The Crosstalk between Melatonin and Sex Steroid Hormones

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Keywords

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Abstract

Melatonin, an indolamine mainly released from the pineal gland, is associated with many biological functions, namely, the modulation of circadian and seasonal rhythms, sleep inducer, regulator of energy metabolism, antioxidant, and anticarcinogenic. Although several pieces of evidence also recognize the influence of melatonin in the reproductive physiology, the crosstalk between melatonin and sex hormones is not clear. Here, we review the effects of sex differences in the circulating levels of melatonin and update the current knowledge on the link between sex hormones and melatonin. Furthermore, we explore the effects of melatonin on gonadal steroidogenesis and hormonal control in females. The literature review shows that despite the strong evidence that sex differences impact on the circadian profiles of melatonin, reports are still considerably ambiguous, and these differences may arise from several factors, like the use of contraceptive pills, hormonal status, and sleep deprivation. Furthermore, there has been an inconclusive debate about the

characteristics of the reciprocal relationship between melatonin and reproductive hormones. In this regard, there is evidence for the role of melatonin in gonadal steroidogenesis brought about by research that shows that melatonin affects multiple transduction pathways that modulate Sertoli cell physiology and consequently spermatogenesis, and also estrogen and progesterone production. From the outcome of our research, it is possible to conclude that understanding the correlation between melatonin and reproductive hormones is crucial for the correction of several complications occurring during pregnancy, like preeclampsia, and for the control of climacteric symptoms.

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Introduction

Melatonin, *N*-acetyl-5-methoxytryptamine, is a pleiotropic hormone known to be associated with the modulation of circadian and seasonal rhythms in many biological body functions [1]. This hormone is rhythmically released at night by the pineal gland, and its synthesis is under the control of the endogenous oscillator in the hypothalamic suprachiasmatic nuclei, which, in turn, is syn-

chronized with the environmental light/dark cycle [2]. Furthermore, as the timing and interval of the nocturnal melatonin production changes according to the duration of the nights, melatonin secretion is the internal reflection of the external photoperiod [3]. Thus, among other actions, melatonin is known to integrate rhythmic alterations to control circadian and seasonal physiology in several processes such as metabolism, immunity, brain function, and reproduction [1, 4, 5]. Particularly, the role of melatonin in the control of reproductive physiology in photoperiodic, seasonally breeding mammals has been documented in the last years. Such control is acquired at the level of hypothalamic pituitary gonadal axis which determines the completion of the reproductive cycle and consequently indicates the best time of the year to breed [6].

Furthermore, when it comes to reproduction, the influence of melatonin in the synthesis and release of the hypothalamic gonadotropin-releasing hormone (GnRH) is well known [3], eventually regulating a variety of physiological responses in gonadal function and activity [7]. However, the first and only review supporting the relationship between melatonin and sex steroid hormones was published in 1999 by Luboshitzky and Lavie [8]. The available data at that time were scarce, and the importance of that relationship was unclear. Here, we review the effects of sex differences and sex hormone background in melatonin circulating levels and update the present knowledge on the crosstalk between sex hormones and melatonin.

Melatonin Synthesis and Function

Melatonin synthesis involves tryptophan as a precursor that is converted into serotonin after hydroxylation and subsequent decarboxylation. Then, arylalkylamine-*N*-acetyltransferase (AANAT) *N*-acetylates serotonin to *N*-acetylserotonin, which is subsequently converted by hydroxyindole-*O*-methyltransferase to finally produce melatonin. The regulation of melatonin synthesis in the pineal gland is controlled by a multisynaptic neural pathway involving the retinohypothalamic tract and the suprachiasmatic nuclei. The information arrives at the pineal gland via peripheral postganglionic sympathetic fibers [9]. Nocturnal elevations in norepinephrine stimulation, via β - and α 1-adrenergic receptors in pinealocytes, activate protein kinase C (PKC) and increases Ca^{2+} movement, leading to an increase in the intracellular concentration of cyclic adenosine monophosphate

(cAMP). The accumulation of cAMP activates protein kinase A (PKA) which in turn increases AANAT activity [10]. Once synthesized, due to the pineal-cerebrospinal fluid interface, melatonin is directly secreted into the cerebrospinal fluid of the third ventricle and into the blood, reaching all body tissues within a very short period of time [11] (Fig. 1). Even so, the complex regulatory mechanism of melatonin release might not only involve simple diffusion but also carrier-like systems [12].

