Melatonin treatment in winter and spring and reproductive recovery in Sarda breed sheep

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Abstract

This study aimed to evaluate the effect of melatonin treatment carried out between late winter and early spring on reproductive response in Sarda breed sheep and whether the photo-refractoriness can influence this reproductive response. The study was conducted on 3,200 adult ewes, aged 3 to 6 years old, with body condition score (BCS) 2.5-4.0, from 16 commercial sheep farms in Northern Sardinia. In each farm 200 animals were enrolled and subdivided into 2 groups (n = 100 each): Group M (treated with one 18 mg melatonin implant), and group C (untreated). The melatonin treatments were performed on 10th February; 10th March; 10th April and on 10th May each time in 4 different randomly selected farms. Adult males, treated with 3 melatonin implants 1 week before females, were introduced in each flock 35 days after ewes' treatment, and removed after 45 days of cohabitation with females. Pregnancy was determined by transabdominal ultrasonography examination between 45th and 90th day after ram introduction. Data showed that melatonin treatment increased the fertility rate significantly ($P<0.05$), with the higher fertility rate in the ewes treated in April and May. The average time in days from male introduction to lambing was shorter in treated than in control ewes ($P<0.05$). Further, at the 160th and 170th day after male introduction the group M showed a higher number of lambed ewes compared to C ($P<0.01$). This effect was observed at 180th and 190th days after ram introduction, also, but with lower significance ($P<0.05$). In conclusion, melatonin treatment improved reproductive efficiency and advanced breeding season in Sarda sheep, especially when ewes were treated in spring.

Keywords: Sarda sheep, seasonal reproduction, melatonin implant, fertility

Introduction

Reproductive seasonality of dairy sheep at the Mediterranean basin latitudes is a major constraint for production (Chemineau et al., 2007). Furthermore, these areas have a mild climate during winter and autumn, rainfall mainly concentrated in spring and a very dry summer. Thus, the sheep production cycle is closely linked to pasture growth which occurs predominantly in autumn and spring (Carcangiu et al., 2011). Therefore, to increase the length of lactation (6-7 months), it is necessary to mate sheep in spring to obtain lambing in autumn. The timing of reproductive seasonality in many small ruminant species is controlled by the shortening of photoperiod (Chemineau et al., 1992) i.e., while the long photoperiods inhibit, the short photoperiods stimulate the reproductive activity (Bittman and Karsh 1984; Boden and Kennaway, 2006). Melatonin shows a low blood-concentration during daylight and a high concentration during darkness, thus it can be considered as an organic informer of the annual photoperiodic trend (Carcangiu et al., 2005, Carcangiu et al., 2013). Melatonin subcutaneous implants are used in Europe and other parts of the world, to advance reproductive activity of sheep in the spring (Abecia et al., 2006; Papachristoforou et al., 2007; deNicolo et al., 2008; Carcangiu et al., 2012). At high latitudes, the administration of melatonin by slow-release implants is usually performed around the summer solstice (Haresign et al., 1990), whereas, in the Mediterranean areas, around the spring equinox (Chemineau et al., 1996; Forcada et al., 1999). Several experiments have been carried out to determine which is the best time for treatment with melatonin, but the responses vary with breed (Staples et al., 1992; Abecia et al., 2007). The Sarda sheep (more than 3.0 million ewes farmed in Sardinia) is the main dairy breed in Italy and its production is parallels the pasture growth pattern (Carta et al., 2009). It exhibits a short anoestrous period, generally from early February until late March (personal observation). Administration of melatonin implants has been reported to improved fertility and advance lambing (Carcangiu et al., 2012; Luridiana et al., 2015). However, even in Sarda sheep the reproductive responses after treatment with melatonin are sometimes unsatisfactory, probably due to treatment fallen in the period of refractoriness (Abecia et al., 2007; Sweeney et al., 1997). This study aimed to evaluate the effect of melatonin treatment carried out between late winter and early spring on reproductive response in Sarda breed sheep and to determine whether photorefractoriness influences this response.

