Control of Luteal Relaxin Release by Prostaglandin $F_{2\alpha}$: Differences in the Sow Cycle and Pregnancy (43067)

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Abstract. **The effect of an *in vivo* prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) challenge in pregnant and cyclic sows was compared to determine whether $PGF_{2\alpha}$-induced release of relaxin (RLX) from the corpus luteum (CL) in late pregnancy is also effective during the cycle. Ovarian venous RLX and progesterone were monitored by radioimmunoassay and RLX localized in the CL by immunohistochemistry. In Day 108 pregnant sows, infusion of $PGF_{2\alpha}$ (100 μg) into the ovarian artery resulted in an immediate and sustained rise in ovarian venous RLX with an initial decline in progesterone levels by 30 min which then returned to pretreatment levels. In Day 13 or 15 cyclic sows with functional corpora lutea (i.e., elevated progesterone), RLX was undetectable in ovarian venous blood after 100 μg of $PGF_{2\alpha}$. Administration of $PGF_{2\alpha}$ via either the jugular vein or intramuscular route was also ineffective in releasing RLX from the CL of the cycle. The intensity of RLX immunostaining of the CL was similar in saline and $PGF_{2\alpha}$-treated sows. These studies indicate that the control of RLX release from the sow CL differs in the estrous cycle and pregnancy.** [P.S.E.B.M. 1990, Vol 194]

Relaxin (RLX) is known as a hormone of pregnancy with high levels produced by the corpus luteum (CL) of the sow. However, RLX also has been localized in luteal tissue of the pseudo-pregnant pig (1) and in the nonpregnant pig throughout the cycle (2). Luteal RLX immunoactivity was highest from Days 7 to 15 of the cycle with a decline by Day 18. This was coincident in time with luteolysis, defined functionally as a decline in serum progesterone and occurring between Days 14 and 18 of the sow estrous cycle (3). Whether prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), postulated as the major signal for luteolysis in swine (4), is also responsible for RLX release from the CL of the cycle is not known.

Administration of $PGF_{2\alpha}$ (10 mg im) to sows during late pregnancy causes luteolysis, as demonstrated by a rapid decline in plasma progesterone, the release of RLX, and parturition (5). However, Nara *et al.* (6) showed that a nonluteolytic dose of $PGF_{2\alpha}$ (i.e., 50 μg iv with no change in plasma progesterone) was effective in releasing RLX in late pregnant pigs. This indicates that RLX release in the pregnant sow may not be linked to the drop in progesterone accompanying luteolysis.

The objective of these studies was to compare the effects of $PGF_{2\alpha}$ in the pregnant and cyclic sow and to determine whether this stimulus for RLX secretion in late pregnancy is also effective during the estrous cycle.

Although bioactive and immunoreactive RLX in ovarian extracts have been shown to be maximal during diestrus in the sow, tissue content is low (7). In the peripheral circulation, RLX immunoactivity was detectable in only 20% of plasma samples (50 pg/ml) obtained from four sows bled every 6 hr throughout the estrous cycle (8). Since tissue levels of RLX are low in the cyclic animal, if RLX was being released in small amounts and diluted in the general circulation, levels of the hormone in peripheral blood might be below the sensitivity of the assay. Therefore, in the present study we chose to sample from the ovarian vein rather than from the jugular in order to maximize our chances of detecting low level RLX release directly from the ovary. Both Day 13 and 15 cyclic sows were used in these

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studies, since it has been shown that luteal RLX immunoactivity is elevated at this time (2, 9), and exogenous $PGF_{2\alpha}$ will induce luteolysis with a fall in serum progesterone after Day 12 of the cycle (10).

Methods

Animals. Multiparous Duroc sows housed at the U.S. Department of Agriculture, Beltsville, MD, were used in these studies. Animals exhibiting at least two estrous cycles averaging 21 days were checked for estrus in the presence of a boar. The day of onset of estrus was designated as Day 0 of the cycle. Animals were used at Day 13 or 15 of the cycle with at least three to four animals per treatment. Gleeson *et al.* (11) monitored progesterone in the utero-ovarian vein of cyclic sows and reported values from 100 to 750 ng/ml in Day 8–13 animals. By Day 16 progesterone dropped to <50 ng/ml, indicating CL regression. To avoid the possibility that CL from the cyclic animals in this study may have already begun regressing, only animals with functional CL, as indicated by elevated ovarian venous progesterone (>50 ng/ml), were used. For the study of pregnant animals, six sows were mated on the first day of estrus (Day 0 of pregnancy) and maintained until Day 108 of pregnancy.

