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Research Article

EFFECT OF PINEALECTOMY AND MELATONIN EFFECT ON GONADAL DEVELOPMENT OF CATFISH (*HETEROPNEUSTES FOSSILIS)* DURING POSTSPAWNING PERIOD

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ABSTRACT

Pinealectomy (Px) and Melatonin treatment effect was examined in the male and female catfish *Heteropneustes fossilis* for 60 days. This experiment was done in post spawning period of the reproductive phase of catfish. The results of this study revealed that pinealectomy (Px) causes inhibitory effects on the GSI and the histology of the male catfish, while in the female catfish stimulatory effects on both was observed. In histological study of the female catfish stage I oocytes were present in the ovary of the control group whereas catfish administered with low (25, 50 mg) and high doses (100, 200, 400 mg) of melatonin very less number of stage I oocytes were seen but the percentage of atretic oocytes were increased when the melatonin doses were increased. The present study showed that the melatonin dose causes follicular atresia in female catfish during the post spawning period. Histological studies of male catfish showed large number of remnants of spermatozoa (rsz) in the testes of the control group while very little elevation of rsz were seen in the somniferous lobules with the increased in the melatonin dose. The result suggested that the effect of pinealectomy and melatonin treatment is mediated through an action of hypothalamo-hypophyseal gonadal axis. In conclusion, the melatonin played an important role to control the gonadal maturation in the catfish *H. fossilis* during the post spawning period.

Keywords: Catfish, Gonads, Melatonin, Pinealectomy.

INTRODUCTION

Reproduction is a part of life cycle and is regulated by the gonadotropin and steroid hormones, seasonal changes in photo-thermal regimes (Shimizu, 2003; Sundararaj & Vasal, 1976). Pineal gland releases the melatonin hormone which has the ability to inhibit the production of gonadotropin from the pituitary gland. A low amount of gonadotropin regulates maturity development. The blood melatonin level increased by the implantation of melatonin pellets and the melatonin surge indirectly regulates the maturation of the ovaries in the blood (Chattoraj *et al.*, 2005). Melatonin production is indirectly regulated by light-dark cycle and melatonin hormone inhibits the maturation of the gonad when released in excessive amount. The changes in the temperature influence the melatonin amplitude rhythms and causes seasonal variations in the melatonin amount (García *et al*., 2001; Iigo & Aida, 1995). From previous studies it has been shown that melatonin administration decreased the food intake (De Pedro *et al*., 2008; Lopez *et al*., 2006; Pinillos *et al*., 2001; Rubio *et al*., 2004) and hence due to this the body weight and growth rate were decreased in *Oncorhynchus mykiss* (Taylor *et al*., 2005). Further it was clear by Pinillos *et al.* (2001) that melatonin hormone treatment inhibited the food intake, but only after i.p. and by modulating noradrenergic metabolism in the hypothalamus decreased

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the specific growth rate and body weight in the goldfish (De Pedro *et al*., 2008).

In *H. fossilis* the ovarian development was retarded in short photoperiods but stimulated under long photoperiods (Sundararaj, 1981) which was contrary to the concept that melatonin secreted during dark hours. Other worker research was in favour of this work where melatonin secretion in all the species is high during night and low during the day (Collin *et al*., 1989). In *Clarias batrachus* melatonin treatment at the dose 100 and 200 mg/fish reduced testosterone levels, but high dose of melatonin 400 mg/fish accelerate the testosterone levels (Singh *et al*.,1994). Pinealectomy effects depending on the season of the reproduction and the species. In resting phase, pinealectomy causes gonadal recrudescence while during preparatory phase increases the gonadal maturation and during prespawning, spawning and early postspawning period pinealectomy had no effects on the gonadal maturation in *Clarias batrachus*. Pinealectomy affect the ovarian activity and vitellogenin level during preparatory and postspawning periods (Garg, 1989). Pinealectomy causes antigonadal, progonadal and no effect when exposed to different photoperiods in the female catfish during the different reproductive seasons (Nath, 2005; Nayak & Singh, 1988). In this study, an attempt has been made to find out the effect of melatonin and pinealectomy on the growth and the gonadal maturation of the catfish *H. fossilis* during the posts pawning period of the reproductive phase.

