Concentrations of progesterone and oxytocin receptors in endometrium of *postpartum* cows expected to have a short or normal oestrous cycle

W. G. Zollers, Jr.^{1*}, H. A. Garverick^{1†}, M. F. Smith¹, R. J. Moffatt¹,
B. E. Salfen¹ and R. S. Youngquist²

¹Departments of Animal Sciences and ²Veterinary Medicine and Surgery, University of Missouri, Columbia, MO 65211, USA

Luteal lifespan is short after first *postpartum* ovulation in early-weaned beef cows unless cows are pretreated with a progestogen. Regression of the short-lived corpus luteum in the *postpartum* beef cow is due to a premature release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus. The premature release of $PGF_{2\alpha}$ may be mediated through lower concentrations of receptors for progesterone, higher concentrations of oxytocin receptors, or both, in the endometrium. Thirty-one beef cows were randomly assigned to four groups at parturition. Calves from cows assigned to the short cycle group ($n = 6$; control) and the short cycle/endometrium group ($n = 10$) were weaned at 30–32 days *post partum*. Cows in the normal cycle group ($n = 5$; control) and the normal cycle/endometrium group ($n = 10$) received norgestomet implants for 9 days beginning 21–23 days *post partum* and calves were weaned at implant insertion. Duration of oestrous cycle ($\bar{x} \pm \text{SEM}$; $P < 0.01$) following first *postpartum* ovulation for the short cycle group was 11.5 ± 1.9 days compared with 18.8 ± 0.6 days for the normal cycle group. On day 5 following first *postpartum* ovulation, cows in the short cycle/endometrium and the normal cycle/endometrium groups were hysterectomized and endometrial tissue collected for measurement of progesterone and oxytocin receptors. Mean number of total progesterone receptors per cell was lower ($P < 0.05$) in the short cycle/endometrium group than in the normal cycle/endometrium group. Mean concentration of oxytocin receptors (fmol mg^{-1} protein) in the short cycle/endometrium group was higher ($P < 0.05$) than that in the normal cycle/endometrium group. In conclusion, uterine receptor populations for progesterone and oxytocin may influence the timing of $PGF_{2\alpha}$ secretion during short oestrous cycles.

Introduction

Subnormal luteal function has been associated with infertility in ruminants (for review see Garverick and Smith, 1986; Hunter, 1991; Lishman and Inskeep, 1991). Corpora lutea with short lifespans have been detected at puberty in cattle (Gonzalez-Padilla *et al.*, 1975) and sheep (Berardinelli *et al.*, 1980) and following the first *postpartum* ovulation in cattle (Odde *et al.*, 1980). In anoestrous ewes, induced ovulation by treatment with GnRH is followed by subnormal luteal function (Haresign *et al.*, 1975).

The following evidence indicates that the short luteal phase in cattle and sheep is due to a premature uterine secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$): (i) hysterectomy prevents regression of corpora lutea anticipated to have short lifespans in prepubertal ewe lambs (Keisler *et al.*, 1983), anoestrous ewes (Southee *et al.*, 1988) and *postpartum* cows (Copelin *et al.*, 1987); (ii)

oxytocin induces a release of $PGF_{2\alpha}$ in cattle on day 5 of a short but not a normal luteal phase (Zollers *et al.*, 1989); (iii) basal and oxytocin-induced secretion of prostaglandins (*in vitro*) from bovine endometrium is greater on day 5 of a short compared with a normal luteal phase (Zollers *et al.*, 1991); (iv) mean concentrations of $PGF_{2\alpha}$ in the vena cava on days 4–9 following first *postpartum* oestrus are higher in cows exhibiting a short compared with a normal oestrous cycle (Cooper *et al.*, 1991); (v) mean concentration of 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ ($PGFM$; the stable metabolite of $PGF_{2\alpha}$) in plasma increases from day 3 to day 5 in ewes expected to have short oestrous cycles (Hunter *et al.*, 1989); and (vi) active immunization of beef cows early *post partum* against $PGF_{2\alpha}$ extends the lifespan of corpora lutea that are expected to be short-lived (Copelin *et al.*, 1989).

