Relaxin Regulates Oxytocin Secretion in Late-Pregnant Beef Heifers[1] (42897)

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Abstract. **The effects of porcine relaxin (3000 units/mg) on oxytocin (OT) and progesterone secretion were studied in beef heifers on Day 274 (10 days before expected parturition). Heifers (n = 11) were randomly assigned to three treatments: relaxin iv infusions combined with im injection (RLX-INF, 9000 units), relaxin im injection (RLX-im, 6000 units), and phosphate-buffered saline-treated controls (PBS). RLX-INF heifers received infusions of PBS and 1000 units of relaxin for 165 min, followed by 2000 units of relaxin im and finally 2000 units of relaxin infusion followed by 4000 units of relaxin im. Endogenous relaxin (immunoreactive) in the PBS-treated group was 0.2–0.9 ng/ml peripheral plasma. For the RLX-im group, peak relaxin was 81 ± 12 ng/ml (±SE) at 45 min after treatment. There were two peaks of relaxin, 18 ± 5.3 ng/ml and 74 ± 7.5 ng/ml, 3.5–4.5 hr apart in the RLX-INF group. Significant peak releases of OT were evident in the relaxin-treated heifers. For the RLX-im group, an OT peak (42 ± 16 pg/ml) occurred within 30 min after relaxin treatment. For the RLX-INF heifers, 2000 and 4000 units of relaxin were associated with major peaks of 14 ± 0.5 and 43 ± 1.7 pg/ml OT, respectively. Basal OT plasma levels in the PBS group were 2.5–3.1 pg/ml. Mean plasma progesterone for all heifers was 6.2 ± 2.11 ng/ml before treatment. There was a significant decrease in progesterone (−2.5 ng/ml) in the RLX-im group within 60 min after relaxin treatment and 45 min after peak OT secretion. The maximum decrease in progesterone (−3.2 ± 0.68 ng/ml) occurred 135 min after treatment in the RLX-im group. In the RLX-INF group, 2000 units of relaxin infusion combined with 4000 units of relaxin im significantly decreased progesterone (−3.2 ± 1.59 ng/ml) in peripheral plasma. These results clearly indicate that relaxin causes an acute peak release of oxytocin within 30 min, followed by a marked decrease in plasma progesterone concentration in late-pregnancy cattle.** [P.S.E.B.M. 1989, Vol 191]

Relaxin is a polypeptide hormone produced in several species, including pigs, cattle, humans, and rats (1). Relaxin induces marked cervical dilation, pelvic relaxation, separation of the pubic symphysis, and interpubic ligament formation (1, 2). Both oxytocin (OT) and relaxin are produced by luteal cells (3–9). It has been postulated that OT of luteal cell origin may play an important role in the development of the bovine corpus luteum as well as in its regression (10). In particular, *in vivo* studies have been interpreted to suggest a role for OT in luteolysis in goats, sheep, and cattle (11–16); OT can stimulate the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and be released by the luteolysin, $PGF_{2\alpha}$ (7, 16–19). The luteolytic effects of OT in heifers require a functional uterus (12, 13). Preliminary evidence suggests that in the cow luteal, and in the rat neurohypophysial OT may be released by exogenous relaxin (20–22). *In vivo* as well as *in vitro* studies also indicate that relaxin alters steroid hormone metabolism (22–25). We speculate that OT and $PGF_{2\alpha}$ may be involved in a feedback interaction with relaxin at the level of the hypophysis, ovaries, and uterus to regulate peripheral blood levels of progesterone in cattle. The objective of this study was to determine the effects of porcine relaxin infused in graded doses and intramuscular administration on peripheral plasma OT and progesterone concentrations in late-pregnancy heifers.

