Prostaglandin $F_2\alpha$ Induced Luteolysis of Aging Corpora Lutea in Pigs

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Summary and Implications

Prostaglandins primarily of uterine origin play an important role in parturition. Hysterectomy of nongravid pigs early in the luteal phase maintains luteal function until about day 150, whereas the duration of normal pregnancy is about 114 days. Precisely timed peak release of relaxin and coincident decrease in progesterone secretion occur in unmated hysterectomized gilts similar to that found a few hours preceding parturition. It is hypothesized that prostaglandin $F_2\alpha$ ($PGF_2\alpha$) in hysterectomized pigs mimics abrupt changes in ovarian and pituitary hormone secretion seen preceding normal parturition and in early lactation. Unmated Yorkshire gilts were hysterectomized on days 6–8 of a normal estrous cycle, and at 1200 hours on day 113, they were given an i.m. injection of 30 mg $PGF_2\alpha$ -THAM salt or phosphate buffer saline (PBS). None of these gilts expressed behavioral estrus immediately after $PGF_2\alpha$ or vehicle treatment. On day 113, $PGF_2\alpha$ increased peak relaxin (60 ng/ml) compared with controls (34 ng/ml; $P<.01$), whereas progesterone decreased abruptly (4 vs. 16 ng/ml in $PGF_2\alpha$ and PBS; $P<.01$). The prolactin (PRL) remained at <5 ng/ml from day 98 to 120 in controls but peaked at 33 ng/ml immediately after $PGF_2\alpha$ treatment on day 113, and then decreased to levels similar to controls on day 120. Sequential bleeding revealed an acute growth hormone (GH) release (4.5 ng/ml) immediately after $PGF_2\alpha$ injection and return to basal levels (<.6 ng/ml) on days 114 to 120. The $PGF_2\alpha$ induced abrupt shifts in progesterone, relaxin, PRL, and GH secretion in hysterectomized gilts that mimic hormone changes seen in late pregnancy, parturition, and early lactation. These findings provide new insight into the role of $PGF_2\alpha$ in abruptly altering hormone secretion by aging corpora lutea and pituitary gland even in the absence of the conceptuses or uterus in the pig.

Introduction

Conception or complete removal of the nongravid uterus interrupts the luteolytic action that defines estrous cycle intervals averaging 21 days in the pig. As pregnancy advances, porcine corpora lutea (CL) become dependent on prolactin to maintain progesterone and relaxin secretion until normal parturition about day 114 (1,2). Corpora lutea are maintained to day 150 in unmated hysterectomized gilts and a programmed peak relaxin release occurs at day 113 and progesterone secretion decreases by half in unmated hysterectomized gilts that mimics hormone changes seen in late pregnancy and parturition (3). The dependence of these aging CL on prolactin was defined by luteal maintenance (progesterone and relaxin secretion) throughout a 10-day period (days 110–120) in hypophysectomized-hysterectomized gilts (1). The role of GH in late pregnancy and early lactation in several species has been identified as supportive of processes that facilitate milk synthesis by the mammary gland. The GH plasma concentrations were similar in hysterectomized and pregnant pigs from day 99 until parturition, but thereafter were consistently greater in lactating than in hysterectomized pigs (4).

The uterus is a major site of prostaglandin production and luteolytic action. Prostaglandins are known to play an important role in parturition in several mammalian species. In cattle and pigs intramuscular injection of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) or potent analogs during late pregnancy induces premature luteolysis and parturition about 30 hours later.

