Effect of Continuous Infusion of Relaxin on Progesterone, Oxytocin, and Relaxin Blood Concentrations and Time of Parturition in Beef Heifers

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Summary

These studies were designed to determine whether continuous intravenous infusion of increasing dosages of porcine relaxin during late pregnancy in beef heifers would influence circulating blood concentrations of relaxin, progesterone, and oxytocin, and time of onset of parturition. Beef heifers were bred by artificial insemination and, on Day 277, fitted with indwelling jugular cannulas for hormone infusion and blood sampling from Day 277 to 286. Intravenous infusion of purified porcine relaxin (pRLX, 3000 U mg^{-1}) was started in heifers (n = 8) at increasing dosages (200 U h^{-1} on Days 277 and 278, 300 U h^{-1} on Days 279 and 280, 500 U h^{-1} on Day 281, 600 U h^{-1} on Day 282, and 700 U h^{-1} on Days 283 to 286). Phosphate buffer saline (PBS, 10 ml h^{-1}) was infused during these same times to control (n = 6) animals. Relaxin treatment steadily increased the circulating plasma concentration of immunoreactive relaxin to more than 120 ng ml^{-1} compared with less than 0.5 ng ml^{-1} in PBS-treated controls. Relaxin infusion in increasing dosages over the treatment time was associated with a significant decrease ($P < 0.01$) in plasma progesterone concentration compared with the PBS controls. Plasma levels of oxytocin at 4-hour intervals remained similar ($P > 0.05$) during the pretreatment period and throughout continuous infusion of pRLX and PBS. Although continuous intravenous infusion of relaxin resulted in a decrease in circulating blood levels of progesterone, it did not significantly reduce the interval between the beginning of pRLX treatment and parturition compared with the PBS-infused control heifers. These results indicate that continuous intravenous infusion of high levels of porcine relaxin resulted in a decrease in progesterone secretion in late pregnant beef heifers.

Introduction

Relaxin (RLX), a peptide hormone with partial structural homology to insulin, is produced in abundant quantities by corpora luteal on the ovaries during late pregnancy in the pig. Relaxin induces marked cervical dilation, pelvic relaxation, separation of the pubic symphysis, and interpubic ligament formation in several mammalian species. Although the pig is a rich source of this hormone, only a single copy of the RLX-like gene with a short open reading frame corresponding to part of the relaxin A-chain is present in sheep; however, there is recent evidence of a RLX-like hormone in granulosa cells of ovarian follicles in cattle. Ruminants do respond to intravenous or intramuscular injection of porcine relaxin, however, by hormone-specific quiescence of uterine muscle activity and by cervical softening and dilation in cattle and sheep.

The objective of the present study was to determine the effects of continuous infusion of increasing dosages of porcine relaxin for several days on hormone secretion profiles associated with late pregnancy and on parturition in beef heifers. These periparturient hormones include progesterone, oxytocin, and immunoreactive relaxin.

Materials and Methods

Animals

Fourteen primiparous crossbred beef heifers approaching their first calving were bred by artificial insemination at estrus (Day 0) from 171 - 189 Julian days. Gestation averaged 283 days in this herd. During the early stages of pregnancy (spring, summer, and fall) heifers were maintained on pasture at the Rhodes Research Farm. The heifers were fed a ration of mixed silage supplemented with hay during the winter months. On Day 275, the heifers were transferred to the Animal Reproduction Laboratory at Ames, acclimated in confinement pens for hormone treatments and calving, and fed corn (2.3 kg $animal^{-1}$ day^{-1}), hay and fresh water *ad libitum*.

Experimental Groups

The experimental design is presented in Figure 1. On Day 275, heifers were assigned randomly to one of two treatment groups: porcine relaxin (pRLX), n = 8; and phosphate buffer saline controls (PBS), n = 6. They were bilaterally fitted with indwelling catheters into the external jugular veins.

