

**INDUCED BREEDING AND REARING OF SILVER CARP**  
**(*Hypophthalmichthys molitrix*, Valenciennes, 1844) BY USING LHRH-A**  
**HORMONE AT FISH DEVELOPMENT AND TRAINING CENTRE**  
**JANAKPURDHAM, NEPAL**



Entry ३१

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**Submitted To:**

**Central Department of Zoology  
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**September, 2019**

## DECLARATION

I hereby declare that the work presented in this thesis entitled “**INDUCED BREEDING AND REARING OF SILVER CARP (*Hypophthalmichthys molitrix*, Valenciennes,1844)BY USING LHRH-A HORMONE AT FISH DEVELOPMENT AND TRAINING CENTRE JANAKPURDHAM, NEPAL** ” has been done by myself, and has not been submitted elsewhere for the award of any degree. All the sources of the information have been specifically acknowledged by reference to the author(s) or institution(s).

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**RECOMMENDATION**

This is to recommended that the thesis entitled “**INDUCED BREEDING AND REARING OF SILVER CARP (*Hypophthalmichthys molitrix*, Valenciennes, 1844) BY USING LHRH-A HORMONE AT FISH DEVELOPMENT AND TRAINING CENTRE JANAKPURDHAM, NEPAL**” for the partial fulfillment of Master’s degree of Science in Zoology with special paper Fish Biology and Aquaculture. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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### LETTER OF APPROVAL

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## **LIST OF ABBREAVATIONS**

<b>Abbreviated form</b>	<b>Details of abbreviations</b>
BW	Body weight
CPE	Carp Pituitary Extract
DoFD	Directorate of Fisheries Development
FAO	Food Agriculture Organization
FDTC	Fish Development and Training Centre
GDP	Gross Domestic Production
GnRH	Gonadotropic Release Hormone
MoAC	Ministry of agriculture and Co-operative

## ABSTRACT

The present study was undertaken to study “Induced Breeding and Rearing of Silver carp by using LHRH-a hormone at Fish Development and Training Centre Janakpurdham, Nepal” .The present field study was carried out for 4 months from May 2019 to August 2019 to study the physico-chemical parameters, Biology of Silver carp-Fecundity, Gonado somatic index (G.S.I), Fertility rate, Hatching rate, embryonic development and growth of Silver carp (fingerlings).Silver carp were spawned successfully in following two successive doses of LHRH-a. Initial dose for female is 2µg/kg and second dose 4µg/kg. For a male single dose is given at time of second dose for female 3µg/kg of the body weight. Latency period ranged between 8-10 hrs. During the study period the range of temperature in different ponds was 24-36<sup>0</sup>C , P<sup>H</sup> of water showed it to be alkaline during the whole study period, Dissolved oxygen were recorded 5.0-8.2 mg/l,Co<sub>2</sub> were recorded 13-17.1mg/l. The total number of egg spawned was found ranged from 324,996 to 606800, fertility rate (72.5-92.5%), G.S.I (16.21-24.44%) and hatching rate (65.21-82.60%) in Silver carp. The development of embryo was noted from three hours onwards. The development of embryo was continued and hatching takes place between 18 to 20 hrs. After five days hatching are transferred in the Nursery ponds, the fry were fed with artificial formulated feed with35- 40% feed regularly. The length and weight of hatchlings was recorded at weekly intervals. Length and weight of fry was noted gradually increasing from first week to fourth week.

# 1. INTRODUCTION

## 1.1 Background of the Study

Aquaculture has relatively short history in Nepal. It was initiated in the mid 1940s on the small scale in the ponds with indigenous Indian major carp seed from India. Further development began in the 1950s with the introduction exotic species of common carp (*Cyprinus carpio*). Its breeding success in the 1960s followed in monoculture practice and gained desirable popularity in the private sector. More significant progress was seen in the 1970s with the introduction and farming of three exotic Chinese carp species. Silver carp (*Hypophthalmichthys molitrix*) Bighead carp (*Aristichthys nobilis*), Grass carp (*Ctenopharyngodon idellus*). Their breeding success in captivity has been a major breakthrough in the development of aquaculture in Nepal. Similarly, the induced breeding of three major indigenous carps, Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), Bhakur (*Catla catla*) were success fully established.

The actual aquaculture production program in Nepal began in 1981/1982 with the execution of National development Program. 1981/82 aquaculture production was estimated to 750 tones. It reached 8,317 tons in 1992/93. Aquaculture production to increase significantly the end of project and reached 20,000 ton by 2003/2004 (MOAC, 2004). From the overall development of aquaculture production trends, ponds fish culture was developed into the major production system in 2003/2004 accounted for more over 95 percent of production. A complication (FAO, 2005 and DOFD, 2005) indicated that in 2003/2004, Silver carp accounted for the major sell (5125 tons i.e. 26%) of total aquaculture production. The major part of the pond fish production takes in the southern part of the country, Terai plain where 94% fish pond are located. More than two third of Nepalese are engaged in agriculture and contribute over 35% of the gross domestic product (GDP).

Aquaculture is one of the fastest growing sectors in Nepal, with an annual growth rate of 9% (FAO, 2016). In recent years, Nepalese government has recognized the potential of aquaculture for improving nutrition and economic opportunities to the citizen. However, despite recent efforts to encourage and promote local aquaculture practice, annual fish productivity from aquaculture remains low (3.89 t ha<sup>-1</sup>) and citizen still lack of fish. Sufficient access to fish, keeping consumption rate low as well (2.1 kg/person, annually; DoFD, 2013). Lower quality feeds often in fish yields considerably lower than possible

with better feeds, generating major concern about under yielding (Iiuma et al., 1999). Improved feeding practices could lead substantial improvement in polyculture production and subsequent increases in access to animal-sources protein food for people living in these regions (da Shiva et al., 2006). As most small-scale farmers lack sufficient financial resource required to sustain their own aquaculture system using only complete feed, developing low cost alternative is essential. Chronic malnutrition and micronutrient deficiency is a major problem in Nepal and other countries trough out southern Asia (UNICEF, 2012). Under 5 years of age are severely or moderately stunted. An estimated 24% of all women are under nourished. Among Nepalese, protein malnutrition is rampant as meat products are expensive to include in regular diets.

There are the different Fish Development and Research Centre in Nepal which involved fry, fingerling and fish production in Nepal. In province one there is one Fisheries centre but there is no fish development centre. In province two there is three Fish Development centre and no Fish Research Centre. There is four Fish Development and two Fisheries centre in province three. There is one Research centre and two Fisheries centre in province number four. In province five there is two Fish development centre and one Fisheries research centre. There is no Fish development and research centre in province six. There is one fish Development Centre and Research in province seven. FDTC, 2018/19

Chinese carps are cultured in ponds where natural food of fish can also be produced. They are usually stocked in polyculture organism of the pond ecosystem more efficiently (Horvanth et al., 1984). Since there is no need to provide supplementary formulated feed, they are not dependent on dietary fish meal and fish oil.

Induced breeding is highly reliable technique to re-establish the declining natural as well as to meet the rising demand of a seed to the farmers. Induced breeding is a technique by which the economically important fish (the fish which do not breed in captive condition) are breed through artificial stimulation. Induced breeding is technique where the ripe fish breeders are stimulated by pituitary or other synthetic hormone introduction to breed in captive condition. The stimulation promotes timely to promote sperm and eggs. Spawn collected from natural water is not pure as because some undesirable wild species may come with them in culture pond. Sorting of pure seed is quite impossible in those stages. In later stages it is possible, but the time consuming. Spawning is the release of sexual products(ova in the case of female and milt in the case of male) to the exterior of the body

(Basaran et al.,2008).Induced breeding is the method in which exogenous hormone are injected into the body of mature parents fish for induction of breeding (Heggberget, 1996). Only fish in advanced gonadal maturation stages were used. Female were selected by external morphological characteristics indicating that they were ready for spawning induction procedure, such as red urogenital papilla and bulging coelomic cavity were selected for the fertilization procedures.

Nowadays fisheries development centre use different synthetic hormone for induced breeding of Indian major carps and Chinese carp.

#### **Advantages of Induced breeding (Jain, 2003; Srivastava, 2005)**

- Any quantity of pure spawn can be made available.
- A pure spawn of desired species is made available. The spawn obtained from the river are not pure .They are mixed with the spawn of other species and sorting pure seed from pure seed from mixed spawn of other species not possible.
- Several carp attains its sexual maturity but they do not breed in confined water. Such fish can be subjected for induced breeding and spawn can be collected.
- Its economical to obtain spawn from induced breeding process in comparison to natural source or riverine sources.
- The technique is very simple and does not need too much technical assistance or knowledge. It can be learn by layman without much training.

#### **1.2 LHRH-A Hormone**

Luteinizing Hormone releasing (LHRH-A): Mammalian gonadotropin that has been used to induce the reproductive cascade in fish. Chinese scientists were the first to examine the feasibility of using LHRH-a to induce ovulation in cultured fish.

