Short Communication

Effects of chronic melatonin administration on pregnancy rate in dairy cows submitted to a fixed-time AI protocol during summer and winter seasons

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Abstract

The aim of this study was to assess the effect of chronic melatonin treatment before calving in summer (long-day photoperiod, LDP, 16 h of light and 8 h of darkness) or winter (short-day photoperiod, SDP, 8 h of light and 16 h of darkness) on pregnancy rate after calving in lactating dairy cows. Before calving 60 cows were treated with melatonin and another 60 cows were not treated with melatonin thus functioning as a control group. In each group, 30 cows were treated during summer and 30 during winter. At 60 days after calving, all animals were submitted to a cosynch-ovsynch plus progesterone protocol and had fixed time artificial insemination (FTAI). The pregnancy rate at day 30 after FTAI was no different between groups (50% and 48% in treatment and in control groups, respectively, P = 0.66). Pregnancy losses during the first trimester of gestation (day 90 after FTAI) were 10% in cows treated with melatonin and 6% in the control group (P = 0.35). Considering the season of treatment there were no differences in either pregnancy rate (P = 0.70) or embryonic losses (P = 0.30) between the treatment and control groups. The results of this study indicate that in dairy cows submitted to a cosynch-ovsynch plus progesterone protocol and FTAI, the treatment with melatonin before calving did not compromise either pregnancy rate or embryo losses in both summer and winter.

Keywords: dairy cow, melatonin, pregnancy rate, season

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Introduction

Seasonal infertility has dramatically increased during the last decades (Lopez-Gatius *et al.* 2003; Pszczola *et al.* 2009) and is likely to continue to rise. Global climate changes cause heat stress in dairy cows and this will become more severe in the future (Battisti *et al.* 2009). During summer in most countries of the northern hemisphere, like Italy, the average temperature goes above 25.0 °C, inducing heat stress in dairy cows that compromises fertility (De Rensis *et al*. 2003; Zimbelman *et al.* 2009; De Rensis *et al.* 2017). Furthermore, besides heat stress, the increased photoperiod during summer also impairs fertility (Dahl *et al.* 2000). Melatonin, a hormone synthesized during darkness by the pineal gland, has a multifactorial effect on animals. Melatonin modulates circadian rhythm (Gilette *et al.* 1999) and the immune system, affects milk yield (Auchtung *et al.* 2005; Velasco *et al*. 2008; Lacasse *et al.* 2014) and growth (Dahal *et al.* 2003) and is involved in follicle development, in the control of oocyte maturation and promotes embryo development (for review see Reither *et al.* 1991; Tamura *et al.* 2009; Tian *et al.* 2014). Furthermore, melatonin is also a powerful free radical scavenger and indirect antioxidant and cytoprotective agent during pregnancy in humans (Tamura *et al*. 2012; Manchester *et al.* 2015). Therefore, administration of melatonin can be a potential tool to boost the cow's immune system, counteract the immuno-depression of the peripartum period and reduce the negative effect of heat stress on fertility in dairy cows. Melatonin supplementation during late lactation reduces prolactin levels and milk yields in grazing cattle (Auldist *et al.* 2007) but there is a limited amount of research in this area involving bovines. In buffaloes, melatonin and melatonin receptors are involved in the control of reproduction (Carcangiu *et al*. 2011; Wang *et al.* 2014; Gunwant *et al.* 2018) and melatonin treatment in buffalo stimulates reproductive activity (Ghuman *et al*. 2010; Kumar *et al.* 2014; Ramadam *et al.* 2014; Kavita *et al.* 2018), increases the number of buffalo cows in oestrus and conception rate during the low breeding season (Kumar *et al.* 2015; Kavita *et al*. 2018). When melatonin treatment is applied during the pre-partum period during the warm season in cows under heat stress conditions, a reduction in embryonic losses has been observed (Garcia-Ispierto *et al.* 2013). The pre-partum period is a period that can be critical for the resumption of cyclic ovarian activity after calving. There is data showing the heat stress during the last trimester of pregnancy can have after calving and the resumption of the complete ovarian activity is significantly delayed with a deleterious effect on fertility (Alves *et al*. 2014). This is because the time that antral follicles take to grow into large dominant follicles and ovulate is approximately 40-50 days (Roth *et al*. 2001). There is also evidence that suggests that factors like backfat thickness during the last trimester of gestation, may influence reproductive activity after calving (Wetteman *et al.* 2003). Thus, administration of melatonin before calving may reduce the effect of heat stress on the antral follicle destined to ovulate 40-50 days later and can increase first service conception rates. Therefore, the aim of this study is to investigate the effect of melatonin treatment on pregnancy rate and embryonic losses during summer, a period of the year in which the photoperiod is increased and cows are under heat stress or winter, when the photoperiod length is short, and cows are not under heat stress.

