



## Effect of pituitary gland extract (PGE) and ovaprim on induced breeding in Rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*)

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### Abstract

The present study was conducted to investigate the induced spawning of Grass carp (*Ctenopharyngodon idella*) and Rohu (*Labeo rohita*) from April to June 2013 at Carp Fish Hatchery Char Banda District Mardan (Khyber Pakhtunkhwa). The weight of brooder fishes was calculated between 2 kg to 4 kg. The fish response was compared between PGE and synthetic Ovaprim. The standard dose for both PGE and synthetic Ovaprim was injected to each brooder and the ovulation period was noted. 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.

**Keywords:** pituitary gland extract (PGE), Ovaprim, (*Ctenopharyngodon idella*), fertilization and Rohu (*Labeo rohita*)

### Introduction

Fishery plays an important role in the world economy. According to the survey conducted by Food and Drug Administration Agency in 2000 fish supplied 10% of animal protein consumed in North America and Europe, 17% in Asia, 26% in Africa and 22% in China. One billion people depend on fish as a primary source of animal Proteins, Fats and Vitamins (A and D). It also consists of Vitamin B which is not present in plants (FAO, 2000) [4]. Hypophysation is a technique in which the fishes are artificially propagated by the injection of pituitary hormones or chemically synthesized hormones. Induced breeding in fish is long and laborious process that may be extend over 20 hours during which large number of worker has to alert in which exogenous hormones are injected into the body of mature parent fish for induction of breeding (Brian, 1979; Heggberget *et al.*, 1996) [6, 8].

During the past two decades, pituitary extract was used for induced breeding. The ever increasing cost of donor pituitary and unmanageable process obliged experts to test alternative hormones such as HCG (Human chorionic gonadotropin), LHRH (luteinizing hormone releasing hormone) and Ovaprim (Cife, 1999; Kather *et al.*, 2002; Jamróz *et al.*, 2008) [3, 5, 9]. Ovaprim is a product that contains salmon gonadotropin releasing hormone analogue (sGnRH; DArg6, Pro9,Net) at a concentration of 20 ug/ml and domperidone, a dopamine antagonist at 10 mg/ml (Hill *et al.*, 2009) [7]. Dopamine antagonists are used for cessation of dopamine activity which acts as an inhibitory factor for the synthesis of gonadotropin (Naeem *et al.*, 2005) [16]. Ovaprim is used to induce ovulation and spermiation in fishes mostly by intramuscular or intra peritoneal route. Reported adverse effects are also observed

which includes bruising, redness, wounds, ulceration, darkened body coloration (hyperpigmentation) and loss of body color (hypopigmentation) at the site of injection and overall 1.3% post spawning mortality occurred (Hill *et al.*, 2009) [7].

Grass Carp (*Ctenopharyngodon idella*) was imported to Pakistan from China for the first time in 1964. The purpose of its introduction in addition to culture was for biological aquatic weed control in natural ways in rivers and manmade lakes (Khan *et al.*, 2004) [12].

The carp culture improves social and economic status of farmer by adopting new scientific technology for breeding (Nandeeshg and Rao, 1989) [22].

Within Pakistan major breakthrough was achieved when Chaudhry and Ali kuanhi succeeded in breeding of Indian carp using pituitary extracts (Alikunhi *et al.*, 1962; Naeem *et al.*, 2005) [1, 16]. Carp culture is rapidly expanding but the major contents in the development of this industry are the non-availability of quality fish seed. "Ovaprim" is used for fertilization stimulation, manufactured by a Canada-based company, M/s Syndel laboratories; Ovaprim contains 201.1g of Salmon gonadotropin releasing hormone (GnRH) and 10 mg of domperidone. The high cost and viscosity of Ovaprim proved a prohibitive factor (Khan *et al.*, 2006) [13]. There is little literature cited about the fertilization and hatching rates of the Grass carp (*Ctenopharyngodon idella*) and Rohu (*Labeo rohita*), Therefore a point study was conducted to determine what type of treatment provides significant result in fish hatching at fish hatchery in Mardan.

## Materials and Methods

### Study Area

The breeding experiments were performed in Fish Hatchery at Char Banda, District Mardan (Khyber Pakhtunkhwa) during the month of April and June 2013. Two fish species were selected for this investigation that was Rohu (*Labeo rohita*) and Grass Carp (*Ctenopharyngodon idella*).

