

Original Research Article

EFFICACY OF DRY PITUITARY GLAND, WET PITUITARY GLAND AND THE SYNTHETIC HORMONE, OVAPRIM IN THE BREEDING OF CATLA, *Catla catla*

ABSTRACT

An induced breeding experiment with Indian major carp, catla (*Catla catla*) was conducted to know the efficacies of three different doses of dry PG and three different doses of wet PG in comparison to that of ovaprim—~~at the Fisheries Field Laboratory Complex, Bangladesh Agricultural University, Mymensingh during February to July, 2020.~~ Catla fish were injected with dry PG low dose (DPL), 1 mg/Kg body weight for first dose of female fish and after 6 hours interval second dose (2 mg/Kg of body weight) was administered; at the same time for female and 1 mg/Kg body weight for male. For dry PG medium dose (DPM), 2 mg/Kg body weight for first dose and after 6 hours interval 4 mg/Kg body weight for female and 2 mg/Kg body weight for male. For dry PG high dose (DPH), 3 mg/Kg body weight for first dose and after 6 hours interval 6 mg/Kg body weight for female and 3 mg/Kg body weight for male fish. Wet PG low dose (WPL), 7 mg/Kg body weight for first dose and after 6 hours interval 10 mg/Kg body weight for female and 7 mg/Kg body weight for male fish. For wet PG medium dose (WPM), 11 mg/Kg body weight for first dose and after 6 hours interval 15 mg/Kg body weight for female and 11 mg/Kg body weight for male fish. And for wet PG high dose (WPH), 15 mg/Kg body weight for first dose and after 6 hours interval 20 mg/Kg body weight for female and 15 mg/Kg body weight for male fish and ovaprim, 5 mg/Kg of body weight for second doses for both male and female. These doses were used for determination of fertilization, hatching and survival rates.

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The fertilization rates were $83.25 \pm 0.85\%$, $92.01 \pm 0.45\%$, $82.45 \pm 1.04\%$, $83.18 \pm 0.68\%$, $88.59 \pm 0.48\%$ and $72.85 \pm 0.43\%$ for the doses of DPL, DPM, DPH, WPL, WPM and ovaprim respectively. The hatching rates were $78.58 \pm 0.94\%$, $85.54 \pm 0.38\%$, $74.8 \pm 0.22\%$, $77.89 \pm 0.82\%$, $81.56 \pm 0.59\%$ and $71.54 \pm 0.48\%$ for the doses of DPL, DPM, DPH, WPL, WPM and ovaprim respectively and the survival rates were $77.24 \pm 0.49\%$, $87.12 \pm 0.46\%$, $73.09 \pm 0.42\%$, $75.8 \pm 0.30\%$, $80.76 \pm 0.64\%$ and $69.2 \pm 0.77\%$ for the doses of DPL, DPM, DPH, WPL, WPM and ovaprim respectively. Among all the doses of dry and wet PG and the synthetic hormone ovaprim, DPM gave the best result in case of fertilization, hatching and survival rate. So, the induced breeding of catla fish may use dry PG at the dose of 2 mg/Kg body weight as first dose and 4 mg/Kg of body weight as second dose.

Keywords: Pituitary gland, Ovaprim, Fertilization, Hatching, Survival rate, Induced breeding, *Catla catla*

1. Introduction

Fish is the second most valuable agricultural crop in Bangladesh, play a crucial role in the livelihoods and employment of 18 millions of people. The culture and consumption of fish therefore has important implications for national income, food and nutrition security. Fisheries sector being one of the most productive and dynamic sectors is playing an increasingly significant role in the economy for the last few decades. Indian major carps are largely cultured in the country. Bangladesh is one of the world's leading fish producing countries with a total production of 4.384 million mt, contributing 3.57% to the GDP and over 25.30% to the agricultural GDP and 1.23% to foreign exchange earnings by exporting fish and fish products in 2020. Fish Now, Bangladesh becomes self-sufficient fish producing country supplements about 60% (with per capita of 62.58 g/day against targeted 60 g/day) of total daily animal protein intake of her people. The country earns BDT 425031.00 lakh (as per EPB) by exporting almost 73.17 thousand MT of shrimps, fish and fishery products [5].

