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## **The Comparative effect of synthetic hormone Ovaprim and Pituitary gland extract (PGE) on the Induced Spawning of Silver carp (*Hypophthalmichthys molitrix*) Khyber Pakhtunkhwa, Pakistan**

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### **ABSTRACT**

The present study was conducted to investigate the comparative effect of Ovaprim and Pituitary gland Extract (PGE) on the induced spawning of Silver carp (*H. molitrix*) from May to July 2024 at Carp Fish Hatchery University of Peshawar, Khyber Pakhtunkhwa Pakistan. Two pairs of healthy, active and sexually matured brooders were selected and calculated their weight between 750 kg to 2100g. The standard dose for brooders, 2mg/kg Pituitary gland extract (PGE) and 0.25 ml Ovaprim were injected once to male and twice to female after 4-6 hours of the administration of the first dose. The Pituitary gland extract (PGE) response was recorded with an average fertilization rate was 62.09 % and the average fertilization rate of Ovaprim was 75.64%. The present study disclose that the synthetic hormone Ovaprim is more effective and efficient as compared to Pituitary gland extract (PGE).

**Key words:** Fish Hatchery, Silver Carp, Standard Dose, Brooders, Fertilization

### **Introduction**

Fisheries and aquaculture play a vital role in food industry and have a fundamental role in nutrition and food security to provide valuable proteins, fats and vitamins to worldwide (FAO, 2000, 2020). Fish is rich source of protein and provide 20% protein to one third population of world (Bene et al., 2007). Fish supplied 24% protein to China, 10% to Europe and North America, 25% to Africa and 20% to Asia. Globally 95% developing countries utilize fats Vitamins, and minerals from fish (Wheeler & Von Braun, 2013). Fish provide good Fates (omega-3-fatty acids), vitamins (A and D) and minerals (zinc, selenium and iron) necessary to cure diseases and improve our health condition (Zuraini et al., 2006). Fisheries is a handy sector, 40 million people directly or in directly depends on fisheries as source of income and employment (Mohanty et al., 2019; Sinha & Khan, 2001). A fisheries not important for food but it boost the economy of the world. Globally fish trade worth is more than 50 billion US\$ per year (Tlusty et al., 2013; Khan, 2025).

Hypophysation is a method that used in the induced spawning of fish to artificially propagate from pituitary gland extract. Pituitary gland is master endocrine gland that stimulate the gametogenesis and promote the function of ovary and testis of fish (Rashid et al., 2020). Hypophysation increase the spawning rate and improve the spawn time and quantity as compared to natural method (Khan & Ali 2021; Shanthanagouda & Khairnar,



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2018). The Induced spawning of fish by Hypophysation is time consuming process that may be extend over 20 hours and required large number of workers to alert to inject hormones into fish body (Heggberget et al., 1996)

Ovaprim is an artificial Spermiating and ovulating hormones use in induced breeding of fish in fish farms, hatcheries and other aquaculture facilities (Hill et al., 2009). Ovaprim is peptide liquid that contain neurotransmitter Dopamine inhibitor and Salmon gonadotropin releasing hormones (GnRH $\alpha$ ) that stimulate to release Gonadotropin hormones from pituitary gland (Naeem et al., 2005). Ovaprim is injected intra muscular or intra peritoneal rout that contain dopamine inhibitor to cease the dopamine activities to induced ovulation (release mature eggs/ oocytes) and spemiation (release mature sperm) (Zohar & Mylonas, 2001). Hypophysation were used from last of two decades but increasing fish demand to obliged the ichthyologist to use synthetic hormones i.e., Ovaprim and HCG( Human chronic gonadotropin) (Ameer et al., 2021; Jamróz et al., 2021; Kather Haniffa & Sridhar, (2002).

Silver carp (*H. molitrix*) is common fresh water species that native to Siberia and China. In natural habitat it is declare that sliver carp is threatened species as its reproduction and habitat is impacted by pollution, over fishing and dam construction (Minder & Pyron, 2018; Irons et al., 2007).The species has been introduced to 88 countries including Pakistan to control weeds, improve water quality and enhanced wild fisheries. The silver carp (*H. molitrix*) is filter feeder and consumed phytoplankton zooplankton detritus and blue green algae. Sometime used to control toxic blue green algae to improve water quality (Kather Haniffa & Sridhar, 2002; Minder & Pyron, 2018). This study was documented investigate the better response pituitary gland extract (PGE) and Ovaprim on the induced breeding of silver carp (*H. molitrix*). The main aim of this study was to document and develop a better understanding of reproduction of silver carp (*H. molitrix*) under the influence of applied hormones.

