



# Genetic mapping of quantitative trait loci for aseasonal reproduction in sheep

R. G. Mateescu\* and M. L. Thonney<sup>†</sup>

\*Department of Animal Science, Oklahoma State University, Stillwater, OK 74078, USA. <sup>†</sup>Department of Animal Science, Cornell University, Ithaca, NY 14853, USA

## Summary

The productivity and economic prosperity of sheep farming could benefit greatly from more effective methods of selection for year-round lambing. Identification of QTL for aseasonal reproduction in sheep could lead to more accurate selection and faster genetic improvement. One hundred and twenty microsatellite markers were genotyped on 159 backcross ewes from a Dorset × East Friesian crossbred pedigree. Interval mapping was undertaken to map the QTL underlying several traits describing aseasonal reproduction including the number of oestrous cycles, maximum level of progesterone prior to breeding, pregnancy status determined by progesterone level, pregnancy status determined by ultrasound, lambing status and number of lambs born. Seven chromosomes (1, 3, 12, 17, 19, 20 and 24) were identified to harbour putative QTL for one or more component traits used to describe aseasonal reproduction. Ovine chromosomes 12, 17, 19 and 24 harbour QTL significant at the 5% chromosome-wide level, chromosomes 3 and 20 harbour QTL that exceeded the threshold at the 1% chromosome-wide level, while the QTL identified on chromosome 1 exceeded the 1% experiment-wide significance level. These results are a first step towards understanding the genetic mechanism of this complex trait and show that variation in aseasonal reproduction is associated with multiple chromosomal regions.

**Keywords** aseasonal reproduction, quantitative trait loci, sheep.

## Introduction

Aseasonal reproduction is a complex trait controlled by many genes and environmental factors. The seasonal lambing pattern that is commonly observed in sheep breeds from temperate climates is a major obstacle to increasing the distribution and intensity of lamb production. The timing of the breeding season to ensure that parturition occurs in the spring depends upon the length of gestation. Thus, sheep (5 months gestation period) are short-day breeders with conception occurring in autumn and winter. Sheep breeds differ in timing and duration of breeding (Nugent *et al.* 1988; Notter 1992; Vincent *et al.* 2000) and, among temperate breeds, Dorset, Finnsheep, and Rambouillet appear least seasonal (Hulet *et al.* 1974; Hall *et al.* 1986; Clarke *et al.* 2003). Heritability estimates

reported for such traits range from 0.03 to 0.32 (Hanrahan & Quirke 1986; Hanrahan 1987; Smith *et al.* 1992). The variability of timing and duration of breeding, as well as successful selection for aseasonal reproduction, indicates that the trait is under genetic control (Notter 1992; Lewis *et al.* 1996; Mateescu *et al.* 2009). The genetic component of sexual activity has long been considered a potential approach to control the onset of the breeding season (Hanrahan 1987), but very little is known about the identity of the genes responsible for this genetic variation. Identification of QTL influencing aseasonal reproduction and implementation of marker-assisted selection would greatly enhance current selection programmes for improving aseasonal reproductive performance (Notter & Cockett 2005).

To systematically map QTL contributing to aseasonal reproduction expression, we developed a pedigree founded on Dorset ewes selected for aseasonal reproduction and East Friesian rams with strong seasonal reproduction. This pedigree consists of backcrosses to the Dorset founder group. The purpose of the project was to identify QTL for aseasonal reproduction traits.

Address for correspondence

R. G. Mateescu, Department of Animal Science, Oklahoma State University, 206D Animal Science, Stillwater, OK 74078, USA.  
E-mail: raluca@okstate.edu

