Melatonin and the ovary: physiological and pathophysiological implications

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Objective: To summarize the role of melatonin in the physiology and pathophysiology of the ovary.
Design: Review of literature.
Setting: University Health Science Center.
Result(s): Melatonin plays an essential role in the pathogenesis of many reproductive processes. Human preovulatory follicular fluid (FF) contains higher concentrations of melatonin than does plasma, and melatonin receptors are present in ovarian granulosa cells (GC). Melatonin has been shown to have direct effects on ovarian function. Reactive oxygen species and apoptosis are involved in a number of reproductive events including folliculogenesis, follicular atresia, ovulation, oocyte maturation, and corpus luteum (CL) formation. Melatonin and its metabolites are powerful antioxidants; the primitive and primary function of melatonin may be its actions as a receptor-independent free radical scavenger and a broad-spectrum antioxidant. A large amount of scientific evidence supports a local role of melatonin in the human reproductive processes. The indole also has potential roles in the pathophysiology of endometriosis, polycystic ovary syndrome (PCOS), and premature ovarian failure (POF).
Conclusion(s): We summarize the current understanding of melatonin's essential functions in the human ovary. Melatonin could become an important medication for improving ovarian function and oocyte quality, and open new opportunities for the management of several ovarian diseases. (Fertil Steril® 2009;92:328–43. ©2009 by American Society for Reproductive Medicine.)

Key Words: Melatonin, follicular development, ovulation, oocyte quality, luteal function, PCOS, endometriosis, POF

The circadian melatonin (*N*-acetyl-5-methoxytryptamine) rhythm with high levels at night is important for the synchronization of the reproductive response to appropriate environmental conditions in photoperiodic animals (1). The recent cloning of melatonin receptor subtypes allowed the characterization of receptor(s) at the molecular level (2, 3). Earlier reports have documented that the hypothalamus and the anterior pituitary play vital roles in the regulation of reproduction by melatonin (4). This is supported in part by the demonstration of melatonin receptors in the suprachiasmatic nuclei in the brain and pars tuberalis of the pituitary (5, 6). Also, the demonstration of melatonin receptors in the ovary indicates multiple sites at which melatonin may influence the reproductive system (7, 8).

Free radicals have a dual role in the reproductive tract and are key signaling molecules for various ovarian functions (9). Specifically, free radicals function in the microenvironments of oocytes, sperm, and in the follicular fluid (FF) (10). Changes in these microenvironments have a direct bearing on follicular development, ovulation, quality of oocytes, sperm–oocyte interaction, implantation, and early embryonic development. Free radicals mediate their actions through a variety of proinflammatory cytokines with these processes having been proposed as a common underlying factor for endometriosis, ovarian cancer, polycystic ovary disease (PCOD), and various other pathologies affecting the female reproductive tract (11).

Melatonin is a documented powerful free radical scavenger and a broad-spectrum antioxidant (12, 13). The use of melatonin as a drug to prevent free radical damage has been widely investigated and its utility as an antioxidant provides opportunities for the management of several diseases including cancer, immunological disorders, Alzheimer's disease, diabetes, and viral infections (14–17). The purpose of current review is to summarize recent developments in the field of melatonin research as they relate to reproduction, focusing on how melatonin directly influences ovarian physiology.

MELATONIN SYNTHESIS

Melatonin is an indoleamine, originally identified in the pineal gland. The initial precursor of melatonin biosynthesis

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is an amino acid, tryptophan. Pinealocytes take up tryptophan from the blood and convert it to serotonin through hydroxylation and decarboxylation; serotonin is then converted to *N*-acetyl-serotonin by the enzyme arylalkylamine *N*-acetyltransferase (NAT). *N*-acetyl-serotonin is subsequently methylated to form melatonin; this step requires the enzyme hydroxyindole-*O*-methyltransferase (HIOMT). The pineal activity of NAT usually exhibits the same circadian rhythm as do blood and pineal melatonin levels with all being suppressed by light. Although NAT was thought to be the rate-limiting enzyme in melatonin biosynthesis, recent findings have shifted the emphasis to HIOMT as possibly being responsible for the circadian melatonin cycle. This idea developed when it was shown that elevated NAT activity, stimulated by an adrenergic-α and adrenergic-β receptor agonist, failed to promote melatonin production, whereas the increased activity of HIOMT was positively correlated with pineal melatonin production (18).

In vertebrates including humans, melatonin is synthesized in the pineal gland, retina, skin, and gastrointestinal tract, but its rhythmic production occurs only in the pineal gland and retina (19). Melatonin is detectable in virtually every bodily compartment including in human preovulatory FF where its concentration is significantly higher than that in peripheral serum (20–22). Although it has been assumed that the melatonin measured in human preovulatory FF is derived from the general circulation (20, 21), it may also be synthesized in the ovary, as both NAT and HIOMT are present in ovarian tissue (23). It seems likely that melatonin in the FF may be produced locally as well as being take up from the blood.

MELATONIN AS AN ANTIOXIDANT

Melatonin functions in a variety of ways to reduce oxidative stress. It is a powerful direct free radical scavenger (13). Free radicals are defined as molecules or molecular fragments containing one or more unpaired electrons in their atomic or molecular orbitals. Radicals and their nonradical related species are referred to as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and are products of normal cellular metabolism (Fig. 1). Melatonin has the capability of quenching both ROS as well as RNS including superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($\cdot OH$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), nitric oxide ($NO\cdot$), and the peroxynitrite anion ($ONOO^-$) (24–26). Not only is melatonin itself a direct free radical scavenger, but metabolites that are formed during these interactions (i.e., cyclic 3-hydroxmelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, and N1-acetyl-5-methoxykynuramine), are likewise excellent scavengers of toxic reactants (26–29).

In addition, melatonin stimulates a number of enzymes that are either involved in metabolizing potentially reactive species to harmless molecules or inducing the synthesis of other endogenously produced antioxidants (30, 31) (Fig. 1). Thus, Ozturk et al. (32) observed elevated levels of superoxide dismutase (SOD) activity after the administration of 10 mg/kg of melatonin for 7 days, whereas Liu and Ng (33) reported enhancement of SOD activity after a single melatonin injection (5 mg/kg). Baydas et al. (34) found that a melatonin deficiency induced by pinealectomy reduced glutathione peroxidase (GPx) activity levels in several tissues of rats. Consistently, melatonin administration increases the activity of SOD, GPx, and glutathione reductase (35–37). Antolín et al. (36) first reported that melatonin causes incremental changes in messenger RNA (mRNA) levels for both Cu, Zn-SOD and Mn-SOD after its exogenous administration (500 μg/kg). Mayo et al. (38) provided an insight into the mechanism by which melatonin regulates antioxidant enzyme gene expression using cultured dopaminergic cells. They found that melatonin induced synthesis of new proteins as a condition for regulation of gene expression of all the three antioxidant enzymes (i.e., CuZn-SOD, Mn-SOD, GPx).

