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Captive Breeding of the stinging catfish, *Heteropneustes fossilis* by using one natural and two analogous synthetic hormones, Ovasis and Ovatide

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Abstract
The performance of various inducing agents in induced breeding of *Heteropneustes fossilis*, the stinging catfish, is compared in this study. Three set of brooders were used for treatment of three hormones, during the disquisition. Pituitary gland extract (PGE) was given at a dose of 5 mg/ kg body weight for males and female in low (0.2 mg), medium (0.4 ml) and high (0.6 ml) in three triplicates and same for other two analogues synthetic hormones. In comparison to PGE and ovasis - convinced individualities, breeding success was shown to be advanced in ovatide - treated individualities in this study in all orders, including latency period, ovulation rate, fertilization rate, hatching rate, and incubation length. The latency period was 15 hours for PGE and ovasis given brooders and within 10 hours for ovatide doses individualities. Likewise, the current study showed that using ovasis at a rate of 0.4 ml/ kg body weight of female fishes is more effective than using other breeding hormones in terms of ovulation, fertilization, and hatching rates. The current study's findings will help hatchery directors in overseeing convinced parentage.

Keywords: *Heteropneustes fossilis*, threatened fishes, induced spawning, pituitary gland extract, ovatide, ovatide, ovulation rate, fertilization rate, hatching rate, survival rate and embryonic development

Introduction
The *Heteropneutes fossilis* (Bloch, 1974) [4] surcharging catfish is an economically significant fish species in India and around the world. Although it can sometimes be lived in muddy gutters, this fish is primarily found in ponds, dikes, bevels, wetlands and morasses (Jha and Rayamajhi, 2010 [11]; Froese and Pauly, 2012). The capability to breathe on air allows surcharging catfish to live in nearly any type of water. Also, it can repel kindly in brackish water. *H. fossilis* frequently inhabits semi liquid or semidry slush throughout the dry season, and indeed as the slush dries out, they move their bodies to the bottom of the cracks and crevices the slush creates. *H. fossilis* can breathe through the air by taking in breaths at different times when the oxygen position is low. While it's extensively used for food and drug over much of its range, overexploitation, niche loss and declination (particularly from pollution), and other factors may pose a trouble, it's presently regarded as hovered (Lakra *et al*.., 2010) [14]. *H. fossilis* is regarded as the perfect fish species for monoculture due to its quick growth, forbearance to high sock consistence, high request value, and capability to survive in oxygen-low waters, low fat, high protein, and iron content (Dehadrai *et al*., 1985 [7]; Alok *et al*..., 1993 [1]; Vijayakumar *et al*, 1998 [25]; Haniffa and Sridhar, 2002 [9]; Froese and Pauly, 2012). Also, monoculture of this species will be helpful not only in adding the overall product but also in the conservation of this important fish species. In India, stinging catfish monoculture is getting decreasingly popular. Still, the culture of any fish species, including *H. fossilis*, depends on a steady force of high-quality fingerlings. Although, major sources of shindig and fingerlings for monoculture were substantially the prisoner fishery due to the limited capacity of the also being hatchery installations in the history, nevertheless, convinced parentage ways have continually perfecting in India.
Therefore, hatchery- produced fry and fingerlings are presently the nation's main force of seed for the monoculture sector.

Due to shy hatchery operation procedures that have mischievous impacts similar negative selection, inbreeding depression, magpie inter specific hybridization, etc., the product of fish seed from hatchery sources has increased mainly, but the quality has not bettered. While there are a many studies on the goods of complaint pituitary gland excerpt, mortal chorionic gonadotropin, and synthetic hormone (ovaprim) boluses on latency parentage, development, and ovulation of *H. fossilis*, (Alok *et al...*, 1993 [1]; Begum *et al...*, 2001 [3] Nayak *et al...*, 2001, Haniffa *et al...*, 2002), there are glaringly no comprehensive studies on the subject. The present study furnishes information on the relative performances of different converting agents on the parentage success of *H. fossilis*.

In northern India, *H.fossilis* naturally spawns during July-August and begetting can be convinced by manipulation of water situations (Fermin, 1992) [8]. Ovulation of the species can be convinced during non-spawning season not only by environmental manipulation but also by hormonal stimulation (Sundararaj and Vasal 1976) [22].

Materials and Methods

The present study was conducted in Aqua lab of St. Andrew's college Gorakhpur, India, during March 2023 to August 2024. The breeding chamber is a modified round plastic trough (3x3 NOS) with a capacity of 25 1itre each (Figure- 2). The breeding chamber contains a removable earthen pipe to allow fish to hide and is covered with a net to prevent them from jumping out during breeding.

The hatchery consists of a spherical plastic bottle (8 cm in periphery) and 20 cm high, with a capacity of 1.00 liter. The bottle has one opening at the bottom for bay of water and one opening at the top for water outlet (5 mm dia.). The opening (2 mm dia.) at the base of the plastic tube is connected to a flexible tube (2 mm dia.) for water inflow The hatchery unit (spherical plastic bottle) was filled with well-conditioned water and was placed inside a plastic trough of 25 liter capacity. The plastic trough had an outlet guarded by a fine net (Figure- 6).

In this trial, a aggregate of thirty six brooders were grazed in cement tank 2 × 1 × 1 m², (Figure- 1) taken from Rapti and Ghaghra river, Gorakhpur and feed.

A mixed feed diet conforming of 25% fish powder, 20% rice bran, 20% wheat flour, 15% mustard oil cake, and 1 vitamin B-complex tablet was given to the fish. For four months, the brooders were raised in the studies, by feeding them twice a day at the rate of 5- 6% of their body weight (Singh *et al..*, 2024) [21].

On the morning of the breeding trials, between 8:00 and 9:00 am, brooders fish were removed from the culturing cement tank using a cast net and incontinently moved to an indirect breeding trough in the hatchery. Male and female were placed in separate troughs with constant water flows maintained at a rate of 10 lit/ min. Water quality parameters were set up as dissolved oxygen 5.2- 5.7 ppm; pH 7.3- 8.5; temperature 27 - 30 °C. Still, no supplementary feed were handed throughout the exertion period.

Commercially available dehydrated complaint pituitary gland extract (PGE), ovasis and synthetic hormone ovatide were used in trial. The body weight (gm) of each brooder was counted on an electronic balance to estimate the needed dosages.

For breeding trial we took three set of, two female and one males of *H. fossilis* for each hormone treatment. First set were treated with low (0.2 mg), medium (0.4 mg) and high (0.6mg) dosage of PGE for female and male, and other two set with similar dosages for other two analogues synthetic hormones like ovasis and ovatide in the breeding chamber (Table 1). For all the treatments, the hormone was administered by intra-muscular injection on muscles beneath the caudal fin slightly above the lateral line (Figure- 3). After injection, the brooders were kept in separate breeding tanks after each treatment.

