https://doi.org/10.48047/AFJBS.6.12.2024.3828-3837

AFJBS
African Journal of Biological Sciences
Journal homepage: http://www.afjbs.com
Research Paper Open Access

# Altered Response of Gonadotrophic Hormones to Non-Invasive Melatonin in Molly Fish (*Poeciliaformosa*)Under Normal Photothermal Condition

**Shivkumar D* and Renuka K****
**(**Corresponding author, Pineal Research Lab, Department of Studies in Zoology, Davangere University, Davangere – 577007 Karnataka, India**
**renukhaple@davangereuniveristy.ac.in,ssugur69@gmail.com,renuckhaple@gmail.com)**

**Article History**
Volume 6, Issue 12, 2024
Received: 02 Jun 2024
Accepted: 25 Jun 2024
doi:
10.48047/AFJBS.6.12.2024.3828-3837

**Abstract:**
Environmental cues are communicated through indoleamine which in turn effects the fish reproductive activities. Seasonally breeders are affected by melatonin where as it effects on aquarium fishes are sparse and ambiguous. Hence aquarium fish Molly (*Poeciliaformosa*), continuous viviparous breeding species was selected to study the effect of indoleamine, Estrogen (E), Progesterone (P) on gonadotropins.
Fish were divided into seven group, 1$^{st}$group as control while remaining 6 groups received 100µg/l: melatonin (MLT), Estrogen (E), Progesterone singly or in combination with MLT (MLT+E or MLT + P, MLT+E+P) for 45 days and were held in L:D 12:12. Hormones assay was done from serum for FSH, LH, GH, E and P.
MLT singly or in combination with E or P or only P reduced FSH levels and fish exposed to MLT +E+P had elevated FSH levels. The inhibitory effect of MLT was combated when the animals received all the three hormones simultaneous. LH levels remained statusco when exposed to E or P and significantly reduced in MLT exposed animals an inhibitory effect on LH also.
GH levels significantly reduced in treated groups with varied intensities. MLT, MLT+E, MLT+P and only E exposures had significantly reduced P levels but this level was reversed in MLT+E+P or P exposed animals. MLT exposed animals had reduced movement, feeding, segregated in a corner of the aquarium and sexual behaviour was almost nil. Thus, it can be concluded that melatonin has altered gonadotropins and induced physiological hibernation in this species.
**Keywords**: Melatonin, Estrogen, Progesterone, Physiological hibernation, Molly fish, Photoperiod

## Introduction:

Reproduction in fishes is effected by numerous factors, among which photoperiod plays an important role. Photoperiod is conveyed to the animal through photic, non-photic receptors (K Renuka and Joshi B N, 2014) found on/in the body which coordinates with the hypothalamus and pineal in signal transduction. The photic input is converted into endocrine output by the pineal gland, a neuroendocrine transducer converting neuronal signals, which are dependent on environmental cues.The pineal gland is influenced by light-dark cycles to regulate the synthesis of melatonin, a compound that acts at distant target organs (Wurtman and Axelrod, 1965). The effect of pineal's indoleamine has been stimulatory in some fish species and inhibitory in others depending on their breeding pattern and these reports are from temperate fish species than tropical or aquarium species. In seasonally breeding animals reproductive activities are influenced by seasonal variation in photoperiod (Sundararaj and Sehgal, 1970, Renuka K and Joshi B N, 2012).

