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# Induction of Spawning in Common Carp (*Cyprinus carpio*) Using LHRHa ([D-Ser (tBu)$^6$, Pro$^9$-NEt]-LHRH) Combined with Haloperidol: Effects of Different Treatment Time and Determination of Latency Period Dependence on Temperature

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**Abstract**

A single injection of 20µg/kg LHRHa ([D-Ser (tBu)$^6$, Pro$^9$-NEt]-LHRH) combined with 0.5 mg/kg of the dopamine receptor antagonist, Haloperidol (LHRHa+HAL) was used for induction of spawning in the common carp under routine hatchery conditions. This treatment caused successful ovulation. Latency in common carp injected with LHRHa+HAL was dependent on water temperature, and minimal latency was 14 h. When LHRHa+HAL was given at various hours of the day it resulted in identical spawning with the same latency. These results show that the predicting latency at a given temperature may provide successful hatchery management for fish farmers.

*Key Words*: induction spawning, LHRHa, haloperidol, common carp.

## Introduction

Induced spawning in common carp is realised by injecting carp pituitary extract (CPE) during the spawning period. Using CPE may be simply processed or calibrated containing a predetermined dose of gonadotropin (GtH) (Yaron *et al*., 1984). CPE is given to broodstocks as priming and resolving doses. CPE must contain enough GtH amount to induce spawning successfully in common carp broodstock. For this purposes, CPE is standardised in respect of the GtH level and presented to aquaculturists commercially. This successful application delayed the introduction of the new approaches into aquaculture.

On the other hand, the increasing of the cyprinid culture in the world caused the problem in the presenting of calibrated CPE to aquaculturists. This led to the development of a new approach in the inducing of spawning for cyprinid fish. In this approach, different LHRH forms and their analogues, stimulating of endogenous GtH release, are used with a dopamine receptor antagonist (DA), which potentiates the response to the peptide (e.g., Lin *et al*., 1986, 1988; Peter *et al*., 1987, 1988, 1993; Zohar, 1986).

Latency of the response (the duration between treatment and ovulation) significantly depends on the form of LHRH and LHRHa, the type of DA, using or not using DA, and also environmental conditions (especially the temperature). (e.g., Rottmann and Shireman, 1985; Peter *et al*., 1987; Glubokov *et al*., 1991: Drori *et al*., 1994). Thus, in order to make a realistic recommendation to aquaculturists, determining the dose and latency period at different water temperatures of LHRHa+DA combinations had to be studied under local conditions (Drori *et al*., 1994).

Ovulation response changed, with CPE treated carp during various hours of the day. (Bieniarz *et al*., 1985). However, the sLHRHa combined with a DA treatment at various hours of the day does not affect the ovulatory response in carp (Drori *et al*., 1994). Therefore, it is essential to clarify whether the ovulatory response of carp to different LHRHa+DA combinations under local conditions differs when treatment is given at various hours of the day.

The goals of this study are;

(1) testing of the LHRHa ([D-Ser (tBu)$^6$, Pro$^9$-NEt]-LHRH) in combination with a DA (Haloperidol) given in a single injection, which would be suitable for spawning induction in common carp under local conditions.

(2) determining the precise latency periods between treatment and ovulation at different water temperatures

(3) and what the ovulatory response is, when LHRHa+ DA (Haloperidol) is given at various hours of the day,

comparing of LHRHa+ DA with CPE partially.

## Materials and Methods

### Fish and fish maintenance

The experiments were conducted on female common carp (*Cyprinus carpio*) in Aksan Carp and Ornamental Fish Hatchery (Manisa, Turkey). Female (1.0-2.25 kg BW) and male broodstocks stocked separately in earthen pond, picked the soft abdomen ones, and than transferred them for acclimation into 750-litre tanks with circulating and aerating water at

different temperatures (20-26 °C). After two or three days ovarian biopsies were obtained, clarified and examined as described in Levavi-Zermonsky and Yaron (1986). The stage of oocyte maturation was determined as follows: Stage I, central germinal vesicle (GV); Stage II, migrating GV; Stage III, peripheral GV; Stage IV, GV breakdown (GVBD); Stage V, ovulated eggs in ovarian lumen. Only fish in which more than 60% of the oocytes were at Stage II were selected for the spawning experiments.

Hormones and drugs were injected into the base of pectoral fin at a volume of 0.5 ml/kg. After 10 hours of injection, the bottom of each tank was controlled every 30 min for the presence of released eggs, which indicates that ovulation has occurred. Each female, emitting some eggs, blotted, and the eggs were then stripped by abdominal pressure. Fertilization and incubation were carried out according to Rothbard (1981).