2. Materials and Methods

2.1 Experimental design

This study was conducted on 3,200 Sarda breed ewes from 16 farms, located in North Sardinia (40°N). In order to minimize the farm effect, farms were selected according to comparable features such as total number of animals, milk production, flock health, veterinary recommendations (deworming, vaccinations and use of other pharmacological treatments), nutrition and reproductive management. All farms were located in the same area, within a 50 km radius of Sassari. During the day the animals grazed on legumes and grasses and they also received 300 g of commercial concentrate feed per head (crude protein 20.4% and 12.5 MJ ME/kg DM) during milking. The sheep were penned during night, and received hay (crude protein 11.1% and 7.2 MJ ME/kg DM) and water *ad libitum*. Each farm reared approximately 400 sheep, and 200 clinically healthy ewes were chosen from each farm for the study, accounting for a total of 3,200 head. Ewes enrolled in the study were lactating, 3 to 6 years old, with body condition score (BCS) 2.5-4.0 and with a single lamb born between 1st November and 10th December, 2013. The individual rumen bolus number was recorded, and each ewe was individually marked with a numbered collar to avoid mistakes in the animals' identification.

2.2 Treatment and registration data

On each farm the enrolled animals were randomly subdivided into 2 groups, each containing 100 ewes. The ewes in group M received one melatonin implant (18 mg) (Melovine, CEVA Sanità Animale, Agrate Brianza, Milano) in the left retro-auricular area; the other group of ewes served as control and remained untreated (group C). In the first 4 randomly selected farms melatonin implants were administered on 10th February, in the second 4 on 10th March, in the third 4 on 10th April and in the last 4 on 10th May; controls and treated animals were kept together all the time. Adult rams (male/female ratio 1/20) were introduced on each farm 35 days after treatment of ewes and remained in the flock for 45 days. Before ram introduction, ewes were separated from rams for at least 3 months. Rams received 3 melatonin implants, in the left retro-auricular area, 1 week before ewes. Gestation was diagnosed starting from 45 days and up to 90 days after the rams introduction by transabdominal ultrasonography examination using Esaote Piemedical Tringa linear equipment (Esaote Europe B.V., Maastricht, The Netherlands) provided with a 5.0 – 7.5 MHz multiple frequency linear probe. For each ewe the lambing date and number of new-born lambs were recorded from 150th to 190th day after ram introduction.

2.3 Statistical analysis

All statistical analysis was performed using the computing environment R, version 3.3.2 (R Core Team, 2016). A logit-link Hierarchical Linear Model (HLM) appropriate for binomial (lambing/not lambing) data was used to analyse the fertility of different treatment time. Variables considered were treatment and treatment time. To analyse the effect of treatment time on period in days from male introduction to lambing a HLM procedure according to the following model was performed.

$Y_{ijk}=\mu+Tm(Pe)_{ij}+\varepsilon_{ijk}$

where Yijk is the period in days from male introduction to lambing, μ is the overall mean, Tm is the fixed effect of treatment, Pe is the nested time effect within treatment, and ε_{ijk} is the error effect. The same model was used to analyse the litter size. A P value <0.05 was considered statistically significant. Multiple comparisons of the means were performed using Tukey's method.

3. Results

The number of ewes diagnosed as pregnant differed from the number of lambed ewes of about 3%. Melatonin treatment increased the fertility rate significantly in the four treatment periods compared to control group ($P < 0.05$). The higher fertility rate was seen in the ewes treated in April and May compared to those treated in the other two periods ($P<0.05$) (Table 1). The average time in days from male introduction to lambing was shorter in the treated animals compared to controls, in all the experimental periods (Table1). Melatonin treatment did not affect litter size, and no difference was observed between the treated groups and controls in different periods of observation. By daily observation of lambing trend, melatonin treated ewes lambed approximately ten days earlier compared to controls. At the 160th and 170th day from male introduction, in all four treatment periods the treated group showed a higher number of lambed ewes compared to control ($P<0.01$). At the 180th and 190th day from male introduction the treated ewes exhibited a higher number of lambed ewes compared to controls ($P<0.05$), although the difference between treated and control group was smaller than in the first 20 days of lambing. In all four treatment times, the lambing peak was registered at day 170, in treated ewes, and at day 180 from male introduction, in controls (Table 2).

