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Successful Induced Spawning of Indian Major Carps Following Injection of Buserelin and Domperidone

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Abstract

The major source of carp seed in India is from induced breeding and most seed farms in Karnataka State use ovaprim for commercial production of fish fry. The present study was conducted to investigate the possibility of employing a combination of buserelin (an analogue of mammalian LHRH) and domperidone (a dopamine antagonist) for the induced spawning of the three Indian major carps, viz. catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). All the three species could be successfully induced bred using a combination of mLHRH-a and domperidone (DOM) through a single injection. Important aspects of induced spawning such as breeding response, fecundity, latent period, fertilization rate and quantity of spawn obtained with mLHRH-a +DOM were compared with those of sGnRH-a and DOM combination (ovaprim), a widely used fish spawning agent in India.

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The breeding response of females of all the three species treated with mLHRH-a+DOM was 100%, while it varied between 86 and 100% for sGnRH-a+DOM treated fish. The fecundity, fertilization rate and quantity of three-day-old spawn realized for the former combination were equal or better than those obtained for the latter. Among the different doses tested, 0.8, 0.7-0.8 and 0.7 ml•kg^{-1} body weight (b.w.) of female produced consistently good results in catla, rohu and mrigal, respectively. Lower doses (0.4-0.5 ml•kg^{-1} b.w.) also led to the mass spawning of mrigal, with fertilization rate ranging between 89 and 93%. In all the trials, the males received either sGnRH-a+DOM at 0.1-0.2 ml•kg^{-1} b.w. or mLHRH-a+DOM at one-third of the dose given to the female. The quality of carp seed produced through mLHRH-a+DOM was good and comparable to that obtained with sGnRH-a+DOM and it is more economical than the former spawning agent.

Introduction

Induced breeding of fish in India is being practised ever since the advent of the technique of hypophysation of the Indian major carps in the late 1950s (Chaudhuri and Alikunhi 1957) and the exotic carps in the early 1960s (Alikunhi et al. 1963). India produced about 17,000 million fish fry in the year 2003 and the lion's share of India's fish seed production came from induced breeding of carps (Basavaraja 2006). The contribution of the other two sources of fish seed, i.e. riverine collection and bundh breeding (breeding fish in special type of seasonal or perennial tank), was negligible (<5%). Carps are the mainstay of Indian freshwater aquaculture and account for nearly 70% of freshwater foodfish production and 95% of freshwater aquaculture production of India (Ayyappan and Biradar 2004). The three Indian major carps, viz. rohu, catla and mrigal are ranked 13th, 14th and 16th, respectively, among the top 40 cultured species in the world (Crespi 2004).

Successful hormone-induced breeding of common carp (*Cyprinus carpio*), Chinese carps (silver carp, grass carp, bighead carp, etc.), Indian carps (catla, rohu and mrigal), Thai carp (*Puntius gonionotus*) and catfish (*Clarias* spp.) have been intensified for more than one and a half decades (Lin and Peter 1996). Currently, emphasis is laid on standardization and reduction of cost of induced breeding by using gonadotropin-releasing hormone (GnRH or LHRH) and their analogues in combination with a dopamine antagonist or alone. The use of fish pituitaries or human chorionic gonadotropin (HCG) is now limited, owing to inconsistent results. The Linpe method of breeding fish with injection of the combination of GnRH agonist analogues and domperidone (DOM) antagonist drugs has

conclusively proved to be a highly effective means of inducing spawning in several cultured freshwater fish (Lin et al. 1986; Peter et al. 1988).

The country-wide field trials on the induced breeding of Indian major and Chinese carps using a commercial preparation of sGnRH-a (D-Arg^6, Pro^9 NEt) and domperidone, marketed under the trade name of ovaprim by Syndel Laboratories Inc., Vancouver, BC, Canada, have shown consistently better results than fish pituitaries (Nandeesha et al. 1990; 1991). Subsequently, ovaprim revolutionized carp seed production activities in India, replacing the traditionally used fish pituitaries. In the southern State of Karnataka, with the exception of one fish seed farm, all other farms produce carp seed solely through the application of ovaprim (Basavaraja et al. 1999) and with ovaprim, even catla, which can not easily be bred, could be spawned with ease. However, breeding carps with ovaprim has disadvantages in that a small percentage of spent fish suffer mortality. Moreover, its high viscosity poses a problem in injecting fish, it needs to be imported and is expensive.

