The seasonality of lambing in performance recorded Dorset flocks

A report for Signet Breeding Services

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Table of Contents

Summary

- There is a global economic demand to exploit the naturally long breeding season of some sheep breeds
- Much of the existing research into the genetic basis for aseasonality in small ruminants has focused on the *MTNR1A* gene but the outcome measurements and experimental designs vary greatly between different studies
- More recent genomic analysis indicates that aseasonal breeding ability is polygenetic
- Aseasonal breeding ability has a low heritability
- Ewe estimated breeding values based on fertility rate during a spring mating period have been successfully used to increase productivity in an autumn lambing flock
- The selection of ewes for aseasonal breeding ability has no apparent detrimental effect on other productivity measures
- The majority of lambing events in the Signet dataset for UK Dorset sheep occurred in September, October and November but this was heavily influenced by a small proportion of flocks contributing a large number of records
- September, October and November lambing events should be considered 'out-of-season' for the purposes of developing an estimated breeding value for aseasonal breeding
- Almost all Signet recorded Dorset flocks recorded lambing events both out of season and in season but the proportion of lambing events falling into each category varied widely from flock to flock
- UK Dorset breeders do not currently submit details of unsuccessful mating opportunities to the Signet database, which would severely limit the accuracy of sire estimated breeding values based on the existing dataset

Genetic and management influences on the seasonality of reproduction in small ruminants

The physiology of reproductive seasonality in small ruminants

Sheep and goats that live in temperate climates are seasonal breeders. The onset of reproductive activity is triggered by shortening daylength (Chemineau*, et al.*, 2010). The resultant spring lambing or kidding period coincides with an abundance of food during lactation and therefore favours the survival of offspring (Chemineau*, et al.*, 2007). In common with other seasonally breeding mammals, photoperiod is only detected by the retina in sheep (Foster*, et al.*, 1994). The absence of light is interpreted by the suprachiasmatic nucleus, which stimulates the nocturnal release of melatonin from the pineal gland (Dardente*, et al.*, 2019b). A complex pathway of local hormone signals culminates in the release of sex hormones from the gonads (**Figure 1**). This pathway involves both up and down regulation of genes dependent on the length of the period of darkness (Dardente*, et al.*, 2019a; Wood*, et al.*, 2020). Melatonin is detected by specific receptors (melatonin receptor subtype 1A) located in the pars tuberalis (PT) region of the pituitary gland where there are abundant thyrotroph cells (Hanon*, et al.*, 2008). The detection of melatonin results in the seasonal expression of many genes including *Tshb.* Thyroid stimulating hormone (TSH) is produced by the thyrotrophs and bound by TSH receptors in tanycytes in the adjacent medio-basal hypothalamus (MBH). This stimulates further seasonal gene expression, including the production of deiodinase 2 (*Dio2*), which converts thyroxine (T4) to triiodothyronine (T3) (Dardente*, et al.*, 2019b). T3 regulates the release of Gonadotrophin Releasing Hormone (GnRH) indirectly through expression of the *Kiss1* gene in the neurons of the arcuate nucleus (Dardente*, et al.*, 2019a). GnRH controls the release of Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH) into systemic circulation from the pituitary, both of which have a role in ovarian follicle development (Campbell*, et al.*, 2007). Recently, it has been demonstrated that the seasonal expression of genes in the PT of ewes is independent of feedback from both thyroid hormones and sex hormones (oestradiol and progesterone), so its function is only to provide circannual rhythm. However, evidence of a feedback effect by these same hormones downstream in tanycytes and in *Kiss1* expression demonstrated that the seasonality of breeding is under more complex influences (Lomet*, et al.*, 2020). In a separate pathway, detection of melatonin in the PT leads to the release of prolactin into systemic circulation from the pituitary (Dardente*, et al.*, 2019b). Prolactin is another hormone with a role in seasonal events in mammals, such as moulting.

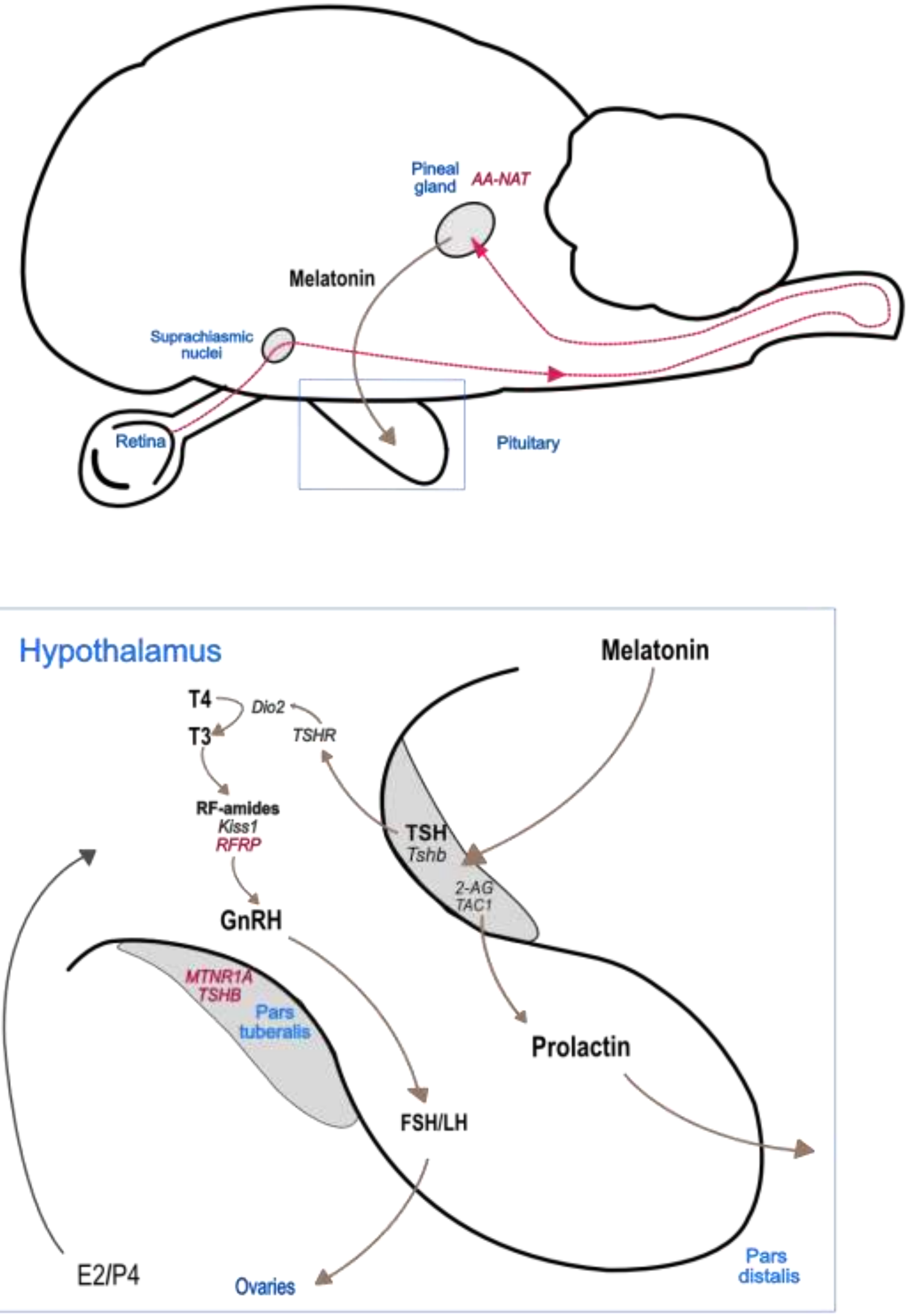


Figure 1 *The neuroendocrine pathway responsible for seasonal breeding in sheep, adapted from Dardente, et al. (2019b). Nocturnal release of melatonin from the pineal gland is detected in the pars tuberalis of the pituitary gland and stimulates a local TSH signal to the hypothalamus where T4 is converted to T3. T3 signals for the production of GnRH via Kisspeptin, and the consequent release of LH and FSH from the pituitary into systemic circulation enabling it to influence ovarian follicular activity. Ovarian produced oestrodiol (E2) and progesterone (P4) have a feedback effect on the hypothalamus. Genes that have been studied for their potential as markers for seasonality are indicated in red at their location in the pathway.*

Variation in the seasonality of reproduction in sheep and the implications for breeding management

The length of the reproductive season and the time of year when the onset of oestrus occurs in sheep varies between breeds (Chanvallon*, et al.*, 2011; Quirke and Hanrahan, 1985). There is a commercial advantage to extending the traditional breeding season of sheep so that market demand for lambs is met all year round (Chemineau*, et al.*, 2007). The 'ram effect', the introduction of a ram to advance the breeding season and synchronise ewes, has a limited impact on shortening the anoestrus period and is most effective in late anoestrus, especially in more seasonal breeds (Chanvallon*, et al.*, 2011). Hormone and light treatments incur additional costs and do not integrate into flocks managed outdoors (Chemineau*, et al.*, 2007; Hanocq*, et al.*, 1999; Notter, 2002). The Poll Dorset has one of the longest breeding seasons of sheep breeds in temperate regions, averaging 294 days in a study by Hall*, et al.* (1986). For this reason the Dorset is used in accelerated lambing systems, for example eight-month lambing intervals or the STAR system (Lewis*, et al.*, 1996). Variation in seasonality also occurs between individuals of the same breed (Hall*, et al.*, 1986; Martinez-Royo*, et al.*, 2017; Vincent*, et al.*, 2000). Hall*, et al.* (1986) reported that only 23% of Poll Dorset ewes, derived from 14 flocks in Australia, ovulated in late spring compared to 99% in Autumn, but 6.8% of ewes did not cease ovulating throughout the year. Breeding strategies that exploit natural differences between breeds and individuals are the most favourable way extend the breeding season. The following section provides evidence that breeding for aseasonality can achieve greater fertility for autumn lambing.

The heritability of breeding seasonality in small ruminants and the impact of selection on fertility and other performance traits

Reproductive traits such as Spontaneous Ovulatory Activity (SOA), spring mating fertility and lambing date are heritable (Al-Shorepy and Notter, 1996; Asadi-Fozi*, et al.*, 2020; Hanocq*, et al.*, 1999; Notter*, et al.*, 2003; Rekik*, et al.*, 2011; Smith*, et al.*, 1992). There is potential to exploit these traits in selective breeding programmes to extend the breeding season of a flock, although the heritability of spring fertility is low (Al-Shorepy and Notter, 1996; Asadi-Fozi*, et al.*, 2020; Notter*, et al.*, 2003; Rekik*, et al.*, 2011), and evidence of SOA is not necessarily a proxy for spring fertility (Hanocq*, et al.*, 1999). Hanocq*, et al.* (1999) measured SOA in April in 933 Merino d'Arles ewes by plasma progesterone assay. The heritability of SOA was estimated as 0.2 and 0.37 using the best linear unbiased predictor (BLUP) and sire threshold models respectively. Ewe age, whether they had lambed the previous autumn, and liveweight in April had significant effects on April ovulatory activity and were included in the models as fixed effects.

The Virginia Polytechnic flock: A controlled, selective breeding programme for autumn lambing

Fertility for spring mating was demonstrated to have both genetic and environmental components and to respond to positive selection in studies on the performance of the Virginia Polytechnic autumn breeding flock over a seventeen-year period (Al-Shorepy and Notter, 1996; Al-Shorepy and Notter, 1997; Asadi-Fozi*, et al.*, 2020; Vincent*, et al.*, 2000). The maintenance of both genetic and environmental control flocks for comparison with the improved flock, and the long-term nature of the study, means that this study provides the strongest evidence for a positive impact of selection for spring fertility using estimated breeding values (EBVs). The base population from which the flock was derived comprised of a 50% Poll Dorset, 25% Rambouillet, 25% Finnsheep crossbreed. A flock of 45 ewes and 5 rams was maintained without selection as a genetic control (GC) and continued to lamb in spring. An environmental control (EC) flock of 55 ewes and 5 rams was lambed in autumn and maintained without selection for fertility. Finally, a third flock of 125 ewes and 10 rams was maintained by selecting and breeding ewe lambs from top performing, autumn lambing dams and any other ewe lambs that achieved conception in the May/June breeding period. These three flocks were maintained for the first two phases of the study, which ran from 1989 to 1993, and 1994 to 1998 respectively. In the second phase of the study rams from the selected flock (S) were used on the EC flock so a more direct comparison could be made of the fertility between the two breeding lines. In the last phase, 1999 to 2005, only the selected flock (S) was maintained but selection was not as intense due to ewes being enrolled in concurrent studies. Throughout the study, ewes were given a score of 1 to signify an autumn lambing or 0 if no lambing occurred as a measure of fertility on which EBVs were calculated. In the first phase of the study, average flock fertility increased with age from 0.11 in 12 month old ewe lambs to 0.59 in ewes that had lambed three times or more, and the heritability of fertility was estimated at 0.07-0.11 (Al-Shorepy and Notter, 1996). In the last year of the study the mean fertility of mature ewes ($\geq$ 3 years old) in the S flock was 0.88 $\pm$ 0.05 (Asadi-Fozi*, et al.*, 2020). Fertility had increased to 0.28 $\pm$ 0.04 in 12-month-old ewe lambs by the end of the study. Heritability estimates for fertility over the whole study were 0.07 $\pm$ 0.01 using a linear model and 0.15 $\pm$ 0.04 for a threshold model. Both models included lambing year and ewe age class at lambing (12 months old, 19 months old, 2 years old or $\geq$ 3 years old) as fixed effects (Asadi-Fozi*, et al.*, 2020).

Ewe spring fertility EBVs increased by 0.0138 per year in the S flock compared to 0.0067 in the EC flock during the initial five-year phase of the study (Al-Shorepy and Notter, 1997). Asadi-Fozi*, et al.* (2020) used both a univariate linear model and a threshold model to calculate spring breeding fertility EBVs and found a correlation of 0.987 between the models. Mean ewe EBVs were regressed onto year for the entire study period. The mean EBV for the S ewe line increased by 0.0093 ± 0.0007 per year compared to -0.0011 ± 0.0007 in the GC line and 0.0021 ± 0.0017 in the EC line. At the end of the study the mean EBV was 0.15 ± 0.03 in the S line ewes.

Vincent*, et al.* (2000) investigated whether ewe spring fertility EBVs correlated with the duration of anoestrus. A proven ability to lamb in autumn was essential for inclusion in the study population. Within this population, the ewes with the highest and lowest EBVs were selected for monitoring, with acknowledgement that 'low' EBV ewes represented an average EBV for the general population because they had already shown aseasonal breeding ability. Ewes were monitored for fresh raddle marks from vasectomised rams twice a week between January and August. Marked ewes were blood sampled for a progesterone assay to confirm ovulation. There was a significant difference in the duration of anoestrus between ewes with a high EBV (28.4 days, mean EBV 12.6%) and low EBV (70.2 days, mean EBV 0.3%). Anoestrus duration reduced over the four years of the study (1992, 1993, 1995, 1997) for both high and low EBV ewes as a result of selection. In the final year of the study nine high EBV ewes and two low EBV ewes were monitored and none showed a true period of anoestrus. Notter*, et al.* (2011) investigated the impact of exposure to light on oestrus expression after the same flock had undergone ten years of selection for autumn breeding. A flock of 67 ewes were housed from February until July. Half the flock was exposed to the equivalent of ambient light conditions whilst the other half were kept under a constant photoperiod of 16 hours of light/day. Ewes were checked twice a week for marks from vasectomised rams and if oestrus was recorded ovulation was confirmed by serum progesterone assay. Serum progesterone was assayed on an additional six occasions between March and July to look for silent ovulations. Photoperiod did not affect the duration of anoestrus or frequency of ovulation, but the duration of anoestrus decreased with ewe age with ewes over 3 years old averaging 34 ± 3 days in oestrus compared to 72 ± 7 days in 2-years-old ewes. When length of anoestrus was regressed onto ewe EBV, a 1% increase in EBV equated to a 1.16 ± 0.41 day decrease in duration of anoestrus, compared to 2.15 ± 0.72 days in the Vincent*, et al.* (2000) study.

Selection for spring fertility was found to have a limited impact on other desirable traits (Asadi-Fozi*, et al.*, 2020). The change in litter size EBV for the S ewe line was +0.020 lambs over the 17-year study. Maternal birthweight for S ewes decreased during the first two phases but the trend reversed in the last phase of the study when ram lambs retained for use on the S ewe line were selected for maternal birth weight EBV. There was a linear increase in 60-day lamb weaning weight EBV in the S line of 0.100 ± 0.006kg per year and a small positive trend for scrotal circumference (SC) EBV, the highest value being for 90-day SC (0.092 ± 0.006).

In conclusion, the Virginia Polytechnic trial provided evidence that selection of breeding replacements based on a binary ewe EBV for autumn lambing resulted in a slow but significant improvement in the spring fertility rate of a flock containing breeds with a naturally short period of anoestrus. Furthermore, increased spring fertility rate was associated with a decrease in the length of anoestrus, an insensitivity to photoperiod, and did not have a detrimental effect on other production traits.

The calculation of EBVs for aseasonal breeding in other flocks or herds

Rekik*, et al.* (2011) analysed the records of sires with five or more daughters across nine Barbarine flocks at one location. A fertility score of 1 or 0 was used to signify whether a ewe lambed in autumn or not. Average spring fertility in 18-month-old, maiden Barbarine ewes was 0.85 ± 0.35 and was significantly affected by year, flock and daily weight gain at 10-30 days of age. The heritability estimate for spring fertility was only 0.05 ± 0.02 but there was a positive relationship between sire EBV and the average fertility of their daughters (P<0.001). Mean spring fertility was 0.97 in the daughters of the 20% top ranked sires compared to 0.75 for the 20% bottom ranked sires.

Smith*, et al.* (1992) used lambing date as an ordered categorical response to estimate heritability and breeding values in a Dorset x Romney flock selected for an advanced breeding season. Heritability was estimated to be 0.23 for standardised lambing date after ewes were exposed to rams in late spring for 8 weeks, then again after a two month break if they were not pregnant. The EBVs were used to select ewes to go into spring or autumn lambing flocks. The authors found negligible phenotypic correlation (0.02) and a small genetic correlation (0.09) between the heritability for standardised lambing date and litter size.

Desire*, et al.* (2018) allocated a numerical aseasonal kidding phenotype to first parity crossbreed does that kidded between 1987 and 2015. A total of 9546 individual records were used from two dairy units with close genetic links. The phenotype value represented the number of weeks either before or after a four-week peak kidding period and therefore ranged from 0 to 24. The goats were housed but the authors do not mention any use of reproductive hormones or light regimes. Univariate analysis of aseasonal breeding capability was performed including herd-year-season and age at first kidding as fixed effects. The heritability estimate was 0.11. Genetic correlation with 520-

day milk yield was small and negative (-0.15) and the phenotypic correlation close to zero so selection for aseasonal breeding ability is not likely to adversely impact productivity. However, the production traits that were tested for correlation against out of season kidding ability are not relevant to the suckled ewe, which has a much shorter lactation.

The impact of selective breeding on plasma melatonin and prolactin concentrations

Notter and Chemineau (2001) compared nocturnal plasma melatonin and prolactin concentrations between the S line ewes and all control ewes in the Virginia Polytechnic flock. Samples were collected in August from 182 ewes of mixed ages. Ewes selected for autumn lambing had significantly lower plasma melatonin ($P<0.02$) and this was negatively associated with the autumn fertility EBV ($P<0.01$). Plasma melatonin concentration tended to increase with ewe age. The estimated heritability for plasma melatonin was 0.43 ($P<0.02$). Melatonin and prolactin concentrations were not correlated but plasma prolactin concentrations were significantly higher in the selected ewe line ($P< 0.001$). Zarazaga*, et al.* (1998) previously observed high variability between individual ewes for nocturnal plasma melatonin concentration in the Ile-de-France breed but did not find that ewe age was a significant factor for the variation. They also found variability in the mean nocturnal plasma melatonin concentration of offspring between the different sires and a high heritability of 0.45, similar to that estimated by Notter and Chemineau (2001). Although melatonin has a crucial role in signalling daylength, the significance of the differences found between the groups in plasma melatonin and prolactin concentrations, and any impact or mechanism by which they might affect the length of the breeding season, is not currently understood.

The genetic origin of variation in seasonality between breeds and individuals

Genetic markers for aseasonal breeding are desirable as a management tool to improve the success of breeding programmes that aim to extend the period of lamb production (Posbergh*, et al.*, 2019). Selection by phenotype alone is limited by being restricted to females, the tendency of ewes not to express aseasonal breeding ability until later in life and the dependence on a breeding system that allows exposure of ewes to rams in the spring (Notter, 2002; Notter and Cockett, 2005). Many of the studies of the genetic variation underlying differences in seasonality between individuals have focused on the *MTRN1A* gene on chromosome 26 despite the complexity of the pathway between photoperiod detection and the control of reproductive activity in small ruminants. More recently, genome sequencing tools have allowed analysis across all chromosomes for quantitative trait loci (QTL) and Single Nucleotide Polymorphisms (SNPs) associated with seasonality. Genomic studies are described in the following section, followed by a summary of the gene specific studies.

Genome wide analysis for aseasonality markers

Most recently, Posbergh*, et al.* (2019) used the Illumina Ovine HD beadchip to test for genotype associations with the number of out of season lambings a ewe had achieved. Out of season lambing (OOSL) was defined as lambing between August and November and the outcome variable took account of the number of opportunities the ewe had to breed out of season and how many of these matings resulted in a successful lambing. A total of 527,139 SNPs were analysed across 257 ewes of seven breeds and crossbreeds. Genes on six chromosomes were significantly associated with OOSL. These included *AGBL1* on chromosome 18, which is involved in the structure of the cornea and *TAF7L* on chromosome X, which is linked with male fertility. A within breed analysis to compare genotypes of 41 Dorsets from US flocks with 48 Dorsets from UK flocks identified further genes associated with OOSL on an additional three chromosomes, including *GnRH1* on chromosome 2. The models used for gene association, both across and within breeds, fitted best with a recessive mode of inheritance. Measurement of allele frequency showed that selection had occurred in the Dorsets from UK flocks in the *PIBF1* region on chromosome 10, which has possible implications for pregnancy maintenance out of season. By analysing the association of OOSL with genomic regions with homozygosity, further candidate genes were identified that are involved in neural development, neural signalling, spermatogenesis and regulation of circadian period length. In summary, the analysis identified multiple candidate genes with varying mechanisms for influencing the ability to lamb out of season. However, none of these genes were located close to *MTNR1A.*

Martinez-Royo*, et al.* (2017) analysed 47206 SNPs in 110 Rasa Aragonesa sheep for association with three indicators of reproductive activity between January and August: total days anoestrus (TDA) measured by progesterone assay, progesterone cycling months (PGCM) and oestrus cycling months measured by marks from vasectomised rams. These researchers also failed to identify SNPs of significance in regions of the genome where previously studied candidate marker genes are located. Five SNPs were significant for one or both of the traits measured by progesterone, but only at chromosome wide level and not genome wide level. Some of these SNPs were located near genes such as *NPSR1* and *HS3ST5* that are potentially involved in regulation of circadian rhythm.

