Attenuation of antepartum relaxin surge and induction of parturition by antiprogesterone RU 486 in sheep

O. S. Gazal¹, Y. Li¹, C. Schwabe² and L. L. Anderson^{1*}

¹Department of Animal Science, Iowa State University, Ames, IA 50011, USA; and ²Department of Biochemistry, Medical University of South Carolina, Charleston, SC 29459, USA

Pregnant ewes were injected with either the antiprogesterone, RU 486 (4 mg kg⁻¹ body weight, i.m.; *n* = 5), 3000 iu relaxin (i.m.; *n* = 9), or diluent (*n* = 8) at 12:00 h on days 144 and 145, to determine its effect on progesterone and relaxin secretion, and on induction of lambing. RU 486 induced earlier lambing (*P* < 0.01) compared with diluent treatment, but relaxin treatment did not significantly reduce the interval to parturition. Mean injection–lambing intervals were 31 ± 2, 109 ± 23 and 121 ± 27 h for the RU 486, relaxin and diluent groups, respectively. There was no incidence of difficult birth (dystocia); all lambs were vigorous at birth; and placenta delivery was rapid (within 207 min) with RU 486 and relaxin treatments compared with diluent treated controls. Plasma progesterone concentrations averaged 11 ng ml⁻¹ during the pretreatment period for all animals. RU 486 had a biphasic effect on progesterone concentrations, causing an initial increase (*P* < 0.05) within 2 h, and then an abrupt drop (*P* < 0.01) to 6 ng ml⁻¹ by 18:00 h on day 145. Progesterone concentrations remained consistently lower (*P* < 0.05) in relaxin-treated ewes than in diluent-treated controls from days 144 to 147 and then began a steady decrease to 4 ng ml⁻¹ on the day of parturition (days 149 and 150) in both groups. Immunoreactive relaxin concentrations in control ewes increased (*P* < 0.05) from 0.6 ng ml⁻¹ to a peak of 3.9 ng ml⁻¹ on day 146, but they were low (0.8 ng ml⁻¹) at the time of parturition (day 150). RU 486 treatment abruptly increased (*P* < 0.05) circulating relaxin concentration, but the amplitude of this antepartum surge was greatly attenuated compared with that of diluent treated controls. Peak RU 486 concentrations in plasma were 7 and 9 ng ml⁻¹ within 2 h after first and second i.m. injection of the hormone, respectively, and stabilized at 4 ng ml⁻¹ at the time of induced lambing (day 145). The results reveal that an antepartum relaxin surge occurs in sheep 4 days before normal parturition (day 150), but that RU 486 greatly attenuates the relaxin surge and abruptly decreases circulating progesterone concentration with an induced parturition (day 145). The results indicate that RU 486 can precisely control the time of parturition in sheep in late pregnancy without detrimental effects of dystocia, retention of placenta or delayed postpartum fertility.

Introduction

Relaxin is a peptide hormone produced in greatest concentrations by female reproductive tissues, particularly during pregnancy (Anderson, 1987; Sherwood, 1988). Maximum peripheral blood concentrations of relaxin occur a few hours before parturition in rats and pigs, but no similar evidence is available for sheep (Sherwood *et al.*, 1980; Felder *et al.*, 1986). Low relaxin immunoreactivity has been reported in placental, endometrial and ovarian tissues (0.05–11.00 ng g⁻¹) of ewes from day 21 to parturition, but no obvious trend was found throughout pregnancy (Renegar and Larkin, 1985; Wathes *et al.*, 1988). Relaxin increases cervical compliance in oestrogen-primed ovariectomized ewes (Nemec *et al.*, 1988) and reduces uterine

contractility (Porter *et al.*, 1981), thus suggesting a role for this hormone in pregnancy.