Sexual dimorphism in growth rate and body size is popular in fish species (Mei and Gui, 2015). Fish sex determination and differentiation is an attractive issue because sex control has great commercial interest in aquaculture (Devlin and Nagahama, 2002). Fish sex is determined both by environmental and genetic factors or a combination of both. Environmental factors, especially sex steroid hormones, could even override the genetic factors and determine gonad fate (Nagahama, 2002). Estrogens are produced by the conversion of androgens through cytochrome P450 aromatase, which was mainly encoded by *cyp19a1a/b* in fish (Simpson *et al.*,1994, Zhang *et al.*, 2014). In teleosts, aromatase and endogenous estrogens are specifically expressed and synthesized in the female gonads during the critical period of molecular sex differentiation and act as natural inducer of ovarian differentiation (Nagahama, 2000, Tao *et al.*, 2013). Studies in several fish species demonstrated that female-to-male sex reversal is associated with a decrease in estrogen levels, while male-to-female sex reversal is associated with an increase in estrogen levels (Guiguen*et al.*, 1993, Guiguen*et al.*, 1999, Chang *et al.*, 1994, Piferrer*et al.*, 1994, Piferrer, 2001). Therefore, estrogens are essential factors for inducing ovarian development in fish. The roles of estrogens in fish gonadal sex differentiation were well reviewed and summarized (Guiguen*et al.*, 2010). It was concluded that Cyp19a1a is not only important for ovarian, but also for testicular differentiation in both gonochoristic and hermaphrodite fish species. Another important hormone i.e., progesterone has no effectson early embryonic development or hatching success. Progesterone may be a significant endocrine-disrupting chemical in fish. Patrick *et al.*, (2011) reported in adult male fathead minnows that continuous exposure for 1 week to low levels of progesterone in vivo had a significant dose-dependent reduction in sperm motility and short-term P4 exposure did not alter sperm swimming characteristics. Progesterone cause dose-dependent decreases in fecundity and fertility and significantly reduced gonadosomatic index and vitellogenin gene expression in fathead minnow (Zacharya*et al.*, 2012). However, the data on the effect of variations in these two hormones among viviparous fishes are sparse.

The effect of melatonin in continuous breeding viviparous fish has not been reported yet. Results of experimental studies on pineal-gonadal interaction in different species of fish are ambiguous and most of the information on this topic is from the species that inhabit the temperate climates. Tropical species have not extensively been investigated in this regard and information on aquarium species is sparse. Hence the present study was undertaken to evaluate the effect of non-invasive administration of melatonin along with gonadotrophs such as estrogen and progesterone on other hormonal profile in general, behavioural changes and sex reversal if any in Molly fish (*Poeciliaformosa*) an aquarium species held under normal photoperiodic condition (L:D 12:12).

**Material and methods:**

Molly fish (*Poecilia Formosa*) were procured from local vendor and were acclimatized for 15 days in glass aquaria in the laboratory in Davangere University, Davangere 14.3934$^{0}$N and 75.9649$^{0}$E. Post acclimatization the animals were divided into seven groups with 10 male and 10 female fishes in 30 litres of water under L:D 12:12. The first group served as control, next group received 100µg/l melatonin (MLT) in water, third group received 100µg/l MLT+100µg/l Estrogen, (MLT+E) fourth group received 100µg/l MLT + 100µg/l MLT + P, fifth group was exposed to 100µg/lMLT +100µg/l Estrogen (E) + 100µg/l MLT+E+P, sixth group was exposed to 100µg/l Estrogen and last group i.e., seventh group received 100µg/l progesterone. The aquariums water was changed daily at 09:00 hrs and exposure of the fish to selected harmones was done by adding 100µg/l of respective hormones to aquaria water daily at 17. 00 hr and the fish were transferred to other aquaria without any hormones in the water at 9.00hr. Thus the exposure of the fish to hormone was for 16.00 hrs. During this period the animals were fed at libidium with commercially available food (Taiyo) at 09:00hrs. The water temperature was maintained at 21 ± 1° C. The fish were held in aquaria and placed in chambers with facility for automatic regulation of photoperiod (L: D 12:12, lights on at 06.00 and off at 18.00).The experiment was carried out for 45 days. Post expermentation, the animals were held in cold anaesthesia and for hormone assay the whole body serum was extracted (for hormone assay 5 female fish from each group where sacrifised) and hormone assay was carried out for FSH, LH, Growth hormone (GH), Estrogen (E), and Progesterone (P) using ImmunoChemiluminescence Instrument: ADVIA Centaure CP on the same day (standard procedures were adopted). Statistical analysis was done using ANOVA and graphs were plotted using MS Office Excel spread sheet.

