Melatonin and the circadian system: contributions to successful female reproduction

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Objective: To summarize the role of melatonin and circadian rhythms in determining optimal female reproductive physiology, especially at the peripheral level.
Design: Databases were searched for the related English-language literature published up to March 1, 2014. Only papers in peer-reviewed journals are cited.
Setting: Not applicable.
Patient(s): Not applicable.
Intervention(s): Melatonin treatment, alterations of the normal light:dark cycle and light exposure at night.
Main Outcome Measure(s): Melatonin levels in the blood and in the ovarian follicular fluid and melatonin synthesis, oxidative damage and circadian rhythm disturbances in peripheral reproductive organs.
Result(s): The central circadian regulatory system is located in the suprachiasmatic nucleus (SCN). The output of this master clock is synchronized to 24 hours by the prevailing light-dark cycle. The SCN regulates rhythms in peripheral cells via the autonomic nervous system and it sends a neural message to the pineal gland where it controls the cyclic production of melatonin; after its release, the melatonin rhythm strengthens peripheral oscillators. Melatonin is also produced in the peripheral reproductive organs, including granulosa cells, the cumulus oophorus, and the oocyte. These cells, along with the blood, may contribute melatonin to the follicular fluid, which has melatonin levels higher than those in the blood. Melatonin is a powerful free radical scavenger and protects the oocyte from oxidative stress, especially at the time of ovulation. The cyclic levels of melatonin in the blood pass through the placenta and aid in the organization of the fetal SCN. In the absence of this synchronizing effect, the offspring may exhibit neurobehavioral deficits. Also, melatonin protects the developing fetus from oxidative stress. Melatonin produced in the placenta likewise may preserve the optimal function of this organ.
Conclusion(s): Both stable circadian rhythms and cyclic melatonin availability are critical for optimal ovarian physiology and placental function. Because light exposure after darkness onset at night disrupts the master circadian clock and suppresses elevated nocturnal melatonin levels, light at night should be avoided. (Fertil Steril® 2014;102:321–8. ©2014 by American Society for Reproductive Medicine.)
Key Words: Circadian rhythms, melatonin, oocyte, placenta, fetus

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To ensure survival of any species, the most critical process is successful reproduction. The challenges to achieving this complex process, however, are becoming increasingly daunting considering the rise in air and water pollutants, food contamination, and possibly particularly due to perturbations of the normal light-dark environment under which humanoids and other vertebrates have evolved over the past 4 million years. This latter issue is frequently overlooked as a factor that compromises successful reproduction. There are, however, physiologic consequences associated with an altered photoperiodic cycle, especially, for example, bright light exposure during the normal period of darkness, i.e., at night.

The widespread use of manufactured light has led to truncation of the duration of darkness with artificial light exposure after sunset or before sunrise and interruption of darkness at night with brief periods of light or light throughout the night, such as for night-shift workers, e.g., hospital staff. These changes in the environmental norm are by no means inconsequential. The central circadian pacemaker, the suprachiasmatic nuclei (SCN) in the hypothalamus, relies on

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regular recurring periods of light and darkness which, when disturbed, initiates abnormal physiology. Moreover, truncating the duration of the daily dark period with artificial light severely limits total pineal melatonin production. Both regular circadian rhythms and ample melatonin are conducive to successful reproduction (1–3).

THE BIOLOGIC CLOCK, PERIPHERAL CIRCADIAN RHYTHMS, AND MELATONIN

The circadian signal for daily changes in physiology originates in the SCN, a small bilateral group of neurons in the anterobasal hypothalamus, that have an intrinsic circadian cycle of slightly greater than 24 hours (4, 5). Because of the lack of preciseness of this self-sustained rhythm, during evolution it became synchronized to 24 hours by the rising and setting of the sun. In current economically developed societies, however, the synchronizing effect of the natural rising and setting of the sun has been subverted by a highly irregular light-dark environment imposed by the ubiquitous use of artificial light sources.

The circadian signal generated in the SCN is transferred to cells in peripheral tissues by means of the central and peripheral autonomic nervous system; in the periphery, this system regulates changes in the physiology of cells over each 24-hour period. Depending on the cell type, up to 20% of the genes in individual cells may exhibit self-sustained rhythms (6, 7). For optimal function, the expression of these peripheral rhythms must be accurately regulated by the SCN. When the master clock signal is disturbed, the peripheral cellular rhythms are likewise negatively affected and their physiologic output suffers accordingly. The circadian output genes in populations of peripheral cells differ according to the tissue in which they reside (8, 9). The rhythms in individual cells must be coordinated with those of neighboring cells but they must also maintain a given phase relationship with more remote organs.