It is well known that a precise prepartum peak release of relaxin occurs about 16 hours before parturition and coincides with the steady prepartum decrease in progesterone blood concentration to basal level at parturition in the pig. Although CL in hysterectomized gilts are capable of remaining large (>450 mg) at least 35 days beyond the time of normal regression, abrupt shifts occur in relaxin and progesterone secretion in complete absence of structural luteal regression but with an obvious abrupt change in functional hormone secretion. It is thought that prostaglandins primarily of uterine origin play an important role in events leading to luteolysis for repetitive 21-day estrous cycle intervals in the pig. Because prostaglandins play an important role in luteolysis (progesterone decrease and relaxin release) and parturition in the pig, the hypothesis to be tested is that, in the absence of the uterus, $PGF_2\alpha$ in gilts with aging corpora lutea induces abrupt shifts in ovarian and pituitary hormone secretion that mimic changes seen at normal parturition. Thus, the objective of this experiment was to determine whether $PGF_2\alpha$ treatment affects ovarian and adenohypophysial function in hysterectomized gilts with aging CL, and whether they mimic hormonal changes seen during late pregnancy and parturition. We determined whether $PGF_2\alpha$ given to hysterectomized gilts at a time equivalent to parturition causes a luteolytic action on the persisting CL by abrupt shifts in progesterone and relaxin secretion and changes in PRL and GH secretion (6).

Materials and Methods

Animals. Sixteen purebred Yorkshire gilts, averaging 130±10 kg BW (±SE), that had exhibited at least one normal estrous cycle averaging 21±1 days were used in this experiment; the first day of estrus was designated day 0. Unmated gilts were hysterectomized between days 6–8 after estrus. Four randomly selected CL were marked on each ovary with a loop of black silk suture for later identification. The state of the ovarian structures was reexamined through midventral laparotomy at the end of the experiment.

Experimental protocol. Hysterectomized gilts were randomly assigned to either the $PGF_2\alpha$ treated group (n=5) or hysterectomized control group with PBS treatment (n=11). Blood samples were collected daily via an indwelling catheter from the anterior vena cava from days 98–107, and twice daily (0800 and 2000 hours) blood was collected from days 108–120. Additionally on days 112, 113, 114, and 115 the gilts were bled sequentially every 20 minutes for 180 minutes beginning at 1200 hours to closely monitor the hormone change upon $PGF_2\alpha$ injection. At 1200 hours on day 113, gilts were given an intramuscular injection of 30 mg $PGF_2\alpha$ THAM salt in PBS (6 ml) or equal volume of PBS. Plasma was decanted and stored at –20°C until required for hormone assay.

Radioimmunoassay (RIA) of relaxin, progesterone, PRL, and growth hormone in peripheral plasma. Relaxin concentration was quantified in duplicate 200-l aliquots of plasma with a homologous double antibody RIA using [^{125}I] monotyrosylated porcine relaxin from C. Schwabe and R6 antibody from B. G. Steinetz. The assay sensitivity was 40 pg/tube. The inter-assay and intra-assay CV were 12.4 (n=3 assays/sample) and 6.3% (n=5 samples) respectively; nonspecific binding was 2.9%.

Plasma progesterone was extracted from 100 µl of plasma in duplicate with a benzene–hexane mixture. A third replicate (100 µl) served as a recovery for determining procedural losses by the addition of 5,000 cpm [2,3,6,7-N-^{3}H] progesterone (97.0 Ci/mmol; NEN Research Products, Boston, MA). The sensitivity of the progesterone RIA was 50 pg/tube. Intra-assay CV was 7.4% (n=6 samples), and the inter-assay CV was 14.8% (n=5 assays/sample). Mean recovery of progesterone after benzene-hexane extraction was 90%.

Prolactin concentration was quantified in duplicate 200-_l aliquots of plasma with a double-antibody homologous RIA. Sensitivity of the assay was .34±.15 ng/ml. Intra-assay CV was 8.9% (two samples per assay; five to eight determinations), and inter-assay CV was 5.3% (two samples, two assays).

Growth hormone concentration was determined in duplicate 200-µl aliquots of plasma with a double antibody homologous RIA. Sensitivity of the assay was 230±32 pg/ml (mean±SE; n=4 assays). Inter-assay and intra-assay CV were 24.4 and 9.5% at a mean concentration of 746 pg/ml (n=4 assays), and 12.2 and 8.3% at a mean concentration of 34 ng/ml (n=4 assays), respectively.

