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Observations on the induced breeding in *Labeo rohita* (Ham.) by hormonal injection of Pituitary Gland Extract and synthetic hormone Ovaprim at FSPC, Paithan, Maharashtra State, India

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Abstract

The present study was undertaken to induce breeding capacity of *Labeo rohita* (Ham.) in the breeding hatchery pool using the technique of induced breeding i.e. hypophysation, The induced breeding brings changes in transforming their physiological activity upon secretion of pituitary hormones in concerning with the physiological interaction with the surrounding abiotic factor and can be expected to determined based upon ovary maturation. Synthetic hormones are the only trustworthy method to procure the pure seed of the fish. Induced breeding is necessary to control timing and synchrony of egg production. The present review focuses on the effect of pituitary gland extract is used in combination with synthetic hormones viz. ovaprim on induced breeding of fishes. Therefore ovaprim is superior than PGE. Hence, to facilitate a steady supply of seeds, oocytes maturation and ovulation need to be induced. The ratio of the male and female (2:1) were selected for each experiment. In the present study, males and females which were healthy and disease free brooders selected for the experiments. The average results during study period i.e. June-August 2013 and June-August 2014 have been tabulated. All the experimental and control fishes treated with synthetic hormonal doses, experiment were conducted in the breeding pool.

Keywords: Induced breeding, *Labeo rohita*, Pituitary Gland Extract and synthetic hormone Ovaprim

Introduction

Synthetic hormonal injection is the most common method for induced breeding in which the pituitary extract injected into the fully ripe brooders both male and female which compel for breeding. These hormones are becoming in vogue now a day and found to be efficient and successful induced breeding in *L. rohita* fish species. Although, according to Basaran and Sabsun 2008 have suggested velocity and mildness while capturing the fish and correct handling in the breeding pool are of utmost importance. Determination of correct dosage of pituitary extract to be given to the breeders depends upon the size and state of maturity of the recipient as well as upon the state of maturity of the donor fish. The basic principle comes from biological mechanism propagation, because the extent ecological condition in ponds cannot satisfy the reproductive requirement of the brood fish (Peter *et al.*, 1998). An investigation upon competency of the pituitary gland extract in fruitful induced breeding substantiated by Nandeesha *et al.*, 1990; Muhammad *et al.*, (2005); Zalina *et al.*, 2012.

A regime of following induced breeding is merely nothing but supplementing the inadequate hormones as suggested by Van Der Hurk, 1983. This is in vogue known that the pituitary gland extract even from same species of fish which gives rise to triggers the best result even though extract from amphibians are seems to be an effective in fishes manifested by Padhi *et al.,* 2015. The induced breeding competencies of *L. rohita* by the application of ovaprim in comparison to PGE have been experimented by many workers Chauhan *et al.* 1999; Reddy and Mathur, 2000; Saini, 2001; Jhajhria, 2002; Dhawan and Kaur, 2004 has unearthed the breeding success of *Labeo rohita* when stimulated to breed along with ovaprim and PGE which is corresponding with the results of present study. The most important reproductive hormone released from the pituitary is gonadotropin releasing hormone (GnRH) that regulates gonadotropin hormone, GtH (Yaron, 1995; peter and Yu, 1977 and Marimuthu *et al.*, 2009). Combinations of GnRH and dopamine antagonists have been used to induce ovulation in several cyprinids including Carassius auratus (Sokolowska *et al.,* 1984), *Labeo rohita,* by (Justus Rutaisire, 2003)

Material and Methods

For the study purpose *L. rohita* male and female were selected on the spot at the breeding pool. A close observation is kept while giving the PGE and ovaprim allotted hormonal injection. The pituitary glands were collected from Indian major carp *L. rohita* in the month of June to August. To gain access to the pituitary, the top of the skull was removed with a knife. Pituitary gland was left behind on the base of the skull. Collected pituitaries were homogenized in 0.6% salt solution or distilled water. The solution was centrifuged and the clear supernatants were used for injection. Sometimes preserved pituitary gland may also be used for extract preparations. Pituitary glands were preserved in absolute alcohol immediately after collection. Each gland was kept in a separate vial with fresh absolute alcohol and stored in a cool shady place at room temperature or under refrigeration until needed. For fish pituitary extract, intramuscular injection is given usually in the region of just below the dorsal fin and above of the lateral line. Brooder fishes were identified and selected for the experiment on the basis of following sexual dimorphism morphological characteristics, such as, the bulging abdomen, soft ventral abdominal region, comparatively larger size, felt pectoral spine, smooth pectoral fin and swelling anal fin with reddish colour of females. But in comparison to males the normal abdomen, milt comes out with gentle pressure on the abdomen, smaller size of similar age, serrated pectoral spine, rough pectoral fin and concave anus from exterior had been observed at the time of selection of brooders.

