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Research Article
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Research Article

Profiles of prostaglandin $F_{2\alpha}$ metabolite in dairy cattle during luteal regression and pregnancy: implications for corpus luteum maintenance†

Megan A. Mezera[1,2], Caleb S. Hamm [1], Caio A. Gamarra[1], Rodrigo S. Gennari[1], Alexandre B. Prata[1,3], Roberto Sartori[1,3] and Milo C. Wiltbank [1,2,*]

[1]Department of Dairy Science, University of Wisconsin-Madison, Madison, Wisconsin, USA; [2]Endocrinology and Reproductive Physiology Program, University of Wisconsin-Madison, Madison, Wisconsin, USA and [3]Department of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Piracicaba, São Paulo, Brazil

*__Correspondence:__ University of Wisconsin-Madison, Department of Dairy Science, 1675 Observatory Drive, Madison, WI 53706. E-mail: wiltbank@wisc.edu

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Abstract

Mechanisms of bovine corpus luteum (CL) maintenance during the second month of pregnancy have not been adequately investigated, despite significant reproductive losses. In the first month, interferon-tau is believed to suppress oxytocin-stimulated prostaglandin $F_{2\alpha}$ (PGF) production, yet there are conflicting reports of circulating PGF metabolite (PGFM). In this study, characterization of PGFM and P4 occurred through continuous bihourly blood sampling in cows undergoing CL regression (day 18–21, n = 5), and during the first (day 18–21, n = 5) and second month (day 47–61; n = 16) of pregnancy. Cattle in the second month were assigned to control (n = 8) or oxytocin treatment (n = 8; three pulses to mimic luteolysis) to evaluate if oxytocin receptors were active. All cows but one (which had elevated PGFM prior to oxytocin treatment) maintained the pregnancy. Basal PGFM concentrations were low (11.6 ± 0.7 pg/mL) in the first month but increased 2.54-fold in the second month. Few (0.26 ± 0.12 pulses/day) PGFM pulses with low peak concentrations (28.8 ± 3.1 pg/mL) were observed during the first month of pregnancy, similar to cows not undergoing regression. However, in the second month, frequency (1.10 ± 0.26 pulses/day) and peak concentration (67.2 ± 5.0 pg/mL) of PGFM pulses increased, displaying similar frequency but lower peak PGFM than seen in regression (1.44 ± 0.14 pulses/day; 134.5 ± 18.9 pg/mL). Oxytocin treatment increased likelihood of PGFM pulses post-treatment and increased peak concentration (89.7 ± 10.1 pg/mL) in cows during the second month. Thus, cows have more PGFM pulses during second than first month of pregnancy, possibly induced by endogenous oxytocin, indicating suppression of PGF production is an important mechanism for CL maintenance during first but not second month of pregnancy.

Summary Sentence

Low basal PGFM concentration and minimal pulsatile activity are observed in first month of pregnancy before increasing during second month of pregnancy, suggesting different mechanisms maintain the CL in the first vs second month of pregnancy.

Key words: ruminants, prostaglandins, corpus luteum, pregnancy, bovine.

Introduction

In cows, normal maintenance of pregnancy requires a functional corpus luteum (CL) through at least day 180–200 of gestation, yet little is known about CL maintenance past day 25 [1]. Instead, focus has been primarily on understanding mechanisms that suppress the luteolytic pathways that occur in nonpregnant cows from day 16–25 of pregnancy, resulting in maintenance of the CL and luteal progesterone (P4) production during this critical time of pregnancy. However, after the first month of pregnancy, mechanisms maintaining the CL are not yet defined, despite substantial pregnancy losses during the second month in dairy cattle (12%) and embryo transfer recipients (16%) [2, 3].

