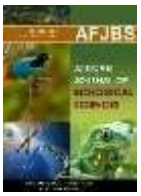
https://doi.org/10.48047/AFJBS.6.13.2024. 3224-3238

African Journal of Biological Sciences



Effect of Exogenous Melatonin on Ovarian Function in Tamoxifen Exposed *Mystus vittatus* Under Varied Photoperiods

K Renuka*

*Pineal Research Lab, Davangere University, Davangere – 577 007 Karnataka India renuckhaple@gmail.com , renukhaple@davangereuniversity.ac.in

Article History

Volume 6, Issue 13, 2024

Received: 18June 2024

Accepted: 02July 2024

doi:*10.48047/AFJBS.6.13.*2024. 3224-3238

Abstract:

Melatonin (MLT) modulates physiological events including reproduction in fishes. However, its mode, time and duration has varied impact on fish reproduction. This study aimed to observe the effect of Tamoxifen citrate (TCT) and / or MLT on *Mystus vittatus* held either long photoperiod or continuous light. Fish were held in long photoperiod (L:D 14:10) and Continuous light (LL or LD: 24:00). Fish were exposed to TCT(Tamoxifen citrate treatment), MLT (Melatonin), MLT+TCT (100 µg /l of water) for 15h daily and MLT for 24h daily with appropriate controls. The GSI (Gondaosomatic index = gonadal wt/100 g body weight) significantly ($P<0.01$) reduced in all the groups in LL compared to L:D 14:10. TCT induced ovarian degeneration irrespective of photoperiods. Further follicular kinetics studies revealed that Pre-Vitellogenic follicle(PVF) was sparse in TCT exposed animals in L:D 14:10, this number increased in LL. PVF reduced ($P<0.01$) in MLT+TCT in long photoperiods but increased ($P<0.01$) in LL. MLT CE in both photoperiod fails to increase PVF counts. MLT+TCT exposure combats the Antiestrogenic effect of TCT which is evident through GSI and increased VF in both photoperiod with higher impact in L:D 14:10 compared to TCT exposures. LL stimulated VF under limited exposure of MLT (15h). Atretic follicle (AF) were fewer in MLT+TCT 17:00 in both photoperiods but MLT CE has higher atresia of follicles compared to MLT (15h) in both photoperiods. This study demonstrates that photoperiodic conditions have effect on TCT as well as MLT depending on duration of exposure/s.

Keywords: Photoperiod, Melatonin, Tamoxifen citrate, Follicular kinetics, *Mystus vittatus*

INTRODUCTION:

Reproduction in most fishes is seasonal or periodic and spawning occurs in an appropriate season to ensure maximum survival of the offspring. The sequence of reproductive events in an annual cycle is largely under the control ofspecies-specific endogenous timing system which essentially relies on a well- equipped physiological response mechanism to changing environmental cues. Reproduction in fishes has been affected by several internal and externalfactors conveyed to the fish through neuroendocrine axis. It is prominent in fishes, that retinal and non-retinal receptors work in signal transduction and gives cues to the species about the environmental conditions: depending onthis information reproductive axis gets stimulated or inhibited (K Renuka and BN Joshi, 2014). Melatonin, an indoleamine secreted by the pineal gland is synthesized by different tissues and organs other than pineal gland and recent findings suggest that melatonin is likely produced in mitochondria of everycell (Reiter *et al.*, 2017). There has been huge data that suggests melatonin entrains the biological rhythms in fishes. Estrogen plays a vital role in the process of reproduction. Alteration in estrogen or its pathway leads to alteration in ovarian function. The effect of melatonin on estrogen or its pathway/s in fish are sparse. Hence the present study was aimed to address this lack of knowledge about the effects of melatonin administration at different timings on Tamoxifen citrate (TCT Antiestrogen) exposed *Mystus vittatus* ovarian function through follicular kinetics study.

MATERIAL AND METHODS:

Mystus vittatus were purchased from local fish vendor and held for acclimatization to laboratory conditions in aquarium for 15days prior to experiment. During the period of acclimatization, the animals were dipped for 2 seconds in $KMnO_4$ (5mg/20l of water) as prophylactic. Post acclimatization, seventy fishes were selected and divided into ten groups and held in aquaria containing 20 litres of water. Fishes selected were of 8 ± 1gm in weight and these fishes were fed with Taiya discovery fish food and water was changed daily twice (at 08:00 hrs and 17:00 hrs). The animals were led in wooden

chamber fitted with chronometer for L:D 14:10 and L:D 24:00 (LL) with proper aeration.

The experimental groups held in L:D (14:10) were divided as follows:

Groups	Experimental conditions	Time of exposure (hrs)	No: of animals
I	Control		7
II	Melatonin (MLT 17:00 100µg/l of water)	17:00	7
III	Tamoxifen citrate treated (TCT 17:00100µg/l of water)	17:00	7
IV	Melatonin+Tamoxifen citrate (MLT + TCT 17:00 100µg/l of water)	17:00	7
V	Melatonin Continuous (MLT CE 100µg/l of water)	Continuous	7

The experimental groups held in L:D (24:00) or LL were divided as follows:

Groups	Experimental conditions	Time of exposure (hrs)	No: of animals
VI	Control		7
VII	Melatonin (MLT 17:00 100µg/l of water)	17:00	7
VIII	Tamoxifen citrate (TCT 17:00 100µg/l of water)	17:00	7
IX	Melatonin+Tamoxifien citrate (MLT+TCT 17:00 100µg/l of water)	17:00	7
X	Melatonin Continuous (MLT CE 100µg/l of water)	Continuous	7

