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Modulation of human ovarian function by melatonin

Seema Rai[1], Hindole Gosh[2]

[1]Department of Zoology, Guru Ghasidas Vishwavidylaya, Bilaspur, Chhattisgarh, India, [2]Department of Zoology, Kalinga University, Raipur, Chhattisgarh, India

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1. ABSTRACT

Melatonin, a hormone which is primarily released by the pineal gland, has a wide range of actions in the female reproductive tract. While the melatonin receptor subtype, MT3, has been identified in amphibian animals and birds, in humans and other mammals, melatonin acts through, MT1 and MT2 receptor subtypes which are expressed in human ovaries. The rhythmic release of melatonin starts at puberty and continues throughout fertile female life, affecting and regulating diverse ovarian functions. Here, we discuss the importance of melatonin in regulating folliculogenesis, oocyte quality, ovulation and luteal function, sex steroid receptor gene expression, ovarian steroidogenesis including the production and steroidogenic enzyme activities in the egg and thecal cells. Melatonin improves the egg quality and increases the chance of success of *in vitro* fertilization (IVF). In view of such extensive actions, melatonin is central to the fertility in females. The objective of this review is to recapitulate the current understanding of the role of melatonin and its receptors.

2. INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine), was identified 60 years ago as a neurohormone chiefly secreted by the pineal gland (epiphysis cerebri). Since then, various pinealogists have revealed the physiology and biochemistry of the pineal melatonin. Reports indicate, that its interaction is not limited to the endocrine organs, rather melatonin interacts with other non-endocrine tissues and hence participates in the regulation of various metabolic, immunologic, reproductive, and, other vital physiologic processes coordinating with neuroendocrine network system (1-4). Melatonin is an indoleamine, a tryptophan derivative, which is an

essential amino acid, synthesized via serotonin and acting as a neurohormone.

The duration of melatonin secretion tracks the dark phase of light /dark cycle but, in the case of the mammals this humoral signal is considered a crucial component of the mechanism governing mammalian photoperiodism. After its release in the circulation, it acts as a chemical messenger of photoperiodic phases of the light / dark cycle and regulates the circadian rhythm, sleep-wake cycle as well as various endocrine processes or factors. Melatonin due to its rhythmic diurnal and/or nocturnal fluctuations is assumed to correspond circadian activities with different photoperiods and hormones of the hypothalamic-pituitary axis (5-7). Moreover, the changes ensure the duration and the magnitude i.e. diurnal (clock) and seasonal (calendar) of circulatory levels of the hormone in the blood. Melatonin throughout the year provides information about the season and hence, it is popularly considered as a clock and calendar. This hormone contributes to various central and peripheral physiological actions related to circadian rhythm and reproduction. It participates in the regulation of reproduction in seasonal breeders and phasing of circadian rhythm in mammalian species (8-10). Pieces of evidence categorize it as an immune-regulatory, antioxidative, anti-stress, antidiabetic, neuroprotective, hepatic, and nephroprotective hormone (11-16). By the virtue of being a highly conserved and dispersed biologically active small lipophilic indoleamine, melatonin can act either via receptor (G-protein coupled melatonin receptors) or non- receptor-mediated pathway (17).

Even though this neurohormone is chiefly synthesized and secreted in the circulation by the pineal gland. There are reports providing evidence of its synthesis by other organs and tissues like the retina, the harderian gland, lymphocytes, gastrointestinal tract, and skin, which is termed as extra pineal melatonin (3, 17 -19). Available literature suggests that melatonin may regulate the reproductive neuroendocrine axis in humans (20). The goal of the present review is to explore the comprehensive information about the new horizon of melatonin research, pointing specifically about its modulatory function in female reproductive physiology, with the hypothesis of a molecular mechanistic approach to stimulate future research. It is expected that this may throw light on therapeutic potentials of melatonin which would be beneficial in medical science for the improvement of human reproductive health and fertility.

