Functional *pleiotropy* of melatonin in the regulation of reproduction : An overview

Palash Kumar Pal, Kazi Nurul Hasan and Saumen Kumar Maitra
Department of Zoology, Visva-Bharati University, Santiniketan - 731 235, India

Summary

This brief review covers recent important findings to form an informative pool depicting unique role of the pineal hormone melatonin in the regulation of reproductive axis in vertebrates. Since last decades, melatonin research witnessed an enormous progress in understanding the nature and mechanism of actions of this tiny, but versatile, product of tryptophan in the regulation of diverse body functions, especially reproduction in different groups of vertebrates. Demonstration of melatonin receptors on different peripheral organs and parts of brain essentially supported the contention of a hormonal effect of melatonin. Several lines of evidence suggest that melatonin regulates, rather modulates, vertebrate reproduction by two pathways- one through controlling the hypothalamo-hypophyseal-gonadal axis and another by inducing direct effect on the gonads through G-protein coupled MT1 and MT2 receptors. The conventional idea of the regulatory actions of melatonin on gonads via the hypothalamo-hypophyseal-gonadal axis involving gonadotrophic releasing hormone (GnRH) and gonadotrophins, has been greatly enriched by the discovery of other hypothalamic peptides like, gonadotrophic inhibitory hormone (GnIH) and kisspeptin. Additional findings that melatonin, due to its lipophilicity and potential antioxidant property, scavenges and detoxifies various free radicals, opened up a possibility of its significant contribution to the physiology of oocytes during growth and maturation. As an obvious outcome of unequivocal evidence suggesting pleiotropic functions of melatonin in the regulation of vertebrate reproduction, studies on the array of molecular events occurring at different sites of melatonin actions have emerged as a new exciting area of investigation.

Keywords **:** Gonads, melatonin, receptors, reproduction, vertebrates

1. Introduction

One of the unique features of almost all organisms is to adapt their behaviors and functions to the daily annual variations of the external cues, of which annual fluctuations in the duration of light or photoperiod appear to play an important role in the regulation or synchronization of annual reproductive cycle in different seasonally breeding animals, including fish (Mayer et al., 1997; Reiter et al., 2009; Maitra, 2011). Melatonin (N-acetyl-5-methoxytryptamine), primarily a pineal hormone, acts as an output signal of vertebrate circadian clocks and synchronizes behaviors and neuroendocrine functions with the daily and annual variations of the photoperiod. Under controlled light-dark conditions, melatonin is synthesized mostly during the darkness and is thereby considered as a 'hormone of darkness' or 'chronobiotic molecule' (Arendt and Skene, 2005). Seasonality in reproduction is in general determined by the functions of the hypothalamo-hypophyseal-gonadal axis, and the pineal gland, through melatonin release, plays important role in operation of such axis (Mazurais et al., 2000).

Though melatonin was first isolated and purified from the extract of bovine pineal gland (Lerner et al., 1958), this indole hormone has also been identified in all major taxa of organisms in different organs or tissues, such as the Harderian gland, extra-orbital lacrimal gland, retina, bone marrow cells, platelets, lymphocytes, skin, enterochromaffin cells of gastrointestinal tract and in bile (Chowdhury and Maitra, 2012). Carefully controlled studies revealed that melatonin plays an important role in the regulation of a wide range of physiological functions ranging from aging to aggression, hibernation to hypertension, sleep to stress, reproduction to tissue regeneration, and scavenging of free radicals to synchronization of body functions with the environmental light-dark cycles. Melatonin has been implicated in the physiology of reproduction in seasonally breeding mammalian (Reiter et al., 2009), as well as non-mammalian vertebrates (Mayer et al., 1997), including the teleosts (Bromage et al., 2001; Maitra, 2011). Most studies investigating the mechanism(s) by which melatonin regulates reproduction have focused the brain and the pituitary (Khan and Thomas, 1996; Popek et al., 2000; Bayarri et al., 2004) as target tissues with little attention to the direct actions of melatonin on the gonad itself. Demonstration of melatonin receptors in isolated human oocytes (Woo et al., 2001), rat ovaries (Soares et al., 2003) and carp ovaries (Chattoraj et al., 2009) opened up a new

Correspondence to be addressed to: Professor Saumen Kumar Maitra, Ph D, Email: dgp_skmaitra@yahoo.co.in

possibility of direct action of melatonin on ovarian functions of the concerned animals.

The melatonin system is characterized by both conservation and diversity: conservation because of its pattern of production and synchronizing properties as a constant among vertebrates; and diversity because regulation of both its synthesis and modes of action have been profoundly modified during vertebrate evolution (Falcón et al., 2007). Various lines of evidence suggest that melatonin acts on its target cells/tissues through transmembrane receptors MT1, MT2 and MT3 or through orphan nuclear receptors (RORα and RZRβ) of the retinoic acid family (Ekmekcioglu, 2006). However, the unique feature that added a unique dimension to the receptor-independent actions of melatonin at cellular level is its lipophilic nature that promoted its free access to all cells, tissues and organs of the body for acting as an antioxidative agent, safeguarding mitrochondrial electron flux and modulation of actions of other hormones or intra-cellular regulators (Hardeland et al., 2006). Melatonin, in order to perform specific receptor binding as well as receptor-independent functions, possesses two functional groups which contribute to its oxidation chemistry (Poeggeler et al., 2002). The result is functional pleiotropy of melatonin in the regulation of various body functions. The purpose of this brief review is to summarize available information and thereto focus pleiotropic functions of melatonin in the regulation of reproduction in vertebrates in general, and in fish in particular.

2. Biosynthesis and release of melatonin

Biosynthetic pathway of melatonin is almost identical in all the animals investigated so far. This process includes four steps. First, its precursor L-tryptophan is taken-up from the circulation into the melatonin synthesizing cells and converted to 5-hydroxytryptophan by tryptophan 5-monooxygenase/hydroxylase and further decarboxylated by L-aromatic amino acid decarboxylase to form 5-hydroxytryptamine (5-HT) or serotonin. Serotonin is acetylated (N-acetylation) to form N-acetyl serotonin by the action of serotonin-N-acetyl-transferase/ arylalkylamine N-acetyltransferase (AANAT) which is the rate limiting enzyme of this pathway. Finally, N-acetyl serotonin is methylated by hydroxyindole-o-methyltransferase (HIOMT) to form melatonin (Chowdhury and Maitra, 2012).

