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REVIEW ARTICLE

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Pleiotropic roles of melatonin in endometriosis, recurrent spontaneous abortion, and polycystic ovary syndrome

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Melatonin is a neurohormone synthesized from the aromatic amino acid tryptophan mainly by the pineal gland of mammals. Melatonin acts as a broad-spectrum antioxidant, powerful free radical scavenger, anti-inflammatory agent, anticarcinogenic factor, sleep inducer and regulator of the circadian rhythm, and potential immunoregulator. Melatonin and reproductive system are interrelated under both physiological and pathological conditions. Oxidative stress, inflammation, and immune dysregulation are associated with the pathogenesis of the female reproductive system which causes endometriosis (EMS), recurrent spontaneous abortion (RSA), and polycystic ovary syndrome (PCOS). Accumulating studies have indicated that melatonin plays pleiotropic and essential roles in these obstetrical and gynecological disorders and would be a candidate therapeutic drug to regulate inflammation and immune function and protect special cells or organs. Here, we systematically review the pleiotropic roles of melatonin in EMS, RSA, and PCOS to explore its pathological implications and treatment potential.

KEYWORDS
antioxidant, endometriosis, immunoregulator, melatonin, polycystic ovary syndrome, recurrent spontaneous abortion

1 | INTRODUCTION

Melatonin (*N*-acetyl-5-methoxytryptamine), a neurohormone synthesized from the aromatic amino acid tryptophan mainly by the pineal gland of mammals. The classic melatonin synthetic pathway in animals needs 4 consecutive enzymatic steps. First, tryptophan is hydroxylated to form 5-hydroxytryptophan (5HTryp) by tryptophan-5-hydroxylase (TPH). 5HTryp is subsequently decarboxylated to 5-hydroxytryptamine (5-HT, also called serotonin) under the catalytic action of aromatic amino acid decarboxylase (AADC). Serotonin is then acetylated to form *N*-acetyl-5-hydroxytryptamine (*N*-acetylserotonin) via arylalkylamine N-acetyltransferase

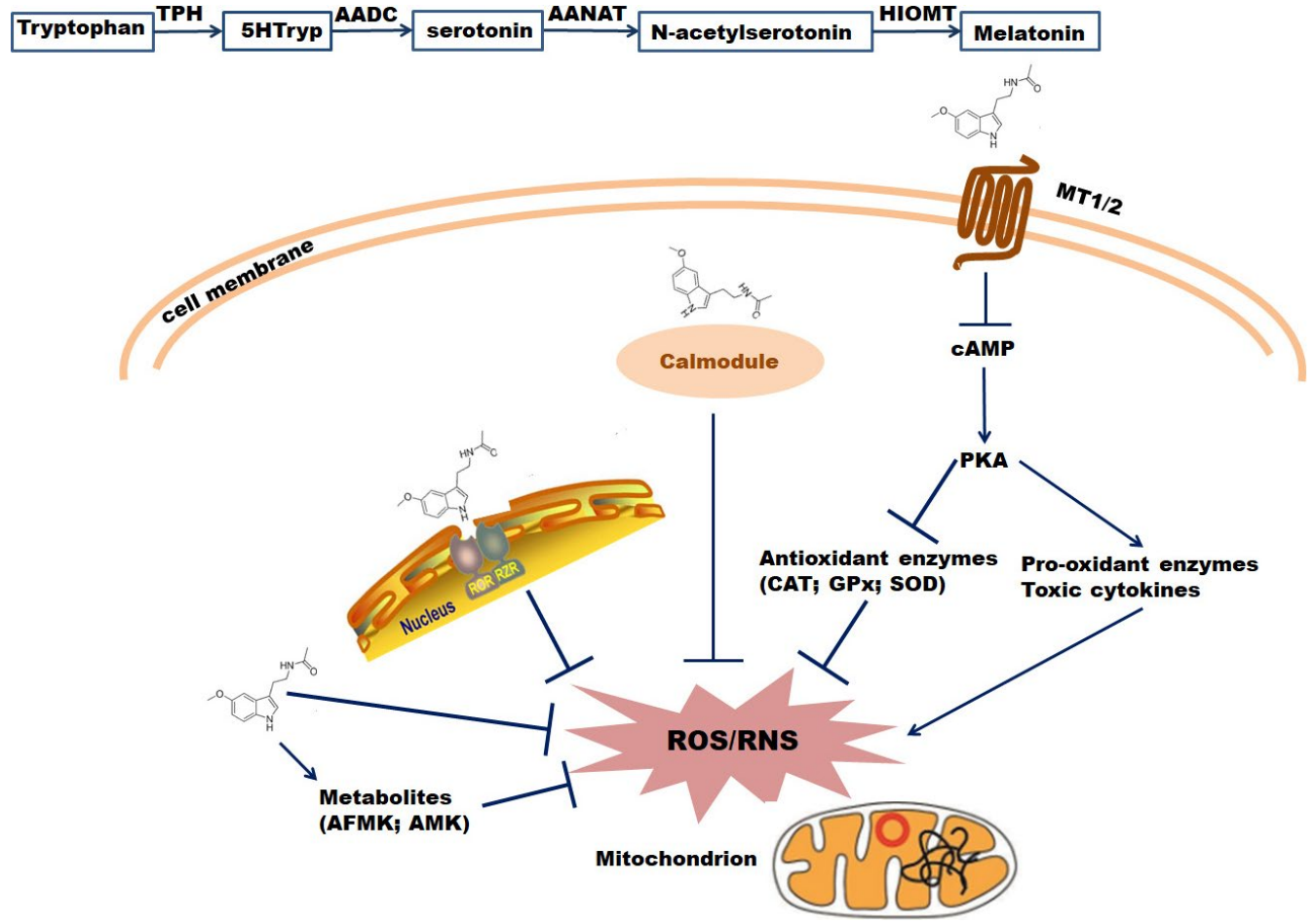

FIGURE 1 The synthesis and secretion characteristics of melatonin and the mechanisms of its powerful capability of free radical scavenger. Melatonin synthesis needs 4 consecutive enzymatic steps. First, tryptophan is hydroxylated to form 5-hydroxytryptophan (5HTryp) by tryptophan-5-hydroxylase (TPH). 5HTryp is subsequently decarboxylated to serotonin under the catalytic action of aromatic amino acid decarboxylase (AADC). Serotonin is then acetylated to form *N*-acetylserotonin via arylalkylamine N-acetyltransferase (AANAT)); and finally, conversion to melatonin by hydroxyindole-O-methyl transferase (HIOMT). Melatonin has antioxidative stress effect mainly through several mechanisms: binding to membrane-bound G protein-coupled receptors MT1/2; binding to nuclear receptors of the orphan family RZR/ROR; binding to intracellular proteins such as calmodulin. Moreover, melatonin and its metabolites are both direct free radical scavengers

(AANAT)); and finally, conversion to melatonin by hydroxyindole-O-methyl transferase (HIOMT), also known as *N*-acetylserotonin O-methyltransferase (ASMT) (Figure 1).[1] It appeared very early during evolution and has then been found in many other extrapineal organs and tissues, such as brain, retina, lens, cochlea, Harderian gland, airway, skin, gastrointestinal tract, liver, kidney, thyroid, pancreas, thymus, spleen, lymphocytes, and reproductive tract.[2] Mitochondria and chloroplasts may be sites of melatonin synthesis within cells. Given that every cell must possess mitochondria to survive, all cells generate melatonin for their local use, likely for cell protection against free radicals.[3] Melatonin secretion is controlled by the endogenous circadian clock located in the suprachiasmatic nucleus (SCN) and regulated by environmental light with low concentrations present during the daytime and high concentrations at night. The shape of the rhythm is roughly sinusoidal.[4]

