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Original article

The characteristics of the melatonin secretory rhythm are not modified by the stage of pregnancy in ewes

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Summary — An experiment was designed to study if, in the same animals, characteristics of the plasma melatonin rhythm vary during pregnancy in ewes. Thirteen Ile-de-France ewes were maintained in natural photoperiod and measurements of the characteristics of the rhythm of melatonin secretion were determined during one estrous cycle and then during pregnancy. Duration and mean plasma concentrations of the nocturnal melatonin elevation were measured at four stages of pregnancy. Melatonin concentrations during the elevation were not significantly affected by the stage of the estrous cycle (mean ± SEM: 227.8 ± 34.9, 263.2 ± 24.2 and 232.4 ± 27.1 pg/mL for follicular phase, early luteal phase and late luteal phase, respectively), or by the stage of pregnancy (292.8 ± 22.2, 268.7 ± 21.7, 267.4 ± 27.9 and 258.3 ± 23.5 pg/mL for first, second, third and fourth month of pregnancy, respectively). A strong individual effect was detected ($P < 0.01$) for melatonin concentrations during elevation (range: 119.2 ± 11.8 to 396.6 ± 26.0 pg/mL of plasma). A highly significant correlation coefficient was observed within individuals for night melatonin concentrations between the different physiological stages. It was concluded that melatonin secretion is unaffected by the stage of pregnancy.

melatonin / pregnancy / secretion / ewe

Résumé — Les caractéristiques du rythme de sécrétion de mélatonine ne sont pas modifiées pendant la gestation chez la brebis. Les caractéristiques du rythme de sécrétion de la mélatonine ont été étudiées pendant la gestation en répétant les mesures sur les mêmes animaux. Treize brebis, soumises à une photopériode naturelle, ont été utilisées et les caractéristiques du rythme de sécrétion de la mélatonine ont été mesurées pendant un cycle œstrien et pendant la gestation. La durée de sécrétion et les concentrations moyennes de mélatonine pendant l'élévation nocturne ont été étudiées à quatre moments différents de la gestation. Les concentrations noc-

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turnes de mélatonine ne sont pas différentes entre les trois stades du cycle étudiés (moyenne ± SEM ; 277,8 ± 34,9 ; 263,2 ± 24,2 et 232,4 ± 27,1 pg/mL pour la phase folliculaire, le début et la fin de la phase lutéale, respectivement), ou les quatre stades de gestation étudiés (292,8 ± 22,2 ; 268,7 ± 21,7 ; 267,4 ± 27,9 et 258,3 ± 23,5 pg/mL pour le premier, deuxième, troisième et quatrième mois de gestation, respectivement). Une variabilité interindividuelle très importante a été observée ($p < 0,01$) pour les concentrations de mélatonine pendant la nuit (écart de 119,2 ± 11,8 à 396,6 ± 26,0 pg/mL de plasma). Un coefficient de corrélation très élevé intra-individus a été observé pour les concentrations de mélatonine entre les différents états physiologiques. Cette étude indique que la sécrétion de mélatonine n'est pas modifiée par la gestation.

mélatonine / gestation / sécrétion / brebis

INTRODUCTION

Diurnal production of the hormone melatonin by the pineal gland mediates the effects of the environmental photoperiod to regulate seasonal cycles of reproduction in a variety of mammals (Bittman et al, 1983; Goldman and Darrow, 1983; Karsch et al, 1984; Hastings et al, 1985). It is still unclear, however, which of the characteristics (amplitude, duration or phase of secretion) of that rhythm conveys the photoperiodic information to the reproductive axis. The amplitude – the difference between melatonin concentrations during the day and the night – has not received much consideration in sheep because it does not differ between long and short days and it appears to be very variable between animals (Malpaux et al, 1987). In contrast, the duration of secretion varies between long and short days, but whether it is the duration of melatonin secretion per se, or its presence at a precise period of the night, which conveys the photoperiodic information to the reproductive axis has yet to be determined (Wayne et al, 1988).