A second pathway by which the central clock imposes itself on the peripheral slave oscillators is via the melatonin rhythm derived from the pineal gland; this cycle is a universal feature of all vertebrates (10). Because many peripheral tissues are not innervated either by the sympathetic or parasympathetic autonomic nervous systems, these cells must receive the circadian message from another source. Presumably this is from the circadian rhythm of melatonin, which reaches every cell via the blood. This may also require that these peripheral cells possess receptors that "read" and respond to this photoperiod-dependent cycle. Two decades ago, the experimental evidence indicated that at least the known membrane receptors for melatonin, MT1 and MT2, had a rather limited distribution (11). More recent studies have revealed, however, that they are more widely distributed than originally supposed (12, 13); our current view is that these receptors may exist on the membranes of all cells whose activity must be synchronized by the melatonin cycle. However, melatonin also affects cellular physiology by processes that are independent from receptors, when it functions as a free radical scavenger (3, 14), so the membrane mediators may not be a requirement for melatonin to influence circadian gene expression in peripheral cells. Melatonin, as well as several of its metabolites, is highly effective in scavenging radicals and reducing oxidative stress (14).

The pineal-derived melatonin rhythm, in either the cerebrospinal fluid (CSF) (15) or the blood (4, 5), strengthens the circadian message that the SCN communicates to other organs via the autonomic nervous system. Perturbations of the cyclic melatonin pattern weaken the SCN message, contributing to faulty information being received by the peripheral organs. This defective signal negatively affects circadian gene expression and physiology in peripheral tissues, which contributes to dysfunction, such as some mood and cognitive disorders.

The earliest identified manifestation of the circadian melatonin rhythm being critical to reproductive physiology was in reference to photoperiod-driven seasonal reproduction (16, 17). In their natural habitat, many species rely on the changing duration of the elevated nocturnal melatonin levels (16) as determined by the length of the night, to signal changes in the physiology of the neuroendocrine-reproductive axis, which in turn alters the function of the peripheral reproductive organs (17, 18). This cyclic message is essential for both long-day and short-day breeders for the timing of the successful coincidence of maximal reproductive fertility in both female and male members of the species to ensure timely mating, fertilization, and delivery of the young (19). Depriving hamsters of the circadian melatonin rhythm by pinealectomizing them renders their reproductive systems unresponsive to the seasonal photoperiod changes (20). Likewise, stabilizing the light-dark cycle to which these animals are exposed often allows them to become continual year-round breeders because the melatonin rhythm is consistent from day to day, i.e., it lacks seasonal information.

In addition to being indispensable for mediating seasonally dependent reproductive capability, it seems safe to assume that clock genes and their related 24-hour rhythms likewise play important roles in peripheral reproductive functions (21). Knowledge related to the beneficial role of melatonin, either from a distance site or locally produced, on the gonads and accessory organs is significantly more advanced than is the information about the role of intrinsic circadian processes in these tissues (22). However, whether the circadian cycle of melatonin and an action on clock genes is essential for melatonin's peripheral effects on the gonads has not been established. Many of melatonin's known functions in the peripheral reproductive system relate to its ability to function as an antioxidant, an action that is not known to be time dependent (22, 23). Moreover, melatonin produced at the level of reproductive tissues themselves provide a portion of the protection against molecular damage resulting from local free radical generation (24, 25).

