

Induced Ovarian Development, Maturation and Ovulation of Domestic Reeves Shad by Hormone Implantation and Injection

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Abstract

Reeves shad, *Tenualosa reevesii* (= *Hilsa reevesii*), one of the most famous anadromous fish in China, is on the verge of extinction. In the face of this crisis, there is an urgent need for conservation of this species by way of propagation and culture. In this study, ovarian maturation and ovulation of the pond-cultured shad is induced for the first time with both ecological and hormonal manipulations. Implantations of testosterone alone were effective in priming ovarian development, although these did not induce ovarian maturation. Multiple injections of LHRH-A following two implantations of testosterone stimulated the brain-pituitary-ovary axis of the pond-reared shad, inducing development and maturation. Induced maturity of 4, 5 and 6 year-old females reached 83.3%, 87.5% and 100%, respectively, while controls remained immature. The mature shad responded better to ovulation to LHRH-A and DOM treatments than to LHRH-A treatments alone. Results are significant in developing shad culture and saving this endangered species.

Introduction

Reeves shad is a highly valued anadromous fish in China. However, this species, which supported a lucrative commercial fishery before the 1960s, is now on the verge of extinction (Wang 1996). In the face of this crisis, there is an urgent need for conservation of this rare-precious species by way of artificial reproduction, culture and stocking.

There have been attempts to propagate Reeves shad in the wild in the past several decades. Artificial fertilization of matured shad was achieved in Qiantan River in 1958 (Lu *et al.* 1964) and Yangtze River in 1982 (Yangtze River Fisheries Institute, unpublished data). However, because the shad is very skittish and easily dies during handling, hormonal induction of maturing shad spawning in the Yangtze River has not been successful (Qiu, pers. comm.).

There are no lack of studies on artificial propagation of other shad (Leach 1925; Raj 1917; Anon 1978; Malhtra *et al.* 1969, Sen, 1990; Hendricks 1995). Mylonlas *et al.* (1995) were successful in inducing spawning of wild American

shad (*Alosa sapidissima*) from fish lift. However, there is no successful report on induced maturation and spawning of cultured shad to date.

It has been proven that commercial culture of Reeves shad is feasible (Wang 1995). Furthermore, a broodstock of shad was established in pond conditions for the first time (Wang 1997), but the domesticated shad remained immature as reproductive migration continued to be blocked. Thus, discovery of a means for inducing ovarian development to prespawning stage, enabling production of fertile eggs and viable larvae, would be a major achievement.

This paper reports on experiments on induced ovarian development, maturation and ovulation of pond-cultured shad.

Materials and methods

Juvenile shad with length of 17.0-37.5mm and body weight of 0.18-0.51g were captured near the mouth of Pear River and transported to Dong Guan Aquacultural Experiment Station in September 1989-1991. Shad were domesticated in 0.067 ha. earth ponds at ambient temperature (annual 14-34°C) and salinity (0.5-12ppt) and under a natural photoperiod. The fish were fed commercial eel feed supplemented with trash fish gruel 2-3 times daily.

17 α -methyltestosterone (T) were purchased from Sigma Chemical Co.U.S.A; LHRH-A and Domperidone (DOM) were purchased from the Ningbo fish hormone factory (Zhejiang Province, China). T was dissolved in molten lacquer butter or incorporated into silastic as described by Pankhurst et al (1986). Crystalline T was mixed with unpolymerized elastomer (Silastic 382 Medical Grade Elastomer, Dow Corning Co.) at 50mg·g⁻¹ elastomer, 5ul of accelerator added, and the mix spread into 2×2×30mm molds to give 0.2mg T/mm pellets. LHRH-A were dissolved in freshwater teleost physiological saline (PS), whereas DOM was suspended in PS.

