The effect of the Booroola (Fec^B) gene on peripheral FSH concentrations and ovulation rates during oestrus, seasonal anoestrus and on FSH concentrations following ovariectomy in Scottish Blackface ewes

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The aim of this study was to investigate the role of FSH in the control of ovulation rate by the Booroola gene. Three Booroola genotypes ($Fec^B Fec^B$, $Fec^B Fec^+$ and $Fec^+ Fec^+$) of the F_2 population, from a cross between Booroola Merino and Scottish Blackface, and two Booroola genotypes ($Fec^B Fec^+$ and $Fec^+ Fec^+$; 25% Booroola Merino and 75% Scottish Blackface), from the backcross of $Fec^B Fec^+$ sires to Scottish Blackface ewes, were compared. During seasonal anoestrus significant differences ($P < 0.05$) in hCG-stimulated ovulation rates were obtained between $Fec^B Fec^B$ and $Fec^+ Fec^+$ ewes from the F_2 population, and $Fec^B Fec^+$ ewes were intermediate. No significant difference in hCG-stimulated ovulation rate was observed in the backcross population between $Fec^B Fec^+$ ewes and $Fec^+ Fec^+$ ewes. There were no significant differences between genotypes in mean serum FSH concentrations during seasonal anoestrus in either backcross or F_2 populations. During the breeding season, two separate experiments confirmed the expected ovulation rate differences between genotypes ($Fec^B Fec^B > Fec^B Fec^+ > Fec^+ Fec^+$). In both experiments, mean peripheral FSH concentrations in the F_2 population were similar in $Fec^B Fec^+$ and $Fec^+ Fec^+$ ewes, but were significantly higher ($P < 0.05$) in $Fec^B Fec^B$ ewes. In the backcross population, mean peripheral FSH concentrations during the oestrous cycle were not significantly different between $Fec^B Fec^+$ and $Fec^+ Fec^+$ ewes, despite significant differences in ovulation rate. Ovariectomy during the breeding season resulted in significantly higher ($P < 0.001$) mean peripheral FSH concentrations in all three genotypes. After ovariectomy, mean FSH concentrations between $Fec^B Fec^+$ and $Fec^+ Fec^+$ ewes, from both the backcross and F_2 populations, were not significantly different. However, mean FSH concentrations in the F_2 population were significantly higher in $Fec^B Fec^B$ ewes than in the other two genotypes. The pattern of differences between genotype in peripheral FSH concentrations and ovulation rates suggest that FSH is not wholly responsible for differences in ovulation rate between genotypes. The results support the hypothesis that the Fec^B gene is operating both within the ovary and at the level of the hypothalamus and pituitary gland.

Introduction

The high fecundity of the Booroola Merino flock (Newton-Turner, 1978) is due to a major gene that is responsible for an increased ovulation rate (Bindon, 1984). The Booroola genotypes have been characterized on this basis, such that $Fec^B Fec^B$ (homozygous carriers for the Booroola fecundity, or Fec^B , gene), $Fec^B Fec^+$ (heterozygous carriers) and $Fec^+ Fec^+$ (non-carriers) have at least one ovulation rate record of ≥ 5 , at least one record ≥ 3 but < 5 , all records < 3 , respectively (Davis *et al.*, 1982).

Despite the finding of genetic markers of the Fec^B gene (Montgomery *et al.*, 1993; Lanneluc *et al.*, 1994), the basis for the increased ovulation rate in the Booroola Merino has not been fully established. The possibility that ovulation rate may be causally related to differences in peripheral concentrations of plasma gonadotrophin and/or hypothalamic and/or pituitary function has been investigated, but the results are equivocal. Studies in ewes with the Booroola gene found no differences in either GnRH concentrations in various areas of the brain, or number of GnRH receptors in the pituitary gland (see reviews by McNatty *et al.*, 1990; Montgomery *et al.*, 1992). The general consensus for a range of breeds, including breeds or strains possessing the Booroola gene, is that peripheral LH concentration is not correlated with ovulation rate (Bindon *et al.*, 1985).

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Although differences in FSH concentration have been found (Bindon, 1984; McNatty *et al.*, 1987), it has not been proved that these are responsible for the differences in ovulation rate between genotypes (Driancourt and Fry, 1990). At about the time of luteolysis, Booroola ewes ($Fec^B Fec^B$) have significantly higher FSH concentrations than have non-carriers ($Fec^+ Fec^+$), while $Fec^B Fec^+$ ewes have intermediate FSH concentrations (McNatty *et al.*, 1987). However, Bindon *et al.* (1985) reported no significant differences between ewes with the Booroola gene and those lacking the gene, between 96 h and 24 h before the preovulatory gonadotrophin surge.