MELATONIN PRODUCTION IN THE PERIPHERAL REPRODUCTIVE SYSTEM

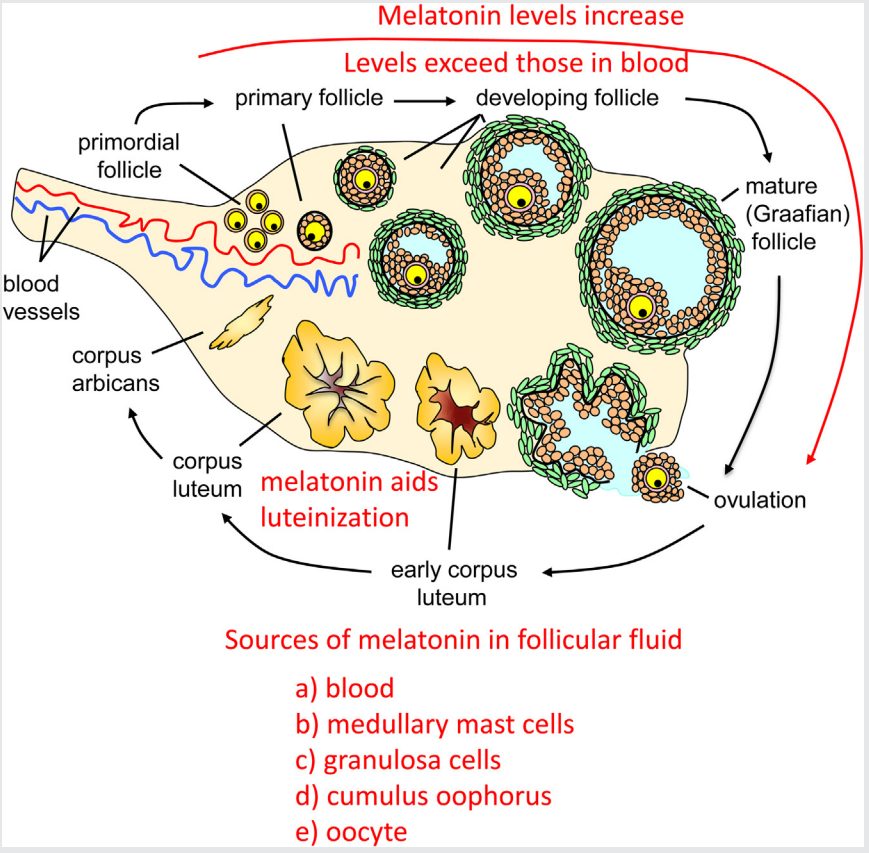
We have proposed that mitochondria may be sites of melatonin synthesis within all cells, not only in pinealocytes (26). Given that every cell must possess mitochondria to survive, the obvious implication is that all cells generate

melatonin for their local use, likely for cell protection against free radicals. Certainly, the idea that the pineal gland is the only organ that produces melatonin is obsolete. Indeed, the list of extrapineal sites of melatonin synthesis is now extensive and expanding (27, 28).

Regarding the peripheral reproductive system, of special interest is that melatonin concentrations in fluid collected from the human ovarian follicle exceed those in simultaneously collected blood samples (29, 30). Besides being in higher concentrations in the follicular fluid than in the general circulation, the levels of melatonin exhibit a 24-hour rhythm in the follicle (31) and the values reportedly increase as the follicle enlarges and ovulation approaches (Fig. 1) (32). Either the vesicular follicle has a means of concentrating melatonin from the blood against a gradient, or all or some of the melatonin in the follicular fluid is from another source. Recent studies support the latter assumption; the ovary as a whole (33, 34), the granulosa cells, including those making up the cumulus oophorus (24, 35), and the oocyte (36) have been reported to synthesize melatonin. Melatonin in the ovary also may be concerned with progesterone production by the transforming granulosa cells after ovulation (37). As with other extrapineal organs that generate melatonin, the ovarian cells do not discharge melatonin into the general circulation. Rather, these cells use the melatonin they produce for their own benefit or for that of their neighboring cells, i.e., as an antioxidant and as an autocrine or paracrine agent (38).

Ovulation, a process supported by the cyclic release of pituitary LH (39), is accompanied by alterations at the ovarian level that culminate in the rupture of the graafian follicle and the discharge of the oocyte. The process of ovulation has been likened to an inflammatory reaction (40). The evidence for this comes from the observations that follicular rupture is accompanied by the locally elevated production of prostaglandins and cytokines, the increased action of proteolytic enzymes and the heightened permeability of small blood vessels in the follicular wall (41, 42). These changes are associated with the generation of reactive oxygen species by macrophages, neutrophils, and endothelial cells, which also contribute to the disintegration of the follicular wall to allow the escape of the oocyte (43).