MATERIAL AND METHODS

Experimental Area

This experiment was performed in the Fisheries / Animal Biology and Behaviour Laboratory, Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana, India.

Experiment Set Up

Male and female *H. fossilis* of (mean body weight 20-50gm) were imported from Sultan Aqua Integrated Education and Research Foundation V.P.O. Nilokheri Karnal, Haryana. The experiment was carried out for 60 days and the fishes were acclimatized under laboratory conditions, photoperiod (LD 12:12) and temperature 25 ± 1˚C for 15 days before the experiment was started. The water of the beach aquarium was changed with the stored fresh tap water every day and then initial weight of the fishes were calculated and the fishes were feed with formulated feed in the morning (at 8: AM) and in the evening (at 5: PM). For this experiment 14 aquariums were used and 15 fishes were placed in each glass aquarium (60×30×30 cms).

Pinealectomy (Px) procedure

Pinealectomy (Px) of fishes was done by following procedure of (Francis *et al*., 2004; Rani & Sabhlok, 2014).

Melatonin solution

Melatonin solution was prepared by following the procedure of (Francis *et al*., 2004; Nath, 2005) for this melatonin was dissolved in (2, 4, 8, 16 and 32 ml) of 100% ethanol and diluted with teleost saline (20 mg Na_2Co_3/100 ml of 0.6% NaCl). Hence melatonin solutions of concentration 25, 50, 100, 200, and 400 mg were prepared. The I. P. injection of melatonin was given to the fishes three times in a week in afternoon and every week fresh solution were prepared.

Experimental Procedure

Total three groups were planned control, pinealectomized and melatonin administered group. Melatonin injection was given to the fishes according to (Singh *et al*., 1994) with small manipulation. The I.P. injection of melatonin was given to the fishes by following the procedure of (de Vlaming *et al*., 1980). The melatonin dose was injected to the fishes with the help of a 0.5 ml syringe three times in a week. After 60 days of the experiment final weight of the fishes were calculated. Following parameters were calculated:

Live Weight Gain (g) = Final weight W_2 - initial weight W_1

Gonadosomatic Index (GSI)

$$GSI = \frac{\text{Gonad weight}}{\text{Body weight} \times 100} \times 100$$

Statistical Analysis

One-way ANOVA (analysis of variance) following by the Tukey's honest significance test were used to analyze the data.

Histological examination

After 60 days of the experiment the fishes were anesthetized and gonads were transferred in 10% formalin solution and then dehydrated in the different grades of ethanol 30%, 70%, 90% and absolute. The gonads were

fixed in paraffin wax and the sections of ovary (7 µm) and testes (5 µm) were cut, dewaxed in xylene and again dehydrated in ethanol. The sections then stained in haematoxylin and again dehydrated in ethanol and stained in eosin. After eosin staining the sections of gonads were mounted in Canada balsam. Histology of ovary and testes were done by following the method of (Singh *et al*., 2012) with small manipulations. Oocyte stages and atretic follicles were characterized by following (Nath, 2005). Spermatogonia were studies by Mokae *et al*., (2013). Spermatozoa were identified by Dziewulska & Domagala, (2003).

RESULTS AND DISCUSSION

In results of this study final body weight of the male and female catfish of the control group were increased whereas in melatonin administered fish it was decreased (Table 1). The GSI of the female catfish of melatonin group increased at low doses (25, 50 and 100 mg) but decreased when the melatonin dose were increased (200 mg and 400 mg) but it had significant value as compared to that of the control group.

The GSI of the male catfish administered with the melatonin dose were significantly high as compared to that of the control group (Table 1). Histological changes in the ovary (Figure 1) and testes (Figure 2) of the melatonin treated, pinealectomized and control group were observed in catfish *H. fossilis* during the postspawning period of reproductive growth.

