Genet. Sel. Evol. 37 (Suppl. 1) (2005) S39–S53

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DOI: 10.1051/gse:2004031

Opportunities for detection and use of QTL influencing seasonal reproduction in sheep: a review

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(Accepted: 16 August 2004)

Abstract – Genetic improvement in traits associated with seasonal breeding in sheep is challenging because these traits have low heritabilities, are generally not expressed until late in life, are commonly recorded only in females, and are expressed only in some lambing seasons and management systems. Detection of quantitative trait loci and their use in marker-assisted selection could therefore substantially enhance selection responses. A population of sheep with an extended breeding season was developed through selection for fertility in spring matings and provides opportunities for further study of candidate genes influencing seasonal breeding. In particular, the *melatonin receptor 1a* gene is polymorphic in many sheep breeds and appears to influence a number of seasonal reproductive responses. In addition, a variety of clock genes have been identified in laboratory mammals and shown to influence biological rhythms. Mutations in these clock genes have been identified and shown to influence circadian periodicities and reproductive patterns in golden hamster and mouse. In sheep, expression of clock genes in the suprachaismatic nucleus and pars tuberalis (PT) suggests that "calendar" cells in the ovine PT play a role in maintaining circannual rhythms. Thus the various clock genes represent potentially important candidate genes that may be involved in control of seasonal breeding.

sheep / seasonal breeding / selection / *melatonin receptor la* / clock genes

1. INTRODUCTION

Seasonal variation in fertility is an important factor limiting efficiency of sheep production. Suboptimal fertility in spring and early summer imposes both a direct biological cost for maintenance of nonpregnant ewes and an opportunity cost associated with inadequate supplies of freshly harvested meat

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to fulfill consumer demands throughout the year. Differences among sheep breeds in timing and duration of the seasonal anestrus are well known, with reviews by Hafez [11], Hunter [12], and Notter [27]. Systems for accelerated lambing began to appear in the literature in the 1960's (*e.g.*, [33, 44]), and indicate that systems involving an 8-mo production cycle (*i.e.*, three lambings in 2 years) are feasible and consistent with the physiology of the ewe. In contrast, more intensive programs involving twice-yearly lambing with a production cycle of 183 d require very rapid rebreeding and appear not to be practical [48].

The STAR accelerated system [16] uses a 7.2-mo (219 d) production cycle with a 30-d breeding season and 73 d from the start of lambing until rebreeding. This is likely the most intensive program that is potentially feasible on a whole-flock basis. Rules proposed for scaling biological intervals to the mature size of the species [41] suggest that an interval between lambings of 7.1 mo for 70 kg ewes would be comparable to an interval between calvings of 12.0 mo for (relatively) nonseasonal 500 kg cows. The STAR system has been used in the Cornell Dorset flock since 1981, but with little evidence for improvement in spring and summer fertility through 1987 [16].

Several studies have reported breed effects on fertility in accelerated lambing systems (*e.g.*, [9, 29]). Among temperate breeds, most studies indicate that breeds of Merino ancestry (Rambouillet, Dorset) have relatively long breeding seasons [27] and are particularly responsive to effects of ram introduction on stimulation of ovulation and estrus [34]. The prolific breeds of Northern Europe such as the Finnish Landrace and Romanov also have been shown to perform well in spring matings [17, 29]. In contrast, the British Down breeds and coarse-wooled breeds appear to both have a shorter breeding season [27] and be less responsive to ram introduction in spring [32].

Over the past half-century, there have been several efforts to develop less-seasonal lines of sheep [7, 21, 39, 45], but most ended before obtaining definitive results. Selection was generally based on fertility in spring or summer matings or in accelerated lambing systems. Heritability estimates for fertility in spring and summer matings are low, the trait is not expressed until late in life, data are generally recorded only in one sex (female), and the trait is expressed in only some lambing seasons and management systems, making genetic improvement through conventional means challenging. Selection schemes to shorten the seasonal anestrus and improve reproductive performance in accelerated lambing would thus benefit from identification of quantitative trait loci (QTL) influencing seasonality and the implementation of marker-assisted selection. This review will therefore focus on the development