While the role of the diurnal rhythm in plasma melatonin concentrations in the ovine foetus is unknown, it has been demonstrated that the reproductive response of the newborn and young to the postnatal photoperiod is markedly affected by the photoperiod experienced by the mother during pregnancy (Horton, 1984; Stetson et al, 1986; Weaver and Reppert, 1986). This information may be used jointly with the postnatal photoperiod to induce seasonally appropriate reproductive and behavioral responses in the young lamb (Zemdegs et al, 1987; Ebling et al, 1989; Foster et al, 1989). Whether melatonin concentrations vary during pregnancy is not clear. Some studies suggest an increase in melatonin concentrations during the last trimester of pregnancy in humans (Birau et al, 1984; Kivelä, 1991). In sheep, an absence of normal melatonin rhythm during pregnancy was suggested by Kennaway et al (1981), but other authors did not report any difference in melatonin concentrations between two different time periods of the last third of pregnancy (Yellon and Longo, 1987; Zemdegs et al, 1987).

Important characteristics of melatonin secretion are its high inter-individual variability (Malpaux et al, 1987) as well as its high repeatability (Chemineau et al, 1996). It is therefore critical to study the effect of potential regulatory factors of melatonin concentrations on the same individuals. Recently, we have shown that melatonin secretion is not modified by steroid administration at concentrations typical of the estrous cycle nor by the stage of the estrous cycle during the seasonal anestrous (Zarazaga et al, 1996). The objective of this work was thus to determine whether melatonin secretion characteristics change during pregnancy by assessing its secretion at four stages during pregnancy and comparing it

to that found at three characteristic times of the estrous cycle of the same animals.

MATERIALS AND METHODS

Thirteen adult Ile-de-France ewes aged 2 to 6 years were studied. During the entire experiment, the ewes were fed daily with hay, straw and corn, and had free access to water and mineral licks. They were maintained outdoors at the INRA research center of Nouzilly, France (48°N) during the study. They were isolated from the rams and treated for 12 days with an intravaginal pessary (30 mg acetate of fluorogestone, FGA; Intervet-AKZO, Boxmeer, the Netherlands), and injected with 400 IU of pregnant mare serum gonadotrophin (PMSG; Intervet-AKZO) at the time of sponge withdrawal to induce synchronized estrus and ovulation. Withdrawal of the pessary was considered as the first day of the estrous cycle (15 September = day 0, d0). In order to obtain a measurement of melatonin plasma concentrations before the establishment of pregnancy (control values), melatonin concentrations were measured at three stages of the estrous cycle preceding fertilization: d1, follicular phase; d5, early luteal phase and d14, late luteal phase. The ewes were subsequently mated with Ile-de-France males at the next natural estrous cycle and melatonin concentrations were measured at four different times during pregnancy: the first (mean ± SEM: 29 ± 0.3 days of pregnancy, range: 27–31 days), second (57 ± 0.3 days of pregnancy), third (90 ± 0.3 days of pregnancy) and fourth (119 ± 0.3 days of pregnancy) months of pregnancy. The stage of pregnancy was calculated from the date of mating. Figure 1 shows the general design of the experiment.

Sunset and sunrise hours were official hours provided by a meteorological station located at about 10 km from the experimental location.

Blood was sampled for melatonin determination by jugular venipuncture at hourly inter-

