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Original article

Amplitude of the plasma melatonin nycthemeral rhythms is not associated with the dates of onset and offset of the seasonal ovulatory activity in the Ile-de-France ewe

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Abstract — We investigated if absolute (nocturnal) or relative (nocturnal/diurnal ratio) plasma melatonin concentrations were associated with the seasonal ovulatory activity in Ile-de-France ewes. Ninety-six and 121 ewes in two different groups of the same flock were used to determine the potential existence of a relationship between melatonin concentrations at the summer and winter solstices, and the dates of onset and offset of the ovulatory activity, respectively. The dates of the first and last ovulations were estimated by assaying progesterone in plasma samples taken once weekly. Mean ± SEM (1) plasma melatonin concentrations at the summer solstice and at the winter solstice were 302.4 ± 19.4 and 412.0 ± 18.7 pg·mL^{-1}, respectively, (2) date of onset of the breeding season 29 Jul. ± 1.6 days, (3) date of offset of the breeding season 24 Jan. ± 2.2 days. In spite of a large variability in the different traits studied here, the analyses of correlation and regression indicated that neither the absolute nor relative melatonin concentrations were significantly related with the dates of onset or offset of ovulatory activity. Therefore, we concluded that absolute or relative plasma melatonin concentrations are not linked to the seasonal breeding activity, in Ile-de-France ewes.

sheep / melatonin / reproduction / seasonality / genetics

1. INTRODUCTION

Seasonality of reproduction is an important feature that limits productivity in small ruminants. In most breeds of sheep originating from high and mid latitudes, the onset of breeding activity occurs in the late summer or autumn when day length is decreasing, while the breeding season stops in the late winter or beginning of spring with increasing day length. This seasonal breeding cycle of the ewe is not directly driven by the

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photoperiod; rather it appears to be controlled by an endogenous circannual rhythm that is entrained by the photoperiod [1]. The reproductive response to photoperiod is mediated by the pineal gland via changes in the daily secretion of its main secretory product, melatonin [1–6]. In sheep, a sudden increase in melatonin release shortly after sunset and a decrease before sunrise, after a continuous nighttime secretion are observed, thus providing a permanently high plasma melatonin during the dark phase [7].

Two main alternative hypotheses have been proposed to explain how nycthemeral melatonin secretion controls the reproductive activity. In sheep and hamsters, the duration of the melatonin secretion versus the temporal position of this rise during the light:dark cycle has been considered [4, 8, 9 versus 10–12]. For each of them, the amplitude of the melatonin secretion (i.e. ratio or difference night/day) may play a specific role in modulating the response. It has been suggested, in sheep, that the nocturnal/diurnal melatonin ratio after implant insertion [13] or in natural conditions [14] could be related to the speed of the resumption of ovulatory activity. In the female Italian Buffalo a significant relationship between the mean nocturnal melatonin concentration and seasonal breeding activity has also been described: animals that show low seasonality, show reduced levels of melatonin concentrations at night [15]. However, whatever the mammalian species and especially in sheep, it is well established that nocturnal melatonin concentrations are highly variable among individuals [16–18], and highly repeatable within individuals [19], a large part of the between-individual variability being under a strong genetic control [20].

Thus, on the one hand, melatonin is a highly repeatable and heritable characteristic and, on the other hand, the dates of (a) the onset of the annual breeding season [21–23] and of (b) the offset of the annual breeding season are very variable among ewes but very repeatable within individuals [23]. Since these last two events (onset and offset of the breeding season) are controlled by changes in the photoperiod via the daily rhythm of melatonin, we tested here the hypothesis that the variability of these two traits (amplitude of melatonin and dates of breeding season) were linked.

2. MATERIAL AND METHODS

2.1. General

This experiment was carried out at the Institut National de la Recherche Agronomique, Research Center of Nouzilly, France (45° North) around the onset of the breeding season (1 June until 30 September, n = 96 ewes) and around the offset of the breeding season (1 December until 30 April, n = 121 ewes). The whole Ile-de-France flock from which the two separate groups of ewes which were subjected to the experimental measurements were obtained, is a large flock of about 600 animals divided into 6 different families. At regular intervals, sires are purchased from various private external flocks and are introduced to prevent inbreeding and maintain genetic connections with the national French scheme of genetic improvement of the Ile-de-France breed.