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Materials and Methods

Experimental Design. Eleven primiparous crossbred beef heifers approaching their first calving were used in this study. On Day 274, heifers were fitted with an indwelling catheter in the external jugular vein (inside diameter, 0.125 cm; outside diameter, 0.225 cm; Tygon Microbore tubing, #14-170-15E; Fisher Scientific Co., Pittsburgh, PA) as described (25). Animals were randomly assigned to three treatment groups of porcine relaxin (3000 units/mg) or phosphate-buffered saline (PBS) on Day 275: heifers were given relaxin infusions with im injection (RLX-INF, 9000 units, n = 3), relaxin im injection (RLX-im, 6000 units, n = 4), and PBS controls (PBS, n = 4) (Fig. 1). RLX-INF heifers received infusions of PBS for 165 min, after 120 min 1000 units of relaxin in 165 min, followed by 2000 units of relaxin im, after 240 min 2000 units of relaxin in 165 min followed by 4000 units of relaxin im. The hormone treatments were designed to generate two peaks of relaxin immunoreactivity, preceded by gradually rising relaxin immunoreactivity, and separated by at least 6 hr of steadily declining and rising levels of relaxin immunoreactivity. Sequential blood samples were collected at 15-, 30-, and 60-min intervals as presented in Figure 1. Blood samples were transferred into heparinized vacutainer tubes maintained on ice and were centrifuged at 2000g at 4°C. Plasma was decanted into siliconized culture tubes immediately, frozen on dry ice, and stored at −20°C until required for radioimmunoassay of relaxin, progesterone, and OT.

Radioimmunoassay of Plasma Progesterone and Relaxin. Peripheral blood plasma levels of progesterone were quantified without modifications by using the procedures described (24, 26). The sensitivity, inter, and intraassay coefficients of variation of the assay were 0.25 ng/ml, 6.8% and 3.6%, respectively. Relaxin immunoactivity in bovine peripheral plasma was quantified by using a homologous radioimmunoassay for porcine relaxin (25). The nonspecific binding, sensitivity, intra-, and interassay coefficients of variations were 3.24% (37 pg/tube), 4.35%, and 6.21%, respectively.

Radioimmunoassay of Plasma Oxytocin. Oxytocin was quantified by using a highly sensitive assay method based upon modifications of two earlier procedures (27, 28). The OT was extracted by using commercial extraction cartridges (Spice C18, #01-10; Analtech Inc., Newark, DE) and tetrahydrofuran (pH 7.2). Plasma (0.5–2 ml) diluted with 0.05 M phosphate buffer (1:1 v/v) was transferred to syringes in a fraction collector-vacuum manifold (Spice). Effluent was discarded and the cartridge rinsed with acetic acid. Tetrahydrofuran (2 ml) was used to elute OT into fresh siliconized borosilicate tubes. Extracts were dried down and reconstituted in an assay buffer (0.05 M phosphate buffer, 50 mM EDTA, and 0.5% human albumin). Extraction efficiency was determined by adding a known amount of ^{125}I-oxytocin to known amounts of OT in plasma (ovariectomized-hypophysectomized calf) to recovery tubes before extraction. The efficiency was calculated as the amount of ^{125}I-oxytocin activity recovered. By using this procedure, the extraction efficiency ranged from 79 to 88%.

