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Melatonin-Implanted Pregnant Ewes Produce Lambs that have Higher Average Daily Growth Rates and Live Weights at Weaning

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Summary

The aim of this study was to quantify differences between lambs from birth to weaning born from ewes that received a melatonin implant before lambing and those that were not implanted. Forty d before lambing, 457 pregnant ewes either did or did not receive a melatonin implant. Subsequently, lambs were divided into two groups: lambs whose mothers received melatonin (MEL, n=248, 166 males, 161 females), and lambs whose mothers were non-treated (CTR, n=327, 128 males, 120 females). Lambs were weighed (kg) at birth (LW0) and at weaning (LWW) and age at weaning (AW, d) was recorded. Average daily growth rate (g/d) (AGR) was calculated as [(LWW-LW0)/AW]. MEL lambs had a mean LWW significantly ($P<0.05$) higher than CTR lambs. In particular, male MEL lambs had a significantly ($P<0.05$) higher LWW and AGR than male CTR lambs. Singleton male MEL lambs had higher LW0, LWW, and AGR, and lower AW at the day of weaning than the other lambs, and differences with singleton male CTR lambs were significant ($P<0.05$). LW0, LWW, and AGR were negatively correlated ($P<0.05$) with the implanting-weaning interval (IWI). Lambs with the shortest IWI had the highest LWW ($P<0.05$) and AGR ($P<0.01$), and the lowest AW ($P<0.01$). Treatment of pregnant ewes with melatonin before lambing increased lamb performance until weaning, and the effect was most pronounced in singleton male lambs, which had the highest growth rate. It remains to be elucidated what is the minimal interval between implantation in the pregnant ewes and parturition that maximizes the growth of lambs during lactation.

Key Words: Lambs, Melatonin, Growth, Live Weight

Introduction

Sheep productivity is constrained by sexual seasonality, which is governed by photoperiod (Yeates, 1949); i.e., the endocrine system receives photoperiodic information from the hormone melatonin, which dictates the timing of reproduction. Melatonin is secreted at night, and subcutaneous melatonin implants can be used to artificially manage estrus in sheep, which causes a brief daytime-like response without inhibiting endogenous production (O'Callaghan et al. 1991; Malpaux et al. 1997). In Spain, the anestrous period covers the late winter/early spring (Feb-Mar) to early- or mid-summer period (June-July); this seasonal breeding pattern results in a clear period of lambing, which in turn causes a seasonal pattern of product prices, with prices being lowest when the supply of meat is the highest (late spring to early fall) and vice versa. If farmers were able to produce products "out of-season", they could take advantage of higher prices for these during the winter by inducing estrous cycles during the seasonal anestrus. In this context, melatonin implants play an important role to obtain out-of-season lambs at high prices. Spain has reached the highest market share of the melatonin implants for sheep in the world, with more than 500,000 treatments per year applied in the ovine population, which is about 14 million heads; it means that around 1 out of 30 ewes in Spain have been treated with melatonin.

Recently, various uses of melatonin have been used in small ruminants, apart from the traditional reproductive control, with most of the focus on the survival and growth of offspring, and the improvement of colostrum and milk quality. Melatonin implants given between 70 and 120 d of pregnancy reduced neonatal mortality and increased survival rates at weaning, which were associated with increases in survival of twins and tolerance for prolonged parturition in sheep flocks that had been intensively managed (Flinn et al. 2020a, 2020b). Melatonin rapidly crosses the blood-brain barrier and the ovine placenta (Yellon and Longo 1987; Aly et al. 2015), which allows for maternal supplementation as a means for providing melatonin to the fetus before birth. Elsewhere (Abecia et al., 2020), we demonstrated that, in the fourth month of pregnancy, melatonin implants in ewes improved the quality of the colostrum produced, and lambs born of ewes that received exogenous melatonin had more IgG than did lambs from ewes that did not receive an implant. Furthermore, treatment with melatonin in ewes at lambing increased the growth rates in their lambs and the fat content of the milk (Abecia et al., 2021), and newborn lambs from ewes that had received a subcutaneous melatonin implant at day 120 of pregnancy had higher rectal temperatures and higher average and minimum body surface temperatures of the shoulder, mid loin, and hips than did control lambs (Canto et al., 2023). Recently, we showed that ewes that received a melatonin implant 40 d before lambing produced colostrum that had higher IgG concentrations, produced more milk, which had a lower somatic cell count (SCC), than did non-implanted ewes. A second melatonin implant prolonged the effect on SCC (Canto et al., 2022). In Lacaune dairy sheep, although exogenous melatonin treatment in late pregnancy did not have an effect on milk yield, it did affect milk composition; specifically, increasing milk fat concentrations and decreasing milk protein and lactose (Canto and Abecia, 2022). Collectively, the evidence indicates that administering melatonin implants to pregnant sheep might increase their economic return by improving the performance of their lambs through an increase in milk quantity and quality, and or an increase in lamb survival.