McCracken *et al.* (1984) hypothesized that progesterone directly inhibits synthesis of uterine oxytocin receptors through the mid-luteal phase of a normal oestrous cycle. Declining progesterone dominance of the uterus near the end of the luteal phase may allow the synthesis of endometrial oxytocin receptors. Thus, the uterus becomes responsive to oxytocin and a positive feedback loop between $PGF_{2\alpha}$ and luteal oxytocin

*Present address: University of Maryland School of Medicine, Physiology Department, Bressler Research Laboratories 11-016, 655 West Baltimore Street, Baltimore, MD 21201, USA.

†Reprint requests.

Received 21 February 1992.

develops. This mechanism may ensure pulsatile secretion of $\text{PGF}_{2\alpha}$ to complete the process of luteal regression (Flint *et al.*, 1990; Silvia *et al.*, 1991).

In cows exhibiting a short oestrous cycle, decreased serum concentrations of oestradiol have been observed at oestrus compared with cows having a subsequent normal luteal phase (Garcia-Winder *et al.*, 1986; Garverick *et al.*, 1988). During a normal oestrous cycle, oestradiol from the preovulatory follicle may induce uterine progesterone receptors which are required to establish progesterone dominance of the uterus following subsequent ovulation (Ottobre *et al.*, 1984). The decreased concentration of oestradiol observed at oestrus before a short luteal phase may therefore result in inadequate uterine progesterone receptor synthesis. Thus, the uterus may lose progesterone dominance earlier following ovulation. This would allow premature synthesis of oxytocin receptors and thereby initiate the positive feedback loop between oxytocin and $\text{PGF}_{2\alpha}$ earlier in the oestrous cycle. The purpose of this study was to investigate mechanisms involved in triggering uterine release of $\text{PGF}_{2\alpha}$ at an earlier time during the short luteal phase. Specifically, the objective was to determine concentrations of endometrial oxytocin and progesterone receptors on day 5 following the first *postpartum* oestrus in cows expected to have a short or normal luteal phase.

Materials and Methods

Animals

Thirty-one crossbred beef cows, expected to have short or normal oestrous cycles, were randomly assigned at parturition to four groups: short cycle ($n = 6$; control), short cycle/endometrium ($n = 10$), normal cycle ($n = 5$; control) and normal cycle/endometrium ($n = 10$). All cows were in moderate to good body condition at calving. Cows in the normal cycle and normal cycle/endometrium groups received norgestomet implants (17 α -acetoxy-11 β -methyl-19-nor pregn-4-ene-3,20-dione; Sanofi Animal Health, Overland Park, KS) inserted into the ear for 9 days beginning 21–23 days *post partum*. Calves were weaned from cows in the short cycle and short cycle/endometrium groups at 30–32 days *post partum* and from cows in the normal cycle and normal cycle/endometrium groups at the time of implant insertion. Cows in the short cycle and normal cycle groups were used to verify that short cycles occurred in untreated animals and that norgestomet pretreatment resulted in a cycle of normal duration (Ramirez-Godinez *et al.*, 1981).

Cows were observed for oestrus twice daily from parturition until the end of the experiment. Blood was collected from each cow via jugular venepuncture three times a week from parturition to weaning and then daily from weaning or implant removal to hysterectomy or second oestrus. Blood samples were kept at 4°C for 6–12 h, and serum was harvested and frozen at –20°C for subsequent measurement of progesterone. Concentration of progesterone in serum was determined by radioimmunoassay as previously described (Copelin *et al.*, 1989). The intra-assay and interassay coefficients of variation were 12.1% and 11.4%, respectively. Concentrations of progesterone were used to identify cows that ovulated prior to

weaning, estimate the time of ovulation in animals that did not exhibit oestrus prior to first ovulation, and determine duration of luteal function and oestrous cycle duration in control animals. The first rise in serum concentration of progesterone was determined to be day 3 of the oestrous cycle. All control animals exhibited behavioural oestrus prior to their second *postpartum* ovulation.

Tissue collection

On day 5 following the first *postpartum* oestrus, cows in the short cycle/endometrium and normal cycle/endometrium groups were hysterectomized via midline incision. Cows were tranquilized by injecting (i.m.) 25 mg xylazine (Haver Lockhart, Shawnee, KS) and 20 mg acepromazine maleate (Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA). Lidocaine hydrochloride (2%; Tech America Group, Inc., Elwood, KS) was used as a local anaesthetic. After hysterectomy, the uterine horns were immediately placed on ice. Aliquots of endometrial tissue, containing caruncular and intercaruncular tissue, were removed from uterine horns within 10 min and rinsed in 0.9% saline. Endometrial samples were frozen in 3 g aliquots in liquid nitrogen and kept at –80°C until contents of progesterone and oxytocin receptors were measured.