The experimental ewes were obtained from 399 ewes that had been used in a previous experiment in which the endogenous melatonin plasma concentration was determined at the June and December solstices [20]. The ewes were maintained under a natural photoperiod, were fed daily with hay, straw, and corn, and had free access to water and mineral licks. In order to obtain data which would eventually be useful for animal production purposes, we chose to use productive animals and, thus, we identified two separate groups of ewes, each group lambing once a year, after estrous synchronization. The first group (96 ewes) lambed in March and was used to measure the date of onset of the breeding season,

while the second group (121 ewes) lambed in October and was used to determine the date of offset of the breeding season. At both lambing seasons, weaning was performed after 2.5 months of suckling. More generally, the two groups were conducted under the same management conditions and were not subjected to any difference in selection procedures. No selection on seasonality was performed in the flock and melatonin measurements were performed in both cases after weaning.

2.2. Blood sampling

Blood samples were taken weekly at the same time of day (between 9:00 am and 11:00 am) by jugular venepuncture and they were assayed for progesterone during the two experimental periods (1 June until 30 September 1995 and 1 December until 30 April 1996). Confirmation of the ovulatory activity was obtained when two consecutive plasma samples showed higher progesterone concentration than the baseline (1 $ng \cdot mL^{-1}$) with a consecutive cyclicity. Cessation of ovulatory activity was considered when 3 or more consecutive plasma samples showed lower concentrations than the baseline. For each ewe, the date of the last plasma progesterone value below the baseline that was part of an extended cyclic pattern, was taken as the onset of ovulatory activity. The date of the last plasma progesterone value above the baseline at the completion of an extended cyclic progesterone pattern was taken as the offset of cyclicity.

Mean nocturnal melatonin plasma concentration of ewes was assessed on 4 plasma samples per ewe, taken at hourly intervals during the night (23:00–2:00 in June 1995, 22:00–1:00 in December 1995). Blood samples were obtained by venipuncture of jugular veins and were collected under dim-red light (less than 1 lux at 20 cm) avoiding any direct illumination of the eyes. Plasma was immediately separated by centrifugation and stored at –20 °C until assay (see explanations in [20]). Mean diurnal melatonin plasma concentrations of the ewes were assessed on four plasma samples per ewe used to determine the progesterone concentrations.

2.3. Radioimmunoassays

Plasma melatonin concentrations were estimated in duplicate aliquots of 100 microliters of blood plasma by radioimmunoassay using the technique described by Fraser et al. [24], with antibody first raised by Tillet et al. [25]. The sensitivity of the assay was 4 $pg \cdot mL^{-1}$. The intra and inter assay coefficients of variation were 12.1% and 2.7%, respectively.

Plasma progesterone concentrations were assayed by radioimmunoassay using the technique described by Terqui and Thimonier [26]. The sensitivity of the assay was 0.125 $ng \cdot mL^{-1}$. The intra and inter assay coefficients of variation were 3.4% and 16.6%, respectively.

2.4. Data analysis

Mean ± SEM were calculated for the dates of the onset and offset of the breeding season (beginning and end of ovulatory activity), absolute levels of plasma melatonin concentrations during the night and day period and relative plasma melatonin concentrations per ewe (night/day ratio). The variances were compared by an F test to detect the differences between seasons regarding the variability in the dates of the onset and offset of ovulatory activity [27]. Analysis of variance was used to test the existence of a difference in absolute or relative melatonin concentrations between solstices and between dates and animals within solstices. A χ^2 test was performed to determine the potential existence of a non-uniform distribution in the cumulative percentage of animals that started their breeding season and those which ended it. A correlation coefficient was calculated by the Spearman test, between the dates of onset and offset of the

breeding season and the night, day and relative plasma melatonin concentrations. Regression analysis was used to evaluate if the absolute or relative plasma melatonin concentrations could be a predictor of the dates of the onset and offset of the breeding season. Statistical analyses of data were performed using the SPSS package [28].