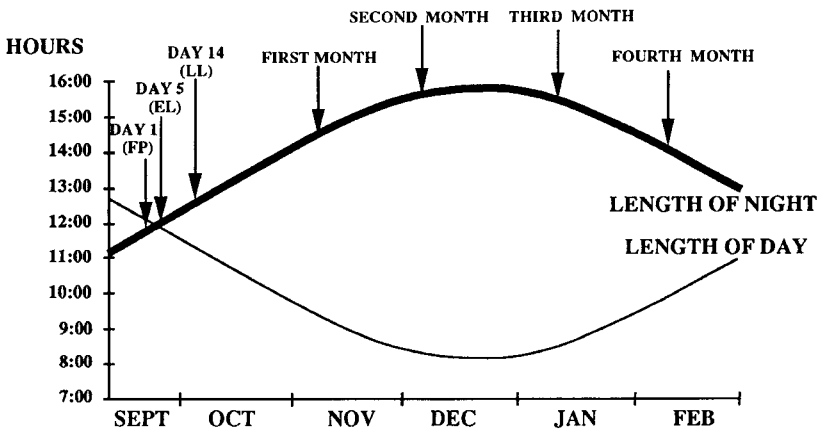

Fig 1. Experimental design. Times of assessment of melatonin plasma concentrations: during the estrous cycle, melatonin concentrations were measured three times: day 1, follicular phase (FP); day 5, early luteal phase (EL) and day 14, late luteal phase (LL). During pregnancy melatonin plasma concentrations were measured four times: first (29 ± 0.3 days of pregnancy), second (57 ± 0.3 days of pregnancy), third (90 ± 0.3 days of pregnancy) and fourth (119 ± 0.3 days of pregnancy) months of pregnancy. The *x*-axis represents the month of the year and the *y*-axis represents the daylength (▬) and nightlength (—) (sunrise to sunset) in hours.

vals, starting and ending 2 h before and after sunrise and sunset, respectively. During the hours of darkness, samples were collected under dim red light (< 3 lux) avoiding any direct illumination of the animal's eyes. Plasma was immediately separated by centrifugation and stored at –20 °C until assay.

In addition, during the estrous cycle ovarian activity was measured by obtaining plasma samples for progesterone determination from d0 to d19, and ovulation rate (OR) was observed on d12 by laparoscopy using the technique described by Oldham and Lindsay (1980).

Hormonal analysis

Plasma melatonin concentrations were assayed by radioimmunoassay using the technique described by Fraser et al (1983) with antibody first raised by Tillet et al (1986), in duplicate aliquots of 100 μL of blood plasma. The sensitivity of the assay was 4 ± 0 pg/mL (two assays) (melatonin Fluka-63610). The coefficient of variation was estimated by assaying three plasma pools (low, medium and high concentrations of melatonin) in duplicate for every 100 unknown samples. The intra-assay coefficient of variation was 10.6%. The inter-assay coefficient of variation was 6.3% (two assays).

Plasma progesterone concentrations were assayed by radioimmunoassay using the technique described by Saumande et al (1985). The sensitivity of assay was 0.125 ng/mL. Intra-assay coefficient of variation was 17.7%. All samples were assayed in the same assay.

Data analysis

For each stage of the estrous cycle and pregnancy and for each animal, a melatonin elevation was defined according to Malpaux et al (1987). Two characteristics of the melatonin elevation were compared: the duration (defined as the time elapsed from the first sample of the elevation to the first sample after the end of the elevation) and the mean melatonin concentration during the elevation.

An analysis of variance with repeated factors was used to test the stage of the estrous cycle and pregnancy (seven stages in total) and individual effects on mean melatonin concentrations and duration of the elevation (Super–ANOVA, Abacus Concepts, Berkeley, CA, USA).

The correlation coefficient between progesterone levels and melatonin concentrations at each stage of the estrous cycle was calculated. The repeatability of mean melatonin concentrations and duration of the elevation from night to night was estimated by the correlation coefficient.

RESULTS

The patterns of mean plasma melatonin concentrations during each stage of the estrous cycle and during each of the four studied stages of pregnancy are presented in figure 2. All ewes presented a clear rhythm in their plasma melatonin concentrations, with a rapid increase after the onset of darkness, a rapid decrease after sunrise and undetectable values during the day.

No significant effect of the different physiological stages was detected on the melatonin concentrations during the period of elevation (mean ± SEM: 277.8 ± 34.9, 263.2 ± 24.2 and 232.4 ± 27.1 pg/mL for follicular phase, early luteal phase and late luteal phase, respectively, and 292.8 ± 22.2, 268.7 ± 21.7, 267.4 ± 27.9 and 258.3 ± 23.5 pg/mL for the first, second, third and fourth month of pregnancy, respectively). A highly significant individual effect was detected for this parameter during the experiment ($P < 0.01$) (range: 119.2 ± 11.8 to 396.6 ± 26.0 pg/mL).

The correlation coefficients for mean night melatonin concentrations between the stages of the estrous cycle as well as the different stages of pregnancy are shown in table I.