LHRH-a hormone was in the white powder form packed inside the small bottles. LHRH-a is a synthetic decapeptide. A bottle contained 100µg of LHRH-a preparation of hormone injection was done right before use. For preparation, hormone was poured into petridish and normal saline water was added and shaken properly for dilution. It and its superactive analogues (LHRH-a) serves as potential ovulating or spawning agents that induced gonadotropin secretion in fishes. Carps when injected with LHRH-a show greater success (78.5%) as against fish pituitary extract. In general, the synthetic analogues like LHRH-a are up to 1000-fold more potent. The LHRH-a treatment involves less handling stress of the fish (brzuska,1999). The use of LHRH-a alone or together with dopamine

antagonists in spawning various species of cultured fish was earlier reviewed by Crim et al.(1987).Synthetic analogues of lutenizing hormone releasing hormone (LHRH-a) are becoming widely used for inducing ovulation and spawning in a variety of teleosts.

### **1.3 Embryonic and Larval Development of Silver carp**

Embryonic and larval stage is very sensitive and critical stages of their life cycle. So, the studies on embryonic and early larval development are essential for successful rearing of larvae, large scale seed production commercial farming. There are 5 basic life stages of fish which are Embryonic Phase, Larval phase, Fry phase, Ripe Phase and senescent Phase. The embryonic phase includes fertilized egg, cleavage, morula, blastula, gastrula, embryonic body formation, optic vesicle and auditory vesicle formation, blastopore closing, tail formation and hatchling stages. After the embryonic phase the hatchling undergoes organogenesis and the larval stage is ended with the appearances like their parents. Therefore, embryonic and larval development studies are essential in fisheries production by reducing fry mortality. Although embryonic and larval development is reported in Nepal, information regarding embryonic and larval development is very useful.

### **1.4 Descriptions of Silver Carp**

Silver carp is a fresh water species living in temperate condition found in static or slow flowing water of Order-Cypriniformes, Family-Cyprinidae, Genus- *Hypophthalmichthys molitrix* and Species *molitrix*. In its natural range, it is, migrating upstream to breed; eggs and larvae float downstream to flood zones. They have laterally compressed body. They are very silvery color when young. When get older they fade greenish color on the back silver on the belly .Silver carp being planktivores, the gill racker being the main means of filtration. Silver carp consume diatoms, dinoflagellates, chrysophytes, xanthophytes, some green algae and cyanobacteria (blue green algae). Because they feed potamodromous plankton they are sometimes successfully used for the controlling water quality, especially in the control of noxious blue-green bacteria.

Silver carp spawning late spring and summer, when the temperature of the water is relatively high. From April to August, either because of the rainstorm or the swollen upper reaches of streams and rivers. Its brood stocks are concentrated in spawning locations where conditions are favorable, and the current swift complicated and irregular. Spawning temperature is generally in between 18°C and 30°C; with an optimum of 22-

28°C. They have matured in farm ponds of Uzbekistan at 3 years when they have attained 17cm and 100-120g in the first year of life (Kamilov, 1985). When silver carp are ready to spawn, ripples have been seen on the water surface from spawners chasing each other. About 40 to 80 minutes later, males and females ascended close to the water surface, shedding eggs and sperm. The egg of Silver carps, like all Chinese carp are non-adhesive. Larvae and small juvenile feed on zooplankton, switching to phytoplankton once a certain size is reached.

#### **1.4.1 Morphology of Silver Carp**

Morphometric measurement of fishes is essential for systemic, taxonomic study and growth variability (Tandon et al., 1993) and gives substantial information with regard to exact identification key for a particular species. Morphometric study is considered a powerful tool characterizing stocks which involves detection of suitable variation of shape (Sharma et al., 2015). They are silvery in color when young and when they get older they fade greenish color on the silver in the belly. Their eyes are located near the ventral side, which make them easily distinguishable from other carp. Both dorsal and anal fin are present but an adipose fin is lacking. They have both 1 to 3 dorsal spines, 1 to 3 anal spines, 6 to 7 soft dorsal rays, and 1 to 14 soft anal rays. The lateral line is approximately 80 to 130 scales in length. They have numerous thin gill rakers (100 or more). Abdominal keel: extending from the base to the anus. Pectoral fin: its terminal tip does not exceed the base of ventral fin. Pharyngeal teeth: one row in 4/4, with fine lines and tiny grooves on the surface. The pharyngeal teeth are in one row (4-4) and are well developed and compressed with a striated grinding surface. Intestinal length: 6-10 times that of body weight. The gills of silver carp have a complex of network and profusion of closely set gill raker.

## **1.5 Objectives**

### **1.5.1 General Objectives**

To evaluate the Induced breeding and Rearing of Silver carp (*Hypophthalmichthys molitrix*) by using LHRH-a hormone at Fish Development and Training Centre, Janakpurdham.

### **1.5.2 Specific Objectives**

1. To investigate Fecundity rate, Gonado-Somatic Index, Fertility rate, Hatching rate of Silver carp (*Hypophthalmichthys molitrix*).
2. To observe various embryonic stages of development of Silver carp.
3. To evaluate growth rate of Silver carp (*Hypophthalmichthys molitrix*) from fry to fingerlings stage.

## **1.6 Significance of Study**

The study of fish like Silver carp highly successful as a food sources but their detail study about breeding, embryonic and rearing details has not been studied. This study will help to fill the knowledge gap to some extent and research of these species.

## 2. LITERATURE REVIEW

### 2.1 History of Induced Breeding

The technique of induced breeding was evolved in Argentina after producing pituitary extract Hussay in 1930 where viviparous fish were injected to the hormone to make premature birth. In the year in 1934, Brazilians were succeeded in induced breeding by pituitary extract.

In India first attempt of induced breeding was made by Khan (1937) on *Cirrhinus mrigala*. Later Choudhuri 1955 applied this technique in minor carps (*Esotrus danricus*) and (*Pseudeotropius atherinoids*). Ramaswamy and Sunderaraj (1956, 1957) reported successful breeding of the cat fishes, *Heteropneustes fossillis* and *Clarias batrachus* during 1955 and 1956, respectively by hormone injection. Chaudhari and Alikunhi in 1957 succeeded in inducing *Labeo rohita*, *Cirrhinus mrigala*, *Cirrhinus reba*, *Labeo bata* and *Puntius sarana* to breed by injecting them with carp pituitary extracts.

The Russian are considered the next to introduce hormone treatment in fish culture after the Brazilians. It was not until 1937 that Gerbilskii (1938) could succeed, and since then this method is utilized with advantages in the Soviet Union for the production of sturgeon eggs in the farms situated along in the farms situated along the lower Volga, Ural, Kuban and others river fishes .

The first hpophysial technique in China was adopted in 1958 by aquaculture researchers of Gungdong province by injecting the hypophysis of Common carp into brood fish of Silver carp and Bighead carp cultured in ponds. In 1960, artificial propagation of Grass carp in china succeeded by pituitary gland of common carp. In China (1963), the artificial propagation of black carp was done similarly by using common carp pituitary.

### 2.2 Role of Hormone in Induced Breeding of Carp

Szabo et al. (2019) they reported 18 spawning season,555silver carp,300 bighead and 1175 grass carp female were selected for experiment .The ovulation ratios were similar between silver carp (80.9%), bighead carp (77.3%) and grass carp (79.1%). Mean PGSI for grass carp Mean (9.3+ 4.26%) was significantly lower than that for silver carp was (10.5 ± 4.85%) and bighead carp (10.1 ± 3.96). In silver carp and bighead carp, there were no significant differences in ovulation ratios and mean PGSI values among.

Khanom et al.(2018)They reported the embryonic and larval development (*Cyprinus carpio* var. *specularis*). They reported the latency period as 8-8.5 hrs after second injection. Embryonic study deals with morula, blastula, gastrula, segmentation, pharyngeal until hatching were visualized at different hours. Hatching observed at 47hr of fertilization. After 2 days yolk sac was greatly reduced and larva started to swim freely.

Naeem et al. (2015) they reported the induced breeding of *Labeo rohita* through single application of; Ovaprim-c. They study fish specimens were spawned successfully following, a single dose of injection of ovaprim-c with 0.4 ml/kg for female and 0.05 ml/kg for male brooders. They reported the rates of fertilization 77.50% and hatchling 81.39%. They reported hatching occurred within 18-30 hr after fertilization. Wet body weight was observed to have a positive influence on absolute ( $r=0.983$ ) and relative fecundity ( $r=0.910$ ) in log-log scale.

Mousa et al.(2015). They reported the effect of water quality on the reproductive performance of Silver carp the fertilized eggs .The youngest eggs collected were at the 32-cell stage, cells were orange and yolk was light yellow. Whereas 128 cell stage, it was light in color and the cytoplasm almost disappeared. The blastodisc flattened more and gradually expanded over the yolk, germ ring was visible. At the gastrula stage the embryo head enlarged and blastoderm covered about three-fourth of the yolk. The embryo continued to elongate, the tail vesicle disappeared, the brain area enlarged, the end of the yolk was colorless. The stage lasted from 60 to 90 days after fertilization.