Mateescu and Thonney (2010) searched for QTL for aseasonal reproduction by measuring a range of direct and indirect indicators of breeding performance and allocating a total score to each of 159 ¾ Dorset x ¼ East Friesian ewes. These indicators included the number oestrus cycles between February and May measured by progesterone, early pregnancy measured by progesterone, pregnancy ultrasound scan at 55 days post mating and number of lambs born. In addition to the breeding ewes, the 132 parent Dorset ewes, paternal grandparents (8 Dorset ewes and 4 East Friesian rams) and 8 F1 rams were genotyped. The aim of crossbreeding was for the breeding flock to carry alleles associated with aseasonality from the Dorset parentage and seasonality from the East Friesian grandsires. The authors selected 120 microsatellite markers distributed across the genome in order to locate potential QTL positions. Putative QTL for one or more of the measured traits were found on seven chromosomes, but not on chromosome 26. Alleles associated with both positive and negative effects on aseasonal breeding were carried by Dorsets. Although 96.3% of ewes had at least one oestrus cycle in the measured period, only 30.6% were scanned pregnant and 20.4% successfully lambed. These results demonstrate the importance of the ability to conceive and maintain a pregnancy, as well as have aseasonal oestrus activity, to be able to achieve an autumn lambing.

Cao*, et al.* (2016) used methylated-DNA precipitation sequencing to locate single nucleotide variations (SNVs) in DNA methylation enriched regions across the sheep genome. In total, ten samples were sequenced across four breeds, the aseasonal and highly prolific Hu sheep and Small-tailed Han sheep, and the seasonal Tan and Ujumqin breeds of low prolificacy. The aim was to identify SNVs associated with fertility based on the characteristics of the breeds. Actual fertility performance was not measured in the sampled sheep. Nineteen of the 359 SNVs they identified were located in the aseasonal reproduction QTL published by Mateescu and Thonney (2010).

Overall, the results of these genome-wide studies suggest that aseasonal breeding is polygenic in nature, with many different mechanisms that have the potential for genetic influence in the pathway from detection of photoperiod to the maintenance of pregnancy. The genes implicated in these mechanisms are located across the genome, but strong evidence of their involvement in aseasonality is currently lacking. Therefore, genomic tools to assist selection of aseasonal flocks are not yet within reach.

Gene specific analysis for association with aseasonality

Calvo*, et al.* (2018) sequenced the *MTRN1A* gene in Rasa Aragonesa sheep and identified 35 SNPs, 13 of which were significantly associated with seasonality in three reproductive traits: total days anoestrus, progesterone cycling months and oestrus cycling months. Four of these SNPs were located in the coding region. SNP rs403212791, was non-conservative and was in strong linkage disequilibrium with the other three SNPs in the region, which did not produce functional mutations. Two of these SNPs corresponded to the *RsaI* and *MnlI* Restriction Fragment Length Polymorphisms

(RFLPs), at positions 606 and 612 respectively, which have been inconsistently associated with seasonality traits in previous studies (Carcangiu*, et al.*, 2009a; Hernandez*, et al.*, 2005; Luridiana*, et al.*, 2015; Martinez-Royo*, et al.*, 2012; Mateescu*, et al.*, 2009; Posbergh*, et al.*, 2017; Teyssier*, et al.*, 2011). Indeed, previous work in Rasa Aragonesa sheep failed to find an association between *Mnl1* genotype and oestrus activity measured during the anoestrus period, although an association was found for *RsaI* genotype (Martinez-Royo*, et al.*, 2012). Martinez-Royo*, et al.* (2012) measured oestrus activity by daily recording of rump marks on ewes left by vasectomised rams and did not confirm ovulation by plasma progesterone assay. Contradictory studies have also been published for the effect of *MTRN1A* genotype in the Merino D'Arles breed. Pelletier*, et al.* (2000) reported that 58.2% ewes with ovulatory activity in early April were genotype *MM* and none were genotype mm. In contrast, 28.5% of ewes that never ovulated in April were genotype MM and 28.5% were genotype mm. Teyssier*, et al.* (2011) also measured ovulatory activity by plasma progesterone and found no association with genotype for the same SNP (*Mnl1 RFLP*). More recently, Mura*, et al.* (2019) enrolled 50 ewes of each genotype for the *Mnl1 RFLP* SNP, now named g.15099485A>G, in each of eight Sarda flocks, a dairy sheep breed. The flocks were located within 20km of each other and management was consistent between flocks. The *MTRN1A* gene was sequenced in 100 ewes from each genotype and eight SNPs were identified that had been detected in previous studies. The G/G (*MM)* genotype at g.15099485A>G, a silent mutation, was always associated with the change g.15099391 G>A, which induces an amino acid substitution. Two flocks were allocated one of four timepoints between March and June for ram introduction so the impact of genotype on the response to the ram during the typical anoestrus period could be assessed. There was a higher rate of lambing in the G/G and A/G genotype ewes compared to the A/A genotype ewes for both the March, April and May joining dates (P <0.01) and June joining date (P <0.05). Furthermore, the average time to successful mating was shorter for ewes carrying at least one G allele for all time periods (P <0.01).

A summary of studies that have looked for associations between genotype and aseasonal breeding activity in small ruminants is presented in Appendix 1. In some of these studies the impact of genotype on litter size was also investigated with no association found for the *MTRN1A, TSHB* nor *RFRP* genes (Carcangiu*, et al.*, 2009a; Huang*, et al.*, 2012; Huang*, et al.*, 2013; Luridiana*, et al.*, 2015; Mura*, et al.*, 2019; Notter*, et al.*, 2003; Teyssier*, et al.*, 2011). The seasonal reproductive traits used as outcome measures for testing genotype associations varies between the studies. In a few studies reproductive performance was not measured in individual animals. Instead, genotypes were compared at the breed level, with reference to the recognised seasonality of the breed being genotyped (Chu*, et al.*, 2006; Ding-ping*, et al.*, 2012; Huang*, et al.*, 2012; Huang*, et al.*, 2013; Wang*, et al.*, 2018). These studies would not have accounted for individual variation in reproductive ability within each breed. In some studies, aseasonal reproductive activity was measured in individuals either indirectly by observing oestrus and/or measuring blood progesterone, rather than a direct measurement of ability to lamb out of season (Hernandez*, et al.*, 2005; Martinez-Royo*, et al.*, 2012; Pelletier*, et al.*, 2000; Teyssier*, et al.*, 2011). The period of indirect measurement of aseasonal reproductive activity varied from two weeks in April (Pelletier*, et al.*, 2000) to twice weekly for a two year period (Hernandez*, et al.*, 2005). Other studies directly measured aseasonal reproductive activity by using the number of successful birth events within a defined period (Carcangiu*, et al.*, 2009a; Lai*, et al.*, 2013; Mateescu*, et al.*, 2009; Mura*, et al.*, 2019; Notter*, et al.*, 2003; Posbergh*, et al.*, 2017). One study does not provide details of spring mating opportunities for the autumn mating cohort used to compare genotype frequencies (Carcangiu*, et al.*, 2009b).

In most studies, individuals of the breed being genotyped were derived from only one flock so some degree of selection for fertility might have previously taken place. This means that the genotype distribution in that flock could differ from the genotype distribution in the breed in general, but not necessarily for the genes being investigated. This could be one explanation for the differences in results between studies using the same breed. Notter*, et al.* (2003) compared ewes that had been bred selectively for autumn lambing with control ewes from the Virginia Polytechnic flock and found a 10% $\pm$ 5.7% mean increase in fertility in older ewes carrying at least one M allele for the Mnl1 SNP (P= 0.09). Teyssier*, et al.* (2011) speculated that the reason for inconsistency between genotype and seasonality between breeds was an undiscovered, non-conservative mutation on an independent gene. The detection of linkage disequilibrium between certain SNPs on the *MTRN1A* gene by Calvo*, et al.* (2018) offers an alternative explanation for the inconsistency. This theory of linkage with non-conservative polymorphisms has been explored previously by Trecherel*, et al.* (2010). An additional non-conservative polymorphism at position 706 in Exon 2 of the *MTRN1A* gene, which occurs with the *mm* genotype, causes a structural change in the melatonin receptor. The in-vitro study demonstrated a difference in melatonin signal interpretation between the two variants, but the relationship has not been established between the polymorphism and reproductive seasonality (Trecherel*, et al.*, 2010). Hernandez*, et al.* (2005) postulated that the seasonality traits associated with the genotypes in the less seasonal breeds were masked by the presence of unidentified seasonality genes in the more seasonal breeds. This theory is consistent with the results of the recent genomic studies that suggest the differences are more likely to be polygenic in nature.

A limitation of a number of the studies is the small number of individuals genotyped, which increases the likelihood of spurious associations, especially given the considerations discussed above of ewes being sourced from the same flock, potentially reducing the variation in genotypes for undetected alleles, and the use of indirect measurements of aseasonal reproductive ability.

In conclusion, the *MTRN1A* gene has been the focus of a number of studies that have compared the genotype of ewes or does with the aseasonal reproductive activity of the individual or breed. The majority of the studies identified a positive effect on aseasonality of carrying a G allele at the *Mnl1 RFLP* site and a C allele at the *Rsal* RFLP site. However, there are a number of limitations to these studies and none of them describes the impact on flock aseasonality of using either or both SNPs as markers for a selective breeding programme.

The implications of the existing evidence for a genetic basis for aseasonality on the development of a breeding programme for UK Dorset breeders

In summary, although there is evidence for the genetic basis of aseasonality in small ruminants, the specific mechanisms for aseasonality have yet to be defined. The ability to breed out of season is likely to be polygenic in origin. Gene markers to assist selective breeding have not yet been identified and there remains the possibility that they would need to be breed specific, which could reduce the prospects for commercial availability in numerically small breeds such as the Dorset. However, there is evidence that the use of an EBV for spring fertility has a gradual but positive effect on the out of season breeding ability of a flock containing breeds with a naturally long breeding season. The EBV can be based on the binary outcome of lambed/did not lamb within a desired time period in autumn but requires the recording of all mating opportunities for individual ewes, successful or not. Environmental factors also influence out of season breeding ability and it is important to note that spring fertility improves with age until ewes become mature.

The seasonality of lambing in performance recorded Dorset sheep flocks

Introduction

Improvements in the productivity of a flock can be accelerated by the selection of sires that are genetically superior for the desired performance traits. In the UK, pedigree flocks from over 30 different breeds undertake performance recording for genetic improvement. Records of parentage, birth events, litter size, lamb weights and measurements of carcass characteristics are collated in a central database managed by Signet Breeding Services (Signet Breeeding Services, 2021). BLUP is then used to evaluate genetically linked sheep managed under different flock conditions and identify rams whose offspring show superiority in the desired performance traits (Quality Meat Scotland, 2019). Genetic superiority is represented by a sire's EBV for a range of performance traits pertinent to each breed. The majority of EBVs are based on variables with continuous measurements, for example eight-week weight and muscle depth, but categorical values are also used to calculate EBVs; For instance, a 'lambing ease' EBV is interpreted as the proportion of additional unassisted births for the offspring of a given sire and is derived from an ordered categorical scale that ranks the amount of intervention for each lamb birth. In the UK, there is not currently an EBV for aseasonal breeding ability in daughter ewes for any breed of ram. Polled and horned Dorset sheep breeders would benefit from being able to preferentially select sheep with the ability to successfully breed out of season (OOS). 'Dorset breed lamb' is a product promoted by the retailer Waitrose & Partners, who favour autumn born lambs to target the early 'new season' British lamb market (Waitrose, 2021). The Dorset Horn and Polled Dorset sheep breeders' association state the objective of their flocks to be the ability to 'lamb regularly out of season' to fulfil the requirements of the meat trade and purchasers of replacement breeding stock (Dorset Horn and Poll Dorset Sheep Breeders' Association, 2021). The definition of 'out of season' is not defined by the breeders' association, but the 'year letter' that prefixes the individual identity number to indicate year of birth refers to the 12-month period commencing on the 1st September. This is in contrast to the Charollais breed society, who define the start of the 'lambing year' as December 1st (Charollais Breed Society, 2021). The latter date is consistent with seasonal breeding in sheep, which as short-day breeders generally commence oestrus cyclicity when daylength starts to decrease (Chemineau*, et al.*, 2010; Quirke and Hanrahan, 1985). The aims of this project were to first, explore the existing Signet performance record dataset for the Dorset breed and to define 'out-of-season' for the calculation of EBVs for aseasonal breeding. Second, to identify patterns and trends in breeding management that indicated whether the existing data were suitable for use in the calculation of a ram EBV for OOSL. Finally, to compare the performance of ewes for certain reproductive traits between those that lambed OOS and those that lambed in season for indications of any negative associations between OOSL and productivity.

Materials and methods

The dataset contained the individual records of lamb births from all Polled or Horned Dorset flocks that had performance recorded through Signet Breeding Services. Data were initially cleaned in MS Excel version 16.39 (Microsoft Corporation, 2020). The dataset was cleaned to remove incomplete and anomalous records, records from flocks that contributed fewer than 100 birth events, records from ewes known to have received artificial breeding manipulation and records for the incomplete 2019-2020 season. In some cases, it was apparent that a birth event had been misallocated to a ewe that was 16 years older with the same individual ID number (arising from the recycling of ID prefix letters every 16 years) and these errors were corrected when the true dam could be identified. R version 4.0.2 (R Core team, 2020) was used for further data cleaning and exploratory data analysis. The dataset was collapsed, and duplicate Dam IDs separated, so that each record represented a single lambing event for each unique ewe. A high proportion of duplicated birth records, including 182/192 (95%) of dam IDs with five or more lambs recorded as born on the same day, were associated with ID prefixes linked to a single flock ID number. This flock was removed from the final dataset. Remaining duplicate entries due to multiple flock or sire IDs assigned to single lambing events were also removed. For every dam, consecutive lambing events were numbered to represent parity, although actual parity was not known for ewes that had already lambed before performance recording was started in their flock. Unrealistically short (<180 days) and long (>3 years) intervals between lambing events accounted for 0.3% and 0.4% of the records respectively and were retained. A separate dataset containing the sire identities of lambs born in the performance recorded flocks was merged with the cleaned dataset. Sire identity was not available for all ewes with birth events because not all ewes were born into performance recorded flocks, or they were born before the performance recording period commenced, therefore details of parentage was not available. This dataset was used to look at the distribution of daughters from the same sires across flocks and to check for variation between sires in the seasonality of when their daughters lambed.

Results

Initial exploration and cleaning of the dataset

The entire dataset contained 230053 lamb birth records from between October 1969 and February 2020. After initial cleaning 224203 birth records were retained. A more detailed summary of discarded records is provided in Figure 26 (Appendix 2). September, October and November were the months with the highest frequency of lamb birth records. The ratio of female lambs to those in the combined male and castrated categories, the latter also used as the default category, was approximately 1:1 for every month. Lamb birth weights were close to normally distributed with a mean and median of 4kg. The median birth weight did not change with month of birth except for a slight increase in July and August (Figure 2) but there were few birth records in these months, so this did not warrant further analysis. Both sex and birth weight records, and the sporadic births recorded in the unfavourable summer months suggested that breeders did not selectively record lambs.

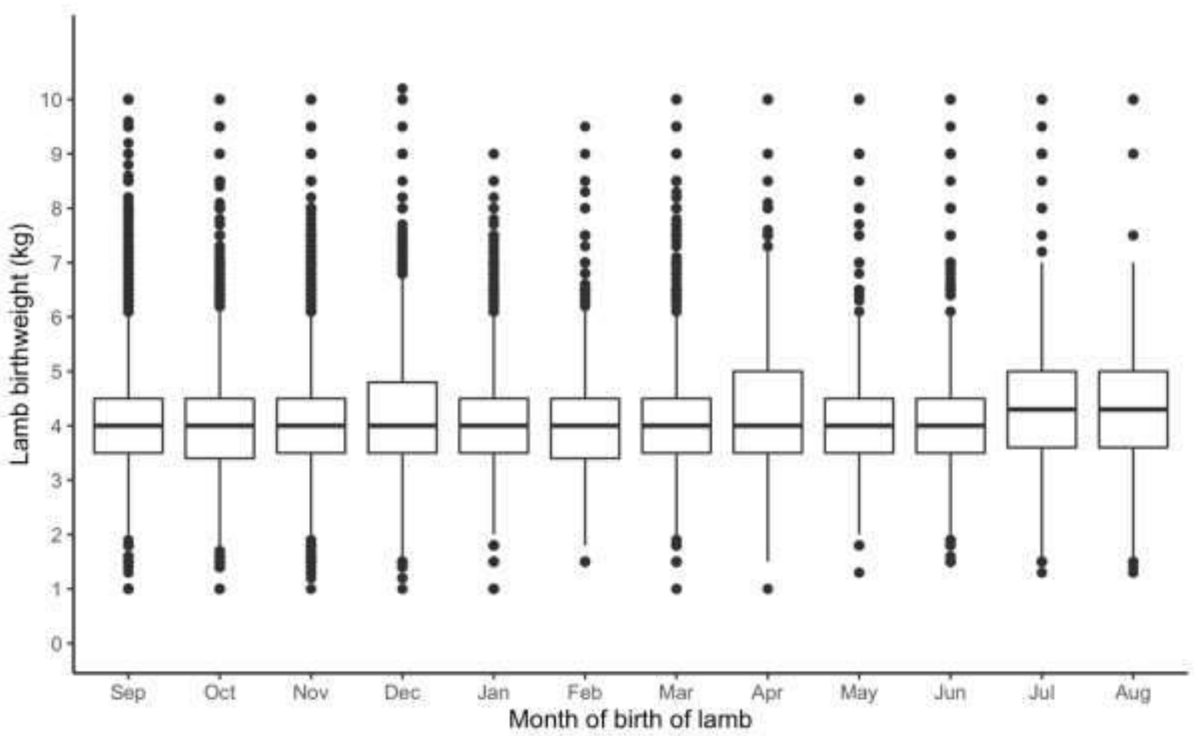


Figure 2 Boxplot for lamb birthweight by month of year. Outliers are intervals beyond 1.5 x the interquartile range (IQR).

The final dataset contained 121936 lambing events from 46911 dams across 102 flocks. There were 12013 ewes (25.6%) that were older than 1000 days at parity 1, and therefore likely to have lambed previously. The number of lambing events per ewe ranged from 1 – 15, with a median of 2. The number of ewes reaching each parity is shown in Figure 3. Only 1% of ewes recorded 8 or more lambing events.

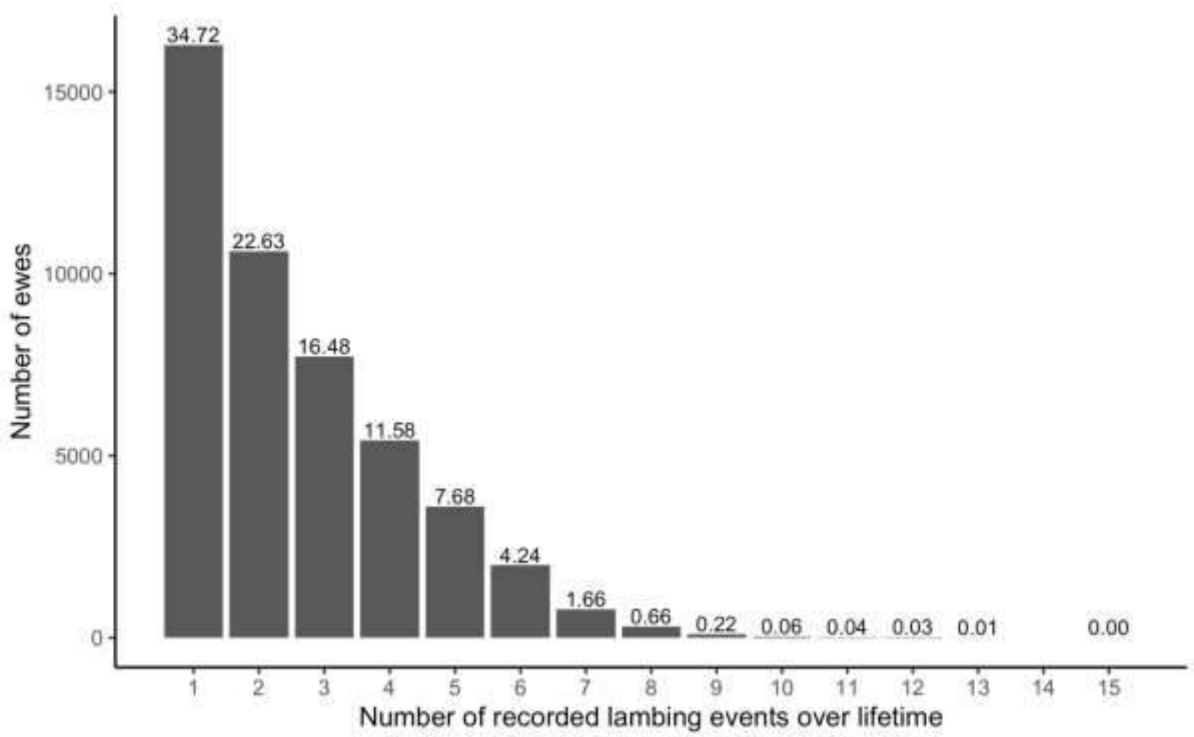


Figure 3 The number of ewes for each maximum recorded parity. The percentage of all ewes in the dataset is indicated above each bar.

The contribution of individual flocks to the dataset

Differences in management between flocks can influence lambing seasonality and performance, hence the need to explore the relative contribution of each flock to the dataset. For the same reason, changes over time in the flocks contributing to the dataset might explain any trends in seasonality and reproductive performance over the entire recording period.

The length of time that flocks were recorded was right skewed with a range of 2 to 40 years, mean of 11 years and median of 9 years (Figure 4). The number of ewes per flock that were in the dataset had a more extreme right skew and ranged from 41 to 3734 with a median of 208 and mean of 475 ewes (Figure 5). Three of the five flocks that had been recorded for the longest time period were also three of the five flocks that had recorded from the most ewes. One of the two flocks that had recorded for 40 years was the only flock to have recorded from over 3000 ewes and had also recorded the most lambing events (10653). 49% of lambing events were attributable to 7/102 (7%) of flocks highlighting that breeding management on these few farms, if different from others, would have a disproportionate influence on seasonality and performance outcomes.

