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GnRH-induced gonadotrophin secretion in ovariectomized Booroola ewes with hypothalamic–pituitary disconnection

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Summary. To test whether the F gene-specific differences in the plasma concentrations of FSH and LH are due to differences in the pituitary responsiveness to exogenous GnRH, ovariectomized Booroola ewes with hypothalamic–pituitary disconnection (HPD–ovx) were treated with GnRH (250 ng i.v.) once every 2 h for up to 5 weeks. In Exp. 1, jugular venous blood was collected once weekly from 13 FF and 14 ++ HPD–ovx ewes for 6 weeks before GnRH treatment and every 2nd, 3rd or 6th day for 5 weeks during treatment. In Exp. 2, jugular venous blood was collected from another 8 FF and 7 ++ HPD–ovx ewes at 5- or 10-min intervals over 4 GnRH pulses (250 ng i.v. once every 2 h) on 3 separate occasions after the animals had been subjected to the GnRH pulse regimen for ~7 days beforehand. Also in Exp. 2, the animals were extensively sampled around a larger (10 µg) i.v. injection of GnRH and the pituitary FSH and LH contents assessed after the animals had been re-exposed to the once every 2 h GnRH (250 ng i.v.) pulse regimen for several days following the larger GnRH bolus. In Exp. 3 the distributions of mean plasma concentrations of FSH and LH in individual GnRH-treated HPD–ovx ewes were compared with those in ovariectomized and ovary-intact FF and ++ ewes.

During the 6 weeks before GnRH treatment (Exp. 1), the plasma concentrations of FSH (~1 ng/ml) and LH ($\leqslant 0.8$ ng/ml) were not different between the genotypes. After GnRH treatment both the mean FSH and LH concentrations increased significantly ($P < 0.01$) above basal values after 2 days with F gene-specific differences being noted for FSH but not LH (FSH; FF > ++; $P < 0.05$). Thereafter, the mean FSH but not LH concentrations increased at a faster rate in FF than in ++ ewes with the overall mean FSH concentrations between the genotypes being significantly different ($P < 0.05$).

In Exp. 2 considerable between-animal variation in the pulsatile pattern of FSH but not LH concentrations was seen in ewes of both genotypes during GnRH treatment. The overall mean FSH concentrations were higher in FF than in ++ ewes ($P < 0.05$) and the mean FSH response to each GnRH pulse was significantly higher in FF than in ++ ewes ($P < 0.05$). For LH a trend towards higher mean peak amplitudes and peak areas was noted in FF than in ++ ewes but no significant differences were noted. Also, no gene differences were observed in the LH or FSH responses to the 10 µg i.v. GnRH bolus or the pituitary contents of FSH and LH.

In Exp. 3, ≥75% of the FF ewes had higher mean FSH concentrations than in 50% of the ++ animals irrespective of treatment (i.e. GnRH–HPD–ovx, ovariectomized or ovary-intact controls; $P < 0.05$). Likewise, ~50% of the FF ewes had higher mean concentrations of LH than did 75% of the ++ ewes for all treatments.

These findings suggest that an association between the Booroola F gene and GnRH-induced FSH secretion exists, but whether such an association also exists for

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LH remains uncertain. These data support the hypothesis that at least part of the expression of the F gene is at the level of the pituitary gland to affect its responsiveness to physiological concentrations of GnRH.

Keywords: GnRH; FSH, LH; Booroola ewes; F gene; hypothalamic–pituitary disconnection; ovariectomy

Introduction

Booroola ewes possess a major gene (F) which influences their ovulation rate (i.e. the number of ovulations per oestrous cycle; see Bindon, 1984, for review). Homozygotes (FF), heterozygotes (F+) and non-carriers (++) of the F gene(s) are segregated on the basis of ovulation-rate recordings of ≥5, 3 or 4 and 1 or 2 respectively (Davis *et al.*, 1982). Previous studies have demonstrated F gene-specific differences in plasma gonadotrophin concentrations in ovary-intact as well as ovariectomized Booroola ewes (McNatty *et al.*, 1987, 1989a). These studies showed that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were more frequently higher in FF than in ++ ovariectomized ewes. However, no evidence has been found from studies of plasma clearance rates of FSH, FSH isoforms or steroid or inhibin feedback effects to suggest that the differences in plasma FSH and LH concentrations are attributable to these factors (Robertson *et al.*, 1984; Fry *et al.*, 1987; McNatty *et al.*, 1989b). Nevertheless, the data from ovariectomized ewes suggest that the F gene may influence gonadal function via some extraovarian site such as the pituitary gland.