**Results and Discussion:**

FSH levels significantly ($P<0.01$) reduced in fish exposed to MLT, MLT+P, MLT+E respectively (Graph -1). However, MLT when treated in combination E and P failed to show such results and rather the FSH level were similar to that of control fish. The same effect was observed in E treated animals also. Progesterone also reduced the levels of FSH when compared with the control group. It is interesting to observe that Estrogen (E) treated as well as the melatonin in combination with E and P (MLT+E+P) treated animals had elevated levels of FSH when compared to other treated groups .The inhibitory effect observed in MLT, MLT+E and MLT+P was combated by the combined treated of all the hormones i.e., MLT+E+P indicating that E plays an important role in FSH levels or in other words the inhibitory effect of MLT has been combated by the combination of MLT+E+P.

MLT, MLT+E, MLT+P had an inhibitory effect on LH levels significantly ($P<0.01$, Graph -2) similar to that of FSH profile. LH levels were higher in MLT+E+P as well as E exposed animals when compared to other treated animals. However, these levels were lower when compared to control group. Exposing animals to P led to significant ($P<0.01$) reduction in LH levels. Both FSH and LH profile data reveals that MLT, MLT+E, MLT+E+P as well as P had an inhibitory effect on both FSH and LH in this fish species. Melatonin is an internal chemical messenger for environmental signals (Renuka K and Joshi B N, 2010). This messenger has drastically reduced the FSH alone or when given in combination with E or P, but the FSH levels remains unaltered when this indoleamine is combined with both E and P in this species. Oliana*et al.*, (2011) reported that melatonin led to an increase of the Gonado Somatic Index (GSI) associated with the increase of eggs production, and the raise of gene and protein levels of vitellogenin (VTG) and estradiol receptor a (ERa) in the liver in the zebra fish *Daniorerio*. Conflicting conclusions on the role of melatonin in the neuroendocrine system of teleost have been achieved by experiments dealing with photoperiod manipulation, pinealectomy and melatonin treatment [Popek et al., 2005, Lopez et al., 2006; Falcon et al., 2006]. In this regard, in the eel, the implant of melatonin induced a decrease in LHb and FSHb [Sebert et al., 2008] while, an increase in LHb secretion was found in croaker [Khan and Thomas, 1996)], in sea bass and in cultured carp pituitary [Popek et al., 2005], showing the ability of this hormone to affect reproduction.The mechanism by which melatonin regulates fish reproduction is one of the major areas that received serious attention in the studies on different fish, but is not yet clearly understood. Possibly, melatonin interacts with the brain–pituitary–gonad axis, or with one or more of a variety of peripheral and/or central sites, including the brain, the pituitary and the gonads. It seems likely that melatonin acts on the HPG axis and on the ovary itself (Carnevali et al., 2011, Lombardo et al., 2012, Lombardo et al., 2014). This conflicting result from different researches through light on the importance of more experiments to be carried out to understand the role of MLT in detailed ways

In general, exposure to all the selected hormones had an inhibitory effect on GH levels (Graph -3) indicating that the animals did not spend their energy in growth parameter. The results of this experiment reveals that the observed inhibitory effect among the exposed group was with varied intensities. Peak inhibition was observed in P exposed animals followed by E, MLT, MLT+E, MLT+E+P and MLT+P respectively.

The levels of E in the animals that received exogenous MLT along with E or P (MLT+E, MLT+P) was similar to that of control group. However, E levels significantly ($P<0.01$) increased in the fish that were exposed 16hrs to MLT+E+P, this increase was similar to that of E exposed animals. The hormone level peaked significantly ($P<0.01$, Graph -4) among the fish held in P when compared to all other groups including control group. Estrogen is used for feminization of teleost fish (Piferrer, 2001). Fish sex could be reversed at the undifferentiated stage of gonad by administration of exogenous estrogen (E2) or blockade of endogenous estrogen synthesis with aromatase inhibitors, which is designated as primary sex reversal (PSR). Recent studies have well demonstrated that gonochoristic fish maintain their sexual plasticity after sex determination/differentiation. The differentiated ovary could be transdifferentiated into functional testis, and vice versa, the differentiated testis could be transdifferentiated into ovary (Piferrer, 2001) but in the present study sex reversal was not prevelant.

