Association between melatonin receptor 1A gene polymorphism and reproductive performance in Dorset ewes[1]

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ABSTRACT: The response to melatonin expression is one way that circadian rhythms of many biological processes are regulated. To evaluate the relationship between the melatonin receptor 1A (*MTNR1A*) gene and reproductive performance, records were compared in Dorset and 3/4-Dorset × 1/4-East Friesian ewes expressing different genotypes at the *MTNR1A* gene in the Cornell University sheep flock. There were 116 ewes with first lambing records, consisting of 91 Dorset and 25 crossbred ewes. Of these, 104 ewes had second lambing records. Genotypes were determined by PCR amplification of a fragment of the ovine *MTNR1A* gene followed by digestion with *MnlI* and *RsaI* restriction enzymes. The effects of breed, year of birth, season of birth or season of first conception, and each polymorphism on days to first lambing and days between first and second lambings were evaluated. Our results show that ewes with at least 1 *M* allele are able to conceive at younger ages, better able to breed and conceive out-of-season, and have shorter intervals between first and second lambings than ewes expressing only the *m* allele. The results presented in this study show, for the first time, an association of the *MTNR1A* gene and lambing frequency and confirm the importance of the *MTNR1A* gene as a potential DNA marker for out-of-season breeding.

Key words: fertility, genotype, reproduction, seasonality, sheep

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INTRODUCTION

The seasonal lambing pattern commonly observed in sheep bred in temperate climates is a major obstacle to increasing the intensity of sheep production. Between and within breed variation in the timing and duration of breeding indicate that lambing out-of-season is partially under genetic control (Notter, 1992; Lewis et al., 1996), but little is known about the identity of the responsible genes. Knowledge of those genes or linked genetic markers would allow more efficient and more intensive selection programs for aseasonal (**AS**) reproduction.

The nocturnal pattern of melatonin secretion, an important endocrine signal controlling seasonal breeding in sheep (Bittman et al., 1983), is mediated by specific melatonin receptors, but only the melatonin 1A receptor (***MTNR1A***) seems to be involved in the regulation of reproductive activity (Weaver et al., 1996). Two polymorphisms were identified (Messer et al., 1997) in the ovine *MTNR1A* gene, and they were found to be segregating in several breeds (Notter and Cockett, 2005). An association with seasonal reproduction was reported in several breeds: Merinos d'Arles (Pelletier et al., 2000); a composite line of 50% Dorset, 25% Rambouillet, 25% Finnsheep (Notter et al., 2003); Small Tail Hansheep (Chu et al., 2003); and Awassi (Faigl et al., 2008). Therefore, the *MTNR1A* gene is a promising candidate for use in marker assisted selection to improve out-of-season fertility. However, Hernandez et al. (2005) failed to detect any association between the same *MTNR1A* polymorphism and reproductive seasonality in Île-de-France ewes. This emphasizes the need to validate the association of the genetic marker with the trait of interest in different breeds and environments, especially when the functional variation is uncertain.

The present study investigates the association between *MTNR1A* polymorphisms and reproductive performance in a population of Dorset and 3/4-Dorset × 1/4-East Friesian ewes managed under an accelerated lambing system.

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MATERIALS AND METHODS

All animal experiments were conducted in accordance with principles and guidelines outlined in Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Animals and Management

Records from 91 Dorset and 25 3/4-Dorset × 1/4-East Friesian ewes from the Cornell University flock were included. First lambing records were available for all ewes, but only 104 ewes had second lambing records (82 Dorset and 22 3/4-Dorset × 1/4-East Friesian).