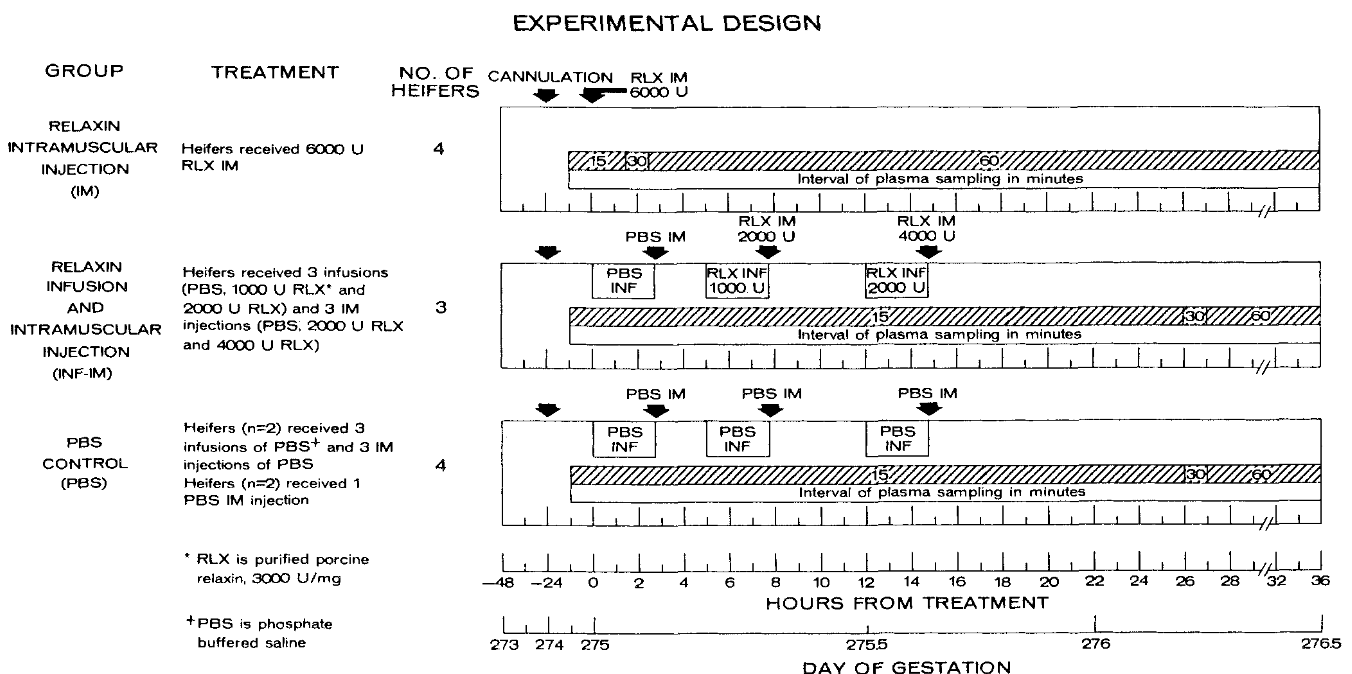

Figure 1. Description of experimental design indicating treatments, day of relaxin or PBS vehicle administration, and day of sequential collection of blood from anterior vena cava.

The OT in reconstituted extracts was determined in duplicate from sample extracts in siliconized borosilicate tubes (10 × 65 mm) by using the double antibody-ammonium sulfate precipitation method. The synthetic OT concentrations (Syntocinon, 10 USP units/ml, #895-J5087; Sandoz Pharmaceuticals, East Hanover, NJ) used for the standard curve were 0.2, 0.4, 0.8, 1.0, 2.0, 4.0, 8.0, 10.0, 20.0, and 40.0 pg/100 μl. Two-hundred microliters of extract or standard were incubated with 100 μl of 1:80,000 OT antibody (from Professor D. Schams, Technische Universität, München, West Germany; Ka #8, E. 15 1.79) for 24 hr at 4°C. Thereafter, 100μl of labeled OT (3000 cpm/100 μl; ^{125}I-OT NEX-187, 2200 Ci/m*M*, NEN Research Products, DuPont, Wilmington, DE) were added and incubated for another 48 hr. Separation of free and bound OT was accomplished in two steps. First, 200 μl of goat anti-rabbit γ-globulin (1:5, #0112-0081, Cappel; Cooper Biomedical Inc., Malvern, PA) were added to each tube and incubated for 4 hr at 4°C; then 100 μl of saturated ammonium sulfate were added to each tube and centrifuged after 20 min. The pellet and supernatant were separated and both fractions counted in a gamma counter. Included in each assay were buffer control tubes (n = 4), plasma blanks (n = 4), two Syntocinon standards (n = 4), and water blank. The maximum binding was 35–45%, nonspecific binding was 2.5–4.2%, and sensitivity was 0.25 pg/tube. The precision and accuracy of the assay were determined by adding known quantities of Syntocinon to plasma samples before extraction. The intra- and interassay coefficients of variation were 8.8% and 10.2%, respectively. The Syntocinon standards and their assay determinations (mean, coefficient of variation) were: 0.00 pg/assay tube (0.05, 3%), 0.80 pg/assay tube (1.2, 2.4%), 2.0 pg/tube (2.30, 4.0%), 4.0 pg/assay tube (5.20, 4.0%), 8.0 pg/assay tube (7.40, 3%), and 20.0 pg/tube (16.0, 3.0%).