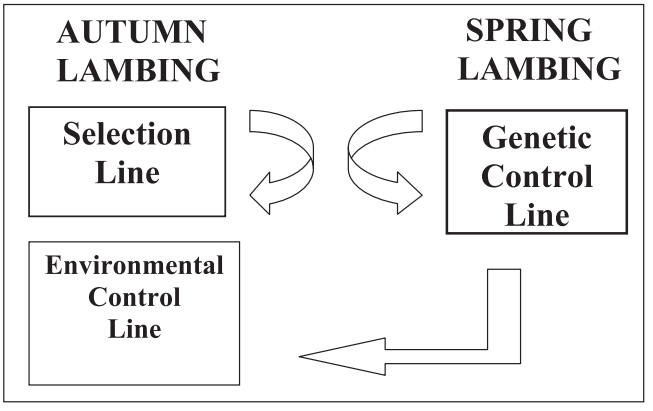

Figure 1. Experimental design of the Virginia Tech selection experiment.

of a population with reduced seasonality of breeding and on opportunities to detect associated QTL.

2. THE VIRGINIA TECH OOS FLOCK

2.1. Design of the experiment

The Virginia Tech out-of-season mating (OOS) line [1,2,30] was developed by selection for fertility in May and June matings. These months correspond to the period of near-minimal fertility for most temperature breeds [27]. Three-way crosses of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding were used to establish the line. Following at least one generation of *inter se* autumn matings among three-way crosses, the base population was divided into three lines in 1988 (Fig. 1).

A selection line (S) of 125 ewes and 10 rams was established in an annual spring mating system. These ewes were mated in single-sire breeding pastures for 60 d starting May 1. In phase 1 of the experiment (1988 through 1993), selection of replacements was based on the mean fertility of the ewes [1], and ewes were exposed to vasectomized rams for 2 weeks before breeding to attempt to induce ovulation and estrus by means of the ram effect [23]. In phase 2 (1994 through 1998), as mean fertility levels improved, teasing was discontinued, ewes were isolated from rams before the start of breeding, and a BLUP system of breeding value estimation was implemented based on a 1 or 0 binomial score for ewes that did or did not lamb in autumn [30]. In both phases, four to seven of the 10 rams and approximately one third of the ewes were replaced annually.

A genetic control (G) line of 45 ewes mated in October and early November was used as a source of unselected replacements. The G line was managed to maximize generation interval and minimize inbreeding. Ewes were replaced only if they became unsound or failed to lamb in two consecutive years. Fourteen foundation sires were identified at the start of the project, and two randomly selected male descendants of these foundation rams were maintained. Five rams from this pool were used in single-sire matings in each year. Rams from eleven of the sire lines remained in the pool at the end of the selection in 1998. In addition, an environmental control line (E) of 55 ewes was maintained contemporary to S with spring mating. Unselected ewe lambs were transferred to E from the G line. In phase 1 of the experiment, E ewes were mated in single-sire pastures to five rams from the G line, but in phase 2, E ewes were mated to the 10 S rams to permit direct comparison of fertility of S and E ewes in the same breeding pastures. This design allowed E ewes to be both unselected and contemporary to S, but also dictated that E ewes be an average of 7 mo older than S ewes.

2.2. Response to selection

The selection experiment ended in 1998 and the E and G lines were terminated at that time. However, selection has continued in the S line. Fertility was variable in the early years of the study, but by 1995, a substantial difference had emerged between lines and continued to increase through 1998 [30]. When mean estimated breeding values (EBV) for fertility of S, E, and G ewes mated in each year were regressed on year, resulting estimates of annual genetic trends were 1.98 ± 0.04, 0.61 ± 0.15, and 0.23 ± 0.10%/year for S, E, and G, respectively [30].

Significant ewe age effects on fertility and response to selection were observed (Tab. I) and highlight the difficulty in evaluating seasonality in young ewes. A cumulative selection response in fertility of 17% was observed in adult ewes, and a similar response of 18% was realized in 2-year-old ewes. However, fertility of 7-mo-old ewe lambs in S exhibited little, if any, response to selection and was essentially unchanged from that observed in 1988. The S ewe lambs had lower fertility than E ewe lambs because of the greater age at first breeding in E lambs transferred from the genetic control line (Fig. 1).

2.3. Characterization of OOS ewes

The timing and duration of anestrus in OOS ewes was evaluated by maintaining high and low breeding value ewes with vasectomized rams from

Table I. Mean fertility (%) of select (S) and environmental control (E) ewes 1995–1997.

