MELATONIN, GESTATION AND FETAL DEVELOPMENT

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SUMMARY

The pineal gland, the transducer of several environmental cues through its hormone, melatonin, is known to play a critical role in the reproduction of several seasonally breeding mammals. The role of pineal gland in the control of male reproduction is already established beyond doubt. The logical hypothesis that the pineal gland would be involved in the aspects of female reproduction, particularly gestation, embryonic development and fetal growth, has been tested in a few seasonally breeding mammals and this paper reviews the outcome as of now. Evidence for establishment of an inverse relationship before pregnancy followed by moderately high activity with a positive correlation during pregnancy and a sudden increase in pineal activity during parturition has been produced, which suggests that high melatonin level has something to do with parturition or early abortion. The pineal gland perhaps maintains the normal physiology during gestation and post-parturition periods. This is further reflected in the prolactin levels of fetal sheep which is modulated by the photoperiod experienced by the mother during gestation. Maternal photoperiodic exposures during gestation and lactation periods alter the neonatal growth and sexual maturation of the Indian palm squirrel *via* the maternal transfer of photoperiodic information through the pineal gland, as aspect known as "pineal programming". The primary source of melatonin for the fetus is the maternal pineal gland, which is transferred across the placenta during conception and, later after parturition, through the milk, which suggests that a maternally generated melatonin rhythm would be expressed in fetal cerebrospinal fluid as well as in the circulation. Further, maternal melatonin is necessary for normal somatic growth and postnatal development of reproductive organs of the offspring. Melatonin ultimately affects reproductive activity by modulating hypothalamic neuroendocrine circuits whose activity is necessary for gonadal function. The influence of melatonin on reproductive development begins during the prenatal period and extends into the postnatal life. Photoperiodic information, mediated *via* the pineal gland may be important for maintaining gestational physiology as well as postpartum recovery in female rodents. Further, melatonin has been shown to play adaptive role in the maintenance of delayed embryonic development in bats. Correlation between a developmental decline in melatonin levels and the timing of puberty in humans led to the speculation that melatonin regulates the timing of puberty in the human. These studies on the influence of melatonin on gestation indicate two major inferences *viz.*, melatonin could be one of the major hormones establishing homeostasis during gestation since any disturbance in the level of melatonin causes abortion, and melatonin is one of the hormones of great adaptive significance for embryonic diapause.

Key words: Embryonic growth, gestation, melatonin, photoperiod, pineal gland, puberty.

INTRODUCTION

Reproductive function in mammals consists of an intricate interplay of hormonal events that are responsible for the development and maturation of gametes, puberty, major events of the reproductive cycle (i.e., ovulation and sexual receptivity) and preparation of the uterus for the possible implantation of embryos.

The pineal gland is known to play a critical role in the reproduction of several seasonally breeding mammalian species (1). The seasonality of reproduction is usually enforced by a dependence on the day length perceived, which in turn regulates the synthesis of melatonin, the pineal hormone. The daily duration and the level of melatonin regulate the secretion of gonadotropins / gonadal steroids. They regulate the breeding in long day breeders such as squirrels, ferrets, voles, hamsters etc. (2-5). In the broadest sense, then, melatonin influences reproduction by restricting the season of conception enabling the offspring to be born under the most favorable environmental conditions.

The role of pineal gland in the control of male reproduction has been quite clearly established. However, its role in female reproduction, especially during gestation, is not clear, although the females allocate the maximum energy towards the begetting and bear most of the cost of reproduction. The pineal gland of the female provides indications of higher activity, with a higher circulating level of melatonin during the reproductive period (6). Therefore, it is conceivable that the pineal gland is involved in the aspects of female reproduction, particularly gestation, embryonic development and fetal growth. Until a little more than a decade ago there was no information on i) the influence of pineal gland on gestation, ii) the influence of pregnancy on the metabolism and function of pineal, and iii) the interrelationship between maternal and fetal pineal glands.

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The influence of melatonin may begin during gestation i.e., the prenatal period, and could extend into the postnatal period (7). The earliest report on such a relationship, published in 1965, was decrease in the weight of pineal during the later part of pregnancy in rats bearing 10 or more fetuses, suggesting the involvement of pineal in gestation and fertility (8). In the present review we analyze the data so far generated by us on the role of pineal gland during gestation and fetal development using tropical mammals (squirrel and bat) and by others in laboratory animals (rat and mouse) and humans.

MATERNAL PINEAL MORPHOLOGY AND MELATONIN

Systematic and detailed studies on pineal gland structure and function in pregnant females are lacking. The cursorial reports on sheep, cattle and rat pineal gland suggest some morphological and hormonal changes during pregnancy in relation to the physiological conditions of the reproductive organs (6).

Ultra-morphometric variation of the pinealocytes of a pregnant female rodent, the Indian palm squirrel, correlating with the functional state, was studied in our lab (9). The pinealocytes of the pregnant females possess two variants of mitochondria, based on size, which form mitochondrial clouds (Fig. 1 D). The Golgi zone appears to be hyperactive. A large number of granular vesicles are noticed in the pinealocyte cytoplasm and in the bulbous ending processes of the pinealocytes (Fig. 1 A-D). A few dense bodies of unknown function are also noticed. The increased abundance of synaptic vesicles