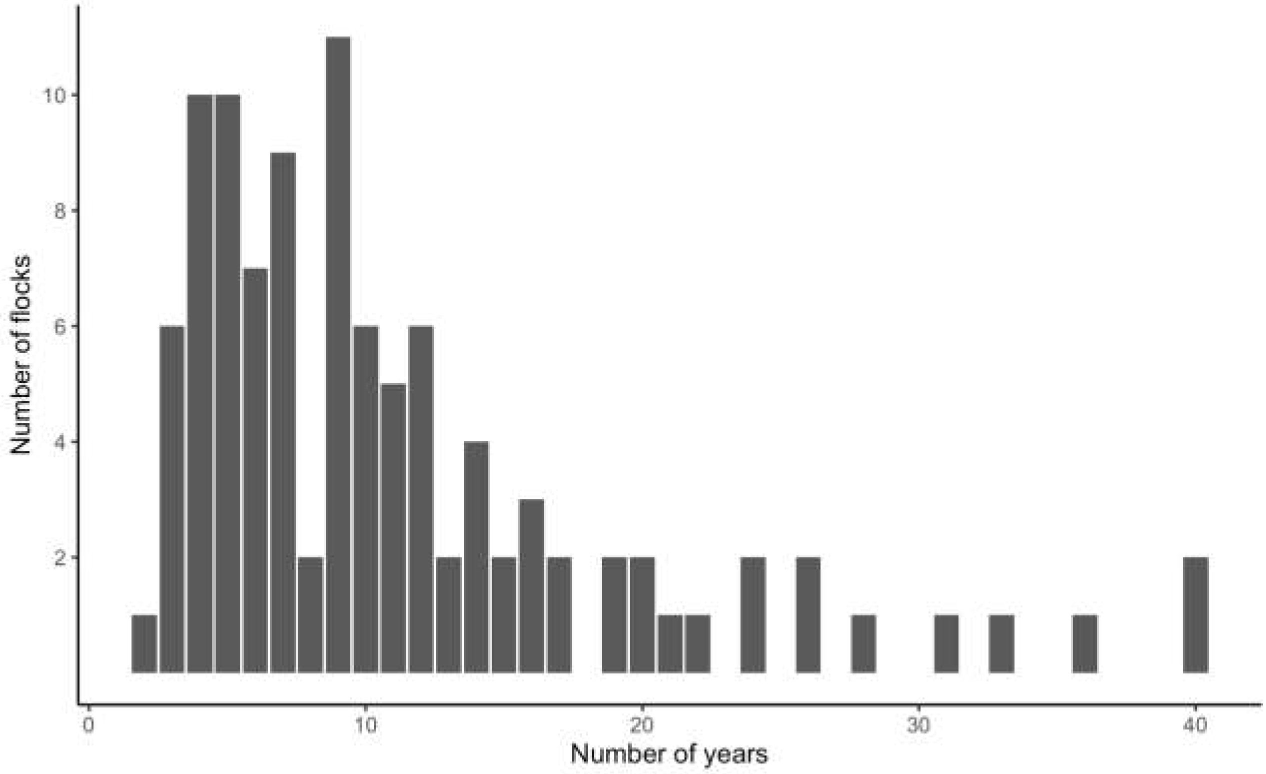


Figure 4 The number of years of participation in the Signet performance recording scheme by Dorset flocks

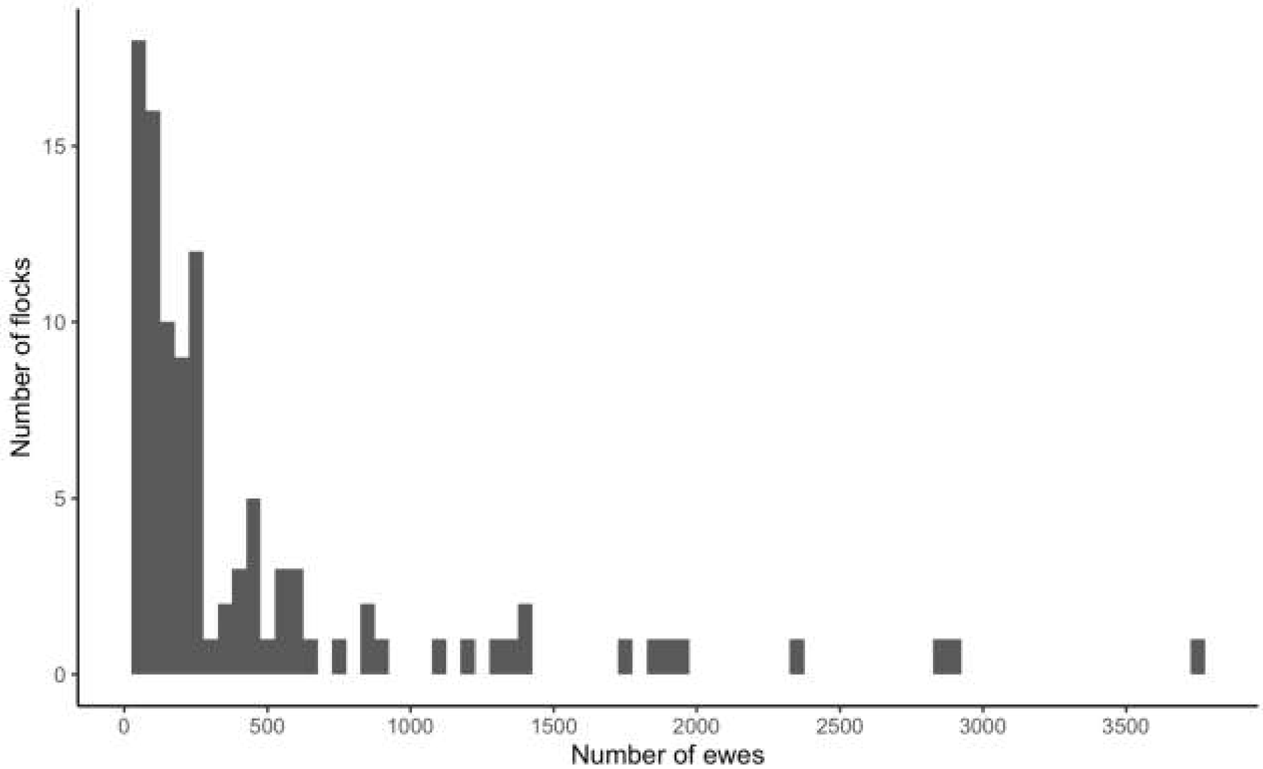


Figure 5 The number of ewes with recorded birth events for performance recorded Dorset flocks

The period of recording by each flock is shown in Figure 6. There is a distinct change between the majority of flocks that performance recorded in the 1980s and those that recorded from the late 1990s onwards. Figure 7 shows that the number of lambing events increased throughout the 1980s before decreasing abruptly at the start of the 1990s. In 1989, when the highest number of lambing events was recorded, 40 flocks contributed to the dataset, compared to only 22 flocks in 1992 when the lowest number of lambing events was recorded (data for 2019 are incomplete). From the late 1990s onwards the number of lambing events recorded remained fairly constant. At the start of the 1990s a change in cost and focus of the performance recording scheme favoured the enrolment of pedigree flocks over commercial flocks (Boon 2020, personal communication). Different breeding objectives between these two flock types might cause a difference in management and this needs to be taken into consideration when interpreting the data. Any trends seen in seasonality and performance over the whole recording period could have been influenced by the change in flocks contributing to the dataset over time. In addition, the flocks recording in the 1980s contributed a larger proportion of the data, and therefore had a greater influence on patterns observed for the combined dataset where year of recording was not taken into account.

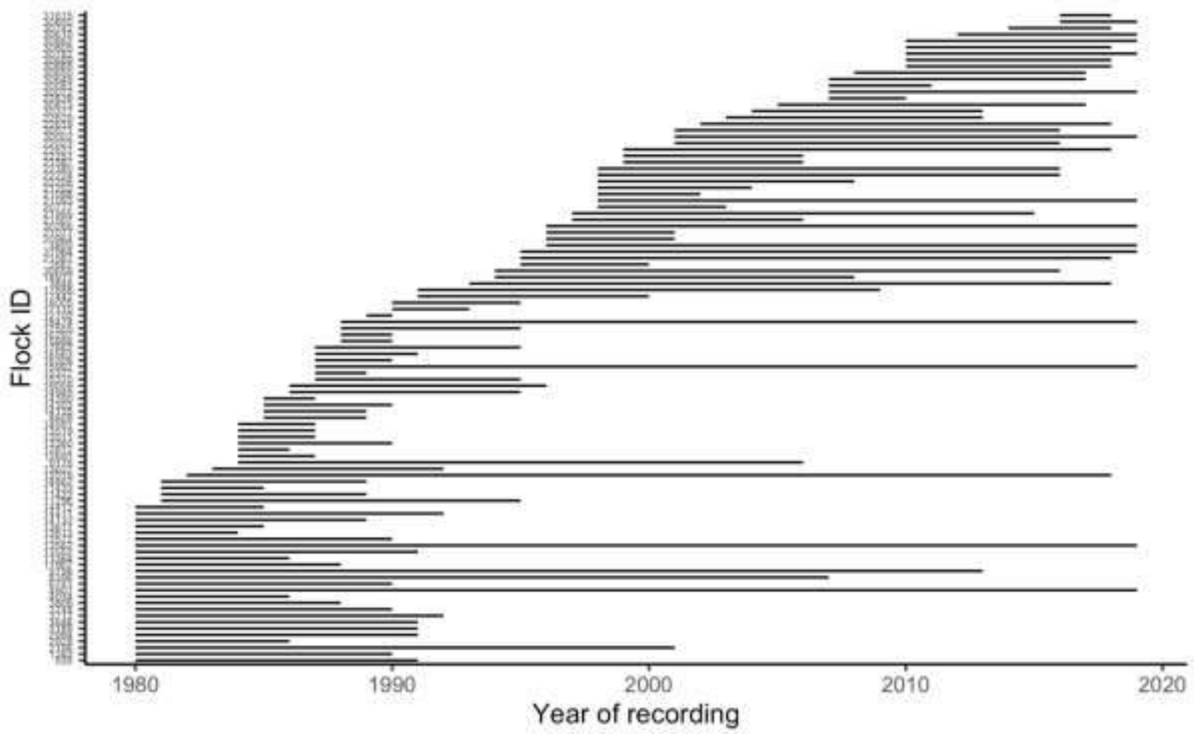


Figure 6 The period of performance recording shown for each flock over the entire recording period

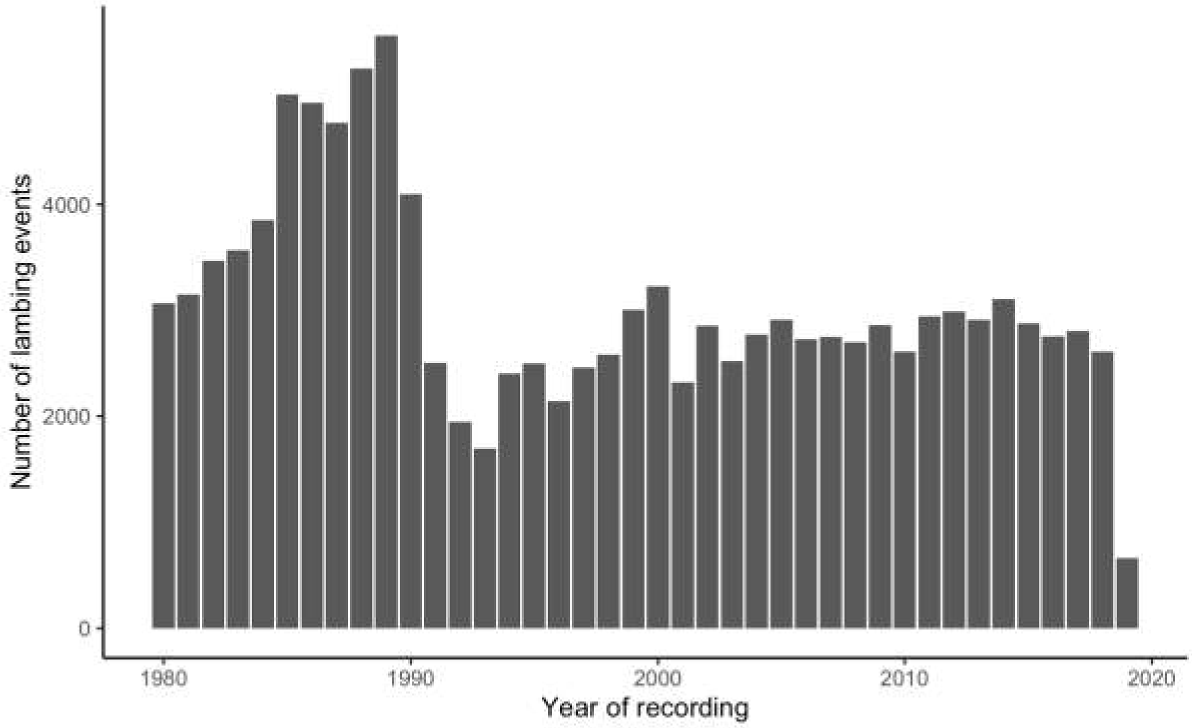


Figure 7 The number of lambing events recorded for each year of the performance recording period

Seasonal variation in lambing time between flocks

In order to estimate a breeding value for a ram to sire daughters with the ability to lamb OOS, the sires of ewes lambing both in and out of season need to be compared. Therefore, the dataset was interrogated to see how the overall seasonal distribution of lambing events compared to the distribution in individual flocks, and whether within flock comparison of sires was likely to be possible. Additionally, the seasonality of lambing over the duration of the recording period was observed, to identify any trends towards OOSL. 'Out of season' was defined as lambing between 1st September and 30th November, based on discussion with four pedigree Dorset breeders. Also, the lambing season of seasonal sheep breeds commences in the winter in accordance with the onset of oestrus cyclicity with decreasing daylight length. Additionally, the months of September, October and November were allocated a value of 3, 2 and 1 respectively. This value represented the extent of non-seasonality and how favourable that month of lambing was to the breeder. All other months were given a value of 0 to represent being in season (or in the case of May-August being undesirable to the breeder). The overall distribution of lambing events showed that they occurred most frequently in the three OOS months and 27.4% (33353/121936) of all lambing events occurred in September. The number of lambing events decreased in each month between September and April, with the exception of March (Figure 8). Only 0.7% to 1.4% of lambing events occurred in the months of May to August. The high proportion of lambing events in September was in part explained by the three flocks that contributed the most lambing events to the dataset. These flocks lambed in September for 49%, 61% and 68% of their records respectively, collectively contributing 47% (15680) of all September lambing records in the dataset. In contrast, the two flocks that contributed the fourth and fifth highest number of records favoured lambing in-season (Figure 9). Only eight flocks recorded more than 25% of lambing events between May and August, a potential indicator of an accelerated lambing system (lambing 3 times/2 years), and collectively their records spanned between 1980 and 2013. The number of OOSL events as a proportion of all lambing records for each farm had a range of 0 – 0.99 (mean 0.54) showing the wide variation in the seasonality of lambing events between flocks (Figure 10). As many of the flocks were exposing ewes to rams both in and out of season, there is the potential to use much of the existing dataset to compare the sires of ewes lambing predominantly OOS with the sires of ewes lambing predominantly in season, provided that the sires had the same opportunities to breed both in and out of season.

Distinct trends in the proportion of all lambing events that were OOS were observed over the recording period, and these reflected the changes that occurred in the number and identity of contributing farms over this period (Figure 11). The percentage of lambing events that occurred OOS decreased over the 1980s from 64% in 1980 to 46% in 1991, whilst the number of recorded lambing events increased over this period. The percentage of OOS lambing events continued to drop to a nadir of 27% in 1991, coinciding with both a sharp decrease in the number of flocks recording, and a change in the flocks contributing to the dataset. The proportion of OOS lambing events increased throughout the 1990s to peak at 80% in 2002, then fluctuated around 70% from the mid 2000s onwards.

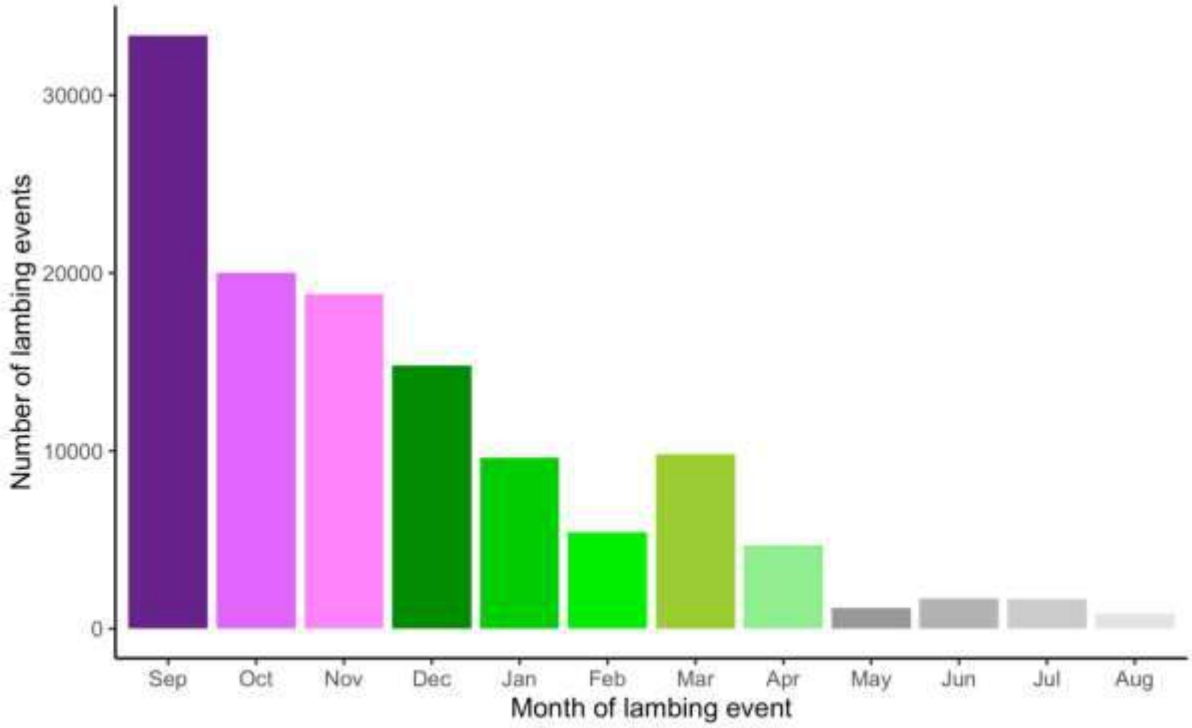


Figure 8 The frequency of lambing events in each month. OOS months are shaded in purple/pink, in season lambing months are shaded in green and undesirable lambing months are shaded in grey.

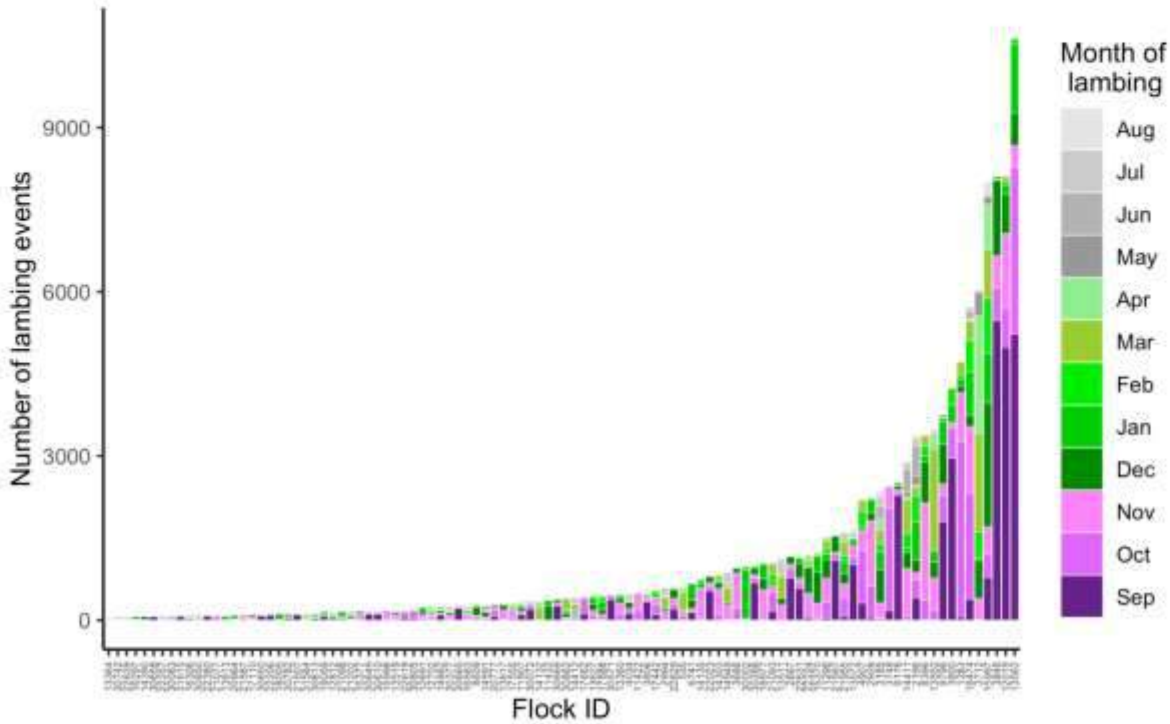


Figure 9 The number of lambing events in each month by flock. OOS months are shaded in purple/pink, in season lambing months are shaded in green and undesirable lambing months are shaded in grey.

