

Effects of Single Injection of Carp Pituitary Extract and Human Chorionic Gonadotropin on Germinal Vesicle Migration and Ovulation in Lampam Sungai, *Puntius schwanenfeldii* (Bleeker): A Preliminary Study

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Abstract

The effects of crude pituitary extract (CPE) and human chorionic gonadotropin (hCG) on germinal vesicle migration and ovulation in lampam sungai *Puntius schwanenfeldii* (Bleeker) were investigated. Sexually mature female lampam sungai were divided into four groups (n=3 per group). In both experiments, fish were injected intramuscularly (im) once and were checked for germinal vesicle migration and sign of ovulation 8 h following injection. In experiment 1, females were treated with CPE at doses of 2, 4, or 6 mg/kg body weight (BW) while controls were administered an equivalent volume of saline. Ovulation occurred in one fish given 2 mg/kg and in another fish that received 6 mg/kg CPE. In a second experiment, female fish were administered hCG at doses of 1000, 2000, or 4000 IU/kg body weight. Similarly, controls received only saline. One female injected with 4000 IU/kg hCG responded positively to the hormone treatment. Clearly, in our study, a single intramuscular injection of CPE and hCG induced spawning in lampam sungai as well as accelerated oocyte development as evidenced by the increasing percentage of oocytes showing germinal vesicle migration.

Introduction

Puntius schwanenfeldii known as lampam sungai in Malaysia is a commonly occurring fish in Southeast Asian region. Adults range in size from 60 to 200 mm in length with average body weight of approximately 300 g (Mohsin and Ambak 1983). An important food fish and aquarium fish in Malaysia, it has a potential to be a major aquaculture species (Mohsin and Ang 1979). However, limited information is

available about the biology and culture of this species. Recently, Siregar (1993) described the reproductive biology of riverine cyprinid found in Indonesia. In general, fry needed for culture still come from the wild. As the fish failed to reproduce in captivity, hormone therapy is one possible way of achieving ovulation and spawning to meet the demand for larvae and fry. We undertook a study to elucidate the reproductive processes of the species for seed production. The study aims to determine the effect of a single injection of CPE and hCG on germinal vesicle migration or ovulation in *Puntius schwanenfeldii* (Bleeker).

Materials And Methods

Sexually mature fish were either caught from Chendoroh Lake in Perak or acquired from a commercial farmer in Pahang in Peninsular Malaysia. Fish were caught by gill net and kept in floating cages in the lake until they were transported to our hatchery in Serdang, Selangor. Upon arrival in Serdang, the fish were stocked in earthen ponds at least two weeks prior to experimentation. Fish were then fed twice daily at 0800 h and 1600 h with formulated diet containing 30% crude protein at 3% of body weight.

At the beginning of the study, experimental fish were selected based on the morphological characters such as distended abdomen with reddish or pinkish cloacal region. In addition, ovarian biopsy was performed prior to hormone injection, using a catheter. Aspirated oocytes were cleared in a solution of alcohol, glycerin and acetic acid (6:3:1, v/v) and the stage of maturity was observed on the basis of the position of germinal vesicle (GV). GV were staged as follows: 1) central, 2) migration, 3) peripheral, and 4) ovulation. Oocytes were measured each time and the mean diameter of the largest size class oocytes (n=25) was determined to the nearest 0.01 mm using an eyepiece micrometer under a dissecting microscope. On termination of the experiment, oocyte diameters and GV position of eggs from unovulated females were determined.

Carp pituitary extract (CPE) (Stoller Laboratories California, USA) and human chorionic gonadotropin (hCG) (Profasi, Serono, Italy) were dissolved in saline (0.6% NaCl). Fish were injected intramuscularly with CPE at 2, 4, or 6 mg/kg body weight while control were administered an equivalent volume of saline. Twenty males were maintained in 2000 l tank and were stripped for milt during the artificial spawning. In the first experiment 12 females (body weight 227.91 ± 11.89 g) were divided into four treatment groups, with three females per treatment. In the second experiment, females (body weight 201.66 ± 19.26 g) were also divided into four treatment groups and received hCG at 1000, 2000, or 4000 IU/kg body weight. Similarly, controls were administered with saline.

Fish were checked for any sign of ovulation 8 h after a single injection by gently massaging the abdomen until a free flow of eggs appeared. Fecundity was determined from the ovulated females by the volumetric method. Eggs were fertilized with pooled milt collected from untreated males. Eggs fertilized with the milt were left to stand for about 2 min before twice washing it with water. Fertilization rate was determined when the eggs were at the closed blastopore stage (approximately 5-6 h after fertilization). Incubation was accomplished in hatching boxes placed afloat in the tank complete with circulation. Hatching rate was determined from subsamples in petri dishes.

Results

Experiment I

Initial mean oocyte diameter of the pre-injected fish groups ranged from 0.67 ± 0.014 to 0.72 ± 0.041 mm and no significant difference ($P > 0.05$) was observed in oocyte diameter among the treatment groups. About 75% of oocytes had GV in the central position. By the end of the experiment, one fish each from the groups given CPE at 2 mg/kg or 6 mg/kg ovulated (Table 1). However, none of the females receiving 4 mg/kg CPE responded to treatment. On termination, mean oocyte diameter of fish in all treatment groups were not significantly different ($P > 0.05$).

Table 1. Fecundity, fertilization rate, and hatching rate of two ovulated females of *Puntius schwanenfeldii* (Bleeker) induced to spawn with a single injection of carp pituitary extract (CPE).

