Differences in gonadotrophin concentrations and pituitary responsiveness to GnRH between Booroola ewes which were homozygous (FF), heterozygous (F+) and non-carriers (++) of a major gene influencing their ovulation rate

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Summary. The mean plasma concentrations of FSH and LH were significantly higher in FF ewes than in ++ ewes with those for F+ animals being consistently in between. These gene-specific differences were found during anoestrus, the luteal phase and during a cloprostenol-induced follicular phase, suggesting that the ovaries of ewes with the F-gene are more often exposed to elevated concentrations of FSH and LH than are the ovaries of ewes without the gene.

The gene-specific differences in LH secretion arose because the mean LH amplitudes were 2–3 times greater in FF compared to ++ ewes with the LH amplitudes for F+ ewes being in between. The LH pulse frequencies were similar. In these studies the pulsatile nature of FSH secretion was not defined.

The pituitary contents of LH during the luteal phase, were similar in all genotypes whereas for FSH they were significantly higher in the F-gene carriers compared to ++ ewes. The pituitary sensitivity to exogenous GnRH (0.1, 0.5, 5.0 and 25 µg i.v.) was related to genotype. Overall the LH responses to GnRH were lower in FF ewes than in ++ ewes with the results for the F+ ewes being in between. The FSH responses to all GnRH doses in the FF genotype were minimal (i.e. <2-fold). In the other genotypes a >2-fold response was noted only at the highest GnRH dose (i.e. 25 µg). Treatment of FF and F+ but not ++ ewes with GnRH eventually led to a reduced FSH output, suggesting that the pituitary responses to endogenous GnRH were being down-regulated in the F-gene carriers whereas this was not the case in the non-carriers.

Collectively these data confirm that peripheral plasma and the pituitary together with the ovary are compartments in which F-gene differences can be observed. In conclusion, these findings raise the possibility that F-gene-specific differences may also extend to the hypothalamus and/or other regions of the brain.

Introduction

High-fecundity Booroola ewes contain a major gene(s) (F) which influences their ovulation rate (see Bindon, 1984, for review). Homozygous (FF), heterozygous (F+) and non-carriers (++) of the putative gene have been segregated on the basis of at least one ovulation rate recording of ≥ 5 , 3 or 4 and 1 or 2 respectively (Davis *et al.*, 1982). The endocrine basis for the high ovulation rate in Booroola ewes has been the subject of several recent reports. It has been established that F-gene carriers have ovarian follicles with higher tissue levels of adenosine cyclic 3',5'-monophosphate

(cAMP) compared to those in similar-sized follicles from non-carriers (++) (McNatty *et al.*, 1986a). Also, ovarian follicles in F-gene carriers are more sensitive than those in ++ ewes to follicle-stimulating hormone (FSH) and luteinizing hormone (LH) with respect to cAMP synthesis (Henderson *et al.*, 1985; McNatty *et al.*, 1986a). Furthermore, in F-gene carriers, granulosa cells synthesize oestradiol, acquire functional LH receptors and the follicles ovulate at a smaller diameter than do those in ++ Booroola ewes (Henderson *et al.*, 1985; McNatty *et al.*, 1985b, 1986c). However, despite these differences at the ovarian level, it is not known whether the principal site(s) of F-gene expression are in the ovary or elsewhere.

It seems reasonable to expect that the anterior pituitary might be an important, if not principal, site of F-gene expression (Bindon, 1984) since FSH is known to exert a major influence on follicular viability, the level of oestradiol synthesis and the ovulation rate (Wright *et al.*, 1981; McNatty *et al.*, 1985a; Henderson *et al.*, 1987). Support for this notion has come from the studies of Robertson *et al.* (1984) and Bindon (1984), who showed that the pituitary FSH contents of Booroola Merinos were higher than in non-Booroola Merino controls. Consistent with these findings are those showing that the plasma concentrations of FSH in 30-day-old Booroola lambs were higher than in similarly aged non-Booroola lambs (Findlay & Bindon, 1976). In contrast, however, the plasma values of FSH in 60- and 90-day-old lambs did not differ between Booroola and non-Booroola Merinos. In mature ewes, the plasma and urine concentrations of FSH in Booroola Merinos have sometimes been found to differ from those in control Merinos (Bindon, 1984; Bindon *et al.*, 1985). However, these authors have also reported that the FSH values were often not different between the Booroola genotypes or between Booroola and control ewes, indicating some uncertainty as to whether FSH has an important role in influencing the ovulation rates of the F-gene carriers. With regard to LH there has been no evidence to indicate gene-specific differences (i.e. FF vs F+ vs ++) in the basal LH concentrations or in the number of LH pulses in ewes during the breeding season (Bindon, 1984).

The aims of this study were to measure the plasma concentrations of FSH and LH in FF, F+ and ++ Booroola Merino ewes during anoestrus and/or at different times during the oestrous cycle in an attempt to confirm or refute the notion that the FSH but not LH concentrations differed between the genotypes. In addition, the pituitary contents of FSH and LH, and the influences of gonadotrophin-releasing hormone (GnRH) in FF, F+ and ++ ewes were assessed.

