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EFFECT OF SYNTHETIC KISSPEPTIN-10 ALONE AND IN COMBINATION WITH DOMPERIDONE ON INDUCED BREEDING OF *CIRRHINUS MRIGALA*

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ABSTRACT
In the present study the Indian major carp, *Cirrhinus mrigala* was induced using synthetic Kissppetin-10 hormone alone and in combination with domperidone. Spawning took place between 8-9 hours of injection. Highest fertilization rate of 91.075% and hatching rate of 86.29% was observed from fishes induced with synthetic kisspeptin alone. In case of fishes induced with a combination of kisspeptin and domperidone maximum fertilization rate of 94.49% and hatching rate of 92.015% was obtained. The effect of synthetic kisspeptin alone on breeding performance was almost similar to the combination of kisspeptin and domperidone

INTRODUCTION

As an additional source of human diet and as a component of various animal feeds, there is now a greater emphasis on fish production. The Mrigal is one of the most widely farmed species among the IMCs which has a good demand and market value.The knowledge of artificial breeding is a key aspect as it permits intensive production of a given species in controlled conditions. This allows continue production of juveniles for restocking natural or artificial water bodies (Montchowui *et al.,* 2011). It has greatly contributed for the rapid development of carp culture in India without having to depend heavily on rive rine spawn collection (Nandeesha *et al*., 1990).

The Mrigal is one of the most widely farmed species among the Indian Major Carps which has a good market value. Mrigal do not breed in confined water hence hormone-induced spawning is the only reliable method to induce reproduction in these fishes. Mrigal have been successfully induced with various hormones like hCG, Carp Pituitary Extract (CPE), ovopel, ovaprim, ovatide (Chaudhuri and Alikunhi, 1957; Vardia, 1954; More *et al.*,2010; Nandeesha *et al*.,1990). Although several hormones have been tried in India with varying degrees of success (Tripathi and Khan, 1990), none has gained acceptance at the farmer's level either due to cost, non-availability or procedural difficulties. Recently it has been demonstrated that the kisspeptin system plays an essential role in the neuroendocrine control of puberty and reproduction by stimulating the GnRH neurons and subsequently releasing gonadotropin hormones. (Parhar *et al*., 2004; d' Anglemont *et al*., 2007; Nocillado *et al*., 2007; Popa *et al*., 2008). In the present study, an attempt has been made to study the effect of synthetic kisspeptin-10 on induced breeding of Mrigal, *Cirrhinus mrigala*. The results of this study will provide information for the development of new induced agent for the breeding.

MATERIALS AND METHODS

All the induced breeding experiments were carried out at National Fish Seed Farm, Manimuthar (Tamil Nadu). Healthy, disease free, fully mature ripe fishes were chosen for the induced breeding.

Hormone treatment

In the first treatment-1 fishes were induced with Synthetic Kisspeptin alone at three concentrations viz., 50, 100,150μg/ kg body weight and in the second treatment-2 they were induced with kisspeptin along with domperidone (at 10mg/kg body weight). Kisspeptin-10 was injected into the fish breeders through intramuscular injections just below the dorasal fin. Hypodermic 2 ml syringe having 0.1 ml graduations with a needle no. 22 was used. Injections were given during the evening between 4-5pm. The injected breeders were released into the hapa for breeding. Next day the spent brooders were collected and transferred to the other pond.

Number of eggs produced

The numbers of eggs were measured by volumetric method. A beaker was used in which number of eggs was counted in

triplicate and the average of the three estimations was taken to know the numbers of eggs per beaker. Multiplying this with the number of beaker measured, total number of eggs was calculated.

Fertilization Rate:

The temperature of water used for incubation was kept around 27-30° C.The fertilization rate of eggs laid by induced fish in each set, under different treatments was estimated. For estimation, three sub samples of water harden eggs were taken from each set and the number of fertilized egg (n) out of total eggs produced (N) in each sub samples was counted. The fertilized egg percentage was then calculated by the following formula (Hogendoorn, 1979) and their mean was determined

$$\text{Fertilization \%} \quad \frac{\text{Total no. of fertilized eggs (n)}}{\text{Total no. of eggs produced (N)}} \quad 100$$

Hatching rate

Hatched eggs are collected and the hatching rate was calculated using the following formula (Olubiyi, *et al*. 2005)

$$\text{Hatching \%} \quad \frac{\text{No. of hatched fry}}{\text{Total no. of fertilized eggs}} \quad 100$$

Statistical analysis

The results found in the experiment were subjected to statistical analysis, ANOVA, (one way) that showed the significance ($P < 0.05$) level of differences between the treatments. This statistical analysis was performed with the aid of the computer software MS Excel program.