Catla (*Catla catla*; Hamilton, 1822) is one of the most important aquacultured freshwater fish species in South Asia. It is a very safe fish because it depends mostly on plankton and for surface feeder it can remain the water body environment well. Due to high market demand of both fingerlings and adult fish people rear catla for meeting protein requirement [10]. Catla do not spawn in confined water bodies such as ponds, lakes etc., but breed in natural running water bodies as in rivers. Environmental factors such as rainfall, temperature, water velocity play an important role in stimulating the fish for reproduction. Catla usually spawn in inundated terrains of rivers and streams during rainy season. On the other hand, the fish seeds collected from

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natural spawning grounds is scanty and does not fulfill the annual requirement for inland aquaculture in the country. Henceforth, efforts have been paved to breed the catla in hatcheries [11].

In 1967, first induced breeding technology of carp fish was developed in Chandpur, Bangladesh. From the beginning the natural sources of rivers were the major source of carp seed production in Bangladesh [7]. Due to the destruction of natural habitats and increased demand, carp seed availability in nature has largely declined and the aquaculture venture are gradually replaced by the hatchery bred fry since early 80's when artificial fish breeding techniques and low-cost hatchery designs have been successfully adapted in Bangladesh [8]. Private fish hatcheries produced about 6,65,535 kg hatchling of 5-6 days old while the public sector farms produced about 13,485 kg hatchling (5). The availability of fish fry is an essential prerequisite for aquaculture. Pituitary gland (PG) hormone usually used for induced breeding of different fish like Indian major carp. The hormone secreted from pituitary gland stimulates growth, development, maturity and ovulation of eggs thus it is important as the production of Indian major carp including catla [4].

The poor breeding response, coupled with a relatively shorter spawning season, results in an inadequate production of hatchery-reared seed, which often fails to cover the entire needs of the farmers. While carp pituitary extract has been the common inducing agent used since the development of the induced breeding technology. Also, several synthetic hormones such as Ovaprim, Ovatide and Wova-FH are used [2].

There is a lack of literature on comparative efficacy of dry and wet PG in induced breeding of catla. To investigate the comparative efficacy of the above mentioned two PGs is the need of time. So, the present study was conducted to determine the effect of dry PG and wet PG with

their different doses on fertilization rate, hatching rate and survival rate of catla, *Catla catla* and to compare their efficacy with that of the widely used synthetic hormone, ovaprim.

2. MATERIALS AND METHODS

2.1 Study site and duration

The experiment was carried out in the brood ponds and hatchery complex of the Fisheries Field Laboratory Complex, Bangladesh Agricultural University, Mymensingh from February to July 2020.

2.2 Collection and selection of brood fish for breeding

For the present study, mature and healthy catla brood were collected for inducing hormone. Main criteria for selection of male and female are given in Table 1.

Table 1. Criteria followed to select broods of catla

Characteristics	Male	Female
1. Scale, Operculum and pectoral fins	Rough touch, particularly the dorsal surface of pectoral fins	Pectoral fins smooth to lubricious
2. Abdomen	Round and firm	Swollen and soft
3. Genital opening	Elongated slit, white in color	Rounded and pink in color
4. When pressure applied on abdomen	Milky white fluid oozes through genital opening	A few ova may ooze through genital pore
5. Shape of body and size	Body linear, smaller	Body stouter, slightly larger

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2.3 Preparation of PG and ovaprim

The amount to be used was calculated using the following formula

$$\text{Weight of PG (mg)} = \frac{Wt \times Pt}{1000}$$

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Where, Wt represents total body weight (g) of the fish to be injected and Pt, represent rate of mg PG to be injected/Kg body weight.

The weighed PG was homogenized with a small volume of distilled water and the homogenate was carefully transferred to a centrifuge tube by using water to ensure complete transfer.

2.4 Doses of PG and ovaprim used for induced breeding

Doses and dosages of PG and ovaprim injected in male and female fish, is shown in Table 2 and Table 3, respectively.