Materials and Methods

Booroola Merino ewes born in 1973–1976, which had been assigned F+ or FF genotypes based on 4–5 laparoscopies according to the criteria of Davis *et al.* (1982), were mated with progeny tested FF or F+ Merino rams. The resultant female progeny that were used in the present experiments were examined laparoscopically each year on 4 or 5 occasions and then assigned to FF, F+ or ++ groups according to ovulation rates. All progeny of FF × FF crosses were found to have had at least one ovulation rate ≥ 5 and were included in the FF group. For all FF × F+ and F+ × F+ crosses the number and type of progeny with each phenotype was consistent with the genotypes expected from those matings (see Davis *et al.*, 1982). Sixteen of the ++ group were offspring of a progeny-tested ++ Merino-type Booroola ram mated with Merino ewes; all had ovulation rates of 1 or 2. The FF, F+ and ++ animals in this study were between 6 and 9 years of age and of proven parity.

Experiment 1 was designed to examine the hourly patterns of FSH and LH secretion as well as the pulsatile nature of LH secretion (at 10-min intervals) before and after a cloprostenol (125 µg s.c.; Coopers Animal Health, Upper Hutt, N.Z.) injection in FF (N = 12), F+ (N = 9) and ++ (N = 12) ewes on Day 10 of the oestrous cycle (May 1984). On the day before blood sampling, the ewes were penned indoors and each was fitted with an intrajugular cannula. When blood sampling began, the animals were bled (2.5 ml) via the jugular cannulae once hourly for 66 consecutive hours as well as for 10-min intervals from –6 to 0 h before or 6 to 12 h and 24 to 30 h after cloprostenol injection. Cloprostenol was injected after the first 6 h of sampling. Oestrous activity was recorded in all ewes by using two vasectomized rams with marking harnesses which were introduced to the ewes 24 h after cloprostenol injection. At 7 days after the end of the intensive blood sampling regimen, all animals were subjected to laparoscopy to determine their ovulation rate. The blood samples which were taken at –6, 0, 6, 18, 24 and 36 h were also assayed for progesterone to investigate the patterns of luteolysis in the different genotypes.

Experiment 2 was designed to examine the FSH concentrations during anoestrus ($N = 10$ ewes/genotype; during January 1984) as well as during the luteal phase (i.e. Days 6 to 10) of the oestrous cycle ($N = 35$ ewes/genotype; during April, 1984). The FF, F+ and ++ ewes were blood sampled via an intrajugular cannula (2 ml/collection) once hourly for 6 consecutive hours. The absence of corpora lutea in the anoestrous ewes was confirmed by laparoscopy 2 days before blood sampling began and also by the fact that the plasma progesterone values were <0.5 ng/ml on the day of blood sampling for FSH.

Experiment 3 was carried out to compare the pituitary FSH and LH contents in FF ($N = 10$), F+ ($N = 7$) and ++ ($N = 7$) ewes slaughtered on Day 10 of the oestrous cycle (i.e. between April and July, 1984). Each freshly dissected pituitary gland was weighed and approximately 50% of the gland (~ 0.4 – 0.5 g) was then finely cut with scissors and homogenized in a 1 mM aqueous solution of EDTA (5 ml) at room temperature. Subsequently, 0.5 ml aliquants of the homogenate were added to 12×75 mm plastic tubes containing an equivalent volume of a solution containing EDTA (1 mM), 0.5% (w/v) egg white and 30 μ l bacitracin (0.02 M). This method of extraction was based on that reported by McIntosh & McIntosh (1983a) for LH. However, the efficiency of this procedure for both LH and FSH was not assessed in our laboratory. These tubes were capped, and frozen to -20°C until assayed for LH and FSH by specific RIA.

Experiment 4 was designed to test the pituitary responsiveness of anoestrous (February, 1984) FF, F+ and ++ Booroola ewes to 0, 0.1, 0.5, 5.0 or 25 μ g GnRH (i.v.; Peninsular Laboratories Inc., Belmont, CA, U.S.A.; 2 ewes/genotype/dose). All animals were fitted with intrajugular cannulae the day before blood sampling began. For each ewe, 7 consecutive hourly blood samples were collected (2.5 ml/collection) before the GnRH injection. Thereafter the animals were bled every 10 min for the first hour after the GnRH injection and then every subsequent hour for another 8 h. The plasmas from the hourly blood samples were assayed for both FSH and LH which was also measured in the 10-min plasma samples. The absence of corpora lutea in the anoestrous ewes was confirmed by laparoscopy 2 days before GnRH treatment and by the presence of <0.5 ng progesterone/ml on the day of GnRH treatment.

Blood samples. All blood samples were centrifuged at 4000 g at room temperature for 20 min within 30 min of collection and the plasma samples were stored at -20°C until assayed.

