naeem et al., (2013) obtained lesser fertilization rate of 75.2%, 60.00% and 77.50% in Koi carp, Siben fish and *Labio rohita* respectively by administration of standard dose (0.5 ml/kg) of Ovaprim. A substantially higher fertilization rate of 97.88% was obtained by Marimuthu et al., (2015) conducted on Catfish by injecting 0.4 ml/kg of Ovaprim. The differences of our results and

other studies are due to selection of species because different fish species can have varying responses to hormonal induction due to differences in their reproductive physiology apart from other reasons. Common carp (*Cyprinus carpio*), Koi carp (*Cyprinus rubrofuscus*), Siben fish (*Leptobarbus hoevenii*), Grass carp (*Ctenopharyngodon idella*), Rohu (*Labio rohita*), and Catfish likely have distinct hormonal requirements for successful induction. Each species has evolved to respond differently to hormone treatments, sample size and different climatic conditions like water quality, temperature, photo period, and other environmental factors can impact the success of induced breeding. These factors can vary between locations and seasons, leading to different outcomes in different studies.

After having ovulation and fertilization while being in the same environment, the hatchability and survival rate may not have been affected in all groups of common carp. Hence the highest hatchability (83%) and survival (91.98%) rates for T_2 were obtained for the standard dose of Ovaprim. These results are like most studies (Malik et al., 2014; Yaesmin et al., 2013; Naeem et al., 2008; Marimuthu et al., 2015) but in contrast to Rehaman et al., 2013; Nuraini et al., 2017. Malik et al., (2014) in line with our results obtained 83.3% hatching rate by injecting 0.5 ml/kg of Ovaprim dose to koi carp. Yaesmin et al., (2013) performed experiment on *Cyprinus carpio* and reported 79.22% hatching rate by using 0.5 $mlkg^{-1}$ dose of Ovaprim which is although slightly lower from current result of T_2 and higher from T_0, T_1, and T_3. Naeem et al., (2008) conducted an experiment on Grass carp and obtained 79.49% hatching rate by using 0.5 ml/kg dose of Ovaprim. Marimuthu et al., (2015) obtained 93.66%, 85.77% and 83.66% hatchability by injecting three doses of Ovaprim i-e 0.4 ml/kg, 0.5 ml/kg and 0.6 ml/kg while studying on African Catfish and. However, Rehaman et al., (2013) obtained much lower; 42.78%, 44.60% and 55.00% hatching rate by using doses of 0.4 ml/kg, 0.7 ml/kg and 1.0 ml/kg of Ovaprim in *Cyprinus carpio*. Nuraini *et al.,* (2017) conducted study on Siban fish (*Cyclocheilichthys apogon)* and reported 25.55%, 62.94%, 56.95%, 76.00% of hatching rate for control and three treatment of various doses i-e 0.3, 0.5 and 0.7 ml/kg. The differences in our results and studies of Rehaman et al., (2013) and Nuraini et al., (2017) may be most probably attributed to the selection of species. After hatching, the similarly highest survival rate of 91.98% was obtained for T_2 followed by T_0 (60%) and T_1 (39.73%). In T_3 there was no survival rate observed. The highest survival rate in T_2 may be attributed to consequential cascade event of acclimatization, optimization ensuring better yield and growth of fish before and after hatching, due to standard dose of Ovaprim. Malik *et al.,* (2014) obtained similar result while conducting experiment on Koi carp in Sindh, Pakistan and found the similar results. Malik *et al.,* (2014) studied Common carp's breeding, in which they obtained 81% survival rate which is lower from current result of T_2 and higher from T_0 and T_1. However, some contrasting literature also exists, like studies of Hakim and Gamal, (2009) and Abdel Hakim *et al*., (2008). Hakim and Gamal (2009) explored Common carp induced breeding and reported 38.70% survival rate of fry which is lower than current result of T_2. Abdel Hakim *et al*., (2008) found 30.83% survival rate while studying Common carp. Difference in the result may be attributed to the sample size, different reproductive physiology of different species and different climatic conditions at which experiment was performed. The water quality parameters were in a suitable range for Common carp (*C. carpio*) throughout the spawning period and similar with the findings of Malik et al., (2014), Ghosh et al., (2012) and Horvath et al., (2015). They recommended that water temperature (18 °C-24 °C), dissolved oxygen (4.0 mg L^{-1}- 6.0 mg L^{-1}), pH (6-8) are suitable for successful breeding of cyprinid species, which we also observed in our study. Overall, the recommended and standard dose of Ovaprim proved to be the most effect dose for induced breeding of Common carp. Hence, it may be similarly used for this specie like the other species to increase the yield and satisfy the consumer preference.

