Effect of sire, breed and age on plasma FSH concentrations in Fec^B Fec^B and Fec^+ Fec^+ Booroola rams before and after castration

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ABSTRACT

It is debatable whether or not genotype influences follicle-stimulating hormone (FSH) secretion in the Booroola ram. Where differences in FSH have been reported, these may have been due to effects of sire, breed and age rather than Booroola genotype. The aim of this study was to determine if these factors do influence Fec^B gene expression in plasma FSH concentrations in the Booroola ram.

A total of 45 Booroola rams of known genotype, breed, age and sire were blood sampled (10 ml by jugular venepuncture) once daily for 11 days, castrated, bled once daily for a further 13 days, and then three times weekly for a further 7 weeks.

There were no gene-specific differences in mean plasma FSH concentrations before or after castration, or in the rate of the post-castration rise in FSH, between Fec^B Fec^B (BB) and Fec^+ Fec^+ (++) rams. This was consistent when data from rams of three ages (1, 2 or 3 years) and of either Booroola Merino or Booroola Romney breeds were combined, or when genotypes were compared within breeds, or within ages. There was, however, a significant effect of sire on plasma FSH concentrations, which was evident before but not after castration. These results suggest that variation in FSH between sire groups may explain differences previously attributed to genotype.

Keywords Booroola, rams, sire effect, FSH, breed, age, castration, heritability.

INTRODUCTION

Ewes carrying the Booroola Fec^B gene are characterized by a higher ovulation rate (Davis *et al.*, 1982) and higher plasma follicle-stimulating hormone (FSH) levels (McNatty *et al.*, 1989) than non-carriers. Studies investigating the expression of the Fec^B gene in plasma FSH concentration in the Booroola ram have, however, been inconclusive. Concentrations are reported to be similar between genotypes in rams as lambs and adults in some studies (Bindon *et al.*, 1985; Bindon *et al.*, 1989; Montgomery *et al.*, 1989; Price *et al.*, 1991a), but not in others (Bindon 1984; Seck *et al.*, 1988). Castration to remove the negative feedback effects of the testes overcomes the difficulty associated with monitoring the low FSH levels characteristic of intact rams. Results from studies on gene-specific differences in FSH in castrated Booroola rams have, however, also been equivocal (Bindon *et al.*, 1985; Price *et al.*, 1991b).

Attempts to identify gene-specific differences in FSH concentrations may be influenced by effects of breed, age and sire. The present study aims to compare FSH concentrations between BB and ++ adult Booroola rams before and after castration, taking these factors into account.

MATERIALS AND METHODS

Animals

A total of 45 Booroola rams of known genotype, breed, age and sire group (Table 1) were used in this study. Booroola Romney (1/2 Merino 1/2 Romney) and Booroola Merino rams were from the flocks at the Invermay Agricultural Centre, Mosgiel, and the Tara Hills High Country Research Station, Omarama, respectively. Rams were classified as BB and ++ based on pedigree and progeny test information from their sires, and ovulation rate records from their dams (Davis *et al.*, 1982).

TABLE 1 Number of experimental animals of each Booroola genotype, classified according to breed, age or sire group.

Booroola genotype	BB	++
Breed		
Booroola Romney (BR)	10	8
Booroola Merino (BM)	16	11
Age		
1 year (BR)	10	8
2 years (BM)	7	3
3 years (BM)	9	8
Sire groups	10 (2-5/sire)	4 (4-5/sire)
Total	26	19

Experimental Protocol

Plasma FSH concentrations were monitored in intact rams to determine endogenous levels, and in the same animals following castration to evaluate FSH concentrations after the removal of feedback influences of gonadal hormones. Rams were blood sampled (10 ml by jugular venepuncture) once daily for 11 days, castrated, bled once daily for a further 13 days, and then three times weekly for a further 7 weeks. The experiment extended from early October to late December (spring-early summer) 1990.

Animals were castrated under general anaesthesia induced with sodium thiopentone (May and Baker Ltd, Dagenham, England) and following premedication with acepromazine (Techvet

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Laboratories Ltd, Auckland, New Zealand). Each testis was removed via an individual ventral incision in the scrotal sac.