Hormone assays

Progesterone. The radioimmunoassay procedure was identical to that described by McNatty *et al.* (1981). The antiserum (WA-26) was raised in an ovariectomized ewe against progesterone-11 α -hemisuccinate conjugated to bovine serum albumin and used at an initial dilution of 1:8000. Major cross-reacting steroids in the assay were 11 α -hydroxyprogesterone (120%), 11 β -hydroxyprogesterone (25%), 20 α -dihydroprogesterone (3.5%) and androstenedione (0.45%). The minimum detectable level of progesterone was 0.15 ng/ml. The intra- and interassay coefficients of variation were 10.6 and 14.0% respectively.

FSH. The radioimmunoassay kit was that supplied by The National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Bethesda, Maryland, U.S.A. The ovine (α) FSH for iodination was NIAMDD-oFSH-1-1, the oFSH reference preparation was NIAMDD-oFSH-RP-1 (biopotency $75 \times$ NIH-FSH-S1) and the oFSH antiserum was NIAMDD-anti-oFSH-1 (AFP-C5288113). At a final FSH antiserum dilution of 1:80 000 this homologous assay had a working range of 0.01 to 5 ng per assay tube. The volume of plasma which was assayed was 0.1 ml and each sample was assayed in duplicate. When assaying the pituitary homogenates for FSH, the homogenates were serially diluted 1/80, 1/160, 1/320, 1/640, 1/1280, 1/2560 in assay buffer (0.01 M-phosphate-buffered saline + 2% normal rabbit serum, pH = 6.8) and 50 μ l aliquants at each dilution in quadruplicate were assayed for FSH content. The intra- and interassay coefficients of variation were 6.3 and 9.6%.

LH. The radioimmunoassay for LH was identical to that described by McNatty *et al.* (1981). The LH antiserum was raised in a rabbit against NIH-LH-S11 and used at an initial dilution of 1:40 000. The antiserum exhibited low cross-reactivity reactions with NIH-P-S12 (0.09%), NIH-TSH-S8 (2.4%), NIH-GH-S11 (0.4%) and NIH-FSH-S10 (0.4%). The volume of plasma which was assayed was 0.2 ml and each sample was assayed in duplicate. When assaying the pituitary homogenates for LH, the homogenates were serially diluted 1/1000, 1/2000, 1/4000, 1/8000, 1/16 000, 1/32 000 in assay buffer (0.01 M phosphate buffer + 2% normal rabbit serum, pH 6.8) and 100 μ l aliquants were assayed for LH content. The minimum detectable level of LH was 0.3 ng/ml plasma. The intra- and interassay coefficients of variation were <10 and $<13\%$ respectively.

Data presentation and statistical analysis

When the raw data were normally distributed (i.e. as assessed from the N-score on Minitab), the results are expressed as means $\pm$ s.e.m., but when not normally distributed the results were either normalized by log transformation and presented as geometric means (and 95% confidence limits) or presented as medians (and 95% confidence limits). The only exception to this was for Fig. 1(a) in which the results are shown as means $\pm$ s.e.m. to enhance the visual presentation even though the data were analysed non-parametrically. Since all the experiments involved ewes between 6 and 9 years of age, the influence of age was tested but none was noted. Therefore, in all of the experiments (i.e. 1–4), the results for each genotype were pooled irrespective of age of the animals.

The FSH, LH and progesterone data between genotypes were compared by analysis of variance (ANOVA) in conjunction with the Neuman-Keuls test (Exps 1, 2 & 3), or by the Kruskal-Wallis test (Exp. 1). It is stressed that all genotypic differences were assessed against the between-animal data. The effects of the different genotypes (FF, F+ and ++ ewes) with respect to doses of GnRH on FSH and LH secretion (i.e. Exp. 4) were tested as follows. For each ewe, the mean FSH or LH value preceding the GnRH injection was obtained (i.e. the Xpre value). Thereafter each LH concentration over every 10 min for the first hour after GnRH injection, and for every subsequent hour for the remaining 8 h, was divided by the Xpre concentration to obtain a scaled value. Scaled values from each hourly FSH concentration after GnRH injection were similarly obtained. The scaled maximum values as well as the scaled areas encompassed by the LH and FSH concentrations after GnRH injection were examined by two-way ANOVA.

The episodic LH data (Exp. 4) were analysed by the Pulsar program of Merriam & Wachter (1982). The LH peaks were identified from the Pulsar program using the values of 3.8, 2.6, 1.9, 1.5 and 1.2 for G(1), G(2), G(3), G(4) and G(5) respectively. The assay s.d. terms for Pulsar were obtained as follows. The s.d. of replicate LH standards equivalent to 0.3, 0.4, 0.6, 0.8, 1.5, 3.3, 4.0, 4.5, 5.0, 6.0, 8.0 and 10.0 ng/ml (10 replicates/dose) were determined. The s.d. of each of the above values was then fitted to the corresponding mean values using least squares regression. This gave constant and linear values for the Pulsar program of 2.6 and 9.8 respectively; no quadratic term was needed. The smoothing time in the program was 12 h. The mean LH values described herein are equivalent to the 'smoothed mean' values from Pulsar (i.e. the baseline values after all the peak samples were omitted). The amplitude of each LH peak was the difference between the peak value and the aforementioned mean LH value.

