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Comparison of Relative Fecundity and Early Survival of *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* through Induced Breeding

Original Article

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Abstract

Induced breeding is also a fundamental capability that ensures aquaculture has a visibly reliable source of seed of aquaculturally valuable species. The relative fecundity and early post-hatch survival of two cyprinid species, the grass carp (Ctenopharyngodon idella) and the silver carp (Hypophtalmichthys molitrix) were the objective of the present study. Ovaprim was injected into the brood fish at three levels of dosage, such as high, standard (0.5 ml/kg in females; 0.3 ml/kg in males), and low. Spawning behavior was noted eight hours after the injection, and the equivalent ovulation, fertilization, and hatching rates were documented systematically. At the same time, the parameters of water quality were measured during the experiment. The variation in ovulation and fertilization was sharp between the subjects who received the low dose and those who received the standard dose; however, the variation between the high dose and the standard dose was not significant. The grass carp under study in the current study hatched four times on average, with a value of 60%. Silver carp hatches, by contrast, have been unsuccessful because temperature inconsistent with both pond and hatchery waters has led to development being halted. These results show that the Grass carp is more resilient to thermal stress in comparison to the silver carp in a similar environment. Studies done in hatchery evidenced that induced breeding of grass carp resulted in good production, but reproduction of silver carp was still impaired by variable thermal regimes. These findings support the need to maintain ideal and consistent environmental settings to increase breeding success of induced aquaculture.

Keywords: Induced Breeding, Ovaprium, Relative Fecundity, Survival Rate, *Ctenopharyngodon,* and *Hypophthalmichthys Molitrix*.

Introduction

Another important farmed freshwater fish species is Grass carp (C. idella), also called white Amur, which had a world production of 5,537,794 tons in the year 2014 (Department, 2018). Grass carp is one of the biggest members of the family Cyprinidae and is the sole species of the genus Ctenopharyngodon (Chilton & Muoneke, 1992). It must not be mistaken with other carp species like silver carp (Hypophthalmichthys molitrix), bighead carp (Hypophthalmichthys nobilis), or mud carp (Cirrhinus molitorella). These other carp species are not good biological control agents in terms of aquatic vegetation management since they feed on other contents of the pond ecosystem. The grass carp originated in southeastern Russia and northwestern China. It is an herbivorous species, hence its deliberate introduction in several countries due to vegetation control. Moreover, grass carp is part of the fish culture and is a significant protein food source for human beings.

Grass carp is a sub-tropical-to-temperate species and spawned in rivers and large lakes of eastern Asia. It inhabits the southern part of Russia to northern Vietnam and along big rivers such as the Amur (China/Russia border), Yang Tze (northern China), Yellow River (central China), and the Min River (spans the border between China and Vietnam). Besides this, grass carp have been exported to numerous locations in the world like Taiwan, Israel, Japan, Philippines, USA, Mexico, India, Malaysia, Netherlands, Switzerland, Czechia, Slovakia, Denmark, Sweden, Romania, Poland, Italy, West Germany, France, United Kingdom, Argentina, Venezuela, Fiji, New Zealand, Australia, and South Africa. Grass carp are uncommon in their natívals range (of the Amur basin), compared to that of other Asian carps. Climatic conditions are widely variable in the native range of grass carp. The two extreme conditions are a mean annual air temperature of 25 C (in the lowest known point of the southernmost hemisphere) and -60 C in the northernmost hemisphere (Asgharnia & Ghasemi, 2021).

The introduction of grass carp (*Ctenopharyngodon idella*) into Pakistan is originally traced to 1964, when it was brought in by the importation of fish stock from China. In addition to culture, it was also introduced to regulate biological aquatic weeds in the natural waterways, rivers, and man-made lakes. Grass carp mature at the age of two years or even more; in the cultured carps, males usually mature a bit earlier than females. Spawning entails the extraction of sexually active products (ovum in the case of females, milt in the case of males) from the body. One of them includes laws of induced breeding in which the adult parent fish is injected with exogenous hormones in the body, resulting in them breeding. Induced breeding using pituitary extract was used in the past 20 years. The cost of donor pituitary was ever increasing, and the cumbersome process forced the expert to experiment with other hormones; HCG (human chorionic gonadotropin), LHRH (luteinizing hormone releasing hormone), and ovaprim-C (Naeem *et al*., 2011).

Ovaprim is a mixture where the amount of gonadotropin-releasing hormone analogue (sGnRH; D Arg 6, Pro 9, Net) is 20 ug/ml, dompridone, which is a type of dopamine inhibitor, is 10 mg/ml. Ovaprim is administered to induce ovulation and spermiation of fish mainly through injection into the muscle or system or through intraperitoneal injection. Adverse effects have also been reported, which include bruising and redness, lacerations, ulcers, hyperpigmentation or darkened body colour, and hypopigmentation (loss of body colour) on the injection site, and an overall post-spawning mortality was observed (approximately 1.3 percent). There was a significant success in Pakistan when Chaudhary and Alikunhi (1957) were successful in inducing breeding of Indian major carp through the call of pituitary extract. The culture of Carps is booming rapidly, and the most significant restraining factor in the development of this industry is the lack of quality fish seed (Rashid, Balkhi, Naiko, & Ahamad, 2014).