Experiment duration: The experiment was conducted for a period of 45 days. After the experimentation period the animals were sacrificed under cold anesthesia and ovaries were dissected out and weighed using 8068 –series of Professional digital scale (weigh machine) readability 0.001g and immediately fixed in Bouin's fluid and later on embedded in paraffin wax for histological studies. Five-micron thick sections were stained in haematoxylin and eosin. Follicular kinetics was studies in histological sections of the ovaries. The

follicles were classified as previtellogenic (PVF), vitellogenic (VF) and atretic (AF) follicles. Seven fishes from each group were used for this purpose. The counting of the follicles was done in twenty slides five from anterior, five from posterior and ten from middle portion of each fish ovary were used for the same. The statistical analysis was done by using Sigma plot 11 software for one – way ANOVA Schiff's Pairwise comparison test. The gondaosomatic index (GSI): Ovarian weight per 100gram body weight) was calculated for each group for comparison.

RESULTS:

In the first experiment where the animals were held in long photoperiod L:D 14:10, the GSI of all the groups decreased significantly ($P<0.01$) Graph -1(a) when compared to control group. The fish exposed to MLT 17:00 (i.e., 15hrs of melatonin) had decreased ($P<0.01$) GSI when compared to control but increased ($P<0.01$) when compared to TCT, MLT+TCT 17:00 and MLT CE. Further follicular kinetics revealed that the PVF count was also highest in control group (Graph -1(b), followed by MLT at 17:00 hrs, MLT CE respectively. The Previtellogenic follicles count was almost nil in the ovaries of fish exposed to with TCT at 17:00hrs. The vitellogenic follicular counts revealed that the highest count was noted in control followed by MLT 17:00hrs, MLT CE and MLT+TCT 17:00 groups respectively and almost no VF in TCT treated group (Graph-1(c)). Atretic follicular count had the similar trend as observed for GSI, PVF and VF (Graph-1 (d)). Date from follicular kinetics in general substantiate the GSI data.

In the second experiment, GSI increased ($P<0.01$, Graph-1(a)) in fish exposed to all melatonin in general compared to control and TCT group. However, melatonin exposure to limited period had significant increase ($P<0.01$) in the GSI when compared continuously available melatonin, which infact had a reduced GSI which is clearly visible from Graph -1(a). Data of follicular kinetics revealed there was no significant variations in the PVF count between control and MLT 17:00hrs (Graph-1(b)). PVF count was reduced in TCT 17:00hrs exposed group fish held in 24:00 compared to its respective control but this number was significantly more when compared to fish exposed to

TCT 17:00hrs but held in L:D 14:10. The Previtellogenic follicles numbers increased significantly (P<0.01) in the fish that were exposed to MLT+TCT 17:00 hrs but this number had decreased (P<0.01) in the fish which were held in melatonin continuously when compared with MLT+TCT 17:00hrs. Vitellogenic follicular count increased (P<0.01) in the fish which were exposed to MLT 17:00hrs when compared to all other groups including controlgroup (Graph-(c)). The TCT exposed ovaries hardly showed any VF and the fish which were exposed to MLT+TCT 17:00hrs had increased number of VF compared to TCT but this number was lower compared to MLT 17:00 hrs as well as MLT CE. MLT CE group fish had reduced VF count compared to control but this number seems to be increased when compared to TCT as well as MLT+TCT 17:00hrs groups.

The observation of Atretic follicles seems to be interesting as this count was significantly higher (P<0.01) in the MLT 17:00hrs group. AF number reduced in the TCT 17:00hrs fish ovaries. It is interesting to note that MLT+TCT 17:00hrs group had fewer AF compared to TCT 17:00 hrs, MLT 17:00 as well as MLT CE groups (graph-1(d)).

LEGEND TO GRAPH

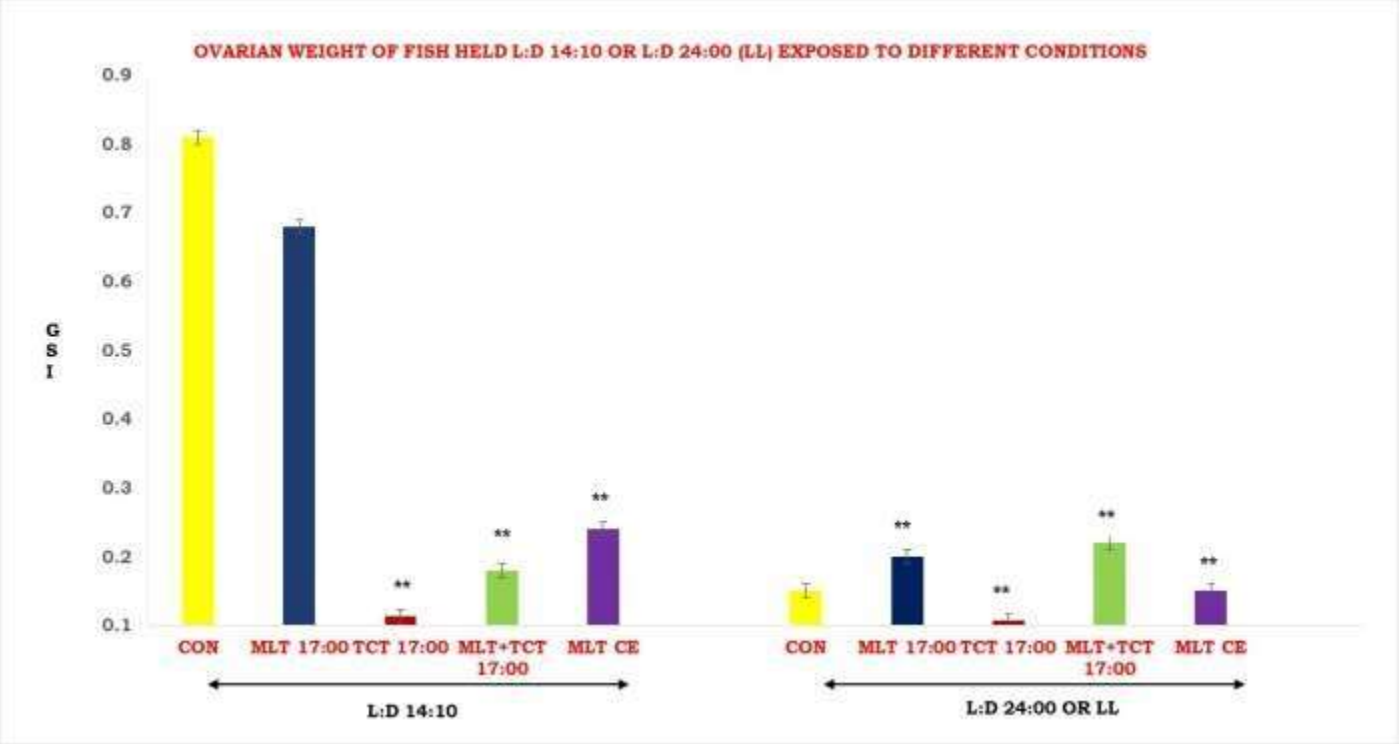
Fish were held in L:D 14:00 or L:D 24:00 OR LL

