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Influence of Human Chorionic Gonadotropin on Maturation in Striped Murrel, *Channa striatus*

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Abstract

Gonadal development, gonadosomatic index (GSI), oocyte diameter, fecundity, histology and level of serum steroid hormones (testosterone and estradiol-17β) were observed in captive striped murrel (*Channa striatus* Bloch) implanted with human chorionic gonadotropin (HCG) capsules and cholesterol capsules as control for a period of five months after implantation.

HCG implants induced a significant increase in the GSI of male and female fish. The GSI values were highest in the fourth month (December) and lowest in the first month (September) in HCG implanted fishes. Variation in ova diameter of control and HCG implanted fishes revealed the presence of large size ova in HCG implanted fishes throughout the study period. Similarly, a significant increase in the fecundity of HCG implanted fishes was also observed. Fecundity was highest in December (15415 eggs) in HCG implanted fish, whereas only 2245 eggs were observed from control fish in the above sampling period. In control fish, only perinucleolar and primary oocytes were observed throughout the study period. Similarly, in testis, spermatogonia and few spermatocytes were observed till the end of the study period. Histological examination of the gonads of HCG implanted fish showed that numerous oogonia were present in the ovary after one month of HCG implantation which continued to develop into vitellogenic oocytes in the third and fourth months. The ovary in the fifth month of sampling of HCG implanted fish consisted of oocytes having completed the process of vitellogenesis. The testicular histology of HCG implanted fish showed primary spermatocytes in the first

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month and testis contained a large reserve of primary spermatocytes and spermatids in the second and third months. A large number of spermatids were observed in the testis in the fourth month of sampling and the testis in the fifth month of sampling predominantly contained spermatozoa. Serum testosterone level was highest ($18 ng \bullet ml^{-1}$) during the fourth month (December) in HCG implanted male fish and serum estradiol-17β level was highest ($61.5 pg \bullet ml^{-1}$) in the third month in HCG implanted female fish. The results of the present work can be used as a reference study for controlled breeding and reproduction of striped murrel, *C. striatus*.

Introduction

Murrels belonging to the Channidae family form unique group of food fishes in freshwater habitats of India (Jhingran 1982). Murrels are commercially cultured in Thailand, Philippines, Vietnam and Cambodia (Wee 1982). However in India, the culture of murrels is still not common due to lack of seed supply, non-availability of quality seeds from the wild, poor recovery from natural collection and limited success of breeding in captive conditions. Moreover, there is no established murrel seed production centre as that of major carps in India.

The striped murrel (*Channa striatus* Bloch), is a native freshwater fish of tropical Africa and Asia (Ng and Lim 1990). It is the most widely cultured species of murrel in many Southeast Asian countries (Ling 1977). It is highly predatory in habit and chiefly feeds on small fishes, tadpoles, insects and live foods in ponds. It grows up to 90 cm in length and sexual maturity is attained at the end of the first year of life. *Channa striatus* attains maturity in about eleven months under natural conditions (Alikunhi 1953). The breeding period of murrels in India is variable. Alikunhi (1953) reported that *Channa striatus* breeds from November to January in Southern India. On the other hand, in Northern India, it is from June to October with the peak from July to August (Qasim and Qayyum 1961). Breeding period of *Channa striatus* is during mid monsoon (ie) from June to August (Jyotsna et al. 1995). Murrels spawn naturally during southwest monsoon (June to August) and also during northeast monsoon (October to December) in flooded rivers and ponds in India (Alikunhi 1957). However breeding in captive conditions requires hormonal treatment for inducing gonadal maturation. Since many cultured species fail to breed under captive conditions, information on the factors affecting the gametogenic process is useful in developing methods to control reproduction under culture conditions. Generally, reproductive parameters and plasma profiles of gonadal steroid hormones are used in understanding the endocrine control of repro-

duction. Gonadosomatic index (GSI), gonad weight relative to body weight is normally used to assess the seasonality of gonad development in teleosts. Histological changes in the growth and development of the gonads are usually examined to assess the various stages. Hormonal induction of the maturation process requires a prolonged period (weeks to months) during which circulating levels of the administered hormone must remain elevated (Lee et al. 1986a). But, the widely used mode of chronically administering the hormone is through a multiple injection protocol. Improvements in slow hormone releasing devices provide an alternative mode of chronically administering hormones and decrease the amount of handling (Crim 1985).