Results

It has been found that the potency of the extract is influenced by the size, age and the sex of the donor fish. Set of brooders for experiment every time usually consists of one female and two males. For almost all the experimental set carried out at the Fish Seed Production Centre hatchery unit similar ratio is kept. Selected brooders of about 1 kg - 4 kg body weight respectively are taken into consideration for study purpose. After selection of males and females of *L. rohita* the ratio 2:1 male and female were brought to circular hatchery. Pituitary gland extract 2mg – 4mg/kg body weight was injected intramuscularly just below the dorsal fin base and slightly above the lateral line in region. The first dose 0.2 - 0.4 ml/kg body weight was administrated to the females for promoting maturation and second dose 0.4 - 0.8 ml/kg body weight was administrated to the females at the same time, the first dose 1.5-0.2 or if further needed 0.2 - 0.4 ml/kg body weight was administrated to the males.

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At the same time for inducing breeding single dose of ovaprim 0.4 - 0.6 ml/kg body weight were administered to the both males and females. Injected brooders were released in a breeding pool. Experimental brooders were observed hatching for 72 hrs after injection; at interval of 4 to 6 hrs the fishes gave responses as a behavioural changes, regarding maturation, ovulation and spawning. Healthy and disease free brooders of *L. rohita* were selected for every experiment in the (2:1) ratio of male and female. For the collection of brooders drag net was used for netting and uplifting the fishes to avoid gill injury.

Table no. 1: Spawning response of female *Labeo rohita* with pituitary gland extract.

Months	**No. of female treated**	**Total wt of female (kg)**	**Average no. of eggs obtained**	**Dose of Pitutary extract ml/kg body weight**		**Average no. of fertilized eggs**	**Total no. of hatchling**	**Average no. eggs Kg^{-1} (Fecundity)**	**Average no. fertilized eggs Kg^{-1}**	**Average no. Hatchling eggs Kg^{-1}**	**Fertilization rate (%)**	**Hatchling rate (%)**
				I st	**II nd**							
June2013	4	12.0	**980000**	0.2-0.4	0.6-0.8	**680000**	**400000**	**79069**	**56666**	**33333**	**69.38**	**58.82**
July 2013	4	16.5	1300000	0.2-0.4	0.6-0.8	1000000	690000	78787	60606	41818	76.92	69
July 2013	4	16.5	1200000	0.2-0.4	0.6-0.8	930000	635000	72727	56363	38484	77.5	68.27
Aug.2013	4	17.5	1300000	0.2-0.4	0.6-0.8	970000	630000	74285	55428	36000	74.61	64.94
Aug.2013	4	21.5	**1700000**	0.2-0.4	0.6-0.8	**1450000**	**1135000**	**81666**	**67441**	**52790**	**85.29**	**78.82**

Table no. 2: Spawning response of female *Labeo rohita* with Ovaprim (Year 2013)

Months	**No. of female treated**	**Total wt of female (kg)**	**Average no. of eggs obtained**	**Dose of ovaprim ml/kg body weight**	**Average no. of fertilized eggs**	**Total no. of hatchling**	**Average no. eggs Kg-1 Fecundity)**	**Average no. fertilized eggs Kg-1**	**Average no. Hatchling eggs Kg-1**	**Fertilization rate (%)**	**Hatchling rate (%)**
June 2013	4	19.0	**2000000**	0.4 - 0.6	**1900000**	**1790000**	**121052**	**92682**	**87317**	**92.00**	**89.31**
July 2013	4	12.0	1450000	0.4 - 0.6	1370000	1230000	95294	114166	102500	94.48	89.78
July 2013	4	16.0	1760000	0.4 - 0.6	1690000	1560000	120833	105625	97500	96.02	92.30
Aug.2013	4	17.0	1620000	0. 4-0.6	1500000	1350000	140000	88235	79411	92.59	90
Aug.2013	4	20.5	**2300000**	0.4 - 0.6	**2116000**	**1890000**	**110000**	**111368**	**99473**	**95.00**	**94.21**

