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Plasma Melatonin and Progesterone Profiles of Suffolk and Romney Marsh Ewes Implanted with Melatonin during Anoestrus Season at Lower Latitudes in Southern Hemisphere

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ABSTRACT

***Background*:** The subcutaneous implants of melatonin are stimulatory and mimic the positive effects of short photoperiod on reproduction in small ruminants. This study investigated the daily plasma melatonin profiles in ewes treated with melatonin implants and kept under natural photoperiod in Southeastern Brazil. The plasma progesterone concentrations were also investigated before and after melatonin implantation.

***Materials, Methods & Results*:** Romney Marsh (n = 11) and Suffolk (n = 10) ewes, which had been isolated from rams for at least 2 months prior to the beginning of the trial, were randomly allocated in two groups based on melatonin implant treatment (with or without melatonin implant). For plasma melatonin concentration, 43 days after melatonin implantation and 3 days before the ram introduction blood samples were collected every 2 hours during 24 hours. For plasma progesterone concentrations, blood samples were collected every once to twice a week for 2 different periods: prior to melatonin implantation and 46 days after the melatonin implantation and at the same day of the introduction of rams. The hormonal concentrations were determined by the radioimmunoassay method (RIA). The data were analyzed according to MIXED procedure (SAS) as repeated measurements for random animal effects. The effect of melatonin treatment on plasma melatonin 24-h period varied according to the breed. At the dark-phase, there were no plasma melatonin differences ($P > 0.05$) between implanted and no-implanted (228.02 ± 58.39 vs. 169.59 ± 48.39) Romney Marsh ewes whereas for Suffolk ewes the plasma melatonin levels were higher in implanted (305.61 ± 68.39 pg/mL) than no-implanted (151.26 ± 38.35 pg/mL) ones. At the light-phase, melatonin treatment effects could be evidenced and these differences ($P < 0.01$) consisted of higher melatonin values for implanted ewes and basal values for no-implanted ones in both breed groups. Before the melatonin implantation, the plasma progesterone levels were < 1 ng/mL for Romney Marsh (0.41 ± 0.02 ng/mL) and Suffolk (0.47 ± 0.02 ng/mL) ewes. During the ram introduction period, no melatonin treatment effect was observed on plasma progesterone concentrations in both breed groups, but 2 days after ram introduction the plasma progesterone concentrations increased the mean values > than 1 ng/mL in implanted and no-implanted Suffolk ewes. In implanted Romney Marsh ewes the elevation of progesterone mean values was weak whereas in no-implanted Romney Marsh ewes the progesterone levels were maintained < 1 ng/mL during all the blood sample collection times.

***Discussion*:** The melatonin treatment also produced a similar model of daily melatonin levels as reported previously by others, which is characterized by high plasma melatonin concentrations during the light phase of the day. The effect of melatonin implants on plasma melatonin profiles interacted with breed confirming an individual response to melatonin implantation which is proportional to genetic individual variation pattern of melatonin secretion. Before the melatonin implantation all Romney Marsh and Suffolk ewes were judged to be in non-ovulatory period (anoestrus) with plasma progesterone mean values lower than 1 ng/mL. The melatonin treatment helped to induce the ovulatory activity in most of the ewes that were in anestrous at the time of melatonin implantation and the efficacy of this treatment depends on the individual variation in ovulatory response to ram introduction. In Southeastern Brazil., melatonin implant altered the daily plasma melatonin profiles of Suffolk and Romney Marsh ewes by increasing the melatonin levels during the light-phase of the day. Melatonin implant also induced an ovulatory response in Suffolk and Romney Marsh after the introduction of the rams. For no-implanted Suffolk ewes, the male effect is sufficient to provoke an ovulatory response.

Keywords: implant melatonin, male effect natural photoperiod, progesterone, sheep.