The ovariectomized ewe with hypothalamic–pituitary disconnection (HPD) prepared as described by Clarke *et al.* (1983, 1984) is an experimental animal deficient in endogenous gonadotrophin-releasing hormone (GnRH) and devoid of ovarian hormones but with a functionally intact pituitary gland. Using this experimental model, it is possible to test whether the F gene-specific differences in plasma FSH and LH concentrations in ovariectomized ewes (McNatty *et al.*, 1989a) are due to differences in the pituitary responsiveness to exogenous GnRH.

The aim of the present studies was to examine plasma FSH and LH concentrations in HPD–ovariectomized Booroola FF and ++ ewes both before and after treatment with GnRH. A secondary aim was to compare the FSH and LH values in GnRH-treated HPD–ovariectomized Booroola ewes with those in ovariectomized and ovary-intact Booroola FF and ++ ewes.

Materials and Methods

Animals and experiments. Booroola × Merino (N = 77) and Booroola × Romney ewes (*N* = 45) were classified as being of the ++ (N = 64) or FF (N = 58) genotypes based on pedigree analysis; the ovulation rates of ≥5·0 for the FF and 1–2 for the ++ genotypes were confirmed by laparoscopy as described by Davis *et al.* (1982). The mean (±s.e.m.) ages of the ++ and FF genotypes were 6·4 (±0·05) and 6·1 (±0·5) years respectively. For each experiment, FF and ++ animals were matched as closely as possible with respect to breed and age. The FF and ++ ewes originated from 28 and 31 different sires respectively, with none of the sires contributing more than 6 and 8 of the FF and ++ animals respectively. In the HPD–ovariectomized experiments (Exps 1 and 2), none of the sires contributed more than 2 and 4 of the FF and ++ animals respectively.

Approximately 2–6 weeks after ovariectomy (ovx), 21 FF and 21 ++ Booroola ewes were subjected to HPD surgery as described by Clarke *et al.* (1983) in accordance with the 1987 Animals Protection (Codes of Ethical Conduct) Regulations of New Zealand after approval was granted by the Animal Ethics Committee at the Wallaceville Research Centre.

Experiment 1 was designed to test the effects of long-term pulsatile GnRH administration on plasma FSH and LH concentrations in 13 FF and 14 ++ HPD–ovx ewes. The GnRH (250 ng per pulse; pulse duration ~1 min) was administered via a jugular venous cannula (Silastic tubing; Cat. No. 602-285; Dow Corning Corporation, Midland, MI, USA). For this experiment all the HPD–ovx animals were blood sampled by venepuncture once weekly for 6 weeks before GnRH administration and then via a jugular venous cannula (contralateral to the GnRH infusion cannula) every 2nd, 3rd or 6th day during GnRH treatment for 5 weeks. Whilst receiving GnRH the animals were bled at random with respect to the time of GnRH administration. On a separate occasion, 6 weeks after the above

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experiment had been completed, some of the above animals (N = 6/genotype selected at random) were given the infusion solution devoid of GnRH for 14 days and all were bled every 2nd or 3rd day for FSH and LH.

Experiment 2 was designed to examine the short-term changes in plasma FSH and LH concentrations in Booroola ewes before and after exogenous GnRH administration. For this experiment, another 8 FF and 7 + + HPD–ovx animals were each fitted with a jugular venous cannula and treated with GnRH (250 ng per pulse) at a frequency of 1 pulse per 2 h beginning 1 day after HPD surgery. After 6 days, a second contralateral (jugular venous) cannula was inserted for blood sampling. This second cannula was kept patent with heparinized (75 U/ml) saline. On Day 7, blood samples (3 ml) were collected over 4 GnRH pulses, administered by hand, 5 min before and at 5, 10, 15 and 20 min and then at 10-min intervals until 110 min after each pulse. This GnRH pulse regimen as well as the blood-sampling regimen were repeated on two further occasions in the same animals at 4-month intervals. At the end of the 3rd intensive sampling period the animals were subjected to a bolus injection of GnRH (10 μg i.v.) with blood samples being collected at 10-min intervals for 30 min before the GnRH bolus and for 90 min afterwards and thereafter at 15-min intervals for a further 3 h. After this test, the animals were put back on the 250-ng GnRH pulse regimen for a further 3 days, at which time they were slaughtered and their pituitaries recovered and stored at −70°C until extraction and assay for FSH and LH contents.