(A) GSI (Gonadosomatic index) following treatment different treatments. Values are Mean + SE; ** P<0.01 Control Vs treated group

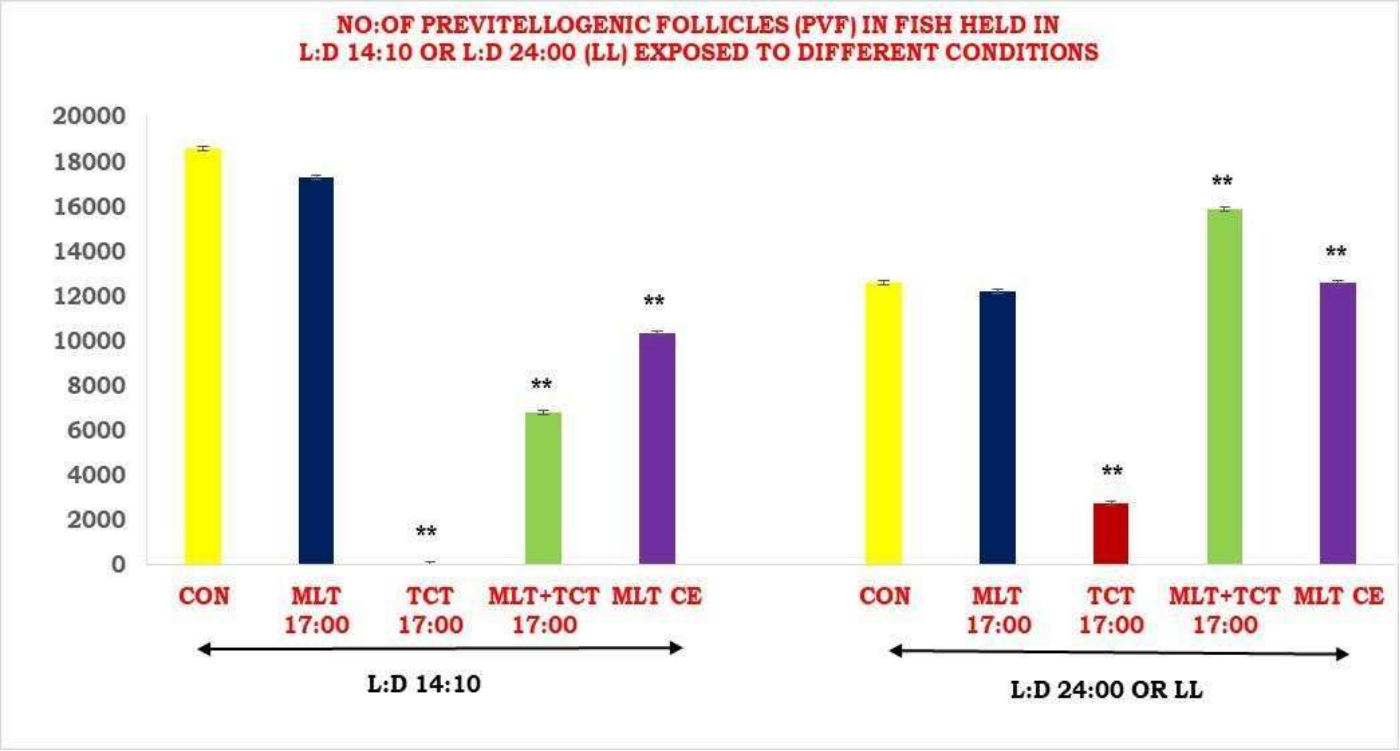
(B) Ovarian follicular kinetics: Previtellogenic follicle numbers following different treatments. Values are Mean ± SE; ** P<0.01 Control Vs treated groups

(C) Ovarian follicular kinetics: Vitellogenic follicle numbers following different treatments. Values are Mean ± SE; ** P<0.01 Control Vs treated groups