In the recent three decades, Chinese major carps consisting of the bighead carp (*Aristichthys nobilis*), the silver carp (*Hypophthalmichthys molitrix*), and the grass carp (*Ctenopharyngodon idella*) have spread slowly. And given the throne that the FAO 2014 database of grass carp and silver carp boasts the highest gross annual production in a single year of more than 4.9 and 5.5 million tons of product respectively, the common carp (*Cyprinus carpio*) was ranked at number 3 as the common carp annual production of > 1 million tons in a single year was listed at 4th rank as far as the total farmed freshwater fishes go in that same year 2014. (Szabo, Urbanyi, Müller, Szabo, & Horvath, 2019). Chinese carp are cultured in ponds where the natural food of fish can also be created. They usually get stocked in polyculture and eat creatures of the pond ecosystem more effectively (Fatima-Zahra, 2021). This is evidenced by the fact that human consumption of food habits has changed over the last 20 years due to the growth in incomes, population growth, and changes in commodity prices. Today, the consumption of meat, fish, and other nutritious meals is popular among people (Patra, Goswami, Ghosh, & Bera, 2025). The two, Ctenopharyngodon idella (grass carp) and Hypophthalmichthys molitrix (silver carp) belong to the Chinese carps of the four key members. These other two are bighead carp *Aristichthys nobilis* and black carp *Mylopharyngodon piceus* (Liu *et al*., 2024). The introduction of the Chinese carps that comprised silver and grass carp into Pakistan was initiated in 1959. They were first introduced into Indian species to study their compatibility in controlling the biological aquatic weed that they were being bio-introduced to control (Azhar, Anjum, & Akhter, 2024). Grass carp feeds on terrestrial grass and water weeds, whereas silver carp is a species that mainly feeds on phytoplankton. Young ones of both species feed on zooplankton (Jawad, 2024). Aquaculture and fisheries industries all around the globe are prospering, and since aquatic food has more than twice the level of availability and has been unceasingly increasing since 1990, or 82.1 million mt (metric ton) in 2018 (Addo *et al*., 2022). This trend has led to a huge paradigm of investments around fish farming and aquaculture in many parts of the world. Carp culture has also developed rapidly in Pakistan, but among the most serious problems in fish farming is the unavailability of high-quality fish seed. To meet the demands of the overpopulated nations throughout the continent, particularly in Asia, aquaculture and the fisheries industry contribute 91 % of the world's production. The overall output of freshwater carbs in the aquaculture sector will go under the family Cyprinidae, which will be approximately 53.1%. In Pakistan, the Aquaculture and fisheries industry is also increasing day by day, and cultivation of almost all the carp fish species in the cultivable category is being performed. The coast in Pakistan that possesses good aquaculture capacity is 1100km, only nine species of carp were the species that are cultivated by the country in commercial fisheries. This is primarily because of improper management and development of this sector (Taheri-Khas, Gharzi, Vaissi, Heshmatzad, & Kalhori, 2024). The inland region of Pakistan covers an aquaculture area of 79,200 km2 and possesses a 1120km coastal strip, yet the number of cultured species of cold and warm water is confined to 31. The primary cause in this region includes a poor management system of aquaculture and fisheries (Umland, 2022). Besides being commonly cultivated in Thailand, China, and Pakistan, bighead carp (*Hypophthalmichthys nobilis*) and common carp (*Cyprinus carpio*) are exotic freshwater fish. Such carps are introduced in 1959 and 1964 to satisfy and threaten the requirement of food stores and nutrients (Paul *et al.,* 2025). By 1974, grass carp fingerlings were free in Halejee Lake, where it is used to regulate vegetation. Grass carp were bred elsewhere in other provinces and subsequently in Punjab public sector hatcheries. It was the second of the 1970s, during which the Karachi Development Authority (KDA) office received another batch of grass carp fingerlings, which were raised in cemented ponds and subsequently released into Halejee Lake, thereby dominating the vegetation (Mao, Shabbir, & Adkins, 2021).