Table 2. Doses and dosages of PG and ovaprim injected in male catla

SI No.	Hormone	Doses and dosages (mg/kg body weight)	Description of the dose	Designation
1 and 2	Dry PG	1 mg/Kg	dry PG low dose	DPL
3 and 4	Dry PG	2 mg/Kg	dry PG medium dose	DPM
5 and 6	Dry PG	3 mg/Kg	dry PG high dose	DPH
7 and 8	Wet PG	7 mg/Kg	wet PG low dose	WPL
9 and 10	Wet PG	11 mg/Kg	wet PG medium dose	WPM
11 and 12	Wet PG	15 mg/Kg	wet PG high dose	WPH
13, 14 and 15	Ovaprim	5 mg/Kg	Ovaprim	Ovaprim

Table 3. Doses and dosages of PG or ovaprim injected in female catla

Fish No.	Hormone	Doses and dosages (mg/Kg body weight)	
		1 st dose	2 nd dose
1 and 2	Dry PG	1 mg PG/Kg (DPL)*	2 mg PG/Kg (DPL)
3 and 4	Dry PG	2 mg PG/Kg (DPM)*	4 mg PG/Kg (DPM)
5 and 6	Dry PG	3 mg PG/Kg (DPH)*	6 mg PG/Kg (DPH)
7 and 8	Wet PG	7 mg PG/Kg (WPL)*	10 mg PG/Kg (WPL)
9 and 10	Wet PG	11 mg PG/Kg (WPM)*	15 mg PG/Kg (WPM)
11 and 12	Wet PG	15 mg PG/Kg (WPH)*	20 mg PG/Kg (WPH)
13, 14 and 15	Ovaprim		5 mg/Kg

*DPL: dry PG low dose; DPM: dry PG medium dose; DPH: dry PG high dose; WPL: wet PG low dose; WPM: wet PG medium dose; WPH: wet PG high dose



2.5 Hormone injection administration

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Hormones were administered according to the experimental design shown in Table 2 and 3. For confirmation of getting sufficient number of ovulated eggs, in each case, 15 females and 15 males fish were injected. During injection, brood fish were covered by soft cloth and handled smoothly. Females brood were given two doses of PG injection. The males were given only one dose at the time of the second dose given to female. Intra- peritoneal injections were given at the base of the pelvic fin. Injections were given to the carps at an angle of 45° of the body. After injection both male and female breeders were released immediately in the same circular tank with showering of water.

2.6 Collection and mixing of eggs and milt

Stripping method was followed for collecting eggs. After 6 hours of 2nd dose, eggs and milt were collected from the ovulated females and males by stripping the abdomen of the fish with a gentle pressure. Eggs were collected at first into a dish and immediately it was weighed. Then milt of the males were stripped over the eggs. The eggs and milts in the dish were mixed together with a soft feather for 2 minutes and distilled water was added to the mixture in order to reduce the stickiness of the eggs and prolong the fertilizing capacity of the milt. Unfertilized eggs were washed away by water. Fertilized and unfertilized egg sample were taken in a vial for counting. After well mixing for two minutes the fertilized eggs were poured into hatching jar or circular hatching tank with care with continuous water flow for aeration and circulation.

2.7 Incubation, hatching and hatchling monitoring and sampling

After transferring eggs into circular or bottle hatching tanks numbers of hatched or unhatched eggs were sampled after 1 hour, 6 hours, 18 hours, 32 hours, 42 hours, 72 hours. In the spawning tank after 24 hours the fertilized eggs were hatched. Hatched larvae were not supplied with any

type of food up to 72 hours. After the yolk sac absorption was completed around 72 hours of hatching, the hatchlings were observed for the total absorption of their yolk sac. All that time, boiled egg yolk diluted in water was provided as the first food for the hatchlings at ambient temperature of 26 to 30°C. At that time, they were little bit of blackish and transparent in colour. After 72 hours fries were transferred into hapa placed in a brood pond. Catla fries at 4 days, 7 days, 10 days, 13 days, and 15 days were sampled and preserved in well written vial with 10% formalin for observation of their successful breeding efficacies.