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INTRODUCTION

In sheep, melatonin is an important photoperiodic modulation of reproductive seasonality [20] and the annual cyclic reproductive activity is initiated by the photoperiodic signal induced by decreasing day-length [15,35]. Melatonin secretion is limited to the dark phase of the light:dark cycle and the duration of its daily secretion is proportional to the length of night [2,20]. The modification of the secretory pattern of melatonin in response to changes in day length transmits photoperiodic information to the hypothalamus-pituitary-gonad axis [36].

Effective mimicking of the temporal information transmitted to the reproductive axis by the progressive increase in the duration of the daily endogenous melatonin rhythm can be achieved by administration of exogenous melatonin [11,39]. It is well documented that subcutaneous implants of melatonin is stimulatory and mimic the positive effects of short photoperiod on reproduction in small ruminants [1,4,17,37].

Although there are some studies describing the characteristic of daily blood melatonin profiles [9,10,28] and also plasma progesterone concentrations [1] in ewes treated with melatonin implants, most of them were performed in high latitudes.

This study investigated the daily plasma melatonin profiles in ewes treated with melatonin implants and kept under natural photoperiod in Southeastern Brazil. The plasma progesterone concentrations were also investigated before and after melatonin implantation.

MATERIALS AND METHODS

Study conditions, locality, animals and management

The study was carried out during the period of September to December (spring and summer) at the Experimental Farm of the Faculty of Animal Science and Food Engineering (FZEA) - University of São Paulo (USP), Pirassununga, SP, Brazil. The city is located at 21°59 southern latitude and 47°26 western longitude from Greenwich at an altitude of 634 m. The climate is subtropical with average annual rain precipitation and temperature of 1,300 mm and 21°C, respectively [18]. The variation between the longest day (13.5 h) and the shortest day (10.5 h) of the year is only 2.6 h [7].

Twenty-one ewe Romney Marsh (n = 11) and Suffolk (n = 10) ewes, which had been isolated from rams for at least 2 months prior to the beginning of the trial, were used. The ewes were 1.2 ± 0.9 years old and the mean live weights of Romney Marsh and Suffolk females were 43.3 ± 0.2 and 47.5 ± 0.8 kg, respectively. To induce male effect, 3 vasectomized adult rams of hair Santa Inês breed with no annual variation in breeding behavior [29], were used during the study. All the animals, previously vaccinated and dewormed, were confined in stalls and received feeding based on commercial ration, grass hay (*Cynodon dactylon*) cost-cross cultivar, twice a day, according to the nutritional requirements for sheep [24], and water *ad libitum*.

The ewes from each breed were randomly allocated in two groups based on melatonin implant treatment (with or without melatonin implant). On 19 October, half of the females of each group (6 Romney March and 5 Suffolk ewes) received a single subcutaneous implant at the base of the left ear containing 18 mg melatonin (Melovine®)[1]. On 4 December (day 0), 46 days after melatonin implantation (day of implant removal), 3 aproned rams were introduced into the 2 groups to induce a ram effect [1].

Blood sampling and hormone assay

Blood samples were collected from the jugular vein contralateral to the site of implantation using vacuum tubes containing heparin as anticoagulant. Plasma was obtained by centrifugation and was stored at -20ºC, before assays to determine plasma concentrations of melatonin and progesterone.

On 01 December, 43 days after melatonin implantation and three days before the ram introduction samples for plasma concentrations of melatonin were collected every 2 h during 24 h. Blood collection during the dark phase was performed using a dim red light (<1 lux). Melatonin was assayed in duplicate 500-µL aliquots of plasma using the direct radioimmunoassay method of Frazier *et al*. [14], modified by English *et al*. [10]. Coefficients of variation were estimated by assaying 2 plasma pools (low and high concentrations of melatonin) of samples in the beginning and in the end of each assay (2 assays). The mean intra- and interassay for two plasma pools were 2.9% and 8.9%, respectively. The detection limit of the assay was 5 pg/mL.