Control group

In histological examinations of the ovary of the female catfish in control group was regressed and contained only the stage I oocyte. The stage II and stage III oocyte were not present in this group. The atretic oocytes were also not recorded in control group (Figure 1). Microscopic studies revealed that the testes of the control group showed large number of spermatogonia and rsz (Remnants of Spermatozoa) (Figure 2).

Melatonin administered group

In the post spawning period as the melatonin dose were increased, the number of stage I oocytes were reduced. The stage III oocytes were present only in the fishes which were treated with the low melatonin dose (25 and 50mg). Different results for atretic oocytes were observed i.e. the number of the atretic oocytes were increased when the melatonin dose were increased (Figure 1). The histological studies of the melatonin treated male catfish testes revealed that no significant changes were observed in melatonin injected group except the rsz were maximum in melatonin injected group as compared to control group.

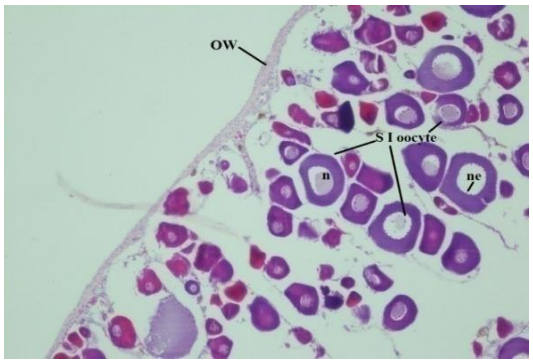
Pinealectomized group

The GSI of the pinealectomized female catfish were increased as compared to the control group. The pinealectomy during the postspawning period in the female catfish stimulate the ovarian development as compared to the control group. The female pinealectomized catfish showed a large number of stages I oocytes and some vitellogenic oocyte (Figure 1). The atretic oocyte was also found in the ovary of the pinealectomized female catfish. The GSI of the pinealectomized male catfish were reduced then that of control group. Histological examinations of the testes of the Pinealectomized group of male catfish the number of spermatozoa were counted lower than that of the control group during posts pawning period (Figure 2).

Table 1. Effect of low and high doses of melatonin and pinealectomy on body weight and GSI of catfish *Heteropneustes fossilis* under laboratory conditions during posts pawning period.

Parameters	Treatments						
	1 (control)	2 (Px)	3 (25 mg) MET/fish	4 (50 mg) MET/fish	5 (100 mg) MET/fish	6 (200 mg) MET/fish	7 (400 mg) MET/fish
Initial weight	23.29	23.86	25.43	27.79	27.15	25.57	27.16
Final weight	23.41	24.45	24.70	27.61	26.29	25.52	26.83
Live weight gain	0.12	0.59	-0.73	-0.18	-0.86	-0.05	-0.33
GSI (Female)	1.78 ± 0.12	2.87±0.05	4.06 ± 0.08	2.46 ± 0.11	1.82 ± 0.05	1.36 ± 0.12	1.14 ± 0.05
GSI (Male)	0.62 ± 0.01	0.60 ± 0.01	4.33 ± 0.06	4.49 ± 0.07	5.40 ± 0.03	5.53 ± 0.04	5.61 ± 0.03

All the values are mean ± S.E. of mean. (Px) Pinealectomized, (MET) Melatonin dose.



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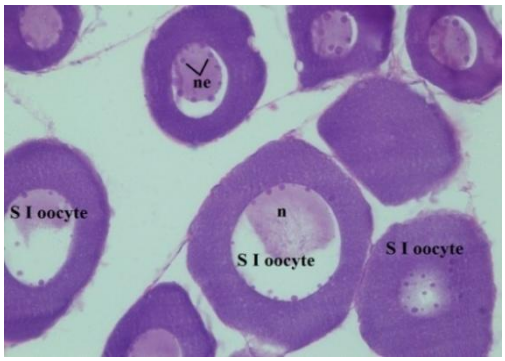