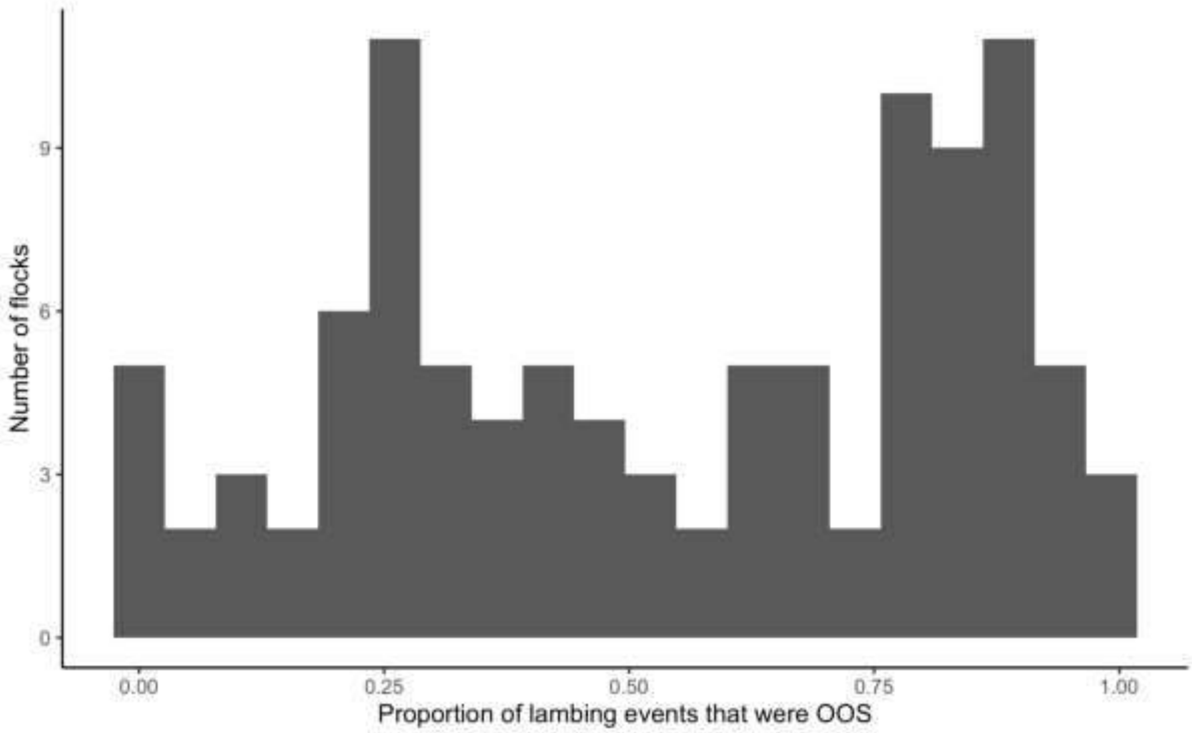


Figure 10 The proportion of lambing events recorded that were OOS as a proportion of all lambing events recorded for each farm

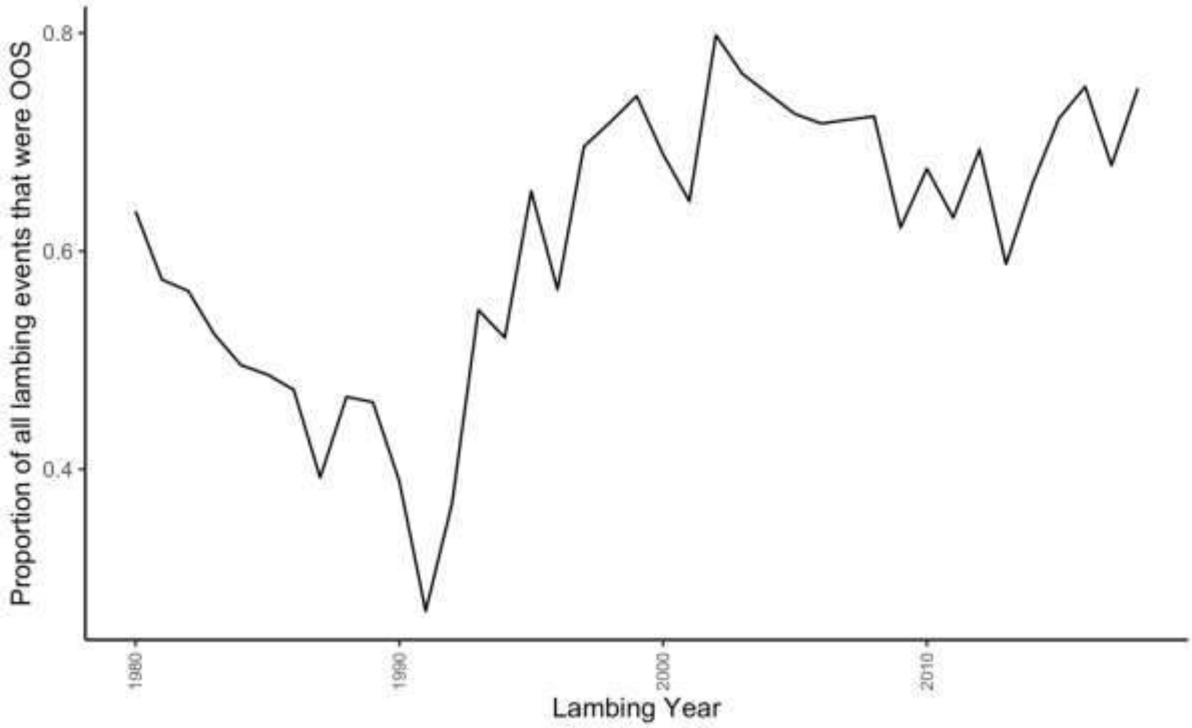


Figure 11 The proportion of all lambing events that were OOS (September, October or November) for each year of the recording period

Age at first successful mating and its impact on the ability to lamb OOS

The impact of age at first lambing was investigated to see if it had an adverse effect on the ewe's ability to lamb OOS. First, the data were examined to see the influence of flock on the age at which ewes first lambed, and to find evidence that farmers were deliberately waiting until ewes were a certain age before mating them. A cut-off age at first lambing of 1000 days was used to exclude the majority of ewes for which a previous lambing history was not available due to recent entry into the recording scheme. Figure 12 shows that in the majority of flocks, ewes lambed for the first time in the lambing season two years after they were born. However, the wide range of ages at first lambing indicates that at least some ewes were exposed to the ram as ewe lambs in most flocks. Without records for the number of unsuccessful exposures to a ram, it is not possible to distinguish between ewe lambs that were not yet reproductively mature when exposed to the ram from those that were not given an opportunity to be mated.

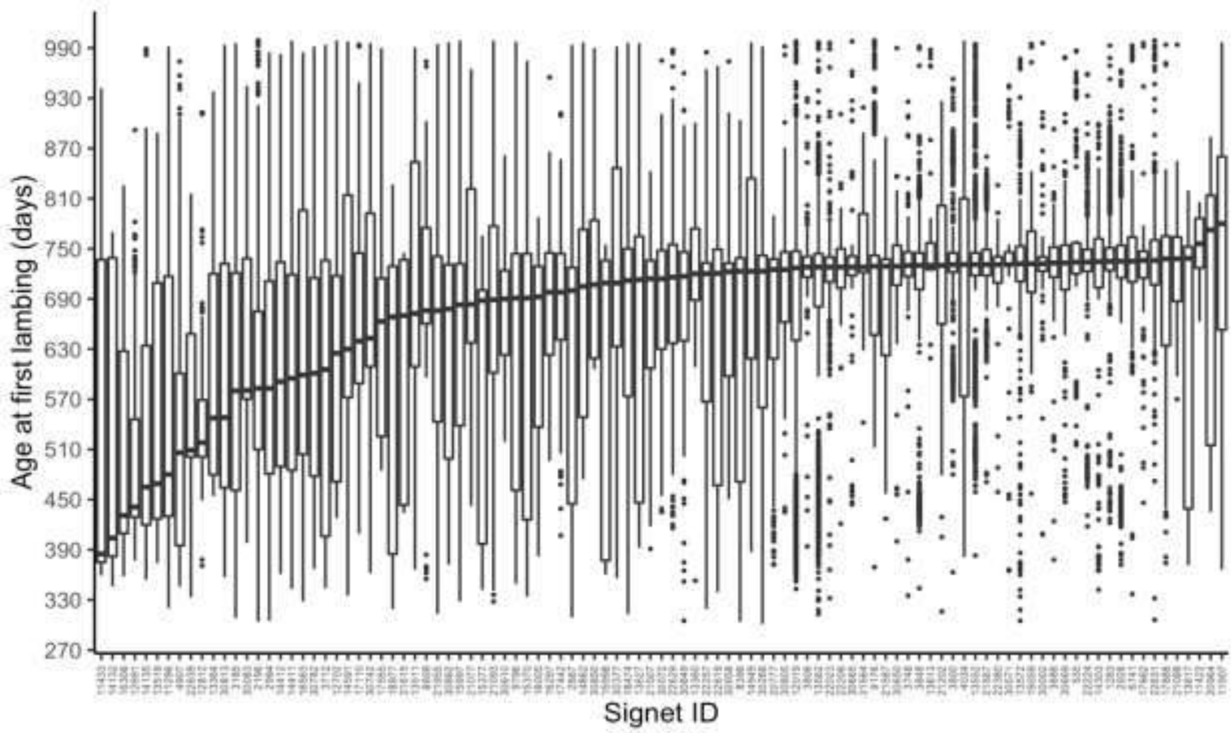


Figure 12 Boxplot of the age at first lambing for ewes under 1000 days old at their first recorded lambing event, outliers are beyond 1.5x the interquartile range

Ewes were grouped into three categories by their age at first successful breeding: "Early ewe lambs", first lambing at less than 18 months old; "Late ewe lambs", first lambing at between 18 and 23 months old; "Shearlings", first lambing at between 24 and 28 months old. The categories were based on the apparent peaks in frequency of lambing events at certain ages at the time of first lambing, shown in Figure 13. The frequency of lambing events was plotted by month of birth of the dam because it was postulated that a farmer would aim to lamb spring-born ewes in the autumn, at around 18-20 months old, whereas they would aim to lamb autumn born ewes in the autumn. There was a greater spread in the distribution of ages at first lambing as the months progressed from autumn to spring, and as postulated the ewes that were born in the spring months tended to be younger than those that were born in the autumn months at the point of lambing.

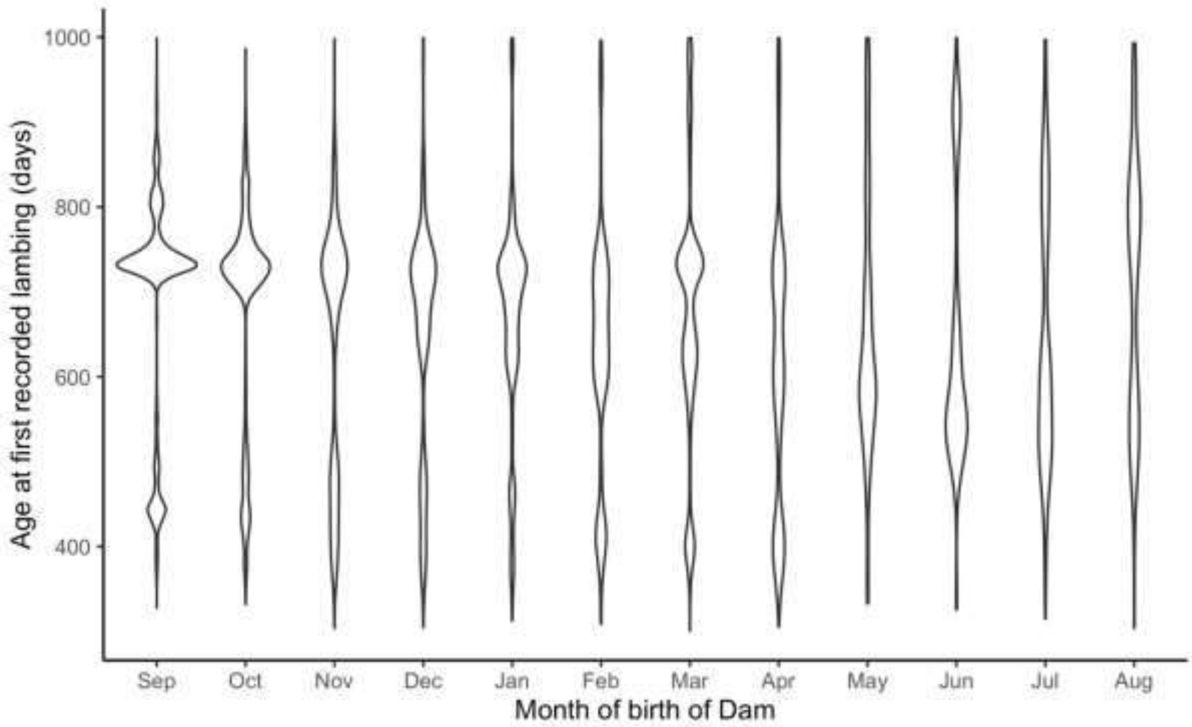


Figure 13 Frequency of lambing events by age at first recorded lambing by month of birth of dam for parity 1 ewes under 1000 days old at the time of first lambing

The age at first lambing categories were used to re-examine the data for the influence of farm on age at first lambing. Summary results for all farms are presented in Table 1. The contribution to all first lambing events from ewes in each category had a wide range between flocks, particularly in the shearling group. All farms had registered birth events from ewes mated as shearlings and late ewe lambs, and only five flocks did not have births registered to ewes mated before they were 18 months old. Trends for age at first lambing by year of lambing event were not investigated for individual flocks, so it is not known if the mixture of age at first lambing categories found on single farms is due to a change in management between years, or due to mixed categories in a single year. However, a mixture of three age at first lambing categories in one lambing year within a single flock is realistic. For instance, when a breeder exposes early ewe lambs to rams with mixed success, and deliberately times the mating of spring born lambs and shearlings unsuccessfully mated 12 months previously for an autumn lambing period. When the flocks were combined, a clear trend was apparent in the proportion of first births from each age at first lambing category by year of birth event. The proportion of births in the shearling category initially followed a similar trend to the proportion of OOS birth events, decreasing throughout the 1980s then increasing from the early to mid 1990s onwards (Figure 14). The proportion in this category continued to increase, although more gradually, until the last recorded year where it reached 68% of all first births (in ewes of 28 months or younger), whilst the proportion of first births from ewes in the early and late ewe lamb categories both decreased. It is not surprising that the proportion of ewes first lambing at 24-28 years old has increased as the trend for lambing OOS has increased. An autumn born ewe lamb that first lambs OOS at 12-15 months old must become fertile before the advent of shortening daylength, the usual physiological trigger for the onset of oestrus.

Table 1 Summary statistics for the proportion of ewes in each age category at first successful mating for all farms

Age at first successful mating	Minimum % ewes in category	Maximum % of ewes in category	Mean (sd) % of ewes in category	Median % of ewes in category
Early ewe lamb	0	75.0	24.8(20.0)	21.8
Late ewe lamb	1	77.8	28.7(22.2)	26.4
Shearling	3.7	92.6	47.7 (16.7)	47.0

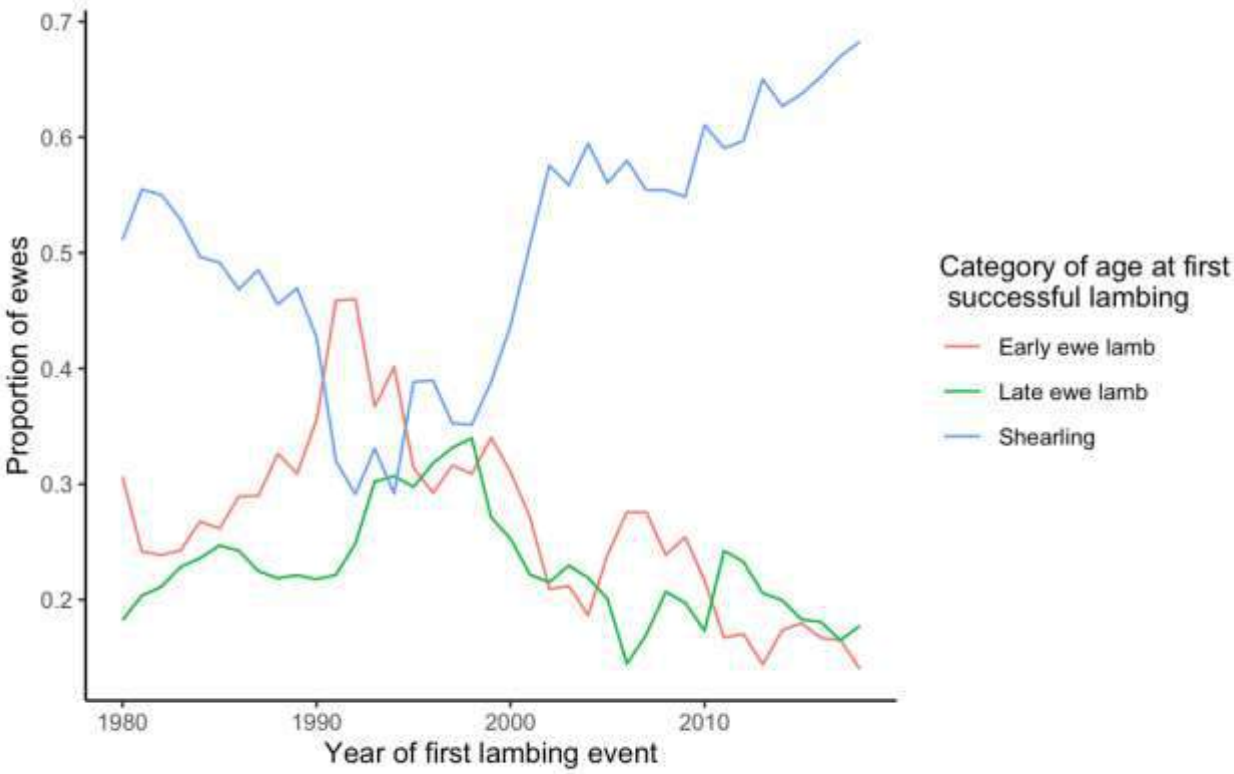


Figure 14 Proportion of ewes in each of three age at first successful mating categories by year of first birth event. Early ewe lambs first lambed at less than 18 months old; Late ewe lambs, first lambed between 18 and 23 months old; Shearlings first lambed between 24 and 28 months old.

The relationship between month of birth, month of first lambing event and age category for first successful mating was explored further to investigate the impact of daylength on OOS breeding in ewes reaching reproductive maturity, and its consequences for flock breeding management. September was the most common month for first parity ewes to have been born (Figure 15). Of the 10506 ewes born in September that lambed within 28 months of age, 2229 (21%) lambed before they were 15 months old. These ewes would have had only a short period of exposure to reducing daylength, or none at all prior to mating. Not surprisingly therefore, in September, when parity 1 lambing events were most frequent (Figure 16), only 136/7507 (1.8%) of ewes were in the early ewe lamb category, compared to 67% (5155) in the shearling category. The proportion of early ewe lambs contributing to parity 1 births increased to 22% (1141/5260) by November and, except in March, continued to increase until May. The number of ewes first successfully mated as shearlings (18119) was more than twice the number of ewes first successfully mated as early (7883) or late (7589) ewe lambs. In the shearling category, 63% (11463) of ewes first lambed OOS, compared to 64% (4842) in the late ewe lamb category and 20% (9463) in the early ewe lamb category (Figure 17). Overall the results showed a tendency for breeders to aim for OOS lambing months in first parity ewes, including those born in the late winter and spring months. However, when early ewe lambs were intentionally mated, they were more likely to lamb later in the year or in spring, coinciding with when they became reproductively mature.

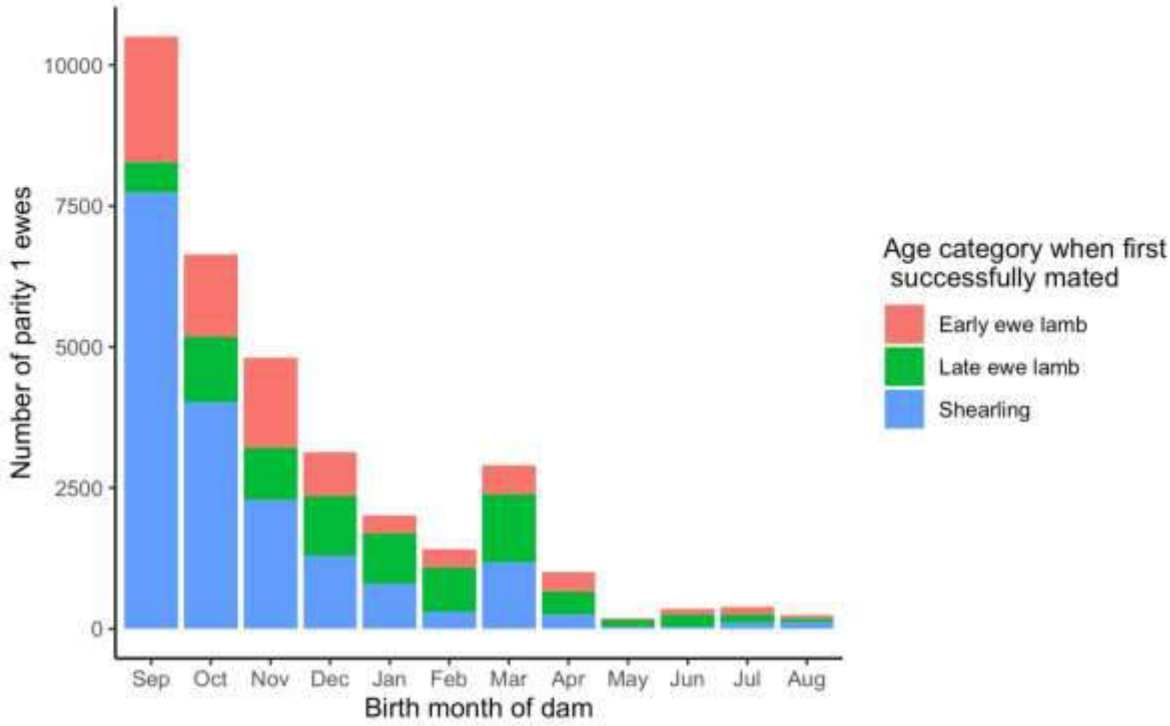


Figure 15 The number of first lambing events for ewes in each age at first successful mating category by month of birth of the ewe. Early ewe lambs first lambed at less than 18 months old; Late ewe lambs, first lambed between 18 and 23 months old; Shearlings first lambed between 24 and 28 months old.