It is possible that the increased ovulation rate of the Booroola reflects a lower sensitivity of the hypothalamus and/or pituitary gland to the negative effects of ovarian hormones. Higher concentrations of ovarian hormones need not, therefore, result in a greater reduction in the release of gonadotrophins (Land, 1976), and there could be differences in ovulation rate without differences in the peripheral concentrations of gonadotrophins. In support of this view, some authors report persistent Fec^B gene effects after ovariectomy, while others find no such effect (see reviews by McNatty *et al.*, 1989; McNatty *et al.*, 1990; Driancourt *et al.*, 1990). In addition, there is no difference in the rate of reduction in ovulation rate in response to increased peripheral oestradiol concentrations between lines of Finnish Landrace sheep selected for differences in ovulation rate (Webb *et al.*, 1992a), suggesting that differences in ovulation rate are not due to differences in the response of the hypothalamus and/or pituitary gland to oestradiol negative feedback.

The above results, from both intact and ovariectomized animals, fail to provide consistent evidence that differences in peripheral FSH concentrations are the cause of high ovulation rates in Booroola ewes. Many of the effects attributed to the Booroola gene could be explained in terms of the effects of the background genotype. Furthermore, problems have been incurred in previous studies by the presence of genetic variation between families and the use of a limited number of sires for each Booroola genotype. The establishment of a new population of Booroola animals (the Booroola Scottish Blackface), from a well-defined and carefully controlled breeding programme, has provided an opportunity to investigate the genetic control of ovulation rate. The Booroola Merino cross Scottish Blackface animals used in these studies were derived from carefully defined experimental lines, with the same genetic background (Haley, 1990), so that the importance of FSH in both genotypic and seasonal differences in ovulation rate could be assessed.

The aims of this study were to examine peripheral serum FSH concentrations and ovulation rate during the breeding season and seasonal anoestrus, and FSH concentrations after ovariectomy in Booroola Scottish Blackface ewes, to determine whether the presence of the Booroola gene and the associated marked increase in ovulation rate is causally related to increased peripheral concentrations of FSH.

Materials and Methods

Animals

In 1984, imported Booroola Merino rams were crossed with native Scottish Blackface ewes. The F_1 population produced

was crossed to produce an F_2 population in which the background genotype of all sheep was, on average, 50% Booroola Merino and 50% Scottish Blackface, but all three genotypes at the Booroola locus would be present (Haley, 1990). The females were genotyped by the segregation criteria of Davis *et al.* (1982), whereas males were progeny tested. Backcross ewes, in which the background genotype was 25% Booroola and 75% Scottish Blackface, were selected from the progeny of F_2 heterozygous males crossed with Scottish Blackface ewes. The animals used in this study were the F_2 and the backcross ewes. All experiments were carried out on the Roslin Institute farms in southern Scotland. Water was available to all ewes *ad libitum* and they were fed on grass supplemented with concentrates, as dictated by normal husbandry practice.

Experiments 1, 3 and 5. Backcross Booroola Merino cross Scottish Blackface ewes, 2–4 years of age, were classified as either the $Fec^B Fec^+$ ($n = 11$) or $Fec^+ Fec^+$ ($n = 12$) genotype on the basis of at least four previous ovulation rate records. The mean ($\pm$ SEM) live masses were 52.7 ± 2.1 kg for $Fec^B Fec^+$ and 55.5 ± 2.0 kg for $Fec^+ Fec^+$ ewes, which are not significantly different, in agreement with Bindon *et al.* (1982).

Experiments 2 and 4. F_2 Booroola Scottish Blackface ewes, 2–4 years of age, were classified as being either $Fec^B Fec^B$ ($n = 9$), $Fec^B Fec^+$ ($n = 9$) or $Fec^+ Fec^+$ ($n = 9$) on the basis of at least five previous ovulation rate records. The mean ($\pm$ SEM) live masses of these ewes were 41.8 ± 2.2 , 39.3 ± 2.4 and 45.6 ± 2.0 kg for $Fec^B Fec^B$, $Fec^B Fec^+$ and $Fec^+ Fec^+$, respectively, which are not significantly different.

Experiment 6. F_2 Booroola Scottish Blackface ewes, 2–4 years of age, were classified as either $Fec^B Fec^B$ ($n = 9$), $Fec^B Fec^+$ ($n = 9$) or $Fec^+ Fec^+$ ($n = 8$) on the basis of at least five previous ovulation rate records. The mean ($\pm$ SEM) live masses of these ewes were 45.4 ± 3.3 , 47.4 ± 1.7 and 49.3 ± 1.5 kg for $Fec^B Fec^B$, $Fec^B Fec^+$ and $Fec^+ Fec^+$, respectively, which are not significantly different.

Experimental procedures

Experiment 1: FSH and ovulation rate during seasonal anoestrus in the backcross population. During seasonal anoestrus (September), twice daily blood samples (10 ml) were obtained by jugular venepuncture for 5 days, with the first day of sampling designated day 1. Hourly samples were also taken in this way for an additional 6 h on day 2. All ewes were given a single i.m. injection of 750 iu hCG (Chorulon: Intervet, Cambridge) on day 3, since this dose has been shown to induce ovulation in Scottish Blackface ewes during seasonal anoestrus (Webb *et al.*, 1992b). On day 5, the ewes were anaesthetized for mid-ventral laparoscopy to assess their ovulation rate (Holland *et al.*, 1981; Webb *et al.*, 1992b).