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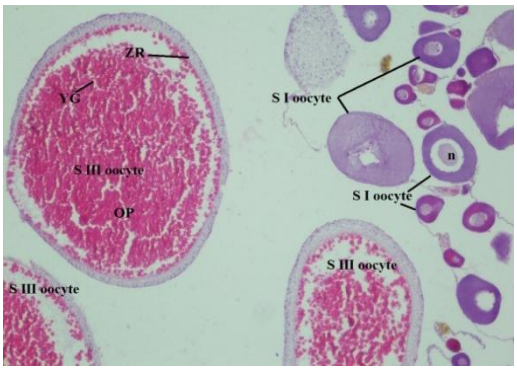
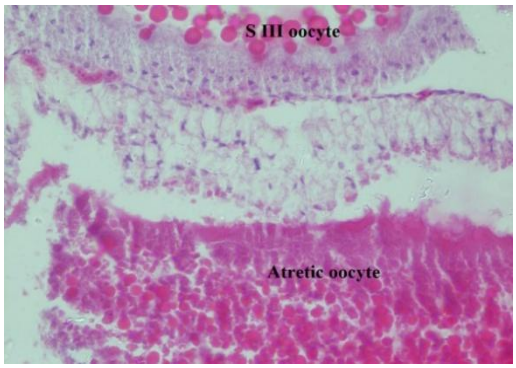


B (i)



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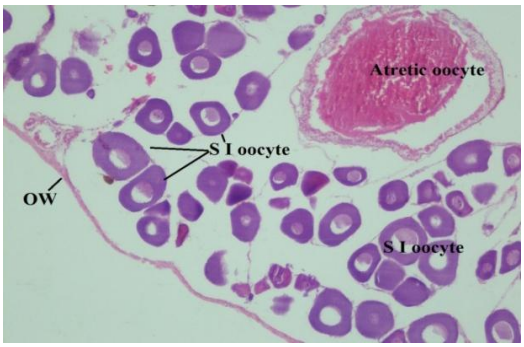

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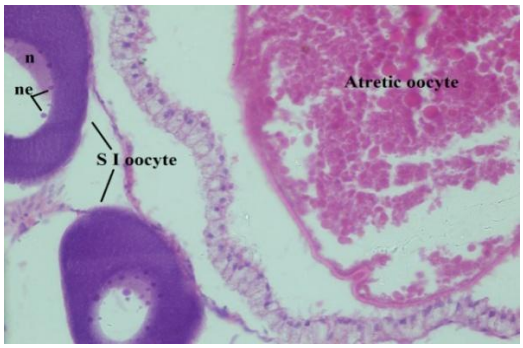
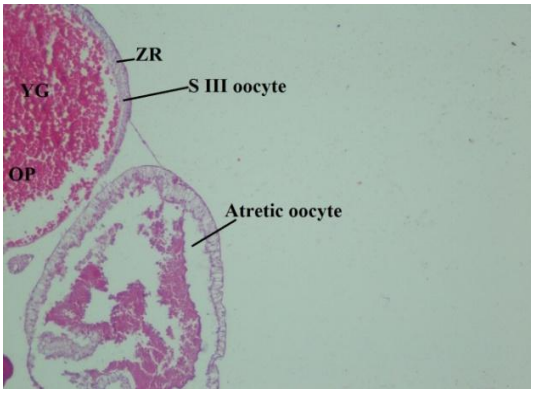
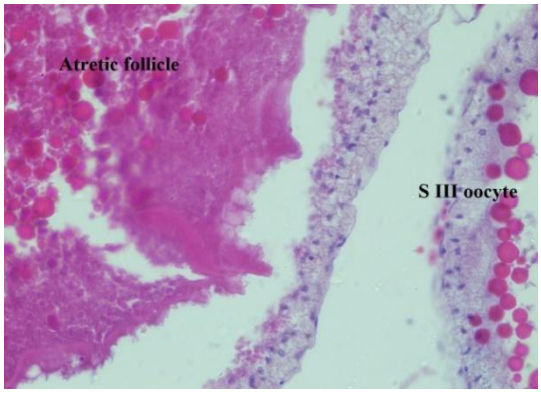


C (ii)



D (i)



D (ii)

