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Effect of Different Synthetic Hormones and/or Their Analogues on Induced Spawning in *Channa marulius*

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Abstract.- Effect of different synthetic hormones and/or their analogues have been evaluated on breeding performance of *Channa marulius* using four different hormonal treatments. Males were injected with various combinations of ovaprim (gonadotropin releasing and dopamine antagonist) with human menopausal gonadotropin (HMG), human chorionic gonadotropin (HCG) and HCG+HMG; whereas, females received combination of ovaprim with HMG and HCG. After 12, 16, 20 and 24 h of first dose, injection of ovaprim were administered and blood testosterone, follicle stimulating hormone and luteinizing hormone were analyzed every 4 h for 48 h. Results indicated that combination of HCG+HMG and ovaprim+HCG were effective and reliable synthetic hormones for induced spawning in *C. marulius*. Fecundity and egg fertilization rate was highest for HCG+HMG at latency period of 43.20-44.45 h. Blood hormonal levels were non-significant ($P<0.05$) and increased gradually till 28 h of post injection and then decreased gradually. Fish injected with ovaprim+HCG does not spawn at all. Eggs obtained were yellow, spherical, non-adhesive and translucent. After fertilization, first cleavage was observed within 2 h, second was between 3-4 h and after 4-6 h, a shield appeared inside and two-layered structure appeared with an outer epiblast and inner hypoblast. However, no further development was observed as the eggs succumb to fungal infection.

Keywords: *Channa marulius*, synthetic hormone, induce breeding, HMG, HCG, Ovaprim.

INTRODUCTION

The snakeheads *Channa marulius* is widely distributed in natural water bodies of Pakistan, India, Bangladesh, Myanmar, Thailand, Philippines, Vietnam and Cambodia. *C. marulius* is a highly priced, valuable fish species and much sought-after group of riverine fishes both for game as well as for food in sub-continent. It is good for its taste, high protein content, low intramuscular spines, high nutritive value, medicinal qualities and recommended as a diet during convalescence (Haniffa *et al*., 2004). It prefers stagnant muddy water bottoms of rivers, lakes, swamps, marshes, canals and ponds. They are voracious carnivore, preying upon live animals. The hatchlings and fry feed mainly on zooplanktons and small insects larvae, while the adults feed on the invertebrates, small fishes and frogs.

Presently it is considered as an important candidate for freshwater aquaculture because of its outstanding resistance to stress, disease and its air-breathing habit (Ponniah and Sarkar, 2000; Ayyappan *et al*., 2001).

Over the past fifty years, wild stock of carnivorous fishes in general and this species in particular is under threat owing to over fishing, deteriorating environmental conditions, habitat losses, pollution, river diversions and acute shortage of water. On account of these factors their population is continuously on decline. Very limited efforts have been made on its conservation through artificial breeding at hatcheries. Some enthusiastic fish farmers collect seed of this fish from the natural spawning grounds for culture in ponds with existing carp varieties but this seed source is neither reliable nor sustainable. This scenario strongly demands captive and controlled breeding of this valuable food resource for production of quality seed in required quantity. Available literature shows no significant research work on the induce breeding of *Channa marulius* in the South East Asia except Hanifa and Sridhar (2002), Hanifa *et al*. (2004), Marimuthu *et al*. (2007). However, their work was

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focused primarily on *C. striatus* and *C. punctatus* but *C. marulius* remained neglected.

Among several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or luteinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists was found to be effective in fish breeding (Lin and Peter, 1996). Different spawning agents have successfully been applied in many fish species including carp. Human chorionic gonadotropin (HCG) is one of them, which has played effective role in inducing ovulation in catfish (Legendre *et al*., 2000; Adebayo and Fagbenro, 2004). Introduction of Ovaprim really revolutionized induced spawning technology and widely used for artificial production of eggs in variety of fish species.

Human menopausal gonadotropin (HMG) a new drug was used for the first time in Pakistan for inducing breeding of *C. marulius* with combination of other hormones. Induced breeding by carp fresh pituitary extract are widely used from the past two decades in the major and exotic carps in Pakistan. All these hormonal combinations have successfully been tested in *Labeo rohita, L. calbasu, Catla catla, Cirrhinus mrigala, Puntius javanicus, Tor putitora, T. musullah* and *T. khudree* (Thakur and Reddy, 1997), bighead carp, *Aristichthys nobilis* (Richardson) (Afzal *et al*. 2008) and *Cyprinus carpio* (Sarma *et al*., 2000).