For plasma progesterone concentrations, samples were collected at the same time in the

morning every once to twice a week for two different periods. The first period (Period 1) collection aimed to monitor the ovulatory status of ewes prior to melatonin implantation and lasted 18 days (beginning on 10 September and ended on 27 September). The second period (Period 2) collection was at the same day of the introduction of rams (Day 0) and 46 days after the melatonin implantation and lasted also 14 days (beginning on 4 December and ended on 21 December). Progesterone concentrations above 1 ng/mL were assumed to indicate the presence of corpora lutea and, therefore, ovulation but, if the concentrations were inferior to 1 ng/mL for a period superior to 10 days, the ewes were considered in anoestrus [23]. Plasma concentrations of progesterone were determined by the radioimmunoassay method (RIA), and samples were analyzed in duplicate using commercial kits (COAT-A-COUNT)2. The sensibility of the assay was 0.02 ng/mL and the inter- and intra-assay variation coefficients were 2.5 and 1.7%, respectively. This RIA assay was previously validated for sheep [22].

Statistical analysis

Values regarding plasma melatonin (pg/mL) were treated according to MIXED procedure [34] as repeated measurements for random animal effects, using Linear Mixed Models. The causes of variation were melatonin treatment (Implanted and No-implanted), breed (Romney Marsh and Suffolk), the time (hour) of sample collection or the 24 h-period (dark period and light- time) and the interaction between them. The same model was used for plasma progesterone with the following causes of variation: the melatonin treatment, breed, the day of sample collection and their interactions.

RESULTS

The statistical model revealed that the melatonin treatment affected significantly the daily plasma concentrations of melatonin of Suffolk and Romney Marsh ewes. The effect of melatonin treatment on plasma melatonin 24-h period varied according to the breed (Figure 1). There was no melatonin implantation effect on 24-h plasma melatonin concentrations in Romney Marsh ewes whereas the plasma melatonin levels were affected by melatonin implantation in Suffolk ewes with higher melatonin profiles during the light-phase of the day. In no-implanted Romney Marsh and Suffolk ewes the amount of nocturnal melatonin detected was related to the extent of the dark-phase of day.

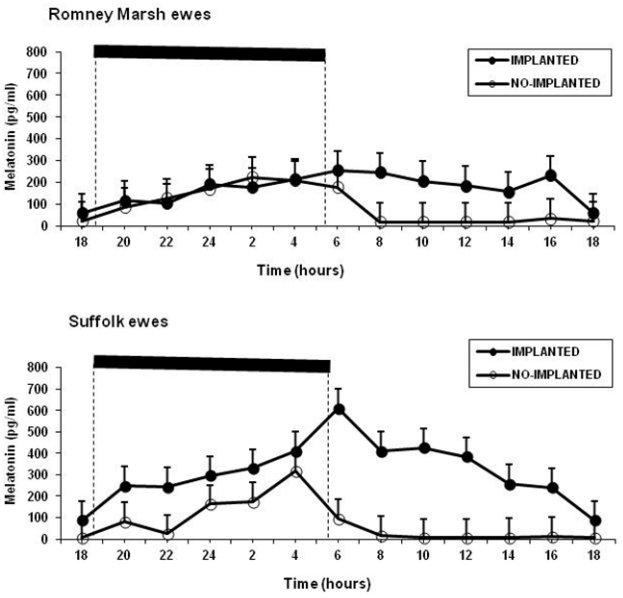

Figure 1. Plasma melatonin concentrations (mean ± S.E.M.) in implanted and no-implanted Romney Marsh and Suffolk ewes determined at 2-hs intervals during a 24-hs period at 43 days after melatonin implantation. The dark-phase of the day is indicated in upper panel. *$P < 0.01$.

When the amount of melatonin detected during the light- and dark-phases was evaluated separately for Suffolk (Table 1) and Romney Marsh (Table 2) ewes, the interaction between melatonin treatment and the period of the day was also significant. At the dark-phase, there were no plasma melatonin differences ($P > 0.05$) between implanted and no-implanted (228.02 ± 58.39 vs. 169.59 ± 48.39) Romney Marsh ewes whereas for Suffolk ewes (Table 1) the plasma melatonin levels were higher in implanted (305.61 ± 68.39 pg/mL) than no-implanted (151.26 ± 38.35 pg/mL) ones. At the light-phase, melatonin treatment effects could be evidenced and these differences ($P < 0.01$) consisted of higher melatonin values for implanted ewes and basal values for no-implanted ones in both breed groups (Tables 1 and 2). Considering the breed factor, in implanted and no-implanted animals the plasma melatonin mean values during the dark-phase were significantly higher than those values observed during the light-phase for Romney Marsh ewes (Table 2) but not for Suffolk ones (Table 1).