3. RESULTS

The mean date of the first ovulation of the breeding season was the 29 July ± 1.6 days ranging individually from 10 July until 18 September. The mean date of the offset of the ovulatory activity was the 24 January ± 2.2 days, ranging from 8 December until 12 March. The onset of the ovulatory activity was significantly less variable than the offset of the ovulatory activity ($P < 0.001$). Figure 1 shows the cumulative percentage of animals that started the breeding season or stopped it. The changes in the cumulative percentage of ewes starting their breeding season occurred significantly faster than a uniform distribution ($P < 0.001$). The distribution of the offset of the breeding season was not different from a uniform one.

The mean nocturnal plasma melatonin concentration was significantly lower at the summer than at the winter solstice and

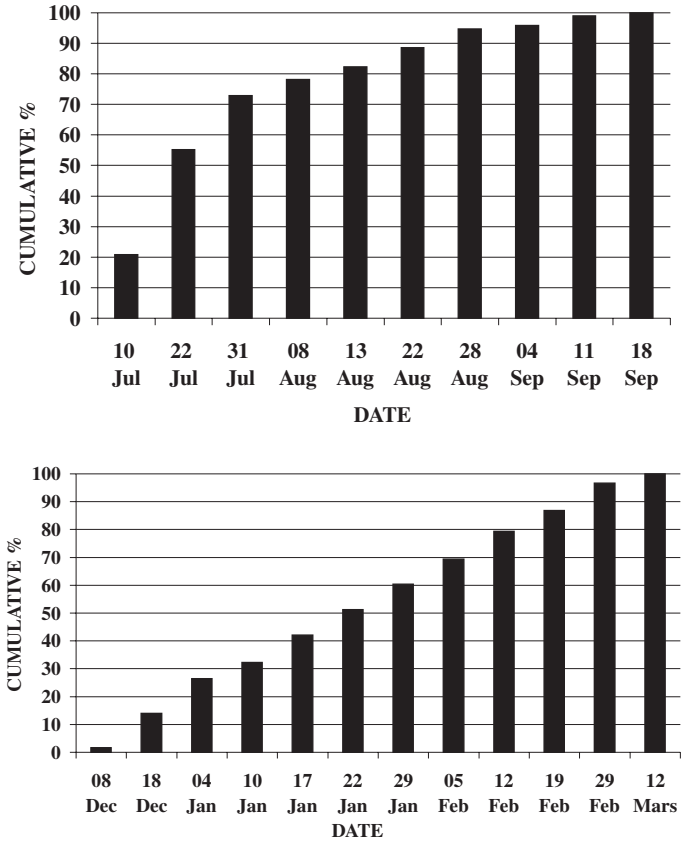

Figure 1. Distribution of the cumulative percentage of animals starting the breeding season (up; 95 ewes) and stopping it (down; 121 ewes).