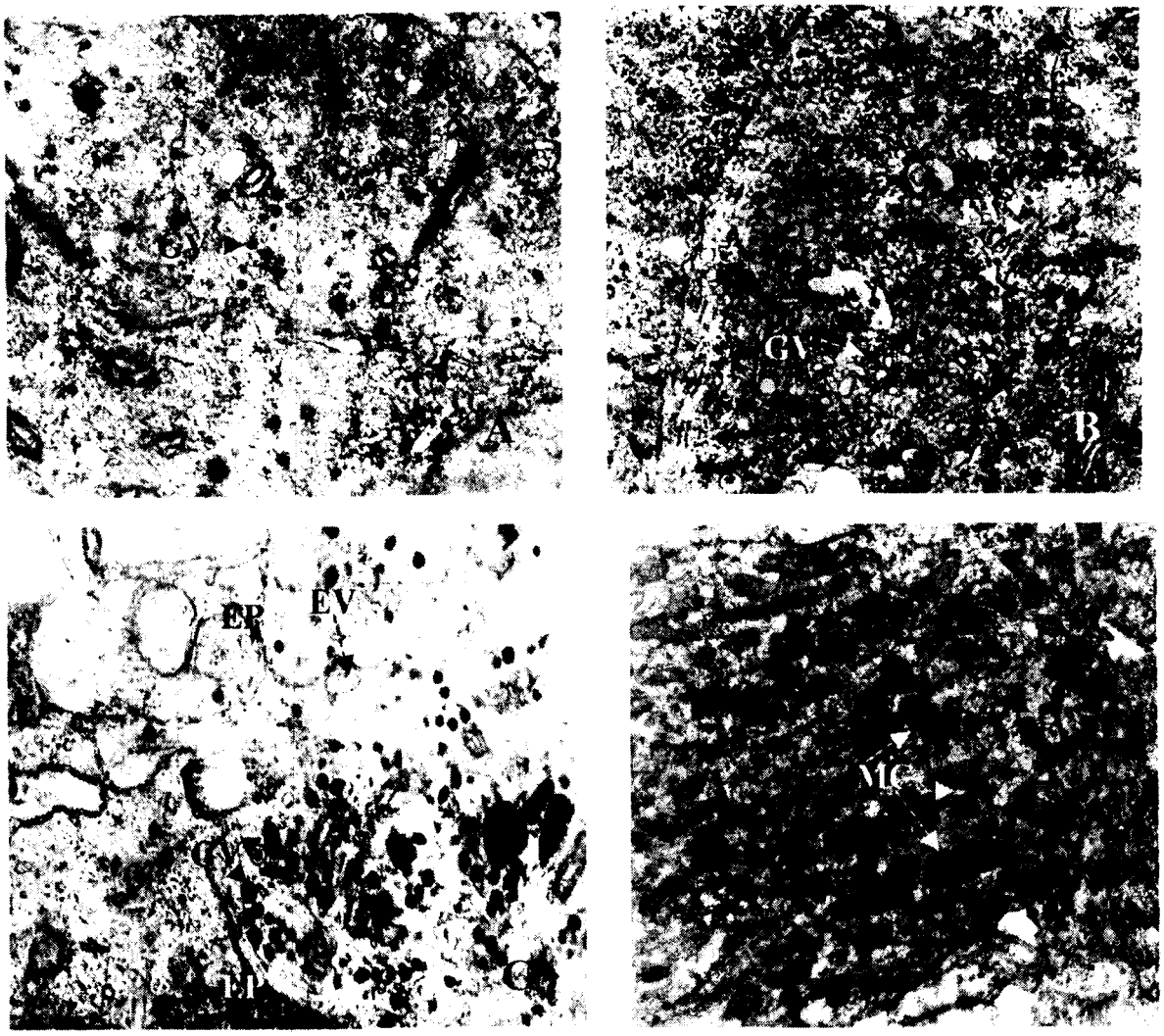

Fig.1. Electron micrograph of pineal gland of non-pregnant and pregnant female squirrels, *Funambulus pennanti* (x 5400). **(A)** Non-pregnant female's pineal gland. Note fewer granular vesicles (GV). **(B-D)**. Pregnant female's pineal gland. Note abundant GV and empty vesicles (EV in Fig. B). **(C)** Abundant GV and EV in ending process (EP) of a pinealocyte. **(D)** Occasionally mitochondrial clouds (MC) were also noticed in EP.

in the cytoplasm and the ending processes of the pinealocytes of pregnant females indicate better cellular communications and transmission than in the non-pregnant females. Such a comprehensive study is not available for any other mammal.

The pineal gland weight and melatonin level, which were high in the non-pregnant female rodents, declined sharply during the beginning of conception, followed by a gradual increase up to the end of gestation, and then a sharp increase during parturition (10). This suggests the establishment of an inverse relationship before pregnancy followed by moderately high activity with a positive correlation during pregnancy and sudden increase in pineal activity during parturition. This may mean that the very high melatonin level has something to do with parturition or early abortion.

PINEAL GLAND, MELATONIN AND GESTATION

Pregnancy is the period during which an animal carries one or more developing embryos. The sequence of events leading to pregnancy is complex and multidimensional. Maintenance of pregnancy is largely dependent on a proper balance between the levels of various hormones, which are maintained by interactions between the mother, the placenta and the fetus.

Whether pregnancy would affect the pineal gland structure and function is not clear but some preliminary studies suggest that the pineal gland is involved in the regulation of certain aspects of gestation (6, 8). An elaborate study on the weight of organs and plasma levels of melatonin, estradiol and progesterone during the gestation period was made in the Indian palm squirrel. The outcome suggested a possible role for pineal gland in maintaining the normal physiology during gestation and post-parturition periods (10). In spite of the inverse relationship between the pineal gland and the gonad in the non-pregnant adult females, the study indicated a direct relationship between the pineal gland activity and the ovarian steroids and uterine function, especially during the gestation period. The inverse relationship between melatonin and ovarian steroids is re-established after parturition and maintained till the next pregnancy (10).

In order to assess the role of melatonin during pregnancy, the hormone was infused at regular intervals into pinealectomized dams (Table 1). Melatonin infusion during the night hours determined the rate of pubertal development. A single gestational melatonin infusion did not influence postnatal reproductive development, but two infusions on consecutive nights provided a sufficient signal. A period of maximum sensitivity to melatonin infusions occurs during late gestation. Outcome of studies using timed injection of melatonin (rather than infusions) supports the conclusion that melatonin is a critical cue for transfer of day length information to the fetus (11).

Table 1. Effect of pinealectomy and melatonin injection / implantation in different mammals

Name of animal	Gestation period (days)	Effect of pinealectomy on gestation	Effect of melatonin injection/ implantation
Rat	21	Spontaneous abortion	Post natal ontogeny of the neuroendocrine reproductive axis
Hamster	16	-	Delayed sexual maturation in offspring
Skunk	31	-	Delays implantation and long term suppression of prolactin levels
Indian Palm Squirrel	45	-	May disrupt the mother's endogenous melatonin rhythm and delay the post-partum recovery process.
Bat	90-120	Abortion	Delayed embryonic development
Sheep	150	-	Delayed sexual maturation

Prolactin levels of fetal sheep can be modulated by the photoperiod experienced by the mother during gestation. Long days resulted in higher prolactin levels (12), while melatonin implants reduced prolactin levels (13, 14) acting via the pars tuberalis (15, 16). In skunks, melatonin treatment delays implantation by several months and a long-term suppression of prolactin levels. Ablation of the suprachiasmatic nuclei (SCN) does not block the effect of exogenous melatonin (17). In contrast, anterior hypothalamic lesions cause precocious termination of diapause, regardless of the melatonin milieu (18, 19). It is not clear whether melatonin acts within or through the anterior hypothalamus. The anterior hypothalamus may be a leg in the final common pathway in prolactin regulation downstream of the melatonin target sites. Considering that high affinity melatonin receptor binding is limited to the pars tuberalis in skunks (20), these data suggest a model in which melatonin regulates production of a factor from the pars-tuberalis that influences prolactin secretion. A similar suggestion has been made to explain the influence of melatonin, mediated by the pars tuberalis on prolactin secretion in sheep (21).