Melatonin is able to regulate several physiological functions from well-known sleep/wake cycles and modulation of the circadian rhythms to neural development and protection and regulation of the immune system and endocrine functions [13]. Those actions are driven through the activation of G protein-coupled membrane-bound melatonin receptors MT1 and MT2 and of a putative cytoplasmic melatonin receptor MT3 [14]. Due to the connection between receptors and Gai/o proteins, modulation of the second messenger cAMP and calcium levels and also the activation of PKC subtypes are the most generally accepted signaling transduction pathways triggered by melatonin [15]. Functional membrane melatonin receptors have been described in several brain structures [16] and also in non-neural tissues, such as the cardiovascular system, the immune system, the endocrine system, the gastrointestinal tract, bone, and reproductive organs [17]. In addition, melatonin also exhibits multiple actions through a melatonin-receptor-independent mechanism [18].

Melatonin and Sex Hormones: Bidirectional Communication

Sex Differences in the Circadian Profiles of Melatonin

One of the first reports of the presence of distinct melatonin profiles between sexes appeared in 1982. The data revealed that the excretion of melatonin in the urine was greater in boys than in girls in the 15–16.9 and 12–14.9 year age-groups, presented no difference in the 11–12.9 year age-group, and was greater in girls than in boys in the 9–10.9 year age-group [19]. Despite these findings of the distinct melatonin profiles between sexes in different age-groups, it became clear that other aspects could also influence melatonin circadian parameters. Several other publications have appeared in recent years documenting some inconsistency regarding the influence of sex on the circadian profiles of melatonin. Earlier onset of salivary melatonin was found in women in comparison with men (19–34 years old) [20]. Similarly, using a constant routine

Fig. 1. Melatonin synthesis. This figure summarizes the pathway for melatonin synthesis. The retina receives photic information from the environment. Light has an inhibitory effect, and darkness promotes the transmission of signals from the SCN to the SCG via the PVN followed by the ILCC. The SCG releases NE that binds with β - and α 1-adrenergic receptors in pinealocytes elevating the intracellular levels of cAMP. cAMP activates AANAT transcription. Serotonin, which is the product of tryptophan's hydroxylation and decarboxylation, is converted to *N*-acetylserotonin by

AANAT and to melatonin by HIOMT. Melatonin is then released by the pineal gland to the cerebrospinal fluid or to the systemic bloodstream via capillary blood vessels. SCN, suprachiasmatic nucleus; SCG, superior cervical ganglion; PVN, paraventricular nucleus; ILCC, intermediolateral cell column; NE, norepinephrine; cAMP, cyclic adenosine monophosphate; AANAT, arylalkylamine-*N*-acetyltransferase; HIOMT, hydroxyindole-*O*-methyltransferase.

protocol to analyze melatonin plasma levels from 28 women and 28 men (18–30 years old), a significantly higher amplitude of melatonin synthesis was observed in women, albeit the presence of similar sleep timing between both sexes [21]. Another noteworthy study, also using a constant routine protocol, determined a possible influence of sex differences in metabolism and excretion of melatonin. The findings indicate that females exhibited a higher amplitude in the rhythm of melatonin plasma levels, but the major melatonin urinary metabolite, 6-sulfatoxymelatonin (aMT6s), levels were not different between groups [22]. A contradictory study in men and women (40–75 years old) showed significantly higher lev-

els of aMT6s in females than in males [23]. Finally, other studies did not report any differences in melatonin parameters [24] and in the circadian amplitude of melatonin rhythms between men and women [25, 26].

Despite the strong evidence for sex differences in the circadian profiles of melatonin, reports are still considerably ambiguous, and this ambiguity may be attributable to several factors. The influence of hormonal birth control pills might be one cause for the significant variability exhibited in females. Indeed, there is evidence that the use of oral contraceptive pills may increase melatonin levels [27], and several studies do not take into account the female hormonal status. Individual differences in weight,

age, bedtime, employment status, caffeine uptake, alcohol, medications, contact lenses, sleep deprivation, and methodological differences in melatonin measurement were also associated with the variability observed in melatonin levels. Although the research presented so far overlooked the contribution of sex hormones to melatonin synthesis, the presence of melatonin receptors in human reproductive organs and evidence of reciprocal sex hormone receptors in the pineal gland suggest a relevant crosstalk between sex hormones and melatonin, which will be discussed in the next sections.

Effects of Melatonin on Reproductive Hormones

Many published reports support a critical role of melatonin on the levels of reproductive hormones, therefore on reproductive activities, through activation of receptors located in the hypothalamic-pituitary-gonadal axis [28]. Below, we explore the effects of melatonin in the regulation of male and female reproductive hormones and the associated mechanisms of action.