Statistical Analysis. The experimental units in this study were the individual heifers. A split-plot experimental design for analysis of variance was based on general linear model (29, 30). For continuous variables Student's t test was used for comparison between treatments (29, 30).

Results

In late-pregnancy beef heifers (Day 275), im injections of porcine relaxin (2000, 4000, or 6000 units) increase ($P < 0.001$) circulating levels of relaxin immunoreactivity in bovine peripheral plasma to peak values within 45–60 min (Figs. 2A and 3A, Table I). Relaxin administration to RLX-INF heifers (Fig. 3A) produces a small and a large relaxin peak separated by at least 6 hr of declining and rising levels of relaxin. Endogenous relaxin immunoreactivity in PBS control heifers (Fig. 4A and Table I) ranged from 180 to 950 pg/ml plasma. High circulating blood levels of relaxin are associated with earlier calving, 3 of 4, 1 of 3, and 0 of 4 RLX-im, RLX-INF, and PBS heifers calved, respectively. RLX-im heifers calved 35 ± 15 hr after treatment on Day 275, whereas the control heifers calved an average of 283 days.

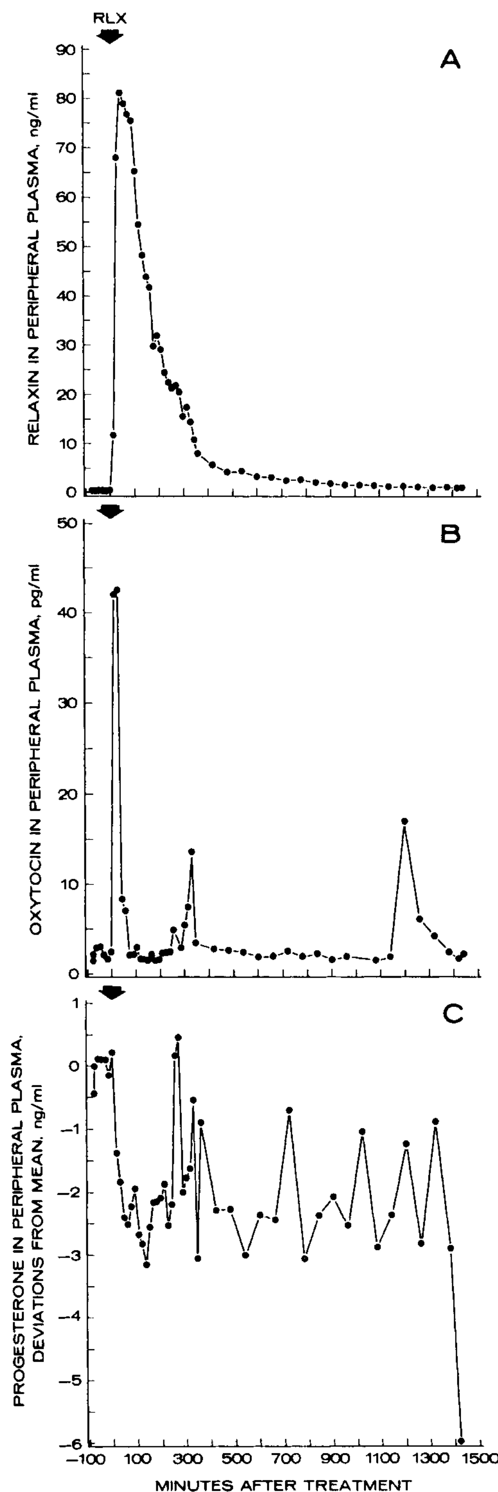

Figure 2. Peripheral blood plasma relaxin immunoreactivity (A), oxytocin (B), and progesterone (C) in primiparous beef heifers (n = 4) receiving 6000 units of purified porcine relaxin (3000 units/mg) on Day 275 of gestation by im injection.