Maintenance of pregnancy in mammals, including sheep, requires the binding of progesterone to its receptor in the uterine endometrium leading to the proliferation of the epithelial and vascular cells. A decrease in circulating progesterone concentrations must precede induced or spontaneous parturition in ewes (Stabenfeldt *et al.*, 1972; Ledger *et al.*, 1985), and this can be achieved by blocking progesterone synthesis with 3 β -hydroxysteroid dehydrogenase inhibitors (Taylor, 1987). RU 486 (11 β -[4-dimethyl-amino phenyl]-17 β -hydroxy-17 α -[1-propynyl]estra-4,9-dien-3-one) is a 19-norsteroid with potent antiprogesterone and antiglucocorticoid activities (Baulieu, 1989), and it reduces maternal progesterone concentrations in several species including pigs, cattle, monkeys (*Rhesus* and *Macaca fascicularis*), and humans (Nieman *et al.*, 1987; Baulieu, 1989; Puri *et al.*, 1990; Li *et al.*, 1991a, b). RU 486 binds competitively to progesterone

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receptors and such binding either blocks the interaction between progesterone receptors and DNA or impairs gene transactivation. Substitution of glycine by cysteine at position 575 in the human progesterone receptor abrogates binding of RU 486, but not that of an agonist (Benhamou *et al.*, 1992). Relaxin administered to cattle late in pregnancy decreases circulating progesterone and also advances parturition (Musah *et al.*, 1986; Bagna *et al.*, 1991) and it decreases progesterone in cyclic ewes (Akinbami *et al.*, 1990). RU 486 can modulate both progesterone and relaxin secretion in pigs, but responses depend on the presence or absence of the uterus (Li *et al.*, 1991b). In pregnant pigs, RU 486 advanced the time and increased the amplitude of peak relaxin release and abruptly decreased circulating progesterone concentrations, whereas in hysterectomized animals the same RU 486 treatment delayed the time of peak relaxin release and abruptly increased progesterone secretion.

Our studies in pigs and cattle indicate that RU 486 is a useful probe for determining the physiological roles of progesterone during pregnancy. The mechanism by which progesterone and relaxin secretion are regulated during late pregnancy in sheep is undefined. It is hypothesized that RU 486 could decrease progesterone secretion in pregnant sheep, but it is not known whether the drug can alter endogenous relaxin secretion. Whether exogenous relaxin could alter progesterone secretion in this species is undefined. This study was designed to determine the effects of both RU 486 and relaxin on antepartum progesterone and relaxin secretion in late pregnant sheep. Furthermore, the effects of RU 486 and relaxin on the time and ease of parturition as well as viability of lambs were determined.

Materials and Methods

Animals

Twenty-two ewes of Suffolk and Hampshire breeds, averaging 97 ± 2 kg body weight (mean $\pm$ SEM) were maintained at the Sheep Teaching Farm, Iowa State University. Animals received 1.8 kg of chopped hay and 0.5 kg of corn daily. Water was provided *ad libitum*. Ewes were naturally mated at oestrus (day = 0). The average duration of gestation in this flock is 150 days.

Experimental groups

The ewes were randomly allocated to one of three treatments: RU 486 group ($n = 5$); relaxin group ($n = 9$); and diluent group ($n = 8$). On day 140 of gestation, one external jugular vein of each ewe was fitted with an indwelling catheter (i.d. 0.127 cm; o.d. 0.299 cm; Tygon Microbore tubing, No. 14-170-15E, Fisher Scientific Co., Pittsburgh, PA) for repeated blood sampling. On days 140–143, a blood sample was collected once a day at 12:00 h. On day 144, beginning at 11:00 h, blood samples were obtained at intervals of 15 min for 1 h; and at 12:00 h, ewes in the RU 486, relaxin, or diluent groups received i.m. injection of either RU 486 (4 mg kg^{-1} body weight in 3 ml of ethanol), relaxin (CM-B fraction, 3000 iu mg^{-1} with 1 mg relaxin in 3 ml of phosphate-buffered saline, PBS), or diluents (PBS and ethanol) in separate sites, respectively.