Accepted for publication 16 December 2009

## Materials and methods

### Animal pedigree

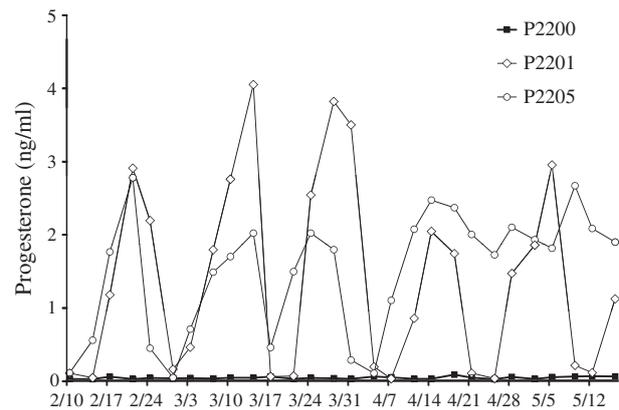
An experimental population of animals was created by crossing Dorset ewes from the Cornell Sheep Farm and East Friesian rams from Old Chatham Shepherding Company (OCSC), a sheep dairy near Albany, NY. The Cornell Dorset ewes were non-dairy sheep that have been selected for aseasality and prolificacy, while the East Friesian dairy breed is known for high milk production but poor aseasality reproduction. During the last 25 years, the Cornell Dorset flock was managed for accelerated lambing under the STAR system (Lewis *et al.* 1998), with ram replacements selected on the ability of dams to lamb every 7.2 months (Thonney 2007). As a result of this selection programme, the proportion of mature Dorset ewes with 2 or more lambing records with aseasality reproduction increased to more than 67%. The OCSC began in 1994 and today is one of the largest dairy sheep farms in the United States, with more than 800 purebred East Friesian and crossbred Lacaune ewes. The East Friesian is one of the world's best milk producing dairy sheep, reported to average 500–700 kg of milk per lactation of 240–260 days (Berger 2007).

The two parental populations were appropriate for this project, with one line likely to carry predominantly favourable alleles and the other line likely to carry predominantly unfavourable alleles with respect to aseasality reproduction traits.

Eight Dorset ewes that expressed aseasality reproduction and four East Friesian rams were used to generate eight F<sub>1</sub> rams which were subsequently mated to 132 Dorset ewes to create 159 backcross ewes (Fig. S1). All sheep were housed, bred and fed under similar conditions at the Cornell Teaching and Research Center Sheep Farm.

### Phenotypes

Ewes in the Spring (March–April and May–June) breeding seasons result in the lowest fertility (Mateescu *et al.* 2009). Therefore, the aseasality reproduction phenotype was assessed using blood progesterone profiles prior to, during and after the Spring (2003 and 2007) breeding season for the backcross to Dorset yearling ewes. Blood samples were collected twice weekly for 14 weeks from 10 February to 16 May. The sampling period included 35 days prior to breeding, with a teaser ram (vasectomized) added to the group for the last 10 days, 32 days with an intact Dorset ram, and 31 days after the intact ram was removed. Oestrous cycles of individual ewes were generated based on dynamic changes in blood progesterone levels over time (Fig. 1). Early conception was indicated by a constant high level of progesterone. At 55 days post-breeding (after the intact ram was removed), transabdominal ultrasound was performed to detect early pregnancy. The final measure of



**Figure 1** Progesterone profile for three backcross to Dorset ewes before, during and after the breeding season. Ewe P2200 showed no ovarian activity, indicated by the constantly low level of progesterone; five regular oestrous cycles (ovulations) were observed for ewe P2201, while the progesterone profile for ewe P2205 showed three ovulations followed by conception.

aseasality reproduction was the actual lambing in the August–September lambing period.

Several component traits were used to describe the aseasality reproduction phenotype. The traits measured and analysed included the number of oestrous cycles and maximum level of progesterone prior to breeding, pregnancy status determined by progesterone level, pregnancy status determined by ultrasound, lambing status and number of lambs born. The regularity of oestrous cycles (oestrous cycles about 17 days apart) based upon the progesterone profile created for each ewe was also considered. A score was created to describe the overall ability to lamb out-of-season (0 = no oestrous cycles; 1 = irregular oestrous cycles; 2 = regular oestrous cycles; 3 = pregnancy determined by progesterone level; 4 = pregnancy determined by ultrasound; 5 = lambed 1 offspring; 6 = lambed 2 offspring).

### Genotyping and linkage maps

Using the latest sheep linkage map available (version 4.7), 120 microsatellite markers were identified to provide uniform coverage of the genome and to maximize the polymorphic information content (IC). These markers were distributed on 26 autosomes at an average marker interval of 29 cM. Genotyping of all 311 animals on the backcross pedigree (140 Dorset and 4 East Friesian founders, 8 F<sub>1</sub> rams and 159 backcrosses to Dorset) was performed at GeneSeek Inc.

### QTL analysis

A QTL interval-mapping analysis was performed using the web-accessible program GRIDQTL (<https://gridqt1.cap.ed.ac>).

uk/gridsphere) (Seaton *et al.* 2002) and was based on a least-squares method (Haley *et al.* 1994). The software allows for the analysis of data from different mating schemes including the combined backcross/ $F_2$  design employed for our crossbreed pedigree.