## Materials and Methods

### Study Area

To study the comparative effect of Ovaprim and pituitary gland extract (PGE) on silver carp (*H. molitrix*), the induced breeding experiments were performed in Carp Fish Hatchery at University of Peshawar, Khyber Pakhtunkhwa, Pakistan from the month of May to July 2024.

### Extraction of Pituitary gland.

For the extraction of pituitary gland, healthy, active and sexually matured donor fish of same species were selected. The selected fishes (male and female) were anaesthetized using phenyl ethanol and decapitated. The cranium were removed with help of bone cutter from posterior to interior to expose their brain. The brain were remove by using forceps and the debris and fatty tissue were washed and finally to expose pituitary gland.

### Storage of pituitary gland extract (PGE) and dose preparation

The fresh pituitary gland extract (PGE) were dried in air inside desiccator having CaCl $_2$ . After that the pituitary gland extract (PGE) were stored in absolute acetone and kept in dark room to protect from sun light and other damages. The pituitary gland extract (PGE) were stored for long term (5 -10 year) in air tight veils containing fresh aliquot instead of acetone. When the pituitary gland extract (PGE) were completely dried inside acetone it was grind and crushed using disinfected mortar and pistol. After crushing and grinding the pituitary gland the distal water were mixed to prepare injections (4mg PGE+1cc



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water).

### **Dose of Ovaprim**

Ovaprim is peptide solution contain Dopamine inhibitor and OvaRH that simulate the endocrine system of fish during spawning cycle. Original 10 ml vial of Ovaprim were ordered from Syndel laboratory USA (WWW.Syndel. Com) (Sales@syndel.com) and stored away from heat and light in 25°C.

### **Brooder selection**

Two pairs of healthy, active and sexually mature brooders of silver carp (*H. molitrix*) (2 male and 2 female) were collected from the hatchery pond. The weight (w) and total length (TL) of brooder were calculated (male1, W=750, male2, W=870g, Female1, W=1100g, Female2, W= 2100g) and released in separate race ways. After the injection of hormones the brooder were shifted to separate breeding tanks.

### **Standard Dose formula for Ovaprim and pituitary gland Extract (PGE)**

The required dose of Ovaprim for 1kg male fish is 0.25ml at once while for the female this dose were injected twice time. The required dose of pituitary extract (PGE) for male fish is 2mg/kg while female required this dose twice time of the female (4mg/kg). For dose preparation, normally 4 mg Pituitary extract (PGE) were mixed with 1cc distal water.

### **Dose injection**

The standard dose were administrated into two rotes i.e. coelomic and inter muscular. The coelomic injection were injected below the pectoral fin. While intra muscular dose were injected between the lateral and dorsal line at 40-50°.

### **Results**

Two pairs of sexually matured and healthy brooders of Silver carp (*H. molitrix*) were captured from hatchery pond in University of Peshawar, Khyber Pakhtunkhwa Pakistan at 25 June where the temperature was 28°C and humidity was 54% at 2.30PM. To ensure the sexual maturity both male and female fish belly were checked by stripping to remove milt and eggs. Female fish were observed slightly large in size and their belly were swollen as compared to male fish. Male and female fish were weighted and measured total to inject standard dose (M1, W=750, Male 2, W=870g, Female1, W=1100g, Female 2, W= 2100g) using digital balance and digital caliper. The brooder were released in separate race ways to recover their strass during capturing. Pituitary gland extract (PGE) were grinded in disinfected crucible by using NaCl solution and add 1cc distal water to 4mg glands. First dose of Pituitary gland extract (PGE) and Ovaprim were injected to female fish at 3 PM. The second dose to female fish were injected 8pm at that time first dose were injected to male. The recommended dose were injected according to their weight (Table1, 2). The selected brooders were shifted to separate Spawning tanks. At next day all brooders were found to spawn and two types of eggs were observed i.e. fertile and unfertile eggs. The number of fertile and unfertile eggs were calculated (Table 3, 4). The fertile eggs were small in size with visible nucleus while unfertile were large in size and without nucleus. Fertile eggs were two types i.e. pre mature and mature eggs. The pre mature eggs nucleus located at center while matured egg nucleus were found a centrally. The fertilization rate of eggs were calculated using formula.