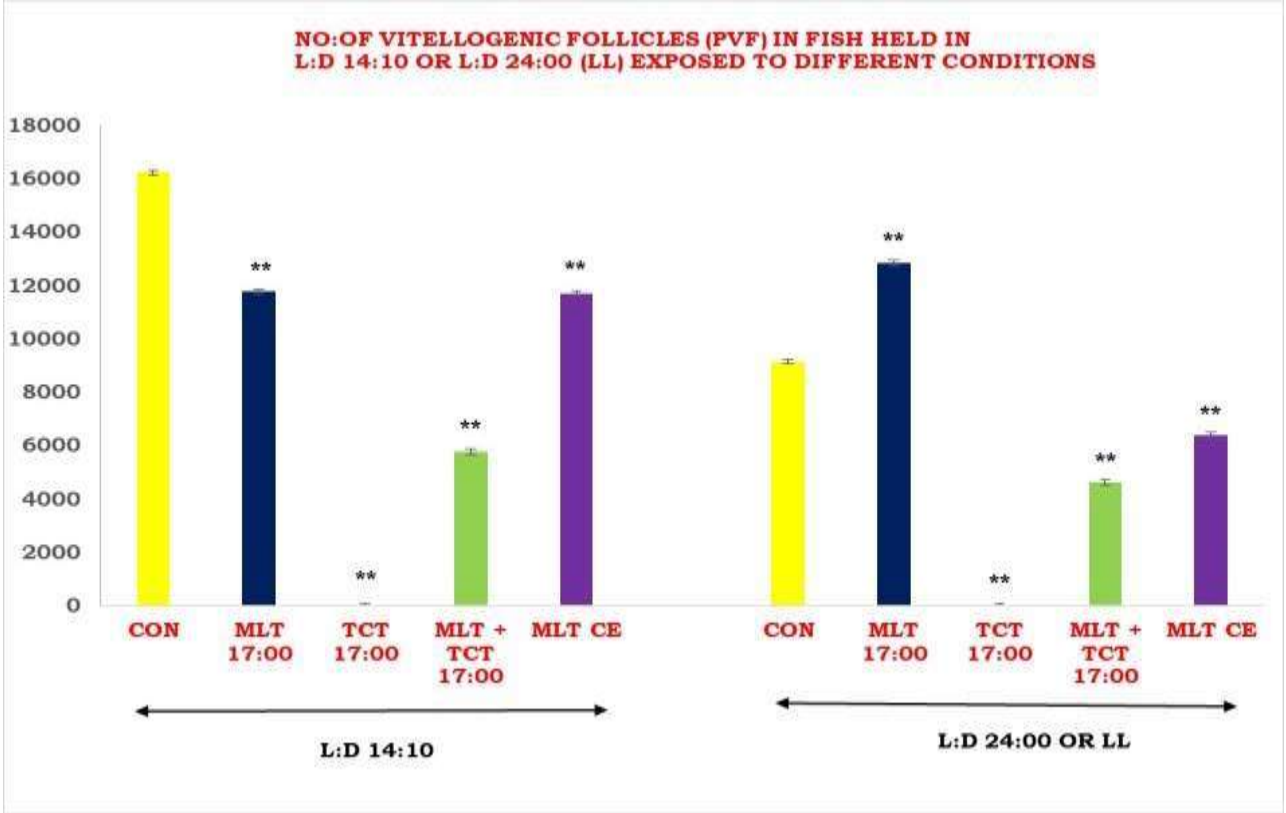
(D) Ovarian follicular kinetics: Atretic follicle numbers following treatment different treatments. Values are Mean ± SE; ** P<0.01


OVARIAN WEIGHT OF FISH HELD L:D 14:10 OR L:D 24:00 (LL) EXPOSED TO DIFFERENT CONDITIONS
GSI
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
CON
MLT 17:00
TCT 17:00
MLT+TCT 17:00
MLT CE
L:D 14:10
L:D 24:00 OR LL

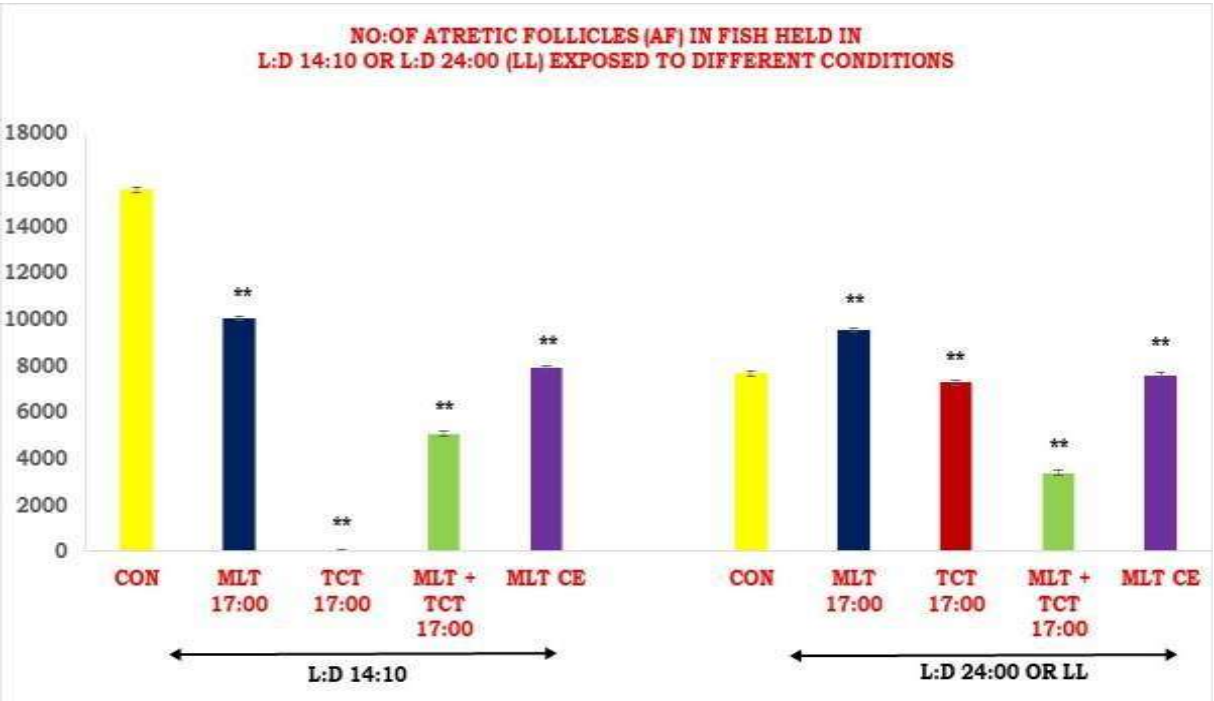
(A)