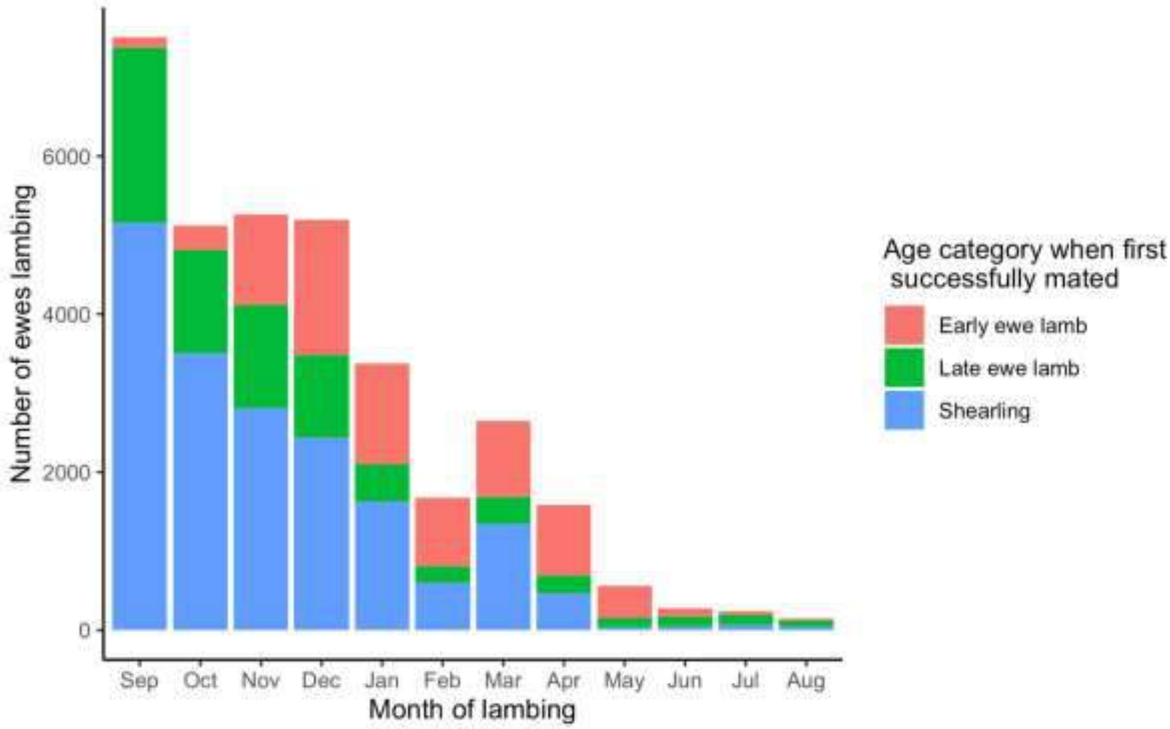


Figure 16 The frequency of lambing events by month of lambing in first parity ewes of 28 months of age or less. Early ewe lambs first lambed at less than 18 months old; Late ewe lambs, first lambed between 18 and 23 months old; Shearlings first lambed between 24 and 28 months old.

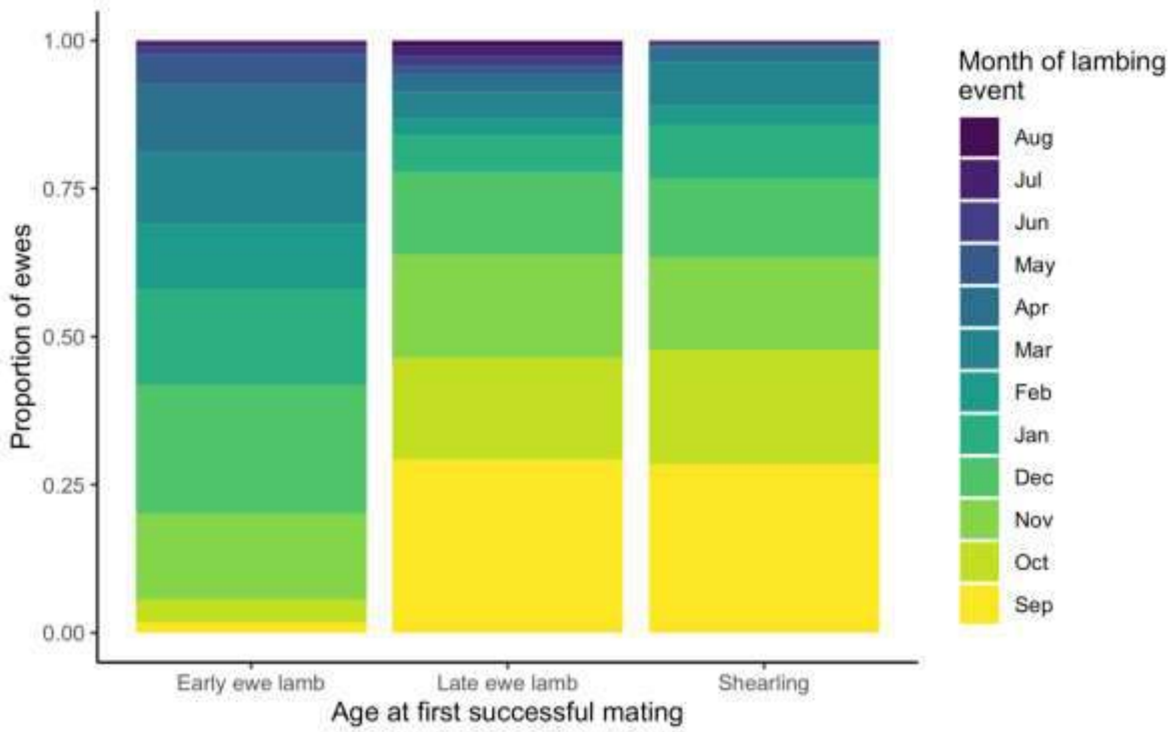


Figure 17 The proportion of parity 1 ewes lambing in each month by age category. Early ewe lambs first lambed at less than 18 months old; Late ewe lambs, first lambed between 18 and 23 months old; Shearlings first lambed between 24 and 28 months old.

The impact of parity, the month of previous lambing event and age at first lambing on the interval between lambing events

The data were explored to look for the impact of lambing OOS on the interval to the next lambing event, and for evidence of short lambing intervals which could indicate an accelerated lambing system (three lambing events in two years), which could be an alternative explanation for OOS lambing. In addition, the data were explored for any impact of age at first lambing on the time taken to the second lambing event. Lambing intervals that were recorded as less than 180 days were removed due to being erroneous or not representing a complete gestation. Lambing intervals of >1095 days were removed as it is likely they were also due to recording errors or missing data. The median interval since the previous lambing event was approximately one year for lambing events occurring between September and April (Figure 18). The interquartile range (IQR) for September lambing events was the narrowest at 30 days, increasing with each month until February (123 days) then decreasing to 41 and 31 days for March and April respectively. The undesirable lambing months (May – August) had shorter median intervals, all below 10 months (range 236-277 days). The median interval of approximately one year between lambing events in September and October, combined with the proportion of lambing events occurring in these months and the narrow IQRs, show the desirability of OOS lambing among Dorset breeders. Additionally, the narrow IQR shows the ability of ewes to repeatedly lamb OOS but could also indicate breeders selecting not to breed ewes that were unable to get back in lamb for the following autumn. In contrast, the wider IQRs for between November and March are likely to be due to breeders either attempting to reduce the lambing interval back to the more desirable autumn months or allowing ewes another chance to lamb in the spring if they have failed to get pregnant for an OOS lambing. In contrast, the much shorter median lambing intervals for ewes lambing in May-August could be the result an accelerated lambing system. The median interval between lambing events by parity was approximately one year up to parity 7, thereafter decreasing to under nine months (Figure 19). Only a small percentage of ewes reached eighth parity or above and the reduced lambing intervals are indicative of an accelerated lambing system, which explains their ability to achieve the higher parities. Lambing intervals between first and second parity were a median of 364, 366 and 331 days for shearling, late ewe lamb and early ewe lamb categories respectively, so there was no evidence of a negative impact of age at first lambing on time taken to second pregnancy (Figure 20). In contrast, there was evidence of a 'catch-up' effect, likely caused by early ewe lambs tending to lamb later in the season at first parity. Median intervals between subsequent lambing events for all parities were approximately one year for all three age at first lambing categories (Figure 21).

In conclusion, most flocks contributing to this dataset favoured OOS breeding, and did not operate an accelerated lambing system. Ewes lambing between December and February were more likely to have lambed OOS previously, suggesting that they were given a second chance to breed in the same management year if they did not achieve a pregnancy for OOSL. March and April also appeared to be favoured lambing months from a management perspective. Both months had a narrow range of lambing intervals around 365 days. This is likely to be explained by economic drivers, with a premium paid for OOS born lambs, and spring grass growth allowing supplementary feed purchases to be minimised for March and April lambing flocks. There was no evidence that breeding successfully for the first time as a ewe lamb increased the interval to the next lambing compared to ewes that were more mature at first lambing.

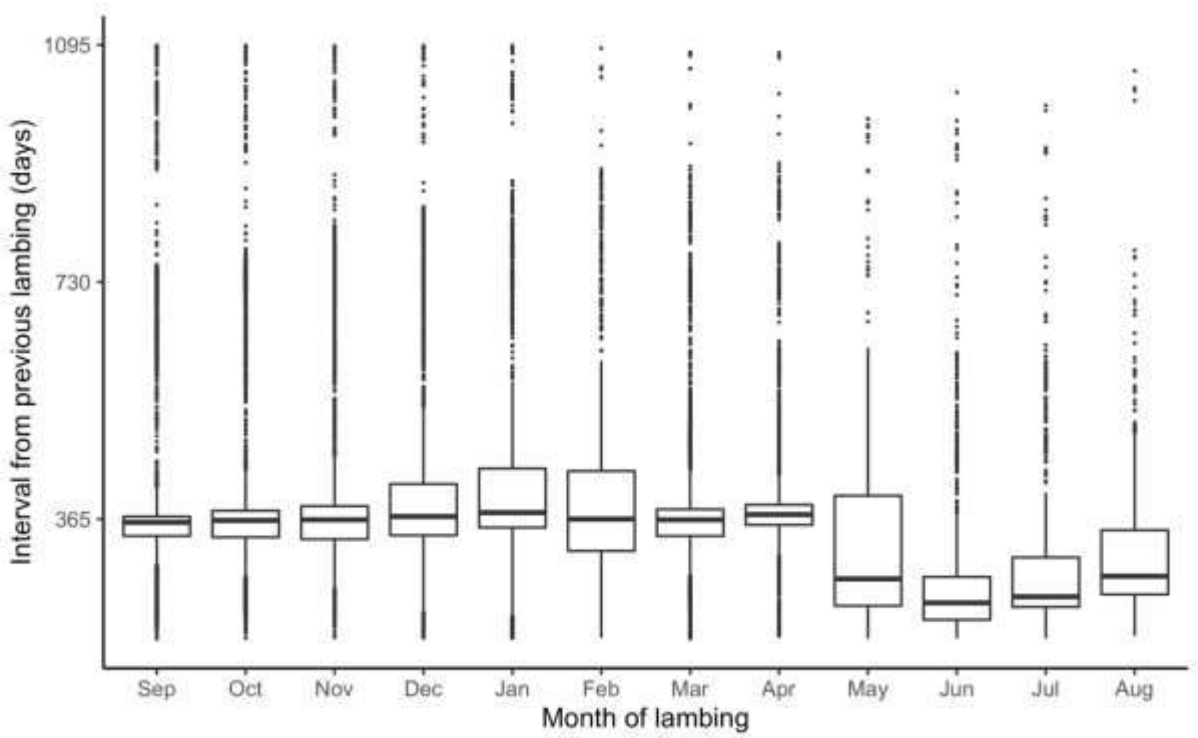


Figure 18 Boxplot of intervals since the previous lambing event for each month that the lambing event occurred. Outliers are intervals beyond 1.5 x the IQR. Records for lambing events at intervals of <180 days and >1095 were removed.

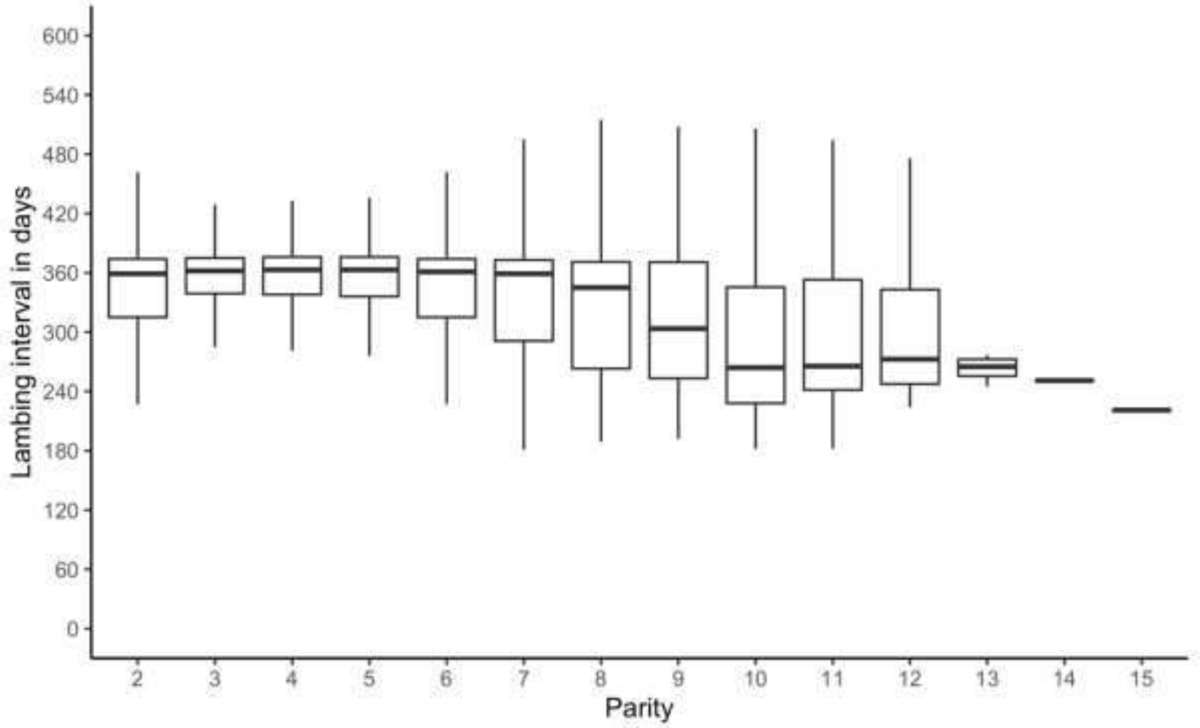


Figure 19 Boxplot of intervals since the previous lambing event for each parity. Outliers (intervals beyond 1.5 x the IQ range) have been removed. Parity was allocated to the records of lambing events in sequential order for each ewe, and did not account for records missing prior to, or during, the period the flock participated in the recording scheme. Records for lambing events at intervals of <180 days and >1095 days were removed.

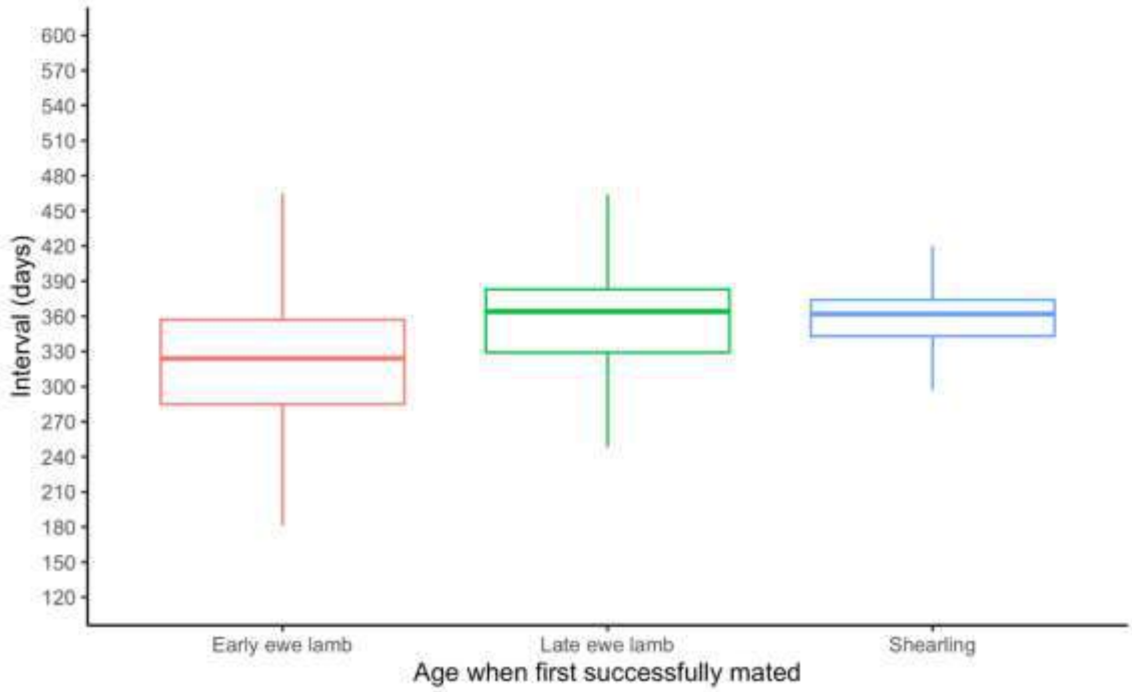


Figure 20 Boxplot of interval in days between first and second parity lambing events by age at first successful mating category. Outliers (intervals beyond 1.5 x the IQ range) have been removed. Early ewe lambs first lambed at less than 18 months old; Late ewe lambs, first lambed between 18 and 23 months old; Shearlings first lambed between 24 and 28 months old.

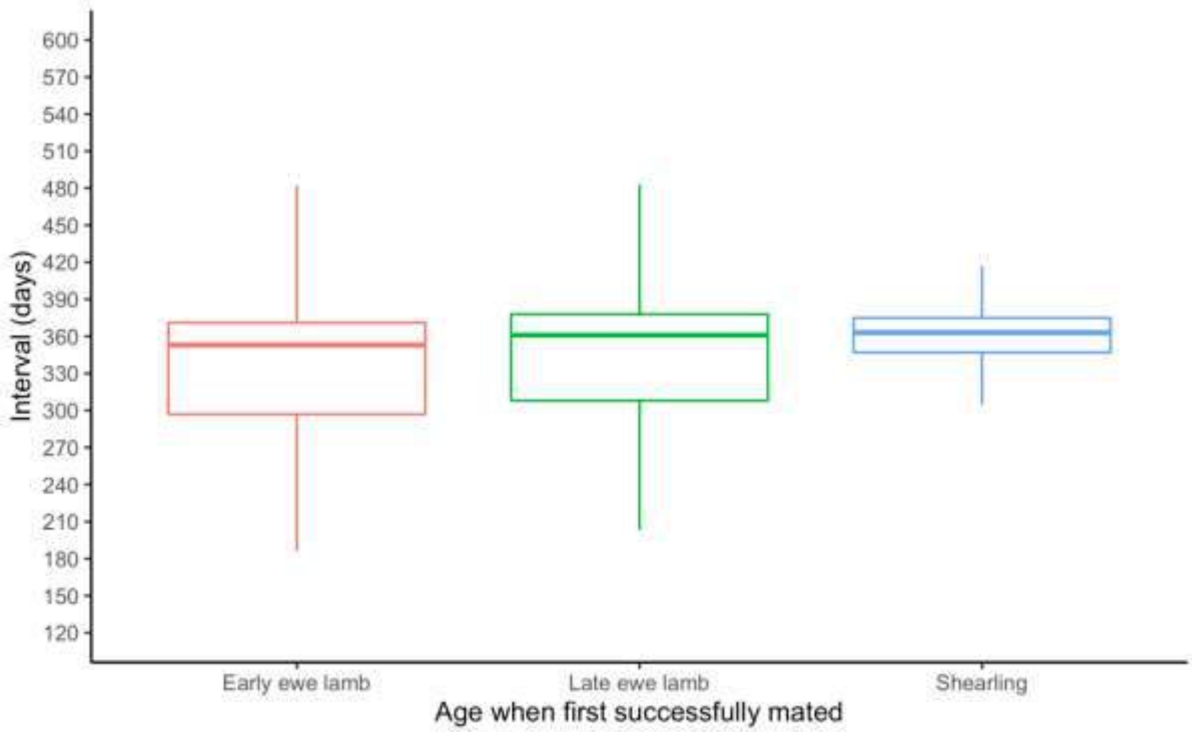


Figure 21 Boxplot of interval in days between sequential lambing events by age at first successful mating category. Outliers (intervals beyond 1.5 x the IQ range) have been removed. Early ewe lambs first lambed at less than 18 months old; Late ewe lambs, first lambed between 18 and 23 months old; Shearlings first lambed between 24 and 28 months old.

The effect of month of lambing and age on litter size

In addition to the ability to lamb OOS consistently every year, reproductive performance can also be measured by litter size. The effect of seasonal lambing compared to OOSL on litter size was investigated in this dataset, along with the effect of age at first lambing and at subsequent lambing events. The aim was to identify potential benchmarks for lambing percentage (number of lambs born per number of ewes lambing), which could be informative for when breeders make management decisions concerning the lambing season and age at first lambing. The overall mean litter size was 1.65 and 53% of births resulted in twins, compared to 41% producing single lambs. Mean litter size peaked at 1.74 in March, but only varied from 1.62 to 1.67 between the months of September and February (Figure 22). Mean litter size was 1.37 (SD = 0.50) for first parity ewes that lambed as ewe lambs compared to 1.57 (SD = 0.55) for ewes that lambed for the first time as shearlings. However, the mean number of lambs produced over a lifetime was higher in ewes first bred as ewe lambs (Table 2). Mean litter size continued to increase until ewes were 4 years old, when it reached 1.74 (Figure 23). In summary, there was no evidence of a detrimental effect on ewe reproductive performance from either OOSL or lambing for the first time as a ewe lamb.

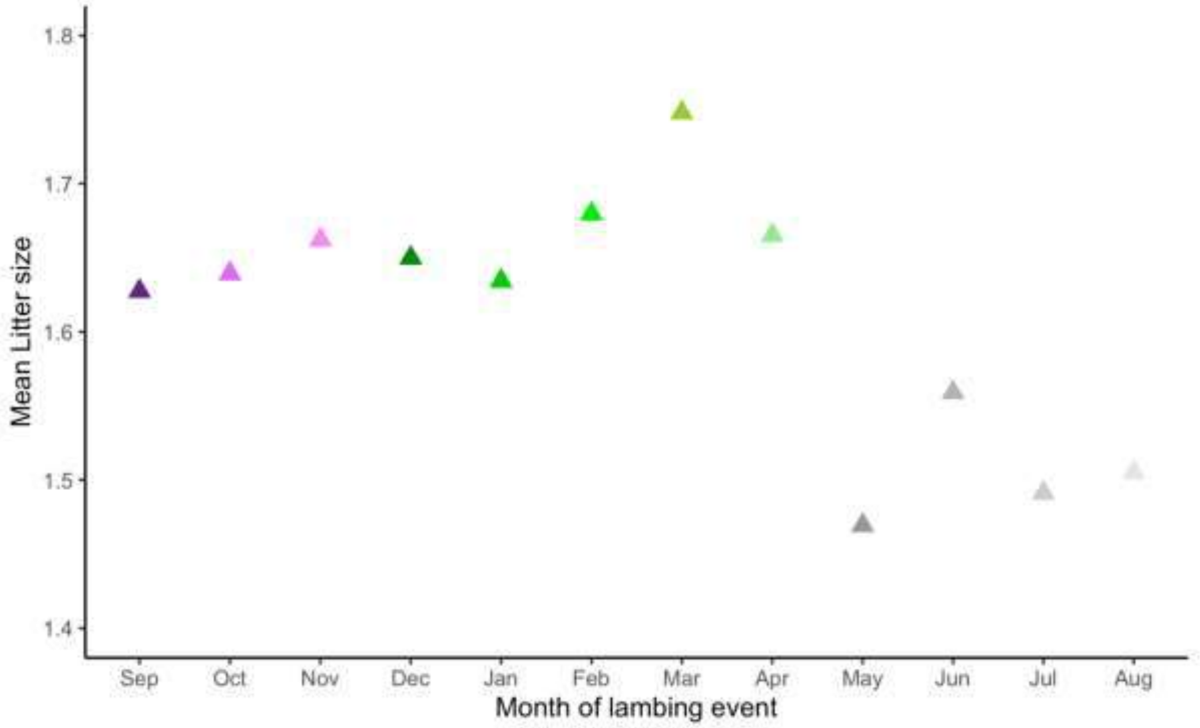


Figure 22 Mean litter size for all lambing events by month in which the lambing event occurred. OOS months are shaded in pink and purple, in season lambing events are shaded in green and undesirable lambing months are shaded in grey.