For Exps 1 and 2, the GnRH solution for infusion was prepared each day and consisted of GnRH (Peninsula Laboratories Inc., Belmont, CA, USA) dissolved in sterile isotonic saline containing ovine serum albumin (0·1% w/v, Sigma Chemical Co., St Louis, MO, USA). The purpose of the serum albumin was to minimize retention of GnRH by the cannula. The flasks containing the infusate were covered with surgical gauze and kept as cool as possible by partial immersion in iced water to minimize the growth of any airborne contaminants. The total volume infused during each pulse (i.e. ~1 min duration) was 2·6 ml which was approximately twice the dead volume of the pump line. The GnRH content of the bottle as well as the GnRH content traversing the infusion lines were monitored periodically and confirmed to be correct by the GnRH radioimmunoassay procedure as described by Li *et al.* (1989). Moreover, at the end of the infusion, some of the bottles containing residual GnRH were screened to check that they were free of bacterial contamination.

The aim of Exp. 3 was to collect data on plasma FSH and LH concentrations from ovary-intact Booroola FF (N = 37) and + + (N = 43) ewes and to compare their means with those obtained for the GnRH-treated HPD–ovx ewes in Exp. 1 and also with those obtained previously for ovariectomized FF and + + ewes (McNatty *et al.*, 1989a). In this experiment the ovary-intact animals were blood sampled via the jugular vein on 3 occasions at 2–3-day intervals during the luteal phase of a spontaneous cycle (i.e. Days 5–13; Day 0 = day of oestrus). All animals in these studies were of similar age and originated from the same breeding flocks at the Invermay Agricultural Research Centre, NZ.

Blood samples. All blood samples were centrifuged at 4000 ***g*** at room temperature for 5–10 min within 20 min of collection and the plasma stored at −20°C until assayed.

Pituitary FSH and LH contents. Approximately 100-mg portions of pituitary were homogenized in 15 ml chilled (4°C) potassium phosphate buffer (5 mM) containing phenylmethyl sulphonyl fluoride (PMSF, 1 mM; Sigma) and bovine serum albumin (0·1% w/v; ICP Ltd, Auckland, New Zealand) (pH 7·4) and then centrifuged for 30 min at 30 000 ***g*** and 4°C. The supernatant was collected and the pellet homogenized once more in the above buffer, centrifuged and the supernatant recovered. The pituitary FSH and LH contents were determined after assaying serial dilutions of the supernatants.

FSH assay. The radioimmunoassay kit was supplied by The National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Bethesda, Maryland, USA. The ovine (o) FSH used for iodination was NIAMDD-oFSH-I-1; the oFSH reference preparation was NIAMDD-oFSH-RP-1 (biopotency 75 × NIH-FSH-S1) and the oFSH antiserum was NIAMDD-anti-oFSH-1 (AFP-C5288113). The volume of plasma used was 0·1 ml and each sample was assayed in duplicate. The internal standard and standard curve samples were prepared in FSH-free hypophysectomized ewe plasma. The overall intra- and interassay coefficients of variation for the internal standards (i.e. 0·5, 1·0, 2·5, 5·0, 10·0 and 15·0 ng/ml) which were included with every standard curve estimation were 6·3 and 10·5% respectively. The minimal detectable concentration of FSH was 0·2 ng/ml.

LH assay. The radioimmunoassay was similar to that described by McNatty *et al.* (1987). The iodination standard was NIDDK-oLH-I-3 (AFP-9598B), the LH antiserum was raised at Wallaceville and its characteristics are described by McNatty *et al.* (1987). The oLH reference preparation was NIAMDD-oLH-S23 (biopotency 2·3 × NIH-LH-S1). The volume of plasma which was assayed was 0·1 ml with each sample being assayed in duplicate. The internal standard and standard curve samples were prepared in LH-free hypophysectomized ewe plasma. The overall intra- and interassay coefficients of variation for the internal standards (i.e. 0·5, 1·0, 2·5, 5·0, 10·0 and 15·0 ng/ml) which were included with every standard curve estimation were 6·1 and 9·9% respectively. The minimal detectable concentration of LH was 0·2 ng/ml.

