Effects of oestradiol-17 β , progesterone or bovine follicular fluid on the plasma concentrations of FSH and LH in ovariectomized Booroola ewes which were homozygous carriers or non-carriers of a fecundity gene

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Summary. The plasma concentrations of FSH and LH were measured in ovariectomized Booroola FF and ++ ewes before and after treatment with subcutaneous implants of oestradiol-17 β (0, 2 or 8 cm Silastic capsules; 5 ewes/genotype per dose) or progesterone (0, 1 or 3 Silastic envelopes; 5 ewes/genotype per dose) or subcutaneous injections of steroid-free bovine follicular fluid (bFF; 0, 0.5, 1.0, 2.5 or 5 ml; 4 ewes/genotype per dose). During the first 50 h after implantation of oestradiol or progesterone, or the first 24 h after bFF treatment, the FSH and LH concentrations in plasma were not different between the genotypes although there were significant effects of the steroids and bFF with respect to dose ($P < 0.05$). At 6 days after steroid implantation, no gene-specific effects were noted for the plasma concentrations of FSH although significant effects of dose of oestradiol ($P < 0.01$) but not progesterone were noted. Also at 6 days after steroid implantation, no gene-specific differences in the pulsatile patterns (i.e. peak frequency or amplitude) of plasma LH concentrations were noted although there were significant effects of steroid dose ($P < 0.05$) on frequency and/or amplitude.

It is concluded that the higher ovulation-rate in FF than ++ Booroola ewes is unlikely to be due to gene-specific differences in the sensitivity of the hypothalamic–pituitary axis to ovarian hormones.

Keywords: Booroola ewes; oestradiol-17 β ; progesterone; bovine follicular fluid; FSH; LH; ovariectomy

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(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 differences both in ovarian activity and plasma gonadotrophin concentrations between ++ and FF ewes (McNatty & Henderson, 1987; Fry *et al.*, 1988; McNatty *et al.*, 1987, 1989). However, it is not known whether F gene-specific differences exist in the sensitivity of the hypothalamic–pituitary axis to oestradiol, progesterone or other ovarian hormones. The highly fecund Finnish Landrace ewe is known to be less sensitive to oestradiol negative feedback than the less fecund Scottish Blackface ewe as assessed by the plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Webb *et al.*, 1985a). Ovarian hormones other than oestradiol or progesterone also contribute to

the feedback control of FSH secretion (Goodman *et al.*, 1981). In this context, steroid-free bovine follicular fluid (bFF) has been used experimentally to lower FSH, and to a lesser extent, LH concentrations in plasma of sheep (Martin *et al.*, 1986) by inhibiting, at least in part, the mRNA for the β -subunit of FSH (Mercer *et al.*, 1987).

The aim of this study was to examine the effects of different doses of oestradiol-17 β , progesterone or bFF on the plasma concentrations of FSH and LH with respect to Booroola genotype.

Materials and Methods

Animals and experiments

Booroola Merino ewes born in 1978–80 were classified as ++ (N = 35) or FF (N = 35) genotypes based on pedigree analysis: these genotypic classifications were confirmed by laparoscopy according to the criteria of Davis *et al.* (1982). All the FF ewes, aged 6–7 years at the time of the study, were recorded as having at least one annual ovulation rate of ≥ 5 whereas the ++ ewes, aged 6–7 years, were never recorded with an ovulation of > 2 . All the ewes were ovariectomized at least 40 days before the first experiment at the start of the breeding season. At the time of ovariectomy the mean $\pm$ s.e.m. liveweights of the ewes were 47.2 ± 1.2 kg for the FF genotype and 49.1 ± 1.5 kg for the ++ genotype; there was no significant difference in liveweight between the genotypes. On the day before the experiments, the animals were penned indoors and each was fitted with an intrajugular cannula under local anaesthesia (Xylocaine 2%: Astra Pharmaceuticals Pty Ltd, NSW, Australia). When blood sampling began, the animals were each bled (2.5 ml) via the jugular cannula.