Results

Hormone concentrations before and after a cloprostenol-induced follicular phase with respect to Booroola genotype (Exp. 1)

The mean $\pm$ s.e.m. ovulation rates in the FF (N = 12), F+ (N = 9) and ++ (N = 12) ewes after the cloprostenol-induced follicular phase were 4.5 ± 0.3 , 2.8 ± 0.1 and 1.3 ± 0.1 respectively.

Progesterone. At -6, 0, 6, 18, 24 and 36 h from cloprostenol injection, the respective mean ($\pm$ s.e.m.) plasma concentrations of progesterone were $2.5 (\pm 0.3)$, $2.3 (\pm 0.3)$, $1.2 (\pm 0.1)$, $0.6 (\pm 0.1)$, $0.4 (\pm 0.06)$ and $0.2 (\pm 0.03)$ ng/ml for the ++ ewes (N = 12), $2.7 (\pm 0.2)$, $2.7 (\pm 0.3)$, $1.1 (\pm 0.1)$, $0.7 (\pm 0.1)$, $0.4 (\pm 0.08)$ and $0.2 (\pm 0.06)$ ng/ml for the F+ ewes (N = 9) and $2.8 (\pm 0.2)$, $2.9 (\pm 0.4)$, $1.2 (\pm 0.1)$, $0.7 (\pm 0.09)$, $0.5 (\pm 0.04)$ and $0.3 (\pm 0.03)$ ng/ml for the FF ewes (N = 12).

FSH. These data are summarized in Fig. 1(a). The overall mean $\pm$ s.e.m. values before cloprostenol injection were 2.4 ± 0.3 , 1.6 ± 0.2 and 1.4 ± 0.2 ng/ml respectively for the FF, F+ and ++ ewes. The mean value for the FF ewes (N = 12) was significantly higher than that for the F+ ewes (N = 9; $P < 0.05$) and for the ++ ewes (N = 12; $P < 0.05$) with the values for the F+ and ++ ewes being not different from one another. For all genotypes, the mean FSH concentrations declined after the cloprostenol injection to reach basal values about 14 h later. The FSH concentrations obtained from the start of blood sampling until the rams were introduced were fitted to a regression line. The median (and 95% confidence limits) of the intercepts of these regression lines for FF, F+ and ++ ewes were 2.0 (1.7, 3.4), 1.5 (1.1, 2.0) and 1.5 (0.9, 1.7) ng/ml respectively; the median FSH value for the FF ewes was significantly higher than those for F+ and ++ ewes (both $P < 0.05$; Kruskal-Wallis test). The median (and 95% confidence limits) of the slopes of the regression lines for FF, F+ and ++ ewes were -0.06 (-0.03, -0.08), -0.03 (-0.02, -0.05) and -0.03 (-0.02, -0.06) ng ml⁻¹ h⁻¹ respectively; the slopes for the FF ewes (n = 12) were significantly steeper than those for F+ ($P < 0.05$; N = 9) or ++ ewes ($P < 0.05$; N = 12). From 36 h after the start of the sampling schedule the differences in the hourly mean FSH concentrations between the genotypes were no longer significantly different from one another.

Asynchronous, FSH peaks around the time of the preovulatory LH surge (see Fig. 1b) were apparent during the final 26 h of continuous blood sampling (i.e. from ram entry). For each animal, the 'maximum FSH value' and the time from cloprostenol injection to that maximum were determined. The median (and 95% confidence limits) of the FSH maximum values for FF, F+ and ++ ewes were 5.7 (3.7, 8.0), 3.4 (2.4, 7.4) and 4.3 (1.3, 10.7) ng/ml respectively; these FSH values were not significantly different from one another (Kruskal-Wallis test). The median (and 95% confidence limits) of the times from cloprostenol injection to the FSH maximum values for the FF, F+

Fig. 1. Changes in the mean plasma concentrations of FSH (a) and LH (b) before and after injection of cloprostenol (PG) with respect to Booroola genotype. All ewes were at Day 10 of the oestrous cycle when injected with PG. For FSH the shaded areas for the F++ and FF ewes represent $\pm$ s.e.m. For LH, the levels represent geometric means and the shaded areas represent the 95% confidence limits. For both hormones the shaded areas are equidistant each side of their respective means. For the sake of clarity the s.e.m. or 95% confidence limits were not shown for the F+ ewes. The number of ewes for both FSH and LH was 12 for FF, 9 for F+ and 12 ++ ewes.

and ++ ewes were 50 (46, 54), 56 (51, 60) and 44 (34, 52) h respectively; the median time for the F+ ewes was significantly longer ($P < 0.05$) than that for the ++ or FF ewes (Kruskal–Wallis test). The median (and 95% confidence limits) with respect to time of onset of oestrous behaviour for FF, F+ and ++ ewes occurred at 52, (40, 64), 57 (50, 74) and 61 (54, 74) h respectively after cloprostenol injection; these values were not significantly different from one another (Kruskal–Wallis test).