The control group received a continuous infusion of 0.01 molar phosphate buffer saline (PBS) pH 7.0 at a rate of 10 ml h^{-1}. Relaxin used in this study was extracted and

purified from ovaries of pregnant pigs. Highly purified porcine relaxin (pRLX, 3000 U mg^{-1}) was dissolved in 0.01 M PBS, pH 7.0, and infused in a graded dosage scheme. Infusion pumps were calibrated to deliver 10 ml h^{-1}. The duration of pRLX or PBS infusion was 10 days, beginning 6 days before expected parturition at 06:00 hours and terminating at either parturition or Day 286, if parturition had not occurred.

The relaxin was infused in graded dosages throughout the time course of the experiment (Figure 1). The dosage schedule for the pRLX group was as follows: 200 U h^{-1} of pRLX on Days 277 and 278; 300 U h^{-1} on Days 279 and 280; 500 U h^{-1} on Day 281; 600 U h^{-1} on Day 282; and 700 U h^{-1} on Day 283 (estimated parturition), until the end of treatment. The control group was infused with PBS from Day 277 through Day 286. The heifers were monitored continuously to precisely determine the time of parturition, dystocia, calf birth weight, and placental delivery. Dystocia was evaluated on the basis of number and percentage of heifers requiring assistance. Dystocia score was defined as: 1 = none, 2 = hand pull, 3 = chain-hand pull, 4 = chain-jack pull, 5 = Caesarean section, and 6 = feteotomy.

Radioimmunoassay of Progesterone, Relaxin, and Oxytocin in Peripheral Plasma

Hourly blood samples (10 ml) were collected via a jugular catheter beginning at 06:00 hours before pRLX or PBS infusion began. Blood was collected in 16 x 100 mm heparinized vacutainors, maintained on ice up to 1 hour, and centrifuged (2000 x *g*). Plasma from these samples was frozen and stored at -20°C for radioimmunoassay (RIA) of progesterone, relaxin, and oxytocin.

Progesterone was determined in duplicate 200-ml aliquots of plasma with RIA by our procedures using a fully characterized antibody. The minimal detectable concentration of progesterone was 0.25 ng ml^{-1}. The inter- and intra-assay coefficients of variation were 8.9 (*n* = 9) and 10.5% (*n* = 36); nonspecific binding was 3.1%.

Immunoreactive relaxin was determined in duplicate aliquots of 25 - 100 ml plasma in a double antibody RIA by using ^{125}I-labeled monotyrosylated pRLX and antiporcine relaxin serum using the procedure we previously described. Assay sensitivity was 35 pg ml^{-1} plasma. Intra-assay variances were 2.90, 1.80, 3.22, 1.18, and 1.15%, respectively. Interassay mean comparisons of relaxin in RLX-treated and PBS-control heifers was 1.10 ± 0.03 ng ml^{-1} with a covariance of 5.90% (*n* = 5).

Oxytocin extracted from plasma was quantified by a sensitive RIA method using synthetic oxytocin for the standard curve, oxytocin antibody (from Professor D. Schams; Technische Universität, München, Germany, K#8, E.15 1.7 g) and labeled oxytocin (100 ml; 3000 cpm 100 ml^{-1}; ^{125}I-OT NEX-187, 2200 Ci mM^{-1}, NEN Research Products, DuPont, Wilmington, DE). The maximum binding was 42 - 44%, nonspecific binding was 1.0 - 1.6%, and assay sensitivity was 0.01 pg $tube^{-1}$. Intraassay coefficients of variation of two internal standards were 13.7 and 18.6% (*n* = 4). Interassay coefficient of variation was 7.0% (*n* = 4).

Statistical Analysis

Experimental units were the individual cows, and they were randomly assigned to treatments. Data were analyzed with a split plot analysis of variance, and the general linear model and Student's *t*-test for continuous variables were used for comparisons between treatment groups. Hormone treatment was the main plot, with time subplots for evaluation of time and time x treatment interaction.

Results and Discussion

Effect of Relaxin on Parturition in Beef Heifers

Continuous infusion of increasing dosages of purified pRLX (range from 4800 - 16 800 U 24 h^{-1}) into primiparous beef heifers during late pregnancy did not affect day of calving as compared with the controls receiving an infusion of PBS (P > 0.05) (Table 1). The duration of delivery was similar in pRLX- and PBS-treated heifers (Table 1). Only two of the fourteen pRLX- and PBS-treated heifers required manual assistance for calf delivery. Dystocia score did not differ between the two groups. There was no incidence of retained placentae beyond 24 hours postpartum in experimental or control heifers. Calves from dams in both treatment groups were considered normal.