Table no. 3: Spawning response of female *Labeo rohita* with Pituitary extract. (Year 2014)

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Months	No. of female treated	Total weight of female (kg)	Average no. of eggs obtained	Dose of Pitutary extract ml/kg body weight		Average no. of fertilized eggs	Total no. of hatchling	Average no. eggs Kg^{-1} Fecundity)	Average no. fertilized eggs Kg^{-1}	Average no. Hatchling eggs Kg^{-1}	Fertilization rate (%)	Hatchling rate (%)
				I st	II nd							
June 2014	4	10.5	**780000**	0.2-0.4	0.6-0.8	570000	380000	74285	54285	36190	73.07	66.66
July 2014	4	7.0	535000	0.2-0.4	0.6-0.8	420000	300000	76428	60000	42857	78.50	70.42
July 2014	4	13.0	900000	0.2-0.4	0.6-0.8	720000	510000	69230	55384	39230	**77.64**	**68.18**
Aug.2014	4	10.5	830000	0.2-0.4	0.6-0.8	650000	430000	79047	61904	40952	78.31	66.15
Aug.2014	4	12.0	**850000**	0.2-0.4	0.6-0.8	**660000**	**450000**	**70833**	**55000**	**37500**	**80**	**71.83**

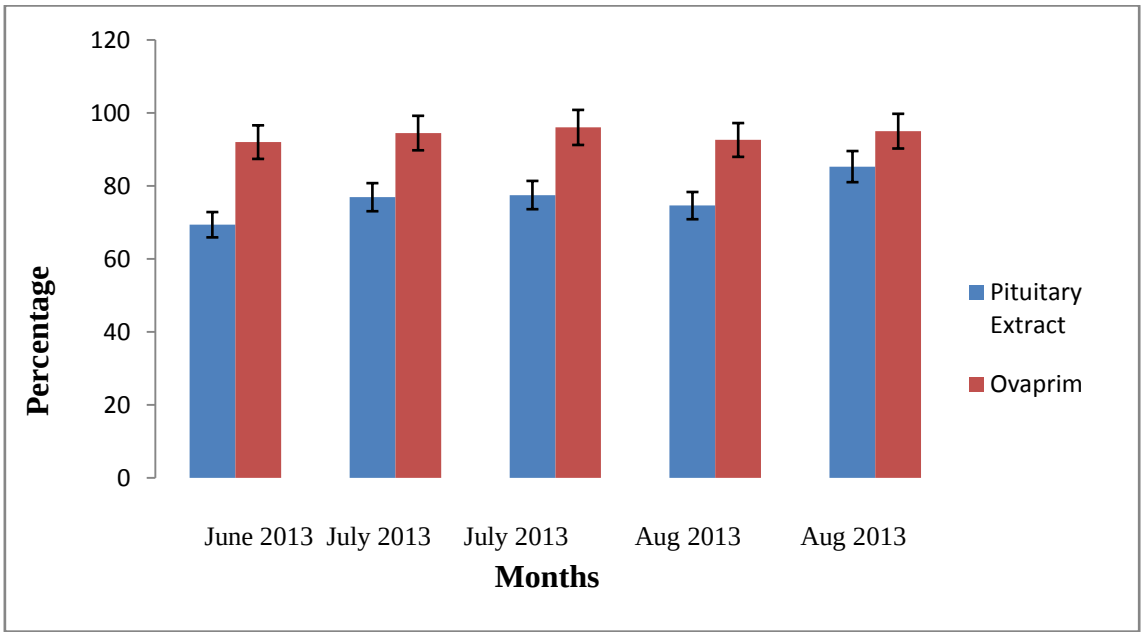
Table no. 4: Spawning response of female *Labeo rohita* with Ovaprim (Year 2014)

Months	No. of female treated	Total weight of female (kg)	Average no. of eggs obtained	Dose of ovaprim ml/kg body weight	Average no. of fertilized eggs	Total no. of hatchling	Average no. eggs Kg-1 Fecundity)	Average no. fertilized eggs Kg-1	Average no. Hatchling eggs Kg-1	Fertilization rate (%)	Hatchling rate (%)
June 2014	4	18.0	1700000	0.4 - 0.6	1595000	1440000	97560	88611	80000	93.82	90.28
July 2014	4	17.5	2015000	0.4 - 0.6	1914000	1800000	94444	109371.	102857	94.98	94.04
July 2014	4	19.5	2200000	0.4 - 0.6	2112000	1964000	122500	108307	100717	**93.06**	92.99
Aug.2014	4	20.0	2450000	0.4 - 0.6	2280000	2170000	115142	114000	108500	**96**	95.17
Aug.2014	4	20.5	2210000	0.4 - 0.6	2080000	1930000	112820	101463	94146	94.11	**92.78**

