Quantitative control of oxytocin-induced PGF_{2α} release by progesterone and oestradiol in ewes

A. P. Beard, M. G. Hunter and G. E. Lamming*

AFRC Research Group on Hormones and Farm Animal Reproduction, Faculty of Agricultural and Food Sciences, University of Nottingham, Sutton Bonington Campus, Leics LE12 5RD, UK

The effect of oestradiol and progesterone concentrations on the uterine PGF_{2α} response to oxytocin was investigated by measuring 13,14-dihydro-15-keto PGF_{2α} (PGFM) secretion. One week after ovariectomy, 27 ewes were administered progestagen for 10 days followed by oestradiol for 2 days. Day 0 was designated as the time of the last 'oestrous' oestradiol injection. Six groups of ewes ($n = 4$) were then treated for 12 days with a high or low dose of progesterone (36 or 12 mg day⁻¹) either alone or with a high or low dose of oestradiol (36 or 12 µg day⁻¹) administered (in 1 ml of corn oil by i.m. injection, at intervals of 8 h) in a pattern designed to simulate a natural oestrous cycle profile. A control group ($n = 3$) was given corn oil alone. Ewes were treated with 1 µg oxytocin (i.v.) on days 4, 8 and 12 of the simulated cycle and plasma was collected for assay of PGFM. An oxytocin-induced PGFM response occurred only on day 12, when the response was suppressed by high doses of progesterone and stimulated by high oestradiol doses. There was a significant effect of progesterone ($P < 0.05$) and a highly significant effect of oestradiol ($P < 0.01$) on the pattern of PGFM release in response to oxytocin. Low progesterone/high oestradiol stimulated the largest and most sustained increase in PGFM following oxytocin. There was a significant relationship between the oestradiol:progesterone ratio and the mean PGFM response on day 12 ($P < 0.05$). This is the first demonstration of a quantitative effect of steroid hormone concentrations on the PGFM response to oxytocin in ewes, and indicates that in early pregnancy, ewes with a high oestradiol:progesterone ratio may generate larger PGF_{2α} episodes thus increasing the risk of a failure of the maternal recognition of pregnancy.

Introduction

Prostaglandin (PG) F_{2α} is the luteolytic substance in ruminants (McCracken *et al.*, 1972) and its pulsatile secretion during early luteolysis is dependent on the binding of ovarian oxytocin to endometrial receptors (Roberts *et al.*, 1976; Flint and Sheldrick, 1983). The rise in the level of uterine oxytocin receptors that occurs at the end of the luteal phase (Roberts *et al.*, 1976; Sheldrick and Flint, 1985) is of primary importance in determining if and when luteolysis occurs. Oestradiol and progesterone have a controlling influence on the oxytocin receptor concentration and the uterine PGF_{2α} response to oxytocin (McCracken *et al.*, 1978; Vallet *et al.*, 1990). A study in cattle (Parkinson *et al.*, 1990) showed a positive correlation between plasma oestradiol concentrations and level of uterine oxytocin receptors, and a negative correlation between plasma progesterone concentrations and level of oxytocin receptors. Further data from cows indicate a positive correlation between the oestradiol:progesterone ratio and the 13,14-dihydro-15-keto PGF_{2α} (PGFM) response to oxytocin, late in the luteal phase (Silvia and Taylor, 1989). The plasma concentration of PGFM (the metabolite of PGF_{2α}) following oxytocin administration

indicates the sensitivity of the uterus to the oxytocin episodes associated with luteolysis.

Experimental manipulation of steroid hormone concentrations has also been shown to influence the luteolytic mechanism. Treatment of ewes with progesterone early in the luteal phase shortened the oestrous cycle (Ginther, 1968; Ottobre *et al.*, 1980). Furthermore, treatment of ewes with high pharmacological doses of oestradiol during the mid-luteal phase induced premature luteal regression (Stormshak *et al.*, 1969; Hixon and Flint, 1987), whereas removal of oestradiol by destruction of the follicles extended the cycle (Karsch *et al.*, 1970), increased the PGFM pulse interval (Zhang *et al.*, 1991) and prevented luteolysis following treatment with PGF_{2α} (Hixon *et al.*, 1975). More recent studies have used steroid hormone treated ovariectomized ewes to investigate the effects of progesterone and oestradiol on oxytocin-induced PGFM release (Homanics and Silvia, 1988; Vallet *et al.*, 1990). Vallet *et al.* (1990) showed that after 12 days of progesterone treatment, oxytocin receptor concentrations were increased and a response to oxytocin was indicated by PGFM production.

The suppression of the late luteal phase rise in oxytocin receptor levels is a major component of the maternal recognition of pregnancy in domestic ruminants (McCracken *et al.*, 1984; Bazer *et al.*, 1991; Flint *et al.*, 1992). Other evidence

*Correspondence and reprint requests.
Received 23 March 1993.