Statistical procedures. For Exp. 1, the mean daily FSH and LH values before GnRH treatment were examined by analysis of variance (ANOVA). After GnRH treatment the FSH values were ranked for each day of sampling for each genotype separately. Correlation analyses were performed using ranked values for each day of sampling to determine whether there were specific sheep effects. Mean concentrations for FSH and LH over the entire sampling period after GnRH were also calculated for each ewe and these values or their rankings were subjected to ANOVA or Kruskal–Wallis testing for sire effects and genotype effects.

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For Exp. 2, the FSH and LH values at each time of sampling relative to the GnRH pulses were averaged for each ewe over the 3 separate experiments. For FSH, a mean value was also obtained for each ewe over all sampling times. The effect of genotype was compared by Student's *t* test on the overall mean values. For LH, the peak areas and peak amplitudes were obtained for each of the 4 peaks and then an average value for each animal was obtained and the effects of genotype examined by Student's *t* test. The LH peak amplitude was the difference in concentration between the peak LH value and the mean baseline value which was obtained after first eliminating all values associated with the peak. For each LH peak the area was calculated as follows: LH pulse duration × LH peak amplitude × 0·5. To test whether GnRH induced an increase in plasma FSH concentrations the mean FSH value in each ewe at 10 min after a GnRH pulse was compared to that at 5 min beforehand by paired *t* test. For each genotype the mean difference in FSH values at 10 min after GnRH treatment minus the value 5 min beforehand (i.e. the amplitude) was also compared by Student's *t* test.

For Exp. 3 the distribution of mean FSH and LH values for each genotype in ovary-intact, ovariectomized or GnRH-treated HPD–ovx animals were compared using box and whisker plots (Velleman & Hoaglin, 1981).

Results

Plasma FSH and LH concentrations in HPD–ovx ewes before and after GnRH administration with respect to genotype and time (Exp. 1)

These data are summarized in Fig. 1. Before GnRH treatment no F gene-specific differences were noted in the plasma concentrations of FSH or LH (ANOVA). For each genotype separately, correlation analyses were performed on the ranked FSH values after GnRH treatment to see if there were any associations between FSH and each time lag. All correlations except those between Days 2 and 29 (for both genotypes), Days 2 and 26, Days 5 and 29 (for + + only) were significant ($P < 0{\cdot}01$), indicating that for each genotype any ewe with a high FSH ranking on one sampling day was likely to have a high value on other sampling days; conversely, ewes with low FSH rankings were likely to retain their low ranking on other days. When the overall mean FSH rankings for each ewe within each genotype were related to their respective sires no significant sire effects were noted for either genotype (ANOVA). After GnRH treatment the mean FSH but not LH concentrations were significantly higher in FF than in + + ewes at the first sampling time 2 days later ($P < 0{\cdot}01$, *t* test). Thereafter the mean FSH values continued to increase at a faster rate in FF than in + + ewes. For example, linear regression analyses of the FSH concentrations over the first 9 days after GnRH treatment produced mean ± s.e.m. slopes for FF and + + ewes of $0{\cdot}68 \pm 0{\cdot}06$ and $0{\cdot}49 \pm 0{\cdot}03$ ng ml^{-1} day^{-1} respectively ($P < 0{\cdot}01$; ANOVA). Higher mean FSH concentrations were observed in FF than in + + ewes throughout the 5 weeks of GnRH treatment. The overall mean FSH concentrations with respect to genotype were significantly different ($P < 0{\cdot}03$; Kruskal–Wallis analysis).

After the initiation of GnRH treatment the mean LH concentrations had increased significantly 2 days later ($P < 0{\cdot}01$) but without any F gene-specific differences being noted. Thereafter, the mean LH concentrations increased at comparable rates in both genotypes. The overall mean LH concentrations with respect to genotype were not significantly different (Kruskal–Wallis analysis).

No significant stimulation of plasma FSH or LH concentrations was noted when the HPD–ovx ewes (N = 6 ewes/genotype) were given the infusion solution devoid of GnRH once every 2 h for 14 days (data not shown).

