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D-Lys^6 Salmon Gonadotropin-Releasing Hormone Analogue-Domperidone Induced Ovulation in *Cl arias batrachus* (L.)

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Abstract -A single intramuscular injection of D-Lys^6 salmon gonadotropin-releasing hormone analogue (sGnRH-A) at a dose of 50 $\mu g \cdot kg^{-1}$ body weight (b. wt.) in combination with domperidone at 5 $mg \cdot kg^{-1}$ b. wt. induced final maturation and ovulation in *Clarias batrachus*. The fish did not spawn spontaneously but released eggs on handstripping. Fertilization and hatching rates were 70-80% and 50-60%, respectively. sGnRH-A alone did not induce ovulation at a dose as high as 500 $\mu g \cdot kg^{-1}$ b. wt. Comparable results in terms of spawning, fertilization and hatching were obtained when pituitary extract was injected at a dose of 40 $mg \cdot kg^{-1}$ b. wt.

Clarias batrachus is a highly priced air-breathing fish in India. In nature, *C. batrachus* breeds once a year during the spawning season (July-August). Like many other teleosts, it does not breed spontaneously in confinement and hence development of reliable methods for induced breeding is needed. Earlier studies evaluated mammalian luteinizing hormone releasing hormone (LHRH) analogue alone or in combination with a dopamine antagonist for induced spawning and ovulation in *Clarias* species (Ngamvongchon et al. 1988; Manickam and Joy 1989; Tan-Fermin 1992; Tan-Fermin and Emata 1993). In studies evaluating the structure-activity relationship of GnRH peptides, analogue(s) of sGnRH exhibited maximum activity (De Leeuw et al. 1988; Habibi et al. 1989). It was observed that proximity of the GnRH variety to the fish species (with respect to its position in the evolutionary ladder) confers higher activity in the fish (Lin et al. 1991). The use of sGnRH-A in combination with a dopamine antagonist has not been evaluated for induced spawning in *C. batrachus*. Therefore, in the present report, sGnRH-A-domperidone combination was used to induce spawning of *C. batrachus*.

The experiments were carried out on a fish farm in Bhopal, India, during the breeding season (July-August). Mature male and female broodfish (200-400 g) were used. Females were selected on the basis of soft and distended belly.

pGlu-His-Trp-Ser-Tyr-D-Lys^6-Trp-Leu-pro-Gly NH_2 sGnRH-A, a decapeptide, was synthesized by the Merrifield solid phase method using tertiary butyl oxycarbonyl (t-boc) chemistry on 4-methyl benz hydroxyl amine (MBHA) resin

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(Rivier et al. 1986). Synthetic D-Lys^6 sGnRH-A was purified, lyophilized and resuspended in degassed saline as described earlier (Alok et al. 1993). Domperidone tablets (Cadilla, India) and lyophilized pituitary extract were resuspended in normal saline.

Female fish were given single intramuscular injections of different doses of D-Lys^6 sGnRH-A with or without domperidone. Corresponding male pairs were given half the dose administered to female fish. Control fish received a standard dose of pituitary extract (40 mg·kg^{-1} b. wt.) or saline.

Experimental fish were checked for ovulation 40 h post-treatment. Testes of male broodfish were biopsied and pooled to avoid variation in fertility. Pooled testes were then macerated in Holtfreter's solution (0.67 mM KCl, 60.2 mM NaCl, 1.3 mM $CaCl_2$, 2.3 mM $NaHCO_3$). The eggs were collected by handstripping in separate plastic troughs for each group. The eggs and milt were gently mixed with a feather followed by gentle rotation of the contents to ensure proper fertilization. Excess milt was removed by washing the eggs several times. The eggs were incubated under a flowthrough system. To prevent fungal infection, dead eggs which turned opaque were removed immediately and scored. Embryos developing to morula stage, in which a compact mass of dividing cells was observed at the animal pole, were taken as an index of fertilization; and embryos reaching larvae stage were taken as an index of hatching. Fertilization and hatching rates were estimated from the ratio of fertilized eggs and the ratio of larvae to the total number of eggs spawned, respectively.