Relaxin for this experiment was extracted from the ovaries of pregnant pigs and purified according to procedures described previously (Büllesbach and Schwabe, 1985). Sequential blood samples were collected at intervals of 15 min for 1 h after treatment and 2, 3, 9 and 15 h later. Ewes not lambing by 11:00 h on day 145 received a second injection of RU 486 or relaxin and were bled using the same regimen as described for day 144. From day 146 to 2 days *post partum*, blood samples were collected once a day at 12:00 h. The catheter was flushed with heparinized saline (100 iu ml^{-1}) after each blood collection. Blood samples (10 ml) were collected in $16 \times 100 \text{ mm}$ borosilicate culture tubes maintained on ice. Tubes were centrifuged at $2000g$ for 10 min and the plasma was immediately harvested into $12 \times 75 \text{ mm}$ culture tubes, frozen on dry ice and stored at -20°C until required for radioimmunoassay of progesterone, relaxin and RU 486.

Ewes exhibiting signs of imminent lambing were kept in the maternity barn for continuous observation. Duration of gestation, exact time of lambing, time of placental delivery and sex and body weights of lambs were recorded at lambing. The interval from first hormone or diluent injection to parturition was calculated to determine the injection–lambing interval.

Radioimmunoassays of RU 486, relaxin and progesterone in peripheral plasma

RU 486 concentrations in the plasma were determined by radioimmunoassay procedures as previously described (Salmon and Mouren, 1985; Heikinheimo *et al.*, 1986) with modifications (Li *et al.*, 1991a, b, c). The specific activity of [^3H]RU 486 was $46.8 \text{ Ci mmol}^{-1}$ (433.59 molecular weight). The anti-RU 486-3-carboxymethylloxime–BSA antiserum was raised in New Zealand White rabbits according to procedures described previously (Raynaud *et al.*, 1974). Plasma samples ($50 \mu\text{l}$) in duplicate were added to 0.25 ml distilled water and extracted with 3.0 ml diethyl ether (spectrophotometric grade; Aldrich, Milwaukee, WI). The recovery of radioactivity was 79.3% ($n = 2$). Total incubation volume was 0.4 ml tube $^{-1}$ in which antiserum was diluted to $1:10\,000$ and total [^3H]RU 486 was $10\,000 \text{ c.p.m.}$ The standard curve contained 0, 20, 40, 80, 125, 250, 1000 and $2000 \text{ pg tube}^{-1}$ RU 486 in duplicate. Separation of unbound fraction was obtained by dextran-coated charcoal. Sensitivity of the assay was 10 pg tube^{-1} , and the intra- and interassay coefficients of variation were 8.0% ($n = 2$) and 18% ($n = 2$), respectively; non-specific binding was 9.0% .

A homologous double antibody radioimmunoassay for porcine relaxin was used as described by O'Byrne and Steinetz (1976) with modifications to quantify relaxin concentration in ovine plasma in duplicate aliquots of $50\text{--}200 \mu\text{l}$. The assay buffer was 0.01 mol phosphate-buffered saline (PBS)– 1% bovine serum albumin (BSA). Monotyrosylated porcine relaxin (Schwabe, 1983) was iodinated with $1 \text{ mCi } ^{125}\text{I}$ (IMS 30; Amersham Corp, Arlington Heights, IL) according to the chloramine T procedure. $100 \mu\text{l}$ of [^{125}I]–porcine monotyrosyl relaxin ($10\,000 \text{ c.p.m.}$ in $0.01 \text{ mol PBS l}^{-1}$ – 1% BSA) was added to all tubes. Antiporcine relaxin antibody (Dual Rabbit R6) was added at a $1:10\,000$ dilution in 0.05 mol ethylenediaminetetraacetic acid l^{-1} –PBS and 6% normal rabbit serum to all tubes except the nonspecific binding and total count tube, which received buffer only. The