FIGURE 1



Presumed association of melatonin with the developing follicle and corpus luteum, based on observations from human and animal studies. Melatonin levels in the follicular fluid gradually increase as the follicle enlarges, and in the graafian follicle melatonin concentrations exceed those in the blood by 2–3 fold. Melatonin in the follicles presumably aids in the development of the oocyte and protects it from oxidative damage by free radicals; this is especially the case at ovulation, when free radical generation is at its highest. Follicular fluid melatonin levels probably derive from several sites (a–e), all of which have been shown to produce melatonin. Blood melatonin levels derive from the pineal gland. After ovulation, luteinization of the developing corpus luteum and the production of progesterone may also be assisted by melatonin.

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At ovulation, the oocyte, because of its proximity to the inflammation in the wall of the rupturing follicle, sits in a highly vulnerable position which could lead to molecular disfiguration of this critical cell by nearby ROS. If successfully fertilized and implanted, a mutilated oocyte would result in pregnancy interruption or in a structurally or functionally impaired fetus. It seems obvious, therefore, that the ovum must be especially well protected from oxidative stress at the time of its extrusion from the follicle. As a result, we have speculated that this is the reason that the cells of the cumulus oophorus (35) and the oocyte (36, 37) produce the potent antioxidant melatonin (2). After the oocyte is shed, it is presumed that melatonin also participates in the maturation of the corpus luteum and aids in progesterone production (44).

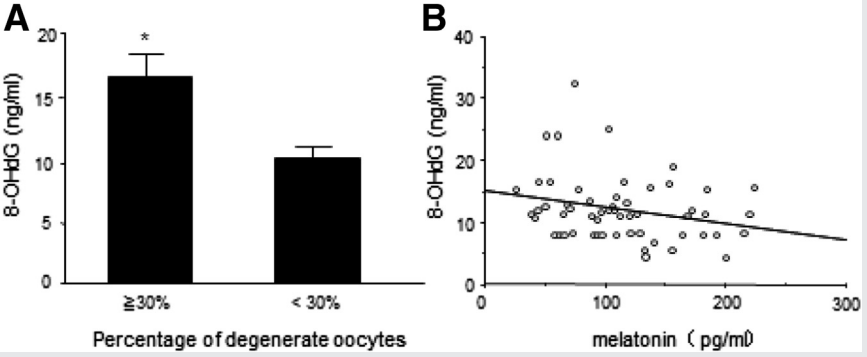
The ability of melatonin to protect oocytes from molecular mutilation by toxic oxygen derivatives is documented. Melatonin reduces the accumulation of oxidatively damaged oocytes incubated with oxidizing agents and improves the outcome of IVF-ET. This is well illustrated by studies performed by Tamura et al. (45) with the use of both mouse and human tissues. Cumulus-free mouse oocytes retrieved from preovulatory follicles were incubated for 12 hours in a solution containing various concentrations of hydrogen peroxide (H_2O_2); in addition to being a strong oxidizing agent, H_2O_2 is also readily converted to the highly damaging hydroxyl radical (·OH). After 12 hours, oocytes containing a first polar body (as an index of a mature oocyte) were counted. H_2O_2, in a concentration-dependent manner, significantly reduced oocyte maturation; however, when melatonin was added to the incubation medium in combination with H_2O_2, a much larger percentage of oocytes remained undamaged and reached maturity (45).

Tamura et al. (45) also extended these studies to the human, where they specifically measured oxidatively damaged lipid and DNA. Both follicular fluid and oocytes were transvaginally collected from gonadotropin-stimulated ovarian follicles in women. The retrieved oocytes were classified, based on morphologic characteristics, as being of either good or poor quality. The follicular fluid corresponding to each of the oocytes was evaluated for the levels of oxidative stress as indicated by the presence of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a damaged-DNA product, and for melatonin. The results showed that the highest levels of 8-OHdG were associated with the poorest quality oocytes. Moreover, the intrafollicular levels of 8-OHdG were negatively correlated with melatonin concentrations in this fluid (Fig. 2). These results are consistent with the antioxidant melatonin having protected oocyte DNA from oxidative damage and cellular degeneration.

Finally, with the intention of improving the outcome of IVF-ET by reducing free radical–mediated damage, 56 women who had a low fertilization rate in an earlier IVF-ET cycle were given melatonin (3 mg) orally each day for about a month before undergoing the procedure again. Compared with the previous IVF-ET cycle, melatonin ingestion doubled the fertilization and successful pregnancy rate in the women (44, 45). Considering what must be considered to be a small amount of natural melatonin, it seems likely that the use of larger daily quantities may further protect the oocytes from free radical damage and greater success of IVF-ET. The technology of protecting oocytes and sperm from oxidative damage with melatonin has also been successively applied in veterinary reproductive science (46).