MELATONIN, PHOTOPERIOD AND GESTATION

In spite of the extensive research on female reproduction, it is not clear how information about the

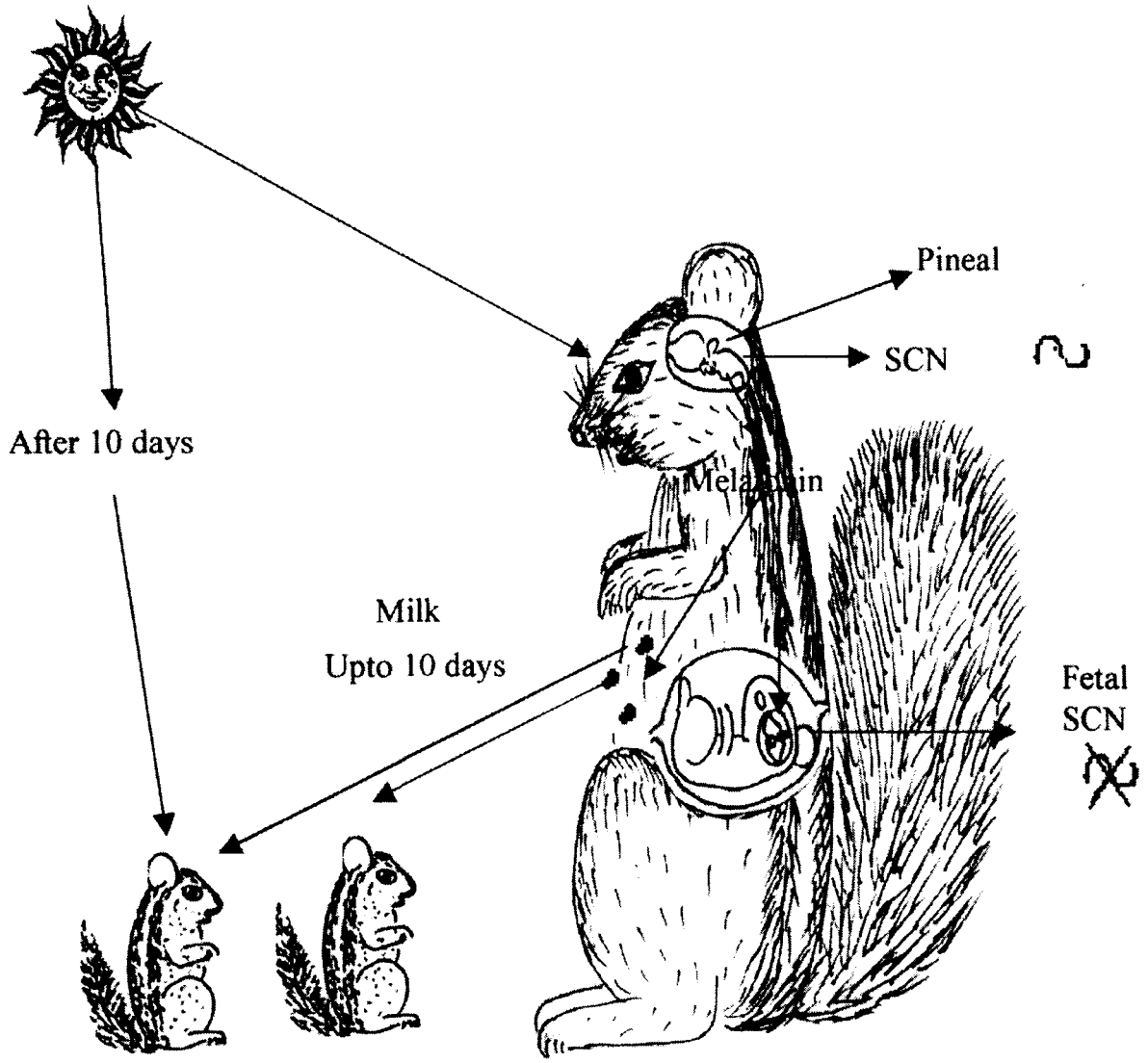

Fig. 2. Diagrammatic representation of transfer of photoperiodic information from mother to fetus

factors of the environment (photoperiod, temperature, humidity etc.) and the others (hormonal and metabolic) are transferred from mother to the fetuses. Only a few reports are available suggesting the phenomenon of maternal transfer of photic information to young ones *via* the pineal gland (Fig. 2). However, the impact of maternal photoperiodic exposures on the growth, pineal functions and sexual development of neonates is lacking. During gestation, the mother provides protects the fetus from the external environment and generates signals which allow the fetus to perceive the length of the day as well as the phase (timing) of the light-dark cycle. It is remarkable that the mother is actively involved in transferring environmental cues to the fetus during the prenatal period.

As pregnancy nears the term, the fetuses race to prepare themselves for life outside the uterus with the development and maturation of various neuronal and humoral systems in a protected intrauterine environment. Studies in several mammalian species show that during fetal life a biological clock in SCN is oscillating in phase (time) with the environmental light-dark cycle and this fetal clock is entrained by reluctant circadian signals (22).

Light-induced neural signals are conveyed to SCN by the mother's retino-hypothalamic pathway (RHP) entraining her circadian rhythm. But, some questions regarding the maternal transfer of photoperiodic / hormonal information still remain unsolved. Photoperiodic information reaches the fetus during prenatal life and can have a dramatic impact on reproductive development. Stetson *et al.* (23) opined that the photoperiodic condition experienced by the mother, especially during gestation is communicated to the fetuses either in a stimulatory or in an inhibitory manner for their pineal gland activity.