LH. The hourly geometric mean concentrations of LH before and after a cloprostenol-induced follicular phase with respect to genotype are summarized in Fig. 1(b). The upper 95% confidence limits for the FF ewes and lower 95% confidence limits for the ++ ewes are also indicated. The 95% confidence limits for the F+ ewes were not included for the sake of clarity. The mean LH

values in the FF ewes were always higher than in the ++ ewes with those in the F+ ewes being in between. However, when the individual mean LH concentrations were compared with respect to genotype throughout the 66-h sampling period, the means were significantly different from one another ($P < 0.05$; ANOVA) on only a few occasions (i.e. 19.4%). For all genotypes the mean LH values increased gradually after cloprostenol injection until the onset of the preovulatory LH surge. From the hourly means there was no obvious convergence of the LH values before the preovulatory LH surge.

The mean $\pm$ s.e.m. times from cloprostenol injection to the onset of the preovulatory LH surge for the 3 genotypes was 44 ± 2 , 48 ± 2 and 46 ± 3 h for FF, F+ and ++ ewes respectively; these times were not significantly different from one another. It was not possible to assess either the mean peak heights or the mean durations of the preovulatory LH surges for each of the genotypes. The

Table 1. Characteristics of plasma LH concentrations before and after cloprostenol (PG) injection (time 0) in Booroola ewes on Day 10 of the oestrous cycle with respect to Booroola genotype

Time from PG injection (h)	Booroola genotype	Mean LH† conc. (ng/ml)	No. of LH peaks/6 h	Amplitude of LH peaks (ng/ml)	Mean peak length (min)
-6 to 0	++	0.7 ± 0.1^a	1.9 ± 0.4^a	1.1 ± 0.1^a	18 ± 2^a
	F+	$0.8 \pm 0.1^{a,b}$	1.9 ± 0.8^a	$1.3 \pm 0.4^{a,b}$	31 ± 7^a
	FF	1.0 ± 0.05^b	2.8 ± 0.6^a	2.3 ± 0.3^b	27 ± 5^a
6 to 12	++	0.9 ± 0.1^d	3.0 ± 0.5^a	1.3 ± 0.1^d	20 ± 2^a
	F+	$1.2 \pm 0.1^{d,e}$	3.2 ± 0.4^a	$1.6 \pm 0.4^{d,e}$	24 ± 4^a
	FF	1.3 ± 0.1^e	3.4 ± 0.6^a	2.8 ± 0.4^e	30 ± 3^a
24 to 30	++	1.1 ± 0.1^a	2.8 ± 0.6^a	1.2 ± 0.1^d	23 ± 5^a
	F+	1.4 ± 0.2^a	4.1 ± 0.7^a	$2.1 \pm 0.6^{d,e}$	17 ± 2^a
	FF	1.4 ± 0.1^a	3.4 ± 0.7^a	3.3 ± 0.6^e	20 ± 3^a

†Mean level (i.e. the 'smoothed mean') was calculated from the baseline values after all the peak samples were omitted.

Values in each column and within each time frame not sharing a common superscript were significantly different from one another. d vs e = $P < 0.05$; a vs b = $P < 0.01$ (ANOVA, Neuman-Keuls test). Values with common superscripts are not different from one another.

Table 2. FSH concentrations (ng/ml) in Booroola ewes with respect to genotype and reproductive status

Reproductive status	Booroola genotype			No. of ewes per genotype
	++	F+	FF	
Days 6-10 oestrous cycle	1.5 ± 0.1^a	$1.8 \pm 0.1^{a,b}$	2.1 ± 0.2^b	35
Anoestrus	1.3 ± 0.1^a	$1.8 \pm 0.2^{a,b}$	2.5 ± 0.3^b	10

Values are means $\pm$ s.e.m.

++, F+, FF are non-carriers, heterozygous carriers or homozygous carriers respectively of the Booroola F-gene. The FSH value for each ewe was the average from 6 consecutive blood samples collected at hourly intervals. For each row a vs b = $P < 0.01$ (ANOVA with Neuman-Keuls test). Numbers sharing the same superscript are not different from one another.

peak LH values exceeded the assay sensitivity and, in some instances, the preovulatory secretory episodes were still elevated at the end of the LH sampling period.

The characteristics of the plasma LH concentrations with respect to mean levels, LH peak, frequency and amplitude and mean LH peak length are summarized in Table 1. At -6 to 0 h and 6 to 12 h from cloprostenol injection (at time 0), the mean concentrations of plasma LH and the mean amplitudes of the LH peaks in FF ewes were significantly higher than in ++ ewes (i.e. both $P < 0.05$; Table 1) with the respective values in F+ ewes being in between the other two genotypes. At 24–30 h, the mean amplitudes of the LH peaks but not the mean LH concentrations in FF ewes were significantly higher ($P < 0.05$) than those in the ++ ewes with those for F+ ewes being in between. In all three time frames, no significant gene-specific differences were noted in the number of LH peaks per 6 h or in the mean peak lengths. For each genotype, the mean LH concentration for the 24–30 h interval after cloprostenol injection was significantly higher than that for the -6 to 0 h interval ($P < 0.05$ for all genotypes) with that for the 6–12 h interval being intermediate for each of the genotypes.

