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ORIGINAL ARTICLE

# A half-yearly aspect of circulating melatonin in pregnancies complicated by intrauterine growth retardation

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**Abstract**

In investigating mechanisms underlying intrauterine growth retardation (IUGR), circulating melatonin and cortisol were radioimmunoassayed. Blood samples were collected every 4 hours during 24 hours on a strict 24-hour standardized routine in hospital from two groups of women in their third trimester of pregnancy. One group consisted of 14 healthy, uncomplicated pregnancies (HAGA); the other group consisted of 11 pregnancies complicated by intrauterine growth retardation (IUGR) confirmed at birth. The circadian characteristics of melatonin and cortisol were assessed for each woman and compared between the two groups by analyses of variance for repeated measures and by parameter tests based on the cosinor. Since a circasemiannual (about half-yearly) component prominently characterizes body weight and length at birth of children with birth characteristics below usual norms, the circadian characteristics of melatonin and cortisol were also analyzed transversely (across women within each group). The 24-hour average and the 24-hour and 12-hour amplitudes of melatonin of women in the IUGR, but not in the HAGA group, were indeed found to be modulated by an about half-yearly component. This study confirms the circadian rhythmicity of melatonin in healthy pregnant women and extends the finding to pregnancies complicated by IUGR, uncovering about half-yearly changes in melatonin in women with IUGR, thereby extending results obtained in healthy non-pregnant women and men. These variations may reflect influences from geomagnetic disturbances also characterized by a prominent half-yearly pattern, to which the pineal has been shown to be sensitive.

**Introductory background**

By isolating melatonin, Aaron B. Lerner (1993), in 1955–1959, placed pineal research on a chemical basis. The ubiquity of melatonin was extended from mammals (Axelrod 1974; Quay 1964; Reiter 1980), including humans (Dollins et al. 1994; Lynch et al. 1975; Wetterberg 1978; Wetterberg et al. 1986) to insects (Wetterberg et al. 1987) and unicells (Balzer and Hardeland 1991). A marked increase in melatonin content during the daily dark span is in keeping with the suggestion that melatonin is involved in the synchronization of about 24-hour (circadian) rhythms with the cycle of light and darkness and circannual rhythms are also documented. Melatonin, however, is characterized by a broader time structure (chronome, consisting of 1. multifrequency rhythms; 2. organizing chaotically-appearing changes; and 3. trends in the characteristics of rhythms and chaos) (Cornélissen and Halberg 1994; Halberg et al. 1991b; Macey 1994). We are here concerned with the first element, the spectrum of multifrequency rhythms characterizing melatonin in vivo as well as in vitro (Cornélissen and Halberg 1992; Cornélissen et al. 1995; Halberg 1983; Halberg et al. 1986, 1988; Sánchez 1993; Sánchez et al. 1983). Among variations with a frequency lower than one cycle per day (infradians), those with 1 or 2 cycles per week (circaseptans and circasemiseptans; Halberg 1995) and 1 or 2 cycles per year (circannuals and circasemiannuals; Tarquini et al. 1997) are prominent.

Among many other components, about-weekly and about-half-weekly variations (Halberg et al. 1991a; Roederer 1995; Vladimirskii et al. 1995) and half-yearly cycles (Fraser-Smith 1972) are also found in the spectrum of indices of geomagnetic disturbance, e.g., in relation to the frequency of geomagnetic solar flare effects (Grafe 1958). Elsewhere, we have reviewed the accumulating evidence on associations of heliogeomagnetics and growth, early in life and at the age of conscription (Halberg et al. 1991a, Cornélissen et al. 1999), Randall (1990; cf. 1988) described the status quo:

*"Data obtained from the literature on the annual pattern of human conceptions and plasma melatonin at high latitudes indicated that simple annual rhythms do not exist. Instead, prominent semiannual rhythms are found, with equinoctial troughs and solsticial peaks. A prominent semiannual environmental event is the magnetic disturbance induced by the solar wind. The semiannual magnetic disturbances are worldwide, but most pronounced in the auroral zones where the corpuscular radiation enters the atmosphere. Magnetic indices that predominantly reflect these events were obtained from the literature and correlated with the melatonin and conception data. Significant and inverse correlations were found for Inuit conceptions and the melatonin data. ..."*

*Melatonin chronomodulates by exerting different, sometimes opposite effects in different stages of the chronome, a role studied in detail for the case of corticosterone synthesis by the murine adrenal in vitro (Cornélissen and Halberg 1992; Halberg 1983; Halberg et al. 1986, 1988; Sánchez 1993; Sánchez et al. 1983, 1988). For instance, as a function of circadian stage, melatonin at one circadian time stimulates the isolated gland to produce more corticosterone than it does without melatonin; at another circadian time it leaves hormone production unchanged, and at a third time it inhibits hormone production, all predictably insofar as rhythmically changing within 24 hours, according to a feedsideward mechanism (Bartsch and Bartsch 1981, 1997; Bartsch et al. 1990a and b, 1995; Halberg E. and Halberg F. 1980; Halberg 1983; Haus et al. 1996; Langevin et al. 1981; Macey 1994; Ronco and Halberg 1996; Sanchez et al. 1988; Walker et al. 1985; Wrba et al. 1990).*

Melatonin can have effects described as feedsidewards, not only directly upon the isolated adrenal but also upon the interaction of the adrenal and the pituitary, the latter represented by ACTH. When these changes in response are circadian stage-dependent, the otherwise uncertain, often confusingly different effect becomes predictable, in terms of a rhythmically recurring sequence of stimulation, no-effect and inhibition, denoted as "chronomodula-tion." This predictably different effect at different specified times should be distinguished from a time-unqualified "modulation." The latter term has been misused to describe changes that are unpredictable both in terms of the presence or absence of an effect, and when the effect happens to be statistically (but not necessarily biologically) significant, there can be the added uncertainty whether it consists of a stimulation or inhibition. Such sequences of opposite effects also characterize other immunomodulators (Sanchez et al. 1988); the effect can vary from a stimulation to an inhibition of DNA labelling in health (Walker et al. 1985) or of cancerous growth (Bartsch and Bartsch 1981; Bartsch et al. 1995; Halberg and Halberg 1980; Langevin et al. 1981; for review see Halberg 1983; Ronco and Halberg 1996; Sanchez 1993). Such a feedsideward interaction within the organism or between the organism and environmental factors can be seen along the circadian and also along the circaseptan and circannual scales (Bartsch et al. 1990a and b; Halberg 1983; Wrba et al. 1990). In studies involving the endpoint of corticosterone production, results obtained with sampling at 4-hour intervals for 24 hours were not observed with 6- hourly sampling (Moore and Eichler 1972, Scheving et al. 1983).

