

# Differential Effects of Glucocorticoid and Antiglucocorticoid Treatment on Ovarian Progesterone and Relaxin Secretion in the Pig

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## Summary and Implications

Pregnancy lasts about 114 days in pigs. Porcine corpora lutea on the ovaries produce not only progesterone but also relaxin (RLX), a peptide hormone that plays a critical role in suppressing uterine motility during pregnancy and in remodeling connective tissues in preparation for imminent parturition. Progesterone concentrations in peripheral blood remain elevated ( $\approx 25 \text{ ng ml}^{-1}$ ) for the major part of pregnancy and decrease just before parturition. The decrease in progesterone coincides with peak prepartum RLX release. Glucocorticoid or antiglucocorticoid steroid, RU 486, administration during late pregnancy can induce parturition in the pig. Peak release of RLX and a coincident decrease of progesterone in the circulating blood also can occur in the complete absence of fetuses and uterus (hysterectomy) in the pig.

The effects of glucocorticoid or antiglucocorticoid-steroid administration to such hysterectomized pigs on the secretion of RLX and progesterone were examined in this experiment. Unmated Yorkshire gilts were hysterectomized on days 6–8 (estrus = day 0) and given a glucocorticoid, dexamethasone, from days 110–118; control animals received vehicle injection during this period. The RU 486 was orally administered once daily (days 111–115) at 0800 hours; placebo-treated controls were given the same amount of feed without the drug at this time. The RLX concentrations in blood were markedly suppressed ( $P < .01$ ) during dexamethasone treatment, whereas a peak release of RLX occurred in the control group on day 113. In contrast, progesterone concentrations were unaffected by dexamethasone treatment compared with the controls. Upon withdrawal of dexamethasone on day 118, RLX concentrations began to increase and peaked at ( $P < .01$ ) on day 120. In contrast, the antiglucocorticoid caused a marked elevation in circulating levels of progesterone and delayed RLX release until after the end of drug treatment on day 115. Results indicate that a synthetic glucocorticoid, dexamethasone, suppresses RLX secretion without causing luteolysis and such suppression is reversible; progesterone secretion remained unaffected. In contrast, the antiglucocorticoid RU 486 raised progesterone plasma concentration and delayed RLX peak release but did not suppress it during treatment. This experiment provides further evidence that relaxin and progesterone secretion

from aging corpora lutea of pigs are regulated through separate mechanisms, and adrenal glucocorticoids may be involved in such a regulation process.

## Introduction

Relaxin (RLX), a peptide hormone with partial structural homology to insulin, and progesterone are produced by corpora lutea of pigs during pregnancy and after hysterectomy. Although the normal duration of the estrous cycle is about 21 days in the pig, the corpora lutea secrete progesterone and small amounts of RLX. During a normal pregnancy of 114 days, circulating progesterone concentrations peak by day 8 and remain high ( $\approx 25 \text{ ng ml}^{-1}$ ) until they decrease just before parturition. Relaxin accumulates in electron dense granules of luteal tissue and increases gradually during pregnancy, and it is released into the blood in peak amounts just before parturition. After hysterectomy of unmated gilts, the corpora lutea are maintained to day 150; these aging corpora lutea continue to secrete RLX and progesterone. Profiles of decreased secretion and simultaneous peak release of RLX from these aging corpora lutea at day 113 were similar to those on the same day just preceding normal parturition. The RU 486 (11 $\beta$ -[4-dimethylamino phenyl] 17 $\beta$ -hydroxy 17 $\alpha$ -[1-propyl]estra-4,9-dien-3-one) acts with high affinity as an antagonist for progesterone receptor; RU 486 also has antiglucocorticoid activity. Oral administration of RU 486 to late-pregnant gilts induces parturition within 34 hours compared with 82 hours in placebo treated controls. Progesterone decreased abruptly to basal levels during the 2 days that RU 486 was given compared with the controls. The RU 486 elevated circulating prolactin (PRL) concentrations in hysterectomized gilts with aging corpora lutea, and PRL maintained luteal function throughout a 10-day period in hypophysectomized-hysterectomized pigs. These results provide strong evidence that the antagonist effect of RU 486 on progesterone/ glucocorticoid receptor results in an abrupt increase in PRL secretion in hysterectomized gilts with aging corpora lutea. In contrast with hysterectomized animals, the acute luteolytic effects of RU 486 depend on the presence of the uterus or conceptuses in the pig. The objective of this study was to determine whether glucocorticoids directly affect RLX and progesterone secretion by aging corpora lutea in hysterectomized gilts.