Though works have been undertaken on the influence of hormone implants on gonadal maturation and spawning of fishes (Crim and Glebe 1984; Sherwood et al. 1988; Lee et al. 1992; Linhart et al. 1995; Sahu et al. 2000; Yeh et al. 2003; Kumakura et al. 2004), the use of HCG implants on gonadal maturity of fishes are very limited (Sahu and Sahoo 2000). Hence, in the present study HCG implants were used to enhance the gonadal maturation of *C. striatus* in captive condition.

Materials and Methods

Fish

Striped murrel, *C. striatus* with body weight ranging from 500 to 600 g were collected from culture ponds and then stocked in circular cement tanks, 3m in diameter and 1m in height. The fishes were maintained at ambient photoperiod (about 12L:12D) and temperature that fluctuated from 29° and 32°C and fed alternately with cooked chicken intestine and clam meat. The male and female fishes were stocked in separate tanks. In female *C. striatus*, urogenital opening is circular in shape while in males, it is elongated in contrast to that of the female (Dehadrai et al. 1973).

Preparation and implantation of HCG implants

HCG implants were prepared by compacting a mixture of 20,000 IU of HCG with 0.5 g of cholesterol (Huat 1980; Lee et al. 1986b). The dosage of HCG used in this study was 1000 IU•kg^{-1} of body weight which was already standardized for murrels (Francis et al. 2000). Based on the individual weight of male and female fishes, the HCG was packed in the

silastic capsules and implanted. Implants consisting of cholesterol only were used in the control male and female fishes.

Human chorionic gonadotropin (trade name Profassi) is in liquid form (20,000 IU). This liquid was mixed with 0.5 gram of cholesterol until a gel like consistency was attained and the resultant paste was incubated at 35^0C for complete drying (Huat 1980; Lee et al. 1986a). After drying HCG, Cholesterol mixture was powdered well for packing in silastic capsule. Since it was in powder form, it was packed in a silastic capsule and implanted.

Before implantation, the fishes were anaesthetized one by one in a 0.1% solution of benzocaine. Following anesthesia, a small incision was made near the dorsal musculature and the implants were implanted intramuscularly on the right side. After implantation, the wound was swabbed with oxytetracycline ointment. The wound healed within four days.

Monthly sampling of gonad from HCG implanted and control fishes (cholesterol implants without hormone) was carried out to assess maturation using various reproductive parameters.

Reproductive parameters

(a) Gonadosomatic index: After sampling, male and female fish from control and treatment (HCG implanted) were weighed on a weighing balance and the ovary and testis were weighed to the nearest 0.1g to calculate the GSI (gonad weight/body weight x100).

(b) Oocyte diameter: Oocytes were fixed in 1% buffered formalin and 100 oocytes from the anterior, middle and posterior ovary of each female fish were measured using ocular micrometry within the same day. The diameter of the oocytes was measured to the nearest 0.01mm. The mean diameter of 300 oocytes represents the oocyte diameter of each fish.

(c) Fecundity: Exactly 1g of the ovary was weighed, fixed in 5% buffered formalin and counted individually on the same day of sampling. Like this three samples were taken and the average value of three samples considered for fecundity calculation. Fecundity is the product of the total number of oocytes and weight of the entire ovary, divided by the body weight of each fish.

(d) Histology: Approximately 0.5 x 1cm mid portions of the ovary and testis were placed in neutral buffered formalin for 24 hours, processed

for routine histology and 5µm sections were stained with haematoxylin and eosin (Humason 1972).

(e) Steroid hormone analysis: Levels of testosterone in male and estradiol-17β in female fish serum were measured following enzyme linked immunosorbent assay kit.

Statistical analysis

Monthly mean values of GSI, ova diameter, fecundity, testosterone and estradiol-17β level of control and HCG implanted fishes were compared using analysis of variance at 1% significance level.