In pregnancy thus far, focus was directed at a circadian pattern of melatonin secretion, characterized

by higher values during nighttime and lower values during the daylight hours in diurnally active, nocturnally resting women (Kivela 1991). The lower melatonin concentrations during the day and their rise in darkness (Bartsch et al. 1994; Lynch et al. 1975; Pelham et al. 1973; Weaver et al. 1993; Wetterberg 1978) led to the suggestion that the pineal may possibly be a neuroendocrine transducer of the effect of darkness.

Melatonin has been shown to mediate the antigonadotropic effect of day length in species with a seasonal (circannual) reproductive cycle. Although day length insofar as the seasonal photofraction is concerned may not seem to be important for human reproduction (Weaver et al. 1993), an inverse relationship in the secretions of the pineal and the ovary is reported at least in northern countries with a strong seasonal contrast in luminosity (Kauppila et al. 1987). Melatonin's role during human pregnancy has not been clarified beyond an increase in melatonin secretion (Kivela 1991). Any effect of this hormone on fetal growth is not known. A direct effect of melatonin on progesterone secretion has been recently suggested on the basis of in vitro studies (Baratta and Tamanini 1992). The topic of melatonin and magnetic fields has been reviewed in relation to spontaneous abortion (Sandyk et al. 1992). The effect of magnetic fields on cell division has also been repeatedly examined (Guzelsu et al. 1994; Levin and Ernst 1995; Moore 1979; Schaarschmidt 1977; Varga 1976).

In this study, we qua ntify the circadian melatonin rhythm in two groups of women in their third trimester of pregnancy, one with healthy uncomplicated pregnancies resulting in the delivery of a baby of appropriate weight for gestational age (HAGA) and another with pregnancies complicated by intrauterine growth retardation (IUGR) confirmed at birth. To better understand the temporal organization of melatonin, we studied the cortisol rhythm of the same subjects, sampled at the same times as a marker of chronome-dependent hormonal dynamics. Using a transverse approach, circasemiannual and circannual variation was also assessed. These components were of particular interest since about half-yearly variations are found to prominently characterize the body length and weight at birth of children with birth characteristics below the usual range, whereas about-yearly changes are also found for children with birth characteristics in the usual value range (Otto and Reissig 1963).

## Subjects and Methods

During the third trimester, 14 healthy pregnancies (HAGA) and 11 pregnancies complicated by intrauterine growth retardation (IUGR) were evaluated in Milan, Italy, at 45°28'N, 9°12'E geographic and 46.31°N, 91.47°E geomagnetic coordinates. The diagnosis of intrauterine growth retardation was made by the growth rate pattern monitored by ultrasonography and was confirmed by a neonatal weight corrected for gender, gestational age and national standards lower than the 10th percentile. There were no malformed fetuses. None of the women had any known disease or received drugs except for their use of iron and vitamin preparations. All women were hospitalized and followed the same daily routine, resting in darkness between 22:00 and 06:00 and eating at about 08:00, 12:00 and 19:00. All women gave informed consent to participate in the study.

Blood was drawn every 4 hours and immediately centrifuged and serum was stored at -40°C until assay. Melatonin was determined by radioimmunoassay (Melatonin I-125 RIA, Eurogenetics, Rivoli-Torino, Italy). Recovery from plasma was between 80 and 95%, as measured with (3H) melatonin. Specificity of the assay was close to 100% (<0.5% 5-methoxytryptophol and <0.01% serotonin or others). Intra- and inter-assay CVs were 11 and 12%, respectively, around means of 55 pg/ml and 20 and 22%, respectively, around means of 7 pg/ml. Free plasma cortisol was radioimmunoassayed and progesterone determined by immunoenzymatic assay after 1:50 dilution. The practical sensitivity of the cortisol assay (Diagnostics Systems Labs., Webster, Texas) was 0.61 $\mu$g/dl. Around means of 5.0, 19.2 and 33.0 $\mu$g/dl, the intra-assay CVs were 8.4, 5.3 and 11.1% and the inter-assay CVs were 9.1, 8.9, and 11.5% around means of 4.8, 19.2 and 35.7 $\mu$g/dl, respectively. Cross-reactivity of the assay with other naturally-occurring steroids is low.

Data were summarized by analysis of variance for repeated measures in order to test for overall differences between groups as well as for mean differences over time and for interactions between groups and timepoints. This analysis was carried out by using the "repeated" procedure of SAS (SAS/STAT Manual). Mean differences in the shape of the circadian patterns were also evaluated using the contrast matrix proposed by the PROFILE option. This approach, adjusting the level for multiple tests, allows comparison of consecutive hourly means. For the visualization of the average circadian profile in each group, timepoint means and standard deviations (SD) were computed and plotted as a function of time. The data from each woman were also analyzed by the one-component single cosinor (Halberg 1969; Nelson et al. 1979). This method involves the least-squares fit of a 24-hour cosine curve to the data ($Y_i$): where **M** represents the **MESOR** (**m**idline-**e**stimating **s**tatistic **o**f **r**hythm), a rhythm-adjusted mean; A is the amplitude, half the extent of predictable


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$$Y_i = Y(t_i) = M + A\cos\left(\frac{2\pi t_i}{24} + \phi\right) + e(t)$$

change within a cycle, $\phi$ is the acrophase, a measure of the timing of overall high values recurring in each cycle, and e is an error term assumed to be independent and normally distributed with zero mean and unknown constant variance. The acrophase is expressed in negative degrees with 360 degrees equated to the period length (24 hours) and the reference time chosen as local midnight. For the transverse assessment of the yearly and half-yearly components, the reference time is chosen as the winter solstice preceding the start of data collection. The error term is used for rhythm detection (by F-test) and for the derivation of 95% confidence intervals of the estimated parameters. Individual circadian estimates were further summarized for each group by population-mean cosinor (Cornélissen and Halberg 1998; Halberg et al. 1967). The rhythmic patterns were compared between the two groups by means of parameter tests (Bingham et al. 1982).