Experiments 1 and 2 were designed to examine the plasma concentrations of FSH and LH with respect to genotype before and after treating ewes with oestradiol-17 β (Exp. 1) or progesterone (Exp. 2). For Exp. 1, 0, 2 or 8 cm Silastic capsules (3.35 mm i.d., 4.65 mm o.d.; Cat. No. 601-335; Dow Corning Corp., Midland, MI, USA), were packed with oestradiol-17 β as described by Karsch *et al.* (1973) and 5 ewes/genotype at each dose of oestradiol were studied. For Exp. 2, 0, 1 or 3 Silastic envelopes (55 $\times$ 75 mm; Cat. No. 500-1; Dow Corning), were packed with progesterone as described by Karsch *et al.* (1977) and 5 ewes/genotype at each dose of progesterone were studied. All steroid capsules or envelopes were presoaked in water for 12–18 h to avoid any potential transient 'peak release' of steroid. The implants were inserted in the subcutaneous fascia of the hind leg(s) while the animals were under local anaesthesia; the time taken to implant each capsule or envelope was approximately 10 min. The ewes in Exp. 1 were the same as those used in Exp. 2 but all were from a different flock from those used in Exp. 3. The period between Exps 1 and 2 was 55–59 days.

The blood sampling regimens were identical for Exps 1 and 2. On the 1st day of the experiment, all animals were blood sampled hourly for 24 h before the implanting of steroids and thereafter, each hour for a further 50 h. On the 6th day after steroid implantation, the animals were blood sampled every 10 min for 12 h. The plasmas from all hourly samples were assayed for FSH and LH whereas the samples taken at 10-min intervals were assayed for LH only.

Experiment 3 was designed to examine the plasma concentrations of FSH and LH with respect to genotype after injecting FF and ++ ewes with 0, 0.5, 1.0, 2.5 and 5.0 ml bFF (s.c.; N = 4 ewes/genotype per dose of bFF). All ewes in this experiment had been ovariectomized 60–65 days previously and had not been pretreated with any exogenous steroid or follicular fluid. On the day of the experiment, hourly blood sampling began at 09:00 h for 29 h with the bFF being administered immediately after the 13:00 h blood sample, and again just after the subsequent 01:00 h blood sample. All ewes were treated with the same pooled batch of bFF (Batch V), which was prepared free of steroids as previously described (Henderson *et al.*, 1986).

At the end of each experiment, all steroid implants and cannulae were removed and the animals were returned to pasture.

Blood samples

All blood samples were centrifuged at 4000 g at room temperature for 8 min within 30 min of collection and the plasma samples were stored at -20°C until assayed.

Hormone assays

FSH. The radioimmunoassay kit was that supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Bethesda, MD, USA. The ovine (o) FSH for iodination was NIAMDD-oFSH-I-1; the oFSH reference preparation was NIAMDD-oFSH-RP-1 (biopotency $75 \times$ NIH-FSH-3) 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. The reference standards and standard curve samples were prepared in hypophysectomized ewe plasma which contained undetectable concentrations of FSH. The intra- and interassay coefficients of variation were < 4 and $< 5\%$ respectively.

LH. 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 $2.3 \times$ NIH-LH-S1). The volume of plasma which was assayed was 0.1 ml and each sample was assayed in duplicate. The minimum detectable level of LH was 0.03 ng/tube. The reference standards and standard curve samples were prepared in hypophysectomized ewe plasma which contained undetectable amounts of LH. The intra and interassay coefficients of variation were <5 and $<8\%$ respectively.

Progesterone. The progesterone radioimmunoassay together with the dilution and specificity of the progesterone antiserum (WA26) were as described by McNatty *et al.* (1981). The volume of plasma assayed was 200 μ l and the detection limit was 20 pg progesterone/tube. All the samples were measured in one assay and the intra-assay coefficient of variation was $<8\%$.