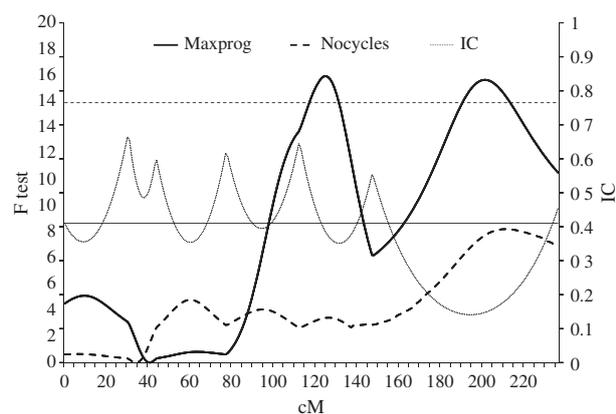
The general linear model allows for fixed effects and covariates to be included. Breed (2 founder groups, the  $F_1$  and backcross groups) and year (1 = 2003; 2 = 2007) were always included in the model as fixed effects.

An  $F$ -ratio of the variance explained by the QTL (after fitting all other effects in the model) to the residual MS was used to test for the significance of the QTL effects. Estimates were obtained for the additive effect of the putative QTL at that location in the backcross population. The location giving the highest  $F$ -ratio statistic was considered to be the best estimate for the position of the QTL. When the plot of the  $F$ -value against the chromosomal location suggested the presence of two QTL, the analysis was repeated with a test for two vs. one QTL (Fig. 2). Threshold values for 5% and 1% chromosome- and experiment-wide significance levels for each trait were determined by a permutation approach with 10 000 iterations (Churchill & Doerge 1994).

## Results and discussion

### Aseasonal reproduction

The aseasual reproduction phenotype was assessed on 163 yearling backcross ewes, 118 in spring-autumn 2003 and 45 in spring-autumn 2007. Of 163 ewes, only six never cycled; 51 ewes had early conception based on progesterone profiles; 48 were validated as pregnant by the ultrasound; and 32 ewes lambed in the autumn lambing season. For



**Figure 2** Profile plot of  $F$ -test statistics (vertical axis) underlying the maximum progesterone level (MaxProg) and number of oestrous cycles (NoCycles) on chromosome 1. The peaks represent positions of the QTL in cM on the x-axis across the chromosome. The solid and dashed horizontal lines indicate the 1% chromosome-wise and 5% genome-wise significance thresholds, respectively. Information content (IC) obtained along the linkage groups is represented on the secondary y-axis.

**Table 1** Distribution of 157 ewes that showed at least one oestrous cycle during the 96-day sampling period with respect to the number of oestrous cycles before introduction of intact rams, and number (%) of ewes identified as pregnant and which subsequently lambed during the autumn season.

Cycles prior to breeding	Ewes	Pregnant		
		Progesterone <sup>1</sup>	Ultrasound <sup>2</sup>	Lambd
0	6	0 (0)	0 (0)	0 (0)
1	21	4 (19.05)	3 (14.29)	3 (14.29)
2	42	12 (28.57)	12 (28.57)	7 (16.67)
3	88	35 (39.77)	33 (37.50)	22 (25.00)

<sup>1</sup>30 days post-breeding.

<sup>2</sup>55 days post-breeding.

157 ewes that cycled at least once during the 14-week sampling period, a detailed distribution with respect to the number of cycles in the 35 days prior to beginning of breeding season is presented in Table 1. As the number of oestrous cycles during this interval increased, a ewe was more likely to be detected as pregnant by the progesterone profile or ultrasound or to actually lamb. With a regular oestrus cycle of about 17 days, it is clear that the ewes with three cycles during pre-breeding period were already cycling at the beginning of the period. Among the 21 ewes with only one cycle during this period, seven cycled at the very end of the period, and that cycle could therefore have been ram-induced.

Examination of 163 yearling ewes before, during and after the breeding season revealed that 96.3% of the ewes showed ovarian activity (at least one estrous cycle), but only 32.5% of the ewes cycling had a successful pregnancy based on the progesterone profile; 30.6% were confirmed pregnant by the transabdominal ultrasound scan 55 days after the end of the breeding season, and 20.4% lambed during the autumn lambing season. These results suggest that the lower ability to conceive and lamb out-of-season is not caused by a lack of oestrous activity, but is related to decreased ability to become pregnant and, to a lesser extent, to maintain the pregnancy. Pope *et al.* (1989) also observed that ability to maintain pregnancy after a spring conception is an important factor contributing to reproductive success of aseasual breeding.