## Results

The two groups were comparable for chronological and gestational age, pre-pregnancy body mass index (BMI) and weight increase during pregnancy, as shown in Tables 1-3. No differences in behavioral or obstetrical characteristics were found between the two groups, except for birth weight and length. A circannual rhythm in birth weight ($P=0.035$) (and length; $P=0.105$) and also in placental weight ($P<0.001$) is demonstrated for the pregnancies complicated by intrauterine growth retardation, maximal values being reached in winter. This result is in keeping with prominent about-yearly and half-yearly rhythms observed in the first year and a half of life, that have been related to both innate and environmental factors (Garcia Alonso et al. 1993). The month-of-birth effects on growth, gauged by body height, reportedly remain prominent at 18 years of age (Weber et al. 1998).

Plasma concentrations of melatonin showed a statistically significant circadian time effect in both groups, Figures 1 and 2.

The circadian pattern in the two groups was similar; no overall inter-group differences were detected by analysis of variance, Table 4. By cosinor analysis, a circadian rhythm is statistically significant for melatonin and cortisol on a population basis, Table 5, despite a large inter-individual variation (not shown). For cortisol, a time effect is also demonstrated in both groups ($P<0.001$), Table 4, without any statistically significant intergroup difference (except for a slight intergroup difference around 05:00 when cor-

**Table 1. Demographic and obstetrical characteristics in the two groups investigated***

| | HAGA (N=14) | | IUGR (N=11) | | Comparison, | |
|---|---|---|---|---|---|---|
| Variable (units) | mean ± SE | (SD) | mean ± SE | (SD) | Student t | P |
| Age (years) | 30.3 ± 1.5 | (5.7) | 28.9 ± 0.9 | (2.9) | 0.727 | n.s. |
| Gestational age (weeks) | 35.4 ± 0.7 | (2.5) | 33.7 ± 0.7 | (2.4) | 1.542 | n.s. |
| Maternal height (cm) | 162.9 ± 2.0 | (7.4) | 164.1 ± 1.6 | (5.4) | 0.462 | n.s. |
| Pre-pregnancy weight (kg) | 54.9 ± 2.1 | (7.8) | 55.5 ± 1.5 | (4.8) | 0.192 | n.s. |
| Maternal weight (kg) | 65.2 ± 1.9 | (7.3) | 66.0 ± 1.7 | (5.8) | 0.302 | n.s. |

*HAGA: clinically healthy uncomplicated pregnancies resulting in the delivery of babies with a body weight appropriate for gestational age; IUGR: pregnancies complicated by intrauterine growth retardation

**Table 2. Comparison of behavioral and obstetrical characteristics of groups investigated***

| | | HAGA (N=14) | | IUGR (N=11) | | Comparison | |
|---|---|---|---|---|---|---|---|
| Characteristic | | n | % | n | % | $\chi^2$ (1 d.f.) | P |
| Smoking | no | 7 | 50 | 7 | 64 | | |
| | yes | 7 | 50 | 4 | 36 | 0.47 | n.s |
| Prior pregnancy, | yes | 6 | 43 | 6 | 55 | | |
| | no | 8 | 57 | 5 | 45 | 0.34 | n.s |
| Prior delivery | yes | 4 | 29 | 3 | 27 | | |
| | no | 10 | 71 | 8 | 73 | 0.005 | n.s. |

*HAGA: clinically healthy uncomplicated pregnancies resulting in the delivery of babies with a body weight appropriate for gestational age; IUGR: pregnancies complicated by intrauterine growth retardation

**Table 3. Pregnancy outcomes***

| | HAGA (N=14) | | IUGR (N=11) | | Comparison |
|---|---|---|---|---|---|
| | incidence | | incidence | | P |
| Cesarean section for fetal distress | 0 | - | 10 | - | <0.001 |
| | mean ± SE | (SD) | mean ± SE | (SD) | |
| Gestational age at delivery (weeks) | 39.2 ± 0.3 | (1.3) | 37.4 ± 0.8 | (2.5) | 0.034 |
| Newborn weight (g) | 3069±104 | (387) | 2048 ± 135 | (449) | <0.001 |
| Newborn length (cm) | 48.8 ± 0.4 | (1.6) | 44.3 ± 1.3 | (4.2) | 0.002 |
| Placental weight (g) | 526.9±29.4 | (109.9) | 433.6 ± 49.8 | (165.1) | 0.026 |
| APGAR score (1 min) | 7.15 ± 0.63 | (2.35) | 6.18 ± 0.50 | (1.66) | n.s. |
| APGAR score (5 min) | 9.46 ± 0.24 | (0.91) | 8.36 ± 0.34 | (1.12) | 0.013 |

*HAGA: clinically healthy uncomplicated pregnancies resulting in the delivery of babies with a body weight appropriate for gestational age; IUGR: pregnancies complicated by intrauterine growth retardation

tisol concentrations are higher in pregnancies complicated by intrauterine growth retardation; P=0.014; not corrected for multiple testing).

Table 6 summarizes the characteristics of the yearly and half-yearly components, assessed transversely. In healthy pregnancies (shown in the top row), the about half-yearly (circasemiannual) variation is not detected with statistical significance, perhaps in view of the relatively small sample size (N=14), with different women opportunistically contributing a single profile at calendar dates that were not systematized a priori. Despite the even smaller sample size of IUGR pregnancies (N=11), the half-yearly variation is detected (P<0.01). Moreover, the circadian (24-hour) and circasemidian (12-hour) amplitudes of circulating melatonin are also found to be modulated by an about-half-yearly variation (P<0.05) in this group.