The pineal gland is known to play an intermediary role between the environment and the endocrine system (Fig. 3) (24, 25). Therefore, a direct influence of the maternal pineal gland on the growth and sexual development of the offspring is conceivable. Horton (26), Stetson *et al.* (27) and Horton *et al.* (28), working with Montane voles and Siberian hamsters, have suggested that the information transfer from the mother to the fetuses occurs during the prenatal period and not during lactation. Maternal photoperiodic exposures during gestation and lactation periods alter the neonatal growth and sexual maturation of the Indian palm squirrel *via* the maternal transfer of photoperiodic information through the pineal gland (10). Our several papers established the above relation in a diurnal rodent, the Indian palm squirrel. The results suggest that prenatal photoperiod has critical impact on postnatal reproductive development. Pups reared in an "intermediate" postnatal photoperiod differ in their rate of reproductive development if the prenatal photoperiods experienced by their mothers differed (23, 29, 30). At more extreme photoperiods, the postnatal photoperiod "overrides" the influence of the prenatal photoperiod (27, 29). Several groups of researchers have shown that the maternal pineal gland is necessary for prenatal communication of day length information and this information profoundly influences postnatal reproductive and somatic development (28, 30). Studies using timed injection of melatonin to pregnant mothers support the conclusion that melatonin is a critical cue for the transfer of day length information to the fetuses (27, 31). Considering the role of pineal gland in photoperiodic regulation, it appeared likely that removal of the maternal pineal gland would prevent prenatal perception of day length. Lee and his colleagues (32-34) have shown that the prenatal development of meadow vole pups (*Microtus pennsylvanicus*) is influenced by the prenatal photoperiods and prenatal melatonin treatment (32-34).

Maternal transfer of photoperiodic information has been referred to as "prenatal programming" of postnatal reproductive development (27, 31). The developing animal takes a reading of the day length (melatonin duration) during the late fetal life and compares this with the melatonin signal derived from the developing pineal around 15-20 days of age (2). This model suggests that a postnatal melatonin pattern is interpreted differently depending on an animal's prenatal photoperiodic history. Melatonin is, thus, an important component of the perceptual world of the fetus. In this context, it may be worth considering melatonin as a pheromone (a chemical substance from one member of a species which communicates information to another) rather than as a hormone.

SOURCES OF MELATONIN FOR THE FETUS

The primary source of melatonin for the fetus is the maternal pineal gland. Robust rhythms in melatonin have been measured in pregnant mammals (35, 36) and although the fetus may have some capacity for melatonin production, any secretion would be low and without rhythmicity. The melatonin rhythm in the maternal circulation is accurately reflected in the fetus. Melatonin levels in the fetal plasma are closely parallel to those in the maternal plasma. Rapid transfer of maternal melatonin across the placenta has been demonstrated in several rodents, sheep and non-human primates (14, 36-40). Furthermore, maternal pinealectomy abolishes the rhythm of melatonin in the fetal circulation (36, 41). It is likely that the fetus has a functional circadian pacemaker in the SCN through out the gestation. Since the fetal pacemaker expresses melatonin-binding sites, it is also likely to be a target of maternal melatonin. Thus, it is possible that the maternal melatonin can affect the fetal SCN with consequences for the prenatal and postnatal expression and entrainment of circadian rhythms.

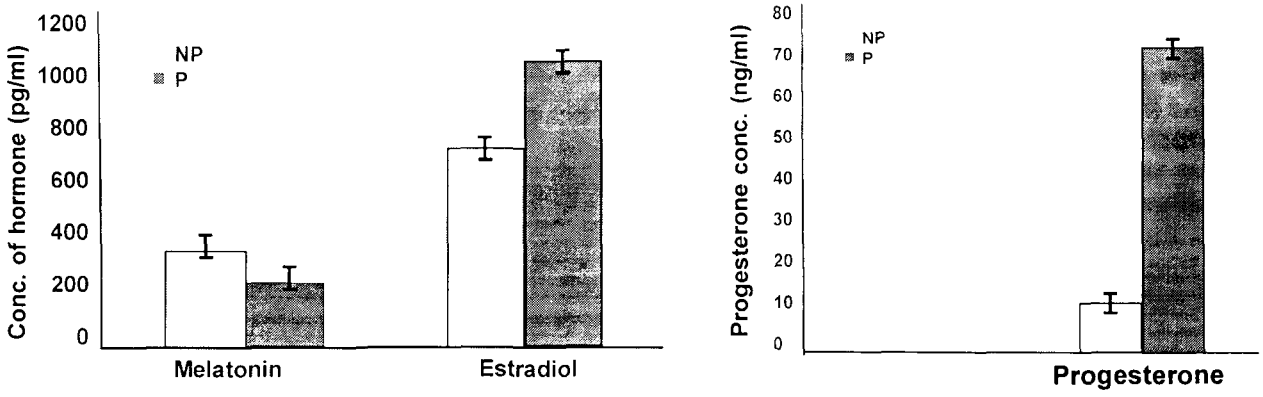

Fig. 3. Hormone levels in pregnant (P) and non-pregnant (NP) Indian palm squirrel, *Funambulus pennanti.*

Some possible role of melatonin in the fetal development of Indian palm squirrel, *Funambulus pennanti,* has been proposed by Bishnupuri and Haldar (9). The inability of the fetal and neonatal rat to synthesize melatonin does not necessarily mean that the developing rat lacks melatonin. Indeed, evidence is available which indicates that the mother is an important source of melatonin for the developing fetus and neonate. Melatonin can be transferred from mother to fetus in two ways:

1. Transfer of melatonin through placental route Placental transfer of melatonin has been demonstrated. First, it was shown that a small amount of (^{3}H) - melatonin injected intravenously into pregnant animals during the later stages of gestation promptly appear in the fetal circulation and that the rates of disappearance of radiolabelled melatonin in the maternal and fetal circulation are parallel. In another experiment, a diurnal change in maternal melatonin, experimentally simulated on a reduced time scale, results in a rapid reflection of the rhythm in the fetal circulation. Rapid placental transfer of melatonin is quite predictable in view of the lipophilic, non-ionized properties of this small molecule (9). Another important finding of the placental transfer study was maternally derived (^{3}H)-melatonin rhythm in fetus. This indicates that a maternally generated melatonin rhythm would be expressed in fetal cerebrospinal fluid (CSF) as well as in the circulation, since it has been shown that in the adult mammal the rhythm in circulating levels is precisely reflected in the CSF. Both the routes may deliver the hormone on a daily basis to the fetal brain where melatonin would potentially exert its effects.