Experiment 2: FSH and ovulation rate during seasonal anoestrus in the F_2 population. During seasonal anoestrus (July), jugular vein blood samples (10 ml) were taken twice a day for 15 days, and hourly samples were collected for 8 h on day 11. On day

15, all ewes were given an i.m. injection of 750 iu hCG. On day 17, the ewes underwent mid-ventral laparoscopy to assess their ovulation rate.

Experiment 3: FSH and ovulation rate during the oestrous cycle in the backcross population. During the breeding season (October), backcross Booroola Scottish Blackface ewes were run with a raddled ram and checked twice daily for oestrus. All ewes were sampled twice a day by jugular venepuncture from day -2 to day 10 of the next cycle (day 0 being the day of behavioural oestrus). Blood samples were also collected every hour for 8 h on day -1 and day 7. All ewes underwent laparoscopy on day 3 to determine their ovulation rate. All ewes then received a single i.m. injection of 750 iu hCG on day 8 and laparoscopy was performed on day 10 to determine the subsequent induced ovulation rate.

Experiment 4: FSH and ovulation rate during the oestrous cycle in the F₂ population. During the breeding season (November), F₂ Booroola Scottish Blackface ewes were run with a raddled ram and checked twice a day for oestrus. All ewes were sampled twice a day by jugular venepuncture from day -3 to day 13 of the oestrous cycle. Blood samples were also collected from all ewes every hour for 8 h during the mid-luteal phase (on day 6, 7 or 8) and all ewes underwent laparoscopy during the luteal phase (days 9–12) to determine their subsequent ovulation rate.

Experiment 5: ovulation rate and FSH, before and after ovariectomy, during the breeding season in the backcross population. During the breeding season (December), all ewes were run with a raddled ram and monitored twice a day for signs of behavioural oestrus (day 0), after being synchronized with a single prostaglandin injection (0.4 ml Estrumate dissolved in 1.5 ml of saline; Coopers Animal Health Ltd, Crewe). Blood samples were collected from all ewes twice a day by jugular venepuncture from day 4 of the second oestrous cycle to day 8. On day 8, they were ovariectomized, and blood samples were collected twice a day by jugular venepuncture for a further 14 days.

Experiment 6: ovulation rate and FSH before and after ovariectomy during the breeding season in the F₂ population. During the breeding season (December), F₂ Booroola Scottish Blackface ewes were synchronized using vaginal progestagen sponges (Veramix: Upjohn Ltd, Crawley) for 12 days. All ewes were run with a raddled ram and checked twice a day for signs of behavioural oestrus (day 0). They were sampled twice a day by jugular venepuncture from the first day of sponge removal (day -2) to day 7, when all ewes were ovariectomized. Blood samples were collected twice a day by jugular venepuncture for a further 14 days after ovariectomy.

In all experiments, blood samples were collected in non-heparinized vacutainers and allowed to clot overnight before being centrifuged at 2000 g for 25 min. The serum samples were stored at -20°C until assay.

Hormone radioimmunoassays

FSH. Concentrations of FSH in peripheral serum were determined in duplicate using the radioimmunoassay validated

by McNeilly *et al.* (1988). The minimum detectable value was 0.6 ng ml⁻¹ ($n = 15$ assays). The interassay coefficient of variation (CV), using six standard quality control samples of known FSH concentrations, was 7.5% and the intra-assay CV was 9.2%.

Progesterone. Concentrations of progesterone in peripheral serum, obtained during seasonal anoestrus, were determined in duplicate using a direct radioimmunoassay (Corrie *et al.*, 1981; Law, 1991). The minimum detectable value was 0.16 ng ml⁻¹ and the intra-assay CV was 8.6%. All the samples analysed from Expts 1 and 2 were found to be less than the minimal detectable level, confirming that the ewes were at anoestrus.

Statistical analyses

Where there were systematic trends in FSH concentrations over time, models were fitted to the data for each individual animal. FSH concentrations for Expts 5 and 6 were analysed by fitting Gompertz curves for each animal separately and averaging the parameter estimates within groups. The three parameters of a Gompertz curve provide estimates for the initial level (preovariectomy), the rate of change and the final concentration (after ovariectomy) of FSH. Differences between groups were tested by comparing the average parameter values for each group using Student's *t* test.

FSH values for Expt 4 were analysed in two parts. Quadratic polynomials were fitted for each animal separately for day -1 to day 3, at about the time of the FSH preovulatory surge. The mean amplitudes of the surge were calculated from the fitted polynomials and averaged within each genotype. The average amplitudes were tested between genotypes using Student's *t* test. The FSH concentrations for days 5–13 were analysed using split-plot analysis of variance because there were no systematic trends over time. FSH data for Expts 1–3 were also analysed in this way. Where comparisons were made between days, within sheep, adjustments to the degrees of freedom for the *F* test were made as necessary (Greenhouse and Geisser, 1959).