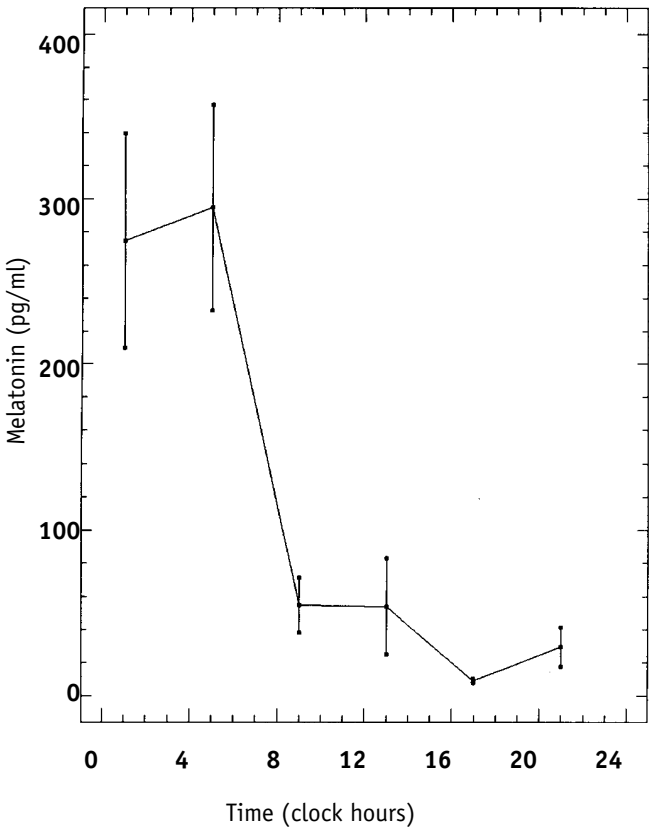


**Figure 1.** Circadian pattern of circulating melatonin in uncomplicated healthy pregnancies. Timepoint means +/- SEs computed for data on 14 women in their third trimester of pregnancy.

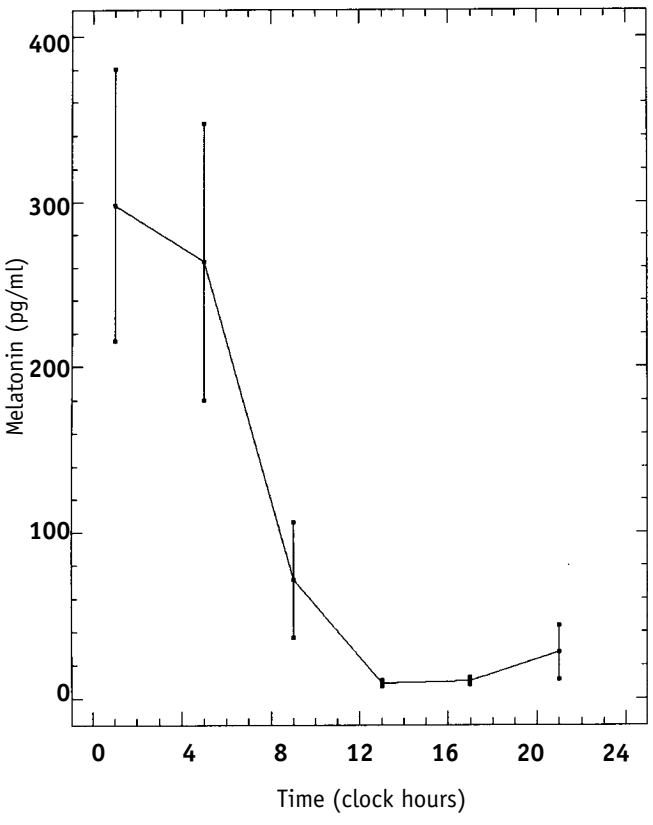


**Figure 2.** Circadian pattern of circulating melatonin in pregnancies complicated by intrauterine growth retardation (IUGR). Timepoint means +/- SEs computed for data on 11 women in their third trimester of pregnancy.



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**Table 4. Analyses of variance on circulating melatonin and cortisol demonstrate time effects but no inter-group difference**

| Clock-hour | | 01:00 | 05:00 | 09:00 | 13:00 | 17:00 | 21:00 |
|---|---|---|---|---|---|---|---|
| Melatonin | | | | | | | |
| HAGA (N=14) | mean | 274.8 | 294.8 | 54.9 | 54.0 | 9.01 | 29.6 |
| | SE | 65.0 | 62.1 | 16.5 | 29.0 | 1.49 | 12.0 |
| IUGR (N=11) | mean | 297.9 | 263.8 | 71.2 | 8.98 | 10.47 | 27.6 |
| | SE | 82.6 | 83.5 | 34.7 | 1.92 | 2.19 | 16.4 |
| Cortisol | | | | | | | |
| HAGA (N=14) | mean | 176.0 | 318.2 | 384.3 | 349.7 | 271.6 | 228.1 |
| | SE | 22.9 | 22.5 | 23.2 | 20.7 | 17.7 | 15.5 |
| IUGR (N=11) | mean | 196.6 | 412.6 | 413.3 | 370.6 | 306.0 | 261.1 |
| | SE | 18.8 | 27.9 | 20.7 | 25.9 | 22.1 | 27.1 |

| | Melatonin | | Cortisol | |
|---|---|---|---|---|
| | F | P | F | P |
| Overall between-groups effect | 0.06 | n.s. | 9.06 | 0.003 |
| Within-subjects time effect | 17.22 | <0.001 | 26.98 | <0.001 |
| Group-time interaction | 0.18 | n.s. | 0.78 | n.s. |

*HAGA: clinically healthy uncomplicated pregnancies resulting in the delivery of babies with a body weight appropriate for gestational age; IUGR: pregnancies complicated by intrauterine growth retardation

In view of prior results suggesting that the about-yearly and half- yearly modulation of circulating melatonin may be circadian stage- dependent (Tarquini et al. 1997), transverse time series were constituted by considering each sampling time separately (taking only the values obtained either around 01:00, 05:00, 09:00, 13:00, 17:00 or 21:00). As seen in Table 6, an about-half-yearly variation characterizes circulating melatonin in blood sampled around 01:00 ($P=0.016$) or 05:00 ($P<0.001$) but not in blood sampled at other times. These are the nightly values found to be modulated by an about-half-yearly variation in 172 clinically healthy subjects of both genders studied earlier at nearly the same latitude (in Florence, Italy, at 43.47°N 11.15°E geographic and 44.26°N 92.86°E geomagnetic coordinates) (Tarquini et al. 1997).

