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Research Article

Potentiality of single dose of pituitary injection for successful induced breeding in Indian major and medium carps

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Abstract: *The present attempt was to generate a primary database on potentiality of single dose pituitary injection for successful induced breeding performance in Indian major and medium carps. In addition to draw a comparative report on induced breeding response by different Indian major and medium carps like Catla, Rohu, Mrigel and Bata in response to different inducing hormones in single and double doses as per requirement. Inducing agents like natural fish pituitary extract, both single (PSD) and double doses (PDD) and two synthetic gonadotropin hormone releasing hormones (sGnRH) namely Wova-FH (W) and Ovaprim (O) in single dose were administered to observe their impact on different induced breeding assessment parameters in Carps during three consecutive rainy seasons from 2019 to 2021. The ovulation rate, fertilization rate and hatching rate were significantly different in four different treatments in all carp species and the best response was recorded in case of single dose pituitary extract (PSD) as compared to others in terms ovulation rate (100%), fertilization rate (87.85%) and hatching rate (84.23%).The result recommends for adoption of single dose pituitary (PSD) to replace double dose pituitary (PDD) extract in carp hatcheries for induced breeding and seed production of Indian major and medium carps as it facilitate to reduce the pain and stress to brood fish due to single pushing as well as potentially to elicit best response towards fish seed (spawn) production.*

Key words: *Fish pituitary extract; Dose of inducing hormone; Induced breeding technique; Indian major carp; Medium carp; Eco-hatchery*

1. Introduction

Carps are the mainstay of freshwater aquaculture sector throughout the country. In the recent years the gap between supply and demand of fish is increasing with increased population need without concomitant increase in fish production [1] because supply of quality fingerling as seed for culture is being compromised. Quality spawn or fry or fish fingerlings are the essential prerequisite for the development of aquaculture (Webber and Riordan, 1976) through scientific package of practice. With passage of time, natural spawning grounds of carps are getting threatened due to construction of different irrigation and flood control dams, river pollution and increased use of pesticides for growing high yielding varieties of rice and consequently the quantity of fries, obtained from the natural spawning grounds are declining. The technology of induced breeding through

hypophysation induced mass scale production of quality seed in the form of spawn under controlled condition and it has reduced the dependence on the natural seed collection from rivers. In [2] for the first time successfully induced the spawning of Indian major carps with pituitary extracts. This technique was spread all over India requiring two times administration with pituitary extract. Later a good number of workers made remarkable contributions in the sphere of induced breeding of different carp species using GnRH analogues and other synthetic hormones. In [3] made a study with pituitary hormone for induced breeding in *Labeo bata*. In [4] expressed a comparative account of the induced breeding in major carp *Cirrhinus mrigala* by pituitary extract and ovaprim. The successful induced spawning was led by [5] using synthetic hormones in Labeo rohita. In [6] worked on induced breeding of Labeo bata (Hamilton- Buchanan, 1822) and Puntius Javanicus (Bleeker, 1855). In [7] worked on comparative study of synthetic hormone ovaprim and carp pituitary extract used in induced breeding of Indian major carp. In [8] used a single dose of intramuscular injection of a synthetic hormone analogue, ovupin for commercial carp seed production in Bangladesh. All of these experiments were done either by double dose of pituitary extract causing stress to female fish or by different synthetic hormones being expensive than pituitary. In the present investigation an attempt has been made to use single dose of natural pituitary extract to induce breeding of different carp species to minimize stress to fish as well as to reduce operational cost to make the technology farmers' friendly. In addition, a comparative study on carp induced breeding performance by four different inducing hormones have been tested to search out their efficacy for quality fish seed production in the form of spawn.