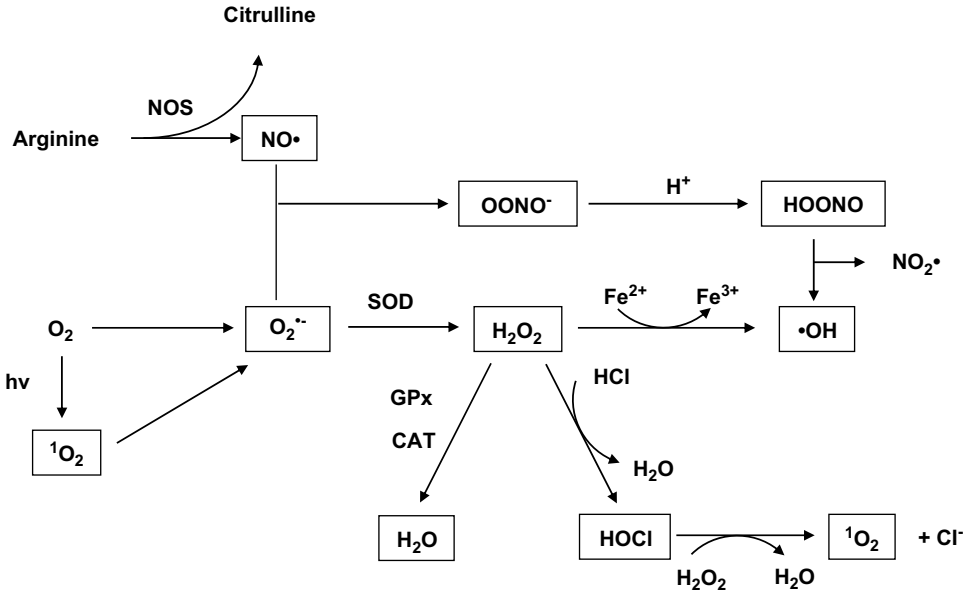
MELATONIN RECEPTORS IN THE OVARY

Some actions of melatonin are mediated through specific membrane receptors, whereas some functions seem to rely on nuclear binding sites; the latter correspond to orphan members of the nuclear receptor RZR/ROR superfamily (39). Three subtypes of mammalian membrane melatonin receptors have been proposed and three proteins have been cloned. Two of these receptors, MT1 and MT2, are members of the 7-transmembrane G protein-coupled receptor family (2, 3, 40). The third receptor, MT3, is an enzyme identified as quinone reductase 2, which possesses, in some animals, features of a melatonin receptor (41, 42). Less is known of the nuclear melatonin binding sites. The signal transduction cascade associated with the activation of MT1 or MT2 in target cells results in the inhibition of adenylate cyclase activity (3). Activation of these receptors reduces forskolin-induced cyclic adenosine 3′5′ monophosphate (cAMP) formation with a subsequent reduction in activated protein kinase A (43). Although this is a general rule in the biochemical pathways for MT1 and MT2 receptors, it is not the only signal transduction mechanism that they can trigger. Depending on the tissue, organ, and species, melatonin activates different second messenger cascades by interacting with the same receptor subtype. Screening studies have shown that various rodent tissues express MT1 or MT2 melatonin receptors. In humans, melatonin receptors are also detected in several organs, including brain and retina, cardiovascular system, liver and gallbladder, intestine, kidney, immune cells, adipocytes, prostate and breast epithelial cells, myometrium, and skin.

Melatonin may directly affect ovarian function. Melatonin is concentrated in human ovarian FF relative to the level in plasma (20–22), and melatonin alters granulosa cell (GC) steroidogenesis and follicular function in hen (44), hamster (45), and humans (8, 46–48). Melatonin binding sites are detected in the membrane fraction of human GCs (7) and both MT1 and MT2 melatonin receptors were identified in human GC/luteal cells (8, 49) and in rat ovaries (antral follicles and corpus luteum [CL]) (50).

FIGURE 1

Free radicals and toxic reactants generated from molecular oxygen (O_2). One-electron reduction of O_2 forms the superoxide anion ($O_2^{\cdot -}$); $O_2^{\cdot -}$ is converted to hydrogen peroxide (H_2O_2) spontaneously by a process termed dismutation or disproportionation. H_2O_2 forms hydroxyl radical ($\cdot OH$) in the presence of transition metals such as iron and copper (Fenton reaction). $\cdot OH$ will react with itself, other reactive oxygen species, or with proteins, lipids, and other biomolecules in close proximity to the site at which it is formed. Thus, $\cdot OH$ can play a role as a localized reaction intermediate, but it generally cannot transduce a signal to a more distant target molecule. $O_2^{\cdot -}$ quickly couples with nitric oxide ($NO\cdot$) to form the highly toxic peroxynitrite anion ($ONOO^-$). $ONOO^-$ can degrade to form the $\cdot OH$. 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. H_2O_2 once formed, is metabolized to innocuous products by catalase (CAT) and glutathione peroxidases (GPx).



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MELATONIN AND FOLLICULAR GROWTH

Ovarian follicle development is a complex process that involves endocrine, paracrine, and autocrine mechanisms. Folliculogenesis begins with the establishment of a finite pool of primordial follicles. Primordial follicles must grow to the primary, preantral, and antral stages before they reach the preovulatory stage and are capable of releasing an oocyte for subsequent fertilization. Growth beyond the late preantral/early antral stage (depending on species) becomes 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. The FF that fills the antral cavity contains water, electrolytes, serum proteins, and high concentrations of steroid hormones secreted by the GCs (51).

High levels of melatonin, which may undergo seasonal variations (48), are found in human preovulatory FF in concentrations that are almost threefold higher than serum levels (20, 21). Melatonin concentrations are higher in the fluid of large follicles than in the fluid of small follicles in patients undergoing IVF-embryo transfer (Table 1). The presence of melatonin and its precursors, serotonin, and *N*-acetylserotonin, have also been documented in extracts of human ovary and activities of the two melatonin synthesizing enzymes, NAT and HIOMT, are present in human ovarian homogenates (23). It is possible that melatonin, synthesized by the ovary, may be released into the FF. However, it is the opinion of the current investigators that the bulk of the melatonin detected in the ovary and preovulatory FF is derived from the circulation (Fig. 2), as the rat and cat ovaries have been shown to take up and retain circulating [^{3}H]-melatonin (52). In addition, melatonin administration (3-mg tablet orally) to infertile women resulted in high melatonin concentrations in their FF (after melatonin treatment: 432 ± 260 pg/mL vs. control: 112 ± 51 pg/mL) (53).

Recruited antral follicles are characterized by induction of expression of mRNAs encoding a range of steroidogenic enzymes, gonadotrophin receptors, and local regulatory factors. As follicles continue to mature, there is a transfer of dependency from FSH to LH, which may be part of the mechanism

TABLE 1

Melatonin, P, T, and E_2 concentrations in large and small follicles of humans.