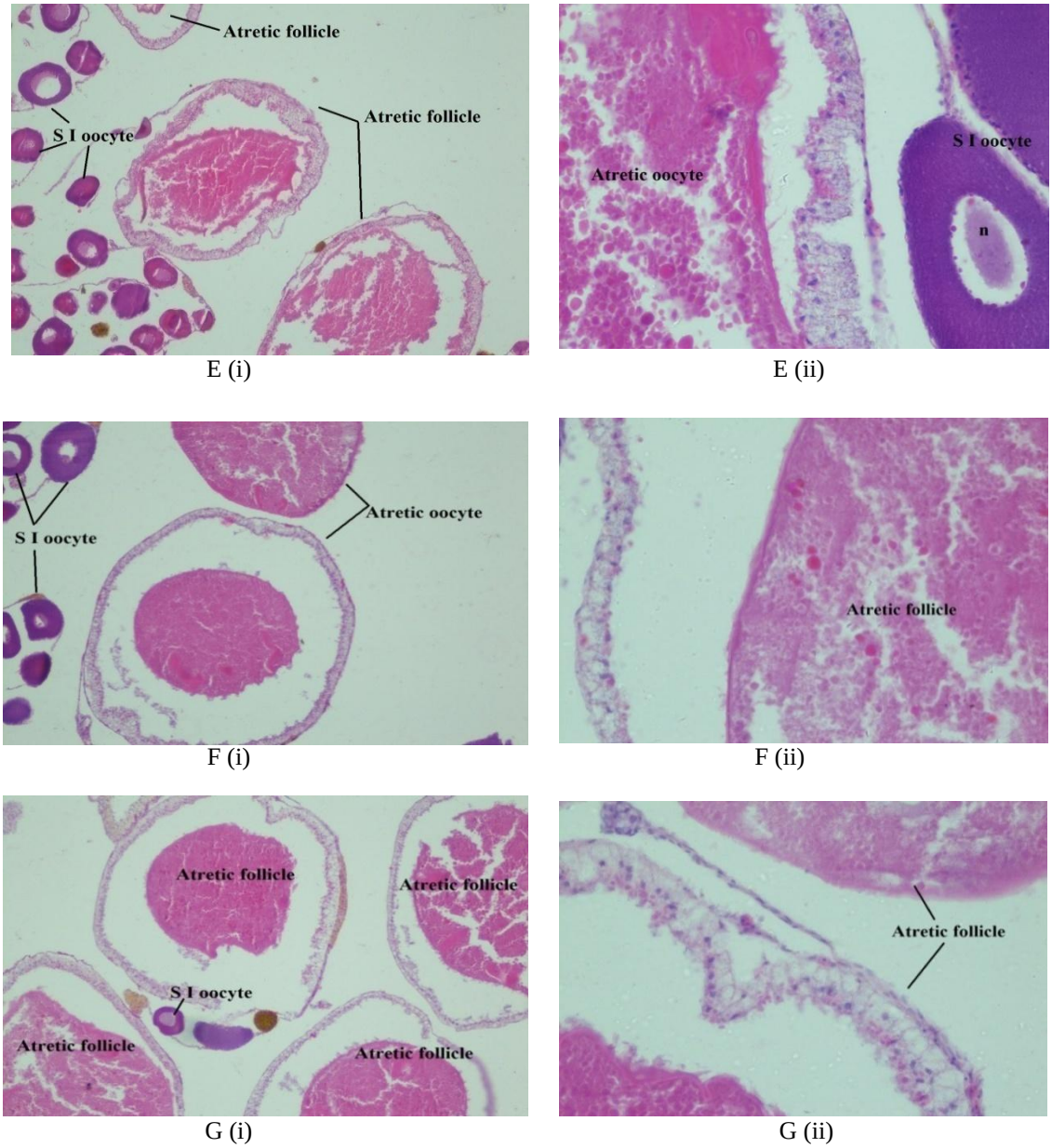


Figure 1. Light micrograph of Px and MET received *H. fossilis* ovaries during post-spawning period shows different stages of development (H&E stain ×100 & 400X).