Conclusion

The recommended dose of Ovaprim affected all stages of reproduction from ovulation till survival and led to higher yield in Common carp in comparison to lower or higher than recommended dose. This is indicating the activation of cascade hormonal system at optimum level than the other doses. If the condition of the brood stock is good, breeding is at right of the season, climatic condition, temperature of the water, and other extrinsic as well as intrinsic conditions are favorable, the tremendous breeding response and results could be achieved from Common carp through semiartificial breeding technique.

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Table 1. Description of Common Carp Brooders with Concentration and Time of Ovaprim Injection

Treatment Groups	**Female Brooders (Body wt[1](kg), no. of Brooders)**	**Male brooders (Body wt (kg), no of Brooders)**	**Inducing agents**	**Hormonal Dosage to Female (ml/kg of wt)**	**Hormonal Dosage to Male (ml/kg of wt)**	**Total amount injected to (in ml)**		**Injection time**
						Female	Male	
T_0	2 , 4	2 , 8	No inducement	No inducement	No inducement	No inducement	No inducement	No time
T_1	2 , 4	2 , 8	Ovaprim	0.3	0.1	0.6	0.2	7:00 pm
T_2	2 , 4	2 , 8	Ovaprim	0.5	0.2	1	0.4	7:00 pm
T_3	2 , 4	2, 8	Ovaprim	0.7	0.3	1.4	0.6	7:00 pm

1; Body weight : 2; Number of brooder fish

Table 2. Water Quality Parameters

Parameters	Values			
	T_0	T_1	T_2	T_3
Water temperature (°C)	19	21.2	22.8	20.4
Dissolved oxygen (mgl^{-1})	3.18	3.58	3.72	3.72
pH	6.9	6.9	6.9	6.9
Free CO_2 (ppm)	11.2	11.2	11.2	11.2
Hardness of water (mgL^{-1})	25	8.2	8.4	8.0
Acidity (mlL^{-1})	0.5	0.46	0.42	0.42
Alkalinity ($g\ L^{-1}$)	0.124	0.174	0.176	0.174
Chlorine ^{1}Conc. (mgL^{-1})	0.25	0.26	0.26	0.28
Nitrogen (mgL^{-1})	0.8	0.7	0.7	0.7

Table 3. Effect of Various Doses of Ovaprim on Latency Period, Fecundity, Fertility, Hatchability and Survival Rates of Common Carp

	T_0 (Control)	T_1 (Low Dose)	T_2 (Standard Dose)	T_3 (High Dose)
Latency period (hrs)	24	8:00	5:00	No
Absolute fecundity (per female)	48000	29892	68000	0
Relative fecundity (per female)	24000	14946	34000	0
Absolute fecundity (total)	192000	119508	272000	0
Relative fecundity (total)	96000	59784	136000	0
No of fertilized eggs (per female)	27360	4484	55597	0
Total no. of eggs laid by a single female	48000	29892	68000	0
No. of fertilized eggs (total)	109440	17936	222388	0
Percentage fertility	57	15	81.76	0
No. of hatchlings	43776	7175	184582	0
Percentage hatchability	40.00	40.00	83	0
Time of hatching (hours)	19	19	22	0
No. of survived fishes up to fry stage	6566	7134	20455	0
Percentage survival	60	39.74	91.98	0