RESULTS

The total number of eggs produced in case of treatment-1 and treatment-2 are shown respectively in Table-1 and Table-2. In Treatment-1, fishes induced with 100 μg/kg of Kisspeptin produced higher number of eggs (160557). On the contrary, intreatment-2, the higher numbers of eggs were produced by fishes induced with150μg/kg + 10mg/kg (181026). In both the treatments lowest number of eggs were produced by fishes induced with 50μg/kg of kisspeptin

Fertilization rate

In the present study, the fertilization rate ranged between 85.115- 95% in all the treatment and are presented in Table 1. Maximum fertilization rate was obtained from the synthetic kisspeptin injected fish at 150 μg/kg body weight followed by 100μg/kg and 50μg/kg body weight. Highest fertilization rate of 94.49% was obtained from fishes induced with combination of Kisspeptin and domperidone at the rate of 100μg/kg + 10mg respectively.

Hatching rate

In the present study, least hatching percentage of 82.125% and a maximum hatching rate of 86.29% were observed from fish induced with 50μg/kg body weight of synthetic kisspeptin-10 (Table 1). The highest hatching percentage of92.015 was recorded from treatment fish induced with combination of synthetic kisspeptin and domperidone at the rate of 100μg/kg + 10mg body weight followed by groups induced with 100μg/kg + 10mg and 50μg/kg + 10mg.

DISCUSSION

Overall, the results of this experiment revealed that there remained non-significant difference between the 2 treatments. In all the fishes induced with synthetic kisspeptin-10 alone and in combination with domperidone, fertilization rate, hatching rate had little variation.This variation was seen probably because they were treated in different doses of hormones. Although, some variations may arise due to the physiological differences in the pair of fishes and experimental error. Similar results were obtained by Basavaraja *et al*. (2007) where a combination of buserelin (an analogue of mammalian LHRH) and domperidone was used for breeding of Mrigal. The result obtained from m-LHRHa + domperidone was better than the result obtained from S-GnRHa + domperidone. Determination of hatching rate of fish is important for various aspects. It can determine the status of how many fry can be produced from a number of fish and how many are lost and why. It helps to improve the hatchery product and thereby production.Various works carried out on mrigal using different hormone like ovatide, ovaprim pituitary extract gave different hatching rate. (Mishra *et al.,* 2001; Saini *et al*., 2001; More *et al.,* 2010)

Recently Synthetic kisspeptin has been widely used to get gonadal maturation in fishes (Francis *et al.,* 2011; Selvaraj *et al*., 2013; Unniappan *et al*., 2011; Benjamin *et al.,* 2012) Even though studies are available on the effect of kisspeptin-10(natural) on the maturity of fish; efficiency of synthetic kisspeptin-10 is very limited. In the present study an attempt was made to assess the effect of synthetic kisspeptin-10 alone

Table 1: Mean number of eggs produced, ferilization rate and hatching rate of *C. mrigala* induced with synthetic kisspeptin-10

Treatment-1	Number of eggs produced	Fertilization Rate (%)	Hatching Rate (%)
50μg/kg body weight	125455 ± 2404.5	85.115 ± 0.79	82.125 ± 0.595
100μg/kg body weight	160557 ± 8983.1	89.495 ± 0.86	85.725 ± 0.285
150μg/kg body weight	156969 ± 2436.4	91.705 ± 0.45	86.29 ± 0.93

Table 2: Mean number of eggs produced, fertilization percentage and hatching percentage of *C. mrigala* induced with a combination of synthetic kisspeptin and domperidone

Treatment-2	Number of eggs produced,	Fertilization rate (%)	Hatching rate(%)
50μg/kg + 10mg/kg body weight	135278 ± 2397.298	87.525 ± 1.015	84.915 ± 0.31
100μg/kg body 10mg/kg weight	171706 ± 10596.62	94.49 ± 0.65195	88.42 ± 0.9
150μg/kg body10mg/kg weight	181026 ± 7026.93	92.05 ± 1.07	92.015 ± 1.34

and in combination with domperi done on induced breeding of mrigal.The addition of domperidone did not show any significant changes in the breeding performance of *C. mrigala*. Hence the present study concludes that kisspeptin alone can be used successfully for the induced breeding of mrigal.

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