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Comparative study on induced breeding of shing, *Heteropneustes fossilis* (Bloch) between HCG and PG with different combination

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Abstract
Induced breeding of shing, *Heteropneustes fossilis* (Bloch, 1794) was carried out by using pituitary gland (PG) and human chorionic gonadotropin (HCG). Twenty pairs of brood fishes were reared up to maturation for spawning operation in a fish farm by providing farm-made artificial diet containing 30% protein for 4-5 months before onset of breeding season. The breeders were induced with hormone HCG (Male 1250 IU/kg and female 2000 IU/kg), PG (Male 10 mg/kg and female 70 mg/kg), HCG 1250 IU/kg for male and PG 70 mg/kg for female, PG 10 mg/kg for male and HCG 2000 IU/kg for female for treatments T_1, T_2, T_3 and T_4, respectively. The brood fishes were injected with a single dose. When the brood fishes were injected with HCG the breeding behavior was exhibited quickly and perhaps males lost most of their milt before the ovulation of female and resulting less fertilization rate (84%) in T_1. Whereas, the male and female fishes were injected with PG the eggs and milt released at the contemporary times, resulting highest fertilization rate (95%) in T_2. In other combination there was some variation of synchronization of releasing eggs and milt. Fertilization rate was 84%, 95%, 80% and 89% and hatching rate was 81.40%, 93%, 89% and 68.90% in treatments T_1, T_2, T_3 and T_4, respectively. The incubation periods for fertilized eggs were 22-24 hrs in all treatments. Higher fertilization rate (95%) and hatching rate (93%) were found in case of PG-PG combinations in T_2. For induced breeding of *H. fossilis* a dose of PG 10 mg/kg body weight of male and 70 mg/kg body weight of female in T_2 was found to be most effective among the combinations of hormones used.

Keywords: Induced breeding, *Heteropneustes fossilis*, pituitary gland (PG) and human chorionic gonadotropin (HCG).

1. Introduction
Among the freshwater fishes, catfish is an important group of fish in our country and the flash of catfish is well known for palatability and great market value. The *Heteropneustes fossilis* (Bloch, 1794), commonly known as Shing or Singhi, is a popular catfish in Bangladesh and generally grows in *haor*, *baor*, *beels*, *swamps* and *marshes*, ditches and floodplains with natural care. It is characterized by an accessory respiratory (air breathing organ) which enables it to exist for hours when out of water or indefinitely oxygen-poor water and even in moist mud. Not only it is able to thrive well in water containing low oxygen but the fish is also extremely hardy with respect to other environmental parameters making it adaptable for shallow and derelict waters. The shing culture holds great promise in rural areas of Bangladesh which abound several thousand hectors of fallow derelict swampy waters unsuitable for carp culture. It requires relatively small area for culture and can be stocked at higher density than many other species. It is also compatible with the carps in mixed culture. It is popular not only for its good taste but is also highly esteemed from nutritional and medical point of view. It is rich in protein and minerals [1]. The chemical composition of the fish is 72% water, 19% protein, 8% fat, 0.15% calcium, 0.25% phosphorus and 0.10% vitamin A, B, C and D [2]. The muscles of the fish have been reported to have very high content of iron (226 mg/l0 gm) and fairly high content of calcium compared to many other freshwater fishes [3]. It may be threatened by over exploitation and habitat loss and degradation (especially from pollution and dams) and subsequently, it is considered least concern at present [4]. Because of its fast growth, tolerance to high stocking densities, high market value, ability to survive in oxygen-low waters, low fat, high protein and iron content and medicinal values, *H. fossilis* is considered as an ideal fish species for aquaculture [5, 6, 7, 8, 9].

Also, aquaculture of this species will be helpful not only in increasing the overall production but also in the conservation of this important fish species. Very recently, the culture of *H. fossilis* has become popular, but fry produced in natural water is not enough to fulfill the demand for its culture. So, appropriate breeding technology must be introduced to the production of fry is demanding for the production of sufficient quantity and quality of shing seed. The technique commonly practiced in the farms is the use of HCG for female and PG for male during induced breeding. But, HCG is responsible for partial ovulation and production of immature eggs which ultimately causes low survival rate and low quality fry (personal communication to farmers). Considering this, the present study has been conducted on induced breeding of the species by using PG and HCG to reduce the use of artificial hormone HCG, to get better survival rate and quality fry of shing.