Mean ovulation rates between genotypes, within season, were analysed using Student's *t* test. Comparisons between the breeding season and seasonal anoestrus were made within sheep. For ovulation rate, the difference between the normal and induced ovulation rates for each sheep during the breeding season and seasonal anoestrus was calculated. For FSH, samples collected before hCG, on days 6 and 7 (breeding season) and days 1 and 2 (seasonal anoestrus) were used. The difference in mean FSH concentrations and in ovulation rates between the breeding season and the non-breeding season was calculated within sheep and the overall mean for each genotype tested, using Student's *t* test, to determine whether they differed significantly from zero.

The influence of body mass upon FSH concentrations and ovulation rates was not significant in a preliminary analysis and was therefore excluded from further analyses.

Table 1. Mean ($\pm$ SEM) ovulation rate and FSH concentration (ng ml^{-1}) from blood samples taken hourly, during seasonal anoestrus, in backcross and F_2 populations of Scottish Blackface ewes with and without the Fec^B gene

Parameter	Population	$Fec^B Fec^B$	Genotype $Fec^B Fec^+$	$Fec^+ Fec^+$
Ovulation rate*	Backcross	—	1.7 ± 0.4 (11)	1.8 ± 0.3 (12)
FSH concentration	Backcross	—	2.5 ± 0.3	2.3 ± 0.2
Ovulation rate†	F_2	2.7 ± 0.6^a (9)	$1.9 \pm 0.4^{a,b}$ (9)	0.9 ± 0.3^b (9)
FSH concentration	F_2	2.7 ± 0.4	2.6 ± 0.2	2.6 ± 0.2

For each population, within rows, values with different superscripts are significantly different ($P < 0.05$).

Number of animals are given in parentheses.

*Mean ovulation rate for all ewes. After 750 iu hCG, 83% of ewes ovulated.

†Mean ovulation rate for all ewes. After 750 iu hCG, 78% of ewes ovulated.

Table 2. Mean ($\pm$ SEM) FSH concentrations (ng ml^{-1}) from blood samples taken twice a day, during seasonal anoestrus, in backcross and F_2 populations of Scottish Blackface ewes with and without the Fec^B gene

Population	$Fec^B Fec^B$	Genotype $Fec^B Fec^+$	$Fec^+ Fec^+$
Backcross	—	2.3 ± 0.2	2.3 ± 0.1
F_2	2.6 ± 0.2	2.3 ± 0.2	2.4 ± 0.2

Results

Experiment 1: FSH concentration and ovulation rate during seasonal anoestrus in the backcross population

Ovulation rate. After the single i.m. injection of 750 iu hCG, 83% of ewes ovulated. Three $Fec^B Fec^+$ ewes and one $Fec^+ Fec^+$ ewe failed to ovulate in response to the hCG treatment. Mean ovulation rates of those ewes that ovulated during seasonal anoestrus were not significantly different between $Fec^B Fec^+$ and $Fec^+ Fec^+$ ewes (Table 1).

FSH concentration. Mean peripheral FSH concentrations in blood samples collected hourly on day 2 of the study (Table 1) and mean peripheral FSH concentrations in blood samples collected twice a day for 5 days did not differ significantly between genotypes (Table 2).

Experiment 2: FSH concentration and ovulation rate during seasonal anoestrus for the F_2 population

Ovulation rate. After a single i.m. injection of 750 iu hCG, 77% of ewes ovulated, and four $Fec^+ Fec^+$ ewes, two $Fec^B Fec^+$ ewes, but no $Fec^B Fec^B$ ewes failed to ovulate. Mean ovulation rates differed significantly ($P < 0.05$) between

Fig. 1. Mean ($\pm$ SEM) serum FSH concentration (ng ml^{-1}), in $Fec^B Fec^B$ (●), $Fec^B Fec^+$ (○) and $Fec^+ Fec^+$ (□) ewes of the F_2 population possessing the Booroola fecundity gene ($n = 9$ per genotype) during 17 days of seasonal anoestrus. All ewes received a single i.m. injection of 750 iu hCG on day 15. Serum samples taken twice a day for individual ewes, from each genotype, were expressed as an arithmetic mean. These daily, within-animal means were used to calculate a group arithmetic mean.

$Fec^B Fec^B$ and $Fec^+ Fec^+$ ewes, and $Fec^B Fec^+$ rates were intermediate (Table 1).

FSH concentration. In blood samples collected hourly, mean peripheral FSH concentrations during seasonal anoestrus did not differ significantly between genotypes (Table 1). $Fec^B Fec^B$ ewes had higher mean ($\pm$ SEM) peripheral FSH concentrations than did the other two genotypes, in samples collected twice a day (Fig. 1), but this difference was not significant. Mean ($\pm$ SEM) FSH concentrations in samples collected twice a day did not differ significantly between $Fec^B Fec^+$ and $Fec^+ Fec^+$ ewes (Table 2). Between groups, FSH concentrations did not vary significantly. Within animals, FSH concentrations did vary, but there was no consistent pattern to this variation. However, 3 of 9 $Fec^B Fec^B$, 1 of 8 $Fec^+ Fec^+$ and 1 of 8 $Fec^B Fec^+$ did exhibit a wave-type pattern in FSH release.

