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Induction of Ovulation in F₁ Malaysian Mahseer, *Tor tambroides* (Bleeker, 1854) by Using Synthetic and Non-synthetic Hormones

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ABSTRACT

Tor tambroides is an important and highly valued freshwater fish. In Malaysia, wild populations of this species has undergone declines in range and abundance due to degradation of their natural habitat. Due to economic important and concerns about their conservation, a culture artificial propagation programme was established to produced seed-stock for aquaculture and conservation purposes. The effectiveness of selected synthetic and non-synthetic commercial hormones was tested on filial 1 generation (F₁) of *Tor tambroides* (Bleeker 1854) females in captivity. A total of 144 matured females were given intramuscular implantation of slow release hormone, Ovaplant (38.6-53.6 µg kg⁻¹ b.wt.), for 5-6 weeks prior to induction. Selected commercial hormones were compared for the induction. The effect of these hormones on breeding performance and larval quality of F₁ *Tor tambroides* were evaluated. The results showed that Ovatide (0.5 mL kg⁻¹ BW) was the most reliable hormone in inducing ovulation of F₁ *T. tambroides* females overshadowed the other tested hormones. Ovaprim (0.5 mL kg⁻¹ b.wt.) was the next best tested hormone followed by a synthetic analogue of salmon gonadotropin releasing hormone (sGnRH_a/OvaRH) (10 µg kg⁻¹ b.wt.). No ovulation occurred in the groups treated with Luteinizing Hormone Releasing Hormone Analogue (LHRH_a) (10 µg kg⁻¹ b.wt.), Human Chorionic Gonadotropin (HCG) (1000 µg kg⁻¹ b.wt.) and Carp Pituitary Extract (CPE) (10 mg kg⁻¹ b.wt.). Ovatide was further chosen to test its effectiveness at various dosage levels in inducing ovulation of *T. tambroides*. Thus, concentration of 0.5 mL kg⁻¹ b.wt. of Ovatide was found to be the optimum dosage for inducing ovulation of F₁ *T. tambroides* in captivity.

Key words: Artificial propagation, breeding performance, conservation, Filial 1 generation, ovulation, commercial hormone, *Tor tambroides*

INTRODUCTION

Malaysian mahseer, *Tor tambroides* (Bleeker, 1854) is locally known as kelah, pelian or empurau belonging to family Cyprinidae and genus *Tor*. It is the most sought after freshwater fish

because of its high quality flesh as food, beautiful body shape as ornamental and aggressive behavior as sport fish. In recent years, due to over exploitation, deforestation, pollution and climate changes, wild populations of this highly prized fish have become dramatically decreased and it is now declared as one of the threatened species in Malaysia. *Tor tambroides* is asynchronous spawners and capable of spawning several times a year, shedding their eggs in small batches (Ingram *et al.*, 2005). Spawning of *Tor tambroides* in the wild takes place after rainy periods, suggesting the influence of low water temperature and monsoon season. However, in general, spawning or ovulation induction in captivity is largely based on captive wild breeders (Ingram *et al.*, 2005, 2007) and takes place mainly during spring while low spawning was observed in autumn and winter (Ingram *et al.*, 2005). In *T. tambroides*, filial 1 generation (F₁) cultured broodstocks (hatched and reared in captivity), however, often fail to ovulate or spawn without hormone induction (Azuadi *et al.*, 2011, 2012). Thus, it is necessary to apply hormone induction for breeding purposes.

For conservation and management of this threatened fish, special techniques to improve propagation have to be undertaken. Successful induced ovulation and multiple spawning in captivity (ponds and tanks) by artificial propagation have been achieved for wild broodstocks of this species (Ingram *et al.*, 2005). Ovaprim is considered the most effective hormone for inducing ovulation and spawning of *T. douronensis* and *T. tambroides* (Ingram *et al.*, 2005). Further improvement was made by Ingram *et al.* (2005) by using Ovaplant implantation to improve gonadal development and oocyte maturation of the fish.

A variety of GnRH analogues have proven to be effective in inducing final oocyte maturation and ovulation, however, their biological responses vary greatly in different fish (Szabo *et al.*, 2007). The most commonly used GnRH analogues in spawning induction are salmon GnRH (sGnRH) analogue and mammalian GnRH analogue (mGnRH_a) or (LHRH_a). sGnRH was found to be more potent than mGnRH in stimulating ovulation in various species such as common carp, *Cyprinus carpio*, Chinese loach, *Paramisgurnus dabryanus* (Lin and Peter, 1996; Lin *et al.*, 1991) and African catfish, *Clarias gariepinus* (Szabo *et al.*, 2007). LHRH_a is still successfully used in inducing final oocyte maturation and synchronize ovulation or spawning in a variety of teleosts such as goldfish, *Carassius auratus*, starlet, *Acipenser ruthenus*, common sole, *Solea solea* and Chinese minnow, *Rhynchocypris oxycephalus* (Chang and Peter, 1983; Horvath *et al.*, 1986; Ramos, 1986; Donaldson and Hunter, 1983; Park, 2002). Originally, Carp Pituitary (CP) either freshly extracted from fish or commercial ready made, is widely utilized to induce spawning in many species such as Chinese carps, major Indian carps, common carp, *Cyprinus carpio* and olive flounder, *Paralichthys olivaceus* (Lam, 1982; Park *et al.*, 1994). Since the 1930s, Human Chorionic Gonadotropin (HCG) has been widely used in inducing ovulation and yielded the best results among the mammalian hormones (Lam, 1982; Kim *et al.*, 1992; Kelly and Kohler, 1994; Park *et al.*, 1994). However, low dosage of CP and HCG failed to induce maturation and ovulation in wild caught *Tor tambroides* (Ingram *et al.*, 2005).