NO:OF PREVITELLOGENIC FOLLICLES (PVF) IN FISH HELD IN
L:D 14:10 OR L:D 24:00 (LL) EXPOSED TO DIFFERENT CONDITIONS
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
CON
MLT 17:00
TCT 17:00
MLT+TCT 17:00
MLT CE
L:D 14:10
L:D 24:00 OR LL

(B)


NO:OF VITELLOGENIC FOLLICLES (PVF) IN FISH HELD IN
L:D 14:10 OR L:D 24:00 (LL) EXPOSED TO DIFFERENT CONDITIONS
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
**
CON
MLT 17:00
TCT 17:00
MLT + TCT 17:00
MLT CE
L:D 14:10
L:D 24:00 OR LL

(C)

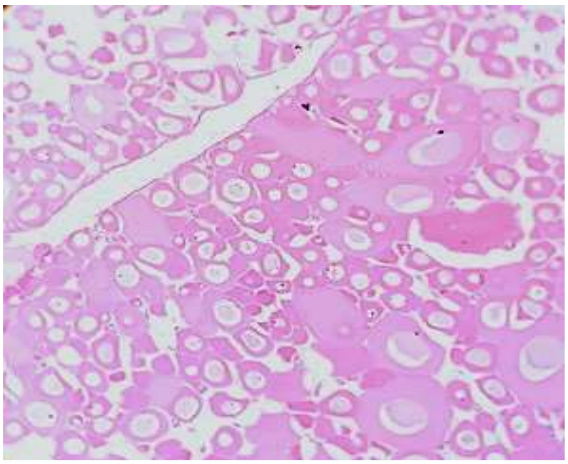

NO:OF ATRETIC FOLLICLES (AF) IN FISH HELD IN
L:D 14:10 OR L:D 24:00 (LL) EXPOSED TO DIFFERENT CONDITIONS
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
**
CON
MLT 17:00
TCT 17:00
MLT + TCT 17:00
MLT CE
L:D 14:10
L:D 24:00 OR LL

(D)

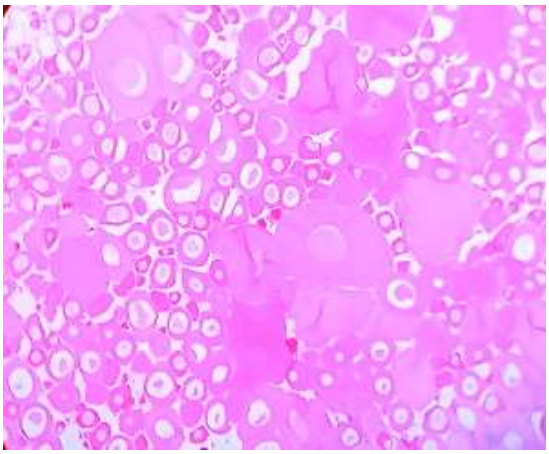
LEGEND TO PHOTOGRAPH

Experimental groups held in L:D 14:00

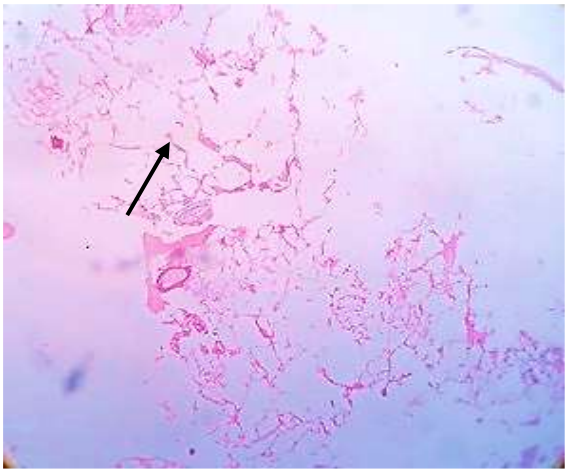
Photographs of cross section of ovaries exposed to (A) Control (B) Melatonin (MLT) 17:00hrs (C) Tamoxifen citrate (TCT) 17:00hrs (Degenerated ovary) (D) Melatonin+Tamoxifen citrate (MLT + TCT) 17:00hrs(Increased no: of PVF) (E) Melatonin (MLT) exposure for 24:00hrs