2. Materials and Methods

2.1 Study site

The experiment was carried out in the Noha Aquafarm Ltd, Kumarghata, Muktagatha, Mymensingh. In this experiment two types of tanks were used. One type for conditioning of the brood fishes and another type were used for spawning purpose. Both conditioning and spawning tanks were with continuous flow of water. Incubation trays were used to study fertilization rate and hatching rate. For rearing of newly hatched spawn, five trays (28 cm × 43 cm × 10 cm) were used.

2.2 Brood stock collection and management

Broods were collected from different market nearby the farm and reared in the earthen ponds at the density of 4000 fish/acre. The brood fishes were fed on farm made artificial balanced diet containing 30% protein. The brooders were reared for 4-5 months before onset of breeding season with feeding at two times a day at the rate of 5-6% of the body weight. Water temperature was measured by thermometer and denoted as °C.

2.3 Brood selection and conditioning

Twenty pairs of broods were collected from the rearing ponds using a cast net in the morning between 8:00-9:00 am on the day of the breeding trials and immediately transferred to a circular tank in the hatchery. Only conspicuous, healthy and uninjured fishes were selected for induced breeding. The male and female fish were determined by eye estimation based on the criteria presented in Table 1. The males and females were kept in separate tanks and continuous water flow was maintained to ensure sufficient aeration. However, no feeding was provided during the conditioning period.

Table 1: Criteria followed to select mature breeders of *Heteropneustes fossilis*

Male	Female
1. Slim and streamlined body.	1. Abdomen is swollen and soft.
2. Genital papilla elongated and pointed.	2. Round and blunt genital opening.
3. Pressing on the belly, small amount of milt comes out.	3. Pressing on the belly, a few eggs comes out.
4. Normal vent.	4. Prominent reddish vent.

2.4 Use of inducing agents

The induced breeding was conducted by using commercially available pituitary gland (PG) and human chorionic gonadotropin (HCG) at different doses and combinations. These two inducing agents are most familiar and widely used among the farm owners all over the country. Four separate treatments were done with different combination of hormone (HCG-HCG, PG-PG, HCG-PG and PG-HCG) to male and female fishes with single dose of injection. The hormone was administered intra-muscularly near dorsal fin and above the lateral line with the 1 ml syringe. For each treatment, a group consisting of 6 male and 5 female were used. For T_1, T_2, T_3 and T_4, the male and female fishes were injected at 09.30 pm, 11:30 pm, 11:45 pm and 12:10 am on the day of breeding trail at the dose of HCG 1250 IU/kg and 2000 IU/kg, PG 10 mg/kg and 70 mg/kg, HCG 1250 IU/g and PG 70 mg/kg, PG 10 mg/kg and HCG 2000 IU/kg body weight of fish, respectively.

2.5 Spawning and fertilization

After hormone injection, both the male and female were kept in the same spawning tank and fishes were allowed to release eggs and milt, fertilize naturally in the tank. All the brooders were found to be ovulated after a period of 7-9 h of injection. The brooders were then transferred from the holding tanks after the completion of ovulation.

2.6 Egg transfer for incubation

The fertilized eggs transferred into mini rectangular hatching trays while taking precaution to avoid damage and fungal/bacterial contamination during the egg collection process. Prior to incubation, fertilized eggs were treated with fungicide and bactericide *i.e.* malachite green and oxytetracycline bath, both at 5 ppm for 10 min considerably prevents the fungal and bacterial infection [10]. The number of eggs transferred into each tray was estimated using gravimetric methods [11, 12]. Eggs were kept under shower of water by piercing a slender pipe. Thereafter, a continuous flow of water was maintained for aeration to ensure the environmental conditions were optimal for the hatching process.

2.7 Determination of ovulation, fertilization and hatching rate

Ovulation, fertilization and hatching rates were calculated using the following formula:

Ovulation rate = No. of egg released/ g of fish

Fertilization rate (%) = (No. of fertilized eggs/ Total no. of eggs) × 100

Hatching rate (%) = (No. of eggs hatched/ Total no. of fertilized eggs) × 100

2.8 Statistical analyses
Data and statistical analyses were performed using Graph Pad Prism 5 software. A Chi-square test was used to check the ovulation, fertilization and hatching rates between PG and HCG treated fishes. All statistical analyses were considered significant at 5% ($p<0.05$).