Cyclic or pregnant sows were anesthetized with a combination of ketamine hydrochloride (Ketaset) and acepromazine for surgery. Anesthesia was maintained for the duration of the experiment by infusing the anesthetics mentioned above in a jugular cannula that was implanted following the initial anesthesia. The right ovary was exteriorized by a midline laparotomy and a cannula (0.75 mm i.d., 2.25 mm o.d., 40 mm in length attached to a 19-gauge adaptor) was inserted into the right ovarian vein. Cannula patency was maintained with a sodium heparin/saline solution. For the infusion of solutions into the ovary, a 25-gauge, ¾-in butterfly needle was inserted into the right ovarian artery. This was immediately withdrawn following the infusion of substances.

Anesthetized animals were given a single bolus injection (1 ml) of $PGF_{2\alpha}$ (10 or 100 μg; Sigma Chemical Co.) or saline via the procedure described above. The dose of prostaglandin used in these studies was based on the observation by Nara *et al.* (6) that 50 μg of $PGF_{2\alpha}$ via the jugular vein stimulated the release of RLX without causing luteolysis in pregnant animals. Since we were using a more direct route via the ovarian artery and looking for effects in a cyclic animal, a lower dose of $PGF_{2\alpha}$ (10 μg) was initially used. When no effect of 10 μg of $PGF_{2\alpha}$ on RLX release was observed, a 10-fold higher concentration (100 μg) was tested in both pregnant and cyclic sows. Blood samples (3 ml) were taken over a 10-sec period by aspiration from the venous cannula at intervals before infusion (5 and 2 min) and at 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 75, and 90 min after infusion. Total blood volume obtained over the sampling period was approximately 51 ml. At the end of blood sampling, the right (treated) and left (untreated) ovaries were collected and processed for immunohistochemistry to localize RLX.

In addition, in preliminary experiments to determine whether the route of prostaglandin administration may have influenced RLX release from the ovarian vein, $PGF_{2\alpha}$ was also administered via the jugular vein (100 μg) or im (1 mg) to cyclic animals. Animals receiving $PGF_{2\alpha}$ via the jugular vein were sampled according to the same schedule as animals receiving $PGF_{2\alpha}$ via the ovarian artery as described earlier. To observe the effects of intramuscular administration of $PGF_{2\alpha}$, sows were laparotomized 12 hr after injection for blood sampling. Both treated ovaries from these animals were processed for immunohistochemistry. Progesterone and RLX were measured in blood samples by radioimmunoassay (RIA).

Immunohistochemistry. Bouin's fixed, paraffin-embedded tissues were immunostained for RLX antigenic sites using the avidin-biotin immunoperoxidase method (12). The antiserum was raised by Dr. Bryant-Greenwood against CM-a porcine RLX and has been characterized (13). The optimum dilution of this antiserum necessary to achieve adequate staining with minimal background has been shown to be 1:10,000 and 1:500 for corpora lutea from pregnant and Day 15 cyclic sows, respectively (2). This reflects the markedly different concentrations of RLX present in luteal tissue in these two reproductive states.

Radioimmunoassay. Relaxin levels were determined with a homologous porcine RLX RIA (14). Relaxin was prepared by Dr. Bryant-Greenwood using the method of Sherwood and O'Byrne (15) and monoacylated using high-pressure liquid chromatography. The monoacylated RLX was radioiodinated directly using chloramine-T (16) and separation of bound and free hormone was carried out by conventional double-antibody procedure. The between and within assay coefficients of variation were 13.1% and 9.2%, respectively. Assay sensitivity was 156 pg/ml and specificity of the assay has been described previously (13).

Concentrations of progesterone plasma samples were measured with the RIA described by Guthrie (17). The between and within assay coefficients of variation were 13.2% and 9.2%, respectively. Assay sensitivity was 100 pg/ml of plasma.

Data were analyzed by analysis of variance followed by Duncan's new multiple range test for comparisons between treatment groups (18).

