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EFFECTS OF MATURATIONAL HORMONE TREATMENT ON SPERMATOGENESIS OF HYBRID CATFISH (*Clarias macrocephalus* x C. *gariepinus*)

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Abstract Hybridization between two different species for the same group of fishes may produce fertile hybrid F_1. However, in hybrid catfish (*Clarias macrocephalus* x *C. gariepinus*) the females are fertile but hybrid males can not be used in producing F2 and backcross hybrid. In order to improve the spermatogenesis, a series treatment with single hormone of ovaprim, ovaplant, human chorionic gonadotropin (hCG), carp pituitary gland (cPG) and combinations of cPG with ovaprim or hCG were tested. Gonado- (GSI) and seminal vesicle-somatic index (SVSI), fertility and histological observation were used to evaluate changes of testis. The GSI increased ($p<0.05$) after treated with cPG or ovaplant. Treatment with ovaplant or hCG also increased the SVSI. However, there was no change in all treatments using combination of cPG and hCG or ovaprim. Histological observation showed 25% of total observed testes producing a few sperm, but backcross with eggs of *C. macrocephalus* still gave negative result.

KEYWORDS: Hybrid catfish, hormone treatment, spermatogenesis

Introduction

Interspecific hybridization in fish has improved or altered valuable characteristics for culture such as growth, reproduction and disease resistance, and has great potential for improvement of quantitative traits. The hybrid between Asian catfish *Clarias macrocephalus* and African catfish *C. gariepinus* has been widely cultured in Thailand and Vietnam for more than a decade (Na-Nakorn, 1999; Kiem, 2003). Due to fast growth and high disease resistance, these hybrid catfish are attractive to farmers who formerly cultivated rice or raised either native catfish or other fish species with a lower market value. In Thailand, more than 80% of catfish farmers currently raise hybrid catfish (Na-Nakorn, 1999) with the annual production estimating about 50,000 tons (Yi *et al.*, 2003). In Malaysia, this hybrid has been evaluated as a good potential for aquaculture (Abol-Munafi *et al.*, 2003). However, mass production of the hybrid was limited because still dependence on source of parental species.

Generally, the hybrid of interspecific hybridization is sterile, caused by the reproductive isolating mechanisms which keep species separate and distinct. Some hybridization produce fertile hybrid as in case of Atlantic salmon x brown trout, red crucian carp x common carp, and channel catfish x blue catfish (Galbreath and Thorgaard, 1995; Bosworth *et al.*, 1997; Liu *et al.*, 2001;

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Argue *et al.*, 2003). The hybrid catfish (*C. macrocephalus x C. gariepinus*) female are fertile and potentially capable of making large numbers of backcross progeny. However, hybrid male is unsuccessfully in producing F2 and backcross hybrid (Abol-Munafi *et al.*, 2004; Na-Nakorn *et al.*, 2004), which could probably due to the problem of spermatogenesis.

Reproductive hormones have been used to stimulate reproductive processes and induce ovulation/ spermiation and spawning. Among various reproductive hormones, pituitary gland (PG), human chorionic gonadotropin (hCG) and GnRHa are the most common used hormones for inducing or maintaining spermatogenesis in many fish species. PG and hCG have been used to induce spermiation in mullet *Mugil cephalus* (Shehadeh *et al.*, 1973), Japanese eel *Anguilla japonica* (Miura et al., 1991), bream *Abramis brama* (Kucharczyk et al., 1997). Carp-PG treatment induced increased volume and sperm cells/fish in *Mystus nemurus* (Christianus et al., 1996). A single hCG injection induced a 13 fold increase in stripped sperm volume in *Pangasius bocourti* (Cacot *et al.*, 2003). Both PG and hCG treatments induced testis hydration and facilitated semen collection from testis of African catfish (Hecht *et al.*, 1982). Treatment with GnRHa has also proven effective in enhancing milt production in fish (review by Zohar and Mylonas, 2001). In the present study, effects of these hormones on the spermatogenesis of hybrid catfish were observed.

Material and Methods

Self produced hybrid catfish (9 - 10 months old) having weight of 218.7 ± 39 g were reared for three months in 2 fiberglass tanks (1.8 x 0.9 x 0.6 m) with a density of 25 fish/m^2 (35 males and 5 females per each tank). Water exchange was carried out twice a week. Fish were fed with commercial pellet (34% crude protein) once a day at daily rate of about 2-3% (dry matter) fish biomass.

