# Clinical significance of melatonin receptors in the human myometrium

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**Objective:** To review and update the research on melatonin receptor expression in the human myometrium, in particular as it pertains to uterine contractility at labor.
**Design:** Summary of previous studies with the addition of new data on the transcriptional regulation of melatonin receptor expression in human myometrial cells.
**Setting:** Not applicable.
**Patient(s):** Late-term pregnant volunteers.
**Intervention(s):** Biopsy collection for in vitro analyses provided the original data. More recently, uterine contractions in late-term pregnant volunteers were assessed before, during, and after acute white-light exposure.
**Main Outcome Measure(s):** Melatonin receptor signaling in myometrial cells and uterine contractions in late-term pregnant volunteers.
**Result(s):** Melatonin acts through the MTNR1B melatonin receptor that is expressed in the myometrium at late term to synergistically enhance oxytocin-dependent signaling and contractions. Acute inhibition of endogenous melatonin levels with light reversibly suppresses uterine contractions.
**Conclusion(s):** These results point to a significant role for circulating melatonin in the timing and degree of uterine contractions in late-term pregnancy. Understanding the regulation of melatonin receptors remains a future objective. (Fertil Steril® 2014;102:329–35. ©2014 by American Society for Reproductive Medicine.)
**Key Words:** Myometrium, melatonin, receptors, contractions, light

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The events leading up to parturition in the human are complex and still poorly understood. Clearly, interactions between hormonal, mechanical, and other signals are key in reversing the quiescent uterine state, which is present throughout most of pregnancy, into a strongly active contractile state capable of expelling the fetus and placenta. Among the physiological events that appear to significantly underlie the lengthy process of uterine activation and ultimately labor onset are increased expression of contractile-associated proteins (calmodulin, caldesmon, phosphorylated myosin light chain kinase, etc.), changes in the expression of membrane ion channels, and enhanced intercellular connectivity due to strong expression of the gap junction protein connexin 43 (1). The precise timing of labor onset in the human largely depends on the duration and strength of these events, as well as the position and number of fetuses, maternal pelvic dimensions, and many other factors.

A feature of human parturition that is often neglected is its strong association with the rest phase of the 24-hour sleep–wake cycle. Both term and preterm human parturition have been reported by several groups to occur most frequently during the late night and early morning hours, i.e. between 10:00 PM and 8:00 AM (2–5). In late pregnancy, uterine contractions are more frequent during the nighttime hours in both human and nonhuman primates (6, 7). These physiological events are not random, and indeed they reflect processes that intersect with the cellular basis for labor onset. In this context, we describe here our findings of an additional contributor to the nocturnal timing of uterine contractions and labor, which may provide a window for exploration into completely novel mechanisms underlying human parturition.

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## LABOR AND DELIVERY ARE TIMED BY THE BRAIN'S CIRCADIAN SYSTEM

Over the course of evolution, most mammals adapted to selective pressures, such

as food availability and predation, by becoming preferentially diurnal or nocturnal (or in rare cases crepuscular). Pregnant females that entered into labor while in a familiar, safe, and secure environment with their kin, rather than in the field, would likely have a selective advantage in terms of successful parturition. Hence, nocturnal and diurnal species would face opposite selective pressures to enter parturition during the daytime or nighttime phases, respectively. This fact may serve to explain why rodents give birth predominantly during the subjective day, even when the light–dark cycles are reversed (8–11). In diurnal primates, the opposite would be adaptive. Indeed, nonhuman female primates enter parturition at night, and this event can be temporally shifted by reversal of the light/dark cycles (12), highly suggestive of input from a circadian clock.

The most likely source of circadian signals for the timing of mammalian parturition is the hypothalamic suprachiasmatic nuclei (SCN), which is recognized as the brain's central circadian oscillator. The SCN is not only directly innervated by retinal ganglion cells that mediate light entrainment but, via both neural and hormonal outputs, the SCN directs rhythmic events, including behavioral, physiological, and cellular rhythms (13, 14). Of note here, the diurnal timing of birth in the rodents has been demonstrated to require an intact SCN (15). In view of its proven role as a circadian clock output signal under SCN control (16, 17), it is not surprising that melatonin has been found to play a key role in the timing of parturition in nocturnal rodents.