Materials and Methods

Animals and herd management: This study was performed in a commercial Holstein-Friesian dairy herd in Northern Italy (45°41′56″04 N 09°40′12″00 E). During the study period, the mean number of lactating cows in the herd was 500 and mean annual milk production was 11,300 kg per cow. The annual culling rate was 25%. Cows were milked twice daily and fed complete rations. Herd management included the use of a pedometer system for oestrus detection and fans and water sprinklers, which were activated automatically when the maximum temperature-humidity index (THI) ≥ 65. The inclusion criteria were utilized to reduce the variability between animals as much as possible. Only reproductive healthy cows (parity numbers between 1 and 3 and BCS 3.0-3.5 on 5 scale system) free from any pathological conditions were selected for study. Exclusion criteria were the following pathologies, i.e., mastitis, lameness, digestive disorders and post-partum disorders of the reproductive tract. A monthly mean of the maximum temperature > 25ºC and/or of the maximum THI > 72 was the threshold to consider the presence of heat stress conditions in the herd (García-Ispierto *et al*. 2007; Zimbelman *et al.* 2009). The cows were milked twice daily at 6:00 AM and 6:00 PM and milk yield was recorded 10 days before dry-off and before FTAI. The feeding of the herd was almost the same throughout the year, with variations in the different groups according to the lactation phase in which the cows were or if they were dry cows or primiparous. This was based on a unifeed diet with a mix of corn silage, wholemeal mash, alfalfa hay, whole cotton seeds, wheat silage, corn flour, nucleus proteins, glycerol, mineral and vitamin supplements and the levels of urea in milk was observed monthly during the study; the value fluctuated between a minimum of 21 and a maximum of 24 mg/dl with an average value of 22.33 mg/dl, calculated over the 9 months considered. Artificial illumination inside the barn was ensured by a sufficient number of neon lamps providing a visibility of about 200 lux, while the ignition and shutdown of the plant was controlled by an automatic system connected to a twilight sensor that was deactivated at the end of daily operations that ended around 8.00 PM. The same veterinarian routinely performed all gynaecological examination and pregnancy diagnoses.

Experimental design and treatment: One hundred twenty cows were utilized in this study and were assigned to 2 groups: melatonin group (the animal receiving melatonin treatment, n = 60) and the control group (without treatment; n = 60). Based on the season of insemination in each group, the cows were further divided into 2 sub-groups of 30 cows: summer group (cows were inseminated between June and August (long-day photoperiod, LDP, 16 hr of light: 8 hr of darkness) and winter group (cows were inseminated between December and February, short-day photoperiod, SDP, 8 h of light: 16 h of darkness). The cows in the melatonin group were treated with Melatonin (Melovine®, CEVA Salud Animal, Barcelona, Spain) administered as 12 slow-releasing subcutaneous implants before calving (day 220 of gestation, Garcia-Ispierto *et al.* 2013). Each implant contained 18.0 mg of melatonin and was administered using an implanter (CEVA Salud Animal, Barcelona, Spain) to an area of free moving skin between the thighs, dorsal to the base of the udder. The dosage per cow had been proved to significantly increase plasma melatonin concentrations three to five times compared untreated animals (Sanchez-Barcelo *et al.* 1991; Garcia-Ispierto *et al.* 2013). The cows in the control group were not treated with melatonin. Mean number of lactations (mean ± SD) at FTAI was 2.8 ± 1.2 and 2.7 ± 1.1 for treated and control cows, respectively. To reduce the variability in the calving to the first insemination interval, and to have a 100% of submission rate, all animals were submitted to a synchronization and FTAI protocol (Figure 1). The protocol consists of a classic presynch and ovsynch plus progesterone protocol followed by FTAI: all cows have been presynch with two doses of PGF2α administered twelve days apart starting at day 33 after calving. Then, 11 days after presynch, it was administrated GnRH (100 µg intramuscularly Gonadoreline acetate; Cystoreline®, CEVA Salute Animale, Agrate Brianza, Italy) and a progesterone-releasing intravaginal device (PRID-DELTA, containing 1.55 g of progesterone; CEVA Salute Animale, Agrate Brianza, Italy) implanted. The PRID was removed 7 days later and two doses of PGF2α (8.0 mg Alfaprostol i.m.; Gabbrostim®, CEVA Salute Animale, Agrate Brianza, Italy) were administered 24 hr apart. Thirty-two hours after the last PGF2α administration, a second GnRH administration was given and the cows were artificial inseminated (FTAI) 16-22 hr later. With this protocol an average of 30% of pregnancy rate at FTAI insemination was expected (De Rensis *et al.* 2015). The same veterinarian inseminated all cows with frozen-thawed semen from a single ejaculate. If a cow after FTAI returned to oestrus, she was re-inseminated. The pregnancy rate was evaluated by ultrasound 28-30 days after FTAI. To determine embryo losses during the first trimester of pregnancy, pregnant cows were rechecked for pregnancy by rectal palpation 90 days after FTAI. Each cow was included only once in the study.