The progesterone levels reduced significantly ($P<0.01$) among the animals exposed to MLT+E ($P<0.01$), MLT ($P<0.05$) as well as E ($<0.05$) when compared with the control group respectively. However, there was no variations observed among the fish that received MLT+P exogenous when compared to its control group. It is interesting to note that the exposure of fish to MLT along with E and P (MLT+E+P) for 16hrs resulted in peak values of P ($P<0.01$ Graph -5).

The effects of P, alone or in combination with E2, on the reproductive-axis of immature rainbow trout (*Oncorhynchusmykiss*) was reported by Christiane A et al., (2003). Liver vitellogenin and estradiol receptor (rtER) mRNA levels increased after E2 treatment, but were unchanged by P treatments as a reflection of peripheral action of steroids. In contrast, at the pituitary level, LH contents increased after E2 and/or P treatments. Focusing on the brain level, a confirmation of a clear up regulation of rtER expression by E2 in sterile triploid females, and also demonstrated a similar stimulating effect of P alone but no cooperative effect together with E2. The immature trout, prior to the beginning of the first reproductive cycle, unlike E2, P is able to stimulate the reproductive brain-pituitary axis without affecting vitellogenin synthesis in the liver. E2 is responsible of a direct stimulating effect on gonadotropin synthesis (Crim*et al.*, 1981) and an indirect (via the brain) inhibitory effect on gonadotropin release (Larsen and Swanson, 1997, Saligaut*et al.*, 1999). However, in this study both E and P were not exposed in combination, hence a general conclusion is not be possible.

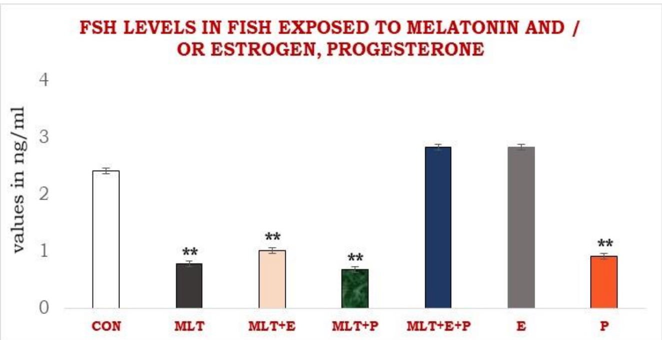
It was observed during the 45 days experiment that the animals which received 16hrs of MLT had reduced movement in the aquaria, the animals segregated in one corner and their food intake also reduced drastically even when the food was available in the aquaria. No sexual behaviour was observed in the fish which received non-invasive melatonin either singly or in combination with E or P. Similar observation was made even in the fish that were exposed to

MLT+E as well as MLT+P groups sexual behaviour or mating was not observed in MLT treated groups. However, the animals that received exogenous MLT+E+P had a reduced activity but fed normally and moved normally in the aquaria. The other two groups i.e., E and P exposed group did not show any variation in their daily activities during experimentation period. The above results reveals that the animals have undergone physiological hibernation which can proved if the experiment is conducted for a longer period such as 90 or 120 days.

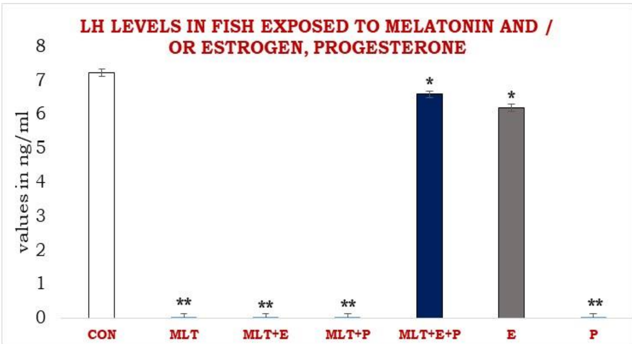
Experimental evidences suggest that the pineal gland plays a vital role in the regulation of both the circadian and seasonal rhythms in apenial variety of species (Morgan and Williams, 1989; Namboodiri*et al.*, 1991; Reiter, 1993) and in the endocrine control of reproductive physiology (Brzezinski and Wurtman, 1988; Matthews *et al.*, 1993). Melatonin is secreted into the blood with an endogenous and individual rhythm synchronized by the LD cycle: the plasma concentration of the hormone reaches a peak during the night hours and lowest amplitude during the day. The persistence of high concentrations of melatonin is proportional to the duration of darkness (Goldman, 1991; Reiter, 1991, 1993; Pevet, 1993). The pineal gland through its hormone secretion 'informs' the animals about the current phase of day and the year (Reiter, 1991).

