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Induction of Ovulation in Tawes, *Puntius gonionotus* Bleeker by Analogue of LH-RH

Uthairat Na-Nakorn[1]

ABSTRACT

A study on effect of LH-RH analogue, [D-Ser (Bu^t)^6] LH-RH (1-9) ethylamide (Hoe 766) on ovulation of Tawes, *Puntius gonionotus* Bleeker was conducted at Aquaculture Department, Faculty of Fisheries, Kasetsart University in June, 1984. The results showed that LH-RH analogue alone could not induce ovulation of Tawes, whereas injection of 100, 150, 210 μg/kg of this hormone preceded the injection of 5 mg/kg progesterone with 6 hours interval yielded successful ovulation. It was concluded that LH-RH analogue was not commercially effective in inducing ovulation of Tawes because two injections were needed compared to only a single injection of pituitary suspension with the same successful ovulation result.

INTRODUCTION

Successful induced spawning of Tawes, *Puntius gonionotus* using hormone injection technique was achieved in 1967 (Boonbrahm *et al.*, 1968). A single injection of crude pituitary suspension of Chinese carp (1.5 kg donors/1 kg brooders) brought about spawning 4-6 hours later. Human Chorionie Gonadotropin (HCG) was also used in combination with pituitary suspension since HCG alone could not induce ovulation of this species (Ngamvongchon, 1981)

Although pituitary suspension has been effectively used through out the country other potential hormones are also looked for to provide more convenient practice and standardized amount of hormone used. LH-RH analogue is one of the promising hormones used in inducing ovulation in various fish species (Donaldson et al., 1981; Fitzpatrick et al., 1984; Doroshov and Lutes, 1984 and Billard et. al., 1984) including *Clarias macrocephalus* which is an economically important fish species in Thailand. (Na-Nakorn, 1987). Therefore, these experiments were conducted in order to study whether LH-RH analogue, [D-Ser (Bu^t)^6] LH-RH (1-9) ethylamide is effective in inducing ovulation of Tawes and to find its potentiality in replacing pituitary suspension in commercial scale of seed production of this species.

MATERIALS AND METHODS

Two experiments were carried out in June, 1984. Gravid female tawes, selected on the basis of their swollen and soft bellies, were injected intramuscularly with [D-Ser (Bu^t)^6] LH-RH (1-9) ethylamide (Hoe 766), with or without Progesterone, and pituitary suspension of Rohu, *Labeo rohita* as was detailed in Table 1.

Male brooders used in the two experiments were not injected because of their plenty of milt.

[1]Aquaculture Department, Faculty of Fisheries, Kasetsart Univ.

Ovulation was checked 4 and 6 hours after injection of the last dose. When ovulation eggs were striped and fertilized with striped, fresh milt using modified dry method. Two replications of approximately 300 fertilized eggs from each treatment were separately incubated in hapas in order to estimate hatching percentage.

The Fisher's exact one-tail test was used in order to compare the number of ovulated fish between experimental groups. (Langley, 1970)

Table 1 Treatment in the various experimental groups of experiment 1 and 2.

	Group no.	No. females per group	Wt. of Females mean	S.D.	Injection protocol (dosage/kg body wt.) Hour 0	Hour 6
1st EXPERIMENT						
14 JUNE, 1984	1	3	470.0	118.5	LH-RHa 100 μg	—
	2	3	426.0	191.0	LH-RHa 150 μg	—
	3	3	413.0	186.6	LH-RHa 210 μg	—
	4	3	451.0	171.3	PG 1.3 kg	—
2nd EXPERIMENT						
15 JUNE, 1984	1	5	465.0	213.4	LH-RHa 100 μg	P 5 mg
	2	5	428.0	184.8	LH-RHa 150 μg	P 5 mg
	3	5	436.0	183.5	LH-RHa 210 μg	P 5 mg
	4	5	550.0	168.9	pG 1.3 kg	—

Note: P-Progesterone

PG-Pituitary Gland (from *Labeo rohita)*

RESULTS

Number of female ovulated, mean hours to ovulation and hatching percentages were given in table 2. A result of the first experiment showed that LH-RH analogue alone could not induce ovulation of Tawes whereas pituitary suspension induced ovulation of all injected females with high hatching percentage.

Table 2 Ovulatory responses of Tawes to treatments with LH-RH analogue with or without Progesterone and pituitary suspension. Injection protocol, numbers of ovulated females, mean hours to ovulation and hatching percentages are presented for each experimental group.