During the studies, all of the brooders ovulated 09 - 21 hours after injection. After the completion of ovulation, the brooders were also moved from the plastic trough. On the other hand, when collecting the fertilized eggs, care was taken to help damage and bacterial or fungal impurity before transferring them into measuring jars. Using gravimetric ways modified from Legender (1986) [15] and examined by Lagler (1992) [13], the number of eggs discharged into the jar was estimated. In order to insure that the environmental conditions were ideal for the hatching process, a constant inflow of water was maintained for aeration. The collected eggs were introduced into the hatching unit from the top, the opening was plugged, and water was pumped through the bay. On average, 2,000 eggs were incubated at a time. For the first 8 hours, water inflow was maintained at 0.5 lit/ min to keep the eggs rotating inside the hatchery jar. After 8 hours, water inflow was increased to 1.00 lit/ min to removing of unfertilized eggs and to increase oxygen force for the developing eggs. The unfertilized eggs came out through the outlet with the water inflow. After 24 hours, the incubated fries propelled themselves to the face of the water and were carried into the plastic trough the incubated fries were collected from the plastic trough of the hatchery unit and transferred to separate plastic troughs of 50 litre capacity covered with bamboo mesh to minimize light.

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

Ovulation rate (%) = (No. of fish ovulated /Total no. of fish egg laid) × 100

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

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

Table 1: Experimental setup for Induced spawning of *H. fossilis* by using 3 types of hormone:

Hormones	Dosage level per kg body weight	Female length (cm)	Female wt (gm)	Male length (cm)	Male wt (gm)	Spawning efficacy	Hatching
Pituitary extract (Two injections at one hr interval)	Low 1.0 mg + 2.0 mg	17.2±1.03	40.3±32.4	15.7±1.01	31.27±3.10	Partial	Normal
	Medium 1.0 mg+ 4.0 mg	18.1±2.04	52.84±41.15	17.5±2.10	40.59±48.3	Complete	
	High 1.0 mg+ 6.0 mg	21.3±1.42	125.52±35.2	21.2±1.54	70.44±24.35	Complete	
Ovasis (Single injection)	Low 0.2 ml	19.4±1.41	78.84±39.24	15.9±2.06	33.35±25.24	Partial	

	Medium 0.4 ml	20.7±2.47	113.5±42.37	20.4±1.26	57.87±38.31	Complete	
	High 0.6 ml	25.3±1.01	151.61±49.23	21.0±2.45	70.15±31.45	Complete	
Ovatide (Single injection)	Low 0.2 ml	20.8±2.41	86.45±41.03	18.3±1.30	61.57±34.21	Partial	
	Medium 0.4 ml	20.4±1.30	115.34±21.20	19.4±2.42	67.52±33.37	Complete	
	High 0.6 ml	23.1±2.10	137.54±47.59	19.8±3.43	68.57±24.85	Complete	
Control (Saline water)	0.6 ml	25.1±2.4	152.64±51.36	21.1±2.64	72.58±49.34	No response	

Statistical analysis

The effect of different doses of Pituitary extract, Ovasis and Ovatide on the latency period, egg number, fertilization rate, incubation period, hatching percentage, survival at first feeding, survival at fry and fingerlings were analyzed by Mean ± SD and ANOVA (Turkey test). The statistical analysis of the data was carried out by using Microsoft Excel 2012.

Result

In present study, one natural (PGE) and two analogues synthetic hormones (Ovasis and Ovatide) (Table 1), were used for induced spawning of *H. fossilis* and total 3 triplicate were done for each experiment hapa (Plastic trough).During present study the male fishes ranged from 15-20 cm (18.96±1.97) in total length and 31-72 gm (57.39±16.16) in body weight while female fishes ranged from 17-25 cm (21.24±2.69) in total length and 40-152 gm (105.45 ±39.48) in to body weight. After 4 hr of injection activities of male was increased and they move around female. They started nudge with its snout at the ventral region of female fish and female makes "U'' shaped and hold the head of male which create pressure on ventral region of female, ova released out at surface of water and male ejaculated sperm simultaneously. Then the eggs slowly fall down to the bottom of tank. The fertilized eggs were black and greenish in color. They were collected by siphoning; unfertilized eggs were white and opaque which float near water surface. Latency period was more in low dosages of PGE (20.93±0.07) while it is less in higher dosages of ovatide (9.8±0.32). Latency period in high and low dosages PGE and ovasis shows significantly same duration. Number of eggs was less in low dosages of PGE (978.66±6.80) while maximum in high dosages of ovatide (2080.33±3.51) and medium dosages of ovasis (2029.66±6.42). Fertilization rate was minimum in low dosages of PGE (45.33±0.57) and also minimum in high dosages of PGE (56.33±0.57). Maximum in medium dosage of ovasis(79.33±0.57) and in high dosages of ovatide (78.33±0.57). Incubation period is maximum in low and high dosages of all three hormones and reduced in medium dosages of these hormones. Hatching period, Survival at first feeding, Survival at fry and Survival at fingerlings stages is minimum in low dosages of PGE and maximum in medium dosages of ovasis and high dosages of ovatide.

Hence, in present study declare that medium dosage of ovasis is best for captive breeding of *H. fossilis.* (Table 2 and Graph 1a-h).

Table 2; a-h: Summary of Captive breeding of *H. fossilis* using various hormones (Mean± SD). Values followed by same alphabets are statistically not significant (Turky test).