Oestradiol-17 β . The extraction of oestradiol-17 β from plasma was similar to that described by Webb *et al.* (1985b). In brief, 3 ml plasma plus [2,4,6,7- 3 H]oestradiol (1000 c.p.m.; sp. act. 94 Ci/mmol) in 100 μ l assay buffer, namely 0.1 M-phosphate buffer + 0.1% gelatin (w/v; pH 7.4), was incubated at room temperature for 30 min. Thereafter 10 ml double glass-distilled (DG-D) water plus 2 ml oestradiol antibody conjugated to Sepharose 4B were added to the mixture and mixed for 2 h at room temperature. The oestradiol antibody (WA-27) used was identical to that described elsewhere (McNatty *et al.*, 1984); 150 μ l of the antibody was conjugated to 9 g Sepharose 4B as described by Webb *et al.* (1985b). At the end of the 2-h incubation the plasma, water, [3 H]oestradiol, oestradiol antibody-Sepharose mixture was poured directly onto a prewashed sintered glass column (1 $\times$ 20 cm; Econo-column, Biorad Labs., Richmond, CA, USA). Before use the column was washed with 3 ml 90% (v/v) methanol and twice with 20 ml DG-D water. The tube from which the incubation contents were transferred to the column was rinsed with 7 ml DG-D water and again with 20 ml DG-D water with each of these rinses being transferred to the column. The residual water in the antibody-Sepharose mixture was then removed by applying slight positive pressure. The bound hormone was then eluted with 3 ml 90% methanol which was evaporated to dryness. Thereafter the assay was performed as previously described by McNatty *et al.* (1984). All aqueous solutions (e.g. buffer, dextran-charcoal solutions) were prepared in DG-D water. The limit of detection for oestradiol was ≤ 3 pg per tube and the intra- and inter-assay coefficients of variation over the range of 3–30 pg were 15 and 22% respectively.

Statistical procedures

Because of between-sheep and genotype variation in the pretreatment concentrations of plasma FSH and LH, both the hourly pre- and post-treatment values for each ewe were normalized by expressing the data as a percentage of the overall pretreatment mean value. Homogeneity of variance was checked using the Bartlett test. The genotype and dose effects as specific time intervals were then analysed by a 1- or 2-way Analysis of Variance (ANOVA) and multiple comparisons were made using Scheffe's test. Because of extensive overlap of the mean percentage data of each time between the genotypes, the data in Figs 1–4 are presented as hourly means without errors or ranges for clarity. In these instances, the variability of the individual means for each genotype are presented as an overall coefficient of variation (CV) which was determined by averaging the CVs over each hour of the study.

The episodic LH data were examined using the method of Van Look (1976) and Backstrom *et al.* (1982). An LH peak was defined as occurring when the hormone concentrations of two consecutive samples was greater than that of the mean of the two previous samples (basal samples) and the value of at least one of the peak samples exceeded the mean basal value by more than twice the coefficient of variation of the assay which was set at 8%. The amplitude of each peak was measured by subtracting the basal from the peak value. The 'smoothed' mean value was defined as the overall mean basal value after all peak values were removed. Genotype and dose differences in LH data were compared by ANOVA.

Results

Effect of different doses of oestradiol on the plasma concentrations of oestradiol, FSH and LH before and after implantation of oestradiol (Exp. 1)

Before implantation, oestradiol was not detectable in the plasma of any individual animal irrespective of genotype. Moreover, in the control animals (i.e. with no oestradiol), oestradiol was not detectable at any time during the experimental period. In the animals given the 2-cm implants, the mean $\pm$ s.e.m. oestradiol concentrations at -24 h, $+5$ h, $+24$ h and $+49$ h from implantation were <1.0 , 2.2 ± 0.8 , 1.9 ± 0.5 and 2.5 ± 0.3 pg/ml respectively for the FF genotype and <1.0 , 1.4 ± 0.2 , 2.6 ± 0.7 and 2.4 ± 0.2 pg/ml respectively for the ++ genotype. For those given the 8-cm implants, the mean $\pm$ s.e.m. concentrations at -24 h, $+5$ h, $+24$ h and $+49$ h from implantation were <1.0 , 17.8 ± 4.2 , 15.8 ± 2.6 and 16.2 ± 1.5 pg/ml respectively for the

FF genotype and <1.0 , 17.2 ± 2.3 , 14.3 ± 1.2 and 14.4 ± 3.0 pg/ml respectively for the ++ genotype. No gene-specific differences in oestradiol concentrations were noted for the 2- or 8-cm implants at any of the above mentioned times.