Melatonin Effects on Male Reproductive Hormones

In men, clinical trials showed no differences in hormonal synthesis of luteotropic hormone (LH), follicle-stimulating hormone (FSH), and testosterone after melatonin administration [29–32]. Furthermore, the levels of plasma testosterone, LH, and FSH decreased in prepubertal animals, but were not affected in pubertal and adult male rats, supporting clear age-related changes in melatonin effects [33]. Indeed, the current research is insufficient to draw any firm conclusions about the role of melatonin in the modulation of male reproductive hormones, and future studies should further enhance and confirm these initial findings.

Melatonin Effects on Female Reproductive Hormones

In opposition to the inconclusive results in men described in the literature, in women, findings indicate a clear response of reproductive hormones to exogenous treatment with melatonin. A report of intravenous administration of melatonin in 3 postmenopausal women was one of the first studies documenting the influence of melatonin in female reproductive hormones, revealing that melatonin decreased the levels of LH [34]. Although showing contradictory results, further reports suggested an important role of melatonin in the modulation of the neuroendocrine-reproductive axis. For example, melatonin treatment enhanced LH pulse amplitude in women

[35] and LH levels only during the follicular phase [36]. In contradiction, daily melatonin administration during 4 months caused a significant decrease in LH, estradiol, and progesterone [37]. A more recent clinical trial confirmed previous studies by showing a decrease in LH levels only in younger perimenopausal women (43–49 years old). In addition, nocturnal administration of melatonin declined FSH levels [38].

The data gathered from animal experiments clearly corroborated an inhibitory action of melatonin in the synthesis of female gonadal hormones. Melatonin administration to elderly acyclic female rats significantly reduced FSH and LH levels to those observed in cyclic control rats [39, 40]; however, no differences in estradiol and progesterone levels were detected [40]. The same authors also found that middle-aged melatonin-treated rats presented increased FSH and LH levels, contradicting the negative influence showed in old rats [40]. More recently, melatonin-treated female rats showed not only a decrease in LH and estradiol levels [7, 41], but also a significant increase in progesterone [41] and dehydroepiandrosterone [42] plasma levels. Overall, despite the different melatonin dosages, time and route of administration, age, and hormonal status, the data yielded by these studies provide convincing evidence of an adjustment of hormones of the female reproductive system by the melatonin.

Mechanisms of Action

Pineal melatonin secretion regulates GnRH, a central component of the hypothalamic-pituitary-reproductive axis, affecting reproductive hormone secretion [43]. Nonetheless, in the past years, there has been an inconclusive debate about possible binding sites of melatonin for its action on the modulation of reproductive hormone synthesis. Based on currently available evidence, some authors state that melatonin does not seem to act on GnRH neurons [44, 45], while others state that melatonin may act directly on GnRH neurons influencing PKA, PKC, and MAPK pathways [46]. In addition, recent results suggested that melatonin might adjust reproductive hormone levels via an indirect circuitry through the KiSS-1/GPR54 system [45, 47]. The consensus view seems to be that melatonin effects on reproductive hormones might be coordinated by 3 different sites of action: (1) the hypothalamic GnRH neurons, (2) the pituitary, and (3) gonads/reproductive organs. Indeed, several theories have been proposed to explain the antiestrogenic action of melatonin in the reproductive system. As reviewed by Cos et al. [48, 49], melatonin reduces the synthesis of bio-

logically active estrogens through the regulation of the activity of different enzymes involved in the synthesis of steroid hormones on the epithelial mammary cells. Such potential highlights the capacity of melatonin to behave as a selective estrogen enzyme modulator [48, 49]. Another direct action of melatonin in the regulation of hormone levels is described in cultured granulosa cells, suggesting an upregulation of estradiol after melatonin stimulus [50].

Sex Hormone Modulation of Melatonin Synthesis

Reproductive hormone receptors are present in the rodent, bovine, and human pineal gland [51–53], suggesting a possible role of reproductive hormones in melatonin synthesis. Indeed, the impact of cyclic oscillations in the levels of sex hormones on pineal melatonin secretion is clear. The effects of male and female reproductive hormones on melatonin synthesis are described in the next sections.

Effects of Male Reproductive Hormones on Melatonin

It is generally accepted that plasma testosterone levels might be indispensable to preserve the nocturnal melatonin peak [54]. Pursuing with clinical trials, it was shown that testosterone treatment of male patients with GnRH deficiency decreased melatonin to basal levels [55]. In addition, using male pineal cultured glands, it was demonstrated that testosterone significantly increased melatonin release [56]. These previous studies thus can be considered a first step towards a more profound understanding of the role of male reproductive hormones in the regulation of melatonin synthesis.

