EXPRESSION OF THE *FecB* GENE IN GAROLE AND CROSSBRED EWES IN MAHARASHTRA, INDIA

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SUMMARY

Ovulation rate (OR) and litter size (LS) records from 227 ¼ Garole ewes genotyped for *FecB* were analyzed. Roughly half of the ¼ Garole ewes were heterozygotes ($FecB^{B+}$) and half were non-carriers ($FecB^{++}$). One copy of *FecB* increased OR from 1.03 to 2.02 and LS from 1.01 to 1.83 (average of second and third parities) in multiparous ewes. The other breeds and crosses studied were largely confounded with *FecB* genotype. However, the results were similar when records of ewes of different genetic backgrounds, i.e. non-carrier Deccani and Bannur ewes and heterozygote Garole and F1 ewes, were added to the analysis. The increase in prolificacy caused by the introduction of the *FecB* gene into a non-prolific breed such as the Deccani is likely to produce a useful outcome with a manageable range of litter sizes in flocks carrying the gene.

Keywords: Booroola, Garole, gene expression, ovulation rate, prolificacy

INTRODUCTION

FecB is an autosomal dominant gene with a large effect on ovulation rate (OR). The existence of *FecB* in the highly prolific Booroola Merino strain of sheep was confirmed through a series of experiments conducted in Australia and New Zealand (summarized by Piper *et al.* 1985). The *FecB* mutation has now been identified as a single-base mutation in the BMP1B receptor (Wilson *et al.* 2001). Turner (1982) suggested that the highly prolific Booroola Merino traced back to an early Australian flock known to include prolific 'diminutive' Bengal sheep that arrived from Calcutta in 1792-93. The prolific Garole sheep of West Bengal, India were postulated to be the same as or closely related to the early Bengal sheep (Nimbkar *et al.* 1998). Davis *et al.* (2002) confirmed that the *FecB* mutation exists in the Garole and hypothesized that the homozygous $FecB^{BB}$ genotype could be the original genotype for the Garole breed. They also found the *FecB* mutation in Javanese sheep.

The Nimbkar Agricultural Research Institute (NARI) at Phaltan, Maharashtra, India (latitude 18^0 N and longitude 74^0 E) has started a breeding program to introgress the *FecB* gene from the prolific (average LS 1.74) Garole (G) into non-prolific (average litter size (LS) 1.02) locally adapted Deccani (D) sheep of Maharashtra, which are mainly used for lamb production (Nimbkar *et al.* 2002). The non-prolific meat breed Bannur (B) known for its blocky conformation was also included in the breeding program. The *FecB* genotyping of ewes in this study was carried out at the National

Chemical Laboratory (NCL) in Pune, Maharashtra, where the direct PCR-RFLP DNA test to identify the *FecB* mutation (Wilson *et al*. 2001) has been established. This paper reports the effect of the *FecB* gene on OR and LS of 227 ¼ G ewes (with ¾ comprised of different combinations of D and B genes), 113 of which were identified heterozygote *FecB* carriers ($FecB^{B+}$) and 114 were non-carriers ($FecB^{++}$). The results of this analysis are compared with OR and LS records of G, F1 (GxD and GxB), D and B ewes also genotyped for *FecB*.

MATERIALS AND METHODS

Animals. Ten F1 rams were produced during 1996-98 by inseminating D and B ewes with fresh diluted semen of 9 different G rams. Each of the 10 F1 rams was single-sire-mated on 3-4 occasions during 1999-2001 to groups of D, B, DxB or BxD ewes, producing progeny groups of 12 to 27 daughters per sire. OR of 210 of these ¼ G ewes were determined 2-3 times before and after first lambing. In addition, OR of 18 ¼ G (born to F1 dams from D or B sires), 58 G, 50 D, 31 B and 40 F1 ewes of various ages, produced in NARI's breeding program were determined 2-3 times during 1999-2002. OR was determined by laparoscopy 4 to 7 days after natural oestrus detected by vasectomized rams. Individual LS of all ewes of all breeds was also recorded. Table 1 shows the breedwise classification of the 407 ewes with OR records, 339 of which also had LS records that were analyzed.

Table 1. Number of records of ovulation rate (OR) and litter size (LS) by breed of ewe

Number of records	Breed of ewes					
	Garole	F1	¼ Garole	Deccani	Bannur	Total
1-3 OR records before first lambing	5	25	228	2	1	261
1-3 OR records after first parity or at later parities	53	18	126	48	30	275
First 1-3 litter size (LS) records	48	34	178	49	30	339

***FecB* genotyping.** All D, B, G and ¼ G ewes and G and F1 sires were genotyped at the *FecB* locus. Five of the 40 F1 ewes were genotyped and the rest were assumed to be $FecB^{B+}$ because their sires and dams were genotyped and were $FecB^{BB}$ and $FecB^{++}$, respectively.