Experimental design

Experiment 1: Effects of single hormone on spermatogenesis namely carp pituitary gland (cPG), hCG, ovaprim, and ovaplant were examined. Five treatments in triplicate with 5 fish per each replicate were tested. Saline (0,9% NaCl) was used as a control. Dosages of hormone and number of injection are shown in Table 1. Mean body weight of experimental fish was 537.17 ± 100 g. Interval between injections was 24 hours. All fish were sampled 24 hours after receiving the last injection except fish with ovaplant treatment which were implanted with ovaplant pellet 10 days before sampling.

Table 1. Dose of hormone (per kg body weight) and number of injection in Experiment 1

Treatment	Injection 1	Injection 2	Injection 3	Injection 4
(1) cPG	8 mg			
(2) Ovaplant	1 pellet			
(3) Ovaprim	1.0 mL			
(4) hCG (2 injection)	1,500 IU	2,500 IU		
(5) hCG (4 injection)	500 IU	500 IU	500 IU	2,500 IU
(6) Saline	0.5 mL			

Hormone: *cPG: dehydrated carp pitutary gland in acetone; hCG: Pregnyl® (Organon, Kloosterstraat, Netrerlands); Ovaplant (Syndel, Vancouver, Canada): pellet containing 75μg GnRHa; Ovaprim (Syndel, Vancouver, Canada): 1 mL containing 20μg salmon GnRHa and 10 mg domperidon*

Experiment 2: Combination of cPG with others hormone were tested. Five fish in each group were received several consecutive injections of cPG (8 mg/kg body weight) and ovaprim or hCG,

with a 24 hours interval (Table 2). Mean body weight of experimental fish was 515.67 ± 87.67 g. Fish were sampled after receiving last injection 24 hours.

Table 2. Dose of hormone (per kg body weight) and number of injection in Experiment 2

Treatment	Injection 1	Injection 2	Injection 3
(1) cPG - cPG - hCG	8 mg	8 mg	2500 IU
(2) cPG - hCG - hCG	8 mg	1000 IU	2500 IU
(3) cPG - Ovp	8 mg	0.5 mL	
(4) cPG - Ovp - Ovp	8 mg	0.5 mL	0.5 mL
(5) cPG - cPG - Ovp	8 mg	8 mg	0.5 mL
(6) Saline (control)	0.5 mL	0.5 mL	0.5 mL

Hormone: *cPG: dehydrated carp pituitary gland in acetone; hCG: Pregnyl® (Organon, Kloosterstraat, Netrerlands); Ovp: Ovaprim (Syndel, Vancouver, Canada)*

Sampling

Fish were anaesthetized in tricaine methanesulphonate (MS 222) before sacrificed. Testes and seminal vesicles (SV) were removed and weighed. Gonado- and SV-somatic index (GSI and SVSI) were calculated as percentage of testis and SV weight related to total body weight. Testis form individual male were divided for histological observation and fertility. Changing of testes after hormone treatment was observed by using histological method. Testis were fixed in Bouin's solution then processed through to paraffin wax (Kiernan, 1990). Sections with a thickness of 5 µm then were stained with Hematoxylin and Eosin (HE).

Fertility test was done by applying dried fertilization method between intra-testicular (IT) semen of experimental male and egg of *Clarias macrocephalus*. IT semen was obtained by squeezing slit testis then diluted 5 times in saline 0.9% NaCl (Legendre *et al.*, 2000). IT semen was kept in refrigerator at 4 °C until fertilization. From each group of treatment, IT semen of 3 males were separately fertilized with 3 samples (about 300 eggs) of pooled eggs that obtained from 3 *C. macrocephalus* females. Fertilization between egg of *C. macrocephalus* and sperm of the same species was kept as control. Fertilization and hatching rates were used to evaluate fertility of experimental fish.

Data analysis

GSI, SVSI, fertilization and hatching rate were calculated by mean and standard deviation (SD). Mean were tested for significant differences by one-way ANOVA followed by Duncan's multiple range test. Statistical analysis was performed with the SPSS software. Spermatozoan stage of testis was not analyzed statistically due to low number of fish per treatment.

Results

Effects of hormone on GSI and SVSI of hybrid catfish

Effects of single hormones on spermiation of hybrid catfish were shown in Table 3. GSI of fish in treatment using cPG injection and ovaplant implantation (for 10 days) were increased significantly ($P<0.05$) compared to control treatment. GSI of fish received injections of ovaprim and hCG increased but there were no differences ($P<0.05$) compared to control treatment. SVSI of fish treated with hormone also increased, however significant increasing ($P<0.05$) of SVSI has observed only in ovaplant and hCG treatments.