The following variables were calculated

$$\text{Fertilization rate\%} = \frac{\text{No of fertilized eggs}}{\text{Total no. of eggs}} \times 100$$

$$\text{Hatching rate\%} = \frac{\text{No. of spawned}}{\text{Total no. of eggs}} \times 100$$

$$\text{Survival rate \%} = \frac{\text{No. of survived hatchling}}{\text{Total no. of eggs}}$$

2.8 Data Analysis

Data analysis was done using Analysis of Variance (ANOVA). The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) (using SPSS 16.0 programme) to test for effects of various type of hormone. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test. For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% ($p < 0.05$).

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3. RESULTS

3.1 Amount of ovulated egg

Total amounts of eggs that could be collected from female breeders were different by using different amounts of dry or wet PG or ovaprim induction. The highest number of eggs could be collected from the dry PG medium dose induced fish, 460 g and the lowest amount, 113 g, from dry PG high dose induced fish and from wet PG high dose induced fish did not lay egg (Table 4).

Table 4. Amount of ovulated egg of PG or ovaprim injected in female catla

Fish No.	Hormone	Average total egg (gram)
1 and 2	DPL	417g
3 and 4	DPM	460g
5 and 6	DPH	113g
7 and 8	WPL	403 g
9 and 10	WPM	227g
11 and 12	WPH	Didn't lay egg
13, 14 and 15	Ovaprim	475g

Numbers of eggs were counted at unfertilized state and at different hours after fertilization is shown in Table 5.

Table 5. Number of eggs at unfertilized state and at different hours after fertilization

Condition of Egg/hr	Dry PG 1mg/Kg	Dry PG 2mg/Kg	Dry PG 3mg/Kg	Wet PG 7 mg/Kg	Wet PG 11 mg/Kg	Ovaprim 5 mg/Kg
Unfertilized	821	856	805	751	790	712
Fertilized (0)	690	708	697	682	700	653
Fertilized (6)	25	28	26	27	24	24
Fertilized (12)	23	24	22	24	22	23
Fertilized (18)	21	22	22	23	21	19

3.2 Hatching condition

It was found that the best result was achieved by the medium dose of dry PG (Table 6)

Table 6. Number of hatched and unhatched eggs/gram

Time (hr)	Dry PG		Dry PG		Dry PG		Wet PG		Wet PG		Ovaprim	
	1 mg/Kg		2 mg/Kg		3 mg/Kg		7 mg/Kg		11 mg/Kg		5 mg/Kg	
	UH**	H**	UH	H	UH	H	UH	H	UH	H	UH	H
1	6	21	8	42	7	21	9	28	5	24	9	21
6	7	23	8	45	9	27	11	33	7	27	11	26
18	9	30	10	60	13	37	12	45	8	34	13	35
32	23	79	15	96	21	65	18	71	19	79	24	61
42	21	85	18	112	24	70	21	78	21	91	25	64
72	19	90	19	117	27	80	24	91	23	112	27	71

**UH= Unhatched; H=Hatched

3.3 Fry sampling after transferred into hapa

After transferring into hapa, fries were counted at different day's interval are shown in Table 7.

Table 7. Number of fry/grams after transferred into hapa

Day	Number of fries					
	Dry PG 1 mg/Kg	Dry PG 2 mg/Kg	Dry PG 3 mg/Kg	Wet PG 7 mg/Kg	Wet PG 11 mg/Kg	Ovaprim 5 mg/Kg
1	418	445	400	431	446	404
4	307	313	278	301	297	293
7	204	218	199	196	199	189
10	184	186	147	158	161	154
13	143	151	107	131	143	139
15	127	138	87	113	129	123

3.4 Fertilization, hatching and survival rate

Variations of fertilization rate, hatching rate and survival rate of *Catla catla* fry in different doses of hormones are shown in Table 8.