Measurement of endometrial cytosolic and nuclear progesterone receptors

Preparation of cytosolic and nuclear fractions. The designations 'cytosolic' and 'nuclear' are defined experimentally, as described below. These terms are used to refer to the two main subcellular fractions into which progesterone receptors redistribute following homogenization of tissue in the buffers used. Our method of tissue preparation was not designed to maintain progesterone receptors at their point of origin (cytosol versus nucleus) in the cell. Various studies have demonstrated that progesterone receptors and other members of the steroid hormone superfamily reside predominantly in the nucleus *in vivo* (Gasc *et al.*, 1984; King and Greene, 1984; Welshons *et al.*, 1985). An aliquot of endometrium from each experimental animal was removed from storage at –80°C, chopped finely with scissors, and allowed to thaw on ice. All procedures were done at 4°C or on ice unless otherwise stated. Tissue was homogenized at a tissue:buffer ratio of 120 mg ml⁻¹ in TEMG buffer (10 mmol Tris-HCl l⁻¹, pH 7.5, 1.5 mmol EDTA Na₄ l⁻¹, 12 mmol monothioglycerol l⁻¹, 10% glycerol, 10 mmol molybdc acid l⁻¹) with glass Duall tissue grinders. Homogenates were centrifuged (1500 g for 30 min) to separate the nuclear pellet from the low-speed cytosol. The nuclear pellet was washed three times with 2.0 ml of TEMG buffer followed by centrifugation for 10 min at 1500 g. The nuclear pellet was resuspended at 120 mg ml⁻¹ in TEMG buffer. The cytosol fraction was further centrifuged (13 000 g) for 30 min before treatment with dextran-coated charcoal (DCC; 0.5% charcoal, 0.05% dextran in TEMG buffer) to remove unbound steroids. The DCC-treated supernatant was centrifuged at 105 000 g for 1 h to obtain a high-speed cytosol. High-speed cytosol was adjusted with TEMG buffer to a tissue concentration of 30 mg ml⁻¹. Nuclear and cytosolic progesterone receptors were measured by nuclear and cytosol exchange assays (Anderson *et al.*, 1972; Katzenellenbogen *et*

Fig. 1. Optimal conditions for measurement of bovine endometrial progesterone receptors. (a) Effects of temperature over time on $[^3\text{H}]$ progesterone binding. (b) Effects of concentration of cytosol fraction added to the assay tube on $[^3\text{H}]$ progesterone binding. (c) Dissociation analysis of $[^3\text{H}]$ progesterone binding in cytosol. (d) Competitive inhibition of $[^3\text{H}]$ progesterone for the progesterone receptor. SB: specific binding; NSB: nonspecific binding.

al., 1973). Both fractions were measured to account for total progesterone receptor concentration.

Cytosolic and nuclear exchange assays. Aliquots (250 μl) of each fraction were added to respective assay tubes. Saturation analysis was conducted using 1–30 nmol l^{-1} $[^3\text{H}]$ progesterone (9 points; New England Nuclear, Boston, MA) in the presence and absence (three tubes each) of a 100-fold molar excess of unlabelled progesterone for the nuclear fraction (4 h at 4°C) and cytosolic fraction (16 h at 4°C) in a total volume of 350 μl . Crude nuclei were washed three times to remove unbound ligand by using 1.0 ml of TEMG buffer per tube and centrifuging for 10 min at 1500 g . Subsequent to the final wash, 1.0 ml of 100% ethanol was added to each crude nuclear pellet to solubilize nuclear-bound $[^3\text{H}]$ progesterone, and tubes were incubated for 30 min at 30°C. Tubes were placed in an ice bath for 5 min and then centrifuged at 1500 g for 10 min. After incubation, cytosol aliquots were treated with DCC to remove unbound ligand. Tubes were immediately centrifuged at 1500 g for 10 min. Aliquots (0.5 ml) of supernatant from both the nuclear and cytosolic exchange assays were measured for $[^3\text{H}]$ progesterone in a liquid scintillation counter. The protein content of high-speed cytosol was determined using the method of Lowry *et al.* (1951). DNA content of crude nuclei was determined by the method of Burton (1965).