(A) T.S. of ovary of control group shows only S I oocyte (large central nucleus bearing ring of nucleoli).
(B) T.S. of ovary of pinealectomized group shows maximum numbers of S I oocyte and some S III oocyte (increasing number of yolk granules throughout the ooplasm) and atretic oocyte.
(C) T.S. of ovary of melatonin treated group 25mg MET/fish shows maximum numbers of S I oocyte and less number of atretic oocyte.
(D) T.S. of ovary of melatonin treated group 50mg MET/fish shows some S III oocyte and increasing number of atretic oocyte.
(E) T.S. of ovary of melatonin treated group 100 MET/fish shows large number of S I oocyte and atretic oocyte.
(F) T.S. of ovary of melatonin treated group 200 mg MET/fish shows decreasing numbers of S I oocyte and more number of atretic oocyte.
(G) T.S. of ovary of melatonin treated group 400mg MET/fish shows very few number of SI oocyte and maximum number of atretic oocyte.
ZR- zona radiata, YG- yolk globules, OP- ooplasm, n- nucleus, ne- nucleoli.

(i) at 100X, (ii) at 400X.

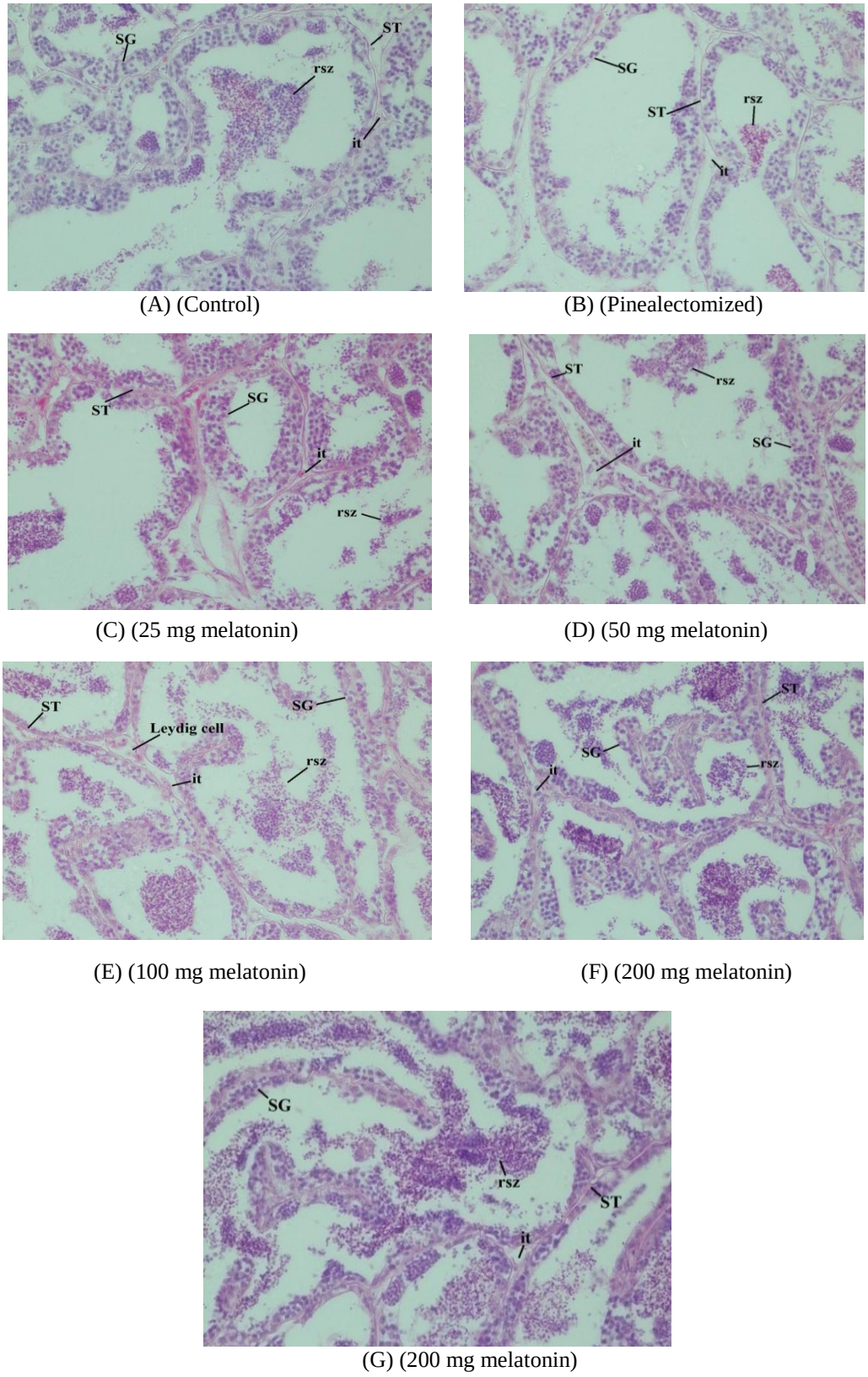


Figure 2. Light micrograph of pineal removal and melatonin dose received *H. fossilis* testes during postspawning period shows different stages of development of testes (H&E stain ×400).
(A) T.S. of testes shows more spermatogonia and remnants of spermatozoa.
(B) T.S. of testes shows reduced number of spermatogonia and remnants of spermatozoa.
(C-G) T.S. of testes shows seminiferous lobules are filled with remnants of spermatozoa.
SG- spermatogonia, ST- seminiferous lobules, rsz- remnants of spermatozoa, it- interstitial tissue.

The final body weight was decreased in melatonin administered fishes when compared to that of the control group in *O. niloticus* (Singh *et al*., 2012). In a study of (Nath, 2005) during posts pawning period the ovary of catfish *Clarias batrachus* had only stage I oocyte and the ovary was regressed and the melatonin causes inhibitory role on the ovarian development The female catfish treated with melatonin showed atresia in findings of (Singh *et al*., 2012; Sundararaj & Keshavanath, 1976) observed that melatonin causes inhibitory effect on the GSI and ovarian maturation in *O. niloticus*. In *Channa punctatus* melatonin dose 100 µg/L for 24 and 17 h daily increased the GSI but when the same dose was given to the fish through injection of melatonin GSI decreased (Renuka & Joshi, 2010). In turbot female melatonin implants 18 mg has been reported to suppress the gonadal maturation (Alvarino *et al.