Table 1. Total daily dose of oestradiol and progesterone administered to ewes

Duration of treatment (days)	High groups total daily dose ^a		Low groups total daily dose ^a	
	Oestradiol (μg)	Progesterone (mg)	Oestradiol (μg)	Progesterone (mg)
0–2	9	0	3	0
2–3	36	9	12	3
3–4	36	18	12	6
4–6	9	27	3	9
6–8	36	36	12	12
8–10	9	36	3	12
10–12	36	36	12	12
12–	9	36	3	12

^aTotal daily dose was divided into three parts administered at intervals of 8 h by i.m. injection in 1 ml of corn oil.

indicates that an abnormal progesterone and oestradiol pattern is often associated with early embryo loss (ewe: Cognie *et al.*, 1975; Parr *et al.*, 1987; Parr, 1992; cow: Lukaszewska and Hansel, 1980; Remsen and Roussel, 1982; Lamming *et al.*, 1989). An abnormal ovarian steroid hormone pattern during the luteal phase could alter the concentration of uterine oxytocin receptors, making these animals more susceptible to the luteolytic mechanism and subsequent embryo mortality. In such cases, the uterine response to oxytocin may be of greater magnitude than normal, so that any embryonic signal designed to maintain the corpus luteum would be too weak to suppress the luteolytic mechanism.

The aim of the present study was to determine the effect of doses of progesterone and oestradiol administered to ovariectomized ewes on oxytocin-induced uterine PGF_{2 α} release.

Materials and Methods

Animals

Twenty-seven mature Finn–Dorset multiparous ewes were housed on straw in a well ventilated building under natural conditions of daylength and temperature (April–May). Ewes were fed concentrates and hay throughout the treatment period with water always available.

Treatments

Ewes were ovariectomized under halothane anaesthesia and administered an intravaginal progestagen sponge (fluorogestone acetate; Chronogest; Intervet UK Ltd, Cambridge) one week after ovariectomy. Ten days later the sponges were removed and all ewes received oestradiol injections ($3 \times 16 \mu\text{g day}^{-1}$) for 2 days to simulate the 'oestrous' oestradiol rise. All steroid hormones were administered in 1 ml of 90% corn oil:10% ethyl alcohol, at intervals of 8 h by i.m. injection. Day 0 was designated as the time of the last 'oestrous' oestradiol injection. Ewes were weighed ($47 \pm 1.8 \text{ kg}$) and allocated at random to the seven treatment groups. Ewes were then treated for 12 days with high doses of progesterone, combined with a high (HPHE), low (HPLE) or zero (HPZE) dose of oestradiol or low doses of progesterone

combined with a high (LPHE), low (LPLE) or zero (LPZE) dose of oestradiol in a pattern previously shown by AP Beard and GE Lamming (unpublished data) to simulate natural cycle profiles including the waves of oestradiol characteristic of follicular development (see Table 1 for daily doses). On days 4, 8 and 12, ewes were treated with $1 \mu\text{g}$ (0.5 iu) oxytocin administered i.v. in 1 ml of saline, given to investigate the uterine prostaglandin response to a physiological dose of oxytocin. Such responses are *in vivo* markers for the presence of functional oxytocin receptors.

Collection of blood samples

The jugular vein of each ewe was cannulated one day before sponge removal. Daily blood samples were collected mid-way between the 08:00 and 16:00 h steroid hormone injections for subsequent assay of progesterone and oestradiol. Blood samples were either allowed to clot overnight at room temperature before centrifugation at 1500 g and collection of serum, or prevented from clotting by the addition of heparin (100 iu ml^{-1}) and centrifuged immediately at 1500 g for the recovery of plasma. Serum or plasma was harvested and stored at -20°C until assayed. Plasma samples were collected for assay of PGFM every 20 min in the hour before each oxytocin injection (pretreatment period) and every 10 min in the following hour (post-treatment period). Blood samples were also collected every 1 or 2 h in the 8 h before each oxytocin challenge, and they were assayed to determine the pretreatment progesterone and oestradiol concentrations (PTP and PTE).

Radioimmunoassay

Plasma concentrations of progesterone were measured in 0.1 ml aliquots using the radioimmunoassay method of Haresign *et al.* (1975) as modified by Hunter *et al.* (1986). The mean extraction efficiency was 81%; the limit of sensitivity was 0.13 ng ml^{-1} ; and the intra-assay coefficient of variation (CV) was 12.0%. The interassay CVs for two samples with concentrations of 1.87 and 2.66 ng ml^{-1} were 14.2 and 12.6%, respectively ($n = 17$ assays).

Plasma concentrations of PGFM were measured in 0.5 ml aliquots using the radioimmunoassay of Kaker *et al.* (1984). The mean extraction efficiency was 87%; the limit of sensitivity was 30 pg ml⁻¹; and the intra-assay CV was 13.7%. The interassay CVs for two samples with concentrations of 97 and 196 pg ml⁻¹ were 21.7 and 15.1%, respectively ($n = 19$ assays).