Table 2 Mean and standard deviation for number of lambs produced over a lifetime by age at first successful mating category. Ewes were removed from the dataset if their final parity was not known. Early ewe lambs first lambed at less than 18 months old; Late ewe lambs, first lambed between 18 and 23 months old; Shearlings first lambed between 24 and 28 months old.

Age at first successful mating	Number of ewes	Mean lifetime number of lambs	SD lifetime number of lambs
Early ewe lamb	5943	5.45	3.78
Late ewe lamb	5904	4.80	3.48
Shearling	13562	4.71	3.06

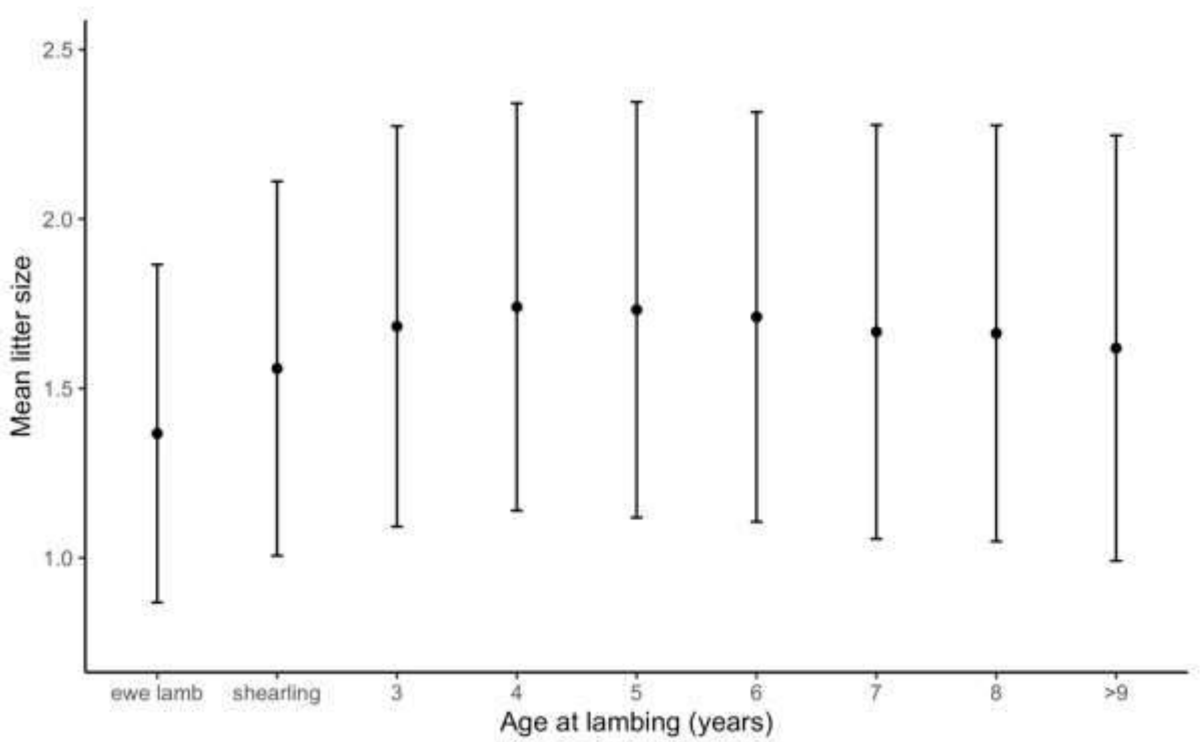


Figure 23 Mean litter size by age of ewe at the time of lambing event. Error bars = sd.

The distribution of sire daughters across performance recorded flocks and lambing events

There were 2322 sire identities available with 31870 daughters and 90470 lambing events in the combined datasets. Summary statistics are presented in Table 3. The median number of flocks in which a sire had daughters was one, with a range of up to 16 flocks and a mean(sd) of 1.6(1.5).

Table 3 Summary statistics for sires with daughters and lambing events in the combined dataset

Per sire	Minimum	Maximum	Mean (sd) %	Median %
Lambing events	1	537	39 (58.6)	18
Daughters	1	183	13.7 (18.2)	8

Constraints were introduced to the dataset so that the feasibility of modelling the impact of sire on OOSB ability could be assessed. Only sires with 25 or more daughters and 50 or more lambing events were retained and the new dataset checked for consistency with the original dataset. The new dataset contained 51468 lambing events involving 16793 dams and 366 sires in 85 flocks. Sires still had daughters in between 1 and 16 flocks but the distribution did not have such an extreme positive skew as the full dataset (Figure 24), with a median of 2 flocks and mean(sd) of 3.3(2.9) flocks.

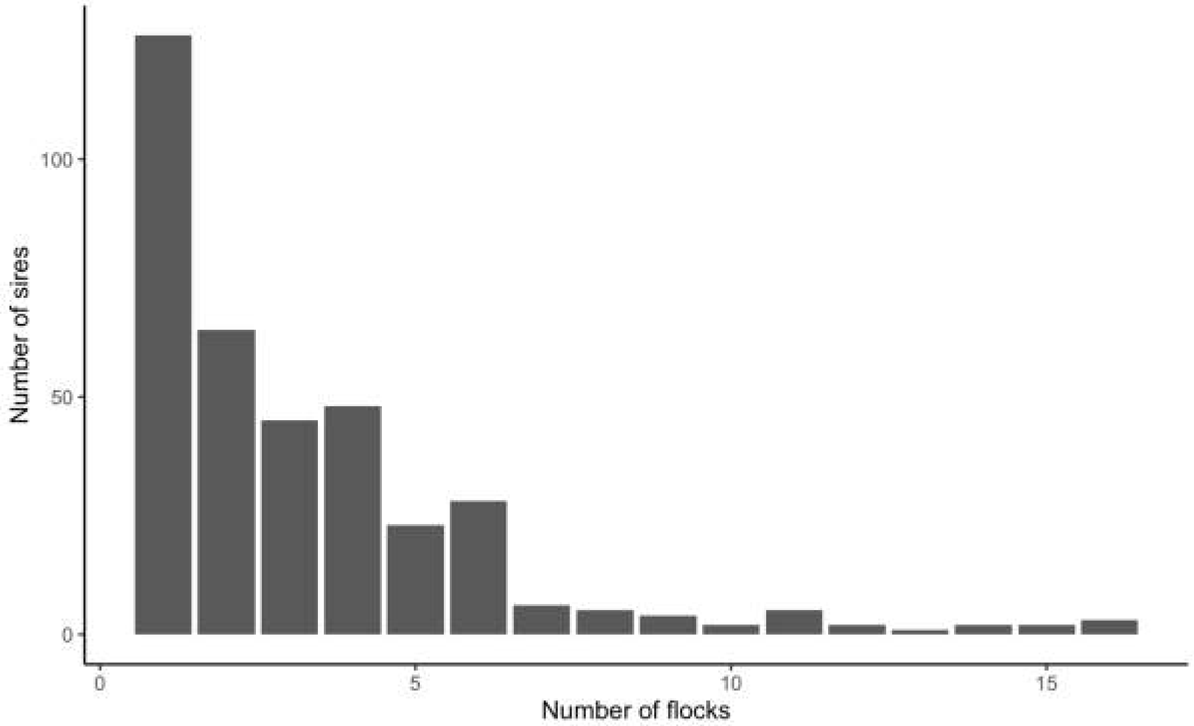


Figure 24 The number of flocks in which sires have daughters with birth events. Dataset constrained to sires with at least 25 daughters and 50 lambing events.

Reproductions of selected figures can be found in Appendix 3 so the restricted 'Sire' dataset can be compared with the cleaned, full performance recorded Dorset dataset. In summary, the dataset restricted to sires with a minimum of 25 daughters and 50 birth events showed a close similarity to the full dataset for distribution of birth events by month (Figure 27). The distribution of birth events across farms was also similar (Figure 28) but does have a higher proportion of OOSL events across the farms in the dataset, as indicated in Figure 29. The number of birth events recorded per year covered the same year range and followed the same trend as the full dataset. However, the number of birth events recorded in the late 1980s was similar to that during the 1990s onwards, and there was a lower relative nadir in birth events in the early 1990s (Figure 30). Overall, the restricted dataset appeared to be representative of the larger dataset in terms of distribution of lambing events. The proportion of all lambing events that were OOS for each sire (Figure 25) showed a negatively skewed distribution compared to that of each farm, with many sires having daughters that lambed predominantly OOS. The mean(sd) proportion of OOS events was 63(27) and median 72.4. The range of proportion of OOS lambing events of 0 to 99% provides scope for comparison between sires, but only provided that these sires were given the opportunity to serve ewes both in and out of season.

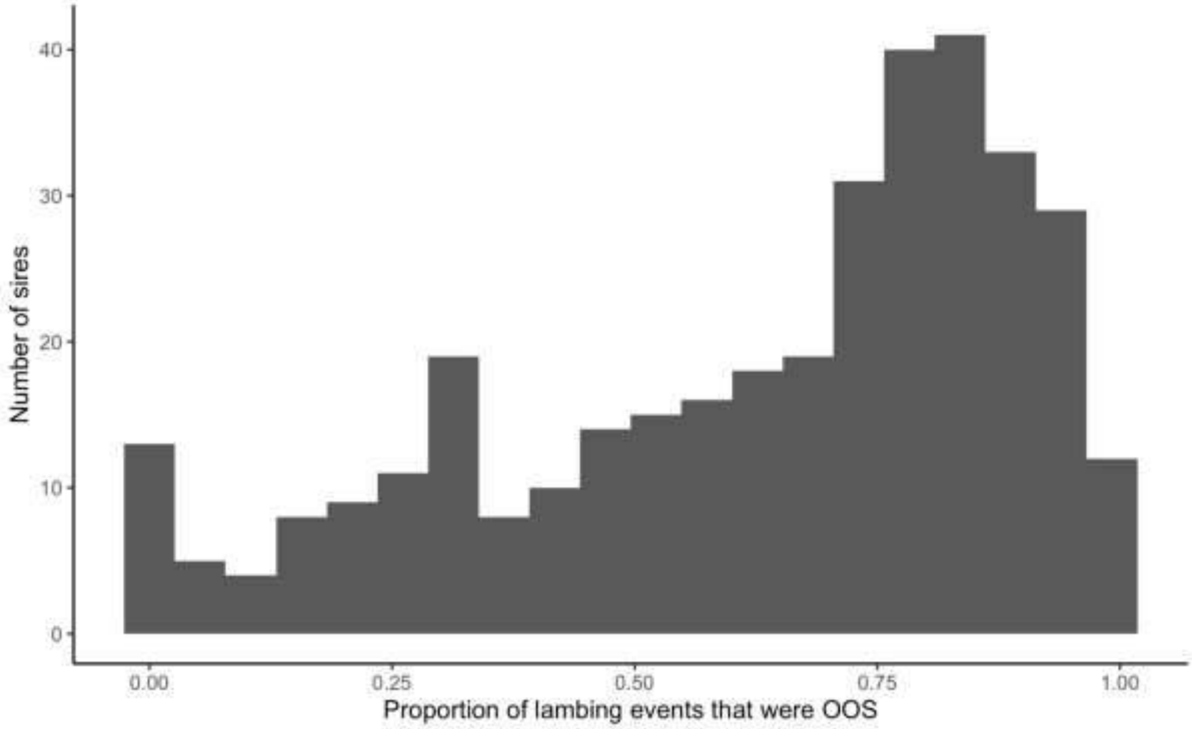


Figure 25 The proportion of lambing events recorded that were OOS as a proportion of all lambing events recorded for each daughter sire in dataset containing rams with >25 daughters and >50 lambing events.

Discussion

The Signet dataset for performance recorded Dorset flocks in the UK showed a clear preference for lambing in the autumn months, particularly during the last two decades. For most of the other numerous sheep breeds in the UK, lambing naturally during the September to November period would be unfeasible for physiological reasons. For this reason, most breed societies register births from a starting date of the 1st of January to reflect the natural anoestrus period of the breed, compared to the September 1st starting date used by the Poll and Horned Dorset Society. Consistent with this, the Dorset dataset shows that August lambing events are unfavourable, therefore it is logical to define 'out of season' lambing as occurring between September and November inclusive. However, Dorset breeders do not necessarily place equal value each of these months when making breeding decisions. A September birth is advantageous in both utilising the autumn grass flush and allowing a longer period of time to reach finishing weight for the new season lamb premium. Despite this some Dorset breeders intentionally lamb predominantly in October or November showing that other factors influence breeding decisions. Although birth events occur most frequently in September in this dataset, this is largely due to the three flocks contributing the most records.

A binary outcome of lambed/did not lamb has been successfully used to calculate a fertility rate for use in estimating EBVs (Asadi-Fozi*, et al.*, 2020; Rekik*, et al.*, 2011). Fertility rates were based on a defined period of exposure to rams, which was possible because the EBVs were calculated for single flocks with consistent breeding management across the years, or flocks with identical mating periods. A binary outcome could be used to calculate OOS fertility rates in UK Dorsets, with a three-month window for lambing from September 1st. The disadvantage of this approach is that it does not account for potential differences in fertility rate in ewes that are able to lamb, for instance, in November but not October, and would therefore diminish the usefulness for some breeders. Selecting a single month within this period to use as a binary response is precluded, with ewes in some flocks never having the opportunity to lamb during certain months for management reasons. An ordered categorical response could be an alternative measure, placing the greatest weight on September births, then October, November and the rest of the year correspondingly. This measure places the most weight on lambing events that occur the longest period before in-season lambing starts. However, Vincent*, et al.* (2000) found that although a high EBV for aseasonal breeding correlated with a shorter period of anoestrus, the lowest months for oestrus activity were May, June and July in both low and high EBV Dorset cross ewes. April matings occurred prior to the onset of anoestrus rather than indicating the absence of anoestrus in an individual ewe. If this is the case in UK Dorset ewes an ordered categorical response weighting October and November above September would permit a more rapid response to selection. Further consultation with the Dorset breeders who are likely to use this EBV, combined with the calculation of existing fertility rates for each of the OOS months (which requires additional data from the breeders) is required to make an informed decision if this approach is to be pursued.

There are a number of variations in breeding management between flocks that affect the ability of a ram to express his genetic potential for OOSL ability in his daughters. Details of most of these management factors are not collected alongside the performance records but could have a positive or negative impact on a ram EBV for OOSL. Most importantly, in order for a ram's daughter to be able to express OOS ability she must be given an adequate period of exposure to a fertile ram, at a realistic ram: ewe ratio, during the corresponding time period in spring. Some of the variations in management between farms can be extrapolated from the existing performance recording dataset for the Dorset breed, for example whether the breeder chooses to lamb ewes OOS or not. However, in almost all flocks in the dataset there was evidence of some OOS lambing. The dataset shows the great variation between flocks in the distribution of birth events across different months, both out of season and in season. This variation shows that it is not possible to make assumptions about a ewe's inability to lamb OOS based on her failure to have lambed within the September to November period. There could have been a deliberate management decision to lamb her later in the year based on, for example, a need to restrict the number of ewes lambing during any one period due to housing constraints. Indeed, it is apparent from the data that some breeders choose to lamb predominantly out of season, whilst others mainly lamb in more conventional months, therefore the opportunities for rams to demonstrate OOSB ability in their daughters varies widely between flocks. Without access to records of unsuccessful matings as well as successful matings, estimates for aseasonal breeding values could be biased, both positively and negatively, towards rams with daughters in flocks that lamb predominantly out of season and allow multiple spring breeding opportunities to ewes. These mating opportunities better allow the genetic variance between sires to be exposed, which results in rams with both high and low EBVs to be identified. In contrast, flocks that aim to lamb only a limited number of ewes in the autumn might not give all ewes the opportunity to be served, for instance withdrawing the ram when a minimum number of ewes have been covered. Whilst an EBV based on these lambing events could identify the sires of ewes with the highest genetic potential (those consistently achieving autumn lambing events), ewes not achieving an autumn lambing could have been unsuccessful due to genetic or environmental reasons, so EBV accuracy would be low, particularly if there is little data to draw upon from other flocks that use related daughters and allow more generous opportunities for mating.

Most ewes in the dataset lambed at an interval close to 12 months but extended intervals were more common for winter and early spring lambing events than autumn and late spring lambing events. It is likely that some ewes that had previously lambed in the autumn but failed to conceive in the spring had been given further opportunities to breed later in the year (Figure 18). This observation was consistent with the pattern of both in season and OOS lambing periods on almost all farms, a practice that reduces the necessity to carry an unproductive ewe for 12 months and permits poorer fertility rates for spring matings than autumn matings. The absence of an equivalent skew in lambing intervals below 365 days for the autumn months suggests that spring lambing ewes were either not given an opportunity to lamb in autumn again once they lambed in spring, or most were unable to get back in lamb with a short post-lambing interval. Conception and maintenance of pregnancy out of season can be negatively influenced by a short interval from lambing to mating. Goff*, et al.* (2014) exposed 24 Dorset cross ewes selected for OOSL to rams in March at an average of 60 days after lambing. All but one ewe had mated within 39 days but only 48% produced lambs. When a further 34 ewes were exposed to rams in May at an average of 40 days after lambing, only 20.6% lambed successfully. However, ewes were lactating at the time of mating in this study.

Encouragingly, Dzabirski and Notter (1989) found no difference in lambing ability or lambing date between spring mated Dorset ewes that had previously lambed in either autumn or winter despite the rams being introduced only 5 weeks after the winter lambed cohort had been weaned. This suggests that short interval breeding management is likely to have only a limited effect on ewes' expression of genetic OOSL ability. In contrast, Lewis*, et al.* (1996) observed lower fertility rates for ewes with shorter intervals between lambing events for March, June and August matings compared to October and January matings in the STAR accelerated breeding management system. It is not possible to know how many winter lambing ewes in the UK Dorset dataset were unsuccessfully mated in the spring but there were some short intervals between lambing events for autumn months, so breeding opportunities had occurred. Consideration would need to be given as to whether winter and early spring lambing ewes that are exposed to rams in late spring/early summer should be included when calculating OOSB EBVs. Access to fertility rates for short interval, spring mated ewes in the UK Dorset population would help inform the decision.

The ability to lamb out of season is not fully expressed until a ewe reaches about 4 years of age, or third parity and above (Asadi-Fozi*, et al.*, 2020; Hanocq*, et al.*, 1999; Lewis*, et al.*, 1996; Teyssier*, et al.*, 2011). The age effect is apparent as an increase in fertility rate for each age cohort that lambs within the designated OOS period, until maturity is reached. The effect of ewe age on aseasonality could not be demonstrated in this dataset because fertility rate data was unavailable. However, ewe age still had a clear effect on the proportion of ewe lambs that lambed OOS, apparent from the difference between ewes that first lambed at 12-15 months of age and those that were older at first parity. Therefore, the age category of the ewes needs to be included in the model for OOSL EBV, and consideration given to which first parity ewes are included for EBV calculation. First parity ewes accounted for a third of the dataset so it is not realistic to disregard them entirely. Ewe lambs that gave birth OOS at under 15 months of age were exceptionally precocious and it could be argued that they should be included because they have the genetic potential that is sought by the breeders. However, to include them might discriminate against equally precocious ewes that were born too late in the autumn to reach sexual maturity in time to lamb OOS. A compromise would be to include only the first parity ewes that are shearlings because they account for the majority of these records and have all had sufficient opportunity to reach sexual maturity.

Vasectomised rams and hormone treatments can be used to advance and synchronise the onset of the breeding season but their use is not recorded in the Signet database. The use of these management tools is likely to vary from flock to flock, although widespread use of hormone treatments is not expected because of the likelihood that Signet recorded Dorset flocks also hold a Waitrose contract for lamb production, which prohibits the use of reproductive hormones. Variation in the use of vasectomised rams and hormone treatments could influence the expression of OOSL ability for some sires, especially as they tend to have daughters in only a small number of flocks. This could be a limitation for calculating sire EBVs using the existing dataset. The 'ram effect' is mainly observed in late anoestrus (Chanvallon*, et al.*, 2011). This short period of response could limit the impact on EBVs in flocks that routinely use this technique but to an extent this depends on the period of time used for the outcome measurement, and whether each month of lambing is weighted in value. Asadi-Fozi*, et al.* (2020) observed that during the Virginia Polytechnic flock study, ewes selected for OOS breeding became refractory to the ram effect because they were already cycling. It would be useful to differentiate natural from induced cyclicity when calculating EBVs. In practice it would be difficult to do this within a single group of ewes exposed to a vasectomised ram and it would be of greater benefit to the breeder to avoid the ram effect when trying to identify ewes with longer breeding seasons. Melatonin implants are licensed in the UK (Regulin®18mg implant; Ceva Animal Health Ltd) for the improvement of the fertility of ewes mating early in their natural season. The product is not marketed for out of season breeding but Mura*, et al.* (2017) observed that Sarda ewes, a dairy sheep breed with a similarly long breeding season to the Dorset, had an increased fertility rate over untreated ewes of 7-9% when ewes were implanted between February and May then mated from 35 days after treatment. The other hormone treatments available to UK sheep breeders are progesterone or progestogen releasing intravaginal devices, which can be used in conjunction with a gonadotrophin injection (PMSG-Intervet® 5000IU; MSD Animal Health). These treatments carry indications for the advancement of the onset of the breeding season in ewes (CIDR OVIS 0.35g; Zoetis UK Limited) or the induction of ovulation during anoestrus (Chronogest CR® 20mg; MSD Animal Health). Cabrera*, et al.* (2019) observed that after introducing rams in June, Targhee ewes that had been selectively bred for an extended breeding season lambed a mean of 11 days sooner than untreated control ewes when treated with a CIDR. The use of hormone treatments to extend the natural breeding system is likely to have a similar impact on the accuracy of EBVs as the use of vasectomised rams, limited but dependent upon the outcome measurement. It would be preferable for breeders to avoid hormonal intervention when trying to accelerate OOSL ability in a breeding programme, and beneficial for the users of an OOSL EBV to have had the use of hormone treatments and vasectomised rams recorded and accounted for in the BLUP model.