Table 1. Plasma melatonin concentrations (mean ± SEM) during the light-phase and the dark-phase of 24-h-period in melatonin implanted or no-implanted Suffolk ewes kept under natural photoperiodic conditions at lower latitudes.

Experimental groups	24-h Periods	
	Light-phase	Dark-phase
Implanted	344.87 ± 32.44^{Aa}	305.61 ± 38.39^{Aa}
No-Implanted	20.48 ± 32.44^{Bb}	151.26 ± 38.39^{Ba}

Values with different superscripts within the same column (A,B) or within the same row (a,b) are significantly different ($P < 0.05$) by Chi-square test.

Table 2. Plasma melatonin concentrations (mean ± SEM) during the light-phase and the dark-phase of 24-h-period in melatonin implanted or no-implanted Romney Marsh ewes kept under natural photoperiodic conditions at lower latitudes.

Experimental groups	24-h Period	
	Light-phase	Dark-phase
Implanted	145.77 ± 45.90^{Ab}	228.02 ± 58.39^{Aa}
No-Implanted	42.64 ± 32.44^{Bb}	169.59 ± 48.39^{Aa}

Values with different superscripts within the same column (A, B) or within the same row (a,b) are significantly different ($P < 0.05$) by Chi-square test.

The plasma progesterone data collected before the melatonin implantation (11 to 27 September) revealed that no breed differences ($P > 0.05$) were observed during this period and the mean values were 0.41 ± 0.02 ng/mL and 0.47 ± 0.02 ng/mL for Romney Marsh and Suffolk ewes, respectively. The Figures 2 and 3 represent the plasma progesterone concentrations 46 days after melatonin implantation and at the same day (Day 0) of ram introduction. During the ram introduction period, no melatonin treatment effect was observed on plasma progesterone concentrations in both breed groups (Figure 2). At the same day and 2 days after ram introduction, the means of plasma progesterone were lower than 1 ng/mL in implanted and no-implanted Romney Marsh (Figure 2A) and Suffolk (Figure 2B) ewes, but after this the plasma progesterone concentrations increased the mean values above than 1 ng/mL in implanted and no-implanted Suffolk ewes (Figure 2B). In implanted Romney Marsh ewes the elevation of progesterone mean values was weak whereas in no-implanted Romney Marsh ewes the progesterone levels were maintained < 1 ng/mL during all the blood sample collection times (Figure 2A). Considering progesterone breed differences, among implanted ewes (Figure 3A), no breed differences on plasma progesterone profiles were observed along the period of blood samples collection, exception in day 9 of ram introduction which plasma progesterone concentrations were higher ($P < 0.05$) in Suffolk than Romney Marsh ewes. Among no-implanted ewes (Figure 3B) after nine days of ram introduction, the Suffolk ewes showed a higher pattern of plasma progesterone concentration with mean values > 1 ng/mL.