Results

The GSI values of control and HCG implanted male and female fishes are tabulated in tables 1 and 2. The GSI values of male and female fishes, before the start of experiment were 0.0767 and 1.32 respectively. In HCG implanted male fishes, it was found to increase gradually from 0.0999 (September) to 0.1845 (December) followed by a small decrease (0.1408) during the month of January. In control male fishes, GSI values were found to increase slightly from 0.0957 (September) to 1.0388 (November) followed by a decrease during December (0.0869) and January (0.0654). From table 2, it is evident that the GSI value of HCG implanted female fishes were found to increase from 1.8 (September) to 6.9 (December). The increase in the GSI value of female fish followed a similar trend as that of HCG implanted male fishes for a period of four months after HCG implantation. In the month of January, the GSI value decreased from 6.9 to 3.7. In control female fishes, GSI values were found to fluctuate during different sampling periods.

Table 1. Gonadosomatic index of control and HCG implanted male fishes

Sl. No.	Sampling period	Male GSI	
		Control (%)	Treatment(%)
1.	August (Initial)	0.0767	-
2.	September	0.0957	0.0999*
3.	October	0.0989	0.1109*
4.	November	0.1038	0.1606*
5.	December	0.0869	0.1845*
6.	January	0.0654	0.1408*

*P < 0.01

Table 2. Gonadosomatic index of control and HCG implanted female fishes

Sl. No.	Sampling period	Female GSI	
		Control(%)	Treatment(%)
1.	August (Initial)	1.32	-
2.	September	1.4	1.8*
3.	October	1.4	2.8*
4.	November	1.3	3.4*
5.	December	1.5	6.9*
6.	January	1.0	3.7*

*P < 0.01

The initial level of testosterone and estradiol-17β in the blood serum of fish were $0.2ng•ml^{-1}$ (Table 3) and $8pg•ml^{-1}$ (Table 4). The level of testosterone in male fish and estradiol-17β in female fish analysed during various sampling periods are presented in tables 3 and 4. Testosterone level in blood serum of HCG implanted male fishes was found to increase from $3.5ng•ml^{-1}$ (September) to $18ng•ml^{-1}$ (December). A sudden increase in the level of testosterone was observed after two months of HCG implantation. An increase in the testosterone level was observed in control male fishes for the first three months, followed by a fall in the level from December ($3.2ng•ml^{-1}$) to January ($2.4ng•ml^{-1}$). Significant differences ($P<0.01$) were observed between control and HCG implanted male fish. The Estradiol-17β level in HCG implanted female fish was found to increase from $34pg•ml^{-1}$ (September) to $61.5pg•ml^{-1}$ (November). A sudden fall in the level of estradiol-17β was observed during the months of December and January. The levels of estradiol-17β in the control and HCG implanted female fish were highly significant ($P<0.01$).

A section of the ovary taken before the start of the experiment (August) showed the presence of oogonia and perinucleloar oocytes, indicating the immature stage of ovary (Fig. 1). The ovary of HCG implanted fish sectioned after one month (September) of HCG implantation revealed the presence of perinucleolar oocytes and previtellogenic oocytes (Fig. 2) which indicated the maturing condition of the ovary. During the same sampling period, the ovary of control fish showed the presence of perinucleolar oocytes (Fig. 3) indicating the immature stage of ovary. Vitellogenic oocytes (Fig. 4) were observed in the ovary of HCG implanted fish during the month of October, but during the same period, late perinucleolar and previtellogenic oocytes (Fig. 5) were observed in the ovary of control fish. This indicated the mature stage of ovary of HCG implanted fish and immature stage of ovary of control fish.

Table 3. Testosterone level in blood serum of control and HCG implanted male fishes

Sl. No.	Sampling period	Testosterone level (ng/ml)	
		Control	Treatment
1.	August (Initial)	0.2	-
2.	September	1.8	3.5*
3.	October	2.2	3.6*
4.	November	6	15*
5.	December	3.2	18*
6.	January	2.4	10.2*

*P < 0.01

Table 4. Estradiol-17β level in blood serum of control and HCG implanted female fishes

Sl. No.	Sampling period	Estradiol-17β level ($pg \cdot ml^{-1}$)	
		Control	Treatment
1.	August (Initial)	8	-
2.	September	28	34*
3.	October	20.4	57*
4.	November	1	61.5*
5.	December	0.12	20.4*
6.	January	2	18*