The results are interesting because the animals selected for this study, the Molly (*Poeciliaformosa)*fish is known as a continuous breeder and there is no evidence that there is any variation in its reproductive activities after its maturation with respect to seasonal variation or photoperiod, while for the first time this experiments shows a variation in activity as well as hormonal profile in this species when exposed to pineal indoleamine led to varied results observed from the general conclusion regarding its gonadotrophic hormonal profile. However, during this experiment there was no reversal of sex in this species even though the animals were exposed to different hormones for 16hrs /day for 45 days. Further this experiment reveals that MLT when administered singly or in combination with E or P had similar results by which can be concluded that MLT has masked effect of E or P when administered in combination with MLT.

**Graphs:**




Graph -1

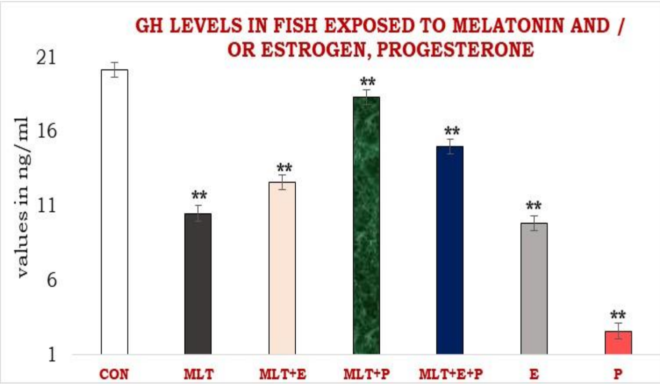



Graph -2

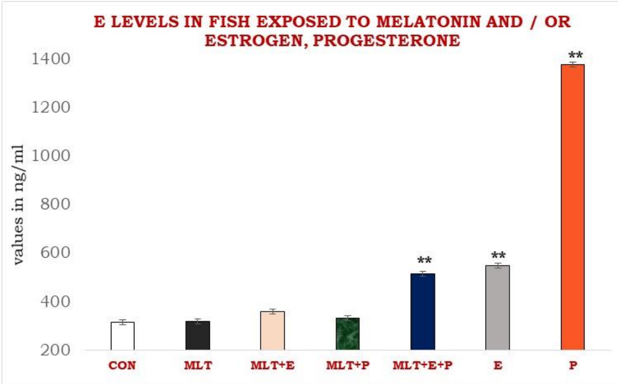
Graph – 1: FSH profile in fish exposed to melatonin and / or estrogen, progesterone held in L:D 12:12 (values expressed in ng/ml. Significance at P<0.01 **) The values are mean ± SE

Graph – 2: LH profile in fish exposed to melatonin and / or estrogen, progesterone held in L:D 12:12 (values expressed in ng/ml. Significance at P<0.01 **, p<0.05*) The values are mean ± SE

Graph -3

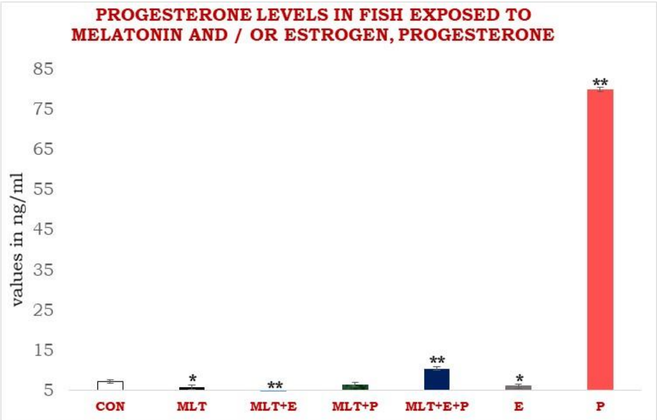



Graphs -4




Graph – 3: Growth hormonal profile in fish exposed to melatonin and / or estrogen, progesterone held in L:D 12:12 (values expressed in ng/ml. Significance at P<0.01) The values are mean ± SE

