Effect of Antiporcine Relaxin Treatment on Parturition in Pigs

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ASL-R1567

Summary and Implications

Antibody against porcine relaxin (antipRLX540; 1:950,000) was produced in sheep and used to determine the effect on relaxin and progesterone secretion, and on parturition in late-pregnant pigs. In group 1, Yorkshire gilts with normal estrous cycles were bred on the second observed estrus and fitted with an indwelling jugular cannula and an intraperitoneal cannula on day 100 of pregnancy. Gilts were infused at 6-hour intervals with antipRLX540 or phosphate buffer saline (PBS) beginning on day 103 until parturition. From days 103 to 120, daily blood samples were collected for radioimmunoassay of relaxin, progesterone, and prolactin. In group 2, bred gilts were randomly assigned to antipRLX540, relaxin, and PBS treatment on days 111, 113, and 115. Blood was collected twice daily from day 108 to 120, and every 20 minutes on days 111, 113, and 115 beginning 60 minutes before treatment and continuing 180 minutes. Parturition in gilts given antipRLX540 occurred on day 112.7 compared with day 114.0 in relaxin-treated gilts and day 114.3 in PBS controls ($P<.05$). Duration of delivery from first to last piglet was greatly delayed in antipRLX540 gilts (240 minutes) compared with PBS controls ([117 minutes] $P<.005$). Average number of stillborns was greater in antipRLX540- than in PBS-treated controls (2.4 vs. 1.0; $P<.05$). Plasma progesterone concentrations were similar in antipRLX540- and PBS-treated gilts throughout the period of the study. By day 113, progesterone decreased in antipRLX540-treated gilts compared with relaxin- and PBS-treated gilts. Prolactin levels were similar in both antipRLX540- and PBS-treated gilts; however, from 1 to 3 days postpartum the antipRLX540 group had higher prolactin concentration ($P<.05$).

The results indicate that antipRLX540 decreased circulating plasma concentrations of unbound or free relaxin during the last 10 days of pregnancy. AntipRLX540 markedly increased both the duration of delivery of piglets and the average number of stillbirths in this litter-bearing species compared with PBS-treated controls. This study provides strong evidence that increasing circulating concentrations of relaxin during late pregnancy is crucial for unimpaired parturition in the pig.

Introduction

Relaxin and progesterone are produced by ovarian corpora lutea of pigs during pregnancy and after hysterectomy. During normal pregnancy of about 114 days, the concentrations of circulating progesterone peak by day 8 and remain high until they decrease just before parturition. Relaxin accumulates in luteal tissue, increases gradually during pregnancy, and is released into the blood in peak quantities just before parturition. Relaxin stimulates growth and softening of the cervix in pigs (1). A primary function of relaxin in mammals during late pregnancy involves the preparation of the reproductive system for delivery of the newborn. This study was designed to (1) determine whether antiserum to porcine relaxin produced in sheep would affect parturition in late pregnant pigs; (2) assess the duration of delivery from first to last piglet, average number of stillborns, and newborn survival rates; (3) compare concentrations of circulating progesterone, relaxin, and PRL in gilts injected with antipRLX540 or PBS continuously from days 103 to 114; and (4) compare concentrations of circulating progesterone and relaxin in gilts injected high doses of antipRLX540, relaxin, or PBS on days 111, 113, and 115.

Materials and Methods

Animals. Forty-four Yorkshire gilts, averaging 120±10 kg BW (± SE), with normal estrous cycles were bred (day 0) at second observed estrus to fertile Yorkshire boars and confirmed pregnant. The expected duration of gestation was 114 days. On day 100 gilts were fitted with an indwelling jugular cannula (i.d. 1.27 mm; o.d., 2.29 mm; Tygon microbore tubing, Fisher Scientific, Pittsburgh, PA) and an indwelling intraperitoneal cannula (in the right lower flank) of the same size and composition.

Experimental protocol group 1. Relaxin was extracted from ovaries of pregnant pigs and purified (pRLX, 3,000 U/mg) according to procedures we described previously. Male sheep were immunized (1 mg/ml injection) with porcine relaxin (3,000 U/mg). Serum from sequential bleedings of sheep #540 at 50% binding affinity with ^{125}I-labeled monotyrosylated porcine relaxin revealed antibody titers of 1:950,000, 1:500,000, and 1:100,000.

Preparations of antiporcine RLX serum #540 were screened for their ability to neutralize porcine RLX in vivo by the mouse interpubic ligament bioassay. Interpubic ligament length in benzopurpurine controls averaged 1 millimeter, and increased in a significant dose-dependent manner at 0.25–2.0 μg pRLX. AntipRLX#540 serum at 1:950,000 significantly inhibited interpubic ligament development in the dose range of pRLX treatments.