Hormone Type	**Hormone dosage (mg)**		
	Low	**Medium**	**High**
PGE	20.93±0.07bA	14.86±0.42abA	11.81±0.37aA
Ovasis	16.90±0.68aA	14.05±0.42aA	11.81±0.51aA
Ovatide	17.92±0.46bA	12.27±0.19abA	9.8±0.32aA
Hormone type	**Hormone dosage**		
	Low	Medium	High
PGE	978.66±6.80 bA	1159.66±4.50abA	1251.66±6.80 abA
Ovasis	1079.33±4.50 bA	2029.66±6.4aA	1642..66±14.18 Ab
Ovatide	1219.33±17.47abA	1753.66±4.Ab	2080.33±3.51 aA

a. Latency period (hrs)

Hormone Type	**Hormone dosage (mg)**		
	Low	**Medium**	**High**
PGE	20.93±0.07bA	14.86±0.42abA	11.81±0.37aA
Ovasis	16.90±0.68aA	14.05±0.42aA	11.81±0.51aA
Ovatide	17.92±0.46bA	12.27±0.19abA	9.8±0.32aA

b. Number of eggs

Hormone type	Hormone dosage		
	Low	Medium	High
PGE	978.66±6.80 bA	1159.66±4.50abA	1251.66±6.80 abA
Ovasis	1079.33±4.50 bA	2029.66±6.4aA	1642..66±14.18 Ab
Ovatide	1219.33±17.47abA	1753.66±4.Ab	2080.33±3.51 aA

c. Fertilization rate (%)

Hormone Type	Hormone dosage		
	Low	Medium	High
PGE	45.33±0.57a	62.66±1.52ab	56.33±0.57ab
Ovasis	45.66±1.54a	79.33±0.57b	66.66±0.57ab
Ovatide	44.66±0.57a	75.33±0.57b	78.33±0.57b

d. Incubation period (hrs)

Hormone Type	Hormone dosage		
	Low	Medium	High
PGE	23.98±1.58a	22.77±0.65a	23.73±1.15a
Ovasis	23.33±0.94a	22.33±1.15a	23.16±0.15a
Ovatide	23.98±0.42a	22.88±1.47a	23.86±0.32a

e. Hatching (%)

Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	43.33±1.15a	68.66±1.15ab	56.33±0.57ab
Ovasis	56.66±2.08ab	76.33±2.08b	68.33±1.15ab
Ovatide	56.33±1.52ab	67.33±0.57ab	77.66±0.57b

f. Survival at 1st feeding

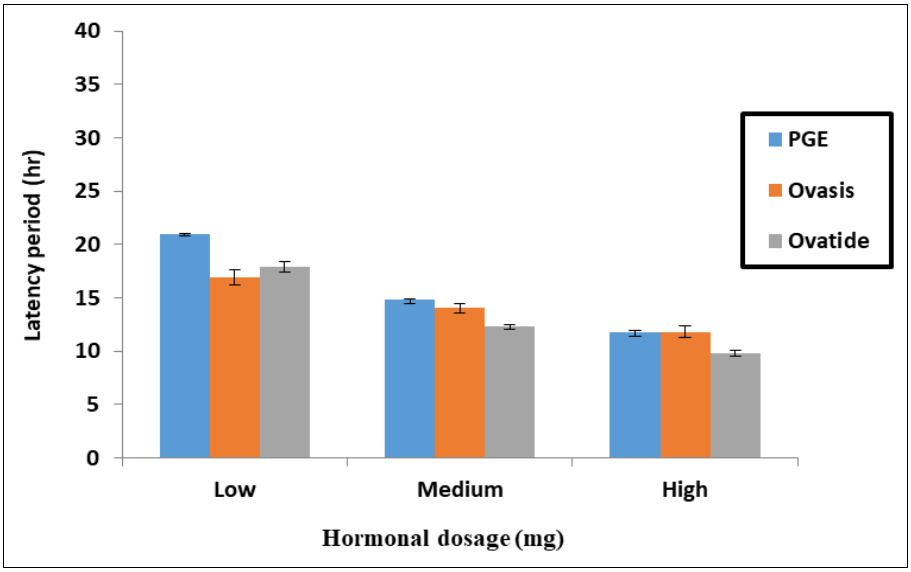
Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	48.66±0.57a	60.33±1.15ab	73.66±0.57ab
Ovasis	55.33±1.15a	80.33±1.52b	75.33±1.52ab
Ovatide	61.33±0.57ab	70.33±2.08ab	80.33±1.15b

g. Survival at fry stage (%)

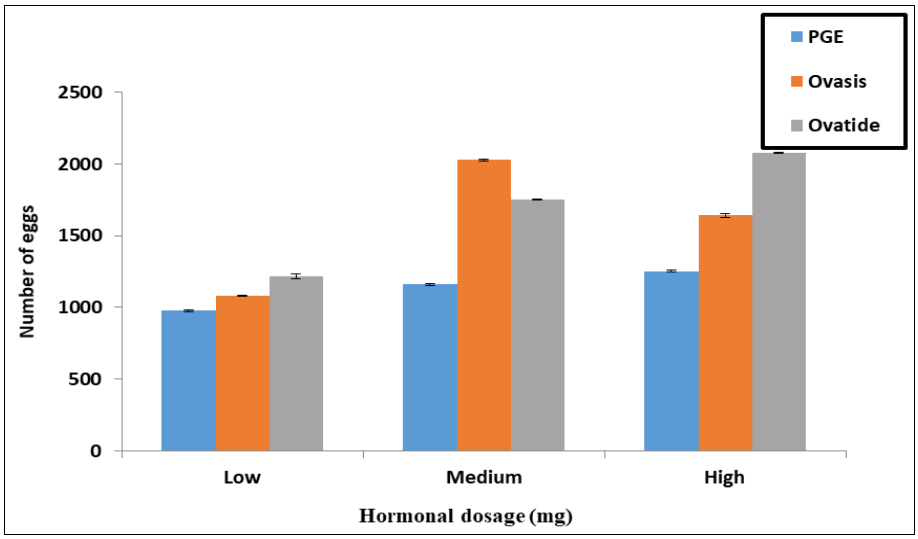
Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	67.66±1.52a	69.66±1.52a	74.33±1.15Aa
Ovasis	73.66±2.51Aa	86.33±2.51ab	79.33±1.15Aa
Ovatide	75.66±1.15Aa	78.33±0.57Aa	84.33±1.52ab

h. Survival at fingerlings stage (%)

