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Induction of Ovulation by HCG, LHRHa and Carp Pituitary in *Rhynchocypris oxycephalus* (Sauvage and Dabry)

I.–S. PARK

Division of Ocean Science
College of Ocean Science and Technology
Korea Maritime University
Pusan 606-791, Korea

Abstract

The effectiveness of human chorionic gonadotropin (HCG), a super active analogue of mammalian luteinizing hormone releasing hormone (LHRHa) and carp pituitary (CP) for inducing ovulation in *Rhynchocypris oxycephalus* (Sauvage and Dabry) was examined, and the effects of the maternal hormone treatment on egg quality were evaluated. None of the control group and sham (saline injected) control group ovulated. The majority (76~96%) of the hormone–treated groups ovulated within 2 days after hormone injection. Studies of egg quality, e.g., fertilization rate and hatching, demonstrated that good quality *R. oxycephalus* (Sauvage and Dabry) offspring can be produced after accelerated spawning by HCG, LHRHa and CP. The increase in egg diameter and number of eggs spawned may have been caused by the increase in the body weight of the females.

Introduction

Environmental and hormonal manipulation of ovulation in the fish have become of practical importance in the fish farming industry for two main reasons: (1) it is important to solve the problem of spawning asynchrony which necessitates frequent broodstock handling (Crim and Glebe 1984, Lin 1996); (2) by accelerating or delaying gametogenesis in captive broodstock, spawning may be scheduled to yield fry whenever needed (Lam 1983). Moreover, induced breeding makes possible hybridization between closely related species (Lam 1982, Kim et al. 1995, Park et al. 1996, 1997a).

Originally, culturists utilized carp pituitary (CP) and this is still widely used particularly for the major Indian carps, Chinese carps and the common carp *Cyprinus carpio* (Lam 1982, Park et al. 1994). Human chorionic gonadotropin (HCG), has been widely used to induce ovulation in fish since the 1930s, and has yielded the best results among mammalian hormones (Lam 1982; Kim et al. 1992, Kelly and Kohler 1994, Park et al. 1994). The superactive

luteinizing hormone-releasing hormone analogue des-Gly_{10}[D-Ala_6]LHRH-Ethylamide (LHRHa) has been sucessfully used to induce final maturation and synchronize ovulation of many commercially cultured fish (Donaldson and Hunter 1983, Park et al. 1997b).

The objective of induced ovulation is to produce, on demand, a large supply of high quality eggs. Egg quality is assessed by characteristics such as egg fertility and hatching (Bromage and Cumaranatunga 1988). Hormonal induction of final oocyte maturation and ovulation, however, can result in reduced egg quality (Mylonas et al. 1992). *R. oxycephalus* (Sauvage and Dabry) is one of the typical freshwater Leuciscinae found in the western and southern area of Korea, living in mountain streams. It is reported that its distribution covers most of the Korea peninsula, extending to the western parts of Japan and to northwestern part of China (Kim 1997; Park et al. 1998a). This species is particularly suitable for use in laboratory studies, as it is hardy and can withstand cold temperature (Park et al. 1998a, 1998b). Furthermore there is good background information on husbandry techniques and aspects of its biology (Park et al. 2000). In the present study we investigated the effects of hormone and time of intraperitoneal injection of HCG, LHRHa and CP on ovulation and egg quality, in order to develop a simple and cost effective method for accelerating and synchronizing ovulation in farmed *R. oxycephalus* (Sauvage and Dabry).

Materials and Methods

Three–year–old mature *R. oxycephalus* (Sauvage and Dabry) were obtained from the Genetics and Breeding Laboratory, Faculty of Marine Life Science, Kunsan National University, Kunsan, Korea. All fish were conditioned to the experimental tanks (800 l water volume), which was supplied with 5 l·min of aerated well water at a temperature of 18±0.5ºC and fed a dry commercial carp feed (crumbles 1 and 2, Agribrand Furina Korea Co., Korea) until the beginning of the experiments when feeding was discontinued. Five groups of female *R. oxycephalus* (Sauvage and Dabry) assumed to be mature but nonspawning, were injected once on day O during the experimental period in April 1998 (spawning season) to determine the effects of administration of HCG (Sigma, USA), LHRHa (Sigma, USA) and powdered CP (Sigma, USA) on ovulation. All hormones were dissolved in sterile physiological saline and the solution was injected intraperitoneally in a total volume of 5 μ*l*·g BW, using a 1 ml syringe fitted with a 19-gauge needle. One group received interperitoneal injections of 1,000 IU·kg BW HCG (n=25), the second group 50 μg·kg BW LHRHa (n=29), the third group 10 mg powdered CP (n=30), the fourth sham control group of sterile physiological saline (n=29) and the fifth control group (n=25). After injection, the groups were placed in individual tanks and maintained at 18.0±0.5ºC.