A newly low cost formulated hormone for inducing ovulation, such as Ovatide, has been commercially introduced, nonetheless not a lot of research has been done to test the effectiveness and potential of this hormone in Malaysia. Ovatide consists of GnRH analogue in combination with dopamine antagonist is effective in inducing spawning (Gupta *et al.*, 2002; Sahoo *et al.*, 2004). Ovatide also less expensive, less viscous, easy to store and simple to use compared to Ovaprim and other hormones used for breeding of fish in the aquaculture sector (Marimuthu *et al.*, 2009). A number of experiments have been done to test the effectiveness of Ovatide in other fishes, such as

Indian major carp, *Catla catla* (Dhawan and Kaur, 2004), walking catfish, *Clarias batrachus* (Sahoo *et al.*, 2005) and spotted snakehead, *Channa punctatus* (Marimuthu *et al.*, 2009). Ovatide has been used as the positive control in inducing ovulation of female F₁ *T. tambroides* and produced highest ovulation rate and egg fecundity (Azuadi *et al.*, 2011, 2012). The effectiveness between Ovatide and Ovaprim was compared among Indian major carps *Cirrhina mrigala* and *Labeo rohita* and the results showed that Ovatide produced higher spawning fecundity and fertilization rate than those of using Ovaprim (Dhawan and Kaur, 2004).

This study aimed to test the effectiveness of various commercial synthetic hormones and suitable dosage of the hormone for inducing ovulation in F₁ generation of *Tor tambroides* females.

MATERIALS AND METHODS

Experimental fish and broodstocks selection: The experiments were conducted between November 2010 and February 2011. Filial one generation (F₁) of Malaysian mahseer *T. tambroides* fingerlings were obtained from Tarat Indigenous Fisheries Production and Research Center (IFPRC), Serian, Sarawak through artificial propagation and reared in concrete recirculating aquaculture system (RAS) tanks for 4-5 years at Lu Thian Tack (LTT) Aquaculture Farm, Asajaya, Sarawak. These fish were fed at 5% body weight in the morning with homemade feed with ingredients consisting of emperang fish, illipenut (*Engkabang*) fruit, vitamin C, squid oil and flour containing 5.3% carbohydrate, 52.6% crude protein, 17.6% crude lipid, 12.7% ash, 1.8% crude fibre and 4770.5 kcal g⁻¹ energy. In the evening, the fish were fed with commercial tilapia pellet (Cargill, Malaysia) containing 16% protein, 4% fat and 6% crude fiber.

A total of 144 sexually matured F₁ female with total length and body weight ranged between 43.9 to 73 cm and 1.4 to 4.39 kg, respectively and 72 males with the average total length and body weight ranged between 42.0 and 54.0 cm and 1.42 to 2.0 kg, respectively were used in this study. The female breeders were selected based on features of having soft and round abdomen with protruding, swollen and reddish papillae (Azuadi *et al.*, 2011), while males were selected when they have running milt after applying a gentle pressure on the abdomen. All breeders were tagged by implanting microchips [American Veterinary Identification Devices (AVID), Norco, CA, USA] for identification purposes.

Hormone preparation and artificial propagation: All the F₁ females were implanted with pellet containing 75 or 150 µg of salmon gonadotropin releasing hormone analogue (sGnRH_a, Ovaplant, Syndel International Inc., Qualicum Beach, BC, Canada; 5.2±0.5 kg b.wt.) for 5-6 weeks prior to induction trials. The Ovaplant was implanted using a RalGun Pellet Injector (Syndel International Inc., Qualicum Beach, BC, Canada). The induction treatments consisted of synthetic hormones, such as mammalian Luteinizing Hormone Releasing Hormone Analogue (LHRH_a), OvaRH (a synthetic analogue of salmon gonadotropin releasing hormone (sGnRH_a)), Human Chorionic Gonadotropin (HCG), Carp Pituitary Extract (CPE) and Ovaprim purchased from Syndel Laboratories, Vancouver, Canada. LHRH_a, sGnRH_a, CPE and HCG were dissolved in sterile physiological saline (0.9% NaCl) solution before administration. Ovatide (Hemmo Pharma, Mumbai, India) supplied in liquid form containing 20 µg of synthetic GnRH_a and 10 mg of dopamine antagonist in 1.0 mL. Twelve females served as control were injected with physiological saline (0.9%; 0.5 mL kg⁻¹ b.wt.; control group; 6.2±0.8 kg b.wt.).