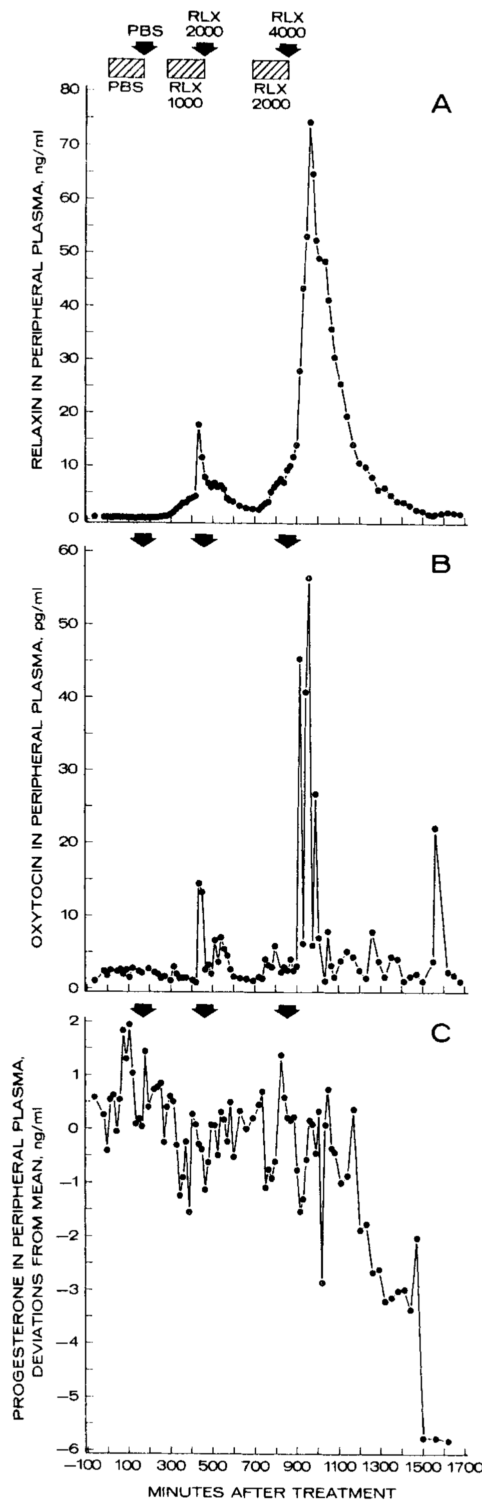

Figure 3. Peripheral blood plasma relaxin immunoreactivity (A), oxytocin (B), and progesterone (C) in primiparous beef heifers ($n = 3$) receiving infusions and im injections of purified porcine relaxin (3000 units/mg) on Day 275 of gestation.

The normal plasma levels of OT before parturition range from 0.3 to 2.9 pg/ml in the control heifers (Fig. 4B and Table I). Administering 6000 units of relaxin im induces a peak release of OT (42 ± 16 pg/ml; mean ± SE) at 15–30 min after treatment (Fig. 2B) which

Table I. Relaxin, Oxytocin, and Progesterone Concentrations in Peripheral Plasma of Heifers Given an im Injection of Relaxin (RLX, 6000 units) or PBS

