Fish Physiol Biochem (2021) 47:1559–1564
https://doi.org/10.1007/s10695-021-01003-x

Comparative efficacy of Ovaprim and hMG (menotropin) to induce breeding in African catfish (*Clarias gariepinus*)

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Received: 22 February 2020 / Accepted: 11 August 2021 / Published online: 18 August 2021

Abstract The applications of exogenous hormones in different species for the induction of oocyte production, final oocyte maturation (FOM), and spawning for their reproduction is getting more attention day by day. The current preliminary research work was carried out to induce breeding in *Clarias gariepinus*, commonly known as African catfish, imported from Thailand. Single doses of two hormones as Ovaprim and human menopausal gonadotropin (hMG) were used and the research work was carried out at Muzaffargarh Fish Hatchery Punjab, Pakistan. A total of twenty-four ($n=24$) *C. gariepinus* were selected having body weight approximately 2 kg and divided into two main groups based on gender as male ($n=12$) and female ($n=12$). For milt collection, all males were treated with Ovaprim 0.5 mg/kg body weight (b.w.) and female fish were divided into three groups as A, B, and C with four ($n=4$) fish in each group. Group A was injected with only normal saline (control group) while fish in group B and group C were treated with hMG at 0.5 mg/kg b.w. and Ovaprim 0.5 mg/kg b.w., respectively. Then, after 6 h of hormone injections until 48 h, spawned eggs, eggs' weight, fertilization rate, hatching rate, survival rate, fecundity, and deformed larvae were investigated. The results revealed that Ovaprim injection significantly ($p<0.05$) modulate the reproductive parameters in group C while no breeding was induced in both control and hMG-treated groups. Hence, it could be concluded that Ovaprim has the potential to induce breeding in African catfish, while in the current study, hMG failed to induce breeding. However, trials at large scales are required to further explore the effect of different doses of both tested hormones by increasing the treated subjects particularly in Pakistani fish farms.

Keywords Reproduction · Ovaprim · hMG · African catfish · Breeding · Pakistan

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Introduction

Reproductive processes of fish species can be expanded by using latest techniques of induced

breeding both qualitatively and quantitatively (Dhawan and Kaur 2004). The natural breeding places of African catfish are flooded rivers, earthen ponds, and paddy fields. In recent years, induced breeding trend has been popular to stimulate reproduction in fish by manipulating different natural and synthetic hormones (Marimuthu et al. 2009). Ovulation in fish can be controlled by both internal hormones and external environment (Peter and Yu 1997; Ali et al. 2015).

To meet the increasing demands of fishes worldwide particularly in developing countries like Pakistan and for the survival of fish species, captive propagation is becoming more popular for fishery resources. So for the survival of aquaculture industry, it becomes essential to find cost-effective and reliable protocols to spawn and culture the fish species of interest (DiMaggio et al. 2013). Fish industry has gain success by the availability of good seeds. To replenish adult stock from market, there is a need to provide young ones and seeds in fish farms. The wild availability of fingerlings is less, and capturing is a difficult, laborious, and time-consuming work. Spoilage, disease infestation, and transport difficulties are some other major factors in the development of this sector (Shepherd and Bromage 1988; Maradun et al. 2018). Readymade available hormones contain GnRH and dopamine blocker receptors. These hormones are very popular for artificial spawning in different fish species. In the past, different hormones were used for induced spawning in African catfish reported during various studies (Olubiyi et al. 2005; Sahoo et al. 2005; Achionye–nzeh and Obaroh 2012; Shinkafi and Ilesanmi 2014; Kasi et al. 2015). Pituitary extract, Ovaprim, Ovatide, Ovulin, Ovopel, Dagin, human chorionic gonadotropin (HCG), aqua spawn, and deoxycorticosterone acetate (DOCA) are some very important hormones under investigations for the artificial and induced breeding in African catfish (Brzuska and Adamek 1999; Cheah and Lee 2000; Zohar and Mylonas 2001; Adebayo and Popoola 2008).