The interacting networks of circadian clock genes located in the suprachiasmatic nucleus (SCN), circadian oscillator/master pacemaker, of the hypothalamus in brain regulates the rhythm of melatonin synthesis. Noradrenalin (NA) or norepinephrine (NE), which plays important role in the control of melatonin synthesis, is released by post-ganglionic sympathetic nerve fibers that terminate at the pineal gland. The dark-induced elevation of nor-adrenergic stimulation via β and α_1-adrenergic receptor of pinealocytes results in an increase in the intra-cellular concentration of cAMP, leading to activation of AANAT which is ultimately responsible for increase in the levels of melatonin at night (Chowdhury et al., 2008; Seth and Maitra, 2010, 2011). Stimulation of α-adrenergic receptors potentiates the β-stimulation and requires participation of other molecules such as Ca^{++} ions, phosphatidylinositol, diacylglycerol (DAG) and protein kinase C (PKC), and thereby darkness stimulates synthesis and release of melatonin (~80% synthesis), while light causes inhibition (Maitra et al., 1986). The 'timenzyme' Arylalkylamine N-acetyltransferase (AANAT), a ~23 kDa cytosolic protein, plays a crucial role in the rhythmic production of melatonin where it becomes activated upon forming a reversible regulatory complex with 14-3-3 proteins and prevents proteosomal proteolysis. AANAT activity increases 10 to 100 fold at night, resulting in increased production and release of melatonin (Klein, 2007).

3. Major influence of melatonin on vertebrate reproduction: An outline

Melatonin is implicated in the photoperiodic control of seasonal reproduction in both mammalian and non-mammalian vertebrates. Initial information on the influences of melatonin came out from studies following exogenous administration, which in most cases appears to be inhibitory to reproductive parameters (Reiter et al., 2009).

In birds, the influence of exogenous melatonin on the gonad appears to vary in relation to the sexual status of concerned species (Maitra et al., 2002). Daily melatonin administration in male roseringed parakeets (*Psittacula krameri*) during different reproductive phases of the annual cycle resulted in either suppression of gametogenesis during the pre-breeding and breeding phases or no response in the remaining part of reproductive cycle (Maitra and Dey, 1992). Actually, removal of melatonin source marginally influences reproduction in bird (Mayer et al., 1997). The role of the pineal gland and its hormone melatonin in the regulation of annual testicular events was investigated in the roseringed parakeets, the testicular responsiveness of which was evaluated following surgical pinealectomy with or without exogenous administration of melatonin and experimental manipulations of the endogenous levels of melatonin through exposing the birds

to continuous illumination (Sengupta and Maitra, 2006). An analysis of the data reveals that the pineal gland and its hormone melatonin may play an inhibitory role in the development of the testis until the attainment of the seasonal peak in the annual reproductive cycle. However, in all probability, the termination of the seasonal activity of the testis or the initiation of testicular regression in the annual reproductive cycle appears to be the function of the pineal gland, but not of melatonin (Sengupta and Maitra, 2006). Melatonin is supposed to act as a modulator in synchronizing different phases of annual reproductive cycle with the environmental factors (Rani et al., 2007).

Exogenous melatonin administration suppresses reproductive parameters in a number of lizards, including *Callisaurus draconoidis* (Packard and Packard, 1977) and the Indian garden lizard, *Calotes versicolor* (Haldar-Mishra and Thapliyal, 1981). A comparison between the melatonin patterns and reproductive functions under different photoperiods in reptiles suggests that the reproductive responses are not controlled by the amplitude or duration of melatonin elevation, but possibly by the phase of the melatonin peak in relation to the period of darkness (Mayer et al., 1997).

Though reports on amphibians are seldom, melatonin administration in toad, *Bufo melanostictus*, resulted in reduced spermatogenic activity (Biswas et al., 1978). It is known that abolishment of the melatonin source influences the normal ovarian and metabolic responses associated with active vitellogenesis, which ultimately leads to an elevation of estradiol, ovarian lipid and plasma and ovarian phosphate (yolk) in amphibians (Mayer et al., 1997).

In most seasonally breeding fish, breeding takes place in most of the favorable time of the annual cycle of the animal as most of the young ones get the utmost chances of development and this annual cycle is governed by certain cue received from the living environment. Though environmental regulation of reproduction in fish is almost same as in higher vertebrates; melatonin in fish, unlike mammals and birds, appears to play an important role of an 'internal calendar', which controls the seasonal breeding pattern (Bayarri et al., 2004). The role of melatonin in the regulation of gonadal growth and maturation in fish has been investigated either by pinealectomy (Popek et al., 1997), or by melatonin administration (Bromage et al., 1995) or both (Joy and Khan, 1991). It is generally agreed that melatonin controls the reproductive seasonality by stimulating the final stages of maturation and by synchronizing the oocyte maturity with optimal timing of spawning (Popek et al., 1991). However, the study of serum melatonin profiles and the effects of melatonin administration during different phases of reproductive cycle in carp (*Catla catla*) generated quite interesting information. In carp, like many other fish species, the annual reproductive cycle comprises of four different phases, i.e., preparatory, pre-spawning, spawning and post-spawning (Dey et al., 2004). In an annual cycle, the highest seasonal value of melatonin is observed during the post-spawning phase and lowest during the spawning phase (Maitra et al., 2005). Notably, exogenous melatonin administration induces variable influences like, pro-gonadal or, anti-gonadal or, no subtle effects on gonads, depending upon the reproductive status of fish in an annual cycle (Bhattacharya et al., 2007). Single administration of melatonin, irrespective of its dose, fails to alter the gonadal status of carp in any part of the annual cycle. However, daily administration of melatonin for 15- and 30 days results in an inhibition in the ovarian activities in a duration-dependent manner during the pre-spawning and spawning phases (Bhattacharya et al., 2007). *In vitro* study of melatonin effects on denuded carp oocytes reveals that addition of melatonin 4 h prior to introduction of maturation inducing hormone (MIH) results in acceleration of the rate of oocyte maturation through formation of maturation promoting factor or MPF (a complex of two proteins, cyclin-B and Cdc2) and, thereby, provides first evidence of a modulatory role of melatonin on the actions of other sex steroids to regulate fish reproduction (Chattoraj et al., 2005). Subsequently, serotonin (5-hydroxy tryptamine) was found to counteract the influence of melatonin in accelerating MIH-induced oocyte maturation in carp (Chattoraj et al., 2008).