Restricted dietary intake and suboptimal body condition has been shown to interact with the effect of photoperiod on breeding season. Menassol*, et al.* (2012) demonstrated a delay in onset and early cessation of oestrus activity in Ile-de-France ewes with restricted dietary intake, shortening the breeding season by more than half without altering the midpoint. Feed restriction was quite extreme in this study and ewes were maintained at condition score 1.5 out of 5 on 80% of their energy requirements over two breeding seasons, conditions unlikely to occur in the pedigree Dorset flocks. Restriction of energy intake to 70% of requirements from February onwards did not affect ovulatory activity during April in Merino d'Arles ewes, another breed with a naturally long breeding season (Teyssier*, et al.*, 2011). Change in bodyweight between January at weaning and April made no difference to ovulatory activity in the same breed (Hanocq*, et al.*, 1999). Therefore, although nutritional status can have an effect on the onset of the breeding season, there is not likely to be sufficient variation in nutritional management within or between flocks to impact on sire EBVs in the Dorset breed.

There was little evidence in either the literature or in the Dorset dataset to indicate that selecting for aseasonal breeding would reduce overall ewe reproductive productivity. The litter size of March lambing ewes was approximately 0.1 lambs larger than autumn lambing ewes, but the economic significance of this must take into consideration the seasonal variation in cost of rearing lambs and the premium paid for lambs reared out of season. Furthermore, the fertility rate for spring breeding is likely to be much lower than that for autumn breeding in an unselected flock (Lewis*, et al.*, 1996). Selection for improved fertility for spring mating will have a greater impact on the number of lambs born per ewe mated than the small reduction in litter size that results from spring mating. Mean litter size for ewes lambing in autumn would only be expected to increase marginally due to the low heritability of this trait (Asadi-Fozi*, et al.*, 2020). The other observation on performance that is notable in this dataset is that lambing at 12-15 months of age is not detrimental to a ewe's ability to lamb again within 12 months. Indeed, ewes that lambed for the first time as yearlings had a higher median parity than ewes that lambed for the first time as shearlings. Further investigation into this aspect of breeding management is recommended because the extra litter gained as a ewe lamb has a positive impact on lifetime productivity, but is against the current trend.

In summary, there is documented evidence for variability between individual ewes in their ability to lamb OOS, and selective breeding can be used to extend the breeding season and increase the fertility rate for OOSL in a flock without causing detriment to other performance indicators. There is the potential to develop an EBV to identify Dorset sires whose daughters have a higher OOS fertility rate. However, due to several currently unrecorded management factors that influence a ewe's ability to express OOSL capability, and the variability in breeding management that occurs between flocks, the dataset in its current form could cause bias (both positive and negative) towards sires used in certain flocks. A minimum requirement would be for the breeders to record each unsuccessful mating. It would also help to increase the accuracy of EBVs if the use of hormones and vasectomised rams were to be recorded.

References

Al-Shorepy, S.A. and Notter, D.R. (1996). Genetic variation and covariation for ewe reproduction, lamb growth, and lamb scrotal circumference in a fall lambing sheep flock. *Journal of Animal Science* **74**, 1490-1498.

Al-Shorepy, S.A. and Notter, D.R. (1997). Response to Selection for Fertility in a Fall Lambing Sheep Flock. *Journal of Animal Science* **75**, 2033-2040.

Asadi-Fozi, M., Bradford, H.L. and Notter, D.R. (2020). Direct and correlated responses to selection for autumn lambing in sheep. *Genetics Selection Evolution* **52**, 56.

Cabrera, C., Maier, G.U., Cuneo, M. and McNabb, B.R. (2019). The use of progesterone intravaginal devices is superior to use of the ram effect at hastening the reproductive performance in transitional Targhee ewes. *Theriogenology* **128**, 17-22.

Calvo, J.H., Serrano, M., Martinez-Royo, A., Lahoz, B., Sarto, P., Ibanez-Deler, A., Folch, J. and Alabart, J.L. (2018). SNP rs403212791 in exon 2 of the MTNR1A gene is associated with reproductive seasonality in the Rasa aragonesa sheep breed. *Theriogenology* **113**, 63-72.

Campbell, B.K., Kendall, N.R. and Baird, D.T. (2007). The effect of the presence and pattern of luteinizing hormone stimulation on ovulatory follicle development in sheep. *Biology of Reproduction* **76**, 719-727.

Cao, J., Wei, C., Zhang, S., Capellini, T.D., Zhang, L., Zhao, F., Li, L., Zhong, T., Wang, L., Du, L. and Zhang, H. (2016). Screening of reproduction-related single-nucleotide variations from MeDIP-seq data in sheep. *Molecular Reproduction and Development* **83**, 958-967.

Carcangiu, V., Mura, M.C., Vacca, G.M., Pazzola, M., Dettori, M.L., Luridiana, S. and Bini, P.P. (2009a). Polymorphism of the melatonin receptor MT1 gene and its relationship with seasonal reproductive activity in the Sarda sheep breed. *Animal Reproduction Science* **116**, 65-72.

Carcangiu, V., Vacca, G.M., Mura, M.C., Dettori, M.L., Pazzola, M., Luridiana, S. and Bini, P.P. (2009b). Relationship between MTNR1A melatonin receptor gene polymorphism and seasonal reproduction in different goat breeds. *Animal Reproduction Science* **110**, 71-78.

Chanvallon, A., Sagot, L., Pottier, E., Debus, N., Francois, D., Fassier, T., Scaramuzzi, R.J. and Fabre-Nys, C. (2011). New insights into the influence of breed and time of the year on the response of ewes to the 'ram effect'. *Animal* **5**, 1594-1604.

Charollais Sheep Society (2021) *Charollais Sheep Society: Registration* [Online]. Available: http://www.charollaissheep.com/members/registration [Accessed: 15/03/2021].

Chemineau, P., Bodin, L., Migaud, M., Thiery, J.C. and Malpaux, B. (2010). Neuroendocrine and genetic control of seasonal reproduction in sheep and goats. *Reproduction in Domestic Animals* **45 Suppl 3**, 42-49.

Chemineau, P., Malpaux, B., Brillard, J.P. and Fostier, A. (2007). Seasonality of reproduction and production in farm fishes, birds and mammals. *Animal* **1**, 419-432.

Chu, M.X., Cheng, D.X., Liu, W.Z., Fang, L. and Ye, S.E. (2006). Association between Melatonin Receptor 1A Gene and Expression of Reproductive Seasonality in Sheep. *Asian-Australian Journal of Animal Science* **19**, 1079 - 1084.

Dardente, H., Lomet, D., Chesneau, D., Pellicer-Rubio, M.T. and Hazlerigg, D. (2019a). Discontinuity in the molecular neuroendocrine response to increasing daylengths in Ile-de-France ewes: Is

transient Dio2 induction a key feature of circannual timing? *Journal of Neuroendocrinology* **31**, e12775.
Dardente, H., Wood, S., Ebling, F. and Saenz de Miera, C. (2019b). An integrative view of mammalian seasonal neuroendocrinology. *Journal of Neuroendocrinology* **31**, e12729.
Desire, S., Mucha, S., Coffey, M., Mrode, R., Broadbent, J. and Conington, J. (2018). Pseudopregnancy and aseasonal breeding in dairy goats: genetic basis of fertility and impact on lifetime productivity. *Animal* **12**, 1799-1806.
Ding-ping, B., Cheng-jiang, Y. and Yu-lin, C. (2012). Association between AA-NAT gene polymorphism and reproductive performance in sheep. *Electronic Journal of Biotechnology* **15**, DOI: 10.2225/vol15-issue2-fulltext-6
Dorset Horn and Poll Dorset Sheep Breeders' Association (2020) *Management of the breed* [Online]. Available: http://www.dorsetsheep.org.uk/index.php/breed/management-of-the-breed [Accessed: 15/03/2021].
Dzabirski, V. and Notter, D.R. (1989). Effects of Breed and Time Since Lambing on Spring Estrous Activity in Mature Ewes. *Animal Reproduction Science* **19**, 99-108.
Fathy, H.A., Gouda, E.M., Gafer, J.A., Galal, M.K. and Nowier, A.M. (2018). Genetic polymorphism in melatonin receptor 1A and arylalkylamine N-acetyltransferase and its impact on seasonal reproduction in Egyptian sheep breeds. *Archives Animal Breeding* **61**, 505-516.
Foster, R.G., Grace, M.S., Provencio, I., Degrip, W.J. and Garcia-Fernandez, J.M. (1994). Identification of Vertebrate Deep Brain Photoreceptors. *Neuroscience and Biobehavioural Reviews* **18**, 541-546.
Giantsis, I.A., Laliotis, G.P., Stoupa, O. and Avdi, M. (2016). Polymorphism of the melatonin receptor 1A (MNTR1A) gene and association with seasonality of reproductive activity in a local Greek sheep breed. *Journal of Biological Research (Thessalon)* **23**, 9.
Goff, K.J., Notter, D.R., Vanimisetti, H.B. and Knight, J.W. (2014). Strategies for rapid rebreeding of lactating ewes in the spring. *Animal* **8**, 968-974.
Hall, D.G., Fogarty, N.M. and Gilmour, a.R. (1986). Seasonality of ovulation and estrus, and the ram effect in poll dorset ewes. *Theriogenology* **25**, 455-461.
Hanocq, E., Bodin, L., Thimonier, J., Teyssier, J., Malpaux, B. and Chemineau, P. (1999). Genetic parameters of spontaneous spring ovulatory activity in Mérinos d'Arles sheep. *Genetics, Selection, Evolution* **31**, 77-90.
Hanon, E.A., Lincoln, G.A., Fustin, J.M., Dardente, H., Masson-Pevet, M., Morgan, P.J. and Hazlerigg, D.G. (2008). Ancestral TSH mechanism signals summer in a photoperiodic mammal. *Current Biology* **18**, 1147-1152.
Hernandez, X., Bodin, L., Chesneau, D., Guillaume, D., Allain, D., Chemineau, P., Malpaux, B. and Migaud, M. (2005). Relationship between MT1 melatonin receptor gene polymorphism and seasonal physiological responses in Ile-de-France ewes. *Reproduction Nutrition Development* **45**, 151-162.
Huang, D.W., Di, R., Wang, J.X., Chu, M.X., He, J.N., Cao, G.L., Fang, L., Feng, T. and Li, N. (2012). Analysis on DNA sequence of goat RFRP gene and its possible association with average daily sunshine duration. *Molecular Biolology Reports* **39**, 9167-9177.
Huang, D.W., Wang, J.X., Liu, Q.Y., Chu, M.X., Di, R., He, J.N., Cao, G.L., Fang, L., Feng, T. and Li, N. (2013). Analysis on DNA sequence of TSHB gene and its association with reproductive seasonality in goats. *Molecular Biology Reports* **40**, 1893-1904.
Lai, P., Wang, P.Q., Chu, M.X., Song, W.J. and Cai, B.J. (2013). Polymorphism of the melatonin receptor genes and its relationship with seasonal reproduction in the Gulin Ma goat breed. *Reproduction in Domestic Animals* **48**, 732-737.
Lewis, R.M., Notter, D.R., Hogue, D.E. and Magee, B.H. (1996). Ewe fertility in the STAR accelerated lambing system. *Journal of Animal Science* **74**, 1511-1522.

Lomet, D., Druart, X., Hazlerigg, D., Beltramo, M. and Dardente, H. (2020). Circuit-level analysis identifies target genes of sex steroids in ewe seasonal breeding. *Molecular and Cellular Endocrinology* **512**, 110825.
Luridiana, S., Mura, M.C., Daga, C., Diaz, M.L., Bini, P.P., Cosso, G. and Carcangiu, V. (2015). The relationship between melatonin receptor 1A gene (MTNR1A) polymorphism and reproductive performance in Sarda breed sheep. *Livestock Science* **171**, 78-83.
Martinez-Royo, A., Alabart, J.L., Sarto, P., Serrano, M., Lahoz, B., Folch, J. and Calvo, J.H. (2017). Genome-wide association studies for reproductive seasonality traits in Rasa Aragonesa sheep breed. *Theriogenology* **99**, 21-29.
Martinez-Royo, A., Lahoz, B., Alabart, J.L., Folch, J. and Calvo, J.H. (2012). Characterisation of the Melatonin Receptor 1A (MTNR1A) gene in the Rasa Aragonesa sheep breed: association with reproductive seasonality. *Animal Reproduction Science* **133**, 169-175.
Mateescu, R.G., Lunsford, A.K. and Thonney, M.L. (2009). Association between melatonin receptor 1A gene polymorphism and reproductive performance in Dorset ewes. *Journal of Animal Science* **87**, 2485-2488.
Mateescu, R.G. and Thonney, M.L. (2010). Genetic mapping of quantitative trait loci for aseasonal reproduction in sheep. *Animal Genetics* **41**, 454-459.
Menassol, J.B., Collet, A., Chesneau, D., Malpaux, B. and Scaramuzzi, R.J. (2012). The interaction between photoperiod and nutrition and its effects on seasonal rhythms of reproduction in the ewe. *Biology of Reproduction* **86**, 52.
Mura, M.C., Luridiana, S., Farci, F., Di Stefano, M.V., Daga, C., Pulinas, L., Staric, J. and Carcangiu, V. (2017). Melatonin treatment in winter and spring and reproductive recovery in Sarda breed sheep. *Animal Reproduction Science* **185**, 104-108.
Mura, M.C., Luridiana, S., Pulinas, L., Di Stefano, M.V. and Carcangiu, V. (2019). Reproductive response to male joining with ewes with different allelic variants of the MTNR1A gene. *Animal Reproduction Science* **200**, 67-74.
Notter, D.R. (2002). Opportunities to Reduce Seasonality of Breeding in Sheep by Selection. *Sheep and Goat research journal* **17**, 20-32.
Notter, D.R. and Chemineau, P. (2001). Nocturnal melatonin and prolactin plasma concentrations in sheep selected for fertility in autumn lambing. *Journal of Animal Science* **79**, 2895-2901.
Notter, D.R. and Cockett, N.E. (2005). Opportunities for detection and use of QTL influencing seasonal reproduction in sheep: a review. *Genetics, Selection, Evolution* **37 Supplement 1**, S39-53.
Notter, D.R., Cockett, N.E. and Hadfield, T.S. (2003). Evaluation of melatonin receptor 1a as a candidate gene influencing reproduction in a fall-lambing sheep flock. *Journal of Animal Science* **81**, 912-917.
Notter, D.R., Smith, J.K. and Akers, R.M. (2011). Patterns of estrous cycles, estrous behavior, and circulating prolactin in spring and summer in ewes selected for autumn lambing and exposed to ambient or long-day photoperiods. *Animal Reproduction Science* **129**, 30-36.
Pelletier, J., Bodin, L., Hanocq, E., Malpaux, B., Teyssier, J., Thimonier, J. and Chemineau, P. (2000). Association Between Expression of Reproductive Seasonality and Alleles of the Gene for Mel1a Receptor in the Ewe. *Biology of Reproduction* **62**, 1096-1101.
Posbergh, C.J., Murphy, R.J. and Thonney, M.L. (2017). Further testing of for out-of-season reproduction in the Cornell flock and allelic frequencies compared with Romney sheep. *Journal of Animal Science* **95**, 1939-1944.
Posbergh, C.J., Thonney, M.L. and Huson, H.J. (2019). Genomic Approaches Identify Novel Gene Associations with Out of Season Lambing in Sheep. *Journal of Heredity* **110**, 577-586.
Quality Meat Scotland (2019) Using EBVs as a Tool to Breed Better Sheep [Online]. Available: https://www.signetdata.com/media/2592/qms-2019-sheep_ebv_guide_2019.pdf [Accessed: 15/03/2021].

Quirke, J.F. and Hanrahan, J.P. (1985) Breed Differences in the Breeding Season in Sheep. In *Endocrine Causes of Seasonal and Lactational Anestrus in Farm Animals. Current topics in Veterinary Medicine and Animal Science.*, vol. 31, pp. 29-43. Editted by Ellendorff, F. and Elsaesser, F.: Springer, Dordrecht.

Rekik, M., Ben Salem, I., Ben Hamouda, M., Aloulou, R. and Ben Sassi, M. (2011). Sources of variation and genetic analysis of fertility of maiden ewes in an out-of-season management system. *Livestock Research for Rural Development* **23**(1).

Signet Breeding Services (2021) *Sheep recording* [Online]. Available: https://www.signetdata.com/technical/sheep-recording [Accessed: 21/01/2021].

Smith, J.F., Johnson, D.L. and Reid, T.C. (1992). Genetic parameters and performance of flocks selected for advanced lambing date. *Proceedings of the New Zealand Society of Animal Production* **52**, 129-131.

Teyssier, J., Migaud, M., Debus, N., Maton, C., Tillard, E., Malpaux, B., Chemineau, P. and Bodin, L. (2011). Expression of seasonality in Merinos d'Arles ewes of different genotypes at the MT1 melatonin receptor gene. *Animal* **5**, 329-336.

Trecherel, E., Batailler, M., Chesneau, D., Delagrange, P., Malpaux, B., Chemineau, P. and Migaud, M. (2010). Functional characterization of polymorphic variants for ovine MT1 melatonin receptors: possible implication for seasonal reproduction in sheep. *Animal Reproduction Science* **122**, 328-334.

Vincent, J.N., McQuown, E.C. and Notter, D.R. (2000). Duration of the seasonal anestrus in sheep selected for fertility in a fall lambing system. *Journal of Animal Science* **78**, 1149-1154.

Waitrose (2021) *About our lamb* [Online]. Available: https://www.waitrose.com/home/inspiration/about_waitrose/about_our_food/aboutourlamb.html [Accessed: 15/03/21].

Wang, K., Yan, H., Xu, H., Yang, Q., Zhang, S., Pan, C., Chen, H., Zhu, H., Liu, J., Qu, L. and Lan, X. (2018). A novel indel within goat casein alpha S1 gene is significantly associated with litter size. *Gene* **671**, 161-169.

Wood, S.H., Hindle, M.M., Mizoro, Y., Cheng, Y., Saer, B.R.C., Miedzinska, K., Christian, H.C., Begley, N., McNeilly, J., McNeilly, A.S., Meddle, S.L., Burt, D.W. and Loudon, A.S.I. (2020). Circadian clock mechanism driving mammalian photoperiodism. *Nature Communications* **11**, 4291.

Zarazaga, L.A., Malpaux, B., Bodin, L. and Chemineau, P. (1998). The large variability in melatonin blood levels in ewes is under strong genetic influence. *American Journal of Physiology - Endocrinology and Metabolism* **274**, 607-610.

Appendix 1: Table 4 A summary of studies of association between aseasonal breeding activity and genotype in small ruminants

Reference	Species	Breed(s)	Number genotyped	Gene	Reproductive trait(s) measured	Results
Mura, *et al.* (2019)	Sheep	Sarda	1200 enrolled in study, 400 of each genotype	*MTNR1A*	Fertility rate and number of days to lambing from four different dates of ram introduction. Litter size	Ewes carrying at least one G allele at g.15099485 (equivalent to *Mnl1* RFLP position 612 of U14109 MTNR1A exon II) had a significantly shorter time to lambing and a higher fertility rate for all four dates of ram introduction compared to A/A genotype ewes. Genotype had no impact on litter size.
Calvo, *et al.* (2018)	Sheep	Rasa Aragonesa	268	*MTNR1A*	Total days of anoestrus (progesterone assay) Progesterone cycling months (progesterone assay) Oestrus cycling months (behavioural assessment)	35 SNPs identified across gene promotor, exon 1 and exon2. SNP rs403212791 (non-synonymous polymorphism) T allele associated with reduced seasonality for all three traits.
Wang, *et al.* (2018)	Goat	Shaanbei White Cashmere (SBWC) Guanzhong Dairy Hainan Black Inner Mongolia Cashmere Jining Gray	3826 (of which 2690 were from SBWC)	*CSN1S1*	Litter size	Genotype II was more frequent in non-seasonal breeds. Genotype II was associated with larger mean litter size in the SBWC (1.73 ± 0.02 compared to 1.55 ± 0.015 for ID and 1.36 ± 0.016 for DD genotypes).
Fathy, *et al.* (2018)	Sheep	Ossimi Rahmani Barki	66 41 19	*MTNR1A* *AA-NAT*	Age at first lambing, litter size, lambing interval from first season of conception.	Differences in allele frequencies between breeds for both genes. Impact of genotype on reproductive trait varied with breed.
Posbergh, *et al.* (2017)	Sheep	Cornell accelerated breeding flock (East Friesian X, Finnsheep x Dorset, Dorset, Finnsheep) Romney	266 54	*MTNR1A*	Lambs per ewe per year in the accelerated lambing system. Success of autumn lambing per spring breeding opportunity.	The *mm* genotype was infrequent compared to the *MM* and *Mm* genotypes in the Cornell flock. Allelic and genotypic frequency was different in the unselected, seasonal breeding Romney. Genotype had no impact on reproductive parameters in the Cornell flock.