Effects of Female Reproductive Hormones on Melatonin

It is well documented that serum melatonin levels decrease with age, attaining minimum levels in menopause [57]. Further, melatonin nocturnal secretion is increased in women using oral contraception [27], and postmenopausal women have diminished nocturnal melatonin levels in comparison with perimenopausal [58]. Clinical trials revealed that administration of estrogens to postmenopausal women suppressed nocturnal melatonin release [59]. In addition, melatonin secretion changed upon administration of GnRH agonists [60]. Delfs et al. [61], on the contrary, argue that circadian melatonin secretion is not regulated by endogenous or exogenous estrogens in women.

Fig. 2. Reproductive hormones and melatonin circadian rhythms relationship. The variation of reproductive hormone concentration during the menstrual cycle influences melatonin circadian concentration. On the other hand, melatonin variations during the day also influence reproductive hormone concentration along the month.

In addition, the function of female reproductive hormones on melatonin synthesis and secretion also comes from animal studies. Estradiol treatment of rat pineal explants significantly increases melatonin production in vitro [62]. As a rebuttal to the increase of melatonin levels in the presence of estrogens, it was further demonstrated that subcutaneous estradiol implantation in female virgin rats significantly decreases melatonin and AANAT activity [63]. Hernandez-Diaz et al. [64] findings lend support to the idea of an estrogen-mediated inhibition of melatonin synthesis in rat pinealocytes, possibly by causing a decrease in AANAT activity. With respect to progesterone, effects are brought about by research that shows a decrease in melatonin synthesis in female perfused pineal glands [65] and in postmenopausal women treated with progesterone during the dark period [66].

Based on currently available evidence and admitting the existence of results that are not so consensual, it is possible to suggest that reproductive hormones affect melatonin nocturnal peak, circulating levels, and duration of secretion. The physiologic consequences for such control are poorly studied and understood. The characteristics of the reciprocal relationship between melatonin and reproductive hormones are misunderstood and based on few publications that show dissenters to the view of melatonin interference on reproductive hormones and vice-versa (Fig. 2). Even though there is some inconsistency, there is a strong probability of a bidirectional communication. Indeed, it is viable that hormonal alterations associated with aging may adjust melatonin levels through regulation of AANAT activity. Besides, it is also plausible that direct or indirect actions of melatonin will support reproductive hormone fluctuations. Future studies on the

current topic are therefore required in order to elucidate the impact during aging and in the control of climacteric symptoms.

Regulatory Role of Melatonin in Gonadal Steroidogenesis: Involvement in Female and Male Reproduction

Male Gonads

Leydig cells are located between the seminiferous tubules of the testis. These cells are responsible for synthesizing and secreting testosterone, which promotes the development of male reproductive tissues and plays a role on spermatogenesis and on the development of secondary sexual features [67]. Testosterone synthesis is induced by luteinizing hormone and cAMP signaling that promote the transfer of cholesterol to the inner mitochondrial membrane involving the steroidogenic acute regulatory protein (StAR). After that, cholesterol is converted into pregnenolone that is transformed into progesterone and subsequently metabolized to testosterone [68]. This similar steroidogenesis pathway occurs in the thecae cells and interstitial cells of the ovaries [69, 70]. Melatonin may act similarly in the Leydig (testis) and thecae cells (ovary – see section below).

Pinealectomy induces alteration in steroidogenic gene expression, such as StAR and cytochrome P450 17A1 (Cyp17) in male gonads [71], and controls androgen production, which is important for male physiology and reproduction. In fact, melatonin regulates testosterone secretion [72–75], enhances the responsiveness of Sertoli cells to FSH during testicular development [76], and has a role in cellular growth, in proliferation, and in the secretory activity of testicular cells [77]. In addition, this hormone may protect the testis against the local generation of reactive oxygen species and inflammatory aggression. Also, melatonin appears to protect human spermatozoa from apoptosis [78, 79] since low melatonin levels are related with some cases of male infertility that include oligoasthenozoospermia or nonobstructive azoospermia [78, 80].

Testosterone production induced by LH stimulation is mediated by the cAMP signaling pathway in the Leydig cells. However, there are some endocrine and paracrine factors that act via cAMP-independent mechanisms and influence the androgen secretion regulation [81]. One of them is the testosterone secretion induced by GnRH stimulation that is mediated by PKC activation and increased cytosolic Ca^{2+} concentrations. Melatonin is able

to block this process by suppressing the GnRH-dependent release of Ca^{2+} from intracellular stores in rat Leydig cells [82].