3. REGULATION OF PINEAL MELATONIN BIOSYNTHESIS

Melatonin is a small (molecular weight 232.3) indoleamine, secreted rhythmically being with the highest peak during the dark period of light/dark cycle. Alterations in environmental lighting are signaled as multisynaptic neural inputs by the central nervous system *via* peripheral nerves into a hormonal output of the pineal gland. In mammals, circadian photoreceptors of the retina convert light and darkness into signals that are sent directly to the SCN through the main pathway, the Retino Hypothalamic Tract (RHT). From the SCN, neuronal projections make synaptic connections in the PVN of the hypothalamus descending onward through the medial forebrain bundle to the intermediolateral cell column of the spinal cord from where preganglionic fibers reach the superior cervical ganglia. Sympathetic postganglionic noradrenergic fibers, from the superior cervical ganglia, innervate the pineal gland through the *nervi conarii*. Interruption of this regulation pathway by a lesion of the SCN or PVN, or by superior cervical ganglionectomy abolishes the pineal gland synthesis. Thus, the production of pineal melatonin occurs in response to nor-adrenergic stimulation which produces a cascade of biochemical events within the pinealocytes. The *N*-acetyltransferase (NAT) activity represents a key regulatory step in melatonin synthesis. Noradrenaline release, from the sympathetic nerves that innervate the pineal gland, is normally high at night and low during the day. In most species, noradrenaline interacts with both beta 1- and alpha1-adrenergic receptors present in the pineal gland. In the rat pinealocyte, stimulation of adrenergic receptors induces a rise in adenylate cyclase, and the cyclic adenosine 3´,5´-monophosphate (cAMP) signaling pathway activates the NAT enzyme that catalyzes the rate-limiting step of melatonin synthesis. Simultaneous activation of alpha1-receptors potentiates the effect

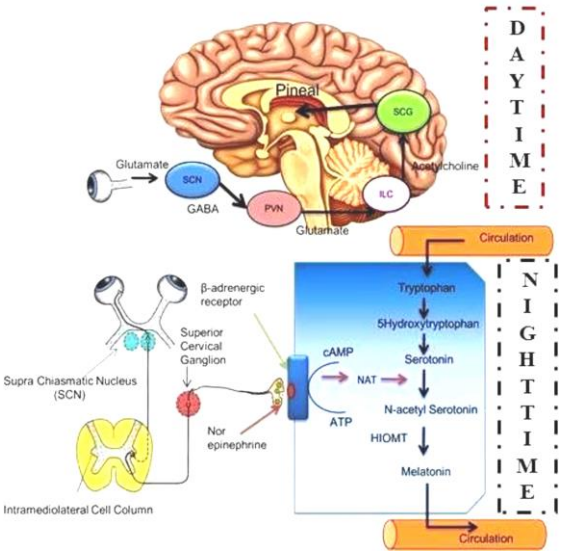

Figure 1. Pathway showing biosynthesis of melatonin and it regulation. Figure 2. Nor–epinephrine (NE) from sympathetic nerve fibre is the key regulator for melatonin synthesis id the key regulator Production of melatonin in the dark and light phases. Gamma-aminobutyric acid (GABA) released by neurons of SCN (suprachiasmatic nuclei) into the paraventricular nucleus (PVN) and switch off the relaease of melatonin from pineal gland during day time (Upper Panel). In night time NE released from superior cervical ganglia (SCG) activating the projection of PVN to the pre ganglionic cell into the intermediolateral cell column (ILC) of the spinal cord. This further, stimulates the β1-adrenergic receptors present in the pinealocytes and promotes protein kinase A (PKA) stimulation, consequently, escalation in the AANAT enzyme activity leads to synthesis and release melatonin in circulation (Lower panel)

mediated through the beta1-receptors (21). Hence, the rhythmic melatonin synthesis is ensured by the oscillating cAMP-dependent transcriptional control mechanism. Melatonin's biosynthetic pathway involves tryptophan, which is hydroxylated and decarboxylated to serotonin, and then serotonin is acetylated by the rate-limiting enzyme NAT and further methylated by hydroxy indole-*O*-methyltransferase to melatonin (5-methoxy-*N*-acetyltryptamine) in the pineal gland. After the synthesis, melatonin is secreted into the blood and cerebrospinal fluid (Figure 1).