4. Diversity in the mechanism of action of melatonin on vertebrate reproduction

4.1 Action of melatonin on hypothalamo-hypophyseal-gonadal axis

Influences of melatonin on reproduction are mediated through an interaction with the hypothalamic control of pituitary function. In different animal species, the hypothalamo-hypophyseal system plays a major role in the photoperiodic regulation of reproduction, where melatonin provides temporal information related to day length and season (Falcón et al., 2007). The preoptic area (POA) possesses neurons that convey monoaminergic (i.e., dopamine, 5-hydroxytryptamine) and/or peptidergic information to the pituitary gland. The peptidergic information is conveyed by peptides or releasing factors that control the activity of the pituitary cells after reaching

the neurohypophysis. The peptides include isotocin and arginine vasotocin, while releasing factors are the pituitary adenylate cyclase-activating peptide, gonadotropin releasing hormone (GnRH), growth hormone releasing hormone (GHRH), and corticotrophin releasing hormone (CRH) (Falcón et al., 2007). Steroidogenic enzyme activity in ovary is stimulated by gonadotropic hormone (GTH) which is secreted by adenohypophysis (Nagahama, 1994). An enhanced activity of two important steroidogenic enzymes (3β-hydroxysteroid dehydrogenase or 3β-HSD and 17β-hydroxysteroid dehydrogenase or 17β-HSD) is detected in fish ovaries under long photoperiod, while 17β-estradiol in turn stimulates the synthesis of vitellogenesis in the concerned animal (Bromage et al., 1995) during the preparatory and the pre-spawning phases of an annual cycle. Exogenous melatonin administration has variable effects in various fishes- it reduced pituitary GnRH and luteinizing hormone (LH) content but stimulated pituitary follicle stimulating hormone content in the masu salmon (*Oncorhynchus masou*), (Falcón et al., 2007), and atlantic croaker (*Micropogonias undulatus*) (Khan and Thomas, 1996). Single dose of exogenous melatonin is effective in influencing the functions of hypophyseal-gonadal axis in laboratory rodents (Maitra and Ray, 2000). Melatonin also contributes to the nocturnal increase and diurnal decrease in fish plasma GH levels (Boeuf and Falcón, 2001). Continuous release of prolactin (PRL) by trout pituitary gland or cells in culture is inhibited by physiological doses of melatonin through a cAMP-mediated process (Falcón et al., 2007).

4.1.1 Hypothalamic actions of melatonin - Possible role of gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH)

Gonadotropin-releasing hormone (GnRH) is a decapeptide that is synthesized and released by hypothalamic neurosecretory cells in a pulsatile pattern into the hypothalamo-hypophyseal portal circulation and plays a pivotal role in the regulation of reproduction. This peptide binds and activates its cognate receptor (GnRH receptor) on the pituitary gonadotrope cells, and in turn stimulates the synthesis and secretion of gonadotropins (GtHs) by acting through G-protein coupled transmembrane receptor having extracellular amino terminal and intra-cellular carboxy terminal domain (Weil et al., 1992). Eleven GnRH structures have been elucidated from different vertebrates and protochordates, among these four different isoforms are isolated from fishes (Bhattacharya, 1999). GnRH in fish structurally varies from other vertebrates, but the amino acid length and in NH_2 terminus and COOH terminus are conserved. The recent discovery of a novel hypothalamic gonadotropin-inhibitory hormone (GnIH) has added a new dimension to the current understanding on the mechanism of hypothalamic control of vertebrate reproduction (Tsutsui, 2009). GnIH inhibits gonadotropin release and synthesis, thus appears to inhibit gonadal development and maintenance. The GnIH receptor, comprising of seven transmembrane domains, that specifically binds to GnIH is expressed in the pituitary and several brain regions including hypothalamus. Anatomically, GnIH fibres remain in close proximity to GnRH neurons in the preoptic area (Ubuka et al., 2008), while GnIH and GnRH are connected in the median eminence (Bentley et al., 2003). So, it is assumed that GnIH may influence the GnRH system at the neuron and fiber terminal levels. As the GnIH receptors (GnIH-R) are expressed in the gonadotropes in the pituitary, it is likely that GnIH acts directly on the gonadotrope cells and inhibits the synthesis and release of gonadotropin. GnIH may also act on GnRH neurons in the preoptic area to inhibit GnRH release. Recent studies provide evidence that melatonin induces GnIH expression in GnIH neurons (Tsutsui et al., 2012). Due to the expression of melatonin receptor (Mel1c) in the GnIH neurons, melatonin can act directly on GnIH neurons to induce GnIH expression and release (Tsutsui, 2009). An *in vitro* study following administration of melatonin demonstrated a dose-dependent increase in GnIH release from the hypothalamus (Chowdhury et al., 2010). The expression of GnIH in brain is increased under short day, when the nocturnal duration of melatonin secretion is also increased, indicating that melatonin may directly induce synthesis of GnIH (Ubuka et al., 2005). Experimental study suggests that avian GnIH, *via* altered melatonin signal, may transduce photoperiodic information to the reproductive axis (Tsutsui et al., 2010). However, it remains to be unraveled if such mechanism operates in lower group of vertebrates also.

4.1.2 Kisspeptin: A hypothalamic candidate in the action of melatonin on reproduction?

Kisspeptin, a key catalyst for the initiation of puberty and regulator in seasonal breeding in mammals (Revel et al., 2007), is supposed to perform functions via melatonin signalling by both directly regulating KiSS-1 expression and changing sensitivity of KiSS-1 to sex steroid feedback (Grieves et al., 2008). Unlike the single protein (KiSS-1) and receptor (GPR54) found in mammals, it is thought that in fishes there are at least two forms of both KiSS-1 and its receptor (Felip et al., 2009). KiSS system is well conserved in fishes and performs identical roles in fishes and mammals. Since kisspeptin is associated with the onset of puberty (Martinez-Chavez et al., 2008), it is

shown to have similar GnRH regulatory abilities (Elizur, 2009). However, the functional interplay between the GnRH, GnIH, kisspeptin and melatonin at hypothalamic level in the regulation of reproduction in different seasonal breeders is far from being completely resolved and remains as an interesting area of future research.