### Marker informativeness

The average number of alleles for the 120 microsatellite marker loci in the entire population was 8.08 (range 1–31), with three markers being fixed for the same allele. Based on the criteria of Botstein *et al.* (1980), 63 markers (52.5%) were highly informative (heterozygosity > 0.6), 36 markers (30%) were moderately informative (0.3 < heterozygosity < 0.6), and 21 markers (17.5%) were uninformative (heterozygosity < 0.3). Although all markers were initially

selected to have a high polymorphic IC and provide a uniform coverage of the genome, 21 markers proved to be less informative in this backcross pedigree.

### QTL analysis

Seven chromosomes (1, 3, 12, 17, 19, 20 and 24) were identified to harbour putative QTL for one or more component traits used to describe aseasonal reproduction. Ovine chromosomes (OAR) 12, 17, 19 and 24 harbour QTL significant at the 5% chromosome-wide level; OAR 3 and OAR 20 harbour QTL that exceeded the threshold at the 1% chromosome-wide level; while the QTL identified on OAR 1 exceeded the 1% experiment-wide significance level. Four of the seven chromosomes harboured putative QTL for more than one component trait. Two distinct QTL regions were identified on OAR 1 for maximum level of progesterone prior to breeding, with a significant *F*-test of 7.8 for the two-QTL model against a one-QTL model.

The QTL locations giving the highest test statistic and the parameter estimates obtained at each location for chromosomes with a significant QTL are shown in Table 2. Additive QTL effects (*a*) correspond to genotype values of +*a* and -*a* for individuals having inherited two Dorset alleles and individuals with two East Friesian alleles, respectively. Positive additive effects indicate that Dorset alleles confer increased ability to breed out-of-season, while negative additive effects indicate that Dorset alleles decreased this ability. QTL for several traits (on OAR 3, 16 and 17) had Dorset alleles with a positive effect (increased the ability to

breed out-of-season) while QTL identified on OAR 1, 12, 19, 20 and 24 had Dorset alleles that decreased the ability to breed out-of-season.

Examination of chromosomes harbouring putative QTL revealed that on most of these chromosomes, many of the other traits analysed indicated the likely presence of an underlying QTL in the same position, even though QTL for these traits did not reach statistical significance (Fig. S2). This pattern, where many traits suggest the presence of a QTL but only one or two traits reach significance thresholds, applied to most of the chromosomes where putative QTL were identified, providing additional support for the existence of QTL in those chromosomal regions.

In recent years, new sheep genomic resources and tools have become available [for a review, see Maddox & Cockett (2007)], allowing comparisons of the ovine, bovine and human genomes. The newly constructed radiation hybrid comparative map of OAR1 (Wu *et al.* 2009) allowed us to further investigate the region containing the two most significant QTL identified on OAR1 between markers *CSSM4* and *BMS2263* (genome-wide *P*-value < 0.05). The region between markers *CSSM4* and *BM6506* containing the first QTL is homologous to the 51.5–80.8 cM region on the bovine chromosome (BTA) 1, while the region between markers *BM6506* and *BMS2263* containing the second QTL is homologous to the 80.8–156.5 cM region on BTA1, and both regions are homologous to different segments on human chromosome 3. A brief exploration of the genes identified on the homologous bovine or human regions revealed two of interest: *progesterin* and *adipoQ receptor family*

**Table 2** Parameter estimates for QTL with *F*-tests significant at *P* < 0.05 (chromosome-wide) for breeding out-of-season traits following a genome-wide screen for QTL. Chromosome (OAR), *F* statistic, log of the odds (LOD) ratio score, and the additive (*a*) effect at the locus are shown.

