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Induced breeding of *Labeo rohita* through single application of ovaprim-C at Faisalabad Hatchery, Pakistan

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A study was conducted to observe the effect of intramuscular injection of ovaprim-C on the number of eggs/kg, fertilization rate and hatching percentage at a private fish hatchery and research center at Faisalabad, Pakistan, during May to June 2008, on *Labeo rohita* (Rohu). Studied fish specimens were spawned successfully following a single dose of injection of ovaprim-C (LH-RH analogue) with 0.4 ml kg^{-1} for female and 0.05 ml kg^{-1} for male brooders. Ova and milt were stripped simultaneously and mixture was stirred for 15 to 30 s during which fertilization occurred. Hatching occurred within 18 to 30 h after fertilization. The experiment was conducted in circular spawning tank with 2 m diameter. If it is impossible to determine the absolute and relative fecundity, then these parameters can be determined from the body weight. Average number of eggs/kg, fertilized eggs/kg and hatchlings/kg was 63574, 49067 and 39952, respectively. Overall fertilization and hatchling %age was 77.50% and 81.39% respectively. 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.

Key words: Induced spawning, ovaprim-C, fecundity, *Labeo rohita.*

INTRODUCTION

The experiments were conducted on males and females of a major Indian carp Rohu, *Labeo rohita,* by injecting ovaprim to observe the efficacy of Ovaprim during induced breeding. Induced breeding is a method in which exogenous hormones are injected into the body of mature parent fish for induction of breeding (Heggberget, 1996). Marte et al. (1987) reported that a drug known as ovaprim has been commonly used as a spawning hormone in fish breeding. Induced breeding through hypophysation has been the common practice since the development of the technology in 1957, several synthetic commercial formulations of purified salmon gonadotropin and dopamine antagonists such as ovaprim, ovatide and wova-FH have also been successfully used in recent years (Jena et al., 1998). Ovaprim is a commercial product used as a spawning aid in fishes and contains a salmon gonadotropin-releasing hormone analog and a dopamine antagonist. Ovaprim has been shown to be effective and safe in numerous ornamental finfish species in the refereed scientific literature. Many years of research investigating hormonal triggers for ovulation and spermiation went into the development of Ovaprim (Peter et al., 1986). In Pakistan, ovaprim was used for the first time in 1991 when it was distributed to all six Government hatcheries in Punjab. In 1991, it was used on experimental basis. In 1992 in all hatcheries of Punjab Govern-

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ment, breeding of carps was done with Ovaprim (Naeem and Salam, 2005). Environmental and hormonal manipulation of ovulation in the fish have become of practical importance in thfish farming industry for two main reasons: to solve the problem of spawning asynchrony which necessitates frequent brood stock handling (Crim and Glebe, 1984) and for accelerating or delaying gametogenesis in captive bloodstock, spawning may be scheduled to yield fry whenever needed (Lam, 1983). Use of exogenous hormones is an effective way to induced reproductive maturation and produce fertilized eggs (Mylonas et al., 2009). Originally, culturists utilized carp pituitary (CP) and this is still widely used particularly for the major Indian carps, Chinese carps and the common carp *Cyprinus carpio* (Lam, 1983; Park et al., 1994). Human chorionic gonadotropin (HCG) has been used to induce final maturation of oocytes and also as a tool for utilization in commercial aquaculture (Kelly and Kohler, 1994; Mylonas et al., 2009). The use of different forms of gonadotropin releasing hormone agonist (GnRHa), which stimulate secretion of endogenous gonadotropin (GTH) (Zohar, 1989; Zohar and Mylonas, 2001), ovaprim and ovatide are a kind of analogue of salmon gonadotropin releasing hormone (sGnRHa) with a dopamine blocker (Syndel International Inc., 2003). The use of sGnRHa resulted in successful stimulation of ovulation in some cyprinids (Drori et al., 1994; Hill et al., 2005) and catfishes (Sahoo et al., 2007). The objective of induced ovulation is to produce, on demand, a large supply of high quality eggs. Egg quality is assessed by characteristics such as egg fertility and hatching (Bromage and Cumaranatunga, 1988).

The aims and objectives of this study were to assess the fecundity, that is, number of egg/kg of wet body weight, by artificial spawning in *L. rohita*.