Specific binding was determined as the amount of total binding minus nonspecific binding. Data were analysed according to the method of Scatchard (1949). Standard curves representing

each $[^3\text{H}]$ progesterone concentration (1 to 30 nmol l^{-1}) were generated and counting efficiencies estimated. Bound counts per minute were converted to fmol progesterone by correcting for counting efficiencies. Results were calculated on a receptor number per cell basis by using a value of 6.5 pg of DNA per bovine somatic cell (Shapiro, 1970).

Validation. Optimal conditions for exchange of specific binding by progesterone receptors were determined by examining several variables using cytosol fractions prepared from endometrium of a cow on the day of oestrus (day 0). The optimal time and temperature incubation period for cytosol was determined as 8–24 h at 4°C (Fig. 1a).

The addition of increasing amounts of cytosol fraction resulted in an increase in $[^3\text{H}]$ progesterone bound (95.0 fmol at 15 mg ml^{-1} to 342.7 fmol at 90 mg ml^{-1}) (Fig. 1b).

An association curve was established for progesterone receptors indicating that approximately 80–90% of maximal association with $[^3\text{H}]$ progesterone was attained by 4 h (Fig. 1c). After addition of unlabelled progesterone, maximum exchange was recorded by 4 h.

Binding specificity of the progesterone receptor was established by examining the ability of a number of steroidal compounds to inhibit specific $[^3\text{H}]$ progesterone-binding (Fig. 1d). All unlabelled compounds were obtained from Sigma Chemical Co. (St Louis, MO), with the exception of promegestone (R 5020, a synthetic progestin) which was from New England Nuclear (Boston, MA). Since the true affinities of these

Fig. 2. Representative (a) and (c) saturation curves and (b) and (d) Scatchard analyses (right panels) of hormone-binding assays of the nuclear progesterone (a, b) and membrane bound oxytocin (c, d) receptors. (○) Data for the normal cycle group; and (●) data for the short cycle group.

compounds for the progesterone receptor were not known, relative binding affinities were estimated as $100\% \times [100\% - \% \text{ binding in the presence of competitor}]/[100\% - \% \text{ binding in the presence of progesterone}]$. Relative binding affinities exhibited by R 5020, oestradiol, testosterone and corticosterone, when present in 100-fold molar excesses, were 98.5, 21.7, 38.1 and 61.7%, respectively. Relative binding affinities of hydrocortisone and dexamethasone were each less than 1%.

Under these conditions, and when measuring content of samples in which progesterone receptor concentrations were below nanomolar, saturation curves were generated and used to estimate progesterone receptor concentrations in subcellular fractions. Two saturation curves and their respective Scatchard analyses, representative of assays of cytosol and nuclei of a short cycle cow, are shown (Fig. 2a and b). Respective estimates of K_d and B_{max} values for the assays shown were 4.2 nmol l^{-1} and $1.28 \times 10^{-10} \text{ mol l}^{-1}$ (for progesterone receptors in cytosol) and $16.96 \text{ nmol l}^{-1}$ and $5.16 \times 10^{-10} \text{ mol l}^{-1}$ (for progesterone receptors in nuclei).

Measurement of oxytocin receptors in endometrial membrane fraction

Preparation of membrane fraction. Subcellular fractions of endometrium were prepared by the method of Sheldrick and

Flint (1985). Briefly, a sample of endometrial tissue was removed from storage at -80°C , minced with scissors, and allowed to thaw on ice. All subsequent procedures were conducted at 4°C unless noted. Tissue was homogenized using a Polytron in TES buffer ($25 \text{ mmol Tris-HCl l}^{-1}$, pH 7.6, $1 \text{ mmol EDTA l}^{-1}$, $0.25 \text{ mol sucrose l}^{-1}$). The homogenates were centrifuged at $2000g$ for 10 min to remove nuclear material and then at $95\,000g$ for 1 h to pellet the membrane fraction. Pellets were resuspended by homogenization in Tris buffer ($25 \text{ mmol Tris-HCl l}^{-1}$, pH 7.6). Protein concentrations in the resuspended membrane fraction were determined by the method of Lowry *et al.* (1951). The membrane fraction was adjusted to $1\text{--}2 \mu\text{g protein } \mu\text{l}^{-1}$.

