

Effects of variations in the melatonin receptor 1A gene on reproductive performance in Dorset ewes

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STORY IN BRIEF

The seasonality of sheep reproduction is a major limiting factor in market lamb production. The objective of this study was to evaluate the relationship between a melatonin receptor (*MTNR1A*) gene and reproductive performance in ewes. Reproductive records of 116 Polled Dorset and ¾-Polled Dorset x ¼-East Friesian crossbred ewes expressing different genotypes for the *MTNR1A* gene were compared. All ewes were genotyped for the *MTNR1A* gene to determine if changes in the gene resulted in changes in reproductive performance. Number of days to 1st lambing and the number of days between 1st and 2nd lambings were used to evaluate ewe performance. The flock was managed according to the STAR breeding system. Season of conception was defined as early favorable (EFV; Jul to Oct), late favorable (LFV; Nov to Feb), or unfavorable (UFV; Mar to Jun). The effect of breed, season of 1st conception, and genotype on ewe reproductive performance was evaluated. Breed of ewe had no effect. Ewes first conceiving in EFV or LFV seasons had 127 fewer ($P = 0.09$) d to 1st lambing and 100 more ($P = 0.07$) d between 1st and 2nd lambings than ewes conceiving in UFV seasons. Ewes conceiving in LFV seasons tended to have 68 fewer days between 1st and 2nd lambings than ewes conceiving in EFV seasons. Days to 1st lambing and days between 1st and 2nd lambings were decreased by 207 ($P = 0.02$) and 140 ($P = 0.03$) d for ewes with *MM* or *Mm* genotypes relative to ewes with *mm* genotype. There was no difference in reproductive performance between ewes expressing *MM* or *Mm* genotypes. These results show ewes that express at least one *M* allele are better able to breed out-of-season and have a shorter interval between 1st and 2nd lambings than ewes expressing only the *m* allele.

Key Words: Melatonin, Sheep, Reproduction, Seasonality

INTRODUCTION

Seasonality of reproduction is an adaptive process utilized by wild animals to deal with seasonal changes in temperature and food availability. Domestication of animals has led to an almost complete loss of this adaptation in cattle and pigs but has been retained in most sheep and goat breeds. The seasonal lambing pattern of most ewes is a major obstacle to increasing the intensity of sheep production. Aseasonal (out-of-season) breeding is an economically important trait to the sheep industry as the market is witnessing an increase in demand for year round lamb. However, natural variation in the timing and duration of breeding for different sheep breeds could offer opportunities for improving the efficiency of market lamb production. Heritability estimates for out-of-season breeding are as high as 0.32, meaning as much as 32% of the variation in this trait is due to genetic factors.

The nocturnal pattern of melatonin secretion represents an endocrine signal controlling seasonal breeding in sheep (Bittman et al., 1983). The effects of melatonin are mediated by specific high affinity melatonin receptors, but only the *MTNR1A* seems to be involved in the regulation of reproductive activity (Weaver et al., 1996). Two regions that differ in the DNA sequence were

reported by (Messer et al., 1997) in the ovine *MTNR1A* gene, and an association between the *MTNR1A* genotype and seasonal reproduction has been reported in several sheep breeds (Pelletier et al., 2000; Chu et al., 2003; Notter et al., 2003; Hernandez et al., 2005).

Although selection has been shown to be somewhat successful, there are challenges due to the fact that many genes as well as environmental factors will affect the out-of-season breeding trait. Knowing the genes that control this trait will be beneficial because this information could be used in a marker assisted selection program which would lead to an increase in the accuracy of selection and help to reduce the generation interval for selection. The overall goal is to increase the rate of genetic change each year to improve out-of-season reproductive performance. The present study was designed to investigate the association between two different patterns in the *MTNR1A* gene and reproductive performance in a population of ewes selected, based on reproductive records, for accelerated and out-of-season lambing.