A new indigenous formulation under the brand name ovatide with a combination of GnRH and domperidone was tested as an alternative to ovaprim (Reddy and Thakur 1998) and it also finds application in the induced breeding of carps in India (Basavaraja 2006). However, information on its efficacy and reliability is limited. With an objective of finding a suitable alternative to ovaprim and ovatide, the present study was undertaken, employing a combination of buserelin and domperidone for the induced breeding of the three Indian major carps.

Materials and Methods

The breeding trials were conducted at Bhadra Fish Seed Farm, Bhadra Reservoir Project, Karnataka, southern India. Buserelin, marketed under the brand name Suprefact by Hoechst AG, Germany, was used in combination with a common dopamine antagonist domperidone (marketed under the brand name Domperon of Johnson & Johnson Inc., USA). Ovaprim-injected fish served as control. The ovaprim used in this study had 20 µg of an analogue of salmon gonadotropin releasing hormone (sGnRH-a: D-Arg^6, Trp^7, Leu^8, Pro^9 NEt)-GnRH and 10 mg of domperidone, per ml, while the new formulation contained 20 µg of buserelin (a synthetic preparation of mammalian LH-RH) and 20 mg domperidone, per ml solution. While ovaprim is a ready-to-use solution, the combination of

buserelin and domperidone had to be prepared every time just before injection. The preparation of the hormone solution for injection was accomplished as follows: the required quantity of DOM tablets was powdered and thoroughly mixed with distilled water as required. The required quantity of buserelin was then added to the solution of DOM, properly stirred and the required quantity of the mixture was injected to the carp brooders.

Mature male and female broodfish was selected based on their external sexual characters (Jhingran and Pullin 1985). The females of the three species were injected intramuscularly with buserelin+DOM solution at doses ranging from 0.4 to 0.8 ml·kg^{-1} b.w., while the males received half or a third of the dosage of female, as a single dose. Lower doses were also attempted. The control females and males were given ovaprim at 0.3-0.5 and 0.1-0.2 ml·kg^{-1} b.w., respectively. In all cases, the females and males were 2-4 years old.

In all cases, both females and males were injected at the same time at around 16-18 h so as to facilitate spawning around midnight or early morning. The injected brooders were released for spawning either in circular concrete tanks (4.0 m dia. x 1.2 m high) or rectangular cloth hapas (2 x 1 x 1 m), with water circulation. The female:male ratio in the breeding pool was around 1:1.5 or 1:2. The following morning, the spawning was ascertained and then the spent brooders were removed from the breeding tank. The spawning response was determined by observing residual eggs in the spent fish and categorized as complete or partial spawning or no response, and was expressed as a percentage. The females with oozing eggs and plugged genital opening were classified under no response category. Mature males freely oozed out milt, including spent males, with a slight pressure on the abdomen. The latent period, relative fecundity and fertilization rate were calculated. The hatching of eggs was carried out in the Chinese type circular concrete incubating tanks with moderate water circulation (80-120 L·minute^{-1}) where the stocking density of eggs varied between 2 and 4 million eggs per tank (4 m dia. x 1.2 m high). The survival from fertilization to three-day-old spawn was calculated. Mann Whitney U-test was used to determine significant difference between any two treatments.

Results

The general weather conditions that prevailed in the months of June, July and August were conducive for the breeding of the carps. Cool

weather (temperature 23-26^{0}C) and overcast sky with drizzles resulted in higher breeding responses.