Trait	OAR	Position (cM)	Flanking markers <sup>1</sup>	<i>F</i>	LOD	<i>a</i>
MaxProg	1	125	<i>CSSM4</i> - <i>BM6506</i>	16.86***	3.46	-1.02 (0.35)
		224	<i>BM6506</i> - <i>BMS2263</i>			-0.99 (0.29)
PregProg	3	248	<i>BM8230</i> - <i>BMS772</i>	9.58*	2.01	0.26 (0.08)
PregUltr	3	245	<i>BM8230</i> - <i>BMS772</i>	12.34**	2.57	0.28 (0.07)
RegulCycle	12	95	<b><i>INRA035</i></b> - <i>HUJ625</i>	6.26*	1.33	-0.29 (0.11)
No.Lambs	16	4	<b><i>RM106</i></b> - <i>BM1225</i>	6.37*	1.35	0.22 (0.08)
PregProg	17	30	<b><i>OARCP49</i></b> - <i>MAF209</i>	7.91*	1.67	0.19 (0.06)
Lambing	17	38	<i>OARCP49</i> - <i>MAF209</i>	6.87*	1.45	0.20 (0.07)
No.Lambs	17	41	<i>OARCP49</i> - <i>MAF209</i>	6.01*	1.27	0.25 (0.10)
Score	17	33	<b><i>OARCP49</i></b> - <i>MAF209</i>	6.26*	1.33	0.68 (0.27)
PregProg	19	26	<i>INRA026</i> - <b><i>OARAE119</i></b>	8.01*	1.69	-0.24 (0.08)
PregUltr	19	25	<i>INRA026</i> - <b><i>OARAE119</i></b>	6.43*	1.36	-0.22 (0.08)
Lambing	20	70	<b><i>BM1818</i></b> - <i>BP34</i>	6.36*	1.35	-0.24 (0.09)
No.Lambs	20	70	<b><i>BM1818</i></b> - <i>BP34</i>	8.28**	1.75	-0.36 (0.12)
No.Lambs	24	51	<i>BP28</i> - <i>ELN</i>	6.34*	1.34	-0.26 (0.10)

MaxProg, maximum progesterone level during the prebreeding season; PregProg, pregnancy status determined by progesterone; PregUltr, pregnancy status determined by ultrasound; RegulCycle, regularity of oestrous cycles; Lambing, lambing outcome; No.Lambs, number of lambs; OAR, *Ovis aries* chromosome.

\*Chromosome-wide 5% significance; \*\*chromosome-wide 1% significance; \*\*\*genome-wide 1% significance.

<sup>1</sup>Markers flanking the position of the maximum *F*-statistic. Markers in bold are <3 cM from the highest *F*-statistic.

member IX (PAQR9) and forkhead box L2 (FOXL2). Human PAQR9 belongs to a large family of membrane progesterin receptors (Lyons *et al.* 2004) potentially involved in mediating the rapid effects of progesterone on cell-signalling pathways independently of transcription (Bramley *et al.* 2002; Losel *et al.* 2003; Thomas 2008). Furthermore, Fernandes *et al.* (2005) suggested that the PAQR9 mRNA could play a role in mediating progesterone actions in those tissues that express low levels of nuclear progesterone receptor. Similarly, FOXL2 is one of the earliest markers of ovarian differentiation (Cocquet *et al.* 2002) and may influence ovarian development and function, with mutations in this gene causing premature ovarian failure (Schmidt *et al.* 2004).

The QTL detected on OAR3 had the second-largest effect on aseasational reproduction traits. The region on OAR3 harbouring the putative QTL is syntenic with the BTA5 region between 90 and 130 cM, where Kirkpatrick *et al.* (2000) mapped a QTL for ovulation rate in cattle. A brief examination of the homologous bovine and human regions revealed the presence of an interesting positional candidate gene similar to the *Pregnancy Zone Protein (PZP)* gene, which encodes a trace protein in the plasma of non-pregnant females that is elevated in pregnancy (Tayade *et al.* 2005).

We recently reported on the association between the *melatonin 1A receptor (MTNR1A)* gene and aseasational reproduction (Mateescu *et al.* 2009), showing that ewes with at least one M allele were better able to breed and conceive out-of-season than ewes expressing only the m allele. Supported by earlier reports about the association with ovarian activity in spring (Pelletier *et al.* 2000) and higher fertility in spring (Notter *et al.* 2003), these results indicated that this polymorphism in the ovine *MTNR1A* gene is a promising genetic marker for the ability to breed and lamb out-of-season. In the current study, our analysis revealed a suggestive QTL for the number of oestrous cycles on OAR26 at position 30 cM, about 9.3 cM away from the location of *MTNR1A*, but this QTL did not reach the 0.05 chromosome-wide significance level of 6.07 (*F*-value for this location was 4.55).