4. INFLUENCE OF RHYTHMICITY OF MELATONIN DURING ONSET OF PUBERTY

There is controversy regarding the effect of melatonin during the pre and post-pubertal stage. As reviewed by Waldhauser and his associates in 1993, before the age of 3 months, the circulatory concentration of melatonin remains either non-detectable or very low. It is only thereafter that, this hormone begins to synthesize (22). During the fetal and the first year of life of all individuals HPG axis remains active; afterward, it becomes quiescent till the age of 10 years. As long as the age of individual advances, the circulatory melatonin gradually drops down to a significantly decreased level and stimulates the enhancement of pulsatile secretion of the gonadotropin-releasing hormone as well as the reproductive axis resulting in the onset of puberty (23). Further, nuclear magnetic resonance (NMR) results also provide shreds of evidence of the independence of the production rate of melatonin with age and growth in pineal size between1 to 15 years of age. Hence, decrement in the circulatory melatonin concentration provides a link between the increase in body mass / and / or due to sexual maturation (24). The hypothesis, regarding the correlation amid endogenous melatonin and puberty, based on the earlier finding suggests that prepubertal high circulatory level of melatonin results in inhibition of secretion of gonadotropin hormones (25). Afterward, as the individual progresses to the age puberty, endogenous melatonin comes down to that critical level, which ultimately triggers or switches on the puberty. This hypothesis recommends that the melatonin influences not only the pubertal sexual maturation but even the gonadal and genital development of offspring in rats. However, such a hypothesis still seeks attention for more investigation to be evaluated further for humans (25). It may, therefore, be ventured that before puberty, melatonin concentrations remain too elevated consequently inhibiting activation of the hypothalamic axis. The important aspect to understand is just before puberty, circulatory melatonin drops even lower than the threshold value hence forming the trigger signals of GnRH from the hypothalamus which results in the onset of pubertal changes (25). In various studies, it has been shown that there is a negative correlation between the melatonin and LH concentration and also that it controls the pulsatile secretion of LH (25). Studies have demonstrated that high nocturnal melatonin secretion in children delays puberty whereas low levels of melatonin are associated with precocious puberty (26). Therefore, it is the decline of melatonin which causes the onset of puberty.