*P < 0.01

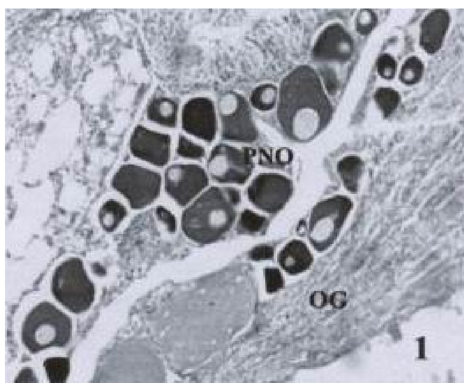

Fig. 1. Section of ovary collected during the month of August (Initial); PNO: Perinucleolar Oocyte; OG: Oogonia

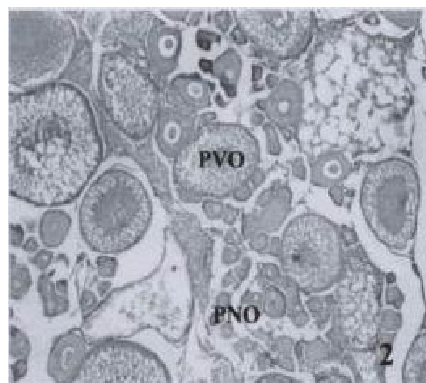

Fig. 2. Section of ovary collected during the month of September (HCG implanted); PVO: Previtellogenic Oocyte; PNO: Perinucleolar Oocyte

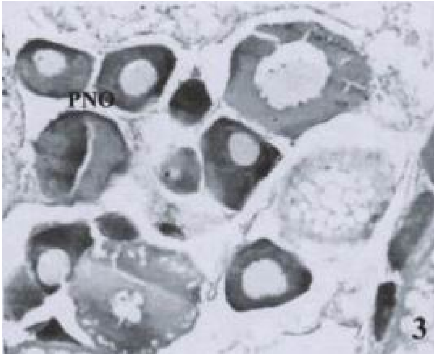

Fig. 3. Section of ovary collected during the month of September (Control); PNO: Perinucleolar Oocyte

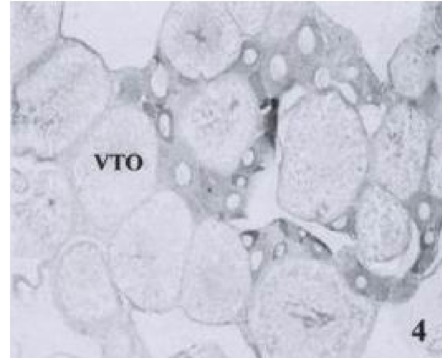

Fig. 4. Section of ovary collected during the month of October (HCG implanted); VTO: Vitellogenic Oocyte

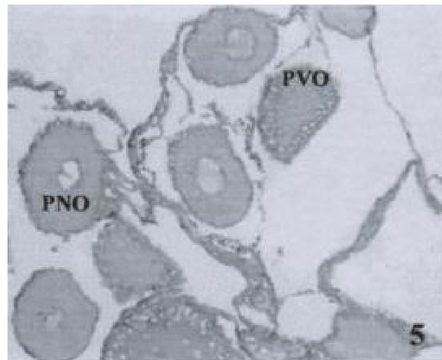

Fig. 5. Section of ovary collected during the month of October (Control); PVO: Previtellogenic Oocyte; PNO: Perinucleolar Oocyte

The histological section of the ovary during the months of November and December revealed the presence of hydrated oocytes and vitellogenic oocytes (Figs. 6 and 7) which indicated the presence of ripe stage of ovary. Ovary of control fish during the above period showed the presence of previtellogenic oocytes with vesicles (Figs. 8 and 9). This indicated the maturing stage of the ovary of control fish. In January, the section of the ovary of HCG implanted fish showed the presence of numerous hydrated oocytes and few post ovulatory follicles (Fig. 10) which indicated the presence of ripe stage of the ovary. Oocytes in the ovary of control fish observed during the same period were found to contain previtellogenic

oocytes of different sizes indicating the maturing stage of the ovary (Fig. 11).