The experiments were conducted between November 2010 and February 2011. The experiments were conducted twice in different day. Six sexually matured female broodstocks were used in each

treatment and kept separately in individual tanks after hormone administration. To avoid any stress and for the ease of handling, these fish were anesthetized with tricaine methanesulfonate MS222 (Syndel International Inc., Qualicum Beach, BC, Canada) before implantation, injection or stripping. Weight and length of the fish were recorded before the hormone administration and the hormone dosages were prepared according to the body weight of the fish. The fish were held in 0.2% acriflavine (10-methylacridine-3, 6-diamine chloride) for injury and infection treatment. All hormone administrations and stripping works were done in the morning. The ovulation response was evaluated 24 h post injection by applying gentle pressure to the female's abdomen. If ovulation occurred, eggs were stripped into a measuring jar with egg volume recorded for the total stripped egg and fecundity estimation. Two matured males having viscous and creamy milt were chosen and stripped into the jar containing eggs for fertilization as modified from the methods described by Joshi *et al.* (2002).

The fertilized eggs were incubated in separate plastic mesh basket according to breeders with 3 replicates. The eggs incubators were placed on water surface in 600 L rectangular fibreglass tanks. During the experiment, the water flow rate into the tanks was 1-2 l per minute to maintain the water level of 5-10 cm above the eggs. Prior to the experiments, the water was treated with UV light, ozone and filtration. To avoid direct exposure to light or UV, the eggs were completely covered with dark plastic perforated sheet for ventilation. To avoid any fungal infection, the opaque unfertilized eggs and dead larvae were removed twice a day (8:00 a.m. and 7:00 p.m.).

Eggs and larval quality measurements: Fertilization rate was evaluated 7 h Post Fertilization (PF) by observing at least 100 eggs at 32-cell stage under stereomicroscope, Motic SMZ-168 (2x magnification, 34.5 mm working distance). From the time of fertilization until 90% of the eggs hatched, hatching rate was determined by placing 200 eggs into a petri disk with 3 replicates and then the numbers of hatched larvae were counted. By counting dead larvae in each plastic mesh incubation tray, live larvae were calculated and recorded, from which survival rate was estimated from day 1 to 7 Post Hatching (PH) in each tray. Deformed larvae were recorded to calculate normal larvae production. Deformed larvae were determined by observed having abnormal head, bent notochord, bent tail and bloated body. During larval rearing period, 50 larvae from each tray were randomly measured for standard length under the stereomicroscope (0.75x magnification, 127 mm working distance) on day 1 to 7 pH.

The following formulae were used to calculate the ovulatory response, eggs and larvae qualities parameters:

$$\text{Ovulatory response (\%)} = \frac{\text{Number of ovulated fish}}{\text{Total number of induced fish}} \times 100$$

$$\text{Fecundity (\%)} = \frac{\text{Number of stripped egg}}{\text{Weight of ovulated fish}} \times 100$$

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs in a batch}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of fertilized eggs in a batch}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Total number of survival larvae until day 7}}{\text{Total number of larvae counted at day 1}} \times 100$$

$$\text{Percentage of normal larvae (\%)} = \frac{\text{Number of normal larvae}}{\text{Total number of larvae counted}} \times 100$$

$$\text{Percentage of abnormal larvae (\%)} = \frac{\text{Number of abnormal larvae}}{\text{Total number of larvae counted}} \times 100$$

Water quality parameters: By using multiparameter water quality checker (WQC 24-1-2), water quality parameters such as dissolved oxygen, conductivity, temperature, pH, turbidity and total dissolved solid were monitored daily.

Statistical analysis: Data were expressed as Mean±standard Error of the Mean (SEM). Total stripped eggs, eggs fecundity, percent of fertilized eggs, survival larvae, normal larvae, deform larvae, eggs diameter and larvae length for each hormone treatment were analyzed using one-way Analysis of Variance (ANOVA), followed by Duncan multiple range test to test differences in the mean between treatments. Statistical significant was inferred at $p < 0.05$. These tests were carried out using SPSS package version 16.0.

RESULTS

Daily water temperature, dissolved oxygen, conductivity, pH, turbidity and total dissolved solid (26.0-28.0°C, 5.45-14.0 mg L⁻¹, 7.1-12.5% FS, 7.21-7.98, 1% FS and 0.1-0.2 g L⁻¹, respectively) were recorded. The ovulatory response, egg and larval quality of *T. tambroides* treated with Ovaprim, Ovatide, OvaRH, LHRHa, HCG, CPE and saline solution are presented in Table 1. Ovulations in female breeders were detected 24 hour post injection. The ovulatory response (75%) was highest in the group injected with 0.5 mL Ovatide per kg body weight, whereby 9 out of 12 fish ovulated. Similar ovulation success (42%) was observed in the group injected with Ovaprim (0.5 mL kg⁻¹ b.wt.) and OvaRH (10 µg kg⁻¹ b.wt.). The total stripped eggs (2457±363 eggs) and

Table 1: Effectiveness of several commercial synthetic hormones on ovulatory response, total stripped eggs, stripped fecundity, fertilization, hatching, survival and larval production of *T. tambroides*