Table no. 5: Overall effect of pituitary gland extract and ovaprim on spawning *Labeo rohita* (2013-2014)

Pituit			
Parar			
No. o			
Total weight of females	137	Total weight of females	180
Total no. of eggs obtained	1037500	Total no. of eggs obtained	1970500
Total no. of fertilized eggs	805000	Total no. of fertilized eggs	1855700
Total no. of hatchlings	556000	Total no. of hatchlings	1712400
Average no. eggs per kg.	75636	Average no. eggs per kg.	112964
Average no. of fertilized eggs per kg.	58307	Average no. of fertilized eggs per kg.	103383
Average no. of hatchlings per kg.	39915	Average no. of hatchlings per kg.	95242
Overall fertilization %	77.12	Overall fertilization %	94.20
Overall hatchlings %	68.25	Overall hatchlings %	92.08

Fig. 1: Fertilization Rate (%) in *Labeo rohita* due to pituitary extract compared with ovaprim (2013)



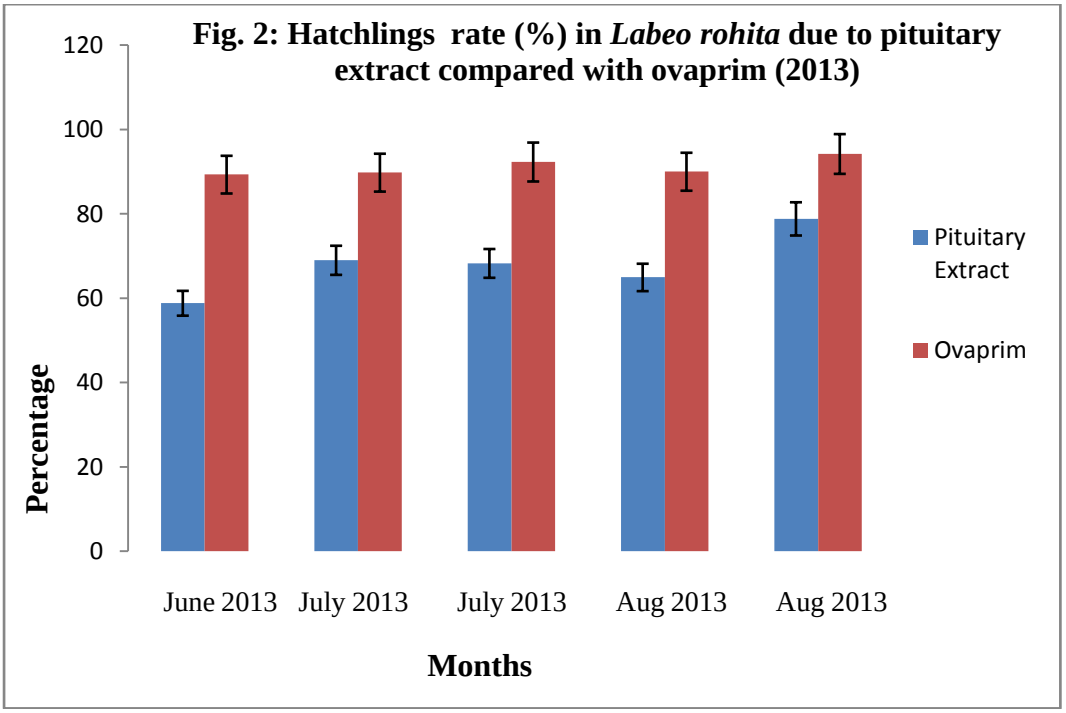


Fig. 2: Hatchlings rate (%) in *Labeo rohita* due to pituitary extract compared with ovaprim (2013)

Fig. 3: Fertilization Rate (%) in *Labeo rohita* due to pituitary extract compared with ovaprim (2014)