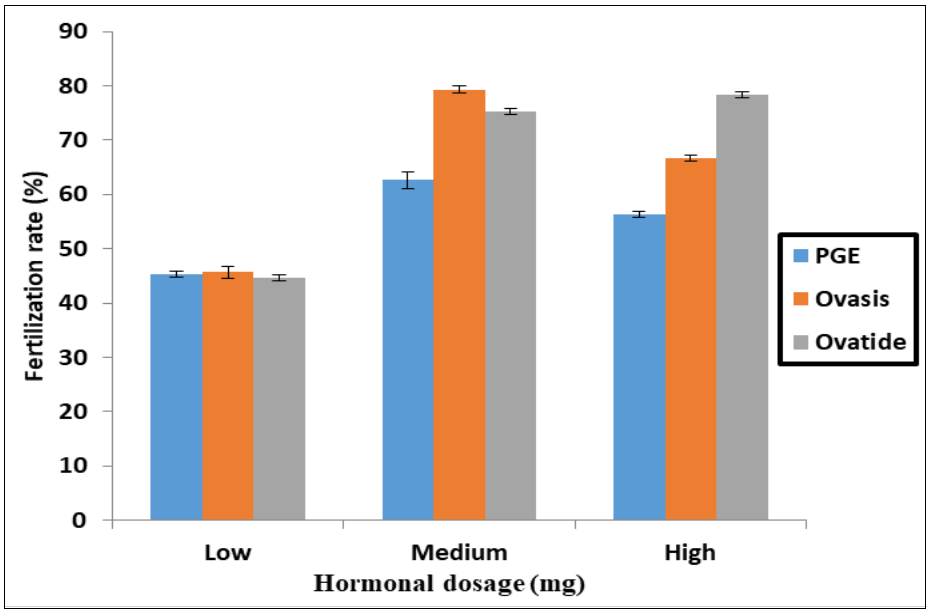
Hormonal Type	Hormone dosage		
	Low	Medium	High
PGE	65.66±1.52ab	73.66±1.52Aa	77.33±0.57Aa
Ovasis	73.66±2.51Aa	87.33±1.52a	78.66±0.57Aa
Ovatide	68.66±0.57ab	78.33±0.57Aa	84.66±1.15a



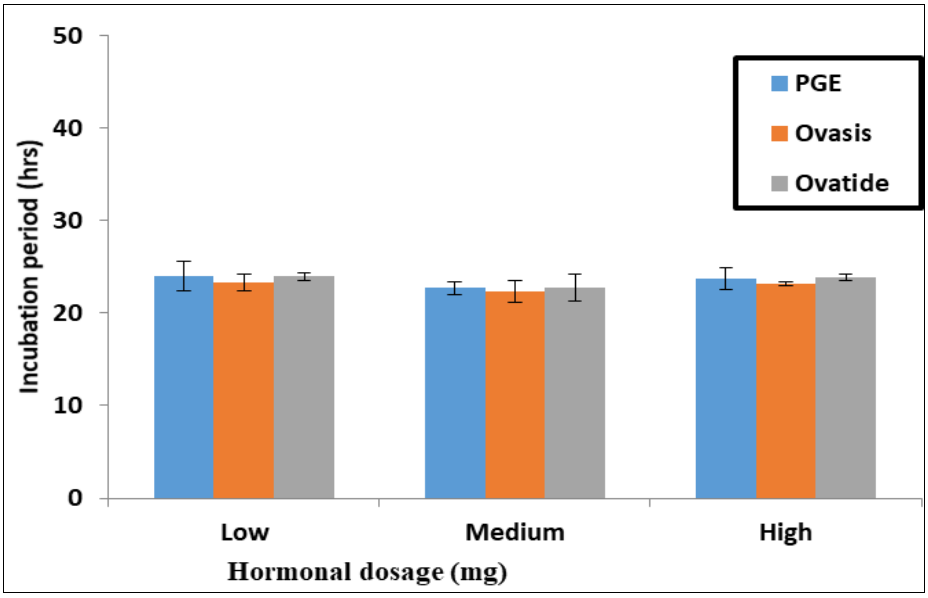
Graph 1.a: Effect of various hormones on latency period (hrs) of *H. fossilis* (vertical bar indicate SD)



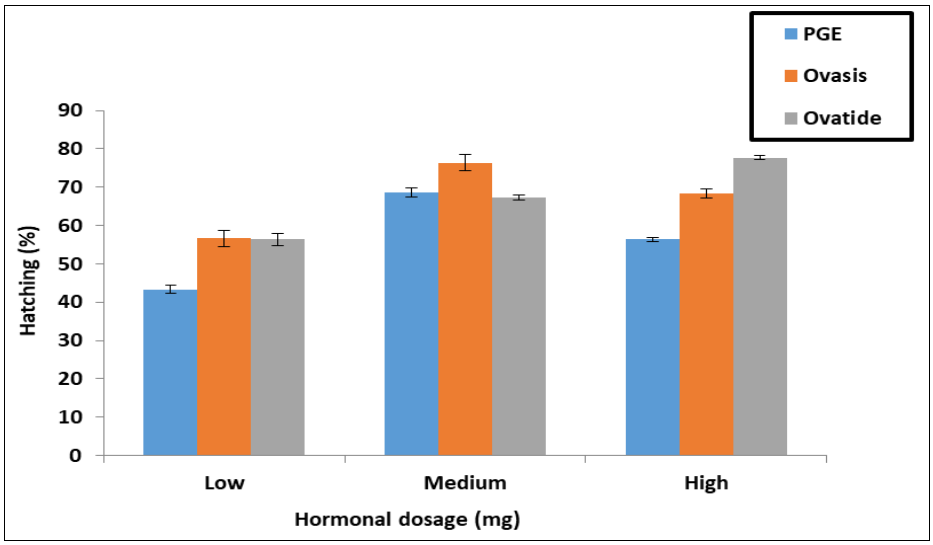
Graph 1.b: Effect of various hormones on number of eggs of *H. fossilis* (vertical bar indicate SD)



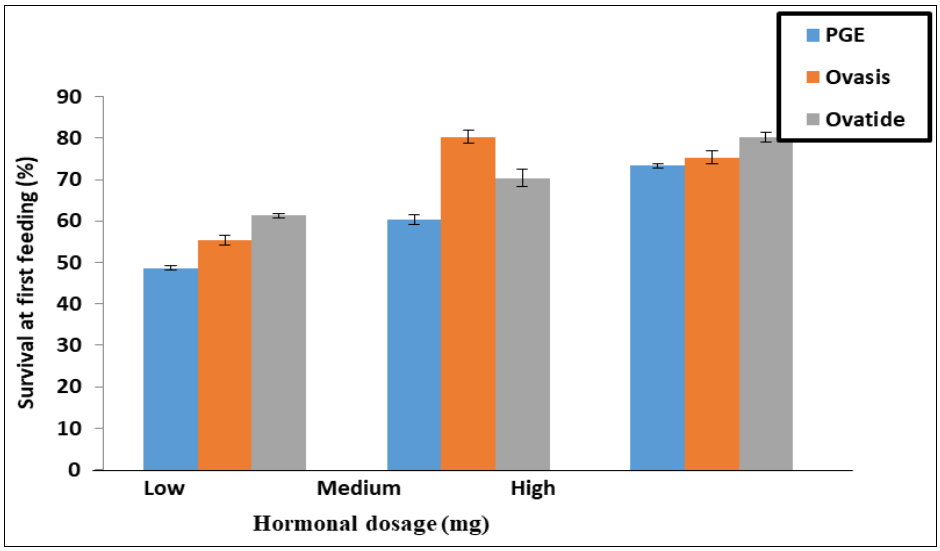
Graph 1.c: Effect of various hormones on fertilization of eggs (%) of H. fossilis (vertical bar indicate SD)