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### Fertilization rate = Total number of fertile eggs\*100/total eggs

A total of four treatment were used and the fertilization rate were calculated. The Ovaprim response were was quite high and more productive as compared to pituitary gland Extract (PGE). The average fertilization rate of eggs under the influence of Ovaprim was 75.64% while under the influence Pituitary gland extract it was 62.09% (Table 5) (Figure 1, 2, 3).

Table 1. Weight of brooders and recommended dose of Ovaprim.

Silver carp ( <i>H. molitrix</i> )	Weight (g)	Ovaprim (ml)
Male	750	0.1875
Female	2100	1.05

Table 2. Weight of brooder and recommended dose of PGE

Silver carp ( <i>H. molitrix</i> )	Weight (g)	PGE (mg)
Male	870	1.74
Male	1100	4.4

Table 3. Number of fertile and unfertile eggs after administration Ovaprim

Treatments	Total eggs	Unfertile egg	Fertile eggs
T1	16	5	11
T2	22	3	19
T3	15	5	10
T4	25	6	19
<b>Total</b>	<b>78</b>	<b>19</b>	<b>59</b>

Table 4. Number of fertile and unfertile eggs after administration of PGE

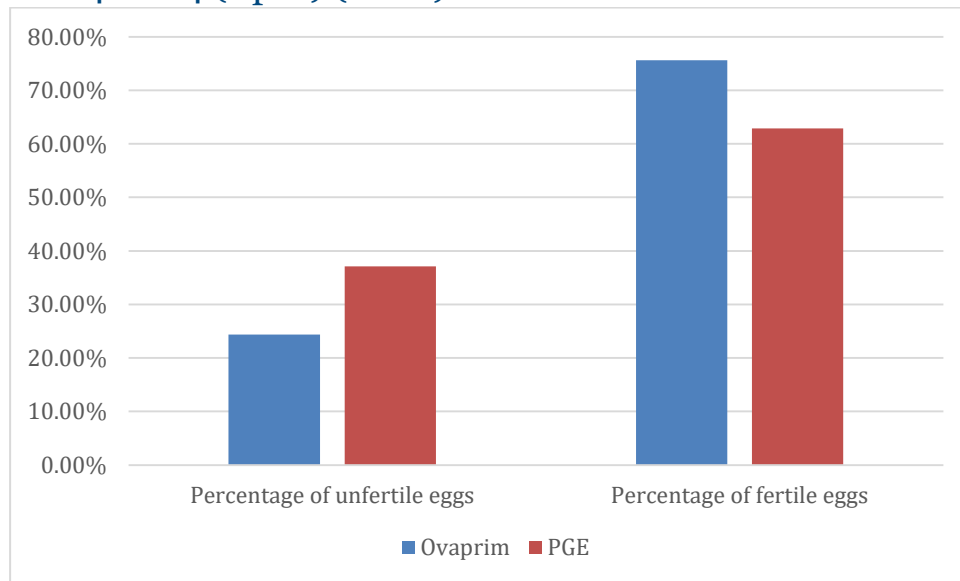
Treatments	Total eggs	Unfertile egg	Fertile eggs
T1	21	8	13
T2	14	6	8
T3	10	3	7
T4	17	6	11
<b>Total</b>	<b>62</b>	<b>23</b>	<b>39</b>

Table 5. Percentage of fertile and unfertile eggs after administration of Ovaprim and PGE