Hormone		Time (min)										
Radioimmuno-assay	Treat-ment	0	15	30	45	60	120	180	240	300	720	1200
Relaxin (ng/ml)	RLX	0.3 ± 0.04[a]	11 ± 2.5	68 ± 13.9	81 ± 12.0	79 ± 4.1	54 ± 4.6	29 ± 2.4	22 ± 5.9	15 ± 3.5	2.3 ± 0.77	1.1 ± 0.21
	PBS	0.3 ± 0.04	0.3 ± 0.05	0.3 ± 0.04	0.3 ± 0.06	0.3 ± 0.02	0.3 ± 0.03	0.3 ± 0.02	0.4 ± 0.06	0.36 ± 0.03	0.5 ± 0.05	0.4 ± 0.09
Oxytocin (pg/ml)	RLX	2.4 ± 0.75	42 ± 24.4	42 ± 16.7	8 ± 4.9	7 ± 3.1	1.6 ± 0.22	1.4 ± 0.26	2.4 ± 1.10	5 ± 3.5	2.4 ± 1.45	17 ± 7.2
	PBS	1.6 ± 0.65	2.2 ± 0.62	1.8 ± 0.26	1.8 ± 0.26	1.7 ± 0.42	1.8 ± 0.68	2.6 ± 1.67	1.9 ± 0.70	2.1 ± 0.64	1.0 ± 1.04	2 ± 1.04
Progesterone[b] (ng/ml)	RLX	0.2 ± 0.38	−1.4 ± 0.24	−1.9 ± 1.04	−2.4 ± 1.21	−2.5 ± 1.11	−2.8 ± 0.91	−2.2 ± 1.13	−2.2 ± 2.56	−1.8 ± 1.79	−0.7 ± 0.19	−1.3 ± 0.24
	PBS	0.2 ± 0.08	0.1 ± 0.27	0.1 ± 0.61	0.1 ± 0.17	−0.3 ± 0.20	−0.8 ± 0.43	−0.1 ± 0.55	−0.1 ± 0.13	0.2 ± 0.22	0.3 ± 0.02	−0.2 ± 0.60

[a] Values are means ± SE, $n = 3$ heifers (RLX); $n = 4$ heifers (PBS).
[b] Mean deviations from a pretreatment mean of 6.2 ng/ml peripheral plasma.

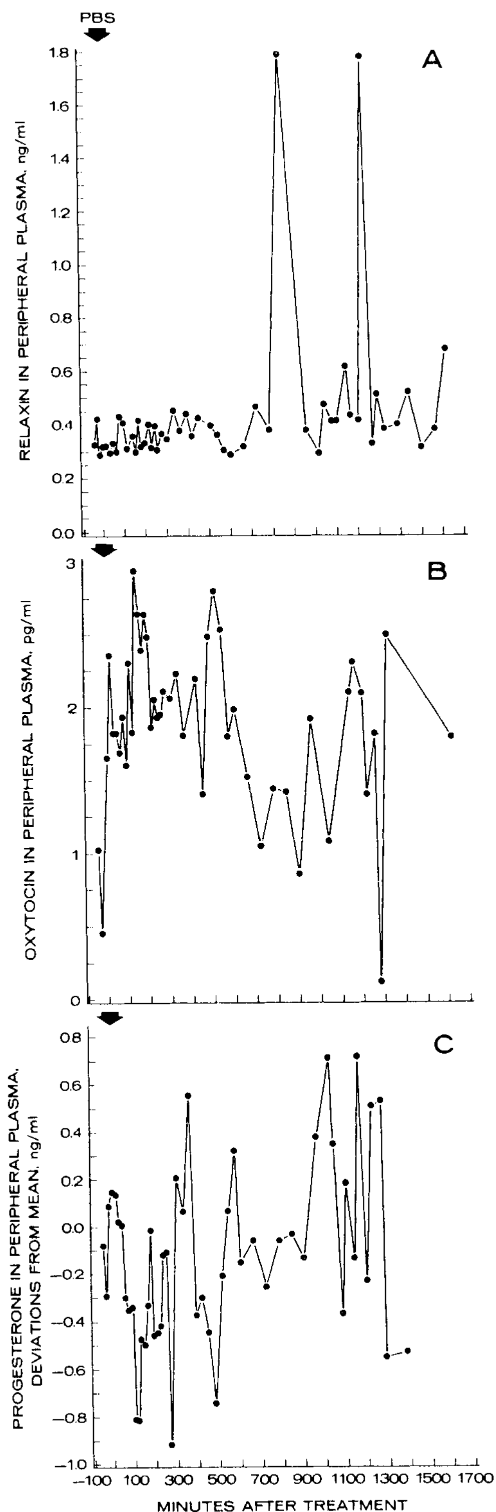

Figure 4. Peripheral blood plasma relaxin immunoreactivity (A), oxytocin (B), and progesterone (C) in primiparous beef heifers (n = 4) receiving PBS on Day 275 of gestation.