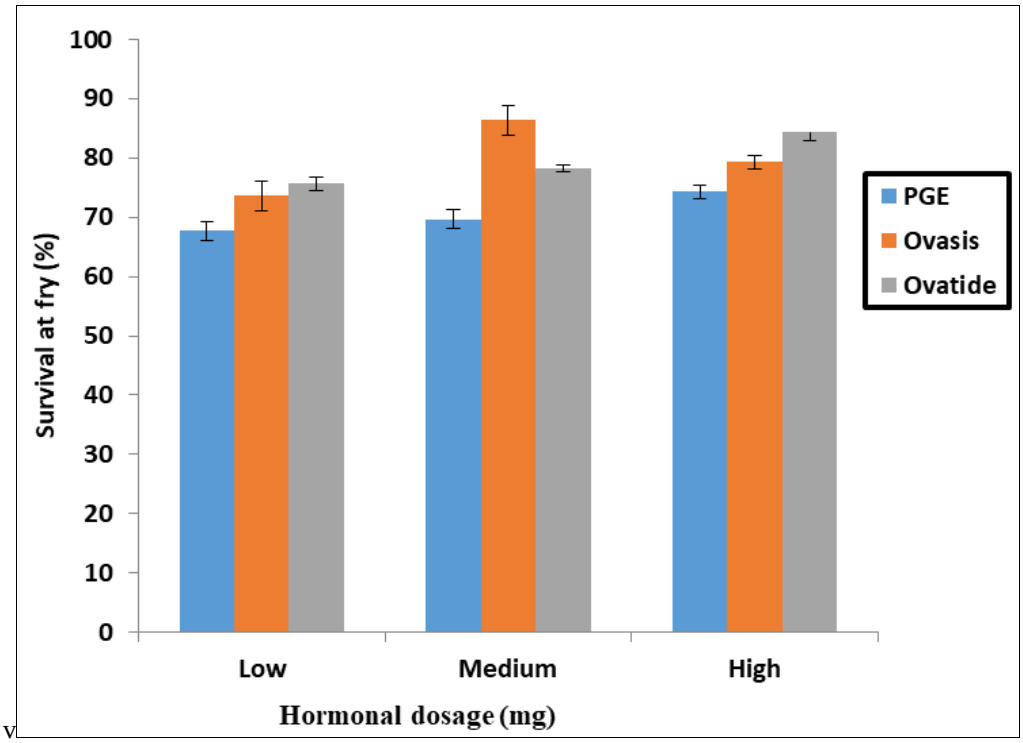
Graph 1.d: Effect of various hormones on incubation period (hrs) of H. fossilis (vertical bar indicate SD)



Graph 1.e: Effect of various hormones on egg hatchability (%) of H. fossilis (vertical bar indicate SD)



Graph 1.f: Survival of H. fossilis at 1st feeding stage (%) from day 4 to 6 after hatching (vertical bar indicate SD)



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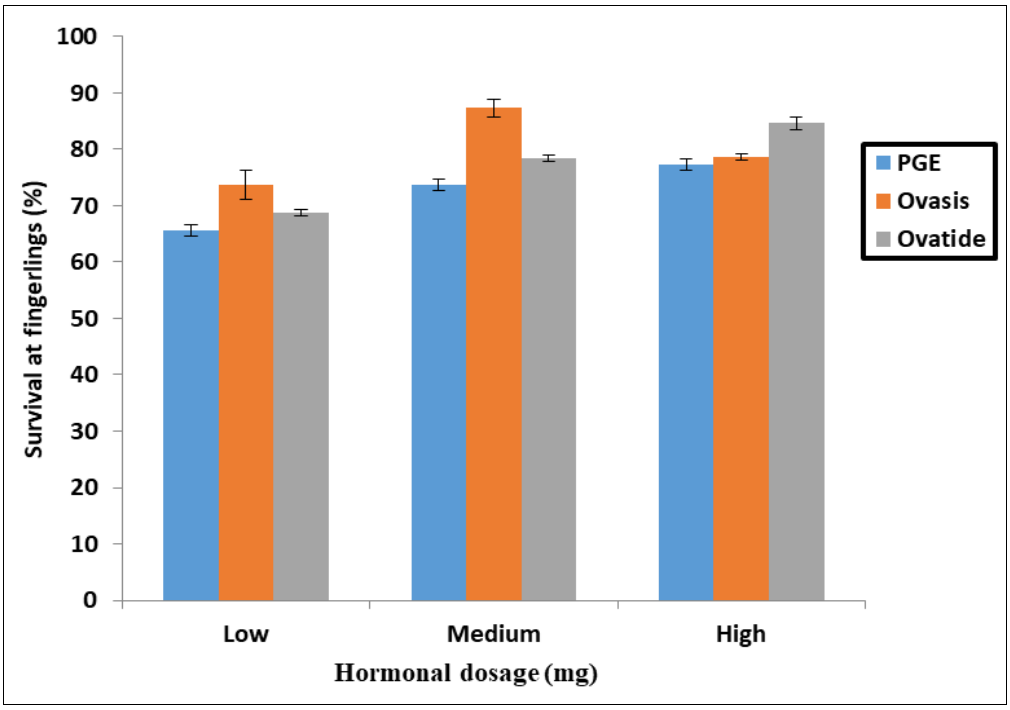
Graph 1.g: Percent survival of H. fossilis fry showed by various hormones (vertical bar indicate SD)



Graph 1.h: Percent survival of H. fossilis fingerlings showed by various hormones (vertical bar indicate SD)