Figure 1
Silver Carp

Figure 2
Grass Carp

In Pakistan, grass carp have occurred in the provinces of Punjab, Khyber Pakhtunkhwa, and Sindh. Fingerlings of grass carp, bighead carp, and silver carp, among other native carp were stocked by the provincial fisheries authorities in Baluchistan province in natural freshwater bodies during July-August 2005 to the tune of around 1.0 million. Grass carp specimens were first caught within the province of Sindh back in 1975 in a Lake called Halejee by the province (Personal communication Khan, M.A., Assistant Warden Fisheries Sindh). In Pakistan, four provinces in the preceding decade, a few thousand fingerlings of grass carp were released into rivers and freshwater lakes to increase fish production as a means of income generation, as the water bodies are sold. It has also been released into the wild in most nations around the world, including the United States and Canada, where it has escaped aquaculture farms (Chaube, 2023). Ctenopharyngodon idella is cultivated across the globe in two different ways, including the production of ironic protein in developing states that have well-planned food sources as well as aquatic weeds, which are also biocontrol (El-Gamal *et al*.,2019). With the overpopulation, there is a growing demand for various foods that are nutritious, including fats, proteins, minerals, vitamins, and carbohydrates (Lam *et al*., 2020). In the aquaculture and fisheries industries, food shortage and poverty have been combated by some form of invention in technologies (breeding systems, vaccines, and Feeds) and mechanization (Yue & Shen, 2022). Fish has a lot of food potential in relieving malnutrition and hence can be expected in countries such as the Republic of Pakistan. It provides a balanced essential amino acid profile, fatty acids, minerals required, and better-quality protein than meat, milk, and eggs (Hossain, 1996).

The composition of some of the possible Bangladesh fish feed ingredients: proximate composition, amino acids. Bangladesh journal of zoology, 24,163-168 (Islam, Mondal, Bhowmik, Islam, & Begum, 2017). In addition to that, fish meat is very digestible and delicious. On top of that, it decreases the risk of cardiovascular ailments and increases life expectancy. A survey conducted by the FDA (Food and Drug Administration) Agency in the year 2000 showed that fish as animal protein (10%) intake in Europe and North America was 26 percent in Africa, 22 percent in China, and 17 percent in Asia.

Fish is one of the major sources of animal Proteins, Vitamins (A and D), and fats, which are relied on by one million people. Fish comprises vitamin B, which does not occur in plants. To cover the shortage of food, some innovations are implemented on the breeding approaches, including induced breeding. The common name by which induced breeding has become locally recognized is the popular form of breeding many domestic fish qualitatively and quantitatively due to their reproductive processes that have been increased in number because of induced breeding. Induced breeding serves the purpose of stimulating fish reproduction through influence on hormonal or environmental conditions. In induced breeding, the exogenous hormones are injected into the body of mature parent fish to invoke breeding. Pituitary extract has been in use in the past 20 years in induced breeding. The whole process is cumbersome and forced the obligation expert to test alternative hormones, including luteinizing hormone-releasing hormone (LHRH), ovaprim-C, and human chorionic gonadotropin (HCG), as well as the consistently increasing rate of donor pituitary. In an aim to effectively bring on ovulation in a selection of the fish, there is a wider variety of artificial regimens on the market today that include GnRH (Nargesi, Falahatkar, & Żarski, 2022).

The research fundamentally seeks to find out the rate of survival of the target species between the initial period of the fertilized ova and fingerling, computation of relative fecundity and preliminary survival, and subsequent survival at the nursery phases. This investigation will sketch the stage-by-stage survival-mortality quotient and assist it in forecasting the potential factors behind the survival habits and will guide us in making our future policy on the induced breeding activities with much higher hatchery management and amplified production. The research can also contribute to the subsequent advancement in the subject matter, which can include initial stages of feed and water as well as pond management, monitoring of biochemical parameters, and to an ideal stage of production. The sharper the handling of the initial phases is, the more scientific allies we would have, to create better production units in the public sector and spread the same in the hatcheries of the private which would widen the spectrum of applications and contributions to the cause very much more.

Literature Review

The experiment conducted by Khan and Ali sought to monitor the impact of ovaprim and pituitary gland extract (PGE) on increasing fertilization and spawning of the grass carp and roho to examine the rate of heightening. It has derived the weight of the brooder as 2-4kg. It is suggested that a dose of 4mg through 1cc of distilled water is appropriate in 1kg of female fish PGE (pituitary gland extract). The female fish were treated to receive two doses. The initial dose was 2mg/kg and was injected into the fish. After 5-6 hours after the initial dose, the second dose was administered to female fish, which was 2mg/kg. The second injection was carried out on the male gender when the females were receiving the second dose, 2mg/kg PGE (pituitary gland extract). A single dose of 0.25 mg/kg was applied to the male fish, along with the second dose of ovaprime, which is 0.25 ml/kg weight to the female fish. The reaction towards the extract of pituitary gland (PGE) was observed between a 53.36 fertilization ratio in grass carp and a fertilization ratio of 75 percent in rohu. The ovaprim fertilization rate is identical among all fish and exists at 86.3 per cent. Due to the demonstrated results, ovaprim is superior to pituitary gland extract (PGE) (Khan & Ali, 2021).