Although ROS may actually aid in oocyte maturation (47) and follicular rupture (42) to permit its extrusion, an excessive level of free radicals would surely damage this critically important cell. Therefore, a delicate balance must be maintained in the follicle to ensure successful maturation of the oocyte and timely follicular rupture. The presence of melatonin, a proven antioxidant, in the ovary likely helps to maintain this balance. In addition to maintaining a high-quality oocyte, melatonin may also prevent ROS from damaging the granulosa cells, which must undergo luteinization and produce progesterone (47). As with damaged oocytes, luteal-phase defects also contribute to infertility.

FIGURE 2



(A) The levels of the oxidatively-damaged DNA product, 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the ovarian follicular fluid of women in which oocytes were judged to be of high quality (<30% damage) or poor quality (>30% damage; based on the amount of the damaged-DNA product). Oocytes manifesting the greatest degenerative changes had significantly higher DNA damage (*P<.01). (B) This illustrates the negative correlation between the intrafollicular levels of 8-OHdG and melatonin. Melatonin is a proven potent antioxidant. The implication is that melatonin protects oocytes from free radical damage. Modified from Tamura et al. (45).

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MELATONIN AND THE PLACENTA

Melatonin, along with the enzymes (arylalkylamine N-acetyltransferase and acetylserotonin methyltransferase) required for its synthesis, were discovered in the human placenta in 2008 (48). Not only is melatonin produced in this tissue, but the villous trophoblasts also possess the known membrane receptors for it, i.e., MT1 and MT2. These studies were performed on normal near-term placentas (37–41 weeks); little is known about the maturation of the placental melatonin-generating system during the earlier stages of pregnancy. The presence of the receptors suggests that melatonin has receptor-mediated actions in the placenta; however, it is also capable of receptor-independent actions when it functions as a radical scavenger (2, 25). The placental primary villous trophoblasts are composed of the mononuclear cytotrophoblasts (vCTB) and the multinuclear syncytiotrophoblasts (STB). Throughout pregnancy, mature vCTB fuse to form STB in a highly regulated process. During this process, it is imperative that the vCTB not undergo apoptosis before the fusion process. This is essential because the STB cells quickly turn over and degenerate and must be continually replaced by fusion of the vCTB (49, 50). Melatonin presumably ensures successful maintenance of the STB by preventing the vCTB from falling victim to apoptosis. This morphogenic action of melatonin aids in ensuring optimal placental function and successfully maintains pregnancy.

In contrast to the benefits of preventing programmed cell death of the vCTB, prolongation of cellular half-life by reducing the likelihood of apoptosis would be counterproductive for the organism in the case of placental cancer cells. Several choriocarcinoma cell lines are available for study, e.g., BeWo (51, 52). These cells are often used as a model for vCTB because they fuse to form a syncytium; likewise, they also synthesize melatonin and contain its receptors (48). Unlike normal vCTB cells, however, BeWo cells undergo apoptosis in response to melatonin (35). As in other cells, in the placenta, melatonin's actions are clearly context specific (52). Thus, melatonin often kills cancer cells by promoting programmed cell death while having the opposite action in normal cells. The mechanisms of these differential actions of melatonin in normal and cancer cells is under investigation (53).

Relevant to a discussion of melatonin in the placenta is its potential association with preeclampsia, an etiologically ambiguous condition. During severe preeclampsia, blood melatonin concentrations are diminished (54) and placental levels of melatonin as well as its receptors are similarly depressed (55). Preeclampsia is a serious disorder in which elevated ROS production is thought to be contributory, and it is known to involve the placenta because after delivery of the placenta, preeclampsia quickly disappears (44). Because melatonin is a potent antioxidant that can be administered easily, its use may be helpful in protection against the free radical–mediated toxicity of preeclampsia. With this in mind, Hobson et al. (56) outlined a clinical trial to test melatonin as a potential treatment for preeclampsia (3, 57). In addition to reducing oxidative stress associated with preeclampsia, melatonin may benefit this condition by limiting the hypertension (58) that accompanies this disorder as well as reducing the likelihood of potential seizures (59), i.e., preventing its progression to eclampsia.