3. Results and Discussion
3.1 Brood maturity study
Brood fishes were reared up to maturation for spawning operation in the farm by providing farm made artificial balanced diet containing 30% protein. During breeding season the broods were found to be fully matured and were ready to spawn. The body colour of the male was prominent but the female was comparatively dull in colour. The abdomen of the female was swollen and soft. The genital aperture of the male was slightly elongated while the genital aperture of the female was round and protruded. From the above criteria the brood fishes were selected for induced breeding.

3.2 Breeding behavior of *Heteropneustes fossilis*
The breeding behavior was observed continuously after the injected shing fishes released into the breeding tank. After 4 hours of injection the activities and movement of male fish was increased. The male started to move around the female and chase her. It started to nudge with its snout at the ventral region of the female fish. This activity was going for a long period. The activities of female were also increased. It started to move and stay at middle of the water column. After that suddenly the male quickly came to the female and the male nudge with its snout at the ventral region of the female. The female makes its body "U" shaped and holds the head of the male inside its "U' shaped structure and on the bending condition the male brought the female at the surface of the water. Pressure was created on the ventral region of the female fish by the male shing with its snout. In this time the female released eggs at the surface of the water column and simultaneously the male ejaculated sperm. Then the eggs slowly fall down to the bottom of the tank. The fertilized eggs were black and greenish blue in colour and they settle on the bottom. They were collected by siphoning from the bottom of the tank. The unfertilized eggs were somewhat white and opaque, and were found floating above the fertilized ones. They were measured 1.4 to 1.6 mm in diameter having a narrow perivitelline space of 0.1 to 0.2 mm width. These activities were observed several times until the total eggs and milt was ejaculated in case of normal spawning allowed.

3.3 Variation to hormone response within the treatments
When the brood fishes were injected with HCG the breeding behavior was exhibited quickly and perhaps males lost most of their milt before the ovulation of female and resulting less fertilization rate (84%) in T_1. Whereas, the male and female fishes were injected with PG the eggs and milt released at the contemporary times resulting highest fertilization rate (95%) in T_2. In other combination there was some variation of synchronization of releasing eggs and milt.

3.4 Ovulation rate
Chi square test showed significant differences in ovulation rates among the treatments. Ovulation rate was higher in the HCG treated fish in T_1 (77.90 eggs/g of fish) compared to ovulation rates (71.40, 61.75 and 47.44 egg/g of fish in T_2, T_3 and T_4, respectively) found in the PG and different combination of HCG treated fish (Table 2). On the other hand, Chi square test revealed no significant differences of the ovulation rates of HCG and PG treated fishes between T_1 and T_2 (Table 2). While the ovulation rates was found 82.67% and 76.51% in *H. fossilis* injected with HCG at 1000 IU/kg of both female and male fish and with pituitary gland extract (PGE) at 6 mg/kg body weight of females and 2 mg/kg body weight of males [13]. Parallel findings were documented by [14] who described that, the ovulation rates in *H. fossilis* injected with PGE at 75 mg/kg body weight were slightly lower, although they recorded 90% ovulation rate when the fish were treated with PGE at 100 mg/kg body weight. Better egg production was observed by using PG at a dose of 8 mg/kg body weight [10]. It was noted that very high doses of PGE hormone often resulted in higher rates of ovulation in the *H. fossils* [8]. Nonetheless, the latency period was significantly shorter in PG treated fish in contrasting to all combination of HCG injected brooders (Table 2). A much longer ovulation period (15h) recorded by using HCG at 1000 IU/kg of both female and male fish and with PGE at 6 mg/kg body weight of females and 2 mg/kg body weight of males [13], unlike that is recorded in the present study. However, it is difficult to clearly spell out the causative factors for the observed differences. A consortium of factors was likely to influence biological experiments particularly those involving hormones thus leading to differences in the observed latency periods [15].

Table 2: Showing details of induced breeding of shing, *Heteropneustes fossilis* (Bloch)

Treatment	Doses of hormone		Latency period (hrs)	Ovulation rate (No. of egg released/g of fish)	Fertilization rate (%)	Hatching rate (%)	Incubation period (hrs)
	HCG (IU/kg)	PG (mg/kg)					
T1	Male-1250 Female-2000	-	9	72.00	84	81.40	23
T2	-	Male-10 Female-70	7	71.40	95	93	24
T3	Male-1250	Female-70	8	61.75	80	89	22
T4	Female-2000	Male-10	7.5	47.44	89	68.90	24