In addition, melatonin regulates the steroidogenesis through the StAR protein. In fact, Wu et al. [73] reported that melatonin inhibited StAR protein expression in MA-10 mouse Leydig tumor cells. Other studies demonstrated that melatonin decreased StAR expression as well as other enzymes such as cytochrome P450 family 11 subfamily A member 1, 3β -hydroxysteroid dehydrogenase, and 17β -hydroxysteroid dehydrogenase type III in vitro and in vivo [72, 75]. There are some pieces of evidence that melatonin may induce the expression of steroid 5α -reductase, which is important for the testicular conversion of testosterone into the active form 5α -dihydrotestosterone. Also, melatonin inhibits the expression of 3α -hydroxysteroid dehydrogenase, an enzyme that catalyzes the interconversion between 5α -dihydrotestosterone and 3α -androstenediol [75]. In addition, melatonin presents a crosstalk with the testicular corticotropin-releasing hormone system, which modulates testosterone production and increases the basal testosterone and cAMP concentration without affecting the maximum human chorionic gonadotropin (hCG)-stimulated testosterone synthesis [83–85].

The expression of MT1 and MT2 receptors has been reported in rat and bovine Sertoli cells [77], which are important for spermatogenesis efficiency and fertility [86]. In fact, Sertoli cells provide energy substrates such as lactate required to fuel germ cell metabolism. Melatonin decreases basal lactate production and upregulates the insulin-stimulated lactate generation, facilitating glucose uptake through the increase in GLUT1 protein levels and glucose consumption in rat Sertoli cells [87]. Melatonin also presents a modulatory role on the oxidant/antioxidant balance that maintains male fertility and spermatogenesis, since the testicular microenvironment is characterized by low oxygen tension and oxidative stress due to the abundance of highly unsaturated fatty acids and the presence of reactive oxygen species-generating systems including the mitochondria and a variety of enzymes that participate in spermatogenesis [88].

Melatonin also affects Sertoli cell growth and proliferation as it upregulates the expression of spermatogenesis-related genes including cyclin D1, cyclin E, platelet-derived growth factor subunit A, desert hedgehog signaling molecule, occludin, and claudin in bovine Sertoli cells. Those genes may interfere in spermatogenesis. In addition, melatonin significantly increases inhibin B [89], considered a marker of Sertoli cell damage. Together, melatonin has multiple transduction pathways for modu-

lating the Sertoli cell physiology and influencing spermatogenesis.

Female Gonads

The rat ovary presents MT1 and MT2 receptors in the secondary and tertiary follicles and luteal bodies, and their expression and capacity to bind melatonin vary along the estrous cycle phases [90]. Estrogens may influence melatonin receptor expression and function [90]. In addition, MT1 and MT2 receptor expression in the granulosa cells of the bovine gonad is regulated by melatonin itself [91].

Melatonin suppresses estradiol and progesterone synthesis on the preovulatory follicle in hamster ovary under hCG treatment [92], and the authors suggested that this effect was dependent on cAMP decrease. In fact, the hCG is a glycoprotein that presents an effect similar to LH, which increases cAMP levels and the production of androgens [93], besides inducing the follicular changes to luteal cells that produce estradiol and progesterone [94]. This effect may have been blocked by the cAMP reduction caused by melatonin treatment.

Melatonin presents several effects on the ovaries. First, it influences the action of LH as its absence through pinealectomy determines a reduction in the ovulation rates [95] as well as intense histological changes, such as low number of luteal bodies, increase in the thecae cells and interstitial cell layers, and presence of microcysts in the periphery of the organ [96, 97]. Those changes are similar to the ones observed in the ovaries of patients with polycystic ovarian syndrome [98]. In pinealectomized rats, melatonin administration may partially reverse those changes and restore the luteal bodies [95, 97, 99]. Also, other studies found that melatonin is important to reduce the thickness of the thecae layer and decrease the proliferation markers on those cells. The authors suggest that melatonin plays a role in the maintenance of a proper follicular morphology, being important for the ovulation process [99, 100].

Melatonin appears to be involved in the follicular dynamics, ovulation, degradation of luteal bodies, quality of oocyte, and steroidogenesis [95, 98, 100]. A recent study showed that melatonin acted on the sheep cumulus-oocyte complex that was important for quality of the oocyte [101]. Taking into consideration that sheep is a seasonal short-photoperiod reproductive species, the authors suggest that estrogen regulates local melatonin metabolism as well as interferes with its receptor expression [90, 101]. In fact, Soares et al. [102] showed that estrogen levels may interfere with the activity of melatonin receptors (MT1

and MT2) in the rat granulosa cells. Therefore, estrogen may change the effect of the melatonin receptor on cAMP signaling [102]. Consequently, melatonin and estrogens seem to be coparticipants in the processes of follicular growth and angiogenesis as well as in the maintenance of the quality of the oocyte [101]. Those pieces of evidence may explain the reason for the follicular concentration of melatonin being 3 times higher than that found in the circulation [103]. In addition, Chuffa et al. [7] described that melatonin significantly reduced LH and estrogen blood levels as well as induced downregulation of estrogen receptor alpha and progesterone receptor beta expression in the ovaries during rat ovulation.