### Removal of Pituitary Gland from donor fish

Sexually mature, active and healthy fishes (male and female) were selected as a pituitary donor. Healthy donor fishes were anaesthetized by using suitable anesthetic chemical such as Phenyl ethanol. Decapitated the fish and removed the roof of cranium with the help of bone cutter from posterior to anterior direction to expose the brain.

Fatty tissues were washed and removed. Brain was removed with the help of fine tweezers or forceps to expose the pituitary gland underlying the brain.

The surrounding tissues debris was washed by using clean water before removing the pituitary gland. It is important to ensure that the gland is not damaged or perforated during collection.

### Storage of Pituitary Gland

After the extraction of PG it was stored in absolute alcohol or acetone in vials in dark room at room temperature or refrigerator and protected from sun light the alcohol was changed several time for its drying. The PG was also dried in air with the help of desiccator containing CaCl<sub>2</sub>. For long term storage the pituitary glands were placed in air tight vials containing acetone for 24 hours and then replaced the acetone with fresh aliquot (used for 6 months to 10 years).

Grass carp (*Ctenopharyngodon idella*) and Rohu (*Labeo rohita*) both are warm water and fresh water species all these fishes breed at the end of June in natural environment and sexually mature in 4 to 5 years. The sexual maturity and growth rate depend upon the temperature. The required temperature is 20°C to 25°C.

### Preparation and injection of Pituitary extract

The pituitary gland was crushed or grinded with the help of crucible. But before grinding the crucible was cleaned with liquid dettol. The pituitary gland was grinded and mixed with distilled water or NaCl solution. There are two types of PGE injection.

### Coelomic Injection

The injections were injected below the pectoral fin in scale less area. It is dangerous because the heart is situated near the pectoral fin which causes heart injury.

### Muscular Injection

This type of injection was injected between the area of the dorsal line and lateral line of scale at an angle of 40° to 50°.

### Selection of brooders

Healthy, active and sexually mature male and female fishes were selected. The male and female fishers were separated by sexual dimorphism.

## Doses

### Recommended standard dose of Pituitary Gland

4 mg of PG mixed with 1cc distilled water is recommended dose for 1 kg female fish. Doses were given to female twice. First dose of 2 mg/kg was given to female fish and the second dose 2 mg/kg was given after 5 to 6 hours of the first dose. At the time of second dose the male was given first dose of 2 mg/kg.

### Recommended standard dose of Ovaprim

Ovaprim is another synthetic hormone which is used nowadays (. It is best and more efficient product as compared to PGE. Female fishes were given 0.25 ml of Ovaprim twice while males received a single dose of 0.25 ml at the time of female second dose. The weight of female Grass carp was 1100 g and the required dose was 4.1 mg which were given twice while the weight of male Grass carp was 1kg and given dose was 2 mg. For Rohu female with 1 kg weight, the required dose was 4 mg whereas for 700 g Rohu male the required dose was 1.8 mg.

After one day observation all eggs were unfertile because of no proper treatment and the males, were already used for spawning, so the result was negative.

## Results

The results were calculated after different treatments of fish extracted pituitary glands. The fertilization rate of Grass carp (*Ctenopharyngodon idella*) and Rohu (*Labeo rohita*) are given in Table 1 and 2. These results show greater variations after changing amount of dose and time. The fertilization rate was decreased with increasing number of isolated eggs. The cited literature revealed greater effect of extracted pituitary glands over fertilization rate but our results showed that its effect was slow and less productive as compared to Ovaprim treatment both for Grass Carp (*Ctenopharyngodon idella*) and Rohu (*Labeo rohita*). The fertilization rate after Ovaprim treatment was quite high and more productive as compared to previous pituitary gland treatment. The fertilization rate of Grass carp (*Ctenopharyngodon idella*) was found to be 52.8% while for Rohu (*Labeo rohita*) it was 74.6%.