Table 8. Fertilization, hatching and survival rate of catla, by different doses of hormones

Dose	Fertilization (%)	Hatching (%)	Survival (%)
Dry PG 1 mg/Kg	83.25 ± 0.85	78.58 ± 0.94	77.24 ± 0.49
Dry PG 2 mg/Kg	92.01 ± 0.45	85.54 ± 0.38	87.12 ± 0.46
Dry PG 3 mg/Kg	82.45 ± 1.04	74.8 ± 0.22	73.09 ± 0.42
Wet PG 7 mg/Kg	83.18 ± 0.68	77.89 ± 0.82	75.8 ± 0.30

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Wet PG 11 mg/Kg	88.59 ± 0.48	81.56 ± 0.59	80.76 ± 0.64
Ovaprim 5 mg/Kg	72.85 ± 0.43	71.54 ± 0.48	69.2 ± 0.77

3.4.1 Fertilization rate

It was observed from this study that the fertilization rates were $83.25 \pm 0.85\%$, $92.01 \pm 0.45\%$, $82.45 \pm 1.04\%$, $83.18 \pm 0.68\%$ and $72.85 \pm 0.43\%$ for the doses of DPL, DPM, DPH, WPL, WPM and ovaprim respectively (Figure 1). The fertilization rate was the best at dry PG medium dose $92.01 \pm 0.45\%$ followed by low dose ($83.25 \pm 0.85\%$) and high dose ($82.45 \pm 1.04\%$). The fertilization rate by wet PG was the highest ($83.18 \pm 0.68\%$) at medium dose followed by low dose ($82.45 \pm 1.04\%$) and ovaprim ($72.85 \pm 0.43\%$).

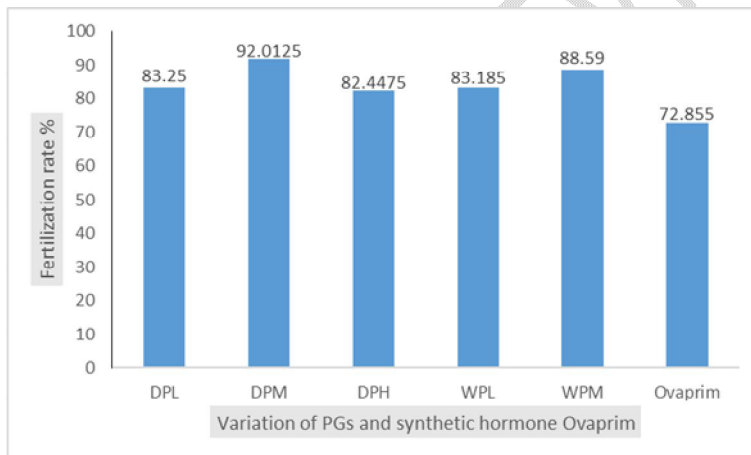


Figure 1. Fertilization rate of catla by different doses of hormones

3.4.2 Hatching rate

The hatching rates were achieved from this study @ $78.58 \pm 0.94\%$, $85.54 \pm 0.38\%$, $74.8 \pm 0.22\%$, $77.89 \pm 0.82\%$, $81.56 \pm 0.59\%$, $71.54 \pm 0.48\%$ for the doses of DPL, DPM, DPH, WPL, WPM and ovaprim respectively (Figure 2). The hatching rate was the best at dry PG medium dose $85.54 \pm 0.38\%$ followed by low dose ($78.58 \pm 0.94\%$) and high dose ($74.8 \pm 0.22\%$). The hatching rate by wet PG was the highest ($81.56 \pm 0.59\%$) at medium dose followed by low dose

(77.89 ± 0.82%) and ovaprim (71.54 ± 0.48%).

