**The Journal of Animal & Plant Sciences, 25(6): 2015, Page: 1585-1590**
**ISSN: 1018-7081**

# EFFECT OF DIFFERENT DOSES OF OVAPRIM (sGnRHa+Domperidone) ON THE EGG FECUNDITY AND REPRODUCTIVE HORMONE LEVELS IN *Channa marulius*

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## ABSTRACT

The study was conducted to determine the efficacy of various doses of Ovaprim on reproductive behavior of *Channa marulius*. Fish was fed on 40% protein containing diet at 5% of its body weight for 4 months. On maturity, males weighed were 1100-1340 g and females 1150-1350 g. Two males and two females were randomly paired (8 pairs in total), the 4 groups which emerged were names as control, treatment 1, 2 and 3 (each in 2 replicate tanks). Each male and female received Ovaprim intramuscularly in two installments with an interval of 24 hours. The blood samples were drawn from each sex with an interval of 2 hour for estimation of testosterone, FSH and LH levels. None of the fish spawned even after 48 hours of hormone administration. Ovaries were removed from each fish, weighed and eggs were counted. Fecundity (No.of eggs) was 870.0 ±75.27, 811.2 ±103.30, 900.0 ±95.56 and 940.0 ±39.79 in Control, $T_1$, $T_2$, and $T_3$ respectively. Mean ova diameter ranged from 1.48±0.02 to 1.54±0.01mm. The lowest mean ova diameter (1.48±0.02 mm) was observed in control tank (0.4 ml Ovaprim/kg b.w) while the highest (1.54±0.01 mm) was in $T_1$ (0.5ml Ovaprim/kg b.w.). The highest testosterone level (2.05±0.11 ng $ml^{-1}$) was observed in $T_3$ after 20 hours of first injection of Ovaprim while the highest FSH level (0.88±0.02 ng $ml^{-1}$) was observed in the same treatement after the 28 hours of hormone injection. These studies discovered that Ovaprim dosages are inadequate for stimulation of breeding.

**Keywords:** Ovaprim, hormone, fecundity, blood chemistry, *Channa marulius.*

## INTRODUCTION

*Channa marulius* is well liked among fish farmers due to its hardiness, fast growth, adaptability to artificial feed and high market price while among consumers due to its high nutriotional value, physical appearance of flesh and tempting taste. Persistent supply of quality and quantity of its seed is crucial for its successful culture. Although, seed of this species is obtained from fishermen folks but is not well appreciated due its potential contamination with undesirable fish species. Sometimes its seed blended with its very close relatives, which is very hard to identify and segregate at very early stages of development. (Haniffa *et al*., 2004)

Like Indian and Chinese major carps, indoor induced spawning of *Channa marulius* can be another fruitful and reliable option. This initiative at least can ensure maximum survival and purity of stock with uniterruped supply of seed to potential stakeholders. Efforts in this direction are quite inadequate and if there are any, are not very successful. It will be quite relevant if we say that its induced spawning is at infancy. Researchers who tried and exhausted their efforts on this venture used different inducing agents for successful ovulation of this valuable species but success remained far away. (Hafeez-ur-Rehman *et al*., 2015). Some of the carnivorous fish species don't readily spawn captivity due to unfavorable environmental conditions which may cause certain stresses or they may not get proper environment which can facilitate their internal and external cascade of breeding activities.

Different hormonal preparations are used to induce spawn numerous fish species (Mylonas and Zohar, 2001; Yousefian and Mousavi, 2011) which otherwise refuse to spawn in captivity. Fishes achieve their different stages of development but get stucked in their ovulation due to absence of external and internal stimuli sometimes it is very vaguely defined as gonadal dysfunction and environmental inhibition (Peter *et al*., 1988; Podhorec and Kouril, 2009). Ovaprim (manufactured by Syndel Laboratories) extensively used in carps as an inducing agent, contains salmon gonadotropin releasing hormone analog (sGnRHa; Arg6-Pro9-NEt) at a concentration of 20µg/ml and domperidone (dopamine antagonist 10 mg/ml). Species-specific dosages of this hormone are commonly delivered to fish interamuscularly or interaperitonially for inducing ovulation in cyprinids and some other fish varieteis (Pinillos *et al*. 2002; Viveiros *et al*. 2002; Hill *et al*. 2005). This inducing agent well accepted among fish breeders due to its easy availability, ease of handling and comfortable administration (Akhtar, 2001). Experiences, however, have shown that reserachers do not stick to the recommended dosages but

it varies from species to species and even under different environmental conditions (Nandeesha *et al*., 1990; 1991).