The results of the different trials on the induced breeding of the three Indian major carps with buserelin+DOM and sGnRH-a+DOM are presented in tables 1 to 4. All the female catla injected with buserelin+DOM (0.8 ml•kg^{-1} b.w.) as well as sGnRH-a+DOM (0.5 ml•kg^{-1} b.w.) spawned completely, as indicated by the breeding response (Table 1). However, catla treated with buserelin+DOM at 0.5 ml•kg^{-1} b.w. failed to ovulate. The number of catla females injected with buserelin was small as sufficient number of broodfish was not available at that time. Buserelin+DOM mixture resulted in a slightly higher fecundity and quantity of three-day-old spawn than sGnRH-a+DOM. However, the fertilization rate was slightly higher with the latter than the former.

All the 35 female rohu injected with buserelin+DOM at 0.7-0.9 ml•kg^{-1} b.w. spawned completely (100% breeding response), while the breeding response was 94.8% for the sGnRH-a+DOM (0.4 ml•kg^{-1} b.w.) injected females (30 kg) (Table 2). The fecundity and the quantity of three-day-old spawn were significantly higher ($p<0.10$) in buserelin-treated fish than the control. On the contrary, the fertilization rate was marginally lower in the former than the latter. Lower doses of buserelin+DOM induced partial spawning or no response, the results of which are not presented here.

Injection of mrigal with buserelin+DOM mixture at 0.7 ml•kg^{-1} b.w. yielded higher breeding response than sGnRH-a+DOM combination (Table 3). The fecundity, fertilization rate and quantity of spawn obtained were not significantly different between the two hormones. The results of mass spawning of mrigal injected with different doses of buserelin+DOM mixture are presented in table 4. Both 0.4 and 0.5 ml•kg^{-1} were found to be equally effective in inducing spawning in mrigal.

In general, the injected rohu and mrigal took more time (latent period: 7-11 h) for spawning than catla (7-9 h). When large-scale spawning of mrigal was undertaken, the latent period was prolonged up to 12 hours.

The cost comparison between sGnRH-a+DOM and buserelin+DOM mixture was as follows: the cost of one bottle of 10 ml of sGnRH-a+DOM was Rs. 350/- (US$1 = Rs. 45/-), while the cost of buserelin+DOM combination worked out to be Rs. 100/- per 10 ml.

Table 1. Breeding response, relative fecundity, fertilization rate and quantity of spawn of catla injected with sGnRH-a+DOM or Buserelin + DOM (Mean ± SD)

Water temperature (^{0}C)	Number/ weight of ♀ brooders (Kg)	Spawning agent	Dosage (ml•kg^{-1} b.w.)	Latent period (hrs)	Breeding response (%)	Relative fecundity (lakh•kg^{-1} b.w.)	Fertilization rate (%)	Quantity of 3-day-old spawn (lakh•kg^{-1} b.w.)
24-26	20/49	sGnRH-a+DOM	0.50	8-9	100 (3)	1.84 ±0.43 (5)	95.8±3.11 (5)	1.38±0.33 (5)
24-26	4/11.5	Buserelin + DOM	0.80	8-9	100 (3)	1.90±0.07 (3)	93.33±1.53 (3)	1.53±0.03 (3)
Value of U-test statistic						6		4

Males were injected with either sGnRH-a+DOM at 0.1-0.2 ml•kg^{-1} b.w. or buserelin + DOM at 0.4 ml•kg^{-1} b.w.
Numbers in parentheses indicate sample size, i.e. no. of sets of breeders, each set consisting of 1 to 8 females. P= 0.10

Table 2. Breeding response, relative fecundity, fertilization rate and quantity of spawn of rohu obtained when injected with sGnRH-a + DOM or buserelin + DOM (Mean ± SD)

Water temperature (^{0}C)	Number/ weight of ♀ brooders (Kg)	Spawning agent	Dosage (ml•kg^{-1} b.w.)	Interval between injection & spawning (hrs)	Breeding response (%)	Relative fecundity (lakh•kg^{-1} b.w.)	Fertilization rate (%)	Quantiy of 3-day-old spawn (lakh•kg^{-1} b.w.)
24-26	30/51	sGnRH-a+DOM	0.40	9-11	94.80±5.15 (3)	1.98 ±0.18(4)	90±3.60 (4)	1.01±0.41 (4)
24-26	35/55.75	Buserelin + DOM	0.7-0.8	7-11	100 ±0 (5)	2.58±0.56 (4)	93.75±0.96 (4)	2.02±0.63 (4)
Value of U-test statistic						2*		1*