2. Materials and methods

2.1. Breeding trial design and set up

Three (3) replications for each fish breeding trials were conducted separately in the chinese hatchery located at fish farm, Uttar Banga Krishi Vishwavidyalaya, Pundibari, Cooch Behar administering four different inducing hormones on four carp species during the month of June-July in three consecutive years (2019-2021) for production of quality spawn (as quality fish seed) from Indian major and medium carps. Inducing agents used for carp breeding were pituitary gland extract, both single (PSD) and double dose (PDD), WOVA- FH (W) and Ovaprim (O). Different carp species like *Catla catla, Labeo rohita, Cirrhinus mrigala, Labeo bata* were bred separately to achieve species-wise data on induced breeding process.

After selection of matured female and male Catla, Rohu, Mrigel and Bata, each fish was injected separately in the evening with pituitary extract (single dose considered as $\mathbf{T_1}$) in 0.6% saline prepared from alcohol preserved pituitary glands. Breeding behaviour was exhibited after 7-8 hours of injection which was followed by spawning and ovulation. Brood fish were removed after

completion of the spawning and fertilized eggs were transferred to the hatching pool after proper hardening. Same procedure was followed for the other inducing agents like PDD (**C**), WOVA-FH (**T2**) and Ovaprim (**T3**). Respective doses of inducing agents are illustrated in **Table 1**.

Table 1: Dose of inducing agents injected to brood fish

Treatment	**Species**	**Dose (ml/kg)**		
		Female		**Male**
T_1 -Pituitary single dose (1mg/0.1ml stock)	*Catla catla*	2.5		1.25
	Labeo rohita	2.0		1.0
	Cirrhinus mrigala	1.5		0.75
	Labeo bata	2.0		1.0
C - Pituitary double dose (1mg/0.1ml stock)	*Catla catla*	0.4	1.0	0.4
	Labeo rohita	0.3	0.8	0.3
	Cirrhinus mrigala	0.2	0.6	0.2
	Labeo bata	0.3	0.8	0.3
T2 - Wova-FH	*Catla catla*	1.0		0.5
	Labeo rohita	0.8		0.4
	Cirrhinus mrigala	0.6		0.3
	Labeo bata	0.8		0.4
T3 - Ovaprim	*Catla catla*	1.0		0.5
	Labeo rohita	0.8		0.4
	Cirrhinus mrigala	0.6		0.3
	Labeo bata	0.8		0.4

2.2. Breeding assessment parameters

Ovulation rate (OR), fertilization rate (FRand hatching rate were calculated for each trial through random sampling before and after hatching events for each set of experiment following the method of More *et al* (2010).

- Ovulation rate (%) = [No. of fish ovulated/Total No. of fish injected] × 100
- Fertilization rate (%) = [No. of fertilized eggs/Total No. of eggs] × 100
- Hatching rate (%) = [No of hatchlings/Total No. of fertilized eggs] × 100

2.3. Statistical analysis

The results obtained were subjected to statistical analysis, ANOVA (one way) that showed the significance ($P<0.05$) level of differences between the treatments. This statistical analysis was

performed with the aid of the computer software MS Excel program. Significant results ($P<0.05$) were further tested using Duncan's New Multiple Range Test (DMRT) to identify significant differences among means. This statistical analysis was performed with the computer software MSTATC program.

3. Results and discussion

General weather conditions prevailed during the month of June-July were conducive for carp breeding. Cool weather with temperature range of 27-28°C and overcast sky with drizzles favored breeding performance (Rokade et al, 2006) as assessed through ovulation rate, fertilization rate and hatching rate in four carp species injected with four different treatments (**Table 2**) including control.

3.1. Gross effect of inducing agents on selected carp breeding parameters

3.1.1. Ovulation Rate (OR)

Highest OR (100%) was found in all the selected carps species induced with PSD (T1) which was trailed by PDD (C) in *Cirrhinus mrigala* (98%) and other fish like *Labeo bata* (95.3%), *Labeo rohita* (92.3%) and *Catla catla* (90.1%). OVAPRIM (T3) induced 92.8% average ovulation rate whereas WOVA-FH (T2) elicited average minimum ovulation rate of 91.6% in four carp species expressing an overall performance trend of T1>C>T3>T2 (**Table 2**).