Breeding opportunity*	Flock	
	S	E
First	10	24
Second	76	58
Third and greater	87	70

*S ewes were first mated at 7 to 8 mo of age whereas E ewes were first mated at 14 to 15 mo of age (Fig. 1).

mid-January through the following July and monitoring their mating behavior [46]. Only ewes that had lambed in the previous autumn (October and November) were used to ensure physiological comparability, and the same rams were used throughout the study to avoid possible induction of ram effect by introduction of novel males. In ewes evaluated in 1992, 1993, and 1995, the seasonal anestrus of high-fertility ewes from the S and E lines (mean EBV of 12.6%) averaged only 28.4 d and was significantly shorter than the 70.1 d of anestrus observed in low-fertility ewes (mean EBV of 0.3%). Vincent *et al.* [46] further reported that 13 OOS ewes evaluated in 1997 all exhibited nearly continuous cyclicity during spring and summer, with a mean period of anestrus of only 11.3 d.

Ewes in the S line were shown to have lower nocturnal levels of circulating melatonin and higher nocturnal levels of circulating prolactin than E ewes [28]. Increases in fertility EBV were associated with declines in circulating melatonin (-2.23 ± 0.79 pg·mL^{-1}·%$^{-1}$) and increases in circulating prolactin (1.23 ± 0.53 pg·mL^{-1}·%$^{-1}$), suggesting that these animals may provide a model for understanding genetic control of secretion of these hormones.

3. *MELATONIN RECEPTOR 1A*: A CANDIDATE GENE INFLUENCING SEASONALITY

Molecular characterization of sequence variants in the ovine *melatonin receptor la* gene (*MTNR1A*) was reported by Barrett *et al.* [3] and Messer *et al.* [24]. Relative to the sequence for ovine *MTNR1A* reported by Reppert *et al.* [37], Barrett *et al.* [3] found a variant form with eight base changes, three of which resulted in amino acid substitutions in the receptor. Subsequent breed comparisons indicated that both forms of the gene were present in samples of six Greyface × Suffolk ewes, six Greyface × Dorset ewes, and six Soay rams (Tab. II). The presence of both forms of the gene was somewhat surprising in

Table II. Allelic frequencies for the *Mln*I and *Rsa*I polymorphisms from the literature.

Breed	Number of animals	*Mln*I		*Rsa*I		Reference
		Allele +	Allele −	Allele +	Allele −	
Merino d'Arles	142	0.63	0.37	-*	-*	[35]
Ile-de-France	29	0.45	0.55	-*	-*	[35]
Suffolk	18	0.67	0.33	0.08	0.92	[24]
Texel	1	0.50	0.50	0.00	1.00	[24]
Coopworth	4	0.75	0.25	0.00	1.00	[24]
Columbia	57	0.84	0.16	0.03	0.97	[49]
Hampshire	79	0.39	0.61	0.57	0.43	[49]
Greyface XB	12	0.71	0.29	-*	-*	[3]
Soay	6	0.75	0.25	-*	-*	[3]
Han	150	0.75	0.25	0.52	0.48	[4]

*Not reported.

Table III. Allelic frequencies for the *Mln*I and *Rsa*I polymorphisms in various populations.

Population	Number	*Mln*I		*Rsa*I	
		Allele +	Allele −	Allele +	Allele −
Virginia Tech OOS	362	0.42	0.58	0.34	0.66
Cornell Dorset	24	0.31	0.69	0.64	0.36
Tisdale Polypay	19	0.47	0.53	0.76	0.24
Israel Assaf	2	0.50	0.50	0.00	1.00
Israel Local Awassi	7	0.07	0.93	0.31	0.69
Israel Improved Awassi	9	0.67	0.33	0.20	0.80

the Soay, given the bottlenecks and small population sizes often postulated for this breed and suggested that these mutations arose early in the development of domestic sheep and had not been lost because of selection or random drift.

Messer *et al.* [24] identified two RFLP polymorphisms in *MTNR1A* in 36 OOS animals. Allelic frequencies were intermediate for both an *Mnl*I polymorphism (0.39 and 0.61) and an *Rsa*I polymorphism (0.39 and 0.61). However, the seasonal Suffolk breed ($n = 18$) was also polymorphic for both RFLP sites. Notter *et al.* [31] subsequently estimated allelic frequencies of 0.42 and 0.58 for the *Mln*I polymorphism and of 0.34 and 0.66 for the *Rsa*I polymorphism in the OOS flock (Tab. III) and further demonstrated that the polymorphisms were not independent in that flock. Only six of nine possible genotypes occurred with frequencies greater that 0.03.