Latency period means time between administration of the drug and ovulation is a valuable parameter to manage any captive breeding trials/experiments. This critical indicator has successfully elucidated in several fish species (Hogendoorn and Vismanas, 1980; Legendre and Oteme, 1995). A suitable combination of proper dose of different hormones and stripping time always give the maximum yield of the eggs during induced spawning. Thus for the production of *C. marulius* at massive scale, a study of its own kind was designed in which variety of hormone sources and /or their analogues were used to induce breeding of this valuable fish.

MATERIALS AND METHODS

Brood stock selection

Males with soft pectoral fins, round genital papilla and coarse lower jaw whereas, females with rough pectoral fins, smooth lower jaw, swollen abdomen and oval genital papilla were harvested from earthen ponds at Department of Fisheries and Aquaculture Fish Pond Facilities, Ravi Campus, Pattoki. Maturity of sexes was further confirmed by microscopic examination after dissection. Eggs were immersed in 70% acetic acid for examination of spatial position of cytoplasm and nucleus. Migration of the nucleus to the periphery region indicated readiness of broodfish for hormonal injection. Brooders were thoroughly bathed in $KMnO_4$ solution (8 ppm) to eradicate infestation and transferred to holding tanks for acclimatization.

Table I.- Dosages (ml. kg^{-1} BW) of different hormone applied for induced spawning of *Channa marulius.*

Treat-ment	Male		Female		Time
	Hormone	Dose	Hormone	Dose	
1st hormone dose (ml.kg^{-1} BW)					
1	Ovaprim + HMG	0.3+0.3	Ovaprim+ HMG	0.5+0.3	
2	Ovaprim + HCG	0.3+0.3	Ovaprim+ HCG	0.3+0.5	
3	HCG+HMG	0.2+0.2	Ovaprim+ HCG	0.5+0.3	
4	Ovaprim + HMG	0.5+0.3	Ovaprim+ HMG	0.3+0.5	
2nd hormone dose (ml.kg^{-1} BW)					
1	Ovaprim	0.2		0.7	12
2		0.2		0.7	16
3		0.2		0.7	20
4		0.2		0.7	24

Induced spawning of C. marulius

Triplicates of 4 hormonal treatments (4 males and 3 females) were stocked randomly. Individual animal was anesthetized (MS-222), dried and weighed carefully for calculation of hormonal dosage. Two doses method applied during induced spawning, intramuscularly on the dorso-lateral side behind dorsal fin. First dose composed of combinations of HCG (LG Laboratories-HCG-5000-PK-0506) and HMG (Massone, FSH-75IU, LH 75 IU) with ovaprim (Syndel Laboratories, Vancouver, BC, Canada) mixed in various proportions (Table I). Second dose consisted of only ovaprim injected at 12, 16,

20 or 24 h post first dose. Fish were released back to their respective spawning tanks (2 m diameter; 2000 L) and monitored continually for spawning developments and readiness of fish for stripping at 28-30°C and 5-6 mg/l dissolved oxygen, respectively. The diameter of egg (20 eggs from each ovary) was estimated by using a calibrated micrometer mounted on the eyepiece of a monocular microscope (1 division = 0.05mm).

Blood collection

The blood samples were taken from the caudal vein after hormonal administration at 4 h interval for 48 h. Blood was centrifuged to separate plasma for 10 min and stored at 4°C for further analysis.

Testosterone assay

Testosterone assay was conducted using Alpha diagnostic international (ADI's) ELISA kit (1880/db120118A), which was developed following kit protocol. The reaction was stopped by adding 50 µl of stop solution. Absorbance was measured at 450 nm using an ELISA reader within 30 min. Standard curve was used to estimate the testosterone level in the sample.

Hormone assays

FSH was determined by using ELISA Kit No. 0200. Absorbance of yellow color developed after following protocol of the kit was measured at 450 nm using ELISA reader within 30 min.

ELISA Kit No. 0100 was used to determine blood LH in females of *C. marulius.* Absorbance of the reaction mixture was measured at 450 nm using an ELISA reader within 30 min.

Statistical analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) using SAS (Statistical Analysis System) 9.1 version. Treatment means were analyzed using the Duncan's Multiple Range Test.