3.1.2. Fertilization Rate (FR)

Similarly, highest FR was observed in Lr x PSD (91.14%) followed significantly by Lr x OP (88.88%) & Cc x PSD (88.28%) having non-significant variations between the two, by Cc x WF (86.24%), Cm x PSD (86.03%), Lb x PSD (85.94%) having non-significant variations between the three and others. Labeo bata showed no fertilization when treated with WF (T2) which may be attributed to undesired level of dopamine activity by Wova-FH (Peter et al, 1986). Cm showed poor performances (74.35%) with PDD (C) which was significantly followed by PDD in Cc (77.50%) & Lb (78.20%), presented in **Table 2**.

3.1.3. Hatching Rate

PSD (T1) when treated on Cc recorded best HR (92.85%) significantly followed by LbxPSD (87.39 %), LrxPSD (82.28%) and others. No performance by WF (T2) on HR in Lb was again reflected. However, OP (T3) when treated on Cm (57.80%) and Lb (61.70%) performed worst followed significantly by CcxOP (60.10%) and CmxWF (60.15%) presented in **Table 2**.

In a nutshell, irrespective of species, maximum gain on OR (100%), FR (87.85%) and HR (84.23%) were achieved in case of single dose pituitary extract (PSD-T1) injected induced breeding whereas minimum gain were recorded with WF (T2) and the difference in results obtained between trials with inducing agents as well as carp species could also be due to environmental factors or varied body size or maturity (Basavaraja et al.,2007).

Table 2: Effect of inducing agents on breeding assessment parameters of Indian major and medium carps

Treatment	Species	Ovulation Rate	Fertilization Rate	Hatching Rate
Pituitary single dose (T1)	*Catla catla*	100.00	88.28	92.85
	Labeo rohita	100.00	91.14	82.28
	Cirrhinus mrigala	100.00	86.03	74.39
	Labeo bata	100.00	85.94	87.39
	Mean	**100.00**	**87.85**	**84.23**
Pituitary double dose ©	*Catla catla*	90.10	77.50	67.61
	Labeo rohita	92.30	83.01	74.13
	Cirrhinus mrigala	98.00	74.35	73.65
	Labeo bata	95.30	78.20	69.67
	Mean	**93.93**	**78.27**	**71.27**
Wova-FH (T2)	*Catla catla*	91.70	86.24	66.90
	Labeo rohita	89.40	84.76	61.53
	Cirrhinus mrigala	95.30	85.60	60.15
	Labeo bata	90.00	0.00	0.00
	Mean	**91.60**	**64.15**	**47.15**
Ovaprim (T3)	*Catla catla*	88.06	84.61	60.10
	Labeo rohita	92.36	88.88	61.70
	Cirrhinus mrigala	95.85	84.84	57.80
	Labeo bata	94.96	82.17	58.70
	Mean	**92.81**	**85.13**	**59.58**
Grand Mean		**94.58**	**78.85**	**65.55**
		Species		
S Em (±)		0.15	0.17	0.25
CD (p = 0.05)		0.42	0.49	0.72
		Treatment x Species		
S Em (±)		0.29	0.34	0.50
CD (p = 0.05)		0.84	0.98	1.45

3.2. Carp species responsiveness to inducing agents

Irrespective of inducing agents, *Cirrhinus mrigala* elicited best range (approx. 95-100%) for OR. FR significantly declined showing the following trend of Lr>Cc>Cm>Lb and HR followed the order of Cc>Lr>Cm>Lb trend.

After considering OR, FR and HR in the form of cumulative quantitative parameter, hatching number per 100 number of ovulated eggs were calculated species -wise as depicted in Table 3 which clearly pointed out that irrespective of inducing agent, Cc and Lr species responded as best (57.00) whereas Cm responded next (48.00) to the above and Lb response was quite poor (46.00). Hence, the order of species responsiveness can be denoted as Cc=Lr>Cm>Lb. Likewise, species responsiveness in terms of FR was assessed from Table 4. It was observed that irrespective of inducing agent, Cc and Lr responded best (61.00) while Cm followed them (55.00) but responsiveness of Lb was quite poor (45.00) (supported by Miah et al.,2008). Therefore, the same response order was revealed for number of fertilized eggs.