Four experiments on induced maturation were conducted using pond-domesticated 4-6 year-old broodfish in 1994-1995 (Table 1). In the first experiment, females received either blank pellets for controls or T-silastic pellets at a dose of 50 mg·kg⁻¹ body weight, using the procedure described by Lee *et al.* (1986a), three times at monthly intervals between April and June. In the second experiment, female shad received lacquer butter and PS, or T-lacquer butter implants followed by injections of LHRH-A. Fish were primed with two implantations of T at a dose of 25mg·kg⁻¹ body weight at biweekly intervals from the middle of March to the middle of April. T, dissolved in molten lacquer butter, was injected intraperitoneally at the base of the pelvic fins at a temperature of 35°C. Following ovary priming and from the fifth week of experiment, the fish were treated with 4-6 injections of LHRH-A at a dose of 10 μ g·kg⁻¹ body weight at 10-15 day intervals between April and June. LHRH-A was injected intraperitoneally at the base of the pectoral fin. In trials on induced spawning, mature fish received two intraperitoneal injections of LHRH-A (10 μ g+20 μ g·kg⁻¹ body weight) plus DOM (1mg+2mg·kg⁻¹ body weight) at an interval of 12 h.

At the time of treatment and on a monthly basis, fish were checked and ovarian samples were collected using the cannulation technique. The ovaries were moved from some experimental fish, weighed to determine GSI (=gonad

Table 1. Summary of experimental design for inducing ovarian development and maturation.

Expt. No.	Age of fish (years)	BW of fish (kg)	N	Hormone	Administration		
					Method	Time	Intervals
1	5	1.2-1.4	7	TS	IM	3	Monthly
	5	1.2-1.4	6	Sham control			
2	5	1.2-1.4	7	TL +LHRH-A	IM	2	Biweekly 10-15d
	5	1.2-1.4	3	Sham control	Inj	4	
3	6	1.4-1.6	7	TL +LHRH-A	IM	2	Biweekly 10-15d
	6	1.4-1.6	2	Sham control	Inj	6	
4	4	0.9-1.2	9	TL +LHRH-A	IM	2	Biweekly 10-15d
					Inj	6	

TS=T-silastic pellet, TL=T-lacquer butter pellet, IM=Implantation, Inj=Injection

weight/body weight \times 100). The samples for histological analysis, fixed in Bouin's fluid, were hydrated and embedded in paraffin. The 4-8 μ m thick sections were stained with Regaud's hemotoxyline, orange G, aniline blue for the males and with Heindenhein's Azan for the female. Diameters of over 1000 eggs were measured with IBAS-2000 image analysis system during each developmental stage. Spawned eggs were collected and their diameters measured.

A method for measuring GtH of Reeves shad serum has not been established yet, so no data is available. GSI and egg diameter data were analyzed by one-way analysis of variance and Duncan's multiple range test following \log_{10} transformation.

Results

Effect of implantation of T at monthly intervals on ovarian development

In experiment 1, on day 80, 20 days following the third implantation, control as well as T-treated animals were immature, but the mean GSI of T-implanted Reeves shad (0.94%) was significantly higher than the blank implanted control(0.15%). Histological examination of the ovaries of the T-implanted animals demonstrated that oocytes were on the early yolk vesicle stage (Fig.1a); in contrast, the oocytes in the blank implanted animals were in the stage of previtellogenesis (Fig.1b).

Effect of injection of LHRH-A every 10 days, following two implantations of T at an interval of 14 days, on ovarian development and maturation

In experiment 2, on day 35, 7 days following the two implantation of T, the development of ovaries was primed and oocytes were in the stage of previtellogenesis (Fig. 2a). At the end of May, after three injections of LHRH-A, the ovaries of T-treated fish were well developed, the oocytes were in

the advanced yolk vesicle stage (Fig. 2b), and average egg diameters reached 508.0 μm . By the end of June, after six injections of LHRH-A, 87.5% (7 of 8) hormone-treated females were mature and possessed vitellogenic ova with an average egg diameter of 691.5 μm (Fig. 2c); in contrast, the oocytes of control animals were only in the previtellogenic follicle stage (Fig. 2d).

Similar to the results of experiment 2, all treated 6-year-old females in Experiment 3, 83.3% (5/6) of treated 4-year-old females in Experiment 4 mature with an average egg diameter of 702.4 μm and 685.3 μm , respectively. All control shad (6-year old) remained immature with a mean oocyte diameter of 263.1 μm .