### Hormones and drugs

[D-Ser (tBu)$^6$, Pro$^9$-NEt]-LHRH used as LHRH analogue was provided by Dr. Fikri Balta as a gift. Haloperidol (HAL) and Domperidon (DOM) used as Dopamine antogonist and was prepared for injection according to Omeljaniuk *et al*. (1987) and Chang *et al*. (1984) respectively. DOM was used for determining the optimal dose of LHRHa (Drori *et al*., 1994). The optimal dose of LHRHa and HAL was determined according to Drori *et al*. (1994).

### Statistical analysis

The significance of differences between groups in the spawning experiments was tested by one-way analysis of variance and Duncan's multiple range tests.

## Results

### The optimal dose of LHRHa

The following experiment was designed to determine the lowest effective dose of LHRHa ([D-Ser(tBu)$^6$, Pro$^9$-NEt-LHRH). Fifty-four female carp were divided into six groups and administered with increasing doses of LHRHa combined with a constant dose of DOM (5 mg/kg). Saline (0.7 %) injected fish were used as a negative control to examine the possibility of spontaneous spawning under the hatchery conditions, while CPE-injected fish served as a positive control (Table 1).

None of the fish injected with saline or the lowest dose of the LHRHa spawned. The spawning rate was similar in CPE-treated fish and in fish injected with either 20 or 50 µg/kg LHRHa+5 mg/kg DOM (Table 1).

### The optimal dose of haloperidol

Thirty three fish injected with LHRHa (20 µg/kg) combined with 0.5 mg/kg HAL spawned

**Table 1.** Spawning induction in mirror carp (1-1.5 kg BW) using graded doses of LHRHa ([D-Ser (tBu)$^6$, Pro$^9$-NEt]-LHRH) alone or combined with a constant dose of Domperidon (DOM). FTS treatment group is the negative control; CPE (0.2 mg/kg for priming and 2.8 mg/ kg resolving dose) treatment group is the positive control group.

| Treatment | Spawning Ratio | Latency (h) | Significance |
|---|---|---|---|
| FTS | 0/8 | - | c |
| LHRHa (50 µg/kg) | 4/10 | 28-30 | b |
| LHRHa (5 µg/kg)+ DOM (5 mg/kg) | 1/9 | 30 | c |
| LHRHa (20 µg/kg)+ DOM (5 mg/kg) | 9/9 | 28-30 | a |
| LHRHa (50 µg/kg)+ DOM (5 mg/kg) | 8/8 | 28-30 | a |
| CPE (II. Injection) | 9/10 | 12-14 | a |

Significance relates to the Spawning ratio only; the same letter refers that there was no significant differences amongst groups ($P$> 0.05). Water temperature: 24°C.

**Table 2.** Spawning induction in common carp using a constant dose (20 µg/kg) of LHRHa ([D-Ser (tBu)$^6$, Pro$^9$-NEt]-LHRH) combined with graded doses of Haloperidol (HAL).

| Treatment | Spawning ratio or oocyte stage |
|---|---|
| FTS | 0/8 |
| LHRHa (20 µg/kg)+ HAL(0.1 mg/kg) | 2/9+7/9 (%90 Stage IV, % 10 Stage II) |
| LHRHa (20 µg/kg)+ HAL(0.5 mg/kg) | 9/9 |
| LHRHa (20 µg/kg)+ HAL(1 mg/kg) | 8/8 |

Water temperature: 20°C.

successfully while lower doses of HAL only led to progress in oocyte maturation but not to complete ovulation (Table 2).

**Using dose of LHRHa+HAL combination**

20 µg LHRHa and 0.5 mg/kg HAL was chosen as a standard treatment dose for further experiments and was given concomitantly to broodstocks for induction of spawning in a single injection.

**Timing of the treatment**

Initial egg release in fish kept at 23 °C and injected with 20 µg LHRHa+0.5mg HAL/kg at 07:00, 12:00 and 19:00 occurred after a latency of 14-16 h, irrespective of the hour of injection. The spawning rate was similar in all groups (Table 3).

**Effects of temperature on latency**

For determining the effects of temperature on the period of latency seven spawning experiments were carried out at various temperatures (20-26 ºC). Latency was negatively correlated with temperature. However, the latency in the range of 22-25 ºC was more or less constant (Figure 1).

## Discussion

Treatment with 20 µg/kg LHRHa ([D-Ser (tBu)$^6$, Pro$^9$-NEt]-LHRH) was effective for spawning induction in carp when given together with a DA. It was reported that this mammalian superactive analogue is also effective for spawning induction in sea bass, sea bream (Arabacı, 2000), and rainbow trout (Arabacı *et al*., unpublished results). The same LHRHa analogue was used for determining the specific LHRH receptors in winter flounder (Crim *et al*., 1988a), and three-spined stickleback (Anderson *et al*., 1989). Biological activity and/or binding affinity was studied in rainbow trout, landlocked salmon, winter flounder (Crim *et al.,* 1988b), goldfish (Habibi *et al*., 1989), and African catfish (Leeuw *et al*., 1988).