Rashid et al.(2014). They reported Fecundity of Grass carp and Silver carp were recorded as 70000-80000 and 1-1.10 lakh egg/kg body wt. of fish respectively. The fertilization percentage of grass carp and silver carp were recorded as 80.03% and 78.12% respectively the hatching percentage of grass carp and silver carp were recorded as 70.10% and 69.71% respectively.

Mir et al. (2014) found that Length-Weight relationships of the Indian major carp, *Labeo rohita* is a geographically widespread and economically important food fish species in tropical freshwater. They studied the Length-Weight relationships of 1033 specimens collected from the main channel of Ganga river and its five major drainages from March 2009 to July 2012. The length of male ranged from 16 to 92 cm and female 16 to 94 cm. The growth is allometric positive ( $b>3$ ) for males, female and pooled sexes. The

coefficient of determination ( $r^2$ ) in Males ranged from 0.978 to 0.989 and Females from 0.958 to 0.985.

Kamilov (2014) reported the age and growth of Silver carp. The ages, total length and weights of silver carp ranged between 1 to 5 years, 15 to 105 cm and 35 to 17,500g, respectively. The mean estimated total length of 1-year old silver carp was 25.25 cm; 2 years old, 49.81 cm; 3-years old, 68.91cm; 4-years old 89.94cm; 5-years old 94.47cm.

Hussian et al. (2013) reported the effect of an organic fertilizer and supplementary feeds on growth performance of silver carp (*Hypophthalmichthys molitrix*) and bata (*Cirrhinus reba*) fry for a period of 90 days. They experimented out under 3 treatments were consisted of mustard oilcake (T1), Cow dung (T2) and ricebran + mustard oil cake (T3) with three replication supplementary feeds. The fries were initially fed at the rate of 30% of the body weight and it was reduced to 15% gradually. During the experimental period, the water parameters were in suitable range. The average weight gain of Silver carp and Bata in T3 followed by T1 and T2.

EL-hawarry et al. (2011) reported that mammalian LHRHa together with dopamine antagonists was more effective in induction of ovulation and increasing fecundity and hatching rate compared to other spawning stimulators. They reported that not only carp pituitary extract and human chorionic gonadotropin but also the mammalian LHRH analogue are available was effective to induce spawning of Silver carp. The fertilization percentage of silver carp 86.5 to 92.5% and hatchling rate is 83.5% to 87.33% respectively.

Naeem et al. (2005) reported the effect of intramuscular injection ovaprim-c on the fertility rate, hatching rate of silver carp. They used a single dose of ovaprim (LH-RH analogue) 0.6ml/kg for female and 0.2ml/kg is given to female. Total no. of obtained egg was  $9.1778\text{kg}^{-1}$ , fertilization and hatching percentage was 72.56% and 71.09% at temperature  $24.8^\circ\text{C}$ - $25.8^\circ\text{C}$  hatching took place 18-32hrs after fertilization.

Arabac et al. (2001) A single injection of  $20\mu\text{g/kg}$  LHRHa ([D-Ser (tBu) 6, pro9-Net]-LHRHa combined with  $0.5\text{mg/kg}$  of the dopamine receptor antagonist, Haloperidol (LHRHa+HAL) was used for spawning in the common carp under routine hatchery conditions. This treatment caused successful ovulation. Latency in common carp injected with LHRHa+HAL was dependent on water temperature, and minimal latency was

14h. When LHRHa+HAL was given at various hours of the day it resulted in identical spawning with the same latency.

Brzuska.E (2001) reported that the ovulation was stimulated in females of grass carp *Ctenopharyngodon idella* and Silver carp *Hypophthalmichthys molitrix* using pimozide at the doses of 15 µg/kg and 5 mg/kg body weight respectively. The results of reproduction after the LHRH-a, the percentage of stripped female was higher than that recorded in case of hypophysation.

Rahman et al. (1988) reported that induced breeding of 30 pairs of *Hypophthalmichthys molitrix* (Silver carp) by different doses of human chorionic gonadotrophin (HCG) and pituitary (PG). The no of egg obtained from a fish by stripping method varied from 145000 to 430000 with an average of 124191 egg/kg body weight of fish. The average percentage of fertilization, hatchling were recorded 78.28 %, 77%, and 69.83% respectively.

There are several methods, preserve fish eggs for study of embryonic development of fish Basak and Basak, 2014; Rath et al., 2002 used distilled water for observation of embryonic development of *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*. Rahman et al., 2009 used Methylene blue for study of embryonic development Mastacembelus.

### **2.3 Artificial breeding of Catfishes**

Nesaet.al (2017). They reported fertilization and hatching rate in hand stripping and non-stripping method  $62.33 \pm 4.51$ ,  $46.67 \pm 5.86$  and  $37.33 \pm 2.52$ ,  $41.33 \pm 5.69$ , respectively. Similarly they reported embryonic developmental mean time for 2-cell, 4-cell, 8-cell, 16-cell, 32-cell, morula, blastula, germinal ring, gastrula, yolk plug, twisting movement, and just before hatching. This study would provide detailed information to the hatchery managers to conduct successful induced breeding of *H. fossilis* as well as other catfishes.

Denson et al. (2007) compared Human Chorionic Gonadotropin (HCG) and luteinizing hormone releasing hormone analogue LHRHa for ovulation induction in Back sea Bass *Centroproistis strita*. Matured Sea Bass (200-800gm) captured during monsoon season were administered hormone. Female receiver a single intramuscular injection of HCG at the rate of 330IU/kg (n=8) or two injection of HCG at 24 hrs intervals. A significant differences infertility was found between HCG ( $75.6 \pm 11.4\%$ ) and LHRHa ( $55.6 \pm 27.6\%$ ). The result of the study indicates the both HCG and LHRHa are effective for ovulation induction in pre spawning Back Sea Bass.

Sial et al. (2005) experimented on *Pangasius sutchi* (sluroid catfish) using carp pituitary extract, ovaprim and ovotide under captive conditions. Out of several doses of carp pituitary extract trail, a dose of 21.0 to 25.0 mg/kg body weight to female brooders induced successful spawning in *Pangasius sutchi* after a latency period of 10-11 hours. Similarly, in case of ovaprim respectively induced ovulation and spawning in the fishes with better fertilization and hatching rate. The result indicated that the response time, rate of fertilization and hatching were found to be better in group administered with ovotide followed by ovaprim and carp pituitary extract.

#### **2.4 Induced Breeding in Nepal**

In Nepal, the first success in induced breeding of fish was made in the 1972 through the hypohysation technique introduced by Woynarovich (1969) in Chinese carp Wagle and Pradhan (2003) estimated effective population size and rate of inbreeding of common carp on the basis of number of new individuals recruited as brood stock each year and the variance of the reproductive success

Sah (2012) reported induced breeding in Common carp (*Cyprinus carpio*) through the application of ovaprim, at Mukhya fish farm Nanupati, Dhanusha. She reported the total number of eggs spawned ranged from 201,000-333,000. The fertilization and hatching rate was recorded 83% and 53.6%. She reported that cleavage of eggs started 4 hrs of fertilization. Length and weight of fry was noted gradually increasing from first week to fourth week.

Banjade (2015) reported ovulation induction of common/ carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) using ovaprim. He reported the percentage fertilization of common carp (75-92.5%) and silver carp (72.6-93.3%) was found higher with ovaprim treatment. Similarly, the percentage of hatchling in Common carp was 44-58% and Silver carp was 44-59% respectively. Cleavage of egg was observed after 4 hrs of fertilization the development of embryo continued at the hatching took place after 48 hrs of fertilization. The fry were feed with artificially formulated feed with 45% protein at the rate 5 to 10% body weight and growth check was done at weekly interval.

Sah (2017) reported induced breeding in Rohu (*Labeo rohita*) and Naini (*Cirrhinus mrigala*) using Ovulin hormone. He reported the percentage fertilization of Rohu (77.77% to 88.33%) and Naini (71.05 to 82.56%) was found with ovulin treatment. Similarly, the hatchling percentage in Rohu was 71.29-82.56% and Naini was 60.86-79.28%.

### 3. MATERIALS AND METHODS

#### 3.1 Study Site

Janakpur is the capital of province number two. The environment condition in this area is ideal for the fish culture purpose. The fisheries Development and training centre (FDTC) lies in the southern region of the Janakpur municipality. The fish ponds and Chinese-ecotype hatchery of Janakpur fisheries Development centre of Janakpur occupies 39.0 hectare in area with 92 pond various size for the purpose of nursing, rearing, stocking, brood management.