Treatment	Body Weight (g)	Fecundity $\times 10^3$	Oocyte diameter (mm)	Fertilization rate (%)	Hatching rate (%)
2 mg/kg	250.00	16.02	0.818	75.00	48.00
6 mg/kg	300.45	25.17	0.830	68.00	40.00

Experiment 2

Mean oocyte diameter of the fish in all treatment groups were similar ($P < 0.05$) at the beginning of this experiment. Except for one female that received 4000 IU/kg hCG, all fish had 70% of oocytes in central position. In this particular fish $58.3 \pm 8.3\%$ of oocytes had GV in the migratory stage. This female ovulated after 8 h post-treatment. Egg-size at ovulation was 0.991 mm. Fecundity was 14,234

eggs, and fertilization and hatching rates were 49% and 28%, respectively. Females receiving 1000 IU/kg groups did not ovulate after the 8 h study. However, females treated with hCG had more than 30% of oocytes having GV in the migratory stage.

Discussion

This experiment demonstrates that a single injection of CPE or hCG is effective in inducing ovulation in lampan sungai. However, the data also suggest that the success of the induced ovulation may partly depend on the germinal vesicle position and oocyte diameters of pre-injected fish.

Maturation of oocytes begin with the movement of germinal vesicle from the center towards the animal pole. In all fish that ovulated after hormone treatment, the GV was either in migratory or peripheral position. In maturing lampan sungai, the ovary contains variable sizes of oocytes. It appears that oocytes would grow to a certain critical size in order to respond to the hormone administration. Our present study indicates that lampan sungai with oocyte diameter above 0.70 mm responded positively to the hormone treatment. The critical oocyte diameter for lampan sungai to respond to hormone treatment have not been determined in detail. In rabbitfish, Juario et al. (1987) have shown that the smaller the initial oocyte diameter, the more hCG is needed to spawn the fish. Thus, female rabbitfish with less than 0.47 mm spawned following hCG treatment whereas fish with oocytes larger than 0.47 mm (GV in migratory stage) spontaneously spawned without hormone administration.

The dosage of hormone administered to female fish may influence the maturation of oocytes. We also observed ovulation in a female receiving low dose (2 mg/kg CPE). This successful ovulation was probably due to the higher percentage of oocytes in this fish with GV in peripheral position (70%) prior to experimentation. It is possible that ovulation occurred even with a low hormone dose because the oocyte may have completed the vitellogenic process. This is consistent with the findings of Tan-Fermin and Emata (1993) who showed that *Clarias macrocephalus* would spawn with lower doses of LHRHa + pimozone when the oocyte contained slightly off-center GV position. On the other hand, fish with 40% of oocytes having GV at peripheral position were also able to ovulate with the highest dose used (6 mg/kg CPE). Another related species, *Puntius gonionotus*, successfully ovulated 6 h after 6 mg/kg body weight CPE administration (Pathmasothy and Jin 1988). Even though none of the females receiving 4 mg/kg CPE ovulated, our study showed that this treatment accelerated oocyte development i.e. a shift in GV position from the pre-injection level although oocyte diameter remained the same size.

Given these preliminary results it is difficult to determine whether hormone-induced ovulation influenced the quality of spawned eggs. Both CPE and hCG accelerated ovulation leading to spawning of lampan sungai but fertilization and

hatching rates were relatively low. The same inconsistent results in fertilization and hatching rates was reported in catfish (Cheah et al. 1990). In conclusion, the results show that less hormone is required to induce spawning if the fish had larger oocyte diameter with GV in the peripheral position. Studies on the use of gonadotropin-releasing hormone analogue and pimozide in induced ovulation of lampan sungai are currently ongoing.

Acknowledgments

I would like to thank SEAFDEC Aquaculture Department for inviting me to participate in the seminar-workshop and also for providing funding for my travel to the Philippines. The authors are also indebted to the Ministry of Science, Technology and Environment, Government of Malaysia for funds to undertake the project through the IRPA grant No. 1-07-05-078.

References

- Cheah SH, Siraj SS, Ang KJ. 1990. A preliminary study on induced spawning of the catfish *Clarias batrachus* (Linnaeus) in Malaysia. *Pertanika* 13(1):63-66.
- Juario JV, Duray MN, Duray VM, Nacario JF, Almendras JME. 1985. Breeding and larval rearing of the rabbitfish, *Siganus guttatus* (Bloch). *Aquaculture* 44:91-101.
- Mohsin AKM, Ambak AM. 1983. Freshwater fishes of Peninsular Malaysia. Universiti Pertanian Malaysia. 284 pp.
- Mohsin AKM, Ang KJ. 1979. Observation on growth of young tinfoil barb, *Puntius schwanefeldii* (Bleeker) fed with pellets. *Pertanika* 2(1):43-46.
- Pathmasothy S, Jin LM. 1988. Induced breeding of *Puntius gonionotus* (Bleeker) utilizing various combinations of pimozide, analogue of LH-RH and common carp pituitary extract. p. 48-52. In: Proc. 11th Ann. Conf. Malaysian Society of Animal Production 1988.
- Siregar M. 1993. Studies of the reproductive biology of the riverine cyprinid, *Puntius schwanefeldii*, from the Kampar river, Sumatra, Indonesia. In: World Fisheries Congress, 3-8 May 1993, Athens, Greece.
- Tan-Fermin JD, Emata AC. 1993. Induced spawning by LHRHa and pimozide in the Asian catfish *Clarias macrocephalus* (Gunther). *J. Appl. Ichthyol.* 9:89-96.