RESULTS

Healthy and sexually matured males and females were paired and four different hormonal treatments were administrated to induce spawning in *C. marulius* at hatchery conditions. Mean average body weights of males, females and hormonal administration is given in Table I. In treatment 2 and 3 brooders of *C. marulius* showed aggressive courtship behavior, after 43 h of second dose. During courtship, female laid eggs and the male bent its body close to the female and sprayed milt on eggs simultaneously fertilizing eggs.

Females that received ovaprim and HMG did not spawn, whereas females that received HCG+HMG and HCG+ovaprim spawned successfully. HCG and HMG induced spawning was very effective and efficient. Egg fertilization rate in this treatment was 81.33% with latency period of 43.20-44.45 h after the second dose. The longest period was 40.25-42.45 h in ovaprim+ HCG dosage with 41.25±0.88 mean latency hours (Tables II and III). HCG and HMG resulted highest fecundity (1386.67±119.27) followed by treatment of HCG+ovaprim with 1291.67±105.71 eggs kg^{-1} body weight. However, differences between the both treatments were non-significant (Table IV). Mean ova diameter ranged from 1.46±0.01 to 1.83±0.025 mm with lowest mean ova diameter (1.46±0.01 mm) observed in ovaprim+HMG injected females (Table III).

The different stages of fertilized eggs showed in Figure 1. The eggs obtained from female *C. marulius* were yellow in color, spherical, non-adhesive and translucent. After fertilization, first cleavage proceeds within 2 h. The second cleavage observed between 3-4 h. After an interval of 4 - 6 h, a shield appeared inside, when anterior and posterior differentiation was obvious. Two-layered structure appeared with an outer the epiblast and inner hypoblast (Table VI). No further development observed as the eggs succumbed to fungal infection although the fertilizing tanks were sterilized with suitable amount of $KMnO_4$.

Blood testosterone levels of male *C. marulius* showed non-significant (P=0.05) differences during first 48 h after hormonal administration (Table III). The highest testosterone level (2.89± 0.70) was observed in 20 h after the second injection of HCG+HMG followed by ovaprim+HCG (2.38± 0.36), Ovaprim+HMG (1.37±0.30) and ovaprim+ HMG (1.03 ± 0.02), respectively.