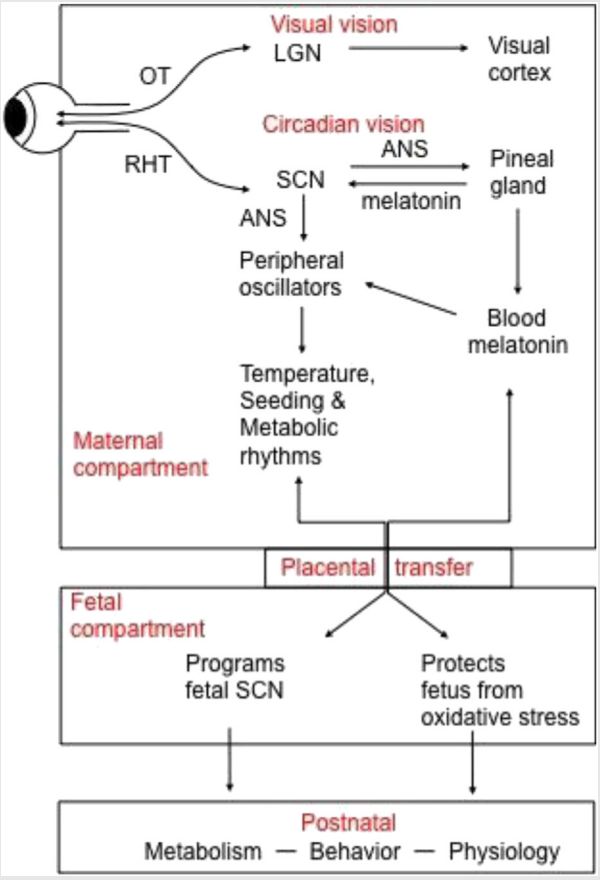
CIRCADIAN MELATONIN AND PROGRAMMING THE FETAL CLOCK

The placenta is not a barrier to melatonin; melatonin is rapidly transferred from the maternal to the fetal circulation (60, 61), and fetal melatonin levels as well as the rhythm are similar to those in the mother (47, 62). Because the fetus is not known to produce melatonin, any melatonin in the fetal circulation is due to its transfer through the placenta.

In the fetus, the master circadian oscillator, the SCN, is morphologically identifiable in the hypothalamus by midgestation. Also, the afferent axons from the retinal ganglion cells are known to terminate in the SCN before delivery (63), and rhythms of mRNAs for both vasopressin and c-Fos are expressed in the nucleus (64, 65). Whether the fetal clock generates any neural output signals, however, has not been documented. The SCN sends no useful neural message to the pineal gland until well after birth (62).

The maturation of the rhythmic expression of the fetal SCN seems to depend on information received from the mother. One reliable signal that passes from mother to fetus is obviously the melatonin cycle. This rhythm may play an essential role in determining the organization and functional architecture of the developing fetal SCN. For example, the exposure of pregnant rodents to constant light, which eliminates the blood melatonin rhythm, provides an inappropriate message to the master circadian oscillator of the fetus. As a consequence, the offspring exhibit disturbances in their behavioral rhythms (65). However, this study was complicated by the fact that the animals were also food restricted. Surgical removal of the maternal pineal gland during pregnancy does distort the drinking rhythms in the offspring (64); this malfunctioning rhythm is prevented if the pinealectomized dams are given daily melatonin injections during pregnancy (66). The authors considered the possibility that the increased frequency of attention deficit/hyperactivity (ADHD) and autism spectrum disorders (ASD) may relate to a prenatal perturbation of the maternal circadian clock given that the melatonin rhythm in these children differs from the norm (67, 68). Collectively, the findings argue that disturbances in the maternal melatonin rhythm may negatively affect the circadian maturation of the fetal SCN. If these relationships exist in humans, it would seem prudent for women in their last trimester to maintain a regular light-dark environment to conserve their normal melatonin cycle. Moreover, perhaps these mothers-to-be should avoid multiple-time-zone transmeridian travel and excessive lengthening of the day with manufactured light, because these behaviors perturb the normal melatonin cycle. Respectively, they cause conventional "jet lag" and what has come to be known as "social jet lag," both of which undoubtedly negatively affect the melatonin rhythm and thereby could alter the development of the fetal master clock (Fig. 3). These potential associations could be tested in pregnant humans, but by necessity the studies would have to be performed under controlled regular or altered