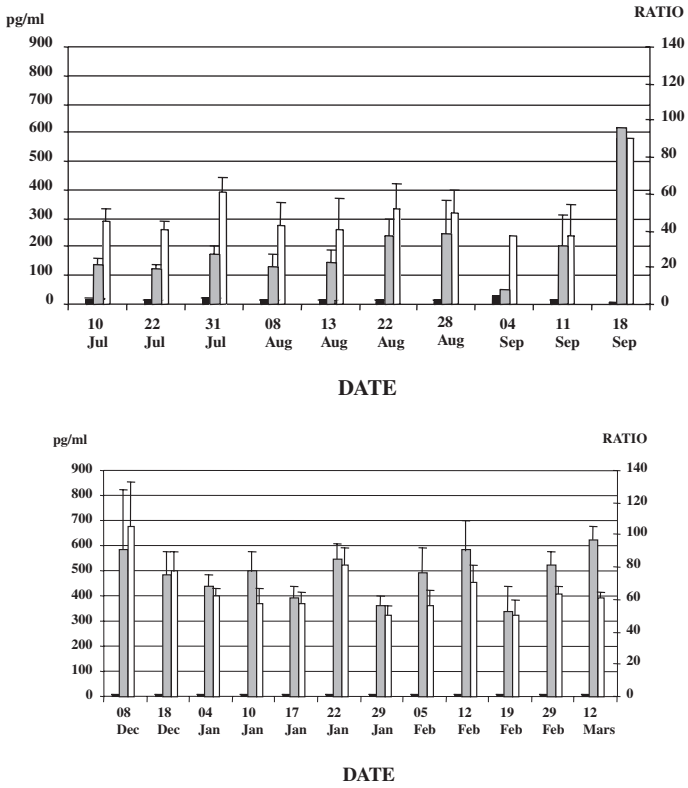

Figure 2. Mean (± standard error) plasma melatonin concentrations (pg·mL^{-1}) during the day (solid bar), during the night (shaded bar) and the ratio between night/day plasma melatonin concentrations (open bar) depending on the date that showed the first (up) or the last (down) elevation of plasma progesterone concentrations.

showed a very high variability among individuals (summer solstice 302.4 ± 19.4 pg·mL^{-1}, range 35 to 842 pg·mL^{-1} vs. winter solstice 412.0 ± 18.7 pg·mL^{-1}, range 61 to 981 pg·mL^{-1}, $P < 0.001$). The mean diurnal plasma melatonin concentration was low compared to nocturnal concentrations, and was significantly higher at the summer than at the winter solstice (summer solstice 14.5 ± 0.7 pg·mL^{-1}, range 4 to 27 pg·mL^{-1} vs. winter solstice 6.5 ± 0.4 pg·mL^{-1}, range 4 to 23 pg·mL^{-1}; $P < 0.001$).

Figure 2 shows the mean plasma melatonin concentrations during the day, during the night and the ratio between mean night and day plasma melatonin concentrations ranked according to the different dates that the ewes started or finished their breeding season. No differences in absolute or relative melatonin concentrations were observed between the dates of the first or last ovulatory activity.

Table I shows the Spearman coefficients of correlation between the interval between 1st June and the first ovulation, the interval between 1st December and the last ovulation, and the melatonin parameters at each solstice. All but one correlation coefficient

Table I. Spearman correlations between the onset of the breeding season (interval 1 June to date of the first ovulation; 95 ewes), the offset of the breeding season (interval 1 December to last ovulation; 121 ewes) and the mean night melatonin concentrations, mean day melatonin concentrations and ratio between night/day melatonin concentrations in the Ile-de-France ewe.

	Onset	Offset
Night Melatonin	0.097	–0.086
Day Melatonin	–0.101	–0.226*
Ratio N/D	0.182	0.072

*$P < 0.05$.

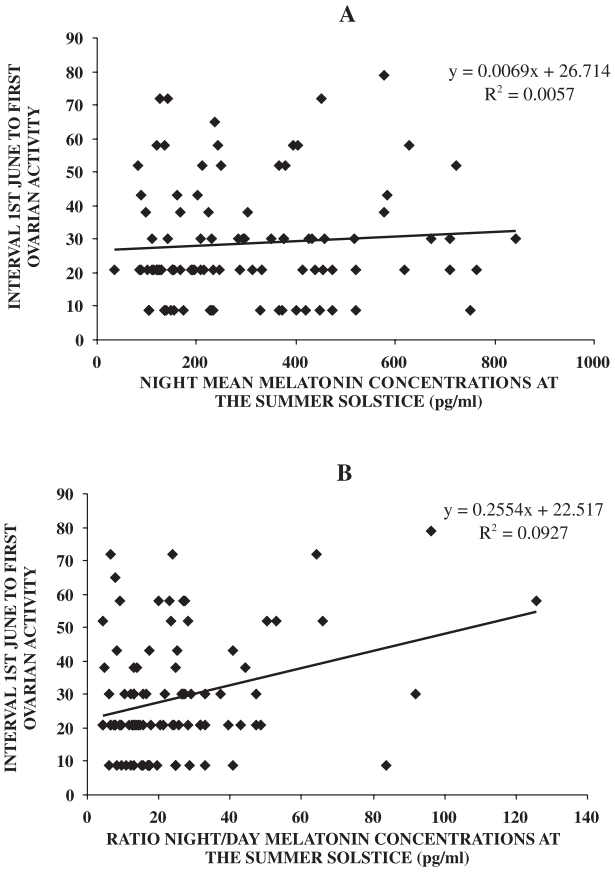

Figure 3. Analyses of regression between the interval from 1st June to first ovulation and the mean nocturnal melatonin concentrations (up) and the ratio between night/day plasma melatonin concentrations (down) at the summer solstice.