The two groups of healthy and IUGR pregnancies were found to differ in their about half-yearly pattern in tests of equality of the $(A,\phi)$ pair. The circadian amplitude ($P=0.028$) and the 05:00 values ($P=0.021$) differ with statistical significance, the MESOR ($P=0.058$) and the 01:00 values ($P=0.072$) with borderline significance. As compared to women with uncomplicated pregnancies, women with intrauterine growth retardation may be more sensitive to geomagnetic disturbance since geomagnetic disturbance also exhibits a prominent about-half-yearly pattern (Grafe 1958). The latter may be involved in the pathogenesis of intrauterine growth retardation.

As another aspect of a putative association of melatonin with an environmental chronome component, the results in the bottom half of Table 6 indicate that only the pregnancies complicated with intrauterine growth retardation show a statistically significant about-yearly component modulating the circadian MESOR of circulating melatonin. The circadian amplitude is modulated by a yearly component in both groups. The amplitude of the circannual component differs with borderline statistical significance between the two groups for the case of the 01:00 ($P=0.067$) and the 09:00 ($P=0.087$) values. These results suggest that any difference between IUGR and uncomplicated pregnancies may involve an about-yearly and/or half- yearly rather than an about-daily variation. They may reflect associations not only with sunlight, temperature and societal schedules, which have a yearly pattern, but also associations with terrestrial magnetism, characterized by a half-yearly pattern of its disturbance.

## Discussion

The results demonstrate, perhaps for the first time, a circadian rhythm in pregnancies complicated by intrauterine growth retardation and confirm earlier work in uncomplicated human pregnancy (Kivela 1991). The circadian melatonin rhythm is statistically significant in each group, and its characteristics do not differ between the two groups investigated. Differences are found, however, in the yearly and half-yearly features assessed transversely. There are precedents for such results in relation to breast cancer risk. Differences in the about-yearly variation of prolactin and TSH were found to differ between groups of clinically healthy women at high vs. low risk of developing breast cancer when their circadian

**Table 5. Circadian rhythm characteristics***

| | | HAGA (N=14) | IUGR (N=11) | Comparison |
|---|---|---|---|---|
| Melatonin | MESOR (95% CI) | 118.9 (69.7; 168.1) | 112.1(46.2; 177.9) | n.s. |
| Amplitude | (95% CI) 146.9 | (72.1; 221.7) 158.4 | (55.7; 261.2) | n.s. |
| Acrophase | (95% CI) -53dg | (-44; -69) -53dg | (-44; -67) | n.s. |
| | P ($H_0$: A=0) | 0.003 | 0.026 | |
| Cortisol | MESOR (95% CI)288.2 | (250.7;325.7) 327.2 | (288.9; 365.5) | n.s. |
| | Amplitude (95% CI) | 95.4 (77.9; 112.9) | 101.4 (82.5; 120.3) | n.s. |
| | Acrophase (95% CI) | -159dg (-148; -168) | -154dg (-138; -171) | n.s. |
| | P ($H_0$: A=0) | <0.001 | <0.001 | |

*HAGA: clinically healthy uncomplicated pregnancies resulting in the delivery of babies with a body weight appropriate for gestational age; IUGR: pregnancies complicated by intrauterine growth retardation; MESOR=rhythm-adjusted mean; amplitude=measure of extent of predictable change within one cycle; acrophase=measure of the timing of overall high values recurring in each cycle expressed in (negative) degrees with 360 dg=period length; 0dg=00:00 on the day preceding data collection; P=P-value from test of zero amplitude (no rhythm) hypothesis.
**The large melatonin amplitude is the artifactual result of overfitting.

rhythm did not show any statistically significant difference (Bulbrook et al. 1987; Halberg et al. 1981; Tarquini et al. 1979). The circannual amplitude of circulating prolactin decreased and that of TSH increased with increasing breast cancer risk (Halberg et al. 1981). A difference in the circannual rhythm of prolactin was also demonstrated in women with fibrocystic mastopathy vs. healthy controls, the circannual rhythm being statistically significant in health, but not in the presence of fibrocystic mastopathy (Tarquini et al. 1979). These findings are in keeping with the suggestion that circannual changes can precede those in the circadian system, as observed in a study in the Channel Islands (Bulbrook et al. 1987). Clinically healthy women had a sample of blood drawn and then stored until a later time when the presence or absence of breast cancer was determined. At that time, retrospectively, two groups could be formed, one of women who did and the other of those who did not develop breast cancer in the interim. In each group, women were sampled in different seasons. To each of the serially independent series from a given group, a one-year cosine curve was fitted. Those who did not have cancer yielded a statistically highly significant circannual prolactin rhythm, while this rhythm was absent in women actually documented as having breast cancer (Bulbrook et al. 1987). The results in Table 6 concerning retarded fetal growth may be viewed in the light of this precedent that a mechanism retarding cancerous growth in the breast or at least a mechanism associated with a low risk of breast cancer when deviant, involves alterations of the about-yearly and/or half-yearly pattern in the absence of a detectable circadian alteration.

The about-yearly and half-yearly results, although obtained transversely on relatively small groups, are in keeping with those of a previous study on 172 subjects of both genders, including 60 non-pregnant women less than 45 years of age (Tarquini et al. 1997). In this study, an about-half-yearly pattern of circulating melatonin was demonstrated by cosinor analysis only in samples collected during the night (dark span, around 00:00 and 04:00). By contrast, an about-yearly rhythm characterized circulating melatonin in samples collected during the daytime. These results were interpreted in terms of a yearly component related to a host of seasonally changing factors, including sunshine and environmental temperature and of a half-yearly component suggesting the effect of geomagnetics (Tarquini et al. 1997).