Mohammadi Avizi *et al*. Presented the results of an experimental study of the influence of a single intramuscular administration of ovaprim-c on the reproductive capacity and hatching rates of silver carp. They study the induction of silver carp spawning. Just one dose of ovaprim is administered, i-e 0.2ml/kg and 0.6ml/kg to males and females, respectively. Hatching occurred 18 to 32 hrs after fertilization. The number of obtained eggs was 91778kg-1, the percentage hatching was 72.56, and that of fertilization was 71.09 (Mohammadi Avizi, Arshadi, & Rahdari, 2023).

Chen *et al*. (2021) explored, when using mammalian LHRHa (luteinizing hormone releasing hormone analogue), why induced fecundity and hatch rate elev weird using dopamine antagonists in increasing the ovulation process, and better than some other spawning process induction. They noted that besides human chorionic gonadotropin and carp pituitary extract, it was also more effective in silver carp inducing spawning, and the mammalian LHRHa (luteinizing hormone releasing hormone analogue). Hatchling and percentage fertilization of silver carp were 83.5 percent-87.33 percent and 86.5 percent-92.5 percent, respectively.

Shaddoud, Saad, & Badran (2023) plotted the quantity of eggs produced against the kilogram of the body weight of grass carp and silver carp, which is reported to be 70000 -80000 and 1-1.10 lakh. There were percentages of 78.12 and 80.03 recorded under silver carp and grass carp, respectively. Hatch percentages of silver carp and grass carp were observed to be 69.71 and 70.10, respectively.

Khanal (2023) they occurred to induce ovaprim ovulation of the common carp (*Cyprinus carpio*) and silver carp (*Hypopthalmichthys molitrix*). It was found that the percentage fertilization of silver carp was found to be 72.6-93.3 percent, and common carp was 75 to 92.5 percent, to have increased by administration ovaprim. The proportion of the hatching silver carp was the same, 44-59 percent, and that of the common carp was the same amount, 44-58 percent, respectively. The resultant embryo continued developing up to the hatching of the same at 48 48hrs following this fertilization, accompanied by cleavage at 4 hours after the fertilization. In the 45% protein fry, the artificially prepared feed was administered at the weekly level that was based on 5-10 % body weight and growth.

Mamun *et al*. (2025) injected goldfish brooders with ovaprim hormone in doses of 0.5,0.7, 1.0, and 1.2ml /kg body weight and held at a temperature of 18-22 º C. Spawning was possible after the second injection dose, and egg fertilization rates were 50.34, 51.47, 47.30, 48.24 percent. The rate of hatchlings was observed to be 36, 39.83, and 43.79 percent. They show that 76hrs were required to incubate eggs at 15 to 20 º C, and full hatching occurred almost 90 hours later, and after this, hatchling was recorded at 1.3 to 1.7 mm.

Urooj, Qayyum, Hadyait, Razzaq, & Anjum (2018) examined intramuscular injection ovaprim-C on the number of eggs/kg, fertilization rate, hatching percentage of silver carp (*Hypophthalmichthys molitrix*) and grass carp (Ctenopharyngodon idella). Single injections of Ovaprim-C at 0.1 ml/kg of the males and 0.5 ml/kg of females also spawned fish successfully. The outcome showed successful spawning of both fish when a constant dosage of Ovaprim was used. They stated that Ovaprim demonstrated the advantage of another commercial pituitary.

Materials and Methods

This research was carried out at the Carp Hatchery Training Centre, Peshawar. The station is in a village named Sherabad, close to Mathra in Warsak Road in Peshawar city, 15 km. It consists of 65 acres of land comprising two compartments of 38 and 27 acres, respectively. The plant was built during the second phase of the second aquaculture development project funded by the Asian Development Bank in November 1999. As the biggest in the province and based in the provincial capital, it remains the heart of warm water fish farming and provides a significant contribution to the supplemental fish farmers and the populace in the province. It also facilitates the aquaculture sector in the district and beyond. The Directorate of Fisheries, Khyber Pakhtunkhwa, Peshawar, is under the control of the facility.

Figure 3
Aerial view of Carp Hatchery & Training Centre, Peshawar

Induced Breeding
Induced breeding can be defined as a process of induction of breeding whereby mature parent fish are injected with exogenous breeding hormones incorporated into the body (Roberts, 2019). Breeding is induced weekly, and in the case of Chinese carps, the process lasted six weeks consecutively. The sampling of healthy brooders every Monday on the brooders' pond was performed with a drag net immediately after the proper inspection of morphological maturity. Patient and well-grown brooders were left in the hatching room in holding tanks to condition in proper oxygenation until the evening before Hypophysation (Malik, Abbas, Jabbar, Sajjad Shah, & Ali Muhammad, 2018).

Figure 4
Netting for selection of Brooders

Figure 5
Netting for selection of Brooders

Figure 6
Ovaprim Injection (Hypophysation)

Figure 7
Selection of Brooders by the hatchery staff.

They underwent measures of their weight after suitable conditioning of about 5-6hrs, before the administration of hormones, to seek the right amount of Ovaprim. Ovaprim (Synthetic form of GnRH) was administered as a dosage of 0.5ml/1.5kg injected at the base of the dorsal or pectoral fin to female and 0.3ml/1.5kg to male brooders, respectively (Susatyo, Setyaningrum, Winarni, & Chasanah, 2018).