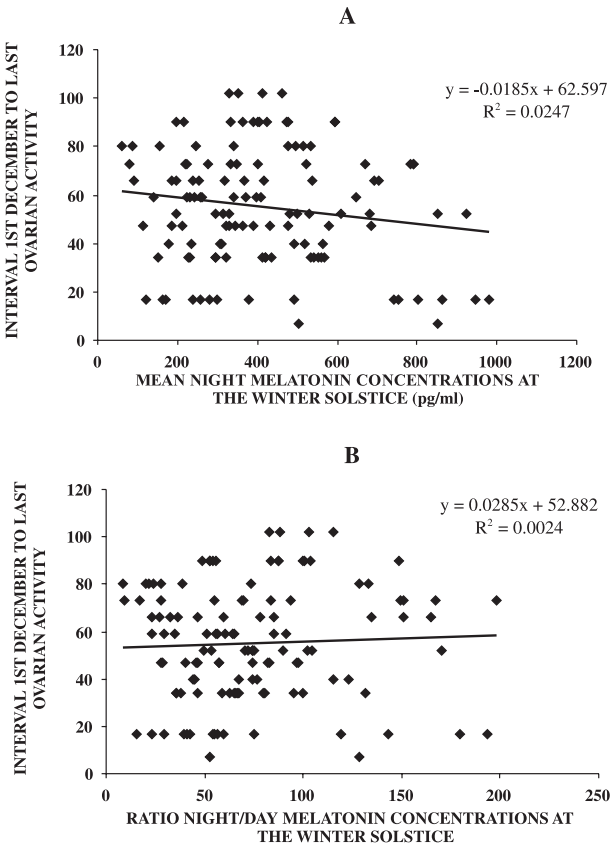

Figure 4. Analyses of regression between the interval from 1st December to last ovulation and the mean nocturnal melatonin concentrations (up) and the ratio between night/day plasma melatonin concentrations (down) at the winter solstice.

were not significantly different from zero; only a negative coefficient of correlation was observed between the plasma melatonin concentrations during the day period and the offset of the breeding season ($r = –0.226$, $P < 0.05$).

Figures 3 and 4 show the absence of a relationship between the interval 1st June–day of the first ovulation, or between the interval 1st December–day of the last ovulation, and the nocturnal melatonin concentrations or the night/day melatonin concentrations ratio at the June and December solstices, respectively.

4. DISCUSSION

In the present study, no relationship was observed between the variability in the onset and the offset of the annual breeding season and the variability in the plasma melatonin concentrations, either absolute or relative to the summer or the winter solstices, respectively.

The mean dates of onset and offset of the ovulatory activity and their range observed in this large set of Ile-de-France ewes are comparable to those found in previous studies performed in the same breed [29]. The

mean duration of the seasonal anestrus was 186 days. These variations of the reproductive activity were described for all breeds outside of the tropics showing increasing seasonality with latitude. In northern Europe, the duration of anestrus can last up to 260 days [30], while in southern Europe (Greece [31]; Spain [32]) or North Africa (Morocco [33]; Tunisia [34]), the mean duration of anestrus is limited to 50–130 days, and they could be considered like reduced seasonality breeds. As stated earlier, the onset of the breeding season appeared to be a less variable trait than its offset, probably because of the synchronization of the first ovulations among ewes due to entrainment among them [23, 29].