Measurement of $[^3\text{H}]$ oxytocin binding to membrane fraction. Membrane fraction ($50 \mu\text{l} = 50\text{--}100 \mu\text{g protein}$) was added to each assay tube. Saturation analysis was conducted using $0.5\text{--}30 \text{ nmol } [^3\text{H}]$ oxytocin l^{-1} (10 points; New England Nuclear, Boston, MA) in the presence and absence (two tubes each) of a 100-fold molar excess of unlabelled oxytocin (Sigma Chemical, St Louis, MO). After addition of all reagents, assay tubes were incubated at 25°C for 20 min. After incubation, tubes were transferred to an ice bath and $2 \text{ ml Tris-BSA buffer}$ ($25 \text{ mmol Tris-HCl l}^{-1}$, pH 7.6, 0.1% bovine serum albumin)

Fig. 3. Optimal conditions for measurement of bovine endometrial oxytocin receptors. (a) Effects of temperature of incubation on $[^3\text{H}]$ oxytocin binding. (b) Effects of time of incubation on $[^3\text{H}]$ oxytocin binding. (c) Effects of protein concentration of membrane fraction added to the assay tube on $[^3\text{H}]$ oxytocin binding. (d) Competitive inhibition of $[^3\text{H}]$ oxytocin for the oxytocin receptors. SB: specific binding; NSB: nonspecific binding.

was added immediately. The buffer was removed from the receptor protein by filtration onto Millipore (Millipore Corp, Bedford, MA) GVWP filters under vacuum. Filters (carrying receptor-bound $[^3\text{H}]$ oxytocin) were rinsed twice under vacuum with Tris-BSA buffer. The filters were then transferred to scintillation vials containing ethylene glycol monoethyl ether, necessary to solubilize the protein bound to the filter. Scintillation fluid was added to each vial and, after 3 h, samples were counted for $[^3\text{H}]$ oxytocin in a liquid scintillation counter.

Specific binding was determined as the amount of total binding minus nonspecific binding. Data were analysed by Scatchard analysis (Scatchard, 1949). Counting efficiencies were estimated using standard curves which were generated from each $[^3\text{H}]$ oxytocin concentration ($0.5\text{--}30\text{ nmol l}^{-1}$). Bound c.p.m. were converted to fmol of oxytocin by correcting for counting efficiencies.

Validation. Conditions for the optimal measurement of oxytocin receptor binding were established using membrane fractions from a cow on the day of oestrus. Effects of temperature and time of incubation on specific oxytocin binding were determined as 20 min to 1 h at 25°C (Fig. 3a, b).

Increasing amounts of protein resulted in an increase in $[^3\text{H}]$ oxytocin bound (46.9 fmol at $10\text{ }\mu\text{g}$ protein to 346.2 fmol at $100\text{ }\mu\text{g}$ protein) (Fig. 3c).

Competitive inhibition of $[^3\text{H}]$ oxytocin for the oxytocin receptor was tested by using several related peptides (Sigma

Chemical Co., St Louis, MO) (Fig. 3d). Oxytocin, isotocin, arg⁸-vasotocin, arg⁸-vasopressin and lys⁸-vasopressin (15.8, 8.8, 14.4 and 27.6% of total binding, respectively) inhibited binding of $[^3\text{H}]$ oxytocin by oxytocin receptors. Tocinoic acid and Tyr-Pro-Leu-Gly amide did not inhibit binding of $[^3\text{H}]$ oxytocin to the oxytocin receptor.

Saturation curves were analysed by the method of Scatchard and used to estimate oxytocin receptor concentrations. A typical saturation curve and Scatchard analysis are presented (Fig. 2c and d). Estimates of K_d and B_{max} values for this assay were 2.51 nmol l^{-1} and $7.59 \times 10^{-11}\text{ mol l}^{-1}$, respectively.

Statistical analyses

Comparison of duration of oestrous cycle (short cycle versus normal cycle) was analysed by a one-tailed Student's *t* test to determine the effects of norgestomet pretreatment (Snedecor and Cochran, 1980). In addition, statistical differences between groups for progesterone and oxytocin receptor data were ascertained by a one-tailed Student's *t* test. Progesterone data were analysed by analysis of variance for a split-plot in time design with repeated measurements (Gill and Hafs, 1971). The general linear model included effects of treatment, time and treatment $\times$ time interaction. Cow within treatment variation was used as an error term for testing treatment effects. Mean differences were ascertained using a protected least significant difference test.