There are several main mechanisms of melatonin's action in mammalian species (Figure 1): binding to (i) intracellular proteins such as calmodulin (an intracellular protein which is involved in second messenger signal transduction); (ii) nuclear receptors of the orphan family; (iii) melatonin receptors localized in plasma membrane; and further playing antioxidative effects.[5] Melatonin's activity is mostly performed through membrane-bound receptors MT1 and MT2, which are members of the superfamily of G protein-coupled receptors.[6] The signal transduction system associated with the activation of MT1 and MT2 in target cells results in the inhibition of adenylate cyclase activity. Activation of them inhibits forskolin-induced cyclic adenosine monophosphate (cAMP) formation with a subsequent reduction in activated protein kinase A (PKA).[7] MT3, the third binding site, was later defined as a melatonin-related receptor as an enzyme quinone reductase was identified recently in mammalians, including humans. It is structurally related to the melatonin receptors, with a 45% homology at the amino acid levels, but is incapable of binding melatonin.[8] Melatonin also appears to be a natural ligand for the retinoid-related orphan nuclear hormone receptor family (retinoid Z

receptor, RZR/retinoid acid receptor-related orphan receptor, ROR), including 3 types. RZR/RORα is expressed in lots of organs, whereas RZRβ is specifically expressed in brain and retina. And RORγ is preferentially expressed in human skeletal muscle. Recent studies have found that the immunomodulatory effects and part of the circadian effects may partly be mediated through the third receptor family.[9,10]

As a neurohormone, melatonin has numerous important physiological functions and regulates varieties of central and peripheral actions related to circadian rhythms and reproduction. It acts as a broad-spectrum antioxidant, powerful free radical scavenger, anti-inflammatory agent, potential immunoregulator, anticarcinogenic effector, sleep inducer, and regulator of the circadian rhythm in the body.[11] Under both physiological and pathological conditions, melatonin and reproductive system are closely related. Oxidative stress, inflammation, and immune dysregulation are associated with the pathogenesis of the female reproductive system which causes endometriosis (EMS),[12] recurrent spontaneous abortion (RSA)[13], and polycystic ovary syndrome (PCOS).[14] In this review, we systematically summarize and evaluate the pleiotropic roles of melatonin in EMS, RSA, and PCOS from the perspectives of neuroendocrine immunity to explore its pathological implications and treatment potential. These findings may indicate a novel therapeutic approach based on modulation of the oxidative stress, inflammation, and immune through melatonin as a possible future immunoregulator and antioxidant in these reproductive diseases.

2 | MELATONIN AS A POWERFUL ANTIOXIDANT

Mitochondria have been identified as a target for melatonin actions. Mitochondrial DNA is a major target for oxygen radicals because of its location near the inner mitochondrial membrane where oxidants are formed and DNA repair activity is lacking. Melatonin can reduce mitochondrial protein damage and mitochondrial DNA damage, and improve electron transport chain activity.[3] Melatonin and its metabolites are both potent direct free radical scavengers. Melatonin's interaction with reactive oxygen species (ROS) is a prolonged process that involves many of its metabolites; this makes melatonin highly effective in protecting cells from oxidative stress.[15,16] Furthermore, melatonin and its metabolites are indirect antioxidants for their ability to modulate gene transcription for antioxidant enzymes.[17]

Oxidative stress, generated by ROS overproduction or myeloperoxidase (MPO) activity, plays a vital role in inflammation.[18] Radicals and their non-radical-related species are referred to as ROS and reactive nitrogen species (RNS) and are products of normal cellular metabolism. As shown in Figure 2, free radicals and toxic reactants generate from molecular oxygen (O_2). One-electron reduction of O_2 forms the superoxide anion ($O_2\cdot-$). Superoxide ($\cdot O_2^-$) is generated on both sides of the inner mitochondrial membrane and hence arises in the matrix or the intermembrane space (IMS). $O_2\cdot-$ is converted to hydrogen peroxide (H_2O_2) mainly by superoxide dismutase enzymes (SOD1 in the IMS or SOD2 in the matrix). H_2O_2 once formed, is metabolized to innocuous products by catalase (CAT) and glutathione peroxidases (GPx).[19] However, H_2O_2 forms hydroxyl radical (·OH) in the presence of transition metals such as iron and copper. ·OH will react with itself, other reactive oxygen species, proteins, lipids, or other biomolecules in proximity to the site at which it is formed. Thus, ·OH plays a role as a localized reaction intermediate, but it cannot transduce a signal to a more distant target molecule. $O_2\cdot-$ quickly couples with nitric oxide (NO·) to form the highly toxic peroxynitrite anion ($ONOO^-$), which can degrade to form the ·OH. MPO can consume nitric oxide (NO) as a physiological one-electron substrate.[20] The photoexcitation of O_2 produces singlet oxygen ($^1O_2$), which is also capable of damaging molecules. Hypochlorous acid (HOCl) is classified either as an oxygen or chlorine-based reactant.[21] MPO generates hypochlorous acid (HOCl) in the presence of chloride (Cl^-) and H_2O_2.[22,23] HOCl not only destroys invading pathogens but also causes damage through its capacity to react with other biomolecules, such as aromatic chlorination, aldehyde generation, chloramine formation, and oxidation of thiols.[24] Moreover, accumulation of HOCl can mediate hemoprotein heme destruction and subsequent free iron release and protein aggregation through a feedback mechanism involving MPO deterioration.[25]

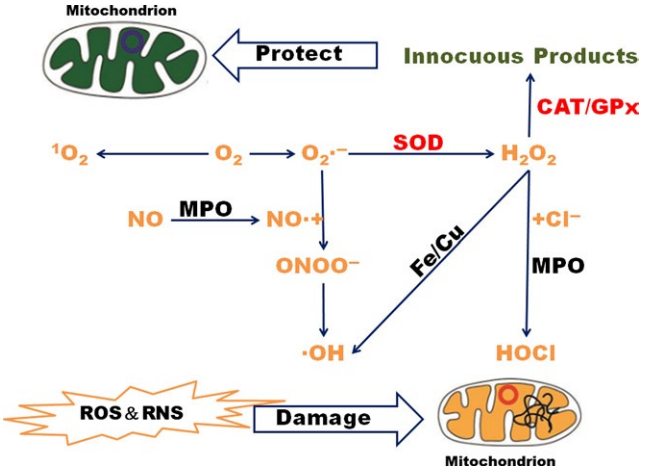

FIGURE 2 The generation and constitution of ROS and RNS and their effect on mitochondrion. Free radicals and toxic reactants generate from molecular oxygen (O_2). One-electron reduction in O_2 forms the superoxide anion ($O_2\cdot-$); $O_2\cdot-$ is converted to hydrogen peroxide (H_2O_2) mainly by superoxide dismutase enzymes (SOD). H_2O_2 once formed is metabolized to innocuous products by catalase (CAT) and glutathione peroxidases (GPx). However, H_2O_2 forms hydroxyl radical (·OH) in the presence of transition metals such as iron and copper. ·OH will react with itself. $O_2\cdot-$ quickly couples with nitric oxide (NO·) to form the highly toxic peroxynitrite anion ($ONOO^-$), which can degrade to form the ·OH. MPO can consume NO as a physiological one-electron substrate. The photoexcitation of O_2 produces singlet oxygen ($^1O_2$), which is also capable of damaging molecules. Hypochlorous acid (HOCl) is classified either as an oxygen or chlorine-based reactant. MPO generates hypochlorous acid (HOCl) in the presence of chloride (Cl^-) and hydrogen peroxide (H_2O_2)