C. gariepinus, also called as African catfish, is a cultivar specie of many Asian countries including Indonesia, Thailand, and Malaysia. It is also commercially cultured in Europeans countries (Netherlands, Germany, and Belgium). According to the best of our knowledge, it was imported in Pakistan for first time and propagated through induced breeding. *C. gariepinus* is a very resistant fish against diseases. This fish has the ability to tolerate wide range of environmental factors and able to reproduce and survive in high population density. The growth rate of this fish is relatively fast and it provides good quality of meat (Kasi et al. 2015; Hogendoorn and Vismans 1980; Henken et al. 1987; Haylor 1991; Henk and Richter 1996). So, the major purpose of this preliminary study was to investigate the impact of single doses of hMG and Ovaprim on the breeding of imported *C. gariepinus* by evaluating the spawning eggs, eggs' weight, fertilization rate, hatching rate, survival rate, fecundity, and deformed larvae.

Materials and methods

Brood stock and study plan

Brood stock of *C. gariepinus* was imported from Thailand and raised at Muzaffargarh Fish Hatchery Punjab, Pakistan. When brood stock gain weight was about 2 kg, it was used for experimental purpose. After gender identification through morphological characteristics, twenty-four ($n=24$) *C. gariepinus* were selected having body weight of approximately 2 kg and divided into two main groups based on gender as male ($n=12$) and female ($n=12$). Then, before starting the experiment, both males and female fishes were acclimatized for 3 weeks in separate concrete ponds, were fed with a standard formulated diet and water quality parameters at breeding site, and were daily monitored to provide suitable environment for breeding (Table 1).

Table 1 Water quality parameters at breeding site of *C. gariepinus*

Aquatic parameters used	Value
Dissolved oxygen (ppm)	5.76 ± 0.01
pH	8.87 ± 0.02
Temperature °C	28.05 ± 0.05
Electrical conductivity (µs)	1854.25 ± 0.76
Salinity (ppt)	0.82 ± 0.01
Total dissolved solids (mg/L)	1080.75 ± 0.75

Hormonal injection

For milt collection, all males were injected with Ovaprim 0.5 mg/kg b.w. and female fishes were divided into three subgroups as A, B, and C with four ($n=4$) fish in each group. Group A was injected with only normal saline (control group) while fish in group B and group C were treated with human menopausal gonadotropin (hMG) at 0.5 mg/kg b.w. and Ovaprim 0.5 mg/kg b.w., respectively. All the groups were injected at the dorsal fin intramuscularly by using a graduated syringe (2 mL) at about 30–45° angle.

Milt and egg collection

After 6 h, ovulation was checked and again checked after continuous intervals of 1 h until eggs are matured (Brzuska 2004). Hand stripping was used to test ovulation in females (Richter et al. 1987). Eggs were collected by pressing the belly. Eggs were collected in a plastic bowl according to the method described by Viveen et al. (1986). Milt was difficult to extract for this purpose so fish was dissected and testes were incised carefully to open milt sacs. A sharp razor blade was used for the incision (Fig. 1C). Milt was squeezed in petri dish (Fig. 1A). After collecting, the eggs (10 g) were placed into specifically labeled bowl and sperms collected from male groups were mixed with eggs to fertilize the eggs. Plastic netting substrate of specific mesh size (2 mm) was used to spread the fertilized eggs in a clean water plastic pond (Fig. 1). Then, the following calculations were done to investigate different reproductive parameters.

Data collection

Data was collected and formulas that were applied are given below.

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

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

Fecundity = total weight of eggs × no. of eggs per gram

$$\text{Larval survival rate (\%)} = \frac{\text{Total number of larvae} - \text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