Graph – 4: Estrogen level in fish exposed to melatonin and / or estrogen, progesterone held in L:D 12:12 (values expressed in ng/ml. Significance at P<0.01) The values are mean ± SE




Graph -5

Graph – 5: Progesterone level in fish exposed to melatonin and / or estrogen, progesterone held in L:D 12:12 (values expressed in ng/ml. Significance at P<0.01 **, p<0.05*) The values are mean ± SE

**References:**

Brzezinski A, Wurtman (1988): The Pineal gland: its possible roles in human reproduction. *ObstetGynecolSurv*. 43: 197-207., https://pubmed.ncbi.nlm.nih.gov/3285261/

Carnevali O, Gioacchini G, Maradonna F, Olivotto I, Migliarini B (2011): Melatonin Induces Follicle Maturation in *Daniorerio*. *PLoS ONE* 6(5): e19978. https://doi.org/10.1371/journal.pone.0019978

Christiane Atteke, Angelique Vetillard, Alexis Fostier, Danièle-Hélène Garnier, Patrick Jego, Thierry Bailhache (2003): Effects of progesterone and estradiol on the reproductive axis in

immature diploid and triploid rainbow trout. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*,Vol (134), 4: 693-705,ISSN 1095-6433,https://doi.org/10.1016/S1095-6433(02)00361-6.
Donald A. Larsen and Penny Swanson (1997): Effects of Gonadectomy on Plasma Gonadotropins I and II in Coho Salmon,*Oncorhynchuskisutch*. *Gen Comp Endocrinol* Vol (108) 1:152-160,ISSN:0016-6480,https://doi.org/10.1006/gcen.1997.6958.
Falco´n J, Besseau L, Sauzet SM, Boeuf G (2006): Melatonin effects on the hypothalamo–pituitary axis in fish. *TRENDS Endocrinol Met*Vol (181): 81–88.https://pubmed.ncbi.nlm.nih.gov/17267239/

Francesc Piferrer (2001): Endocrine sex control strategies for the feminization of teleost fish, Editor(s): Cheng-Sheng Lee, Edward M. Donaldson, *Reproductive Biotechnology in Finfish Aquaculture*, Elsevier, pp: 229-281, ISBN 9780444509130, https://doi.org/10.1016/B978-0-444-50913-0.50014-X.

Goldman BD (1991): The circadian timing system and reproduction in mammals. *Steriods*. 64(9): 679-685

Guiguen Y, Baroiller JF, Ricordel MJ, Iseki K, Mcmeel OM(1999): Involvement of estrogens in the process of sex differentiation in two fish species: the rainbow trout (*Oncorhynchusmykiss*) and a tilapia (*Oreochromis niloticus*). *Mol Reprod Dev* 54: 154–162., https://pubmed.ncbi.nlm.nih.gov/10471475/

Guiguen Y, Fostier A, Piferrer F, Chang CF. (2010): Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. *Gen Comp Endocrinol*. 1;165(3):352-66. doi: 10.1016/j.ygcen.2009.03.002. Epub 2009 Mar 14. PMID: 19289125.,https://pubmed.ncbi.nlm.nih.gov/19289125/

Khan IA and Thomas P (1996) Melatonin influences gonadotropin II secretion in the Atlantic croaker (*Micropogonias undulatus*). *Gen Comp Endocrinol* 104: 231–242., https://pubmed.ncbi.nlm.nih.gov/8930614/

K Renuka and B N Joshi (2012): Photoperiodic regulation of ovarian function in the teleost fish *Channa punctatus* (Bloch). *International Journal of Life Sciences* Vol. 1(4): 104-107, https//www.crdeepjournal.org/wp-content/uploads/2012/10/Vol-144-IJLS.