External and particulary environmental changes convey the onset of breeding stage to nervous which inturn triggers qualitative and quantitative hormonal changes, which ultimately stimulate fish to spawn which does not happen spontaneously in captivity but works well in natural environment (Hochachka and Mommsen 1995; Luskova 1997; Svoboda *et al*. 2000). So in captivity fish do need stimulus to break this barrier and reinitiate the stagnancy of egg devlopemnt. Biochemical hormonal estimation during gonad devlopement can reasonably give good estimation about maturity and time of induction of Ovaprim. Therfore, objectives of the study was to induce spawning in this highly neglected fish by using varying doses of Ovaprim and to study the peak of reproductive hormones after injection to ascertain the ovulation. It also aimed to provide future guidelines to the breeders for successful breeding of this fish with little hassle, without wastage of precious time and expensive hormones and breeders.

## MATERIALS AND METHODS

**Management of Broodstock and Selection for Induced Spawning:** Broodstock was purchased from the Hamalya Fish Hatchery, Muridky and raised for one year in pond facility located in Fish Farm Complex, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi campus, Pattoki, Pakistan. The fish fed regularly at 5% of their body weight containing 40% crude protein. At the start of breeding season, mature males (1220 ± 121 g & 40.2 ± 5.5 cm) and females (1250 ± 100 g & 38.5 ± 4.7 cm) were selected for induce spawning. However, maturity symptoms were hard to ascertain as compared to carps, which are quite apparent, nevertheless the best males with most distinctive maturity signs such as soft pectoral fins, dull lower jaw and slightly dull rounded anal genital papilla while females with soft distended belly, swollen and oval shaped genital papilla selected for hormonal administration.

**Hormonal Administration:** Ovaprim (Syndel International Inc., Canada) is a liquid preparation and contains 20µg of salmon gonadotropin releasing hormone (D-Arg$^6$, Pro$^9$, Net-sGnRH) and 10 mg of domperidone, a dopamine antagonist (Brzuska and Adamek, 1999). The recommended dose from the manufacturer is 0.5 ml/kg body weight of the fish. However, the dose varies from species to species and depends on the location and physical condition of fish. The experiment conducted in four circular tanks having a 6' diameter with 1600 L water holding capacity. Each tank randomly received two males and two females from the bulk stock. All the four tanks indiscriminately divided among 4 experimental groups. Brood stock present in tank 1 served as control and injected a standard dose of Ovaprim (0.2 ml/Kg) intramuscularly at the base of dorsal region in two installments after an interval of 24 hours (Table-1). Immediately after administration of the hormone, the brooders shifted into their respective tanks. During the course of the trial temperature remained 27-29$^o$C, dissolved oxygen 5.5-6.0 mg/l, pH 7.9-8.2, salinity 0.9 ppt, Electrical Conductivity 2.5 and Total dissolved solids 817 mg/l. None of the female spawned even after 48 hours of first injection. Female *Channa marulius* taken out from the tanks, its belly excised out, ovaries removed, and eggs physically examined and then counted for determination of fecundity kg$^{-1}$ of the body weight.

**Determination of Hormone Levels in Blood:** Blood samples were drawn at regular intervals of 4 hours after administration of second dose of Ovaprim for 24 hours. Blood was collected by inserting 3 cc syringe in caudal vein and then blood was stored in vacutainer, centrifuged (Model No YM-1522) at 3000 rpm for 20 minutes and then stored for further determination of concentrations of hormone in both male and female blood samples. Blood serum was used for the determination of testosterone levels in males and follicle stimulating hormone (FSH) and luteinizing hormones (LH) levels in females. Bio-Merieux (VIDAS), an automated quantitative test, was run in VIDAS analyzer for the quantitative measurement of testosterone, while FSH, and LH in blood serum was determined by Enzyme Linked Fluorescent Assay (ELFA) technique.

**Statistical analysis:** Data collected on fecundity, and hormones from different treatments were analyzed using one-way analysis of variance (ANOVA) using SAS software (9.1 Version). The treatment means were compared by Duncan multiple range test ($P \leq 0.05$) to test their significance among various experimental groups.