Table 3: Species- wise calculated hatching number/100no.of ovulated eggs

	PSD	PDD	WF	OP	Avg
Cc	82	47	53	45	57
Lr	75	56	46	51	57
Cm	64	53	49	48	48
Lb	75	52	00	46	46
Avg.	74	52	28	51	51

Table 4: Species-wise calculated number of fertilized eggs/100no.of ovulated eggs

	PSD	PDD	WF	OP	Avg
Cc	82	52	58	51	61
Lr	75	61	52	55	61
Cm	64	55	52	49	55
Lb	75	55	00	48	45
Avg.	74	56	41	51	56

3.3. Effect of Inducing agents on breeding performances of specific Carps

3.3.1. Effect of Pituitary Single Dose

Non-significant variation was observed in all species for Ovulation rate (OR) as it was found 100% in all the selected carps. Significantly highest Fertilization rate (FR) was recorded in *Labeo rohita* (91.14%) followed by *Catla catla* (88.28%), *Cirrhinus mrigala* (86.03%) and *Labeo bata* (85.94%) having non-significant difference between the last two. *Catla catla* showed best Hatching rate (HR) (92.85%) followed significantly by *Labeo bata* (87.39%), *Labeo rohita* (82.28%) and *Cirrhinus mrigala* (74.39%), presented in **Table 2** and **Figure 1a**.

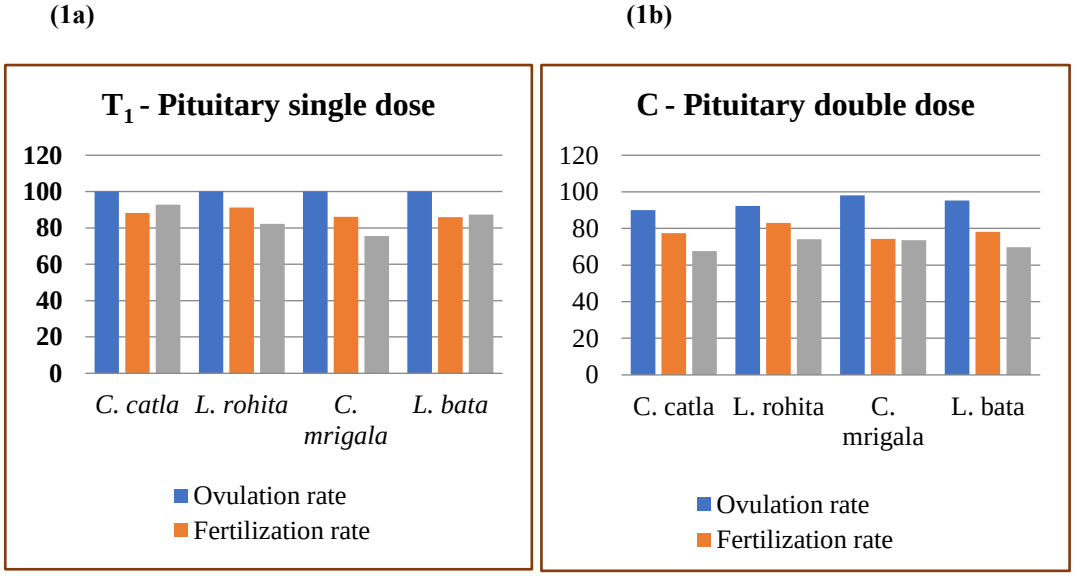

Figure 1 (a-b): Graphical representation on comparative account of fish ovulation rate, fertilization rate and hatching rate treated with different inducing agents; **(1a)** T1- Pituitary single dose and **(1b)** C- Pituitary double dose)