Table 3. Testicular quality of hybrid catfish treated with single hormone including cPG, hCG, GnRHa (ovaprim and ovaplant) in Experiment 1

Treatment	GSI (%)	SVSI (%)	Spermiation (No. of fish)	
			Spermatocyte	Spermatozoa
(1) Carp PG	1.01 ± 0.25^{a}	0.15 ± 0.09^{bc}	4	1
(2) Ovaplant	0.98 ± 0.16^{a}	0.27 ± 0.06^{a}	4	1
(3) Ovaprim	0.91 ± 0.07^{ab}	0.14 ± 0.05^{bc}	3	2
(4) hCG (2 injection)	0.90 ± 0.15^{ab}	0.22 ± 0.05^{ab}	3	2
(5) hCG (4 injection)	0.83 ± 0.10^{ab}	0.28 ± 0.09^{a}	3	2
(6) Saline	0.70 ± 0.18^{b}	0.09 ± 0.03^{c}	4	1

[a,b] *Means in each column having the different superscript are significantly different (P < 0.05).*

In the Experiment 2, combination of cPG with hCG or ovaprim also caused an increase in GSI and SVSI (Table 4), however there were no significantly differences (P<0.05) compared to control treatment. SVSI was high in treatments involving with hCG.

Table 4. Testicular quality of hybrid catfish treated with the combination of cPG with hCG and GnRHa (ovaprim) in Experiment 2

Treatment	GSI (%)	SVSI (%)	Spermiation (No. of fish)	
			Spermatocyte	Spermatozoa
(1) cPG - cPG - hCG	1.07 ± 0.52^{a}	0.14 ± 0.08^{a}	4	1
(2) cPG - hCG - hCG	0.93 ± 0.24^{a}	0.19 ± 0.16^{a}	4	1
(3) cPG - Ovp	0.98 ± 0.22^{a}	0.14 ± 0.04^{a}	4	1
(4) cPG - Ovp - Ovp	1.00 ± 0.32^{a}	0.13 ± 0.04^{a}	4	1
(5) cPG - cPG - Ovp	0.94 ± 0.15^{a}	0.14 ± 0.05^{a}	5	0
(6) Saline (control)	0.64 ± 0.06^{a}	0.09 ± 0.03^{a}	3	2

[a,b] *Means in each column having the different superscript are significantly different (P < 0.05).*

Spermatogenesis

Histological observation showed that most of testes samples in Experiment 1 and Experiment 2 had well developed seminiferous tubules with cells in the early stage of spermatogenesis (Fig. 1). However, only 25% of total experimental fish possess testes containing a few spermatozoa (Fig. 2) including fish in control treatment (30% in experiment 1 and 20% in experiment 2). Size of hybrid sperm was 2-3 times larger than sperm of *C. macrocephalus* (Fig. 3) and *C. gariepinus* (Fig. 4).

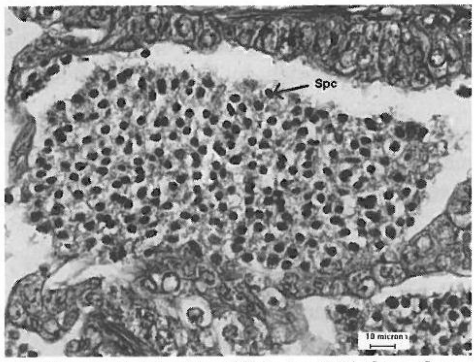

Figure 1. Testis of fish in cPG-Ovp-Ovp treatment (Spc : spermatocyte)

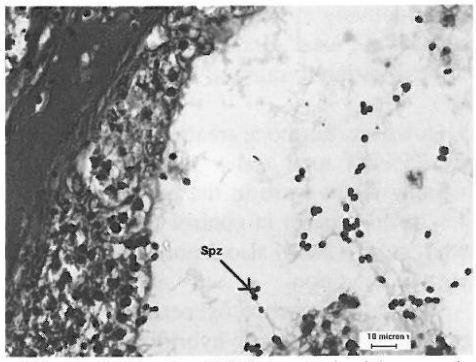

Figure 2. Testis of fish treated with ovaprim (Spz: spermatozoa)

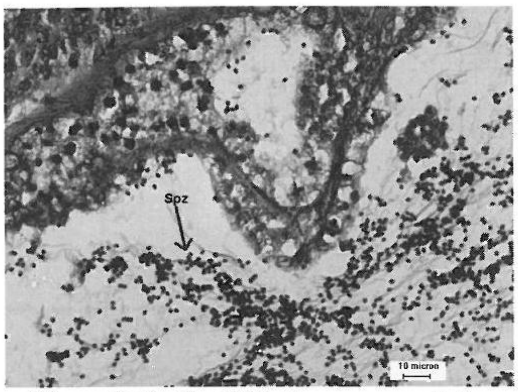

Figure 3. Testis of *Clarias macrocephalus* (Spc : spermatocyte)