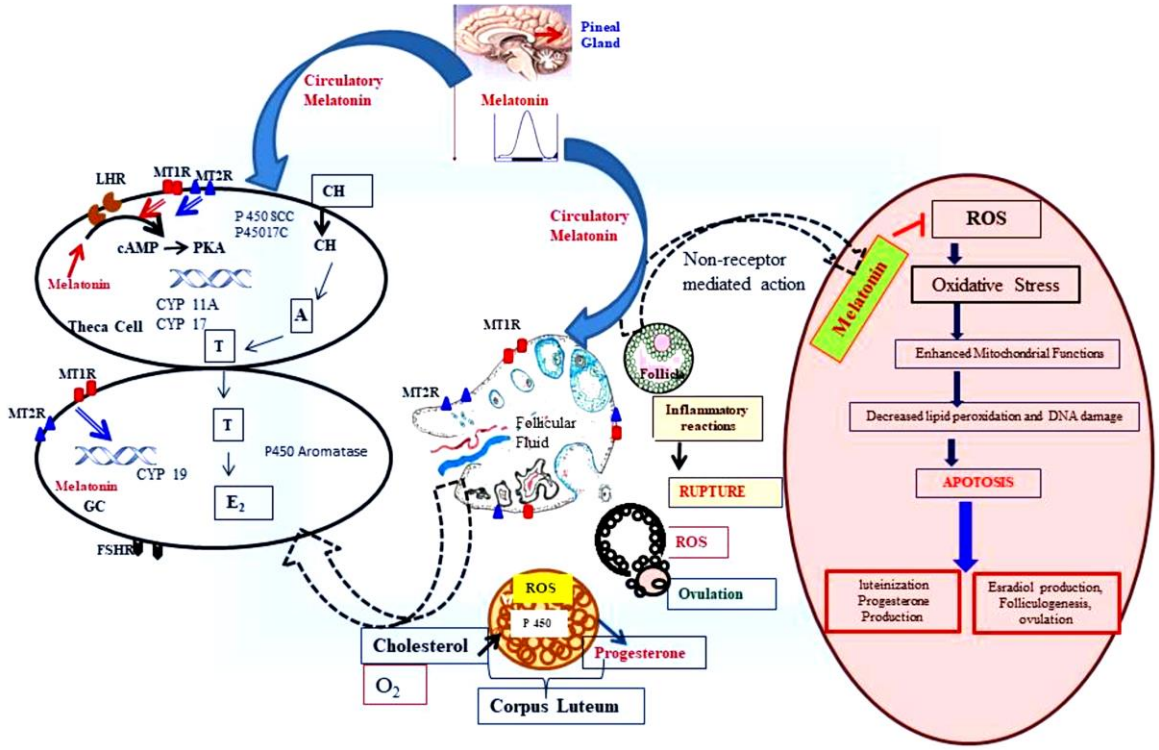

Figure 2. Diagrammatic representation of the proposed mechanism of action of melatonin following receptor and non-receptor mediated pathway. Melatonin in circulation taken up by TC, GC, enters to follicular fluid stimulated the LH, FSH mRNA expression and maturation inducing hormone activity via interacting with its own receptors (MT1R and MT2R) which in turn enhancing and regulating steroidogenic enzyme activities. ROS generation during follicular rupture results in ovulation and steroidogensis (monooxygenage reaction for synthesis of Progesterone) in corpus luteum in because of inflammatory reaction . Circulatory melatonin released from pineal gland, acts as free radical scavenger and maintaining balance between ROS generation and antioxidative enzymes hence promoting mature ovum

5. MODULATION OF OVARIAN FUNCTION BY MELATONIN

A high concentration of melatonin in follicular fluid (FF), relative to the circulatory plasma level and expression of its both the receptors MT1 and MT2 in granulosa cells, luteal cells, antral follicles and corpus luteum of rats, indicates that it plays many essential roles in the regulation of mammalian reproductive processes (27-31). Further, literature provides evidence that melatonin alters granulosa cell steroidogenesis and follicular function in avians, rodents, and humans (32-35) (Figure 2).

5.1. Distribution of melatonin receptors in the ovary

The available report strongly suggests that neurohormone melatonin is neither antigonadotrophic nor progonadotrophic. Therefore, it is very clear that change in nocturnal circulatory melatonin act as a messenger for passive signal updating the hypothalamic-pituitary-gonadal (HPG)-axis regarding the information about the time of year (36 -37). Pineal melatonin is also documented for its participation in the regulation of reproduction in polyestrous mammals such as laboratory rats and human females. This documentation has been supported by the results of the decrement on gonadotrophins and impairments in the periodicity of estrous cycles (38-39). The melatonin actions are mediated through specific membrane receptors which are either G-protein-coupled receptors (GPCRs) or on nuclear binding sites; the latter corresponds to orphan members of the nuclear receptor RZR/ROR superfamily (40). Among the proposed three subtypes of mammalian membrane melatonin receptors, MT1 and MT2, are members of

the 7-transmembrane G protein-coupled receptor family (41-43). MT3, the third receptor which has all the features of a melatonin receptor, is an enzyme called quinone reductase 2, which has been identified only in a few animals (41- 42). The signal transduction cascade associated with the activation of MT1 or MT2 in target cells results in the inhibition of adenylate cyclase activity (42). Activation of these receptors results in the reduction of forskolin-induced cyclic adenosine 3050 monophosphate (cAMP) formation leading to the subsequent reduction in activated protein kinase A (44).MT1 and MT2 receptors trigger the physiological activity following the conventional rule of biochemical pathways of signal transduction. Melatonin may activate different second messenger cascades by interacting with the same receptor subtype depending on the tissue, organ, and species. The literature report, therefore, demonstrates that melatonin may directly affect ovarian function as its receptor binding sites (MT1 and MT2 receptors) are present human GCs as well as on luteal cells (36-37).