3.3.2. Effect of Pituitary Double Dose

Significantly best performance was observed in *Cirrhinus mrigala* (98%) on OR that decreased significantly in *Labeo bata* (95.3%), *Labeo rohita* (92.3%) and *Catla catla* (90.10%). FR record depicted highest in *Labeo rohita* (83.01%) and declined significantly in Lb(78.2%)>Cc(77.5%)>Cm(74.35%). HR was highest in Lr (74.13%) and Cm (73.65%) followed significantly by Lb (69.67%) & Cc (67.61%), presented in **Table 2** and **Figure 1b**.

3.3.3. Effect of Wova-FH

A decreasing trend of OR was found in the order of Cm (95.3%)> Cc (91.7%) >Lb(90%)>Lr (89.4%) order. FR was found highest in Cc (86.24%) followed significantly by Cm (85.6%) & Lr (84.76%) having non-significant variation between each other and significant variation with Lb(0%). HR obtained from fertilized eggs showed the decreasing trend like Cc (66.9%)> Lr (61.53%)>Cm (60.15%), presented in **Table 2** and **Figure 2a**.

3.3.4. Effect of Ovaprim

Highest OR was recorded in Cm (95.85%) and significantly decreased as Lb(94.96%)>Lr(92.36%)>Cc(88.06). FR was found best in Lr (88.88%) followed significantly by Cm (84.84%), Cc (84.61%) and (82.17%). *Labeo rohita* exhibited best response to OP and FR. HR declined significantly following the order Lr (61.7%)>Cc (60.10%)>Lb (58.70%)>Cm (57.80%) presented in **Table 2** and **Figure 2b**.

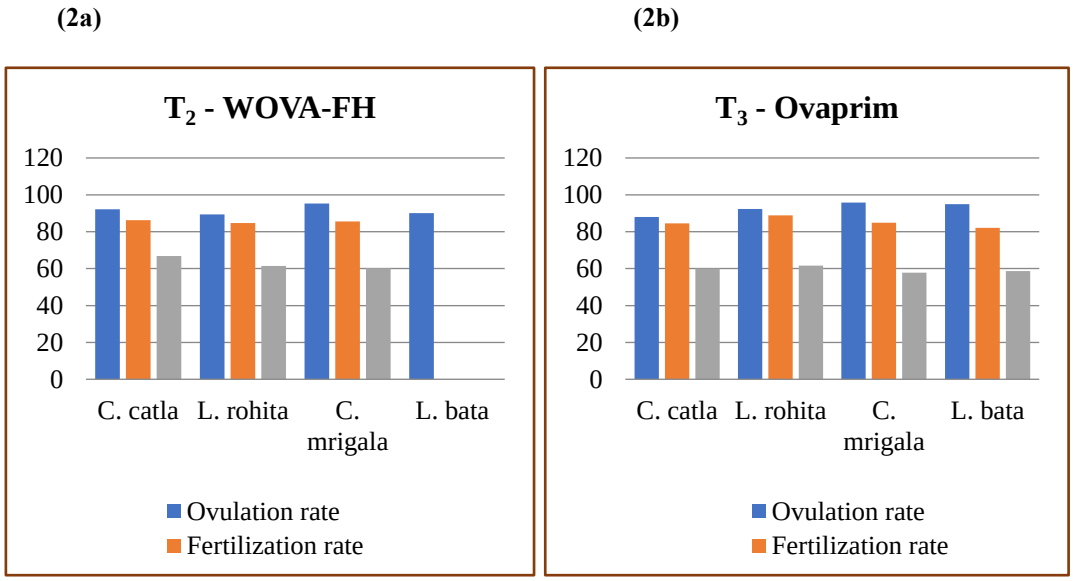

Figure 2 (a-b): Graphical representation on comparative account of fish ovulation rate, fertilization rate and hatching rate treated with different inducing agents; **(2a)** T_2- Wova –FH and **(2b)** T_3- Ovaprim