Table 1. Effect of antiprogesterone RU 486 and relaxin on pregnancy and parturition in sheep

	Ewe		Pregnancy duration (days)	First treatment to first lambing (h)	Time from lambing to placental delivery (min)	Lamb			
	(n)	Body weight (kg)				Birth weight (kg)	Number per ewe	Sex	
								Male (n)	Female (n)
RU 486	5	94 ± 6	145 ± 0.2*	31 ± 2**	204 ± 17	5.1 ± 0.4	1.8	6	3
Relaxin	9	100 ± 5	149 ± 0.7	109 ± 23	183 ± 21	4.1 ± 0.2	2.3	8	13
Diluent	8	96 ± 4	150 ± 0.8	121 ± 27	193 ± 17	4.3 ± 0.2	2.0	9	7

Ewes were bred by natural service with a ram and given: RU 486 (4 mg kg⁻¹ body weight) by i.m. injection at 12:00 h on days 144 and 145; relaxin (3000 i.u.) by i.m. injection at these same times on days 144 and 145; and diluent by i.m. injection on days 144 and 145.

Values are means ± SEM. **P* < 0.05 and ***P* < 0.01 compared with diluent-treated controls.

tubes were incubated for 24 h at 4°C, and 200 µl of 1:10 goat rabbit γ globulin antiserum (No. 0112-0081 Lot No. 33091; Organon Teknika Co., PA) was added to all tubes except total count tubes, which received the buffer (0.01 mol PBS l⁻¹). After incubating for 18–24 h, 3 ml of 0.01 mol PBS l⁻¹–1% BSA was added to all tubes except total count tubes and centrifuged at 2000 *g* for 30 min. The pellets were counted for 2 min in an automatic gamma counter (Packard Cobra Auto Gamma 5003). Accuracy and precision of the assay were determined by adding known amounts of standard porcine relaxin (purified pig CM-B relaxin, 3000 iu mg⁻¹, to plasma (200 µl) from ovariectomized hysterectomized sheep, as follows: 0.10, 0.25, 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 ng ml⁻¹ in quadruplicate aliquots. The plasma blank values were assayed and the values subtracted from those tubes containing known amounts of relaxin. Concentrations (ng ml⁻¹) of relaxin averaged 0.12 ± 0.020, 0.24 ± 0.030, 0.48 ± 0.010, 1.1 ± 0.03, 1.9 ± 0.06, 2.3 ± 0.07, 4.1 ± 0.13 and 5.2 ± 0.06, respectively. A pool of sheep blood plasma with high relaxin immunoreactivity was assayed in quadruplicate at volumes of 50, 100, 200 and 400 µl and showed parallelism in a dose-dependent manner with porcine relaxin added to plasma from ovariectomized ewes. Intra- and interassay coefficients of variation were 5.6% (*n* = 3) and 9.7% (*n* = 3), respectively. The nonspecific binding of the assay was 2.08% (*n* = 3), and the maximum binding of the first antibody was 33%.

Progesterone was determined in 200 µl aliquots of plasma, as described by Musah *et al.* (1986), using the progesterone antibody GDN-337 (Niswender, 1973). Plasma was extracted with a benzene:hexane mixture (1:2, v/v). Precision and accuracy were validated by adding known quantities of standard progesterone, 0.00, 0.50, 1.25, 2.50, 3.75, 5.00, 7.50, 12.50 and 25.00 ng to 200 µl of ovariectomized sheep plasma in quadruplicate aliquots. These standard samples were assayed and the concentrations of progesterone (mean ± SEM) were 0.00, 0.50 ± 0.002, 1.31 ± 0.020, 2.6 ± 0.03, 3.0 ± 0.09, 4.5 ± 0.33, 6.0 ± 0.24, 11.7 ± 0.33 and 24.8 ± 0.95 ng, respectively. The sensitivity of the assay was 0.25 ng ml⁻¹. The nonspecific binding of all assays was 3.0% (*n* = 4) and the maximum antibody binding was 34.3% (*n* = 4). The overall extraction efficiency was 84.8% (*n* = 4) and the intra- and inter-assay coefficients of variation were 4.8% (*n* = 4) and 8.1% (*n* = 4), respectively.