## Materials and Methods

*Animals.* Purebred Yorkshire gilts (8 months old;  $\approx 125 \text{ kg}$  body weight) that had exhibited at least one normal estrous

cycle (18–23 days; estrus = day 0) were used in this experiment. Unmated gilts were hysterectomized on days 6–8 of the estrous cycle. The number of corpora lutea and diameter of each were recorded. Four randomly selected corpora lutea were marked on each ovary with a loop of black silk suture for later identification. Reexamination of the state of the ovarian structures was performed at the end of the experiment through midventral laparotomy.

**Experimental groups.** On day 98, two days before the start of blood collection, gilts were anesthetized and catheterization (i.d., 1.27 mm; o.d., 2.29 mm; Tygon® microbore tubing, Fisher Scientific, Pittsburgh, PA) of the cranial vena cava was performed to allow for repeat blood sampling. The catheter was tunneled under the skin and exteriorized at the junction of the scapulae. Blood samples were collected via the cranial vena cava beginning on day 100 to 124. Sequential samples were collected (0800 and 2000 hours) from day 100 to 124. Blood samples were immediately centrifuged (2,000 X g, 4°C, 10 minutes). Plasma was stored at –20°C for radioimmunoassay of RLX and progesterone. Dexamethasone (total of 30 mg day<sup>-1</sup>) was injected intramuscularly at 0800 and 1600 hours from days 110–118 (n=5); control animals received vehicle injection twice daily during this period (n=6). The RU 486 (4 mg kg<sup>-1</sup> body weight) was administered orally once a day from days 111–115 (n=7). The RU 486 was mixed with a small amount of feed and given to the pigs at 0800 hours. All feed mixed with RU 486 was consumed by the pigs within 20 minutes; placebo-treated controls were given the same amount of feed without the drug at this time (n=6).

**Radioimmunoassays (RIA).** The RLX concentrations were quantified in duplicate in aliquots of 100 and 200 µl of plasma with a double antibody RIA by using [<sup>125</sup>I] monotyrosylated porcine RLX and R6 antibody from B. G. Steinetz by procedures we described previously. The assay sensitivity was 40-pg tube<sup>-1</sup>. The inter- and intra-assay coefficient of variance (mean ±SE) were 12.4% (n=3 assays sample<sup>-1</sup>) and 6.2% (n=5 samples), respectively; nonspecific binding was 2.8%.

Plasma progesterone was extracted with a benzene-hexane mixture (1:2 vol/vol). Plasma samples (100 µl) were extracted in duplicate. Plasma extracts were assayed for progesterone by using the fully characterized antibody (GDN antiprogestosterone-11-BSA 337). Recovery after extraction with benzene-hexane was 89%, and the sensitivity of the progesterone RIA was 50 pg tube<sup>-1</sup>. The inter- and intra-assay coefficient of variance was 13.3% (n=5 assays sample<sup>-1</sup>) and 9.8% (n=6 samples), respectively; nonspecific binding was 3.2%.

**Statistical analyses.** Experimental units were the individual pigs, which were assigned to treatments at random. Data

were analyzed by split-plot analysis of variance. A one-way analysis of variance and a Student's *t*-test for continuous variables was used for comparisons between treatment groups.

### Results and Discussion

**Effects of dexamethasone and RU 486 on progesterone concentrations in peripheral plasma.** Progesterone plasma concentrations in the placebo-treated gilts decreased abruptly at days 111–113 but were maintained at 18–20 ng ml<sup>-1</sup> to day 124 (Figure 1A). Progesterone plasma concentrations (14±2.5 ng ml<sup>-1</sup>) were unaffected by twice daily injection of dexamethasone for 8 days compared with the vehicle-treated controls (15±2.0 ng ml<sup>-1</sup>; P>.82). In contrast, progesterone concentrations in RU 486-treated gilts were markedly increased (P<.01) during 5 days of treatment compared with the decreasing hormone concentrations in the hysterectomized controls (Figure 1B). Following RU 486 treatment, progesterone concentrations decreased but remained consistently greater than seen in hysterectomized controls from days 116–124.

**Effects of dexamethasone and RU 486 on relaxin concentrations in peripheral plasma.** In the placebo-treated hysterectomized control groups, RLX plasma concentrations increased steadily (5–8 ng ml<sup>-1</sup>) from days 100–110 (Figure 2A,B). A peak release of RLX (17±2.8 and 18±2.5 ng ml<sup>-1</sup>) occurred at day 113 in the hysterectomized controls. Thereafter, RLX remained significantly elevated (5–9 ng ml<sup>-1</sup>) to day 124 in both control groups. In marked contrast, dexamethasone treatment for 8 days consistently suppressed (P<.01) RLX concentration (3±.9 ng ml<sup>-1</sup>) compared with peak RLX release in hysterectomized controls (Figure 2A). Upon the withdrawal of dexamethasone at the end of the treatment on day 118, RLX plasma concentrations began to increase and peaked at 14±2.1 ng ml<sup>-1</sup> (P<.01) on day 120.