## Conclusions

To our knowledge, this is the first QTL mapping experiment for aseasational reproduction in sheep. Seven chromosomes exhibited evidence for the presence of putative QTL based on the number of markers and the statistical methods used. The power of this pedigree for successful detection of QTL was due to diversity in founder trait characteristics and advancement in ovine mapping tools. Advantages of the pedigree include the measurement of multiple traits, which improves the trait identification, as well as the specially constructed, crossbred nature of the pedigree, and the controlled environment in which the backcross ewes were bred and reared to maturity. The

crossbred nature of the pedigree optimized the informativeness of the microsatellites, maximized the spread of the trait measures compared with that expected for a pure breed pedigree (e.g. of only Dorset), and introduced alleles at the QTL contributing to trait expression that clearly increase or decrease the ability to breed out-of-season. Future QTL fine-mapping will be essential to narrow the chromosomal regions identified as harbouring putative QTL, and in-depth comparative genomic studies will facilitate the investigation of positional candidate genes for the QTL identified.

## Acknowledgements

This project was supported by National Research Initiative Grant no. 2005-35205-17680 from the USDA Cooperative State Research, Education, and Extension Service; by Oklahoma Agricultural Experiment Station; and by New York Agricultural Experiment Station Hatch Project 470. We appreciated the help of Brian Magee and John Knowlton for caring for the sheep and Dr Mary Smith for performing the ultrasound assessment of pregnancy.

## References

- Berger Y.M. (2007) The East Friesian breed of sheep in North America. *Proceedings of the 3rd Spooner Dairy Sheep Day*, Spooner Agricultural Research Station, University of Wisconsin, Madison, WI, August 25, 2007, pp. 10–7.
- Botstein D., White R.L., Skolnick M. & Davis R.W. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* **32**, 314–31.
- Bramley T.A., Menzies G.S., Rae M.T. & Scobie G. (2002) Non-genomic steroid receptors in the bovine ovary. *Domestic Animal Endocrinology* **23**, 3–12.
- Churchill G.A. & Doerge R.W. (1994) Empirical threshold values for quantitative trait mapping. *Genetics* **138**, 963–71.
- Clarke I.J., Rao A., Chilliard Y., Delavaud C. & Lincoln G.A. (2003) Photoperiod effects on gene expression for hypothalamic appetite-regulating peptides and food intake in the ram. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **284**, R101–15.
- Cocquet J., Pailhoux E., Jaubert F. *et al.* (2002) Evolution and expression of FOXL2. *Journal of Medical Genetics* **39**, 916–21.
- Fernandes M.S., Pierron V., Michalovich D. *et al.* (2005) Regulated expression of putative membrane progesterin receptor homologues in human endometrium and gestational tissues. *Journal of Endocrinology* **187**, 89–101.
- Haley C.S., Knott S.A. & Elsen J.M. (1994) Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* **136**, 1195–207.
- Hall D.G., Fogarty N.M. & Gilmour A.R. (1986) Seasonality of ovulation and estrus, and the ram effect in poll dorset ewes. *Theriogenology* **25**, 455–61.
- Hanrahan J.P. (1987) Genetic variation in seasonal reproduction in sheep. *Proceedings of the 38th Annual Meeting of the E.A.A.P.* p. 14.