Serum concentrations of oestradiol were determined by double-antibody radioimmunoassay using a diagnostic kit (E₂ MAIA: Serono Diagnostics, Woking, Surrey) with the following adaptations. The lyophilized ¹²⁵I-labelled oestradiol was dissolved in assay buffer (phosphate-buffered saline 0.1 mol l⁻¹, pH 7.6, 0.1% gelatin, 0.2% NaN₃, 0.01 mol EDTA l⁻¹) to a dilution giving approximately 10 000 c.p.m. in 50 µl buffer. The first antibody was diluted in assay buffer (0.6 ml of solution provided plus 4.4 ml of assay buffer). The second antibody (separation reagent) was used in the solution provided without dilution. Standard concentrations (0.0625 – 16 pg in 50 µl) were prepared in assay buffer. Serum or plasma samples (250 µl) were dispensed in duplicate into diethyl ether washed extraction tubes and vortexed with 3 ml diethyl ether for 5 min, snap frozen, poured into borosilicate glass assay tubes, evaporated (under a stream of air in a water bath at 35°C) and reconstituted in 250 µl of assay buffer. Duplicate aliquots of standard (50 µl made up to 250 µl with assay buffer) and sample extracts (250 µl) were incubated with the first antibody (50 µl) and ¹²⁵I-labelled oestradiol (50 µl) at room temperature for 2 h. Total bound ($n = 5$), and non-specific binding tubes ($n = 3$) also received assay buffer, first antibody (except nonspecific binding tubes) and ¹²⁵I-labelled oestradiol. Total count tubes received ¹²⁵I-labelled oestradiol alone. Free and antibody-bound tracer were separated by incubation with the second antibody (100 µl) for 10 min, followed by addition of 1 ml of 0.9% saline, centrifugation (6°C for 10 min at 1500 g), and finally aspiration. Tubes were counted for 1 min (1277 Gamma Master: LKB Wallace, Stockholm).

The assay was validated for use with both ovine serum and plasma. Known amounts of oestradiol (2.5, 5, 10, 20 pg ml⁻¹) added to serum and plasma samples were accurately recovered in five separate assays (mean ± SEM serum: 1.8 ± 0.1, 4.2 ± 0.3, 8.8 ± 0.7, 17.8 ± 0.9 pg ml⁻¹ recovered, plasma: 2.5 ± 0.2, 4.3 ± 0.3, 9.9 ± 0.7, 17.8 ± 0.7 pg ml⁻¹ recovered). These samples were also measured using a well established assay (Webb *et al.*, 1985) and gave similar results again over five separate assays (serum: 2.4 ± 0.3, 5.5 ± 0.3, 13.1 ± 0.7, 26.5 ± 1.1 pg ml⁻¹ recovered, plasma: 2.8 ± 0.4, 5.3 ± 0.4, 12.6 ± 0.9, 22.1 ± 2.1 pg ml⁻¹ recovered). Serial dilutions of both serum and plasma samples ($n = 4$) ran parallel to the standard curve. Serum and plasma samples assayed at volumes of 62.5, 125, 250 and 500 µl, gave the following results, serum: 8.5, 7.5, 7.1, 6.3 pg ml⁻¹ and plasma: 8.2, 7.2, 7.5, 7.9 pg ml⁻¹. Specificity data are supplied with the kit (crossreactivity of oestrone 1.77% and oestradiol 0.47%). The mean extraction efficiency was 88%; the limit of sensitivity was 0.6 pg ml⁻¹, and the intra-assay CV was 12.5%. The interassay CVs for two samples with concentrations of 3.5 and 16.3 pg ml⁻¹ were 13.7 and 9.2%, respectively ($n = 6$ assays). The assay blank was consistently below the limit of sensitivity of the assay.

Statistical analysis

The progesterone, oestradiol and PGFM data were analysed by ANOVA and in each set the residuals were normally distributed and the magnitude of the residuals remained approximately constant over the range of values recorded.

Effects of hormone dose administered on the concentration of progesterone and oestradiol produced throughout the simulated cycle were determined by split-plot ANOVA with individual sheep used as plots and samples as subplots. Orthogonal contrasts were used to make comparisons of interest between treatment groups (Lowry, 1992). Effects of treatment on the PTP and PTE values were also determined by ANOVA using a similar model. In addition, mean values for the progesterone and oestradiol measurements before the oxytocin challenge on day 12 (mPTP, mPTE) were also obtained and used in regression analysis with PGFM response characteristics.

A PGFM response was defined as an increase in two points during the post-treatment period above the mean + 2 SD values of the pretreatment period. Peak height (PGFM peak) was defined as the greatest increase during the post-treatment period above the pretreatment mean. In addition, mean values (PGFM mean) during the post-treatment period above the pretreatment mean were also obtained.

Effects of treatment and day on the PGFM concentrations were determined by split-plot ANOVA with individual sheep used as plots and samples as subplots. Pretreatment (prior to oxytocin) and post-treatment values were analysed separately. Differences in treatment means and polynomial regression lines fitted to post-treatment values were evaluated by orthogonal contrasts. For example, patterns of PGFM concentrations after oxytocin were compared using tests of homogeneity of regression over the post-treatment period. Two further non-orthogonal contrasts were performed to compare (1) LPZE and HPZE groups and (2) HPHE and LPLE groups. Analysis of all PGFM data in the above manner revealed that the effects of steroid hormone concentration on the PGFM response to oxytocin were dependent on the day oxytocin was administered. To ease interpretation of the results, data from individual days were then analysed separately.

Relationships between PGFM responses on day 12 (PGFM peak, PGFM mean) and mPTE, mPTP or the ratio of mPTE: mPTP were determined by linear regression analysis (control group ewes were omitted from this analysis because progesterone values were below the limit of sensitivity of the assay).