The mean FSH and LH data expressed as a percentage of the pretreatment mean value with respect to Booroola genotype are summarized in Fig. 1 (FSH) and Fig. 2 (LH). The pretreatment mean $\pm$ s.e.m. FSH concentrations for the FF (N = 15) and ++ (N = 15) genotypes were 7.8 ± 0.8 ng/ml and 7.5 ± 0.7 ng/ml respectively and the corresponding LH concentrations were 5.1 ± 0.4 and 5.4 ± 0.5 ng/ml respectively; for each hormone the pretreatment mean values were not significantly different. In the ewes not receiving oestradiol, there were no appreciable changes after 0 h (i.e. the time corresponding to implantation of oestradiol in the treated groups) in the plasma concentrations of FSH or LH in either genotype compared to the pretreatment values. Moreover, when the hourly values after 0 h for each of the untreated genotypes were compared, no gene-specific differences were noted for either FSH or LH.

After receiving 2- or 8-cm oestradiol capsules, there was a progressive decline in the mean FSH and LH concentrations in ewes of both genotypes to a nadir around 4–10 h after implantation followed by a rise to peak values at 15–16 h. From 17 h onwards, the mean FSH and LH values declined once more to reach basal values between 30 and 50 h after oestradiol implantation. No gene-specific differences were observed in the time to the onset of the FSH 'peak' values or in the FSH peak areas for either dose (Fig. 1). For 2-cm implants the mean $\pm$ s.e.m. times were 15.4 ± 0.4 and 14.8 ± 0.4 h for the ++ and FF genotypes respectively, whereas for 8-cm implants the mean $\pm$ s.e.m. times were 15.0 ± 0.5 h for both genotypes. Moreover, no difference was noted with respect to peak areas or amplitudes with respect to genotype or dose of oestradiol. Likewise, no gene-specific differences were observed in the time of steroid implantations to the onset of the LH 'surge': 2-cm implants; mean $\pm$ s.e.m. = 11.8 ± 0.8 h for ++ ewes and 10.6 ± 0.4 h for the FF ewes; 8-cm implants; mean $\pm$ s.e.m. = 10.0 ± 0.6 h for the ++ ewes and 9.4 ± 0.5 h for the FF ewes. Moreover, no differences were noted with respect to peak areas or amplitude either with respect to genotype or dose of oestradiol. When the FSH or LH means (one mean value for each ewe) from 30 to 50 h for each oestradiol dose for each genotype were examined by 2-way ANOVA, no gene effects were observed but for both the 2-cm and 8-cm oestradiol treatment groups values were significantly lower than in the controls ($P < 0.01$) but not significantly different from one another.

Effect of different doses of progesterone implants on the plasma concentrations of progesterone, LH and FSH before and after implantation of progesterone (Exp. 2)

The mean LH data together with the changes in the mean plasma concentrations of progesterone with respect to Booroola genotype are summarized in Fig. 3. Before progesterone implantation, progesterone was not detectable in the plasma of any individual animal irrespective of genotype. Moreover, in the control animals, progesterone was not detectable at any time during the experimental period. In the animals given 1 or 3 implants, the progesterone concentrations were above the detection limits in all animals after 2 h and the concentrations in both treatment groups continued to increase rapidly over the first 11 h and then more gradually over the next 38–39 h. There were no significant differences between the genotypes in the concentrations of progesterone at any of the sampling times.