Melatonin has the capability of scavenging both ROS and RNS including $O_2\cdot-$, ·OH, $^1O_2$, H_2O_2, HOCl, NO·, and $ONOO^-$ via

receptor-independent (MT1/MT2) actions thereby reducing mitochondrial damage and the apoptotic cascade.[26,27] Melatonin's ability to inhibit the chlorinating activity of MPO or scavenging neutrophil- or macrophage-driven HOCl has been also reported.[28] Melatonin acts via membrane receptors (MT1/MT2) to stimulate a cascade of events which increase transcriptional activity, which leads to an upregulation of antioxidant enzymes and a downregulation of pro-oxidant enzymes as well as a reduction in toxic cytokine synthesis. It influences both antioxidant enzyme activity and cellular mRNA levels for these enzymes, such as Cu-superoxide dismutase (SOD), Zn-SOD, Mn-SOD, and glutathione peroxidase (GSH-Px). Not only is melatonin itself a direct free radical scavenger, but its metabolites that are formed during these interactions and are likewise excellent scavengers of reactive species, such as cyclic 3-hydroxmelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), and N1-acetyl-5-methoxykynuramine (AMK).[15,16,29] These metabolites are generated from melatonin via several pathways including enzymatic, pseudo-enzymatic and because of interaction with a variety of ROS.[16] Melatonin's interaction with ROS is a prolonged process that involves many of its metabolites, which assist melatonin in protecting cells from oxidative stress in a high efficient way. Melatonin also binds to calmodulin to modulate NO production and acts on cytosolic quinone reductase (MT3) to eliminate free radicals and reduce oxidative damage. In addition, nuclear binding sites may also be involved in some of these actions described above.[30]

3 | MELATONIN AS A POTENTIAL IMMUNOREGULATOR

Melatonin is also an effective regulator of immune reactions. A previous study of diurnal rhythmicity of human lymphocyte subpopulations and cytokine production revealed a strong positive correlation between some characteristics of leukocyte subset structures and plasma melatonin.[31] Meanwhile, the immunoregulatory function of melatonin is daily and seasonally dependent. The daily and seasonal variations in immune system status seem to determine the outcome of infectious challenges, as well as predisposition and progression of immune-related morbidities.[32]

As an established and well-known notion, the nervous and endocrine systems can interact with the immune system to modulate its function.[33] Accordingly, melatonin can act as a regulator of circadian rhythms in a hormone-like fashion by modulating other functions and by affecting target cells, for instance, regulating photoperiodic oscillations of the immune or inflammatory response.[34,35] Melatonin receptors were detected in various immune cells of humans (MT1, and nuclear receptor RZR/RORα) and mice immune system (MT1, MT2).[36] Virtually all types of immune cells possess melatonin-specific receptors, providing the molecular basis for their sensitivity to the hormone. There are reports showing that pineal ablation, or any other experimental procedure which reduces melatonin synthesis and secretion, such as exposure to constant illumination or pineal denervation, depresses both cellular and humoral immunity, which is counteracted partly by exogenous melatonin.[37]

Besides having its systemic effects on immune system as a hormone by neuroendocrine mechanism, melatonin, which can be synthesized and secreted by human lymphocytes, also acts through autocrine/paracrine mechanisms in the immune system. A recent research has revealed that the RelA/cRel nuclear factor-κB (NF-κB) dimer, which is crucial for inflammation resolution, mediates the transcription of the key enzyme in melatonin synthesis in RAW 264.7 macrophages. The effects of exogenous melatonin in the resolution phase of inflammation are paralleled by the effects of locally synthesized melatonin in immune cells.[38] Endogenous melatonin is associated with production of interleukin (IL)-2 and is mediated by binding to the low-affinity targets in paracrine/autocrine immunoregulatory manners. Moreover, melatonin leads to transient activation of phospholipase A2 and lipoxygenase activation by combining to calmodulin.[39,40]

The immunostimulatory and antiapoptotic roles of melatonin are exerted mainly through its action on T helper (Th) lymphocytes (Figure 3). At supraphysiological concentrations, melatonin induces T-cell proliferation and upregulation of pro-inflammatory cytokines.[37] Increasing concentrations of melatonin induce T-cell proliferation in a dose-dependent way.[41] The study of diurnal rhythmicity of human lymphocyte subpopulations and cytokine production revealed plasma melatonin is a strong positive correlation with interferon (IFN)-γ/IL-10 peak, suggesting a melatonin/Th1 causality.[31] However, there are studies supporting melatonin's immunosuppressive function and anti-Th1 activity.[42] Th17 subpopulation has been recently identified as a distinct T helper cell lineage with the unique set of cytokines produced, which involve in tissue inflammation, such as IL-17, IL-17F, and IL-22.[43,44] It is well known that Th17 differentiation is mediated by lineage-specific transcription factors and the first to be identified was RORγ.[45] RORα, along with RORγ, has been proved both in vivo and in vitro to play a key role in Th17 lineage differentiation.[46] RORα, as mentioned above, also serves as a high-affinity nucleus melatonin receptor, suggesting direct melatonin involvement in the induction of Th17 cell development. On the one hand, melatonin may be involved in Th17 differentiation along with circadian as well as seasonal variations of immune system activity. On the other hand, the function of Th17 must undergo alterations under the conditions associated with the melatonin level alterations.[47]

It has become increasingly clear that melatonin also affects innate immune function, including monocytes/macrophages, dendritic cells, polymorphonuclear granulocytes, neutrophils, eosinophils, basophils, mast cells, and natural killer (NK) cells.[48] What deserves our attention is the role of melatonin in monocyte/macrophage system and NK cells (Figure 3). Melatonin can stimulate monocyte's proliferation and inhibit its apoptosis and promote production of IL-1, IL-6, and IL-12 and inhibit secretion of IL-10, IL-2, and tumor necrosis factor α (TNFα) by monocytes.[48] Whereas, LPS-stimulated gene expression and Toll-like receptor (TLR)3- and TLR4-mediated signals and production of IL-6, IL-8, IL-10, NO, Prostaglandin E2 (PGE_2),

