EXPRESSION OF REPRODUCTIVE AND PRODUCTION TRAITS IN COMMERCIAL MERINO EWES HAVING 0, 1 OR 2 COPIES OF THE *FecB* MUTATION

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SUMMARY
Analysis of 3 years of performance data from a commercial flock of Merino ewes having 0, 1 or 2 copies of Booroola fecundity (*FecB*) mutation determined by direct DNA test showed that the mutation does not have deleterious effects on growth and wool production beyond those associated with a higher incidence of multiple births. Consistent with earlier studies, the effect of *FecB* on ovulation rate was shown to be approximately additive with a dominant effect on litter size. There was a non-significant decrease rather than increase in litter size with the second copy. Homozygous *BB* ewes also had lower fertility rates and increased lamb losses between scanning and lamb marking relative to heterozygote and wild-type ewes, independent of litter size. This represents a significant limitation on the utility of the gene for increasing reproductive rate in Australian Merinos.

INTRODUCTION
The discovery of the Booroola fecundity (*FecB*) gene in the Australian Merino and its subsequent detailed characterisation as a mutation of the BMPR-1B gene (Wilson *et al.* 2001) proved a boon for fundamental studies into the processes governing ovulation rate. However, it has had little practical application for improving the reproductive rate in Merinos due to initial difficulties in identifying homozygous and heterozygous carriers (particularly rams), unacceptable lamb mortality rates, and perceived negative associations with production traits. The development of a direct DNA test for the gene (Wilson *et al.* 2001) removed the first of these barriers. Following this, numerous sheep populations have been tested and the gene has been found to be the basis of prolificacy in several prolific breeds. The gene was probably introduced to Australia via the Garole breed from India (Davis *et al.* 2002). Interestingly the effect of the *FecB* gene on ovulation rate and litter size in Garole and Garole crossbred sheep in India appears to be considerably dampened relative to that reported for the Booroola Merino (Nimbkar *et al.* 2003; 2007), offering the prospect of manageable litter sizes in some sheep carrying the *FecB* gene. In light of the above, we used DNA testing to genotype sheep for the *FecB* gene in a commercial Merino flock, and examined the effect of the mutation on ovulation rate, scanned litter size, lamb survival and growth and wool traits.

MATERIALS AND METHODS
The resource flock for the study was located at "Allandale", Rand between Wagga Wagga and Albury in Southern NSW and comprised a self-replacing flock of approximately 3500 fine wool (19.5μm) plain-bodied Merino ewes. There had been significant infusions of Booroola Merino via purchases of 6 Booroola Merino rams from CSIRO between 1982 and 1991 (2 rams in 1982, 3 rams in 1985 and 1 ram in 1991). An "experimental" flock was established in 2002 to enable comparison of the 3 *FecB* genotypes in the same environment (target of 60 ewes/genotype). It was initiated by genotyping all of the 2002-drop lambs born to maiden ewes from the main commercial flock. Due to

insufficient numbers of *BB* lambs, an additional 64 triplet born lambs from older ewes were included. The initial structure of the experimental flock by genotype and birth type is summarized in Table 1.

Table 1. Initial structure of the experimental flock and numbers pregnancy scanned each year

Genotype	Initial structure by birth type and genotype				Ewes pregnancy scanned each year			Total records
	1	2	3	Total	2004	2005	2006	
BB	3	8	12	23	23	23	38	84
B+	26	26	39	91	91	85	95	271
++	47	19	18	84	84	82	78	244
Total	76	53	69	198	198	190	211	599

The experimental flock ewes were joined as one mob to *B+* rams in late February-early March in 2004, 2005 and 2006. The first replacement ewes were mated in 2006. In June ewes were ultrasound scanned to determine litter size. Ewes were managed as one group until just prior to lambing (late July) before being separated into lambing groups on the basis of litter size, with increased pasture feed allocations for multiple bearing ewes. Lambs were marked in mid September, shorn in October and weaned in late November to mid December. Maiden ewes were shorn at 15 months (October) and weighed at 18 months (January). In 2006, ovulation rate was measured in the cycle of conception and lamb survival assessed by mothering up ewes with their lambs at lamb marking.