Data and analysis. Table 2 shows that ewe breed and *FecB* genotype were largely confounded in these data apart from within the ¼ G ewes.

Table 2. *FecB* genotypes of ewes of different breeds

Ewe breed	$FecB^{BB}$	$FecB^{B+}$	$FecB^{++}$	Total
Garole (G)	52	5	1	58
Deccani (D)	-	-	50	50
Bannur (B)	-	-	31	31
F1 (GxD, GxB)	-	40	-	40
¼ Garole (¼ G)	1	113	114	228
Total	53	158	196	407

OR before and after first lambing and LS of ¼ G ewes were analyzed separately (analysis A) to get an estimate of the effect of the *FecB* gene with the same background breed. Ovulation rates at later parities of all ewes of all breeds were analyzed together (analysis B) to see if the effect of the gene changed with a different genetic background. All analyses were done by fitting a repeated measures model using ASReml (Gilmour *et al.* 1999). *FecB* genotype was fitted as a fixed effect. Year of measurement and birth type as fixed effects and the covariables age and weight of ewe, the proportion of D genes (0, 0.25, 0.5 or 0.75), the interaction between proportion of D genes and *FecB* genotype, individual heterosis (both analyses) and the proportion of G genes (analysis B) were tested for significance.

RESULTS AND DISCUSSION

FecB genotype was highly significant for OR and LS in both analyses ($P<0.01$) and explained most of the variation. Year of measurement and birth type of ewe were not significant. The proportion of D genes and heterosis as covariables and the interaction between proportion of D genes and *FecB* genotype were found not to be significant for any of the traits in both analyses. The proportion of G genes was significant for OR in analysis B.

Table 3. Least Squares Mean (LSM) ovulation rate (OR) and litter size (LS) of heterozygote *FecB* carrier (*FecB*$^{B+}$) and non-carrier (*FecB*$^{++}$) ¼ Garole ewes

	FecB^{++} ewes			*FecB*$^{B+}$ ewes		
	No.	LSM	Std. error	No.	LSM	Std. error
OR before lambing	114	1.03	0.04	113	1.76	0.04
OR after first lambing	57	1.03	0.04	69	2.02	0.07
First LS	92	1.01	0.04	86	1.53	0.04
Second LS	50	1.02	0.05	53	1.63	0.05
Third LS	19	1.00	0.09	18	2.03	0.09

Table 3 shows that one copy of *FecB* increases OR in ¼ G ewes by 0.73 in maiden ewes and by 0.99 after first lambing. The effect of one copy of the *FecB* gene on OR was 1.01 in analysis B (mean OR of *FecB*$^{B+}$ ewes being 2.09 ± 0.1 compared to 1.08 ± 0.06 in *FecB*$^{++}$ ewes). The effect of one copy of the *FecB* gene on LS in ¼ G ewes was 0.52, 0.61 and 1.03 for the first, second and third parities respectively. The effect appears to increase with parity. However, the third parity LS is based on a small number of records. Again the mean LS from analysis B were very similar when data from ewes of all breeds and all *FecB* genotypes were analyzed together (average of second and third parity mean LS was 1.76 ± 0.06 for *FecB*$^{B+}$ ewes and 1.03 ± 0.03 for *FecB*$^{++}$ ewes). The ratio of LS to OR decreases as mean OR increases because of the negative relationship between the number of embryos in the uterus and the probability of survival for the individual embryo.

The effect of the first copy of the *FecB* gene on OR in this study (close to 1) was lower than the 1.65 ova per copy reported by Piper *et al.* (1985) for the Booroola Merino. This might be due to the low average fecundity of the base breeds D and B or to the effect of regulatory genes or to environmental factors such as nutritional status. Plane of nutrition effects, indicated by highly significant

differences between years of measurement, in the mean difference in ovulation rate between putative $FecB^{B+}$ and $FecB^{++}$ Merino ewes were reported by Piper *et al.* (1985). However, the effect on LS here is similar to the 0.9 reported by them. The results of this study agree very closely with the effects of the *FecB* gene in Javanese sheep documented by Bradford *et al.* (1990). Practical utility of the *FecB* gene has been questioned because of the high proportion of triplets and higher order litters it causes. However, results indicate that the average litter size of D sheep carrying the *FecB* gene is likely to be more manageable.

The LSM for OR and LS of homozygote *FecB* ($FecB^{BB}$) ewes in this study which were all G except one ¼ G ewe, were 3.37 ± 0.26 and 2.01± 0.07, respectively, compared to the mean OR of 5.7 and LS of 2.6 in the CSIRO Booroola Merino flock with a high frequency of the *FecB* gene (Piper and Bindon, 1996). There are indications that the second copy of the gene increased OR by 3.37-2.02=1.35 and LS by 2.01- ½(1.63+2.03)=0.18. This points to an additive effect of the gene on OR and a partially dominant effect on LS. However, we cannot conclude this with confidence since the base breeds carrying one and two copies of the gene are different.

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