Table 3. Mean ($\pm$ SEM) ovulation rate and FSH concentration (ng ml^{-1}) in blood samples collected hourly during the oestrous cycle in backcross and F_2 populations of Scottish Blackface ewes, with and without the Fec^B gene

Parameter	Population	$Fec^B Fec^B$	Genotype $Fec^B Fec^+$	$Fec^+ Fec^+$
Ovulation rate	Backcross	—	3.5 ± 0.4^a (11)	1.7 ± 0.1^b (12)
Follicular phase FSH	Backcross	—	1.3 ± 0.1	1.2 ± 0.1
Luteal phase FSH	Backcross	—	1.9 ± 0.2	1.9 ± 0.2
Ovulation rate	F_2	4.4 ± 0.4^a (9)	2.4 ± 0.2^b (9)	1.6 ± 0.2^c (9)
Luteal phase FSH	F_2	3.5 ± 0.5^d	$2.7 \pm 0.2^{d,e}$	2.4 ± 0.3^e

Values within rows with different superscripts are significantly different, ^a versus ^b, ^b versus ^c, ^a versus ^c; $P < 0.001$; ^d versus ^e; $P < 0.05$.

Numbers of animals are given in parentheses.

Table 4. Mean ($\pm$ SEM) FSH concentrations (ng ml^{-1}) in blood samples taken twice a day during the oestrous cycle from backcross and F_2 populations of Scottish Blackface ewes, with and without the Fec^B gene

FSH	Population	$Fec^B Fec^B$	Genotype $Fec^B Fec^+$	$Fec^+ Fec^+$
Concentration	Backcross	—	1.8 ± 0.6	1.8 ± 0.6
Concentration peak amplitude*	F_2	4.6 ± 0.2^a	2.9 ± 0.3^b	3.0 ± 0.3^b
Concentration mean†	F_2	3.2 ± 0.3^c	2.5 ± 0.2^d	$2.7 \pm 0.2^{c,d}$

Values within rows with different superscripts are significantly different: ^a versus ^b; $P < 0.01$; ^c versus ^d; $P < 0.05$.

*Peak height was calculated from a quadratic equation fitted to time points (days $-1, 0, 1, 2$ and 3) for each ewe. Amplitude of the surge was calculated from a fitted curve for each sheep and averaged over genotype.

†Mean FSH concentration for days 5–13 of the oestrous cycle in each ewe was used to calculate the overall mean and SEM for each genotype.

Experiment 3: FSH concentration and ovulation rate during the oestrous cycle in the backcross population

Ovulation rate. Mean ovulation rates differed significantly ($P < 0.001$) between the genotypes (Table 3). A single injection of hCG, administered on day 8 of the oestrous cycle, resulted in an approximately 70% increase ($Fec^B Fec^+$ ovulation rate: 5.00; $Fec^+ Fec^+$ ovulation rate: 2.50) in the ovulation rate for each genotype, maintaining the significant ($P < 0.001$) genotypic difference.

FSH concentration. For each genotype, mean FSH concentrations in hourly blood samples were significantly lower ($P < 0.001$) on day -1 of the oestrous cycle than on day 7 (luteal phase) (Table 3). Between genotypes, there was no significant difference on day -1 or day 7 in mean peripheral FSH concentration in the hourly blood samples (Table 3). Mean serum FSH concentrations in blood samples collected twice a day from day -1 to day 10 also did not differ significantly between the genotypes (Table 4), but there was significant variation between days within sheep and between sheep within genotype.

Experiment 4: FSH concentration and ovulation rate during the oestrous cycle in the F_2 population

Ovulation rate. Mean ovulation rates differed significantly ($P < 0.001$) between genotypes (Table 3).

FSH concentration. Mean FSH concentrations in blood samples collected hourly during the luteal phase (day 6, 7 or 8) differed significantly ($P < 0.05$) between $Fec^B Fec^B$ and $Fec^+ Fec^+$ genotypes (Table 3). Mean ($\pm$ SEM) FSH concentrations for each genotype, in samples collected twice a day, from 3 days before until 13 days after behavioural oestrus (day 0), are shown in Fig. 2. In all three genotypes, FSH varied according to stage of the oestrous cycle, with the expected fall before day 0, and an increase around day 0, coincident with the expected timing of the preovulatory gonadotrophin surge. Mean FSH concentrations at about the time of the preovulatory gonadotrophin surge (day -1 to day 3) were significantly higher ($P < 0.01$) in $Fec^B Fec^B$ ewes than in the other two genotypes, whereas FSH concentrations in samples collected from day 5 to day 13 of the oestrous cycle were significantly higher ($P < 0.05$) in $Fec^B Fec^B$ ewes than in $Fec^B Fec^+$ ewes, and $Fec^+ Fec^+$ ewes were intermediate (Table 4).