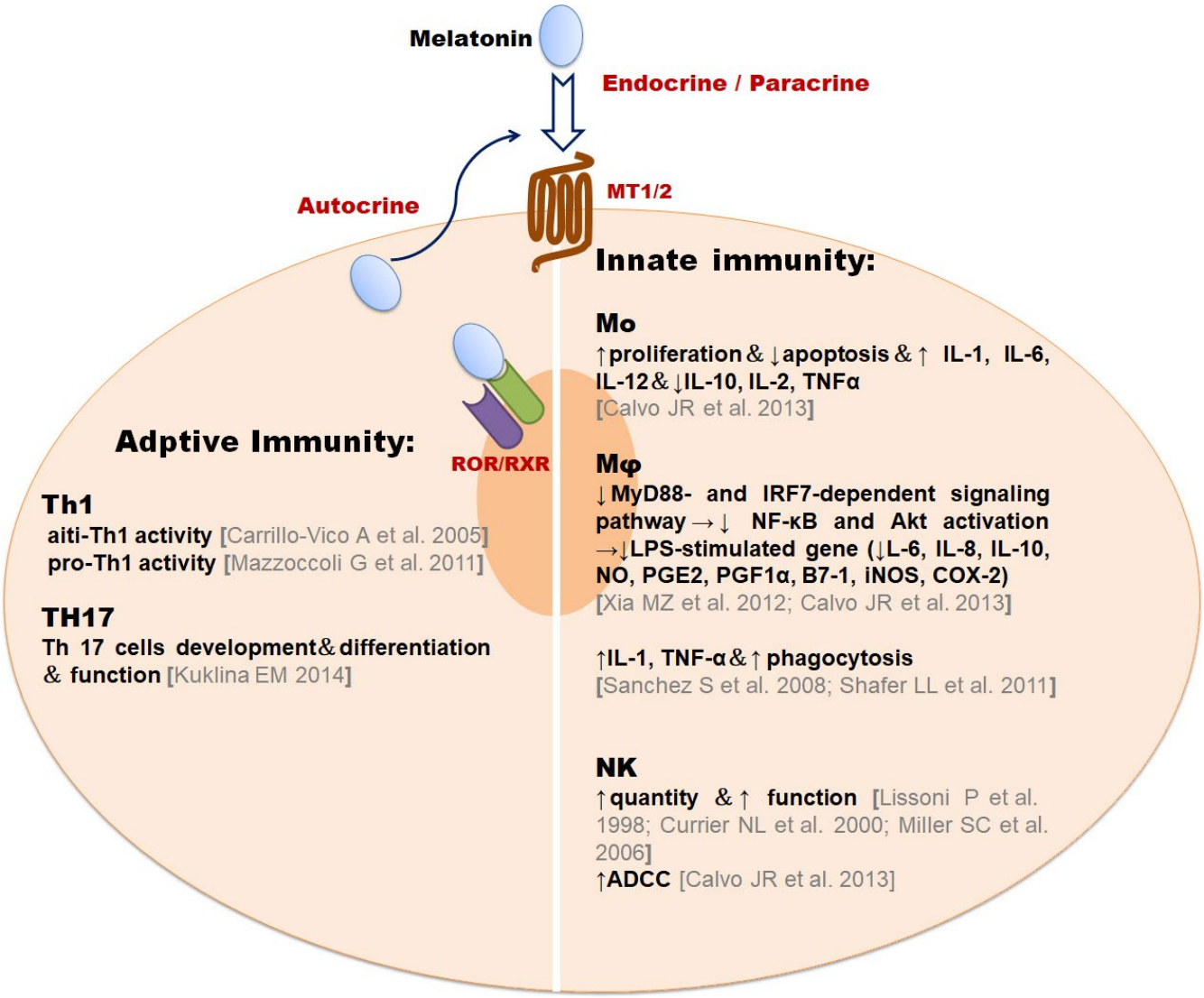

FIGURE 3 Melatonin is an effective regulator of immune reactions. As a potential immunoregulator, melatonin affects both innate immune function (monocytes, macrophages, and NK cells are shown here) and adaptive immune function (Th1 and Th17 are shown). Melatonin receptors are in various immune cells of humans (MT1, and nuclear receptor RZR/ROR) and mice immune system (MT1, MT2). Besides having its systemic effects on immune system as a hormone by neuroendocrine mechanism, melatonin, which can be synthesized and secreted by human lymphocytes, also acts through autocrine/paracrine mechanisms in the immune system

Prostaglandin F1α (PGF1α) in macrophages can be inhibited by melatonin. And melatonin downregulates B7-1, inducible nitric oxide synthase (iNOS), Cyclooxygenase-2 (COX-2) expression in macrophages.[48] Melatonin inhibits TLR4-mediated inflammatory genes in macrophages to exert its anti-inflammation effect. In addition, melatonin alleviates TLR4-mediated NF-κB and Akt activation in macrophages. It inhibits not only myeloid differentiation primary response 88 (MyD88), the key signaling adaptor for MyD88-dependent signaling pathway, but also interferon regulatory factor 7 (IRF7), which is involved in TRIF-dependent signaling pathway, in lipopolysaccharide (LPS)-stimulated macrophages.[49] However, exogenous melatonin stimulates the pro-inflammatory cytokines IL-1 and TNF-α production and enhances phagocytosis of macrophages.[50,51] Numerous researches have demonstrated that melatonin increases the number of NK cells under a variety of conditions.[52] Melatonin administration to both normal and leukemic mice resulted in a quantitative and functional enhancement of NK cells.[53] The increased production of cytokines (IL-2, IL-6, IL-12, and IFN-γ) by melatonin-stimulated T helper cells may partly contribute to the elevated NK cell number and function.[54] Regarding the effect of melatonin on the cytotoxic activity of NK cells, several reports with different results have been published. It seems that melatonin squints toward enhancement of the antibody-dependent cell-mediated cytotoxicity (ADCC) of NK cells.[48]

4 | MELATONIN AND ENDOMETRIOSIS

EMS is a complicated gynecologic disease that affects approximately 5%-15% of all women of reproductive ages and 20%-50%

of all infertile women. The pelvic fluid of women suffering from EMS has high concentrations of inflammatory cytokines, such as IL-6, IL-8, and TNFα.[55] Neutrophil activity with expression of MPO, the source of HOCl during inflammation, is higher in advanced EMS compared to earlier stages secondary to suppression of phagocytic property or establishment of neovascularization.[55] Oxidative stress in the peritoneal cavity is one of the causes of EMS-associated infertility associated with causing detrimental effects on cells through lipid peroxidation, protein oxidation, and DNA damage.[56] Additionally, EMS is strongly associated with chronic pelvic pain (EACPP), which presents with an intense inflammatory reaction.[57] EMS lesions produce pain by compressing or infiltrating the nerves near the lesions. The presence of nerve growth factors (NGFs) in lesions is correlated with hyperalgesia and the growth of sympathetic and sensory neurons of ectopic endometrial growths.[58,59] EMS is also an estrogen-dependent disease, and estrogen increases brain-derived neurotrophic factor (BDNF) during the estrous cycle, and BDNF has received attention as a neuromediator of hyperalgesia and spinal central sensitization in pain states.[60]

Accumulating studies have provided evidence of the potential therapeutic effect of melatonin to facilitate the regression of endometriotic lesions. For example, melatonin effectively decreased endometriotic explant volumes and weights in a rat model.[61] In the melatonin-treated group, the levels of malondialdehyde (MDA) and COX-2 of endometriotic explants and tissue were significantly decreased; the activation of SOD and CAT was significantly increased. Melatonin protected and caused regression of peritoneal EMS in mice by downregulating the activity and expression of matrix metalloproteinase (MMP)-9, MMP-3, and by increasing tissue inhibitor of metalloproteinase (TIMP)-1 expression.[62,63] MMP-9/TIMP-1 expression ratio is identified as a novel diagnostic marker for judging disease progression and severity.[62] In addition, melatonin induces apoptosis and regresses endometriosis through a caspase-3 mediated pathway.[63] Compared with letrozole, melatonin caused more regression of endometriotic foci. Melatonin caused significant increases in SOD and CAT levels, and the recurrence rate was also lower in melatonin group than letrozole group after cessation of treatment.[64] Similarly, pinealectomy was associated with significant growth of endometrial explants and decreased antioxidant activity in a rat model.[65] The growth of endometrial explants and oxidative stress could be decreased by exogenous melatonin supplementation via reducing the explant level of MDA and increasing the levels of SOD and CAT. Activity of SOD and TIMP-2 staining in melatonin group was significantly higher, while there were significant reductions in implant levels of vascular endothelial growth factor (VEGF) and MMP-9 in melatonin group than control group.[66] In another study, different doses of melatonin treatment on endometrial implants (10 or 20 mg/kg/day) resulted in the regression of endometriotic lesions by improving histologic scores in the oophorectomized rat experimental models. And higher levels of melatonin treatment tend to be more effective.[67] Thus, melatonin might be an alternative treatment for EMS with obvious effects on minimizing ectopic lesions and with possible effects on reducing the recurrence rates or increasing the lesion differentiation after testing in a clinical setting.