Parameters	Treatments						
	Ovaprim (0.5 mL kg ⁻¹)	Ovatide (0.5 mL kg ⁻¹)	OvaRH (10 µg kg ⁻¹)	LHRHa (10 µg kg ⁻¹)	HCG (1000 iu kg ⁻¹)	CPE (10 mg kg ⁻¹)	Saline (0.5 mL kg ⁻¹)
Weight of females (kg)	2.30±0.07	2.48±0.37	2.47±0.10	2.45±0.31	2.45±0.16	2.29±0.14	2.38±0.15
Total length of females (cm)	55.1±0.7	53.6±1.0	53.7±0.9	54.0±1.8	58.5±1.6	55.1±2.8	57.9±2.0
Ovulatory response (N)	5/12	9/12	5/12	0/12	0/12	0/12	0/12
Total stripped eggs (N)	1519±363 ^a	2457±363 ^d	489±203 ^b	000±0 ^a	000±0 ^a	000±0 ^a	000±0 ^a
Stripped fecundity (eggs kg ⁻¹ b.wt.)	501±80 ^c	850±170 ^d	153±62 ^b	000±0 ^a	000±0 ^a	000±0 ^a	000±0 ^a
Fertilization rate (%)	69.6±2.66 ^b	89.6±2.8 ^c	57.7±0.9 ^a	-	-	-	-
Hatching rate (%)	61±3.2 ^b	76.9±5.6 ^b	26.4±0.9 ^a	-	-	-	-
Survival rate (%)	53.3±4.4 ^b	80.6±3.6 ^c	33.3±8.8 ^a	-	-	-	-
Deformed larvae (%)	23.3±0.9 ^b	10.7±1.0 ^a	69.4±5.3 ^c	-	-	-	-
Normal larvae (%)	65.7±5.2 ^b	82.7±5.7 ^b	30.6±5.3 ^a	-	-	-	-

OvaRH: A synthetic analogue of salmon gonadotropin releasing hormone (sGnRH), LHRHa: Luteinizing hormone releasing hormone analogue, HCG: Human chorionic gonadotropin, CPE: Carp pituitary extract, Mean values with different superscripts were significantly different ($p < 0.05$) evaluated using one-way ANOVA followed by Duncan's *post-hoc* test. The observation are based on n = 3 of twelve replicates

fecundity (850±170 egg kg⁻¹ b.wt.) was significantly highest (p<0.05) in the group treated with Ovotide compared to other treatments. The next highest total stripped eggs (1519±363 eggs) and fecundity (501±80 egg kg⁻¹ b.wt.) were observed in the group treated with Ovaprim. The lowest (p<0.05) total stripped eggs (489±203 eggs) and fecundity (153±62 egg kg⁻¹ b.wt.) were observed in the group administered with OvaRH. No ovulatory responses were observed in the group treated with LHRHa (10 µg kg⁻¹ b.wt.), HCG (1000 iu kg⁻¹ b.wt.), CPE (10 mg kg⁻¹ b.wt.) and 0.9% saline solution (0.5 mL kg⁻¹ b.wt.) that lead to no egg being produced.

The highest fertilization (89.6±2.8%) and hatching (76.9±5.6%) rates were observed in the groups injected with Ovotide followed by the Ovaprim and OvaRH treatments. The results are presented in Table 1. The fertilization and hatching rates in the group injected with Ovaprim were 69.6±2.66 and 61±3.2%, respectively. The fertilization rate (57.7±0.9%) and hatching rate (26.4±0.9%) of the group treated with OvaRH were significantly lower (p<0.05) than those in other groups. The significant highest (p<0.05) larval survival rate was observed in the groups treated with Ovotide followed by Ovaprim and OvaRH with the values of 80.6±3.6, 53.3±4.4 and 33.3±8.8%, respectively. The survival rate from the OvaRH group was significantly lower (p<0.05) than the other groups. The percentage of normal larvae (82.7±5.7%) was highest while the percentage of deformed larvae (10.7±1.0%) was significantly lower (p<0.05) in the group treated with Ovotide than those injected with OvaRH and Ovaprim. The OvaRH treatment had the lowest percent of normal larvae (30.6±5.3%) and the highest percentage of deformed larvae (69.4±5.3%) compared to other treatments. Slightly higher percentage of normal larvae (65.7±5.2%) and slightly lower deformed larvae (23.3±0.9%) were found in the group injected with Ovaprim. In these experiments, there were no size differences of larvae observed, despite of variation in hatching and survival rates among the hormone treatments.

The hormone dosages of most effective hormone from previous experiment were tested in this study. The ovulatory response, egg and larvae qualities of *T. tambroides* induced at different Ovotide dosages are presented in Table 2. The ovulation response (67%) was highest in the group treated with 0.5 mL Ovotide per kg body weight. Similar ovulation response (33%) was observed in the group administered with 0.25 and 1.0 mL kg⁻¹ b.wt. of fish. The group treated with

Table 2: Effectiveness of various doses of Ovotide on ovulatory response, total stripped eggs, stripped fecundity, fertilization, hatching, survival and larval production of *T. tambroides*

