EFFECT OF HCG IMPLANTS ON GONADAL MATURITY OF MRIGAL, *CIRRHINUS MRIGALA* (HAMILTON, 1822)

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ABSTRACT

The present study was undertaken to induce gonadal maturation of Mrigal, Cirrhinus mrigala using Human Chorionic Gonadotropin (HCG) implants in captive conditions. The male and female fishes of C. mrigala were implanted intramuscularly with hCG implants at the dosage of 1000 IU/kg body weight, to study the effect of hCG hormone. Hormone implants were given once in two months during the experimental period of February to June. Histological observation in the ovary of C. mrigala revealed the presence of four types of growing oocytes namely perinucleolar, previtellogenic, vitellogenic and hydrated oocytes. Histological observation of the testis of C. mrigala showed the presence of five types of spermatogenic cell types namely spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Hence, the present study recommends that hCG implants can successfully be used to induce sustained maturation of C. mrigala in captivity even during off season.

Key Words*:* hCG implants, Gonadal maturation, Histology, Oocytes, Spermatocytes

INTRODUCTION

Fish is one of the major components of the aquatic ecosystem. Carp culture is the largest and the most wide spread practice of aquaculture in the world (Desilva, 2003). Mrigal, a carp endemic to Indo – Gangetic Riverine systems is one of the three Indian Major Carp species cultivated widely in Southeast Asian countries. Mrigals are the members of the family Cyprinidae, comprising more than 400 sp of fishes. It is also known as white carp.The rapid growth rate coupled with its compatibility with other carps has helped in establishing this species as one of the principal component species in pond culture (Hamilton, 1822). Mrigal has become an important component in the fish culture systems of Bangladesh, Pakistan, Myanmar, Thailand and Nepal. Maturation may occur once or many times a year, depending on species and conditions. Maturation of gonads during puberty is closely accompanied by the proliferation and differentiation of germ cells. Correspondingly, there is proliferation of somatic cells (in the ovary), cyst or sertoli cells (in the testis) and steroid producing interstitial cells in both the ovary and testis (Sardul, 1994).

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Mrigal attains maturation within two years in captive condition. Captive breeding in hatcheries has been made possible through induced breeding by hypophysation and the use of synthetic hormones. As Mrigal doesn't breed in confined waters, injection of pituitay extract and other synthetic hormones have been successfully used in recent years.

Hormone manipulations play a vital role in commercial aquaculture, especially for fish that do not undergo final oocyte maturation and spermiation spontaneously. Human Chorionic Gonadotropin (HCG) is widely used for growth, maturation and ovulation in fish and it is a glycoprotein having a molecular weight of around 25.7 kDa. It's chemical structure and properties are similar to that of Luteinising Hormone (LH), though it has higher carbohydrate content such as mannose, galactose, glucosamine, galactosamine, fructose and N – Acetyl Neuraminic Acid [NANA]. The presence of NANA is essential for the biological activity and because of which it is relatively resistant to metabolic degradation and hence has a much longer biological shelf life than LH and has a stimulating effect on the gonads of both the sexes (Rath, 2000).Hence, the present study was carried out to know the effect of HCG implants on histological changes in gonads of Mrigal*, Cirrhinus mrigala.*

MATERIALS AND METHODS

Mrigal, *Cirrhinus mrigala* used in the present study was collected from local freshwater fish farm. The fishes were transported to the National Fish Seed Farm, Manimuthar and acclimatized in the fish culture tanks. During this period, the fishes were fed with formulated feed. After acclimatization, both male and female were segregated and stocked in separate ponds.

Preperation of hCG implants

Human Chorionic Gonadotropin (Trade name, Sifassi) of 10,000 IU (International units) was mixed with 0.025g of cholesterol until a gel like consistency was attained and the resultant paste was incubated in incubator at 35°C till it dried up (Huat, 1980; Lee et al., 1986). After drying the hCG-cholesterol mixture was powdered well for packing in silastic capsule.

Specifications of silastic capsule

Length of the empty silastic capsule	:	1.0cm
Diameter of the empty silastic capsule	:	0.3cm
Weight of the empty silastic capsule	:	0.037g

Based on the individual weight of fish, hCG-cholesterol mixture was packed in the silastic capsule at the concentration of 1000 IU hCG/kg of body weight (Francis et al., 2000). The average weight of hCG implant used was 0.082g.