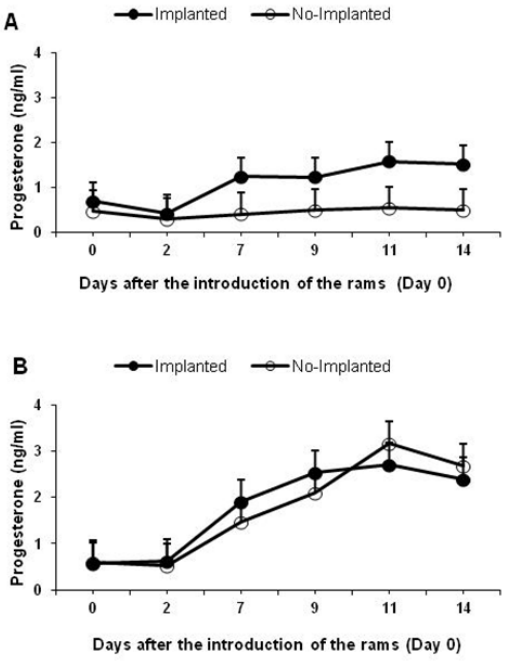

Figure 2. Plasma progesterone concentrations (mean ± S.E.M.) in implanted and no-implanted Romney Marsh (A) and Suffolk (B) ewes determined at the same day of the introduction of the rams (Day 0) and 46 days after melatonin implantation. $P > 0.05$.

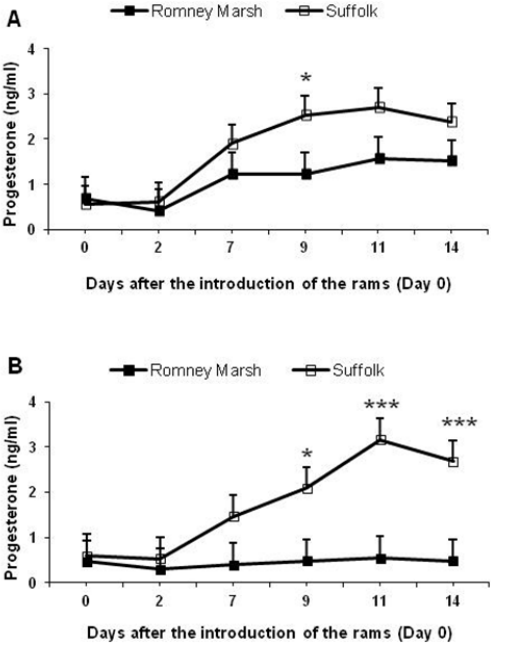

Figure 3. Plasma progesterone concentrations (mean ± S.E.M.) in implanted (B) and no-implanted (B) Romney Marsh and Suffolk ewes determined at the same day of the introduction of the rams (Day 0) and 46 days after melatonin implantation. *$P < 0.05$; ***$P < 0.001$.

Regarding to plasma progesterone concentrations of individual implanted and no-implanted Suffolk (Figure 4) and Romney Marsh (Figure 5) ewes, four of five implanted and all of no-implanted Suffolk ewes showed a significant increased of progesterone levels > 1 ng/mL (Figure 4). Progesterone mean values above 1 ng/mL were observed only in three of six implanted Romney Marsh ewes. No elevation of plasma progesterone concentrations was observed in no-implanted Romney Marsh ewes (Figure 5).