Results

Progesterone and oestradiol

Progesterone concentrations of three ewes, one from each of groups HPZE, LPZE and HPHE, were above normal baseline values for ovariectomized ewes, before treatment (normal baseline values were below the limit of sensitivity of the assay). Data from these animals were excluded from the results. Upon examination at slaughter all ewes were shown to be

Fig. 1. Mean plasma progesterone (○) and oestradiol (●) profiles from ewes during the simulated cycle in (a) the high progesterone/low oestradiol group ($n = 4$) and (b) the low progesterone/high oestradiol group ($n = 4$). Time zero was designated as the time of the final 'oestrous' oestradiol injection. Arrows indicate the time of the oxytocin injections. Standard error of the difference, progesterone = 0.22, oestradiol = 0.85.

completely ovariectomized, and therefore the cause of the high progesterone concentration is unknown.

The oestradiol concentrations of the four ewes in the HPLE group (Fig. 1a) rose on days 2–4, 6–8, 10–12 before each oxytocin challenge and corresponding to follicular oestradiol waves normally seen in cyclic ewes (Smith *et al.*, 1976). The hormone pattern for the LPHE group was similar (Fig. 1b), although during the treatment period the concentrations of oestradiol were approximately three times higher than in the LE group, and the progesterone concentrations were approximately a third of those of the HP group. There was a significant difference in the plasma progesterone and oestradiol concentrations when high and low dose treated groups were compared ($P < 0.001$) and a significant difference in the concentration of progesterone in plasma when low and zero treated groups were compared ($P < 0.001$). Progesterone and oestradiol concentrations in samples taken before and after a steroid hormone injection on day 12 (PTP and PTE samples) from ewes in the HPHE group are shown (Fig. 2). Groups treated with zero oestradiol had circulating endogenous concentrations of oestradiol of approximately 1 pg ml⁻¹.

Fig. 2. Mean plasma progesterone (○) and oestradiol (●) concentrations from ewes following a progesterone and oestradiol injection (time zero) in the high progesterone/high oestradiol group on day 12 ($n = 4$). Standard error of the difference, progesterone = 0.44, oestradiol = 0.49.

PGFM

None of the ewes in the control group showed a PGFM response to oxytocin on any day. No ewe, in any group, showed a PGFM response to the oxytocin treatment administered on day 4, but two ewes showed a small but significant PGFM response on day 8 (one ewe in each of groups LPZE and HPLE). The percentage of ewes in each group exhibiting a PGFM response to the oxytocin challenge given on day 12 is shown (Fig. 3) with the mean PGFM response characteristics for each group.

Analysis of all PGFM post-treatment data revealed that the day of oxytocin administration determined whether the steroid hormone concentration affected the PGFM response to an oxytocin challenge. There was no significant difference in the pattern of PGFM production following oxytocin on day 4 compared with day 8 ($P > 0.05$); however, there was a difference on day 12 compared with days 4 and 8 ($P < 0.001$). On days 4 and 8 the PGFM concentration did not rise above the pretreatment baseline value, whereas on day 12, oxytocin caused an increase in the PGFM concentration. The mean PGFM pretreatment concentration on day 12 was significantly greater than on days 4 and 8 ($P < 0.01$) but was not significantly affected by steroid hormone concentration.

The analysis of day 12 PGFM post-treatment data revealed a significant negative effect of progesterone concentration ($P < 0.05$) and a highly significant positive effect of oestradiol concentration ($P < 0.01$) on the pattern of PGFM release in response to oxytocin. Mean day 12 PGFM data from all oestradiol-treated groups is shown (Fig. 4). The LPHE group showed the largest and most sustained increase in PGFM following oxytocin. The high value in the first post-treatment sample in the HPLE group is due to a large increase in only one ewe. The pattern of PGFM response in the HPHE and LPLE groups (similar ratio, different concentrations) did not differ significantly. The pattern of PGFM response in the HPZE and LPZE groups did not differ significantly, although it should be noted that all ewes in the LPZE group and no ewes in the

Fig. 3. Mean $\pm$ standard error of the mean (SEM) 13,14-dihydro-15-keto PGF_{2 α} (PGFM) response characteristics and the percentage of ewes responding to oxytocin from all steroid hormone treated groups ($n = 4$) on day 12. PGFM peak (■): the greatest increase in plasma PGFM concentrations (pg ml⁻¹) during the period after oxytocin treatment, above the mean in the period before oxytocin treatment. PGFM mean (▨): the mean plasma PGFM concentration (pg ml⁻¹) during the period after oxytocin treatment above the mean before oxytocin treatment.

HPZE group showed a defined response to the oxytocin challenge (see Fig. 3).

On day 12, there was a positive relationship between mPTE:mPTP and PGFM mean response ($P < 0.05$; R value from linear regression analysis = 0.445, $n = 21$ points per line).

Discussion

This study demonstrated that the oxytocin-induced PGFM response in the ovariectomized ewe is under the quantitative control of progesterone and oestradiol. The concentration of steroid hormone administered determined the level of the PGFM response to an oxytocin challenge given late (day 12) in the simulated cycle.