The Spanish sheep and goat sector accounts for approximately 10% of Spain's final livestock production, when considering the entire meat and dairy subsector. With a sheep population of around 14 million in the last five years, our country holds the top position in importance within the European Union (MAPA, 2023).

The objective of this study was to quantify the differences from birth to weaning between Rasa Aragonesa lambs born from ewes that had received an exogenous melatonin implant before lambing and those that were not.

Material and Methods

The experiment followed a protocol (PI29/21) approved by the Ethics Committee of the University of Zaragoza, which met the requirements of the European Union for Scientific Procedure Establishments.

The study involved 575 Rasa Aragonesa lambs (294 males, 281 females; 199 singles, 307 twins, 69 triplets) born on two commercial sheep farms (Farm1, n=354; Farm2, n=221) in Zaragoza, Spain. The farms were members of the Cooperative "Oviaragón", which produce the local lamb "Ternasco de Aragón" under the European Protected Geographical Indication (PGI). The Rasa Aragonesa sheep breed is a native breed of Spain that has been traditionally raised in Northeastern Spain; this breed has been recognized for its resistance, adaptability to various environments and its role in meat and wool production. After weaning in the farms, at an age of 45 d, lambs are housed in feed lots to achieve the slaughter weight (18-24 kg; 70-90 d of age). The farms applied the same management of the animals for the breed (Rasa Aragonesa), because farms that are involved in the production of that PGI lamb must meet several quality standards.

Approximately 40 d (mean ± S.D.= 39 ± 7 d; range 18-60 d) before the expected time of lambing (lambing season: 2 May-9 Jun), 457 pregnant ewes were either treated or not with a single melatonin implant (18 mg melatonin; Melovine, CEVA Salud Animal, Barcelona, Spain), which produced lambs that were assigned to one of two groups for analytical purposes: lambs whose mother had received a melatonin implant (group MEL, n=248) and lambs whose mothers were non-treated (group CTR, n=327). Lambs were weighed (kg) at birth (LW0) and at weaning (47 ± 8 d of age) (LWW). Age at weaning (AW, d) was recorded, and the average daily growth rate (g/d) (AGR) was calculated as [(LWW-LW0)/AW]. The weaning date is decided by the farmer when the lambs begin to reach a LW of about 12 kg, and the whole group of lambs is weaned. The effects of farm, sex of the lamb, type of parturition (single or multiple), and treatment with melatonin (MEL or CTR), were evaluated statistically based on a multifactorial model. It included farm, sex of the lamb, type of parturition, and treatment as fixed effects, and the Least Squares Method of the GLM procedure in SPSS v.26 (IBM

Corp. Released, 2019) was used. Sire effects were not considered since no information about mating is available in our farms when natural mating is used. Within fixed effects, significant differences were identified by an ANOVA. Pearson correlation coefficients among the implantation-weaning interval (IWI) and the lamb performance (LW0, LWW, AGR and AW) were calculated. A regression analysis was conducted between IWI and AGR.

To identify the optimal time before parturition to insert a melatonin implant in the ewes, the interval between melatonin implantation and lambing (IIL) was divided into four quartiles based on 'visual binning' (SPSS), which provides an interactive means of choosing how to transform a quantitative variable into a categorical variable. Differences in LW0, LWW, AGR, and AW among quartiles and the control group were assessed statistically by an ANOVA and the Least Squares Method.

Results and Discussion

Farm, sex of the lamb, type of parturition, and treatment with melatonin of the mothers had a significant (at least $P<0.05$) effect on LW0 and LWW (Table 1), and the interaction between type of parturition and treatment with melatonin of the mothers had a significant ($P=0.01$) effect on LW0. Farm, type of parturition, and their interaction had a significant ($P<0.001$) effect on AGR, and the interaction between sex and treatment was significant ($P=0.05$). Farm and type of parturition, but not melatonin treatment of the mothers, had a significant effect on AW.