Fig 1: Brooder stocking tank

Fig 2: Breeding chamber

Fig 3: Intramuscular injection Hormones

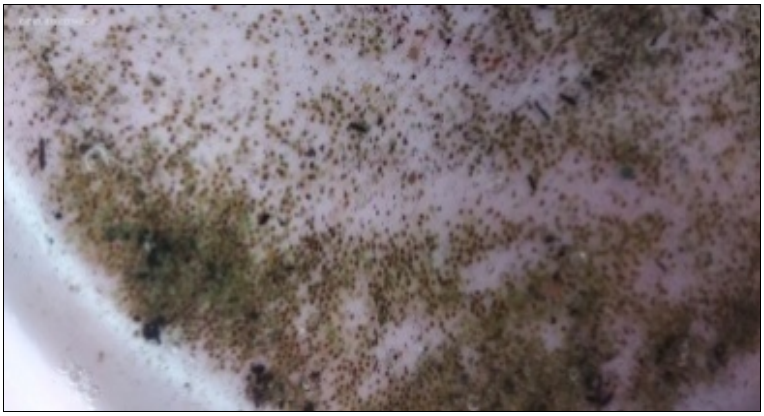


Fig 4: Ovulation after 11:00 hrs of 1st dose

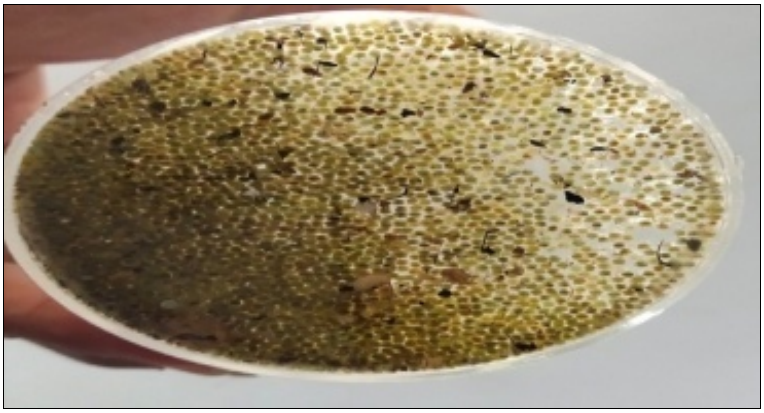


Fig 5: Fertilization after 14;30 hrs after 1st dose

Fig 6: Hatching chamber

Developmental biology of *Heteropneusts fossils*

Embryonic development

The eggs were transparent, spherical, adhesive,brownish green color. The outer egg membrane was separated from the rest of the egg by a small perivitelline space.

Fig.1 (a) Unfertilized eggs: Looks dull, opaque, white and nucleus disintegrated within one hour (1.1-1.2 mm) (Fig. 1 a)

(b) Fertilized egg: Round, clear, reddish spot on one pole (1.3-1.5 mm) (Fig 1 b).

(c) Cleavage: At 1st cleavage, the upper reddish region divides discoidal and meroblastically into two blastomeres in 15-20 min. At 2nd cleavage four cells formed within 40 minutes (Fig. 1 c), 32-cells formed within 125 minutes.

(d) Morula stage: After 2.30 hrs blastomeres decreased in size. The crown of blastomeres start spread over the yolk in the form of thin layers. Anterior and posterior ends became differentiated (Fig. 1 d).

(e) Blastula (3.20-4.15 hrs of fertilization)

At this stage, cellular materials flattened and embryo goes into blastula stage. After this stage, germinal ring formed (Fig. 1 e) at the middle of the blastula.

(f) Gastrula (6.30-6.50 hrs of fertilization)

In this stage, formation of blastopore has completed. Head and tail ends of embryo clearly were seen transparently. After 30 min, yolk invasion takes place due to which blastopore almost closed (1 f).

(g) After 11.30-12.30 hrs: At this stage 7-8 somites are formed, pigmentation was noticed on the somites and optic cup is visible. Notochord were clearly seen anterior end. Fore, mid and hind brain seen at lower end (Fig. 2 a).

(h) After 22-23 hrs: In tthis stage, 20-25 somites are devopeded. In this time, the rudimentary heart lying anterior to yolk sac and yolk completely encircled by embryo and tail end where two somites are separated. Mobility in the embryo was observed at the rate of 22-25 contractions per minute (Fig. 2 b).

Hatchlings

Fig.2 (a) newly hatched larva: It is transparent, light brown in color and 2.3±0.5 mm in length with laterally compressed body (Fig. 3 a). Eye unpigmented. Mouth and fins were not distinct. Head is small and it is not separated from the yolk sac. Yolk sac is oval in shape and pale greenish in color. The larvae inhabited at the bottom of the tank and swam with rapid movements using their tail.

(b)Four hr old larva: It is about 3.6±0.2 mm long and brownish in color. Anus invagination was seen at the mid ventral part of the body. Mouth was yet to develop. (Fig.3b).Eyes and mouth unpigmented. Heart became distinct and two chambered, circulation of body fluid observed. A tube like structure emerges from posterior dorsal side of yolk sac which represents digestive tract. Barbells have not developed yet. Pigmentation were dark at their anterior region.

(c) Eight hrs old larva: They were about 3.6±0.1 mm in length. The bulged yolk became gradually elongated and

buccal invagination was noticed in anterior region. The larva shows a dorso-ventral unpaired fin, few melanophores appeared on the head region, ventral side of notochord and dorsal side of body (Fig. 3 c).

(d) 24 hr old larva: The average length of the larva was 4.1±0.2 mm with a reduced yolk sac. Dark pigmented eye spot has appeared on the anterior part of the head and 32 myotomes were seen at this stage. Buccal invagination appeared in the mouth where upper and lower jaws were formed. Pectoral fin buds were seen as a small outgrowth on it innerside. Mouth was not opened yet. Heart was visible in front of yolk. Pigmentation appeared on both dorsally and ventrally sides. Melanophore scattered on the dorsal fin fold and trunk regions (Fig. 3 d).

(e) 36 hr old larva: The average length of the larva was 4.4±0.2 mm and yolk sac much reduced in size. The eyes were darkly pigmented and spherical in shape. Pectoral fin is in oval shape which used for actively swimming. Heart was clearly located behind the head and beat regularly. Mouth formed with small opening. Rudimentary gill opening and olfactory pit were differentiated in these larvae. Thick melanophore formed on the base of pectoral fin (Fig. 3 e).

(f) 48 hr old larva: The average length of 48 hrs old larva was 4.7±0.1 mm**.** The eye ball has dark prominents, mouth opening well formed on the well-developed lower jaw. The barbells became elongated and it is prominent around the mouth. The yolk reserve further became reduced much. Pectoral fin became paddle shaped. Anal opening well formed. Blood circulation was observed in heart and tail regions. Melanophores much concentrated at head region than other body regions. The alimentary canal became short, straight and distinct and the larva started feeding exogenously so that a pouch like stomach formed (Fig. f).

(g) 3 Day old larva: The average length of 3 day old larva was measured at 5.1±0.3 mm with a post anal length of 2.6 mm. The body brownish in color and the mouth and anus became fully functional. The size of the stomach was larger than intestine which is coiled. Pectoral fin vascularied and move vigorously. The head was well developed and free movement of eye ball takes place and the four pair of barbells noticed at this stage. The size of barbells has slightly increased than the previous one. The reserved yolk material has completely absorbed. The caudal fin has 5 rudimentary rays. The larvae have shows vigorous movements to the water surface and sometime sink to bottom (Fig. 3 g).