Melatonin, at least in part, exerts anti-EMS via hormone pathway. According to previous studies, melatonin inhibits steroidogenesis by altering cyclic adenosine monophosphate levels through direct action on the theca or granulosa cells of the follicles.[15] It decreases the luteinizing hormone surge and increases progesterone without affecting follicle-stimulating hormone or estrogen levels.[5] Additionally, the treatment of rats with melatonin resulted in reduced plasma levels of luteinizing hormone and 17 beta-estradiol and promoted differential regulation of the estrogen, progesterone, and androgen receptors in the reproductive tissues.[68]

Additionally, melatonin has an effect on EMS directly via biological behavior of uterine endometrium and indirectly by reducing the formation of intraperitoneal adhesions. Many findings indicate that melatonin receptors were present in the rat uterine endometrium, suggesting that melatonin plays an integral part in uterine physiology.[69] Melatonin may act directly on the MT1 receptors in the rat uterine antimesometrial stromal cells to inhibit their proliferation.[70] Its action may be mediated through a pertussis toxin-sensitive adenylate cyclase-coupled Gi-protein. Oxidative stress may also be involved in the formation of intraperitoneal adhesions.[71] The effects of different routes (intraperitoneal or subcutaneous) and treatment schedules (10 mg/kg; single dose or 5 days) of melatonin on postoperative adhesion formation were investigated in a rat uterine horn model. The results indicated that a significant reduction in postoperative adhesion formation in rats treated with melatonin, regardless of application procedure and duration of the agent. Even a single dose of melatonin therapy was effective in the prevention of postoperative intraperitoneal adhesion formation.[72] Melatonin also significantly reduced adhesion formation in an experimental pericardial adhesion model in dogs.[73]

Melatonin may be a therapeutic agent for alleviating EMS-associated chronic pelvic pain (Figure 4). It appears that antioxidant vitamins C and E are biologically plausible treatments to consider for EMS-associated pain unrelated to menses.[74] Of note, a phase II, randomized, double-blind, placebo-controlled trial has been carried out. Melatonin has been demonstrated to be one of the few medications which have proven useful in the treatment of EMS-associated pelvic pain. Treatment with melatonin of 10 mg/d is more effective than placebo for ameliorating daily pain, dysmenorrhea, dysuria, dyschezia, and sleep in women with biopsy-proven EMS and chronic pelvic pain.[57]

5 | MELATONIN AND RECURRENT SPONTANEOUS ABORTION

Recurrent spontaneous abortion, defined as 3 or more consecutive pregnancy losses before twenty-four weeks of gestation, affects 0.5%-3% of women at the reproductive age.[75] In 50%-60% of RSA patients, the causative agent cannot be identified except the known causes, including chromosomal and metabolic abnormalities,

	Melatonin	Effects	Reference
Animal experiment	10 mg/kg/d ip	↑SOD CAT ↓MDA COX-2 ↓Lesion volume &weight	Guney M et al. 2008
	48 mg/kg/d b.w.	↑TIMP-1 ↓MMP-9	Paul S et al. 2008
	10 mg/kg/d ip & 10 mg/kg/d s.c.	↑SOD CAT ↓Histopathologis Scores ↓Lesion volume	Yildirim G et al. 2010
	10 mg/kg/d ip	↑SOD CAT ↓MAD ↓Histopathologis Scores	Koc O et al. 2010
	10 mg/kg/d ip	↑SOD MDA TIMP-2 ↓VEGF MMP-9 ↓Histopathologis Scores ↓Lesion weight	Yilmaz B et al. 2015
	10 mg/kg/d OR 20 mg/kg/d	↓SOD ↓Lesion volume ↑Histopathologis Scores	Cetinkaya N et al. 2015
Clinical trial	10 mg/d p.o.	↑Sleep quality ↓BNDF ↓Daily pain scores ↓Dysmenorrhea dysuria dyschezia	Schwertner A et al. 2013

FIGURE 4 Melatonin is a therapeutic agent for endometriosis. Melatonin is an effective therapeutic candidate for EMS due to its antioxidant and anti-inflammation ability or its endocrine modulation function via hormone pathways. Accumulating animal experiments have proved that exogenous melatonin application can suppress EMS ectopic lesions, relieve pelvic pain, and improve sleeping quality of women with EMS

uterine anatomic anomalies, blood clotting disorders such as hyperhomocystinemia, immunologic disorders such as systemic lupus erythematosus or antiphospholipid syndrome, infectious diseases, endocrinopathies, PCOS, and sperm DNA fragmentation.[75] RSA is related to the presence of inflammatory cytokines and high levels of ROS.[76] Accumulating studies have provided evidences in support of the occurrence of an imbalance between antioxidant levels and ROS generation, which could be responsible for the start and progression of pathological processes related to RSA.[77] Elevated generation of the superoxide free radical ($O_2^{\cdot-}$) by placental mitochondria and polymorphonuclear leukocytes from pregnant women in their first trimester of pregnancy has been detected.[78] Biochemical markers of ROS-induced membrane damage such as lipid peroxidation products have been shown to increase before abortion.[79] The impaired placental development or degeneration of syncytiotrophoblast in early pregnancy may be caused by oxidative stress that leads to RSA.[80] Furthermore, the significantly decreased GPx and CAT activities and selenium levels as well as increased lipid peroxides and malondialdehyde levels were detected in serum and/or placental tissue of RSA patients. A recent study has also found the enhanced ROS in blood and placental tissue of RSA patients.[81]

Although reports suggest that the use of antioxidant supplements is beneficial in vitro fertilization,[82] studies related to their use in preventing miscarriage are very rare. Concerning the current research results, we speculate strongly that supplementary antioxidant therapy including Se, Zn, Cu, Mn, ascorbate, GSH, and α-tocopherol, as well as melatonin, may be beneficial to such patients during pre-conception and the first-trimester post-conception. In normal pregnant women, melatonin levels increase with gestation, which would aid in reducing oxidative stress.[83] It has been further demonstrated that, under specific condition, melatonin treatment could significantly improve fertilization and pregnancy rates.[84,85] Of note, the oral supplementation of melatonin has a beneficial effect on the improvement of fertilization and embryo quality, likely due to a reduction in oxidative damage.[86] Recently, it has been reported that melatonin system is expressed in human placental tissues throughout pregnancy with greater expression of MT1 receptor in the first trimester. During the differentiation of villous cytotrophoblast into syncytiotrophoblast, MT1 receptor expression is increased, while MT2 is decreased, suggesting different roles of these receptors during trophoblast syncytialization. Moreover, melatonin plays an essential role in enhancing villous trophoblast differentiation and human chorionic gonadotropin (hCG) secretion, as well as in pregnancy well-being and fetal development.[87]

Melatonin regulates endometrial morphology and embryo implantation, and is beneficial to a successful pregnancy possibly via

hormone regulation.[88] Progesterone is essential to achieve and maintains a healthy pregnancy. It is secreted naturally by the corpus luteum during the second half of the menstrual cycle and by the corpus luteum and placenta during early pregnancy. Progesterone prepares the endometrium for the implantation of embryo. If implantation occurs, the corpus luteum continues to produce progesterone, but between 8 and 12 weeks of gestation, the placenta takes over this role and maintains the pregnancy thereafter.[89] In the human, melatonin-binding sites have been detected in granulosa-luteal cells. Melatonin enhances hCG-stimulated progesterone production in human granulosa and/or luteal cells.[90] Melatonin also increases prolactin secretion[91] and inhibits oxytocin release.[92] All of studies suggest that melatonin is important in maintaining progesterone production and luteal function.

Maternal-fetal interface immune tolerance is responsible for the survival of the fetus within the maternal uterus via preventing it from being attacked by the cells of the maternal immune system despite their direct contact. In recent years, a wider appreciation of how the maternal immune system recognizes and even nurtures the developing trophoblast has been established, not only the concept of specific immune rejection, or tolerance of the genetically dissimilar fetus.[93] In the first trimester of pregnancy, 30%-40% of decidual stromal cells are leukocytes, which are prominent at the implantation site where they are in close contact with the invasive extravillous trophoblast cells, spiral arteries, and each other.[94] These leukocytes primarily include uterine natural killer (uNK) cells, macrophages, and T lymphocytes. Other less abundant but functionally important endometrial leukocyte populations are also present including dendritic cells, natural killer T (NKT) cells, and regulatory T cells.[95] Retrospectively, melatonin as a potential immunoregulator can modulate both innate and adaptive immune responses through autocrine, paracrine, or incretion mechanisms. Thus, function of melatonin in maternal-fetal immune microenvironment becomes quite remarkable and interesting.