Fish were inspected daily, after anesthetizing by bathing them in a solution of 300 ppm lidocaine-HCl·$NaHCO_3$ at 20ºC (Park et al. 1998b), for signs of ovulation according to abdominal swelling, softening of the body wall and finally the release of free eggs from the egg pore upon application of gentle abdominal pressure. Increases in body weight (a sign of ovarian hydration)

were also daily monitored. From ovulated eggs, number of eggs per BW (g), egg diameter (mm) and gonadosomatic index (GSI) were calculated. Mean egg diameters were calculated from a measurement, to the nearest 0.01 mm using Vernier calipers, of 100 eggs from each sample. Fertilization was performed artificially by mixing eggs stripped from each with spermatozoa from one male. Fertilization was assessed at the 4~16 cell stage according to the method described by Park et al. (1998a). Percentage data were arcsine transformed. Data were analyzed by two-way analysis of variance followed by Duncan's multiple range test to determine the differences in GSI, fertilization rate, hatching and egg diameter between groups. Data were considered significant at the 0.05 probability level.

Table 1 demonstrates the result of ovulation after injection of saline 1,000 IU HCG·kg BW, 50 μg LHRHa·kg BW and 10 mgCP·kg BW to mature *R. oxycephalus* (Sauvage and Dabry). During the experiment, no mortality were recorded in control group and sham control group. Neither control group nor sham control group ovulated during the experiment. Considering the concept of environmental factors on ovulation in fish, Lin and Peter (1996) pointed out that fish integrate their reproductive activities with seasonal environmental cycles; and certain environmental factors, such as temperature, photoperiod and rainfall, act as cues for the approaching season, which is favorable for reproduction. Environmental stimuli are further necessary for the induction of ovulation in this species. In contrast to the result of control and sham control group, the majority of HCG, LHRHa and CP-treated group had ovulated (cumulative percentage of 76~97) within 2 days after injection, with no mortality. Ovulation rates of HCG– and LHRHa–treated groups 1 day after injection, 52% and 31%, changed to 24.0% and 51.7% at 2 days after injection, respectively. Twenty eight out of 29 fish injected with CP underwent ovulation (93.0%) one day after injection. After 3 days no ovulatory response occurred in all hormone-treated groups. Similar to our results, the synthetic nonapeptide analogue of LHRH compound typically more powerful and long-lived, and easier to obtain and work with, has been used successfully to induce ovulation in the four Chinese carps (Cooperative Team for Hormonal Application in Pisciculture: CTHAP, 1977).

Donaldson et al. (1983), summarizing the progress made in the use of LHRHa to induce ovulation in teleosts of commercial significance, said that the

Table 1. The response of ovulation in mature *Rhynchocypris oxycephalus* (Sauvage and Dabry) following injection on day 0 with saline, HCG (1,000 IU·kg BW), LHRHa (50 μg·kg BW) or CP (10 mg·kg BW)*

Treatment females	No. of	No. of ovulated (%)			
		Day 1	Day 2	Day 3	Total
Control	25	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sham control	27	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
HCG	25	13 (52.0)	6 (24.0)	0 (0.0)	19 (76.0)
LHRHa	29	9 (31.0)	15 (51.7)	0 (0.0)	24 (82.8)
CP	30	28 (93.0)	1 (3.3)	0 (0.0)	29 (96.7)

*HCG: human chorionic gonadotropin; LHRHa: luteinizing hormone releasing hormone analogue; CP: powdered carp pituitary.

hope held out at the FAO World Conference on Aquaculture in Kyoto for the use of LHRHa as a practical tool is well on the way to being fulfilled. Traditional methods of induced spawning for cultured fish are based on the injection of crude extract of CP and HCG (Lin and Peter 1996). Though the ovulation rate of 93.0% in CP–treated group at 1 day after injection was higher than those of HCG- and LHRHa-treated group; their ovulation rate decreased sharply to 3.3% at 2 days after injection. Treatment with HCG and CP resulted in a significant synchronization of ovulation compared to LHRHa treatment at 1 day after injection. These results are similar to those previously reported, where HCG, at the same dosage reported here, induced ovulation either alone, e.g., in channel catfish *Ictalurus punctatus* (Sneed and Clemens 1959), goldfish *Carassius auratus* (Sneed and Clemens 1959), striped bass *Morone saxatilis* (Stevens 1996), redear sunfish *Lepomis microlophus* (Smitherman and Hester 1962) or in combination with fish pituitary preparations, e.g., in the grass carp *Ctenopharyngodon idella* (Lam 1982). In this experiment, HCG is successfully used to induce spawning of *R. oxycephalus* (Sauvage and Dabry), but is ineffective in others, such as grass carp (*Ctenopharyngodon idellus*, Cyprinidae) and black carp (*Mylopharyngodon piceus*, Cyprinidae) (Lin and Peter 1996).