Results

Day 108 Pregnant Sow. $PGF_{2\alpha}$ treatment induced an immediate release of RLX from the right ovary with levels remaining significantly elevated above that in

pretreatment samples for the duration of sampling (pre-$PGF_{2\alpha}$ = 25.6 ± 8.0 ng/ml, post-$PGF_{2\alpha}$ = 202.8 ± 47.21 ng/ml ($P < 0.05$); Fig. 1B). Relaxin may have been released from the contralateral ovary; however, its contribution to the total amount of RLX released following $PGF_{2\alpha}$ treatment would most likely have occurred in the later stages of sampling. Although RLX levels showed a small rise 5 min after saline treatment (from 40 to 70 ng/ml), this was not statistically significant (Fig. 1A). Thereafter, RLX concentrations continued unchanged in the saline-treated sows (Fig.1A). In spite of the elevated venous RLX levels in $PGF_{2\alpha}$-treated sows, immunocytochemistry indicated no decline in RLX immunostaining of CL. Staining intensity was similar in treated (right) and untreated (left) ovaries.

Pulsatile secretion of progesterone was observed in both saline- and prostaglandin-treated pregnant sows (Fig. 1, A and B). The initial decline in progesterone during the first 5 min after saline infusion was a part of this pattern with a return to preinfusion levels by 20 min (Fig. 1A). Ovarian venous progesterone levels declined linearly within 30 min of $PGF_{2\alpha}$ treatment and