The use of oestradiol and progesterone injections in this study produced an episodic-like pattern of plasma hormone concentrations similar to that in intact ewes (Baird *et al.*, 1976; Sheldrick and Flint, 1990). Plasma steroid hormone measurements made following injections on days 4, 8 and 12 demonstrated that the concentrations produced remained within physiological limits (well below values quoted by Smith *et al.*, 1976).

A criticism of many previous studies investigating the role of ovarian steroid hormones in the luteolytic mechanism is that the pattern of hormone administration often did not create plasma concentrations that closely mimicked the typical oestrous cycle steroid hormone profile. For example, progesterone was often administered at a constant rate and not in steadily increasing amounts (Caldwell *et al.*, 1972; Ford *et al.*, 1975; Louis *et al.*, 1977; Vallet *et al.*, 1990). In other studies, oestradiol was administered in a constant rather than a fluctu-

ating manner (Homanics and Silvia, 1988; Zhang *et al.*, 1992). The administration regimen used in the present study produced steroid hormone concentrations resembling endogenous secretion, in both temporal pattern and absolute amount (Smith *et al.*, 1976; Hauger *et al.*, 1977; Pant *et al.*, 1977).

Pretreatment baseline PGFM values were significantly higher on day 12 than on days 4 and 8, in all treated groups, including those not administered additional oestradiol. Prolonged exposure to progesterone appears to increase PGFM concentrations possibly by increasing the amount of PGH synthase (Raw *et al.*, 1988) or by stimulating the accumulation of lipid droplets in the uterine epithelium (Brinsfield and Hawk, 1973). However, an effect of oestradiol on the temporal changes in basal PGFM secretion cannot be excluded, since in the groups not treated with oestradiol, endogenous secretion accounted for concentrations of approximately 1 pg ml⁻¹ (presumably from adrenal secretion of oestrogenic hormones or their precursors (Adams *et al.*, 1990). Oestradiol may stimulate the action of PGH synthase or alter the functioning of an oxytocin pulse generator in the neurohypophysis (McCracken *et al.*, 1991).

The role of oestradiol and progesterone in the control of the oxytocin-stimulated PGFM release was also investigated. On days 4 and 8, there was a lack of response to oxytocin, irrespective of the steroid hormone concentration administered (the two responses detected on day 8 were probably large fluctuations in the baseline value rather than an actual PGFM release in response to the oxytocin administered). This result is in agreement with findings of Homanics and Silvia (1988) and Vallet *et al.* (1990) who found a similar lack of response on days 5 and 10 in their steroid hormone treated ovariectomized ewe models.

Fig. 4. Mean plasma 13,14-dihydro-15-keto PGF_{2α} (PGFM) response profiles on day 12 from ewes in four groups treated with progesterone and oestradiol ($n = 4$). Time zero is the time of the oxytocin injection. Treatment groups are (a) high progesterone, high oestradiol, (b) high progesterone, low oestradiol, (c) low progesterone, high oestradiol and (d) low progesterone, low oestradiol. Standard error of the difference = 28.1.

The attainment of a response to oxytocin on day 12 is in agreement with the results of previous studies (Homanics and Silvia, 1988; Vallet and Lamming, 1991; Lau *et al.*, 1992). Vallet and Lamming (1991) detected a PGFM response to oxytocin and increased oxytocin receptor concentrations on day 10 of progesterone treatment, equivalent to day 12 in this study (as progesterone was administered from day 0 by Vallet and Lamming, and from day 2 in the present study). Homanics and Silvia (1988) demonstrated that the response to oxytocin developed between days 10 and 15 in their model. Lau *et al.* (1992) first detected a response on day 14, which is also roughly equivalent to day 12 in this study, as progesterone concentrations in the model used by Lau *et al.* (1992) did not rise significantly until 4 days after oestrous oestradiol treatment, compared with day 2 in this study. In intact ewes a response to oxytocin was apparent on day 13 (Fairclough *et al.*, 1984) or day 14 (Raw *et al.*, 1990) after oestrus, equivalent to day 12 or 13 in this model, as some ewes began to show signs of behavioural oestrus approximately 24 h before the final 'oestrous' oestradiol injection. The development of the oxytocin-induced secretion is probably a reflection of the induction of oxytocin receptor synthesis, as Vallet and Lamming (1991) demonstrated that oxytocin receptor levels increased sharply between days 8 and 10 of progesterone treatment. In intact ewes, oxytocin receptor concentrations begin to increase on days 13–14 after oestrus (Sheldrick and Flint, 1985). The results provided by studies using intact and steroid hormone treated ovariectomized ewes investigating the

development of the oxytocin-induced PGFM response appear to be highly correlated.