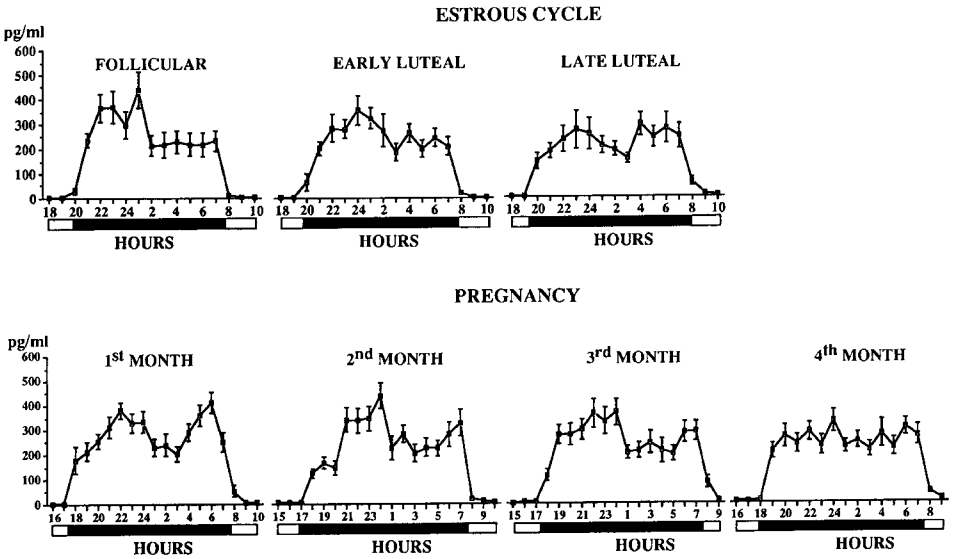

Fig 2. Plasma melatonin concentrations (mean ± SEM) in 13 Ile-de-France ewes when sampled at 1 h intervals. The top panel shows the pattern of plasma melatonin concentrations during follicular, early luteal and late luteal phases (11h47', 12h01' and 12h33' of darkness, respectively). The bottom shows the pattern of plasma melatonin concentrations of the same animals when sampled during the first, second, third and fourth months of pregnancy (14h29', 14h37', 13h10' and 13h32' of darkness, respectively). The period of darkness is indicated by a solid bar.

Table I. Correlation coefficients for mean night melatonin concentrations between the different stages of the estrous cycle (follicular phase, FP; early luteal phase, EL and late luteal phase, LL) and the different stages of pregnancy (first, second, third and fourth months).

	EL	*LL*	*1st*	*2nd*	*3rd*	*4th*
FP	0.75^{c}	0.91^{c}	0.64^{b}	0.91^{c}	0.86^{c}	0.88^{c}
EL		0.81^{c}	0.55^{b}	0.79^{c}	0.67^{c}	0.76^{c}
LL			0.51^{a}	0.89^{c}	0.78^{c}	0.80^{c}
Months						
1st				0.75^{c}	0.36^{NS}	0.39^{NS}
2nd					0.73^{c}	0.71^{c}
3rd						0.89^{c}

[a] $P < 0.07$; [b] $P < 0.05$; [c] $P < 0.01$; NS: not significant : $P > 0.07$.

A significant effect of the photoperiod (ie, stage of the experiment) on the duration of melatonin elevation was observed (mean ± SEM: 11.0 ± 0.1, 10.9 ± 0.1 and 11.9 ± 0.2 h for follicular phase, early luteal phase and late luteal phase, respectively, and 13.7 ± 0.2, 13.5 ± 0.2, 13.8 ± 0.2 and 12.8 ± 0.2 h for first, second, third and fourth months, respectively, $P < 0.05$). No individual effect was detected for this parameter (range: 12.0 ± 0.5 to 13.0 ± 0.6 h). Only the correlation coefficient between the follicular and early luteal phase was significantly different from zero: 0.83 ($P < 0.01$).

Figure 3 shows changes in plasma progesterone concentrations during the estrous cycle with respect to the time of determination of melatonin levels. Plasma progesterone concentration was low during the follicular phase (0.13 ± 0.00 ng/mL), increased during the early luteal phase (0.31 ± 0.05 ng/mL) and was high during the late luteal phase (3.91 ± 0.36 ng/mL). Correlation coefficients between progesterone levels during the estrous cycle and melatonin concentrations during the elevation at the different stages of the estrous cycle were low and not significantly different from zero.