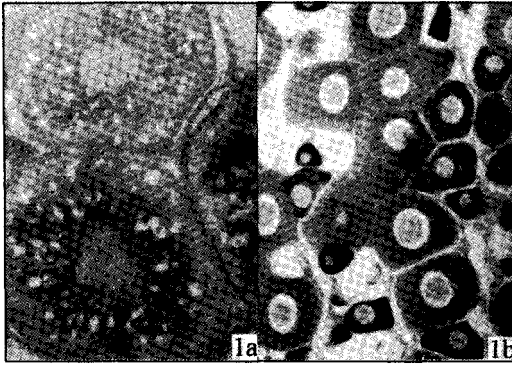


Fig. 1. Effect of implantation of T at monthly intervals on ovarian development. T-implanted shad had GSI=0.94% and oocytes were in the early yolk vesicle stage (Fig. 1a). The blank implanted shad had GSI=0.15% and oocytes were in the stage of previtellogenesis (Fig. 1b)

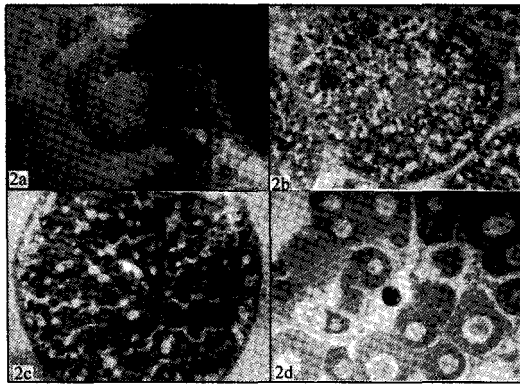


Fig. 2. Effect of injection of LHRH-A every 10 days, following two implantations of T at interval of 14 days, on oocyte development and maturation. See text for details.

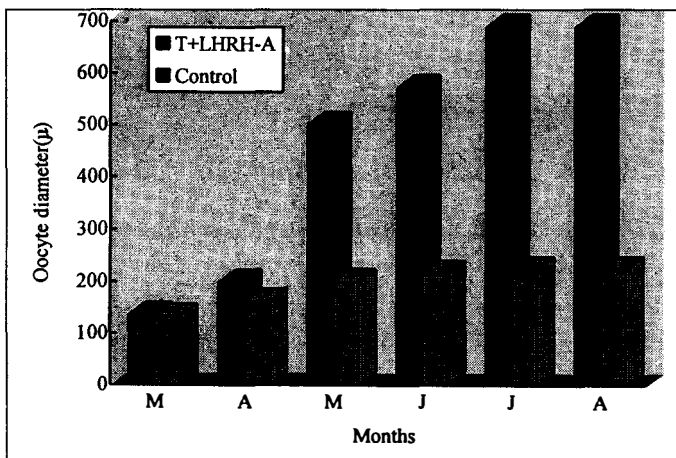


Fig. 3. Effect of six injections of LHRH-A following two implantations of T on oocyte diameters in cultured shad.

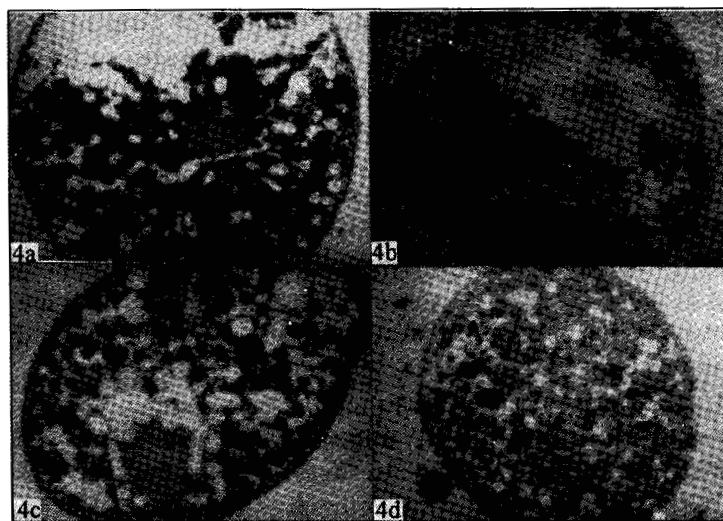


Fig. 4. Effects of two injections of LHRH-A alone or combined with DOM at injection interval of 12 h on induced spawning of Reeves shad.