In cycling cattle, ovulation provides an opportunity for conception. However, should pregnancy fail to be initiated, regression of the CL in response to oxytocin-induced uterine prostaglandin F2α (PGF) [4] decreases circulating P4, allowing subsequent ovulation and another opportunity to conceive [5, 6]. In early pregnancy, interferon-tau (IFNT) from the elongating embryo blocks the normal luteolytic process through a variety of mechanisms [7–9]. Early research indicated that IFNT reduced uterine PGF production by the uterus, preventing luteolysis by lack of exposure to the luteolytic substance [10, 11]. Another mechanism that has been proposed is that increased secretion of PGE exerts an anti-luteolytic effect on the CL [12, 13], while a third proposed mechanism, evaluated in sheep, is that there is an endocrine effect of IFNT directly on the CL to promote luteal maintenance [14, 15]. There is evidence supporting all three mechanisms, with the possibility that multiple mechanisms occur simultaneously.

In support of the first mechanism, hysterectomy of ruminants results in maintenance of the CL indefinitely due to lack of uterine-secreted PGF [16]. Thus, pregnancy could act similarly by preventing uterine PGF production. Indeed, intrauterine infusion of IFNT or the presence of an elongating embryo reduces expression of the estradiol receptor, ESR1, which prevents the upregulation of endometrial oxytocin receptors seen in nonpregnant ruminants near the end of the estrous cycle [17, 18]. Thus, the absence of uterine oxytocin receptors during early pregnancy can prevent the pulses of PGF in ruminants that would cause luteolysis during this time in nonpregnant animals [19]. Early studies of circulating PGF (uterine vein) and PGFM (systemic) in cattle are somewhat consistent with reduced PGF secretion from the uterus during early pregnancy [20–23]. However, continual blood sampling for PGFM was reported in only one heifer that was pregnant by artificial insemination [22] and four cows that remained pregnant after transferring older embryos (day 16 embryos) [23]. In these early pregnant animals, there was a relative lack of PGFM pulses compared to the distinct PGFM pulses that were present in nonpregnant animals during this same time period. In ewes, there are reports of low circulating PGF or PGFM in pregnant animals [24–27] and one report of elevated basal PGF in early pregnant ewes [28]; however, these studies did not monitor PGF or PGFM with sufficient frequency to monitor pulses. Additionally, there is one report of elevated basal PGFM and distinct PGFM pulses in early pregnant ewes during continual blood sampling [29]. In cattle, a recent study [30] used 8-h windows of continual blood sampling on day 16, 17, and 18 post-ovulation to compare PGFM patterns in pregnant and nonpregnant heifers, and reported similar frequency but reduced amplitude of PGFM pulses during early pregnancy. Hence, there is still substantial discrepancy in results among studies, with few reports using continual sampling of pregnant ruminants during the period of normal luteolysis. Thus, in our study, we monitored PGFM concentrations in multiple cows continuously for 3 days during early pregnancy to directly compare basal and pulsatile PGFM in pregnant and noninseminated animals.

The evidence for IFNT in maintenance of the CL during the first month of pregnancy is convincing [7, 8]. However, most studies indicate an absence of IFNT during the second month of pregnancy in ruminants [31–33] due to a dramatic decrease in embryonic IFNT mRNA associated with trophectoderm attachment to the uterus [34, 35]. Further, in contrast to the lack of uterine oxytocin receptors and oxytocin responsiveness reported during early pregnancy, oxytocin receptor was detected on day 50 of pregnancy and exogenous oxytocin treatment increased circulating PGFM on day 50 and 150 of pregnancy [36–38]. Therefore, it appears lack of IFNT in the second month of pregnancy may allow acquisition of the molecular machinery involved in producing uterine PGF pulses.

Another relevant observation is that accessory CL, induced on the contralateral side from the pregnancy, generally regress, providing evidence for a local effect of pregnancy on maintenance of the CL [39]. The timing of CL regression is particularly intriguing as the majority (74.4%) of contralateral CL that regressed underwent regression between day 33 and 67 of pregnancy, after having maintained both original and contralateral CL through the first month. This leads to the intriguing hypothesis that the contralateral CL is maintained during the first month due to the actions of IFNT, whereas during the second month another mechanism maintains the original CL through local mechanisms but fails to maintain the contralateral accessory CL in many cows [3, 39].