Beginning on days 103 to 120, twice daily blood samples (10 ml) were collected at 12-hour intervals from each gilt (n=20) for radioimmunoassay (RIA) of progesterone, relaxin, and prolactin (PRL). Using antipRLX540 produced in sheep with high antibody titer (1:950,000) or PBS (n=10 for each group), gilts were infused (0.1 ml) through intraperitoneal cannula at 6-hour intervals beginning on day 103 (12 days before expected parturition) to parturition.

Experimental protocol group 2. Gilts were randomly assigned to antipRLX540 (n=11, 1 ml with antibody titer of 1:950,000), relaxin (n=5; 3,000 units), and PBS (n=8) treatment on days 111, 113, and 115 through an intraperitoneal cannula. Blood was collected twice daily from day 108 to 120 for RIA of progesterone, relaxin and prolactin in the plasma, and every 20 minutes on days 111, 113, and 115 beginning 60 minutes before treatment and continuing to 180 minutes for determination of relaxin and progesterone. Gilts were observed for exact time of parturition to establish the interval between delivery of individual piglets, total delivery time (from first to last piglet), incidence of stillborns, and neonatal survival.

RIA of relaxin, progesterone, and prolactin in peripheral plasma. Relaxin was quantified in duplicate 200-µl aliquots of plasma by using a homologous double-antibody RIA for porcine relaxin we described previously (2). The sensitivity of the assay was 40 pg/tube. The inter- and intra-assay coefficients of variation were 12.4% (n=3 assay/sample) and 6.2% (n=5 samples); nonspecific binding was 2.8%.

Progesterone was extracted with a benzene-hexane mixture (1:2 vol/vol) and 100 µl of plasma was extracted in duplicate. Recovery after extraction with benzene was 89%, and the minimal detectable concentration of progesterone was 50 pg/tube. The inter- and intra-assay coefficients of variation were 11.3% (n=5 assays/sample) and 8.5% (n=6 samples), respectively; nonspecific binding was 3.1%.

The PRL concentration in plasma was determined by double antibody homologous RIA. The sensitivity of the assay was .7±.25 ng/ml (mean±SD; n=5), and the inter- and intra-assay coefficients of variation were 14±3.7% (mean±SD; 2 samples; 5 assays) and 7.4±5.5% (mean±SD; 2 samples per assay; 2-4 determinations).

Statistical analysis. Experimental units were the individual pigs that were assigned to treatments at random. Data were analyzed by one-way analysis of variance for samples collected each day during hormone or vehicle infusion. Student's *t*-test for continuous variables was used for comparisons between treatment groups.

Results and Discussion

Effect of antipRLX540 on parturition. In gilts given the lower dosage (.1 ml ip every 6 hours of antipRLX540 [1:950,000] from day 103, parturition occurred significantly earlier than in the PBS-treated controls (Table 1, Group 1). The acute effects of a higher dosage (1 ml ip on days 111, 113 and 115) of the same antipRLX540 serum resulted in a significantly earlier parturition compared with PBS-treated controls (Table 1, Group 2). Parturition in pRLX-treated gilts was similar to the PBS-treated controls (Table 1, Group 2). The duration of delivery was greatly prolonged in antipRLX540-treated gilts compared with PBS controls in both Groups 1 and 2 (Table 1), but duration of delivery in pRLX-treated gilts was similar to the controls (Group 2). The prolonged duration of delivery as well as more immature development of the piglets at the time of parturition in the antipRLX-540 treated gilts probably was related to the significantly greater incidence of stillborns compared with the PBS-treated controls (Table 1). Data on significantly greater intervals between delivery of each piglet in antipRLX540-treated gilts compared with PBS-treated controls are shown in Figure 2.

Plasma concentration of relaxin and progesterone in Yorkshire gilts treated with antipRLX540, relaxin, or PBS at days 111, 113, and 115. Sequential bleedings revealed that ip injection of pRLX on days 111, 113, and 115 consistently maintained elevated peripheral plasma relaxin concentrations of about 30–40 ng/ml throughout 180 minutes, whereas treatment with antipRLX540 significantly decreased ($P<.05$) circulating relaxin concentration on these days (Figure 1). Although circulating concentrations of relaxin were consistently increased in pRLX-treated gilts on day 111, 113, and 115, they did not shift normal time of parturition. In contrast, antipRLX540 treatment decreased circulating unbound relaxin concentration and delivery by day 112.7. On day 113, the elevated plasma relaxin concentration in five of the eight PBS-treated gilts reflected an endogenous release of the hormone; by day 115, relaxin was at basal levels in all PBS-treated animals. Progesterone plasma concentration in pRLX-treated gilts averaged approximately 12 ng/ml on day 111 and 8 ng/ml on day 113 during the 180-minute sequential bleeding period, whereas progesterone concentration was consistently decreased (averaging 8 ng/ml on day 113 and 4 ng/ml on day 113) throughout the 180 minutes in the antipRLX540-treated gilts (Figure 2). By day 115, circulating progesterone was basal in all three groups of gilts.