4.2 Direct action of melatonin on gonads

4.2.1 Receptor mediated action of melatonin

Demonstration of melatonin binding sites in mammalian reproductive organs (Woo et al., 2001) raised a possibility of direct action of this indoleamine on the gonad. Studies employing molecular techniques revealed the existence of three different subtypes of melatonin receptors, namely, MT1, MT2 and Mel1c (Dubocovich et al., 2000). Melatonin is concentrated in human ovarian follicular fluid relative to the level in plasma, and it alters granulosa cell steroidogenesis and follicular function in hen, hamster and humans. Both MT1 and MT2 melatonin receptors are identified in human granulosa cell/luteal cells and in rat ovaries (antral follicles and corpus luteum) (Tamura et al., 2009). An early study in fish suggested that melatonin and its receptors play a positive role in the regulation of vitellogenesis, during which the yolk precursor protein (vitellogenin) is synthesized in the liver under the influence of estrogens followed by its active incorporation into the developing oocytes. Further support to this contention is available from the report that melatonin treatment and pinealectomy failed to induce gene expressions of estrogen receptors and vitellogenin in rainbow trout (Mazurais et al., 2000). Though a few *in vitro* studies indicated that melatonin alone does not exert any action on oocyte maturation in fish (Popek et al., 1996; Cerda et al., 1997), evidence is available to suggest that melatonin can modulate the action of MIH or maturation inducing hormone (17α, 20β-dihydroxy-4-pregnen-3-one) on the maturation of oocytes (Chattoraj et al., 2005). In teleosts MIH by acting on its receptors on the oocyte membrane, induces the activation of maturation promoting factor (MPF) in the oocyte cytoplasm to initiate final maturation of oocytes characterized by morphological changes associated with progression of meiotic cell cycle leading to breakdown of oocyte nuclear envelope or germinal vesicle breakdown (GVBD) occurring at the prophase/metaphase transition (Nagahama, 1997). GVBD is usually regarded as a signal of the progress of oocyte maturation (Tokumoto et al., 2004). Detection of Mel1a melatonin receptor on both the membrane and cytosolic fractions of the carp ovarian follicles supports the conjecture that melatonin may have direct action on oocyte maturation (Chattoraj et al., 2009; Maitra, 2011). A recent study revealed that photoperiodic response of ovary varies with the plasma profiles of melatonin, but not on its MT1 and MT2 receptors in the carp ovary (Moniruzzaman and Maitra, 2012).

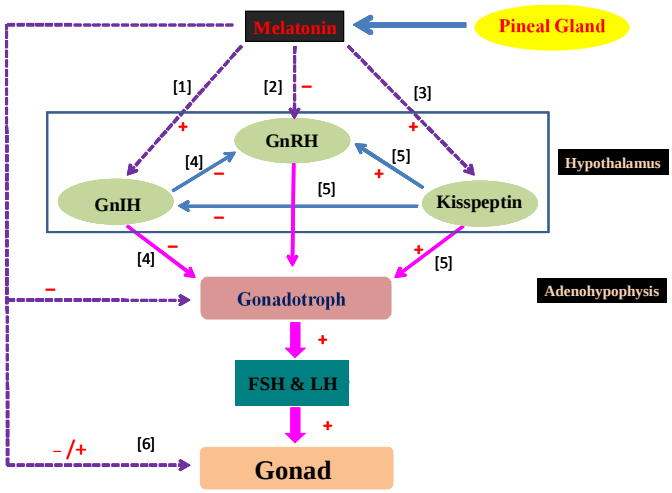

Figure 1: Schematic representation of the presumed role of melatonin and hypothalamic hormones in the regulation of gonadal functions in vertebrates. In brief, the figure represents several steps- melatonin induces gonadotropin inhibitory hormone (GnIH) production in the hypothalamus that may inhibit gonadotropin releasing hormone (GnRH) release, and at the same time may inhibit pituitary gonadotropes to regulate the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). On the contrary, melatonin appears to inhibit the production of kisspeptin and GnRH, where kisspeptin may induce GnRH release from the hypothalamus and stimulate the gonadotropes to increase the FSH and LH release from the adenohypophysis, and ultimately regulate the developmental status of the oocyte. It is likely that melatonin itself can directly influence the oocyte maturation and modulate functions of gonadotropes to regulate FSH and LH release. Numerical values within parenthesis represent the literature base of the proposed diagram; [1] Chowdhury et al., 2010, [2] Tsutsui, 2009, [3] Revel et al., 2007, [4] Ubuka et al., 2008, [5] Tsutsui et al., 2010, [6] Maitra et al., 2005 (for details, see the text).

4.2.2 Receptor independent action of melatonin on ovarian functions

One of the unique properties of melatonin is that it plays a significant protective role against a wide array

of conditions (e.g., ischemia/reperfusion injury, aging and age-associated diseases, toxin exposure, and lipopolysaccharide exposure) resulting from free radical damage (Claustrat et al., 2005). Due to its amphiphilic nature, melatonin can easily cross the cellular barrier and act as a highly ubiquitous direct free radical scavenger and indirect antioxidant. It detoxifies the highly reactive free radical molecules by direct scavenging mechanism (Reiter et al., 2005) without an interaction with specific receptor, or by stimulating the synthesis of a number of antioxidant enzymes that are involved in conversion of harmful reactive molecules to harmless molecules (Barlow-Walden et al., 1995).

4.2.2.1 Free radicals and ovarian functions

Free radicals are defined as molecules or molecular fragments containing one or more unpaired electrons in their atomic or molecular orbits or as an open shell configuration and these unpaired electrons cause the free radicals to become highly chemically reactive. Radicals and their non-radical related species are referred to as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and are by-products of normal cellular metabolism (Tamura et al., 2009). One-electron reduction of O_2 forms the superoxide anion ($O_2^{-\cdot}$) which is converted to hydrogen peroxide (H_2O_2) spontaneously by a process termed dismutation and ultimately forms the most toxic ROS i.e., hydroxyl radical ($^{\cdot}OH$), in the presence of transition metals such as iron and copper (Fenton reaction) (Konturek et al., 2007). Hydroxyl radical may react with itself, other reactive oxygen species, or with proteins, lipids, and other biomolecules in close proximity to the site at which it is formed. Thus, $^{\cdot}OH$ can play a role as a localized reaction intermediate, but generally cannot transduce a signal to a more distant target molecule. $O_2^{-\cdot}$ quickly couples with nitric oxide ($NO^{\cdot}$) to form the highly toxic peroxynitrite anion ($ONOO^-$) due to the enhanced activity of iNOS system accompanied by the overproduction of NO (Hirota et al., 1990). Peroxynitrite anion ($ONOO^-$), the potent RNS, causes change in the protein structure by cysteine oxidation and tyrosine nitration. It can degrade to form the $^{\cdot}OH$. The photoexcitation of O_2 produces singlet oxygen ($^1O_2$), which is also capable of damaging biomolecules. Once H_2O_2 is formed, it is metabolized to innocuous products by catalase (CAT) and glutathione peroxidases (GPx) (Tamura et al., 2009). Because of excessive reactivity, free radicals cause the unwanted side reaction resulting in cellular damage and oxidative stress which ultimately lead to a number of major disorders (Rajamani et al., 2011). They are key signalling molecules for various ovarian functions mediating actions through a variety of pro-inflammatory cytokines (Agarwal et al., 2006). Specifically, free radicals function in the microenvironments of oocytes, sperm, and in the follicular fluid. Changes in these microenvironments have a direct effect on follicular development, ovulation, and quality of oocytes, sperm-oocyte interaction, implantation, and early embryonic development (Agarwal et al., 2006).