NK cells are the predominant immune cells present in the endometrium in the luteal phase and in early pregnancy, accounting for 50%-70% of the total number of immune cells after 9-12 weeks of pregnancy.[96] The decidual NK (dNK) cell and peripheral blood NK (pNK) cell show distinct phenotypes, defined by their expression of the surface antigen CD56. More than 90% of pNK cells are $CD56^{dim}$, whereas dNK cells ($CD56^{bright}CD162$) account for 70% of first-trimester decidual stromal leukocyte.[97] The $CD56^{bright}$ cells, dNK cells, which are a rich source of a range of cytokines and angiogenic growth factors and have low killing ability, do not lyse the trophoblast in vitro and can promote trophoblast growth and proliferation.[98] Women with RSA have more $CD56^{dim}$ cells and fewer $CD56^{bright}$ cells.[99] Therefore, it may be hypothesized that the ratio of cytotoxic $CD56^{dim}$ cells to cytokine-producing $CD56^{bright}$ cells, rather than the numbers of NK cells present, may be significant. As a potential regulator of the immune system, melatonin increases NK cell levels and NK cell activity.[52] However, whether melatonin regulates the ratio of $CD56^{dim}$ cells to $CD56^{bright}$ cells remains a mystery, which requires further studies.

Macrophages are the second largest category of immune cells in decidual tissue, accounting for approximately 20% of all the immune cells. Decidual macrophage cells constitute a special group that performs special functions, being involved in organizational recasting, renovation of apoptotic cells, inducing a locally tolerant microenvironment during early pregnancy, and starting the delivery in late pregnancy.[95] Decidual macrophages in early pregnancy can be divided into 2 groups: $CD209^{+}$ macrophages, which account for 70% of decidual macrophages and are responsive toward infection in the decidua and chorionic inflammation, and $CD209^{-}$ macrophages, which express higher levels of IL-10 at the basic level or from stimulation with LPS than $CD209^{+}$ macrophages and whose features tend to be the M2 type.[100] Decidual macrophages are also key immunoregulators at the maternal-fetal interface under local environmental cues from different lymphocyte populations. They not only monitor innate NK cell responses, but are also proficient in regulating adaptive T-cell responses.[94] The production of important anti-inflammatory substances such as IL-10, PGE_2, and indoleamine 2,3-dioxygenase (IDO)[101] by the decidual macrophages plays key immunosuppressive roles in fetal antigen tolerance throughout gestation.[102,103] The early decidua has previously been characterized as a place of immune privilege that contains repressed or suppressed immune cells. However, a recent study has investigated 2 unique human decidual macrophage populations-$CD11c^{HI}$ decidual macrophages and $CD11c^{LO}$ decidual macrophages precisely and suggested that fetal-placental development may require a necessary state of inflammation. $CD11c^{LO}$ decidual macrophages may be important in extracellular matrix formation and cell-cell communication, as well as muscle cell development; $CD11c^{HI}$ decidual macrophages may be important in inflammatory processes including lipid metabolism and lipid Ag presentation. Together, these decidual macrophages populations do not fit the conventional M1/M2 paradigm but produce both pro-inflammatory and anti-inflammatory molecules, thereby contributing to the balance that is necessary for tissue remodeling and growth, as well as for fetal-maternal tolerance.[104] Retrospectively, the influences of melatonin toward macrophages are inconsistent according to the current studies.[48] Therefore, whether melatonin can influence the maternal-fetal interface immune balance through the effect of decidual macrophages and if so, how it will take place need further investigations.

Th1/Th2 cytokine balance with Th2 predominance at maternal-fetal interface is an important mechanism determining the survival of the fetus in the maternal uterus.[105] Physiologically, the melatonin rhythm correlates with rhythmicity in the Th1/Th2 ratio. It seems that melatonin stimulates Th2 immune activity and inhibits Th1 immune activity in experimental mice of septic shock.[42,106] $CD4^{+}CD25^{+}$ regulatory T cells (Treg) were claimed to be important players in the tolerance toward the fetus bearing alloantigens. They are a unique subpopulation of T cells and are confirmed to play a key role in preventing autoimmunity and tolerating allogeneic organ grafts.[107] It has been reported that melatonin increases the number of $CD4^{+}$Th lymphocytes and T-lymphocyte proliferation.[108,109] However, the relation between melatonin and Treg production and

function is obscure. Further studies will be necessary to clarify these relationships, clarifying that association could help in understanding the regulation of successful pregnancy. Meanwhile, a pre-requisite for the successful pregnancy is forming a temporary immune tolerance toward the semiallogenic fetus. A balance of Tregs and Th17 cells plays a key role in this process. These 2 T-cell populations carry out diametrically opposing functions, namely suppression or propagation of inflammation, respectively. So, the abundant activation of Th17 by melatonin must disrupt the balance and consequently provoke abortions.[110]

6 | MELATONIN AND POLYCYSTIC OVARY SYNDROME

Polycystic ovary syndrome is a gynecological endocrine disorder which is a common cause of female anovulatory infertility and menstrual irregularities and affects 6%-8% of women of reproductive age. It has been defined as a syndrome involving polycystic ovaries, hyperandrogenism, hyperinsulinemia, and chronic anovulation. Polycystic ovaries beneath the tunica albuginea contain numerous small antral follicles (so-called cysts) that have stopped growing and developing.[111] High levels of oxidative stress were found in patients with PCOS, indicating an association between PCOS and an increase in oxidative stress in humans, as well as the increase of these radicals occurred in women affected by the syndrome irrespective of body weight.[112] One of the experimental models used in researches is the induction of PCOS in rats through constant illumination, which causes a deficiency in the production of melatonin by the pineal gland, damage to the reproductive system and an increase in oxidative stress.[113] The PCOS rats induced by constant illumination had an increase in the number and diameter of ovarian cysts, thickening of the tunica albuginea, a lack of primary and growing follicles, and numerous atretic follicles.[114] They also presented degeneration and diffuse hyperplasia of the Kupffer cells in livers, as well as alterations in the plasmatic levels of cholesterol, triglycerides, enzymatic alterations, and alterations of the cytokines including iNOS, IL-1β, and TNFα.[115] TNFα may induce oxidative stress and decreases melatonin levels in the follicle.[116] Therefore, the organisms with PCOS have an unstable milieu with an increased oxidative stress.