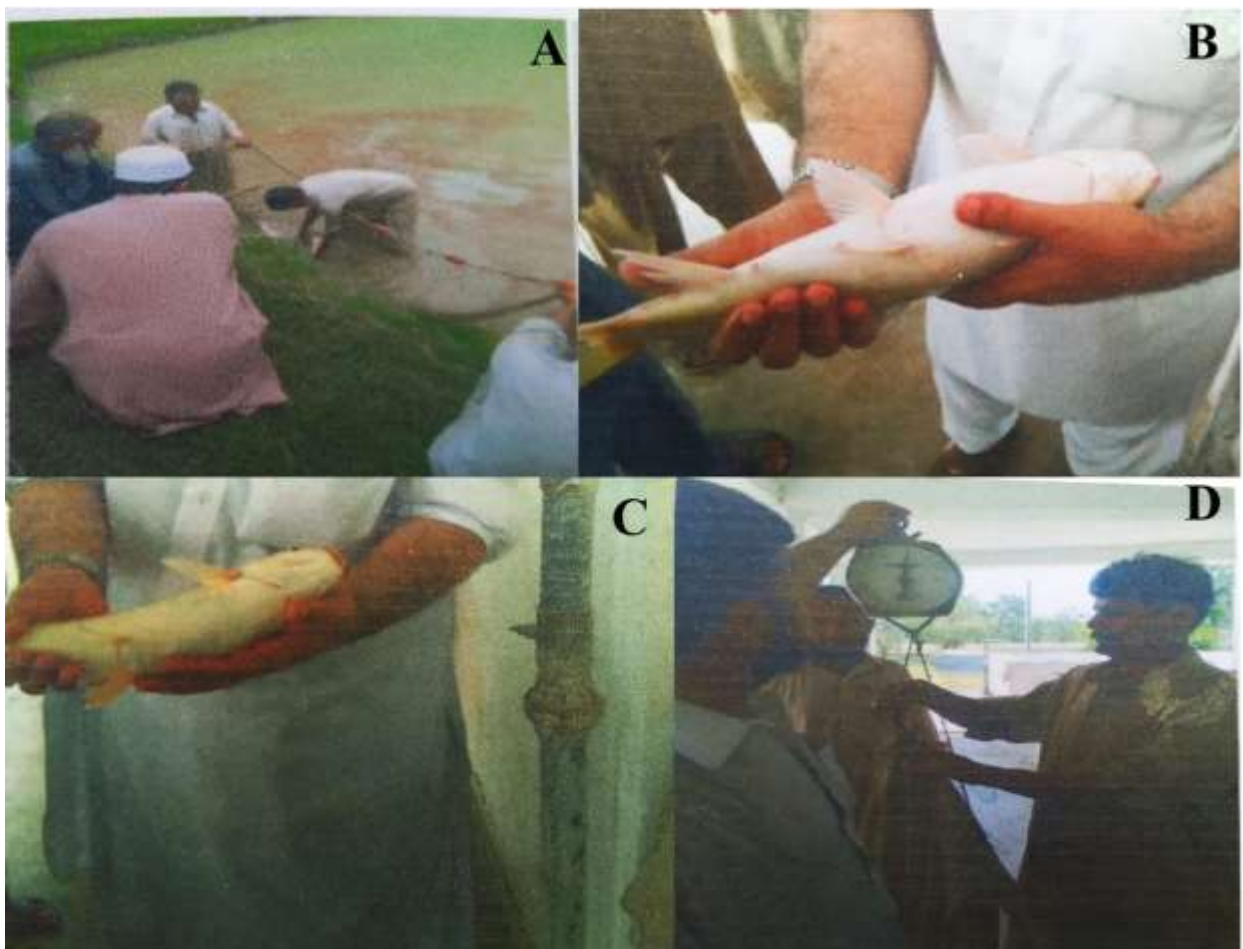
Dose	Total eggs	Percentage of unfertile eggs	Percentage of fertile eggs
<b>Ovaprim</b>	78	24.35%	75.64%
<b>PGE</b>	62	37.09%	62.90%