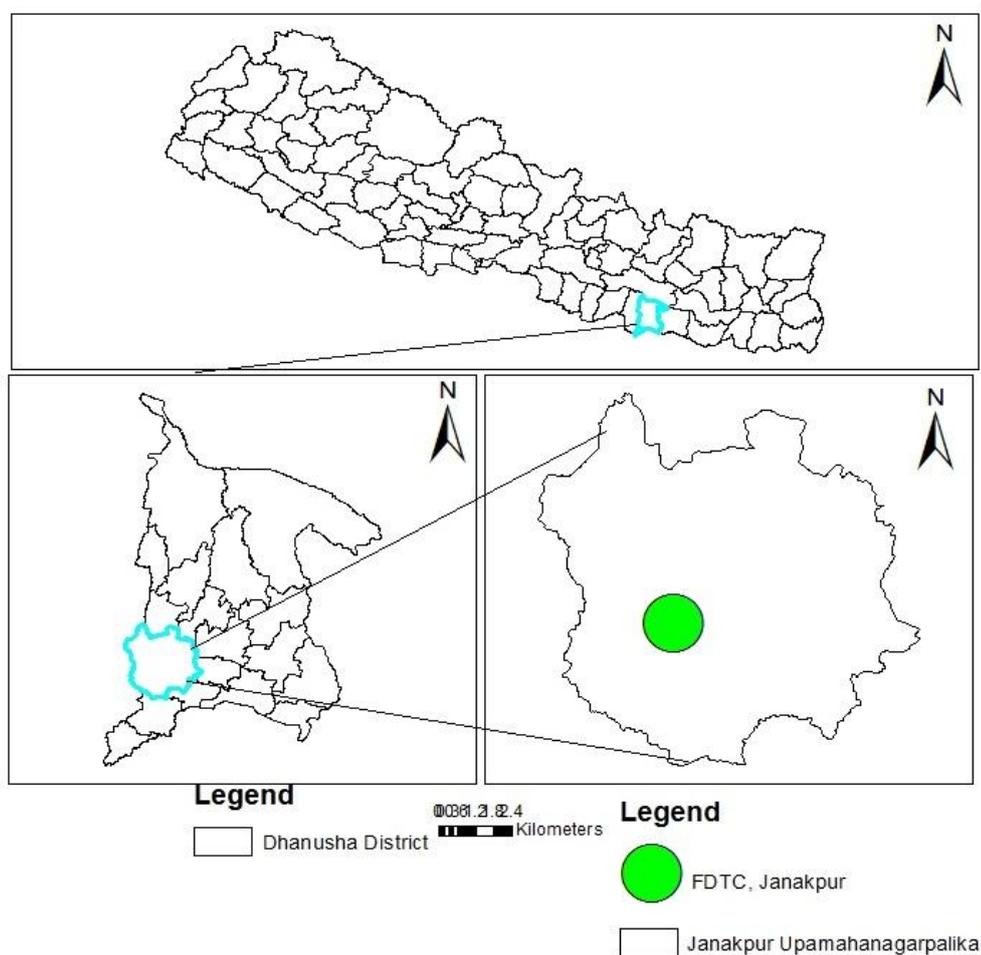


Figure 1: Map of the Study Area

**Table 1: The Number of Pond and their Area in Study Site FDTC**

SN	Name of pond	Number	Area(ha)	Used pond	Unused pond
1	Brood stock pond	11	2.1	All	-
2	Nursing pond	16	1.0	All	-
3	Rearing pond	28	3.2	18	10
4	Production pond	23	6.5	19	4
5	Other pond	14	0.4	2	12
	Total pond	92	13.2	10.5	27

There are 92 ponds having 11 Brood stock ponds, 16 Nursing ponds, 28 rearing ponds, 23 production ponds and other 14 ponds. Nearly 27 ponds are unused in this farm. (Table1)

### **3.2 The Availability Silver Carp Seed**

Hatchling in the month of (April-August), fry in the month of (April-September) and fingerlings in the month of (July- November).

### **Study Period**

The field study was carried out for 4 months from May 2019 to August 2019. Breeding was conducted from May, 2019 – July, 2019 (silver carp). Larval rearing and growth check-up fry was done from 27 May, 2019.

### **3.3 Physico-chemical Parameters**

#### **3.3.1 Physical Parameters**

The physical parameter of studied during experimental period was follows:

**Nature of day:** By visual observation. It was recorded in the field during working.

**Color of the water:** The pond water was taken in a Petridish over white paper and then the color of water was observed.

**Temperature:** The water temperature was measured by using a standard digital meter.

Measured directly by dipping the thermometer bulb onto the water body for two minutes and the reading was recorded in °C.

### **3.3.2 Chemical Parameters**

The chemical parameters studied during present study were as follows:

**PH:** The pH of water in hatchery was measured in study sites by pH meter (EZDO). First pointer of PH meter was dipped into the water for two minutes and reading was recorded.

**Dissolved oxygen (DO):** The dissolved oxygen was measured by using DO meter (LUTRON). Pointer of the DO meter is dipped into the water for two to three minutes and reading was recorded.

**Free carbon dioxide (CO<sub>2</sub>):** Free carbon dioxide was determined by titration method.

### **3.4 Fish Species**

Silver carp was selected for the study. The fishes were widely cultured in fresh waters along with other carp for polyculture.

### **3.5 Collection and Maintenance of Brood Fish**

For conducting breeding experiments on Silver carp, healthy and disease free, farmed reared brooders were selected. The brooders were of 2 to 4+ years of age group and weighing 3.5 to 6.5kg.

“Broods” refers to the mature male and female ready for spawning. Male and female were stocked in different ponds to avoid self-fertilization. Each of the ponds had prepared according to standard pond management practices for stocking and rearing of brood stocks. The specimens selected were healthy without any deformity of the body. In the initial stage of brood maintenance, they were kept in stocking ponds. Due to insufficient macro-vegetation or filamentous algae in the ponds, broods were fed with decayed vegetable matter containing bottom dwelling organisms. The fecundity regime of brood stocks is species specific and requires consideration of timing and composition of the food protein, lipid, and fatty acid composition is particularly important. The quantity of food intake can be altered to influence spawning and maturity.

### **3.6 Identification of Male and Female Brooders**

At the time of breeding the male and female brood fishes were identified on the basis of their secondary sexual characters. The ripe male was identified by roughness of the dorsal surface on the pectoral fin, which on the contrary was very smooth in the female. The roughness in pectoral, fins was felt by touching the surface of fin close to the body. The

mature male and female were also distinguished from the shape of their body, condition of the vent and secretion of milt in males. The distinguishing characters for the identification of the mature male and female brooders are explained in table.

**Table 2: Characteristics Features of Sexually Mature Male and Female Fishes Used for Induced Breeding Trails**

S.No.	Character	Male	Female
1	Scale	Rough with sandy texture, especially on the flanks and anterior dorsal side and nape.	Scales of female are smooth and silky.
2	Pectoral fin	Rough with sandy touch. Particularly on the dorsal side pectoral fins slightly stouter and longer.	Pectoral fins very smooth and slippery. Slightly smaller in length.
3	Abdomen	Round and firm. Not very soft to touch.	Bulging out on both sides. Soft and palpable. A distinct cleavage is formed mid - ventrally along the abdomen when fish is placed on it's back.
4	Vent	Elongated silt, introvert (concave), white in color.	Round silt extrovert (convex) fleshy and pinkish in color, papillae prominent.
5	Putting pressure on abdomen	On slight pressure above the vent on the abdomen milky white fluid (milt) runs out through the vent.	Pressure slight yellowish discharge or a few ova may come out through the vent.

Source, Sharma 2008

Medium size fish were selected for breeding trails as they are easier to handle. At the time of selection the brooder were taken one by one in a hand net. After administration of sex hormone the breeding sets of matured brooders were kept in breeding tank. The breeding set of fishes comprised 2:1 ratio of male and female of approximately similar weight and size (Malik et al., 2014)

### **3.7 Hormonal Doses and Schedule of Administration**

#### **3.7.1 Doses of Administration**

Silver carp were injected intramuscularly with different doses of LHRH-a hormone according to the weight of the body weight. Female broods were injected with LHRH-a hormone in two successive doses, while male were given a single doses at the time of second injection of female. Initial dose for female is 2 $\mu$ g/kg and second dose is 4 $\mu$ g/kg. For male 3 $\mu$ g/ kg of the body weight.

### **3.8 Steps of Breeding**

#### **3.8.1 Collection of Brood Fish for Injection**

Selected fish brooders were collected from the breeding pond using a hand net cloth in the shape of a bag. After selection of male and female broods they were marked separately on the head of bamboo twig each of fish was numerically and serially marked as A, B, C, D, E according to sets. Length and weight of every fish were measure with measuring tape and recorded. They were then wrapped in a soft towel and weighed one by one in weighting machine.

#### **3.8.2 Method of Hormone Injection**

Two persons were required at the time of injection, one of them hold the head of the fish pressed gently against the cushion while the second person pressed the tail of fish with one hand and with other hand gave the intramuscular injection on the caudal peduncle, above the lateral line. For the hormonal preparation a hypodermal syringe of 3ml was used with graduation of 0.1ml. The needle used in syringe was BDH needle number 24. After, injecting the brooders were immediately very quickly i.e. within 1-5 minutes. Times care was taken to hold the fish gently while injecting the hormone so that maximum stress is laid on the fish.