4. Discussion

The results from this study show that melatonin was able to advance the reproductive activity in a dairy sheep. This finding is in agreement with other observations where melatonin improves the reproductive efficiency in different sheep breeds (Chemineau et al., 1991; Haresign, 1992; Staples et al., 1992; Carcangiu et al., 2012). However, in many experiments melatonin treatments were performed from the end of April till September, and thus are not completely comparable to our experiment (Haresign et al., 1990; Forcada et al., 1995; Scott et al., 2009).

However, our data indicate that the reproductive response is influenced by the treatment time; in fact, fertility was higher when the animals were implanted in April and May (early spring) than in February-March (late winter). This result does not agree with the findings of Abecia et al. (2007) in three sheep breeds reared in Spain, where fertility of the Rasa Aragonesa breed was not improved when the administration of melatonin was performed in January and February (winter) compared to April and May (spring). This finding from Abecia et al. (2007) is also in agreement with previous study carried out by Forcada et al. (2002) and Zuniga et al. (2002) always on the Rasa Aragonesa breed .

In the Assaf breed melatonin administration in January, February (winter) and May (spring) increased fertility, but not when it was administered in April (spring). In the same experiment the administration of melatonin in the Merino breed in February (winter) and April (spring) led to increased fertility but not in January (winter) and May (spring) (Abecia et al., 2007). This finding is presumably due to the different sensitivity to photoperiod in the different sheep breeds. Indeed, after a short-day period, in several sheep breeds, a photo-refractory period occurs, corresponding to the deep anestrous that can have a different length among sheep breeds (Chemineau et al., 2010). Therefore the breed differences in response to melatonin and the male effect will impact on reproductive. Our results show that in the four treatment times, reproductive response was different, the more effective treatments being those performed in spring, compared to those performed in winter.

The onset of the breeding season is a consequence of the number of long days experienced by the ewes (Malpaux et al., 1989), and it is possible that our Sarda sheep experienced a number of long or increasing days sufficient to respond to a short-day treatment such as that resulting from melatonin implants (O'Callaghan et al., 1994; Malpaux et al., 1997). Sweeney et al. (1997) postulated that ewes are able to reinitiate reproductive activity in response to 35 long days (photorefractoriness period) followed by short days or melatonin treatment at any stage between the winter and summer solstices. In fact in the present study the first melatonin treatment was performed on February 10, thus approximately 50 days after the winter solstice. It is therefore plausible to think that the ewes were already at a late-stage of their photorefractoriness period. It is reasonable to think that the progressive increase in the fertility rate in the other three doses was due to an increasing sensitivity of the reproductive system to melatonin.

In different experiments the reproductive response to the male effect in melatonin treated ewes was affected by the farm/flock as well (Chemineau et al., 1991; Haresign, 1992; Scott et al., 2009).

The differences in fertility rate found among treated and control ewes are mainly mediated by the direct effect of melatonin on the ovary (where are located melatonin receptors) (Yie et al., 1995), as well as by its effect on gonadotropin secretion at pituitary-hypothalamic level (Viguié et al., 1995). Moreover, in all four treatment times, implanted sheep exhibited a reduced number of days from ram introduction to lambing than the controls. Subsequently, the treated animals had a more ready response to the male effect compared to controls. In our experiment a difference between the melatonin-treated and untreated groups was registered in the lambing periods. That difference was attributable to the fact that treated ewes were mated within the first 17 days of the mating period, and had a higher proportion of cyclic ewes at ram introduction than the untreated group. Then the administration of melatonin was able to stimulate the resumption of reproductive activity, and this result agrees with the findings from Abecia et al. (2006). Lambing records showed that the melatonin treated animals exhibited the onset of reproductive activity approximately one week before the untreated ones. In fact, at the 160^{th} and 170^{th} day after male introduction the number of lambed ewes was much higher in treated than in control animals. Conversely, at the 180^{th} and 190^{th} day from male introduction the number of lambed ewes increased considerably in control compared to treated group. This trend probably is related to the "carry-over" effect, that occur when the treated and control animals are kept together (Abecia et al., 2006). When melatonin treated and untreated ewes are kept together the onset of reproductive activity occurred earlier in control ewes as well compared to isolated controls (Kennaway et al. 1987). Moreover, the introduction of estrus ewes, exposed to controlled photoperiod, to a flock of anestrous ewes triggers reproductive activity, indicating that female—female social interactions can influence the timing of reproductive transitions in ewes (O'Callaghan et al., 1994). Establishing the number of sheep in the flock to be treated to obtain the triggering effect on reproductive activity could be of considerable importance for farmers, since the treatment of a part of the flock is able to improve the reproductive efficiency of the entire flock. However, even without an economic calculation, it is clear that in the present research, the treated ewes had an advance in the onset of lactation compared to controls, and this results in an economic profit for farmers. In fact, the treated animals exhibited on average 10 days shorter time between male introduction and lambing than controls.