In contrast both fishes responded with same fertilization rate with Ovaprim which was 81.5%.

$$\text{Fertilization rate} = \frac{\text{Fertilized eggs} \times 100}{\text{Total eggs}}$$

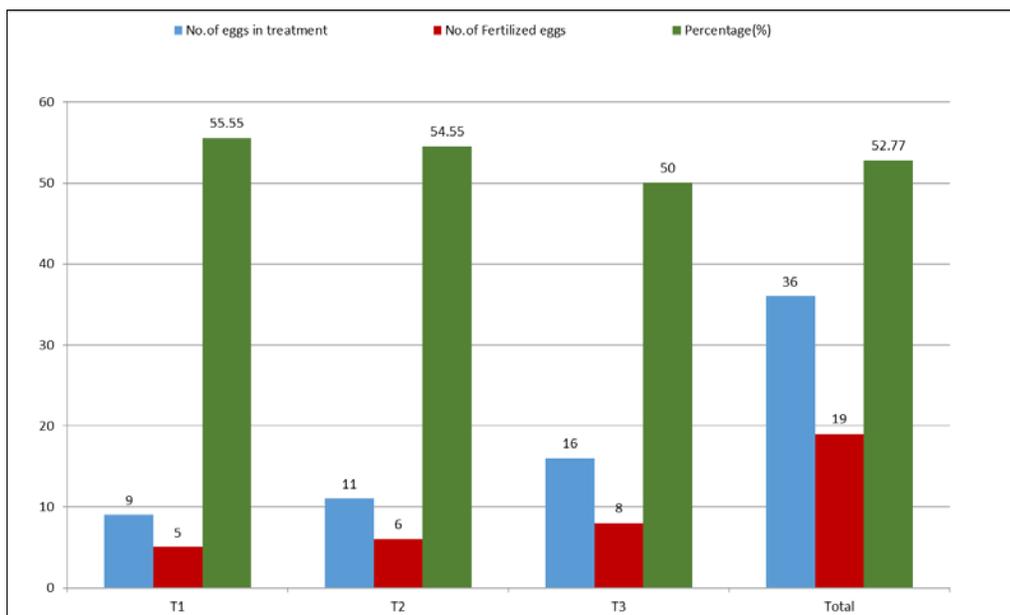
After spawning we studied that the size and location of nucleus was different in different eggs. If the nucleus is present in the mid of an egg then it is pre mature. If the nucleus is acentric then it is ready and well natured. If the nucleus becomes large then it would occupy most of an egg space it indicates that it is over mature. Further the hatching of eggs and survival of lings depend upon water quality and temperature. For example if the temperature reaches 32°C the sliver carp and Grass carp (*Ctenopharyngodon idella*) eggs would denature while Rohu (*Labeo rohita*) and Mori (*Cirrhinus marigala*) mature at this temperature. Collected data is loaded in given formula and the results are shown in (Fig.1 and 2 and Table 1 and 2).

**Table 1:** Showing the fertilization rate of Grass Carp induced by administered pituitary gland extract

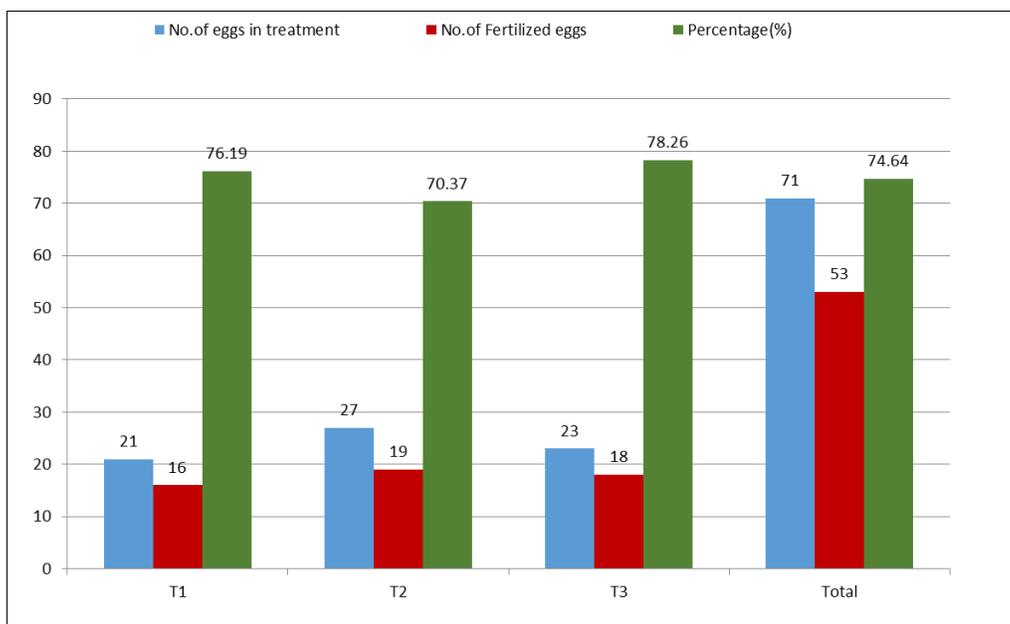
Treatments	Total No. of Eggs in treatment	Fertilized Eggs	Percentage (%)
T1	9	5	55.55
T2	11	6	54.54
T3	16	8	50
Total	36	19	52.77