Genotyping for *FecB* status using DNA extracted from blood was by the PCR-RFLP method of Wilson *et al.* (2001) and was carried out by commercial laboratories in New Zealand, or at the University of Melbourne or the University of New England.

The traits measured and their abbreviations were weights (kg) of the ewes at weaning (WWT) and as 18 month old hoggets (HWT), fleece traits at ewe hogget shearing (15 months) including greasy fleece weight (GFW, kg) and mean fibre diameter (MFD, μm), ovulation rate in the cycle of conception or last ovulation for ewes ovulating (OR), scanned litter size (LS), lambs weaned and lamb weaning weight (LWWT, Kg). Conception rate (CR) was defined as ewes pregnant/ewes scanned, litter size was expressed as either lambs scanned/pregnant ewes (LS1) or lambs scanned/all scanned ewes (LS2), and lamb weaning rate (WR) was defined as lambs weaned/ewes scanned.

All data were analysed using JMP 6.0 (SAS Institute, NC, USA). Data measured once only (eg. production traits and traits measured only in 2006) were analysed using a general linear model fitting the effects of ewe genotype, ewe birth type and year. Data for which there were repeated measures (eg. reproductive traits measured in multiple years) per animal were analysed using a mixed REML model with ewe genotype, ewe birth type and year fitted as fixed effects and the ewe fitted as a random effect. Main effects and significant 2-way interactions were retained in the model.

RESULTS AND DISCUSSION

Unadjusted means by genotype and year are presented in Table 2. Analysis of performance traits is summarised in Table 3. When adjusted for the effects of birth type and year, there was no significant effect of genotype on any of the ewe production variables (WWT, HWT, GFW and MFD) although there was a strong trend towards higher MFD in ewes carrying the *FecB* gene. The lack of an effect on productivity is consistent with several earlier studies but contrasts with the report of adverse effects on productivity associated with *FecB* in Assaf ewes (Gootwine *et al.* 2006).

Table 2. Unadjusted means of selected traits for ewes in the experimental flock by ewe *FecB* genotype (GEN) and year of birth (YOB) for production traits, or year of lambing (YOL) for reproductive traits. Traits and their units are defined in the text.

YOB	GEN	WWT	HWT	GFW	MFD	YOL	GEN	OR	CR	LS1	LS2	WR	LW WT
2002	++		43.3	1.94	18.0	2004	++		0.99	1.16	1.14		
	B+		43.5	1.87	18.4		*B*+		0.98	2.11	1.97		
	BB		42.6	1.84	18.3		*BB*		0.83	2.05	1.70		
2004	++	20.6	39.2	2.33	18.1	2005	++		0.94	1.45	1.37		
	B+	20.0	37.7	2.44	18.3		*B*+		0.96	2.60	2.51		
	BB	18.4	35.7	2.38	18.7		*BB*		0.78	2.39	1.87		
2005	++	24.1	40.8	3.14	19.2	2006	++	1.26	0.83	1.12	0.97	0.81	22.0
	B+	23.4	40.0	3.09	19.5		*B*+	2.43	0.79	2.27	1.71	1.07	19.0
	BB	23.2	39.7	3.00	19.9		*BB*	4.15	0.76	1.87	1.42	0.71	18.2

Table 3. Least squares means (±SEM) for the effect of *FecB* genotype on selected traits and significance (P value) of other effects (G = genotype, B= ewe birth type, Y=year).