Different species of fish respond differently when induced with GnRH or their analogue(s) (Breton et al. 1990; Fermin 1991). The variation in response can be attributed to species specificity, amino acid sequence of the GnRH or their analogue(s) (Peter et al. 1987), maturation stage of oocytes (Tan-Fermin 1992; Tan-Fermin and Emata 1993), dose of the inducer, and presence of inhibitory mechanisms (Fermin 1991; Lin et al. 1991). In the present study, the efficiency of D-Lys^6 sGnRH-A in combination with domperidone on the induced spawning of *C. batrachus* was demonstrated. D-Lys^6 sGnRH-A-domperidone administration induced ovulation as indicated by the release of a stream of eggs on gentle abdominal pressure. A single dose of D-Lys^6 sGnRH-A at 50 μg·kg^{-1} b. wt. in combination with domperidone at 5 mg·kg^{-1} b. wt. induced ovulation in all the fish injected. However, fish administered with D-Lys^6 sGnRH-A alone at different doses neither spawned spontaneously nor released eggs upon handstripping at least until 40 h after injection. The minimum dose required to induce ovulation in *C. batrachus* was 50 μg·kg^{-1} b. wt. of sGnRH-A in combination with domperidone. This dose is similar to that reported earlier in *Clarias* species (Manickam and Joy 1989; Tan-Fermin and Emata 1993). Eggs were released only upon handstripping even when fish were injected with higher doses of sGnRH-A (100-200 μg·kg^{-1} b. wt.) in combination with domperidone (5 mg·kg^{-1} b. wt.; Table 1). The inability of sGnRH-A alone to induce ovulation in *C. batrachus* may have been due to the presence of a relatively strong dopamine-mediated inhibitory mechanism as reported in other fishes (Fermin 1991; Lin et al. 1991). The present study indicates that, as in carps, a dopamine antagonist is indispensable in order to induce ovulation in *C. batrachus*.

Table 1. Induced ovulation of *C. batrachus* at various doses of D-Lys^6 sGnRH-A alone and in combination with a constant dose of domperidone.

Treatment	No. of fish	Dose		No. of fish spawned	Fertilization[2] (%)	Hatching (%)
		sGnRH-A ($\mu g \cdot kg^{-1}$ b. wt.)	Domperidone ($mg \cdot kg^{-1}$ b. wt.)			
D-Lys^6 sGnRH-A	4	500	—	0	—	—
"	4	200	—	0	—	—
"	4	100	—	0	—	—
"	4	50	—	0	—	—
"	4	10	—	0	—	—
D-Lys^6 sGnRH-A + domperidone	4	200	5	4	70-80	50-60
"	4	100	5	4	70-80	50-60
"	4	50	5	4	70-80	50-60
"	4	10	5	0	—	—
Pituitary[1] extract	4	—	—	4	70-80	50-60

[1]40 $mg \cdot kg^{-1}$ b. wt. of pituitary extract was injected in a single injection.
[2]Eggs released after handstripping were mixed with the testes homogenate for fertilization.

The released eggs fertilized by testes homogenates of control males or of males injected with the analogue resulted in comparable rates of fertilization and hatching (data not shown). Pituitary extract at a dose of 40 $mg \cdot kg^{-1}$ b. wt. also induced ovulation and the fish spawned on handstripping within 40 h, releasing fertilizable eggs. The percentage fertilization and hatching were comparable when fish were injected with D-Lys^6 sGnRH-A and domperidone or pituitary extract. A seasonal, diurnal and sexual variation in gonadotropic potency of pituitary extracts has been reported (Lam 1982). The potency is also affected by the method of storage and preparation of these extracts. Thus, the standardization of pituitary extract dosage becomes important. The use of sGnRH-A analogue overcomes the problem of variability in gonadotropic potency of pituitary extracts as well as the inadequate supply and ever increasing cost of pituitary glands.

Earlier studies by Manickam and Joy (1989) have reported fertilization and hatching rates of 90% and above. A lower percentage of fertilization and consequent hatching in the present study is probably because the fish were handstripped after 40 h and some of the eggs released may have been overripe, leading to a greater number of non-viable eggs. Earlier studies have demonstrated the importance of the time of stripping to obtain fertilizable eggs (Zonneveld et al. 1988; Kestemont 1988; Tan-Fermin 1992).

In conclusion, the present study proposes the use of D-Lys^6 sGnRH-A along with domperidone as an efficient method for artificial breeding of *C. batrachus*.

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