Follicle size	Melatonin (pg/mL)	P (μg/mL)	T (ng/mL)	E_2 (ng/mL)
Large follicles (>18 mm)	123 ± 39	10.3 ± 0.7	5.2 ± 0.5	512 ± 39
Small follicles (<10 mm)	54 ± 11[a]	3.3 ± 0.7[b]	7.5 ± 0.8[c]	299 ± 30[b]

Note: Data are the mean ± SEM of 18 patients.
All significance values are as compared with large follicles.
From Nakamura et al. (22).
[a] $P<.05$.
[b] $P<.05$.
[c] $P<.05$.

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involved in selection of follicles for continued growth. The mechanism of selection of the ovulatory follicle seems to be linked to the timing of mRNA expression encoding LH receptors in GCs (54). Melatonin treatment (10 pM–100 nM) remarkably increased mRNA expression of LH (but not FSH) receptors in human GCs (8).

Sex steroids also play an important role in the growth and differentiation of ovarian cells. The two-cell, two-gonadotropin model describes the role of theca cells and GCs in the production of steroids, highlighting the cooperation between the two cell types, which is necessary for estrogen (E) production (55). The steroidogenic enzymes, namely, P450 side-chain cleavage enzyme (P450scc), P450 17alpha-hydroxylase/C17, 20 lyase (P450c17), P450 aromatase, are known to regulate the biosynthesis of P, androstenedione (A), and E_2, respectively. These enzymes are activated by cAMP in thecal cells and GCs, with the cAMP level being regulated by FSH or LH through their membrane receptors (56). The mRNA expression of three major steroidgenic genes in the porcine theca, namely, CYP 11A, CYP 17, and CYP 19, are regulated by cAMP, and the protein products of these genes are, respectively, P450scc, P450c17, and P450 aromatase.

Estrogens exert effects on GC growth and differentiation in association with gonadotropins (55). The role of P in follicular growth and development is limited, but it has a regulatory effect in GC function and follicle rupture during ovulation (57). Consistent with these findings, P receptor knockout mice are infertile because they do not ovulate (58). Androgens have been shown to promote early follicular growth (59), but also to impede follicular development by stimulating atresia and apoptosis (60).

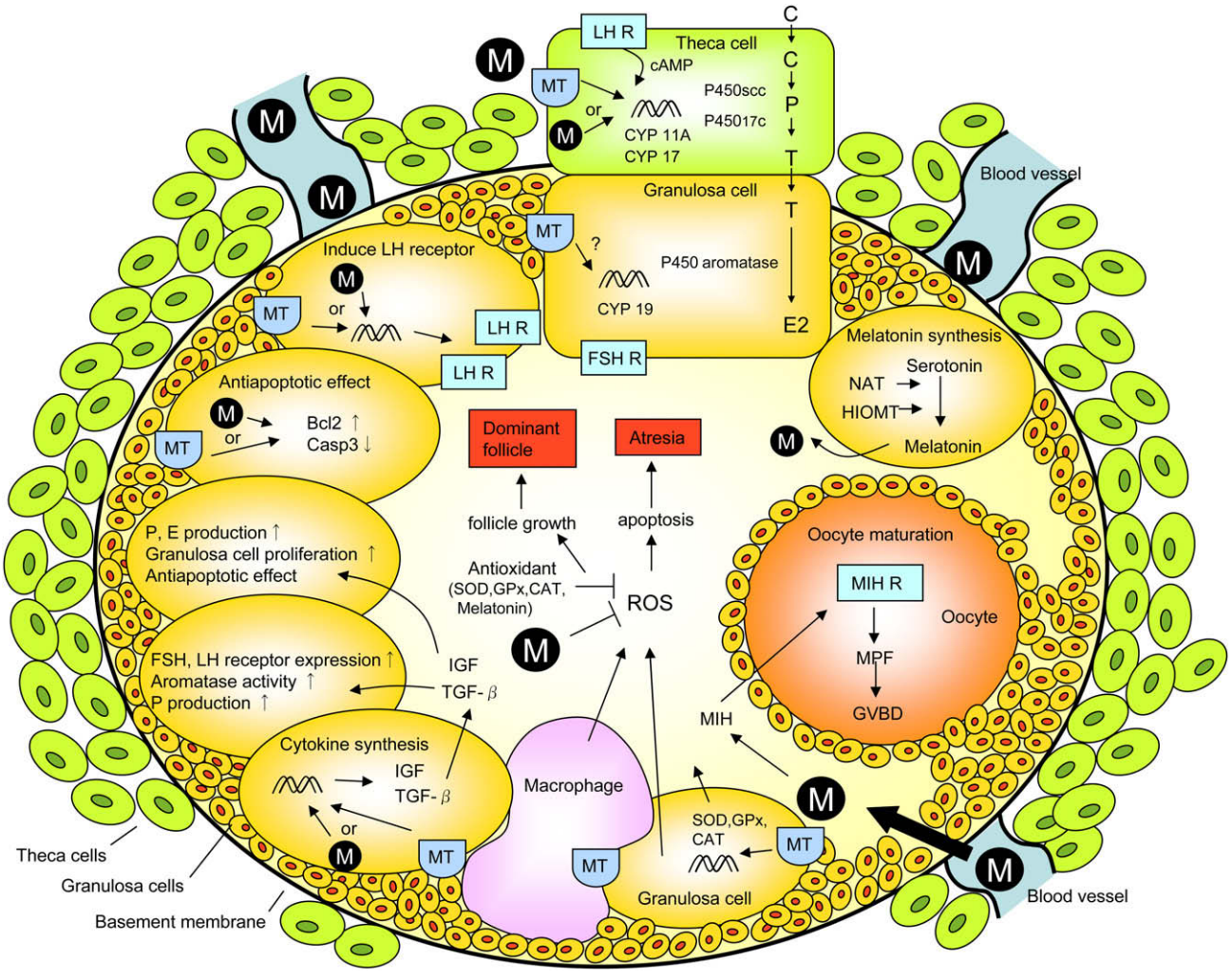
Melatonin influences sex steroid production at different stages of ovarian follicular maturation. Recently, Adriaens et al. (61) demonstrated that melatonin (100 μM) increased P and A production in mouse preantral follicles after incubation for 12 days. Melatonin (100 ng/mL) stimulated P and A production in 30-hour cultures of porcine antral follicles, whereas E_2 levels were not changed (62). This group of investigators also showed that melatonin inhibited CYP 11A and CYP 17 expression but not that of CYP 19. Despite an increase in P production, the expression of CYP 11A was significantly inhibited. The investigators insisted that, at the time point studied, CYP 11A is transcriptionally inhibited as a result of feedback inhibition by high levels of P secreted into the culture medium.