Takayama and colleagues (18) demonstrated that female rats whose endogenous melatonin was eliminated by pinealectomy had no difficulties in becoming pregnant or maintaining their pregnancies; however, they failed to deliver their young exclusively during the daytime (the normal birthing phase for nocturnal rodents). Instead, the rats gave birth randomly across the 24-hour light–dark cycle. However, evening administration of melatonin (i.e., to restore the normal endogenous secretory pattern) was effective in restoring the daytime birth pattern. Melatonin was ineffectual when given in the morning or continuously. This strongly points to the timing of birth in the rat being under circadian control, and implicates melatonin as a key circadian "gating" signal for this event.

In diurnal species, such as the human, little attention has been paid to the circadian dimensions of labor onset and nocturnal parturition. The proposed roles of functional progesterone withdrawal, increased prostaglandins, placental corticotropin-releasing hormone, or oxytocin levels in regulating the events that lead to parturition do not apply to the circadian phasing of human parturition, as these processes are nonrhythmic (19–25).

Endocrinological research on the function of melatonin has long been a part of reproductive physiology. Melatonin is a small lipid-soluble hormone generated and released into the circulation primarily by the epithalamic pineal gland in response to circadian drive from the SCN. Melatonin synthesis and secretion occurs almost entirely during the dark phase of the 24-hour day/night cycle in all vertebrate species. This secretory pattern is considered to serve as a neuroendocrine code for night phase and duration. Melatonin binds to G-protein–coupled receptors in the brain (26) and in peripheral tissues (27, 28) to inhibit cyclic AMP signaling and to stimulate phosphatidylinositol turnover (17).

The precise mode of action of melatonin in the mammalian uterus, while still not completely understood, is clearly species-specific. Earlier reports with rodents (29, 30) showed direct inhibitory effects of melatonin on uterine contractility. Later studies with rodents have confirmed inhibitory effects of melatonin on uterine contractility in vitro following stimulation by oxytocin (31, 32). Inhibitory effects of melatonin on prostaglandin synthesis in various rodent tissues have also been reported (31, 33–36). In view of the diurnal timing of parturition in these nocturnal rodents, these inhibitory effects of melatonin on uterine contractions are apparently important in reducing the likelihood of parturition during the night, i.e., during the animals' active phase. As discussed below, the situation in diurnal mammals, such as humans, is understandably quite the opposite.

## CONTROL OF UTERINE CONTRACTIONS INVOLVES SYNERGISM BETWEEN MELATONIN AND OXYTOCIN

In 2007, we reported that melatonin and oxytocin recruit similar intracellular signaling mechanisms in human myometrium smooth muscle cells (37), many of which are known to play a pivotal role in the induction or facilitation of labor. Additionally, a significant positive synergistic action of melatonin and oxytocin on human myometrial cell contractions in vitro was discovered (38, 39). When applied to human myometrial cells that are treated with oxytocin, melatonin at physiological doses leads to striking increases of oxytocin-induced IP3 (inositol 1,4,5-triphosphate) signaling and oxytocin-induced contractions. Thus, in the presence of 1 nM melatonin, even an oxytocin dose equivalent to only 1% of that normally needed for maximal contraction is fully effective (38, 39).

Oxytocin is an important tool in obstetrical practice. Continuous infusion of oxytocin is commonly used to induce labor, whereas oxytocin antagonists (e.g., atosiban) are now part of the armamentarium to prolong pregnancy in cases of preterm labor. However, prolonged labor induction by application of continuous oxytocin is only effective when high amounts of the hormone are given (due to receptor "desensitization") (40, 41). Not surprisingly, continuous oxytocin administration is often accompanied by side effects, including fetal distress, uterine rupture, postpartum atony, and bleeding. The discovery of a strong synergism between oxytocin and melatonin signaling opens new horizons for the potential development of melatonin + low-dose oxytocin combinations for labor induction without the side effects of high oxytocin levels.

## MELATONIN RECEPTORS ARE UPREGULATED AT LABOR

Using defined human myometrial biopsies and receptor-specific antibodies, it was found that the MTNR1B (MT2)