4.2.2.2 Melatonin as an antioxidant agent

Melatonin functions as a direct free radical scavenger and detoxifies the highly reactive hydroxyl radical ($^{\cdot}OH$) proving its broad spectrum antioxidant property (Reiter et al., 2009). This tiny tryptophan derivative has the capability of quenching both ROS and RNS including superoxide anion, hydroxyl radical, singlet oxygen, hydrogen peroxide, hypochlorous acid, nitric oxide and peroxynitrite anion. Melatonin itself is not only a free radical scavenger, but the metabolites produced during these interactions (i.e., cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, and N1-acetyl-5-methoxy-kynuramine) are excellent scavengers of toxic reactants (Tan et al., 2007) as well. Melatonin stimulates a number of antioxidant enzymes that are either involved in metabolizing potentially reactive species to less reactive or harmless molecules, or inducing the synthesis of other endogenously produced antioxidants (Pablos et al., 1995). Further investigation reveals that exogenous melatonin administration leads to elevated activity of superoxide dismutase (SOD) and also glutathione peroxidase (GPx) and glutathione reductase (Tomás-Zapico and Coto-Montes, 2005), whereas in rat melatonin deficiency induced by pinealectomy reduced GPx activity (Baydas et al., 2002). Melatonin also causes incremental changes in messenger RNA (mRNA) levels for Cu, Zn-SOD and Mn-SOD after its exogenous administration (Antolín et al., 1996). The intrafollicular concentration of bio-markers of oxidative stress such as 8-hydroxy-2-deoxyguanosine (8-OHdG), and hexanoyl-lysine adduct (HEL) are reduced by melatonin administration (Tamura et al., 2008).

4.2.2.3 Melatonin and oxidative stress in reproduction

Both ROS and RNS are produced inside the follicular microenvironment, i.e., follicular somatic cells and oocyte. Oxidative stress is one of the major factors responsible for poor oocyte quality. *In vitro* study revealed that the increase of intracellular concentration of H_2O_2 induces the resumption of meiosis from diplotene arrest in denuded rat oocyte (Chaube et al., 2009), while further increase of ROS causes meiotic cell cycle arrest and apoptosis. Free radicals are responsible for deterioration

of cell membrane lipids, damaging DNA, and induce apoptosis, two-cell block and inhibition of fertilization (Kowaltowski and Vercesi, 1999).

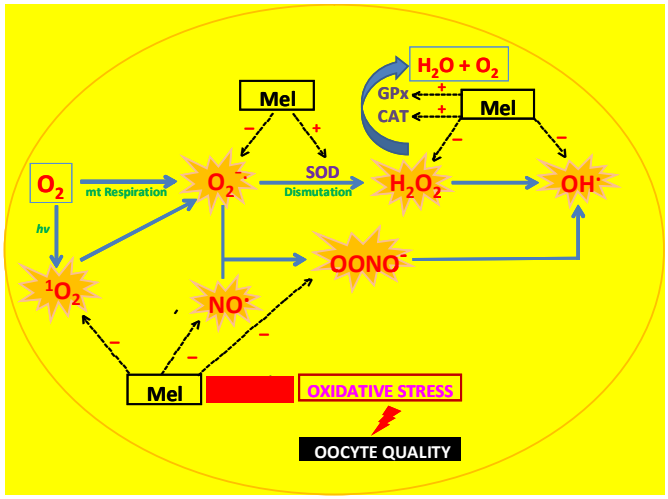

Figure 2: Diagrammatic representation of the interaction between melatonin, antioxidative enzymes and stress-inducing free-radicals within an oocyte. Current information base indicates that melatonin scavenges the toxic free radicals and reactants (marked as explosion) where positive (+) sign signifies its stimulatory effect and negative (-) sign denotes inhibitory effect of melatonin on it. Melatonin (Mel) reduces the production of hydrogen peroxide (H_2O_2) from molecular oxygen (O_2) through regulating the enzymatic action of superoxide dismutase (SOD) under a process known as dismutaion. On the other hand, it is likely that melatonin stimulates antioxidative enzymes (e.g., glutathione peroxidase and catalase) which ultimately induce the splitting rate of H_2O_2 to molecular oxygen. Melatonin may also inhibit the production of singlet oxygen ($^1O_2$)- precursor of superoxide anion ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$) and peroxinitrite anion ($ONOO^-$), to ultimately regulate the hydroxyl radical ($OH^{\cdot}$) production, which is the most powerful reactant in this pathway. Collectively, melatonin appears to reduce the oxidative stress within an oocyte to regulate its physiological status.

Melatonin, due to its lipophilicity and broad spectrum antioxidant property, has been implicated to the optimal function of cells and organs, including those of reproductive system. Generally, the direct free radical scavenging actions of melatonin are accomplished without an interaction with specific receptors. However, evidences are available to suggest that stimulatory effects of melatonin on antioxidative enzymes are likely to be mediated either by membrane receptors or by nuclear or cytosol-binding sites (Reiter et al., 2009). The protective actions of melatonin against toxic free radicals may also be manifested at the time of normal ovulation. The shedding of oocytes from a Graafian follicle involves processes similar to a local inflammatory response in the wall of the follicle. As a consequence, both ROS and RNS are produced in excess by local inflammatory cells, increasing the likelihood that the oocyte could be oxidatively abused during ovulation. Given that melatonin and its metabolites effectively reduce molecular damage due to ROS and RNS, the high endogenous concentrations of melatonin in the ovarian follicular fluid may serve to protect both the oocytes and the steroid-secreting granulosa cells from toxic oxygen and nitrogen-based by-products. These radicals act not only in the regulation of ovulation but also induce apoptosis of ovarian cells (Reiter et al., 2009). The presence of high melatonin levels in ovarian follicular fluid and the presence of melatonin receptors in granulosa cells (Woo et al., 2001) suggest that this indoleamine may be highly beneficial to the follicle. Obviously, available data strongly suggest a protective role of melatonin against free-radical-induced damage of mammalian oocytes. Nonetheless, it remains to be known whether melatonin plays a similar role in the process of oocyte growth and maturation in seasonally breeding sub-mammalian vertebrates, as carefully controlled studies on them have not yet reached momentum.