Soares et al. (63) demonstrated that ovaries had an increase in the number of atretic follicles after pinealectomy, and animals lacking their pineal gland had elevated levels of E_2 but reduced P. In addition, we observed a positive relation between P and melatonin concentrations in FF of human preovulatory follicles (22). In contrast, melatonin (0.1–10 ng/mL) decreased P, E_2, and cAMP production by hamster preovulatory follicles after 24 hours of incubation with hCG (45). When theca cells and GCs were separated, melatonin reduced P production by theca, whereas it did not influence the GCs. Melatonin may directly suppress follicular (thecal) steroidogenesis at an early stage in the steroid synthesis pathway through cAMP modulation (Fig. 2). Our results are consistent with a report demonstrating that melatonin blocks the expression of steroidogenic acute regulatory protein (64). It is believed that steroidogenic acute regulatory protein determines the translocation of cholesterol across the intermembrane space into the inner membrane where P450scc cleaves 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. However, the direct effect of melatonin on follicular steroid production is complicated; it seems to depend 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.

Locally produced growth factors, such as the insulin-like growth factors (IGFs) and members of the transforming growth factor β (TGF-β) superfamily (inhibins, activins, and bone morphogenetic proteins [BMPs]), work in concert with gonadotropins throughout the follicular growth continuum. Insulin-like growth factors are produced by GCs and theca cells during follicle development (65). Insulin-like growth factors are mitogenic and antiapoptotic peptides

FIGURE 2

Schematic representation of the presumed roles of melatonin in ovarian antral follicle. Melatonin in the follicular fluid (FF) may be produced by granulosa cells (GC) but in addition, it is taken up from the blood through the basement membrane. Melatonin regulates sex steroid production by regulating steroidogenic enzyme activities or their gene expression in thecal cells and GCs. Melatonin also regulates LH mRNA expression, *Bcl2* and *Casp3* production, IGF and TGF-β activity, and gene expression and maturation-inducing hormone (MIH) activity. Macrophage and GCs produce ROS and melatonin modulates SOD, GPx, and CAT gene expression in GCs. Excessive ROS induces apoptosis and results in follicular atresia; however, increased levels of melatonin in FF scavenges ROS through its direct and indirect antioxidant actions and prevents atresia. The follicle may be rescued by melatonin and continues its growth to become a dominant follicle. M = melatonin; MT = melatonin receptors; LH R = LH receptor; FSH R = FSH receptor; C = cholesterol; NAT = *N*-acetyltransferase; HIOMT = hydroxyindole-*O*-methyltransferase; MIH = maturation-inducing hormone; MIF = maturation-promoting factor; GVBD = germinal vesicle breakdown; ROS = reactive oxygen species; SOD = superoxide dismutase; GPx = glutathione peroxidase; CAT = catalase; IGF = insulin-like growth factor; TGF-β = transforming growth factor β.



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that promote differentiation and also have insulin-like metabolic effects mediated by binding to specific high-affinity membrane receptors. The IGF-I and IGF-II stimulate DNA synthesis and E_2 and P secretion by human GCs and granulosa-luteal cells (65). Insulin-like growth factor-I is antiapoptotic in ovarian follicles, whereas ovarian apoptosis is enhanced by IGF binding protein (66). Melatonin (0.01–10 μg/mL) stimulates IGF-I production by cultured human GCs (67). Recently, Picinato et al. (68) demonstrated that melatonin (0.1 μM) induces 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.

The TGF-β superfamily is expressed by ovarian cells and oocytes in a developmental, stage-related manner and function as intraovarian regulators of follicle development. In humans, TGF-β is produced by both thecal cells and GCs (69). The TGF-β stimulates FSH receptor expression (70), amplifies FSH-induced aromatase activity, and P production and LH receptor induction in GCs (71–73). Interestingly, melatonin enhances synthesis of TGF-β1 in human benign prostate epithelial cells (74). Similarly, TGF-β1 immunostaining of multinuclear chondrocytes was dramatically increased in degenerated intervertebral disk tissue after exogenous melatonin application (30 mg/kg of body weight daily at 5 PM to 6 PM for 4 weeks) in rats (75). Melatonin treatment (5 mg/kg intraperitoneal injection) up-regulates the level of gene expression of TGF-β in mouse peritoneal cells (76). There is increasing evidence to support a critical role of TGF-β superfamily members BMPs and growth and differentiation factor-9 (GDF-9) in growing antral follicles. The BMP-15 and GDF-9, exclusively produced by oocytes, may exert their effects by regulating the actions of gonadotropins. The BMP-15 has been shown to attenuate FSH actions on rat GCs by suppressing FSH receptor expression (77). The GDF-9 reduced FSH-stimulated P and E_2 production and attenuated FSH-induced LH receptor formation (78). Given these findings it seems important to study the relationship between melatonin and BMP-15 and GDF-9 in the growing follicle.

Atresia is an apoptotic process that is highly regulated by proapoptotic and antiapoptotic factors. We previously demonstrated a relationship between follicular atresia, apoptosis, and nitric oxide (NO) generation in follicular development in various sized follicles. Although there were no differences in the concentrations of nitrite and nitrate, the percentage of apoptotic cells in small follicles was significantly higher than in large follicles (79). Small follicles, which are poorly responsive to gonadotropins, may undergo atresia through apoptosis. Zhang et al. (80) also documented an apoptotic mechanism for atresia of human follicles during oxidative stress. Phagocytic macrophages, which increase in number in the follicle during its growth (81), and endothelial cells generate ROS in ovaries (82). Steroidogenically active cells, such as GCs of antral follicles, require high levels of energy production and thus generate large amounts of ROS (83). The reduced levels of antioxidant enzymes, SOD, GPx, and catalase, mRNA expressions may contribute to oxidative stress-mediated apoptosis in atretic follicle (84). These enzymes would normally protect GCs from free radical damage and suppress atresia (85).

Members of the *Bcl2* family are important for regulating the atretic degradation of antral follicles. Deletion of *Bcl2* reduces healthy follicle numbers and increases abnormal follicle numbers compared with wild-type (*Bcl2* +/+) ovaries (86). Targeted overexpression of *Bcl2* in GCs of growing follicles results in reduced apoptosis of these cells (87). Caspases (*Casps*) also influence folliclar atresia because *Casp3*-/- follicles fail to be eliminated by apoptosis (88). Recently, several reports demonstrated that melatonin prevented the induction of the mitochondrial pathway of apoptosis by inducing *Bcl2* expression and reducing *Casp3* activity (Fig. 2). Melatonin (10 mg/kg injection) remarkably prevents hepatocyte apoptosis in mice induced during malarial infection by inhibiting *Casp 3* activity (89). Aged rats exhibit increases in the liver apoptotic changes and increases in cytochrome c mitochondrial release, *Bax* to *Bcl-2* relative expression, and activity of *Casp3*, whereas melatonin, provided in the drinking water (20 mg/L) for 4 weeks, abrogated these changes (90).