Effect of Continuous Infusion of pRLX on Plasma Concentrations of Immunoreactive Relaxin

Peripheral plasma concentrations of immunoreactive relaxin in these late pregnant beef heifers are presented in Figure 2. Pretreatment concentrations of immunoreactive relaxin remained similar (P > 0.05) between pRLX and PBS groups (0.4 ± 0.07 and 0.3 ± 0.02 ng ml^{-1}). Immunoreactive relaxin in the PBS control heifers did not change 1 hour after treatment (0.3 ± 0.01 ng ml^{-1}) until the end of the PBS infusion 216 hours later (0.3 ± 0.01 ng ml^{-1}). However, continuous infusion of pRLX resulted in increased immunoreactive relaxin concentration in peripheral plasma (P < 0.01) as the dosage of pRLX (U h^{-1}) was increased. Mean plasma concentrations of immunoreactive relaxin during each successive pRLX dosage infused were significantly different (P < 0.001; 4.4 ± 0.35 ng ml^{-1} at 300 U h^{-1}; 9.3 ± 0.75 ng ml^{-1} at 300 U h^{-1}; 21 ± 2.0 ng ml^{-1} at 500 U h^{-1}; 33 ± 4.3 ng ml^{-1} at 600 U h^{-1}) with a peak of 77 ± 4 ng ml^{-1} during the infusion of 700 U h^{-1} of pRLX. Mean concentrations of immunoreactive relaxin in PBS control heifers were significantly lower during infusion of pRLX at dosages of 200 - 700 U h^{-1}.

Effect of Continuous Infusion of pRLX on Peripheral Plasma Progesterone Concentrations

Changes in progesterone concentrations in peripheral blood plasma are presented in Figure 3. During the pretreatment period, mean (± SE) progesterone concentrations were 4.2 ± 0.38 ng ml^{-1} with a range of 2.2 to 7.2 ng ml^{-1} in the 14 beef heifers. Mean deviations of progesterone from pretreatment means remained similar ($P > 0.05$) among both groups before the infusion of pRLX or PBS. Progesterone decreased ($P < 0.05$) during continuous infusion of 500 - 700 U h^{-1} pRLX compared with PBS controls. The rate of change in progesterone levels between 300, 600, and 700 U h^{-1} pRLX and PBS groups differed ($P < 0.05$).

Effect of Continuous Infusion of pRLX on Peripheral Plasma Oxytocin Concentrations

Oxytocin levels at 4-h intervals remained similar ($P > 0.05$) during the pretreatment period and throughout continuous infusion of pRLX and PBS (Figure 4). Oxytocin in control heifers peaked at 0.95 ± 0.10 pg ml^{-1} during the 72 hours before parturition, corresponding to infusion of 500 - 600 U h^{-1} in the pRLX-treated group, which peaked at 0.77 ± 0.07 pg ml^{-1}. Plasma oxytocin increased simultaneously during the corresponding infusion period of 200 - 500 U h^{-1} in the PBS-control heifers ($P < 0.05$), and during the same period in the pRLX-treated heifers ($P < 0.01$). In one control heifer, a spike release of oxytocin (3.8 pg ml^{-1}) was detected, but no peak releases of oxytocin were determined in any other heifers in that group.

The main finding was that continuous intravenous infusion of purified pRLX at increasing dosages ranging from 200 - 700 U h^{-1} over several days significantly decreased plasma progesterone concentration but did not result in earlier parturition compared with PBS-infused control heifers. Continuous infusion of pRLX into late pregnant beef heifers maintained greatly elevated circulating relaxin concentrations to parturition. This imposed RLX profile in cattle mimicked the endogenous prepartum profiles of circulating concentrations of relaxin found in other species, such as the pig. Increasing circulating concentrations of endogenous relaxin during late pregnancy have been shown to be associated with unimpaired normal parturition in this species, as evidenced by an increased number of stillborns when pigs are treated with antiporcine relaxin antibody. In cattle, relaxin given intramuscularly at a rate of 6000 U on Day 275 resulted in a transient decrease in circulating progesterone concentration and earlier calving. The continuous infusion of high dosages of pRLX in the present study may have decreased uterine myometrial activity. In contrast to previous findings, gestation was not shortened in the present study. Thus a single injection of pRLX too many days before expected parturition or continuous infusion of high dosages of pRLX for several days did not result in earlier calving. In the present study, there was no incidence of retained placentae in either group, and calving was similar in both RLX- and PBS-treated heifers.