Males were injected with either sGnRH-a+DOM at 0.1-0.2 ml•kg^{-1} b.w. or buserelin + DOM at 0.35-0.4 ml•kg^{-1} b.w.
Numbers in parentheses indicate sample size, i.e. no. of sets of breeders, each set consisting of 2 to 10 females. P= 0.10

Table 3. Breeding response, relative fecundity, fertilization rate and quantity of spawn of mrigal obtained when injected with sGnRHa+DOM or buserelin + DOM (Mean ± SD)

Water temperature (^{0}C)	Number/ weight of ♀ brooders (Kg)	Spawning agent	Dosage (ml•kg^{-1} b.w.)	Interval between injection & spawning (hrs)	Breeding response (%)	Relative fecundity (lakh•kg^{-1} b.w.)	Fertilization rate (%)	Quantiy of 3-day-old spawn (lakh•kg^{-1} b.w.)
24-26	12/19.25	sGnRH-a+DOM	0.30	7-9	86±12.77 (3)	1.57 ±0.39(3)	91.67±1.53 (3)	1.24±0.41 (3)
24-26	8/14.50	Buserelin + DOM	0.7	7-9	100 ±0	1.41±0.266 (3)	92.33±2.52 (3)	1.15±0.27 (3)
Value of U-test statistic						3		4

Males were injected with either sGnRH-a+DOM at 0.15 ml/kg b.w. or buserelin + DOM at 0.3 ml/kg b.w.
Numbers in parentheses indicate sample size, i.e. no of sets of breeders, each set consisting of 1 to 5 females.P= 0.10

Table 4. Mass spawning of mrigal injected with buserelin (20 µg•ml^{-1}) and DOM (20 mg•ml^{-1})

Trial	Age of breeder (yr)	Number and weight of ♀brooders (Kg)	Number and weight of ♂ brooders (Kg)	Water temperature (^{0}C)	Dosage (ml•kg^{-1} b.w.)	Latent period (hrs)	Fertilization rate (%)	Total quantity of eggs obtained (lakhs)	Total quantiy of 3-day-old spawn (lakhs)	Remarks
I	2$^+$	20 21	30 29	24.6	♀: 0.5 ♂: 0.2	10-12	93	18.00	16.50	20% of spent fish suffered mortality
II	2$^+$	6 6.9	9 9	24.8	♀: 0.5 ♂: 0.2	10-12	92	6.70	4.80	10% of spent fish died after 2 days
III	3$^+$	10 18.75	20 24.75	25	♀: 0.4 ♂: 0.2	10-12	92	24.00	18.00	20% of spent fish suffered mortality
IV	2$^+$	10 11.2	15 22	24.5	♀: 0.4 ♂: 0.2	10-12	89	9.79	8.00	No mortality of spent fish

Discussion

The results of the present study indicate that a single injection of buserelin+DOM mixture to the three Indian major carps resulted in successful ovulation and spawning. The breeding response, fecundity, fertilization rate and spawn survival were comparable, even better in some cases, with those obtained through single administration of sGnRH-a+DOM. Nandeesha et al. (1990) reported successful breeding of several species of carps with single injection of sGnRH-a+DOM (ovaprim) vis-à-vis fish pituitaries. They have standardized and recommended the dosages of ovaprim for the large-scale production of carp seed in India. The dosages for different species of carps are: catla - 0.5 ml, rohu - 0.4 ml and mrigal - 0.3 ml (Nandeesha et al. 1991).