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 Vol. 4(4): 1-4. https://www.worldwidejournals.com/indian-journal-of-applied-research-(IJAR)/recent_issues_pdf/2014/April/April_2014_1396588475_cafe4_201.

Lombardo F, Giorgini E, Gioacchini G, Maradonna F, Ferraris P, Carnevali O. (2012): Melatonin effects on *Fundulusheteroclitus* reproduction. *ReprodFertilDev* 24:794–803. https://pubmed.ncbi.nlm.nih.gov/22781930/

Lombardo F, Gioacchini G, Fabbrocini A, Candelma M, D'Adamo R, Giorgini E, (2014): Melatonin-mediated effects on killifish reproductive axis. *Comp BiochemPhysiol* 172:31–8. doi: 10.1016/j.cbpa.2014.02.008 41. https://pubmed.ncbi.nlm.nih.gov/24548909/

Lo´pez LC, Escames G, Tapias V, Utrilla P, Leo´n J. (2006): Identification of an inducible nitric oxide synthase in diaphragm mitochondria from septic mice: its relation with mitochondrial dysfunction and prevention by melatonin. *Int J Biochem Cell Biol* 38: 267–278.
https://www.researchgate.net/publication/7541982
L.W. Crim, R.E. Peter, R. Billard (1981): Onset of gonadotropic hormone accumulation in the immature trout pituitary gland in response to estrogen or aromatizable androgen steroid hormones.*Gen Comp Endocrinol*, 44(3): 374-381,ISSN 0016-6480,https://doi.org/10.1016/0016-6480(81)90015-0.

Matthews CD, Guerin MV, Deed JR (1993): Melatonin and photoperiodic time measurement: seasonal breeding in sheep. *J Pineal Res* 14: 105-116., https://pubmed.ncbi.nlm.nih.gov/8336272/

Mei Jie and Gui Jian-Fang (2015): Genetic basis and biotechnological manipulation of sexual dimorphism and sex determination in fish. *Sci. China Life Sci*., 58: 124-136, https://pubmed.ncbi.nlm.nih.gov/25563981/

Morgan, J.P. and Williams, L.D. (1989): Central melatonin receptors: implications for a mode of action. *Experientia* 45: 955–965.,https://pubmed.ncbi.nlm.nih.gov/2553471/

Nagahama, Y. (2002): Ontogeny and plasticity of sex determination/gonadal differentiation in fishes, in: Biology of reproduction. *Soc study reproduction* 1603 Monroe ST, Madison, WI 53711–2021 USA, pp. 75–76., https://journals.physiology.org/doi/full/10.1152/physrev.00044.2019

Nagahama Y (2000): Gonadal steroid hormones: major regulators of gonadal sex differentiation and gametogenesis in fish. In: Norberg B, Kjesbu OS, Taranger GL, Andersson E, Stefansson SO, editors. Proc 6th International Symposium on Reproductive Physiology of Fish. Bergen, Norway: Institute of Marine Research and University of Bergen. pp. 211–222.
https://link.springer.com/article/10.1023/A:1023343023556

Namboodiri MA, Valivullah HM, Moffett JR. (1991): Regulation of melatonin synthesis in the ovine pineal gland. *AdvExp Med Biol*. 294:137-48. doi: 10.1007/978-1-4684-5952-4_12. PMID: 1772063.,https://pubmed.ncbi.nlm.nih.gov/1772063/

Patrick J Murack [1], John Parrish, Terence P Barry (2011): Effects of progesterone on sperm motility in fathead minnow(Pimephalespromelas). *AquatToxicol*. 104(1-2):121-5. doi: 10.1016/j.aquatox.2011.04.006. Epub 2011 Apr 15., https://pubmed.ncbi.nlm.nih.gov/21569753/

Pevet P (1993): Present and future of melatonin in human and animal reproduction functions. *ContraceptFertil Sex*. 21: 727-732.,https://pubmed.ncbi.nlm.nih.gov/8269019/

Popek W, Uszczek-Trojnar E, Drng-Kozak E, Fortuna-Wroska D. (2005): Effects of the pineal gland and melatonin on dopamine release from perifused hypothalamus of mature female carp during spawning and winter regression. *AIchthyolPiscator*35: 65–71., https://www.researchgate.net/publication/236021450

Robert H. Devlin and Yoshitaka Nagahama (2002): Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influence. *Aquaculture*, 208(3-4): 191-364,ISSN 0044-8486,https://doi.org/10.1016/S0044-8486(02)00057-1.