Statistical analyses. Experimental units in this study were the individual gilts, which were assigned to treatments at random. Data were analyzed by split-plot analysis of variance. The main plot effect was treatment tested against pig within treatment as the whole plot error term. A one-way analysis of variance and a Student's *t*-test for continuous variables was used for comparisons between treatment groups. Data are presented as geometric mean ± standard error, and statistical significance was concluded when $P<.05$.

Results and Discussion

Progesterone concentrations in peripheral plasma. In hysterectomized gilts given PBS, progesterone blood level gradually decreased from day 108 (31 ng/ml) to day 114 (17 ng/ml) (Figure 1a). This programmed decrease by half in progesterone blood level occurs in hysterectomized gilts at the expected time of normal parturition (day 114) in pregnant animals. The CL in hysterectomized gilts can be maintained to day 150. None of the hysterectomized gilts given $PGF_2\alpha$ or PBS expressed behavioral estrus by day 125. The CL in both groups were maintained, abut they appeared less vascular in animals given $PGF_2\alpha$ treatment than those in the PBS injected controls. Aging corpora lutea in hysterectomized gilts are acutely responsive to the luteolytic action of $PGF_2\alpha$ (Figure 1a). The $PGF_2\alpha$ treatment on day 113 caused an abrupt decrease ($P<.01$) in plasma progesterone (4.8 ng/ml) on day 113, and a further decrease to basal level (.7 ng/ml) by day 116 in these hysterectomized animals that similar to hormone levels seen in the early postpartum period.

Relaxin concentrations in peripheral plasma. From days 100–108, relaxin plasma concentration in hysterectomized gilts in this experiment, as well as our earlier study, is consistently greater than that seen in pregnant animals from the previous study (>4 ng/ml). At day 113, a programmed peak release of relaxin (range 26–36 ng/ml) occurs in the unmated hysterectomized gilts (Figure 1b) that coincides with the prepartum relaxin release seen in normal pregnancy (3). From days 114–120, relaxin remains elevated (10–12 ng/ml) in hysterectomized gilts given PBS, whereas it is at basal levels in $PGF_2\alpha$ injected animals (Figure 1b). The $PGF_2\alpha$ treatment at day 113 caused peak relaxin release (range 38–142 ng/ml) similar to that seen in pregnant gilts (3), followed by an abrupt decrease ($P<.01$) to basal levels from days 114–120 (Figure 1b).

Prolactin concentrations in peripheral plasma. Prolactin plasma concentration remains consistently low (i.e., 5–8 ng/ml) from days 100–120 in unmated hysterectomized gilts

given PBS (Figure 1c), whereas it increases markedly (i.e., 30–60 ng/ml) during late pregnancy and early lactation (4). The $PGF_2\alpha$ treatment on day 113 in hysterectomized gilts causes prolactin increase (peak 32 ng/ml) similar to that seen in late pregnancy (Figure 1c) ($P<.01$).

Growth hormone concentrations in peripheral plasma. Growth hormone plasma concentration remains consistently low (1–2 ng/ml) from days 100–120 in unmated hysterectomized gilts given PBS (Figure 1d). There was an abrupt increase in GH (>5 ng/ml) ($P<.05$) in $PGF_2\alpha$ treated group, but return to basal levels from day 115–120.