The integration of extraovarian signals and intrafollicular factors determine whether a follicle will continue to develop or be diverted into atretic pathways. Melatonin is likely to scavenge RNS and ROS and stimulate antioxidant enzyme activities in growing follicle. In addition, melatonin regulates the antioxidant enzymes and antiapoptotic/proapoptotic protein gene expression. The increase in follicular melatonin concentration in the growing follicle could be an important factor in avoiding atresia. This would allow a preovulatory follicle to fully develop and provide an oocyte for fertilization (Fig. 2).

MELATONIN AND OVULATION

Administration of exogenous melatonin in combination with P to women reportedly induces a reduction in LH secretion, blocks ovulation, and in the luteal phase increases P without affecting FSH or inhibiting E_2 (91). Acute suppression of LH levels was also observed in men after melatonin treatment (92). These effects may be mediated by melatonin's ability to influence hypothalamic gonadotropin release (93). However, it has been shown that melatonin can also exert effects on this axis by directly binding to GCs in the ovary (7). A study in human GCs showed that both types of membrane melatonin receptors (i.e., MT1 and MT2) are present and that melatonin can up-regulate LH mRNA receptor (8). The LH is essential for the initiation of luteinization.

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, NO, prostaglandins, and peptides in a time-dependent manner. The LH surge triggers structural and biochemical changes that lead to rupture of the Graafian follicles, resulting in expulsion of the oocyte and subsequent development of a CL. After hCG injection, follicular steroidogenesis quickly shifts from E_2 dominance to P dominance by the inhibition of 17α-hydroxylase-$C_{17\text{-}20}$ lyase activity (94). This acute increase of P production is essential for luteinization and ovulation. Progesterone and E_2 concentrations are significantly higher in the large follicles than in the small follicles of humans, and likewise melatonin concentrations are also higher in the large follicles compared to smaller follicles (Table 1). Interestingly, there was a positive correlation between

follicular P and melatonin concentrations (22). Elevated concentrations of melatonin in preovulatory follicle may be involved in P production resulting in luteinization and ovulation.

At the time of ovulation, local increases in the concentration of the ovarian prostaglandin, angiotensin II (95), and NO synthase (NOS) (96) have been observed. These vasoactive substances have major roles in the ovulatory process by controlling follicular blood flow. Degradation of collagen in the follicular wall is accompanied by increased vascular dilatation and permeability, processes that are necessary for follicular rupture (97). Successful ovulation requires elevated follicular prostaglandin E_2 levels. Melatonin treatment (20 mg/kg body weight) significantly increases prostaglandin E_2 concentrations in rat gastric mucosa (98), and melatonin (20 mg/kg body weight intraperitoneal injection) also increases prostaglandin E_2 in rat esophageal tissue (99). On the contrary, physiological concentrations of melatonin inhibit the norepinephrine-induced activation of prostaglandin E_2 in rat medial basal hypothalamus (100). Although the relation between melatonin and prostaglandin E_2 in the ovulation process is unknown, it would be of importance to address this relationship.

Ovulation is similar to a local inflammatory response (101), with both RNS and ROS being generated in this process. Both endothelial NO synthase (eNOS) and inducible NO synthase (iNOS) are present in the oocytes and thecal cells of the mouse (96). The major source of ROS appears to be inflammatory cells including macrophages and neutrophils, as they are present in the ovary at ovulation (102, 103) and they generate tremendous numbers of free radicals. These radicals act not only in the regulation of ovulation but also induce apoptosis of ovarian cells (84, 104). Melatonin, as well as its metabolites, are broad-spectrum antioxidants and free radical scavengers (13, 25), and melatonin and its derivatives quench ROS as well as RNS (26, 28). Elevated melatonin in preovulatory follicles is likely to protect GCs and the oocyte from free radicals that are induced during ovulation.

MELATONIN AND OOCYTE QUALITY

Poor oocyte quality remains a profound problem for female infertility. The ROS are produced within the follicle, especially during the ovulatory process (10). Oxidative stress may be a cause of poor oocyte quality; ROS such as ·OH, $O_2^{\cdot -}$, H_2O_2 are known to be detrimental to the oocyte (105). They cause deterioration of cell membrane lipids, destroy DNA, and accelerate apoptosis (106) and induce two-cell block, apoptosis, and inhibition of fertilization (107). Reduced antioxidant enzyme levels, such as GPx, are reported in the FF of women with unexplained infertility (108). Also, higher levels of the oxidant H_2O_2 in fragmented embryos compared with nonfragmented embryos and unfertilized oocytes have been reported (109). Elevated utilization of antioxidants, which is suggestive of increased ROS levels, during incubation of poor quality embryos has been reported (110). The balance between ROS production and the scavenging ability of antioxidants is an important factor for oocyte maturation and fertilization.

Drugs that protect the oocyte and its surrounding feeder cells from damage are of great importance. The presence of high melatonin levels in FF (20, 21) and the presence of melatonin receptors in GCs (7, 8) suggest that this indoleamine may be a molecule that is highly beneficial in the follicle. Intrafollicular concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG, a biomarker of damaged DNA products) in women with poor oocyte quality are significantly higher than those with normal oocyte quality in patients undergoing IVF-embryo transfers, and intrafollicular concentrations of 8-OHdG and hexanoyl-lysine adduct (HEL, a biomarker of lipid peroxidation) are significantly reduced by melatonin (3 mg/day) or vitamin E (600 mg/day) treatments (53). In addition, the fertilization success of the women with a low fertilization rates ($\leq$50%) in the prior IVF-embryo transfer cycle was improved by melatonin treatment compared to the prior IVF-embryo transfer cycle (53). Melatonin also positively influences both antioxidant enzyme activity and gene expression. Melatonin administration (5 mg/kg body weight) enhances SOD activity (33) 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) (38). Melatonin may 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 (61). A progestational steroid, 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DP), has been identified as the maturation-inducing hormone (111), which acts on receptors located on the oocyte membrane and induces the activation of maturation-promoting factor in the oocyte cytoplasm to initiate final maturation (112). Under the influence of maturation-promoting factor, oocytes undergo drastic morphological changes associated with progression of the meiotic cell cycle, in which breakdown of the oocyte nuclear envelope or germinal vesicle breakdown occurring at the prophase/metaphase transition is usually regarded as a hallmark of the progress of oocyte maturation (113). Amazingly, a recent report demonstrated the influence of melatonin on the action of maturation-inducing hormone on the oocyte maturation in carp. Melatonin (50–500 pg/mL) accelerated the action of maturation-inducing hormone on the formation of maturation-promoting factor and germinal vesicle breakdown of oocytes (114) (Fig. 2).

Melatonin's epigenetic efficacy has been proven in several diseases including cancer (115) and hypertension (116), and melatonin may also induce epigenetic changes in oocytes (117). Melatonin could exert DNA methyl transferase inhibitory effects by masking target sequences or by blocking the active site of the enzyme (115). The epigenetic modifications may result from the interaction of melatonin with nuclear melatonin receptors. Melatonin significantly increases the transactivating effects of these receptors (118), and nuclear

melatonin receptors appear to have a functional role in DNA bending (119). These findings suggest that melatonin induces epigenetic modifications by affecting nuclear melatonin receptors, which can, in turn, change the superstructure of DNA. Therefore, melatonin seems to be a mediator that transfers the environmental stimuli to oocytes and interactions between environmental factors and the epigenetic inheritance system.