A temporal relationship exists between the graded rise in plasma relaxin concentration and the abrupt decrease in progesterone in response to infused porcine relaxin. This is consistent with our earlier findings in which a significant decrease in circulating progesterone occurred as early as 24 hours after deposition of relaxin in a gel into the cervical os on Day 278, and again, at 90 minutes after intramuscular injection on Day 273. The dosage, time, and route of relaxin treatment are crucial in determining whether complete luteal demise or temporary suppression of luteal progesterone secretion without induction of parturition will occur in cattle.

It has been postulated that oxytocin of luteal cell origin may play a role in the regression of the bovine corpus luteum. In the present study, plasma oxytocin concentrations increased near parturition but were similar when compared with PBS-treated controls. No spike releases of oxytocin were detected as compared with our prior studies, in which a bolus intravenous or intramuscular injection of relaxin (i.e., up to 9000 U) induced single and multiple peaks of oxytocin that ranged from 10-42 picograms per milliliter; peak relaxin concentrations occurred within 30 minutes after hormone treatment. However, oxytocin peaks in the present study may not have been detected because of the infrequency of plasma sampling. Furthermore, the infusion of increasing dosages of relaxin may have subdued oxytocin peak releases during the 216 hours of continuous pRLX treatment. It has been shown that oxytocin is released intermittently, and in cattle plasma oxytocin levels appear to be low or undetectable during late pregnancy but high during the expulsive phase of labor. The discrepancies between these results may relate to the origin of endogenous oxytocin, whether the source is hypothalamic or luteal. In summary, the results presented here indicate that the continuous infusion of porcine relaxin during late pregnancy results in a decrease in progesterone secretion in cattle.

Implications

Although continuous intravenous infusion of increasing dosages of purified porcine relaxin over several days significantly decreased plasma progesterone concentration, it did not induce earlier parturition compared with phosphate buffer saline-infused control beef heifers.

Figure 1. Experimental design for late pregnant beef heifers intravenously infused with porcine relaxin (pRLX) or phosphate-buffered saline (PBS).

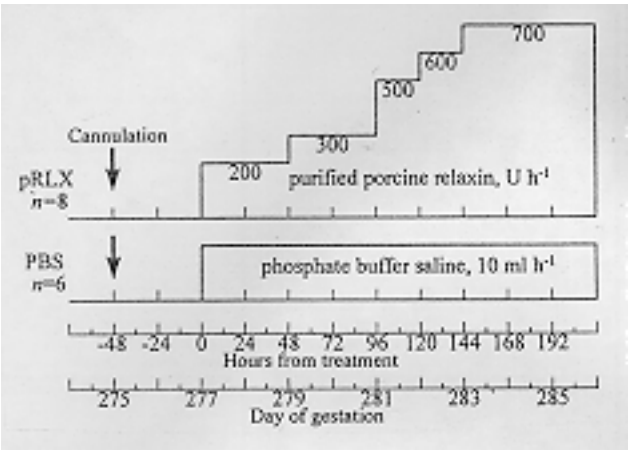

Figure 2. Immunoreactive relaxin concentration (ng ml^{-1}) in peripheral plasma of beef heifers during continuous intravenous infusion of porcine relaxin (pRLX, 3000 U mg^{-1}, *n* = 8) or phosphate buffer saline (PBS, *n* = 6) from Days 277 to 286 of pregnancy. Values are mean ± SE.