Parameters	Treatments (mL kg ⁻¹)				
	Ovotide (0.25)	Ovotide (0.5)	Ovotide (0.75)	Ovotide (1.0)	Saline (0.5)
Weight of females (kg)	3.08±0.16	2.85±0.21	2.72±0.20	2.81±0.21	2.67±0.18
Total length of females(cm)	55.8±1.1	53.8±1.4	54.6±1.1	56.5±1.4	55.6±1.6
Ovulatory response (N)	4/12	8/12	3/12	4/12	0/12
Total stripped eggs (N)	835±74 ^b	2461±111 ^d	1296±137 ^c	986±65 ^b	000±0 ^a
Stripped fecundity (eggs kg ⁻¹ b.wt.)	271±33 ^b	439±16 ^c	286±27 ^b	281±16 ^b	000±0 ^a
Fertilization rate (%)	82.4±2.8 ^b	88.2±2.2 ^b	70.2±1.7 ^a	67.8±1.2 ^a	-
Hatching rate (%)	38.4±25.1 ^a	83.6±1.8 ^c	64.3±1.3 ^b	35.9±1.7 ^a	-
Survival rate (%)	45.6±2.3 ^b	80.9±1.9 ^c	50.8±1.4 ^b	27.4±1.3 ^a	-
Deformed larvae (%)	12.1±0.5 ^b	8.0±0.6 ^a	12.7±0.5 ^b	20.2±1.5 ^c	-
Normal larvae (%)	40.2±2.2 ^b	74.4±1.6 ^d	54.2±1.6 ^c	23.1±1.4 ^a	-

Mean values with different superscripts were significantly different (p<0.05) evaluated using one-way ANOVA followed by Duncan's *post-hoc* test. The observation are based on n = 3 of twelve replicates

0.75 mL kg⁻¹ b.wt. of Ovotide produced 25% of ovulatory response. No ovulatory response was observed in the group injected 0.9% saline solution. The total stripped eggs and egg fecundity were significantly higher ($p < 0.05$) in females injected with 0.5 mL Ovotide per kg body weight than those injected with 0.25, 0.75 and 1.0 mL of Ovotide per kg body weight.

No significant differences ($p > 0.05$) of fertilization rate (82.4 ± 2.8 and $88.2 \pm 2.2\%$) were found between different dosages of Ovotide, 0.25 and 0.5 mL kg⁻¹ b.wt. The results are presented in Table 2. The fertilization ($67.8 \pm 1.2\%$), hatching ($35.9 \pm 1.7\%$) and survival rates ($27.4 \pm 1.3\%$) were significantly lower ($p < 0.05$) in the group injected with 1.0 mL kg⁻¹ b.wt. of Ovotide. The significant highest ($p < 0.5$) hatching ($83.6 \pm 1.8\%$) and survival rates ($80.9 \pm 1.9\%$) were observed in the females administered with 0.5 mL kg⁻¹ b.wt. of Ovotide. Next highest hatching ($64.3 \pm 1.3\%$) and survival rates ($50.8 \pm 1.4\%$) were observed in the group treated with 0.75 mL kg⁻¹ b.wt. of Ovotide. The highest percentage of normal larvae was observed in the group injected with 0.5 mL kg⁻¹ b.wt. of Ovotide ($74.4 \pm 1.6\%$) followed by the groups injected with 0.75 mL kg⁻¹ b.wt. (54.2 ± 1.6), 0.25 mL kg⁻¹ b.wt. ($40.2 \pm 2.2\%$) and 1.0 mL kg⁻¹ b.wt. of Ovotide ($23.1 \pm 1.4\%$). The significant lowest ($p < 0.05$) percent of deformed larvae ($8.0 \pm 0.6\%$) was observed in the group treated with 0.5 mL kg⁻¹ b.wt. of Ovotide followed by the group administered with 0.25 mL kg⁻¹ b.wt. ($12.1 \pm 0.5\%$), 0.75 mL kg⁻¹ b.wt. (12.7 ± 0.5) and 1.0 mL kg⁻¹ b.wt. of Ovotide ($20.2 \pm 1.5\%$).

DISCUSSION

Commercial synthetic hormones, such as LHRHa, HCG and CPE, failed to induce the ovulation of *T. tambroides* females. Similar result was noted by Ingram *et al.* (2005) in which HCG and CP was ineffective to induce ovulation in the wild captive *T. tambroides*. The failure of using these hormones to induce ovulation in the fish probably due to insufficient stimulation to induce GtH increment which can lead to no Final Oocyte Maturation (FOM). Lutenizing Hormone Releasing Hormone Analogue (LHRHa) was not able to induce FOM in gilthead seabream *Sparus auratus* because LHRHa was found to be degraded faster than sGnRH_a by the enzyme activity (Cytosolic enzyme) of the pituitary, kidney and liver, resulting in no spawning (Zohar *et al.*, 1990). However, the HCG, LHRHa and CP have successfully induced ovulation in *Rhynchocypris oxycephalus* and produced good larval quality (Park, 2002). This success could be due to the administration of HCG exogenous gonadotropins that directly delivered into the body while Ovotide and Ovaprim are known to act on the pituitary level that leads to secretion of fish's own endogenous gonadotropin (Habibi *et al.*, 1989; Zairin *et al.*, 1992; Goswami and Sarma, 1997). Nonetheless, HCG failed to induce ovulation in *T. tambroides* and other cyprinids such as grass carp *Ctenopharyngodon idellus* and black carp *Mylopharyngodon piceus* (Lin *et al.*, 1988). Ovotide was the most reliable and effective in inducing ovulation of *T. tambroides* and resulting in higher ovulation success, egg and larval qualities compared to Ovaprim. Dhawan and Kaur (2004) reported that Ovotide gives higher fecundity and fertilization rates in *Labeo rohita* and *Cirrhina mrigala* than those using Ovaprim. OvaRH containing active ingredient of sGnRH_a without domperidone, has successfully induced ovulation but had lowest percentage of egg and larval qualities. Considering the effect of dopamine antagonist domperidone, the egg quality has improved which leads to good larval quality and normal larvae production. In general, Ovotide was most effective hormone in this experiment considering having highest ovulatory response, total stripping eggs, fecundity, egg and larval quality compared to other tested hormones.