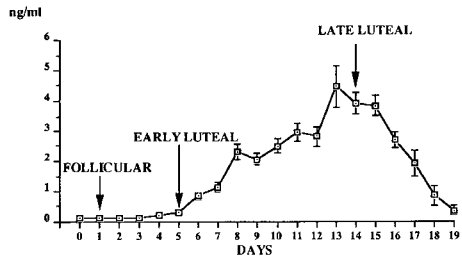

Fig 3. Plasma concentrations of progesterone (mean ± SEM) during the estrous cycle are shown in 13 Ile-de-France ewes. The periods when the melatonin determinations were carried out are indicated by the arrows.

DISCUSSION

These results demonstrate that melatonin concentrations studied by repeating measurements at seven different time periods in the same animals are not modified by the physiological stage of the animal; melatonin characteristics are not modified during the estrous cycle nor during pregnancy.

The results presented here concerning the effect of the stage of the estrous cycle during the breeding season on melatonin characteristics, confirm our previous observations on the absence of significant effects of administration of steroids (estradiol and progesterone) at concentrations typical of the estrous cycle and the absence of a significant effect of the stage of estrous cycle during seasonal anestrous (Zarazaga et al, 1996). We can conclude that melatonin characteristics are not modified by the stage of the estrous cycle in ewes, irrespective of the season (spring and autumn).

Concerning pregnancy, there is some evidence in the literature for increased function of the pineal gland compared with nonpregnant animals, which could suggest an increase in melatonin concentrations in peripheral blood. For example, morphological and histochemical changes of the pineal gland have been described during pregnancy: Pevet and Smith (1975) showed an increase in the quantity of Golgi apparatuses in the mole and Nesic and Kadic (1979) showed changes in the nuclear volume of pinealocytes in the ewe pineal gland. However, Huang and Everitt (1965) showed a decrease in pineal weight of rats with ten or more fetuses.

Concerning the melatonin secretion during pregnancy, Birau et al (1984) and Kivelä (1991), reported an increase in melatonin concentrations in humans, in the last trimester of pregnancy compared to the first and second trimester and Zimmermann et al (1989) observed an increase in melatonin concentrations at 30 weeks of pregnancy in contrast to 10 and 20 weeks. One study in pregnant ewes

found no normal nightime rise in the plasma melatonin levels (Kennaway et al, 1981). In the present study, all ewes had a clear diurnal rhythm in plasma melatonin concentrations during pregnancy, as is generally observed during other physiological stages. In contrast to the above observations in humans, Zemdegs et al (1987) and Yellon and Longo (1987) did not find differences in melatonin concentrations between two different times of the last trimester of pregnancy (120 vs 135 and 114 vs 142 days of pregnancy, respectively) in sheep. Our study extends these observations by showing that until d120 of pregnancy melatonin concentrations do not vary.

Melatonin secretion shows a very high inter-individual variability (Malpaux et al, 1987). It is possible that the lack of consistent results on the effect of the stage of estrous cycle or pregnancy on melatonin concentrations or pineal characteristics could have resulted from the inter-individual comparisons. Therefore, in the present experiment, we studied the influence of physiological conditions on the melatonin characteristics by repeating the measurements in the same individuals. Under these conditions, we found that melatonin concentrations at three critical stages of the estrous cycle and four stages of pregnancy were not different. These results suggest that melatonin secretion in the ewe is not influenced by reproductive physiological stage.

Regarding duration of melatonin secretion, it should be stated that the design of the present experiment was not fully adequate to monitor its variation during pregnancy, as the animals were maintained in open barns, and not in controlled photoperiodic conditions. The animals were maintained outdoors on a natural photoperiod from September to February, a period during which daylength changed from about 12.5 to 10.0 h. Therefore, the effects of the physiological conditions of the animal are potentially confused with effects of changing daylength.

In conclusion, the overall results and those of our previous study (Zarazaga et al, 1996), show that melatonin concentrations are not modified during the estrous cycle and demonstrate that the amplitude of the rhythm of melatonin secretion is not modified during pregnancy in ewes. Moreover, the high inter-individual effect detected on melatonin concentrations suggest that this parameter is an individual characteristic of each animal which may have a strong genetic basis.

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