Reiter RJ (1991): The chemical expression of darkness. *Mol Cell Endocrinol* 79(1-3): C153-8. doi: 10.1016/0303-7207(91)90087-9.,https://pubmed.ncbi.nlm.nih.gov/10503728/

Reiter R J (1993): The melatonin rhythm: Both a Clock and a Calender. *Experientia* 49: 654-664.,https://pubmed.ncbi.nlm.nih.gov/8395408/

Renuka K, Joshi BN (2010) Melatonin-induced changes in ovarian function in the freshwater fish *Channa punctatus* (Bloch) held in long days and continuous light. *Gen Comp Endocrinol* 165: 42–46.,https://pubmed.ncbi.nlm.nih.gov/19501093/

Saligaut C, Linard B, Breton B, Anglade I, Baihache T, Kah O, Jego P (1999): Brain aminergic systems in salmonids and other teleosts in relation to steroid feedback and gonadotropin release. *Aquaculture*. 177:13–20.,https://www.sciencedirect.com/science/article/abs/pii/S0044848699000654

Se´bert ME, Legros C, Weltzien FA, Malpaux B, Chemineau P, et al. (2008): Melatonin activates brain dopaminergic systems in the eel with an inhibitory impact on reproductive function. *J Neuroendocrinol* 20: 917–929. https://pubmed.ncbi.nlm.nih.gov/18445127/

Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD. (1994): Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev.*;15(3):342-55. doi: 10.1210/edrv-15-3-342. PMID: 8076586.,https://pubmed.ncbi.nlm.nih.gov/8076586/

Sundararaj BI, Sehgal A (1970): Effects of a long or an increasing photoperiod on the initiation of ovarian recrudescence during the preparatory period in the catfish, *Heteropneustesfossilis* (Bloch). *BiolReprod*. 2: 413-424,https://pubmed.ncbi.nlm.nih.gov/5527838/

Tao W, Yuan J, Zhou L, Sun L, Sun Y, Yang S, Li M, Zeng S, Huang B,Wang D (2013): Characterization of gonadal transcriptomes from Nile tila-pia (*Oreochromis niloticus*) reveals differentially expressed genes. *PLoS One8*:e63604,doi:10.1371/journal. pone.0063604,
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0063604

Wurtman, R.J. and Axelrod, J. (1965): The Pineal Gland. *Sci. Amer*. 213: 50.
https://www.scientificamerican.com/article/the-pineal-gland/

Y. Guiguen, B. Jalabert, E. Thouard, A. Fostier (1993): Changes in Plasma and Gonadal Steroid Hormones in Relation to the Reproductive Cycle and the Sex Inversion Process in the Protandrous Seabass, Lates calcarifer. . *Gen Comp Endocrinol*. 92(3): 327-338,ISSN0016-6480,https://doi.org/10.1006/gcen.1993.1170.

Zachary A DeQuattro , Evan J Peissig, Dagmara S Antkiewicz, Erica J Lundgren, Curtis J Hedman, Jocelyn D C Hemming, Terence P Barry (2012): Effects of progesterone on reproduction and embryonic development in the fathead minnow (*Pimephalespromelas*) *Environ Toxicol Chem*.31(4):851-6., https://pubmed.ncbi.nlm.nih.gov/22374535/

Zhang Y, Zhang S, Lu H, Zhang L, Zhang W. (2014): Genes encoding aromatases in teleosts: evolution and expression regulation. *Gen Comp Endocrinol*. 1; 205:151-8. doi: 10.1016/j.ygcen.2014.05.008. Epub 2014 May 20. PMID: 24859258., https://pubmed.ncbi.nlm.nih.gov/24859258/