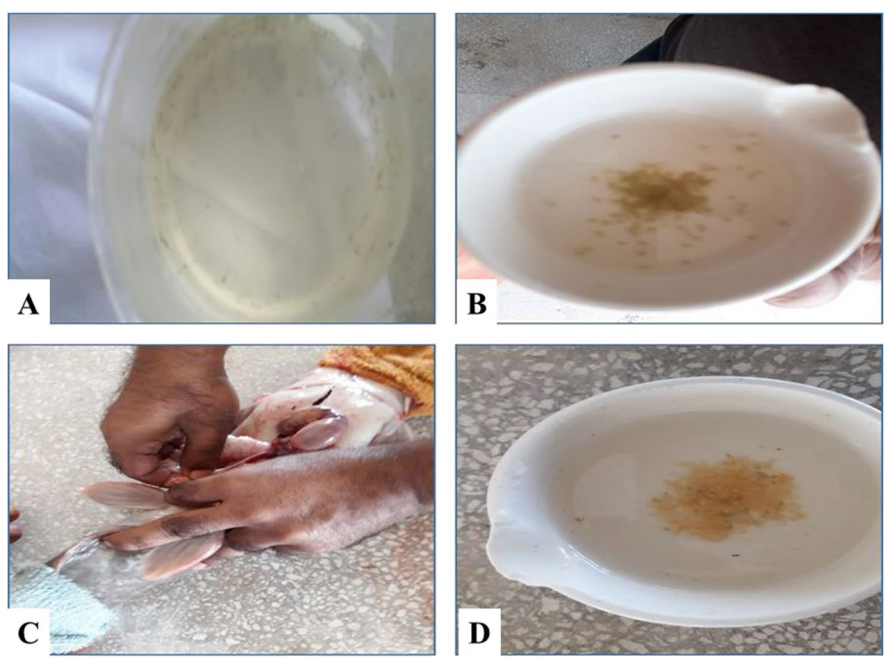

Fig. 1 Eggs and milt extraction from *C. gariepinus*. **A** Represent the milt collected from male fish. **B** Eggs collected from female fish. **C** Represent the collection of milt from male fish. **D** Fertilized and unfertilized eggs after mixing milt and eggs

Data analysis

All the results were expressed as mean ± SEM and one-way analysis of variance (ANOVA) was applied using SPSS version 20 (Trial version). Tukey's test was used to make pairwise study and significant values were considered with p value < 0.05.

Ethical approval

Study was approved by the Ethical Committee for Animal Research and Board of Advance Study, Government College University Faisalabad (DZ-GCUF-107–2018).

Results and discussion

Seed collection from the wild is a very harsh, time-consuming, costly, and laborious task for human beings and to overcome these problems, one of the alternative solutions is induced breeding and this technique is attaining attention day by day (Bruton 1979; Uys 1989). This study was designed to check the induced breeding by Ovaprim and hMG. Several previous studies as on stinging catfish, *H. fossilis*, *Ompok bimaculatus*, *Snakehead murrel*, *Neosilurus ater*, and *C. batrachus* explored successful induced breeding with Ovaprim in different regions of the world (Nandeesha et al. 1990; Vijaykumar et al. 1998; Sridhar et al. 1998; Haniffa et al. 2003; Francis et al. 2000; Sahoo et al. 2008). Change in body weight was non-significant ($p > 0.05$) in this study after hormonal injection because no effect was seen on fish, but after egg extraction weight was slightly decreased in the Ovaprim-injected group. This may be due to egg extraction because the weight of eggs was minus from the total body weight, but the weight of fish was remaining constant (Fig. 2). In the present study, latency period was calculated after hormonal injection in each group; in group A and group B, latency period was zero because no breeding was induced in these groups. The results of our finding revealed that hMG failed to induce breeding in African catfish (group B), but in group C artificial breeding was successful after single dose of Ovaprim showed 13.42 ± 0.08 h of latency period (Table 2). Kasi et al. (2015) reported 14 to 18 h of latency period in different species of catfish by administration of Ovaprim in

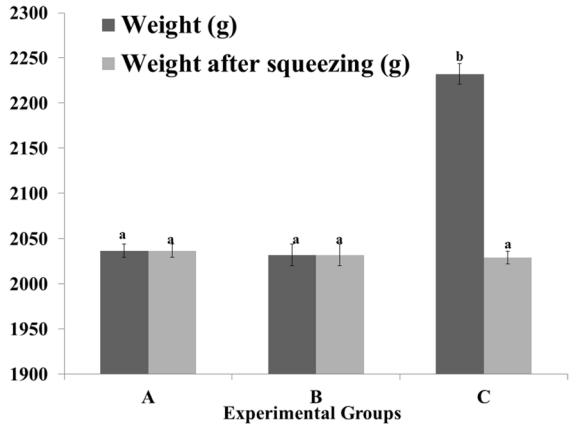

Fig. 2 Difference between body weight of *C. gariepinus* before and after squeezing to extract eggs treated with same concentration of Ovaprim and hMG for induced breeding. **A** Saline treatment. **B** hMG 0.5 mg/kg b.w. **C** Ovaprim 0.5 mg/kg b.w. Bars sharing different letters represent the significant ($p < 0.05$) difference

accordance to our study. It was also found using *C. batrachus* as experimental fish that Ovaprim induced the best breeding with 14–17 h latency period (Sahoo et al. 2008).