MEL lambs had a higher mean (± S.E.) LWW (12.26 ± 0.10 g/d) than did CTR lambs (12.00 ± 0.08 g/d) ($P<0.05$). In particular, male MEL lambs had a higher LWW and AGR than did male CTR lambs ($P<0.05$), but there were no significant differences between female MEL and CTR lambs (Table 2). Singleton male MEL lambs had the highest LW0, LWW, and AGR, and the lowest AW, and the differences with singleton male CTR lambs were significant ($P<0.05$) (Table 3). Male MEL and CTR lambs with littermates, and female MEL and CTR lambs with or without littermates did not differ significantly (Table 3).

LW0, LWW, and AGR were significantly negatively correlated with IIW ($P<0.01$ for LW0, AGR, and AW, and $P<0.05$ for LWW) (Table 4). The linear regression analysis between IIW and AGR had a high coefficient of determination with AGR (0.3512), and a negative slope (-2.3459) (Figure 1), which reflected the negative relationship between the IIW and the AGR of the lambs. The lambs that had the shortest IIW had the highest LWW ($P<0.05$) and AGR ($P<0.01$), and the lowest AW ($P<0.01$) (Table 5).

The experiment in this study demonstrated that lambs born from ewes that had received a melatonin implant in the last third of pregnancy had the highest LW during lactation and grew faster than did lambs born of non-implanted ewes. Their mother's milk was the lamb's only source of nourishment; therefore, the melatonin implants increased the quantity and or quality of the milk. Previously, we (Canto et al., 2022) demonstrated that melatonin implants in pregnancy had a significant effect on milk quality; specifically, ewes that had received a melatonin implant 40 d before lambing produced the most milk, which had the highest fat content. In another study (Abecia et al., 2021), ewes that had received a melatonin implant at lambing produced milk that had the fattest content, and their offspring had the highest growth rate In goats, melatonin implants inserted seven weeks before kidding had a significant effect on milk production in the subsequent lactation and improved the daily weight gain of their suckling kids (Avilés et al., 2019). Melatonin membrane receptors MT1 and MT2 are expressed in the mammary glands of goats throughout lactation (Zhang et al., 2019), which suggests that melatonin has a direct role in the regulation of mammary physiology.

In our study, the effects of treating with melatonin ewes in pregnancy were

Table 1. *P*-values in each of the factors affecting live weight at birth (LW0) and weaning (LWW), and the average daily growth rate (g/d) (AGR) and age at weaning (AW) in Rasa Aragonesa lambs.

	LW0	LWW	AGR	AW
Farm	0.046	<0.001	<0.001	<0.001
Sex	0.002	0.002	0.169	0.638
Type of parturition	<0.001	<0.001	<0.001	<0.001
Treatment	0.049	0.029	0.181	0.681
Farm x Sex	0.787	0.259	0.092	0.240
Farm x Type of parturition	0.579	0.198	<0.001	0.130
Farm x Treatment	0.733	0.565	0.092	0.806
Sex x Type of parturition	0.946	0.637	0.356	0.130
Sex x Treatment	0.918	0.100	0.050	0.956
Type of parturition x Treatment	0.010	0.175	0.397	0.217

Table 2. Mean (±S.E.) live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of male and female Rasa Aragonesa lambs that were born of ewes that either did (MEL) or did not (CTR) receive a melatonin implant in the last third of pregnancy.

Sex	Group	LW0 (kg)	LWW (kg)	AGR (g/d)	AW (d)
Male	CTR (128)	4.15±0.05	12.13±0.12^{a}	174.31±3.33^{a}	46.9±0.6
	MEL (166)	4.20±0.07	12.62±0.15^{b}	188.59±4.12^{b}	46.2±0.8
Female	CTR (120)	3.93±0.06	11.87±0.11	173.82±3.45	47.0±0.7
	MEL (161)	3.94±0.07	11.87±0.12	170.29±3.25	47.4±0.6

Means within an effect with no common superscript are different $P<0.05$.

Table 3. Mean (±S.E.) live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of singleton and multiple Rasa Aragonesa lambs born of ewes that either did (MEL) or did not (CTR) receive a melatonin implant in the last third of pregnancy.