MATERIALS AND METHODS

The experiments were conducted at Fish Hatchery Satayana Road, Faisalabad, Pakistan during the months of May to June 2008. Sixteen specimens of *L. rohita* were used for investigation, they weighed about 1.6 to 3.0 kg. These artificial breeding tests were conducted in circular spawning tanks with 2 m in a diameter with optimum water quality variables. Ovaprim-C was injected in a single dose because trails by Kaula and Rishi (1986) had found the effectiveness of a single and simultaneous injection. So, risk was not taken to try varied doses due to shortage of Ovaprim-C and brooders. These experiments were done to produce fish fry for sale for farmers and achieve targets by the following procedures:

Selection and handling of bloodstocks

Good quality and well-matured male and female brood fish were collected because according to Muir and Robert (1985), healthy parental fish are prerequisite for successful artificial propagation. So, the most suitable size of spawners is 4 to 6 kg, because of difficulty of handling of bigger fish and requirement of high doses of expensive hormones. The female fish with a bulging abdomen and swollen soft, pink red genital papilla were selected. The males that released milt when subjected to gentle pressure on the abdomen were selected. These were then transferred to the cemented holding tanks of the hatchery and anesthesia with 100 to 200 ppm 2-phenoxy ethanol in one ton capacity fiberglass tanks half filled with tap water. The sex ratios of one female to two males were used, spawning run so as to get best results.

Hormone injection

The brood fish were weighed and dosage of Ovaprim-C solution was calculated according to Nandeesha et al. (1991) by the following formula:

Quantity to be injected (ml) = weight of brood fish (kg) x dosage of ovaprim-C (ml)

The males were injected ovaprim-C with about 0.05 ml/kg, while the females were injected ovaprim-C at a rate of 0.4 ml/kg. It was ensured that all the equipments were cleaned and needle of the hypodermic syringe was cleaned by cotton swab, soaked in alcohol before injection. Required amount of Ovaprim-C was withdrawn from the bottle by keeping the needle upward and syringe was squeezed gently to expel any trapped air. During injection, a brood fish was placed in a cloth bag, lying laterally in water, the upper half of the fish was held above the water surface. At the inner side of the basal part of the pectoral fin, where it was scale less, the syringe needle was inserted gently towards the head at an angle of 45° to the body's longitudinal axis to a depth of about 1.5 cm and fluid was injected slowly. Then, both males and females were released in the fresh aerated water of circular tank.

Estrus

After injection, the spawning behavior was observed in the brood fish as the males started chasing the females after 8:30 h. This phenomenon is called estrus in which the female shows restlessness and its abdomen and tail becomes extremely constricted. The activity lasted for about 30 to 60 min after which fish were netted out for stripping.

Stripping

For spawning, the belly of the female was pressed. The eggs were released from the genital pore and were collected in the dry plastic bowl. The same process was repeated with the males and milt was put into the bowl having eggs.

Fertilization

Following the semidry fertilization method, milt was mixed with the eggs using a bird feather for two minutes. The significance of this method was that, the travel distance for the sperm to the micropyle of the egg was short. Then eggs were washed with water for 10 min, they absorbed water and attained the size of 1 to 1.4 mm in diameter.

Assessment of fecundity

The total number of eggs spawned was counted by the volumetric method. In this method, approximately 1 g egg sample was weighed out three times. The total number of eggs spawned was calculated by multiplying the average number of eggs from three one gram samples with the total weight of eggs sampled. So, the total number of eggs/kg was counted by the following formula:

Table 1. Effect of Ovaprim-C on spawning of *L. rohita.*

Parameter	Ovaprim treatment
No. of females treated .	16
Total weight of females	41.00 kg
Total no. of eggs	2630000
Total no. of fertilized eggs	2020000
Total no. of hatchling	1647000
Overall fertilization percentage	77.50%
Overall hatching percentage	81.39%
Average no. of eggs/kg	63574
Average no. of fertilized eggs/kg	49067
Average no. of hatching/kg	39952

Table 2. Spawning response of female *L. rohita.*

Month	Temperature (°C)	Number of female	Total weight of females (kg)	Dose of Ovaprim (ml kg^{-1})	Total Number of egg	Total Fertilization rate	Total number of hatching
May, 2008	33/29	4	11.0	0.4	0.70	0.49	0.390
June, 2008	33/29	3	8.3	0.4	0.46	0.37	0.297
June, 2008	34/30	4	10.5	0.4	0.67	0.60	0.480
June, 2008	33/30	5	11.2	0.4	0.80	0.56	0.480

Number of eggs/kg = Total number of eggs/total weight of fish

Assessment of fertilization

After 3 to 4 h of fertilization, the fertilized and unfertilized eggs were distinguished. In the fertilized eggs, the division of cells was so regular that the size and shape of blastomeres was equal. So, the percentage of fertilized eggs was calculated according to Muir and Robert (1985); the percentage of eggs beyond morula stage expressed as percentage of the total number of eggs was the fertilization rate, using the following formula:

Percentage of fertilization = (Number of fertilized egg/total number of eggs) x 100

Incubation and hatching

As the breeding tests were conducted in the circular spawning tanks with 2 m diameter, hatching occurred after 18 to 24 h at water temperature (20 to 24.5°C). The newly hatched larvae were kept in the circular spawning tanks with bolting cloth for three days until yolk was absorbed. Then the percentage of hatchling was calculated by the following formula:

Percentage of hatchlings = (Total number of hatchlings/total number of fertilized eggs) x 100