Giantsis, *et al.* (2016)	Sheep	Non specified Greek dairy breed	60	*MTNR1A*	Reproductive activity – not specified	Ewes that were reproductively active in spring had a higher frequency of the C/C genotype at position 606 (28/30), compared to ewes that weren't reproductively active until summer (16/30). The T/T genotype was only found in the latter group (2/30).
Luridiana, *et al.* (2015)	Sheep	Sarda	255 enrolled in study, 85 of each genotype	*MTNR1A*	Fertility rate and number of days to lambing from date of ram introduction for three consecutive years Litter size	Ewe lambs of all three genotypes for position 612 had equivalent days to lambing after ram introduction on July 30th. In the second and third years, rams were introduced on the 1st May. Ewes with G/G genotype had shorter intervals to lambing than A/G or A/A genotypes ($p<0.05$). Fertility rates and litter size were equivalent between genotypes.
Lai, *et al.* (2013)	Goat	Gulin Ma	57	*MTNR1A* *MTNR1B*	Distribution of kidding between Sept-Dec, Jan-Apr, May-August	Five polymorphic mutations found for *MTNR1A* and two for *MTNR1B.* Distribution of kiddings in each season similar for each genotype.
Huang, *et al.* (2013)	Goat	Aseasonal: Jining Grey, Guizhou White, Anhui White, Boer Seasonal: Liaoning Cashmere, Taihang, Saanen Dairy	 234, 59 91, 59 78, 56, 54	*TSHB*	Distribution of genotypes between seasonal and aseasonal breeds Litter size in Jining Grey	Five nucleotide mutations were identified. Genotypic differences between the seasonal and aseasonal breeds were observed, with the most frequent genotypes for the five foci AA, -/-, +/+, AA and AA in the three seasonal Chinese breeds and CA, ±, ±, AC and TA for the aseasonal breeds. The aseasonal Boer had genotype frequencies opposite to the aseasonal breeds: CC, +/+, -/-, CC and TT. Litter size was unaffected by genotype.
Huang, *et al.* (2012)	Goat	Aseasonal: Jining Grey, Guizhou White, Boer Seasonal: Taihang, Liaoning Cashmere, Saanen Dairy	 241, 56, 33 55, 82, 60	*RFRP*	Distribution of genotypes between seasonal and aseasonal breeds Litter size in Jining Grey	Three nucleotide mutations were identified, one resulting in a conservative amino acid change, the other two each resulting in three genotypes. Pairwise comparison of seasonal and non-seasonal breeds did not find consistent differences in genotypes at the three loci. Litter size was unaffected by genotype.
Martinez-Royo, *et al.* (2012)	Sheep	Rasa Aragonesa	80	*MTNR1A*	Oestrus activity February-July (considered the anoestrus period)	28 SNPs identified across Exon1, Exon 2 and promotor region. The percentage of oestrous cyclic ewes was significantly greater for genotype TT (77.5%) than genotype CC (55.6%) at SNP 606 (*Rsal* RFLP). At SNP 612 (*Mnl1* RFLP)

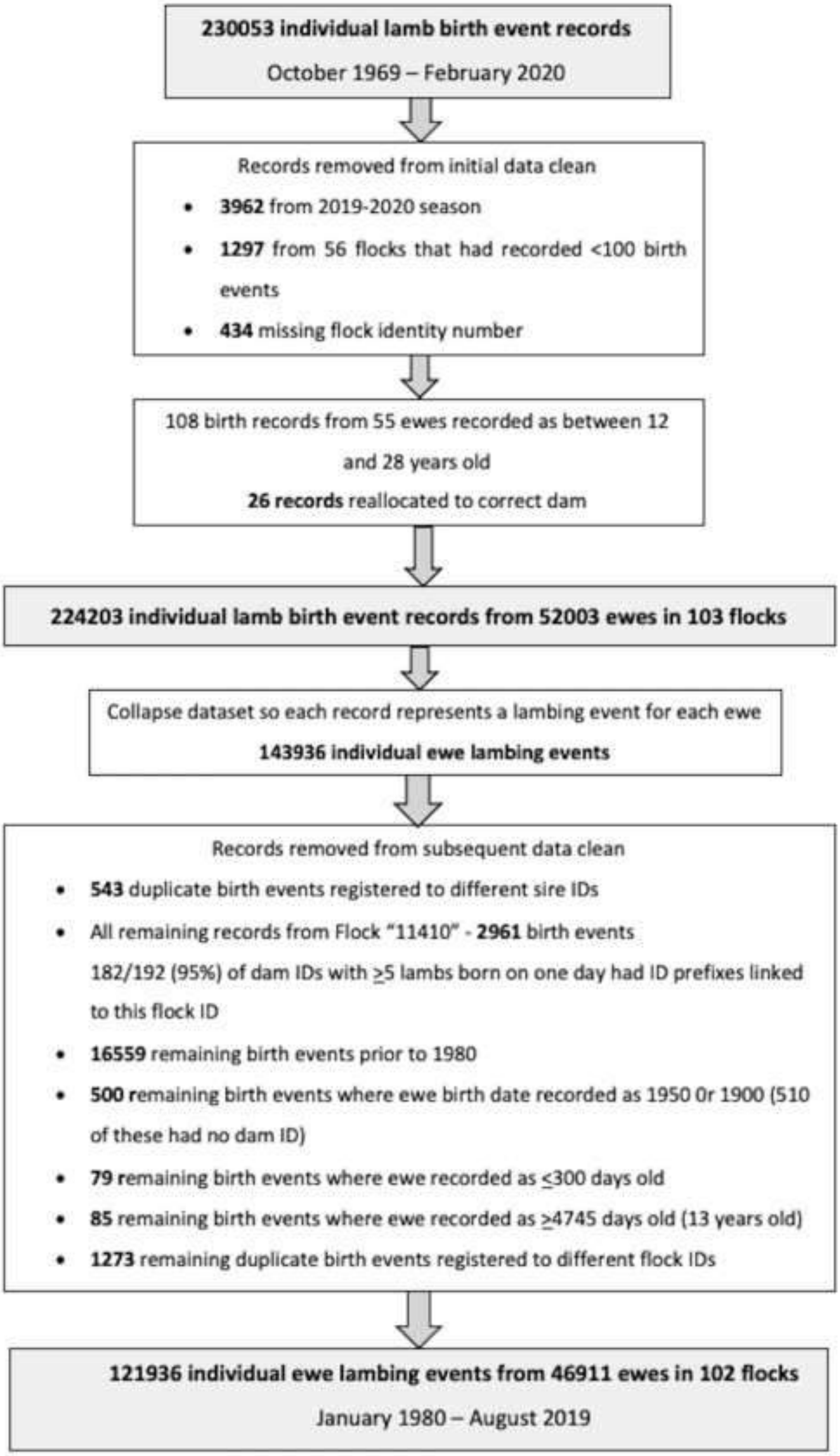
					Polymorphisms initially identified in 6 ewes with extreme seasonality, and 3 rams.	no association was found between oestrous cyclicity and GG and GA genotypes. Associations were found between genotype and oestrus activity for five SNPs in the promotor region.
Ding-ping, *et al.* (2012)	Sheep	Seasonal: Xinjiang Fine Wool sheep, Altay Fat-rumped sheep Aseasonal: Small Tail Han sheep, Dolang	 58, 30 60, 31	*AA-NAT*	Distribution of genotypes between seasonal and aseasonal breeds	A novel SNP/*Sma1* RFLP site leading to a functional mutation (Arginine to Glycine) was found in exon 3. The GG genotype was more common in both aseasonal breeds and the AA genotype was absent. The GA genotype was most frequent in seasonal breeds.
Teyssier, *et al.* (2011)	Sheep	Merinos d'Arles	314	*MTNR1A*	Spontaneous ovulatory activity by plasma progesterone. Fertility at spring mating by percentage of ewes lambing in autumn Litter size	There was no association between *MM*, *Mm* and *mm* genotypes for the *Mnl1* RFLP and cyclicity in April for two consecutive years. For 30 ewes of each homozygous genotype there was no difference in cyclicity between January and April for two consecutive years. Genotype did not affect fertility or litter size.
Carcangiu, *et al.* (2009a)	Sheep	Sarda	400	*MTNR1A*	Distribution of lambing between September-December and January-April Litter size	Ewes with the *MM* genotype most frequently lambed Sept-Dec (80% of births compared to 22% for *mm* and 49% for *Mm* genotypes). Ewes with the C/C *Rsa1* genotype most frequently lambed Sept-Dec (65% of births compared to 45% for *T/T* and 51% for *C/T* genotypes). Genotype did not affect litter size.
Carcangiu, *et al.* (2009b)	Goat	Sarda Saanen Chamois coloured Maltese Nubian	225 30 30 30 30	*MTNR1A*	Distribution of genotypes between goats with spring reproductive activity and autumn reproductive activity	Nine silent mutations were identified. All goats were genotype *MM* for the *Mnl1* RFLP. No goats were r/r for the *Rsa1* RFLP and the *R/r* genotype was present in only nine (4%) of the Saarda goats.
Mateescu, *et al.* (2009)	Sheep	Dorset ¾ Dorset X ¼ East Friesian	91 25	*MTNR1A*	Number of days from birth to first lambing Number of days between first and second lambing	Ewes with *MM* or *Mm* genotypes were 136 days younger at first lambing and lambed after an interval 124 days shorter than ewes of mm genotype in an accelerated lambing system. This was attributed to the ability to lamb OOS. *Rsa1* genotype did not affect reproductive traits.

					Season of conception (Aseasonal, early or late)	
Chu*, et al.* (2006)	Sheep	Small tail Han Hu Dorset Suffolk German Mutton Merino	137 27 30 24 21	*MTNR1A*	Distribution of genotypes between seasonal and aseasonal breeds	The frequency of the *MM* genotype for the *Mnl1* RFLP cleavage site and the *RR* genotype for the *Rsa1* RFLP was greater in the non-seasonal breeds than the seasonal breeds. The frequency of the *mm* and *rr* genotypes was lower in the non-seasonal breeds.
Hernandez*, et al.* (2005)	Sheep	Île-de-France	21	*MTNR1A*	Length of breeding season (plasma progesterone) Plasma melatonin and prolactin	No difference in length or date of onset of breeding season, plasma prolactin or melatonin concentration was found in a comparison between *MM* and *mm* half siblings
Notter*, et al.* (2003)	Sheep	Virginia Polytechnic flock derived from 50% Dorset, 25% Rambouillet and 25% Finnsheep	362	*MTNR1A*	Fertility (ewes lambing per ewes exposed to ram) Litter size	Ewes of parity 3 and above carrying at least one M allele had a mean increase in fertility of 10% (+ 5.7%, p=0.09) but this difference was not apparent when all ewe ages were included. Overall heritability for fertility (all ages) estimated to be 0.09. Genotype had no significant effect on litter size.
Pelletier*, et al.* (2000)	Sheep	Merino d'Arles (MA) Île-de-France (IF)	71 29	*MTNR1A*	Plasma progesterone measured twice 8-10 days apart in the first two weeks in April over 1-3 years.	MA ewes with ovulatory activity in April were significantly more likely to have the *MM* genotype than ewes never cycling in April (58.2% compared to 28.5%, p<0.05). The reverse was true for the *mm* genotype (0% compared to 28.5%, p<0.001). 28% of IF ewes, a seasonal breed, had the *MM* genotype compared to 38% with the *mm* genotype.

MTNR1A gene, exon 2 on chromosome 26: genotype notation

Mutation position Genbank U14109	Mutation position genome version OAR4.0	Base change	RFLP cleavage site	Genotypes
606		C > T	*Rsa1*	*RR, Rr, rr = C/C, C/T, T/T*
612	g.15099485	G > A	*Mnl1*	*MM, Mm, mm = G/G, G/A, A/A*

Appendix 2: Figure 26 Records discarded during the data cleaning process



Appendix 3: Additional figures for the restricted sire dataset

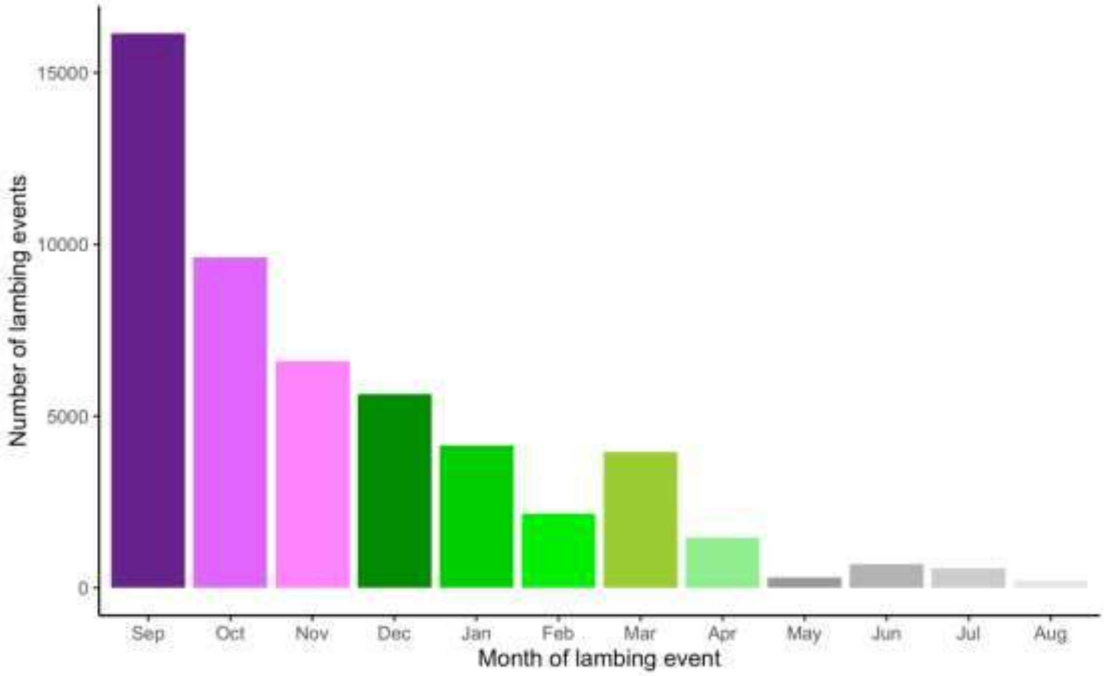


Figure 27 The frequency of lambing events in each month for rams with >25 daughters and >50 lambing events. OOS months are shaded in purple/pink, in season lambing months are shaded in green and undesirable lambing months are shaded in grey.

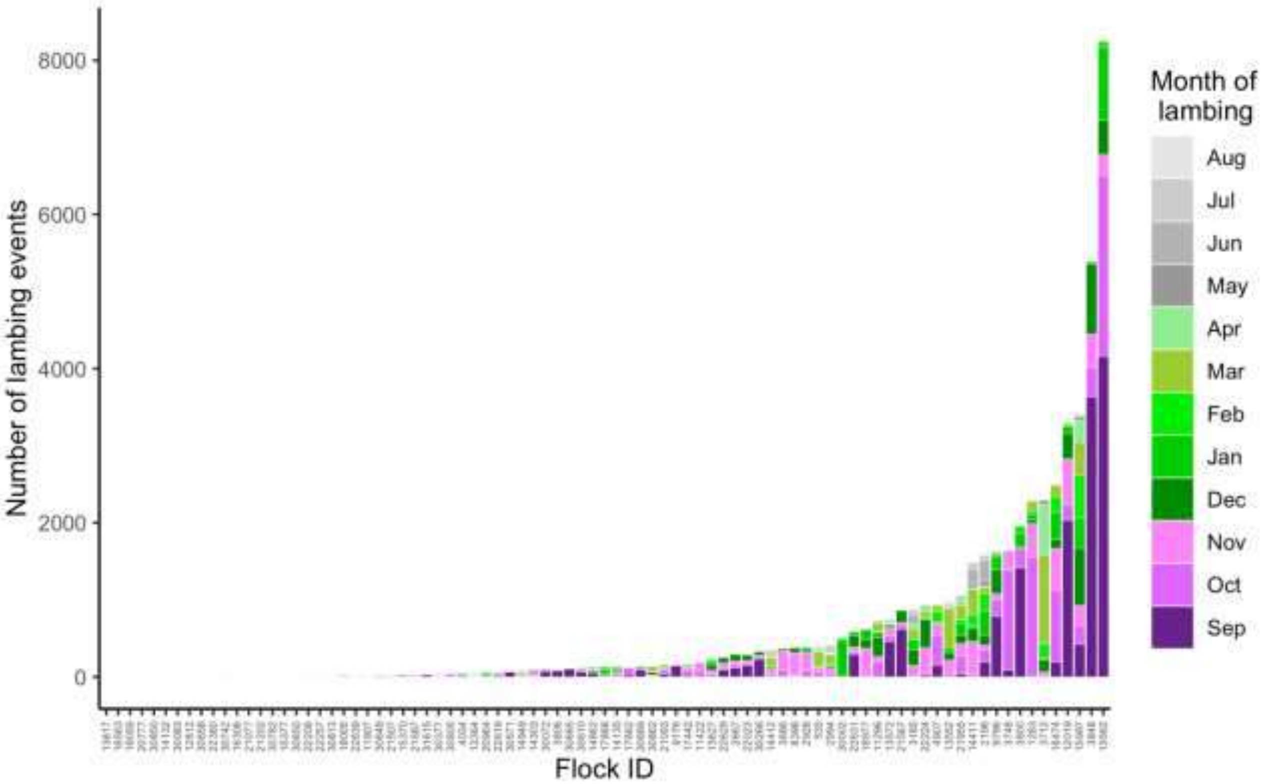


Figure 28 The number of lambing events in each month by flock for rams with >25 daughters and >50 lambing events. OOS months are shaded in purple/pink, in season lambing months are shaded in green and undesirable lambing months are shaded in grey.

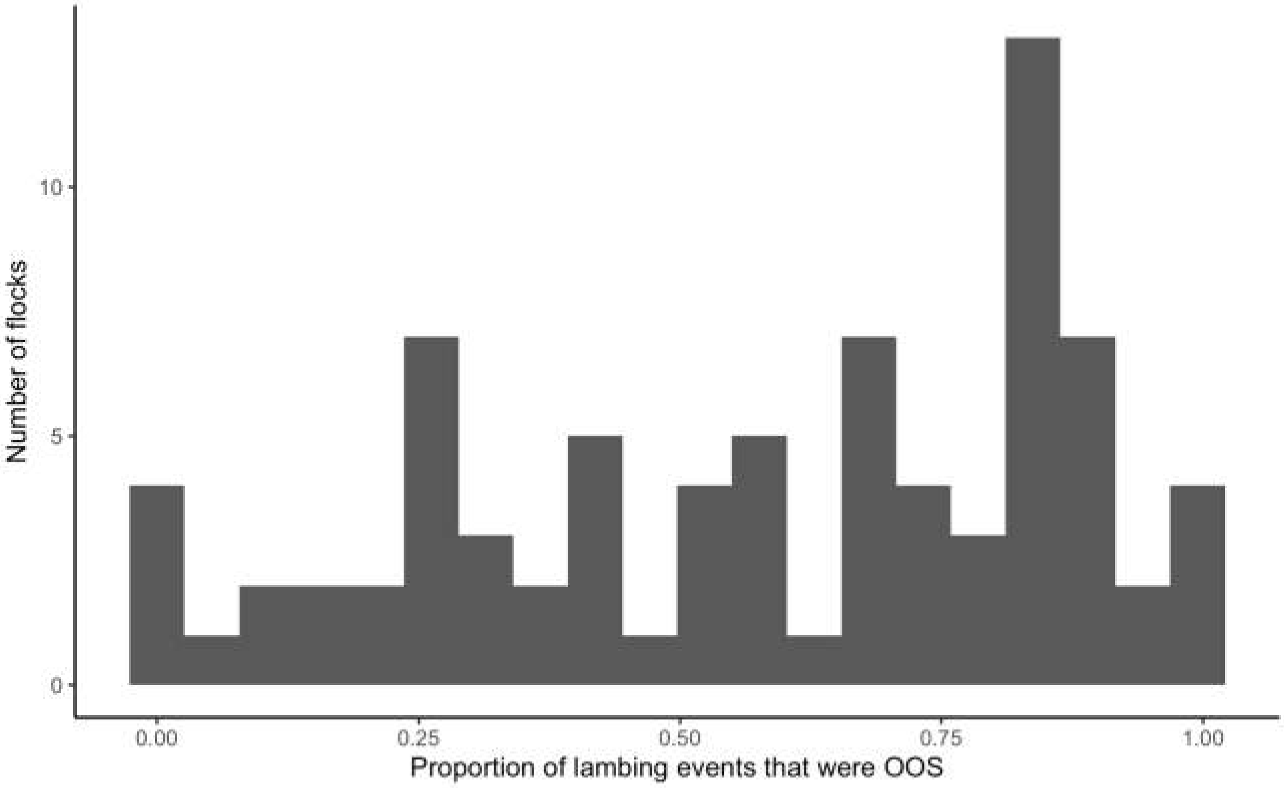


Figure 29 The proportion of lambing events recorded that were OOS as a proportion of all lambing events recorded for each farm in dataset containing rams with >25 daughters and >50 lambing events

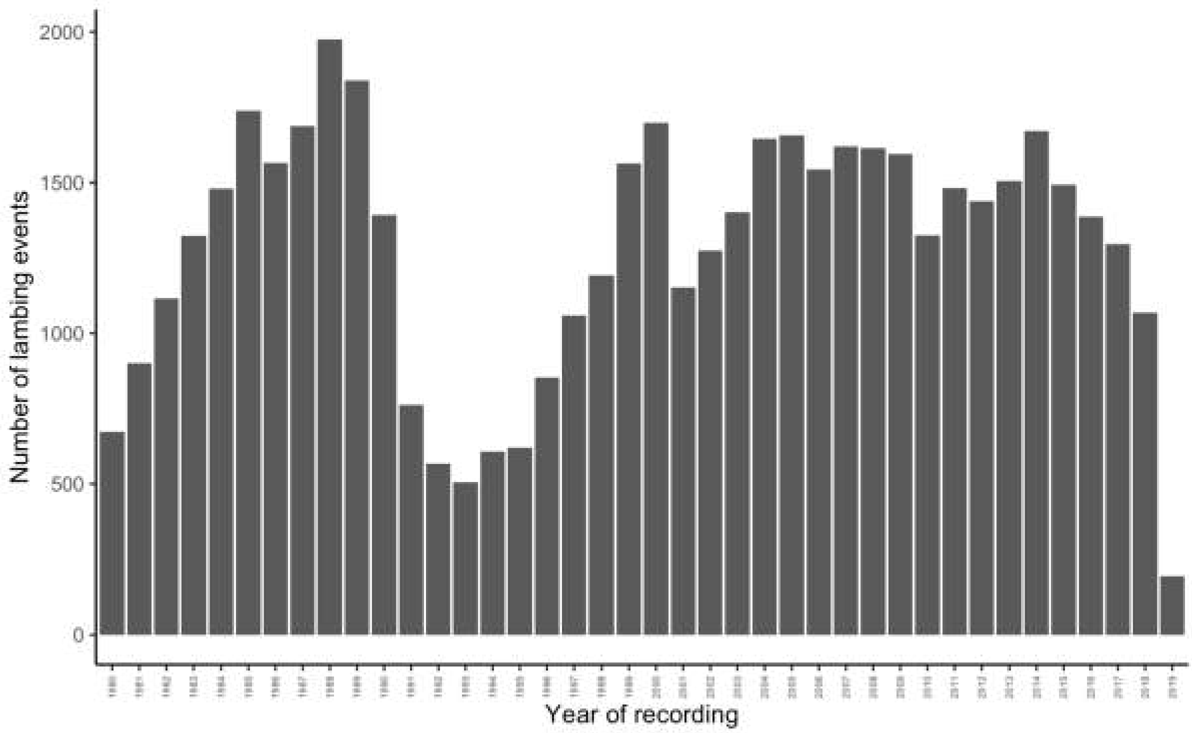


Figure 30 The number of lambing events recorded for each year of the performance recording period in dataset containing rams with >25 daughters and >50 lambing events