Melatonin in the culture medium supports not only mouse fertilization but early development of embryonic tissue (120), probably by functioning as a potent free radical scavenger. Recently, Rodriguez-Osorio et al. (121) reported that melatonin (10 nM) has a positive effect on porcine embryo cleavage rates and blastocyst total cell numbers. In addition, melatonin in the culture medium improved the rate of development of thawed blastocysts with higher hatching rate after 24 hours of culture (122). No negative effects of melatonin on embryo development were observed at any of the concentrations (1 pM–100 μM) tested (123), or even when administered in high doses during pregnancy (124).

MELATONIN AND LUTEAL FUNCTION

In the female reproductive tract P plays key roles in ovulation, implantation, and the maintenance of pregnancy by regulating GC function and follicle rupture during ovulation (57). Activation of the LH receptor in follicular cells by the preovulatory LH surge causes ovulation and rapidly initiates a program of terminal differentiation of the ovulated follicle into a CL through a process termed luteinization. Formation of the CL is initiated by a series of morphological and biochemical changes in cells of the theca interna and granulosa of the preovulatory follicle. Remarkably, transformation of GCs into luteal cells occurs within a few hours (125). Not only structural changes but genomic alterations lead to the terminal differentiation of follicular cells into P-producing luteal cells. Progesterone receptor (PR) and cyclooxygenase-2 (COX-2) gene expression are induced after LH/hCG surge in GCs of ovulating follicles (126, 127). When the LH surge is blocked, PR mRNA is not induced. Mice deficient in PR or COX-2 are infertile; they develop preovulatory follicles that fail to ovulate (127, 128).

There are reports of increased melatonin levels in the luteal phase compared with the follicular phase of the menstrual cycle (129, 130). In humans, melatonin binding sites have been detected in GCs–luteal cells (7, 8), and melatonin directly stimulates the secretion of P by human GCsor luteal cells (8, 47). Melatonin may act at the level of the ovary to modify its function, especially luteal function (Table 2). In addition to melatonin's stimulation of P production by GCs–luteal cells (8, 46–48), it (10 pM–100 nM) also remarkably increases mRNA expression of the LH (but not FSH) receptor in human GCs/luteal cells, while inhibiting expression of GnRH and the GnRH receptor (8). Melatonin 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 (22, 44, 67, 131, 132). Sirotkin (131) documented that melatonin (1 ng/mL–100 μg/mL) inhibited P and cAMP secretion by GCs isolated from porcine ovaries.

Melatonin presumably suppresses steroidogenesis by inhibiting cAMP, which is a key second messenger in the steroidogenesis pathway. As summarized in Table 2, short-term incubation (within 48 hours) resulted in a negative effect of melatonin on P secretion. However, long-term incubation showed a positive effect. We hypothesize that, initially, the suppressive effect of melatonin on cAMP is dominant, but thereafter the other effects of melatonin such as induction of mRNA expression for the LH receptor and supportive effect on GCs becomes dominant. Melatonin might rescue GCs from free radical cytotoxicity in long-term cultured cells by its direct and indirect antioxidant ability. The ROS suppress P production and induce CL regression (9). Melatonin is likely to protect CL from ROS and has a important role in maintaining CL function. Recently, Dair et al. (133) documented the essential effects of melatonin on endometrial morphology and embryo implantation. They 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 body weight). Elevated melatonin in the luteal phase and early pregnancy may induce P production by luteal cells, which is necessary for successful pregnancy.

Much information exists relative to various biochemical and endocrine factors that impact P production by luteal cells. The hCG, LH, PRL (134), cytokines (135), and growth factors (136) promote P production, whereas prostaglandin F-2α (137), oxytocin (138), cytokines (139), and ROS (9) suppress P production. Prostaglandin F-2α is of particular importance because of its potential autocrine/paracrine actions that induce CL regression. Melatonin (10 mM) diminished prostaglandin F-2α secretion from rat uterus (140). Melatonin (0.1–1 mM) also inhibits COX-2 gene expression, which is the synthesizing enzyme of prostaglandin F-2α, in a murine macrophage cell line (141). Melatonin also increases PRL secretion (142) and inhibits the oxytocin release from the rat's hypothalamo-neurohypophysial system (143), suggesting that the indoleamine is essential for maintaining P production and luteal function by making the best use of various mechanisms.

MELATONIN AND POLYCYSTIC OVARY SYNDROME

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder that causes infertility due to anovulation in women of reproductive age. Besides infertility, women with PCOS manifest clinical features such as hyperandrogenism, hyperinsulinemia, insulin resistance, hirsutism, obesity, chronic anovulation, and polycystic ovaries (PCO). Not only anovulation but also decreased oocyte and embryo quality may be a cause of infertility in women with PCOS (144). The ROS-induced oxidative stress may be responsible for poor oocyte quality. The ROS generation from mononuclear cells is

TABLE 2

Effects of melatonin on P production in the granulosa cell.

Ref.	Sample	Species	Method	Incubation period	Melatonin dose	Effects
Woo 2001 (8)	Granulosa luteal cell	Human	Cell culture (w/wo hCG)	D4–D5	10 pM–100 nM	P ↑, LH receptor ↑, GnRH receptor ↓
Nakamura 2003 (22)	Growing granulosa cell granulosa luteal cell	Human	Cell culture (w/wo hCG)	24 h	10 pg/mL–1 ng/mL	P →
Yie 1995 (48)	Granulosa luteal cell	Human	Cell culture (hCG or FSH)	D1–D7	10 pg/mL–1 ng/mL	FSH stimulated E2 →, hCG stimulated P ↑
Brzezinski 1992 (47)	Granulosa luteal cell	Human	Cell culture (w/wo hCG)	D2–D10	10 pM–100 nM	hCG stimulated P ↑
Welbey 1986 (46)	Granulosa luteal cell	Human	Cell culture (wo hCG)	D1–D4	12.5–800 pg/mL	E2 →, P↑
Sirotkin 1994 (131)	Granulosa luteal cell	Porcine	Cell culture (wo hCG)	12–48 h	1 ng/mL–100 μg/mL	E2↑, P↓, cAMP↓, cGMP↑
Murayama 1997 (44)	Growing granulosa cell	Hen	Cell culture (LH)	4 h	0.5–5 nM	P↓
Bodis 2001 (132)	Granulosa luteal cell	Human	cell culture (LH or FSH)	48 h	10 nM–1 mM	E2↓, P↓
Schaeffer 1997 (67)	Growing granulosa cell	Human	Cell culture (wo hCG)	48 h	10 ng/mL–10 μg/mL	P↓

wo = without; w/wo = with or without; D = day(s).