A more detailed molecular analysis of *MTNR1A* was carried out by Pelletier *et al.* [35]. Ten mutations were observed in sequences from 30 Merino d'Arles ewes. Five of these corresponded to mutations identified by Barrett *et al.* [3] (and one of these was responsible for the *Mnl*I polymorphism), another corresponded to the *Rsa*I polymorphism of Messer *et al.* [24], and four were newly discovered mutations. Two mutations resulted in amino acid substitutions, only one of which had been identified by Barrett *et al.* [3]. Animals homozygous for the absence of the *Mnl*I site ($n = 16$) were also homozygous for three other mutations, including one that resulted in an amino acid substitution. In contrast, individuals homozygous for the presence of the *Mnl*I site ($n = 26$) had three different arrangements of mutant alleles, one of which resulted in a change in amino acid sequence (at a different location than that observed for ewes that were homozygous for the absence of the *Mnl*I site).

Recent data from additional populations confirm that *MTNR1A* is polymorphic at both the *Mln*I and *Rsa*I restriction sites (Tab. III). Frequencies of both alleles at *Mln*I were greater than 0.30 in the Cornell University Dorset flock, in a commercial Polypay flock in the United States, and in an improved Awassi flock in Israel. Frequencies of *Rsa*I alleles were likewise between 0.20 and 0.80 in all populations except an Assaf flock in Israel (with a sample size of only two). The Dorset breed contributed to the formation of both the Polypay breed and the OOS population. Notter *et al.* [31] speculated that intermediate frequencies observed for *Mln*I alleles in the OOS flock may have resulted from the contribution of different alleles from different founder breeds, but results from the Cornell Dorset flock suggest that this was not the case.

Results in Tables II and III reveal substantial allelic diversity at the *Mln*I restriction site in *MTNR1A* in a number of breeds. All the breeds listed in Table III are under at least some selection for reduced seasonality. The Dorset and Polypay flocks have been selected for performance in accelerated lambing. The Cornell flock has had few introductions of outside breeding stock, although there was a link to a Dorset flock that also contributed to formation of the OOS flock. The Awassi and Assaf flocks are also maintained in frequent-lambing systems [10]. However, Table II reveals similar intermediate frequencies in the very seasonal Suffolk [24], Soay [3], and Ile-de-France [35] breeds. An alternative hypothesis of some form of heterozygote superiority involving these polymorphisms or the presence of unknown, very closely linked alleles naturally emerges but has not been tested. In goats, Migaud *et al.* [25] identified seven mutations with the caprine *MTNR1A* gene in 16 goats of the seasonal Alpine breed in France and 14 goats of the less seasonal Creole type

from Guadeloupe. One amino acid changes was identified in the receptor, but no differences in allele frequencies were observed between these breeds.

Evidence for an association between *MTNRIA* and seasonal reproduction comes mainly from two sources. Pelletier *et al.* [35] compared genotypic frequencies for the *Mnl*I polymorphism in 36 Merino d'Arles ewes with a history of spontaneous ovulatory activity in April (H ewes; identified by circulating progesterone levels in two jugular blood samples collected 8 to 10 d apart) and 35 ewes of the same breed that had not ovulated in this period (L ewes). The frequency of homozygotes for the presence of the *Mnl*I restriction site (genotype ++) was significantly higher in H ewes (52.8 *versus* 28.5%; $P < 0.001$). Homozygotes for the absence of the restriction site (genotype −−) were correspondingly less frequent in H ewes (0.0% *versus* 28.5%). An analysis of genotypic frequencies within half-sibs (21 H and 29 L ewes) yielded similar results, with frequencies of genotype −− of 0.0% in H ewes and 24.1% in L ewes ($P < 0.01$). Ewes of the seasonal Ile-de-France breed ($n = 29$) also had relatively low frequencies of genotype ++ (28% *versus* 38% for −−). Differences in genotypic frequencies between the H and L Merino d'Arles ewes for the *Rsa*I polymorphism were not significant.

In the OOS flock, sampling of DNA began in 1997, and 362 ewes were genotyped for the *Mnl*I and *Rsa*I polymorphisms between 1997 and 2000 [31]. Genotypic means for spring fertility and litter size were estimated for six high-frequency haplotypes. Genotypic effects on fertility were observed only when the analysis was restricted to records of adult (3 years old and older) ewes, which was not surprising given the low fertility of OOS ewe lambs (Tab. I). Among adult ewes, mean fertility varied among genotypes from 65.5 to 85.3%. Ewes with at least one copy of the + allele at the *Mnl*I restriction site had 11.2 ± 5.1% higher spring fertility than ewes that were homozygous for the – allele ($P = 0.03$). Independent effects and interactions involving the two polymorphisms were difficult to assess given observed linkage disequilibrium, but there was no evidence for a direct impact of the *Rsa*I polymorphism on fertility. Effects of *MTNR1A* genotype on litter size were not significant.