FSH assay

Plasma FSH concentrations were measured using the double-antibody radioimmunoassay described by McNatty *et al.* (1989). The coefficient of variation was less than 10% both between and within assays and the limit of detection was 0.1 ng/ml plasma. The limit of detection is defined as twice the standard deviation of the error associated with the zero value.

Analysis of data

Plasma FSH concentrations were log transformed. Comparisons were made between geometric mean FSH concentrations averaged over the 11 pre- and 35 post-castration observations for each animal. Post-castration FSH concentrations followed a characteristic line plus exponential curve, and were fitted by the equation $\log FSH(t) = a + br^t + ct + \varepsilon(t)$, where t is time in days, r is a parameter for the rate of approach of FSH to the asymptote following castration ($0 < r < 1$), c is the asymptotic slope of the curve, a and b are linear parameters and $\varepsilon(t)$ is normally distributed error, for each animal and for genotype, breed, age and sire group.

The above summary statistics for each animal were then analyzed by residual maximum likelihood (Patterson and Thomson, 1971), with sire as a random effect and ram genotype, age/breed class and their interaction as fixed effects. From this, the ratios of the between-to-within sire variances (variance ratio; VR) and heritability estimates were calculated to assess the presence of a sire effect. Geometric means are significantly different when the ratio of one to another is greater than the square of the standard error associated with that ratio (SER).

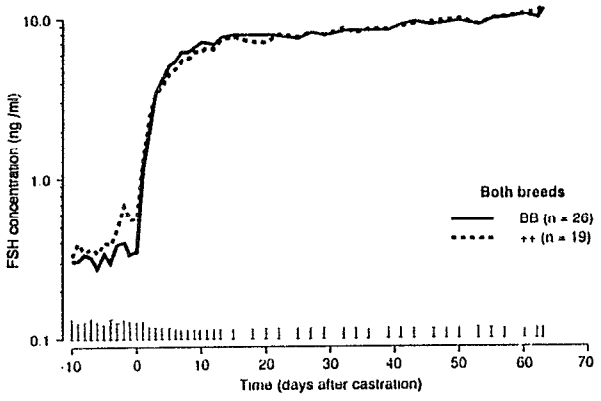
RESULTS

Prior to castration mean FSH concentrations in individual rams ranged from 0.1-2.4 ng/ml. There was a marked increase in FSH immediately after castration (Figure 1). For example, when combining BB and ++ rams of all breeds and ages (determined from the fitted curve), geometric mean FSH concentrations increased from 0.5 ng/ml on day 0 to 7.1 ng/ml on day 13. Thereafter there was a more linear but less rapid increase in FSH levels, to reach 10.6 ng/ml at day 65.

Genotype

When Merino and Romney breeds aged one to three years were combined, there were no gene-specific differences in the geometric mean plasma FSH concentrations either prior to or following castration, or in the rate parameter for the post-castration rise in FSH (Figure 1; Table 2). In addition, there were no significant differences between BB and ++ rams in any of these variables within the breed/age groups (Table 2).

FIGURE 1 Geometric mean plasma FSH concentrations in Fec^B Fec^B (BB) and Fec^+ Fec^+ (++) Booroola Merino and Booroola Romney rams before and after castration. (Vertical lines represent SED's.)



Age/breed effects

Geometric mean FSH concentrations were not significantly different between two and three year old Booroola Merino rams before castration (0.43 and 0.66 ng/ml respectively; SER 1.27) or after castration (7.61 and 8.52 ng/ml; SER 1.17), nor were there differences in the rate parameter for the post-castration rise in FSH (0.60 and 0.65 ng/ml respectively; SED 0.037; Figure 2). In comparison, one year old Booroola Romney rams had significantly lower geometric mean pre- (0.19 ng/ml; SER 1.27) and post-castration (5.29 ng/ml; SER 1.17) FSH concentrations, and a significantly slower approach to the asymptote of the fitted curve (0.71 ng/ml; SED 0.037), than the older rams (2 and 3 year olds). Age/breed effects are, however, confounded as all two and three year old rams were Booroola Merino and all one year old rams Booroola Romney (Table 1).