Fig. 2. Mean ($\pm$ SEM) serum FSH concentration (ng ml^{-1}) from day -3 until day 13 of the oestrous cycle (day 0 is the day of oestrus), in $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ ($\bullet$), $\text{Fec}^{\text{B}}\text{Fec}^{+}$ ($\circ$) and $\text{Fec}^{+}\text{Fec}^{+}$ ($\square$) of the F_2 population possessing the Booroola fecundity gene ($n = 9$ per genotype). Serum samples taken twice a day for individual ewes were expressed as an arithmetic mean and then used to calculate a group arithmetic mean.

Experiment 5: ovulation rate and FSH concentration before and after ovariectomy during the breeding season in the backcross population

Ovulation rate. Mean ovulation rate on the day of ovariectomy (day 8 of the oestrous cycle) was significantly greater ($P < 0.05$) in $\text{Fec}^{\text{B}}\text{Fec}^{+}$ ewes than in the non-carriers (Table 5).

FSH concentration. From the parameters of Gompertz curves fitted to the data of each animal, mean peripheral FSH concentrations before or after ovariectomy (blood samples collected twice a day) did not differ between genotypes (Table 5). Within a genotype, FSH concentrations (blood samples collected twice a day) were significantly higher ($P < 0.001$) after removal of the ovaries (Fig. 3).

Experiment 6: ovulation rate and FSH concentration before and after ovariectomy during the breeding season in the F_2 population

Ovulation rate. On the day of ovariectomy (day 7 of the oestrous cycle), $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ ewes had significantly higher ($P < 0.01$) mean ovulation rates than did either $\text{Fec}^{\text{B}}\text{Fec}^{+}$ or $\text{Fec}^{+}\text{Fec}^{+}$ ewes; and the $\text{Fec}^{\text{B}}\text{Fec}^{+}$ ewes had significantly higher ($P < 0.05$) ovulation rates than did $\text{Fec}^{+}\text{Fec}^{+}$ ewes (Table 5).

FSH concentration. Mean peripheral FSH concentrations in blood samples collected twice a day before ovariectomy were significantly ($P < 0.05$) greater in $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ ewes than in $\text{Fec}^{\text{B}}\text{Fec}^{+}$ ewes (Table 5), but differences were not significant between $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ and $\text{Fec}^{+}\text{Fec}^{+}$ ewes. Mean serum FSH concentrations after ovariectomy, in blood samples collected twice a day, were significantly greater ($P < 0.001$) in $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ ewes than in the other two genotypes (Table 5), but were not significantly different between $\text{Fec}^{\text{B}}\text{Fec}^{+}$ and $\text{Fec}^{+}\text{Fec}^{+}$ ewes. Within each genotype, mean peripheral FSH concentrations increased significantly ($P < 0.001$) after ovariectomy (Fig. 4).

Seasonal comparisons between FSH concentration and ovulation rate in the backcross population

Ovulation rate. The hCG-induced ovulation rate during seasonal anoestrus was significantly ($P < 0.01$) lower than the non-induced ovulation rate during the breeding season in the heterozygous ewes. However, the hCG-induced ovulation rate during seasonal anoestrus in homozygous non-carriers was not significantly different from the non-induced ovulation rate during the breeding season.

FSH concentration. Mean FSH concentrations from blood samples collected twice a day, taken for the 2 days before hCG treatment, in heterozygous and non-carrier ewes were significantly greater ($P < 0.05$ and $P < 0.01$, respectively) during seasonal anoestrus than during the breeding season.

Seasonal comparisons between FSH concentration and ovulation rate in the F_2 population

Ovulation rate. Mean non-induced ovulation rates were significantly ($P < 0.05$) higher during the breeding season, compared with hCG-induced ovulation rates during seasonal anoestrus in $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ ewes. There was no seasonal difference in ovulation rate in either the $\text{Fec}^{\text{B}}\text{Fec}^{+}$ or $\text{Fec}^{+}\text{Fec}^{+}$ genotypes.

FSH concentration. Similarly, in the $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ genotype, serum FSH concentrations, from blood samples collected twice a day, were significantly ($P < 0.05$) higher during the breeding season compared with seasonal anoestrus. No such seasonal differences were present in the $\text{Fec}^{\text{B}}\text{Fec}^{+}$ or $\text{Fec}^{+}\text{Fec}^{+}$ genotypes.

Discussion

This series of experiments supports the hypothesis that differences in peripheral FSH concentrations are not wholly responsible for the genotypic differences in ovulation rate in Booroola Scottish Blackface ewes. In the breeding season, the mean FSH concentrations in the F_2 population were nearly always higher in the $\text{Fec}^{\text{B}}\text{Fec}^{\text{B}}$ line than in the other two genotypes, whereas they did not differ significantly between the heterozygous and the homozygous non-carriers, despite significant differences in ovulation rate. In the backcross population, mean FSH concentrations did not differ between the heterozygous and homozygous non-carriers (Expts 3 and 5) despite significant differences in ovulation rate. During seasonal anoestrus (Expt 2), ovulation rate differences between homozygote carriers and non-carriers in the F_2 population were not accompanied by significant differences in peripheral FSH concentrations. As in the F_2 population, there were no significant differences between heterozygotes and homozygous non-carriers in the backcross population in either ovulation rate or peripheral FSH concentrations.