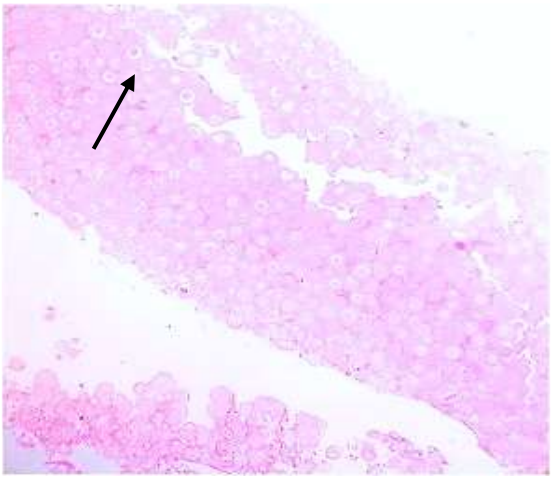
(A)



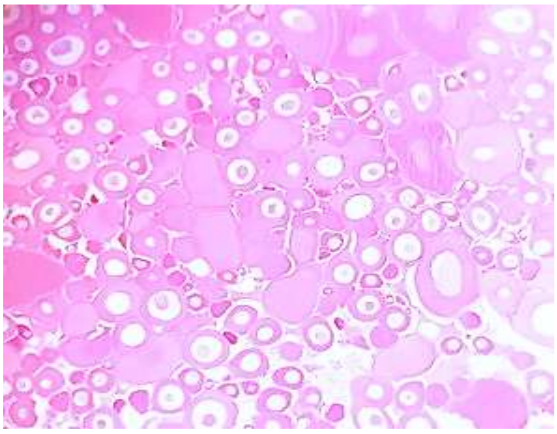
(B)



(C)



(D)

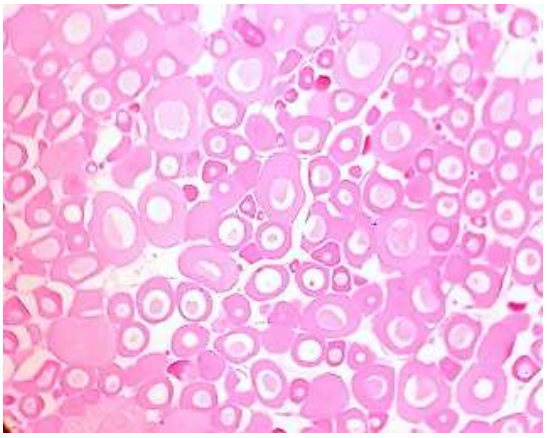


(E)

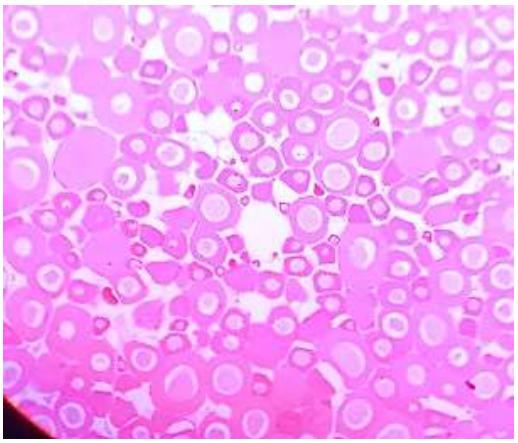
LEGEND OF PHOTOGRAPHS

Experimental groups held in L:D 24:00

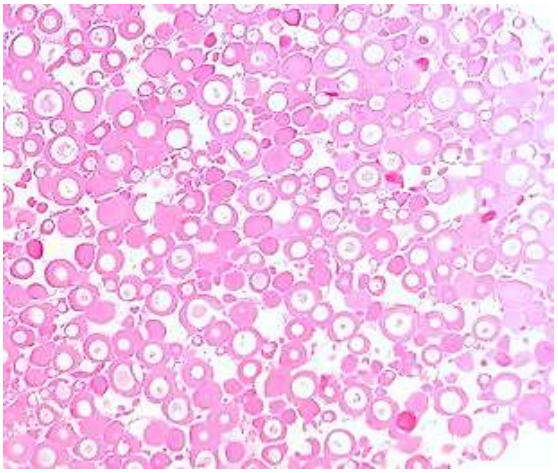
Photographs of cross section of ovaries exposed to (F) Control (G) Melatonin (MLT) exposure for 24hours (H) Melatonin (MLT) 17:00hrs (I) Tamoxifen citrate (TCT) (J) Melatonin+Tamoxifien citrate (MLT+TCT) at 17:00hrs



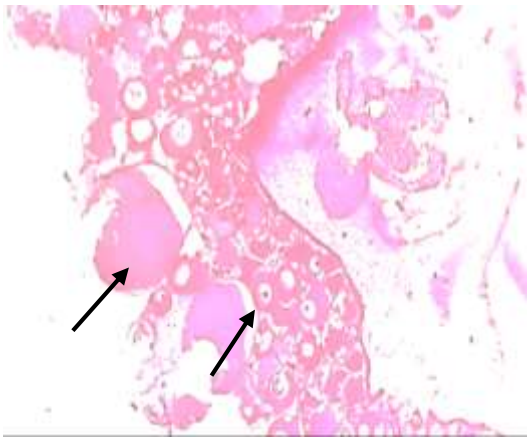
(F)



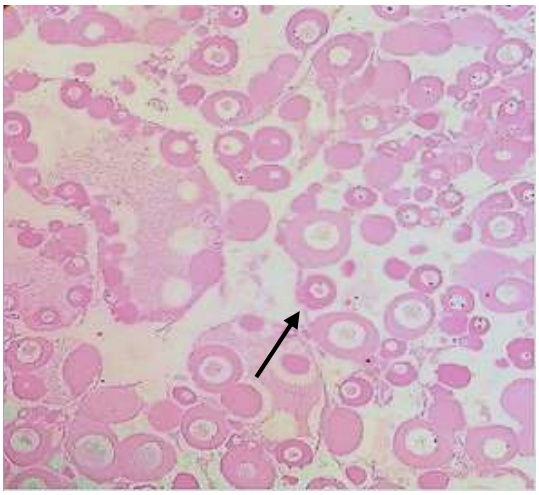
(G)



(H)



(I)



(J)

DISCUSSION:

Melatonin has myriad functional importance. Melatonin when treated with scotophase / photophase has varied results. In this study exposure of animals to limited period of melatonin has drastically improved GSI in the fish held in continuous light when compared to other groups held in LL condition.