For LH, the pretreatment mean $\pm$ s.e.m. values for the FF (N = 15 animals) and ++ (N = 15 animals) genotypes were 5.4 ± 0.4 and 5.1 ± 0.5 ng/ml respectively. Concomitant with the rapid rise in progesterone over the first 11 h after implantation of progesterone there was a precipitous fall in the plasma concentrations of LH with no gene-specific differences noted. In the animals treated with 1 or 3 implants, the LH concentrations fell to the lowest recorded values at 5 h afterwards, remained at that level for the next 5 h and then partly recovered. This pattern of partial recovery occurred in a similar manner in ewes of both genotypes. When the overall means of the

Fig. 1. Hourly changes in the mean plasma concentrations of FSH before and after implantation of 0 (a), 2-cm (b) and 8-cm (c) Silastic capsules of oestradiol-17 β in ovariectomized FF ($\circ$ — $\circ$) and ++ ($\bullet$ — $\bullet$) ewes (5 animals/genotype per dose of oestradiol). Implants were inserted within 30 min of 0 h. For clarity the hourly s.e.m. values for each genotype are omitted. The overall coefficients of variation of the means are shown within the square brackets for each treatment group.

individual mean values for each sheep between 20 and 50 h were examined by 2-way ANOVA with respect to genotype and number of progesterone implants, a significant effect of dose ($P < 0.01$) but not genotype or genotype $\times$ dose interactions was observed, with the values from each of the progesterone dose levels being significantly different from one another ($P < 0.05$).

In contrast to the effects of progesterone on plasma LH, there were no effects of dose of progesterone or genotype on plasma FSH concentrations during the first 50 h after implantation.

Fig. 2. Hourly changes in the mean plasma concentrations of LH before and after implantation of 0 (a), 2-cm (b) and 8-cm (c) Silastic capsules of oestradiol-17 β in ovariectomized FF (○—○) and ++ (●—●) ewes (5 animals/genotype per dose of oestradiol). Implants were inserted within 30 min of 0 h. For clarity the hourly s.e.m. values for each genotype are omitted. The overall coefficients of variation of the means for each genotype are shown within the square brackets for each treatment group.

FSH and LH concentrations 6 days after implantation of oestradiol or progesterone (Exps 1 and 2)

The mean $\pm$ s.e.m. plasma concentrations of oestradiol in the ovariectomized ewes with 0, 2-cm or 8-cm implants of oestradiol-17 β were <1.0 , 2.4 ± 0.4 and 16.1 ± 3.1 pg/ml respectively for the FF genotype (5 ewes/dose) and <1.0 , 2.3 ± 0.4 and 16.3 ± 4.1 pg/ml respectively for the ++ genotypes (5 ewes/dose). The mean $\pm$ s.e.m. plasma concentrations of progesterone in the ovariectomized ewes with 0, 1 or 3 Silastic envelopes of progesterone were <0.1 , 1.1 ± 0.1 and

Fig. 3. Hourly changes in the mean plasma concentrations of LH before and after implantation of 0 (a), 1 (b) or 3 (c) Silastic envelopes of progesterone in ovariectomized FF (○—○) and ++ (●—●) ewes (5 ewes/genotype per progesterone treatment). Implants were inserted within 30 min of 0 h. The overall coefficients of variation of the means for each genotype are shown within square brackets for each treatment group. Changes in the mean $\pm$ s.e.m. (vertical bars) plasma concentrations of progesterone before and after insertion of the Silastic envelopes of progesterone are also shown for the FF ($\triangle$ — $\triangle$) and ++ ($\blacktriangle$ — $\blacktriangle$) genotypes in (b) and (c). In (a), the progesterone concentrations were undetectable.

3.0 ± 0.4 ng/ml respectively for the FF genotype (5 ewes/dose) and <0.1 , 0.9 ± 0.1 and 2.9 ± 0.4 ng/ml respectively for the ++ genotype (5 ewes/dose).

The mean $\pm$ s.e.m. ($N = 5$ sheep/steroid dose) concentrations of FSH and LH with respect to Booroola genotype (the gonadotrophin value for each ewe was averaged over time) and dose of oestradiol or progesterone 6 days after implantation are summarized in Fig. 4. When the oestradiol implant data were analysed by a 2-way ANOVA (genotype and dose of oestradiol), there were significant effects of dose of oestradiol for both FSH ($P < 0.01$) and LH ($P < 0.01$) but no significant effects of genotype for either hormone and no dose $\times$ genotype interactions were noted. The effect of the 2-cm oestradiol capsule was to lower the FSH ($P < 0.01$) and LH ($P < 0.05$) concentrations with respect to the untreated controls while the 8-cm capsules lowered the LH ($P < 0.05$) but not the FSH concentrations beyond those in the ewes with the 2-cm implants. When the progesterone implant data were examined by a 2-way ANOVA (genotype and number of progesterone envelopes), there was a significant effect of dose of progesterone for LH ($P < 0.01$) but not for FSH, and there were no dose $\times$ genotype effects for either LH or FSH. The effect of 1 envelope of progesterone significantly lowered the mean LH concentration ($P < 0.05$) with respect to the untreated control while 3 envelopes lowered the mean LH concentrations significantly below those found in the ewes with one implant ($P < 0.01$).