Injection protocol (dosage/kg. body wt.)		Number of females ovulated	Hours to ovulation mean	S.D.	Hatching percentage mean	S.D.
1st EXPERIMENT (14 JUNE, 1984)						
Hour 0						
LH-RHa 100 μg		0/3	-	-	-	-
LH-RHa 150 μg		0/3	-	-	-	-
LH-RHa 210 μg		0/3	-	-	-	-
PG 1.3 kg (donor)		0/3	-	-	-	-
2nd EXPERIMENT (15 JUNE, 1984)		3/3	4h. 15m.	10m.	80.10	13.21
Hour 0	Hour 6					
LH-RHa 100 μg	P 5 mg	3/5	4h. 20m.	52m.	63.03	17.09
LH-RHa 150 μg	P 5 mg	5/5	4h. 38m.	27m.	66.29	24.15
LH-RHa 210 μg	P 5 mg	4/5	4h. 45m.	10m.	83.87	11.44
PG 1.3 kg (donor)	—	5/5	4h. 18m.	7.5m.	72.99	11.12

Note: P - Progesterone

PG - Pituitary Gland (from *Labeo rohita)*

In the second experiment injection of 5 mg/kg Progesterone following 3 different doses of LH-RH analogue could induce ovulation. Al though there is a slight difference among number of ovulated females in each experimental group, that difference was, however, not statistically significant. Means and standard deviations of hatching percentage were shown in figure 1. Mean hatching percentage seemed to increase as higher doses of LH-RH analogue were injected with even the lowest hatching value (63.03%) was still acceptable for commercial seed production practice.

DISCUSSION

When induced spawning by injection of pituitary suspension is practised Tawes seems to show the best response among various fish species in Thailand for very low dose in only one injection is needed and shorter latency period of about 4-6 hours is required (Boonbrahm *et al.*, 1968). On the contrary, a negative result was shown when using LH-RH analogue alone. In experiments on inducing ovulation of *Carassius auratus* and *Cyprinus carpio* which belong to the same family as Tawes limited success was achieved using LH-RH analogue al though blood gona-

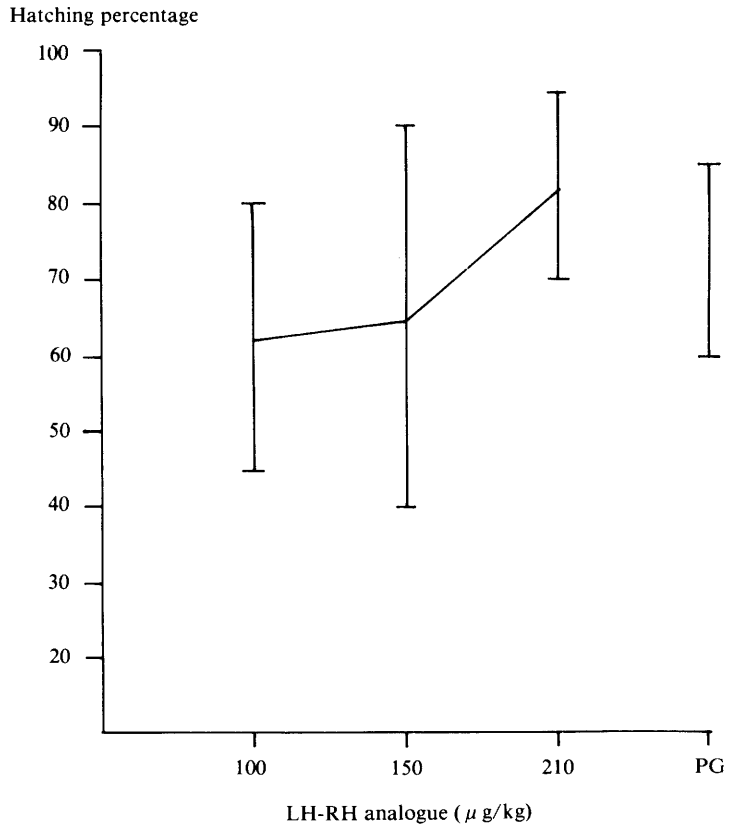

Figure 1 Mean and S.D. of hatching percentages of Tawes eggs injected with 100, 150, 210 μg/kg LH-RH analogue +5 mg/kg Progesterone and pituitary gland.

dotropin level was increased. (Wiel et al., 1980 and Sokollowska et al., 1980). Some authors suggested that the presense of GRIF (Gonadotropin Releasing Inhibitory Factor) in those species might be the cause of the negative result (Peter et al., 1978). Tawes might have GRIF, therefore, when LH-RH analogue was administered its action was inhibited to some extent. Consequently, surge of serum gonadotropin level could not be reached and then resulted in no ovulation response.

Ovulation of some fish species was found following a sharp rise in plasma level of 17∞-20β Progesterone (Fostier and Jalabert, 1982). Based on this observation it can be supposed that injection of Progesterone following LH-RH analogue in this experiment caused the increase of 17 ∞-20 β Progesterone which probably was maturation steroid in serum of this species and finally triggered ovulation. The study of Jalabert et al. (1987) also supported this explanation for they found that successful ovulation induced by steroid hormone required previously increas of serum gonadotropin level which stimulated the synthesis and storage of ovulation mediator.

The conclusion was drawn, however, that LH-RH analogue alone was not effective in inducing ovulation of Tawes Though LH-RH

analogue together with *Progesterone* gave successful induced spawning result, but not economicly feasible because two injection were needed compared to only a single injection of pituitary suspension with the same successful ovulation result.

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