MATERIALS AND METHODS

Animals and Management. Reproductive records from 91 Dorset and 25 $\frac{3}{4}$ -Dorset x $\frac{1}{4}$ -East Friesian ewes from the Cornell University flock were utilized for this study. First lambing records were available for all ewes, while second lambing records were only available for 104 of the ewes. The Cornell University flock has been managed under the STAR breeding system since 1981 (Lewis et al., 1998). In the STAR system, each year is divided into five 73-d periods that begin on January 1, March 15, May 27, August 8, and October 20. Breeding in January, August, and October falls within the normal breeding season for sheep and were referred to as the favorable season. March and May exposures are outside of the normal breeding season and were referred to as the unfavorable season. All open ewes were exposed to rams for the first 30 d of each period in the STAR system. Following lambing, ewes nursed lambs from 40 to 73 d, with lambs being weaned on d 73 of each period. Three days following weaning, ewes are again exposed to rams for rebreeding. 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) for a longer lambing cycle (Thonney, 2007).

Genotyping. Blood (10 mL) was collected from each ewe by jugular venipuncture into sodium heparanized tubes. Genomic DNA was extracted from whole blood using the Qiagen DNeasy blood and tissue kit (Qiagen, Valencia, CA). An 824-bp fragment of the ovine *MTNR1A* gene was amplified, as described by Messer et al. (1997), separated into two equal samples, and subsequently digested with either *MnII* or *RsaI* restriction enzyme. Different forms of the gene were identified based on their fragment pattern following digestion. Gene fragments that contained a restriction cut site for the *MnII* restriction enzyme were identified as allele *M* and resulted in a 236-bp and a 67-bp fragment, while gene sequences not digested by the *MnII* restriction enzyme resulted in a 303-bp fragment and were identified as allele *m*. Likewise, gene sequence that contained the restriction cut site for the *RsaI* restriction enzyme was labeled *R* allele and *r* allele denoted gene sequence lacking the *RsaI* restriction cut site. When digested the *R* allele produced a 290-bp and a 5-bp fragment, while the *r* allele produced only a 295-bp fragment. Possible ewe genotypes were as follows: mm, Mm, MM for *MnII* sequence differences and rr, Rr, RR for *RsaI* sequence differences.

Statistical Analysis. The effect of gene sequence on the number of days to 1st lambing and the number of days between 1st and 2nd lambing was evaluated. The GLM procedure of SAS (SAS Inst., Inc. Cary, NC) was used to make all comparisons. Breed was defined as Dorset (DORSET) or crossbred (CROSS). Season of conception associated with 1st lambing was defined as unfavorable (UNFV; conception in the months March to June), early favorable (EFV; conception in the months July to October), or late favorable (LFV; conception in the months November to February). Ewe genotypes for the *MTNR1A* gene sequence for *MnII* and *RsaI* cut patterns 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 two variable sites included the overall mean and the fixed effects of breed (DORSET or CROSS), season of first conception (EFV, LFV, or UNFV) and *MTNR1A* genotype (0, 1, or 2). The two-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 1st conception (EFV and LFV vs. UNFV and EFV vs. LFV) and genotypes (0 vs. 1 and 2; 1 vs. 2).

RESULTS AND DISCUSSION

Gene frequencies for the *MnII* and *RsaI* restriction cut sites in the ovine *MTNR1A* gene are shown in Table 1. Among the 116 ewes, genotypic frequencies were 0.43, 0.44 and 0.13 for *MM*, *Mm* and *mm* genotypes at the *MnII* restriction site and 0.13, 0.43 and 0.44 for *RR*, *Rr* and *rr* genotypes at the *RsaI* restriction site. When the two restriction sites were considered separately in the present population, genotypic frequencies did not differ from those expected under random mating.

The accelerated lambing system used in this population rewards ewes with the ability to breed out-of-season and penalizes ewes with a strong seasonal breeding pattern. If the *MTNR1A* gene sequence is associated with this trait, then the relatively lower frequency of *m* allele and *mm* genotype in our study population could be a consequence of selection based on the STAR system for the ability to breed out-of-season.