Plasma LH and FSH concentrations in HPD–ovx ewes over 4 exogenous GnRH pulses with respect to genotype (Exp. 2)

These data are summarized in Fig. 2. Each GnRH pulse was followed by a rapid increase in plasma LH concentrations to peak values after ~10 min followed by an exponential decay to baseline values ~100 min later. There was a trend towards lower mean LH peak amplitudes and peak areas (see box and whisker plots; Fig. 2a) in + + compared with FF ewes, but no significant F gene-specific effects were measurable. The basal LH values in ewes of both genotypes were similar. For FSH, the mean concentrations over time in FF ewes were consistently higher than in + + ewes

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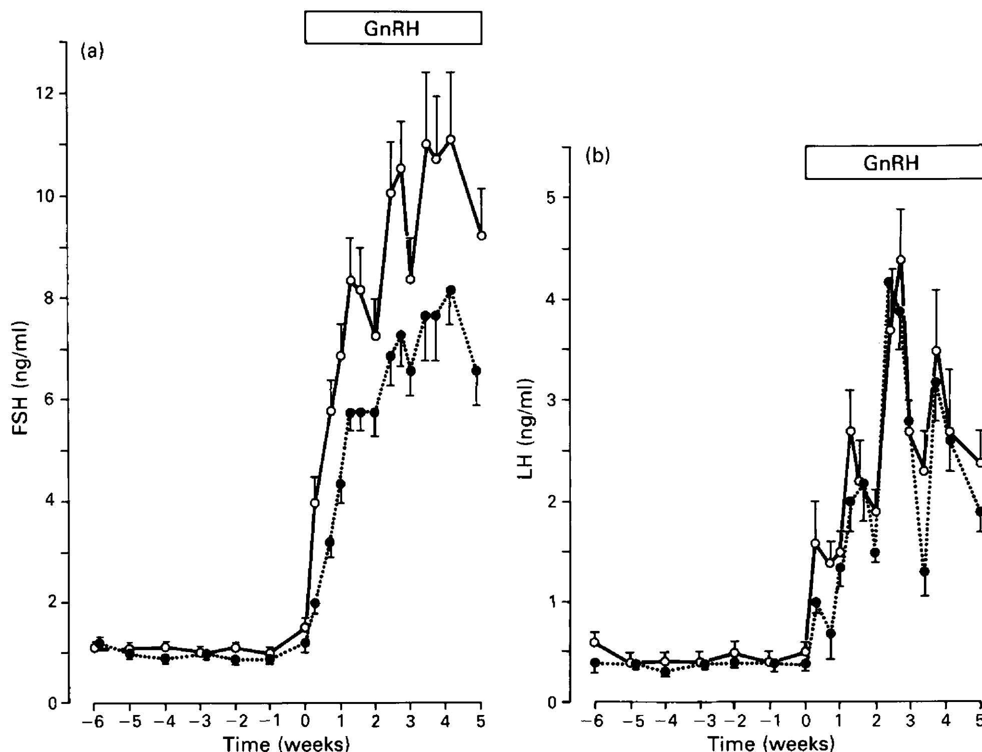

Fig. 1. Mean plasma FSH (a) and LH (b) concentrations in hypothalamic–pituitary disconnected–ovariectomized (HPD–ovx) Booroola FF (N = 13; ○——○) and ++ (N = 14; ●---●) ewes before and after GnRH treatment. GnRH (250 ng) was administered i.v. as a pulse lasting ~1 min. Vertical bars = 1 s.e.m.

with the overall mean concentrations (i.e. one mean value/ewe) between the genotypes being significantly different ($P < 0{\cdot}05$): >75% of the FF animals had higher overall mean FSH values than in 50% of the ++ animals (see box and whisker plots; Fig. 2b).

In contrast to LH, each GnRH pulse was followed by a smaller and more variable increase in FSH concentration. Irrespective of genotype, the mean FSH concentration 10 min after a GnRH pulse (i.e. the FSH pulse amplitude) was significantly higher than the concentration 5 min beforehand ($P < 0{\cdot}001$; paired t test). Moreover, the mean FSH pulse amplitude in FF ewes (i.e. 1·3 ± 0·1 ng/ml) was significantly higher ($P < 0{\cdot}05$) than that in ++ ewes (i.e. 0·8 ± 0·1 ng/ml).

Effect of a large GnRH bolus injection (10 μg) on plasma FSH and LH concentrations (Exp. 2)

No gene-specific differences were noted in the height of FSH or LH release or in the areas under the FSH or LH response curves to GnRH injection (ANOVA; data not shown). After subtracting the pretreatment FSH values, the mean (±s.e.m.) GnRH-induced FSH peak heights were 5·8 (±0·5) and 5·2 (±0·5) ng/ml for the FF and ++ genotypes respectively, whereas for LH the mean (±s.e.m.) peak heights were 13·0 (±1·6) and 10·4 (±0·9) ng/ml respectively.