		Singleton				Multiple			
Sex	Group	LW0 (kg)	LWW (kg)	AGR (g/d)	AW (d)	LW0 (kg)	LWW (kg)	AGR (g/d)	AW (d)
Male	CTR (128)	4.49±0.07[a]	12.36±0.19[a]	184.82±5.91[a]	43.7±0.9[a]	3.93±0.07	11.98±0.14	167.38±3.78	49.1±0.8
	MEL (166)	4.79±0.11[b]	13.19±0.25[b]	211.83±6.69[b]	40.6±0.8[b]	3.87±0.08	12.29±0.18	175.55±4.66	49.3±0.9
	Total	4.61±0.06[a]	12.70±0.16[a]	195.91±4.59[a]	42.4±0.6	3.90±0.0[a]	12.12±0.1[a]	171.06±2.9[a]	49.2±0.6
Female	CTR (120)	4.38±0.09	12.12±0.22	188.97±7.25	42.6±1.2	3.73±0.06	11.75±0.13	166.32±3.28	49.2±0.7
	MEL (161)	4.53±0.10	12.22±0.27	173.82±6.09	44.9±1.0	3.71±0.05	11.73±0.13	168.92±3.85	48.4±0.8
	Total	4.43±0.07[b]	12.12±0.11[b]	183.22±5.10b	43.5±0.8	3.72±0.0[b]	11.74±0.0[b]	167.46±2.4[b]	48.9±0.5
	CTR (327)	4.44±0.06[a]	12.25±0.15	186.68±4.59	43.2±0.7	3.82±0.05	11.86±1.00	166.83±2.48	49.2±0.5
	MEL (248)	4.68±0.08[b]	12.78±0.19	195.95±5.09	42.4±0.7	3.79±0.05	12.01±0.11	172.18±3.02	48.8±0.6

Means within an effect with no common superscript are different *P<0.05*.

strongest in male lambs. Similarly, Abecia et al. (2021) reported that the effects of melatonin implants in the mothers was significant in male lambs, only; specifically, male lambs reared by melatonin-treated ewes had significantly higher LW at weeks 2, 3, and 4 than did male lambs that had been reared by untreated ewes. In goats, melatonin implants in the dry period increased milk yield and the weight gain of male offspring, only (Avilés et al., 2019). Wallace et al. (2014) reported that, in early postnatal life, lamb sex had a significant effect on adipose tissue gene expression in favor of male lambs because female lambs had lower IGF1, IGF2, IGF1R, IGF2R, and hormone-sensitive lipase mRNA expression levels, which are associated with growth and reflect the sexual dimorphism in body composition.

The effects of melatonin implants in the mothers on the growth of male lambs might have been because they consumed the most colostrum, or the colostrum had the best quality. Elsewhere (Canto et al., 2022), we showed that ewes that received a melatonin implant 40 d before lambing produced colostrum that had higher IgG concentrations than did the colostrum from non-implanted ewes, and that ewes that had singleton male lambs had higher colostrum IgG concentrations (54.57 ± 5.37 mg IgG mg/mL) than ewes that had singleton female lambs (34.66 ± 4.30 mg/mL) (Abecia et al., 2020). In sheep, colostrum is important in the development of the immune system, post-natal growth, and thermoregulation, and mediates the formation of the ewe-lamb bond (Agenbag et al., 2021). In addition to increasing neonate survival, access to colostrum in the neonatal period can have a positive effect on future production, development, and reproductive efficiency of lambs through growth factors that facilitate neonatal growth and development. Öztabak and Özpinar (2006) reported that, from the

Table 4. Matrix of correlations between the interval between the insertion of a melatonin implant in Rasa Aragonesa ewes in the last third of pregnancy and weaning (IIW), live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of lambs (*$P<0.01$; ** $P<0.001$).

	IIW	LW0	LWW	AGR	AW
IIW		-0.240**	-0.162*	-0.593**	0.763**
LW0	-0.240**		0.293**	0.183**	-0.492**
LWW	-0.162*	0-293**		0.727**	-0.102*
AGR	-0.593**	0.183**	0.727**		-0.627**
AW	0.763**	-0.492**	-0.102*	0.627**	

Figure 1. Linear regression between the interval between the insertion of a melatonin implant in Rasa Aragonesa ewes in the last third of pregnancy and weaning (d) and the average growth rate of their lambs (AGR) (g/d).