Melatonin plays a role in the ovary morphology and follicular dynamics [95] as well as in the integrity of the ovarian follicle. In fact, Wang et al. [104] demonstrated that melatonin may maintain follicular health through the inhibition of the apoptosis of granulosa cells that are important for the interaction between the granulosa cells and oocytes [105]. This action involved the proapoptotic protein Bcl-2-interacting mediator of cell death-extra long (BimEL) since melatonin at 10^{-9} M downregulated BimEL expression in in vitro porcine granulosa cells [104]. Moreover, BimEL controls the follicular atresia and the apoptosis of granulosa cells. In addition, high dose (30 µg/kg), but not low dose (3 µg/kg), of oral melatonin determines increase in antral follicles in murine ovaries [106]. Therefore, it seems that high local melatonin concentration in the follicles is important for the protective action of this indolamine.

Circulating melatonin ablation after pinealectomy increases the expression of CYP11A1, CYP17A1, and CYP19A1, and melatonin replacement determines a decrease in CYP17A1 expression, mainly in the theca interna and interstitial cells [107]. Also, melatonin activation of the StAR and PI3K/AKT pathways in theca cells influences androgen production. The MT2 receptor may be involved in the regulation of steroidogenesis by melatonin [108], and the PI3K/AKT pathway is important for steroidogenesis and apoptosis [109]. Therefore, melatonin may modulate the LH action in steroidogenesis and apoptosis through this pathway, at least, in ovarian theca cells. A recent study showed that melatonin increased the expression of inhibin beta and follistatin in the ovaries of pinealectomized female rats, playing a role in the gonadotropin regulation, mainly on FSH, and in ovary steroidogenesis [110].

Other authors described that oral melatonin could improve the quality of oocytes in aged mice [111]. The age is a major factor for reducing the fertility and oocyte qual-

ity in women undergoing in vitro fertilization-embryo transfer. In both situations, melatonin treatment showed positive effects [112, 113].

The high levels of melatonin in preovulatory follicles may have a role in progesterone synthesis by the human granulosa cells. Also, melatonin may interfere with the amount of the steroid receptor in the ovaries, mainly the progesterone receptor [99]. In summary, melatonin affects the ovaries in many ways: (a) increasing progesterone production in vitro and in vivo; (b) increasing estrogen production; (c) antagonizing estrogen action; (d) improving theca cell quality, resulting in improved embryo and higher pregnancy rates; (e) improving cell proliferation via MAPK; (f) reducing free radicals; and (g) reducing the precocious follicular atresia.

Melatonin Involvement in Hormonal Control in Females

Effects of Melatonin in Placental Hormones

Throughout pregnancy, women undergo a myriad of hormonal and physiological adjustments which include the formation of a new, transient organ – the placenta. This autonomous organ displays unique features besides providing fetal nourishment and oxygenation. The placenta synthesizes many steroids and peptide hormones and acts as an autocrine, paracrine, and endocrine organ that contributes to the formation of a perfect environment for life development [114].

A healthy gestation depends, among other factors, on placental production of neuropeptides, growth factors, adipokines, pituitary-like hormones, and steroids. The present section focuses on melatonin involvement in pregnancy only. As previously described, the circulating melatonin pattern reflects the pineal synthesis rhythm, providing the organism far-reaching internal time cues [11, 13]. However, a plethora of organs synthesize this indoleamine for local use [115, 116]. In the female reproductive tract, extrapineal synthesis of melatonin occurs in the ovary [117], oocytes [118], granulosa cells [119], and placenta [120, 121].

Extrapineal sources of melatonin cause different melatonin concentrations, which diverge even among cellular compartments [115], hindering a consensus about what a melatonin “physiological concentration” is [122]. In the ovary, both syncytiotrophoblasts and villous cytotrophoblasts synthesize melatonin, as well as other 2 in vitro trophoblast models, JEG-3 and BeWo choriocarcinoma cells [121]. Moreover, placentas obtained from healthy wom-

en in the first trimester and also near term presented functional AANAT and hydroxyindole-O-methyltransferase/ASMT enzymes [120, 121].

Besides its autocrine and paracrine effects, melatonin acts as a direct and indirect antioxidant [119, 123, 124]. Its molecular structure consists of a pyrrolic ring containing electrons that easily quench oxidative and nitrosative reactive species. These reactions generate other stable antioxidant molecules, such as cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, and N1-acetyl-5-methoxykynuramine, whose free radical scavenging potentials are even higher than the one observed for melatonin [125, 126]. Regarding its indirect antioxidant effects, melatonin increases the expression of antioxidant enzymes such as superoxide dismutase [59], glutathione peroxidase [123], and catalase [127]. Furthermore, this amphipathic molecule freely crosses all organism membranes, even reaching mitochondria where its role is crucial to quench free radicals in the electron transport chain, a highly oxidative process [128, 129].