Effects of two injections of LHRH-A alone or combined with DOM at injection interval of 12h on inducing ovulation

Two injections of LHRH-A alone induced ovulation in one of four fish with injection interval of 12h. Fish responded better to LHRH-A and DOM treatment with the same injection interval. At 6 h after the first injection of LHRH-A and DOM, the nucleus of oocytes began to migrate (Fig. 4a); after 12 h, the nucleus migrated to half of the radius (Fig. 4b); 5 h after the second injection, the nucleus was near to the membrane (Fig. 4c); within 8 to 12 h from the second injection, all 6 treated fish, and none of the control fish, ovulated (Fig. 4d).

Discussion

The results presented demonstrate that pelleted 17α -methyl-testosterone is effective in inducing gonadal development in cultured Reeves shad. In another word, this sex steroid has some stimulatory effects on the brain-pituitary axis leading to an accumulation of GtH in the pituitary. This effect of 17α -methyl-testosterone on the brain-pituitary axis has been reported by several authors in immature salmonid (Crim and Evans 1979, 1982, 1983a), milkfish (Lee *et al.* 1986b) and Japanese silver eel (Lin *et al.* 1991).

Serial injection of LHRH-A on T-primed shad cultured in pond can induce gonadal development up to the maturation stage. Mclean (1991) reported that oral delivery of LHRH-A to estradiol-primed coho salmon results in a significant release of GtH when compared to control, saline-treated fish. Single injection of LHRH-A alone stimulate GtH release in female Japanese eel pretreated by estradiol (Lin *et al.* 1991). These results confirm that GtH release can be induced in T or E2-treated fish. Evidence that LHRH-A stimulates GtH secretion in teleost fish has been presented previously for carp (Weil *et al.* 1980), trout (Crim *et al.* 1981) and goldfish (Peter 1980).

LHRH-A may also play a role in stimulating synthesis of GtH in the fish pituitary gland. Fang *et al.* (1981) noted nucleoplasmic changes in basophilic cells of the Tilapia pituitary following LHRH-A treatment, which were interpreted to signify the stimulation of GtH synthesis. In the landlocked salmon, LHRH-A administration resulted in marked increases in pituitary GtH content of fish in the regressed or early phases of gonadal recrudescence (Crim 1983b). In more mature salmon, when pituitary GtH content is already elevated, no augmentation of pituitary GtH in response to LHRH-A treatment could be demonstrated (Crim 1983b). These results suggested LHRH-A can influence synthesis of pituitary GtH, especially during the period of the seasonal reproductive cycle when pituitary GtH levels are very low.

Two injections of LHRH-A alone showed some effects in inducing ovulation in female Reeves shad. However, LHRH-A in combination with DOM was more effective in inducing ovulation. These results suggested that the neuroendocrine regulation of GtH secretion in shad also involves dual control, with GtH release stimulated by GnRH and inhibited by dopamine acting as a GRIF.

The reproductive cycle of the Reeves shad is characterized by a long delay before sexual maturation, and, even at the early phase of the reproductive migration to the river, the gonads remain immature; furthermore, if the reproductive migration is blocked, such as by captivity, sexual maturation will never occur (Wang 1998a, 1998b). The most important finding from the present study is that serial injection of LHRH-A following ovary priming by implantations of T can stimulate the brain-pituitary-ovary axis of domesticated Reeves shad to induce gonadal development to maturation. Furthermore, injection of LHRH-A and DOM can induce spawning. To date there is no report on shad culture to adulthood as there is no report on induced maturation and ovulation in cultured shad. The results show the importance of induced maturation and ovulation in developing shad culture and saving this endangered species.

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