*, 2001). Nayak *et al*. (1987) stated that the pinealectomy increased the GSI during January-may in *Clarias batrachus* which co-exist with *H. fossilis* and causes stimulatory effect on the ovarian development. Pinealectomy of the male catfish inhibited the testes weight increase as compared to the control. The histological results of the testes of the pinealectomized catfish decreased the number of spermatozoa and cause inhibitory effect on the testes development of the *H. fossilis* during the post-spawning phase of the reproductive cycle when compared to the control (Garg, 1987). In the present study the follicular atresia was maximum in the melatonin administered female catfish and the number of atretic oocyte was increased in the posts pawning period of the female catfish treated with the melatonin dose. The male catfish treated with melatonin dose showed no significant changes in the histological examination during the posts pawning season in *Catla catla* (Bhattacharya *et al*., 2007) these results are in agreement to the present study. In (Aripin *et al*., 2015) find out that *C. macrocephalus*, when given melatonin dose enhances the maturation of testes and spermatozoa. No significant changes in the testes of *Catla catla* was noted in any group among the natural photoperiod, long photoperiod and short photoperiod during the posts pawning period (Bhattacharyya *et al*., 2005).

CONCLUSION

The results of the present study provide the information that melatonin treatment regulates the ovarian development. The pinealectomy causes inhibitory effect in the testes maturation whereas pinealectomy causes stimulatory action on ovary. The melatonin administration in male catfish causes no significant changes in the testes development but the low doses of melatonin in female catfish causes stimulatory effects on the ovary while the high doses of melatonin causes the follicular atresia during the post spawning period of the reproductive growth.

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