The Linpe method, a technique of inducing ovulation and spawning with a combination of a mammalian LHRH analogue (mLHRH-a) or a salmon GnRH-a and a dopamine antagonist, domperidone or pimozide, has proved to be highly effective in a number of cultured teleosts (Lin and Peter 1996). In the present study, the doses of buserelin+DOM attempted indicate that the minimum dosage required for successful spawning of Indian major carps is higher than that of sGnRH-a+DOM, which is probably attributed to the higher potency of the latter as reported by Peter et al. (1985; 1987) who observed sGnRH-a (D-Arg^6, Pro^9, Net) to be 17 times more potent than mLHRH-a (D-Ala^6, Pro^9 NEt). Lin et al. (1988) also observed better results with sGn RH-a than LHRH-a in silver carp and loach. The differential dose response rate of the three species to buserelin+DOM may be attributed to differential dopamine activity in them. Lin and Peter (1996) reviewed dopamine activity in various fish species and indicated that it may vary considerably between species. Das (2000) successfully induced spawning in Indian major carps and a catfish with a combination of mammalian GnRH-a (D-Ala^6, Pro^9 NEt) and metoclopramide, marketed under the trade name ovopel. The structure of the analogue of mLHRH present in buserelin (used in the present investigation) is not known. However, De Leeuw et al. (1988) had earlier used buserelin which contained [(a synthetic preparation of mammalian LH-RH; D-Ser (TBU)-LHRH (1-9) nonapeptide-ethylamide]. They compared the efficacy of sGnRH-a (D-Arg^6, Pro^9 NEt) with mGnRH-a (buserelin) and opined that the *in vitro* activity of both sGnRH-a and buserelin was similar in the African catfish (*Clarias gariepinus*). Published information on the use of buserelin as a carp spawning agent is lacking. To date, several spawning

agents and drugs have been tested for the breeding of fishes, with great success (Harvey and Hoar 1979; Chang and Peter 1983; Billard et al. 1984; Sokolowska et al. 1984; Lin et al. 1988; Peter et al. 1988; Nandeesha et al. 1990; Harvey and Carolsfeld 1993; Lin and Peter 1996).

Buserelin+DOM (14-16 µg+ 14-16 mg•ml^{-1}) treatment to rohu resulted in better breeding response, fecundity, fertilization rate and higher quantity of spawn than did sGnRH-a+DOM treatment. One significant feature of the present study is that rohu injected with buserelin yielded double the spawn that was obtained with ovaprim. The effective dose of buserelin required for successful ovulation in mrigal was slightly less, i.e. 0.4-0.5 ml•kg^{-1} b.w. and the results obtained with both the hormones were comparable. The dosage of buserelin+DOM mixture could possibly be further reduced if trials are conducted a little earlier in the natural spawning season of the major carps, but before August, particularly for catla and rohu which undergo resorption earlier than mrigal. Nandeesha et al. (1990) reported poorer spawning response and fertilization rate in trials that were carried out towards the end of the breeding season. Although there is no concrete evidence about the relationship between the dose of hormone and temperature and gonadal state of fish, Rao and Gnaneshwar (1988) found a linear relationship between dose and temperature. On account of this, fish breeders inject generally a higher dose towards the end of the carp breeding season.

The interval between injection and spawning did vary a little between different species, with rohu and mrigal taking two hours more than catla. The ovulation time was short and it was always predictable. The results of the present investigation clearly demonstrate that buserelin+DOM combination is as effective as (even better in rohu) sGnRH-a+DOM mixture in inducing spawning of major carps. The marginally higher dose of buserelin+DOM combination will be compensated by its much lower cost, apart from being less viscous and easy to inject. The mortality of mrigal observed following mass spawning may be attributed to poor broodstock handling. The results obtained with single injection are also significant because this saves considerable amount of time, labour and reduces handling stress on broodfish.

The cost of induced spawning of Indian major carps with buserelin+DOM has been found to be less as compared with sGnRH-a+DOM. However, further work is needed to reduce the dose of buserelin+DOM, dose of DOM in particular, by conducting trials during the peak breeding season and evaluate the viability of spent fish and the resultant fry.

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