3.4. Inducibility of agents on induced breeding

Irrespective of carp species, the overall inducibility was recorded best by PSD (T1) for all the parameters viz. OR, FR and HR and the significant pattern like PSD>PDD>OP>WF for OR; PSD>OP>PDD>WF for FR and PSD>PDD>OP>WF for HR may be drawn, presented in **Table 2**. After considering OR, FR and HR as cumulative quantitative breeding parameters, hatching number per 100 number of ovulated eggs calculated from FR and it was observed that with PSD

was administration (T1) , all four selected carps showed better responsiveness (64.00–82.00) having an average value of 74.00 with a declining trend as Cc>Lr=Lb>Cm whereas PDD (C), OP and WF yielded an average FR of 56.00, 51.00 and 41.00), presented in **Table 4**.

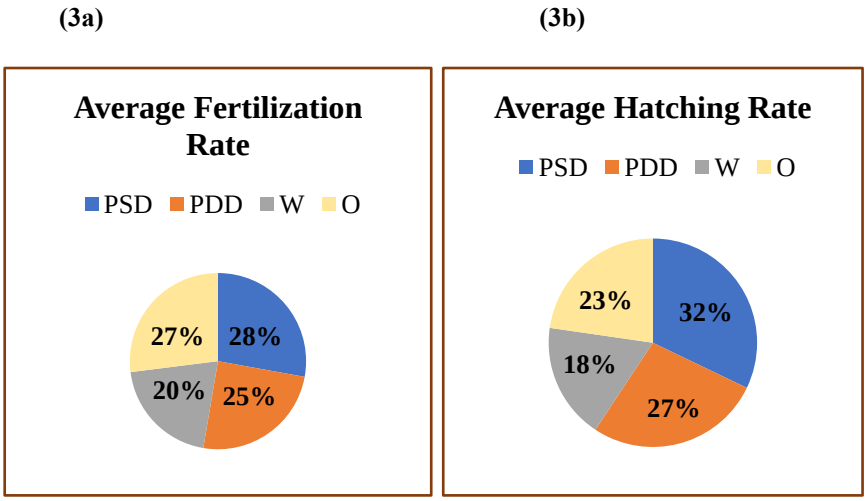

Figure 3 (a-b): Comparative account of average fertilization rate and hatching rate of fish treated with different inducing agents. (**PSD:**Pituitary single dose; **PDD:**Pituitary double dose; **W:**Wova-FH; **O:**Ovaprim)

Figure 4: Some representative images of Brood fish, applying pituitary injection, fertilized eggs in hatchery, and spawn in hatchery

It may be interpreted from the results that PSD (T1) elicited best response for all three breeding assessing parameters like OR, FR and HR in the four fish species and minimum response was generated by WF (T2). Performance of PSD was the most satisfactory on all the selected carp species with a little variation in between three species like *Catla catla, Labeo rohita and Labeo bata but Cirrhinus mrigala* performed superior over all the other species.

4. Conclusion

Our present observation reveals more potentiality with the cheaper but live pituitary gland extract to induce breeding in different carp species. The market value of synthetic hormones like Wova-FH and Ovaprim are comparatively higher (approx. 400/- per 10 ml vial) than the market value of pituitary (Rs. 3/- per pc and approx. 100 pc is required to prepare 10 ml extract). Interestingly it is observed that even single dose of pituitary injection can be more efficient than double dose. Hence, single dose pituitary administration to major carps and medium carp like Bata may strongly be recommended for the hatchery owners to save their brood fish from pushing stress as well as to get more healthy hatchlings (spawn) out of each breeding cycle. As quality fish spawn or hatchlings are the essential pre-requisites for successful fish farming, so hatchery owners may adopt the technology to improve their livelihood through entrepreneurship development and socio-economic up gradation. The present study may also illuminate further researches on the improvement of artificial breeding of variety of high value fish through optimization of hormone application, environmental manipulations and other technology improvisation.

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