Melatonin has inhibitory effect on ovary when exposed in the evening in long photoperiod while stimulates in LL condition whereas K Renuka and BN Joshi (2010) reported that 24h MLT in long day or in LL conditions stimulates and restricted period exposure inhibits the gonadal growth in *Channa punctatus*. Other studies by Sundararaj BI and Keshavanath (1976) reported that melatonin inhibits the stimulatory effects of long photoperiods on gonadal activity in *Heteropneustes fossils*. Ghosh and Nath (2005) showed that matonin reduces GSI in the catfish *Clarias barachus*; melatonin had an inhibitory effect on the gonads as reported for other species. All these studies show variations which indicates that the administration of melatonin has varied effect depending on species and geographical areas.

However melatonin administered for 15h had stimulatory effect on GSI of fish held in L:D 24:00 when compared to all other groups, this differential response of the gonads could possibly be attributed to diurnal changes in the sensitivity of the melatonin receptors, continuous availability of light might have induced a condition similar to pinealectomy where the animals have lost the photic input through pineal/melatonin, while 15h of MLT in such condition gives the animal cue about photoperiod and leads to observed change. It is interesting to note that melatonin when treated with TCT masked the anti-estrogenic effect of TCT and enhanced gonadal growth, which is evident from both the photoperiod experiment.

Fish exposed continuously to melatonin-containing water had a decreased GSI and the number of PVF, VF as well as AF decreased significantly (graph-1(a, b, c, d), whereas the reports by K Renuka and BN Joshi (2010) had revealed that GSI, PVF and VF count increased in the fish held in LL + 24h MLT. On the other hand, this study exhibits that exposure of fish to water-containing melatonin for a restricted period (17.00-08.00 h) and held in LL condition daily resulted in increased GSI, VF and AF indicating a stimulatory effect of melatonin on the gonads. This result suggests that continuously available melatonin inhibits and periodically available melatonin, stimulates the gonads in this species irrespective of the photoperiod when compared to continuously available MLT. Arendt J (2006) reported that melatonin profile in the blood represents biological night', exposure to melatonin for 15-h/day (i.e, 17.00-08.00 h) perhaps mimics a LD 09:15 situation-that is a short photoperiod condition and that may precipitate a gonadal change to exposure to short days. On the other hand, continuously available melatonin (24:00) perhaps fails to provide any clue regarding photoperiod, due either to lack of periodicity in melatonin rhythm or to the down regulation of melatonin receptors. Therefore, a long day situation prevails leading to the variations in gonadal activity. The inhibitory or stimulatory response of the animals depended on daily duration of exposure to melatonin. The determination of the diurnal variations in the plasma levels in the fish in both the experiments was not possible. Bornestal *et al.*, (2001) in their study on three-spined sticklebacks measured plasma melatonin after exposure to low (20 g/l) and high (80 g/l) melatonin via water and found that the morning levels of plasma melatonin on the high-dose group almost matched normal nocturnal values (455 kg/ml) for this fish. Though it is not possible to speculate on the plasma melatonin profile in this study, the results are unambiguous and do suggest melatonin's involvement in the transaction photoperiodic signal in *Mystus vittatus* and it also reveals that MLT has combated the effect of TCT which is evident by the increased GSI and PVF in the fish that received MLT+TCT (graph-1 (a and b)). It is interesting that TCT exposed fish had almost nil PVF or VF either but the number of AF was more showing the anti-estrogenic effect of TCT which is efficiently combated by when animals receive both TCT and MLT simultaneously. It is observed that the number of PVF increased significantly in the ovaries of fish which received MLT+TCT irrespective of photoperiod. Factors like species

variation, geographic distribution, habitat and times of experimental studies could account for the apparently different results in this species when compared to other researches. The difference in GSI of fish held in L:D 14:10 or 24:00 and exposed melatonin continuously is probably due to the intervention of a dark phase in the former and lack of it in the later. Since light suppresses endogenous melatonin (Reiter RJ, 1986) exposure to continuous light might mimic a condition of physiological pinealectomy. Under such conditions periodically available melatonin may provide a hormonal signal for biological night. The species may thus use the duration of melatonin formeasuring the length of dark phase. Continuously available melatonin on the other hand may fail to provide a signal for dark periodicity of period as stated above due either to absence of or to the down regulation of melatonin receptors. In the frog *Rana cyanophlyctis* continuously available melatonin from subcutaneous implants did not affect GSI but affected follicular kinetics (Udaykumar K and Joshi BN, 1997) and in the golden hamsters continuously available melatonin from subcutaneous implants did not affect reproduction (Tamarkin *et al.,* 1976), obviously their reproduction responses seem to be species specific in gonadal response to continuously available melatonin.