Effect	Level /sig.	Trait									
		WWT	HWT	GFW	MFD	OR	CR	LS1	LS2	WR	LWWT[2]
G	P =	0.809	0.383	0.949	0.066	0.001	0.005	0.001	0.001	0.006	0.191
	++	20.7	40.2	2.37	18.34	1.16	90.8	1.31	1.19	0.79	17.4
		±1.0	±0.6	±0.05	±0.17	±0.20	±0.03	±0.05	±0.07	±0.09	±0.8
	B+	20.7	39.9	2.38	18.58	2.17	90.3	2.27	2.06	1.05	16.2
		±0.63	±0.6	±0.05	±0.15	±0.17	±0.03	±0.05	±0.06	±0.09	±0.8
	BB	21.2	39.4	2.39	18.80	3.96	0.79	2.12	1.64	0.70	15.8
		±0.70	±0.7	±0.06	±0.19	±0.24	±0.04	±0.10	±0.11	±0.12	±1.2
B	P =	0.001	0.258	0.001	0.237	0.260	0.395	0.164	0.703	0.90*	0.210
Y[1]	P =	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.651	0.008
GxB	P =	ns	ns	ns	ns	ns	0.087	ns	ns	ns	ns
GxY	P =	ns	ns	ns	ns	0.013	ns	ns	ns	ns	ns
BxY	P =	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]Y= year of measurement effect for WWT, HWT, GFW, MFD; Year of birth (parity) effect for OR, WR and LWWT; Confounded effect of year of birth and parity for CR, LS1, LS2.
[2]No of lambs weaned fitted as a covariate ($p<0.0001$). *Effect removed from final model.

Ovulation rate was somewhat lower than in earlier studies in Merino sheep genotyped on the basis of ovulation rate. Piper *et al.* (1985) reported OR of 1.3, 2.95 and 4.6 for ++, *B*+ and *BB* respectively while Dodds *et al.* 1991 reported values of 1.91, 3.18 and 4.6 respectively. Nevertheless OR was substantially higher than that reported by Nimbkar *et al.* (2003) in Garole sheep and their crosses with values of 1.03, 2.02 and 3.37 for ++, *B*+ and *BB* respectively. Litter size of pregnant ewes was closer to earlier reports of litter size per ewe lambing (1.3, 2.2 and 2.6 respectively by Piper *et al.* 1985; 1.51, 2.39 and 2.56 respectively by Dodds *et al.*, 1991) although lower in *BB*s. Values were only slightly higher than the 1.01, 1.83 and 2.01 respectively reported by Nimbkar *et al.*, (2003).

The reduction in LS1 in *BB* ewes relative to *B*+ ewes observed in each year of the study, although

non-significant, is a novel finding as all other studies have reported an increase in litter size. There was a significant reduction LS2 in *BB* ewes relative to *B+* ewes of 20%, reflecting both a lower conception rate and slightly lower LS1 of *BB* ewes. In this study reproductive wastage between ovulation and weaning in *BB* ewes under commercial conditions was very high. Analysis of loss between scanning and lamb marking revealed losses of 20, 64, and 89% for ++, *B+* and *BB* respectively. Fitting scanned litter size as a covariate did not remove the effect of genotype (P=0.004), with a significant difference remaining between *BB* and *B+* ewes. On the other hand, fitting OR in the cycle of conception as a covariate removed the effect of genotype (P=0.57). This is indicative of a deleterious maternal effect of *BB* on lamb viability, independent of litter size, but related to high ovulation rate. This is consistent with the report of lower birth weights of *BB* lambs by Gootwine *et al.*, (2006) and our understanding of the effects of ovulation rate on prenatal well being. It may also explain the lower fertility rate in the *BB* ewes, although it was not clear from our study that this was associated with the highest ovulation rates.

CONCLUSIONS

This study has confirmed that the *FecB* mutation does not have deleterious effects on growth and wool production beyond those associated with a higher incidence of multiple births. Consistent with earlier studies, the effect on ovulation rate was additive and the effect of litter size dominant. An important finding was that homozygote *BB* ewes have lower fertility and increased lamb losses between scanning and lamb marking independent of litter size. These effects are probably mediated by high ovulation rate in these ewes and represent a significant limitation on the utility of the gene for increasing reproductive rate. Practical ulitisation of the mutation in Merino sheep requires understanding and overcoming this phenomenon, understanding the basis for the more moderate effect in breeds such as the Garole, development of practical methods for reducing excessive ovulation in *FecB* carriers, and ongoing research into practical methods for enhancing lamb survival.

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