2. Transfer of melatonin through mother's milk. A part of (^{3}H) melatonin injected intravenously to lactating female was also found in the mammary gland and in the stomach of suckling pups. This is because the melatonin rhythm in the fetus is established after 1 week or 10 days postnatally. The melatonin rhythm in the fetus is driven up to one week by the maternal melatonin present in the milk (42).

MELATONIN IN EMBRYONIC GROWTH AND SURVIVAL

Melatonin is generally viewed as remarkably nontoxic (43, 44) and this also appears to be the case during fetal life. Melatonin is without effects on the development of mouse embryos *in vitro* (45). Another study reports that melatonin does produce toxicity to embryos (46). An influence of melatonin injection on embryonic survival has been reported in meadow voles (47). Melatonin injections (10µg) reduced survival rates of female (but not male) pups when given prior to blastocyst implantation. Injections later in gestation were without effect. This effect of melatonin may underlie seasonal changes in litter size and, perhaps, seasonal changes in sex ratios. The mechanism by which melatonin modulates prenatal mortality in a sex-specific manner is not known. However, the melatonin binding and the density of melatonin binding sites are more in the fetal than the adult brain.

Pinealectomy of the mother produced an altered developmental pattern in the offspring of rat. During the infantile period when pups are lacking maternal melatonin due to pinealectomy and their own melatonin rhythm is yet to be established, a delayed growth of body was observed. Significantly greater growth rate was observed in pinealectomized offspring during the pubertal period than in the control offspring, which could be due to the increase in LH secretion up to normal values observed in the pinealectomized offspring. Melatonin treatment during pregnancy produced minor alterations in postnatal development of the reproductive tract. Maternal melatonin is necessary for normal somatic growth and postnatal development of reproductive organs of the offspring (48).

MELATONIN AND PUBERTY

Melatonin ultimately affects reproductive activity by modulating hypothalamic neuroendocrine circuits whose activity is necessary for gonadal function. The influence of melatonin on reproductive development begins during the prenatal period and extends into the postnatal life. Melatonin influences the other neuroendocrine parameters in the developing animals prior to puberty.

In many seasonally breeding species, the timing of initial reproductive development (sexual maturity or puberty) is strongly influenced by day lengths experienced during the postnatal period. The most well studied species include Siberian hamsters, Indian palm squirrel and sheep. Administration of melatonin in the appropriate temporal (durational) patterns can influence the timing of puberty in these species. Exposure to long days stimulated postnatal reproductive development. Infusion of long day patterns of melatonin, similarly, stimulates gonadal growth, while short day patterns of melatonin suppress puberty. While the details are species-specific, the same general phenomenon exists in the other photoperiodic species. Specific photoperiodic requirements must be met to allow rapid pubertal development, and these photoperiodic requirements are transduced to the neuroendocrine axis by melatonin. In general, puberty occurs at about the same time of the adult maturing season and, therefore, is also a photoperiodic reproductive phenomenon. In species with rapid development (e.g. rodents), animals born early in the year mature rapidly and reproduce within the same season, while in animals born at the end of breeding season, puberty is delayed until the beginning of the next season.

The photoperiod experienced by females during their gestation period may influence the pineal physiol-

ogy of fetus but nothing has been reported about the growth and sexual development of the young ones. Studies on the maternal transfer of photoperiodic information in mammals indicate that the daily photoperiod perceived by the mother during the gestation and lactation periods is communicated to the fetus either through the placenta or via the milk. In a seasonally breeding Indian tropical rodent *Funambulus pennanti*, constant light (LL: 24L: 0D) and long day length (LDL; 14L: 10D) exposure to gestating females conveyed opposite information to the fetuses and inhibited fetal pineal function. Short photoperiod (SDL; 10L: 14D) exposure to gravid females stimulated pineal function of the pups. Altered pineal functions of the pups ultimately interfered with their growth and sexual maturation. We, therefore, suggested that photic information perceived by the mother during gestation alters her own normal melatonin level, which passing through placenta or through milk influences sexual maturation of the young ones (10). The maternal pineal gland can affect fetal development because the main pineal hormone, melatonin, can cross the placental barrier. Melatonin treatment during gestation in the rat produced delayed sexual maturation of the female offspring (49). The maternal pineal gland participates in the cellular and nuclear volumes of prepubertal oocyte development. Melatonin treatment during pregnancy resulted in a redirected postnatal oocyte development (49).

Alterations in photoperiodic condition during late gestation and lactation altered the postpartum recovery process. Pineal gland activity, as assessed by pineal mass, protein content and plasma melatonin, was the lowest during the breeding phase, but increased gradually after parturition until the next breeding phase. During gestation and lactation, constant light, long day length and short day length conditions were less effective, while constant dark condition had a profound effect in depressing pineal gland activity, which subsequently advanced postpartum recovery. Hence, lactating females under constant darkness prepared themselves for the next mating much earlier than females under natural day length (12h light: 12h dark) conditions. Therefore, photoperiodic information, mediated *via* the pineal gland may be important for maintaining gestational physiology as well as postpartum recovery in female rodents.

The offspring of melatonin-treated rats showed later vaginal opening than did control and pinealectomized offspring, which was accompanied by a lower LH concentration, showing statistically significant differences with the control levels and with the pin-x group. The percentage of rats in proestrus was higher in the offspring of pinealectomy (78.6%) compared to control offspring (30%) and offspring of melatonin-treated rats (11.8%). The pituitary weight was significantly lower in the offspring of pin-x rats than in the other two groups (50).

Menstrual cyclicity is associated with fluctuations in melatonin production but whether they are related to ovulation or menstruation is not established. Menopause is associated with a reduction in melatonin, which may relate to the changing gonadotropin levels. In males of the same age melatonin levels also drop with no significant alteration in reproductive physiology (51).

MELATONIN AND EMBRYONIC DEVELOPMENT IN BATS

A fascinating and ecologically important role for melatonin in regulating reproduction during early fetal life is in the initiation and maintenance of seasonal embryonic diapause (52). The period of delayed implantation (embryonic diapause) is regulated by the environmental light cycle *via* melatonin. Seasonal embryonic diapause occurs in many different mammalian species, including mustelids (eg., skunks, ferrets, badgers, weasels, mink), pinipeds (e.g. Australian sea lions and Antarctic fur seals, harbor seals), insectivores (several bat species), canids (wolves and coyotes), bears, and marsupials. Western spotted skunks, tammar wallabies and mink have been studied most extensively with respect to the role of the pineal and melatonin. In each of these three species, pinealectomy or denervation of the pineal prevents seasonal embryonic diapause, and melatonin treatment influences the length of diapause (17, 18, 53-56). The data from all these species are consistent with the interpretation that melatonin acts in the pregnant female to influence neuroendocrine function, particularly prolactin secretion and that diapause is caused by alterations in the uterine environment. Melatonin does not appear to affect the embryo directly.