Figure 8
Ovaprim injection in the base of pectoral fin

Table 1
Weekly Ovaprim Dosage for Grass Carp

No	Week 1						Week 2					
	FEMALE				MALE		FEMALE				MALE	
	Wt. Kg	Dose (ml) High	Standard	Low	Wt. Kg	Dose ml	Wt. (Kg)	Dose ml High	Standard	Low	Wt. Kg	Dose ml
	2	1.5	1	0.7	2	0.6	2	1.5	1	0.7	2.2	0.4
2	2	1.5	1	0.7	1	0.3	2	1.5	1	0.7	1.7	0.3
3	1.5	1	0.7	0.5	2	0.6	2	1.5	1	0.7	1.1	0.2
4	1.5	1	0.7	0.5	1.5	0.45	1	0.7	0.5	0.3	2.2	0.4
5	1.5	1	0.7	0.5	1	0.3	2	1.5	1	0.7	2.2	0.4
6	2	1.5	1	0.7			2	1.5	1	0.7	2	0.4
7							2	1.5	1	0.7	2	0.4

8							2.5	1.7	1.2	0.9	2.2	0.4
9							2.5	1.7	1.2	0.9	1.7	0.3
10							2	1.5	1	0.7		

Table 2

Weekly Ovaprim Dosage for Grass Carp

S. No	Week 3						Week 4					
	Female				Male		Female				Male	
	Wt. (Kg)	Dose (ml)			Wt. (Kg)	Dose (ml)	Wt. (Kg)	Dose (ml)			Wt. (Kg)	Dose (ml)
		High	Standard	Low				High	Standard	Low		
1	2	1.5	1	0.7	2	0.4	2	1.5	1	0.7	1.5	0.3
2	2.5	1.7	1.2	0.9	2	0.4	2	1.5	1	0.7	1	0.2
3	1.5	1	0.7	0.5	2	0.4	2.5	1.7	1.2	0.9	1.5	0.3
4	2	1.5	1	0.7	1	0.2	1.5	1	0.7	0.5	1.5	0.3
5	1.5	1	0.7	0.5			2	1.5	1	0.7	1.5	0.3
6	1.5	1	0.7	0.5			1.5	1	0.7	0.5	1.7	0.34
7							1.5	1	0.7	0.5	1.4	0.28
8											2	0.4
9											1.5	0.3
10											1.5	0.3
11											2	0.4
12											2	0.4

Induced Spawning

Spawning is when the body of the brooders pumps their milt or eggs through the water (Amezawa, Yazawa, Takeuchi, & Yoshizaki, 2018). The brooders were placed in the Chinese circular tanks after Ovaprim injection, maintaining the approximately 1:2 (Female: Male) ratio and moderate flow of water to ensure an optimum flow of water during spawning. They were maintained in a continuous state around the clock, both overnight and without disruption, until spawning occurred and monitored in terms of spawning and subsequent fertilization approximately 12 hours later. The presence of fertilized eggs was checked in tanks the following morning, and fecundity in every weekly breeding test has been calculated (Begum *et al*., 2022).

Figure 9

Brooders are released in circular tanks for spawning

Figure 10

Fertilized eggs after 12 hours of Brooders Hypophyzation

Total Fertilization and Early Survival (Hatching Rate)

The volume of the Chinese circular tank was also computed and was discovered to hold 3325 liters. Random sampling was used by counting fertilized eggs randomly using the assistance of a 500ml beaker in each corner of the tank. The number of eggs was counted manually in the samples, and the total fertilization was counted as (Arts *et al.,* 2016).

Total Fertilized Eggs = Average No. of eggs per Liter × Total volume of the tank in liters.
The fertilized eggs were then checked for the hatchlings within two days following fertilization. Fertilized eggs, as well as the hatchlings, were seen and counted the same way in the third period after fertilization. There was a total number of post-hatch larvae (Arts *et al.,* 2016).

Total Hatchlings = Average No. of hatchlings per Liter × Total volume of the tank in liters.

Hatching Rate (%) = Total Hatchlings / Total fertilized eggs × 100
The fertilized eggs were compared with the numbers regarding the early survival ratio. On the fourth day of hatching, the post-hatch larvae were moving towards the nursery pond. All the breeding tanks were used to spawn post-hatch larvae into the same nursery pond, in which the final estimation of survival of the fry would occur.

Figure 11

Eggs Sampling with a 500ml Beaker

Survival in Nursery Pond

Table 3
Two Months the Nursery Pond Sample

For two months the nursery pond was sampled, and the fry counted, the later survival percentage on that basis, until the pond was exhausted.