For spawning induction in different fish species different Dopamine antagonists were used. Although Haloperidol is also a nonsoluble DA in water as in Domperidon and Pimozid, it is not very common. Haloperidol was used in goldfish (Peter *et al*., 1986; Omeljaniuk, 1987) and Atlantic croaker (Copeland and Thomas, 1989). As Atlantic croaker does not have dopaminergic inhibition, Haloperidol was non-effective in the spawning induction. The HAL dose of 0.5 mg/kg combined with LHRHa had to be chosen because lower doses only advanced oocyte maturation in most of broodstocks but does not lead to complete ovulation (Table 2). The same dose of Haloperidol (HAL), when given together with LHRHa (20 µg/kg), was realised complete ovulation (Table 2).

Latency between injection and initial egg release in fish treated with LHRHa+DOM was 28-30 h. However, the fish administered with LHRHa+HAL (in this study; at 24 °C water temperature) and LHRHa+MET (Drori *et al*., 1994; at 23 °C water temperature) the latency was shorter at 14-16h. It is interesting that HAL, directly non-soluble in water, resembles MET (soluble in water) in respect of the latency period. It is possible that the absorption speed from the injection site of HAL and MET may be equally fast, which would account for the faster spawning response. A High dose (1mg/kg) of HAL is also effective and tolerable (Table 2).

These results show that treating common carp with LHRHa+HAL at any time during the day realises successful ovulations with similar latency period. These results show similarity with the results of Drori *et al*. (1994) who worked on common carp treated

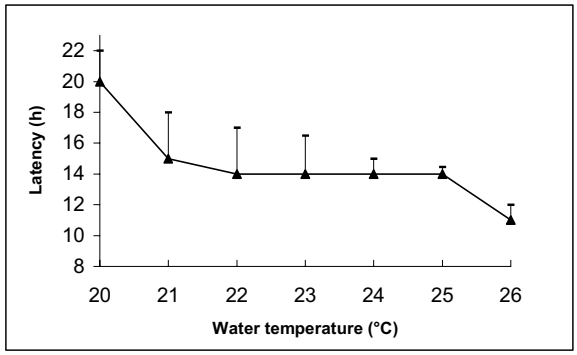


**Figure 1.** Time interval at various temperatures between injection of LHRHa+HAL (LHRHa, 20 µg/kg+HAL, 0.5 mg/kg) and initial egg release. Bars represent the time range for each.

**Table 3.** Spawning induction in mirror carp by 20 µg/kg LHRHa+0.5 mg HAL/kg given at various hours during the day.

| Hour of Treatment | Hour of initial egg release(h) | Spawning Ratio | Latency (h) | Significance |
|---|---|---|---|---|
| 07:00 | 21:00 | 4/4 | 14-16 | a |
| 12:00 | 02:00 | 4/4 | 14-16 | a |
| 19:00 | 09:00 | 5/5 | 14-16 | a |

Water temperature; 24°C.

with sLHRHa+MET. However, in CPE treated common carp at various hours of the day, ovulation response changed (Bieniarz *et al*., 1985). The situation is different for different fish species. While, *Sparus aurata,* using LHRHa treatment at 10 p.m. was effective in inducing spawning (Zohar, 1986) in, *Dicentrarchus labrax*, using LHRHa treatment at 10 a.m. resulted in a shorter latency (Alvarino *et al*., 1992).

We have observed that the latency was negatively correlated with water temperature in LHRHa+HAL injected fish. The longer latency in LHRHa+HAL treated fish (Figure 1), as compared to the CPE (second injection) treated fish (Table 1), which was also observed at the same water temperature (24 °C). Similar findings were reported by Drori *et al*. (1994). This is expected in poikilothermic organisms, as their rate of metabolic processes was dependent on the ambient temperature. That LHRHa combination has a hypophysiotropic effect, but the CPE has a direct gonadotropic effect that may be linked to the longer latency in LHRHa combination treated fish, as compared with CPE-treated fish (Drori *et al*., 1994).

There are several reports about the relationship between latency and temperature in CPE treated common carp, and in other cyprinid species it was found to be linear in the range of l9-26 °C (Horvath, 1978, 1985; Zonneveld, 1984; Peter *et al*., 1988). In LHRHa treated grass carp, linear negative correlation was reported in the range of 25-31 °C (Rottmann and Shire-man, 1985). In this study, a constant minimal latency of 14 h in the temperature range of 22-25 °C was seen in LHRHa+HAL treated Broodstocks (n=51). Interestingly, Drori *et al*. (1994), also reported that common carp injected with sLHRHa+MET showed a constant minimal latency of 14 h in the temperature range of 22.5-25°C. This range corresponds to the optimal temperature range for spawning in the common carp (Horvath, 1978; Peter *et al*., 1988).

As a result, only a single injection of LHRHa+HAL given at any time during the day and predicting the latency according to the water temperature is very important for the fish breeder. The use of LHRHa (20 µg/kg) and HAL (0.5 mg/kg) together will provide healthy hatchery management.

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