In the present study, the complete ovulation was obtained when at 0.5 and 0.75 mL kg⁻¹ b.wt. of Ovotide were used, while 0.25 mL kg⁻¹ b.wt. of Ovotide showed partial ovulation. Partial

spawning has been reported using suboptimal dosage of 0.2 mL kg⁻¹ b.wt. of Ovotide in spotted snakehead *Channa punctata* (Marimuthu *et al.*, 2009). The suboptimal dosage of 0.25 mL kg⁻¹ b.wt. of Ovotide was insufficient to induce the complete ovulation in *T. tambroides*. It might be due to insufficient release of gonadotropin to effect complete ovulation in the fish, agreeing to earlier studies (Billard *et al.*, 1984; Sahoo *et al.*, 2005). Some of the breeders injected with high dosage of Ovotide (1.0 mL kg⁻¹ b.wt.) produced over ripened and ruptured eggs upon stripping. The higher dosage resulted in early ovulation and the ovulated eggs remained in the ovarian lumen for longer time causing over ripeness. Lam *et al.* (1978) reported that over ripe eggs did not form perivitelline space in fresh water because of changes or reduction in the permeability of chorion lead to abnormal embryonic development (Sahoo *et al.*, 2005), low quality eggs and larvae. Deterioration of egg quality was observed in the group treated with 0.25 and 1.0 mL kg⁻¹ B.wt. dosage leading to low hatching and survival rates. Protein from ruptured eggs and blood on the stripped egg will coagulate and clog the micropile (Sahoo *et al.*, 2005), which leads to poor fertilization (Piper *et al.*, 1982).

A high percentage of deformed larvae was observed in the group treated with 0.25 and 1.0 mL kg⁻¹ b.wt. More deformity in larvae at lower or higher dose probably due to the fertilization of unripe or over ripe ova (Sahoo *et al.*, 2005). Higher deformed larval production was observed in walking catfish *Clarias batrachus* after being induced with lower and higher doses of pituitary (Rao and Ram, 1991; Goswami and Sarma, 1997). However, in this study, the highest production of good larvae was obtained when administered with 0.5 mL kg⁻¹ B.wt. followed by 0.75, 0.25 and 1.0 mL kg⁻¹ b.wt. Of ovotide. This could be due to higher numbers of good egg quality, higher fertilization, hatching, survival and low larval deformity rates.

In this study, a single intramuscular injection of synthetic hormone, ovotide (0.5 mL kg⁻¹ b.wt.) was considered the most reliable and effective hormone to induced ovulation of *Tor tambroides*. Results of induced ovulation and the success rate were compared among different fish species using Ovotide as summarized in Table 3. This is the first successful attempt to compare the effectiveness of several commercial peptide hormones to induce ovulation in F₁ females of *T. tambroides* in a controlled condition. Successful ovulation or spawning using ovotide (doses from 0.2 to 2.0 mL kg⁻¹ b.wt.) has been reported in catla, *Gibelion catla* and grass carp, *Ctenopharyngodon idella* (Thakur and Reddy, 1997), walking catfish, *Clarias batrachus* (Sahoo *et al.*, 2005), snakehead, *Channa striatus* (Marimuthu *et al.*, 2007), Malaysian mahseer, *T. tambroides* (Azuadi *et al.*, 2011) and in several cyprinid species such as common carp, *Cyprinus carpio*, tench, *Tinca tinca* and silver carp, *Hypophthalmichthys molitrix* (Horvath *et al.*, 1997).

In general, response of fish to Ovotide was found to be better than other commercially available synthetic hormones tested in this study. This is based on the ovulatory response, number of released eggs, fertilization, hatching and larval survival rates. Implantation of slow releasing

Table 3: Comparative account on induced breeding by Ovotide in different fish species

Species	Ovotide dose (mL kg ⁻¹ b.wt.)	Latency period (h)	References
Rohu, <i>Labeo rohita</i>	0.2-0.4	8.45	Thakur and Reddy (1997)
Java barb, <i>Puntius javanicus</i>	0.3-0.6	8.0	Thakur and Reddy (1997)
Snakehead murrel, <i>Channa striatus</i>	0.4	24.0	Marimuthu <i>et al.</i> (2007)
Spotted snakehead, <i>Channa punctatus</i>	0.2-0.6	25.0-30.5	Marimuthu <i>et al.</i> (2009)
Malaysian mahseer, <i>Tor tambroides</i>	0.5	24.0	Azuadi <i>et al.</i> (2011)

salmon gonadotropin releasing hormone analogue (sGnRHa) pellet has greatly improved the breeding performance and synchronized the ovulation time in females of *T. tambroides*. Sustained GnRHa delivery has greatest potential in inducing long term spawning in fish species with asynchronous ovarian development which requires long term elevation in plasma gonadotropin hormone II (GtH II) in order to reach their full fecundity potential (Mylonas *et al.*, 1998).