Statistical analyses

Results are expressed as means ± SEM. The experimental units in this study were the individual animals randomly assigned to treatments. Data were analysed by split-plot design using both the general linear model and Student's *t* test for comparisons between treatment groups (Blair *et al.*, 1979; Snedecor and Cochran, 1989).

Results

Effects of RU 486 and relaxin on induction of parturition and survival of lambs

Intramuscular injections of RU 486 to late pregnant ewes induced a markedly earlier lambing than did diluent treatment (Table 1). RU 486 ewes lambed 31 h after the first RU 486 injection, but the injection–lambing interval was variable and not statistically different between relaxin-treated (109 h) and diluent-treated (121 h) ewes. The time interval between the first lamb and the expulsion of the last placenta was similar among the three groups, averaging 207, 183 and 193 min in the RU 486, relaxin and diluent groups, respectively. Birth weights were similar among groups. At birth, all single-, twin-, and triplet-born lambs could breathe, walk and suck. Hence, the survival and vigour of the lambs were unaffected by RU 486 or relaxin treatment. The ewes were successfully bred in the next breeding cycle.

Effects of RU 486 and relaxin on maternal peripheral progesterone concentration

The progesterone concentration averaged 11 ± 0.6 ng ml⁻¹ during the pretreatment period. During the post-treatment period, overall plasma progesterone concentrations were significantly lower in RU 486- (8 ± 0.7 ng ml⁻¹; *P* < 0.05) and relaxin- (8 ± 0.4 ng ml⁻¹; *P* < 0.05) groups compared with diluent-treated controls (11 ± 0.6 ng ml⁻¹; Fig. 1) as determined by general linear model analysis. RU 486 had a biphasic effect on blood progesterone concentrations with an initial

Fig. 1. Effect of (a) the antiprogesterone RU 486 and (b) relaxin on progesterone concentrations in peripheral plasma during late pregnancy in sheep. On day 140, an indwelling catheter was inserted into a jugular vein for repeated blood sample collection from days 140–143 and day of lambing to 2 days *post partum*. Blood was collected once a day at 12:00 h; on days 144 and 145 at 12:00 h, the ewes were injected with (a) RU 486 (4 mg kg^{-1} body weight, i.m.), and (b) relaxin (3000 iu, i.m.). The controls received i.m. injections of 3 ml diluent. Blood was collected at -60 , -45 , -30 , -15 min before treatment and immediately after treatment at 15, 30, 45, 60 min and at 2, 3, 9, and 15 h. Progesterone concentrations (ng ml^{-1}) are indicated during the pretreatment period ($\circ$, $n = 22$); after the RU 486 treatment ($\blacktriangle$, $n = 5$), after the relaxin treatment ($\triangle$, $n = 9$), and after the diluent treatment ($\bullet$, $n = 8$). Times of hormone and diluent treatments are indicated by open arrows. The solid arrows indicate the mean and the bars indicate the standard error for the time of parturition in the RU 486-treated ewes (▨), relaxin-treated ewes (▩), and diluent-treated controls (▧). Results are expressed as means $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ compared with diluent-treated controls.

marked increase ($P < 0.05$) within 2 h compared with the pre-treatment period, and a consistent decrease ($P < 0.05$) by 9 h compared with diluent-injected controls (Fig. 1a). Progesterone averaged 6 ng ml^{-1} in the RU 486 treated ewes at induced lambing on day 145 compared with 11 ng ml^{-1} in the controls ($P < 0.01$) at that time. Up to day 147, progesterone was consistently lower ($P < 0.05$) in the relaxin-injected ewes than in the controls and then it decreased steadily until parturition occurred on days 149 and 150 in both groups (Fig. 1b). At

parturition, progesterone averaged 4 ng ml^{-1} in relaxin- and diluent-treated ewes (Fig. 1b).