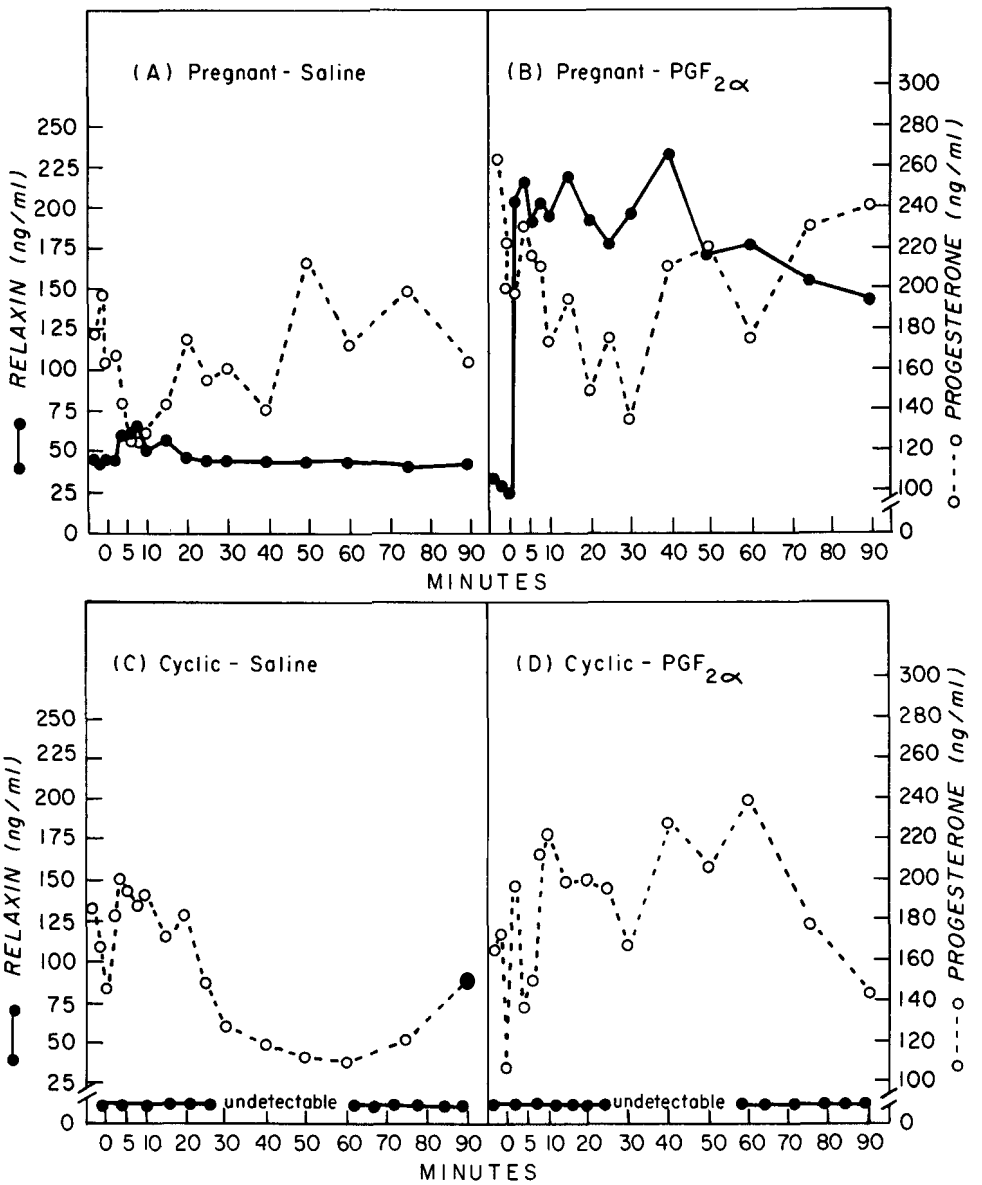

Figure 1. (A) Ovarian venous progesterone (O- - -O) and relaxin (●—●) levels after administration of saline via the ovarian artery at 108 days of pregnancy. Each point represents mean of three to four animals with a pooled SEM of 6.45 and 4.70, respectively. (B) Ovarian venous progesterone (O- - -O) and relaxin (●—●) levels after administration of $PGF_{2\alpha}$ (100 μg) via the ovarian artery at 108 days of pregnancy. Each point represents mean of three to four animals with a pooled SEM of 12.88 and 20.95, respectively. (C) Ovarian venous progesterone (O- - -O) levels after administration of saline via the ovarian artery on Day 13 or 15 of the estrous cycle. Each point represents mean of three to four animals with a pooled SEM of 9.26. Relaxin levels were undetectable (assay sensitivity 156 pg/ml). (D) Ovarian venous progesterone (O- - -O) levels after administration of $PGF_{2\alpha}$ (100 μg) via the ovarian artery on Day 13 or 15 of the estrous cycle. Each point represents the mean of three to four animals with a pooled SEM of 18.24. Relaxin levels were undetectable (assay sensitivity 156 pg/ml).

then returned to pre-$PGF_{2\alpha}$ levels (Fig. 1B) which were not significantly different when compared with saline-treated control sows (Fig.1A).

Day 13 and 15 Cyclic Sows. $PGF_{2\alpha}$ (100 μg) administered via the ovarian artery to Day 13 or 15 cyclic sows failed to release RLX over a 90-min period. Relaxin was undetectable in ovarian venous blood from both saline- and $PGF_{2\alpha}$-treated animals (Fig.1, C and D).

Ovarian venous progesterone was monitored to ascertain whether our inability to detect a change in RLX secretion in the Day 13 and 15 cyclic sows was due to the CL already having undergone regression. Three of the Day 15 cyclic sows had ovarian venous progesterone levels from 10 to 20 ng/ml, indicating that luteolysis was underway, and these animals were excluded from the study. Thirteen midcyclic sows (seven animals at Day 13 and six animals at Day 15) had pretreatment ovarian venous progesterone values from 103 to 398 ng/ml and were considered to be in the range previously reported for animals prior to luteolysis (11). Progesterone concentrations in both the saline- and $PGF_{2\alpha}$-treated animals were variable and fluctuated over the 90-min sampling period. However, there was no evidence for a significant change in progesterone levels after treatment (Fig.1, C and D).

In some cyclic animals, $PGF_{2\alpha}$ was administered via the jugular vein to more closely compare results with the original route of administration described by Nara *et al.* (6) in pregnant animals. Even with 100 μg of $PGF_{2\alpha}$ infused via the jugular vein, there was no evidence of RLX release into the ovarian vein or change in RLX immunostaining. Ovarian venous progesterone also remained unchanged after $PGF_{2\alpha}$ challenge into the jugular vein (pre-$PGF_{2\alpha}$ = 188.4 ± 3.71, post-$PGF_{2\alpha}$ = 191.8 ± 7.5).

In preliminary work in the cyclic sow, the effect of an im injection of $PGF_{2\alpha}$ was investigated. Twelve hours after the im injection (1 mg) of $PGF_{2\alpha}$, RLX could not be detected in the ovarian venous blood and no change in immunostaining intensity was noted (data not shown). Progesterone levels in the ovarian vein after intramuscular administration of $PGF_{2\alpha}$ were 195.3 ± 9.2 ng/ml, which were similar to progesterone levels observed after either ovarian artery (Fig.1, C and D) or jugular vein administration and not significantly different from saline controls ($P < 0.05$).