The results of spawning success in Ovaprim injection showed 74.03 ± 0.69% which is a significant ($p < 0.05$) value as compared to the control and hMG-treated groups (Table 2). Our results are in agreement with the findings of Mahapatra et al. (2000), Basu et al. (2000), and Sahoo et al. (2008) who injected different doses of Ovaprim hormone in *C. batrachus* to explore spawning success of African catfish. Moreover, in the Ovaprim-injected group egg weight was 203.24 ± 1.01 g but no egg was produced in groups A and B (Fig. 2; Table 2). Fecundity was also zero in both A and B groups but in group C fecundity was significantly ($p < 0.05$) increased which was 145,981.75 ± 1517.75. Egg mass was reported in the range of 155 to 212 g by different doses of Ovulin hormone in *C. gariepinus* by Maradun et al. (2018). The findings of Maradun et al. are also in agreement of our study in *C. gariepinus* brood stock (Table 2).

Fertilization is an important parameter to assess the accuracy of a hormone to induce the ovulation and spermatozoa production. As no ovulation in the experimental groups A and B was observed, no fertilization occurred in these studied groups. On the other hand, fertilization in group C was significantly

Table 2 *C. gariepinus* treated with the same concentration of Ovaprim and hMG for induced breeding

Experimental parameters	Group C
Latency period (H)	13.42 ± 0.08^{b}
Spawning success (%)	74.03 ± 0.69^{b}
Total egg weight (g)	203.24 ± 7.01^{b}
Fecundity (no)	$145{,}981.75 \pm 1517.75^{b}$
Fertilization rate (%)	73.72 ± 1.16^{b}
Incubation period (hours)	23.52 ± 0.14^{b}
Survival rate (%)	75.42 ± 1.57^{b}
Hatching (%)	81.77 ± 0.64^{b}
Deformed larvae (%)	2.50 ± 0.28^{b}

C: Ovaprim 0.5 mg/kg b.w. Superscript letters represent the significant ($p < 0.05$) difference on comparing groups A and B having no value to mention (zero results)

($p < 0.05$) high ($73.72 \pm 1.16\%$). The incubation period for the hatch after laying was also investigated and the results revealed that in this study the incubation period required was 23.52 ± 0.14 h and our finding are comparable with previous finding as 72 to 88% of fertilization rate was reported by Maradun et al. (2018) in *C. gariepinus*. Sixty to 86% of fertilization was reported by Marimuthu et al. (2015), (Adebayo and Popoola 2008) which is in consistent with this research. The results of survival rate explored that significant ($p < 0.05$) percentage (75.42 ± 1.57) of larvae had survived after hatching in the Ovaprim-treated group. Hatching is the release of larvae from fertilized eggs and results showed significant ($p < 0.05$) hatching percentage as 81.77 ± 0.64. Deformed larvae were reported significantly ($p < 0.05$) very low after hatching as only 2.5% (Table 2). The hatching percentage reported by Basu et al. (2000) was as 60% during their research.

Conclusions

The applications of exogenous hormones in different species for the induction of oocyte production, final oocyte maturation (FOM), and spawning for their reproduction is getting more attention day by day. The current preliminary research work was carried out to induce breeding in African catfish using hormones as Ovaprim and human menopausal gonadotropin (hMG). Significant ($p < 0.05$) response by Ovaprim was observed in the present study considering the fecundity, spawning success, egg mass, fertilization, and hatching etc., but hMG failed to induce ovulation in African catfish. Hence, it could be concluded that Ovaprim has the potential to induce breeding in African catfish, while in the current study, hMG failed to induce breeding. However, trials at large scales are required to further explore the effect of different doses of both tested hormones by increasing the treated subjects particularly in Pakistan fish farms.

Author contribution All authors contributed equally.

Data availability Data will be provided on request.

Code availability Not applicable.

Declarations

Ethics approval Ethical approval for this research was taken from the Ethical Research Board for Animal Government College University Faisalabad (GCUF-DZ-2018).

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

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