During 5 days of antiglucocorticosteroid treatment from days 111–115 in the hysterectomized gilts, RLX peak release was inhibited (P<.01) compared with the controls (Figure 2B). Upon withdrawal of RU 486 treatment a delayed RLX peak release (16±1.9 ng ml<sup>-1</sup>) occurred on day 117; however, the amplitude of this delayed RLX peak did not differ from that in the controls (18±2.5 ng ml<sup>-1</sup>; P>.20). Following the relaxin peak, the hormone concentration remained elevated (6–7 ng ml<sup>-1</sup>) to day 124 in both groups.

The main finding was that glucocorticoid (dexamethasone) administration for several days in hysterectomized gilts with aging corpora lutea markedly suppressed peak relaxin release without affecting progesterone secretion compared with vehicle treated controls. It is well known that an inherent peak release of relaxin occurs in unmated hysterectomized gilts at day 113

that coincides with the prepartum peak relaxin release seen at normal parturition (1). The programmed relaxin release 113 days after estrus does not depend upon hormonal signals from the uterus or conceptuses in this species. Although RLX secretion was suppressed during dexamethasone treatment, it peaked soon after hormone withdrawal. Furthermore, glucocorticoid treatment in these hysterectomized gilts did not induce luteolysis as indicated by sustained RLX and progesterone secretion to day 124. Results from this experiment reveal that dexamethasone at the given dose suppresses RLX secretion without causing luteolysis and such suppression is reversible. In contrast, in late-pregnant pigs and cattle, dexamethasone treatment induces luteolysis and premature parturition.

The RU 486 acts with high affinity as an antagonist for progesterone receptor and also has antiglucocorticoid activity. In the mammalian uterus, progesterone binds with its receptor to cause endometrial proliferation of epithelial, glandular, mesenchymal, and vascular cells, and such binding is required to maintain pregnancy to normal term. The abortifacient actions of RU 486 in late pregnant pigs may result from an interplay of prostaglandins, glucocorticoids, and oxytocin on the maternal uterus and cervix for expulsion of the conceptuses. An acute decrease in circulating progesterone concentrations is a prerequisite for parturition in this species, and RU 486 given during late pregnancy abruptly decreases progesterone secretion and induces parturition within 34 hours (2).

In the absence of the nongravid uterus, porcine corpora lutea are maintained for prolonged periods by secreting greater amounts of progesterone and lower concentrations of estradiol-17 $\beta$ , estrone, and prolactin (PRL) compared with pregnant animals. In sharp contrast with pregnant animals, the progesterone/glucocorticoid antagonist RU 486 promotes both PRL and progesterone secretion and delays peak RLX release in hysterectomized pigs. It is well known that daily injection of purified pPRL in hypophysectomized-hysterectomized pigs maintains progesterone secretion throughout a 10-day period (3). Thus, the divergent effects of the progesterone/glucocorticoid antagonist on progesterone and RLX secretion by aging corpora lutea in pregnant pigs may involve receptors in the uterus that induce parturition with premature RLX release and luteal demise, whereas in nongravid hysterectomized animals receptors in hypothalamus-pituitary-ovarian (corpus luteum) axis that delay RLX release and promote progesterone secretion (2). The RU 486 binds to glucocorticoid receptors and appears to at least partly stabilize the untransformed 8-9S glucocorticoid receptor in several systems.

Activators of protein kinase A unmask latent transcription activating potential in the RU 486-glucocorticoid receptor complex. In contrast, RU 486 promotes dissociation of the progesterone receptors from heat shock proteins, receptor dimerization, and receptor-DNA binding as effectively as the progestin agonist, R5020. Thus, type I steroid antagonists are defined as ligands that

bind receptor but do not promote conversion of receptor to a form capable of high affinity binding to DNA. Type II steroid antagonists are defined as ligands that recapitulate most of the early steps of agonist action, including stimulation of DNA-binding activity of receptor. A significant fraction of glucocorticoid receptor appears activated and binds to DNA. Thus, RU 486 is classified as a type II glucocorticoid antagonist.