Discussion

Nara *et al.* (6) reported that jugular vein infusion of $PGF_{2\alpha}$ in the Day 108 pregnant sow caused a rise in peripheral RLX, which peaked at 90 ng/ml within 10 min after infusion. The present work correlates well with those studies by demonstrating that direct injection of $PGF_{2\alpha}$ into the ovarian artery of Day 108 pregnant sows elicited an abrupt rise in ovarian venous RLX that was 2-fold higher than previously described in peripheral plasma (6). These findings also support the study by Stone *et al.* (19) who monitored relaxin in utero-ovarian venous blood of sows prior to parturition. They reported peak RLX levels ranging from 140 to 280 ng/ml which correlates well with the $PGF_{2\alpha}$-induced RLX surge of 200 to 230 ng/ml observed in this study. Higher ovarian venous RLX levels might be expected based on the dilution effect inherent in peripheral blood sampling in the sow. The explanation for lower than expected RLX levels in ovarian venous blood is not clear, but may be due in part to the blood sampling methods used. In the present study and that of Stone *et al.* (19), RLX was monitored in the venous effluent from a single ovary and thus represents only half of the RLX detected in the periphery.

The present data indicate that fundamental differences exist in the control of RLX release from the ovary of the pregnant versus cyclic sow. In the midcyclic animal, exogenous $PGF_{2\alpha}$ treatment failed to generate a rise in ovarian venous RLX when administered via the ovarian artery and on a time scale similar to that used to demonstrate an immediate response in the pregnant sow. Since the route of $PGF_{2\alpha}$ administration may have affected the RLX response, $PGF_{2\alpha}$ was administered to cyclic animals either intramuscularly or via the jugular vein as described previously in pregnant sows (5, 6). Neither route of administration was effective in releasing RLX from the CL of the cycle. The possibility exists that the 1 mg im dose of $PGF_{2\alpha}$ used in cyclic animals was too low to be effective, since 12 hr after treatment there was still no decline in serum progesterone and relaxin immunostaining was unchanged. Sherwood *et al.* (5) used a 10-fold higher dose to induce luteolysis in pregnant animals. However, even when 100 μg of $PGF_{2\alpha}$ was administered to cyclic sows via the jugular vein there was no effect on luteal RLX.

Luteal regression occurs in the cyclic sow between Days 13 and 18 with ovarian venous progesterone declining to basal levels by Day 17 (20). Peripheral progesterone levels in cyclic sows are highest before luteolysis, averaging 27 ng/ml and decline after Day 15 to 0.5–2 ng/ml (10, 21). As expected, in blood draining the ovary, progesterone levels are considerably higher than levels measured in the peripheral circulation. Ovarian venous progesterone in cyclic animals before and after $PGF_{2\alpha}$/saline treatment ranged from 101 to 572 ng/ml. This correlates well with a report by Gleeson *et al.* (11) who monitored progesterone in the utero-ovarian vein of cyclic animals throughout the cycle and found levels ranging from 100 to 750 ng/ml before luteolysis. Gomes *et al.* (20) reported peak ovarian venous progesterone levels of 1 μg/ml whereas Masuda *et al.* (22) found 3-fold higher levels at Day 8 of the sow cycle. The explanation for these differences in ovarian venous progesterone levels reported in the lit-

erature is not clear; however, it may be related to differences in blood collection and sampling techniques. In the report by Masuda *et al.* (22), blood was collected by free flow from both ovaries, whereas in the present study and in the others cited (11, 20) blood was collected by aspiration from a single ovary. Thus, our inability to detect RLX in blood draining the ovary was not likely due to a blood flow or sampling problem since progesterone was elevated as expected in the same samples.