We have demonstrated the same pattern of hormonal change in hysterectomized gilts given $PGF_2\alpha$ as seen during normal late pregnancy and parturition. Increasing production and secretion of $PGF_2\alpha$ could signal decreasing production and secretion of progesterone and the release of relaxin. Whether the progesterone decrease signals relaxin release is unresolved. Whether ovarian progesterone secretion signals the timing of relaxin release in hysterectomized gilts is unknown. When RU 486, a progesterone antagonist, was orally administered to hysterectomized gilts from days 111 to 115, however, circulating blood concentrations of progesterone were significantly increased in a dose-dependent manner and relaxin release was significantly delayed (5). The results from the current study reinforce the tightly regulated patterns of ovarian relaxin and progesterone secretion induced by $PGF_2\alpha$ treatment in hysterectomized pigs. Thus, an acute decrease in circulating progesterone concentration is a prerequisite for parturition in the pig, and this can be mimicked by $PGF_2\alpha$ treatment in unmated hysterectomized animals (Figure 1a). The $PGF_2\alpha$ -induced premature luteolysis is not mediated by an increase in luteal $PGF_2\alpha$ receptor concentration but a decrease in luteal progesterone concentration. There are important differences in the inherent abilities of aging CL to secrete relaxin and progesterone from days 100–120 in pregnant and hysterectomized pigs. The current study indicates not only higher plasma levels of progesterone after hysterectomy but also an abrupt decrease by half (≈16 ng/ml) that coincides with the decrease seen in pregnant animals from days 110–114, which remains consistently greater than seen in normal pregnant gilts or in hysterectomized gilts given $PGF_2\alpha$. Furthermore, in the current study and a previous one (3), progesterone concentrations decrease in hysterectomized gilts from as early as day 109 onward.

The results of this study indicate that the CL in hysterectomized gilts secrete peak quantities of relaxin on about day 113 (Figure 1b), the same time as do pregnant gilts, even though these corpora lutea can persist to day 150. The aging porcine CL may be genetically programmed to release relaxin as a result of an inherent lifespan, defined through evolutionary development of the reproductive cycle as the duration of gestation of about 114 days. How the aging porcine luteal cell autonomously signals itself for the timed release of relaxin and coincident decrease in progesterone, however, remains to be further investigated. The $PGF_2\alpha$ treatment at day 113 in these hysterectomized gilts causes a relaxin release at a peak (range 38–142 ng/ml) similar to that seen in pregnant sows, and followed by an abrupt decrease to basal levels from days 114–120 (Figure 1b). The results clearly indicated a trigger mechanism of $PGF_2\alpha$ on parturition because one injection of $PGF_2\alpha$ on day 113 in hysterectomized animals simulated the pattern of progesterone and relaxin secretory change as seen at normal parturition.

The $PGF_2\alpha$ treatment on day 113 causes an abrupt increase in prolactin secretion that is sustained throughout 180 minutes in hysterectomized animals, which explains the peak of prolactin on day 113. There is a prepartum rise in PRL blood levels, which is temporally associated with the prepartum peak relaxin levels (Figure 1b,c); however, PRL does not seem to be required for the release of relaxin because the administration of bromocriptine prevented the PRL rise, but not the relaxin peak before farrowing.

Pituitary GH concentrations remain similar in pregnant and hysterectomized gilts until day 80, but decrease by day 110 in pregnant, but not hysterectomized, animals indicating hormones release into peripheral blood. The GH concentrations, however, were consistently greater in lactating than in hysterectomized pigs indicating GH may be involved in lactation (4). The $PGF_2\alpha$ causes a transient increase in GH secretion that was not sustained throughout the 180-minute sampling period.

This experiment shows that ovarian and adenohypophysial function are acutely responsive to exogenous $PGF_2\alpha$ in hysterectomized gilts with aging CL. Additionally, the $PGF_2\alpha$ treatment immediately shifts relaxin peak release and decreased progesterone production in these hysterectomized gilts in a manner that mimics the events seen in late pregnancy, parturition, and early lactation.

References

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Figure 1. Effect of i.m. injection of $PGF_{2\_}$ (30 mg) or vehicle (PBS, 5 ml) at 1200 hours on day 113 on progesterone (**a**), relaxin (**b**), PRL (**c**), and GH (**d**) secretion in hysterectomized gilts. Days indicate 1200 hours; values are means_SE. The value after $PGF_{2\_}$ treatment on day 113 is the average value of sequential bleeding.