The same litter size was observed in treated and control animals in all four treatment times in this study, as established also in our previous works (Carcangiu et al., 2012; Luridiana et al., 2015). Our data did not agree with Abecia et al. (2002) and Scott et al. (2009), who found an increase in litter size in the treated animals. Some other studies found no effect of melatonin treatment on litter size (Rajkumar et al., 1989; Schoeman and Botha, 1995; Gates et al., 1998). Moreover, Abecia et al. (2007) after melatonin implants found an increased litter size in Rasa Aragonesa, but not in Assaf breed. Thus, since Sarda and Assaf are both dairy breeds, we can hypothesize that the absence of melatonin effect on litter size could be due to the genetic features of these breeds.

5. Conclusion

Melatonin treatment improved the reproductive efficiency and advanced the breeding season in dairy sheep. These effects were more pronounced when ewes were treated in spring than in winter. So, to obtain an optimal reproductive efficiency in Sarda ewes melatonin implants should be placed after the spring solstice. The present data also indicate that the period of photorefractoriness in this breed could be confined between January and early February since the melatonin administration on February 10 is able to stimulate the reproductive activity.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

References

Abecia, J.A., Forcada, F., Zuniga, O., 2002. The effect of melatonin on the secretion of progesterone in sheep and on the development of ovine embryos in vitro. Vet. Res. Commun. 26, 51-58.

Abecia, J.A., Palacın, I., Forcada, F., Valares, J.A., 2006. The effect of melatonin treatment on the ovarian response of ewes to the ram effect. Domest. Anim. Endocrinol. 31, 52-62.

Abecia, J.A., Valares, J.A., Forcada, F., Palacìn, I., Martìn, S., Martino, A., 2007. The effect of melatonin on the reproductive performance of three sheep breeds in Spain. Small Rum. Res. 69, 10-16.

Bittman, E.L., Karsch, F.J., 1984. Nightly duration of pineal melatoninsecretion determines the reproductive response to inhibitory daylength in the ewe. Biol. Reprod. 30, 585–593.

Boden, M.J., Kennaway, D.J., 2006. Circadian rhythms and reproduction. Reproduction 132, 379-392.

Carcangiu, V., Mura, M.C., Bini, P.P., Vacca, G.M., Daga, C., Luridiana, S., 2012. Can advance of first lambing induced by melatonin implants influence the next lambing time in Sarda breed sheep? Can. J. Anim. Sci. 92, 67-71.

Carcangiu, V., Mura, M.C., Parmeggiani, A., Piccione, G. , Bini, P.P., Cosso, G., Luridiana, S., 2013. Daily rhythm of blood melatonin concentrations in sheep of different ages. Biol. Rhythm Res. 44, 908-915.

Carcangiu, V., Luridiana, S., Vacca, G.M., Daga, C., Mura, M.C., 2011. A polymorphism at the melatonin receptor 1A (MTNR1Aa) gene in Sarda ewes affects fertility after AI in the spring. Reprod. Fert. Dev. 23, 376-380.

Carcangiu, V., Vacca, G.M., Parmeggiani, A., Mura, M.C., Bini, P.P., 2005. Blood melatonin levels relating to the reproductive activity of Sarda does. Small Ruminant Res. 59, 7-13.

Carta, A., Casu, S., Salaris, S., 2009. Invited review: current state of genetic improvement in dairy sheep. J. Dairy Sci. 92, 5814-5833.

Chemineau, P., Guillaume, D., Migaud, M., Thiéry, J.C., Pellicer-Rubio, M.T., Malpaux, B., 2008. Seasonality of reproduction in mammals: Intimate regulatory mechanisms and practical implications. Reprod. Dom. Anim. 43 (Suppl. 2), 40–47.