5.2. Modulation of follicular growth by melatonin

Folliculogenesis is a complex process involving endocrine, paracrine, and autocrine mechanisms. It begins with the formation of numerous primordial follicles. Primordial follicles progressively develop into the primary, preantral, and antral stages, ultimately reach the pre-ovulatory stage and are capable of releasing an oocyte for subsequent fertilization. Depending upon the species, the process of folliculogenesis is critically dependent on circulating levels of FSH. Interestingly, of the vast number of follicles that are recruited from the ovarian follicular reserve to develop during each reproductive cycle, few (arguably<0.1%) of these follicles will ever fully mature and shed their ovum. Instead, all but the so-called selected follicles die due to atresia. The antral follicle is a small fluid-filled space, which culminates in the fully mature follicle. Human preovulatory follicular fluid FF has been reported with an almost threefold higher circulatory level of melatonin than normal [26-27, 34]. Further, the presence of melatonin, its precursors, serotonin, and N-acetylserotonin, have also been documented in extracts of human ovary. Enzyme activities of NAT and HIOMT required for melatonin synthesis, are also reported in human ovarian homogenates (45). These findings support the possible root cause of the presence of a higher concentration of melatonin in ovarian follicular fluid, suggesting that after its synthesis by the ovary, melatonin might have released into the FF. Nevertheless, the current investigations narrate the opinion that the majority of the detected ovarian and preovulatory FF melatonin is derived from the endogenous circulation (46). The scenario becomes a bit more apparent and it can be explained that the follicular melatonin is taken up from the circulation following its synthesis from the pineal gland. Reportedly, the process of folliculogenesis produces a lot of ROS because of ongoing functional and morphological changes in the two major gears ovarian follicle viz the theca and granulosa cells. Since melatonin is present in the vicinity of the ovarian follicle, therefore, and being a strong antioxidant, it protects the female gamete from the oxidative mutilation and hence regulates healthy folliculogenesis.

5.3. Influence of melatonin on ovarian steroidogenesis

The two-cell theory explains a tuned model of co-operativity of theca and granulosa cells in the process of ovarian steroid hormone synthesis, important for the estradiol production. Recruitment of antral follicles is characterized by the induction of expression of mRNAs encoding a range of steroidogenic enzymes, gonadotropin receptors, and local regulatory factors. The mechanism of selection of the graafian follicle seems to be linked to the timing of mRNA expression encoding LH receptors in GCs (47). *In vitro* melatonin supplementation (10 pM–100 nM) resulted in a remarkable increase in mRNA expression of LH (but not FSH) receptors in human GCs [31]. When theca cells and GCs were separated, melatonin supplementation resulted in the decrement of the production of P by theca, irrespective of its influence on the GCs. It may be assumed that melatonin might be involved in suppressing follicular (thecal) steroidogenesis via modulating the cAMP level. Further, reports demonstrate that melatonin blocks the expression of the steroidogenic acute regulatory protein (48). It is believed that steroidogenic acute regulatory (StAR) protein

determines the translocation of cholesterol across the intermembrane space into the inner membrane where P450scccleaves cholesterol into pregnenolone. Melatonin (10 nM) treatment for 3 hours reduced the steroidogenic acute regulatory protein expression stimulated by hCG in mouse Leydig tumor cells (48). Further, pinealectomy and/or/ administration of melatonin influences CYP17 expression and therefore the process of steroidogenesis as well (38). It seems that there is a complex effect of melatonin on follicular steroid production depending on the cell type (theca cell or GC), duration of treatment (acute or long-term response), experimental model (cell culture or follicle culture), species, and dose (48).

Melatonin treatment with a dosage of 0.01–10 mg/ml stimulates IGF-I production, reported by cultured human GCs (49). Picinato and his associates during 2008 demonstrated that melatonin (0.1 mM) induces the IGF-I receptor and activates two intracellular signaling pathways, the PI3 K/AKT, which is mainly involved with cell metabolism, and MEK/ERKs that participate in cell proliferation, growth, and differentiation (50). Insulin-like growth factors are produced by GCs and theca cells during follicle development (51). Insulin-like growth factors are mitogenic and antiapoptotic peptides that promote differentiation and also possess insulin-like metabolic effects mediated by binding to specific high-affinity membrane receptors. The IGF-I and IGF-II stimulate DNA synthesis and E2 and P secretion by human GCs and granulosa- luteal cells (51). Insulin-like growth factor-I is antiapoptotic in ovarian follicles, whereas ovarian apoptosis is enhanced by IGF binding protein (52). Available research findings suggest that melatonin influences the process of steroidogenesis at the molecular level especially mRNA expression of CYP 11A and CYP17 gene which are the rate-limiting steps in this biosynthesis pathway (38).