The contention that progesterone alone controls the timing of the response to oxytocin is apparently supported by the results of the groups treated with progesterone only. The assumption that such results indicate that the timing of luteolysis depends solely on the actions of progesterone must be tempered by the observation that even ewes not administered oestradiol have a basal value of endogenous oestradiol that may be sufficient to increase the oxytocin receptor concentration without the addition of progesterone (Vallet *et al.*, 1990; Lau *et al.*, 1992); however, without progesterone, PGF_{2α} release in response to oxytocin does not occur (Vallet *et al.*, 1990). The present study, therefore, confirms and refines the working hypothesis of McCracken (1980), by providing a quantitative as well as a qualitative role for both progesterone and oestradiol, acting in concert or cascade. McCracken (1980) suggested that oestradiol stimulates, and progesterone inhibits, oxytocin receptor development, or put more precisely, that progesterone appears to inhibit the oestrogenic stimulation of functional oxytocin receptor formation. The period of progesterone inhibition is proposed to determine the duration of the luteal phase (Lamming *et al.*, 1991). The inhibitory influence of progesterone is lost late in the luteal phase when progesterone receptor levels decline, and progesterone is unable to prevent oestradiol receptor formation. The significant effect of the oestradiol concentration administered, and the positive correlation between the ratio of pretreatment plasma oestradiol:progesterone concentration and the level of PGFM response to oxytocin, confirms the importance of oestradiol in the control of the luteolytic mechanism. Throughout the luteal phase, oestradiol secretion by waves of developing follicles may provide the stimulatory drive leading to the onset of luteolysis (Karsh *et al.*, 1970; Cox *et al.*, 1972; Zhang *et al.*, 1991).

A high progesterone concentration significantly reduced the PGFM response to oxytocin supporting the 'progesterone block' hypothesis of McCracken (1980) and extending this by suggesting that the progesterone block is less effective when the uterus is exposed to low concentrations of progesterone. There is supporting evidence in intact ewes in which progesterone supplementation significantly reduced PGFM peak amplitude, observed in response to endogenous oxytocin surges, in comparison with control ewes (Fairclough *et al.*, 1983). Ott *et al.* (1992) demonstrated that withdrawal of progesterone treatment from ovariectomized ewes on day 10 increased the oxytocin-induced PGFM response on days 13 and 14, in comparison with ewes in which progesterone treatment was maintained until day 15. However, Vallet *et al.* (1990) suggested that the luteolytic mechanism requires progesterone. It is possible that only a low effective concentration of progesterone (that is low concentration or low receptor level) is required to maintain the prostaglandin precursor supply, but the inhibitory actions of progesterone on the binding of oestrogen to its receptor may require a higher effective concentration of progesterone (that is, high concentration or high receptor level).

It has therefore been established that the level of response to luteolytic signals is held in balance, with the inhibitory effect of progesterone acting on one side and oestrogenic stimulation

providing a positive influence on the other. An increase in the effective concentration of oestradiol could tip the balance and induce luteolysis. The positive correlation between the oestradiol:progesterone ratio and the level of PGFM response represents a summation of the positive effects of oestradiol and the negative effects of progesterone on oxytocin receptor development. The similar response in the HPHE and LPLE groups indicate that it is the ratio of oestradiol:progesterone that is important rather than the absolute concentration of each individual steroid hormone. Corroborative evidence is provided by Silvia and Taylor (1989) who showed a similar correlation between the oestradiol:progesterone ratio and the response to oxytocin in cyclic cows. Furthermore, Parkinson *et al.* (1990) demonstrated a positive correlation between plasma oestradiol concentrations and uterine oxytocin receptor levels, and a negative correlation between plasma progesterone concentrations and oxytocin receptor levels. Together these studies establish the working hypothesis that cyclic ewes and cows with a high oestradiol:progesterone ratio may generate stronger luteolytic signals. Further extrapolation to pregnant cows and ewes indicates that the abnormal steroid hormone patterns associated with embryo loss (Lamming *et al.*, 1989) may be the cause of the failure to maintain a pregnancy, through their influence on the luteolytic mechanism.

A. P. Beard was in receipt of a MAFF postgraduate scholarship. The authors thank J. L. Vallet for his help in the design and execution of this study; G. E. Mann for expert advice concerning the establishment of the oestradiol assay; M. Jones and L. Hill for their excellent assistance in the laboratory; and staff of JABU for their help with the animal work; and J. Craigmiles for statistical advice and H. Dobson for PGFM antiserum.