(h) 10 Day old larva: The average length of 10 day old larva was 8.92±0.2 mm with post anal length of 4.1 mm. Dorsal and anal fins were clearly seen and almost separated from the caudal fin. Eight caudal fin rays were clearly seen. The larvae shows frequent surface movements and aerial breathing observed at this stage. Larvae were actively swim and the foraging behavior of larvae noticed.

(i) 20 Day old larva: The average length of 20 day old larva was 16.4±1.5mm with post anal length of 12.2 mm. Pigmentation appeared on all over the body of the juvenile. At this stage, organogenesis was completed and juvenile were morphologically almost similar to the adult except for their color pattern.

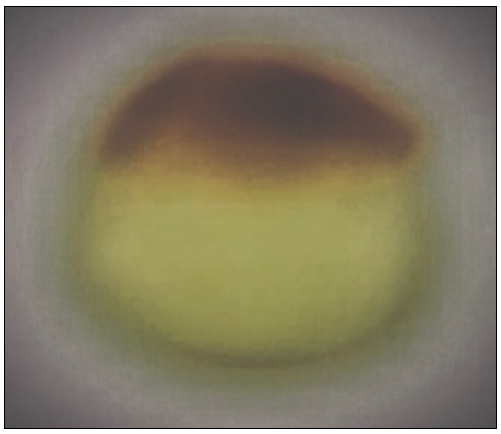
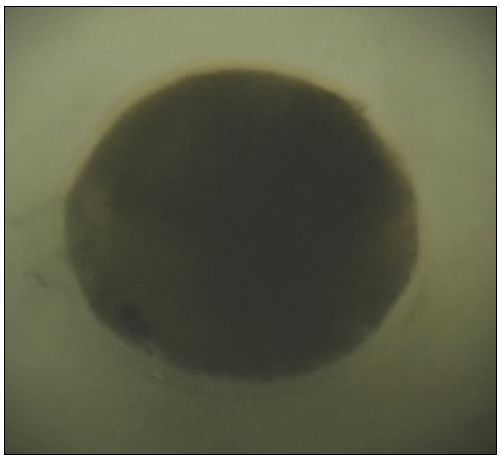
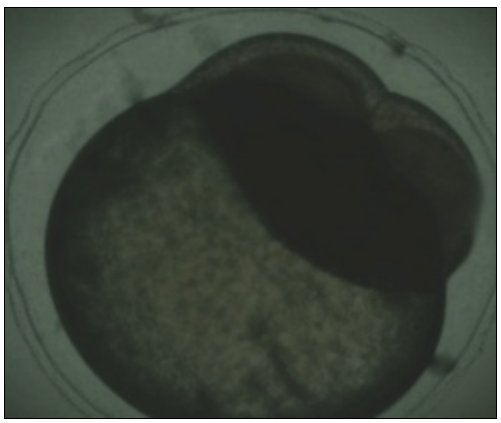