Fig. 4. Effects of length of Silastic capsules of oestradiol-17 β (0, 2- or 8-cm) or number of Silastic progesterone envelopes on the mean plasma concentrations of FSH and LH in ovariectomized ewes with respect to genotype. Values are mean $\pm$ s.e.m. (5 ewes/genotype per steroid implant).

Pulsatile plasma LH characteristics 6 days after implantation of oestradiol or progesterone (Exps 1 & 2)

These data are summarized in Table 1. Significant effects were noted on the overall mean, the smoothed mean or LH peak frequency with respect to the length of the oestradiol capsule

($P < 0.01$) or number of progesterone envelopes ($P < 0.01$) but not for genotype and, moreover, no interactions were noted. Significant effects were also noted with respect to oestradiol ($P < 0.01$) but not progesterone or genotype on LH peak amplitude. With respect to the length of the oestradiol capsule the overall mean LH concentration and the smoothed mean LH concentration in each of the treatment groups were significantly different from one another ($P < 0.05$). The mean values for LH peak frequency and LH peak amplitude in ewes with 2-cm capsules were not different from those with 8-cm capsules, but the values in ewes with no capsules were significantly higher than those in either of the other two groups ($P < 0.05$), indicating that the oestradiol implants led to a suppression of both LH peak frequency and amplitude. With respect to the number of progesterone envelopes both the overall mean and the smoothed mean LH concentration for each of the dose groups were different from one another ($P < 0.05$). For LH peak frequency, the mean values in ewes with 1 envelope were not significantly different from those without implants but both the aforementioned had significantly higher frequencies than those with 3 envelopes (3 vs 1 or 3 vs 0) envelopes; ($P < 0.01$).

Table 1. Effect of dose of oestradiol (length of capsule, cm) or progesterone (no. of implants) on the pulsatile characteristics of LH with respect to genotype in ovariectomized Booroola ewes (N = 5 ewes/genotype per treatment)

Genotype		Overall mean (ng/ml)	Smoothed mean (ng/ml)	LH peak frequency (peaks/12 h)	LH peak amplitude (ng/ml)
Oestradiol					
0	FF	3.7 ± 0.6	3.0 ± 0.5	11.2 ± 1.2	2.9 ± 0.7
	++	3.4 ± 0.2	2.8 ± 0.2	11.8 ± 2.1	2.4 ± 0.1
2	FF	1.8 ± 0.2	1.7 ± 0.2	5.8 ± 0.5	1.7 ± 0.3
	++	2.0 ± 0.5	1.9 ± 0.5	7.0 ± 0.7	1.0 ± 0.2
8	FF	0.6 ± 0.1	0.6 ± 0.1	5.2 ± 0.7	0.6 ± 0.1
	++	0.6 ± 0.1	0.6 ± 0.1	6.4 ± 0.8	0.6 ± 0.1
Progesterone					
0	FF	4.0 ± 0.6	3.4 ± 0.4	12.4 ± 1.9	2.9 ± 0.5
	++	4.0 ± 0.5	3.6 ± 0.4	11.8 ± 0.4	2.5 ± 0.6
1	FF	2.9 ± 0.3	2.6 ± 0.3	9.4 ± 1.2	3.1 ± 0.5
	++	2.6 ± 0.3	2.3 ± 0.3	8.2 ± 1.2	3.6 ± 0.8
3	FF	0.9 ± 0.1	0.8 ± 0.1	2.8 ± 0.4	3.3 ± 0.6
	++	0.7 ± 0.2	0.6 ± 0.2	2.2 ± 0.4	3.3 ± 0.9

For each treatment group, there were no significant effects of genotype with respect to each LH secretory characteristic.