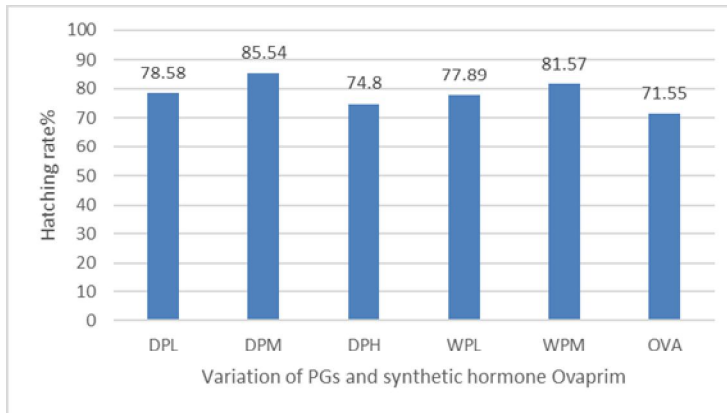


Figure 2. Hatching rate of catla by different doses of hormones

3.4.3 Survival rate

The survival rates were recorded in this study @ 77.24 ± 0.49%, 87.12 ± 0.46%, 73.09 ± 0.42%, 75.8 ± 0.30%, 80.76 ± 0.64%, 69.2 ± 0.77% for the doses of DPL, DPM, DPH, WPL, WPM and ovaprim respectively (Figure 3). The survival rate was the best at dry PG medium dose 87.12 ± 0.46 followed by low dose (77.24 ± 0.49%) and high dose (73.09 ± 0.42%). The survival rate by wet PG was the highest at medium dose 80.76 ± 0.64% followed by low dose (75.8 ± 0.30%) and ovaprim (69.2 ± 0.77%).

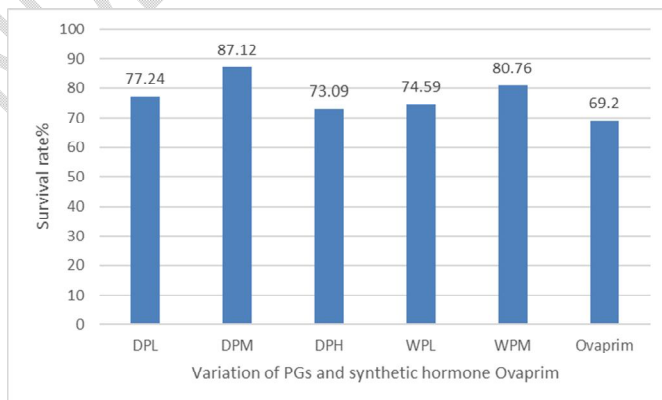


Figure 3. Survival rate of catla by different doses of hormones

4. DISCUSSION

During the present study of induced breeding of catla, in case of dry PG low dose (DPL), 1 mg/Kg body weight for first dose of female fish and after 6 hours interval 2 mg/Kg of body weight for female and 1 mg/Kg body weight for male. In case of dry PG medium dose (DPM), 2 mg/Kg body weight for first dose and after 6 hours interval 4 mg/Kg body weight for female and 2 mg/Kg body weight for male. For dry PG high dose (DPH), 3 mg/Kg body weight for first dose and after 6 hours interval 6 mg/Kg body weight for female and 3 mg/Kg body weight for male fish. In case of wet PG low dose (WPL), 7 mg/Kg body weight for first dose and after 6 hours interval 10 mg/Kg body weight for female and 7 mg/Kg body weight for male fish. For wet PG medium dose (WPM), 11 mg/Kg body weight for first dose and after 6 hours interval 15 mg/Kg body weight for female and 11 mg/Kg body weight for male fish and for wet PG high dose (WPH), 15 mg/Kg body weight for first dose and after 6 hours interval 20 mg/Kg body weight for female and 15 mg/Kg body weight for male fish. The fertilization rate of catla, *Catla catla* were $83.25 \pm 0.85\%$, $92.01 \pm 0.45\%$, $82.45 \pm 1.04\%$, $83.18 \pm 0.68\%$, for the doses of DPL, DPM, DPH, WPL and WPM respectively. The hatching rate of catla, *Catla catla* were $78.58 \pm 0.94\%$, $85.54 \pm 0.38\%$, $74.8 \pm 0.22\%$, $77.89 \pm 0.82\%$, $81.56 \pm 0.59\%$ for the doses of DPL, DPM, DPH, WPL, WPM respectively and the survival rate of catla, *Catla catla* were $77.24 \pm 0.49\%$, $87.12 \pm 0.46\%$, $73.09 \pm 0.42\%$, $75.8 \pm 0.30\%$, $80.76 \pm 0.64\%$ for the doses of DPL, DPM, DPH, WPL and WPM respectively which were similar to the findings of **Almamun** [1] though the species were different. **Almamun** [1] studied on induced breeding of *Labeo bata* and reported that the effective dose for induced breeding was 0.5, 1.0, 1.5, and 2.0 mg PG/Kg body weight for first dose for female while the second doses were 4.0, 4.6, 5.0 and 5.5 mg/Kg body weight for female