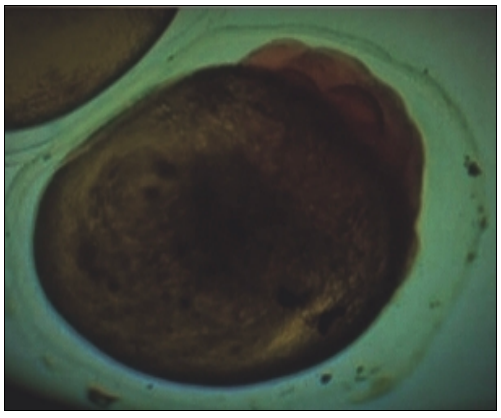
Fig 1: (a) Unfertilized egg



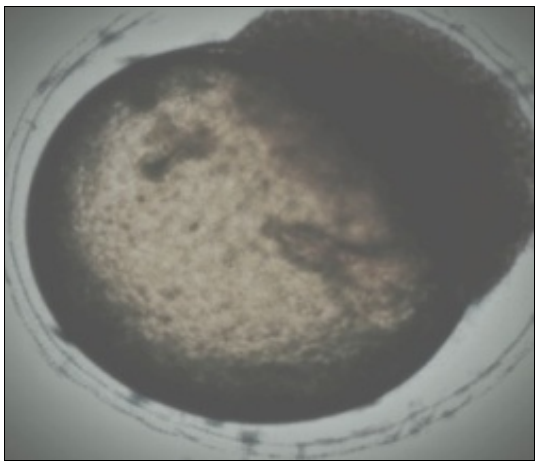
(b) Fertilized Eggs



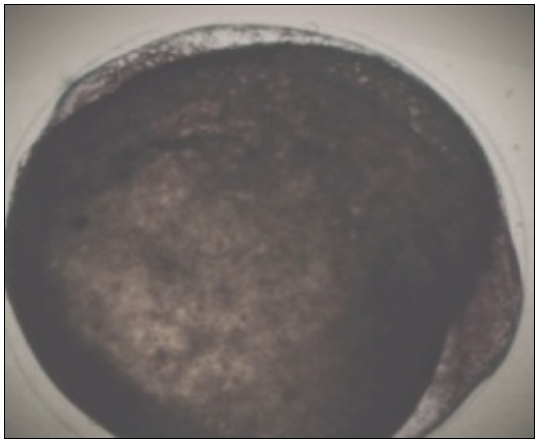
(c) 4-cell stage



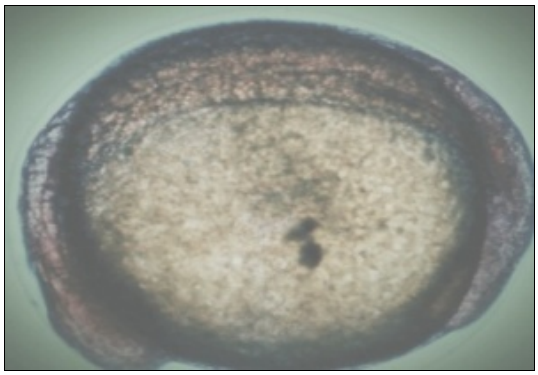
(d) Morula stage



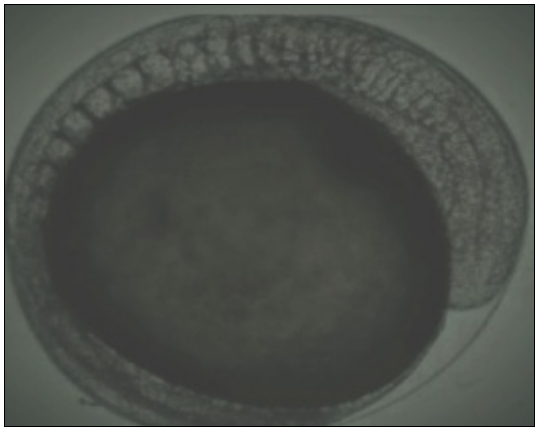
(e) Blastula stage



(f) Gastrula stage

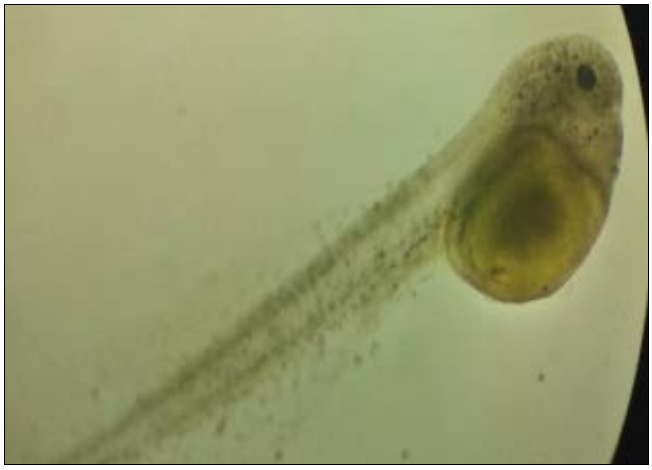


(g) Hatching after 11.30-12.30hrs



(h) Hatching after 22-23 hrs

Fig 2: (a): Newly hatched larva

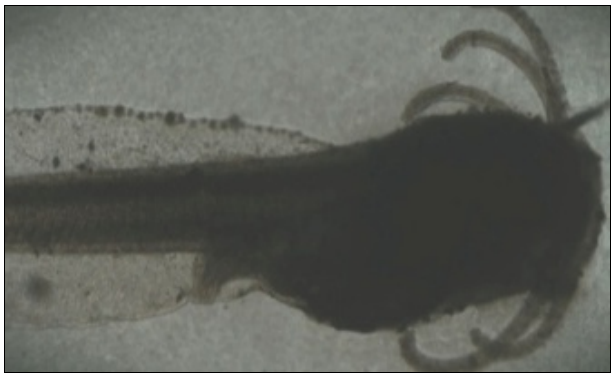


(b) 4 hrs old larva

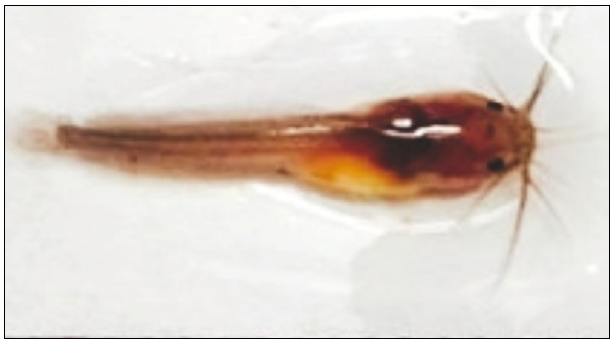
(c) 8 hrs old

(d) 36 hrs old larva

(e) 48 hrs old larva



(f) 3 Day old larva



(g) 10 Day old larva

(h) 20 Day old larva

Discussion

At present, Indian people are solely dependent on the natural sources for fry and fingerlings *H. fossilis* but natural sources alone cannot supply for commercial scale. Therefore, the breeding of fish was carried out by using one natural and two synthetic hormones. In present study we use three different hormones, previously PGE and ovasis used for captive breeding of *H. fossilis* (Vijayakumar *et al.*, 1998) [25] which result similar to present study. In current study, each female was reported to have produced between 1900-2100 sticky eggs in a single spawn which was less than previous study (Nesa *et al.*, 2017) [18] but fertility rate maximum than the previous study. Furthermore, to expand the aquaculture of *H. fossilis*, knowledge of early larval development of this fish is scanty and only few studies have been made (Nesa, *et al.*, 2017) [18]. Therefore, present study conducted. Diameter of unfertilized egg ranged from 1-1.1 mm and fertilized egg 1.3-1.4 mm which is similar to other study (Marimuthu *et al.*, 2009) [16]. It may also depend upon water quality, environmental factors, food availability (Thakur *et al.*, 1980) [23]. Fertilization rate (63%) and hatching rate (68%) of *H. fossilis* in this experiment were low when we inject medium dosage of PGE. Fertilization rates (79%) and hatching rate (76%) of *H. fossilis* in this experiment were high when we inject medium dosage of ovasis in present study and almost similar result also reported by previous study (Rehman *et al.*, 2013) [20]. Fertilization rates (78%) and hatching rate (77%) of *H. fossilis* in this experiment was high when we inject high dosage of ovatide in present study. The mode of cleavage recorded in the present study was similar to other cat fish species such as *Pangasius sutchi* (Islam, 2005) [10]. The observation of early development and pre-hatching behavior of *H. fossilis* agrees well with previous study (Arockiaraj *et al.*, 2003) [2]. In present study, the hatching time 21 to 22 hrs but in previous study its about 20 to 24 hrs (Marimuthu *et al.*, 2000) [16]. Present study conducted at 30-32 ºC where as Marimuthu *et al.*, 2000 [16] carried out their work at 29 ºC. The temperature has a significant effect on the embryonic development. Kohli and Vidyarthi (1990) [12] reported the incubation period of 16-18 hrs in *H. fossillis* at 26 ºC and in present study, incubation period 22-23 hrs for medium dosage of ovasis and ovatide. The morula stage was reached between 2-2.35 hrs, blastula stage 3.25-4.1 hrs, gastrula stage 6.2-6.5 hrs after fertilization. The morula stage 140 min, gastrula stage was reached 7 hrs after fertilization (Marimuthu *et al.*, 2000) [16]. In *Channa punctus* the blastula stage appeared after 2-3 hrs and yolk invasion was completed at about 10 hrs after spawning (Munshi *et al.*, 1991) [17]. Just before 1-2 hrs of hatching, the embryo of *H. fossilis* showed twisting movements and similar behavior was reported in same fish species (Thakur *et al.*, 1974) [23]. In present study, it was found that the mouth was opened at 36 hrs, hatchlings feed at 48 hrs after hatching but in *H. longifils* mouth opened at 3-4 hrs, hatchlings feed at 48 hrs (Ogunji *et al.*, 1999) [19]. In the present study complete yolk sac absorption of *H. fossilis* was observed on the 3 day and similar event found in other study also (Ogunji *et al.*, 1999) [19].

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of the present paper.

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