The mean plasma melatonin concentrations and their range, such as the change observed in the amplitude of the melatonin rhythm between seasons, with higher concentrations in December than in June, were also very comparable to those found in previous studies in the same breed [20]. These results are in agreement with those obtained by Malpaux et al. [17] suggesting that the seasonal change in amplitude reflects the action of non-photoperiodic environmental variables such as temperature. An alternative hypothesis is that light would exert a stronger inhibitory influence because of its probably higher intensity around the summer solstice; however, this possibility is not supported by the observation that diurnal melatonin concentrations were higher at the summer than at the winter solstice. This difference between solstices, could also be explained by the fact that different animals were studied at the two solstices. Because of the high variability between individuals, it could be possible that the difference between solstices originated in a difference between individuals. Thus, in spite of these minor differences, the two sets of ewes studied here could be considered as being representative, regarding the variability in plasma melatonin concentrations, of the general population of Ile-de-France ewes.

As exposed earlier, the individual dates of the onset and offset of the breeding season and the mean plasma melatonin concentrations are repeatable traits; it was thus interesting to know if these two types of traits were phenotypically linked or not. The present data clearly showed that these two types of traits are not linked and that the variability in plasma melatonin concentrations is independent from the variability in the dates of the onset or offset of the breeding season.

The absence of a correlation between the absolute or relative melatonin concentrations and the onset of the reproductive activity contrasts with three previous experiments in sheep and one in Buffaloes. In the first experiment done in sheep, using melatonin-treated Ile-de-France ewes after long day exposure, the interval between the insertion of melatonin implants and the onset of the ovulatory activity was positively correlated with the relative melatonin concentrations [13]. In the second one, performed in non-implanted ewes of a low seasonal breed (Rasa Aragonesa, which starts earlier and stops its breeding activity later compared to Ile-de-France sheep), maintained under a natural photoperiod, a high correlation ($r = 0.84$) between the night/day ratio and the date of the first estrous activity in non implanted ewes was found [14]. In the third experiment, selection for fertility following mating in the spring affects nocturnal melatonin secretion with lower melatonin plasma concentrations in ewes selected for higher spring fertility [35]. Altogether, these data suggest that ewes with reduced seasonality present a lower night/day plasma melatonin concentration ratio. Similarly, in Italian Buffaloes, low amplitude of night/day plasma melatonin concentrations is associated with a low seasonal ovulatory activity [15].

These three results seemed to associate an early onset or a low seasonality of the breeding activity to relatively low nocturnal

concentrations of endogenous melatonin; this constituted the rationale for the present experiment.

However this was not the case in the present study in which ewes which stopped their breeding season late, did not have reduced melatonin concentrations. This divergence with the results obtained in the initial studies [13, 14] may be attributable to the low number of animals generally used, which differed strongly with the present study. The large number of ewes used here at each solstice reinforces the idea that the variability in plasma melatonin and the dates of onset/offset of the breeding season are not linked.

On the contrary, the present results confirmed previous results obtained in mares in which melatonin amplitude varies, although not significantly, across seasons [36] without any association between this variability in amplitude and reproductive activity during the nonbreeding season [37].

The major influence of melatonin on the reproductive axis is exerted on the pulsatile secretion of LHRH/LH and this effect could be sufficient itself to explain the regulation of seasonality [4, 8]. One explanation of the absence of a relationship between the variability in plasma melatonin concentrations and variability in dates of the breeding season may be found in the route taken by pineal melatonin to control LHRH/LH pulsatility. Melatonin synthesized in the pineal gland is secreted directly into the cerebrospinal fluid (CSF) of the III Ventricle [38], from which it may diffuse to reach the premammillary hypothalamus where melatonin receptors controlling the LH pulsatile secretion are located [39]. According to this, the rest of the pineal melatonin flowing into the general circulation via the Galen vein would not be the one which controls the central effects of melatonin. Therefore it is possible that the main role of CSF melatonin may be the control of seasonality of reproduction, whereas the role of melatonin in the peripheral circulation may be to control other traits like moult, thermoregulation [16] and/or embryo survival [40], depending on the action of melatonin on peripheral rather than central receptors.

In conclusion, relative or absolute blood melatonin concentrations are not related with the onset or offset of the ovulatory activity in Ile-de France ewes.

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