3.5 Fertilization rate

Chi square test revealed significantly higher fertilization rates (95%) was recorded in T_2 of the PG treated brooders compared to 3 others treatments (Table 2 and Fig. 1). The highest rate of fertilization (98%) recorded in *H. fossilis* injected with pituitary gland extract (PGE) at 75 mg/kg which is higher than found in the present study of PG injected fishes [14]. Whereas, lower fertilization rates tabulated than the present study as 75.33% and 70.45% in *H. fossilis* injected with HCG at 1000 IU/kg of both female and male fish, and with PGE at 6 mg/kg body weight of females and 2 mg/kg body weight of males [13]. Differences in the fertilization rate can be attributed to the huge differences of hormonal doses, size of the brood fishes, seasonal variations [8, 15, 16]. The quality of the PG hormone could be ruled out as factor influencing the fertilization rates.

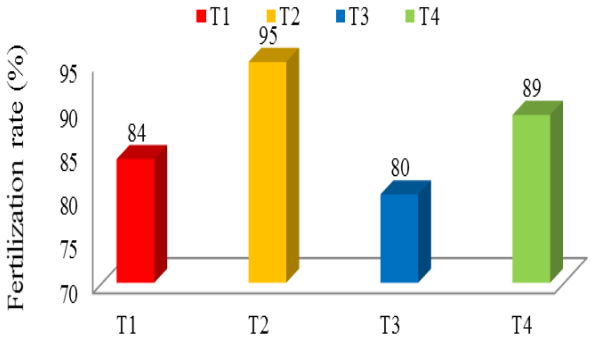

Fig 1: Fertilization rate (%) of *H. fossilis* by using different doses of HCG and PG.

3.6 Hatching rate

In case of T_2, the hatching rates was found to be slightly higher (93%) for eggs compared to 3 other treatments that of different combination of HCG and PG treated fishes (84, 80 and 89% in T_1, T_3 and T_4, respectively) at 30°C (Table 2 and Fig. 2). Chi square test showed significant differences in hatching rates between PG and other combination (HCG and PG) of hormone treated fishes. While comparing among the treatments it is quite clear that hatching rate was higher in T_2 than all other treatments. Much lower hatching rates than the present study as 66.58 and 70.25% in *H. fossilis* injected with HCG at 1000 IU/kg of both female and male fish, and with pituitary gland extract (PGE) at 6 mg/kg body weight of females and 2 mg/kg body weight of males and obtained a hatching period of 5 h cited by [13]. Moreover, a slight lower hatching rate of 72.72 and 76.92% in *H. fossilis* administered PGE at 6 mg/kg of body weight for females and 2 mg/kg of body weight for males, and ovaprim at 0.3 ml/kg body weight and 0.1 ml/kg of body weight for females and males, respectively has been recorded earlier [17]. In this experiment the incubation period of eggs was 22-24 hrs after fertilization at 29-30 °C water temperature. A similar hatching time of *H. fossilis* ranges from 20-24 hrs at 25 °C reported by [18]. The hatching period of shing continued from 18 to 20 hrs at temperature ranging from 26 °C to 29 °C noted in the past [19]. The embryo hatched out after 21-24 hrs of fertilization stated by [20]. Even though, the incubation period varied from 16-19 hrs at 28-30 °C [21]. All these studies to some extent support the findings of the present study.

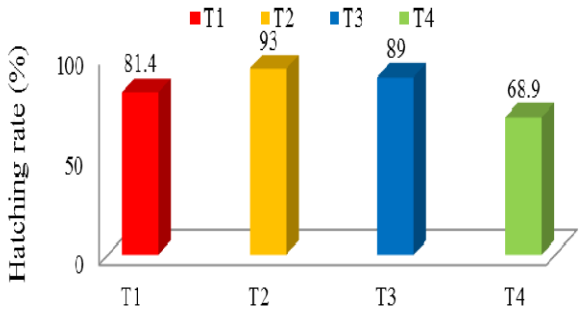

Fig 2: Hatching rate (%) of *H. fossilis* by using different doses of PG and HCG.

Finally, from the experiment it was observed that for *H. fossilis* a single dose of PG 10 mg/kg for male and 70 mg/kg for female gave the best result in both case of fertilization (95%) and hatching (93%) compared with other combinations (HCG-HCG, HCG-PG and PG-HCG) and doses. The induced breeding of *H. fossilis* by using HCG and PG has been conducted to develop a successful artificial breeding of the species, which will helpful in producing good quality fry.

4. Conclusion

Although the experiment was conducted with limited facility the consequence is hopeful and would serve up to the fish farmer, hatchery owner and researcher for further studies.

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