Estimates of breeding values and dominance deviations for spring fertility were used to determine the proportion of the total additive variance that could be attributed to this gene and to partition the genotypic variance at the locus into additive and dominance components [13]. In adult ewes, estimates of total additive and permanent environmental variances for fertility were 151 and 148%^{2}, respectively. Corresponding estimates of additive and dominance variances for the *MTNR1A* locus were 35.7 and 13.9%^{2}, respectively. Thus these *MTNR1A* markers accounted for 23.8% of the additive variance for

fertility, and dominance effects at this locus could account for 9.3% of the permanent environmental variance. These values highlight both the potential value of markers such as these and the challenges inherent in their identification. The heritability of spring fertility was 11% in adult matings. Thus while the markers account for nearly 25% of additive variance, they account for only about 2.5% of phenotypic variance, emphasizing the difficulty that will be involved in detection and accurate estimation of marker effects in other populations.

In a related study of the effects of these polymorphisms on reproduction, Chu *et al.* [4] reported effects of *MTNR1A* markers on litter size in Small-tailed Han sheep in China. The Small-tailed Han is a prolific breed [8] that expresses nonseasonal ovulatory activity and has been shown to have a high frequency of the *FecB* mutation at the *bone morphogenic protein receptor IB* gene [22]. In this study, ewes that were homozygous for the absence of the *Rsa*I restriction site had larger litters at second parity than ewes that were homozygous for the presence of the restriction site (3.19 ± 0.13 *versus* 2.25 ± 0.12 lambs/litter) and larger litters than heterozygous ewes at both first and second parity. Genotypic frequencies at the *Mln*I site in this lowly seasonal breed revealed that animals of genotype −− were not present in this sample of 150 ewes, even thought heterozygotes were relatively frequent (50.9%). Prior selection for fertility in spring matings or preferential use of ++ sires may have led to this unexpected distribution of genotypes at the *Mln*I restriction site.

4. CLOCK GENES: POSSIBLE EFFECTS ON SEASONAL REPRODUCTION

Identification of mutations that disrupt circannual reproductive patterns and the introgression of such alleles into other populations could provide an effective means to reduce seasonality. Knowledge of genetic mechanisms controlling circannual rhythms remains limited, but information on genetic control of circadian rhythms in mammals is expanding rapidly, even though most studies involve laboratory rather than livestock species. Extension of these studies from circadian to circannual rhythms is far from trivial, but the various clock genes involved in circadian timekeeping may function as QTL influencing seasonal reproduction.

Eight major clock genes have been identified in mammals [14]: *Clock*, three *Period* genes (*Per1, Per2,* and *Per3*), *BmalI, Timeless,* and two *Cryptochrome* genes (*Cry1* and *Cry2*). These genes are distributed throughout the genome; in the mouse they are located on chromosomes 6, 11, 1, 4, 7, 10, 10, and 2, respectively [38]. Discussion of gene expression and the interactions involved

in the establishment and maintenance of circadian rhythms at the cellular level can be found in Reppert and Weaver [37]. Briefly, autoregulatory feedback loops and the resetting and entrainment of these feedback loops by external stimuli (primarily, but not exclusively, light) are central components of the circadian clock. In the absence of external stimuli, these feedback loops, located primarily in the suprachiasmatic nucleus (SC) of the anterior hypothalamus, can maintain a daily circadian rhythm of approximately 24 h. This circadian rhythm can be observed in a number of responses, including feed intake and sleep-wake patterns, but is most often measured by the daily period of locomotor activity which is commonly measured by wheel-running behavior in rodents and represented as τ.