The main finding was that long-term (days 103 to 114; 0.1 ml at 6-hour intervals/day) and short-term (days 111, 113, and 115; 1 ml/day) intraperitoneal administration of antipRLX540 in late-pregnant pigs markedly prolonged the duration of delivery of piglets, increased the average number of stillborns, and reduced the number of live piglets. The results presented herein suggest a physiological requirement for endogenous relaxin at term for normal delivery of the newborn. Relaxin is a peptide hormone found in high concentration during late pregnancy that probably contributes to uneventful births in the pig; in most mammals, it has biological actions on pelvic and cervical

connective tissue as well as uterine smooth muscles. These actions include growth-promoting effects, extensibility, and increase in lumen diameter of these organs. In the pig, relaxin deficiency at parturition results in prolonged delivery time with a high incidence of stillbirths (1). The time interval of delivery from the previous piglet and the overall duration of delivery were longer in antipRLX540- compared with relaxin- or PBS-treated gilts. The observed delay in the duration of delivery of piglets in this study may be attributable to lack of cervical remodeling in antipRLX540-treated gilts compared with relaxin- or PBS-treated controls. The physiological changes experienced by antipRLX540-treated pigs resulted in prolonged durations of straining and increased time of delivery in this group compared with diluent-treated animals. The antipRLX540-infused group had a greater incidence of stillbirths and decreased survival rates of newborns, whereas relaxin- or PBS-infused gilts had significantly fewer incidences of stillbirths and greater neonatal survival rates. The greater number of stillbirths and low incidence of live births in antipRLX540-treated pigs may result, at least in part, from the increased duration of straining time in the unexpanded reproductive tract. Furthermore, because relaxin has been shown to have no influence on the onset of lactation in the pig, the decreased piglet survival rates shortly after delivery in antipRLX540-treated gilts were probably not due to lack of milk production by the mammary gland. Physically weak piglets after a delayed delivery period may have contributed to greater neonatal losses because some piglets from antipRLX540-treated gilts appeared to lack energy to immediately stand up and suckle soon after delivery.

The second finding was that antiporcine relaxin in this study decreased circulating plasma concentration of unbound or free relaxin during the last 10 days of pregnancy in these Yorkshire gilts. It is likely that dystocia observed in this group was associated with the decrease in free relaxin because relaxin- or PBS-treated controls had a low incidence of dystocia. The expected increase in relaxin secretion that occurs on day 113 (2) was observed in both antipRLX540- and PBS-treated controls.

In summary, antipRLX540 markedly prolonged the duration of delivery of piglets, increased the average number of stillborns, and reduced the number of live piglets after delivery in this litter-bearing species compared with relaxin- or PBS-treated controls. This study provides strong evidence that increasing circulating concentrations of relaxin during late pregnancy are crucial for unimpaired normal parturition in the pig.

References

1. Kertiles, L.P. and L. L. Anderson. 1979. Effect of relaxin on cervical dilation, parturition and lactation in the pig. Biol. Reprod. 21:57–68.
2. Felder, K.J., J. R. Molina, A. M. Benoit and L. L. Anderson. 1986. Precise timing for peak relaxin and decreased progesterone secretion after hysterectomy in the pig. Endocrinology 119:1502–1509.

Table 1. Effect of antiporcine relaxin- and relaxin-treatment on parturition in Yorkshire gilts.

Treatment	No. of gilts	Average duration of pregnancy (days)	Average duration of delivery (min)	Average no. of piglets born total	live	stillborn
Group 1						
antipRLX540[a]	10	112.2[d]	240[c]	9.8	8.1	1.7[d]
PBS	10	114.0	104	10.5	9.5	1.0
Group 2						
antipRLX540[b]	11	112.7[d]	214[c]	8.6	6.8	2.4[d]
pRLX[c]	5	114.0	133	11.2	9.6	1.6
PBS	8	114.3	117	11.2	10.2	1.0

[a].1 ml i.p. injected at 6-hour intervals from day 103 to parturition.

[b]1.0 ml i.p. injected on days 111, 113, and 115.

[c]3,000 U.i.p. injected on days 111, 113, and 115.

[d]P<.05 compared with PBS control.

[e]P<.01 compared with PBS control.