Immunoreactive relaxin plasma concentrations

In the control ewes, immunoreactive relaxin concentrations increased ($P < 0.05$) with advancing gestation, reaching a peak of 3.9 ng ml^{-1} on day 146 (Fig. 2). On the day of parturition (day 150), relaxin concentrations were low, averaging

Fig. 2. Effect of RU 486 on relaxin concentrations in peripheral plasma during late pregnancy in sheep. On day 140, an indwelling catheter was inserted into a jugular vein for repeated blood sample collection from days 140–143 and day of lambing to 2 days post partum. Blood was collected once a day at 12:00 h; on days 144 and 145 at 12:00 h, ewes were injected with RU 486 (4 mg kg^{-1} body weight, i.m.). The controls received i.m. injections of 3 ml diluent. Blood was collected at -60 , -45 , -30 , -15 min before treatment and immediately after treatment at 15, 30, 45, 60 min and at 2, 3, 9, and 15 h. Relaxin concentrations (ng ml^{-1}) are indicated during the pretreatment period ($\circ$, $n = 22$); after the RU 486 treatment ($\triangle$, $n = 5$) and after the diluent treatment ($\bullet$, $n = 8$). Times of RU 486 and diluent treatments are indicated by open arrows. The solid arrows indicate the mean and the bars indicate the standard error for time of parturition in RU 486-treated ewes (hatched box) and diluent-treated controls (white box). Results are expressed as means $\pm$ SEM. * $P < 0.05$ compared with diluent treated control ewes.

0.8 ng ml^{-1} . RU 486 caused a small but significant increase ($P < 0.05$) in circulating relaxin within 8 h after treatment, but relaxin remained at basal concentrations at the time of induced parturition (Fig. 2). Relaxin concentrations remained consistently less ($P < 0.01$) in the RU 486-treated group (0.5 ng ml^{-1}) than in the controls (1.5 ng ml^{-1}) from 5 days before and up to the day of parturition. During the overall post-treatment period, immunoreactive relaxin was lower ($P < 0.05$) after RU 486 ($0.6 \pm 0.03 \text{ ng ml}^{-1}$) than after diluent treatment ($1.2 \pm 0.17 \text{ ng ml}^{-1}$).

Relaxin i.m. injection abruptly increased ($P < 0.01$) the circulating relaxin concentration from 0.6 to 45 ng ml^{-1} on day 144 (Fig. 3b). Relaxin increased ($P < 0.01$) within 15 min, peaked within 1 h, and basal concentrations were reached 24 h later. The second i.m. injection of relaxin, given 24 h after the first, increased circulating relaxin within 15 min and reached a peak of 49 ng ml^{-1} within 2 h; basal values were reached 48 h after the second hormone injection. During the post-treatment period, relaxin averaged $20 \pm 1.7 \text{ ng ml}^{-1}$ ($P < 0.01$) in the relaxin-treated ewes compared with $1.2 \pm 0.17 \text{ ng ml}^{-1}$ in diluent-treated controls. As observed in both the RU 486- and diluent-treated controls circulating relaxin was also low on the day of parturition in this group.

RU 486 concentrations in peripheral plasma

Intramuscular injection of RU 486 abruptly increased ($P < 0.001$) circulating RU 486 within 15 min, reaching a peak

value of 7 ng ml^{-1} within 2 h (Fig. 3a). During the next 24 h, RU 486 concentrations were maintained at 4 ng ml^{-1} and the second injection of the drug, 24 h after the first, caused a further increase ($P < 0.001$) to 8.0 ng ml^{-1} within 15 min and a peak of 9 ng ml^{-1} by 45 min. RU 486 averaged $5 \pm 0.7 \text{ ng ml}^{-1}$ at the time of induced parturition.