Tamura. Melatonin and the ovary. Fertil Steril 2009.

elevated in women with PCOS (145), and significantly increased serum lipid peroxidation products in women with PCOS has been shown (146). The lipid peroxidation product malondialdehyde is increased in the FF of women with PCOS (147), and the ratio of apoptotic GCs is greater in women with PCOS (148). Oxidative stress may cause GCs and oocyte damage by lipid peroxidation, protein oxidation, and DNA damage in the follicle.

Levels of urinary 6-sulfatoxymelatonin, the major enzymatic metabolite of melatonin, is reportedly higher in women with PCOS compared to women without this condition (149). Elevated melatonin enhances LH secretion (150), LH pulse amplitude (150), and LH response to GnRH (151). Furthermore, melatonin may reduce peripheral tissue sensitivity to insulin (152). On the contrary, the reduction in melatonin levels due to pinealectomy and continuous light exposure induces the development of some aspects of PCOS in rats (153).

We hypothesize that there may be a reduction in the uptake of melatonin into the ovarian follicle. In fact, we showed that intrafollicular melatonin concentrations were significantly lower in PCOS than those of control in women undergoing IVF-embryo transfer (PCOS group: 74.3 ± 6.6 pg/mL, n = 9 vs. non-PCOS group: 104.9 ± 9.5 pg/mL, n = 27) (Tamura et al., unpublished data). Even if there are high concentrations of melatonin in serum, women with PCOS have a deficiency of this indoleamine in their ovarian follicles. Increased serum melatonin in PCOS may be a feedback response to the deficient levels of melatonin in the ovary. As mentioned previously, high levels of melatonin in the FF is essential for follicle growth, ovulation, and oocyte quality, whereas reduced follicular melatonin concentrations may be responsible for anovulation and poor oocyte quality in PCOS.

The 16-kDa protein hormone leptin is mainly produced in adipose tissue and reaches high serum levels in obese humans (154). In the circulation, leptin binds to protein(s) (155), which may modify its biological activity (156). Leptin regulates metabolic homeostasis and food intake, and affects the reproductive system by binding to specific cellular receptors (154, 157). The leptin system disorder is related to reproductive pathologies of PCOS (158). Physiological levels of leptin stimulate steroidogenesis and follicle maturation, whereas supraphysiological concentrations of leptin may produce the opposite effect (159). Leptin levels in serum of women with PCOS are significantly higher than those of control women (160). Furthermore, FF contains leptin in concentrations

similar to serum levels (161), and ovarian cells, including GCs, theca, and interstitial cells, express specific leptin receptor (162). Leptin alters steroid production by GCs and theca cells in vitro (163), suggesting a direct intraovarian effect in vivo. Increased leptin levels in FF are reported in women with PCOS (160).

Daily melatonin administration to rats suppresses body weight, intraabdominal adiposity, and plasma leptin levels (164). Whereas pinealectomy increases and exogenous melatonin reduces serum leptin (165). On the contrary, melatonin enhances leptin expression by rat adipocytes in the presence of insulin (166). Melatonin acts directly on adipocytes through specific G protein-coupled receptors, MT1 and MT2 (167). The activation of these receptors might exert a modulatory effect on leptin production by lowering cAMP levels. However, the relation between decreased melatonin and increased leptin concentrations in the FF of women with PCOS is obscure. Further studies will be necessary to clarify these relationship, which may be main point in understanding the pathophysiology of PCOS.

MELATONIN AND ENDOMETRIOSIS

Endometriosis is a chronic inflammatory disease that is characterized by implantation and growth of endometrial tissue outside the uterine cavity. It is a common gynecological problem that has a progressive and recurrent nature and has been estimated to affect 21%–44% of infertile and 4%–22% of fertile women (168–170). It is associated with chronic pelvic pain, progressive dysmenorrhea, dysapareunia, and infertility. Most commonly, the extrauterine implantation site is in the dependent portions of the pelvis, specifically the ovaries, the pelvic walls, and posterior cul-de-sac.

The cause of endometriosis is unknown, but it is believed to be a multifactorial disease associated with a general inflammatory response in the peritoneal cavity. One theory suggests that during menstruation, shedding endometrial fragments might pass through the fallopian tubes and reach the peritoneal cavity in a retrograde manner. These endometrial fragments may implant on the serosal surfaces of the peritoneal cavity, and with each subsequent menstrual cycle they may undergo proliferation and bleeding. At these sites, inducers of oxidative stress may include erythrocytes, apoptotic endometrial cells, and undigested endometrial cells in the menstrual effluent (171).

The ROS have been shown to be closely related to the inflammatory process and pathophysiology of disease. Peritoneal fluid (PF) volume in women with endometriosis is increased, and the number of macrophages in the PF is greater than in normal women. Activated macrophages induce oxidative stress, lipid peroxide formation, and other by-products resulting from the interaction of apolipoprotein with peroxides. Elevated ROS production by PF macrophages in endometriosis patients occurs (172). The ROS lead to a localized pelvic inflammatory reaction, resulting in increased concentrations of cytokines, growth factors, prostaglandins, and other proinflammatory mediators. Free iron and heme also play an important role in the production of ROS. Their deposition is increased in close proximity to the endometriotic implants of the peritoneum (171). Consistently, iNOS activity and NO production by the peritoneal macrophages are significantly elevated in endometriosis (173).

Intraperitoneal administration of melatonin, which is a powerful free radical scavenger, reduces adhesions (174). Although the role of melatonin in endometriosis is unknown, two interesting articles demonstrated the involvement of melatonin in pathogenesis of endometriosis. Güney et al. (175) confirmed the antioxidant, anti-inflammatory, and immunomodulatory effects of melatonin on endometrial explants in the rat endometriosis model. Melatonin treatment (10 mg/kg a day injected intraperitoneally) significantly reduced explant volumes compared with the control group (129.4 ± 28.7 vs. 42.9 ± 14.0 mm^3), and COX-2 positive cells from endometrial explants were significantly decreased in melatonin-treated rats (91% vs. 18.1%). The endometrial explant levels of malondialdehyde were significantly reduced and activities of SOD and catalase (CAT) were elevated in the melatonin-treated rats. This suggested that melatonin caused regression and atrophy of the endometriotic lesions by oxidative stress reduction (175).

In another study, Paul et al. (176) showed the role of melatonin in prevention and regression of endometriosis in mice. They identified a novel diagnostic marker, matrix metalloproteinases (MMP-9)/tissue inhibitors of metalloproteinase (TIMP-1) expression ratio in judging disease progression and severity, and melatonin administration (48 mg/kg, intraperitoneally) arrested lipid peroxidation and protein oxidation in peritoneal endometriosis. Melatonin also down-regulated the activity and expression of proMMP-9 and increased TIMP-1 expression. This findings indicate a role for melatonin in preventing and inducing regression of endometriosis through the regulation of MMPs.