Various mutations in clock genes are known to interrupt this 24-h circadian activity rhythm. The *tau* mutation in the hamster *Clock* gene shortens the daily period of locomotor activity by 2 h in *tau*/+ individuals and by 4-h in *tau/tau* homozygotes [36]. In contrast, the $Clock^{M1Jt}$ mutation in mice lengthens the circadian period in heterozygotes and abolishes it in homozygotes [47]. Two studies utilized mouse strains with abnormal circadian activity patterns to attempt to identify QTL associated with this behavior. Suzuki *et al.* [40] utilized F_2 offspring of CS and C57BL/6J mice. Mice of the inbred CS strain are active in both the light and dark period, have a free-running circadian period of greater than 24 h in constant darkness, and have been shown to differ from C57BL/6J [5]. A QTL was identified on chromosome 19 and confirmed to influence the activity pattern in a second F_2 family derived from crosses with MSM, a line derived from Japanese wild mice (*Mus musculus molossinus*). This locus accounted for 10.4% of F_2 phenotypic variance in circadian period. Three additional suggestive QTL were detected: one on chromosome 12 and two others on chromosome 19. However, none of these QTL mapped to the location of a known clock gene. In a second study, Shimomura *et al.* [38] used F_2 offspring of C57BL/6J and BALB/cJ mice, which have a circadian period of less than 23 h, to identify 14 loci on 10 chromosomes with apparent effects on some aspect of circadian behavior. Markers that specifically affected the length of the circadian period were found on chromosomes 4, 5, and 12, but again did not correspond to the location of any known clock genes.

In sheep, gene expression studies are beginning to explore molecular regulation of the circadian clock. Expression of *PER1* in the pars tuberalis (PT) has been shown to be under photoperiodic control [26]. Expression of seven clock genes in the ovine SC and PT revealed effects of melatonin on phase relationships between *PER* and *CRY* genes in the PT but not the SC [18], suggesting a role for these genes in translation of melatonin signals into

physiological responses. Lincoln *et al.* [20] further hypothesized the presence of "calendar cells" in the brain and pituitary gland that express a full complement of clock genes and provide a molecular basis for seasonal phenomena. In the ovine PT, activation of *PER* occurs early in the light phase, while activation of *CRY* occurs early in the dark phase [19]. In the SC, dimerization of the protein products of *PER* and *CRY* in the cytoplasm is required for translocation into the nucleus [15], so the interval between expression of *PER* and *CRY* may affect opportunities for dimerization of their gene products in cells of the PT, with potential effects on expression of other genes involved in reproductive function.

These results suggest that mutations in clock genes can affect circadian behavior patterns and may likewise influence more complex circannual rhythms. However, other QTL affecting quantitative variation in circadian behavior appear to exist independently of clock genes, a result that is perhaps not surprising, given the complex interactions and regulatory events involved in expression of these behaviors.

5. PROSPECTS

Development of populations with reduced seasonality is clearly possible through selection. However, identification of QTL and molecular markers could substantially augment selection responses. Studies to identify QTL affecting seasonal reproductive patterns in small ruminants are feasible given the very large between-breed variation in these patterns. Techniques to characterize the timing and duration of the seasonal anestrus [46] are labor-intensive but straightforward and informative and have modest facility and laboratory requirements. However, a substantial investment will be required to generate sufficient numbers of animals to allow accurate detection of QTL. Time requirements for such studies are also substantial. Fertility in spring and summer appears to be uniformly low in ewe lambs of all breeds. Useful variation among F_2 ewes in seasonal fertility is expected only at the second and subsequent lambings. New techniques for QTL detection in outbred populations are emerging [42, 43] to compliment older approaches involving segregation analysis [6] and may be used in selection lines and commercial populations, but the complex management effects and interactions common in accelerated lambing systems will limit accuracy of individual-animal evaluation. Also, if fertility in spring and summer is the main phenotypic characteristic to be measured, issues of scaling arise. Crosses between seasonal and nonseasonal lines need not be intermediate to parent lines in realized fertility, and performance of F_1

animals may need to be carefully evaluated in order to help choose between F_2 and backcross designs.

A search for, and characterization of effects of, mutants and sequence variants in candidate genes such as *MTNR1A* and the various clock genes should continue, since loss of function (*i.e.*, loss of seasonality) relative to the wild type is desired and could possibly be induced by mutations in genes involved in regulation of circadian and circannual cycles. Study of these genes in livestock and laboratory studies will likely continue to expand, providing new information on mechanisms of gene expression and regulation in these complex systems, and with associated opportunities for their manipulation.

ACKNOWLEDGEMENTS

We would like to thank Elisha Gootwine for providing DNA from the Awassi and Assaf animals, Mike Thonney for providing DNA from the Cornell University Dorset flock, and Dwight and Sharon Tisdale for providing DNA from their Polypay animals.

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