The histological section of the testis showed the presence of numerous spermatogonia (Fig. 12) before the start of the experiment (August). During the months of September and October, the testis of HCG implanted fish showed the presence of spermatogonia, primary spermatocytes and secondary spermatocytes (Figs. 13 and 14). During the above sampling periods, the testis of control fish showed the presence of spermatogonia only (Figs. 15 and 16). The presence of spermatogenic cell types indicated a maturing stage of the testis of HCG implanted fish and immature stage of the testis of control fish.

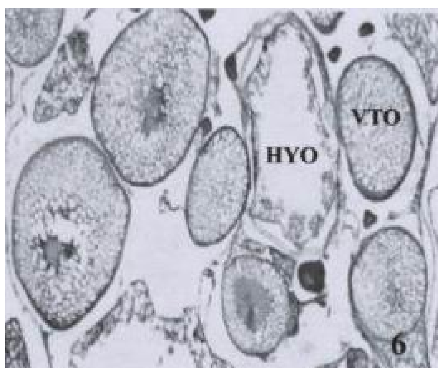

Fig. 6. Section of ovary collected during the month of November (HCG implanted); VTO: Vitellogenic Oocyte; HYO: Hydrated Oocyte

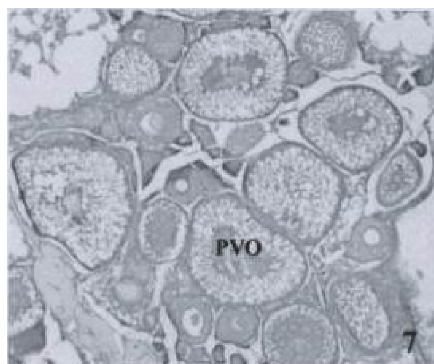

Fig. 7. Section of ovary collected during the month of December (HCG implanted); PVO: Previtellogenic Oocyte

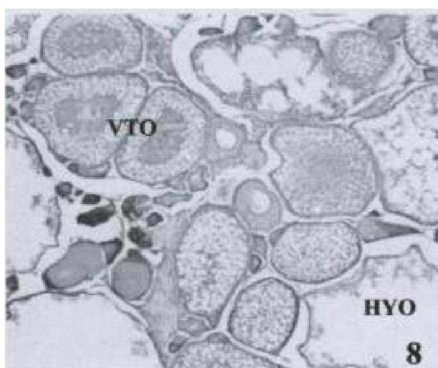

Fig. 8. Section of ovary collected during the month of November (Control); VTO: Vitellogenic Oocyte; HYO: Hydrated Oocyte

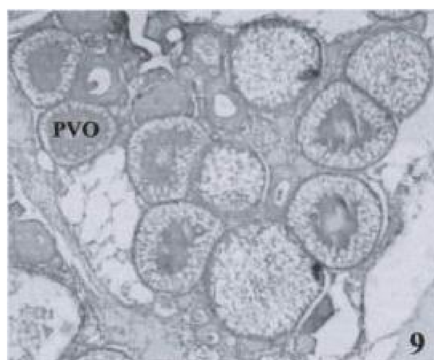

Fig. 9. Section of ovary collected during the month of December (Control); PVO: Previtellogenic Oocyte

The histological section of the testis of HCG implanted fish during the month of November showed the presence of secondary spermatocytes and spermatozoa (Fig. 17) indicating the mature stage of testis. In control fish, testis possessed spermatogonia and few primary spermatocytes (Fig. 18) during the same period which indicated the immature stage of the testis. Spermatids and spermatozoa were abundant in the testis of HCG implanted fish sectioned during the months of December and January (Figs. 19 and 20). During these two months, the testis of control fish showed the presence of spermatocytes (Figs. 21 and 22). This indicated the mature stage of the testis of HCG implanted fish and the maturing stage of the testis of control fish.