Moreover, there is a greater oxidative stress and more ROS are produced within the follicle, especially during the ovulatory process. Ovulation is a complex process by which a preovulatory follicle ruptures and releases a fertilizable oocyte into the oviductal lumen. It occurs as a result of a dynamic interaction between the luteotropic hormone (LH) surge and local factors including steroids, NO, PGs, and peptides in a time-dependent manner. Local increases in the concentration of the ovarian PGs, angiotensin II, and NOS have been observed at the time of ovulation.[117] The deleterious actions of activated macrophages, the major source for ROS and MPO, could migrate to any site in the female genital tract and cause their cellular effects at the level of the oocyte. Activated macrophages are found in the cumulus cell mass within the cumulus-oocyte complex (COC) under normal and inflammatory conditions.[118,119] High levels of MPO have been found in the collected peritoneal fluid samples of patients with PCOS and the follicular fluid of women with chronic anovulation, which correlated to a decline in their fertility.[101,120] It is believed that oxidative stress may be a cause of poor oocyte quality. The ROS generation from mononuclear cells is elevated in women with PCOS, and significantly increased serum lipid peroxidation products in women with PCOS have been detected.[121] The ratio of apoptotic granulosa cells (GCs) is greater in women with PCOS, and the lipid peroxidation product malondialdehyde is increased in the follicular fluid of women with PCOS.[122] Oxidative stress may cause GCs and oocyte damage by lipid peroxidation, protein oxidation, and DNA damage in the follicle. The harmful effects of H_2O_2 on oocyte maturation show that oxidative stress induces apoptosis of human oocytes.[85]

The ovary, as a whole, the granulosa cells, the oocyte, and those making up the cumulus oophorus have been reported to synthesize melatonin.[123] The binding sites of melatonin are detected in the membrane fraction of human GCs, and both MT1 and MT2 melatonin receptors were identified in rat ovaries (antral follicles and corpus luteum [CL]) and in human GC/luteal cells.[90,124] The ovarian cells do not discharge melatonin into the general circulation. Rather, these cells use the melatonin they produce for their own benefit or for that of their neighboring cells as an antioxidant or as an autocrine or paracrine agent.[125] Melatonin concentrations in the ovarian follicular fluid of normal women are reported to be 3 times higher than that in the serum.[21] Moreover, the concentration of melatonin is higher in the fluid of larger follicles than that of smaller follicles in women undergoing in vitro fertilization (IVF)-embryo transfer. Elevated melatonin in preovulatory follicles is likely to protect granulosa cells and the oocyte from free radicals that are induced during ovulation. On the contrary, they showed that intrafollicular melatonin concentration was significantly lower in PCOS patients than those in women undergoing IVF-embryo transfer, possibly accounting for the anovulation and poor oocyte quality seen in PCOS.[21] Results in another study showed that the highest levels of 8-OhdG (8-hydroxy-2 deoxyguanosine, a sensitive indicator of DNA damage as a result of oxidative stress) were associated with the poorest quality oocytes. Moreover, the intrafollicular levels of 8-OHdG were negatively correlated with melatonin concentrations in this fluid.[85] Interestingly, a study has reported that total aMT6s (urinary 6-sulfatoxymelatonin, the major enzymatic metabolite of melatonin, and a good indicator of pineal melatonin secretion) values were significantly increased in PCOS women compared with control women. In PCOS, mean serum LH, testosterone, and insulin levels were higher than the mean values of those hormones control women. However, aMT6s inversely correlated with testosterone, and only testosterone was an important determinant of aMT6s values in PCOS. These findings demonstrate that PCOS women have increased melatonin secretion, which is associated with their testosterone levels.[126] A study has reported a reduction in melatonin levels due to pinealectomy, and continuous light exposure induces the development of PCOS in rats.[114] There may be a reduction in the uptake of melatonin into the

ovarian follicle. And it can be deduced that pineal melatonin secretion levels would elevate for self-regulation and feedforward due to the milieu with an increased oxidative stress. This mechanism was also considered to be involved in the increased sleep disturbances and abnormal sleep architecture of women with PCOS. In a recent study, serial urine collections over a 24-hours period have revealed novel observations that nighttime melatonin and 8-OHdG levels are significantly elevated in PCOS women compared with the non-PCOS controls. The elevated nighttime levels of melatonin in women with PCOS could potentially be acting as a free radical scavenger for the increased oxidative stress. This indicates that PCOS women with high 8-OHdG levels, and thus high oxidative stress levels, are producing more melatonin, possibly in an attempt to neutralize excess ROS.[127] As far as Jain et al[128] concerned, melatonin concentration in serum of women with PCOS was found to be higher than that of control women, also indicating a feedback mechanism due to reduced melatonin concentrations at the level of ovarian follicles. To sum up, melatonin could be one of the factors in the pathogenesis of PCOS.

Accumulating studies have given evidence of the potential role of melatonin as a therapeutic agent in PCOS. Within the ovary, melatonin regulates steroidogenesis, folliculogenesis, and oocyte maturation.[129] First of all, the increase in follicular melatonin concentration in the growing follicle could be an important factor in promoting oocyte mature and avoiding atresia. Macrophage and GCs produce ROS, and excessive ROS induces apoptosis and results in follicular atresia; however, increased levels of melatonin in follicular fluid scavenge ROS directly, regulate the antioxidant enzymes and antiapoptotic/proapoptotic protein gene expression, and prevent atresia. It can also modulate SOD, GPx, and CAT gene expression in GCs. The follicle may be rescued by melatonin and continues its growth to become a dominant follicle.[21] MPO has been known to have a detrimental effect on oocyte quality through its chlorination activation. There is the intimate link between MPO (purified and naturally secreted from macrophages and neutrophils) and oocyte quality deterioration, which can be prevented by melatonin. Similarly, stimulated macrophages and neutrophils were also found to deteriorate oocyte quality independent of cumulus cells presence in a time-dependent fashion, which could be also prevented by melatonin.[28] The oral supplementation of melatonin raises its concentration in the follicular fluid,[130] which defines a follicle containing high-quality oocytes. In this study, it should be highlighted that melatonin intrafollicular concentrations in the group A (treated with myo-inositol, melatonin, and folic acid) were 3 times higher than in group B (treated with myo-inositol and folic acid) and almost 4 times higher than in ctrl group (treated with only folic acid). These findings indicated that myo-inositol and melatonin behaved synergistically at ovarian level, improving ovarian response to gonadotropin stimulation, with the result to increase oocyte and embryo quality. On the one hand, melatonin could significantly improve nuclear maturation of PCOS oocytes. The cleavage rate was significantly higher in 10^5 mol/L or 10^6 mol/L concentrations of melatonin compared to untreated oocytes in PCOS, indicating that melatonin has the potential to induce oocyte nuclear maturation and guarantee fertilization potential.[131] On the other hand, supplementation of in vitro maturation medium with melatonin may facilitate the cytoplasmic maturation of human immature oocytes and improve subsequent clinical outcomes.[131] According to Esteghamati et al[132] metformin hydrochloride is an excellent reducer of oxidative stress markers in diabetic individuals. A combination of metformin hydrochloride and melatonin was more effective against liver toxicity produced by PCOS, allowing a normalization of biochemical parameters during pregnancy, than monotherapeutic treatment with these drugs. Although intrafollicular 8-OhdG concentrations were significantly reduced by melatonin treatment, the reduction in intrafollicular HEL (Hexanoyl-Lysine, a useful biomarker for the initial stage of lipid peroxidation) was not statistically significant. Therefore, the main role of melatonin within the follicle may be a free radical scavenger which reduces oocyte DNA damage.[85]

Melatonin is beneficial to treatment of PCOS through its effects on steroidogenesis, thereby regulating ovulation, overcoming dyslipidemia and insulin resistance, preventing hyperplastic changes in the endometrium, and protecting against the development of endometrial cancer.[133] Melatonin in the ovary also may be concerned with progesterone production by the transforming granulosa cells after ovulation.[134] It can regulate sex steroid production by regulating steroidogenic enzyme activities or their gene expression in thecal cells and GCs. Melatonin regulates LH mRNA expression; elevated melatonin was reported to enhance LH secretion, LH pulse amplitude, and LH as well as follicle-stimulating hormone (FSH) response to gonadotropin-releasing hormone (GnRH) in the follicular. These effectors are all essential for ovulation and the initiation of luteinization.[90] Melatonin directly regulates progesterone production, LH receptor gene expression, and gonadotropin-releasing hormone receptor gene expression in human granulosa-lutein cells via the mitogen-activated protein kinase pathway and activation of Elk-1.[90] Interestingly, ultralong GnRHa therapy increased the melatonin concentrations in the follicular fluid. Reduced oxidative stress and increased antioxidant activities by melatonin in follicular fluids by ultralong GnRHa therapy may also have contributed to the improvement of implantation rate and pregnancy rate.[116]