Ovarian venous progesterone was also used to assess the functional state of the CL since after luteolysis progesterone levels decline to 50 ng/ml (11). In the cyclic sows with elevated progesterone before $PGF_{2\alpha}$ treatment, no decline in ovarian venous progesterone was observed within 90 min after administration of $PGF_{2\alpha}$. This is in agreement with other studies in the cyclic pig that indicate that it takes at least 4–6 hr before a decline in progesterone occurs in response to a $PGF_{2\alpha}$ challenge via the utero-ovarian vein (10, 22). Thus, although progesterone levels could not be used as an indicator of the acute effects of a bolus dose of $PGF_{2\alpha}$, they were useful as an indicator of the functional status of the corpus luteum before prostaglandin treatment.

In vitro studies also support the *in vivo* studies by Nara *et al.* (6) by showing that $PGF_{2\alpha}$ directly stimulates RLX secretion from dispersed large luteal cells derived from pregnant pigs (24). Similar studies using cells from the CL of the cycle have not been reported. However, even in the pregnant pig there is a subpopulation of large luteal cells that do not release RLX despite prolonged stimulation by $PGF_{2\alpha}$ (25). The results here suggest that luteal cells of cyclic animals are similar to the non-RLX-releasing subpopulation of luteal cells of pregnancy. Although it is considered that $PGF_{2\alpha}$ is the natural luteolysin in swine (4), the $PGF_{2\alpha}$ receptor has not been characterized in luteal tissue from cyclic or pregnant pigs. Further work is needed to characterize these luteal cell subpopulations and determine how this insensitivity to a $PGF_{2\alpha}$ challenge is mediated.

The difference in tissue content of RLX in the CL of pregnancy and the cycle is considerable. Anderson *et al.* (9) reported that levels of RLX bioactivity in extracts of ovaries from cyclic sows were more than 100-fold lower than in late pregnancy. Although immunoactive RLX is detectable in the CL between Days 11 and 15 of the sow cycle, a 20-fold greater concentration of antibody was needed to localize RLX at this time when compared with the pregnant animal (2). Based on these observations, if endogenous $PGF_{2\alpha}$ was indeed involved in the release of RLX from the CL of the cycle, one would expect serum RLX levels to be up to 100-fold lower after an exogenous $PGF_{2\alpha}$ challenge when compared with similar treatment in the pregnant animal. Using the peripheral RLX levels reported by Nara *et al.* (6) within 90 min after $PGF_{2\alpha}$ treatment (30–90 ng RLX/ml), one would expect picogram concentrations of RLX/ml to be released into the systemic circulation from the CL of the cycle. However, RLX was not detected in blood draining the ovary of cyclic animals using an RIA with a sensitivity of 156 pg/ml. Therefore, the lower tissue content of RLX in the cycle when compared with pregnancy does not fully explain our inability to demonstrate a release of RLX into circulation after a $PGF_{2\alpha}$ challenge.

Attempts to show depletion of immunoreactive RLX by immunohistochemistry in the pregnant or cyclic sow CL after prostaglandin administration were not successful irrespective of whether RLX was detected by RIA in the ovarian vein. Our inability to show a decline in RLX immunostaining during pregnancy is likely due to a combination of short sampling time used in this study (90 min) and high tissue content of RLX in the CL of pregnancy. Depletion of electron-dense granules in luteal cells has been observed a few hours before parturition and coincides with a final surge of RLX into blood (26, 27). However, serum RLX is actually elevated for a 2- to 3-day period preceding the prepartum peak (28, 29). In the present study, ovaries were collected for immunostaining after only 90 min of RLX release following the $PGF_{2\alpha}$ challenge. Since RLX is elevated for several days before parturition, it is unlikely that this short sampling time would be sufficient to deplete the pregnancy CL of RLX immunostaining.

During the cycle, bioactive and immunoactive RLX in porcine ovarian extracts decline after Day 18 (7) and correlate well with a decrease in RLX immunostaining of the CL after Day 18 (2). Thus, there is a decline in immunoreactive RLX associated with regression of the CL accompanying luteolysis. The sequence of events that result in this depletion of RLX from the CL of the cycle are unknown. The present studies indicate that a $PGF_{2\alpha}$ challenge to induce luteolysis at midcycle does not result in a detectable release of RLX into blood or induce the clearance of immunoreactive RLX from cells of the CL. These results in the cyclic sow are in contrast to the rise in plasma RLX associated with luteolysis occurring naturally or induced with exogenous $PGF_{2\alpha}$ in the late pregnant sow (5, 30). Thus, there appears to be a different control of RLX secretion from the CL of the cycle and the CL of pregnancy.

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