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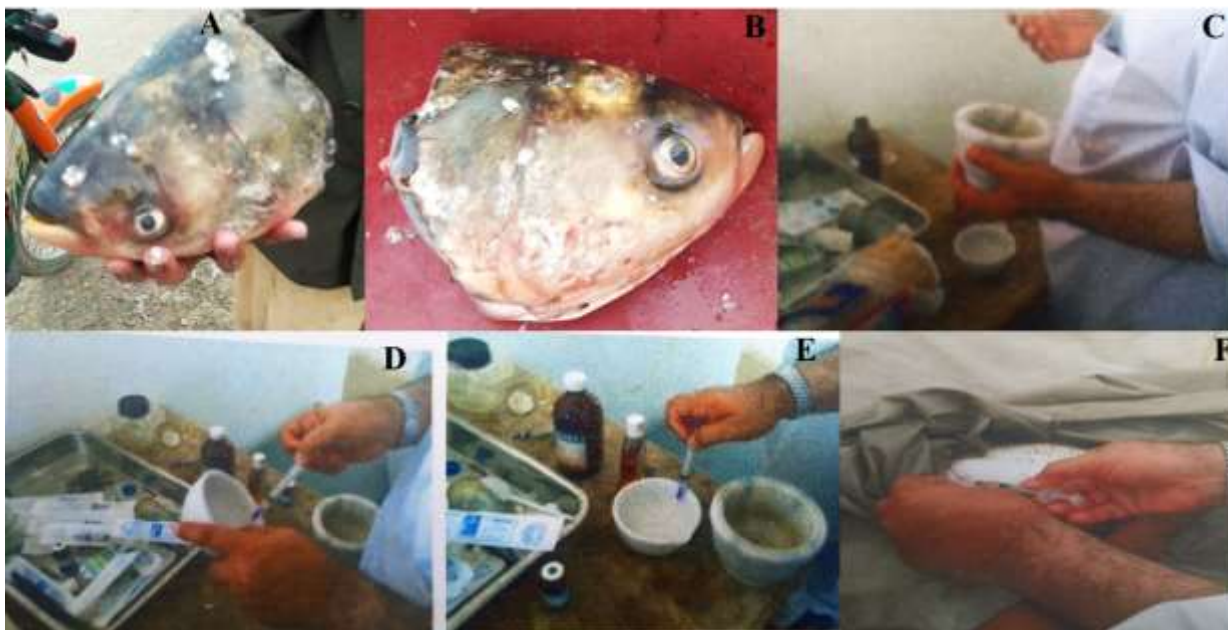
**Figure 1.** The percentage of fertile and unfertile eggs by administration of Ovaprim and PGE



**Figure 2.** A) Brooders collection B) Female brooder C) Male brooder D) weight of brooders



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**Figure 3.** A-B) Mature heads for pituitary gland extract C-E) Dose preparation F) Coelomic injection

### Discussion

The present study was conducted to investigate the breeding effect of Ovaprim and Pituitary gland extract (PGE) on induced breeding of silver carp (*H. molitrix*). Many ichthyologist used synthetic hormone i.e. HCG (Human Chorionic Gonadotropin) LH (Luteinizing hormones), Ovaprim and semi purified fish gonadotropin to stimulate spawning and increase quality and time of spawns. Khan and Ali (2021) studied the effect of Ovaprim and PGE on Grass carp (*Ctenopharyngodon idella*) and Rohu (*Labeo rohita*). The PGE response in Grass carp (*Ctenopharyngodon idella*) was recorded with an average fertilization rate of 53.36 % and in Rohu (*Labeo rohita*) a fertilization rate of 75 %, respectively. The fertilization rate of Ovaprim in all fishes remained the same as 86.3%. This study revealed that the synthetic Ovaprim response is more effective as compared to PGE. Asghar et al. (2024) compared Ovaprim with PGE and investigate that Ovaprim is more effective as compared to PGE. In the present study we applied Ovaprim and Pituitary gland extract (PGE) on silver carp their response were 75.64% and 62.90% respectively. Our finding was similar with Khan and Ali (2021), Asghar et al. (2014), Naeem and Salim, (2005a), Naeem et al. (2005) and Naeem et al. (2011) use different analogous of LH and RH without pituitary gland which result in failure of spawning and clearly indicates that the dopamine block the action of LHRH on the secretion of gonadotropin. Syndal laboratory in Canada synthesize Ovaprim containing Dopamine inhibitors which increase the releasing of Gonadotropin, induced maturation, increase milt and egg production, reduce stress and improve spawn date and time. The studies conducted for first time Khan et al. (1992) in Pakistan and Nandeesh and Rao (1989) in India. Hafeez ur rehman et al. (2015) mixed Ovaprim with HMG and HCG and injected to *C. marulius*. It was observed that the fecundity and eggs fertilization rate were highest and the spawning was more effective and efficient. Nazir et al. (2022) injected GnRH and dopamine inhibitor to *C. marlius* and the outcome showed highest GSI (Gonado-Somatic index) during spawning season. Similar result were find by Gaiwad et al, (2009) in *C. punctatus* and Narejo et al, (2018) in *C. striatus*. Our result provide useful basis that Ovaprim is more affective and showed good response as



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compared to Pituitary gland extract (PGE).

### Conclusion

In the present study we induced Silver carp (*H. molitrix*) by using synthetic hormones Ovaprim and pituitary gland extract (PGE). The study shows that Ovaprim performed better response as compared to Pituitary. It is concluded that the synthetic hormone, Ovaprim can be used successfully to induce spawning in fishes.

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