5.4. Effect of melatonin on ovulation

Ovulation is a complex process by which a preovulatory follicle ruptures and releases a fertilizable oocyte into the oviductal lumen. This process occurs as a result of a dynamic interaction between the LH surge and local factors including steroids, nitric oxide (NO), prostaglandins, and peptides in a time-dependent manner. The LH surge activates basic structural and biochemical variations leading to rupture of the graafian follicles, and, therefore, resulting in the exclusion of the oocyte with subsequent development of a corpus luteum (CL). Combined treatment of melatonin and progesterone (P) into women resulted in a reduction of LH secretion and finally block ovulation. It is also reported that such combined therapy led to an increase in circulating P without effecting FSH or inhibiting LH secretion (53). This effect might have mediated by the influence of melatonin on hypothalamic gonadotropin release via activation of the HPG axis (54). Nonetheless, the experiment showed that large follicles showed higher melatonin concentration as compared to smaller follicles therefore, strongly suggesting a positive correlation between follicular P and melatonin concentrations [31]. A Higher concentration of circulatory melatonin during the preovulatory phase is suggested to be involved in the production of progesterone, consequently leading in luteinization, followed by successful ovulation [31]. In this connection, research findings confirm that melatonin treatment (20 mg/kg body weight) resulted in a significant increment of prostaglandin E2 concentrations in gastric mucosa as well as esophageal tissue in rats (55-56). On the other hand, the inhibitory effect of melatonin on prostaglandin E2 in medial basal hypothalamus has also been documented by researchers (57). Here it becomes important to address that although the relationship between Prostaglandin E2 and melatonin received enough experimental pieces of evidence by the researcher even then the mechanism towards their exact involvement is still not properly established. Nevertheless, the process of ovulation appears very similar to a local inflammatory response because of the generation of both RNS and ROS (58). Mammalian ovulation is comparable to the inflammatory reaction as successful ovulation requires elevated follicular prostaglandin E2 levels which is one of the inflammatory components. Elevation in follicular prostaglandin levels activates proteolytic enzymes and enhancement of vascular permeability (58). Both endothelial NO synthase (eNOS) and

inducible NO synthase (iNOS) are present in the oocytes and thecal cells of the mouse (59). The major source of reactive oxygen species (ROS) appears to be inflammatory to cells including macrophages and neutrophils, as they are present in the ovary during ovulation. They generate tremendous numbers of free radicals leading to the induction of apoptosis in ovarian cells (6063). Melatonin, as well as its metabolites, is broad-spectrum antioxidants and free radical scavengers that quenches ROS as well as reactive nitrogen species (RNS) (64- 66). Elevated circulatory level of plasma melatonin of preovulatory follicles is presumed to protect GCs and the oocyte from free radicals stress and thus helping in the induction of ovulation [67-68].

5.5. Effect of melatonin in maintenance of Oocyte quality

Poor oocyte quality remains a profound problem for female infertility and hence contributes and participates in the process of fertilization. Oxidative stress, due to ROS generation during the ovulatory process within the follicle, may be a cause of poor oocytes quality (69). They accelerate apoptosis cause deterioration of cell membrane lipids and destroy DNA etc. (70). The balance between ROS production and the scavenging ability of antioxidants is an important factor during the process of oocyte maturation and till fertilization. Further, drugs that protect the oocyte and its surrounding feeder cells from damage, are of great importance and in this connection, melatonin is considered on the top among the basic pinealogist. In this review it is previously discussed that follicular fluid (FF) showed a significantly high level of endogenous melatonin] and its receptors in GCs suggesting a highly beneficial role of this indoleamine and neurohormone in the follicle at the molecular level [26-27,31,35]. Melatonin administration (5 mg/kg body weight) enhances SOD activity and at physiological serum concentrations (1 nM), induces gene expression of all the three antioxidant enzymes (i.e., Cu, Zn-SOD, Mn-SOD, and GPx) (71- 72). Hence, melatonin positively induces both antioxidant enzyme activity as well as the expression of their corresponding genes. Therefore, it may be suggested that melatonin could become the medicine of choice for improving oocyte quality for women who are unable to become pregnant because of poor oocyte quality. Melatonin also regulates the oocyte maturation capacity (73).