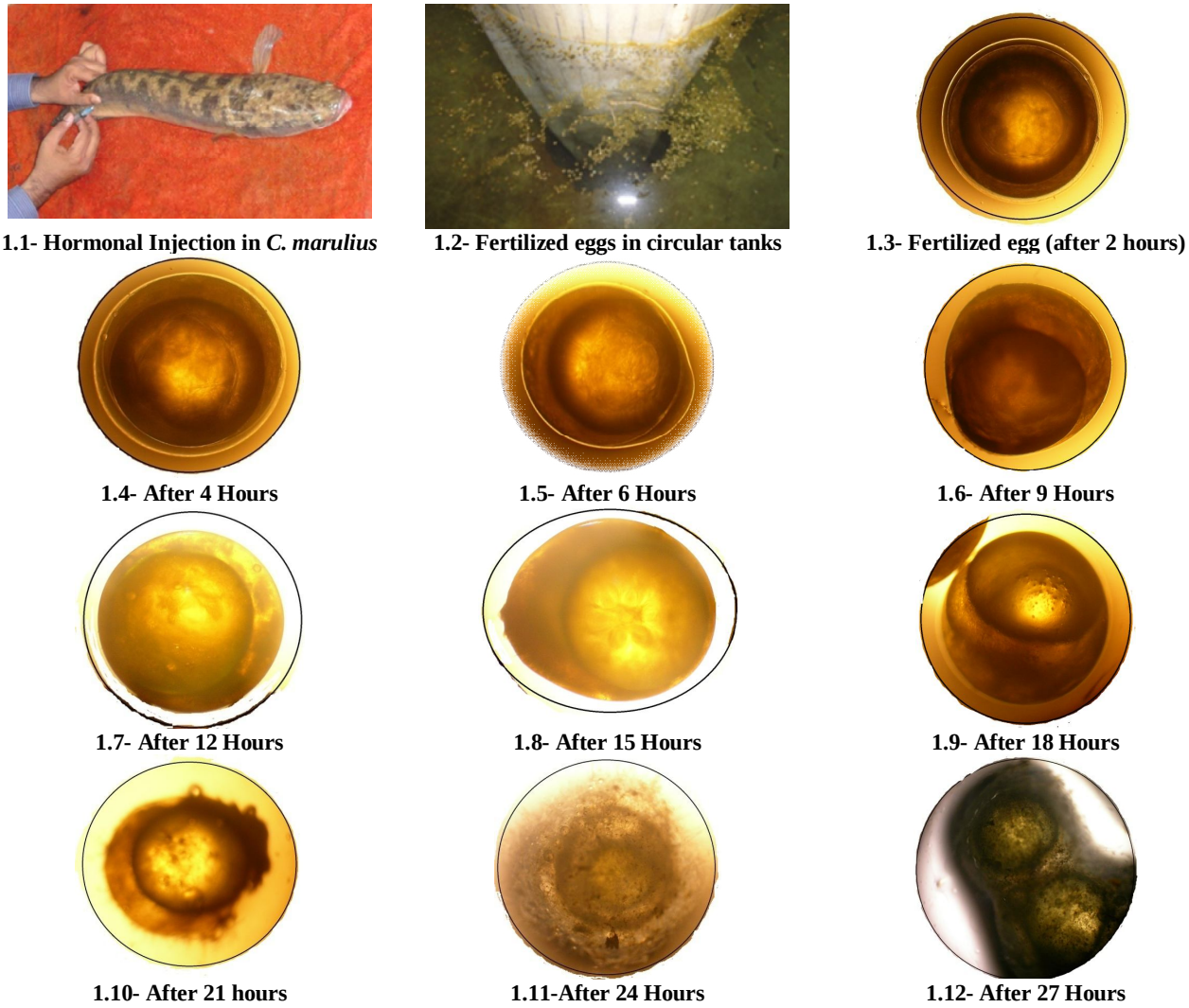

Fig. 1. Different developmental stages of fertilized eggs of *Channa marulius.*

The FSH and LH levels in blood serum of female *C. marulius* during 12-48 h with different hormonal treatments are given in Tables IV and V. FSH and LH level showed non-significant difference (P=0.05). The highest follicle stimulating hormone level (1.22±0.25) was observed in HCG+HMG treated group in 20 h after the second injection followed by ovaprim+HCG and ovaprim+HMG, respectively. Hormonal levels in the blood of males and females of *C. marulius* first increased till 20-24 h of second injection then the hormone level decreased with the passage of time.

Temperature, dissolved oxygen, pH, total dissolved solid, electrical conductivity and salinity ranged from 29.0-30.4ºC, 5.5-6.5 mg/l, 7.6-8.20, 815-820 mg/l, 2.05-2.10 ms/cm, and 0.9 ppm, respectively, in the spawning tanks.

DISCUSSION

This study represents significant advances toward induce breeding of *C. marulius.* HCG, HMG, ovaprim and fresh carp pituitary glands (extracted from *Cyprinus carpio)* were injected to both male and female breeders. The experimental fish spawned, eggs produced were fertilized artificially; cell division started but stopped later on and failed to hatch. In another trial, fish were

Table II.- Fecundity, latency period, spawning success in *Channa marulius* breeders injected with at different doses of synthetic hormones (Mean + SD).

Treatments	Body weight (g)		Latency period (h)	Spawning success	Fertilization rate (%)	Incubation period (h)	Fecundity Rate (Natural)	Fecundity (after dissection)	Ova diameter (mm)
	Male	Female							
T_1	1318.75±162.95b	1283.3±53.16a^{b}	Nil	Nil	Nil	Nil	Nil	984.16±124.03^{a}	1.48±0.02c
T_2	1620.0±271.66a	1430.0±237.82a^{b}	41.25±0.88^{b}	Complete	67.0±3.89^{b}	Nil	1291.67±105.71^{a}	Nil	1.74±0.01^{b}
T_3	1352.50±187.67^{b}	1470.0±290.37^{a}	43.80±0.52^{a}	Complete	81.33±1.21^{a}	Nil	1386.67±119.27^{a}	Nil	1.83±0.025^{a}
T_4	1233.75±82.62^{b}	1216.67±20.65^{b}	Nil	Nil	Nil	Nil	Nil	955.33±57.50^{a}	1.46±0.01^{c}

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

Table III.- Testosterone (ng ml^{-1}) level in the blood of the male *Channa marulius* after administration of second dose of hormones at different time intervals (Mean ± SD).