Effect of bFF on the plasma concentrations of FSH and LH (Exp. 3)

The pretreatment mean concentrations of FSH in the FF (N = 20) and ++ ewes (N = 20) were 16.2 ± 1.4 and 11.6 ± 1.0 ng/ml respectively whereas those for LH in the FF and ++ ewes were 3.5 ± 0.4 and 2.8 ± 0.3 ng/ml respectively. The mean concentrations for FSH but not LH in FF ewes were significantly different from those in ++ ewes ($P < 0.05$). The higher mean FSH values in Exp. 3 compared to Exp. 1 are probably due to time after ovariectomy (McNatty *et al.*, 1989). The data summarizing the mean FSH and LH concentrations over 6–12 h and over 18–24 h from the first bFF injection with respect to genotype and dose of bFF injected are shown in Fig. 5. When the effects of genotype (i.e. ++, FF) and dose (i.e. 0, 5 ml, 1 ml, 2.5 ml and 5 ml bFF) were examined by 2-way ANOVA, at 6–12 h or after 18–24 h there were significant effects with respect to dose ($P < 0.05$) but no effects of genotype for both FSH and LH and no significant interactions were noted.

Fig. 5. Effect of different doses of bovine follicular fluid (bFF) on the plasma concentrations of FSH and LH in ovariectomized ewes with respect to Booroola genotype (FF, ○—○; ++, ●---●; 4 ewes/genotype per dose of bFF) at 6–12 h (a, b) and 18–24 h (c, d). Values are mean $\pm$ s.e.m. for 5 ewes/genotype per bFF dose.

Doses of bFF at 2.5 ml or 5 ml s.c. suppressed FSH concentrations in ewes of both genotypes by $\sim 40\%$ between 6 and 12 h and by $\sim 65\%$ between 18 and 24 h. Moreover, the highest dose of bFF (i.e. 5 ml) suppressed LH concentrations in both genotypes by $\sim 20\%$ between 6 and 12 h and by $\sim 45\%$ between 18 and 24 h (Fig. 5). Overall bFF was effective even at the lowest dose (i.e. 0.5 ml) in suppressing FSH concentrations compared to the zero dose ($P < 0.05$). In contrast, bFF was only effective in suppressing LH concentrations at the 2 highest doses (i.e. 2.5 ml and 5.0 ml; both ($P < 0.05$) relative to the zero dose.

Discussion

In this study we have examined the effects of different doses of oestradiol, progesterone or bFF in modulating the plasma concentrations of FSH and LH over time (i.e. ≤ 6 days) in ovariectomized FF and ++ Booroola ewes. The major finding is that the FF and ++ genotypes respond in a similar manner to each of the above substances. That is, no overall genotype differences were observed even though there were significant effects of the steroids or bFF with respect to dose. Thus the high ovulation rates in Booroola FF ewes compared to ++ ewes are unlikely to be related to gene-specific differences in the sensitivity of the neurohypophysis to ovarian hormones.