### **3.9 Latency periods**

It is the latency period between injection of inducing hormone i.e. 8 to 10 hrs and the time of spawning in experimental fishes. If the injected hormone affects the induced fish normally, and then the fish will begin chasing each other excitedly this phenomenon is called estrus. The phenomenon of onset of estrus was noticed when ripples appeared on the surface of the water caused by chasing of the breeders in the middle or bottom layer. When brooders start chasing at that time they are selected for stripping.

### **3.10 Hand Stripping and Incubation**

Once eggs were identified in the tank (Drori et. al., 1994, Brzuska, 2004) fish were examined every 30 minutes and ovulated fish were stripped by sight pressure on abdominal region. Female were gently pressed in the abdominal region, by hand stripping method from the LHRH-a treated female. To ensure fertilization mixture of sperm and eggs was gently mixed with feathers of birds. While stirring water was added for hardening of eggs. After 3-5minutes, fertilized eggs were washed carefully with water. Repeated the washing of eggs several time. When fertilized eggs swell and started to float then transferred in incubation tank.

Malachite green (2-5 gm in 10 liter water) was treated in water before transferring eggs to prevent from attack of bacteria and fungus. Soon after the loading of eggs, water was treated with potassium permanganate (5-8 gm in 10 liter water). 40 fertilized eggs were kept in three floating sieves in the incubation for an experiment to study fertility rate and hatching rate .The hatchings were given appropriate food a little before total absorption of the yolk sacs. They were fed with milk powder by simply scattering it over the water slowly. This helps the food not to be immediately throughout the outlet. In the same way egg yolk mix with water were also given twice a day.

### **3.11Determination of Fecundity, Gonado-Somatic index (G.S.I), Fertility and Hatching Rate**

The fecundity, fertility and hatchling rate can be determined by following formula, (Kaur and Dhawan, 1997).

#### **3.11.1 Determination of Fecundity Rate**

Fecundity was estimated separately by sampling one gram egg and multiplying with the total weight of brood female fish. one gram of the stripped out dry eggs were on an electric weighting machine .Three such sample will be taken and will be emerged in salt solution separately counting eggs with the help of brush .

Fecundity = No. of egg per gram × Wt. of .total egg (gm) spawned by female fish

### 3.11.2 Gondo Somatic Index (G.S.I)

GSI express the weight of the gonads as the total weight of the body. Stage of maturity is reflected by the weight of gonads, which are related to the female fish .GSI increase with the maturation of fish being maximum during peak period of maturity and decline abruptly after spawning .GSI (%) of all female fish carps was determined by the following formula.

$$GSI = \frac{\text{Gonadial weight of broods}}{\text{Weight of brood fish}} \times 100$$

### 3.11.3 Determination of Fertility Rate

Fertility rate was calculated for every female separately by sampling eggs at early morula stage, it could estimated .Fertility rate was calculated for every female separately by sampling eggs at the early morula stage. It could be estimated properly after four hours of embryonic development. The eggs in the sieve were taken out in a plastic trough and checked for the fertility. Fertilized eggs were observed as clear crystals balls whereas the unfertilized ones were dull and opaque. Its fertility was calculated in average of the total sample eggs.

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

### 3.11.4 Determination of Hatchling Rate

Hatchings number was determined by taking out the net bowl in a plastic trough and number of hatched ones and unfertilized eggs were counted.

$$\text{Hatching rate} = \frac{\text{total no. of hatchling}}{\text{total no. of fertilized egg}} \times 100$$

### 3.12 Embryonic Stage of Silver Carp

The sample of fertilized eggs was collected for the study of the different embryonic stages of the fish .The egg was taken and several time washed into the distilled water. Various stages of eggs were preserved in 1% formalin solution and the study was done under Binocular microscope in 10X magnification and photographs were taken in with Samsung Jalaxy J5. Methyl orange and Eosine was used for the colour and observed in the lab of Fish Development and Training Centre Janakpurdham.

### **3.12.1 Fertilized and Unfertilized Eggs of Silver Carp**

Fertilization of eggs took place as soon as the sperm enters into the eggs. The eggs deposited singly and were highly adhesive throughout the incubation period. These became translucent as development progressed. Fertilized egg of Silver carp looks like fresh. Unfertilized egg of Silver carp looks dull in nature.

### **3.13 Rearing of Hatchling Till Fry Stage**

After nursing of hatchlings for 5 days inside the hatchery in the incubation tank, one week old hatchlings were transferred into the prepared nursery ponds in the farm. Collections of hatchlings were done through the outlet of the incubation tank by putting a piece of cotton cloth on the mouth of the incubation tank outlet. The hatchlings were then measured in measuring cup (at the rate of 50,000 per cup). The hatchlings were transferred to the nursery ponds for rearing in plastic bags containing sufficient amount of water. Fry were fed two times a day with 35-40 % crude protein feeds. This experiment was continued for 40 days. Hatchling after being transferring to the nursery pond with the help scoop net and the length and weight of the hatchings were measured, up to 40 days. The hatchlings are stocked in the nursery pond. The stocking density directly affects the survival rate. When it is higher, survival will be low. The expected survival rate is 70-80 %. If it is well managed pond, survival rate can get up to 90%.

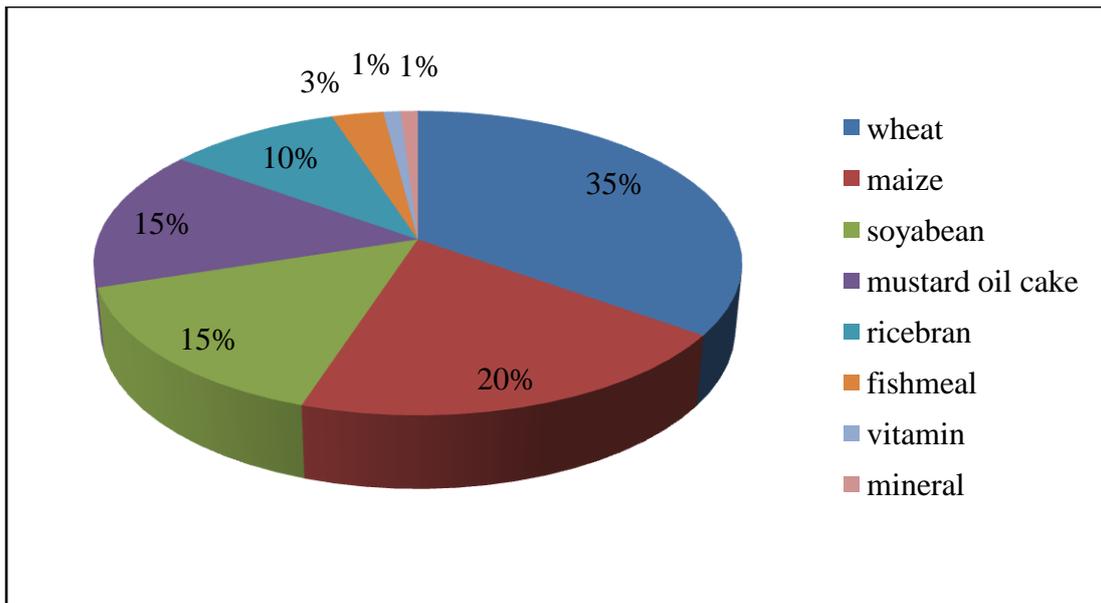
### **3.14 The Routine Management of Nursery Pond**

**3.15** Morning and afternoon observation is necessary to fry activity and changes of water colour, in order to decide on the quantity of fertilizer and food, or whether water replacement is necessary.

We must be careful to exclude harmful insects, frog eggs and tadpoles, birds, snake and remove aquatic weeds.

### **3.16 Feed Composition for Fry and Fingerlings**

Wheat (30%), Maize (20%), Soyabean(15%), Pinna (15%), Ricebran (10%). Fishmeal (13%), Vitamin(1%), Minerals (1%). These are ingredients used in the preparation of feed for fry and fingerlings. Feed are mixed properly made ball and put in the fixed place in fixed time. Feed having 35- 40% protein at the rate of 5-10% body weight .Healthy and proper protein containing feed is very necessary for their proper growth and weight.



**Figure 2: Composition of feed for fry rearing in nursery pond up 40 days in percentage**

## 4. RESULTS

### 4.1 Biology of Silver Carp

The fecundity of silver carp ranged from 324,996 to 698,500 and G.S.I ranged from 16.21 to 24.44 (Table3).

**Table3: Fecundity and Gonado-somatic Index (G.S.I) of Silver Carp at FDTC, Janakpur.**

No. of female	Weight of female (kg)	Fecundity	Weight of total eggs per/gm	G.S.I (100%)
1.	4.7	606,800	740	17.44
2.	4.5	698,500	635	24.44
3.	3.7	324,996	541.6	16.21
4.	4.3	596,490	718.6	19.30
5.	4	361,200	602	17.5
6.	4.1	576,720	712	19

#### 4.1.1 Fertility Rate and Hatching Rate (%)

Fertility rate of Silver carp ranged from 72.5% to 92.5% and Hatching rate 65.21% to 82.60% (Table4).