Placenta-derived fetal nourishment, oxygenation, and excretion processes generate a significant amount of free radicals [130]. Oxidative stress is a physiological aspect needed for placental development, but yet there must be a balance between pro-oxidant molecules and antioxidant defenses [131].

Melatonin exerts its actions, including the antioxidant effects, by receptor-dependent and receptor-independent mechanisms. Placental villous trophoblasts express both melatonin MT1 and MT2 transmembrane receptors, although their precise role during pregnancy remains to be studied [121]. Other pregnancy-classical hormones might also interact with melatonin throughout this period.

In vitro studies reported an increase in progesterone synthesis by human luteal cells and rat granulosa cells when low concentrations of melatonin were added to the medium [132, 133], while estradiol synthesis remained unchanged. Nonetheless, when the media contained hCG and melatonin combined, estradiol concentrations increased [133]. Another experiment tested melatonin ability to increase either estrogen or progesterone in cell cultures of bovine and human granulosa cells. While low concentrations of melatonin did not influence either progesterone or estradiol outcome, incubation with higher doses stimulated progesterone, but not estradiol, secretion, and a dose/response concentration outcome was absent [134].

Towards the term, healthy pregnancies physiologically express higher levels of estrogen and progesterone [135].

In vivo experiments with pinealectomized rats showed even higher levels of plasma progesterone on gestational days 18 and 19 and also of estrogen on days 20 and 21 [136]. This study opposes the findings obtained in vitro, as previously described. Noteworthy, all plasmatic measurements were performed during the day, which could have misled some interpretations. Literature about the relation between melatonin and classical pregnancy-induced hormones is still scarce. However, there is plenty of evidence that melatonin participates in many processes throughout gestation, from embryo implantation to birth [137].

The relevance of melatonin during pregnancy, a remarkable characteristic of this hormone, is still not fully understood. Studies performed in humans and rats reported a significant increase in plasma melatonin concentrations towards the end of gestation, reaching the peak right before delivery and returning to basal rates in the following evening [135, 138–140]. Human maternal melatonin levels increase approximately 4 times in comparison with their pregestational nocturnal concentrations [139]. Meanwhile, daytime variation in melatonin concentrations is still a matter of controversy. One group reported a decrease in early pregnancy followed by an increment on late gestational weeks [135]. Another study showed a daytime increase during the third semester [138], while the most recent one did not find any evidence of diurnal rise [139]. From our understanding, it seems unlikely to detect a rise in diurnal concentrations in this period; nonetheless, this question needs further investigation.

The last trimester increase in maternal melatonin positively relates to the number of children borne, both in humans and rats. Twins pregnancy presented a significant earlier surge in melatonin increase – 24 weeks of gestation versus 28 weeks in singleton pregnancies. Additionally, circulating melatonin levels are higher in mothers of twins [139]. Rats also synthesize more melatonin towards the end of pregnancy, which is also relatable to the offspring quantity. Tamura et al. [140] tested the relation between the offspring quantity and melatonin increase. They reported a noticeable increase in this indolamine concentration in dams with 10 or more offspring, while single-offspring dams (surgically induced by fetectomy) showed secretion profiles comparable to control rats. Afterward, they collected, washed, and cultured placentas from 10+ offspring pregnancies to obtain aliquots of the placental incubation medium supernatant. After 3 consecutive days of medium injection, the single-offspring pregnant dams prominently increased nocturnal

melatonin levels to the same of the 10+ offspring group. Performing the same procedure after charcoal addition to the medium resulted in unchanged basal melatonin levels. That points to a hypothesis in which some placental factors might trigger pineal melatonin increase towards the term [140].

The physiological increase in melatonin synthesis throughout pregnancy can be related to healthy outcomes, since maternal melatonin levels are reduced in the presence of gestational complications such as in intra-uterine growth restriction, preeclampsia, and eclampsia [139, 141]. Moreover, alterations in the circadian rhythm of melatonin reportedly increase the risk of preeclampsia in addition to altering this hormone secretion pattern [142].

Preeclampsia is an exclusive human dysfunction affecting 3–8% of gestations worldwide. It can be defined as *de novo* pregnancy-induced hypertension in addition to proteinuria, among other complications that appear after 20 weeks of gestation, compromising the mother and the fetus [143]. Its pathogenesis is related to placental increased oxidative/nitrosative stress and apoptosis, which consequently augment the release of free radical species in the bloodstream, damaging the endothelium and leading to blood pressure elevation [144]. Considering melatonin direct and indirect antioxidant effects, its association in pregnancy was investigated.