Implantation of hCG implants

A small incision was made near the dorsal musculature and the implants were implanted intramuscularly. After implantation, the wound was swabbed with oxytetracycline ointment. The wound got healed within 5 days.

Experimental design

The fishes were divided into two groups. Each group consisted of male and female fishes stocked in separate tanks. Both males and females were implanted with hCG implants. After the start of experiment, the fishes were fed with the formulated feed. Monthly sampling of gonad from the above two groups were carriedout to study the effect of hCG implantation on gonadal maturity. Hormone implantation was carried out once in two months in the present study. The monthly sampling of gonad was carried out. Sampling of gonad was continued till the end of experimental period (5 months).

Sampling of gonad

Monthly sampling of gonad from hCG implanted and control fishes were carried out to assess the maturation using histological changes in the gonads. Totally 20 fish samples were drawn during the study period.

Histology of gonad

After dissection of fish, 0.5x1cm mid portions of the ovary and testis were placed in Bouin's fixative for 24 hours and then, the tissue was processed for routine histology (Humason, 1972) work. The processing of tissue in different grades of alcohol was carried out in Automatic tissue processor (Thermo scientific make) and the processed tissues were embedded in cassettes using tissue embedder instrument. Thin sections (5 µm sections) were taken from the processed tissue using microtome (Thermo scientific make).

RESULTS AND DISCUSSION

Effect of hCG implants on histological changes in gonads

Histological changes in the ovary and testis of control and hCG implanted male and female fishes were assessed during from February 2015 to June 2015 to study the maturity stages of gonads during the experimental period.

Maturity stages of ovary of *C. mrigala*

The maturity stages of ovary of *C. mrigala* were classified based on the growing oocytes present in the ovary.

a) Immature/Resting stage

Presence of Oogonia and perinucleolar oocytes throughout the ovary.

b) Maturing stage

At this developmental stage, most of the oocytes present were previtellogenic oocytes of different sizes and small vitellogenic oocytes, with a few larger ones.

c) Mature stage

The dominant oocytes in this stage were in an advanced or active stage of vitellogenesis but perinucleolar and small vitellogenic oocytes were also present.

d) Ripe stage

The histological section of the ovary of this stage revealed oocytes of two developmental stages and sizes: hydrated oocytes that appeared translucent with an oil globule and vitellogenic oocytes in an advanced or active stage of development.

e) Spawned stage

Oocytes of all stages of development were found in the ovary. Histological sections at this stage showed numerous post-ovulatory follicles. Some vitellogenic and previtellogenic oocytes were also present.

Histological changes in the ovary of control and hCG implanted fish

During the start of experiment (February) the ovary of control and hCG implanted fish showed the presence of perinucleolar oocytes and oogonia (Fig. I A & B) indicating the immature stage of ovary.

During the month of March, the presence of perinucleolar oocytes (Fig. I C) in the ovary of control fish indicated the immature stage of ovary. During the same sampling period the ovary of hCG implanted fish showed the presence of previtellogenic and vitellogenic oocytes (Fig. I D) which is indicative of the maturing condition of ovary.

In the month of April, the ovary has reached the matured stage with the presence of vitellogenic oocytes (Fig. I E & F) in hCG implanted and control fish.

During the month of May, the ovary of control fish had mature stage of oocyte only as it showed the presence of same vitellogenic oocytes (Fig. II A). During the same sampling period, the ovary of hCG implanted fish showed the presence of enlarged vitellogenic oocytes and hydrated oocytes (Fig. II B) which indicates that the ovary is fully matured.

In the month of June, the control fish showed the advanced vitellogenic oocytes (Fig. II C), which indicated the matured stage. The ovary of hCG implanted fish indicated the presence of large size vitellogenic oocytes and hydrated oocytes (Fig. II D) which resulted the spawning stage of ovary (Table 1).

Similar observations were reported by Marino et al. (2003) in the histology of ovary using GnRHa implants were used in Dusky grouper, *Epinephelus marginatus*.In an attempt to study the pattern of reproduction in the Dusky grouper, *E. marginatus*, Marino et al. (2001) have carried out histological observation of ovary. Ovary was categorized histologically into seven stages. The GnRHa implants were able to promote the completion of vitellogenesis and induce final oocyte maturation and ovulation which was confirmed by migration of germinal vesicles towards the periphery of ova. Similarly Ya-Ju Tsai et al. (2011) observed oogonia, few primary oocytes, cortical alveoli and vitellogenic oocytes in aromatase inhibitor implanted *E. coioides*. In Swordfish, *Xiphias gladius* and the oocytes were classified into four stages of development based on histological examination as perinucleolar oocytes, previtellogenic oocytes, vitellogenic oocytes and hydrated oocytes (Arocha, 2002).