Sire effects

Variation in the mean pre-castration (days -10 to 0) FSH concentration was significantly greater between than within sires (Figure 3; Table 3). Following castration no significant sire effects were evident during either the immediate (days 1-13) or late (14-65) post-castration periods. Sire effects did not differ

TABLE 2 Influence of Fec^B genotype (BB versus ++) on geometric mean pre- and post-castration plasma FSH concentrations (ng/ml), and rate parameter (r) for the post-castration rise in FSH from the fitted curve $\log FSH(t) = a + br^t + ct + \varepsilon(t)$, for Booroola Merino and Booroola Romney rams aged 1-3 years combined, and Booroola Merino and Booroola Romney rams within breed/age groups. (SER: standard error of the ratio).

Breed/Age	Pre-castration			Post-castration			r		
	BB	++	SER	BB	++	SER	BB	++	SED
Merino + Romney (n = 45)	0.33	0.43	1.25	7.05	6.95	1.13	0.65	0.66	.031
Romney (1 yr old) (n = 18)	0.19	0.19	1.46	5.37	5.21	1.22	0.72	0.71	.047
Merino (2 yr old) (n = 10)	0.37	0.50	1.48	8.36	6.94	1.27	0.59	0.61	.064
Merino (3 yr old) (n = 17)	0.53	0.83	1.35	7.81	9.27	1.19	0.63	0.67	.045

FIGURE 2 Geometric mean plasma FSH concentrations in two and three year old Booroola Merino rams and one year old Booroola Romney rams before and after castration. (Vertical lines represent SED's.)

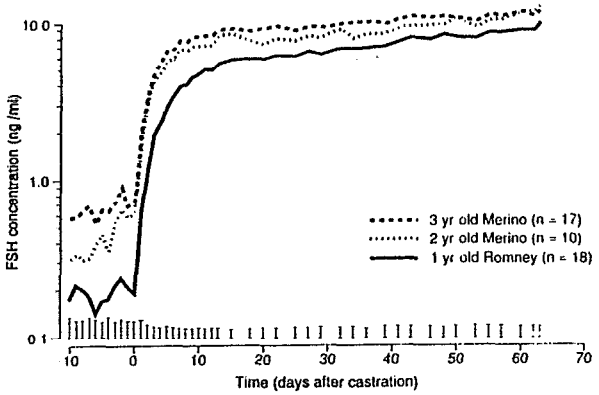


FIGURE 3 Geometric mean pre-castration plasma FSH concentrations for individual rams within sire groups. (Perpendicular lines link individuals with the same sire.)

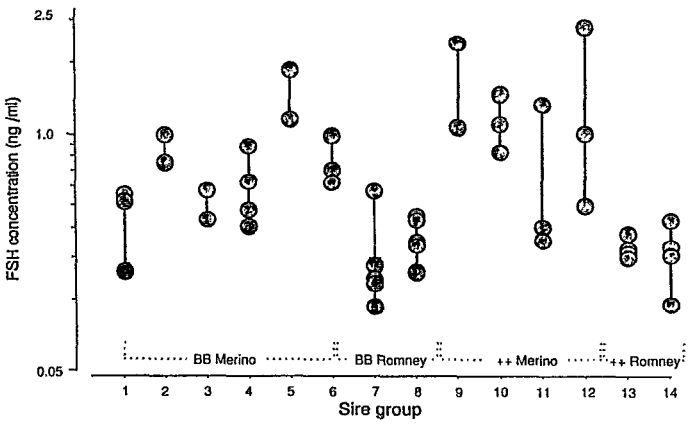


TABLE 3 Estimates of the between-to-within sire variance ratio (VR) and heritability (h^2) of plasma FSH concentrations before castration (days -10 to 0), immediately following castration (days 1-13; Imm post-cast), and later following castration (days 14-65; Late post-cast)

Period	Variance ratio	Heritability	+ SE
Pre-castration	2.20	1.16	± 0.79
Imm post-cast	1.85	0.89	± 0.77
Late post-cast	1.44	0.50	± 0.76

(VR's greater than $F_{10,29} = 2.18$ are significant at $P<0.05$)

with genotype. Although not significant, heritability estimates for all three periods were high, with the pre-castration period being highest (Table 3).