RESULTS

Mean values and ranges of absolute fecundity, fertilization and hatchlings of Rohu (*L. rohita*) following the intramuscular injection of ovaprim-C are given in Table 1. Fish given single injection of Ovaprim-C was successfully induced to spawn. Sixteen (16) females were injected with ovaprim-C. Spawning response of female *L. rohita* is given in Table 2. Ovulation of fish in this treatment was 100%. Average no. of eggs/kg, fertilized eggs/kg and hatchlings/kg was 63574, 49067 and 39952 respectively. Overall fertilization and hatchling %age was 77.50 and 81.39%, respectively (Table 1). Regression analyses of absolute and relative fecundity with body weight were found highly correlated ($P < 0.001$). When these parameters were transformed into log-log scale, highly significant correlation was obtained for absolute ($r=0.983$) and relative fecundity ($r = 0.910$) in *L. rohita* (Table 3).

DISCUSSION

Dose of ovaprim-C for females in carps were comparable with other studies from different locations (Table 4). In the present study, a single intramuscular injection of synthetic hormone, ovaprim-C resulted in successful spawning of *Labeo rohita*. The results of the current work in the hormonal stimulation are similar to the effectiveness and usefulness by using Ovaprim-C (Jamroz et al., 2008). Although, according to Basaran and Sabsun (2008), speed and gentleness during fish capture and handling are of utmost importance. Different analogues of LH-RH, without pituitary gland, which of results in

Table 3. Regression analyses of body weight versus total No. of eggs of *L. rohita*.

Relationship	r	a	b	S. E. (b)
Wet body weight (x) Total no. of eggs (y)	0.972***	--410769	104221.4	17806.6
Wet body weight (x) No. of eggs/kg, (y)	0.898***	18449.98	4402.347	1528.803
Log wet body weight, (x) Log total no. of eggs (y)	0.983***	4.115355	1.68	0.22
Log wet body weight (x) Log total no. of eggs/kg (y)	0.910***	4.115355	0.68	0.22

r = Correlation coefficient; a = intercept; b = regression coefficient; S.E (b) = standard error of b; *** = P < 0.001, n = 16 in each case.

Table 4. Dosage of Ovaprim-C for carps at different locations.

Fish species	Dose of ovaprim-C for (♀)	Reference
Catla catla	0.4 - 0.5	Nandeesha et al. (1990a)
Labeo rohita	0.3 - 0.4	Nandeesha et al. (1990a)
Labeo rohita	0.4	Khan et al. (1992)
Cirrhina mrigala	0.25 - 0.3	Nandeesha et al. (1990a)
Cirrhina mrigala	0.4	Khan et al. (1992)
Hypophthalmichthys molitrix	0.4 - 0.7	Nandeesha et al. (1990a)
Ctenopharyngodon idella	0.4 - 0.8	Khan et al. (1992)
Aristichthys nobilis.	0.4 - 0.5	Nandeesha et al. (1990a)
Aristichthys nobilis.	0.6	Naeem and Salam (2005)
Hypophthalmichthys molitrix	0.6	Naeem et al. (2011a)
Ctenopharyngodon idella	0.6	Naeem et al. (2011b)
Labeo rohita	0.4	Present study

failure of spawning indicates that dopamine blocks the action of LH-RH on the secretion gonadotropin reported by Naeem and Salam (2005). However, the use of dopamine antagonists like pimozide or doperidon, potentiate the action of LH-RH, resulting in successful spawning (Chang and Peter, 1983). Major breakthrough in the history of aquaculture happened when extensive research on Chinese carp (Peter et al., 1988) and a new Linpe method was used in which LH-RH analogue is combined with a dopamine antagonist. Then, Canada introduced the ovaprim-C containing the analogue of salmon gonadotropin releasing hormone (D-Arg6, Pro9, Net) and dopamine antagonist, and studies conducted in India (Nandeesha et al., 1990a) and Pakistan (Khan et al., 1992; Naeem and Salam, 2005; Naeem et al., 2005a; b) revealed the superiority of Ovaprim-C in induced spawning.

Dose of Ovaprim-C used in *L. rohita* in the present experiment is 0.4 ml/kg, while experiments conducted by Nandeesha et al. (1990a) and Khan et al. (1992) reported the dose rate of 0.3 to 0.4, 0.4 ml/kg, respectively. So, the present study reveals that Ovaprim-C use is more economical in commercial carp seed production, as it saves a considerable amount of time and avoids the excessive handling of brood fish. The positive response of Rohu to ovaprim indicated the higher potency of this drug in inducing the spawning. *L. rohita* have been reported to spawn with 10 mg of Pimozide (Kaul and Rishi, 1986) and the results obtained from Ovaprim is supported by the results of the trials in 1988 and 1989 (Nandeesha et al., 1990a; b). Moreover, reliable estimates for absolute and relative fecundity can be attained by using the predictive equations with a fair amount of accuracy.

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