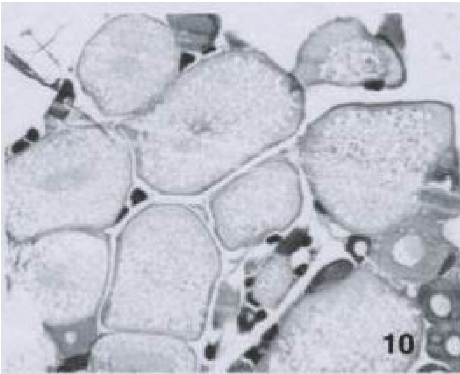

Fig.10. Section of ovary collected during the month of January (HCG implanted)

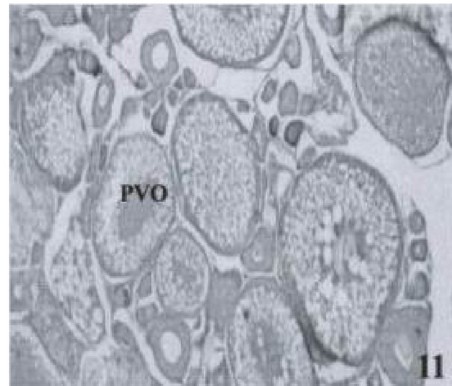

Fig.11. Section of ovary collected during the month of January (Control); PVO: Previtellogenic Oocyte

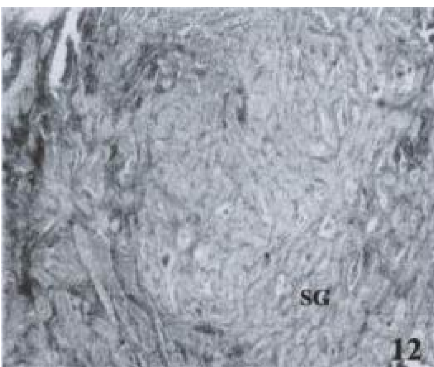

Fig.12. Section of testis collected during the month of August (Initial); SG: Spermatogonia

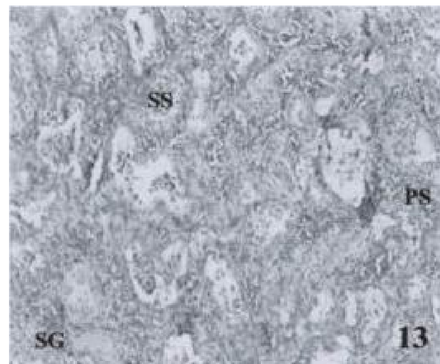

Fig.13. Section of testis collected during the month of September (HCG implanted); SG: Spermatogonia; SS: Secondary Spermatocyte; PS: Primary Spermatocyte

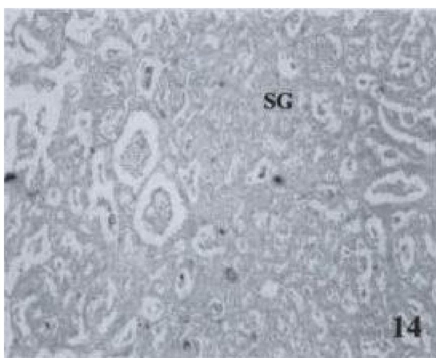

Fig.14. Section of testis collected during the month of October (HCG implanted); SG: Spermatogonia

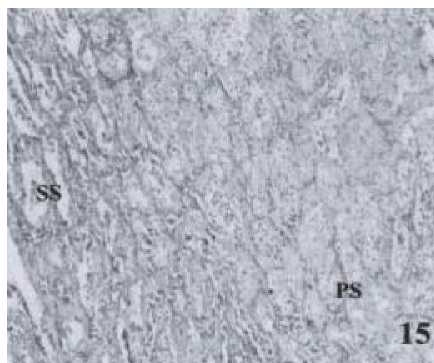

Fig.15. Section of testis collected during the month of September (Control); SS: Secondary Spermatocyte; PS: Primary Spermatocyte

Fig.16. Section of testis collected during the month of October (Control); SG: Spermatogonia

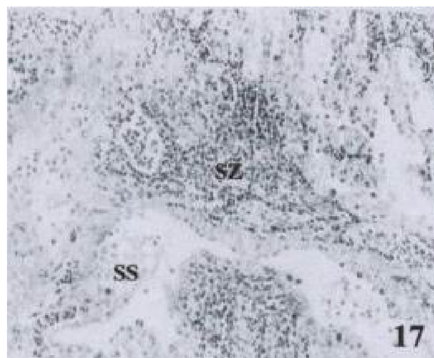

Fig.17. Section of testis collected during the month of November (HCG implanted); SZ: Spermatozoa; SS: Secondary Spermatocyte