The results of these studies suggest that the control of the genotypic differences in ovulation rate occurs at the ovary, since there was no general correspondence between mean FSH concentrations and ovulation rates, in the F_2 and backcross populations, during either the breeding season or seasonal

Table 5. Mean ($\pm$ SEM) ovulation rate and FSH concentrations (ng ml^{-1}), in blood samples collected twice a day, before and after ovariectomy, in Scottish Blackface ewes, with and without the *Fec^B* gene

Parameter	Population	<i>Fec^BFec^B</i>	Genotype <i>Fec^BFec⁺</i>	<i>Fec⁺Fec⁺</i>
Ovulation rate	Backcross	—	3.3 ± 0.5^a (<i>n</i> = 11)	1.7 ± 0.2^b (<i>n</i> = 12)
FSH concentration before ovariectomy*	Backcross	—	2.4 ± 0.2	2.6 ± 0.2
FSH concentration after ovariectomy*	Backcross	—	8.2 ± 0.3	8.5 ± 0.6
Ovulation rate	<i>F</i> ₂	3.9 ± 0.6^a (<i>n</i> = 9)	2.3 ± 0.2^b (<i>n</i> = 8)	1.3 ± 0.2^c (<i>n</i> = 8)
FSH concentration before ovariectomy	<i>F</i> ₂	2.7 ± 0.2^d	2.0 ± 0.2^e	$2.1 \pm 0.2^{d,e}$
FSH concentration after ovariectomy*	<i>F</i> ₂	13.0 ± 0.9^f	9.7 ± 1.19^g	10.0 ± 0.5^g

Within rows, values with different superscripts are significantly different. ^d versus ^e, ^f versus ^g; *P* < 0.05; ^a versus ^b, ^a versus ^c, ^b versus ^c; *P* < 0.01. Mean FSH concentrations were estimated from parameters of the Gompertz curve.

*For all genotypes, the FSH concentrations before and after ovariectomy are significantly different (*P* < 0.001).

Fig. 3. Mean ($\pm$ SEM) serum FSH concentration (ng ml^{-1}) before and after ovariectomy, in *Fec^BFec⁺* ($\circ$) and *Fec⁺Fec⁺* ($\square$) ewes of the backcross population (*n* = 11–12 per genotype). The arrow indicates the day of ovariectomy (day 8 of the oestrous cycle). Twice daily serum samples for individual ewes were expressed as an arithmetic mean and then used to calculate a group arithmetic mean.

Fig. 4. Mean ($\pm$ SEM) serum FSH concentrations (ng ml^{-1}) before and after ovariectomy, in *Fec^BFec^B* ($\bullet$), *Fec^BFec⁺* ($\circ$) and *Fec⁺Fec⁺* ($\square$) ewes of the *F*₂ population (*n* = 8 per genotype) from day -9 to day 14 of the oestrous cycle. Arrow indicates the day of ovariectomy, equivalent to day 7 of the oestrous cycle. Twice daily serum samples for individual ewes, from each genotype, were expressed as an arithmetic mean and then used to calculate a group arithmetic mean.

anoestrus. However, in agreement with a number of other studies, the expected falls in FSH concentrations during the follicular phase occurred. The comprehensive results for the *Fec^BFec⁺* and *Fec⁺Fec⁺* ewes from the study reported here extends and supports the results obtained by Driancourt and Fry (1990), who suggested that, despite genotypic differences in ovulation rate, FSH concentrations in samples taken at 8 h intervals, starting 24 h before prostaglandin administration on day 12 of the oestrous cycle for 60 h, did not differ between genotypes. Furthermore, Driancourt *et al.* (1985a) did not observe any significant difference in mean peripheral FSH concentration 72 h before oestrus (i.e., at the time of follicle recruitment) between genotypes. If the major gene is acting at the ovary, further study is required to determine the precise site of action, since no genotypic differences have been found in the ovarian response to FSH, FSH or LH receptor binding characteristics of granulosa cells, LH receptor binding characteristics to thecal cells, gonadotrophin-sensitive components of

the cAMP-generating system or LH-induced cAMP and steroid synthesis (see McNatty *et al.*, 1990).

Although gonadotrophins are required for growth of follicles, particularly those greater than 2 mm in diameter (Dufour *et al.*, 1979; McNeilly *et al.*, 1986), differences in the pattern of gonadotrophin secretion within the physiological range may not be responsible for differences in ovulation rate. For example, it is not known whether differences in FSH are the cause or the effect of differences in ovulation rate. Two studies in hypophysectomized ewes, one in Booroola Merino ewes (Fry *et al.*, 1988) and the other in Romanov and Ile de France ewes (Driancourt *et al.*, 1988), demonstrate that differences in ovulation rate can be maintained despite similar exogenous gonadotrophin treatment. These results suggest that high prolificacy may be due primarily to ovarian rather than to pituitary factors. A number of FSH isoforms have been identified that may differ in biological potency. However,

Robertson *et al.* (1984) reported no qualitative differences in pituitary FSH between Booroola Merinos and control Merinos of a different strain on day 3 of the oestrous cycle and Phillips *et al.* (1993) confirm that there are no overall differences in FSH isoforms between Booroola genotypes. However, these authors do report differences over days 13–16 of the oestrous cycle, so this explanation cannot be ignored completely.

