



Manipulation of Reproduction in Farmed Fish

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Introduction

Farmed fish fail to complete the reproductive cycle in captivity and exhibit different forms of reproductive dysfunction. In females, the two major problem encountered are failure to complete the vitellogenesis and the other is failure to undergo final oocyte maturation and ovulation, after completion of vitellogenesis. In few species, captive male fails to undergo spermiation. In addition, few species of captive females and males take longer duration to undergo gonadal growth and maturation. To overcome these problems, several hormonal preparations have been used and found to show prominent results.

Gonadotropin-releasing hormone (GnRH) producing neurons, localized in specific regions of the brain, particularly preoptic-hypothalamic region innervate anterior pituitary regions, where pituitary gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) synthesizing cells are localized. These FSH and LH are released into the blood streams, which act on the gonadal somatic cells to produce sex steroids. These sex steroids act on gonadal somatic cells to regulate germ cell proliferation, growth and maturation. Most of the commercial hormone preparations such as human chorionic gonadotropin (hCG), GnRH analogue (GnRHa), Ovaprim, and Ovatide target the above hormonal pathways to affect gonadal growth and maturation. In recent years, kisspeptins have been shown to act as novel upstream regulator of GnRH and few in-vivo studies indicated their possible application for aquaculture.

This paper presents the application of different hormones through different administration methods for manipulation of gonadal growth and maturation in different farmed fishes.

Materials and methods

Experiment 1: Koi carp (*Cyprinus carpio* var *koi*) were purchased from commercial aquarium dealer and stocked in 500 l tanks for a week. After a week, the animals (total length = 12.6 to 12.77 cm, body weight = 50.95 to 51.28 g) were subcutaneously injected with hCG at 0.6, 0.8, 1 and 1.2 IU/g of body weight and sampling was performed after 45 days to monitor the gonadal growth and maturation.

Experiment 2: Freshwater prawn (*Macrobrachium rosenbergii*) were purchased from a commercial farm and transferred to the laboratory for acclimatization. Prawns were fed with probiotic incorporated feed at 6, 8, 10, and 12% and the experiment performed for 60 days.

Experiment 3: Striped murrel (*Channa striatus*) were purchased from a farmed fish supplier and stocked in 3 ton tanks for two weeks. The animals were surgically implanted with silastic capsules loaded with hCG. The dose used was 1000 IU/

kg body weight. Sampling of fish was performed each month for 10 months to monitor the gonadal growth and maturation.

Experiment 4: Efficacy of newly found kisspeptin peptides to induce gonadal growth was evaluated in prepubertal and adult chub mackerel (*Scomber japonicus*). Synthetic kisspeptin peptides (Kiss1-15, Kiss1 pentadecapeptide and Kiss2-12, Kiss2 dodecapeptide and GnRHa) were administered subcutaneously. In prepubertal fish, peptides were mixed with cocoa butter and administered every two weeks for 42 days. In adult fish, peptides were administered via mini-osmotic pumps and sampling was performed on 45th day post-implantation. The peptide dose used for prepubertal fish was 250 ng/g fish. For adult fish, peptides at a concentration of 2 µg/µl were loaded in the pumps.

Gonadosomatic-index (GSI) values were calculated and gonadal histology was performed to screen the gonadal growth stages.

Experiment 5: Natural and artificial stimulation of maturation and spawning of pearl spot (*Etroplus suratensis*) under captivity was performed.

Results and Discussion

Experiment 1: GSI values of hCG injected male and female koi carp at 1.2 IU/g showed higher values (6.62 and 5.30), in comparison to control (4.78 and 2.97) and other treatments (0.6 IU, 5 and 3.18; 0.8 IU, 5.51 and 4.06, 1.2 IU, 5.6 and 4.29). Histologically, active spermiation and several late vitellogenic oocytes were found in the testes and ovaries of hCG injected koi carp at 1.2 IU/g. These results indicated hCG at 1.2 IU/g as the most effective dose for the acceleration of gonadal growth and maturation in captive reared koi carp (Selvaraj et al., 2006).

Experiment 2: Probiotic inclusion at 8% in the feed accelerated maturation in the prawn.

Experiment 3: GSI values of hCG implanted male and female striped murrel showed first peaking at 4th month post-implantation (0.18 and 6.93) and subsequently, peaked again at 8th month post-implantation (0.28 and 4.62). During the above periods, control male and female fish showed lower values (0.09 and 1.47; 0.06 and 0.75). Histologically, presence of spermatozoa and advanced vitellogenic oocytes were recorded during increased GSI values in hCG implanted male and female fish, in comparison to slow growth of gonads in control fish. These results indicated application of silastic capsules for hormone delivery in striped murrel and could advance gonadal development in captive reared fish (Selvaraj et al., 2007).

Experiment 4: GSI values in prepubertal males, administered

with Kiss1-15 showed significant increase, in comparison to control, Kiss2-12, and GnRHa. Histologically, spermatozoa count was significantly higher in Kiss1-15 treated fish. In females, GSI values did not show any significant differences between treatments; however, an increase in mean oocyte diameter was recorded. Continuous administration of Kiss1-15 peptides using mini-osmotic pumps induced spermiation in adult immature males and vitellogenic onset in immature females. Interestingly, Kiss1-15 showed superiority over GnRHa on inducing spermiation in males. These results indicated for the first time, possible application of Kiss1-15 for inducing gonadal development in captive reared fish (Selvaraj et al., 2013a,b,c).

Experiment 5: Natural spawning of pearl spot recorded in small FRP tanks, for the first time (Selvaraj et al., 2016a, communicated). Subsequently, we have recorded multiple spawning in the same tank, when maintained in group; in contrast to the paired fish maintained separately (Selvaraj et al., 2016b, communicated).

Future perspectives

Based on the above experiences, experiment for mass scale seed production of pearl-spot (*Etroplus suratensis*) under captive conditions using kisspeptin, neurokinin B, GnRH and hCG will be performed.

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