5. Conclusion

Since melatonin serves as a physiological messenger of darkness, its role of a synchronizer of environmental light-dark cycle in the regulation of periodic reproductive events in photoperiodic animals is well documented. But the mechanisms by which melatonin regulates/modulates the functions of gonad remain speculative largely due to lack of convincing data from experimental studies. In recent years, substantial progress has been made in understanding the mechanisms of the actions of melatonin at the central as well as peripheral levels. It is clear that more than one hypothalamic peptide are involved in mediating melatonin actions on reproductive axis. Moreover, the evidence that melatonin functions through multiple receptors, both membrane and nuclear, on different components of hypothalamo-hypophyseal-gonadal axis and also as a free radical scavenger, a process that requires no receptors, is unequivocal. Obviously, data generated in recent studies collectively favor an idea of functional *pleiotropy* of melatonin in the regulation of reproduction. However, emergence of a unifying hypothesis on the physiology of melatonin in the

regulation of reproduction has suffered a major setback due to lack of information from carefully controlled study of both receptor-dependent and -independent molecular mechanism of melatonin actions on individual regulatory components of reproductive system in both males and females of the same animal species. Thus, an optimistic idea is that future meaningful studies covering hitherto neglected aspects of melatonin actions would surely prove the worth of versatile nature of this wonder molecule on the reproductive physiology in vertebrates in general, and in seasonally breeding animals in particular.

Acknowledgments

Financial assistance from the Department of Biotechnology (DBT), Government of India, (BT/PR11423/AAQ/03/421/2008) and Council of Scientific and Industrial Research (CSIR), New Delhi (37(1220)/05/EMR-II) to SKM, the Department of Science and Technology (DST), Government of India, (DST/INSPIRE Fellowship/2011/IF110070) to PKP and the University Grants Commission (UGC), Government of India,(F.4-1/2006(BSR)/5-132/2007 to KNH), is thankfully acknowledged.

References

Agarwal A, Gupta S, Sikka S (2006) The role of free radicals and antioxidants in reproduction. *Curr Opin Obstet Gynecol.* **18:**325-332.

Antolín I, Rodríguez C, Saínz RM, Mayo JC, Uría H, Kotler ML, Rodríguez-Colunga MJ, Tolivia D, Menéndez-Peláez A (1996) Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. *FASEB J.* **10:**882-890.

Arendt J, Skene DJ (2005) Melatonin as a chronobiotic. *Sleep Med Rev.* **9:**25-39.

Barlow-Walden LR, Reiter RJ, Abe M, Pablos M, Menendez-Pelaez A, Chen LD, Poeggeler B (1995) Melatonin stimulates brain glutathione peroxidase activity. *Neurochem Int.* **26:**497-502.

Bayarri MJ, Garcia-Allegue R, Munoz-Cueto JA, Madrid JA, Tabata M, Sanchez-Vazquez FJ, Iigo M (2004) Melatonin binding sites in the brain of European sea bass (*Dicentrarchus labrax*). *Zool Sci.* **21:**427-434.

Baydas G, Gursu MF, Yilmaz S, Canpolat S, Yasar A, Cikim G, Canatan H (2002) Daily rhythm of glutathione peroxidase activity, lipid peroxidation and glutathione levels in tissues of pinealectomized rats. *Neurosci Lett.* **323:**195-198.

Bentley GE, Perfito N, Ukena K, Tsutsui K, Wingfield JC (2003) Gonadotropin-inhibitory peptide in song sparrows (*Melospiza melodia*) in different reproductive conditions, and in house sparrows (*Passer domesticus*) relative to chicken gonadotropin-releasing hormone.*JNeuroendocrinol.***15:**794-802.

Bhattacharya S (1999) Recent advances in the hormonal regulations of gonadal maturation and spawning in fish. *Current Sci*. **76:**342-349.

Bhattacharya S, Chattoraj A, Maitra SK (2007) Melatonin in the regulation of annual testicular events in carp *Catla catla*: evidence from the studies on the effects of exogenous melatonin, continuous light and continuous darkness. *Chronobiol Intl.* **24:**629-650.

Biswas NM, Chakraborty J, Chenda S, Sanyal S (1978) A basic experimental approach in perspective of pineal and melatonin involvement in photoperiod-induced alteration of spermatogenesis in toad (*Bufo melanostictus*). *Endocrinologie* **71:**143-148.

Boeuf G, Falcón J (2001) Photoperiod and growth in fish. *Vie Milieu.* **51:**237-246.

Bromage NR, Randall CF, Porter MJR, Davis B (1995) How do photoperiod, the pineal, melatonin and circannual rhythms interact to co-ordinate seasonal reproduction in salmonid fish? In: Goetz F, Thomas O (Eds) *Proc Fifth Intl Symp Reprod Physiol. Fish Symp.* Austin. **95:**164-166.

Bromage NR, Porter MJR, Randall CF (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* **197:**63-98.

Cerda J, Petrino TR, Greenberg MJ, Wallace RA (1997) Pharmacology of the serotonergic inhibition of steroid-induced reinitiation of oocyte meiosis in the teleost *Fundulus heteroclitus*. *Mol Reprod Dev.* **48:**282-291.

Chattoraj A, Bhattacharya S, Basu D, Bhattacharya S, Bhattacharya S, Maitra SK (2005) Melatonin accelerates maturation inducing hormone (MIH): induced oocyte maturation in carps. *Gen Comp Endocrinol.* **140:**145-155.

Chattoraj A, Seth M, Maitra SK (2008) Influence of serotonin on the action of melatonin in MIH-induced meiotic resumption in the oocytes of carp *Catla catla. Comp Biochem Physiol. A.* **150:**301-306.

Chattoraj A, Seth M, Maitra SK (2009) Localization and dynamics of Mel1a melatonin receptor in the ovary of carp *Catla catla* in relation to serum melatonin levels. *Com. Biochem Physiol.* **152:**327-333.

Chaube SK, Tripathi A, Khatun S, Mishra SK, Prasad PV, Shrivastav TG (2009) Extracellular calcium protects against verapamil-induced metaphase-II arrest and initiation of apoptosis in aged rat eggs. *Cell Biol Int.* **33:**337-343.

Chowdhury I, Maitra SK (2012) Melatonin time line: From discovery to therapy. In: Watson BR (Ed) *Melatonin in the Promotion of Health*. Taylor & Francis, Boca Raton, Florida, USA. pp 1-60.

Chowdhury I, Sengupta A, Maitra SK (2008) Melatonin: Fifty years of scientific journey from the discovery in bovine pineal gland to delineation of functions in human. *Ind J Biochem. Biophys.* **45:**289-304.