The flying mammalian group, Chiroptera, is known for its various reproductive delays. There may be stressfull situations in the wild (for example, lack of support food or roosting sites) in which case the ability to delay pregnancies would be of considerable adaptive value. The delayed embryonic development (DED) has been described by various workers as a phenomenon of adaptation to unfavorable environmental conditions. Since pineal gland is the mediator between the environmental changes and adaptation to seasonal reproduction, a role for pineal gland was suspected in delayed embryonic development of chiropterans. According to Sandyk *et al.* (57) melatonin stimulates progesterone secretion that prevents the immunological rejection of the trophoblast.

It has been demonstrated that melatonin levels in the plasma increase during gestation, reaching high values at the end of this period, suggesting that the hormone plays an important role in the maintenance of the gestation (10, 35, 57-60). However, little is known about the importance of the melatonin on the implantation process.

We found a high melatonin and estrogen ratio during DED beside the high androgen, as reported earlier in fruit bats (61). Any disturbance in this ratio (following pinealectomy (Px), melatonin injection or photoperiodic exposure, which may disturb internal melatonin level, leads to termination of DED either as immature delivery or abortion. Furthermore, Sandyk *et al.* (57) suggested a functional deficiency in the melatonin production at the beginning of the gestation may cause spontaneous abortion in cases where chromosomal anomalies and structural abnormalities of the uterus were excluded. The pinealectomy, the constant illumination or association of these factors induce a reduction of the blastocysts number implanted in female rats besides stimulating the development of the implantation sites .This would indicate that the melatonin can have an important function in the viability of implantation of the blastocyst and in the process of formation of the placenta in these animals. Pinealectomy followed by immediate high dose of melatonin maintains pregnancy in short-nosed fruit bats (62). It could be suggested that high maternal melatonin flow via the placenta reached the fetus and retarded the growth and development of the fetus since melatonin is having an inverse relationship with growth hormone. Therefore, melatonin may play an adaptive role in the maintenance of DED.

The target of melatonin on the uterus is the myometrium, where we found receivers for the MT1 and MT2 melatonin receptors, through which they can modulate myometrial operation, inhibiting the spontaneous contractions induced by the oxytocin (63-65). However, Zhao *et al.* (66) reported the presence of the MT1 receptor in the endometrial stroma, which decreases progressively during the decidualization, staying in this state until the end of the gestation.

MELATONIN IN HUMAN GESTATION AND FETAL DEVELOPMENT

Correlation between a developmental decline in melatonin levels and the timing of puberty in humans led to the speculation that melatonin regulates the timing of puberty. Subsequent investigation indicated that this developmental decline in melatonin levels is due at least in part to developmental changes in body mass (and thus volume of distribution) and is without a strict relationship to pubertal development. While endogenous melatonin does not appear to play a role in timing human puberty, data are not available to draw a conclusion with respect to the effects of exogenous melatonin on puberty in humans. In the case of humans (pregnant women), pineal concretions were found in a much higher percentage than in women who had never been pregnant.

Serum melatonin levels during the third trimester of pregnancy (76.5+/- 38.3pmol/l) were significantly higher than those during the first (29.7+/-9.9 pmol/l) and the second trimesters (39.1+/-11.2 pmol/l) and those of non-pregnant control women (41.7+/-15.5 pmol/l). There was a positive correlation between the week of gestation and serum melatonin at 11.00 h with a clear diurnal rhythm in serum melatonin concentrations both in early and late pregnancies. The amplitude and duration of the nocturnal rise of melatonin were higher during late pregnancy, but there was no clear phase shift. Increased serum concentration of melatonin in late pregnancy may be due to increased synthesis and secretion or retarded metabolism of melatonin during late pregnancy (35).

In the case of human newborns, melatonin levels in the umbilical artery and veins were no different from maternal serum melatonin (67, 68). Chiidren with precocious puberty have lower nocturnal serum concentrations of melatonin than age-matched prepubertal children (69), whereas children with delayed puberty present higher nocturnal melatonin concentrations than age-matched normal children.

It is likely that melatonin may exert an inhibitory effect on hypothalamic GnRH secretion in humans. It has been postulated that, before puberty, even if they progressively decrease, melatonin concentrations are too elevated to allow hypothalamic activation; however, at 9 or 10 years of age the decline of serum melatonin below a threshold value (500 pmol/l -115 pg/ml) represents the activating signal for the hypothalamic pulsatile secretion of GnRH and hereafter the onset of pubertal changes (70).

CONCLUSIONS

Studies on melatonin influence on gestation indicate two major observations:

i) Melatonin could be one of the major hormones establishing homeostasis during gestation since any disturbance in the level of melatonin causes abortion.

ii) Melatonin is one of the hormones of great adaptive significance for embryonic diapause.

Both the results/effects of melatonin are of high clinical value for treatment of frequent abortion or maintenance of gestation. This is one of the novel branch offs of melatonin physiology that warrants attention of researchers and clinicians.

ACKNOWLEDGMENTS

We thank the University Grants Commission (UGC) and the Council of Scientific and Industrial Research (CSIR), New Delhi, for financial support and Alexander von Humboldt foundation, Bonn, Germany, for the equipment facility.