Discussion

The results indicate that RU 486 can precisely control parturition in sheep in late pregnancy. RU 486 had a biphasic effect on concentrations of progesterone in peripheral blood, with an initial increase 1 h in duration that was followed by a marked and consistent decrease 9 h after RU 486 injection. A similar biphasic response has been observed in cultured human syncytiotrophoblasts incubated with RU 486 (Das and Catt, 1987). The initial increase in circulating progesterone concentration in sheep may result from the disinhibition of pituitary luteinizing hormone (LH) secretion arising from RU 486 blockage of progesterone receptor. RU 486 can induce changes in the frequency of LH pulsatility (Garzo *et al.*, 1988; Swahn *et al.*, 1990). RU 486 administration to women during the follicular, early luteal and midluteal phases of the menstrual cycle increases LH secretion leading to increased circulating progesterone concentrations (Herrmann *et al.*, 1985; Shoupe *et al.*, 1987; Garzo *et al.*, 1988). In hysterectomized pigs, RU 486 caused a dose-dependent increase in progesterone concentration that lasted

Fig. 3. Comparison of (a) RU 486 and (b) relaxin in peripheral plasma during late pregnancy in sheep. On day 140, an indwelling catheter was inserted into a jugular vein for repeated blood sample collection from days 140–143 and day of lambing to 2 days *post partum*. Blood was collected once a day at 12:00 h; on days 144 and 145 at 12:00 h, the ewes were injected with (a) RU 486 (4 mg kg⁻¹ body weight, i.m.), and (b) relaxin (3000 iu, i.m.). The controls received i.m. injections of 3 ml diluent. Blood was collected at -60, -45, -30, -15 min before treatment and immediately after treatment at 15, 30, 45, 60 min and at 2, 3, 9, and 15 h. (○), pretreatment ($n = 22$); (■), treatment with RU 486 (4 mg kg⁻¹ body weight; i.m., $n = 5$); (●), treatment with 3000 U relaxin (i.m., $n = 9$); (▲), diluent-treated controls ($n = 8$). Values are means $\pm$ SEM. Times of hormone and of diluent treatments are indicated by open arrows.

throughout the 5-day treatment (Li *et al.*, 1991b). Furthermore, intracerebroventricular infusion of RU 486 increased prolactin plasma concentration within 20 min in hysterectomized pigs (Li *et al.*, 1992), and it is known that prolactin is required for

progesterone and relaxin secretion by ageing corpora lutea in this species (Li *et al.*, 1989). In sheep, placental progesterone synthesis accounts for up to 80% of circulating progesterone in the last two-thirds of pregnancy. How RU 486 compromises

placental steroidogenesis is undefined. In both monkeys (*Macaca fascicularis*) and humans, transplacental transfer of RU 486 occurs from the maternal blood to the fetal circulation (Wolf *et al.*, 1988; Hill *et al.*, 1990). RU 486 inhibited placental protein synthesis within 6 h of *in vitro* incubation (Ogle, 1988), and decreased human chorionic gonadotrophin (hCG) production by explants of human trophoblast (Swahn *et al.*, 1990), as well as production of progesterone, hCG and oestrogen by dispersed human syncytiotrophoblast (Bischof *et al.*, 1986). Thus, RU 486 may act acutely within the central nervous system via gonadotrophin-releasing hormone to increase progesterone secretion and then act chronically, possibly at the level of the trophoblast, to decrease progesterone secretion through enzymatic pathways.