Egg quality is a very important concern following successful induction of spawning in teleosts (Harmin and Crim 1992). The influences of induced ovulation in mature *R. oxycephalus* (Sauvage and Dabry) following injection on day 0 with 1,000 IU HCG·kg BW, 50 μg LHRHa·kg BW or 10 mg CP·kg BW on GSI, fertilization rate and hatching rate are presented in table 2. The GSI at 1 day after injection was 13.7% for the HCG–treated group and 8.5% for the LHRHa–treated group, decreasing to 11.2% for the HCG–treated group and 12.3% for the LHRHa–treated group, respectively, at 2 days after injection. Although the CP- treated group had a 10.7% GSI at 1 day after injection, the GSI declined to 7.8% at 2 days after injection. Overall means of fertilization rate for HCG-, LHRHa- and CP-treated groups at 1 day after injection were

Table 2. Influence of induced ovulation in mature *Rhynchocypris oxycephalus* (Sauvage and Dabry) following injection on day 0 with HCG (1,000 IU·kg BW), LHRHa (50 μg·kg BW) or CP (10 mg·kg BW) on GSI, fertilization rate and hatching[*1]

Treatment	Days after injection					
	1[*2]			2[*2]		
	GSI (%)[**3]	Fertilization rate (%)	Hatching (%)	GSI (%)[**3]	Fertilization rate (%)	Hatching (%)
HCG	13.7±0.9(13)[a]	89.3±6.7[a]	80.8±4.3[a]	11.2±0.5(6)[a]	83.6±5.7[a]	70.8±6.1[a]
LHRHa	8.5±0.3(9)[b]	77.7±5.9[b]	68.7±5.3[b]	12.3±0.7(15)[a]	85.6±4.9[a]	74.1±5.7[a]
CP	10.7±0.5(28)[ab]	92.4±5.1[a]	86.4±4.7[a]	7.8±0.3(1)[b]	81.8±5.0[a]	76.8±6.5[a]

*1 HCG: human chorionic gonadotropin; LHRHa: luteinizing hormone releasing hormone analogue; CP: powdered carp pituitary.
*2 Values represent means±SD. Same superscript letter within each experiment and part denotes groups that were not significantly different.
*3 Gonadosomatic index (GSI)=(b×a)×100, a: weight of fish before injection; b: weight of stripped eggs. Parenthesis indicate number of ovulated females.

89.3%, 77.7% and 92.4%, respectively; however, there were no significant differences in fertilization rate which ranged from 81.8% to 85.6% at 2 days after injection ($p<0.05$). At 1 day after injection, fish that received HCG or CP had higher average hatching than LHRHa–treated fish; however, at 2 days after injection fish in all treatments had similar hatching ($p<0.05$). At 1 day and 2 days after injection, relatively high and constant GSI were observed in the groups treated with HCG, LHRHa or CP. These result indicate that these fish possessed oocytes which had already completed vitellogenesis. Our results with HCG, LHRHa or CP induction of spawning and production of normal larvae in this experiment are in agreement with the results of a study performed on catfish *Clarias batrachus* with Gn-RHa (Manickam and Joy 1989). Harmin and Crim (1992) reported that good quality egg indicated by high hatching rates and good quality larvae were obtained from female winter flounder, *Pseudopleuronectes americanus* (Walbaum) treated with GnRH-A.

The mean total number of eggs spawned, the mean number of eggs spawned·g BW, and the egg diameter for each of the five body weight classes after HCG, LHRHa and CP injection are shown in table 3. The changes in mean egg diameter ($p<0.05$) and number of eggs spawned appeared to be correlated with body weight class between 4.1~8.0 g and 20.1~24.0 g. In number of eggs per body weight, however, no such correlation was observed, as shown in table 3. The increase of mean egg diameter in the body weight class of 20.1~24.0 g compared to those of previous body weight class are probably due to aging in this species. Similar findings to those described in the present study were reported from experiments performed on the cyprinid loach *Misgurnus anguillicaudatus* by Suzuki (1983) and the mud loach *M. mizolepis* by Kim et al. (1992).

It is clear that major progress has occurred in recent years towards the development of reliable and economic procedures for the induction of ovulation in economically important teleosts (Donaldson and Hunter 1983, Park et al. 1994). Further research is required for successful induction of ovulation in mature *R. oxycephalus* (Sauvage and Dabry) by hormone treatment, to establish optimal dosages, mode of administration (e.g., solution, oil emulsion, or by pellet), and location of treatment with regard to treatment costs since multiple injections may require less HCG, LHRHa and CP; however, single injections or pellet implantation although requiring more hormone involve less handling of the fish. Further studies are also required to establish which are the most effective hormones for induction of ovulation in this species.

Table 3. Relationship between egg numbers and body weight treated with hormones in mature *Rhynchocypris oxycephalus* (Sauvage and Dabry)

Body weight class (g)	No. of eggs spawned*	No. of eggs×g BW*	Egg diameter (mm)*
4.1 ~ 8.0	359.0±53.3	52.7±3.9	1.58±0.12[a]
8.1 ~ 12.0	513.7±62.7	50.1±4.8	1.61±0.09[a]
12.1 ~ 16.0	721.8±43.2	44.2±5.3	1.68±0.10[b]
16.1 ~ 20.0	987.0±57.9	32.6±2.7	1.71±0.13[b]
20.1 ~ 24.0	1127.3±70.4	34.2±3.5	1.70±0.14[b]

*Values represent means±SD. Same superscript letter within each experiment and part denotes groups that were not significantly different.

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