**Table 2:** Showing the fertilization rate of Rohu fish induced by PGE injection

Treatments	No. of Eggs in treatment	Fertilized Eggs	Percentage (%)
T1	21	16	76.19
T2	27	19	70.37
T3	23	18	78.26
Total	71	53	74.64



**Fig 1:** Showing fertilization rate of Grass carp induced by pituitary gland extract injections



**Fig 2:** Showing fertilization rate of Rahu fish induced by PGE injection

**Discussion**

Various scientists have conducted investigations on various aspects of synthetic hormones to check their efficacy over the fertilization rate, fish growth rate and fish physiology etc. In the last few decades the artificial stimulation of fish

fertilization rate has gained great attention of scientists. As fishes are major source of instant proteins in the local Asian fish market. Commercially important tropical freshwater and marine finfishes were investigated by Marte, (Marte *et al.*, 1989) [15]. They were spawned with pituitary homogenate,

human chorionic gonadotropin (HCG) and semi-purified fish gonadotropins. These hormones were injected into two doses, a lower priming dose followed a few hours later by a higher resolving dose. The present study on the pituitary gland and Ovaprim showed that the Ovaprim is better product than pituitary gland. A single coelomic injection of pituitary gland into Rohu (*Labeo rohita*) and Grass carp (*Ctenopharyngodon idella*) indicated that the pituitary gland is less effective as compared to Ovaprim.

Certain hormones are used individually like 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 (Billard *et al.*, 1983; Naeem and Salim, 2005a; Naeem *et al.*, 2005c; Naeem *et al.*, 2011) [2, 17, 18, 19]. In Canada the researchers introduced the Ovaprim containing the analogue of salmon gonadotropin releasing hormone (DArg<sup>6</sup>,Pro<sup>9</sup> Net) and dopamine antagonist. The studies conducted in India (Nandeeshha *et al.*, 1990) [20] and Pakistan (khan *et al.*, 1992; khan *et al.*, 1990; Naeem *et al.*, 2005) [11, 14, 16] revealed the superiority of Ovaprim in induce spawning. The current study was conducted to work out the efficacy and usefulness of both the hormones using Rohu (*Labeo rohita*) at Government Fish Seed Hatchery, Bahawalpur and Punjab. A comparison was carried out for both the drugs for fecundity, fertilization, and hatching rate during the induced spawning of *Labeo rohita* by a single dose of each hormone. Ovatide performed much better than Ovaprim.

In the present study 4 mg pituitary gland for one kg fish showed a fertilization rate of 53.6% for Grass carp (*Ctenopharyngodon idella*) and 75% for Rohu (*Labeo rohita*). Dose of Ovaprim used in Grass carp (*Ctenopharyngodon idella*) in the present experiment is 0.5 ml/kg for female and 0.25 ml/kg for male which is half of female while experiment conducted by Nandeeshha and Peter reported a dose rate of 0.7 ml/kg. The present study reveals that the Ovaprim use is more economical in commercial carp speed production as it saves a considerable amount of time and avoids the excessive handlings of brooder fishes (Nandeeshha *et al.*, 1990; Sokolowska *et al.*, 1986) [10, 21]. Grass carp (*Ctenopharyngodon idella*) and Rohu (*Labeo rohita*) preferably spawn during mid of April to June with fertilization rate of 53.6% and 75% respectively with hatchlings percentage of 63.88% at favorable temperature of 20°C to 25°C.

The present study indicates that pituitary gland injection gives low production than Ovaprim.

### Conclusion

In this experiment we studied spawning in Rohu and Grass carp induced by pituitary gland extract (PGE) and Ovaprim. Both PGE and Ovaprim induced fertilization in both fishes. The result shows that the Ovaprim performed better than pituitary gland extract. It is concluded that the synthetic hormone, Ovaprim can be used successfully to induce spawning in fishes.

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### Author contribution

JK conceived and designed the project and experiments. JK performed the experiments and FA analyzed the data. JK and FA wrote the paper.

### Conflict of interest

The authors declare no conflict of interest.

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