Treatments after (h)	Male body weight (kg)	12 h	16 h	20 h	24 h	28 h	32 h	36 h	40 h	44 h	48 h
12	1300±192.52^{ab}	0.375±0.05^{a}	0.59±0.11^{b}	1.37±0.30^{b}	1.32±0.24^{b}	1.28±0.25^{b}	1.15±0.27^{b}	0.92±0.26^{b}	0.75±0.28^{b}	0.59±0.25^{b}	0.36±0.12^{b}
16	1627.5±322.01^{a}		2.09±0.46^{a}	2.38±0.36^{a}	2.32±0.55a	2.23±0.41a	2.04±0.40^{a}	1.81±0.41a	1.58±0.35a	1.37±0.32^{a}	1.12±0.18^{a}
20	1350±197.65^{ab}			2.89±0.70^{a}	2.62±0.69a	2.61±0.65a	2.31±0.54a	2.10±0.52^{a}	1.84±0.42^{a}	1.17±0.20^{a}	1.27±0.32^{a}
24	1187.5±94.64^{b}				1.03±0.02^{b}	1.01±0.01^{b}	0.89±0.04^{b}	0.65±0.05^{b}	0.45±0.06^{b}	0.35±0.03^{b}	0.22±0.03^{b}

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

Table IV.- Follicle Stimulating Hormone (FSH) level (mIU/ml) in the blood of the female *Channa marulius* after the administration of second dose of hormones at different time intervals (Mean ± SD).

Treatments after (h)	Female body weight	12 h	16 h	20 h	24 h	28 h	32 h	36 h	40 h	44 h	48 h
12	1266.67±57.73^{a}	0.56±0.04a	0.82±0.01^{a}	1.08±0.01^{a}	1.01±0.01^{a}	1.02±0.002^{a}	0.78±0.08^{b}	0.63±0.08^{b}	0.54±0.10^{b}	0.32±0.02^{c}	0.23±0.04^{b}
16	1433.33±292.80^{a}		0.98±0.15^{a}	1.13±0.10^{a}	1.07±0.06^{a}	1.10±0.05^{a}	0.98±0.07^{a}	0.88±0.09^{a}	0.75±0.05^{a}	0.64±0.04^{a}	0.32±0.02^{a}
20	1456.67±366.65^{a}			1.22±0.25^{a}	1.10±0.12^{a}	1.10±0.12^{a}	0.98±0.15^{a}	0.88±0.10^{a}	0.75±0.08^{a}	0.60±0.06^{a}	0.33±0.02^{a}
24	1210.0±17.32^{a}				0.95±0.09^{a}	1.00±0.09^{a}	0.91±0.05^{ab}	0.77±0.03^{ab}	0.61±0.01^{b}	0.43±0.04^{b}	0.26±0.01^{b}

Table V.- Luteinizing Hormones (LH) (mg ml^{-1}) level in the blood of the female *Channa marulius* after the administration of second dose of hormones in different time intervals (Mean ± SD).

Treatments after (h)	Female body Weight	12 h	16 h	20 h	24 h	28 h	32 h	36 h	40 h	44 h	48 h
12	1266.6±57.73^{a}	0.81±0.11^{a}	0.90±0.03^{a}	1.00±0.02^{a}	0.95±0.02	0.94±0.02^{ab}	0.86±0.04^{a}	0.74±0.04^{ab}	0.64±0.06^{ab}	0.53±0.05^{ab}	0.44±0.03^{a}
16	1433.3±292.80^{a}		0.94±0.05^{a}	1.06±0.05^{a}	1.03±0.05	1.03±0.04^{a}	0.93±0.07^{ab}	0.84±0.06^{ab}	0.73±0.06^{ab}	0.62±0.07^{ab}	0.54±0.11^{a}
20	1456.6±366.65^{a}			1.10±0.11^{a}	1.06±0.09	1.05±0.09^{a}	0.97±0.11^{a}	0.86±0.11^{ab}	0.77±0.12^{a}	0.65±0.12^{a}	0.58±0.10^{a}
25	1210.0±17.32^{a}				0.90±0.04	0.89±0.05^{a}	0.80±0.005^{b}	0.71±0.005^{b}	0.59±0.005^{b}	0.49±0.01^{b}	0.43±0.11^{a}