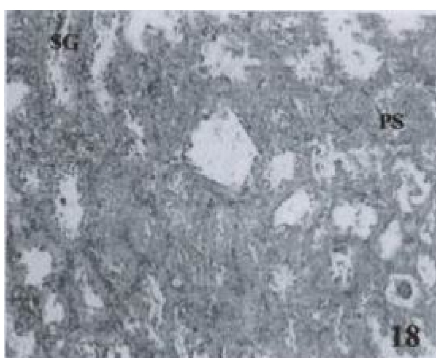

Fig.18. Section of testis collected during the month of November (Control); SG: Spermatogonia

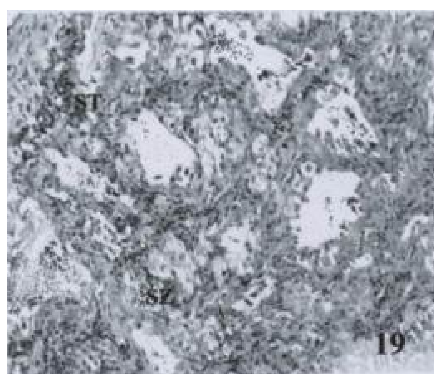

Fig.19. Section of testis collected during the month of December (HCG implanted); ST: Spermatid; SZ: Spermatozoa

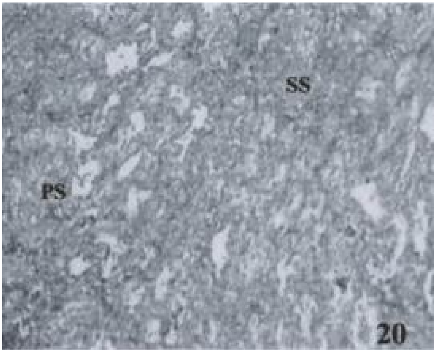

Fig.20. Section of testis collected during the month of January (HCG implanted); PS: Primary Spermatocyte; SS: Secondary Spermatocyte

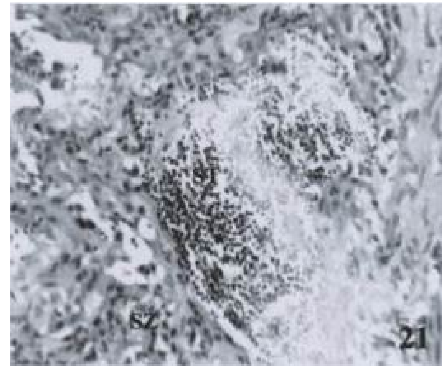

Fig.21. Section of testis collected during the month of December (Control)

Fig.22. Section of testis collected during the month of January (Control); SS: Secondary Spermatocyte; PS: Primary Spermatocyte

The fecundity of control and HCG implanted fish calculated for different sampling periods are presented in table 5. The fecundity of fish before the start of the experiment was 2714. Fecundity reached its highest during the month of December (15415) in HCG implanted fish. An increasing trend was observed in the fecundity of HCG implanted fish during the first four months of the sampling period. A highly significant ($P<0.01$) difference between the fecundity of HCG implanted and control fish (Table 5) was observed. The mean ova diameter of control and HCG implanted fish during different sampling periods are tabulated in table 6. The mean ova diameter of fish before the start of the experiment was 0.0755mm (August). Mean ova diameter of HCG implanted fish reached its peak during the month of December (1.0572mm). In HCG implanted fish, mean ova diameter was always higher than control fish during different sampling periods. The control fish attained its highest mean ova diameter during the month of November (0.9881). Significant ($P<0.01$) difference between the mean ova diameter of control and HCG implanted fish was observed (Table 6).