In summary, these experiments provide evidence that relaxin and progesterone secretion from aging corpora lutea of pigs are regulated through separate mechanisms, and adrenal glucocorticoids may be involved in this regulation process not only during pregnancy but also in unmated hysterectomized pigs.

### References

1. 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.
2. Li, Y., C. Huang, J. Klindt and L. L. Anderson. 1991. Divergent effects of antiprogesterone, RU 486, on progesterone, relaxin, and prolactin secretion in pregnant and hysterectomized pigs with aging corpora lutea. *Endocrinology* 129:2907-2914.
3. Li, Y., J. R. Molina, J. Klindt, D. J. Bolt and L. L. Anderson. 1989. Prolactin maintains relaxin and progesterone secretion by aging corpora lutea after hypophysial stalk transection or hypophysectomy in the pig. *Endocrinology* 124:1294-1304.

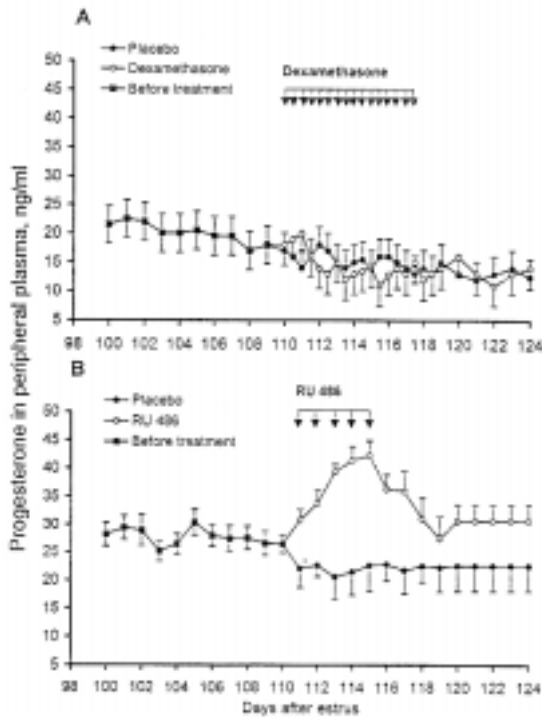


Figure 1. Progesterone concentration in peripheral plasma ( $\text{ng ml}^{-1}$ ) in unmated, hysterectomized gilts (A) given i.m. injection of dexamethasone (total of  $30 \text{ mg day}^{-1}$ ) at 0800 and 2000 hours ( $n=5$ ) or vehicle injection ( $n=6$ ) from days 110–118 and (B) oral administration of RU 486 ( $4 \text{ mg kg}^{-1}$  body weight) at 0800 hours ( $n=7$ ) or placebo-treated controls given the same amount of feed at this time ( $n=6$ ) from days 111–115. Values are mean  $\pm$  SE.

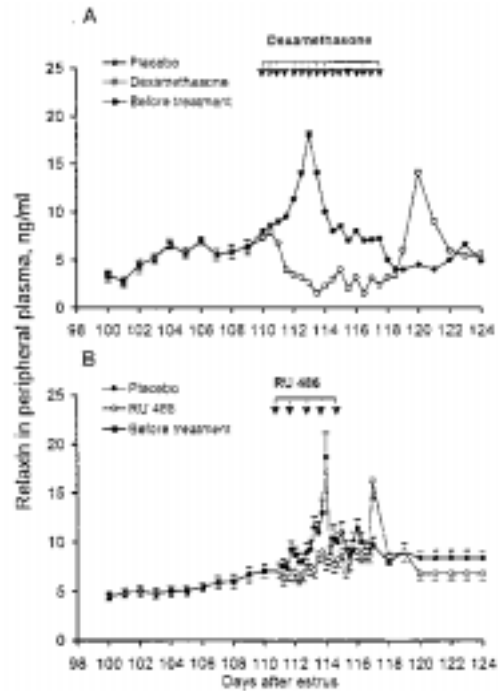


Figure 2. Immunoreactive relaxin concentration in peripheral plasma ( $\text{ng ml}^{-1}$ ) in unmated, hysterectomized gilts (A) given i.m. injection of dexamethasone (total of  $30 \text{ mg day}^{-1}$ ) at 0800 and 2000 hours ( $n=5$ ) or vehicle-injection ( $n=6$ ) from days 110–118; and (B) oral administration of RU 486 ( $4 \text{ mg kg}^{-1}$  body weight) at 0800 hours ( $n=7$ ) or placebo-treated controls given the same amount of feed at this time ( $n=6$ ) from days 111–115. Values are mean  $\pm$  SE.