Maturity stages of testis of *C. mrigala*

The maturity stages of testis of *C. mrigala* were classified based on the spermatogenic cell types present in the testis.

a) Immature stage

Testis predominantly occupied by spermatogonia.

b) Spermatogenesis

The numbers of spermatocytes and spermatids continued to increase and a reduction in the number of spermatogonia were observed in the testis.

c) Spermiation

Testis contains predominantly spermatozoa.

d) Post spawning

Residual spermatozoa were observed in the testis.

Histological changes in the testis of control and hCG implanted fish

In the month of February, March and April the testis of control fish showed the presence of spermatogonia (Fig. III A, C & E), which indicates the immature stage of testis. During February, the hCG implanted fish revealed the presence of spermatogonia (Fig. III B), which also resulted the immature stage of testis.

During the month of March, the hCG implanted fish showed the presence of spermatogonium and primary spermatocytes (Fig. III D) indicating the maturing stage of testis. In the month of April the hCG implanted fish showed the presence of primary spermatocytes, secondary spermatocytes and spermatids (Fig. IV F) indicating the mature stage of testis.

In the month of May, the control fish showed the presence of primary spermatocytes, secondary spermatocytes (Fig. IV A) which indicates the matured stage of testis. In the same sampling period the hCG implanted fish showed secondary spermatocytes, spermatids, spermatozoa (Fig. IV B) which resulted in the matured stage of testis. In the month of June the control fish showed the presence of secondary spermatocytes, spermatids, spermatozoa (Fig. IV C) which indicates the matured stage of testis. During the same sampling period, the hCG implanted fish revealed the presence of spermatids and spermatozoa (Fig. IV D) which showed the spawning stage of testis (Table 2).

Similar results have been reported in histology of testis of Brown Bullhead catfish, *Ictalurus nebulous* with active spermiogenesis and the spermatids were seen filled with mature spermatozoa by Rosenblum et al. (1987), the histological changes in the testis of freshwater catfish, *Clarias macrocephalus* over a period of 12 months without hormone implantation was observed by Mollah. (1988) and he classified the testis into seven stages based on histological characteristics. Hence, HCG implantation induced faster maturation in female and male *C. mrigal*.

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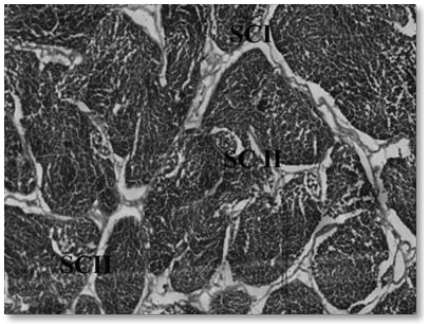
Table 1: Different stages of ovary in hCG implanted fishes during different sampling periods

S. No	Month	Changes	Stage of Ovary
1	February	Presence of perinucleolar oocytes and oogonia	Immature
2	March	Presence of previtellogenic and Vitellogenic oocytes	Maturing
3	April	Presence of vitellogenic oocytes	Matured
4	May	Presence of enlarged vitellogenic oocytes and hydrated oocytes	Fully Matured
5	June	Presence of large size vitellogenic oocytes and hydrated oocytes	Spawned

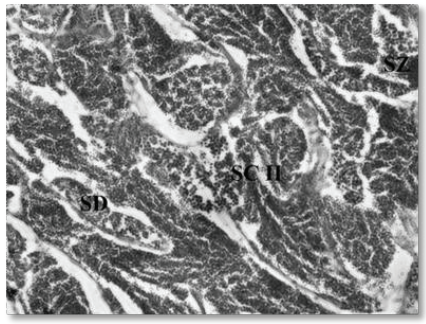
Table 2: Different stages of testis in hCG implanted fishes during different sampling periods