Fig. 4. Mean concentration of progesterone from day 0 to day 5 during the first *postpartum* oestrous cycle in beef cows. ($P > 0.05$ among groups.)

Results

Oestrous cycle durations and progesterone concentrations

Oestrous cycle duration ($\bar{x} \pm \text{SEM}$) was shorter ($P < 0.01$) for the short cycle group (11.5 ± 1.9 days) than for the normal cycle group (18.8 ± 0.6 days). Mean concentration of progesterone was similar ($P > 0.10$) for all groups from day 0 to day 5 (Fig. 4). On day 6, mean concentration of progesterone ($\bar{x} \pm \text{SEM}$) was lower ($P < 0.05$) for the short cycle group ($1.6 \pm 0.4 \text{ ng ml}^{-1}$) than for the normal cycle group ($3.3 \pm 0.6 \text{ ng ml}^{-1}$). Progesterone concentration decreased thereafter to oestrus in the short cycle group and increased in the normal cycle group (peaking at 4.9 ng ml^{-1} on day 12) and remained high until luteolysis (0.1 ng ml^{-1} on day 18; data not shown). In addition, concentrations of progesterone were higher in all cows at the time of hysterectomy than they were on day 4. Functional luteolysis had, therefore, not begun at the time of collection of endometrial tissue.

Endometrial content of progesterone receptors

Mean number ($\bar{x} \pm \text{SEM}$) of total progesterone receptors per cell and cytosolic progesterone receptors per cell were lower ($P < 0.05$) in the short cycle/endometrium group ($67\,596 \pm 9367$; $41\,879 \pm 7138$, respectively) than in the normal cycle/endometrium group ($97\,953 \pm 15\,042$; $74\,502 \pm 10\,389$, respectively; Fig. 5). However, numbers ($\bar{x} \pm \text{SEM}$) of nuclear progesterone receptors per cell were similar in the short cycle/endometrium group ($25\,718 \pm 3807$) and in the normal cycle/endometrium group ($21\,200 \pm 4748$; Fig. 5). The dissociation constant (K_d ; $\text{nmol l}^{-1} \pm \text{SEM}$) estimates of progesterone receptors measured in crude nuclei were greater ($P < 0.05$) in the short cycle/endometrium group ($15.9 \pm 1.8 \text{ nmol l}^{-1}$) than in the normal cycle/endometrium group ($10.3 \pm 0.9 \text{ nmol l}^{-1}$). Estimates of K_d values of pro-

Fig. 5. Number of progesterone receptors per cell in bovine endometrial tissue on day 5 of a short or normal oestrous cycle; (□) cytosolic; (■) nuclear. A,B Means ($\pm \text{SEM}$) having different letters are significantly different ($P < 0.05$).

Fig. 6. Oxytocin receptor content ($\text{fmol mg}^{-1} \text{ protein}$) in bovine endometrium on day 5 of a short or normal oestrous cycle. (■) Short cycle/endometrium; (□) normal cycle/endometrium. A,B Means ($\pm \text{SEM}$) having different letters are significantly different ($P < 0.05$).

gesterone receptors in the cytosolic fraction did not differ between the short cycle/endometrium group (2.9 ± 0.2) and normal cycle/endometrium group (3.0 ± 0.2). The reported difference in apparent K_d values is difficult to explain. Although the statistical difference in K_d values due to luteal status was significant, these values imply that 50% occupancy of receptors occurs when progesterone concentrations are approximately 3.2 ng ml^{-1} (for normal cows) as opposed to 4.9 ng ml^{-1} (for cows with short luteal phases). Because plasma progesterone concentrations on day 5 were between 1.5 and 2 ng ml^{-1} for

cows in all groups, the absolute difference in per cent receptor occupancy between groups was probably small and of little biological significance. However, if such a difference occurred in conjunction with fewer progesterone receptors available for hormone binding, it would tend to render uterine target cells less responsive to effects of circulating progesterone.