precedes a significant decline in plasma progesterone (Table I). Subsequently, spontaneous OT spikes of 10 pg/ml are seen at random in the relaxin-treated groups (Figs. 2B and 3B). In RLX-INF heifers, the period (time, 0–240 min) of buffer infusion is associated with 1.8 ± 0.20 pg/ml OT (Fig. 3B). During the period of low level relaxin infusion (1000 units/165 min), OT levels remained low from 1.1 ± 0.20 to 1.4 ± 0.27 pg/ml. High blood levels of relaxin, as a result of im injection of the hormone, induced spikes of plasma OT (Fig. 3B). The amplitude of these spikes, however, was dose dependent (Fig. 3B). The post peak levels of OT returned to basal levels in spite of the relatively high circulating concentrations of relaxin. In all heifers, parturition was associated with peak release of OT (15–20 pg/ml) (Figs. 2B and 3B and Table I).

Progesterone concentrations in peripheral plasma are presented as deviations from the pretreatment mean for each heifer (Table I). In these heifers mean progesterone concentration before the treatments was 6.2 ± 2.11 ng/ml. The results indicated that administration of 6000 units of relaxin depressed progesterone levels (Fig. 2C and Table I). At the time of hormone treatment, mean progesterone deviation was 0.2 ± 0.38 ng/ml, and by 135 min a maximum decrease of −3.2 ± 1.51 ng/ml was evident in RLX-im heifers (Fig. 2C). There was a rebound in progesterone levels from −3.1 ± 2.15 ng/ml to −0.9 ± 0.21 ng/ml. Of greater significance is the pattern of progesterone in the plasma of RLX-INF group (Fig. 3C). Infusion of low dosages of relaxin elevated levels of progesterone at time 0, and mean progesterone deviation was −0.4 ± 0.18 ng/ml; at 60–150 min, however, posttreatment progesterone deviation was −1.9 ± 1.36 to 0.5 ± 0.84 ng/ml.

Discussion

The results presented here indicate that high levels of relaxin stimulate spike release of OT in late-pregnancy beef heifers. In this study, peak release of OT could be mediated by large doses of relaxin, but the continual presence of relatively high circulating levels of relaxin did not maintain OT levels at peak amplitudes. This suggested that either the source of OT was depleted or the system becomes refractory to relaxin after the OT spike response. It could also suggest dual sources of OT, from ovarian and neurohypophysial origins, but responding differently to a hormone such as relaxin. High progesterone blood levels are preceded by relatively low levels of OT and moderate levels of relaxin, whereas relatively low or rapidly declining levels of progesterone in the plasma are associated with a prior surge of relaxin and peak activity of OT. The decrease in progesterone in peripheral blood plasma as seen here is consistent with our earlier studies (25, 26). This study however presents for the first time the temporal relationship between relaxin, OT, and progesterone in late-pregnancy cattle.

Relaxin immunoactivity in PBS control heifers suggests that endogenous bovine relaxin in peripheral blood during late pregnancy may not reach such high concentrations as achieved in this study by administering porcine relaxin. The results thus presented for re-

laxin-treated heifers may very well be a pharmacologic rather than a physiologic response. However, *in vivo* and *in vitro* evidence suggest that OT has a role in bovine luteal integrity (10, 17, 31, 32). We have hypothesized that luteal relaxin, OT, and $PGF_{2\alpha}$ may be involved in a feedback interaction at the hypophysial, uterine, and ovarian levels in regulating luteal function in periparturient beef heifers (24, 25). Relaxin is a member of the insulin-like growth factor family, including insulin, IGF-1, and IGF-2, and some members of this family, such as IGF-1, can stimulate the release of OT from bovine granulosa cells (33). Relaxin also stimulates, in a dose-dependent fashion, OT release from cultured bovine luteal cells (22). The findings in the present study are consistent and further strengthen the possibility that OT may be involved in luteal regression in cattle (17). We have not distinguished luteal OT from neurohypophysial OT nor shown how the endogenous relaxin might mediate these changes. We have however demonstrated that exogenous relaxin causes an acute peak release of oxytocin followed by a marked decrease in plasma progesterone secretion in late-pregnancy beef heifers.

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