Together, the incidence of contralateral accessory CL regression during the second month, combined with the increase in oxytocin responsiveness of the uterus, led to interest in PGFM profiles in the second month of pregnancy in comparison with the first month. Therefore, two experiments were designed to investigate PGFM patterns during the first and second months of pregnancy, as well as during luteal regression. The first experiment investigated the first month of pregnancy, with blood samples collected from day 18–21 in cows that received AI (pregnant cows in some cases) or did not receive AI (nonpregnant cows either undergoing or not undergoing CL regression in the study period). In the second experiment, PGFM and P4 profiles were determined in cows during the second month of pregnancy in animals that were treated or not treated with an oxytocin challenge designed to mimic the pattern of PGFM pulses observed during luteolysis. We hypothesized that (1) nonpregnant animals would display multiple distinct PGFM pulses during CL regression, associated with a decrease in circulating P4; (2) there would be low PGFM secretion and no PGFM pulses in animals

during the first month of pregnancy; (3) there would be greater basal PGFM concentrations accompanied by PGFM pulses during the second month of pregnancy; and (4) cows in the second month would respond to oxytocin administration with PGFM pulses indicating the presence of responsive uterine oxytocin receptors in the second month of pregnancy.

Materials and methods

All procedures were approved by the University of Wisconsin-Madison Animal Care and Use Committee. All cows were housed in a tie stall barn and milked twice per day.

Experiment 1: Characterization of PGFM in CL regression and early pregnancy

Multiparous Holstein cows were synchronized with an Ovsynch [40] or a Double-Ovsynch [41, 42] protocol during a total of 32 cycles, and either bred or not according to the assigned group. Cows were no longer used in the same group (bred or nonbred) after having successfully become pregnant or undergoing regression during the sampling window. The day of breeding (day after GnRH) was considered day 0. Cows were only included in the pregnant group if pregnancy was confirmed via ultrasound on day 28. Cows were considered part of the regressed group if they had not been bred and achieved P4 concentrations below 1 ng/mL by the end of the sampling period.

Jugular catheters were placed in all cows on day 17 of the cycle. Catheters consist of 0.07-inch exterior diameter and 0.04-inch interior diameter Tygon tubing (14171287, Saint-Gobain PPL Corp., Courbevoie, France). These were threaded through a 12 g sterile needle (J-174EE, JorVet, Loveland, CO, USA) into the jugular vein. Eighteen-gauge luer stub adaptors (427563, Becton, Dickinson, and Company, Franklin Lakes, NJ, USA) were attached to facilitate sample collection, and catheters were routinely flushed with heparinized saline (10,000 IU per 1 L 0.9% sodium chloride, NDC 0409-7983-09, Hospira Inc., Lake Forest, IL, USA) to prevent blood clots from forming.

Blood samples were collected every 2 h into 10 mL vacutainers containing K2 EDTA (368589, Becton, Dickinson, and Company) from jugular catheters starting at 7 AM on day 18 of the estrous cycle and continuing for 74 h (9 AM on day 21 in most cows). For one cow in the nonbred group blood sampling continued through 5 PM on day 21 since luteolysis appeared imminent via ultrasound. All blood samples were centrifuged, and serum were collected and stored at –20°C until assayed.

Experiment 2: Characterization of PGFM in the second month of pregnancy

Sixteen Holstein cows were bred and confirmed pregnant on day 32, and again on the first day of sample collection by ultrasound evaluation of fetal heartbeat. Cows were between days 47 and 61 of pregnancy on the first day of sample collection. Cows were paired with another cow on a similar day of pregnancy, and one cow from each pair assigned to the treatment group.

All cows received a jugular catheter 1 day before the beginning of sample collection, in the same manner as in experiment 1. Blood samples were collected every 2 h from jugular catheters starting at 9 AM and continuing for 52 h of continuous sampling.

Cows in the treatment group received three IM administrations of oxytocin: the first of 15 IU 8 h after blood collection had begun, the second of 15 IU 24 h later, and a third of 30 IU approximately 12 h after the second treatment. Dose of oxytocin and timing of oxytocin administration were selected to match physiologic PGF pulses in cyclic animals undergoing CL regression that were observed in experiment 1. An additional blood sample was collected 1 h after administration of oxytocin to better monitor responses to oxytocin. All blood samples were centrifuged, and serum were collected and stored at –20°C until used in assays.