That a half-yearly pattern may be influenced by geomagnetics is suggested further by the meta-analysis of data by Martikainen et al. (1985). Blood was obtained longitudinally, at monthly intervals for a year, on 11 clinically healthy subjects. The study was conducted at a geographic latitude of 65.00°N and a geomagnetic latitude of 61.75°N. By contrast to the data obtained in Florence at mid-latitude showing an about-yearly pattern of melatonin during the daytime, an about-half-yearly pattern of melatonin is seen up north in samples drawn at midday. This difference can be anticipated in a geographic comparison where the contribution of interacting synchronizers differs in their relative strength. The yearly pattern of sunshine at mid-latitudes dominates over the half-yearly pattern of geomagnetics, whose effects are relegated to the night. By contrast, near the pole, geomagnetics are more intense and the half-


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**Table 6: About-yearly (circannual) and about-half-yearly (circasemiannual) population patterns* of circulating melatonin differ between 14 healthy (HAGA) vs. 11 IUGR-complicated human pregnancies: The half-yearly patterns indicate an association of IUGR with geomagnetic disturbance?**

| IUGR vs HAGA ** | Period (yrs) | | M P | M ϕ | 24-h A P | 24-h A ϕ | 12-h A P | 12-h A ϕ | 01:00 P | 01:00 ϕ | 05:00 P | 05:00 ϕ | 09:00 P | 09:00 ϕ | 13:00 P | 13:00 ϕ | 17:00 P | 17:00 ϕ | 21:00 P | 21:00 ϕ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.5 | HAGA | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| | | IUGR | **0.013** | -344° | **0.005** | -340° | **0.024** | -357° | **0.016** | -342° | **<0.001** | -338° | . | . | . | . | . | . | . | . |
| 0,5y (A,ϕ) pair: | | | 0.058 | | **0.028** | | | | 0.072 | . | **0.021** | | | | | | | | | |
| | 1.0 | HAGA | . | . | **0.036** | -327° | . | . | . | . | **0.023** | -328° | **0.021** | -345° | . | . | . | . | . | . |
| | | IUGR | **0.008** | -23° | **0.019** | -21° | . | . | **0.009** | -20° | 0.094 | -19° | **0.022** | -46° | 0.092 | . | . | . | . | . |
| | | | | | | | | | | | | | | | | -36° | | | | |
| 1.0y A: | | | | | | | | | 0.067 | | 0.087 | | | | | | | | | |

*Assessed by the analyses of original values at specified clock-hours and by circadian characteristics that in themselves did not differentiate the groups investigated, until they were scrutinized for infradian patterns. Since the data cover only one idealized year, we speak of patterns rather than rhythms, but it should be noted that the rhythms of a sufficient number of members in a group must be sufficiently concordant to allow the (transverse or cross-sectional) demonstration of the rhythmic pattern. Each pregnant woman was sampled at 4-hour intervals for 24 hours (with serial dependence as to individuals). These series were fitted by cosinor (1) with a 24-hour and a 12-hour cosine curve to obtain circadian means (MESORs, M) and amplitudes (24-h A and 12-h A). To these Ms and separately to the 24-h and 12-h As, and also separately to data from each of 6 clock-hours of sampling, 1.0- and 0.5-year cosine curves were fitted again by cosinor (Halberg et al. 1972) to test the zero circannual or zero circasemiannual amplitude assumptions. These series were serially independent in terms of subjects, but serially dependent in terms of environment. Among the results, those P-values below 10% are listed; those below 5% are in **boldface**. For the acrophase, ϕ, also given for each test, 360° is equated to 0.5 year (top) or to 1 year (bottom) and zero ϕ is midnight on December 22 of the year preceding data collection. Generally used in particular for the cases when both groups showed a circannual pattern, the relative proximity of acrophases about winter is noted. A yearly component characterizes the spectrum of many socio-ecological phenomena, including sunshine and temperature.

¶ A half-yearly component also characterizes geomagnetic disturbance, gauged by the planetary index, Kp (Fraser-Smith 1972; Grafe 1958). At 43.47° N latitude the about half-yearly pattern is seen only in samples during darkness (Tarquini et al. 1997), whereas at 65°N, where geomagnetics is more intense and sunlight is weaker, the about half-yearly pattern is seen at noon (Tarquini et al. 1997; cf. Martikainen et al. 1985).

**HAGA: uncomplicated pregnancy with baby **a**ppropriate for **g**estational **a**ge; IUGR; **i**ntra**u**terine **g**rowth **r**etardation, studied in Milan, Italy.


Fraser-Smith AC (1972) Spectrum of the geomagnetic activity index Ap. J Geophys Res. 77: 4209-4220

Grafe A (1958) Einige charakteristische Besonderheiten des geomagnetischen Sonneneruptionseffektes. Geofisica Pura e Applicata 40: 172-179

Halberg F, Johnson EA, Nelson W, Runge W, Sothern R (1972) Autorhythmometry – procedures for physiologic self-measurements and their analysis. Physiol Tchr 1: 1-11

Martikainen H, Tapanainen J, Vakkuri O, Leppaluoto J, Huhtaniemi I (1985) Circannual concentrations of melatonin, gonadotrophins, prolactin and gonadal steroids in males in a geographical area with a large annual variation in daylight. Acta endocrinol (Copenhagen) 109: 446-450

Tarquini B, Cornelissen G, Perfetto F, Tarquini R, Halberg F (1997) Chronome assessment of circulating melatonin in humans. In vivo 11: 473- 484

yearly pattern of geomagnetics emerges in melatonin of blood samples drawn during daylight.

Against this background, the study of geomagnetics in relation to melatonin in general, and with a view of a contribution by geomagnetics to the pathogenesis of intrauterine growth retardation, possibly involving melatonin, deserves further study. The spectrum of Kp, a planetary index of geomagnetic disturbance, obtained for the years of the intrauterine growth retardation study reported herein, revealed a prominent about half-yearly pattern, Figure 3. A much sharper peak at half a year when all Kp data (for the span from 1932 to 1997) are analyzed is also shown in Figure 3.