## RESULTS AND DISCUSSION

The study focused on the induced spawning of highly important fish species for mass production of its seed using commercial breeding practices. Fishes were injected conventional Ovaprim doses as well as enhanced dosages to check hormonal changes at different time inervals. Ovaprim dosages applied on fishes in different experimental groups have given in Table 1. Breeding behaviors of both male and female breeders were observed closely. The male neither chased the female nor showed the aggressive behavior. Both males and females in all the treatments remained under water supply pipes during this whole period. After expiry of 48 hours of Ovaprim injection when *Channa marulius* were not spawn then all the fishes were dissected, and ovaries were removed, weighed and eggs were counted. The average weighs of ovaries were recorded as 4.29±0.27, 4.13±0.48,

4.46±0.45 and 4.70±0.14 g .in control, $T_1$, $T_2$ and $T_3$, respectively Number of eggs counted were, 870.0 ±75.27, 811.2 ±103.30, 900.0 ±95.56 and 940.0 ±39.79 in control, $T_1$, $T_2$ and $T_3$, respectively. Number of eggs obtained remained insignificant among treatments. The ova diameter appeared slightly dependent on Ovaprim dosage; the lowest ova diameter (1.48±0.02 mm) was observed in control, which received 0.4ml ovaprim/kg body weight, and the highest ova diameter (1.54±0.01 mm) was observed in $T_2$, which received 0.5ml Ovaprim/kg. Ova diameter was slightly lower in $T_2$ and $T_3$ but both groupd did not differ significntly (Table 2). These findings are quite in line with that of Zairin *et al*., (1992) suggesting that ovulation is related to Ovaprim and/or its analogues; though this did not happen in the current scenario but it did enhanced the level of reproductive hormones in female blood. The preveious researchers tried to induced spawn this fish @ of 0.3 ml/kg to 0.6 ml/kg body weight (Nandeesha *et al*., 1993; Francis, 1996; Haniffa *et al*., 1996). Tan-Fermin *et al*. (1997) and Pagador & Chavez (1997) have reported similar observations on *Clarias macrocephalus*. The increased level of sGnRha and domperiodone (Ovaprim) may stimulate the pituitary for secretion of sufficient gonadotropin-II, which current hormone analogues could perform and on-account of which fish failed to spawn. Some other workers also have different view points and do not seem fully satisfied with its performance. Yanong *et al*. (2009) reported that Ovaprim alone did not warrant successful spawning, fertilization, development, and hatching of fry. Other factors like poor husbandry and hatchery management, unreliable genetic background, and improper nutrition, have significant bearing on egg and milt quality, hatching of eggs, and demand very close monitoring. Though due importance was gien to all these factors during rearing of broodstock and induced spawning of fish but there may be some flaws which were not taken care off and ended up in non-spawning of fish. Studies of Goetz (1983) and Guraya, (1986) do support and partially agree with our findings that Ovaprim do play role in ovulation, fecundity enhancement and egg size. It could be assume that Ovaprim stimulation might have helped eggs to absorb more water for timely development and release from ovary. Although, water quality have unique role in induce spawning especially temperature, remained in suitable range (27-30ºC) has been already confirmed by Clemens and Sneed (1962), who worked on optimum temperature for successful spawning of fishes.

Testosterone levels at the start were approximately same in control, $T_1$ and $T_2$ but quite higher in $T_3$. $T_3$ showed its persistent and distinct superioroity over all the other groups during 48 hours of observation ($P \leq 0.05$). Testeronoe level remained on the rise upto 20 hours of injection then it started to decline and after 48 hours receeded to the original level (Table 3). The highest level of testosterone in Ovaprim induced male *Channa marulius* to release milt, was observed after 20 hours of first injection. Metwally and Fouad (2008) however, observed the highest testosterone level after 8-10 hours in pregnyl and Ovaprim induced male grass carp (*Ctenophryngodon idella*) after the first injection. This variation is quite evident due to differences in fish species.

In the beginning all the female breeders had same FSH level but over the passage of time differences magnified ($P<0.05$) in proportionate to the colume of Ovaprim dosage used. It remained the highest in $T_3$ (0.82±0.03ng/ml) and the lowest in control (0.53±0.01 ng/ml). Like testosterone levels FSH also showed peak after 20 hours and after 48 hours of exposure it approached the level observed at the beginning of experiment (Table 4).

Like FSH level of LH remained highest in $T_3$ throughout the experiment. The peak of LH concentration was observed after 20 hours, which then declined in all treatment groups. LH concentrations varied among different groups but it was more prominent in former groups than later where level of significance decreased. The highest level (0.94±0.36 ng/ml) was observed in treatment 3, followed by 0.87±0.04 in $T_2$, then, 0.68±0.07 in $T_1$ and the lowest in control (0.65±0.01) (Table 5).