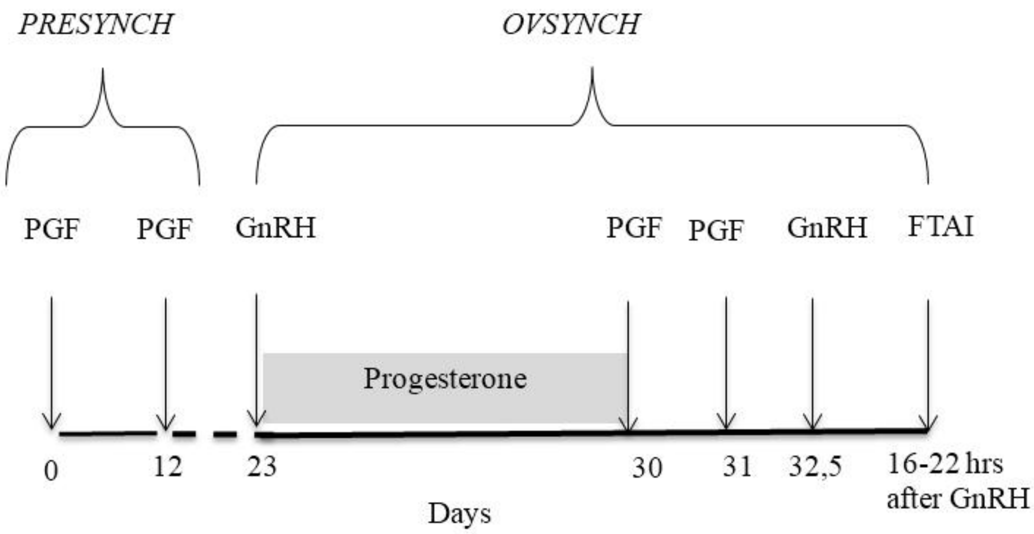

Figure 1 The Fixed Time Artificial Insemination Protocol (FTAI) utilized in the study. The protocol started at day 33 after calving.

Statistical analysis: The statistical analyses were carried out using SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA). The main outcome of the study was pregnancy rate at day 28-30 after FTAI and embryonic losses during the first trimester of pregnancy. The data was recorded for each animal: treatment (control and melatonin), season (summer or winter), pregnancy rate at day 28-30 after FTAI and embryonic losses during first trimester of gestation (i.e., day 90 after FTAI). The data was analysed using logistic regression analyses using GENMOD procedure of SAS. The statistical models included treatment (control and treatment), season (summer and winter) and interaction between treatment and season. The goodness-of-fit of the statistical model was around 1.0 indicating that the sample size was appropriate for the analysis. Least square means were obtained from each class of the factors and were compared by using least significant difference test. The effects of treatment and season on milk production of cows were analysed using multiple analysis of variance using general linear model (GLM) procedure of SAS. Least square means were obtained from each class of the factors and were compared by using a least significant difference test. A value with $P < 0.05$ was regarded to be statistically significant.