The Cornell University flock has been managed under the STAR system since 1981 (Lewis et al., 1998). In this STAR system, the year is divided into five 73-d periods that begin on January 1, March 15, May 27, August 8, and October 20. Breedings in January, August, and October fall within the normal breeding season and are referred to as seasonal. March and May exposures are outside the normal breeding season and are referred to as AS. In the STAR system, all open ewes are placed with breeding rams for 30 d at the start of a STAR period. After lambing, ewes lactate for 40 to 73 d, and lambs are weaned before d 73 of the period, when ewes again are placed with breeding rams to begin the next season. In the STAR system, the shortest possible mean interval between lambings is 219 d (7.2 mo). Failing this, ewes could lamb after 292 d (9.6 mo) or a longer lambing cycle (Thonney, 2007).

Genotyping

Blood (10 mL) was collected into a heparinized (143 USP) tube from a jugular vein of each sheep. Genomic DNA was extracted from whole blood, using a commercial kit (Qiagen, Valencia, CA). A fragment of ovine *MTNR1A* gene was amplified, as described by Messer et al. (1997), and subsequently digested with *MnlI* or *RsaI* restriction enzymes. Allele *M* contained the restriction site for *MnlI* and resulted in 236- and 67-bp fragments, whereas the absence of the restriction site in the *m* allele resulted in a single 303-bp fragment. Similarly, allele *R* contained the restriction site for *RsaI* and resulted in 290- and 5-bp fragments, whereas the absence of the restriction site in the *r* allele resulted in a single 295-bp fragment.

Statistical Analysis

The effect of each polymorphism on the number of days from birth to 1st lambing and number of days between 1st and 2nd lambing was evaluated using the GLM procedure (SAS Inst. Inc., Cary, NC). Breed was defined as Dorset or crossbred. Year of birth was coded 1 through 6, representing animals born in year ≤1996, 1997, 1998, 1999, 2000, and 2001 with 12, 18, 11, 33, 16, and 26 ewes in each group, respectively. Season of birth and season of conception associated with 1st lambing were defined as AS (birth or conception in mo 3, 4, 5, or 6), early seasonal (**ES**; birth or conception in mo 7, 8, 9, or 10), or late seasonal (**LS**; birth or conception in mo 11, 12, 1, or 2). Genotypes for *MTNR1A Mn1I* and *RsaI* polymorphisms were coded as 0, 1, or 2, representing the genotypes *mm*, *Mm*, and *MM*; or *rr*, *Rr*, and *RR*; respectively.

The statistical model used in the analysis for each of the 2 polymorphic sites included the overall mean and the fixed effects of breed (Dorset or crossbred), year of birth (1, 2, 3, 4, 5, or 6), season of birth for number of days from birth to 1st lambing or season of 1st conception for number of days between 1st and 2nd lambing (ES, LS, or AS), and *MTNR1A Mn1I* or *RsaI* genotype (0, 1, or 2). The 2-way interactions between the fixed effects in the model were tested, but none were significant.

Orthogonal 1 degree of freedom contrasts were constructed to compare the season of birth or 1st conception (ES and LS vs. AS and ES vs. LS), *MnlI* genotypes (0 vs. 1 and 2, and 1 vs. 2), and *RsaI* genotypes (2 vs. 1 and 0, and 1 vs. 0).

Table 1. Genotypic frequencies for *MnlI* and *RsaI* polymorphisms[1,2]

	RsaI genotype		
MnlI genotype	*RR*	*Rr*	*rr*
MM	0	2.59	39.66
Mm	0.86	39.66	3.45
mm	12.93	0.86	0

[1]Total number of animals = 116.

[2]Genotypes for 6 additional animals (1, 2, and 3 of genotype *RR*, *Rr*, and *rr*, respectively) were available for the *RsaI*, but not *MnlI* polymorphism.