FSH concentrations with respect to Booroola genotype and time of year (Exp. 2)

These data are summarized in Table 2. During both the luteal phase of the oestrous cycle and anoestrus, FF ewes contained significantly higher mean plasma FSH concentrations compared to those in ++ ewes with the concentrations in the F+ animals being in between the other two genotypes. Although the overall FSH means were significantly different with respect to genotype, the range of mean FSH values between sheep within each genotype was 0.8–4.3, 0.6–3.0 and 0.6–3.5 ng/ml for the FF, F+ and ++ animals respectively. The likelihood of determining Booroola genotype in an individual ewe from one or more blood samples during anoestrus or the luteal phase is therefore minimal. In the ewes during the luteal phase, the mean $\pm$ s.e.m. number of corpora lutea in the FF, F+ and ++ genotypes ($N = 35$ ewes/genotypes) were 4.7 ± 0.2 , 3.1 ± 0.1 and 1.2 ± 0.07 respectively.

Immunoreactive FSH and LH contents in the pituitary glands of Booroola ewes (Exp. 3)

The geometric mean (and 95% confidence limits) for the FSH pituitary contents in ++ ($N = 8$), F+ ($N = 7$) and FF ($N = 7$) ewes were 26 (20,32), 52 (34,82) and 36 (22, 60) μ g/pituitary gland respectively. The FSH content in F+ ewes was significantly higher than in ++ ewes ($P < 0.05$) but not different from that in FF ewes. The geometric mean (and 95% confidence limits) for the LH pituitary contents in the above ++, F+ and FF ewes were 590 (473, 735), 493 (302, 804) and 889 (578, 1366) μ g/gland respectively. For LH there were no significant differences between the genotypes.

The mean $\pm$ s.e.m. number of corpora lutea in the aforementioned ++, F+ and FF ewes were 1.4 ± 0.2 , 3.3 ± 0.3 and 4.9 ± 0.3 respectively.

Effects of GnRH on the plasma concentrations of LH and FSH with respect to GnRH dose and Booroola genotype (Exp. 4)

The mean $\pm$ s.e.m. concentrations for LH in the FF, F+ and ++ ewes before GnRH injection were 1.7 ± 0.1 , 1.1 ± 0.1 and 0.9 ± 0.05 ng/ml ($N = 10$ /genotype). The concentrations in FF ewes were significantly higher than those in F+ ($P < 0.05$) and ++ ewes ($P < 0.01$; ANOVA, Neuman-Keuls test); the concentrations in F+ and ++ ewes were not significantly different from one another. At 10 min after GnRH injection, and irrespective of GnRH dose, all LH values were elevated (> 2.5 -fold) with respect to the pretreatment values. Thereafter, depending on the GnRH dose, the LH values remained elevated for between 0.5 and 5.0 h; after these times the plasma LH concentrations returned to the pretreatment levels.

Fig. 2. Changes in the scaled LH peak values (a) and scaled LH peak areas (b) in individual ++ (□), F+ (○) and FF (△) ewes with respect to GnRH dose. The lines (---, ++ ewes; ---, F+ ewes; —, FF ewes) are drawn through the midpoints of the results for the 2 ewes at each GnRH dose. The results were scaled to normalize the results between genotypes (see 'Statistical procedures') since the pretreatment values were related to genotype.

The scaled LH peak values and peak areas following GnRH injection are shown in Fig. 2. The values were scaled as described in 'Materials and Methods' to normalize the results between genotypes because the pretreatment LH values were related to genotype. For the ++ ewes, 7 out of 8 produced higher scaled peak and area values than those for the FF ewes with the results for the F+ ewes being in between. When a two-way ANOVA was performed on these data (i.e. genotype vs GnRH dose; Fig. 2), significant effects of genotype ($P < 0.01$), GnRH dose ($P < 0.01$) and genotype $\times$ dose interaction ($P < 0.01$) were noted with respect to both the scaled LH peak values and scaled LH peak areas.

The mean $\pm$ s.e.m. concentrations for FSH in the FF, F+ and ++ ewes before GnRH injection were 2.5 ± 0.1 , 1.8 ± 0.1 , 1.3 ± 0.1 (N = 10 sheep/genotype). The concentrations in FF ewes were significantly higher than those in F+ ($P < 0.05$) and ++ ewes ($P < 0.01$; ANOVA, Neuman Keuls test); the mean concentrations for F+ and ++ ewes were not significantly different from one another.