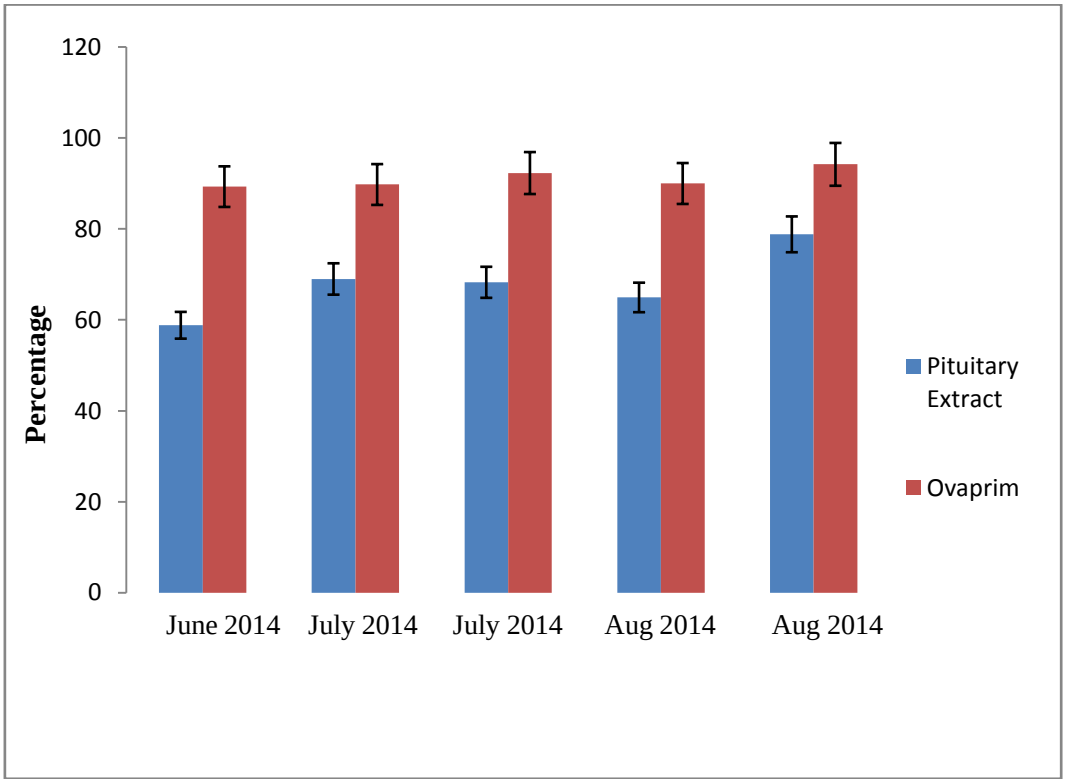


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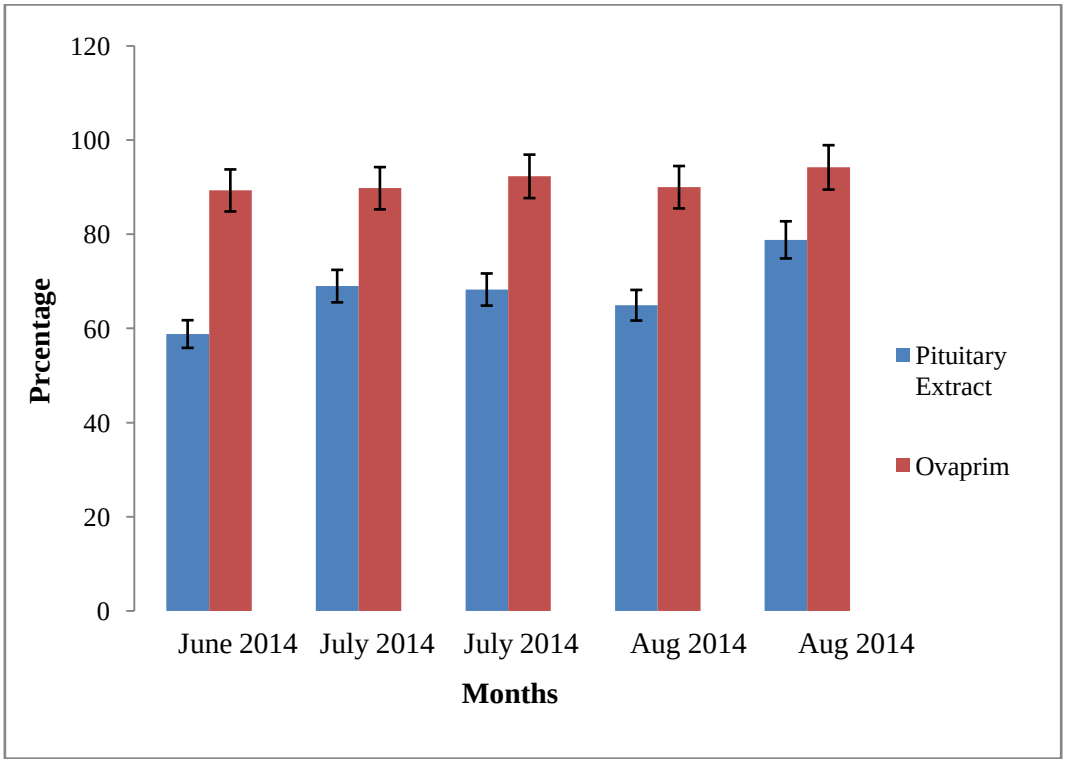
Fig. 4: Hatchlings rate (%) in *Labeo rohita* due to pituitary extract compared with ovaprim (2014)