Chemineau, P., Malpaux, B., Delgadillo, J.A., Guerin, Y., Ravault, J.P., Thimonier, J., Pelletier, J., 1992. Control of sheep and goat reproduction: use of light and melatonin. Anim. Reprod. Sci., 30, 157–184.

Chemineau, P., Bodin, L., Migaud, M., Thiery, J.C., Malpaux, B., 2010. Neuroendocrine and Genetic Control of Seasonal Reproduction in Sheep and Goats. Reprod. Dom. Anim. 45 (Suppl. 3), 42-49.

Chemineau, P., Malpaux, B., Brillard, J. P., Fostier, A., 2007. Seasonality of reproduction and production in farm fishes, birds and mammals. Animal 1, 419–432.

Chemineau, P., Malpaux, B., Pelletier, J., Leboeuf, B., Delgadillo, J.A., Deletang, F., Pobel, T., Brice, G., 1996. Emploi des implants de melatonine et des traitements photoperiodiques pour maıtriser la reproduction saissoniere chez les ovins et les caprins (use of melatonin implants and photoperiodic treatments to resuce seasonal reproduction in sheep and goats). INRA Prod. Anim. 9, 45–60.

Chemineau, P.,Vandaele, E., Brice, G., Jardon, C., 1991. Utilisation des implants de mélatonine pour l'amélioration des performances de reproduction chez la brebis (use of melatonin implants to improve sheep reproductive performances). Rec. Méd. Vét. 167, 227-239.

deNicolo, G., Morris, S.T., Kenyon, P.R., Morel, P.C., Parkinson, T.J., 2008. Melatonin-improved reproductive performance in sheep bred out of season. Anim. Reprod. Sci. 109, 124-133.

Forcada, F., Abecia, J.A., Lozano, J.M., Ferrer, L.M., Lacasta, D., 1999. The effects on reproductive performance in the short and medium term of the combined use of exogenous melatonin and progestagen pessaries in ewes with a short seasonal anoestrous period. Vet. Res. Commun. 23, 257-263.

Forcada, F., Abecia, J.A., Zuniga, O., Lozano, J.M., 2002. Variation in the ability of melatonin implants inserted at two different times after the winter solstice to restore reproductive activity in reduced seasonality ewes. Aust. J. Agric. Res. 53, 167–173.

Forcada, F., Zarazaga, L., Abecia, J.A., 1995. Effect of exogenous melatonin and plane of nutrition after weaningon estrous activity, endocrine status and ovulation rate in Salz ewes lambing in the seasonal anoestrus. Theriogenology43, 1179–1193.

Gates, P.J., Henningsson, T., Tengroth, G., Forsberg, M., 1998. Effects of melatonin, progestagens, and the ram on out-of-season reproduction in Swedish Landrace finewool sheep. Acta Vet. Scand. 39, 499-510.

Goldman, B.D., 2001. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J. Biol. Rhythms 16, 283-301.

Haresign, W., 1992. The effect of implantation of lowland ewes with melatonin on the time of mating and reproductive performance. Anim. Prod. 54, 31–39.

Haresign, W., Peters, A.R., Staples, L.D., 1990. The effect of melatonin implants on breeding activity and litter size in commercial sheep flocks in the UK. Anim. Prod. 50, 111–121.

Kennaway, D.J., Dunstan, E.A., Staples, L.D., 1987. Photoperiodic control of the onset of breeding activity and fecundity in ewes J. Reprod. Fert. (Suppl.) 34, 187-199.

Luridiana, S., Mura, M.C., Daga, C., Farci, F., Di Stefano, M.V., Zidda, F., Carcangiu, V., 2015. Melatonin treatment in spring and reproductive recovery in sheep with different body condition score and age. Anim. Reprod. Sci. 160, 68-73.

Malpaux, B., Robinson, J.E., Wayne, N.L., Karsch, F.J., 1989. Regulation of the onset of the breeding season of the ewe: importance of long days and of an endogenous reproductive rhythm. J. Endocrinol. 122, 269–278.