Table 1. Allele frequencies, SE, and 95% confidence limits for *MnII* and *RsaI* sequences differences in the ovine melatonin receptor 1A gene

Locus	Allele	Frequency	SE	95% Confidence Limits	
<i>MnII</i>	<i>M</i>	0.64	0.03	0.58	0.69
<i>MnII</i>	<i>m</i>	0.36	0.03	0.30	0.42
<i>RsaI</i>	<i>R</i>	0.35	0.03	0.29	0.40
<i>RsaI</i>	<i>r</i>	0.65	0.03	0.59	0.71

Genotypic Effects on Fertility. The effect of the *RsaI* restriction site on the two fertility measures evaluated was not statistically significant. Therefore, only the results for *MnII* polymorphism are presented. The ewe reproductive performance data for days to 1st lambing and days between 1st and 2nd lambing are presented in Table 2. Crossbred ewes had 17 fewer days to 1st lambing and 71 fewer days between 1st and 2nd lambing relative to Dorset ewes, but these differences were not statistically significant. Ewes with 1st conception in EFV and LFV

seasons tended to have 127 fewer ($P = 0.09$) d to 1st lambing and 100 more ($P = 0.07$) d between 1st and 2nd lambing relative to ewes with 1st conception in UNFV season. Ewes conceiving for the first time in EFV or LFV seasons were younger relative to ewes conceiving first in the UNFV season, probably because most ewes conceiving first in the UNFV season had previously failed to conceive in a favorable 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 UNFV season are more likely to conceive sooner after lambing because their next breeding season will be in a favorable season.

The ewes with *Mm* or *MM* genotypes needed 207 fewer ($P = 0.02$) d to 1st lambing and 140 fewer ($P = 0.03$) d between 1st and 2nd lambings relative to ewes with the *mm* genotype. Ewes carrying the *M* allele 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.

Table 2. Contrasts evaluating the effect of breed, season of 1st conception, and *Mnll* genotype on the number of days from birth to 1st lambing and the number of days between 1st and 2nd lambings in Dorset and $\frac{3}{4}$ -Dorset x $\frac{1}{4}$ -East Friesian ewes

Contrast	Days to 1st lambing			Days from 1st to 2nd lambing		
	Constant	SE	<i>P</i> -value	Constant	SE	<i>P</i> -value
Effect of breed						
Crossbred vs. Dorset	-16.87	73.33	0.82	-70.90	55.40	0.20
Effect of 1st conception season						
EFV and LFV vs. UNFV	-127.27	73.86	0.09	99.80	53.80	0.07
EFV vs. LFV	82.83	69.98	0.24	-67.68	53.54	0.20
Effect of <i>Mnll</i> genotype						
Mm vs. Mm and MM	207.19	87.10	0.02	139.68	63.15	0.03
Mm vs. MM	-79.43	63.50	0.21	40.42	48.53	0.40

The ability to breed and lamb out-of-season is a trait controlled by many genes and is important for improving profits in the sheep industry, by either accelerated lambing or by increasing the proportion of the flock that lambs out-of-season. While accelerated lambing can improve flock efficiency, both accelerated lambing and 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 its economic importance, several attempts have been made to improve out-of-season breeding using traditional breeding methods, but with limited success. Selection of breeding individuals was based on fertility in spring and summer or ability to perform in an accelerated lambing management system (Al Shorepy and Notter, 1997; Notter, 1992). The genetic improvement of this trait through a conventional breeding program is difficult because: fertility out of season has a moderate heritability; the trait is expressed in ewes; the trait is expressed only after sexual maturity has been reached. The availability of molecular techniques to determine variation between animals at the DNA level has led to a 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, while Notter et al. (2003) showed higher 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 $\frac{3}{4}$ -Dorset x $\frac{1}{4}$ -East Friesian ewes, indicating that the DNA sequence surrounding the ovine *MTNR1A* gene influences reproductive performance and the ability to breed out-of-season in our flock. The trait 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 sequence differences in the *MTNR1A* gene influences out-of-season reproduction has not been established. The DNA sequence differences in the *MTNR1A* gene evaluated in this study do not result in amino acid substitutions in the melatonin receptor (Pelletier et al., 2000), and functional differences in the receptor are not anticipated for different genotypes. 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 flock. For the Cornell flock and perhaps other Dorset flocks exposed to an accelerated lambing management program, it seems appropriate to genotype the young rams for the *MTNR1A* gene and select breeding rams with an MM genotype. Before using the *MTNR1A* gene in a marker assisted selection program in other breeds or flocks, this association should be validated in the target flock and its effects on other economically important traits should be explored.

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