RESULTS AND DISCUSSION

Gene and Genotypic Frequencies

Gene frequencies were 0.64 and 0.36 for the *MnlI* polymorphism (alleles *M* and *m*, respectively) and 0.35 and 0.65 for the *RsaI* polymorphism (alleles *R* and *r*, respectively). Among the 116 ewes, genotypic frequencies were 0.43, 0.44, and 0.13 for *MM*, *Mm*, and *mm* genotypes at the *MnlI* polymorphism and 0.13, 0.43, and 0.44 for *RR*, *Rr*, and *rr* genotypes at the *RsaI* polymorphism. When the 2 restriction sites were considered separately in the present population, genotypic frequencies did not differ from those expected under random mating. However, the 2 polymorphisms were not in joint equilibrium. In our population, individuals of genotypes *mmRR*, *MmRr*, and *MMrr* had greater frequencies than would be expected with linkage equilibrium (Table 1), whereas the other genotypes were absent or at reduced frequency.

Crossbred ewes had 113 more days to 1st lambing and 155 fewer days between 1st and 2nd lambing rela-

Table 2. Least squares means (LSM) and SE for the effect of year of birth on number of days from birth to first lambing

	Year of birth					
Item	≤1996	1997	1998	1999	2000	2001
LSM	628.55	690.57	741.81	676.10	693.79	610.35
SE	111.10	96.69	110.93	88.71	97.14	89.64

tive to Dorset ewes, but these differences were not statistically significant. The year of birth effect on days to 1st lambing or days between 1st and 2nd lambing was not statistically significant. The least squares means for age (in days) at 1st lambing for the 6 birth year groups are presented in Table 2. The youngest was the group of ewes born in 2001 with 610 d, and the oldest was the group of ewes born in 1998 with 742 d at 1st lambing.

Season of Birth, Season of 1st Conception, and Genotypic Effects on Fertility

The estimates for each contrast, SE, and probability > | t| for days to 1st lambing and days between 1st and 2nd lambing are presented in Table 3. Ewes born in ES and LS seasons had 141 fewer days to 1st lambing relative to ewes born in AS season, and this difference was significant with probability of 0.02. Ewes conceiving for the first time in ES and LS seasons had 89 more days between 1st and 2nd lambing relative to ewes with 1st conception in AS season, and this difference was significant with probability of 0.1. The effect of season of birth on age at 1st lambing was probably due to the fact that ewes born in ES or LS seasons should reach breeding age (8 to 12 mo of age) in the ES or LS season the following year and, therefore, are more likely to conceive earlier relative to ewes born in the AS season. The effect of season of 1st conception on days between 1st and 2nd lambing can also be explained when the sequence of breeding seasons in a STAR system is considered. Ewes that conceive in the AS season are more likely to conceive sooner after lambing because their next breeding season will be in a seasonal period.

The ewes with *Mm* or *MM* genotypes needed 136 fewer days to 1st lambing and 124 fewer days between 1st and 2nd lambing relative to ewes with the *mm* genotype, both differences significant with probabilities 0.05 and 0.04, respectively. The effect of *RsaI* genotype on days to 1st lambing or days from 1st to 2nd lambing, also shown in Table 3, was not statistically significant.

These results indicate that ewes carrying the *M* allele (containing the restriction recognition site for *MnlI* enzyme) were able to conceive at younger ages and had shorter intervals between 1st and 2nd lambing. In the STAR accelerated lambing system under which the ewes were given the chance to lamb every 219 d, these effects were mostly due to the ability to conceive and lamb out-of-season.

The ability to breed and lamb out-of-season is a quantitative trait important for enhanced economic prosperity of the sheep industry, by accelerated lambing, or by increasing the proportion of the flock that lambs out-of-season. Whereas accelerated lambing can improve flock efficiency, accelerated lambing or breeding a proportion of the flock to lamb out-of-season allows a more continuous supply of fresh, young, market lambs. This should allow for improved market development and enhanced prices.