The data from follicular kinetics revealed a decrease in PVF in LL condition and in all the melatonin-exposed groups except MTL+TCT irrespective of the duration of exposure to melatonin wherein MTL has enhanced their number indicating stimulatory effect. The results indicate that melatonin probably does not interfere with the recruitment of PVF into VF. If melatoninadministration designed to mimic prolonged darkness enhances follicular atresia in LL conditions remains to be confirmed by further experimental studies. Probably this is first study dealing with follicular kinetics in a fish following treatment with TCT and melatonin.

There has been a debate as to how melatonin influences gonadal function. One hypothesis proposes that duration of melatonin peak in the blood represents biological dark phase of the diurnal cycle (Goldman *et al.,* 1984). According to this duration hypothesis', in long day breeding species a regimen of melatonin treatment that augments nocturnal peak of melatonin is believed to signal prolonged dark period or a short photoperiodic condition. The signal for short

photoperiod by alerting the neuroendocrine reproductive axis inhibits reproductive function in long day breeders. An alternate hypothesis assumes existence of circadian sensitivity to melatonin and the gonadal atrophy ensues when the exogenously administered melatonin coincides with the sensitivity rhythm (Reiter RJ, 1993). Though results of this study tend to favor theduration hypothesis, was posed primarily for mammalian species. This highlights the importance of extending these studies to a greater number offish species both from temperate and tropical regions.

CONCLUSIONS:

The data suggest that melatonin plays an important role in counter acting the anti-estrogenic effect of TCT and exposure to MLT can initiate the gonadal growth which is evident by increased GSI and PVF counts and decreased AF count. These two experiments throw light on importance of photoperiod along with the timing of melatonin exposure, which clearly indicates that melatonin administration at dawn has positive effect on follicular kinetics. This experiment was focused on fish but the results obtained may be utilized for females undergoing oncogenic treatment where antiestrogen are used and treatment couple with melatonin may help stop atresia of ovaries in patients suffering from cancer.

ACKNOWLEDGMENTS:

I would like to dedicate this work to my teacher Prof. Bhaskar N. Joshi who introduced me to this fascinating field of Neuroendocrinology.

FUNDING:

This work was supported by IQAC, Davangere University, Davangere under DUSMYTR PROJECT (NO: DU/HRM/2020-21/6045).

REFERENCE:

Arendt (2006): Melatonin and Human Rhythms. *Chronobiology International*. 23(1-2): 21-37. doi: 10.1080/07420520500464361. PMID: 16687277.

Bornestaf C., Mayer, Ian, Bertil, B. (2001): Melatonin and maturation pace in female three-spinned stickleback, *Gasterostus aculeatus*. *Gen. Comp. Endocrinol.* 122 (3):341–8. doi: 10.1006/gcen.2001.7640. PMID: 11356046.

Ghosh and Nath (2005): Seasonal effects of melatonin on ovary and plasma gonadotropin and vitellogenin levels in intact and pinealectomized catfish *Clarias batrachus* (Linn). *Indian Journal of Experimental Biology*. 43:224- 232. PMID: 15816408.

Goldman B.D., Darrow J.M. and Yogev L. (1984): Effects of timed melatonin infusions on reproductive development in the Djungarian hamster (*Phodopus sungorus*). *Endocrinology*. 114: 2074-2083. doi: 10.1210/endo-114-6-2074. PMID: 6426935.

K. Renuka and B.N. Joshi (2010): Melatonin-induced changes in ovarian function in the fresh water fish *Channa punctatus* (Bloch) held in long days and continuous light. *Gen. Comp. Endocrinol*. 165(2010):42-46. doi: 10.1016/j.ygcen.2009.05.020. Epub 2009 Jun 6. PMID: 19501093.

K. Renuka and B.N. Joshi (2014): Significance of retinal and the pineal photoreceptors in the photoperiodic regulation of reproduction in the bony fish *Channa punctatus* (Bloch). *Indian Journal of Applied Research*. 4(4): 1- 4. doi:10.36106/ijar

Reiter R J. (1986): Normal patterns of melatonin levels in the pineal gland and body fluids of humans and experimental animals. *Journal of Neural Transmission. Supplementum.* 21: 35-54. PMID: 3018145.

Reiter R J. (1993): The melatonin rhythm: both a clock and a calendar. *Experientia*. 49:654–664. doi: 10.1007/BF01923947. PMID: 8395408.

Reiter R J, Rosales- Corral S, Tan D X, Jou MJ, Galano A, Xu B. (2017): Melatonin as a mitochondria targeted antioxidant: one of evolution's bestideas. *Cell Mol life Sci.* 74(21): 3863- 3881. doi: 10.1007/s00018-017-2609-7. Epub 2017 Sep 1. PMID: 28864909.

Sundararaj BI and Keshavanath P (1976): Effects of Melatonin and prolactin treatment on the hypophysial-ovarian system in catfish, *Heteropneutes fossils* (Bloch). *Gen. Comp. Endocrinol*. 29: 84-96. doi: 10.1016/0016-6480(76)90010-1. PMID: 939419.

Tamarkin L., Westrom, W.K. Hamill, A.1. and Goldman B.D. (1976): Effect of melatonin on the reproductive system of male Syrian hamsters: A diurnal rhythm in sensitivity to melatonin. *Endocrinology*. 99, 1534-1541. doi: 10.1210/endo-99-6-1534. PMID: 1033827.

Udaykumar K and Joshi BN (1997): Effect of exposure to continuous light and melatonin on ovarian follicular kinetics in the skipper frog, *Rana cyanophlyetis. Biol Signals*. 6:389- 342. doi: 10.1159/000109110. PMID: 9266093