Malpaux, B., Viguié, C., Skinner, D.C., Thyéry, J.C., Chemineau, P., 1997. Control of the circannual rhythm of reproduction by melatonin in the ewe. Brain Res. Bull. 44, 431–438.

O'Callaghan, D., Donovan, A., Sunderland, S.J., Boland, M.P., Roche, J.F., 1994. Effect of the presence of male and female flockmates on reproductive activity in ewes. J. Reprod. Fertil. 100, 497-503.

Papachristoforou, C., Koumas, A., Photiou, C., 2007. Initiation of the breeding season in ewe lambs and goat kids with melatonin implants. Small Ruminant Res. 73, 122-126.

Rajkumar, R.R., Argo, C.M., Rodway, R.G., 1989. Fertility of ewes given either melatonin or progesterone sponges. Vet. Rec. 124, 215-217.

Schoeman, S.J., Botha, M.A., 1995. The effect of melatonin treatment on reproductive efficiency in an accelerated lambing system. J. S. Afr. Vet. Assoc. 66, 230-234.

Scott, P.R., Sargison, N.D., Macrae, A.I., Gough, M.R., 2009. Melatonin treatment prior to the normal breeding season increases fetal number in United Kingdom sheep flocks. Vet. J. 182, 198-202.

Staples, L.D., McPhee, S., Kennaway, D.J., Williams, A.H., 1992. The influence of exogenous melatonin on the seasonal patterns of ovulation and oestrus in sheep. Anim. Reprod. Sci. 30, 185–223.

Sweeney, T., Donovan, A., Roche, J.F., O'Callaghan, D., 1997. Variation in the ability of a long day followed by a short-day photoperiod signal to initiate reproductive activity in ewes at different times of the year. J. Reprod. Fertil. 109, 121–127.

Viguié, C., Caraty, A., Locatelli, A., Malpaux, B., 1995. Regulation of luteinizing hormone-releasing hormone (LHRH) secretion by melatonin in the ewe: II. Changes in N-Methyl-

D,L-aspartic acid induced LHRH release during the stimulation of LH secretion by short days. Biol. Reprod. 52, 1156-1161.

Yie, S.M., Niles, L.P., Younglai, E.V., 1995. Melatonin receptors on human granulose cell membranes. J. Clin. Endocrinol. Metab. 80, 1747-1749.

Zuniga, O., Forcada, F., Abecia, J.A., 2002. The effect of melatonin implants on the response to the male effect and on the subsequent cyclicity of Rasa Aragonesa ewes implanted in April. Anim. Reprod. Sci. 15, 165–174.

Table 1

Fertility rate, litter size and distance in days from rams introduction to parturition (DRIP) in the treated (M) and control (C) ewes in the four period (total ewes n = 3,200).

	M	C		M	C	M	C	
Time	Fertility rate	Fertility rate		Litter size	Litter size	DRIP	DRIP	
10th Febr.	68%[a]	61%[a]	*	1.29	1.18	164.96	175.57	*
10th March	75%[a]	66%[a]	*	1.20	1.22	165.66	174.77	*
10th April	81%[b]	72%[b]	*	1.18	1.17	165.62	175.07	*
10th May	83%[b]	76%[b]	*	1.19	1.22	165.90	175.02	*

Means within a column with different lowercase are significantly different (P<0.05); In group comparison means within a row with * differ significantly for P<0.05.

Table 2

Cumulative number of lambed ewes from 150th to 190th day after rams introduction (every 10 days) in the treated (M) and control (C) Sarda breed sheep, in the four observed periods (total ewes n=3,200).

	150-160 DARI[a]			161-170 DARI[a]			171-180 DARI[a]			181-190 DARI[a]		
	M	C		M	C		M	C		M	C	
10th February	83	17	**	213	57	**	253	177	*	272	244	*
10th March	77	21	**	230	68	**	276	201	*	300	264	*
10th April	83	22	**	246	75	**	217	226	*	324	288	*
10th May	83	20	**	248	78	**	302	231	*	332	304	*

[a]DARI = days from rams introduction to parturition. In group comparison means within a row with * or ** differ significantly for P<0.05 and for P<0.01 respectively.

Conflict of interest

None of the authors have any conflicts of interest to declare.