S. No	Month	Changes	Stage of Testis
1	February	Presence of spermatogonia	Immature
2	March	Presence of spermatogonium and primary spermatocytes	Maturing
3	April	Presence of primary spermatocytes, secondary spermatocytes and spermatids	Mature
4	May	Presence of secondary spermatocytes, spermatids, spermatozoa	Matured
5	June	Presence of spermatids and spermatozoa	Spawned

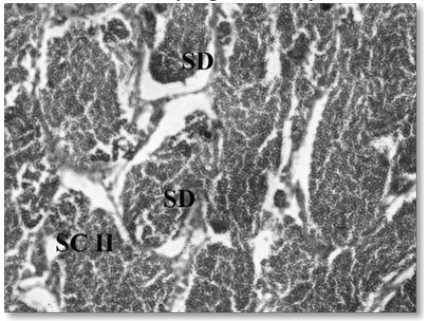
Fig. IV. Photomicrograph of histology of testis (20 X) from control and hCG implanted Mrigal *C. mrigala*



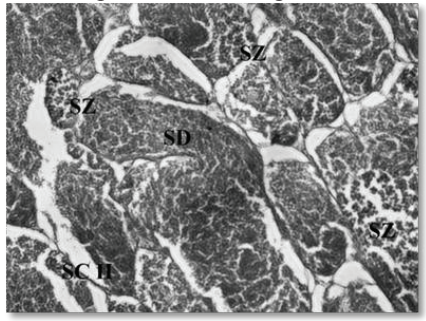
A. Testis-Control (May 2015)
SC I- Primary Spermatocytes; SC II- Secondary Spermatocytes



B. Testis- hCG Implanted Fish (May 2015)
SC II -Secondary Spermatocytes; SD- Spermatids; SZ- Spermatozoa



C. Testis-Control (June 2015)
SC II- Secondary Spermatocytes; SD- Spermatids



D. Testis- hCG Implanted Fish (June 2015)
SD- Spermatids; SZ- Spermatozoa

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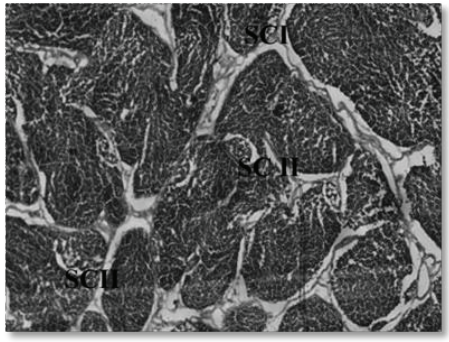

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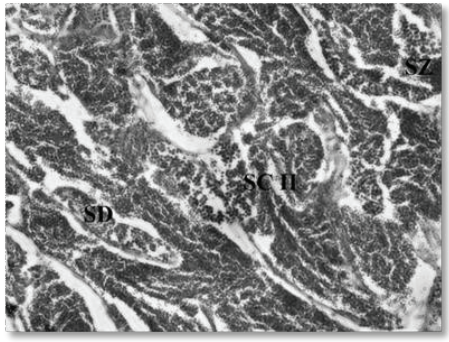

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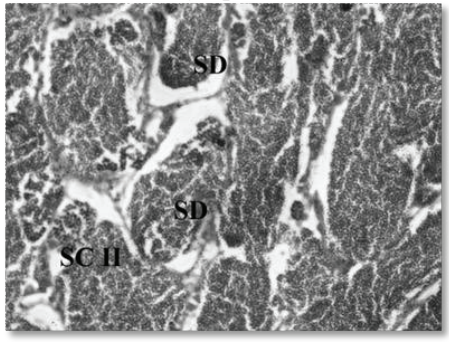
SC II -Secondary Spermatocytes; SD- Spermatids; SZ- Spermatozoa



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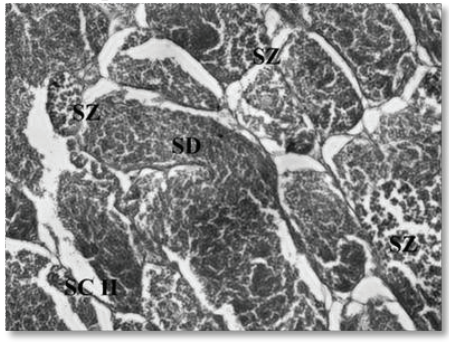