The limited size of the present study may have prevented the detection of a half-yearly component for the MESOR, the circadian amplitude or the separate circadian stages (even for the data collected at night) of melatonin in the group of women with uncomplicated pregnancies. Whether the failure to detect such a modulation in health is the result of the limited sample size or whether it is primarily a feature of intrauterine growth retardation, as partly suggested by the statistically significant difference in pattern between the two groups, deserves further investigation to examine whether pregnancies complicated by intrauterine growth retardation may be more sensitive to the effect of geomagnetic disturbance. The question whether manipulating geomagnetics, or rather shielding from their effect, is a countermeasure of merit in cases of intrauterine growth retardation also deserves further testing. As to operating values assessed by averages, in keeping with Kivela (1991), we also find that the nighttime values are higher in pregnancy than in clinically healthy non-pregnant women less than 45 years of age. A similar difference by comparison to non-pregnant women is found both in healthy pregnancies and in pregnancies complicated by intrauterine growth retardation. The peripheral melatonin is largely derived from the pineal gland and is considered to be an index of pineal function (Arendt 1985; Grota et al. 1982).

Components with frequencies higher than one cycle per day (ultradians) characterizing the secretion of melatonin have been reported by Trinchard-Lugan and Waldhauser (1989). This six-timepoint study at 4-hour intervals assesses the circadian variation and detects about-yearly and about-half-yearly modulations of circadian features. But with four-hourly sampling, only the 24-hour and 12-hour components could be assessed.

Exposure during the night for half an hour or one hour to artificial light may partly suppress the melatonin secretion. In this study, exposure to light for blood drawing took only a few minutes. Whether or not it affected the melatonin rhythm unduly, it did not interfere with its demonstration, as is apparent from the tabulated results.

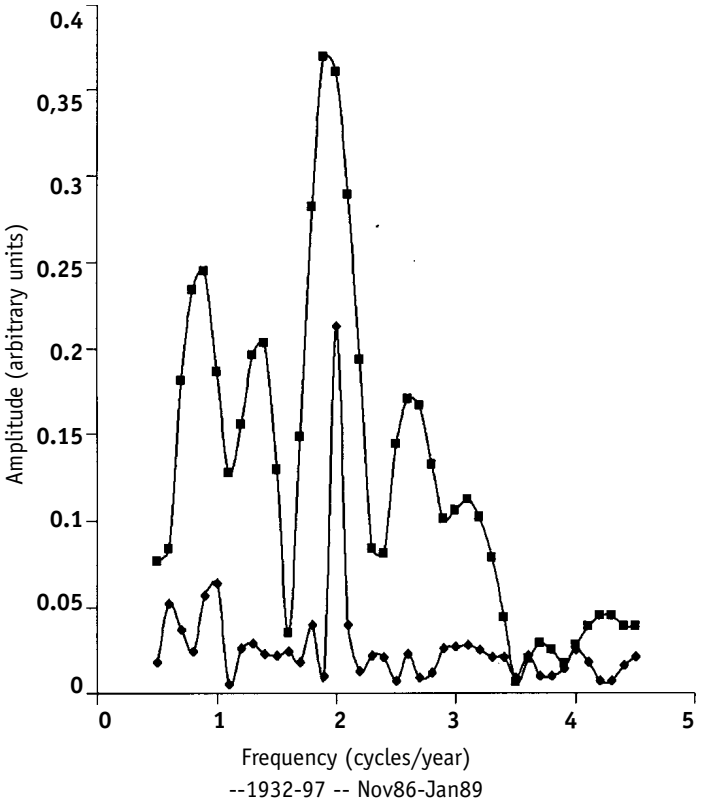


**Figure 3.** Spectra of geomagnetic index Kp during study span and during 66 years (since its recording in 1932).

Increased enzymes of pinealocytes during gestation and increased melatonin values compared to non-pregnant values were reported by Kivela (1991) and Birau et al. (1984). In humans, increased melatonin concentrations by a factor 4 are reported in anorexia nervosa (Tortosa et al. 1989) and in hypothalamic amenorrhea (Berga et al. 1988; Brzezinski et al. 1988).

Some changes during the menstrual cycle with a nadir during ovulation and increased concentrations during the luteal phase are reported by some authors (Brun et al. 1987; Wetterberg et al. 1976), but not by others (Brzezinski et al. 1988; Kivela et al. 1988). Increased serum melatonin concentrations are unable to prevent or delay the preovulatory LH surge (Zimmermann et al. 1990), but it is possible that prolonged elevated serum melatonin concentrations may be required for the manifestation of altered LH pulses (Bittman and Karsch 1984). The role of melatonin in development more broadly is discussed by Davis (1997).

The persistence of a circadian cortisol rhythm and the elevated free and total cortisol are reported in a healthy pregnancy (Nolten and Rueckert 1981; Okamoto et al. 1989). In the present study we found that


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**Table 7. Circadian variation in sensitivity of adrenals to stimulation by melatonin of mouse DHEA production***

| Endpoint | P | MESOR ± SE (ng/ml) | 2A ± SE | ϕ (95 %CI) |
|---|---|---|---|---|
| Least squares plot: | | | | |
| $_{log}$DHEA (50 μM dose) | 0.009 | -1.117 ± 0.002 | 0.476 ±0.004 | -80° (-73; -87) |
| Dose Response (log) | | | | |
| intercept | 0.126 | -2.748 ± 0.061 | 1.360 ± 0.172 | -84° ( ) |
| slope | 0.145 | 0.964 ± 0.030 | 0.572 ± 0.084 | -265° ( ) |
| correlation coefficient | 0.057 | 0.992 ± 0.001 | 0.012 ± 0.001 | -347° ( ) |

*Data taken off Figure 1 of a publication by E. Haus et al. (1996). Light (L) from 07:00 to 19:00. DHEA production in vitro by 5 pairs of random-bred "Parhon" mouse adrenals removed at 08:00, 14:00, 20:00 or 02:00 (at 1, 7, 13 and 19 hours from light-on). Circadian stage-dependence observed for low 50 μM melatonin dose (top row), is not statistically significant at higher dosages of 150 and 450 μM (P>0.50; not shown). Characteristics of the dose response curve, however, approach borderline statistical significance when for samples obtained at each of the test times separately, the log value of adrenal DHEA, observed at a given log dose, is linearly regressed to log dose; the intercept of the regression line is on the average largest around 05:36 (-84° from local midnight), a timing similar to that observed for the lowest 50 μM dose; this time also corresponds to the time of the least steep slope, while the best fit (largest correlation coefficient) occurs around 23:08. The detection of the effect of the 50 μM dose is one of the examples for the power of the cosinor method here used, as compared to the analysis of variance used solely in the original publication (Haus et al. 1996).

the melatonin rhythm maintains its lead in phase versus the cortisol rhythm, although cortisol is elevated.