Irrespective of GnRH dose (i.e. 0, 0.1, 0.5, 5.0 and 25.0 μg) there was little or no increase (i.e. < 2 -fold) in plasma FSH concentrations in the FF ewes (N = 10) relative to the pretreatment values. Similarly, in the F+ (N = 10) and ++ ewes (N = 10), the FSH responses were minimal (i.e. < 2 -fold) for all but the highest GnRH dose (i.e. 25.0 μg). After the 25 μg injection, the scaled peak FSH values in the ++ and F+ ewes (N = 2 ewes/genotype) increased 2.5- to 4.3-fold; in these animals FSH was elevated for the first 3 h after GnRH treatment. The scaled FSH peak areas followed a pattern similar to those described for the peak values. Thereafter, in these 4 animals and

in all the others, the FSH concentrations either returned to the pretreatment values or to concentrations lower than those before treatment. At 5–8 h after GnRH injection, the FSH values sometimes declined to concentrations which were only 30% of pretreatment concentrations. When the frequency of these low values was compared with respect to genotype and GnRH dose, a significant effect of genotype ($P < 0.05$) but not dose or genotype $\times$ dose interaction, was observed. The average numbers of low FSH values after GnRH were 3.7, 2.5 and 1.0 (s.e.d. = 0.94; $N = 10$ ewes/genotype) for FF, F+ and ++ ewes. Therefore, compared to ++ but not F+ ewes, treatment of FF ewes with GnRH eventually resulted in a greater frequency of low FSH concentrations relative to those before treatment.

Discussion

These studies show that the mean plasma concentrations of FSH and LH are significantly higher in FF Booroola ewes than in ++ Booroola ewes with those of F+ animals being consistently in between. These gene-specific differences in mean values were found during anoestrus, the luteal phase and during a cloprostenol-induced follicular phase. These differences in plasma FSH and LH were noted for the overall means for each genotype but only after frequent blood samplings from each animal. However, as can be noted from the results of Exps 1 and 2, these differences are not always obvious in individual animals. Nevertheless, it seems likely that the ovaries of ewes with the F-gene are more often exposed to high concentrations of FSH and LH than is the case in ++ ewes. These studies have confirmed that peripheral blood and the pituitary together with the ovary are compartments in which F-gene differences can be observed (Bindon, 1984). However, they do not establish whether the F-gene differences in pituitary function are a cause or a consequence of F-gene expression in some other tissue such as the ovary or hypothalamus.

It is possible that some of the ewes were misclassified with respect to genotype. Davis *et al.* (1982) found that 3% of New Zealand Merino ewes with a mean $\pm$ s.e.m. ovulation rate of 1.39 ± 0.02 may have a triple ovulation at least once in their lifetime and 7% of F+ ewes from the same flock were misclassified as FFs. Ewes in the present study were also generated from the same sources of animals reported in the above study. It therefore seems reasonable to assume that the numbers of misclassified ewes in the present study were small. Moreover, errors of the aforementioned magnitudes are unlikely to affect the overall conclusions of this study since they would tend to reduce the observed differences between the three groups.

In Romney ewes, exogenous FSH is known to increase oestrogen synthetase (aromatase) activity in granulosa cells from 3–4.5 mm diameter follicles to levels similar to those in cells from preovulatory follicles (i.e. ≥ 5 mm diam.) of the controls (McNatty *et al.*, 1985a). Perhaps the exposure of ovaries in FF and F+ ewes to higher FSH concentrations compared to that in ++ ewes is the reason why peak levels of aromatase activity as well as LH receptors are observed in the granulosa cells of 3–4.5 mm diameter follicles in F-gene carriers whereas in ++ ewes these characteristics are not observed unless the granulosa cells are recovered from follicles ≥ 5 mm diameter (Henderson *et al.*, 1985; McNatty *et al.*, 1986a). If this is so, then the elevated FSH concentrations in F-gene carriers may be partly responsible for the maturation of follicles at smaller diameters (i.e. 3–5 mm) than in ++ ewes (i.e. ≥ 5 mm).

In contrast with previous reports (see Bindon, 1984, for review), the present studies have shown a consistent correlation between plasma LH concentrations and Booroola genotype. In the earlier studies, the blood sampling frequencies were at 20-min intervals and so possible gene-specific differences in LH amplitude may have been missed. Moreover, it is difficult to discern from the earlier studies the accuracy of the genotypic classifications. And, as mentioned earlier, errors due to misclassification may mask any putative gene-specific differences in gonadotrophin secretion. In the present study, the gene-specific differences in LH secretion arose because the amplitude of each LH pulse was 2–3 times greater in FF compared to ++ ewes, with those for F+ ewes being in

between. In all three genotypes, the LH pulse frequencies were similar. The amplitudinal differences were recorded during the luteal and cloprostenol-induced follicular phases. It is likely that the differences in LH amplitude also occur during anoestrus as there were gene-specific differences in mean LH values at this time (see 'Results' for Exp. 4). The reasons why the LH amplitude differed between the genotypes are unclear. LH pulse amplitude (but not LH pulse frequency) as well as FSH secretion has been reported to be inhibited after the administration of sheep follicular fluid to ovariectomized ewes (Clarke *et al.*, 1986). It has been suggested that differences in the production of ovarian inhibin, a protein present in high concentrations in follicular fluid, may lead to F gene-specific differences in FSH secretion and thereby the ovulation rate in Booroola ewes (Cummins *et al.*, 1983; Bindon, 1984). Perhaps differences in the production of a follicular protein (i.e. inhibin?) may also be responsible for the gene-specific differences in LH pulse amplitude. However, an alternative explanation might be that the above differences in LH pulse amplitude are indicative of gene-specific differences in the amplitudes and/or the widths of the hypothalamic GnRH pulses (McIntosh & McIntosh, 1983b), independent of inhibin feedback.