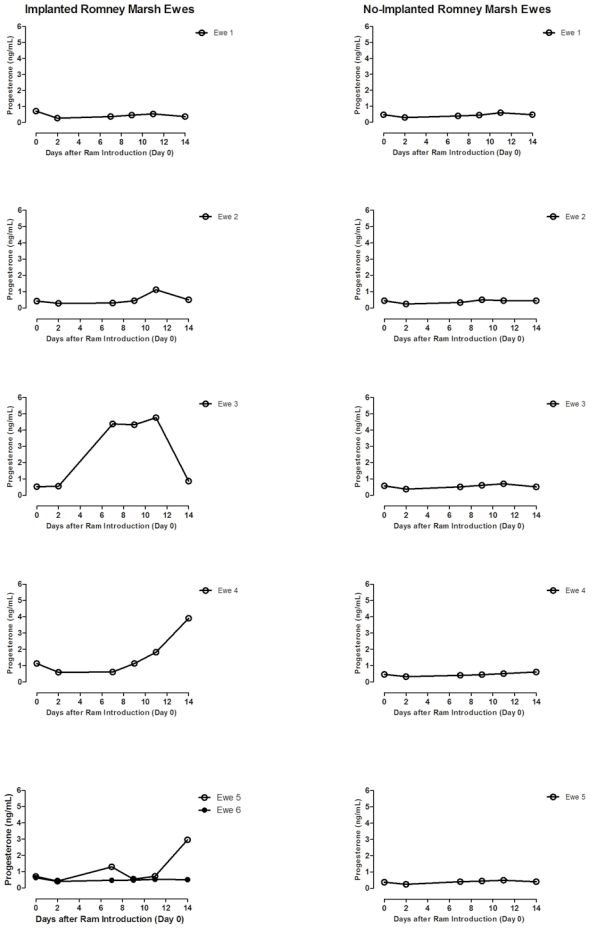

Figure 4. Individual plasma progesterone concentrations in melatonin implanted and no-implanted Suffolk ewes determined at the same day of the introduction of the rams (Day 0) and 46 days after melatonin implantation.

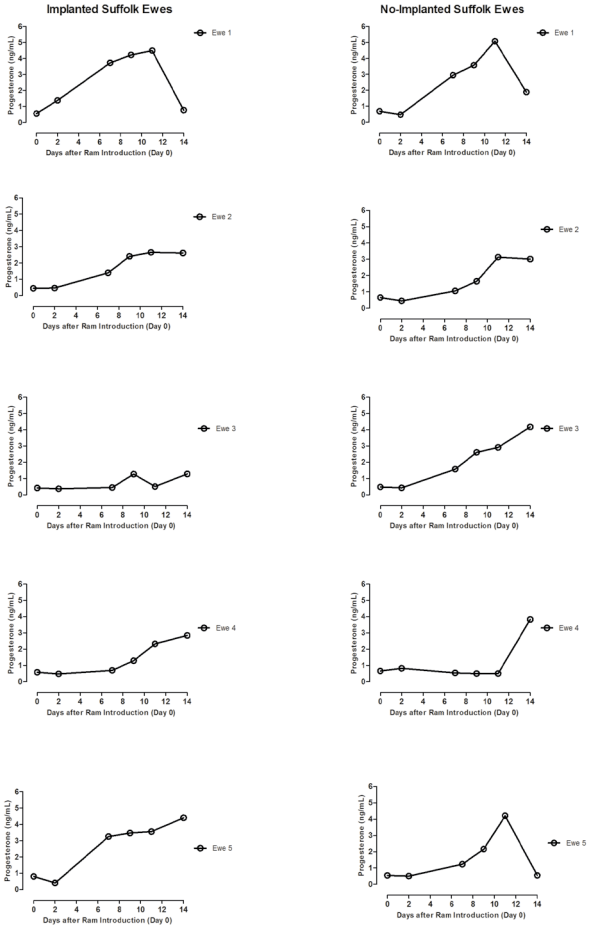

Figure 5. Individual plasma progesterone concentrations in melatonin implanted and no-implanted Romney Marsh ewes determined at the same day of the introduction of the rams (Day 0) and 46 days after melatonin implantation.

DISCUSSION

The present study describes the daily changes in plasma concentrations of melatonin in ewes living under natural light conditions at 21o59'S which received melatonin implants during the spring season. This study also describes the plasma progesterone concentration before and after melatonin implantation.

The daily plasma melatonin pattern in Romney Marsh and Suffolk no-implanted ewes seems to be the same of those raised under natural or artificial conditions in lower, intermediate or higher latitudes in southern or northern hemispheres [2,16,21,30]. The melatonin treatment also produced a similar model of daily melatonin levels as reported previously by others (9, 12,13,17,28,33,39], which is characterized by high plasma melatonin concentrations during the light phase of the day.