References

- Adams NR, Atkinson S, Hoskinson RM, Abordi JA, Briegel JR, Jones M and Sanders MR (1990) Immunization of ovariectomized ewes against progesterone, oestrogen or cortisol to detect effects of adrenal steroid on reproduction *Journal of Reproduction and Fertility* **89** 477–483
- Baird DT, Swanston I and Scaramuzzi RJ (1976) Pulsatile release of LH and secretion of ovarian steroids in sheep during the luteal phase of the estrous cycle *Endocrinology* **98** 1490–1496
- Bazer FW, Thatcher WW, Hansen PJ, Miranda MA, Ott TL and Plante C (1991) Physiological mechanisms of pregnancy recognition in ruminants *Journal of Reproduction and Fertility Supplement* **43** 39–47
- Brinsfield TH and Hawk HW (1973) Control by progesterone of the concentrations of lipid droplets in epithelial cells of the sheep endometrium *Journal of Animal Science* **36** 919–922
- Caldwell BV, Tillson SA, Brock WA and Speroff L (1992) The effects of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes *Prostaglandins* **1** 217–228
- Cognie Y, Hernandez-Barreto M and Saumande J (1975) Low fertility in nursing ewes during the non-breeding season *Annales de Biologie Animale Biochimie Biophysique* **15** 329–343
- Cox RI, Thorburn GD, Currie WB, Restall BJ and Schneider W (1972) Prostaglandin F group (PGF), progesterone and estrogen concentrations in the uteroovarian venous plasma of the conscious ewe during the estrous cycle *Advances in Biosciences* **9** 625–630
- Fairclough RJ, Moore LG, Gowan LT, Smith JF and Watkins WB (1983) Effect of exogenous progestogens on plasma concentrations of the oxytocin-associated neurophysin and 13,14-dihydro-15-keto-prostaglandin F in intact and ovariectomized ewes over the time of expected luteal regression *Biology of Reproduction* **29** 271–277
- Fairclough RJ, Peterson AJ, Moore LG and Watkins WB (1984) The endocrine control of luteal oxytocin and uterine PGF_{2α} release in the ewe. In *Reproduction in Sheep* pp 125–126 Eds DR Lindsay and DT Pearce. Australian Academy of Science, Canberra
- Flint APF and Sheldrick EL (1983) Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep *Journal of Reproduction and Fertility* **67** 215–225
- Flint APF, Stewart HJ, Lamming GE and Payne JH (1992) Role of oxytocin receptor in the choice between cyclicity and gestation in ruminants *Journal of Reproduction and Fertility Supplement* **45** 53–58
- Ford SP, Weems CW, Pitts RE, Pexton JE, Butcher RL and Inskeep EK (1975) Effects of estradiol-17β and progesterone on prostaglandins F in sheep uteri and uterine venous plasma *Journal of Animal Science* **41** 1407–1413
- Ginther OJ (1968) Influence of exogenous progesterone and the uterus on ovarian activity in sheep *Endocrinology* **83** 613–615
- Haresign W, Foster JP, Haynes NB, Crighton DB and Lamming GE (1975) Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone *Journal of Reproduction and Fertility* **43** 269–279
- Hauger RL, Karsch FJ and Foster DL (1977) A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion *Endocrinology* **101** 807–817
- Hixon JE and Flint APF (1987) Effects of a luteolytic dose of oestradiol benzoate on uterine oxytocin receptor concentrations, phosphoinositide turnover and PGF_{2α} secretion in sheep *Journal of Reproduction and Fertility* **79** 457–467
- Hixon JE, Gengenbach DR and Hansel W (1975) Failure of prostaglandin F_{2α} to cause luteal regression in ewes after destruction of ovarian follicles by X-irradiation *Biology of Reproduction* **13** 126–135
- Homanics GE and Silvia WJ (1988) Effects of progesterone and estradiol-17β on uterine secretion of prostaglandin F_{2α} in response to oxytocin in ovariectomized ewes *Biology of Reproduction* **38** 804–811
- Hunter MG, Southey JA, McLeod BJ and Haresign W (1986) Progesterone pretreatment has a direct effect on GnRH-induced preovulatory follicles to determine their ability to develop into normal corpora lutea in anoestrous ewes *Journal of Reproduction and Fertility* **76** 349–363
- Kaker ML, Murray RD and Dobson H (1984) Plasma hormone changes in cows during induced or spontaneous calvings and the early post partum period *Veterinary Record* **115** 378–382
- Karsch FJ, Noveroske JW, Roche JF, Norton HW and Nalbandov AV (1970) Maintenance of ovine corpora lutea in the absence of ovarian follicles *Endocrinology* **87** 1228–1236
- Lamming GE, Darwash AO and Back HL (1989) Corpus luteum function in dairy cows and embryo mortality *Journal of Reproduction and Fertility Supplement* **37** 245–252
- Lamming GE, Vallet JL and Flint APF (1991) Progestational control of endometrial oxytocin receptor determines cycle length in sheep *Journal of Reproduction and Fertility Supplement* **43** 53–54
- Lau TM, Gow CB and Fairclough RJ (1992) Differential effects of progesterone treatment on the oxytocin-induced prostaglandin F_{2α} response and the levels of endometrial oxytocin receptors in ovariectomized ewes *Biology of Reproduction* **46** 17–22
- Louis TM, Parry DM, Robinson JS, Thorburn GD and Challis JRG (1977) Effects of exogenous progesterone and oestradiol on prostaglandin F and 13,14-dihydro-15-oxoprostaglandin F_{2α} concentrations in uteri and plasma of ovariectomized ewes *Journal of Endocrinology* **73** 427–439
- Lowry SR (1992) Use and misuse of multiple comparisons in animal experiments *Journal of Animal Science* **70** 1971–1977
- Lukaszewska J and Hansel W (1980) Corpus luteum maintenance during early pregnancy in the cow *Journal of Reproduction and Fertility* **59** 485–493
- McCracken JA (1980) Hormone receptor control of prostaglandin F_{2α} secretion by the ovine uterus. In *Advances in Prostaglandin and Thromboxane Research* Vol 8 Ed. B Samuelsson, PW Ramwell and R Paoletti. Raven Press, New York
- McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K and Samuelsson B (1972) Prostaglandin F_{2α} identified as a luteolytic hormone in sheep *Nature* **238** 129–134
- McCracken JA, Cammell LM, Glew ME and Underwood LF (1978) Hormone receptor control of prostaglandin secretion from the ovine uterus *Endocrinology* **102** A440
- McCracken JA, Schramm W and Okulicz WC (1984) Hormone receptor control of pulsatile secretion of PGF_{2α} from the ovine uterus during luteolysis and its abrogation in early pregnancy *Animal Reproduction Science* **7** 31–55