**Table 3: Fertility Rate and Hatching Rate of Silver Carp in FDTC Janakpur**

Number of eggs	Number of viable fertilized egg	Fertility rate	Hatching rate
40	37	92.5%	73.91%
40	31	77.5%	65.21%
40	29	72.5%	69.56%
40	35	87.5%	78.26%
40	33	82.5%	82.60%
40	36	90%	76%

### 4.2 Embryonic Development of Fertilized Eggs

The eggs of Silver carp were observed under the binocular microscope 10X magnification in the lab of FDTF Janakpur. The observations were continuous from the time of fertilization till the hatching period. The study of embryonic structure were made at 3 hr,

4hr, 6hr, 10hr, 12 hr, 16 hr, 17 hrs and 18 hour. After hatching the observation were made at 4hr, 8hrs, 16hr, 24hr, 96 hr for this specimen is preserved in 1% formalin.

#### **4.2.1 Different Embryonic Stages**

**Unfertilized egg:** Unfertilized egg of Silver carp looks dull in nature eggs are opaque white and the nucleus disintegrate within one hour 10X.(PL I, Photo 1)

**Fertilized egg:** The fertilized egg were found floating, dispersal and brownish in colour. Slight swelling was observed after adding the water. After water hardening, the egg of silver carp were obviously transparent (PL I, Photo 2).

**1-cell stage (3 hrs):** Fertilization activated cytoplasmic movement. No yolky cytoplasm began to stream towards animal pole, segregating the from the yolk granules rich vegetal pole (PL I, Photo 3, 4).

**Morula stage of silver carp development (4hrs):** Morula stage, cells become smaller, blastodisc highly raised above yolk, cytoplasm disappears (PL II, Photo5).

**Germ ring formation (6hrs):** The germ ring appeared around the entire circumferences of the blastoderm. The yolk cell remained about half covered by the blastoderm. The middle of the embryonic shield projecting into the germ ring (PL II, Photo 6).

**Blastopore closure stage of Silver carp (10hrs):** Front of head square, yolk round (PLII, Photo7).

**At optic-primordium stage (16 hrs):** The optic primordium was long oval shaped and clearly visible, the embryo embraced about three –fourth of the yolk ,the uncircled margin of the yolk was convex ,and there were four to six pairs of somites ( PL III , Photo8 ).

**Muscular effect 17 hrs:** The embryo continued to elongate and lashed slightly, the bumb of brain enlarged, but was not highly raised, the end of yolk was colorless, and there were about 26-28 pairs of somites (PL III, Photo 11).

**Hatching stage (18-20 hr after fertilization):** The length was 6.2mm at 18 to 20 hrs after fertilization. The tail length was 28%of the total length. The yolk sac was light in colour and slightly transparent. The anterior or portion of the yolk sac was large and oval. Small optic capsule was seen. The back apparent eye was not apparent (PL IV, Photo12).

**Xanthic eye stage, 8hrs after hatching:** At the xanthic eye stage larva was approximately 7.2mm, indentation of mouth (PL IV, Photo13).

**Gill arch stage 16 hrs after hatching:** The total length was 7.6mm at 28h after fertilization. The tail length was 33% of the total body. The head and body straightened. The eyes of hatchlings increased (PL IV, Photo14).

**24 hrs hatchling:** After one day of hatchlings the yolk sac become thinner prominent pectoral fins fold (PL IV, Photo15).

**One -chamber gas bladder stage 96 hrs:** After 4 days of hatching total length was 8.5mm. The gas bladder appeared and was oval and olive shaped. The mouth was terminal. Feeding began the pectoral fin enlarge and covered the front third of gas bladder (PL V, Photo16).

**PLATE I**

**Embryonic Development of Silver Carp at 10X**



Photo1. Unfertilized egg 10X

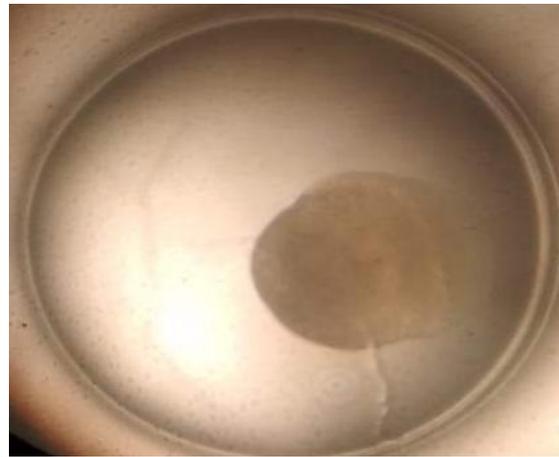


Photo 2: Fertilized egg 10X

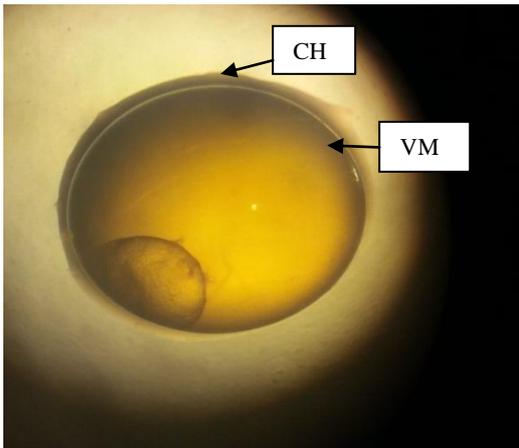


Photo3: 1 cell stage at early 3hr 10X

CH-Chorion, VM-Vitelline membrane

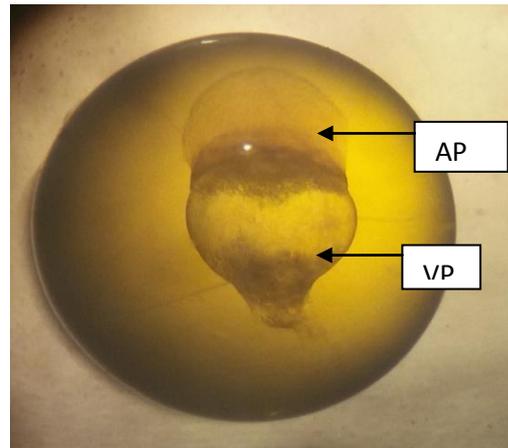


Photo 4: Cytoplasmic movement at 3hr 10X.

AP – Animal pole , VP- Vegital pole

**PLATE II**

**Embryonic Development of Silver Carp at 10X**



Photo 5: Morula stage . At 4 hrs10X

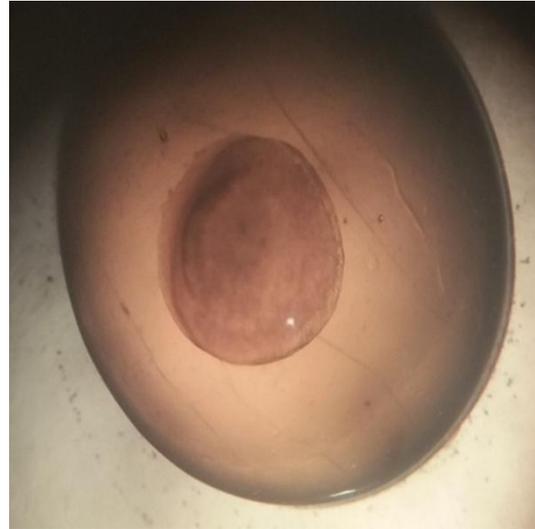


Photo 6: Six hours germ ring formation 10 X

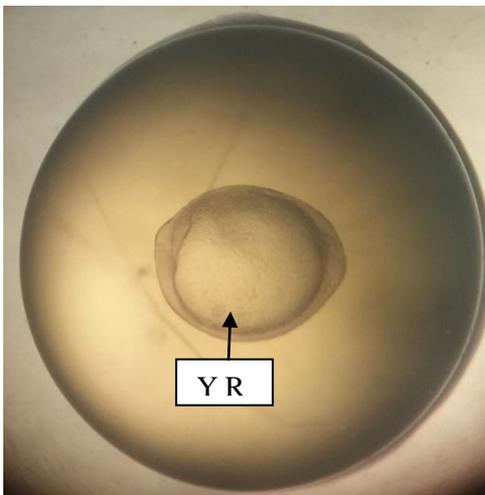


Photo 7: 10 hrs Blastopore closure stage 10X

YR-Yolk round

**PLATE III**

**Embryonic and Hatching stages of Silver carp at 10X**



Photo 8: Embryonic stage at 12 hrs 10X



Photo 9 : Optic primordium stage at 16 hr 10X

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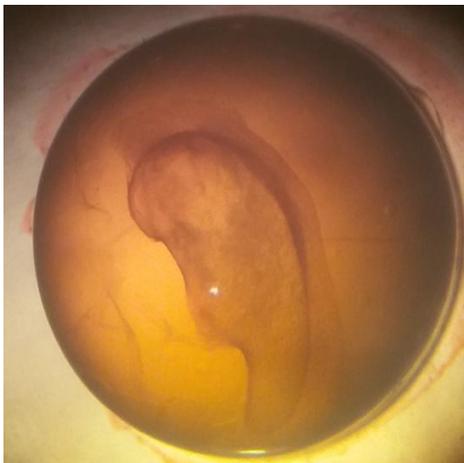