MELATONIN AND PREMATURE OVARIAN FAILURE

Premature ovarian failure (POF) is diagnosed when sex steroid deficiency, elevated gonadotropins, and amenorrhea are found in women less than 40 years old (177). Premature ovarian failure arises from a genetically predetermined reduced number of ovarian follicles at birth, accelerated follicular depletion (atresia), or follicular dysfunction (178). There are a variety of causes of POF including chromosomal and genetic abnormalities, autoimmune disease, viral infections, and iatrogenic therapy (e.g., pelvic surgery, chemotherapy, radiotherapy). Chemotherapy and radiotherapy for treatment of malignant disease are the most commonly known causes of POF. Although improved chemotherapy and radiotherapy regimens for cancers in young people have led to increased long-term survival, one consequence has been a diminution in ovarian reserve and, thus, an increased incidence of POF. The risk of treatment leading to POF increases with age after puberty, with various high-dose chemotherapy

regimens and with combined chemotherapy and radiation therapy (179).

The damaging effects of ionizing radiation are brought about by direct and indirect mechanisms. The direct action produces disruption of sensitive molecules in the cells, whereas the indirect actions of ionizing radiation occur when it interacts with water molecules in the cell, resulting in the production of highly reactive free radicals, such as ·OH, ·H, aqueous electrons (e^-aq). An estimated 60%–70% of tissue damage and cellular DNA damage induced by ionizing radiation is believed to be a consequence the ·OH (180). Gonadotoxicity is pronounced if both ovaries are exposed to radiation (181). There is dose-related depletion of primordial follicles after increasing radiation doses of 0.1, 0.2, and 0.3 Gy (182).

Numerous studies have confirmed the high efficacy of melatonin in protecting against ionizing radiation (180). When melatonin interacts with the ·OH, it is converted to an intermediate indolyl (melatonyl) radical. This reactant has very low toxicity, therefore there is a net gain when melatonin scavenges the ·OH with a highly toxic reactant being replaced by a radical with low toxicity (183). This intermediate molecule then scavenges a second ·OH to generate cyclic 3-hydroxy melatonin. Melatonin's radioprotective action is likely achieved by its ability as a scavenger of free radicals generated by ionizing radiation (184). Pretreatment with melatonin reduces plasma and erythrocyte malondialdehyde levels, which are a result of radiation-induced oxidative damage after total body irradiation. At the same time, melatonin also increased the levels of SOD and GPx (185). Thus, in addition to its direct scavenging effects, melatonin may protect against the molecular destruction produced by radiation by the up-regulation of antioxidant enzymes. Melatonin administration protects against damage inflicted by radiation when it is given before exposure to irradiation and not after the exposure has occurred (184). This indicates that melatonin must be inside the cell at the time of exposure to radiation.

The structure and function of GCs and oocytes are affected by chemotherapeutic agents given for various malignant diseases in young women. Marked loss of primordial follicles is seen in ovarian biopsies after chemotherapy (186). The gonadotoxic effect of chemotherapy is largely drug- and dose-dependent and is related to age. Some studies have suggested that oxidative stress also plays an important role in chemotherapy-induced cytotoxicity. Some chemotherapeutic medications including alkylating agents (e.g., cyclophosphamide, ifosfamide), platinum agents (e.g., cisplatin), antitumour antibiotics (e.g., doxorubicin, daunorubicin, bleomycin) induce generation of ROS in mitochondria. Melatonin counteracts chemotherapy toxicity by acting as an antioxidant agent, and promotes apoptosis of cancer cells, thereby reducing chemotherapy cytotoxicity (187, 188). The concomitant administration of melatonin with several chemotherapeutic combinations in cancer patients reduced the frequency of thrombocytopenia, neurotoxicity, cardiotoxicity, stomatitis, and asthenia (7). Melatonin co-administration (10 mg/kg body weight) with doxorubicin decreased malondialdehyde levels and glutathione levels in cardiac tissues compared to the doxorubicin-treated rats (189). Other studies have also shown that melatonin is highly effective in protecting against doxorubicin-induced cytotoxicity (190). Melatonin would likely protect follicles and oocytes from free radicals generated by these agents.

Some cases of POF may be due to an abnormal self-recognition by the immune system. Autoimmune mechanisms are likely involved in pathogenesis of up to 30% of cases of POF (191). The evidence takes the form of a clinical association of POF with other autoimmune diseases, demonstration of ovarian autoantibodies against GCs, thecal cells, as well as against the zona pellucida (ZP) (192–194). Autoimmune lymphocytic oophoritis is commonly associated with the increased activity of peripheral T lymphocytes (195) and may be isolated or associated with other endocrine disorders such as Addison's disease, diabetes mellitus, hypothyroidism, myasthenia gravis, systemic lupus erythematosus (SLE), rheumatoid arthritis, and autoimmune hypothyroidism (196, 197). The modulatory roles of melatonin on immune system are also widely known (198, 199).

The direct effect of melatonin on the human immune system is supported by the existence of specific melatonin binding sites on lymphocytes (200) and monocytes (201). Through these receptors, melatonin regulates lymphocyte and monocyte function (202) and Th1/Th2 cytokine balance (203, 204). Melatonin suppresses autoimmune diseases by reducing lymphocyte physiology in autoimmune target organs (205). A recent report documented that melatonin protects against immune ovarian failure induced by antiovarian antibodies in mice (206). This report showed that melatonin treatment (5 mg/kg body weight, IV injection 1 hour before antibodies administration) restored meiotic maturation and survival of the oocytes by means of its anti-inflammatory and antiapoptotic effects. Thus, melatonin would be expected to exert beneficial actions on immune-mediated ovarian pathology.

CONCLUDING REMARKS

The vast amount of research conducted on the mammalian pineal and melatonin has led to great progress in understanding the molecular processes of its synthetic and secretory abilities and the mechanisms of its major hormone, melatonin, at the peripheral level. The evidence that melatonin functions through multiple receptors, both membrane and nuclear, and also as a direct free radical scavenger, a process that requires no receptors, is unequivocal. As reviewed, numerous data suggest the involvement of melatonin in ovarian physiology including follicular development, ovulation, oocyte maturation, and luteal function. In addition, a melatonin deficiency seems to be involved in the pathophysiology of PCOS, endometriosis, and POF.

The clinical use of melatonin in humans has been explored and the findings open new opportunities for the management of several diseases including cancer, immunological

disorders, Alzheimer's disease, diabetes, and viral infections (14–17). In humans, the safety of exogenous melatonin has been revealed in many studies (207, 208). We have demonstrated that melatonin treatment for patients with infertility improves oocyte quality. Furthermore, melatonin increases fertilization rates and reduces oxidative damage in the FF. However, the usage of melatonin for ovarian diseases, such as PCOS, endometriosis, and POF, is limited. Clinical trials of melatonin for these conditions may prove highly beneficial.

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