

SPLIT PATERNITY IS HIGH IN TWINS BORN FROM SYNDICATE-MATED MERINO EWES

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SUMMARY

Split paternity rates in multiple born lambs of syndicate-mated Merino flocks have previously not been reported, primarily due to the cost of genotyping. Pedigree data from litters born to genetically diverse syndicate mated ewes in three Merino Lifetime Productivity flocks across Australia were analysed to examine rates of split paternity, or heteropaternal superfecundation. Over three joinings at three sites, 1082 twin or triplet litters were marked, of which 577 were heteropaternal (53.3%). There was no effect of age of dam, year of birth, sire or maternal grandsire on heteropaternal rates. These high rates of heteropaternal confirm the need to genotype all progeny from multiple births in syndicate mated flocks to ensure accurate genetic evaluation.

INTRODUCTION

Split paternity, or heteropaternal superfecundation, occurs when females are successfully mated by multiple males during the same oestrus cycle. Decreased costs of determining paternity using DNA means that it is now feasible to obtain pedigrees from syndicate mating which may have some practical advantages over single-sire mating. Being able to syndicate mate sheep and determine parentage using DNA requires less paddocks than single sire joining and reduced labour in terms of feeding, labour for lambing rounds (preparation for lambing through identification of dams and mothering up) together with less disturbance during the lambing period as well less risk of failed matings with infertile single-sire mated rams.

Very little data about heteropaternal superfecundation has been published for either sheep or cattle. In cattle, where twinning rates are traditionally very low (1-5% depending on breed), McClure *et al.* (2017) examined rates of heteropaternal superfecundation in Irish herds, citing data from the Irish Cattle Breeding Federation database. They reported that, with an average of 1.7% twin rates, 0.98% of these were heteropaternal. By contrast, in syndicate-mated sheep, surprisingly high levels of heteropaternality, detected by DNA technology, have recently been reported for flocks in Ireland under relatively intensive conditions (Berry *et al.* 2020). The only information on the occurrence or frequency of heteropaternality in multiple-born lambs in naturally mated Merinos showed even higher levels (46-59%) in lambs born in 2012-2015 as part of the Merinolink Genomic Validation Project (Martin 2016).

We predicted that heteropaternality would also be high in extensively run Merino flocks in different parts of Australia with varying genotypes. We tested this in three naturally mated Merino flocks in Western Australia and New South Wales over 2-3 years. We also examined the effect of heteropaternality on total weaning weight of litters to see if there was any difference in the weaning weights of single-sired twin lambs compared to heteropaternal twins.

MATERIALS AND METHODS

Pedigree data were collected from F2 Merino lambs born between 2018 and 2020 in the Merino Lifetime Productivity (MLP) Project (Ramsay *et al.* 2019). These data are from three MLP sites located at Pingelly in WA and at Trangie (Macquarie) and Armidale (New England) in NSW and consist of lambing records of ewes born to genetically diverse sires representative of the Australian Merino population. Lambs included in this analysis were from paddock-mating of MLP ewes to a team of Merino rams at each site. The ewe to ram ratio was approximately 50 ewes per ram and 8 to 16 rams were used in each flock, depending on flock size. All flocks had a five-week joining period and paddock sizes for joining ranged from 10 to 65 hectares. The ewes were pregnancy scanned for litter size about 80-90 days after the start of joining.

Tissue samples were taken from all F2 lambs alive at marking and tested using an 800K SNP chip. No DNA or data were collected on lambs that did not survive to marking. Parentage was verified against the ewe and ram genotypes, all of which had been previously genotyped using a 50K SNP chip. Lambs that were assigned a birth type as twin or triplet and rear type as single were removed from the analysis as birth type was inferred from pregnancy scanning results and there were no data on litters that were scanned multiple but had less lambs survive to rearing. The pedigree assigned using tissue samples collected at marking and rear type at marking were used to determine if a lamb was a single or multiple and if heteropaternal or not. Data were available for 1,082 litters where sire, dam and lamb's pedigree were available.