- Hanrahan J.P. & Quirke J.F. (1986) Breeding season and multiple births in small ruminants. *Proceedings of 3rd World Congress on Genetics Applied to Livestock Production* pp. 11–30.
- Hulet C.V., Shelton M., Gallagher J.R. & Price D.A. (1974) Effects of origin and environment on reproductive phenomena in Rambouillet ewes. I. Breeding season and ovulation. *Journal of Animal Science* **38**, 1201–17.
- Kirkpatrick B.W., Byla B.M. & Gregory K.E. (2000) Mapping quantitative trait loci for bovine ovulation rate. *Mammalian Genome* **11**, 136–9.
- Lewis R.M., Notter D.R., Hogue D.E. & Magee B.H. (1996) Ewe fertility in the STAR accelerated lambing system. *Journal of Animal Science* **74**, 1511–22.
- Lewis R.M., Notter D.R., Hogue D.E., Magee B.H. & Bergmann J.A.G. (1998) Lambing frequency in the STAR accelerated lambing system. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production* **27**, 51–4.
- Losel R.M., Falkenstein E., Feuring M., Schultz A., Tillmann H.C., Rossol-Haseroth K. & Wehling M. (2003) Nongenomic steroid action: controversies, questions, and answers. *Physiological Reviews* **83**, 965–1016.
- Lyons T.J., Villa N.Y., Regalla L.M., Kupchak B.R., Vagstad A. & Eide D.J. (2004) Metalloregulation of yeast membrane steroid receptor homologs. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 5506–11.
- Maddox J.F. & Cockett N.E. (2007) An update on sheep and goat linkage maps and other genomic resources. *Small Ruminant Research* **70**, 4–20.
- Mateescu R.G., Lunsford A.K. & Thonney M.L. (2009) Association between *melatonin receptor 1A* gene polymorphism and reproductive performance in Dorset ewes. *Journal of Animal Science* **87**, 2485–8.
- Notter D.R. (1992) Genetic improvement of out-of-season breeding through selection. *Proceedings of the Out of Season Breeding Symposium*, Ames, IA, June 19–20, 1992, 55–81.
- Notter D.R. & Cockett N.E. (2005) Opportunities for detection and use of QTL influencing seasonal reproduction in sheep: a review. *Genetics Selection Evolution* **37**(Suppl. 1), S39–53.
- Notter D.R., Cockett N.E. & Hadfield T.S. (2003) Evaluation of *melatonin receptor 1a* as a candidate gene influencing reproduction in an autumn-lambing sheep flock. *Journal of Animal Science* **81**, 912–7.
- Nugent R.A., Notter D.R. & Beal W.E. (1988) Effects of ewe breed and ram exposure on estrous behavior in May and June. *Journal of Animal Science* **66**, 1363–70.
- Pelletier J., Bodin L., Hanocq E., Malpoux B., Teyssier J., Thimonier J. & Chemineau P. (2000) Association between expression of reproductive seasonality and alleles of the gene for Mel(1a) receptor in the ewe. *Biology of Reproduction* **62**, 1096–101.
- Pope W.F., McClure K.E., Hogue D.E. & Day M.L. (1989) Effect of season and lactation on postpartum fertility of Polypay, Dorset, St. Croix and Targhee ewes. *Journal of Animal Science* **67**, 1167–74.
- Schmidt D., Ovitt C.E., Anlag K., Fehsenfeld S., Gredsted L., Treier A.C. & Treier M. (2004) The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiation and ovary maintenance. *Development* **131**, 933–42.
- Seaton G., Haley C.S., Knott S.A., Kearsey M. & Visscher P.M. (2002) QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics* **18**, 339–40.
- Smith J.F., Johnson D.L. & Reid T.C. (1992) Genetic parameters and performance of flocks selected for advanced lambing date. *Proc. New Zealand Soc. Anim. Prod* pp. 50–2.
- Tayade C., Esadeg S., Fang Y. & Croy B.A. (2005) Functions of alpha 2 macroglobulins in pregnancy. *Molecular and Cellular Endocrinology* **245**, 60–6.
- Thomas P. (2008) Characteristics of membrane progesterin receptor alpha (mPRalpha) and progesterone membrane receptor component 1 (PGMRC1) and their roles in mediating rapid progesterin actions. *Frontiers in Neuroendocrinology* **29**, 292–312.
- Thonney M.L. (2007) Sheep reproduction: accelerated lambing systems. In: *Encyclopedia of Animal Science* (Ed. by W.G. Pond & A.W. Bell), pp. 1–4. Marcel Dekker, New York.
- Vincent J.N., McQuown E.C. & Notter D.R. (2000) Duration of the seasonal anestrus in sheep selected for fertility in a fall-lambing system. *Journal of Animal Science* **78**, 1149–54.
- Wu C.H., Jin W., Nomura K., Goldammer T., Hadfield T., Dalrymple B.P., McWilliam S., Maddox J.F. & Cockett N.E. (2009) A radiation hybrid comparative map of ovine chromosome 1 aligned to the virtual sheep genome. *Animal Genetics* **40**, 435–55.

## Supporting information

Additional supporting information may be found in the online version of this article.

**Figure S1** Diagram of the backcross pedigree used to identify QTL associated with aseasonal reproduction.

**Figure S2** Profile plots of *F*-test statistics underlying the maximum progesterone level (MaxProg), number of estrus cycles (NoCycles), pregnancy status by progesterone (PregProg), pregnancy status by ultrasound (PregUltras), lambled (Lamb), number of lambs (NoLambs) and the overall score (Score); and information content across all ovine chromosomes.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.