Given the economic importance of out-of-season breeding, several attempts have been made to improve out-of-season breeding using traditional breeding methods, but with limited success. Selection of individuals to

Table 3. Effect estimates, SE, and probability > | t| for contrasts evaluating the effect of season of birth or season of 1st conception and *MnlI* and *RsaI* genotype on the number of days from birth to 1st lambing and the number of days from the 1st to the 2nd lambing

	Days to 1st lambing			Days from 1st to 2nd lambing		
Contrast[1]	Constant	SE	$P >$ | t|	Constant	SE	$P >$ | t|
Effect of season of birth[2] or 1st conception[3]						
ES and LS vs. AS	−140.76	58.61	0.02	89.46	53.52	0.10
ES vs. LS	30.7	77.86	0.70	−90.49	54.05	0.10
Effect of *MnlI* genotype						
mm vs. *Mm* and *MM*	135.76	70.1	0.05	123.59	59.75	0.04
Mm vs. *MM*	−78.77	51.7	0.13	19.83	46.33	0.70
Effect of *RsaI* genotype						
RR vs. *Rr* and *rr*	−2.20	54.25	0.97	−55.48	45.85	0.23
Rr vs. *rr*	−127.18	77.86	0.11	25.28	64.74	0.69

[1]ES = early season; LS = late season; AS = aseasonal.
[2]Season of birth was included in the statistical model analyzing days from birth to 1st lambing.
[3]Season of 1st conception was included in the statistical model analyzing days from 1st to 2nd lambing.

be parents was based on fertility in spring and summer or ability to perform in an accelerated lambing management system (Notter, 1992; Al Shorepy and Notter, 1997). The genetic improvement of this trait through a conventional breeding program is difficult because a) fertility out of season has a low heritability; b) the trait is expressed only with management systems designed for year-round breeding or deliberate spring and summer breeding and is measured in only one sex; and c) the trait is expressed late in life. Since the availability of molecular techniques to determine variation among animals at the DNA level (microsatellites, RFLP, SNP), there has been growing interest in utilizing this information in selection, particularly for traits difficult to improve through conventional programs.

The study of Pelletier et al. (2000) demonstrated an association between the *mm* genotype and ovarian inactivity in spring in Merino d'Arles ewes, whereas Notter et al. (2003) showed greater spring fertility (ewes lambing per ewe exposed) for crossbred ewes carrying at least one *M* allele. Our study is consistent with these findings, showing this association also exists in a population of Dorset and 3/4-Dorset × 1/4-East Friesian ewes, indicating that the region surrounding the ovine *MTNR1A* gene influences reproductive performance and ability to breed out-of-season in our population. The phenotype of interest in this study was the ability of a ewe to perform in an accelerated lambing system, and our results show, for the first time, an association of the *MTNR1A* gene and lambing frequency, confirming the importance of the *MTNR1A* gene as a potential DNA marker for out-of-season breeding.

The mechanism by which the polymorphism in the *MTNR1A* gene influences out-of-season reproduction has not been established. The polymorphisms evaluated in this study do not result in AA substitutions in the melatonin receptor (Pelletier et al., 2000), and functional differences in the receptor are not anticipated for different genotypes. Several other polymorphisms were identified within the *MTNR1A* gene (Pelletier et al., 2000), 4 of these in linkage disequilibrium with the *MnlI* polymorphism. Only 1 of these polymorphisms results in a change in the AA sequence, but it appears that this change alone could not be responsible for the phenotypic differences reported in the present and previous studies. It is possible that the effect is due to regulatory sequences or other genes closely linked with the *MTNR1A* gene. It is also possible that the effect of melatonin is mediated via other hormones. Nevertheless, our results confirm that the *MTNR1A* gene is a potential DNA marker for breeding out of season in our study population. For the Cornell flock, and perhaps other Dorset populations managed in an accelerated lambing management program, it seems appropriate to genotype the young rams for the *MTNR1A* gene and use this information in the selection process. However, because this polymorphism is not the causative mutation, its effectiveness as a DNA marker, even in the study population, should be monitored because its association with ability to breed out of season can be lost due to recombination. Even more importantly, before using it in a marker assisted selection program in other breeds or populations, this association should be validated in the target population and its effects on other economically important traits should be explored.

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