Table 5. Changes in fecundity of control and HCG implanted fishes during different sampling periods

Sl. No.	Sampling period	Fecundity	
		Control	Treatment
1.	August (Initial)	2714	-
2.	September	2978	3160*
3.	October	2730	9171*
4.	November	2554	10028*
5.	December	2245	15415*
6.	January	1239	5839*

*P < 0.01

Table 6. Changes in mean ova diameter of control and HCG implanted fishes during different sampling periods

Sl. No.	Sampling period	Mean ova diameter	
		Control	Treatment
1.	August (Initial)	0.0755±0.0541	-
2.	September	0.0786±0.1338	0.4545±0.0884
3.	October	0.3777±0.1345	0.9672±0.0873*
4.	November	0.7070±0.1311	1.0510±0.0529*
5.	December	0.9881±0.1121	1.0572±0.1407*
6.	January	0.4353±0.1098	0.8239±0.0887*

*P < 0.01

Discussion

It is interesting to note that the GSI values of HCG implanted male and female fishes were higher during all sampling periods. The GSI value in male and female fishes increased in similar trend for four months after HCG implantation. After HCG implantation, the GSI value of both male and female fishes attained its peak during the month of December which gradually decreased in the succeeding month (January). The slow increase in GSI value of male and female fishes is attributed to the slow release of HCG hormone from silastic capsule. The increase in GSI value may be due to the relatively slow growth of fish leading to the accumulation of fat and protein in the gonad as suggested by Basavaraja et al. (1989) in common carp, *Cyprinus carpio*. Changes in testosterone and estradiol-17β levels in the blood serum of control and HCG implanted and control fishes were analysed to understand the endocrine control of maturation in fish (Zairin et al. 1992). After HCG implantation, testosterone and estradiol-17β levels were high during the months of December and November and then slowly decreased. In HCG implanted fish, testosterone and estradiol-17β levels

were always higher than the control fish. This study confirms the findings of Zairin et al. (1992) and Zairin et al. (1993).

Histological examination of the ovary of *C. striatus* showed the presence of four types of oocytes namely perinucleolar, previtellogenic, vitellogenic and hydrated oocytes. Based on the presence of growing oocytes in the ovary, maturity stages of ovary were classified as immature, maturing, mature, ripe and spawned. From the above, it was observed that the HCG implantation induced maturation in female *C. striatus* after two months. The HCG implanted female *C. striatus* attained a mature condition in the second month (October) after HCG implantation and the presence of ripe stage of ovary was observed till the months of November, December and January. During the above sampling periods, control fish ovary was found to be in maturing condition. Induced breeding can be carried out when the fish ovary is in full ripe condition. Similar observation was reported by Marino et al. (2003) using GnRHa implant in captive reared dusky grouper, *Epinephelus marginatus*. The same observation was also reported by Morehead et al. (1998) in striped trumpeter, *Latris lineata*.

Histological examination of the testis of *C. striatus* showed the presence of five types of spermatogenic cells namely spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Based on the type of spermatogenic cells present in testis, maturity stages of testis were classified as immature, spermatogenesis, spermiation and post spawning. From the above classification, it was inferred that HCG implantation induced sustained maturation in male *C. striatus*. The HCG implanted *C. striatus* were found to be in mature condition for the first four months after HCG implantation. The above four month period also coincided with the mature condition of the HCG implanted female fish. Breeding and seed production of *C. striatus* can be successfully carried out throughout the year if the broodstock are developed by HCG implantation. In comparison, the gonadal maturation process in control male fish is observed to be slow. Similar observation was also reported in common tench (*Tinca tinca*) induced with implantation of GnRH anlogues (Linhart et al. 1995). Zanuy et al. (1999) made an similar observation in seabass, *Dicentrarchus labrax* induced by testosterone implantation.

Conclusion

The present investigation was undertaken to ascertain the influence of human chorionic gonadotropin (HCG) implants on gonadal maturation of striped murrel, *Channa striatus* in captivity. The implantation of human chorionic gonadotropin implants at 1000 $IU \bullet kg^{-1}$ of body weight in *C. striatus* accelerated maturation significantly compared to control. The HCG implanted fishes attained maturation within two months and continued to be in matured condition till the end of the experimental period. The ovary of HCG implanted fish showed the presence of advanced stage of growing oocytes throughout the experimental period when compared to control. Similarly, the advanced stage of spermatogenic cell types were observed in the testis throughout the experimental period in HCG implanted fishes when compared to control. Hence, HCG implantation induced faster maturation in female and male *C. striatus*.

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