Endometrial content of oxytocin receptors

Mean concentration of oxytocin receptors ($\bar{x} \pm \text{SEM}$; fmol oxytocin receptors mg^{-1} protein) was greater ($P < 0.05$) in the short cycle/endometrium group (151.8 ± 18.8) than in the normal cycle/endometrium group (98.0 ± 17.8 ; Fig. 6). In addition, estimates of K_d values ($\text{nmol l}^{-1} \pm \text{SEM}$) of oxytocin receptors in the short cycle/endometrium group (2.2 ± 0.2) were lower ($P < 0.05$) than in the normal cycle/endometrium group (3.9 ± 0.9).

Discussion

Pulses of oxytocin occur synchronously with pulses of $\text{PGF}_{2\alpha}$ at the time of luteolysis in cattle (Vighio and Liptrap, 1986) and sheep (Hooper *et al.*, 1986). Oxytocin stimulates the uterus to secrete $\text{PGF}_{2\alpha}$ (Sharma and Fitzpatrick, 1974; Lafrance and Goff, 1985; Homanics and Silvia, 1988). In turn, $\text{PGF}_{2\alpha}$ causes release of oxytocin from the ovary (Heap *et al.*, 1989; Lamsa *et al.*, 1989). Thus, it has been proposed that uterine $\text{PGF}_{2\alpha}$ and luteal oxytocin develop a positive feedback loop that ensures that $\text{PGF}_{2\alpha}$ is secreted in high amplitude pulses during luteal regression (see review by Flint *et al.*, 1990; Silvia *et al.*, 1991).

The acquisition of uterine responsiveness to oxytocin may determine when release of $\text{PGF}_{2\alpha}$ occurs during the oestrous cycle in cattle and sheep. Uterine responsiveness to oxytocin appears to develop slowly under the influence of progesterone, and is enhanced by oestradiol (Homanics and Silvia, 1988; Lafrance and Goff, 1988; Vallet *et al.*, 1990). Oestradiol appears to enhance uterine responsiveness to oxytocin by increasing synthesis of endometrial oxytocin receptors (Hixon and Flint, 1987). In cattle and sheep, concentrations of oxytocin receptors in endometrium are low during the mid-luteal phase and increase at the time of luteolysis until they reach peak concentrations at oestrus, a time when oestradiol concentrations are increased owing to follicular growth (Sheldrick and Flint, 1985; Jenner *et al.*, 1991). In addition, Vallet *et al.* (1990) and Lau *et al.* (1992) suggested that progesterone and oestradiol may control factors associated with the coupling of oxytocin receptors to $\text{PGF}_{2\alpha}$ synthesis.

Progesterone may exert a negative effect on the concentration of oxytocin receptors in uterine tissue. Progesterone blocks the ability of oestradiol to increase endometrial oxytocin receptors in rabbits (Nissenson *et al.*, 1978) and sheep (Koligian and Stormshak, 1977). In addition, concentration of oestradiol receptors and oxytocin receptors increased in endometrium of ovariectomized ewes following progesterone withdrawal (Leavitt *et al.*, 1985). McCracken *et al.* (1984) proposed that progesterone, through binding to its receptor, may inhibit synthesis of oxytocin receptors early in the oestrous cycle when the uterus is progesterone dominant. As the oestrous

cycle progresses through the mid-luteal phase, concentration of progesterone receptors decreases and the uterus loses progesterone dominance. This loss allows synthesis of endometrial oestrogen receptors. Oestrogen, secreted from developing follicles, through binding to its endometrial receptor, stimulates the synthesis of endometrial oxytocin receptors. The uterus is then responsive to oxytocin and a positive feedback mechanism is established between luteal oxytocin and $\text{PGF}_{2\alpha}$.

Oestradiol may play a role during the follicular phase to establish progesterone dominance of the uterus and, subsequently, the time of $\text{PGF}_{2\alpha}$ secretion. Oestradiol induces synthesis of endometrial progesterone receptors *in vitro* and *in vivo* in rats (Kassiss *et al.*, 1984) and sheep (Zelinski *et al.*, 1980), respectively. Also, oestradiol increases progesterone receptors in whole uterus of ovariectomized ewes and progesterone inhibits this effect (Stone *et al.*, 1978). Ottobre *et al.* (1984), therefore, suggested that the high concentrations of oestradiol associated with oestrus may induce synthesis of uterine progesterone receptors and establish the appropriate time of $\text{PGF}_{2\alpha}$ secretion. In fact, progesterone receptors in endometrium are highest at oestrus and decrease by mid-luteal phase in cows (Meyer *et al.*, 1988) and ewes (Miller *et al.*, 1977).