Notwithstanding the underlying mechanisms leading to gene-specific differences in LH pulse amplitudes these might be important for stimulating a level of steroid synthesis in F-gene carriers comparable to that in ++ ewes. The ovarian secretion rates of androstenedione, testosterone and oestradiol are not significantly different between the genotypes during the luteal- or cloprostenol-induced follicular phase (McNatty *et al.*, 1985b; unpublished data). Moreover, it is known that granulosa cell oestradiol synthesis is critically dependent on theca interna androgen synthesis (Baird, 1977) and that, under in-vitro conditions, thecal androgen output is directly proportional to the wet weight of tissue (McNatty *et al.*, 1984). The respective mean $\pm$ s.e.m. wet weights (mg) of theca interna recovered from all follicles ≥ 1 mm in diameter in FF, F+ and ++ ewes were 10.0 ± 0.6 (N = 10 sheep), 12.0 ± 0.7 (N = 12) and 15.5 ± 1.0 (N = 15) (K. P. McNatty, unpublished data). On a per unit wet weight basis, the androgen output from theca interna was found to be the same for all genotypes (McNatty *et al.*, 1985b, 1986b). Although the amount of thecal tissue is smaller in the FF or F+ ewes than in ++ ewes, the steroidogenic responses in the former might be compensated by the higher amplitude LH pulses than those delivered to the thecae in ++ ewes. In part, this notion is supported by the finding that, under in-vitro conditions, the output of thecal androstenedione from ovine theca interna is directly correlated with LH pulse amplitude (McNatty *et al.*, 1986d).

An unexpected result in the present study was that the pituitary sensitivity to exogenous GnRH in anoestrous Booroola ewes was influenced by genotype. With regard to the LH responses to GnRH, the FF ewes did not respond as well as the ++ ewes, with the F+ ewes being in between. With regard to FSH, none of the FF ewes produced an appreciable FSH response (i.e. > 2 -fold) to any of the GnRH doses. For the other genotypes (F+, ++) only the highest GnRH dose (i.e. 25 μ g) produced an appreciable FSH response (i.e. > 2 -fold). The finding that GnRH caused a decrease in FSH output in the FF ewes, and to a lesser extent in F+ ewes, suggests that their pituitary responses to endogenous GnRH were being down-regulated whereas this was not the case in the ++ ewes. The lower FSH and LH outputs in the FF and F+ ewes are unlikely to have been due to an exhaustion of pituitary reserves since, in luteal-phase ewes at least, the pituitary contents of LH and FSH were either similar in all genotypes or higher in the F-gene carriers compared to those in ++ ewes (see 'Results' for Exp. 3; and also Bindon, 1984; Robertson *et al.*, 1984). Collectively, these findings from the GnRH experiments suggest that the pituitary glands in F-gene carriers may experience a different pattern of endogenous GnRH secretion relative to that in ++ ewes. However, the possibility cannot be discounted that the pituitary glands in the F-gene carriers have a different level of sensitivity to GnRh than is the case in the ++ ewes.

In conclusion, the present studies have shown that significant gene-specific differences exist in the plasma concentrations of FSH and LH and for LH, at least, that these differences are due to the pituitary release of LH at higher amplitudes in F-gene carriers than in ++ ewes. These findings, together with those showing gene-specific differences in pituitary sensitivity to GnRH raise the

possibility that gene-specific differences between Booroola ewes with and without the F-gene may also extend to the hypothalamus and/or other regions of the brain.

We thank the National Hormone and Pituitary Program, and the National Institute of Diabetes and Digestive and Kidney Diseases, Maryland, U.S.A. for supplying the ovine FSH assay kit and ovine LH standards; our colleagues at the Invermay Agricultural Research Centre, Mosgiel, N.Z., and especially Mr G. Davis, Dr J. Owens and Ms J. Armstrong for supplying the Booroola ewes and providing the lifetime reproductive records of these animals, Dr P. Truman and Mr G. Davis for advice during preparation of the manuscript, Dr D. Thurley, Mr D. Heath, Mrs L. O'Keeffe, Mr S. Lun, Mrs J. McDiarmid, Miss M. Prisk and Mrs F. Collins for assistance with the blood sampling, laparoscopy, animal care and/or hormone assays, and Mrs P. Cattermole for typing the manuscript.

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Received 20 November 1986