In the present study, the effect of melatonin implants on plasma melatonin profiles interacted with breed. In Suffolk implanted ewes there was no difference between dark-phase and the light-phase periods on plasma melatonin levels, but the exogenous melatonin from implant was additive to the endogenous dark-phase production (Table 1). In Romney Marsh implanted ewes the plasma melatonin levels were higher during the dark-phase than the light-phase of the day, as reported previously in literature [2,9,13,26]. However, the implanted

Romney Marsh ewes showed a weak response to melatonin implantation by increasing the plasma melatonin profile during the dark-phase (Table 2). In those animals the effect of melatonin implantation inducing a significant high melatonin levels was observed only the light-phase of the day. In general, the alterations of the 24-h blood melatonin profiles by melatonin treatment also seem to be varied according to the breed (Figure 1). The present findings do not allow definitive conclusions regarding breed differences on daily melatonin profiles in response to melatonin implantation because only 5 to 6 animals per breed were studied but, they do suggest a individual response to melatonin implantation which is proportional to genetic individual variation pattern of melatonin secretion [25,38]. In fact, it has been demonstrated a repeatable individual variability on amplitude and duration of the plasma melatonin concentration melatonin in ewes [6], but the plasma concentrations of melatonin are quite stable within the same individual [19].

The results regarding plasma progesterone concentrations showed that before the melatonin implantation all Romney Marsh and Suffolk ewes were judged to be in non-ovulatory period (anoestrus) with plasma progesterone mean values lower than 1 ng/mL. Peripheral progesterone concentrations have been used to monitor the cyclic reproductive activity in sheep [5,22,23,39]. Ewes were judged to be in anovulation if plasma progesterone concentration never exceeded 1 ng/mL for more than 2 consecutive samples or during a 10-d period [22,23]. After 7 days of ram and 46 days after melatonin implantation, an increase in plasma progesterone mean values above 1 ng/mL was observed in implanted Romney Marsh and implanted and no-implanted Suffolk ewes. In spite of the implanted Romney Marsh ewes showed an elevation of progesterone mean levels, this increase was more pronounced in implanted Suffolk ewes. This event probably happened because 3 of 6 implanted Romney Marsh ewes whereas 4 of 5 implanted Suffolk ewes showed an ovulatory response with progesterone levels above 1 ng/mL. Our results suggest that melatonin treatment helped to induce the ovulatory activity in most of the ewes that were in anestrous at the time of melatonin implantation. It was also clear that efficacy of melatonin treatment in advancing the reproductive activity depends on the individual variation in ovulatory response to ram introduction. In fact, there is evidence that the melatonin treatment modifies the response of male effect in ewes [1] and goats [4,37]. Abecia *et al*. [1] reported that the proportion of ewes exhibiting ovarian activity in response to introduction of ram after melatonin treatment was 42%. Others have observed ovarian activity 66 days [27] and 77 days [8] after the onset of melatonin treatment. In the present study, the plasma progesterone concentrations to monitoring the ovulatory activity were evaluated only 60 days after the beginning of melatonin treatment.

On the other hand, all the 5 no-implanted Suffolk ewes presented an ovulatory response by increasing the plasma progesterone concentrations suggesting that for these ewes the male effect without melatonin treatment is sufficient to induce the ovulatory activity. The introduction of male to previously separated anovulatory females can induce female estrous activity [31] and this strategy is an important component of reproductive management to anticipate the breeding season under field conditions at lower latitudes [32]. It has been demonstrated that the efficacy of the ovulatory response to male effect varied according to the degree of reproductive seasonality of the ewe [31]. In our previous studies, it was indicated that the breeding season of Romney Marsh ewes was more restricted than the breeding season of Suffolk ones [7,29]. In the present study, it seems that no-implanted Suffolk ewes appear to have a slight anoestrus [31] and could respond to introduction of rams by increasing the plasma progesterone concentrations.

In conclusion, under natural lighting at 21o59'S, melatonin implant altered the daily plasma melatonin profiles of Suffolk and Romney Marsh ewes by increasing the melatonin levels during the light-phase of the day. Melatonin implant also induced an ovulatory response in Suffolk and Romney Marsh ewes by increasing the plasma progesterone concentrations after the introduction of the rams. For no-implanted Suffolk ewes, the male effect is sufficient to provoke an ovulatory response with progesterone levels above to 1 ng/mL.

MANUFACTURERS

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***Ethical approval*.** All the procedures were performed in accordance with local Ethics Committee for Animal Experiments (CEUA No. 613818115) and Brazilian guidelines for the protection of experimental animals (CONCEA).

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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