Accelerated action of maturation-inducing hormone for the formation of maturation- promoting factor was reported, following administration of melatonin (50–500 pg/mL) and germinal vesicle breakdown of oocytes. It exerts DNA methyltransferase inhibitory effects by masking target sequences and/or by blocking the active site of the enzyme (74). Melatonin's epigenetic efficacy has been proven in several diseases including cancer and hypertension (75-76). However, this miraculous neurohormone may also induce epigenetic changes in oocytes (77). These epigenetic modifications may result from the interaction of melatonin with nuclear melatonin receptors (78). The nuclear receptors of melatonin seem to show a functional role in DNA bending because it may result in the changes of DNA superstructure following epigenetic modifications by affecting nuclear melatonin receptors (79). Therefore, melatonin seems to be a mediator, to oocytes and interaction between environmental factors and the epigenetic inheritance system, for the environmental stimuli. Melatonin being a lipophilic molecule as well as an effective free radical scavenger in the culture medium supports not only mouse fertilization but also to the early development of embryonic tissue (80). Earlier it has been reported that melatonin supplementation (10 nM) consequences a positive effect on porcine embryo cleavage rates and blastocyst total cell numbers (81). Besides, melatonin in the culture medium improved the rate of development of thawed blastocysts with a higher hatching rate after 24 hours of culture (82). No negative effects of melatonin were observed on embryo development, at any of the concentrations 1 pM–100 tested, or even when administered in high doses during pregnancy (83-84).

6. MODULATION OF LUTEAL FUNCTION BY MELATONIN

The formation of the CL is initiated by a series of morphological and biochemical changes in cells of the theca interna and granulose of the preovulatory follicle (85). Melatonin may act at the level of the ovary to modulate its luteal function. Not

only structural changes but genomic alterations lead to the terminal differentiation of follicular cells into P-producing luteal cells. Research reports deliver information regarding the increase in melatonin levels in the luteal phase compared with the follicular phase of the menstrual cycle (86-87). In humans, melatonin binding sites have been detected in GCs–luteal cells which consequently stimulate the secretion of P by human GCs or luteal cells [26, 31, 35]. This, in turn, remarkably increases mRNA expression of the LH (but not FSH) receptor in human GCs/luteal cells, while inhibited expression of GnRH therefore, the GnRH receptor indicating a direct involvement in the regulation of the luteal function[26,31,35]. Further, this hormone also enhances hCG-stimulated P secretion from these cells, possibly by the elevated expression of the LH receptor. On the contrary, some reports showed no effects or negative effects of melatonin on P production in the growing and luteinized GCs [28, 29, 50,89] Nonetheless, literature also suggests the inhibitory action of melatonin (1 ng/mL–100 mg/mL), P and cAMP secretion by GCs isolated from porcine ovaries (89). Interestingly, the effects of melatonin such as induction of mRNA expression for the LH receptor and supportive effect on GCs are more dominant than its inhibitory actions. Melatonin might rescue GCs from induced free radical cytotoxicity in long-term cultured cells by its direct and indirect antioxidative ability.

The essential effect of melatonin on endometrial morphology and embryo implantation has been well described by Dair and their associated research team (90). The reports demonstrated that the implantation rates and serum P levels were decreased in the pinealectomized rats, whereas the reduced serum P levels were restored to normal by daily melatonin injections (2 mg/kg). Nevertheless, melatonin also increases PRL secretion inhibits the oxytocin release from the rat's hypothalamic-neurohypophysial system, suggesting that the indoleamine is essential for maintaining P production and luteal function by making the best use of various mechanisms (91-92). Elevated melatonin in the luteal phase and early pregnancy may induce P production by luteal cells, which is necessary for a successful pregnancy.

7. PROTECTIVE EFFECT OF MELATONIN IN EMBRYO CULTURE

Oocytes and embryos face the stress of high concentrations of reactive oxygen species (ROS) during gamete and embryo culture in the Assisted reproductive technology (ART) technique. Nowadays, infertility research working on assisted reproductive technology (ART) has developed an intense interest in the effects of oxidative stress on the success rates of ART. The retrieval of the micro-environment for cultured gametes and embryos is taken as an essential determinant factor for fertilization and consequently successful implantation. The treatment of melatonin has been reported beneficial in previous *in vivo* studies when it was supplemented in corresponding culture media of bovine, murine, and porcine embryo development (93). Ranges of melatonin concentration between 10−5M and 10−11 M showed an increase in 8-celled embryo yield and hence an increased number of blastocysts and blastocyst hatching in embryos with a higher cleavage rate in bovine (94). Findings from the previous experiment lead to the hypothesis that lower concentration of melatonin has a significant impact on the in-vitro supplementation of embryo culture media with its higher impact on the development and quality of embryos and also reported to be less harmful than higher doses (94).