In some other studies these hormones helped quite lot in improving the spermatogenesis with a series of inducing hormones and /or their analogues such as Ovaprim, Ovaplant, HCG, cPG and comibination of cPG with Ovaprim or HCG (Abol-Munafi *et al*. 2006, Tu *et al*., 1995 ) in silver carp (*Hypophthalmicthys molitrix*). On the other hand, Zvi Yaron, (1995) stimulated sperm duct by gonadotropin, or by 17, 20-dihydroxy-4-pregnen-3-one (17, 20-P) in common carp (*Cyprinus carpio*). When bass (*Dicentrarchus labrax* L.) and white bass (*Morone chrysops*) were injected with gonadotropin-releasing hormone antagonist (GnRHa) and then exposed to higher temperature, enhanced milt production was observed. (Lucinda *et al*., 2001, Costadinos *et al*., 1997).

However, species variation, reproductive physiology, behavure and geographical conditions facilitaes or inhibits the egg release but at present it is hard to entertain any claim at present. Indian and Chinese major carps, (Khan *et al*.,1992, Naeem *et al*., 2005a, Naeem *et al*., 2005b), *Aristichthys nobilis* (Naeem and Salam, 2005), and spotted snakehead, *Channa punctatus* (Marimuthu, 2000), *Neosilurus ater* (Cheah and Lee, 2000), did respond well to this miracle product. Therefore, the same notion still dominates here dicussed in former paragraphs i.e. species difference is the major governing factor controlling this whole process. So lot more need to be done to successfully spawn this species which will be given due importance in our next trials.

**Table 1. Doses of Ovaprim for Induce Spawning of *Channa marulius***

| Treatment | Male body weight (g) | Female body weight (g) | 1st dose Ovaprim (ml kg$^{-1}$ BW) | | 2nd dose Ovaprim (ml kg$^{-1}$ BW) | |
|---|---|---|---|---|---|---|
| | | | Male *Channa murulius* | Female *Channa marulius* | Male *Channa marulius* | Female *Channa murulius* |
| Control | 1275±25 | 1225±25 | 0.2 | 0.4 | 0.3 | 0.7 |
| $T_1$ | 1125±25 | 1175±25 | 0.2 | 0.5 | 0.3 | 0.7 |
| $T_2$ | 1275±25 | 1325±25 | 0.2 | 0.6 | 0.3 | 0.7 |
| $T_3$ | 1300±40 | 1270±30 | 0.2 | 0.7 | 0.3 | 0.7 |

**Table 2: Effect of different doses of Ovaprim on the induced spawning of *Channa marulius***

| Treatments | Female body weight (g) | Average No. of ggs (after removal of ovary) | Weight of the ovary (g) | Ova diameter (mm) |
|---|---|---|---|---|
| Control | 1225±28.86 $^{a}$ | 870.0±75.27 $^{a}$ | 4.29±0.27 $^{a}$ | 1.48±0.02$^{b}$ |
| $T_1$ | 1200±70.71 $^{a}$ | 811.2±103.30 $^{a}$ | 4.13±0.48 $^{a}$ | 1.54±0.01$^{a}$ |
| $T_2$ | 1270±67.82 $^{a}$ | 900.0±95.56 $^{a}$ | 4.46±0.45 $^{a}$ | 1.53±0.03$^{ab}$ |
| $T_3$ | 1260±27.08 $^{a}$ | 940.0±39.79 $^{a}$ | 4.70±0.14 $^{a}$ | 1.53±0.01$^{a}$ |

Data figures with different superscript letters are significantly differen from each other at P<0.05

**Table 3. Testosterone (ng ml$^{-1}$) levels in blood of male *Channa marulius* at different time intervals after hormonal administration.**