- McCracken JA, Smith TT, Lamsa JC and Robinson AG (1991) The effect of estradiol 17 β - (E) and progesterone (P) on the oxytocin (OT) pulse generator in the ovariectomized sheep *Biology of Reproduction* **44** (Supplement 1) Abstract 137
- Ott TL, Miranda MA, Davis MA and Bazer FW (1992) Effects of ovine conceptus secretory proteins and progesterone on oxytocin-stimulated endometrial production of prostaglandin and turnover of inositol phosphate in ovariectomized ewes *Journal of Reproduction and Fertility* **95** 19–29
- Ottobre JS, Lewis GS, Thayne WV and Inskeep EK (1980) Mechanism by which progesterone shortens the estrous cycle of the ewe *Biology of Reproduction* **23** 1046–1053
- Pant HC, Hopkinson CRN and Fitzpatrick RJ (1977) Concentration of oestradiol, progesterone, luteinizing hormone and follicle stimulating hormone in the jugular venous plasma of ewes during the oestrous cycle *Journal of Endocrinology* **73** 247–255
- Parkinson TJ, Jenner LJ and Lamming GE (1990) Comparison of oxytocin/prostaglandin F_{2 α} interrelationships in cyclic and pregnant cows *Journal of Reproduction and Fertility* **90** 337–345
- Parr RA (1992) Nutrition–progesterone interactions during early pregnancy in sheep *Reproduction Fertility and Development* **4** 297–300
- Parr RA, Davis IF, Fairclough RJ and Miles MA (1987) Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep *Journal of Reproduction and Fertility* **80** 317–320
- Raw RE, Curry TE and Silvia WJ (1988) Effects of progesterone and estradiol on the concentration and activity of cyclooxygenase in the ovine uterus *Biology of Reproduction* **38** (Supplement 1), Abstract 104
- Raw RE, Hayes SH and Silvia WJ (1990) Development of oxytocin-induced secretion of prostaglandin F_{2 α} during the estrous cycle and early pregnancy in ewes *Biology of Reproduction* **42** (Supplement) Abstract 172
- Remsen LG and Roussel JD (1982) Pregnancy rates relating to plasma progesterone levels in recipient heifers at the day of transfer *Theriogenology* **18** 365–372
- Roberts JS, McCracken JA, Gavagan JE and Soloff MS (1976) Oxytocin-stimulated release of prostaglandin F_{2 α} from ovine endometrium *in vitro*: correlation with estrous cycle and oxytocin receptor binding *Endocrinology* **99** 1107–1114
- Sheldrick EL and Flint APF (1985) Endocrine control of uterine oxytocin receptors in the ewe *Journal of Endocrinology* **106** 249–258
- Sheldrick EL and Flint APF (1990) Effect of continuous infusion of oxytocin on prostaglandin F_{2 α} secretion and luteolysis in the cyclic ewe *Reproduction Fertility and Development* **2** 89–99
- Silvia WJ and Taylor ML (1989) Relationship between uterine secretion of prostaglandin F_{2 α} induced by oxytocin and endogenous concentrations of estradiol and progesterone at three stages of the bovine estrous cycle *Journal of Animal Science* **67** 2347–2353
- Smith JF, Drost H, Fairclough RJ, Peterson AJ and Tervit HR (1976) Effect of age on peripheral levels of progesterone and oestradiol-17 β , and duration of oestrus in Romney Marsh ewes *New Zealand Journal of Agricultural Research* **19** 277–280
- Stormshak F, Kelley HE and Hawk HW (1969) Suppression of ovine luteal function by 17 β -estradiol *Journal of Animal Science* **29** 476–478
- Vallet JL and Lamming GE (1991) Ovine conceptus secretory proteins and bovine recombinant interferon α_1 -1 decrease endometrial oxytocin receptor concentrations in cyclic and progesterone-treated ovariectomized ewes *Journal of Endocrinology* **131** 475–482
- Vallet JL, Lamming GE and Batten M (1990) Control of endometrial oxytocin receptor and uterine response to oxytocin by progesterone and oestradiol in the ewe *Journal of Reproduction and Fertility* **90** 625–634
- Webb R, Baxter G, McBride D, Nordblum GD and Shaw MPK (1985) The measurement of testosterone and oestradiol-17 β using iodinated tracers and incorporating an affinity chromatography extraction procedure *Journal of Steroid Biochemistry* **23** 1043–1051
- Zhang J, Weston PG and Hixon JE (1991) Influence of estradiol on the secretion of oxytocin and prostaglandin F_{2 α} during luteolysis in the ewe *Biology of Reproduction* **45** 395–403
- Zhang J, Weston PG and Hixon JE (1992) Role of progesterone and oestradiol in the regulation of uterine oxytocin receptors in ewes *Journal of Reproduction and Fertility* **94** 395–404