Chowdhury VS, Yamamoto K, Ubuka T, Bentley GE, Hattori A, Tsutsui K (2010) Melatonin stimulates the release of gonadotropin-inhibitory hormone by the avian hypothalamus. *Endocrinology* **151:**271-280.

Claustrat B, Burn J, Chazot G (2005) The basic physiology and pathophysiology of melatonin. *Sleep Med Rev.* **9:**11-24.

Dey R, Bhattacharya S, Maitra SK (2004) Temporal pattern of ovarian activity in a major carp *Catla catla* and its possible environmental correlate in an annual cycle. *Biol Rhythm Res.* **35:**329-353.

Dubocovich ML, Cardinalli DP, Delagrange PRM, Krause DN, Strosberg D, Sugden D, Yocca FD (2000) Melatonin receptors. In: Girdlestone D (Ed) *The IUPHAR Compendium of Receptor Characterization and Classification.* Second ed, IUPHAR Media, London. pp 270-277.

Ekmekcioglu C (2006) Melatonin receptors in human: Biological role and clinical relevance. *Biomed Pharmacother.* **60:**97-108.

Elizur A (2009) The KiSS1/GPR54 system in fish. *Peptides* **30:**164-170.

Falcón J, Besseau L, Sauzet S, Boeuf G (2007) Melatonin effects on the hypothalamo-pituitary axis in fish. *Trnd Endocrinol Metabol.* **18:**81-88.

Felip A, Zanuy S, Pineda R, Pinilla L, Carrillo M, Tena-Sempere M, G´omez A (2009) Evidence for two distinct *KiSS* genes in non-placental vertebrates that encode kisspeptins with different gonadotropin-releasing activities in fish and mammals. *Mol Cell Endocrinol.* **312:**61-71.

Grieves TJ, Humber SA, Goldstein AN, Scotti MA, Demas GE, Kreigsfield LJ (2008) Photoperiod and testosterone interact to drive seasonal changes in kisspeptin expression in Siberian hamsters (*Phodus sungorus*). *J Neuroendocrinol.* **20:**1339-1347.

Haldar-Mishra C, Thapliyal JP (1981) Responses of reptilian gonad to melatonin. *Neuroendocrinol.* **33:**328-332.

Hardeland R, Pandi-Perumal SR, Cardinali DP (2006) Molecules in focus melatonin. *Int J Biochem Cell Biol.* **38:**313-316.

Hirota M, Inoue M, Ando Y, Morino Y (1990) Inhibition of stress-induced gastric mucosal injury by a long acting superoxide dismutase that circulates bound to albumin. *Arch Biochem Biophys.* **280:**269-273.

Joy KP, Khan IA (1991) Pineal-gonadal relationship in the teleost *Channa punctatus* (Bloch): Evidence for possible involvement of hypothalamic serotonergic system. *J Pineal Res.* **11:**12-22.

Khan IA, Thomas P (1996) Melatonin influences gonadotropin II secretion in the Atlantic croaker (*Micropogonia sundulatus)*. *Gen Comp Endocrinol.* **104:**231-242.

Klein DC (2007) Arylalkylamine N-Acetyltransferase: "the Timezyme". *J Biol Chem.* **282:** 4233-4237.

Konturek SJ, Konturek PC, Brzozowski T, Bubenik GA (2007) Role of melatonin in upper gastrointestinal tract. **58:**23-52.

Kowaltowski AJ, Vercesi AE (1999) Mitochondrial damage induced by conditions of oxidative stress. *Free Radical Biol Med.* **26:**463-471.

Lerner AB, Case JD, Takahashi Y, Lee TH, Mori N (1958) Isolation of melatonin, pineal factor that lightens melanocytes. *J Am Chem Soc.* **80:**2587-2589.

Maitra SK (2011) Strategic management of photoperiodic schedule and melatonin profile in the regulation of carp reproduction. In: Sanders JD, Peterson SB (Eds) *Carp: Habitat, Management and Diseases*. Nova Science Publishers, Inc., New York, USA. pp 1-62.

Maitra SK, Dey M (1992) Testicular responsiveness to exogenous melatonin during different phases of the annual testicular cycle in rose-ringed parakeet, *Psittacula krameri* (Neumann). *Eur Arch Biol*. **103:**157-164.

Maitra SK, Ray AK (2000) Role of light in the mediation of acute effects of a single afternoon melatonin injection on steroidogenic activity of testis in the rat. *J Biosci*. **25:**253-256.

Maitra SK, Huesgen A, Vollrath L (1986) The effects of short pulses of light at night on numbers of pineal "synaptic" ribbons and serotonin N-acetyl transferase activity in male Sprague-Dawley rats. *Cell Tissue Res.* **246**:133-136.

Maitra SK**,** Dey M, Sengupta A, Haldar C (2002) Functional organisation and implication of pineal gland and its hormone melatonin in the regulation of avian reproduction: An overview. In: Haldar C, Singaravel M, Maitra SK (Eds) *Treatise on Pineal Gland and Melatonin*. Science Publishers, Inc., Enfield (NH), USA, Plymouth, UK. pp 299-321.

Maitra SK, Chattoraj A, Bhattacharyya S (2005) Implication of melatonin in oocyte maturation in Indian major carp *Catla catla*. *Fish Physiol Biochem.* **31:**201-207.

Martinez-Chavez CC, Minghetti M, Migaud H (2008) GPR54 and rGnRH I gene expression during the onset of puberty in nile tilapia. *Gen Comp Endocrinol.* **156:**224-233.

Mayer I, Bornestaf C, Borg B (1997) Melatonin in non-mammalian vertebrates. Physiological role in reproduction? *Comp Physiol Biochem.* **118:**515-531.

Mazurais D, Porter M, Lethimonier C, Le Drean G, Le Goff P, Randall C, Pakdal F, Bromage N, Kah O (2000) Effects of melatonin on liver estrogen receptor and vitellogenin expression in rainbow trout: an *In vitro* and *In vivo* study. *Gen Comp Endocrinol.* **118:**344-353.

Moniruzzaman M, Maitra SK (2012) Influence of altered photoperiods on serum melatonin and its receptors (MT1 and MT2) in the brain, retina, and ovary in carp *Catla catla. Chonobiol Intl.* **29:**175-188.

Nagahama Y (1994) Molecular biology of oocyte maturation in fish. In : Davey KG, Peter RE, Tobe SS (Eds) *Perspectives in Comparative Endocrinology.* National Research Council of Canada, Ottawa, Canada, pp. 193-198.