Implantation of oestradiol or of progesterone caused a rapid lowering of plasma LH concentrations after 2–5 h coincident with a measurable increase in the plasma concentrations of oestradiol or progesterone. Implantation of oestradiol but not progesterone provoked the release of LH to peak concentrations some 10 h later. The timing of this 'surge' and the peak characteristics (i.e. area and height) revealed no differences between the genotypes. These findings are consistent with those reported by Bindon *et al.* (1984) who showed that the timing of the preovulatory LH surge and LH peak concentrations were similar in Booroola and control Merino ewes, notwithstanding their different ovulation rates. Although implantation of progesterone did not provoke an LH 'surge' there was at least a partial recovery of the acute suppressive effects of progesterone some 20 h later; this was much more obvious after the implantation of 1 progesterone envelope than with 3 envelopes. From 30 to 50 h after progesterone implantation, the plasma LH concentrations, although fluctuating, had stabilized to some extent but were significantly lower overall than the untreated controls ($P < 0.01$; Fig. 4). The lower but variable plasma LH concentrations are probably the result of the exogenous progesterone reducing LH peak frequency without altering LH peak amplitude (Table 1). In contrast, the lower and much less variable LH concentrations 30–50 h after oestradiol implantation are probably due to the oestradiol inhibiting LH peak amplitude as well as LH peak frequency (Table 1; Martin, 1984). Although there was no overall effect on genotype, there was a divergence in the mean LH concentrations between the genotypes at 30–50 h after implantation of the 8-cm but not the 2-cm oestradiol capsules. The higher mean values in the FF genotype were due to 2/5 animals showing a level of LH suppression of ~90% of the pretreatment mean whereas the remaining 3 animals displayed a level of suppression similar to that in the ++ genotype (i.e. 35% of the pretreatment mean). When the LH concentrations in these 5 animals were examined 6 days after implantation (Fig. 3), the above-mentioned discrepancy had completely disappeared. It seems unlikely that the apparent but not statistically significant difference at 30–50 h after implantation has any physiological significance.

Implantation of oestradiol caused a similar overall pattern of suppression for FSH as that observed for LH. However, there was a slower rate of suppression of FSH compared to LH, and the magnitude and duration of the subsequent FSH 'surge' were both smaller than were observed for LH. Implantation of progesterone had no significant suppressive effect on the plasma concentrations of FSH. This finding supports those of Hamernik *et al.* (1987) and Martin *et al.* (1988) and suggests that at concentrations ≤ 3 ng/ml, progesterone has no influence on FSH secretion in female sheep. Again, there were no significant gene-specific differences.

The effects after 6 days of steroid implantation were generally similar to those observed after 30–50 h. The major difference between these times was that the plasma concentrations of LH after oestradiol or progesterone implantation or FSH after oestradiol implantation were lower after 6 days than after 30–50 h (see text and Figs 1–4). Nevertheless, the longer exposure to progesterone or oestradiol did not unmask any gene-specific differences, in tonic LH and FSH secretion or in the pulsatile characteristics of LH secretion.

Bovine follicular fluid at all doses between 0.5 and 5 ml caused a significant suppression of FSH concentrations at 6–12 h and also at 18–24 h after treatment. Moreover, ewes of the FF and ++ genotypes responded similarly, in contrast to the data of Cummins *et al.* (1983) who reported that the plasma FSH concentrations were more readily suppressed in Booroola Merino ewes compared to those found in control Merino ewes (not of the Booroola strain) during the first day of treatment with charcoal-treated ovine follicular fluid. However, these data may not be related to the F gene. With regard to plasma LH concentrations, high doses of bFF (i.e. 2.5 ml and 5 ml per injection) also caused a significant suppression, but, as with FSH, no F gene-specific effects were observed.

In the present study most groups of FF animals had slightly higher mean hourly gonadotrophin concentrations than did those of the ++ genotype before steroid or bFF treatment but they were only significantly higher on one occasion (FSH in Exp. 3). This, at first glance, seems inconsistent with the results of previous studies (McNatty *et al.*, 1989) in which mean daily FSH and LH concentrations were found overall to be significantly higher in FF than in ++ ovariectomized

ewes. However, in these animals the genotypic differences were obvious only in the frequency of higher FSH and LH values in FF compared to ++ ewes over a long (i.e. 30-day) sampling period. The present experiments, which were designed to detect differences in gonadotrophin concentrations over relatively short periods of time, are therefore inappropriate for testing subtle differences which might become apparent in long-term sampling. Nevertheless, the present study has demonstrated the absence of any measurable genotypic differences in the positive and/or negative feedback effects to ovarian hormones at the level that can be observed between Finnish Landrace and Scottish Blackface ewes (Webb *et al.*, 1985a). Although Booroola FF ewes are more likely to have a higher frequency of elevated FSH and LH concentrations compared to ++ ewes (McNatty *et al.*, 1987, 1989), as is the case for Finnish Landrace compared to Scottish Blackface ewes (Webb *et al.*, 1985a), the mechanisms which underly these differences may not be the same.

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