Withdrawal of ovarian steroids by removal of the ovaries in ewes results in profound chronic and time-dependent changes in gonadotrophins. The effect of ovariectomy on peripheral LH concentrations has been well documented, with concentrations increasing after ovariectomy and then exhibiting a circorhal pattern (Butler *et al.*, 1972; Diekman and Malven, 1973; Davis and Borger, 1974; Joseph *et al.*, 1992). Similarly, there is a rapid rise in FSH concentration following ovariectomy (see Goodman and Karsch, 1981 for review; Driancourt *et al.*, 1987) which is independent of season (Webb *et al.*, 1985; Montgomery *et al.*, 1987; Joseph *et al.*, 1992). The relative increases in LH and FSH concentrations after ovariectomy are reported to be of similar magnitude within a population of sheep (Bolt, 1981). The data from this study demonstrated that there were no significant genotypic differences, in either the backcross or F_2 populations, in the time taken for FSH to reach a plateau, although, as in intact ewes, mean peripheral concentrations of FSH after ovariectomy were higher in $Fec^B Fec^B$ ewes compared with $Fec^B Fec^+$ and $Fec^+ Fec^+$ ewes, which had similar concentrations. Other studies demonstrate no differences in GnRH concentrations in hypothalamic and extra-hypothalamic areas of the brain in intact and ovariectomized Booroola ewes (Gale *et al.*, 1988) and in hypophyseal portal blood (McNatty *et al.*, 1993). The similarity in FSH concentration between $Fec^B Fec^+$ and $Fec^+ Fec^+$ ewes in the F_2 population was also observed in the backcross population. Although the ovary is involved in regulating the secretion of FSH within a specific genotype (Martin *et al.*, 1988), the genotypic differences within a population, in terms of peripheral FSH concentrations, in these studies were maintained in the absence of the ovary. This supports the suggestion of an action of the Booroola gene at the hypothalamus or pituitary gland, in addition to the ovaries. However, further study is required, since the pattern of FSH secretion changes significantly with time after ovariectomy (Webb *et al.*, 1985).

In agreement with results in other breeds of sheep (Webb and Gauld, 1985; Driancourt *et al.*, 1988, 1990; Webb *et al.*, 1989), the study reported here shows that ewes in Booroola Scottish Blackface populations can be induced to ovulate, during both seasonal anoestrus and the luteal phase of the oestrous cycle, with a single challenge of hCG (750 iu). The number of induced ovulations was generally representative of the genotype and supported our previous hypothesis that the mechanism controlling the number of follicles maturing to the ovulatory stage is operative throughout the year in sheep, including seasonal anoestrus (Webb *et al.*, 1985). There does appear to be some seasonal effect, since in the F_2 population, despite maintaining genotypic differences in ovulation rate, the hCG-induced ovulation rate and FSH concentrations were significantly lower during seasonal anoestrus than during the breeding season. However, this positive correlation between ovulation rate and FSH concentration was not supported by similar comparisons in the backcross population, in which a

lower hCG-induced ovulation rate during seasonal anoestrus in the $Fec^B Fec^+$ ewes was associated with significantly higher concentrations of FSH than in the breeding season.

The Booroola ewes in this study were approximately the same age, of similar body mass and received the same plane of nutrition, so as to minimize environmental effects on ovulation rate (Scaramuzzi and Radford, 1983). It is acknowledged, however, that there is substantial genetic variation between individuals such that, within a population, peripheral gonadotrophin concentrations may vary randomly. Even between genotypes, care in comparison must be exercised because of genetic variation between half-sibling families (the so-called 'sire effect') that may be independent of the trait of interest. The presence of such sire effects led us in these experiments to use the progeny from many F_1 sires. In addition, the female progeny used were all from heterozygous sires, thus genotypic comparisons were, in effect, all within sires. Hence there was no requirement to consider the genetic structure of the groups by analysing the data using Restricted Maximum Likelihood (REML; Patterson and Thompson, 1971).

In conclusion, the results of this study support the hypothesis that the Booroola gene exerts its effect both at the ovary and at the hypothalamus or pituitary gland. It is known that FSH acts synergistically with insulin and insulin-like growth factor I (IGF-I) to stimulate granulosa cell proliferation (Webb and McBride, 1991). Hence synergism between gonadotrophins and other peripheral hormones or locally produced factors such as IGF-I, may be of central importance (see Campbell *et al.*, 1995). From the analysis of ovine follicular growth patterns (Driancourt *et al.*, 1985b, 1986; McNatty *et al.*, 1990), it appears that high ovulation rate can be achieved through different pathways. The characterization of these pathways and demonstration of their importance will be achieved only by more detailed study of the control of follicular growth in Booroola genotypes that are of a known genetic background.

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