Discussion

Generally, the female is given an initial dose of 2-3 mg of pituitary gland extract per kg body weight and another dose of 5-8 mg per kg body weight after an interval of 6 hours. Males are given only a single dose of 2-3 mg per kg of their body weight. There are several ways of hormone administration to mature carp such as intracranial, intraperitonial and intramuscular. We have practiced intramuscular injections in our experiment during June-August 2013 and June-August 2014 applying appropriate doses of the hormones. Aggressiveness in the brooders was noticed after 4-6 hrs, of the second dose 0.6 - 0.8 ml/kg body weight of pituitary hormones to female and first dose 0.2 - 0.4 ml/kg body weight of pituitary extract to male. The first dose given to the female was 0.2 - 0.4 ml/kg body weight of pituitary extract. Whereas the single dose 0.2 - 0.4 ml/kg body weight of ovaprim were administrated to both male and female *L. rohita*

Dynamic activity on the part of brooders was observed thereafter 4-6 hrs, for the second dose determined was 0.6 - 0.8 ml/kg body weight of pituitary extract to female and first dose 0.2 - 0.4 ml/kg body weight of pituitary extract to male. The first dose was given to the female was 0.2 - 0.4 ml/kg body weight of pituitary extract. While the single dose 0.2 - 0.4 ml/kg body weight of ovaprim were administered to for male and female fishes

The ratio of the male**:** female (2:1) were selected for each experiment. In the present study, males and females which were healthy and disease free brooders selected for the

experiments. The average results during study period i.e. June-August 2013-2014 have been tabulated.

Induced breeding spawning response due to pituitary gland extract (2013)

There were four female fishes used for experiment purpose in particular in June 2013, weight of fishes was recorded 12 kg. The dwindled response was recorded an average number of eggs procured 980000 and average number of fertilized eggs were 680000, average number of hatchlings were 400000 and average number of eggs per kg body weight of the fish was 79069, average number of fertilized eggs per kg body weight of the fish was 56666 and average number of hatchlings per kg body weight of the fish was 33333, fertilization rate in % was 69.38% and hatchlings rate in % was 58.82% in the *Labeo rohita.*
While in the month of August 2013 the number of treated females fish were four, total weight of fish recorded was 21.5 kg. maximum response was recorded such as the average number of eggs obtained 1700000 and average number of fertilized eggs were 1450000 average number of hatchlings were 1135000 and average number of eggs per kg body weight of the fish was 81666 and average number of fertilized eggs per kg body weight of fish was 67441, average number of hatchling per kg body weight of the fish was 52790 and fertilization rate (%) was 85.29% and hatchling rate (%) was 78.82 % of the *Labeo rohita.*

Induced breeding spawning response due to ovaprim (2013)

During June, 2013 the number of treated female fish were four, total weight of fish recorded was 19.0 kg. the minimum response was observed such as the average number of eggs obtained 2000000 and average number of fertilized eggs were 1900000, average number of hatchlings were 1790000, average number of eggs per kg body weight of fish was 121052, average number of fertilized eggs per kg body weight of fish was 92682 and average number of hatchling per kg body weight of fish was 87317, fertilization rate (%) was 92.00% and hatchling rate (%) was 89.31% of *Labeo rohita.*
In the month of August 2013 the number of experimented females fish were four and total weight of the fishes recorded was 20.5 kg, the maximum response was noticed such as the average number of eggs gained to the tune of 2300000 and average number of fertilized eggs were 2116000, average number of hatchling were 1890000, on the whole of it was an average number of eggs per kg body weight of fish was 110000 and average number of fertilized eggs per kg body weight of fish was 111368, average number of hatchling was 99473, per kg body weight of fish and fertilization rate in % was 95.00% and hatchling rate in % was 94.21% of *Labeo rohita* exhibited in the table no. 4.47. Fertilization and hatchling rate (%) which was noticed because of pituitary gland extract plus ovaprim are shown in Fig no. 1 & 2.