REFERENCES

1. Bartness TJ, Powers JB, Hastings MH, Bittman EL, Goldman BD (1993) The timed infusion paradigm for melatonin delivery: what has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? J Pineal Res 15:161-190.
2. Tamarkin L, Reppert SM, Orloff DJ, Klein DC, Yellon SM, Goldman BD (1980) Ontogeny of the pineal melatonin rhythm in the Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorus*) hamsters and in the rat. Endocrinology 107: 321-326.
3. Reiter RJ (1986) Pineal melatonin production: photoperiodic and hormonal influences. In: Reiter RJ and Karasek M (eds) *Advances in Pineal Research*, pp 77-87, John Libbey, London.
4. Haldar C, Saxena N (1989) Antigonadotropic activity of pineal gland of the Indian palm squirrel, *Funambulus pennanti.* Indian J. Exp Biol 27: 421.
5. Haldar C (1996) In: Haldar C (ed) *The Pineal Gland: Its Molecular Signals*, pp 94-117, HPLC, New Delhi.
6. Lew GM (1987) Morphological and biochemical changes in the pineal gland during pregnancy. Life Sci 41: 2581-2596.
7 Attanasio A, Borelli P, Gupta D (1985) Circadian rhythms in serum melatonin from infancy to adolescence. J Clin Endocrinol Metab 61: 388-390.
8 Huang CY, Everitt AV (1965) The effect of pregnancy on pineal weight in the rat. J Endocrinol 32: 261-262.
9 Bishnupuri KS, Haldar C (1999) In: Gpupta PD and Yamamoto H (eds) *Electron Microscopy in Biology*, pp 33-40. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.
10 Bishnupuri KS, Haldar C (2000) Profile of organ weights and plasma concentrations of melatonin, estradiol and progesterone during gestation and postparturition periods in female Indian palm squirrel *Funambulus pennanti.* Indian J Exp Biol 38: 974-981.
11 Thomas L, Drew JE, Abramovich DR, Williams LM (1998) The role of melatonin in the human fetus. Int J Mol Med 1: 539-543.
12 Ebling FJ, Wood RI, Suttie JM, Adel TE, Foster DL (1989) Prenatal photoperiod influences neonatal prolactin secretion in the sheep. Endocrinology 125: 384-391.
13 Bassett JM, Curtis N, Hanson C, Weeding C (1989) Effects of altered photoperiod or maternal melatonin administration on plasma prolactin concentrations in fetal lambs. J Endocrinol 122 633-643.
14 Serone-Ferre M, Vergara M, Parraguez VH, Riquelme R, Llanos AJ (1989) The circadian variation of prolactin in fetal sheep is affected by the seasons. Endocrinology 125: 1613-1616.
15 Houghton DC, Young IR, McMillen IC (1997) Photoperiodic history and hypothalamic control of prolactin secretion before birth. Endocrinology 138: 1506-1511.
16 McMillen IC, Houghton DC, Young IR (1995) Melatonin and the development of circadian and seasonal rhythmicity. J Reprod Ferti Suppl 49: 137-146.
17 Berria M, DeSantis M, Mead RA (1988) Effects of suprachiasmatic nuclear ablation and melatonin on delayed implantation in the spotted skunk. Neuroendocrinology 48: 371-375.
18 Berria M, DeSantis M, Mead RA (1989) Lesions to the anterior hypothalamus prevent the melatonin-induced lengthening of delayed implantation. Endocrinology 125: 2897-2904.
19 Kaplan JB, Berria M, Mead RA (1991) Prolactin levels in the western spotted skunk: changes during pre- and peri-implantation and effects of melatonin and lesions to the anterior hypothalamus. Biol Reprod 44: 991-997.
20 Duncan MJ, Mead RA (1992) Autoradiographic localization of binding sites for 2-[^{125}I] iodo-melatonin in the pars tuberalis of the Western spotted skunk (*Spilogale putorius latifrons*). Brain Res 569: 152-155.
21 Lincoln GA, Clark IJ (1994) Photoperiodically-induced cycles in the secretion of prolactin in hypothalamo-pituitary disconnected rams: evidence for translation of the melatonin signal in the pituitary gland. J Neuroendocrinol 6: 251-260.
22 Viswanathan N, Davis, FC (1993) The fetal circadian pacemaker is not involved in the timing of birth in hamsters. Biol Reprod 48: 530-537.
23 Stetson MH, Elliot JA, Goldman BD (1986) Maternal transfer of photoperiodic information influences the photoperiodic response of prepubertal Djungarian hamsters (*Phodopus sungorus sungorus).* Biol Reprod **34:** 664-669.
24 Carter DS, Goldman BD (1983) Anti-gonadal effect of timed melatonin infusion in pinealectomized male Djungarian hamsters (*Phodopus sungorus sungorus*): Duration is the critical parameter. Endocrinology 113: 1261-1267.
25 Reiter RJ (1991) Pineal metabolism: Cell biology of its synthesis and its physiological interactions. Endocrine Rev 12: 151-180.
26 Horton TH (1985) Cross-fostering of voles demonstrates in utero effect of photoperiod. Biol Reprod 33: 934-939.
27 Stetson MH, Ray SL, Creyaufmiller N, Horton TH (1989) Maternal transfer of photoperiodic

information in Siberian hamsters. II. The nature of the maternal signal, time of signal transfer, and the effect of the maternal signal on peri-pubertal reproductive development in the absence of photoperiodic input. Biol Reprod 40: 458-465.

28 Horton TH, Stachecki SA, Stetson MH (1990) Maternal transfer of photoperiodic information in Siberian hamsters. IV. Peripubertal reproductive development in the absence of maternal photoperiodic signals during gestation. Biol Reprod 42: 441-449.

29 Shaw D, Goldman BD (1995) Influence of prenatal and postnatal photoperiods on postnatal testis development in the Siberian hamster (*Phodopus sungorus*). Biol Reprod 52: 833-838.

30 Weaver DR, Reppert SM (1986) Maternal melatonin communicates day length to the fetus in Djungarian hamsters. Endocrinology 119: 2861-2863.

31 Horton TH, Ray SL, Stetson MH (1989) Maternal transfer of photoperiodic information in Siberian hamsters: Melatonin injection programs post-natal reproductive development expressed in constant light. Biol Reprod 41: 34-39.

32 Lee TM, Zucker I (1988) Vole infant development is influenced perinatally by maternal photoperiodic history. Am J Physiol 255: R 831-838.

33 Lee TM, Smale L, Zucker I, Dark JD (1987) Influence of day length experienced by dams on postnatal development of young meadow voles (*Microtus pennsylvanicus*). J Reprod Fertil 81: 337-342.

34 Lee TM, Spears N, Tuthill CR, Zucker I (1989) Maternal melatonin treatment influences rates of neonatal development of meadow vole pups. Biol Reprod 40: 495-502.

35 Kivela A (1991) Serum melatonin during human pregnancy. Acta Endocrinol 124: 233-237.

36 Yellon SM, Longo LD (1988) Effect of maternal pinealectomy and reverse photoperiod on the circadian melatonin rhythm in the sheep and fetus during the last trimester of pregnancy. Biol Repro. 39: 1093-1099.

37 Klein DC (1972) Evidence for the placental transfer of 3 H-acetyl-melatonin. Nat New Biol 237: 117-118.

38 Reppert SM, Chez RA, Anderson A, Klein DC (1979) Maternal-fetal transfer of melatonin in the non-human primate. Pediatr Res 13: 788-791.