Melatonin's function is mainly mediated by the melatonin receptor 1A (MTNR1A) gene and the melatonin receptor 1B (MTNR1B) gene, both of which belong to the G protein-coupled receptor superfamily. Several findings suggest an important role of the MTNR1A and MTNR1B genes in the etiology and pathophysiology of PCOS. The polymorphisms rs2119882 in the MTNR1A gene and rs10830963 in the MTNR1B gene may play a common causative role in the pathogenesis of PCOS.[135,136] A family-based study also showed a significant difference in the transmission of rs2119882 among Han Chinese women, which indicates that rs2119882 was a risk marker for PCOS. Furthermore, the clinical and metabolic characteristics of women with PCOS were evaluated according to the genotypes of SNP rs2119882.[137] Thus, the MTNR gene, which is a novel candidate gene for type 2 diabetes, could be a plausible candidate gene for PCOS. The MTNR1A gene is mainly expressed in alpha cells, while the MTNR1B gene is mainly expressed in beta

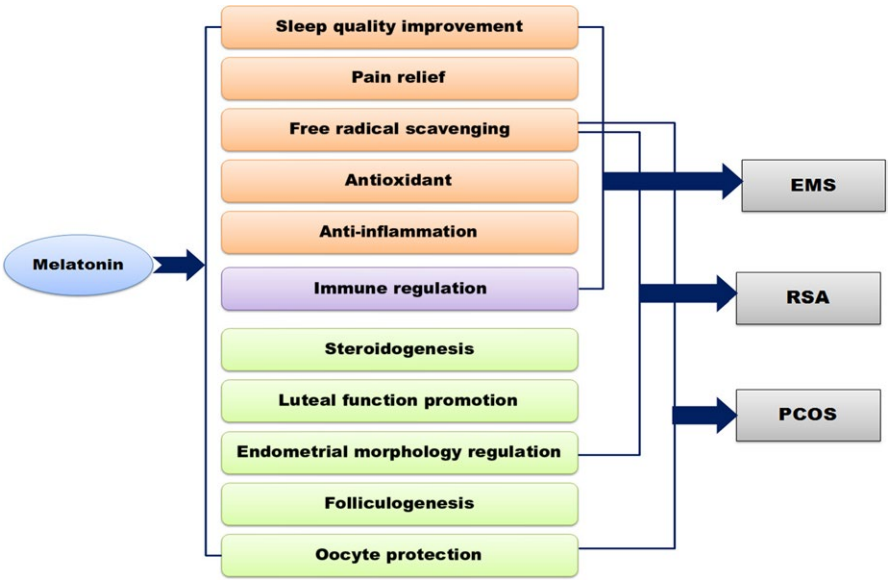

FIGURE 5 The representation of pleiotropic roles and treatment potential of melatonin in EMS, RSA, and PCOS. As a powerful antioxidant, melatonin has strong ability of scavenging free radicals and anti-inflammation. Immune regulation capacity and endocrine modulation function via hormone pathways make it a multifunctional molecule. Melatonin is an effective therapeutic candidate for EMS and PCOS due to these pleiotropic roles. Moreover, exogenous melatonin application can suppress ectopic lesions, relieve pelvic pain, and improve sleeping quality of women with EMS and regulate endometrial morphology, promote folliculogenesis, and protect oocytes of women with PCOS. For women with RSA, melatonin may also have therapeutic potential via alleviating oxidative stress, endocrine-immune disorders, corpus luteum, and endometrial behavior dysfunction

cells. Melatonin can reduce peripheral tissue sensitivity to insulin.[138] However, whether the supplementation of melatonin would remit insulin resistance in PCOS women and, if so, how would it conduct can be hypothesis, which need further research.

7 | CONCLUSION AND DISCUSSION

Melatonin is a hormone secreted mainly by the pineal gland. Melatonin regulates a variety of central and peripheral actions related to circadian rhythms. It is a multitasking molecule as a powerful free radical scavenger, a broad-spectrum antioxidant, or a pleiotropic immunoregulator. Female reproduction is under the control and regulation of the neuroendocrine-immune axis. Thus, melatonin has been considered to play a vital role in female reproduction and be involved in many gynecological and obstetrical pathology.

It seems that melatonin is an effective therapeutic candidate for EMS due to its antioxidant and anti-inflammation ability or its endocrine modulation function via hormone pathways (Figure 5). Accumulating animal experiments have proved that exogenous melatonin application can suppress EMS ectopic lesions, relieve pelvic pain, and improve sleeping quality of women with EMS. The multiple effects have been summarized that melatonin exerts at different steps of the inflammatory response, indicating a pro-inflammatory role at an early phase, and an antagonist role at later phases.[139] This evokes a smart behavior where melatonin may favor the inflammatory healing processes while contrasting pathologically chronic or deregulated inflammation, thus potentially being an ideal compound to treat EMS. However, the melatonin levels in peripheral blood as well as in the local ectopic environment have not been evaluated; whether altered melatonin concentrations are involved in the occurrence, development, and severity of EMS is unclear. The aberrant biological behavior of endometrial stromal cells plays key roles in establishment and maintenance of ectopic lesions. The directly and indirectly regulatory mechanisms of melatonin on the viability, proliferation, apoptosis, autophagy, migration, and implantation of endometrial stromal cells, require more investigation. Moreover, the dysfunction of the immune cells in the microenvironment of the peritoneal cavity, including neutrophils, macrophages, dendritic cells, NK cells, B cells, and T helper cells, is considered to contribute to the pathogenesis and progression of EMS via mediating immune escape of ectopic lesions and improving the proliferation, adhesive, and invasive of the endometrial cells, as well as enhancing angiogenesis of endometriotic tissues.[140–144] As a immunomodulator, how melatonin affects the ectopic immune microenvironment is an important but unclear question to answer.

The immune regulatory function of melatonin seems most disputable and complicated. It would affect the function and status of different immunocytes under different physiological or pathological conditions. RSA is closely associated with oxidative stress and immune dysregulation. How the melatonin levels in maternal peripheral blood and at the maternal-fetal interface influence embryo implantation and pregnancy outcomes is unclear. Whether abnormal melatonin secretion would break the maternal-fetal immune tolerance also needs further investigation. The effect of melatonin on different melatonin receptor subtypes is a promising area of research

that would provide greater mechanistic details. Based on current findings, there is still a long way to apply melatonin to RSA, which need to be corroborated by multidisciplinary basic researches and clinical studies.

The association between PCOS and an increase in oxidative stress in humans makes melatonin a probable drug for women with PCOS. Melatonin may benefit patients with PCOS by promoting oocyte maturation and improving oocyte quality (Figure 5). Melatonin would be expected to exert beneficial actions on immune-mediated ovarian pathology. A previous report documented that melatonin protects against immune ovarian failure induced by antiovarian antibodies in mice. In this research, melatonin treatment (5 mg/kg body weight, IV injection 1 hour before antibodies administration) restored survival and meiotic maturation of the oocytes by means of its anti-inflammatory and antiapoptotic effects.[145] Therefore, searching the role of melatonin in PCOS or other ovarian complicated pathology in the aspect of inflammation and immunity would be a promising direction. Melatonin is a protective molecular for oocytes; however, the uptake of melatonin into the ovarian follicle reduces in PCOS. Ultralong GnRHa improves the function of the follicle by reducing inflammation of the ovary so that the follicle can effectively take up melatonin.[116] It is worthy of finding more ways to enhance the uptake of melatonin into the ovarian follicle from serum.

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