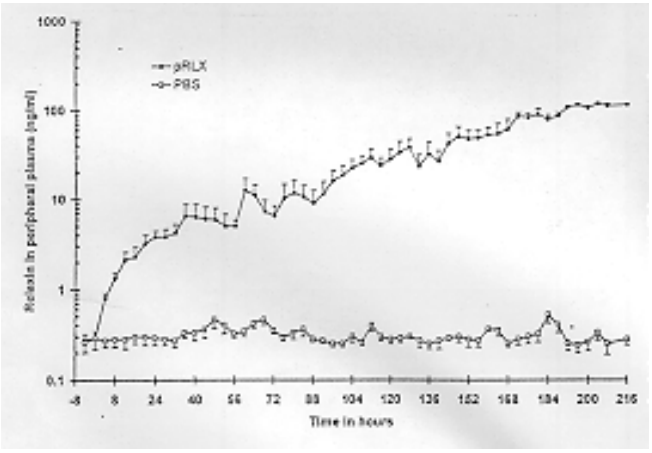

Figure 3. Progesterone concentration in peripheral plasma (ng ml^{-1}) during 100 h before parturition in relaxin (pRLX, 3000 U mg^{-1}, *n* = 8) and phosphate buffer saline (PBS, *n* = 6) intravenous infusion. Values are mean ± SE.

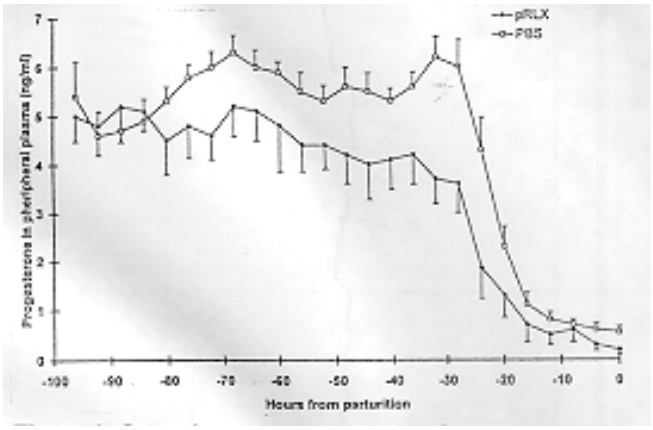

Figure 4. Oxytocin concentration (pg ml^{-1}) in peripheral plasma during continuous intravenous infusion of increasing dosages of porcine relaxin (pRLX, 3000 U mg^{-1}, *n* = 8) and phosphate buffer saline (PBS, *n* = 6) in late pregnant beef heifers from time of parturition (0 h). Values are mean ± SE.

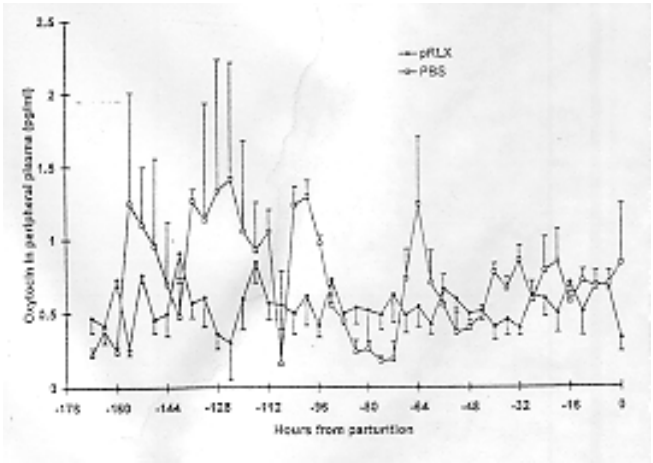

Table 1. Effect of continuous infusion of porcine relaxin on duration of pregnancy in beef heifers.

Treatment	No. of heifers	Pregnancy (days)	First treatment to calving[a] (h)	Duration of delivery (min)	Dystocia No.	Dystocia Score
pRLX	8	282 ± 1.4	131 ± 20	87 ± 15	2	2.6 ± 0.42
PBS	6	281 ± 1.3	102 ± 26	76 ± 18	0	2.5 ± 0.34

[a]Porcine relaxin (3,000 U mg^{-1}) dissolved in 0.01 M PBS was infused continuously from Day 277 to parturition at increasing dosages from 200 - 700 U h^{-1}. Controls received a continuous infusion of PBS at 10 ml h^{-1}. Values are mean ± SE.