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

Table VI.- Developmental stages of the fertilized eggs of *Channa marulius.*

Spawning time (h)	Developmental stages	Interpretation of the cell division
0	Fertilized egg	Eggs were free-floating, spherical, non-adhesive, transparent and straw yellow in color. The diameter of the fertilized eggs varied from 1.20 mm-1.40 mm.
2	2 Cell stage	Cleavage, the first cell division were started from the fertilized eggs may became into two cells
3	16 Cell stage	Fourth Cleavage, moved towards the blastulation
4	Morula	Multicelluar blastodisc were produced as result of Blastulation process
6	Blastula	In this stage a shield was formed, more than half the yolk entered inside, anterior and posterior differentiation was obvious.
8	Gastrula	Two layered structure was appeared with an outer the epiblast, and inner one the hypoblast

Further developmental stages could not be preceded due to fungus that encircled the whole eggs. The fertilized eggs were become in cluster form.

injected with different doses of ovaprim but fishes did not spawn (Hafeez-ur-Rehman, 2013). Different inducing agent used in this study have been used in major carps (Leelapatra, 1988; Thalathiah *et al*., 1988) and Chinese carps (Peter *et al*., 1988; Weil *et al*., 1986; Kumarasini and Seneviratne, 1988) extensively to produce high yields. However, the spawning success among these fishes varied from species to species. In the present study, two treatment regimes resulted in successful spawning of *Channa marulius*. Haniffa and Sridhar (2002) results were in lined with our study who reported Induced spawning of the spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) by using ovaprim and HCG at varying dosages. Similarly, Haniffa *et al*. (2000) injected natural hormones (pituitary extract and human chorionic gonadotropin) to murrel *Channa striatus*. The fertilization rate was 81.33% in fish that received HCG+HMG and 67% in Ovaprim+HCG receiving group. These results are in line with those of Haniffa and Sridhar (2002) who used HCG Hormone (3000 IU) in the induced breeding of *Channa punctatus*, produced fertilized eggs (1253±126 eggs) weighing 65-85 g . The differences in egg outputs observed previously (Haniffa and Sridhar, 2002; Yaakob and Ali, 1992) may be due to different experimental species, of spawners and the source of hormones used. It may be true in low egg production rate per kg of female body weight.

Failure of Ovaprim+HMG synthetic hormones may be due to hormone type, manufacturing process, administration procedures and extraction of acquirement of gonads procedure may vary from species to species and depending on the reproductive biology as obseverd by Mylonas *et al*. (2009). The understanding of the endocrine gland control gametogenesis, final maturation and spawning played key role for the proper management of the broodstock fish.

Mean egg diameter of all fish, which ovulated in the four experiments ranged from 1.46 to 1.83 mm. Germinal vesicle, broke down and an increase in the size of oocytes due to hydration indicated the changes in the nucleus and cytoplasm during final maturation (Goetz, 1983; Guraya, 1986). The increase in ova diameter might be an early action of steroidogenesis through hormonal influence. Accordingly, decrease in egg diameter may be an early steroidogenesis due to which oocyte batch have not obtained sufficient yolk.

Non-spawning response of two treatments injected with ovaprim+HMG might be due to the non-stimulation of the male and female due to less testosterone level in the blood in males and FSH and LH level in the female *C. marulius* blood. Other factors might be that the fish were heavily conditioned, and the eggs not staged prior to the spawning attempt.

The highest level of testosterone in male *C. marulius* by Ovaprim obtained after 20 h of second injection. These results are very similar to Metwally and Fouad (2008) who reported that the highest testosterone level in induced male grass carp (*Ctenophryngodon idella*) by pregnyl and Ovaprim obtained in 8-10 h after the first injection. Other

studies indicated improving the spermatogensis with a series of treatment hormone of Ovaprim, Ovaplant, HCG, cPG and comibination of cPG with Ovaprim or HCG (Abol-Munafi *et al*., 2006; Tu *et al*., 2012) in white silver carp (*Hypophthalmicthys molitrix*). On the other hand ZviYaron, (1995) reported that stimulation of sperm duct by gonadotropin or by 17, 20-dihydroxy-4-pregnen-3-one (17, 20-P) in common carp (*Cyprinus carpio*). In white bass (*Morone chrysops*) were exposed to an increase in temperature and treated with a gonadotropin-releasing hormone antagonist (GnRHa) enhancing milt production in white bass (Costadinos *et al*., 1997) and on bass (*Dicentrarchus labrax* L.) (Lucinda *et al*., 2001).

CONCLUSIONS

It conclude that combination of HCG+HMG and the ovaprim+HCG were effective and reliable synthetic hormones for induce breeding of *Channa marulius*. Hence *Channa* breeding can be induced and these findings can be helpful in future induced spawning and sustainability attempts of this valuable species.

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