Statistical analysis. Estimates of heteropaternal effects were assessed by fitting General Linear Mixed Models (GLMM; Genstat VSN International 2017). The approach used a logit transformation and binomial distribution. Using additive models, logits were predicted as a function of syndicate group (a combination of flock, year of birth and joining group) as a fixed effect, and sire and maternal grandsire as random effects.

Estimates of differences between litters for total weaning weight (sum of littermate weaning weights) were restricted to twin-born litters (triplets were excluded). Total weaning weight was analysed using the restricted maximum likelihood method (REML, Genstat, VSN International 2017). Syndicate group and heteropaternal status were fitted as fixed effects, and sire and maternal grandsire were fitted as random terms.

RESULTS AND DISCUSSION

The average percentage of heteropaternal lambs across the three MLP sites over 2 or 3 joinings was 53.3% or 577 of the 1082 multiple born litters (Table 1). This included three of the four sets of triplets. The frequency of heteropaternality was greater than previous estimates (Berry *et al.* 2020), but similar to that reported by Martin (2016) who studied 349 litters. The estimates of heteropaternality in our data are possibly underestimated as only those litters where at least two lambs survived to marking are included in the analysis. It is reasonable to expect that heteropaternal lambs could have greater variation in weight and other behaviours at birth and hence have lower survival than single sired twins. There were no significant differences between fixed effects of dam year of birth, syndicate group, or sire and maternal grandsire, suggesting no differences between maiden or later joinings in the production of heteropaternal litters.

Over two or three joinings, the Merino flocks in this study showed higher levels of heteropaternality than a similar study of six commercial but smaller flocks of crossbred Irish sheep (Berry *et al.* 2020). This is surprising because our study included a lower ratio of rams to ewes (1:50 v 1:22-39) and was on a much larger and more extensive scale with larger paddocks. The lower ram to ewe mating ratios and larger paddock sizes would be expected to provide less interaction between rams and ewes during the oestrus period (Croker and Lindsay 1972). Nevertheless, the consistently high rates of heteropaternality (42% to 65%) across the three genetically and environmentally diverse flocks suggest these could be typical for rates of

heteropaternality in twin lambs in syndicate-mated Merino flocks across Australia. While factors that influence this surprisingly high level of heteropaternality in syndicate mating are largely unknown this reinforces the need to determine parentage of all multiple born lambs included in genetic evaluation using DNA.

Table 1. Percentages of heteropaternal lambs marked between 2018 and 2020, at each of three MLP sites

Site	Dam year of birth	Progeny year of birth	No. heteropaternal litters	No. multiple marked litters	Percent heteropaternal lambs
Pingelly	2016 drop	2018	31	48	64.6%
	2016 drop	2019	60	108	55.6%
	2017 drop	2019	46	86	53.5%
	2016 drop	2020	74	142	52.1%
	2017 drop	2020	66	109	60.6%
Macquarie	2017 drop	2019	47	83	56.6%
	2017 drop	2020	62	113	54.9%
	2018 drop	2020	27	63	42.9%
New England	2017 drop	2019	18	43	41.9%
	2017 drop	2020	107	219	48.9%
	2018 drop	2020	39	68	57.4%
Total			577	1082	53.3%

There were no significant differences between single sire and heteropaternal litters in total litter weaning weight ($P = 0.764$) despite Australian Sheep Breeding Values for weaning weight ranging from -1.6 to 9.9 kg (Merino Select analysis run date 21/02/2021) in rams used over the three sites (up to 6.9 kg range within site). There were, however, significant differences between syndicate group ($P < 0.001$) for total weaning weight.

CONCLUSIONS

Extensively run Merino flocks have not previously been candidates for large scale flock genotyping but, as the technology becomes more cost-effective for ram breeding and commercial sheep flocks, it is becoming feasible to genotype progeny from large syndicate-mated flocks. High rates of heteropaternal litters unequivocally confirm the need to genotype all offspring of syndicate matings to ensure pedigree is correctly assigned. The increased use of genotyping in Merino flocks that syndicate mate will allow for greater access to genetic evaluation with accurate pedigree data that has previously not been available for syndicate mated flocks.

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