The induced parturition by RU 486 may result primarily from abrupt progesterone withdrawal. RU 486 blocks the progesterone receptor at the level of the trophoblast and downregulates nuclear progesterone receptors in decidualized cells (Vij *et al.*, 1989). The withdrawal of progesterone would permit uterine contractions by stimulating oestrogen receptor synthesis or enhancing endometrial prostaglandin synthesis (Kelly *et al.*, 1984, 1986; Haluska *et al.*, 1990). The decrease in circulating progesterone with a coincident increase in oestrogen may stimulate gap junction formation. In late pregnant rats, RU 486 stimulates extensive development of gap junctions, which is soon followed by birth of pups (Garfield *et al.*, 1987). Gap junctions permit coordinated myometrial contractility and convert the uterus into a functional syncytium responding to the stimulatory actions of prostaglandins and oxytocin. RU 486 markedly decreased circulating progesterone concentrations within 24 h in late pregnant cows and pigs (Li *et al.*, 1991a, b), which was similar to that seen in late pregnant sheep in this study. The abortifacient actions of RU 486 may result from an interplay of prostaglandins, glucocorticoids, and oxytocin on the maternal uterus and cervix for expulsion of the conceptus(es). Acting also as an antiglucocorticoid compound, RU 486 disrupts the negative feedback loop of the hypothalamus–pituitary–adrenal axis and causes increased blood concentrations of cortisol and adrenocorticotrophic hormone in pregnant laboratory animals and in women (Gaillard *et al.*, 1984; Healy *et al.*, 1985; Heikinheimo *et al.*, 1990). The extent to which the antiglucocorticoid activity of RU 486 contributed to its parturition inducing ability in this study with sheep was not determined. RU 486 may also block the glucocorticoid negative feedback at the fetal pituitary gland and therefore induce compensatory increases in plasma adrenocorticotrophic hormone and in cortisol.

This is the first report of an antepartum relaxin surge in late pregnant sheep, as has been found in rats and pigs (Sherwood *et al.*, 1980; Felder *et al.*, 1986). In these ewes, immunoreactive relaxin in the circulating blood peaked 4 days before normal parturition at day 150, but the modest amplitude of this relaxin surge differs greatly from that seen in pigs and rats. The significance of this antepartum relaxin release is not clear; however, it occurred earlier in sheep than in rats and pigs. RU 486 treatment in late pregnant pigs caused a greater peak relaxin release that occurred significantly earlier than that seen in placebo-treated controls (Li *et al.*, 1991b). Relaxin concentration in peripheral plasma of both RU 486- and placebo-treated pigs was low at the time of induced or normal parturition. The antepartum increase in circulating relaxin concentrations in ewes in

this study preceded a significant decrease in circulating progesterone concentrations. RU 486 treatment greatly attenuated the antepartum relaxin surge, as seen in the control ewes; peak circulating concentrations of relaxin were lower but occurred earlier compared with the diluent-treated controls. The lower peak release of immunoreactive relaxin soon after RU 486 treatment of sheep may result from reduced relaxin synthesis and release at this time compared with greater relaxin synthesis and release seen a few days later in the diluent-treated animals. Regardless, immunoreactive relaxin concentrations are low at induced or normal parturition in sheep, as shown in this study, as well as in pigs. In spite of this, however, the RU 486-treated ewes lambed without complications of dystocia. There is a cotyledonary-caruncle type of placentation in this species, but there was no incidence of retained placenta during the *postpartum* period as a result of either RU 486 or relaxin treatment.

The circulating concentrations of RU 486 observed in this study were lower than those reported for pigs and cattle in which the drug was fed, or injected *i.m.* at lower doses, although the same pharmacokinetics was observed (Li *et al.*, 1991a, b, c). The differences among these species may result from an increased metabolism of RU 486 in sheep, or from an increased transplacental diffusion of the drug to the fetus. A rapid transplacental transfer of RU 486 has been reported in monkeys and humans (Wolf *et al.*, 1988; Hill *et al.*, 1990).

In summary, the results reveal that an antepartum relaxin surge occurs in sheep 4 days before normal parturition. RU 486 attenuates the relaxin surge, abruptly decreases circulating progesterone concentration, and advances an early, safe lambing. The results indicate that RU 486 precisely controlled the time of parturition in sheep in late pregnancy without detrimental effects of dystocia, retention of placenta, or delayed *postpartum* fertility.

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