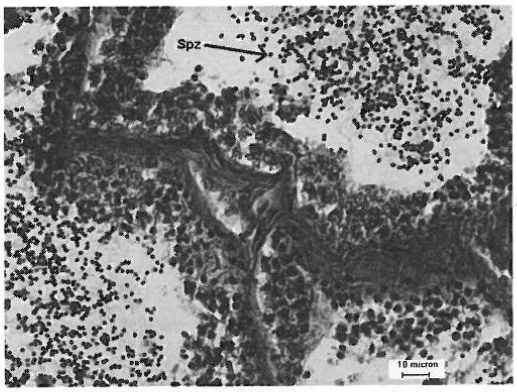

Figure 4. Testis of *Clarias gariepinus* (Spz: spermatozoa)

Fertility

Fertilization between egg of *C. macrocephalus* and sperm of the same species gave very high fertilization rate (89.35%). However, fertilization between the same egg and intra-testicular semen obtained from males in all treatments of 2 experiment was failed resulting in 0% fertilization rate.

Discussion

Increase of GSI in treatments using cPG and ovaplant was caused by an increased seminal fluid production as a result of testicular hydration. Testicular hydration after hormone treatment was also reported for *C. macrocephalus* after ovaprim treatment (Tambasen Cheong *et al.*, 1995), for rabbitfish *Siganus guttatus* after mGnRHa treatment (Garcia, 1991). However, it was not observed in Experiment 2 though combinations of cPG with hCG or ovaprim were used. For *C. gariepinus*, single injection or two injections of cPG increased SVSI but SVSI did not increased in treatment using combination of cPG and ovaprim (Viveiros *et al.*, 2001). Perhaps, cPG do not stimulate a long-term elevation of seminal fluid production, and testes returned to pre-treatment stage after 24 hours. hCG was considered more appropriate hormone for inducing spermiation and spawning because its act fast, via direct stimulation of the gonad. It induced an increase of milt volume in *Pangasius* catfish from 5.4 to 13 times for *P. hypophthalmus* and *P. bocourti*, respectively (Cacot, 1999; Cacot *et al.*, 2003). Several injections of hCG (3-10 times) induced maturation and final oocyte maturation of *P. bocourti* (Cacot, 2002). In the present study, 3-4 injections of hCG also caused an increase of SVSI. Single injection of GnRHa did not induce testicular hydration, but GnRHa-delivery system caused an increase of GSI and SVSI for hybrid catfish. GnRHa-delivery systems were used successfully to induce both spermatogenesis and spermiation or increase milt volume of Atlantic salmon, brown trout, rainbow trout, white bass (review by Zohar and Mylonas, 2001).

However, hormone treatment did not improve the spermatogenesis in male of hybrid catfish. Only 25% of total testes samples in two experiments developed to spermatozoan stage, the remaining testes were in the early stage of spermatogenesis. In Experiment 2, the percentage of testes having sperm in control treatment was even higher than those in hormone treatments. Na-Nakorn *et al.*, (2004) also reported that about 50% hybrid male possess testis at spermatozoan stage in culture condition.

Problem of spermatogenesis in hybrid catfish may due to genetic aspect. Usually, hybrids produced by interspecific hybridization are sterile because of genetic incompatibility during meiosis (Tave, 1993). It functions as reproductive isolating mechanisms which keep species separate and

distinct. In this study, although fertilization did not occur between sperm of hybrid and egg of *C. macrocephalus*, 25% male hybrid catfish presented well-developed testes with spermatozoa. Well-developed testis was also reported in other hybrid catfish, the *Heterobranchus longifilis* x *Clarias gariepinus* hybrid, and this hybrid can produce viable F2 generation (Legendre *et al.*, 1992). However, mechanism causing fertility or sterility in male hybrid catfish was still unclear. Gorshkov *et al.*, (2002) have attributed the sterile of hybrid to the systematic distance between the parental species. The more distantly related between two species, the greater likelihood of hybrid being sterile. The closed of identical chromosome number of *C. gariepinus*, 2n = 56, (Teugels *et al.*, 1992) and *C. macrocephalus*, 2n = 54, (Poompuang and Na-Nakorn, 2003) seems to be enough for the normal gonadal development. Other reason may due to the different of sex-determination system. *C. macrocephalus* follows the XY system (Na-Nakorn, 1995). *C. gariepinus*, on the other hand, follows the ZW system (Ozouf-Costaz *et al.*, 1990). The complexity in the interaction between two sex-determination systems may cause differences in fertility within male hybrid catfish.

Conclusion

Hormone treatment can produce testicular hydration in the hybrid catfish but did not improve the spermatogenesis process. Problem of spermatogenesis may be due to genetic incompatibility during meiosis process or sex-determination system differences between the two parental species.

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