8. EFFECT OF MELATONIN IN SUCCESS RATE OF IVF

Improvement in the success rates of IVF remains a focus of infertility research. As discussed earlier regarding the increment in the reactive oxygen species during the process of assisted reproductive techniques like *in vitro* fertilization (IVF) which undesirably may cause cell death and hence negatively affects the success rate of IVF outcome (95). The participation of the important role of melatonin during the luteal phase is also reported in patients undergoing IVF treatment. In 25 women with luteal phase defect and between them 14 women given with 3 mg/day of melatonin calculated with time-dependent delivery to match the HCG trigger time throughout the luteal phase it was found in a prospective study that melatonin supplementation significantly increased progesterone levels (11.0

ng/ml vs 8.9 ng/ml, $p < 0.05$) (96). A survey-based on for IVF-embryo transfer suggests that melatonin concentration is higher in the fluid of large follicles than in the smaller follicles [48]. Also, the fertilization success of the women with a low fertilization rate (%50%) in the prior IVF-embryo transfer cycle was improved by melatonin treatment compared to the prior IVF-embryo transfer cycle (97). Hence, melatonin, because of being a strong antioxidant molecule, may enhance the improvement of egg quality which further can ensure the increased success rate for IVF and could be a good *in vitro* drug for the ARTs infertility clinics (97).

9. CONCLUSION

Melatonin has received a lot of attention in recent years with a major focus on its free radical scavenging capacity. These findings have widened the understanding of its pleiotropic physiological effects. Among these physiological actions, few are receptor-dependent whereas others are receptor-independent. The presence of melatonin has been reported in FF which might be taken up from the circulation after being released from the pineal gland. During the entire process of ovulation free radicals are produced. Melatonin because of its, antioxidative and free radical scavenging property scavenge produced free radicals; diminishes oxidative stress thereby participating in ovulation, oocyte maturation, and development of an embryo. Findings also indicate its potential role in ART and hence participating as an antioxidant for women having IVF with the aim of possible improvement of egg quality. The safety evaluation of *in vitro* and *in vivo* melatonin treatment has been broadly demonstrated by various researchers, suggesting without any unfavorable impact. However, before adopting the administration of melatonin it becomes important to further evaluate pharmacological consequences and therapeutic potentials. On a closing note, it is expected that melatonin or its novel analog will certainly find a good place among reproductive medicine in the near future.

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Abbreviations: SCN: Suprachiasmatic nucleus., LD: Light/dark cycle, LH: Luteinizing hormone, PCOS: Polycystic ovary syndrome , RZR: Retinoid Z receptor, ROR: Retinoic acid orphan receptor, MT1: Melatonin receptor 1, MT2: Melatonin receptor 2, cAMP: Cyclic adenosine 3050 monophosphate , FF: Follicular fluid , GC: Granulosa cell , FSH: Follicle stimulating hormone, IVF: In-vitro fertilization, NAT: N-acetyltransferase, HIOMT: Hydroxyindole-o-methoxytryptamine, E: Estrogen, P450scc: P450 side-chain cleavage enzyme , C17: P450 17a-hydroxylase,, P: Progesterone , E2: Estradiol , CYP: Cytochrome P, hCG: Human Chorionic gonadotrophin , IGF: Insulin like growth factor, PI3 K/AKT: Phosphatidylinositol 3- kinase/ protein kinase B , MEK: Mitogen activated protein kinase, ERK: Extracellular signaling regulated kinase, NO: Nitric oxide , ROS:

Reactive oxygen species, GPx: Glutathione peroxidase , RNS: Reactive nitrogen species , CL: Corpus luteum , eNOS: Endothelial NO synthase , iNOS: Inducible NO synthase (iNOS), CL: Corpus leutium, PR: Progesterone receptor , GnRH: Gonadotropin regulatory hormone , PRL: Prolactin, PGF2a: ProstaglandinF-2a , LPO: Lipid peroxide , SOD: Superoxide dismutase , CAT: Catalase, ERa: Estrogen receptor a , ART: Assited Reproductive technology

Key Words: Pineal gland, Melatonin, Reproduction, Ovary, *In vitro* fertilization, IVF, Review

Send correspondence to: Seema Rai, Department of Zoology, Guru Ghasidas Vishwavidylaya, Bilaspur Chhattisgarh, India, Tel: 07752-260472, Fax:,07752-260468 E-mail: seemarai.72@ggu.ac.in