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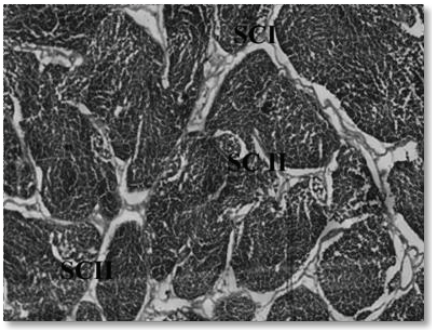
SD- Spermatids; SZ- Spermatozoa

Fig. IV. Photomicrograph of histology of testis (20 X) from control and hCG implanted Mrigal *C. mrigala*



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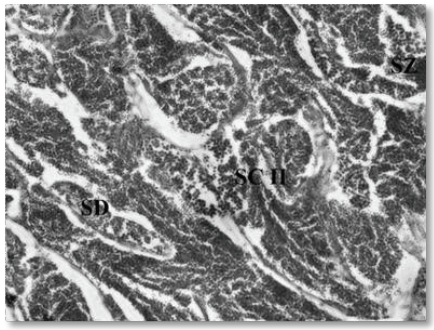

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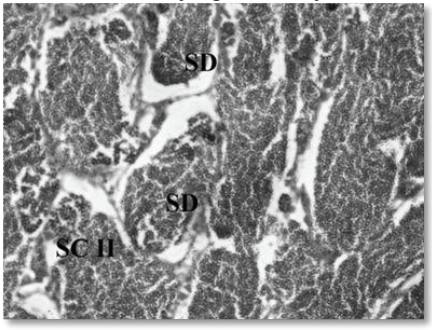
SC II -Secondary Spermatocytes; SD- Spermatids; SZ- Spermatozoa



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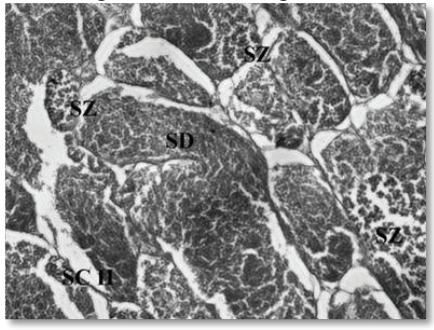

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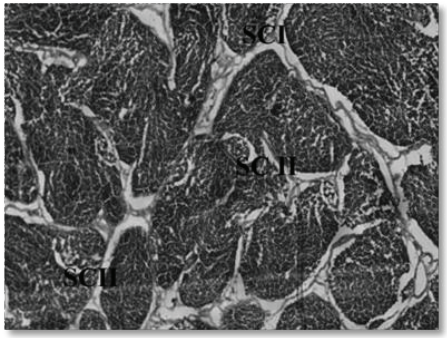
SD- Spermatids; SZ- Spermatozoa

Fig. IV. Photomicrograph of histology of testis (20 X) from control and hCG implanted Mrigal *C. mrigala*



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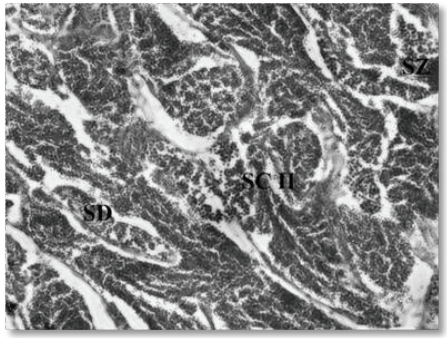

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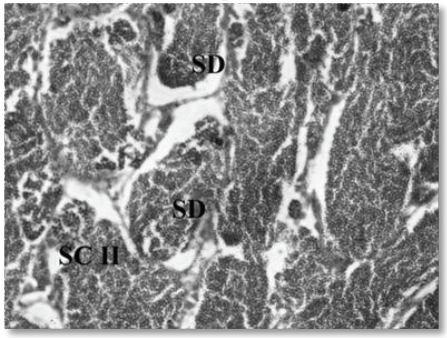

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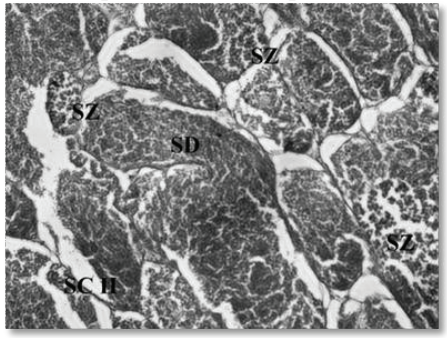

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