No of Female Brooders	Relative Fecundity (Week 1)			Relative Fecundity (Week 2)		
	High Dose	Standard Dose	Low Dose	High Dose	Standard Dose	Low Dose
1	104312	103866	83236	69431	67164	62306
2	102946	102552	81652	70412	67632	62112
3	79743	77898	63415	68934	65986	61342
4	79201	77204	65634	69321	67314	60918
5	78316	76412	66641	67954	65918	58402
6	103412	102892	86746	67802	66498	57912
7				68216	66934	56214
8				85942	83955	72316
9				84514	82646	70863
Total Fecundity	**547930**	**540824**	**447324**	**652526**	**634047**	**562385**

No of Female Brooders	Relative Fecundity (Week 3)			Relative Fecundity (Week 4)		
	High Dose	Standard Dose	Low Dose	High Dose	Standard Dose	Low Dose
1	114369	111840	86416	81326	78776	60316
2	69342	67104	42312	82016	78126	61121
3	93616	89472	64946	99631	98470	79436
4	72346	67232	43269	62412	59082	42163
5	68142	65314	41964	83164	79067	60346
6	89692	88632	66426	62212	58641	41924
7				63124	57964	40326
Total Fecundity	**507507**	**489594**	**345333**	**533885**	**510126**	**385632**

Statistical Analysis

Statistically, the results were analyzed by applying One-way ANOVA in SPSS every week the process was done. This was done by establishing the confidence interval of 95 percent or the value of P = 0.05 and the findings are presented below.

Results and Findings

By randomly sampling and counting manually the number of fertilized eggs in the circular tank of each dosage group with the help of a glass slide after approximately 12 hours (overnight) Ovaprim was injected, the relative fecundity of each dosage group was calculated by computing the total fertilization (the total number of eggs as stated in Methodology). The resulting total counts (relative fecundity) are tabulated in Tables 4.1 and 4.2.

Table 4

Relative fecundity of Grass Carp for the third and fourth week

ANOVA for Week 1

No of Female Brooders	Relative Fecundity (Week 1)			Relative Fecundity (Week 2)		
	High Dose	Standard Dose	Low Dose	High Dose	Standard Dose	Low Dose
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4	72346	67232	43269	62412	59082	42163
5	68142	65314	41964	83164	79067	60346
6	89692	88632	66426	62212	58641	41924
7				63124	57964	40326
Total Fecundity	**507507**	**489594**	**345333**	**533885**	**510126**	**385632**

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2744835137.333	2	1372417568.667	8.397	.004
Within Groups	2451575300.667	15	163438353.378		
Total	5196410438.000	17			

The overall distinction between the dosage groups is P = 0.004 which is rather considerable.

The detailed comparison is demonstrated in the following table where it is shown that a significant difference ($P< 0.05$) exists between standard and low dose and non-significant ($P > 0.05$) difference between high and standard dose i.e. P = 0.986.

Table 5
Relative Fecundity with Ovaprim Dosage

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
High Dose	Standard Dose	1184.333	7381.020	.986	-17987.65	20356.31
	Low Dose	26767.667*	7381.020	**.007**	7595.69	45939.65
Standard Dose	High Dose	-1184.333	7381.020	.986	-20356.31	17987.65
	Low Dose	25583.333*	7381.020	**.009**	6411.35	44755.31
Low Dose	High Dose	-26767.667*	7381.020	.007	-45939.65	-7595.69
	Standard Dose	-25583.333*	7381.020	**.009**	-44755.31	-6411.35

**. The mean difference is significant at the 0.05 level.*

Table 6
ANOVA for Week 2
The overall difference among the dosage groups is **P = 0.000,** which is quite significant.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2746836354.296	2	1373418177.148	36.245	.000
Within Groups	909414599.778	24	37892274.991		
Total	3656250954.074	26			

Table 6
Multiple Comparisons within groups

The comparisons are described by the table below and clearly, the resultant difference between standard and low dose is significant (P< 0.05) whereas, the difference between high and standard dose is insignificant (P > 0.05) i.e. P = 0.761.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
High Dose	Standard Dose	2053.222	2901.811	.761	-5193.43	9299.88
	Low Dose	22349.000*	2901.811	**.000**	15102.35	29595.65
Standard Dose	High Dose	-2053.222	2901.811	.761	-9299.88	5193.43
	Low Dose	20295.778*	2901.811	**.000**	13049.12	27542.43
Low Dose	High Dose	-22349.000*	2901.811	.000	-29595.65	-15102.35
	Standard Dose	-20295.778*	2901.811	**.000**	-27542.43	-13049.12

**. The mean difference is significant at the 0.05 level.*

Table 7
ANOVA for Week 3

The overall difference among the dosage groups is P = 0.016 which is quite significant.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3023115998.111	2	1511557999.056	5.480	.016
Within Groups	4137671022.333	15	275844734.822		
Total	7160787020.444	17			