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and 2 mg/Kg for male. Dose of 1 mg/ Kg and 4.6 mg PG showed the best performance when the fertilization rate was 84%, hatching rate was 85% and survival rate was 84%. The doses of synthetic hormone ovaprim was 5 mg/Kg body weight on both male and female for only first dose and the result of injecting ovaprim were, the fertilization rate was $72.85 \pm 0.43\%$ the hatching rate was $71.54 \pm 0.48\%$ and the survival rate was $69.2 \pm 0.77\%$. **Hasan** [6] reported that female catla were given a dose of HCG 200IU/ kg body weight (BW) and 500IU HCG + PG 3mg/kg BW as a resolving dose was induced after 6.0 hours of 1st dose and PG 2mg/kg BW in male fish. The fertilization rate was 88%.

Mudnakudu [9] observed that a new drug, ovaprim-cin Indian major carps, viz. catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). All the three species could be bred with a single intramuscular injection of ovaprim at 0.5 ml/Kg body weight. mrigal responded positively to even lower dosages of 0.3 and 0.4 ml/Kg, but 0.4 ml/Kg was found to be the minimum dosage required for rohu. In all these trials, males were injected with carp pituitary extract at 3-4 mg/ Kg, 6 hours. after injecting ovaprim to females. In another trial, wherein female and male rohu were injected simultaneously with ovaprim at 0.4 and 0.15 ml/Kg, respectively. The percentage of fertilization in most cases ranged from 70 to 99%.

Bhuiyan [3] reported that an experiment was conducted to study the induced spawning of rajputi (*Puntius gonionotus*) in the months of April, May, June and July 2005. Five different doses of PG were used in the experiment. *Puntius gonionotus* all the different doses (doses D₁-3.00 mg/Kg, D₂-6.00 mg/Kg, D₃-9.00 mg/Kg, D₄-12.00 mg/Kg and D₅-15.00 mg/Kg) were effective equally (100%) in egg release. The fertilization rates were $70.10 \pm 2.25\%$ in April (dose D₄), $75.00 \pm 2.10\%$ in May (dose D₃), $80.00 \pm 2.14\%$ in June and $79.20 \pm 2.65\%$ in July (dose D₃). The hatching and survivability rates were 61.50 ± 3.10 and 55.00 ± 2.13 in April (dose D₄),

68.00 ± 3.5 and 63.00 ± 2.20 in May (dose D₄), 71.80 ± 2.00 and 68.50 ± 2.60 in June (dose D₂), and 70.25 ± 3.50 and 65.70 ± 2.16 in July (dose D₃) respectively. The month of June and the dose D₂ (2 mg in first injection and 4 mg in second injection) were found to be most effective for induced spawning of *Puntius gonionotus*.

5. CONCLUSION

Aquaculture has diversified, intensified, and advanced technologically. Aquaculture depends on the availability of fry. In this experiment based on using different doses of PGs hormone and synthetic hormone ovaprim. The fertilization, hatching and survival rate were best in the medium dose of dry pituitary gland (2 mg/Kg body weight of fish) than those of wet pituitary gland and the synthetic hormone, ovaprim. Therefore, 2 mg/Kg body weight of dry pituitary gland can be used as first dose of female and 4 mg/Kg body weight of fish as second dose of female and 2 mg/Kg body weight of fish as first dose of male catla. The findings of this experiment will be helpful of hatchery owners for using the dose of PGs and synthetic hormone for getting desirable output of fertilization, hatching and survival rate and thus overall fish seed production of catla fish.

7. REFERENCES

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