Results of the study reported here suggest that the uterus may lose progesterone dominance and be more responsive in terms of $\text{PGF}_{2\alpha}$ secretion on day 5 of a short oestrous cycle. In the present study, the concentration of endometrial progesterone receptors was greater on day 5 of a normal oestrous cycle than on day 5 of a short oestrous cycle. In addition, the concentration of oxytocin receptors was lower in endometrium on day 5 of a normal oestrous cycle versus a short oestrous cycle.

After exposure of the uterus *post partum* to progesterone, the first ovulation is followed by normal luteal function. At this time, there is no evidence to implicate a direct effect of progesterone priming on secretion of $\text{PGF}_{2\alpha}$ during the subsequent cycle. However, progestogen pretreatment may indirectly coordinate timing of $\text{PGF}_{2\alpha}$ release during the subsequent oestrous cycle by altering endometrial oxytocin receptor concentrations. Progesterone priming of ovariectomized ewes followed by oestradiol replacement for 2 days and then exogenous progesterone for 5 days decreased oxytocin receptor concentrations compared with non-primed ewes receiving oestradiol for 2 days followed by progesterone for 5 days (Vallet *et al.*, 1990). Oestradiol further enhanced endometrial oxytocin receptor concentrations in primed and non-primed ewes when administered on days 4 and 5 of progesterone treatment. In addition, oxytocin induced an increase in plasma concentrations of PGFM only in ewes that did not receive progestagen pretreatment and subsequently received progesterone treatment for 5 days and oestradiol treatment on days 4 and 5 (Vallet *et al.*, 1990). In addition, Hunter (1991) reported that although concentration of endometrial oxytocin binding sites was high on day 1, they were similar to those of anoestrous ewes expected to have a short versus normal luteal phase. However, on days 3 and 5, numbers of endometrial oxytocin receptors were greater in ewes having a short oestrous cycle compared with a normal oestrous cycle. In this study, numbers of endometrial oxytocin receptors were greater on day 5 of cows expected to have a short luteal phase than in cows receiving progestogen pretreatment and expected to have

a normal luteal phase. These results suggest that progesterone priming enhances the effect of subsequent progesterone on inhibition of oxytocin receptor concentrations in endometrium and therefore decreases uterine responsiveness to oxytocin.

Concentration of oestradiol in serum was lower at oestrus in cows exhibiting a subsequent short versus normal luteal phase (Garcia-Winder *et al.*, 1986; Garverick *et al.*, 1988). The role oestradiol plays before ovulation in determining subsequent luteal lifespan is not clear. Prolonged exposure to exogenous oestradiol during the *postpartum* interval extended luteal lifespan following first ovulation in beef cows (Day *et al.*, 1990). Conversely, inhibition of synthesis of oestradiol prior to ovulation did not result in subfunctional corpora lutea but delayed oestrus and onset of the luteal phase in sheep (Benoit *et al.*, 1991). The increase in oestradiol around ovulation in cows receiving progesterone priming may be a direct result of the progestogen pretreatment used in these studies. However, since increased concentrations of oestradiol have been associated with induction of a full complement of endometrial progesterone receptors required for progesterone dominance of the uterus (Zelinski *et al.*, 1980), the decreased concentrations of oestradiol preceding ovulation of a short oestrous cycle may have resulted in decreased progesterone receptor synthesis leading to a loss of uterine progesterone dominance earlier following ovulation.

In conclusion, concentrations of progesterone receptors in endometrium was greater on day 5 in cows having a normal oestrous cycle rather than a short oestrous cycle. In addition, concentration of oxytocin receptors in endometrium was lower in cows having a normal versus a short luteal phase. Thus, the uterus may lose progesterone dominance at an earlier time during a short oestrous cycle. This would allow the synthesis of oxytocin receptors and initiate the positive feedback loop between oxytocin and $\text{PGF}_{2\alpha}$ which leads to a premature release of $\text{PGF}_{2\alpha}$.

Contribution from the Missouri Agricultural Experiment Station Journal Series No. 11,581. This research was partially supported by USDA Grant, USDA CSRS 90-37240-5777.

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