Table 8
Multiple Comparisons within groups

The comparative analysis in details is given below in the following table indicating that there is a significant difference between standard dose and low dose and insignificant difference between high dose and standard dose i.e. P = 948.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
High Dose	Standard Dose	2985.500	9588.965	.948	-21921.55	27892.55
	Low Dose	28862.333*	9588.965	**.023**	3955.28	53769.38
Standard Dose	High Dose	-2985.500	9588.965	.948	-27892.55	21921.55
	Low Dose	25876.833*	9588.965	**.041**	969.78	50783.88
Low Dose	High Dose	-28862.333*	9588.965	.023	-53769.38	-3955.28
	Standard Dose	-25876.833*	9588.965	**.041**	-50783.88	-969.78

**. The mean difference is significant at the 0.05 level.*

Table 9
ANOVA for Week 4

The overall difference among the dosage groups is **P = 0.003** which is quite significant.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2903022672.667	2	1451511336.333	7.964	.003
Within Groups	3280730850.000	18	182262825.000		
Total	6183753522.667	20			

Table 9
Multiple Comparisons within groups

The elaborate contrast is described in the table below which reveals that there is a substantial difference (P< 0.05) between standard dose and low dose, whereas there is no such difference (P > 0.05) or rather P = 886 between high dose and standard dose i.e. standard dose and high dose.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
High Dose	Standard Dose	3394.143	7216.307	.886	-15023.05	21811.34
	Low Dose	26464.714*	7216.307	**.005**	8047.52	44881.91
Standard Dose	High Dose	-3394.143	7216.307	.886	-21811.34	15023.05
	Low Dose	23070.571*	7216.307	**.013**	4653.38	41487.77
Low Dose	High Dose	-26464.714*	7216.307	.005	-44881.91	-8047.52
	Standard Dose	-23070.571*	7216.307	**.013**	-41487.77	-4653.38

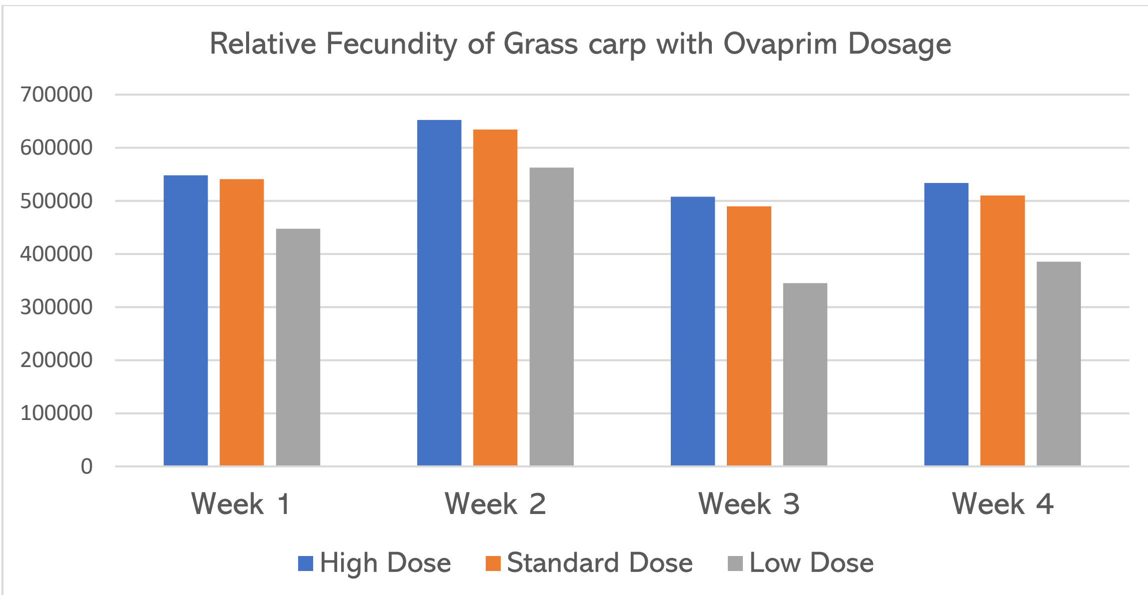
**. The mean difference is significant at the 0.05 level.*

Cumulative Fecundity Trend

Statistically, when all the four independent variables are considered cumulatively, it indicates that the difference between the number of males parenting, compared to standard and low dose of Ovaprim is significant and very large but there was no significant difference between standard and high dose. This indicates the comparative success of standard dose with minimal dose range of standard increment that is not representative on the cost effectiveness of the Ovaprim hormone as shown in the figure below.