Photo 10: Embryo at 17 hr 10X

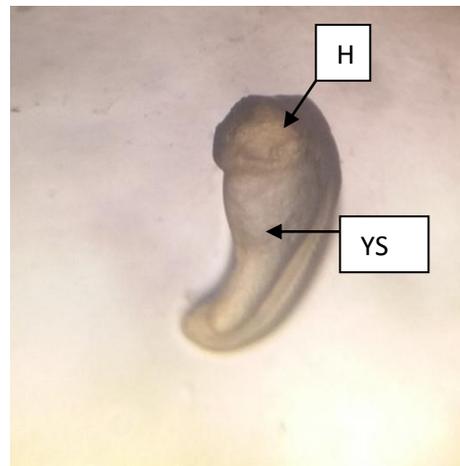


Photo: Hatching stage stage10X at 18 hr

H-Head ,YS-yolk sac

**PLATE IV**

**Hatching Stages of Silver Carp**



Photo 12: 4 hrs hatching stage 10X



Photo 13: Xanthic eye stage at 8 hr hatching 10X

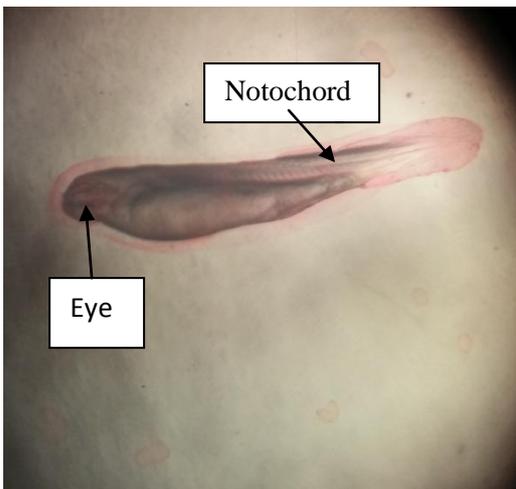


Photo 14: 16 hrs hatching 10X



Photo 15: 1Days hatching 10X

**PLATE V**

**HATCHING AND GROWTH RATE OF SILVER CARP**



Photo 16: 4 days hatching , Gas bladder stage 10X magnification



Photo 17 : 21days after hatching



Photo 18 : 33 days after hatching

**PLATE VI**

**Growth rate of Silver carp**

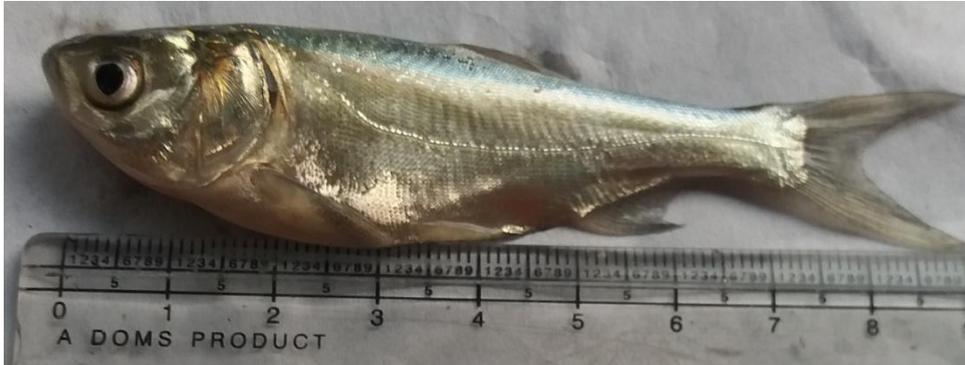


Photo 19: 40 days after hatching



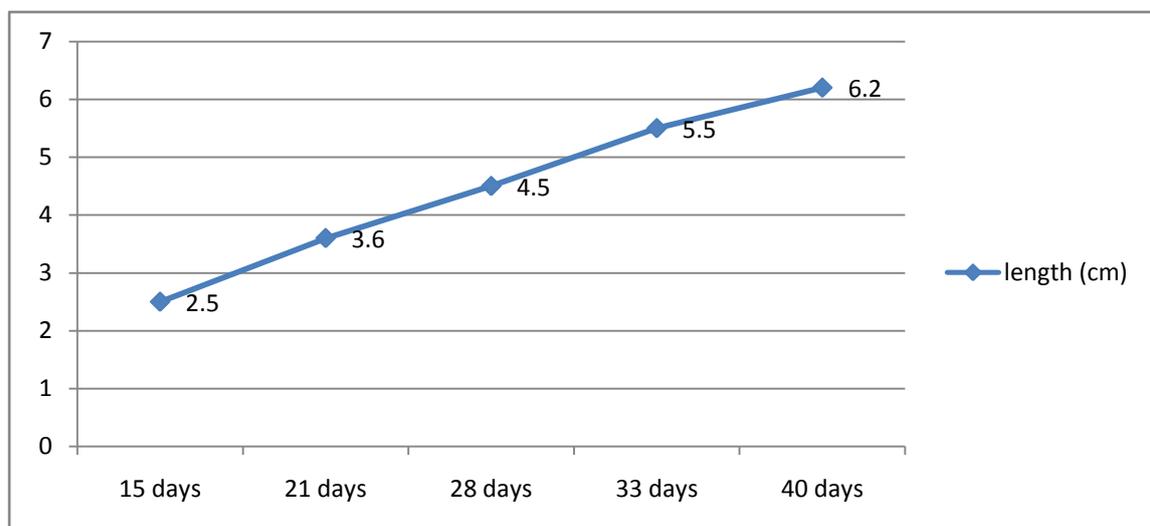
Photo 20: Growth check from right to left fry to fingerlings.

## Growth of Length and Weight of Hatchlings

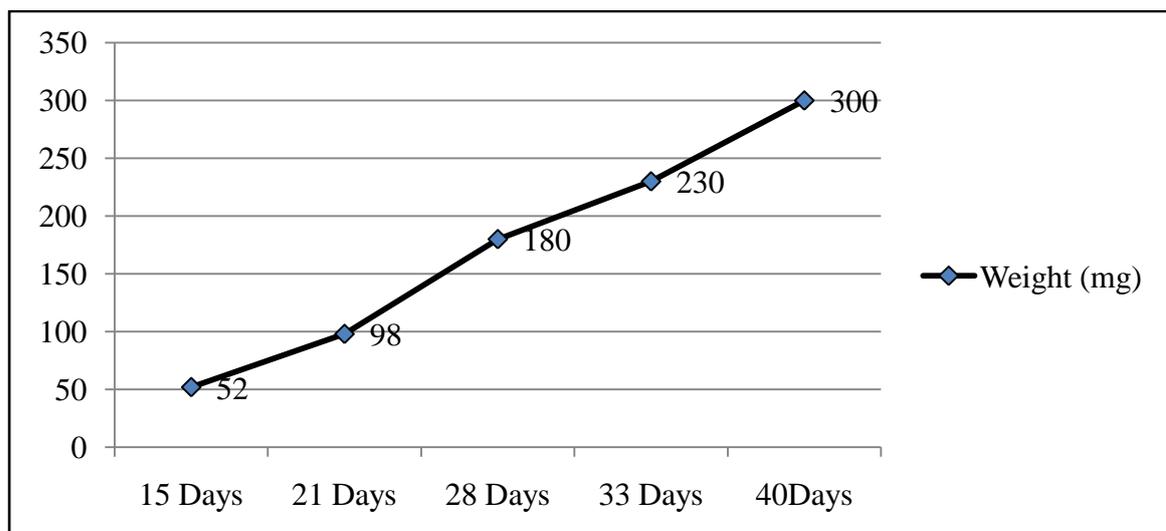
The length and weight of hatchlings were recorded gradually

### 4.2.2 Growth-check of Fry Up to 40 Days

After 5 days hatchlings were transfer to the nursery ponds, the fry were fed with artificially formulated feed with 35-40% protein at the rate of 5-10% body weight. Healthy and proper protein containing feed is very necessary for their proper growth and weight. The growth check of silver carp was done at weekly intervals. Length and weight of fry was noted gradually increasing



**Figure 3: Growth Performance (Length) in the Fry Under the Influence of Commercial Feed at Regular Interval**



**Figure 4: Growth Performance (Weight) of the Fry of Under the Influence of Commercial Feed at Regular Interval**

### **4.3 Physico –chemical Parameter**

#### **4.3.1 Physical Parameter**

The physical parameter had been studied from the sub-surface of the water bodies.

##### **4.3.1.1 Nature of the Day**

During the study period nature of the day was observed within the 4 months of study period 9 days were recorded cloudy, 8 days partially cloudy, 4 days rainy and rest day sunny (clear sky).

##### **4.3.1.2 Color of Water**

The color of water at FDTC was noted greenish during the whole study period.

##### **4.3.1.3 Temperature**

The range of temperature of brood ponds was 26-30°C in the morning (7-10 am) and 29-34°C during the day. Incubation tank 24-28°C in the morning (7-10 am) during the (27-31°C) day. That of nursery ponds was 25-32°C in the morning (7-10 am) and 28-36°C during day.