| Treatments | Male body weight (g) | 0 hours (before 1st dose) | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 hours (before 2nd dose) |
|---|---|---|---|---|---|---|---|---|
| Control | 1240±69.76 $^{a}$ | 0.11±0.008 $^{b}$ | 0.21±0.01 $^{b}$ | 0.32±0.02 $^{c}$ | 0.48±0.01 $^{c}$ | 0.62±0.02 $^{c}$ | 0.81±0.05 $^{c}$ | 0.75±0.05 $^{c}$ |
| $T_1$ | 1155±42.03 $^{b}$ | 0.10±0.02 $^{b}$ | 0.20±0.06 $^{b}$ | 0.36±0.05$^{bc}$ | 0.57±0.02$^{bc}$ | 0.70±0.05$^{bc}$ | 0.85±0.05 $^{c}$ | 0.88±0.05 $^{c}$ |
| $T_2$ | 1258±38.37 $^{a}$ | 0.13±0.04 $^{b}$ | 0.28±0.09 $^{b}$ | 0.49±0.16 $^{b}$ | 0.66±0.15 $^{b}$ | 0.79±0.11 $^{b}$ | 1.13±0.17 $^{b}$ | 1.05±0.04 $^{b}$ |
| $T_3$ | 1280±51.63 $^{a}$ | 0.41±0.20 $^{b}$ | 0.93±0.25 $^{a}$ | 1.28±0.10 $^{a}$ | 1.56±0.90$^{a}$ | 1.79±0.12 $^{a}$ | 2.05±0.11 $^{a}$ | 1.79±0.17 $^{a}$ |

| Treatments | Male body weight (g) | 28 hours (after 2nd dose) | 32 hours | 36 hours | 40 hours | 44 hours | 48 hours |
|---|---|---|---|---|---|---|---|
| Control | 1240±69.76 $^{a}$ | 0.71±0.05 $^{d}$ | 0.65±0.06$^{c}$ | 0.56±0.08$^{c}$ | 0.40±0.07$^{c}$ | 0.27±0.05 | 0.16±0.01$^{c}$ |
| $T_1$ | 1155±42.03 $^{b}$ | 0.88±0.07$^{c}$ | 0.79±0.05$^{bc}$ | 0.65±0.07$^{bc}$ | 0.50±0.07$^{bc}$ | 0.35±0.028 | 0.31±0.01$^{c}$ |
| $T_2$ | 1258±38.37 $^{a}$ | 1.11±0.04 $^{b}$ | 0.91±0.07 $^{b}$ | 0.77±0.10 $^{b}$ | 0.64±0.09$^{b}$ | 0.50±013 | 0.42±0.01 $^{b}$ |
| $T_3$ | 1280±51.63 $^{a}$ | 1.73±0.16$^{a}$ | 1.17±0.15 $^{a}$ | 1.02±0.15 $^{a}$ | 0.83±0.13 $^{a}$ | 0.73±0.08 | 0.61±0.02 $^{a}$ |

**Table 4. Follicle Stimulating Hormone (FSH) levels (mIU/ml) in blood of induced female *Channa marulius*.**

| Treatments | Body weight (g) | 0 hours (before 1st dose) | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 hours (before 2nd dose) |
|---|---|---|---|---|---|---|---|---|
| Control | 1225±28.86 $^{a}$ | 0.11±0.005 $^{a}$ | 0.17±0.005$^{b}$ | 0.28±0.02$^{b}$ | 0.38±0.03$^{b}$ | 0.46±0.03 $^{c}$ | 0.53±0.01$^{d}$ | 0.48±0.009$^{d}$ |
| $T_1$ | 1200±70.71 $^{a}$ | 0.11±0.009 $^{a}$ | 0.20±0.03$^{b}$ | 0.28±0.02$^{b}$ | 0.37±0.06$^{b}$ | 0.45±0.03 $^{c}$ | 0.64±0.03 $^{c}$ | 0.57±0.12 $^{c}$ |
| $T_2$ | 1270±67.82 $^{a}$ | 0.12±0.009 $^{a}$ | 0.33±0.05$^{a}$ | 0.42±0.08$^{a}$ | 0.50±0.07$^{a}$ | 0.58±0.07$^{b}$ | 0.75±0.02$^{b}$ | 0.67±0.02$^{b}$ |
| $T_3$ | 1260±27.08 $^{a}$ | 0.11±0.02 $^{a}$ | 0.36±0.09$^{a}$ | 0.44±0.09$^{a}$ | 0.54±0.03$^{a}$ | 0.72±0.05$^{a}$ | 0.82±0.03$^{a}$ | 0.79±0.04$^{a}$ |