Figure 12

The Cumulative Comparison of Relative Fecundity among weekly groups



Early Survival Trends (Hatchability)

The fertilized eggs were further tracked by counting the proportion that hatched after 48 hours, to find out early survival to hatchlings and subsequently a monthly tracking to find out late survival to fry stage in the nursery pond. The findings obtained have been presented in table 10 and graphically depicted in Figure 12

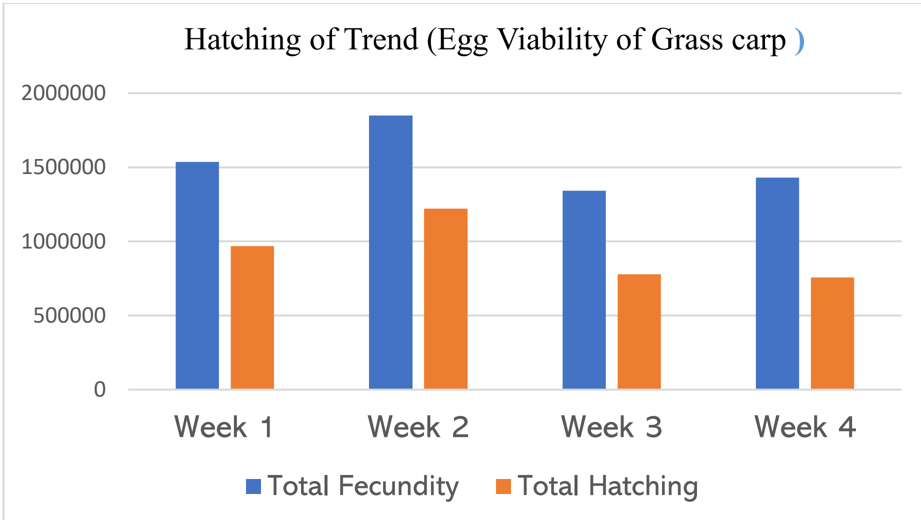
Table 10

Weekly Hatching Rates and Early Survival ratio of Grass Carp

Weeks	Total Fecundity	Total Hatching	Hatching Rate	Total Fry	Survival Rate
1	1536078	967729	63 %		
2	1848958	1220312	66 %	633141	17%
3	1342434	778611	58 %		
4	1429643	757710	53 %		

Figure 13

Weekly Hatching trend among groups



Induced Breeding of Silver Carp

These induced breeding experiments of silver carp were two weeks long as well with the same dosage of ovaprim as before. This led to the eggs being hatched although not all the eggs would live the duration of both the weeks. This can perhaps be attributed to the temperature shock as a result of varying water temperature of ponds and hatcheries since this species is so sensitive that other hatcheries had reported the same. It is therefore advisable that silver carp ought to be placed under the season breeding trial to search the possible temperature window to intersect its optimum temperature range that is usually missed when it is done alongside any other species.

Discussion and Conclusion

Similar study conducted in the article has also determined that Ovaprim had a comparable work in relation to induced spawning of grass carp with 79.49 hatching rate compared to this paper 60%. The divergence is hampered with the insignificant variation in the environmental situation (Naeem *et al.,* 2011). Likewise, fertilization and vibration that brought about induced spawning in grass carp through the use of Carp Pituitary Extract was also exercised and the average fertilization rate attained of 79.3 % which is around the range of the present study and therefore validates the overall conclusion of induced spawning and hormonal treatment (Bozkurt & Ogretmen, 2012). An instant experiment involved categorical spawning of carps by combining Human chorionic Gonadotropin and Carp pituitary extract in a mixture and hatching was 70 as compared to the instant study i.e. 60 and this can be considered as a difference (Akar *et al.,* 2010). They had carried out other studies on the relative efficacy of Ovaprim and Ovatide on grass carp in the same premise where the current one is being conducted at back and had an observation of fertilization rate of 56.5 and 48 percent in Ovatide and Ovaprim respectively back in 2006. The distinction between the two is minimal and could not be compared as it can only be expressive to a level of justifying our results (Ali *et al.,* 2015). The records of large-scale breeding successes of the Chinese carps that have been recorded in Hungary over the years were also sampled and arrived at the same conclusion as those in the current study. The variation is not very large to be used to describe it but it can be explained by the use of the non-synthetic carp Pituitary Extract in such research (Szabo *et al.,* 2019).

The study report explained a high extent of trust regarding the regular dosage of Ovaprim that is administered in the hatcheries. The relative fecundity and total fertility also have a fair chance in early stages as indicated by the outcome of the hatching ratio. The work shows however that the actual challenge becomes rather alarming in the present induced breeding processes because it is easily defeated and only 17 percent of the total hatched emerges as fry. Hence, the question of knowing how to address scientific and managerial methods to confront the phenomena of such a stunted ratio of mortality to fry is proposed to be very well-conceived in the form of a study.

Recommendations

Research on early development and hatching within outdoor nurseries is highly required regarding nursery preparation and fertilization along with management procedures like disinfection and use of chemicals. Much attention is devoted to research on early planktonic nutrition of hatchlings production and observation of early months' growth.

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Informed consent was not required, as secondary data available on websites was obtained for analysis. All procedures performed in this study were by the ethical standards of the Helsinki Declaration.

Consent for Publication: The authors give their consent for publication.

Availability of Data and Materials: Data will be made available upon request from the corresponding author.

Competing Interest: The authors declare that they have no competing interests.

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