#### **4.3.2 Chemical Parameter**

##### **4.3.2.1 PH**

The PH remained alkaline during the whole study period, it ranged from 8.1 to 10.5 showing highest in May 10.5 and lowest in June 8.1 at the brood pond and that of the Incubation tank 8-8.1 in the morning (7-9a.m) and 8.7-8.9 during day (2-4p.m) from and that of nursery pond is ranged from 8.1 to 10.5 21 may to 18<sup>th</sup> June.

##### **4.3.2.2 DO (dissolved oxygen)**

The range of DO concentration of brood pond was 5.0-6.1 mg/l during (7-10a.m) in the morning and 6.1-7.5mg/l during day (2-5p.m) from 10<sup>th</sup> may to 23<sup>rd</sup> June. In nursery ponds it ranged from 7.5-8.1mg/l during 7-10a.m and 7.3-8.2mg/l during (2-5p.m) from 25<sup>th</sup> to 29<sup>th</sup> July. In the incubation tank 5.4-7.1 in the morning and 7.3-8.2 in day time. The concentration of dissolved increased from 7a.m to 5p.m.

##### **4.3.2.3 Free CO<sub>2</sub>**

The CO<sub>2</sub> for the brood pond is 14.2 to 17 mg/l. In nursery pond 13.2 -14.6mg/l and incubation tank 13.5 to 14.2mg/l.

**Table 4: Physico -chemical Parameter of Brood Ponds, Incubation Tank and Nursery Ponds**

Average →	Temperature(°C)		DO (mg/l)		P <sup>H</sup>		Free co <sub>2</sub> (mg/l)
	7-10 a. m	2-4 p.m.	6-10a.m	2-5p.m	7-10 a.m	2-5 p.m	
Brood ponds	26-30	29-34	5.0-6.1	6.1-7.5	8.6-10.5		14.2-17
Incubation tank	24-28	27-31	5.7-6	7.5-8.1	8-8.1	8.7-8.9	13.2-5.6
Nursery pond	25-32	28-36	5.4-7.1	7.3-8.2	8.6-9		13.5 -14.2

## 5. DISCUSSION

### 5.1 Latency Time

Concerning the latency period, Silver carp (*Hypophthalmichthys molitrix*) began spawning 8 to 10 hrs after LHRH-a hormone injection. The present result is consistent with the following several studies respective to induced spawning of many cyprinids including Silver carp (Sah 2017), (Khanom et al.2018).

### Fecundity, G.S.I, Fertility Rate, Hatching Rate and Embryology of Fish

Induced breeding of Silver carp (*Hypophthalmichthys molitrix*) with administering a dose of LHRH-a hormone were conducted to find out the effectiveness of hormone in the fish and optimum sizes of female broods for induced breeding cycle of Silver carp with LHRH-a. It was reported that same LHRH-a hormone is also effective for spawning in black Sea Bass, (Denson et al., 2007), common carp (Arabacet al., 2001) ,(Brzuska., 2001).

The fecundity in the present study ranged from 324,996 to 606800 adhesive eggs was reported in a single spawning by each female. The present result is consistent with the results of Banjade (2015). Naeem et al. (2011) in grass carp. (Kamilov and Salikhov 1916) fecundity of silver carp was 299,000-5,400,000 eggs per female .Dhawan and Kaur (2004) experimented that a relative fecundity of 62500 and 100805 in female fish of *Cirrhinus mrigala*.

G.S.I (Gonado somatic Index) in the present study ranged from 16.21 to 24.44% by using Luteinizing releasing hormone these results were comparatively similar to the result reported by Banjade (2015) in silver carp the GSI ranged from 14.1-15.15%. The present result is similar to Sah (2017) in *Cirrhinus mrigala* GSI (10.52-12.52%) and *Labeo rohita* GSI (11.25-15.45%).

In the study, it was found that fertility rate (72.5 % to 92.5%) was good in the present study. The similar result is reported by EL-hawarry et al. (2012) reported that the fertilization percentage of silver carp 86.5 to 92.5% and hatchling rate is 83.5% to 87.33% respectively. Szabo et al. (2019) - ovulation ratios - silver carp (80.9%), bighead carp (77.3%) and grass carp (79.1%) – a little lower to present study. Rahman(1998). The average percentage of fertilization, hatchling were recorded 78.28 %, 77%, and 69.83%. Rashid et al., 2014 in silver carp and grass carp by using Ovotide. The

fertilization percentage of grass carp and silver carp were recorded 80.03% and 78.12%. LHRH-a was more effective in induction of ovulation and increasing fecundity and hatching rate in the present study.

Embryonic development starts at 3hrs after fertilization at 32-36<sup>0</sup>c. Contrary to the present study, Banjade (2015) reported that development starts after 4hrs of fertilization at 28-30<sup>0</sup>c. Embryonic and larval development stages are most delicate part of their life cycle and resulting in mass mortality due to unavailability of appropriate husbandry.

In the present study hatching took place about 18 to 20 hrs after fertilization at temperature 32-36<sup>0</sup>c. Contrary to the present study Mousa et al., 2015 in the silver carp hatching took place at 37 hrs at temperature 28<sup>0</sup>c. (Khanom et al.2018) in the *Cyprinus carpio* var. *Specularis* hatching took place at 47 hrs at 29<sup>0</sup>c.

In the present study the normal developmental stages of silver carp under controlled condition, into two successive periods: Embryonic and larvae period. At the blastula stage, the blastodisc flattened more and gradually expanded over the yolk, germ ring was visible at six hours. The similar result is reported by Basak et al. (2014) in Silver barb.

In the present study optic- primordium is observed at 16 hrs, the optic primer was along and clearly visible. The similar result were reported carp, Mousa et al. (2015) in the silver carp the optic primodium was long oval and clearly visible. Hatchling stage, the tail length was 28.5% of the total length. The yolk sac transparent, juvenile stage the total length was about 35mm. The similar result was reported by Mousa et al. (2015) and George et al. (2013)

## **5.2 Growth Rate of Fingerling**

The growth performance of fish directly depends upon the surrounding aquatic environment, stocking density and food availability, food quality, the number fish utilizing the same food source and also the age, size and sexual maturity of fish.

The hatchlings were scooped out from the pond with the help of scoop net and the length and weight of hatchlings were measured up to 40 days. The 1<sup>st</sup> measurement was done in 15 days of rearing and length and weight was recorded 2.5 cm and 52 mg respectively. Second measurement was done at 21 days where hatching gain 3.6cmlength and 98 mg weight. Thoroughly, 3<sup>rd</sup> measurement in 28 days the length was recorded 4.5cm and weight was 180mg. Fourth (4<sup>th</sup>) measurement in 33 days where length was measured 5.5 cm and weight was 230mg .Final measurement indicates the peak length and weight at 40

days of rearing under the influences of artificial feed and reaches 6.2cm length and 300mg in weight under the suitable physicochemical and environmental factor. The common carp and silver carp showed rapid growth in artificially formulated diet and similar outcome reaching 10 to 13 centimeters in length within the first year was reported by Steiner (2000), Spataru et al. (1980) and Schroeder (1983) reported Common carp and Silver carp naturally depended on plankton and benthic macro invertebrates but when artificial food is applied, they will readily accept artificial feed. Contrary to the present study, Banjade (2015) observed a length 4.1cm and gain weight 250gm after 40 days of rearing of Silver carp by using Ovaprim hormone.

### **5.3 Physico-chemical Parameters**

Physico-chemical parameters of water play a significance role in the biology and physiology of fish. During the present study it was observed that water quality parameters of the brood fish pond, hatchery tank and nursery ponds were found to be within the suitable ranges as reported by Jhingran (1991), Hossian et al. (2013) in the growth response of silver carp.

## **6. CONCLUSIONS AND RECOMMENDATIONS**

### **6.1 Conclusions**

The synthetic hormone LHRH-a have been found to be more effective for induced breeding of Silver carp. LHRH-a was more effective in induction of ovulation and increasing fecundity and hatching rate in the present study. LHRH-a hormone resulted in significantly increased survival and growth of spawn during rearing period of Silver carp. Embryonic and larval development stages are most delicate part of their lifecycle and resulting in mass mortality due to unavailability of appropriate husbandry. Maximum growth and survival of fry and fingerlings is observed on artificial feed. So, Information on early development of Silver carp generated from this will help sustainable development of aquaculture as well as management Silver carp have fast growth rate i.e 6months so it can be good in collecting revenue.

### **6.2 Recommendations**

1. The healthy and mature fish should be selected for breeding.
2. Induced breeding of Silver carp may be more beneficial preferably with the use LHRH-a hormone.
3. The water quality should maintain for the better breeding performance, survival and growth of the spawn produced.
4. It is mandatory to select fully mature, ripe and gravid brood fish for breeding.
5. Further study on embryonic development, survival rate hatching's rate of silver carp is should be done.
6. Several training, workshop, seminar should be organized both at National and International level to bring this industry globally competent by GN, NGO, INGO.

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