CONCLUSION

In conclusion, amongst the synthetic hormones used in this study, Ovatide was found to be the best hormone for induced ovulation of F₁ females of *Tor tambroides*. Ovatide is less costly, easy to store and less viscous than Ovaprim and other tested hormones. The results of this study provide information which can lead to production of good quality seeds from F₁ generation of Malaysian mahseer.

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REFERENCES

- Azuadi, N.M., S.S. Siraj, S.K. Daud, A. Christianus, S.A. Harmin, S. Sungan and R. Britin, 2011. Enhancing ovulation of Malaysian mahseer (*Tor tambroides*) in captivity by removal of dopaminergic inhibition. *J. Fish. Aquatic Sci.*, 6: 740-750.
- Azuadi, N.M., S.S. Siraj, S.K. Daud, A. Christianus, S.A. Harmin, S. Sungan and R. Britin, 2012. Sex steroid levels and breeding performance of F₁ generation Malaysian Mahseer, *Tor tambroides* (Bleeker, 1854) by removal of dopaminergic inhibition. *Asian. J. Anim. Vet. Adv.* 10.3923/ajava.2012
- Billard, R., K. Bieniarz, R.E. Peter, M. Sokolowka, C. Weil and L.W. Crim, 1984. Effects of LHRH and LHRH-A on plasma GtH levels and maturation/ovulation in the common carp, *Cyprinus carpio*, kept under various environmental conditions. *Aquaculture*, 41: 245-254.
- Chang, J.P. and R.E. Peter, 1983. Effects of pimozone and des-Gly10 (D-Ala6) Lutinising hormone-releasing hormone ethylamide on serum gonadotropin concentration, germinal vesicle migration and ovulation in female gold fish *Carassius Auratus*. *Gen. Comp. Endocrinol.*, 52: 30-37.
- Dhawan, A. and K. Kaur, 2004. Comparative efficacy of ovaprim and ovatide in carp breeding. *Indian J. Fish.*, 51: 227-228.
- Donaldson, E.M. and G.A. Hunter, 1983. Induced Final Maturation, Ovulation and Spermiation in Cultured Fish. In: *Fish Physiology V9B*, Hoar, W.S., D.J. Randall and E.M. Donaldson (Eds.). Academic Press, New York, pp: 351-403.
- Goswami, U.C. and N.N. Sarma, 1997. Pituitary dose optimization for induced ovulation, *in vitro* fertilization and production of normal fry of *Clarias batrachus* (Linn.). *Asian Fish. Sci.*, 10: 163-167.