In other hypercortisolemic states such as Cushing disease, melatonin concentrations are decreased (Soszynski et al. 1989). In patients with hyper-cortisolism of different etiology, the melatonin rhythm is reported to be abolished (Soszynski et al. 1987) or to remain unchanged (Piovesan et al. 1990). An increased melatonin production during daytime and a "usual" rhythm are reported by Fevre-Montange et al. (1983) in idiopathic hemochromatosis with a "usual" cortisol rhythm. Plasma concentrations of melatonin are reported as reduced or unaltered in adrenal hyperplasia (Waldhauser et al. 1986) and apparently unchanged after bilateral adrenalectomy. Cortisol and melatonin secretion are reportedly linked (Kennaway et al. 1982); however, the rhythms can be readily dissociated (Fevre-Montange et al. 1983). An analysis of ultradian components of cortisol and the effect of external stimuli (such as food intake or loads) on the cortisol and melatonin rhythms was interpreted to suggest that any correlation between the peaks of these two hormones is not causal and depends on a third factor (Rivest et al. 1989).

Pregnancy is a combination of high cortisol and melatonin concentrations with usual cortisol and melatonin circadian rhythms. These findings are similar in pregnancy and hypothalamic amenorrhea (Berga et al. 1988; Biller et al. 1990; Brzezinski et al. 1988; Tortosa et al. 1989) or anorexia nervosa (Estour et al. 1990) associated with an increased cortisol and melatonin secretion, an abnormal cortisol response to CRH and tissue refractoriness to cortisol (Berga et al. 1988; Estour et al. 1990; Loucks et al. 1989; Nolten and Rueckert 1981). Melatonin could play a role in anabolic processes, under special "loading" conditions, in the face of a need to increase the body mass index.

The placental CRH production is not the only coordinator of maternal cortisol (Allolio et al. 1990). The high cortisol concentrations during pregnancy may not result from increased production of progesterone and its antiglucocorticoid activity (Abou-Samra et al. 1984); progesterone during pregnancy exhibits a different circadian phase (Allolio et al. 1990; Nakajima et al. 1990) as compared to the non-pregnant state (Carandente et al. 1989), while the cortisol phase remains the same. In our study, we found the same nocturnal phase for progesterone (not shown) in both groups, opposite to the rise of cortisol which occurs in the morning. Studies on laboratory animals suggest that melatonin is not directly involved in the coordination of parturition time or in the uterine contractile activity rhythm (Matsumoto et al. 1991).

Melatonin may inhibit locomotor activity. It may inhibit platelet aggregation and induce analgesia. It reportedly has immunosuppressive effects (Maestroni et al. 1986, 1987) and also antigonado-tropic and progonadotropic effects. Yet its more general role may be in feedsidewards, multiple interactions documented in vitro for corticosterone[1] but not for DHEA (Haus et al. 1996): it seems possible that a 6-hourly sampling may not allow the detection of both inhibition and stimulation in a rhythmically repro-

ducible pattern. Alternatively, feedsideward interactions can involve only quantitative changes rather than reversals in sign, i.e., changes from stimulation to inhibition, Table 7. To paraphrase Davis (1997), melatonin is the mammalian fetus' window to the chronomes of the outside world, those of geomagnetism in particular. Davis rightly recommends caution not only concerning "the use of exogenous melatonin during pregnancy and lactation, but also concerning any behavior that might disrupt the mother's endogenous melatonin rhythm." Geomagnetic disturbances, a behavior only of our natural environment, may modulate, if not disrupt, the mother's melatonin rhythm. Whether it does so in an interaction with the suprachiasmatic nuclei coordinating the amplitude and acrophase of several rhythms, including those in DNA labelling and mitosis essential to growth (Cornélissen and Halberg 1994), and perhaps the adrenal cortex, which reaches peak size at the end of human pregnancy and via these structures contributes to intrauterine growth retardation, is a topic for further study.

## Conclusions

The circadian rhythm of melatonin is maintained during pregnancy, whether uncomplicated as noted earlier by Kivela (1991) or complicated as recorded herein for intrauterine growth retardation. An increased plasma concentration of melatonin and cortisol also characterizes intrauterine growth retardation, as found in uncomplicated pregnancies. A circadian rhythm is detectable for both hormones, but a 12-hourly component is also found only for melatonin (not shown), in the 4-hourly sampling during 24 hours of this study.

The circadian time relations of melatonin to cortisol are statistically validated. The increased melatonin values in intrauterine growth retardation, here shown apparently for the first time, as well as in healthy pregnancy, suggest a possible neuro-endocrine involvement in the coordination of pregnancy, although there is no evidence to infer that melatonin spontaneously and directly affects the in utero development of the fetus. Conceivably, the circadian melatonin rhythm participates in the synchronization of maternal rhythms with environmental conditions. About-yearly and about-half-yearly changes are here shown, also for the first time for pregnancies complicated by intrauterine growth retardation, while a very slightly larger group of women with uncomplicated pregnancies did not (suffice to?) document an about half-yearly melatonin pattern.

An about half-yearly modulation is demonstrated for women with intrauterine growth retardation for the 24-hour mean, for the extent (amplitude) of circadian change and in particular also for melatonin in blood collected during the hours of darkness. Around 01:00 and 05:00, synchronization by geomagnetic disturbance, gauged by the planetary index Kp, may override the about-yearly synchronizing effect of sunshine, temperature and other seasonally varying factors. If we consider that melatonin crosses the placenta and thereby influences the time structure of the fetus, these results on external geomagnetic effects open new perspectives concerning relationships, not only between fetus and mother, but also with a heretofore neglected geomagnetic environment to which pregnancies with intrauterine growth retardation may be particularly sensitive.

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