Pituitary contents of FSH and LH with respect to genotype in the GnRH-treated HPD–ovx ewes (Exp. 2)

The mean (±s.e.m.) pituitary weights in the FF (N = 8) and ++ genotypes (N = 7) were 0·78 (±0·10) and 0·85 (±0·09) g respectively. The mean (±s.e.m.) pituitary contents for FSH and LH

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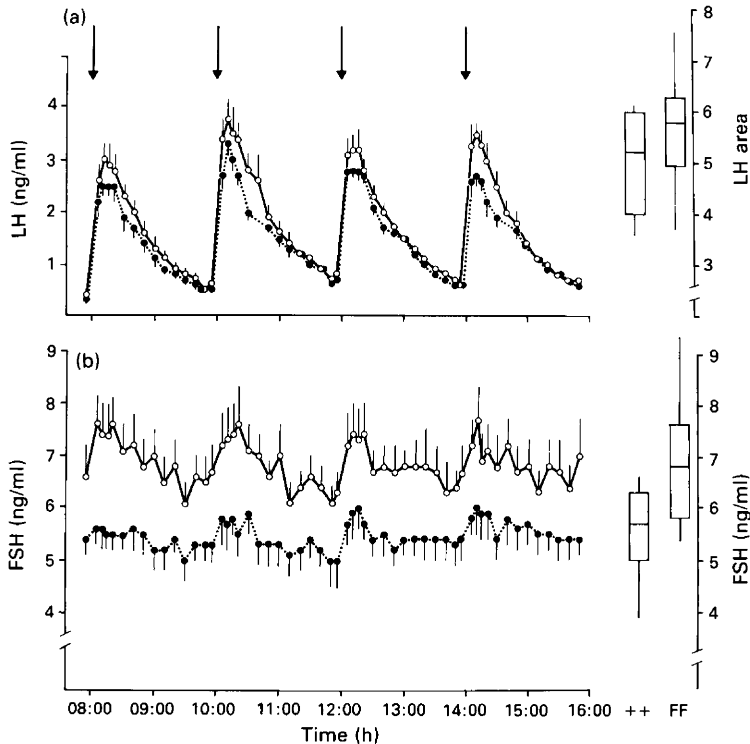

Fig. 2. Mean plasma LH (a) and FSH (b) concentrations every 5 or 10 min before and after 4 exogenous administered GnRH (250 ng i.v.) pulses (arrowed) in HPD–ovx Booroola FF (N = 8; ○——○) and ++ (N = 7; ●- - -●) ewes. Vertical bars = 1 s.e.m. Also shown are the box and whisker plots of the mean FSH concentrations and mean LH peak areas for each ewe. The extreme ends of the vertical (whisker) lines represent the maximum and minimum values. The upper and lower horizontal lines of the box refer to the upper and lower quartiles and the lines through the box represent the median values.

were 178 ± 24 and 181 ± 59 μg respectively for the FF genotype and 211 ± 22 and 97 ± 47 μg respectively for the ++ genotype. No F gene-specific differences were noted for any of these parameters.

Distribution of mean plasma FSH and LH concentrations in GnRH-treated HPD–ovx ewes compared with those in ovariectomized and ovary-intact ewes (Exp. 3)

These results are summarized in Fig. 3. Values for LH in ovary-intact ewes were excluded because the mean concentrations during the luteal phase in most animals was ≤0·2 ng/ml. In the ovary-intact, as well as the ovariectomized and GnRH-treated HPD–ovx ewes, the mean FSH but not LH concentrations were significantly higher in FF than in ++ animals (ovary-intact, $P < 0{\cdot}001$; ovariectomized, $P < 0{\cdot}05$; GnRH-treated HPD–ovx, $P < 0{\cdot}05$). For all treatments, ≥75% of the FF ewes had higher overall mean FSH concentrations than did 50% of the ++ animals. This difference in distribution of FSH means between the genotypes was also evident in Exp. 2 (see Fig. 2). For LH, ~50% of the FF ewes had higher overall mean LH concentrations than did 75% of the ++ ewes, but these differences never reached statistical significance.