- Gupta, S.D., B.C. Mohapatra, S.K. Sahoo and D.K. Verma, 2002. Breeding response of rohu, *Labeo rohita* induced with different inducing agents. Proceedings of the 6th Indian Fisheries Forum, December 17-20, 2002, Mumbai.
- Habibi, H.R., T.A. Marchant, C.S. Nahomiak, H. Van Der Loo, R.E. Peter, J.E. Rivier and W.W. Vale, 1989. Functional relationship between receptor binding and biological activity for analogue of mammalian and salmon gonadotropin-releasing hormones in the pituitary of goldfish (*Carassius auratus*). Biol. Rep., 40: 1152-1161.
- Horvath, L., A. Peteri and J. Kouril, 1986. Successful sterlet, *Acipenser ruthenus* L., propagation with synthetic LH-RH hormone. Aquacult. Fish. Manage., 17: 113-116.
- Horvath, L., T. Szabo and J. Burke, 1997. Hatchery testing of GnRH analogue-containing pellets on ovulation in four *Cyprinid* species. Polish Arch. Hydrobiol., 44: 221-226.
- Ingram, B., S. Sungan, G. Gooley, S.Y. Sim, D. Tinggi and S.S. De Silva, 2005. Induced spawning, larval development and rearing of two indigenous Malaysian mahseer, *Tor tambroides* and *T. douronensis*. Aquacult. Res., 36: 983-995.
- Ingram, B.A., S. Sungan, D. Tinggi, Y.S. Sim and S.S. De Silva, 2007. Breeding performance of Malaysian mahseer, *Tor tambroides* and *T. douronensis* broodfish in captivity. Aquacult. Res., 38: 809-818.
- Joshi, P.L., T.B. Gurung, S.R. Basnyat and A.P. Nepal, 2002. Domestication of wild golden mahseer (*Tor putitora*) and hatchery operation. FAO Fish. Tech. Pap., 431: 173-178.
- Kelly, A.M. and C.C. Kohler, 1994. Human chorionic gonadotropin injected in fish degrades metabolically and by cooking. J. World Aquacult. Soc., 25: 55-57.
- Kim, D.S., J.H. Kim and I.S. Park, 1992. Induced and multiple spawnings by human chorionic gonadotropin injection of the loach, *Misgurnus mizolepis* (Teleostomi; Cobitidae). J. Aquacult., 5: 109-115.
- Lam, T.J., 1982. Applications of endocrinology to fish culture. Can. J. Fish. Aqua. Sci., 39: 111-137.
- Lam, T.J., Y. Nagahama, K. Chan and W.S. Hoar, 1978. Overripe and post-ovulatory corpora lutea in the threespine stickleback, *Gasterosteus aculeatus* L., form *Trachurus*. Can. J. Zool., 56: 2029-2036.
- Lin, H. R. and R.E. Peter, 1996. Hormones and spawning in fish. Asian Fish. Sci., 9: 21-33.
- Lin, H.R., G. Van Der Kraak, X.J. Zhou, J.V. Liang, R.E. Peter, J.E. Rivier and W.W. Vale, 1988. Effects of (D. Drg) Try, Leu, Pro, Net.) Lutinising hormone-releasing hormone (LH-RH-A) in combination with pimozide or doperidone on gonadotropin release and ovulation in the Chinese loach and Common carp. Gen. Comp. Endocrinol., 69: 31-40.
- Lin, H.R., X.J. Zhou, G. Van Der Kraak and R.E. Peter, 1991. Effects of gonadotropin-releasing hormone agonists and dopamine antagonists on gonadotropin secretion and ovulation in Chinese loach, *Paramisgurnus dabryanus*. Aquaculture, 95: 139-147.
- Marimuthu, K., D. Kumar and M.A. Haniffa, 2007. Induced spawning of striped snakehead, *Channa striatus*, using Ovatide. J. Appl. Aquacult., 19: 95-103.
- Marimuthu, K., M.A. Haniffa and M.A. Rahman, 2009. Spawning performance of native threatened spotted snakehead fish, *Channa punctatus* (ActinopterygII: Channidae: Perciformes), induced with ovatide. Acta Ichthyol. Piscatoria, 39: 1-5.
- Mylonas, C.C., L.C. Woods, P. Thomas and Y. Zohar, 1998. Endocrine profiles of female striped bass (*Morone saxatilis*) during post-vitellogenesis, and induction of final oocyte maturation via controlled-release GnRH delivery systems. Gen. Comp. Endocrinol., 110: 276-289.

- Park, I.S. H.B. Kim, H.J. Choi, Y.D. Lee and H.W. Kang, 1994. Artificial induction of spawning by Human Chorionic Gonadotropin (HCG) or Carp Pituitary Extract (CPE) in olive flounder, *Paralichthys olivaceus*. *J. Aquacult.*, 7: 89-96.
- Park, I.S., 2002. Induction of ovulation by HCG, LHRHa and Carp pituitary in *Rhynchocypris oxycephalus* (Sauvage and Dabry). *Asian Fish. Sci.*, 15: 387-393.
- Piper, R.G., I.B. McElwain, L.E. Orme, J.P. McCraren, L.G. Fowler and J.R. Leonard, 1982. *Fish Hatchery Management*. U.S. Department of Interior, Fish and Wildlife Service, Washington, DC., USA., Pages: 517.
- Ramos, J., 1986. Luteinizing hormone-releasing hormone analogue (LH-RH) induces precocious ovulation in common sole (*Solea solea* L.). *Aquaculture*, 54: 185-190.
- Rao, G.R.M. and K.J. Ram, 1991. An effective dose of pituitary for breeding *Clarias*. *J. Aquacult. Trop.*, 6: 207-210.
- Sahoo, S.K., S.S. Giri and A.K. Sahu, 2004. Induced breeding of *Clarias batrachus* (Linn.): Effect of different doses of Ovatide on breeding performance and egg quality. Proceedings of the National Seminar on Responsible Fisheries and Aquaculture, February 12-13, 2004, Orissa, India, pp: 2.
- Sahoo, S.K., S.S. Giri and A.K. Sahu, 2005. Effect on breeding performance and egg quality of *Clarias batrachus* (Linn.) at various doses of ovatide during spawning induction. *Asian Fish. Sci.*, 18: 77-83.
- Szabo, T., F. Radics, T. Barth and L. Horvath, 2007. *In vivo* activity of native GnRHs and their analogues on ovulation in the African catfish, *Clarias gariepinus* (Burchell). *Aquacult. Res.*, 38: 140-146.
- Thakur, N.K. and A.K. Reddy, 1997. Repeat field trials with new hormonal preparation Ovatide for fish breeding. Final Report, CIFE, Mumbai, India.
- Zairin, Jr. M., K. Furukawa and K. Aida, 1992. Induction of ovulation by HCG injection in the tropical walking catfish *Clarias batrachus* reared under 23-25°C. *Nippon Suisan Gakkaishi.*, 58: 1681-1685.
- Zohar, Y., A. Goren, E. Fridkin, E. Elhanati and Y. Koch, 1990. Degradation of gonadotropin-releasing hormones in the gilthead seabream, *Sparus aurata*. II. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary, kidney and liver. *Gen. Comp. Endocrinol.*, 79: 306-319.