Nagahama Y (1997) 17á, 20â-dihydroxy–4–pregnen–3–one, a maturation-inducing hormone in fish oocytes: mechanisms of synthesis and action. *Steroids* **62:**190–196.

Pablos MI, Agapito MT, Gutierrez R, Recio JM, Reiter RJ, Barlow-Walden L, Acuña-Castroviejo D, Menendez-Pelaez A (1995) Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. *J Pineal Res.* **19:**111-115.

Packard MJ, Packard GC (1977) Antigonadotropic effects of melatonin in male lizards (*Callisaurus draconoides*). *Experimentia* **33:**1665-1666.

Poeggeler B, Thuermann S, Dose A, Schoenke M, Burkhardt S, Hardeland R (2002) Melatonin's unique radical scavenging properties- Roles of its functional substituents are revealed by a comparision with its structural analogs. *J Pineal Res.* **33:**20-30.

Popek W, Bieniarz K, Epler P (1991) Role of pineal gland in the sexual cycle in common carp. In: Surowiak JM, Lewand, H (Eds) *Chronobiology and Chronomedicin*. Verlag Peter Lang, Frankfurt. pp 99-102.

Popek W, Epler P, Sokolowska-Mikolajczyk M (1996) Melatonin does not affect steroidogenesis or maturation of carp oocytes *in vitro* during the pre-spawning phase. *Pol Arch Hydrobiol.* **43:** 379-385.

Popek W, Epler P, Bieniarz K, Sokolowska-Mikolajczyk M (1997) Contribution of factors regulating melatonine release from pineal gland of carp (*Cyprinus carpio L.)* in normal and in polluted environments. *Arch Ryb Pol.* **5:**59-75.

Popek W, Luszczek-Trojnar E, Epler P (2000) Effects of pineal gland and melatonin on maturation gonadotropin (GtH2) secretion from perifused pituitary glands of mature carp during spawning. *Electronic J Pol Agri Uni Fisheries* **3:**1-10.

Rajamani K, Manivasagam T, Anantharaman P, Balasubramanian T, Somasundaram ST (2011) Chemopreventive effect of Padinaboergesenii extracts on ferric nitrilotriacetate (Fe-NTA)-induced oxidative damage in Wistar rats. *J Appl Phycol.* **23:**257-263.

Rani S, Singha S, Kumar V (2007) Photoperiodism, pineal clock and seasonal reproduction in the Indian Weaver Bird (*Ploceus philippinus*). *J Ornithol.* **148:**601-610.

Reiter RJ, Tan D-X, Maldonado MD (2005) Melatonin as an antioxidant: physiology versus pharmacology. *J Pineal Res.* **39:**215-216.

Reiter RJ, Tan D-X, Manchester LC, Paredes SD, Mayo JC, Sainz RM (2009) Melatonin and reproduction revisited. *Rev Biol Reprod.* **81:**445-456.

Revel FG, Ansel L, Klosen P, Saboureau M, P´evet P, Mikkelsen JD, Simonneaux V (2007) Kisspeptin: a key link to seasonal breeding. *Rev Endocr Metabol Disorders* **8:**57-65.

Sengupta, A, Maitra SK (2006) The pineal gland, but not melatonin, is associated with the termination of seasonal testicular activity in an annual reproductive cycle in rose-ringed parakeet *Psittacula krameri*. *Chronobiol Intl.* **23:**915-933.

Seth M, Maitra SK (2010) Neuronal regulation of photo-induced pineal photoreceptor proteins in carp *Catla catla*. *J Neurochem.* **114:**1049-1062.

Seth M, Maitra SK (2011) Neural regulation of dark-induced abundance of arylakylamine N-acetyltransferase (AANAT) and melatonin the carp (*Catla catla*) pineal: an *in vitro* study. *Chronobiol Int.***28:**572-585.

Soares JM Jr, Manson MI, Ersahin C, Dubocovich ML (2003) Functional melatonin receptors in rat ovaries at various stages of the oestrous cycle. *J Pharmacol Exp Ther.* **306:** 694-702.

Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K, Morioka H, Ishikawa H, Reiter RJ, Sugino N (2008) Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. *J Pineal Res.* **44:**280-287.

Tamura H, Nakamura Y, Korkmaz A, Manchester LC, Tan D-X, Sugino N, Reiter RJ (2009) Melatonin and the ovary: physiological and pathophysiological implications. *Fertil Steril.* **92:**328-343.

Tan D-X, Manchester LC, Terron MP, Flores LJD, Reiter RJ (2007) One molecule, many derivatives: a never ending interaction of melatonin with reactive oxygen and reactive nitrogen species? *J Pineal Res.* **42:**28-42.

Tokumoto T, Tokumoto M, Horiguchi R, Ishikawa K, Nagahama Y (2004) Diethylstilbestrol induces fish oocyte maturation. *Proc Natl Acad Sci. USA.* **101:**3686-3690.

Tomás-Zapico C, Coto-Montes A (2005). A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *J Pineal Res.* **39:** 99-104.

Tsutsui K (2009) A new key hormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH): Biosynthesis, mode of action and functional significance. *Progr Neurobiol.* **88:**76-88.

Tsutsui K, Bentley GE, Bedecarrats G, Osugi T, Ubuka T, Kriegsfled LJ (2010) Gonadotropin inhibitory hormone (GnIH) and its control of central and peripheral reproductive function. *Front Neuroendocrinol.* **31:**284-295.

Tsutsui K, Ubuka T, Bentley GE, Kriegsfeld LJ (2012) Gonadotropin-inhibitory hormone (GnIH): Discovery, progress and prospect. *Gen Comp Endocrinol.* **177**:305-314.

Ubuka T, Bentley GE, Ukena K, Wingfield JC, Tsutsui (2005) Melatonin induces the expression of gonadotropin-inhibitory hormone in avian brain. *Proc Natl Acad Sci. USA.* **120:**3052-3057.

Ubuka T, Kim S, Huang Y C, Reid J, Jiang J, Osugi T, Chowdhury VS, Tsutsui K, Bentley GE (2008) Gonadotropin - inhibitory hormone neurons interact directly with gonadotropin-releasing hormone-I and II neurons in European starling brain. *Endocrinology* **149:**268-278.

Weil C, Crim LW, Wilson CE, Cauty C (1992) Evidence of GnRH receptors in cultured pituitary cells of the winter flounder (*Pseudopleuronectes americanus* W). *Gen Comp Endocrinol.* **155:**126-140.

Woo MMM, Tai CJ, Kang SK (2001) Direct action of melatonin in human granulosa-luteal cells. *J Cli Endocrinol Metab.* **86:** 4789-4797.