FIGURE 3



The proposed mechanisms by which the photoperiod acting via the suprachiasmatic nucleus (SCN; the master circadian clock) and the pineal gland influence circadian rhythms in the host and in the fetus. Typical ganglion cell axons from the retina project through the optic nerve and optic tract (OT) to the lateral geniculate nucleus (LGN), the axons of which pass to the visual cortex. This system subserves vision or what is referred to as visual vision. A second group of specialized ganglion cell (intrinsically photoreceptive ganglion cells) axons pass through the optic nerve as the retinohypothalamic tract (RHT) and terminate in the SCN; these subserve circadian vision. The SCN eventually sends a neural message to the pineal gland via the autonomic nervous system (ANS) to control melatonin production, which in the pineal gland is produced and released only at night. The SCN, also via the ANS, synchronizes circadian oscillators in peripheral tissues, an action likely supported by the circadian melatonin rhythm in the blood. Melatonin also acts on the SCN to regulate the master clock. Melatonin, as well as other blood-borne signals, passes through the placenta to program the fetal SCN, and, additionally, the antioxidant melatonin protects the fetus from oxidative stress. Proper programming of the fetal SCN and protection of the fetus improves postnatal metabolism, behavior, and physiology.

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light-dark cycles over an extended period of time. This makes human studies impractical.

In addition to advancing the maturation of the fetal SCN, melatonin has an additional benefit in the fetus as in the mother, i.e., its ability to reduce oxidative stress. When pregnant rats were subjected to bilateral utero-ovarian artery occlusion for 20 minutes on day 19 (normal pregnancy duration is 21 days), the brain of the fetuses endured massive lipid peroxidation and DNA damage as well as compromised mitochondrial function 30 minutes later (69, 70). However, when melatonin was exogenously administered to the mothers in advance of the arterial occlusion, it attenuated the damage to both lipids and DNA and preserved mitochondrial physiology in the fetal brain. The benefits of melatonin were predictably related to its ability to detoxify ROS and to stimulated antioxidative enzymes (70).

In fetal sheep as well, a 10-minute hypoxic episode induced by obstruction of the umbilical cord vessels led to an elevation in extracellular brain ·OH generation and augmented fragmentation of lipid and DNA/RNA in the brain (71, 72). The infusion of melatonin into the pregnant ewes prior to interruption of the blood flow in the umbilical blood vessels depressed neural ·OH levels and mitigated lipid and DNA/RNA destruction. These protective actions of melatonin were attributed to its ability as a radical scavenger and suggested that melatonin may be useful in situations where there is a heightened risk of fetal brain damage (72), e.g., in periventricular leukomalacia. This has been preliminarily verified by Fulia et al. (73), who found that melatonin reduced lipid damage and nitrite/nitrate levels in human newborns who had suffered transitory asphyxia during delivery.

CONCLUSIONS AND PERSPECTIVES

Clearly, both regular circadian rhythms and cyclic melatonin availability are beneficial in assuring optimal reproductive physiology in female mammals. For successful ovulation, regular cyclic events at the hypothalamopituitary level ensures properly timed gonadotropin release which leads to extrusion of the mature oocyte from the ruptured graafian follicle. Melatonin is synthesized at several sites in the ovary, and melatonin from these sources and from the blood probably assist in the maturation of the oocyte and protect it from free radical damage, especially at ovulation. Melatonin may also enhance development of the corpus luteum and progesterone production.

Throughout pregnancy, the circadian melatonin rhythm passes the placenta and influences the development and synchronization of the fetal master oscillator, the SCN. The implication of this finding is that, during pregnancy, a relatively regular undisturbed light-dark cycle should be maintained to enhance and strengthen circadian rhythms and to preserve the melatonin cycle. Regular perturbations of the light-dark environment by contaminating the night with artificial light undermines the maternal circadian clock and suppresses the nocturnal melatonin rise, both of which are normally important for the developing fetus. Considering these findings, it would seem judicious that, particularly during the last trimester, pregnant women maintain a stable light-dark environment.

Finally, exogenously administered melatonin during pregnancy may be helpful in protecting both the mother and the fetus from oxidative stress and mitochondrial dysfunction. Because of this, melatonin, considering its virtual absence of toxicity, should be considered for clinical

trials where the placenta is malfunctional and where excessive free radical generation is likely, e.g., in preeclampsia.

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