Induced breeding by synthetic hormone spawning response due to pituitary gland extract (2014)

During June 2014 the numbers of treated female fish were four; total weight of fish recorded was 10.5 kg. minimum spawning response was found such the average number of eggs obtained were 780000 and average number of fertilized eggs were 570000, average number of hatchling were 380000, average number of eggs per kg body weight of fish was 74285, average number of fertilized eggs per kg body weight of fish was 54285, average

number of hatchling per kg body weight of fish was 36190, fertilization rate (%) was 73.07% and hatchling rate (%) was 66.66 % of *Labeo rohita.*

In the month August, 2014 the number of treated female fish were four, total weight of fish recorded 12.0 kg and maximum response was found such as the average number of eggs gained were 850000 and average number of fertilized eggs were 660000, while an average number of hatchling were 450000, average number of eggs per kg body weight of fish was 70833 average number of fertilized eggs per kg body weight of fish was 55000, average number of hatchlings was 37500 per kg body weight of fish fertilization rate (%) was 80.00% and hatchling rate (%) was 71.83 % of *Labeo rohita.*

Induced breeding by synthetic hormone spawning response due to ovaprim (2014)

During the month of June 2014 the number of experimented female fish were four and in total weight of fish recorded was 18.0 kg and dwindled spawning response was observed such as the average number of eggs procured were 1700000, moreover an average number of fertilized eggs were 1595000 and average number of hatchling were1440000, average number of eggs per kg body weight of fish was 97560, average number of fertilized eggs per kg body weight of fish was 88611 and average number of hatchling was 80000 per kg body weight of fish, fertilization rate in % was 93.82% and hatchling rate in % was found to be 90.28 % of *Labeo rohita* shown in the table no. 4.49 and fertilization and hatchling rate (%) which were observed due to pituitary extract and ovaprim are presented in Fig no. 3 & 4.

During the month of August 2014 the numbers of experimented female fish were four and in total weight of fish recorded was 20.5 kg and at the most of an optimum response was noticed such as an average number of eggs gained 2210000 and an average number of fertilized eggs were to the tune of 2080000, average number of hatchling were 1930000, average number of eggs per kg body weight of fish was 112820, average number of fertilized eggs per kg body weight of fish was 101463, average number of hatchling per kg per kg body weight of fish was 94146, fertilization rate in % was 94.11% and hatchling rate in % was 92.78% of *Labeo rohita.*

Conclusion

The present study connoted that the overall performance of the induced breeding major carps as mature brooders *L. rohita* were injected by synthetic hormonal doses upon administration of PGE plus later other hormonal doses viz. ovaprim. As a consequence of this study suggest that ovaprim hormone was found to be better as collated with the PGE. All the experiments were performed and conducted in the breeding pool subsequently hormonal injection is given. Work seems to be very difficult but with the kind cooperation of in-charge manager as fish breeder plus by the courtesy of Fishery Development Officer at FSPC, Paithan, the investigation was undertaken very correctly and honestly, however there was difficulty initially in handling, selection of brooders, care taking act of all selected fishes. For the experiment throughout the study all the fishes were used by administration of pituitary gland extract plus in combination with other synthetic hormone viz. ovaprim.

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circular hatchery, stocking ponds, breeding pool, incubation pools and nursery ponds were made available to us during the course of study. I afford special thanks to my beloved research guide **Dr. Quadri S. A**., Assistant Professor, Maulana Azad College of Arts, Science & Commerce, Aurangabad who has been always behind me and for his invariable encouragement and scientific support in the analysis of scientific study and kind cooperation in the completion of research work extremely successfully throughout the study period. Thanks to Department of Zoology, Maulana Azad College of Arts, Science & Commerce, Aurangabad for providing laboratory facilities.

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