| Treatments | Body weight (g) | 28 hours (after 2nd dose) | 32 hours | 36 hours | 40 hours | 44 hours | 48 hours |
|---|---|---|---|---|---|---|---|
| Control | 1225±28.86 $^{a}$ | 0.45±0.01$^{d}$ | 0.40±0.010$^{d}$ | 0.33±0.01 $^{c}$ | 0.27±0.005$^{d}$ | 0.18±0.01$^{d}$ | 0.12±0.009 $^{d}$ |
| $T_1$ | 1200±70.71 $^{a}$ | 0.52±0.17 $^{c}$ | 0.48±0.23 $^{c}$ | 0.44±0.21$^{b}$ | 0.37±0.21 $^{c}$ | 0.31±0.009 $^{c}$ | 0.26±0.01 $^{c}$ |
| $T_2$ | 1270±67.82 $^{a}$ | 0.62±0.03$^{b}$ | 0.57±0.03$^{b}$ | 0.52±0.03$^{b}$ | 0.46±0.04$^{b}$ | 0.39±0.03 $^{b}$ | 0.33±0.02$^{b}$ |
| $T_3$ | 1260±27.08 $^{a}$ | 0.76±0.06$^{a}$ | 0.70±0.06$^{a}$ | 0.62±0.10$^{a}$ | 0.57±0.01$^{a}$ | 0.52±0.08 $^{a}$ | 0.45±0.03$^{a}$ |

**Table 5. Luteinizing Hormone (LH) (mg ml$^{-1}$) levels in the blood of induced female *Channa marulius.***

| Treatments | Body weight (g) | 0 hours (before 1st dose) | 4 hours | 8 hours | 12 hours | 16 hours | 20 hours | 24 hours (before 2nd dose) |
|---|---|---|---|---|---|---|---|---|
| Control | 1225±28.86$^{a}$ | 0.34±0.009$^{b}$ | 0.40±0.03$^{bc}$ | 0.46±0.02$^{b}$ | 0.52±0.04$^{b}$ | 0.57±0.03$^{b}$ | 0.65±0.01$^{b}$ | 0.50±0.02$^{d}$ |
| $T_1$ | 1200±70.71$^{a}$ | 0.26±0.05$^{c}$ | 0.32±0.10$^{c}$ | 0.40±0.10$^{b}$ | 0.48±0.10$^{b}$ | 0.58±0.10$^{b}$ | 0.68±0.07$^{b}$ | 0.60±0.02$^{c}$ |
| $T_2$ | 1270±67.82$^{a}$ | 0.35±0.03$^{b}$ | 0.47±0.05$^{bc}$ | 0.59±0.03$^{a}$ | 0.72±0.06$^{a}$ | 0.81±0.05$^{a}$ | 0.87±0.04$^{a}$ | 0.77±0.01$^{b}$ |
| $T_3$ | 1260±27.08$^{a}$ | 0.41±0.01$^{a}$ | 0.54±0.03$^{a}$ | 0.61±0.02$^{a}$ | 0.76±0.07$^{a}$ | 0.87±0.03$^{a}$ | 0.94±0.36$^{a}$ | 0.85±0.02$^{a}$ |

| Treatments | Body weight (g) | 28 hours (after 2nd dose) | 32 hours | 36 hours | 40 hours | 44 hours | 48 hours |
|---|---|---|---|---|---|---|---|
| Control | 1225±28.86$^{a}$ | 0.53±0.03$^{d}$ | 0.46±0.02$^{d}$ | 0.39±0.01$^{c}$ | 0.30±0.01$^{c}$ | 0.19±0.06$^{c}$ | 0.15±0.02$^{d}$ |
| $T_1$ | 1200±70.71$^{a}$ | 0.64±0.03$^{c}$ | 0.54±0.03$^{c}$ | 0.46±0.04$^{c}$ | 0.36±0.07$^{c}$ | 0.29±0.06$^{b}$ | 0.23±0.04$^{c}$ |
| $T_2$ | 1270±67.82$^{a}$ | 0.80±0.01$^{b}$ | 0.69±0.01$^{b}$ | 0.60±0.06$^{b}$ | 0.49±0.04$^{b}$ | 0.40±0.04$^{a}$ | 0.33±0.02$^{b}$ |
| $T_3$ | 1260±27.08$^{a}$ | 0.88±0.02$^{a}$ | 0.78±0.02$^{a}$ | 0.68±0.04$^{a}$ | 0.58±0.06$^{a}$ | 0.49±0.03$^{a}$ | 0.43±0.02$^{a}$ |

**Conclusion:** Different Ovaprim doses used in current studies for *Channa marulius* are quite inadequate for induced spawning of this fish. Some other hormone sources singly or in combination with ovaprim can be tried. Environmental parameters are another consideration, which also demands serious future negotiations.

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