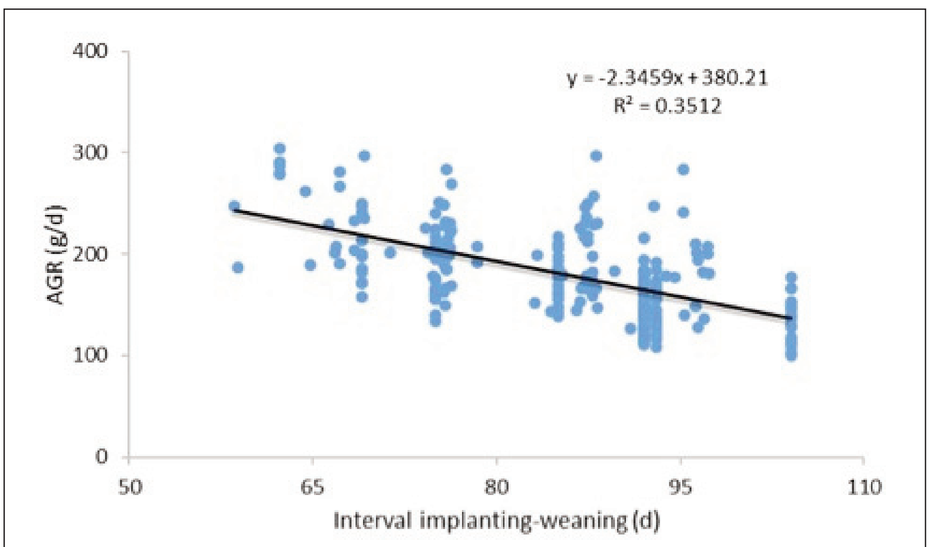

Table 5. Mean (±S.E.) live weight at birth (LW0) and at weaning (LWW) (kg), average growth rate (AGR) (g/d), and age at weaning (AW) (d) of Rasa Aragonesa lambs born of ewes that either did (MEL) or did not (CTR) receive a melatonin implant in the last third of pregnancy, and the interval between implantation and lambing (IIL) (IIW: interval between implantation with melatonin and weaning).

Group	IIL (d)	AW (d)	IIW (d)	LW0 (kg)	LWW (kg)	AGR (g/d)
CTR (n=329)	--	47.0±0.5[a]	--	4.05±0.04	12.00±0.08[a]	174.07±2.36[a]
MEL 1 (n=63)	30.34±0.34	44.1±1.3[b]	74.48±1.23[a]	3.94±0.11[a]	12.49±0.20[b]	200.37±5.92[b]
MEL 2 (n=61)	36.77±0.12	47.4±1.1[a]	84.19±1.11	4.04±0.09	12.24±0.21	177.64±5.07[a]
MEL 3 (n=62)	40.31±0.17	48.3±0.7[a]	88.64±0.65	4.09±0.09	12.13±0.19	169.71±4.64[a]
MEL 4 (n=60)	47.61±0.68	47.2±0.8[a]	94.83±0.86[a]	4.22±0.10[b]	12.16±0.21	170.87±5.09[a]

Means within an effect with no common superscript are different *P<0.05*.

second week onward, rearing method has an effect on body weight gain; specifically, lambs that were reared with their mothers and received colostrum had a higher mean body weight in lactation than did lambs that were reared without colostrum or artificially.

In our study, the correlations between implantation-weaning interval and the LW and AGR of lambs, indicated that the efficacy of the melatonin implants in improving lamb performance was greatest for individuals in which the melatonin implant was inserted closest to parturition. However, the later in pregnancy that the ewe was implanted, the smaller the effect on the development of mammary tissue because milk fat and total solid content were higher in ewes that had been implanted immediately after parturition than they were in control ewes at day 45 of lactation, only, which was close to weaning, and had no effect on the amount of milk produced. Although milk production and quality was not assessed, apparently, a single melatonin implant can affect the mammary gland of the ewes until the implant is exhausted. The implants can release melatonin for up to 100 d (Forcada et al., 2002), therefore, probably, the poorer performances of the lambs of mothers that had been implanted > 30 d before lambing was due to the earlier absorption of the implant such that the beneficial effects of melatonin on milk production diminished earlier in lactation.

Conclusions

Melatonin treatment of pregnant ewes before lambing increased lamb performance until weaning and, in particular, the effects were observed in singleton male lambs, who had the highest LW at birth and weaning, and the highest growth rate. These results, and our previous findings on the effect of melatonin treatment at the end of pregnancy, open new possibilities to optimize lamb performances during lactation. It remains to be elucidated what is the minimal interval between implantation in the pregnant ewes and parturition that maximizes the growth of the lambs during lactation.

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