The severity of preeclampsia relates negatively to circulating melatonin levels, meaning that women suffering from severe preeclampsia present less melatonin when compared to mild preeclampsia or healthy pregnant ones [139]. Based on this evidence, Lanoix and colleagues [145] investigated melatonin and its receptors in pre-eclamptic placentas. They showed that both hormone production and receptor expression are diminished when comparing this group to healthy controls. Preeclamptic women produce less melatonin and present diminished placental AANAT expression and a significant decrease in MT1 and MT2 placental receptors. Noteworthy, pre-eclamptic placentas display increased serotonin levels, pointing to a defective molecular cascade in melatonin production rather than a lack of the substrate for hormone conversion.

The presented data indicate a pivotal role of melatonin in the maintenance of healthy pregnancies. Low levels of melatonin during pregnancy are not only a complication to the mother, but also influences fetal programming in terms of fetus development, energy metabolism, cognition, and behavior until later in life [146–148].

Fig. 3. Melatonin's effects on menopause and/or postmenopause. Melatonin therapy is useful in preventing obesity and high cholesterol by reducing food intake in women undergoing menopause or postmenopause. Metabolic changes, dyslipidemia, and climacteric symptoms are also regulated by melatonin therapy.

Melatonin assessment through the nocturnal excretion of its metabolite, aMT6s, is a practical and noninvasive method. As low concentration of melatonin could be a signal of preeclampsia, aMT6s dosage throughout pregnancy might be useful as a marker to be followed. This potent antioxidant could also be considered for clinical trials in women suffering from such conditions.

Protective Actions of Melatonin in Menopausal and Postmenopausal Women

Endocrine transition-menopause is characterized by disruption of estrogen-regulated systems such as thermoregulation, sleep, and circadian rhythms [149]. The potential reciprocal relationship between melatonin and reproductive hormones lends support to the use of pineal melatonin in the control of disruption of such systems, influencing sleep and mood. It is well documented that among menopausal and postmenopausal women, one of the major problems resides in deregulation of sleep/wake cycles, probably as a result of a reduction of not only estrogens but also melatonin levels [150]. The consensus view seems to be that melatonin administration resynchronizes circadian rhythms, affecting symptoms associated with hormonal fluctuations. In ovariectomized rats, melatonin treatment reduced food intake and prevented the increase of body weight and cholesterol, metabolic changes associated with menopause and postmenopause [151]. Further, another animal study reported that mela-

tonin was more efficient than the combination of estrogens and melatonin in the regulation of dyslipidemia, suggesting a therapeutic alternative to control symptoms associated with transition-menopause [152]. Based on a clinical trial, melatonin administration to menopausal women significantly changed their reproductive hormone levels. Such regulation of FSH and LH levels was followed by a tendency to a reduction in climacteric symptoms [153]. Recent data contradicting previous studies suggested that melatonin administration to postmenopausal women does not alter FSH and estradiol plasma levels. Yet, melatonin therapy exerts real effects on the climacteric symptoms such as sleep, mood, and vasomotor dysfunctions [154]. However, other studies did not support a direct action of melatonin on climacteric symptoms [155].

Based on currently available evidence, it seems reasonable to suggest that melatonin is a therapeutic alternative to control the complexity of climacteric symptoms regardless of the deficiency of steroid hormones in menopausal and postmenopausal women (Fig. 3). Nonetheless, as other studies failed to observe a modulation of reproductive hormones by melatonin, the view of a different mechanism uncoupled to female reproductive hormones to justify the melatonin role in the improvement of climacteric symptoms may also be considered [154].

Conclusion

It is becoming clear that melatonin interacts with sex steroid hormones, in particular cases reciprocally, whereby melatonin action influences but is also being influenced by the sex steroids, thus a bidirectional relationship exists. This communication depends on the melatonin levels, age, hormonal status, tissue, and environmental conditions. Furthermore, the direct or indirect mechanistic pathways involved also bring to this interaction a high level of complexity.

Clinically, the relationship between melatonin and sex steroid hormones is of particular importance in female and male reproduction and in pregnancy in the case of treatment or prevention of preeclampsia as well as in the potential use of melatonin during the menopausal transition and beyond. It is clear, however, that the current knowledge is just the tip of the iceberg in a complex network of interactions in the crosstalk between melatonin and sex steroid hormones.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J.C.N., F.G.A., J.M.S., C.C., and T.Q. conceived the review, collected and analyzed the data, and wrote the paper. A.F., I.G., J.E.C., and C.S. revised the manuscript.

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