DISCUSSION

These studies clearly demonstrate that the effect of the Fec^B gene is not apparent in plasma FSH concentrations in Booroola rams. There were no differences in FSH levels between BB and ++ rams prior to castration in either breed, or at any of the ages. Similarly, there were no gene-specific differences in the rate of increase in FSH immediately following castration, or in mean FSH concentrations either over the immediate post-castration period, or in the late post-castration period. These results are in agreement with most, but not all, earlier comparisons between genotypes (see Introduction). For example Price *et al.* (1991b) found a significantly faster post-castration rise in FSH in BB than ++ rams (0.55 and 0.29 ng/ml/day respectively). Comparable values in the present study are 0.39 and 0.41 ng/ml/day, which are in between the rates reported in the former study.

While it is possible that differences between studies are attributable to strains within Booroola rams, sampling protocols and misclassification of genotypes; time of year and sire influences appear the most likely cause of these differences. For example, Price *et al.* (1991b) castrated rams in autumn (March), when the testes are most active, and found significantly higher post-castration plasma FSH levels in BB than ++ rams. In the present study rams were castrated in spring (October), the period of testicular development, and no such differences were found. Photoperiod has previously been shown to influence plasma FSH concentrations in rams (Lincoln *et al.*, 1977). Although time of year did not significantly influence gene differences in FSH in intact animals, BB rams tended to have higher mean FSH levels than ++ rams from spring to early autumn (Price *et al.*, 1991a). The influence of time of year on gene-specific differences in plasma FSH in castrated Booroola rams has yet to be established.

Evidence of an influence of sire on FSH concentration is of considerable interest. Although sire effect was significant only during the pre-castration period in the present study, this probably reflects the small number of sires and offspring within each sire group, and not that castration has removed the sire effect. In support of this suggestion, Price *et al.* (1991b) reported significant effects of sire on FSH concentrations following castration, although only in BB and not ++ rams. Even considering the high standard errors of the estimates, the high heritability coefficients add convincing support to the evidence of a sire effect both pre- and post-castration. Further evidence of the influence of sire on plasma FSH concentrations comes from a comparable study involving 51 entire ram lambs from 5 sire groups (G.B. Martin *pers. comm.*). It is of interest to note that the putative gene-specific differences in FSH concentrations reported by Price *et al.* (1991b) were also associated with sire effects. In the current study, sire effects were present prior to castration where gene differences were not apparent. In the study of Price *et al.* (1991b) it is possible that the apparent effect of genotype was an artifact due to variation between sires, although the influence of time of year cannot be ruled out. This highlights the importance of accounting for sire in such investigations.

That there was a significant sire effect on FSH in intact rams is consistent with the relatively high heritability estimate (h^2) of 0.63 ± 0.22 cited by Bindon and Piper (1987). This is supported by the results of the present study which also indicate that plasma FSH concentrations in rams are highly heritable.

There were no significant differences in mean FSH concentrations between ages in the Booroola Merino rams either before or after castration. There were, however, significant differences in FSH concentrations between the one year old Booroola Romney rams and the two and three year old Booroola Merino rams. This may suggest developmental changes, perhaps in the presence of different isoforms of FSH (see Wide, 1985 for data in humans), or in gonadotroph cell development in the pituitary. Alternatively, FSH concentrations in rams may differ between breeds. Breed differences in seasonal cycles of FSH secretion have been reported (G.B. Martin and S. Tjodronegara *pers. comm.*), although Barrell and Lapwood (1978/1979) reported seasonal changes in seminal characteristics and in plasma LH, prolactin and testosterone to be similar in Merino and Romney rams.

In the present experiment, removal of the negative feedback effects of the testes did not result in gene differences in FSH

concentrations, indicating that the Booroola gene may not be expressed via the pituitary-gonadal axis in the ram. An important outcome of this is that attempts to identify differences in FSH concentrations between Booroola genotypes in the ram should always consider the possible effects of sire and time of year.

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