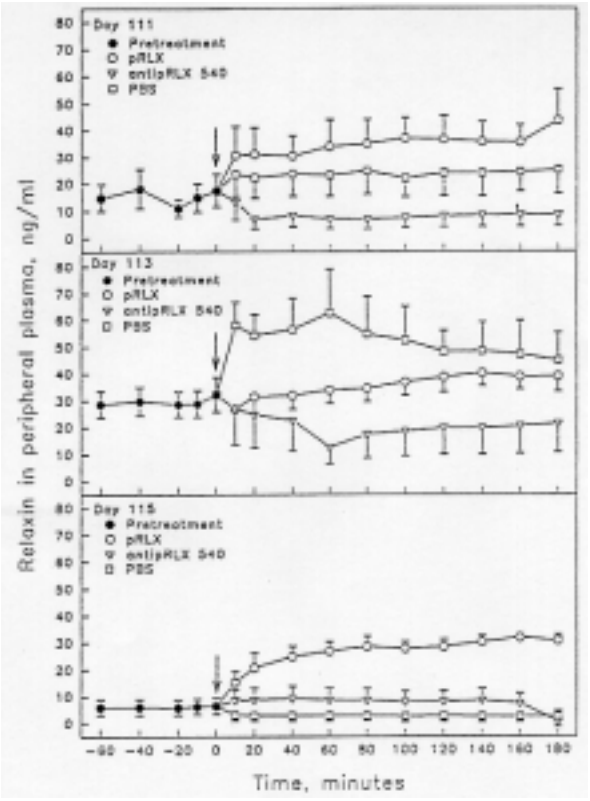

Figure 1. Relaxin plasma concentration in sequential blood samples at 20-min intervals of bred gilts ip injected with 3,000 U pRLX (n=5), 1 ml antipRLX540 (n=11), or PBS (n=8) on days 111, 113, and 115. Values are mean ±SE.

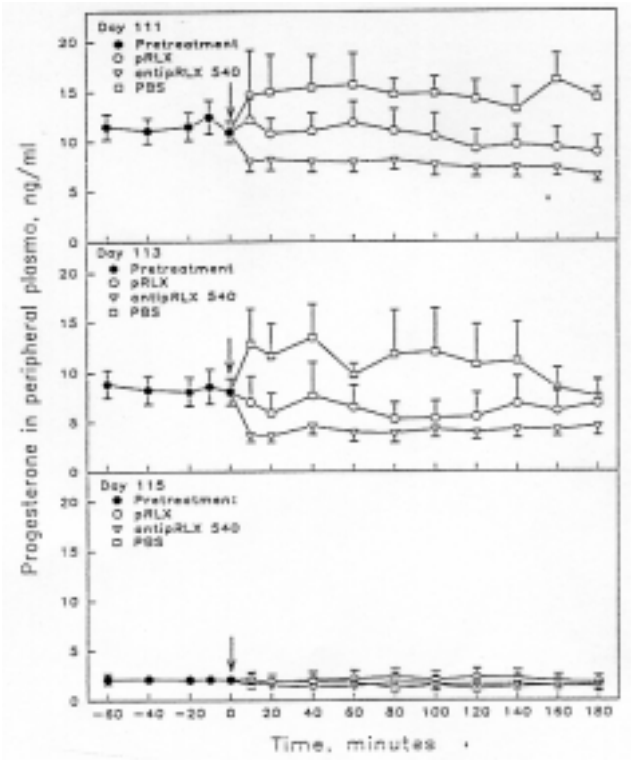

Figure 2. Progesterone plasma concentration in sequential blood samples at 20-min intervals of bred gilts ip injected with 3,000 U pRLX (n=5), 1 ml antipRLX540 (n=11), or PBS (n=8) on days 111, 113, and 115. Values are means ±SE.

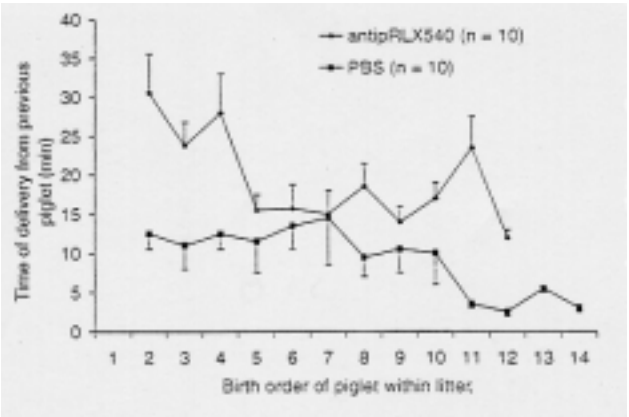

Figure 3. Effect of antiporcine relaxin antiserum (antipRLX540; 0.1 ml ip at 6-h intervals from days 103 to 114) and phosphate buffered saline (PBS; 5 ml ip at 6-h intervals from days 103–114) on the interval for the delivery of each piglet within the litter. The duration of delivery was increased (P<.01) in antipRLX540-treated gilts compared with PBS-treated controls. Values are means ±SE.