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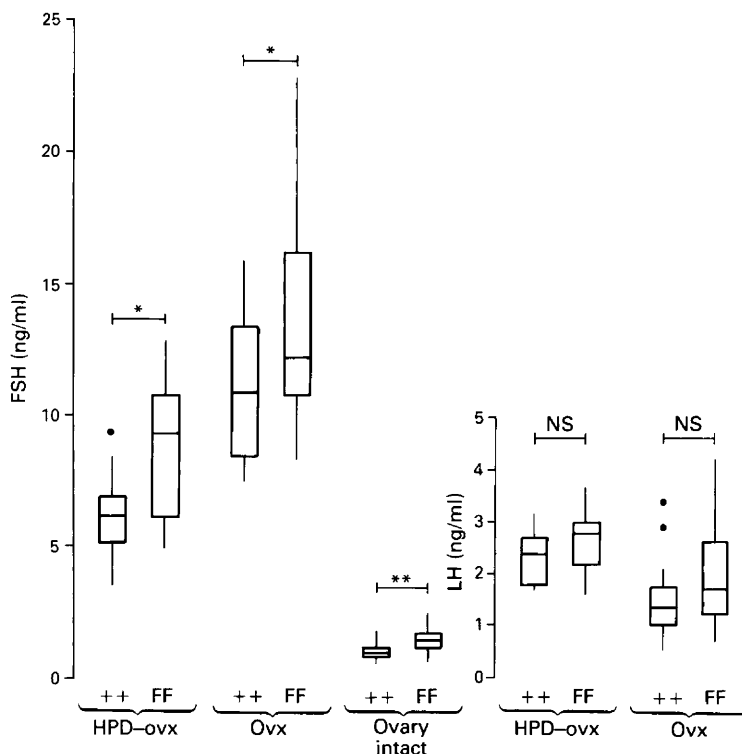

Fig. 3. Box and whisker plots of the mean FSH and LH data for: HPD–ovx Booroola FF (N = 13) and ++ (N = 14) ewes pulsed with GnRH (250 ng i.v. once per 2 h) for 35 days, Booroola FF (N = 21) and ++ (N = 21) ewes during the first 35 days after ovariectomy (from McNatty *et al.*, 1989) and for Booroola FF (N = 37) and ++ (N = 44) ewes during the luteal phase. The extreme ends of the vertical (whisker) lines represent the maximum and minimum values. The solid circles refer to outlier values. The upper and lower horizontal lines of the box refers to the upper and lower quartiles and the lines through the box represent the median values. The data for LH in ovary-intact ewes were excluded since most animals had mean concentrations $\leqslant 0{\cdot}2$ ng/ml. **P* < 0·05; ***P* < 0·001; NS = not significantly different.

Discussion

The major findings from these studies are (1) that GnRH stimulates significantly higher mean plasma FSH but not LH concentrations in FF than in ++ HPD–ovx ewes; and (2) that the genotypic distributions of mean plasma FSH and LH concentrations in GnRH-treated HPD–ovx ewes are similar to those in ovariectomized and ovary-intact sheep. In the GnRH-treated HPD–ovx ewes, a significant 'sheep effect' was noted for both genotypes with the ranking of FSH values in individual animals remaining unchanged on most sampling days. However, in contrast to the 'sheep effect', no sire effects were noted. Collectively, therefore, these findings support the hypothesis that some association exists between the Booroola F gene and plasma FSH concentrations at the level of the pituitary gland. However, the GnRH-induced F gene difference in plasma FSH concentration cannot be attributed to differences in pituitary gonadotrophin content or to differences in the overall size of the releasable 'pools' of FSH or LH as assessed from the pituitary response to the large (i.e. 10 μg i.v.) GnRH bolus. Also, Montgomery *et al.* (1990) have shown that the F gene can be excluded from close linkage to the FSH-β region of chromosome 15 and that the F gene has no influence on the size or number of FSH-β gene transcripts in pituitary mRNA

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preparations. However, it is possible that the F gene-specific differences in FSH secretion are related to post-translational processing of FSH (e.g. glycosylation; see Chappel *et al.*, 1983). Other studies have been unable to show any F gene-specific differences in hypothalamic GnRH concentrations (Gale *et al.*, 1987), GnRH–receptor binding in pituitary tissue (Fleming *et al.*, 1990) or in the GnRH secretory characteristics in hypophysial–portal blood (K. P. McNatty & I. J. Clarke, unpublished findings). Although a number of studies in gonad-intact Booroola ewe-lambs as well as in adult rams and ewes report differences between F gene carriers and controls in pituitary FSH and/or LH output to exogenous GnRH (McNatty *et al.*, 1987; Braw-Tal & Gootwine, 1989; Price *et al.*, 1991; C. A. Price & K. P. McNatty, unpublished findings), it has not been established whether these putative F gene-specific differences in pituitary response are exclusive to GnRH, or whether they also occur in response to other hypothalamic-releasing factors.