39 Velazquez E, Esquifino AI, Zueco JA, Ruiz Albusac JM, Blazquez E (1992) Evidence that the circadian variations of circulating melatonin levels in fetal and suckling rats are dependent on maternal melatonin transfer. Neuroendocrinology 55: 321-326.

40 Yellon SM, Longo LD (1987) Melatonin rhythms in fetal and maternal circulation during pregnancy in sheep. Am J Physiol 252: E799-802.

41 Nowak R, Young IR, McMillen IC (1990) Emergence of the diurnal rhythm in plasma melatonin concentrations in newborn lambs delivered to intact or pinealectomized ewes. J Endocrinol 125: 97-102.

42 Reppert SM, Klein DC (1978) Transport of maternal [^{3}H] melatonin to suckling rats and fate of [^{3}H] melatonin in the neonatal rat. Endocrinology 102: 582-588.

43 Sugden D, (1983) Psychopharmacological effects of melatonin in mouse and rat. J Pharmacol Exp Ther 227: 587-591.

44 Weaver DR (1997) Reproductive safety of melatonin: a "wonder drug" to wonder about. J Biol Rhythms 12: 682-689.

45 McElhinny AS, Davis FC, Warner CM (1996) The effect of melatonin on cleavage rate of C57BL/6 and CBA/Ca preimplantation embryos cultured *in vitro*. J Pineal Res 21: 44-48.

46 Chan WY, Ng TB (1995) Changes induced by pineal indoles in post-implantation mouse embryos. Gen Pharmacol 26: 1113-1118.

47 Gorman MR, Ferkin MH, Dark J (1994) Melatonin influences sex-specific prenatal mortality in meadow voles. Biol Reprod 51: 873-878.

48 Diaz B, Diaz E, Colmenero Mf M, Arce A, Esquifino A, Marin B (1999) Maternal melatonin influences rates of somatic and reproductive organs postnatal development of male rat offspring. Neuro-Endocrinol Lett 20: 69-76.

49 Fernandez B, Diaz E, Colmenero MD, Diaz B (1995) Maternal pineal gland participates in prepubertal rats' ovarian oocyte development. Anat Rec 243: 461-465.

50 Colmenero MD, Diaz B, Miguel JL, Gonzalez ML, Esquifino A, Marin B (1991) Melatonin administration during pregnancy retards sexual maturation of female offspring in the rat. J Pineal Res 11: 23-27.

51 Reiter RJ (1998) Melatonin and human reproduction. Ann Med 30: 103-108.

52 Sandell M (1990) The evolution of seasonal delayed implantation. Q Rev Biol 65: 23-42.

53 May R, Mead RA (1986) Evidence for pineal involvement in timing implantation in the western spotted skunk. J Pineal Res 3: 1-8.

54 McConnell SJ, Tyndale-Biscoe CH, Hinds LA (1986) Change in duration of elevated concentrations of melatonin is the major factor in photoperiod response of the tammar, *Macropus eugenii*. J Reprod Fertil 77: 623-632.

55 Murphy BD, DiGregorio GB, Douglas DA, Gonzalez-Reyna A (1990) Interactions between melatonin and

prolactin during gestation in mink (*Mustela vison*). J Reprod Fertil 89: 423-429.

56 Renfree MB, Lincoln DW, Almeida OF, Short RV (1981) Abolition of seasonal embryonic diapause in a wallaby by pineal denervation. Nature 293: 138-139.

57 Sandyk R, Anastasiadis PG, Anninos PA, Tsagas N (1992) The pineal gland and spontaneous abortions: implications for therapy with melatonin and magnetic field. Int J Neurosci 62: 243-250.

58 Bishnupuri KS, Haldar C (2001) Maternal transfer of melatonin alters the growth and sexual maturation of young Indian palm squirrel *Funambulus pennanti*. Biol Signals Recent 10:317-325.

59 Nakamura Y, Tamura H, Kashida S, Takayama H, Yamagata Y, Karube A, Sugino N, Kato H (2001) Changes of serum melatonin level and its relationship to feto-placental unit during pregnancy. J Pineal Res 30: 29-33.

60 Haldar C, Yadav R, Alipreeta (2006) Annual reproductive synchronization in ovary and pineal gland function of female short-nosed fruit bat, *Cynopterus sphinx*. Comp Biochem Physiol 144 : 395-400.

61 Krishna A, Dominic CJ (1982) Differential rates of fetal growth in two successive pregnancies in the emballonurid bat, *Taphozous longimanus* Hardwicke. BiolReprod 27: 351-353.

62 Alipreeta (1998) Physiology of the pineal organ of short- nosed fruit bat *Cynopterus sphinx*. Ph.D dissertation, Banaras Hindu University, Varanasi, India.

63 Matsumoto T, Hess DL, Kaushal KM, Valenzuela GJ, Yellon SM, Ducsay CA (1991) Circadian myometrial and endocrine rhythms in pregnant rhesus macaque: effects of constant light and timed melatonin infusion. Am J Obstet Gynecol 165:1777-1784.

64 Ayar A, Kutlu S, Yilmaz B, Kelestimur H (2001) Melatonin inhibits spontaneous and oxytocin-induced contractions of rat myometrium in vitro. Neuroendocrinol Lett 22:199-207.

65 Schlabritz-Loutsevitch N, Hellner N, Middendorf R, Müller D, Olcese J (2003) The human myometrium as a target of melatonin. J Clin Endocrinol Metab 88:908-913.

66 Zhao H, Pang SF, Poon AMS (2002) Variations of mt1 melatonin receptor density in the rat uterus during decidualization, the estrous cycle and in response to exogenous steroid treatment. J Pineal Res 33: 140-145.

67 Lang U, Beguin PC, Sizonenko JD (1986) Fetal and maternal melatonin concentrations at birth in humans. J Neural Tradin Suppl 21: 479-480.

68 Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y (1998) Maternal-fetal transfer of melatonin in pregnant women near term. J Pineal Res 25: 129-139.

69 Waldhauser F, Boepple PA, Schemper M, Mansfield MJ, Crowley WF Jr (1991) Serum melatonin in central precocious puberty is lower than in age-matched prepubertal children. J Clin Endocrinol Metab 73: 793-796.

70 Silman AJ (1991) Melatonin and the human gonadotropin-releasing hormone pulse generator. J Endocrinol 128: 7-11.