Results and Discussion

From June to August (summer period with LDP), mean monthly a maximum temperature was higher than 25 ºC and maximum THI were higher than 72. Both values are indicative of heat stress conditions in the herd. Body condition score at FTAI (mean ± SD) was 2.36 ± 0.17 and 2.41 ± 0.22 for treatment and control groups, respectively. Mean number of lactations at treatment was 2.8 ± 1.2 (mean ± SD). Mean milk production at the time of FTAI was 42.2 ± 1.1 kg and 42.9 ± 0.9 kg for the treatment and control groups, respectively, during summer, and 44.9 ± 1.1 kg and 44.8 ± 0.9 kg for the treatment and control groups, respectively, during winter. Neither treatment nor season had an effect on milk production ($P > 0.05$). Considering the treatment, no difference ($P = 0.66$) in pregnancy rate (50% and 48% in melatonin and in control animals, respectively) was detected and there was no interaction between treatment and season. Embryonic losses during the first trimester of gestation were no different between treatments (10% and 6% in the treatment and control groups, respectively, $P = 0.35$).

Table 1 Number of lactations, body condition score (BCS) and milk production at fixed-time artificial insemination (FTAI), pregnancy rate at 30 days after FTAI and embryonic losses at 90 days after FTAI in dairy cows treated before calving with melatonin (treatment) or not treated (control) and inseminated after a cosynch-ovsynch plus progesterone protocol and FTAI (means ± SD).

Variables	Control	Treatment	P value
Number of lactations	2.7 ± 1.1	2.8 ± 1.2	NS
BCS	2.40 ± 0.22	2.36 ± 0.17	NS
Milk production (kg)			
Summer	42.9 ± 0.9	42.2 ± 1.1	NS
Winter	44,9 ± 1.1	48.8 ± 0,9	NS
Pregnancy rate (%)			
Summer	14/30 (46%)	14/30 (46%)	
Winter	15/30 (50%)	16/30 (53%)	
Total	29/60 (48%)	30/60 (50%)	0,66
Embryonic loss (%)			
Summer	1/14 (7%)	2/14 (14%)	
Winter	1/15 (6%)	1/16 (6%)	
Total	2/29 (6%)	3/30 (10%)	0,35

In this study we expected to observe the effect of melatonin treatment on pregnancy rate and embryonic losses during the first trimester of gestation in cows. In fact, a previous observation in humans indicated that melatonin antioxidant effects can counteract recurrent pregnancy loss (Simsek *et al.* 1998) and can induce inhibition of prostaglandin synthesis (Tamura *et al.* 2012; De Rensis *et al.* 2015). This can improve placental well-being (Milczarek *et al*. 2010). Moreover, in rats, pinealectomy increases the frequency of spontaneous abortions. When melatonin treatment has been applied in dairy cows, pregnancy rate was improved from 29.6% to 55.6% (Yildz 2015). Our result did not show any effect of melatonin on pregnancy rate. However, there are substantial differences between our study and the previous study. In our study, the cows were submitted to a cosynch-ovsynch plus progesterone protocol and FTAI while in Yildz *et al.* (2015), study, the cows were inseminated after detecting oestrus naturally without FTAI. Furthermore, in Yildz *et al.* (2015),' study, melatonin treatment was applied after calving. In a previous study, pregnancy losses were reduced after melatonin treatment before calving from 17.9% to 4.1% during summer (Garcia-Ispierto *et al.* 2013). It is well established that heat stress and, in part, increased photoperiod may impair fertility during summer (Dahl *et al*. 2000; Dahl and Petitclerc 2003; De Rensis *et al*. 2003; López-Gatius 2003; De Rensis *et al.* 2017; Pszczola *et al*. 2017). Melatonin regulates the circadian rhythm and is a powerful free radical scavenger and antioxidant (Tamura *et al*. 2012; Manchester *et al*. 2015). Therefore, we expected to observe a positive effect of melatonin on pregnancy rate during summer. In the area where our study was done, THI in June, July and August (summer) was indicative of heat stress in the herd (Collier *et al*. 1982; García-Ispierto *et al*. 2007; Zimbelman *et al*. 2009), while from December to February (winter) the cows were under normal thermic conditions. The lack of a seasonal effect observed in our study could be related to the application of a cosynch-ovsynch plus progesterone and FTAI protocol that could have masked the negative effects of heat stress on fertility. In conclusion this study indicates that in dairy cows inseminated using FTAI after a cosynch-ovsynch plus progesterone protocol, treatment with melatonin before calving does not compromise either pregnancy rate or embryo losses in both summer and winter.

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