In the present study on HPD–ovx ewes, the pattern of FSH secretion following GnRH administration was variable compared to that for LH. Nevertheless, the results show a clear pulsatile pattern of FSH release following GnRH treatment (see Fig. 2). For both genotypes the plasma FSH concentrations increased significantly above basal values 10 min after a GnRH pulse and the increase in concentration at this time was 1·6-fold greater in FF than in ++ ewes. The faster rate of increase in plasma FSH observed in FF than in ++ ewes after the initiation of GnRH treatment to the HPD–ovx animals might be attributable to this difference in FSH output to each GnRH pulse. Overall, the mean FSH but not LH concentrations were lower in the GnRH-treated HPD–ovx ewes than in ovariectomized ewes. The FSH measurements in the HPD–ovx ewes were made over the 5 weeks of GnRH treatment which was initiated some 8–12 weeks after ovariectomy. In contrast, the FSH measurements in the ovariectomized ewes were made during the first 5 weeks after ovariectomy. Previous studies by Clarke *et al.* (1984) have shown that the rate of FSH increase in response to GnRH is influenced by the time lapsed after ovariectomy and/or HPD surgery. Other factors such as frequency (and amount) of GnRH administered are also important. For example, the GnRH pulse frequency in ovariectomized Booroola ewes is probably >1 pulse every 60 min (McNatty *et al.*, 1989b), whereas in the GnRH-treated HPD–ovx ewes, GnRH pulses were given every 120 min.

These studies do not unequivocally answer the question of whether F gene-specific differences occur with respect to LH secretion. In previous studies of ovary-intact and ovariectomized ewes, differences have sometimes been observed with respect to LH pulse amplitude (McNatty *et al.*, 1987,1989a, b). In the present study, GnRH pulses stimulated clearly defined LH pulses of uniform amplitude. No statistically significant differences between the genotypes were noted although there was a consistent tendency for the FF animals to have higher mean pulse amplitudes. In addition, the GnRH-treated HPD–ovx ewes of the FF genotype showed greater between animal variability in mean plasma LH concentrations or mean LH peak areas relative to the ++ animals and this characteristic was similar to that observed in intact or ovariectomized ewes (Figs 2 & 3; McNatty *et al.*, 1987, 1989a).

Previous studies suggest that the F gene influences ovarian follicular growth before antrum formation, resulting in fewer granulosa cells at all phases of antral growth as well as smaller follicular diameters at ovulation (see McNatty & Henderson, 1987, for review). In essence the ≥ 5, 3–4 or 1–2 presumptive preovulatory follicles in FF, F+ or ++ ewes are respectively 2·5–4·5 mm, 4–5 mm and >5 mm in diameter, but the total populations of oestrogen-secreting follicular or progesterone-secreting luteal cells in the different genotypes are the same (McNatty & Henderson, 1987; Niswender *et al.*, 1990). The Booroola F gene seems to effect these changes without altering gonadotrophin receptor binding characteristics, cAMP synthesis or catabolism or steroid or inhibin biosynthetic functions in follicular cells (McNatty *et al.*, 1990; K. M. Henderson, unpublished data). FSH is thought to be important for stimulating preantral follicular growth *in vitro* (Ryle, 1969; Quist *et al.*, 1990) and has been shown to stimulate oestrogen synthesis from small antral follicles *in vivo* (i.e. 2–4·5 mm diameter; McNatty *et al.*, 1985). Also FSH treatment during the follicular phase leads to the formation of multiple corpora lutea at smaller diameters

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(Henderson *et al.*, 1988). Thus, in many respects FSH is a suitable hormone to be linked to the F gene. However, it remains to be proved whether small increases in plasma FSH after the administration of exogenous FSH to ++ Booroola ewes over a long period of time would be sufficient to modify the population of granulosa cells in individual antral follicles and stimulate multiple ovulation without altering the plasma steroid concentrations.

The present results show that the previously reported gene differences in FSH concentration in ovary-intact and ovariectomized ewes can be replicated by GnRH therapy to HPD–ovx ewes. These findings suggest that some association between the Booroola F gene and GnRH-induced FSH secretion exists. Furthermore they support the hypothesis that at least part of the expression of the F gene is at the level of the pituitary gland to affect responsiveness to physiological concentrations of GnRH.

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