Hormone assays

Concentrations of PGFM were measured via an ELISA based on the method used by Ginther et al., with some modifications [43]. Plates were coated with goat anti-rabbit IgG (4050-01, Southern Biotech, Birmingham, AL, USA) (stock 2 μg/mL diluted 1:1000 in a 0.05 M sodium carbonate buffer, pH 9.6), and left overnight at 4°C. Standards from 9.8 to 625 pg/mL were prepared in prostaglandin-free plasma from flunixin meglumine-treated cows [44]. All samples and standards were adjusted to pH < 3 with 3M hydrochloric acid, and 250 μL of sample was combined with diethyl ether (2 mL), vortexed for 3 min, and allowed to separate for 3 min before submerging vials in a methanol-dry ice bath to freeze the aqueous layer before ether was poured into new tubes. Subsequently, ether was evaporated at room temperature overnight, and 250 μL of assay buffer added to re-suspend the extracted material. Samples were then placed on a plate shaker for an hour. The coated ELISA plate was washed four times, primary antibody was added (1:16,000 dilution, gift of Dr Thatcher, University of Florida), and plate was placed on a shaker for 1.5 h at room temperature. The plate was washed four times, and 100 μL of sample was added in duplicate to wells and incubated for 20 min at room temperature before addition of 50 μL of PGFM-HRP conjugate (1:20,000 dilution). After 1-h incubation at room temperature, the plate was washed four times and 125 μL of substrate solution added. Color developed for 15 min and the reaction was stopped with 50 μL 0.5 M sulfuric acid before optical density was read at 450 and 600 nm wavelengths. The intra- and interassay CV were 6.7% and 15.0%, and sensitivity was 9.9 pg/mL.

Progesterone concentrations were evaluated using a commercial kit, CT Progesterone (07-270105, MP Biomedicals LLC, Solon, OH, USA), following the manufacturer's instructions. The intra- and interassay CV were 7.7% and 3.7%, and sensitivity was 0.04 ng/mL.

PGFM and P4 normalization

Progesterone and PGFM concentrations were normalized to account for baseline animal variation. Concentrations of PGFM were adjusted to percentage of basal PGFM. Progesterone was adjusted to percentage of baseline. The mean basal PGFM concentration was calculated as the average concentration of the lowest 50% of samples. When comparing cows in experiment 1 to controls in experiment 2, baseline consisted of the average P4 in all samples from the first 24 h of the experiment. In experiment 2, baseline consisted of the average P4 in all samples prior to the time when treated animals received oxytocin.

Pulse identification and statistical analyses

All PGFM values that were below the sensitivity of the assay were set at sensitivity (9.9 pg/mL). Analyses of whether a sample was part of a pulse were based on statistical criteria that compared the coefficient of variation for a given sample to three times the interassay CV. The interassay CV was 0.15 for the PGFM assays in this study; therefore, three times the interassay CV was 0.45. A sample was considered

part of a pulse if two criteria were met. First, the interassay CV for the mean concentration of the sample and the two preceding samples needed to be greater than 0.45. Second, the interassay CV for the sample and basal concentration of PGFM for the individual animal needed to be greater than 0.45. In both these criteria, the mean value of the concentration was used and duplicate values that were evaluated in the assay for each sample were not included in the calculations.

In other words, if Z is the concentration of the sample being evaluated to determine if it is part of a pulse, Y and X are the two samples preceding Z, and B the basal PGFM concentration for the individual animal being evaluated, then if both of the following were true the sample was considered part of a pulse.

$$0.45 < \frac{\mathrm{SD(X, Y, Z)}}{\mathrm{Average(X, Y, Z)}} \qquad 0.45 < \frac{\mathrm{SD(Z, B)}}{\mathrm{Average(Z, B)}}$$

Statistical differences were analyzed using PROC MIXED in SAS. The F statistic was used to determine if there were differences between groups, and Student *t* test was used to determine differences within the groups if the F statistic was significant.

All tests were two sided for experiment 1 and comparisons between experiments 1 and 2. In experiment 2, number of pulses per day, basal PGFM, and number of animals displaying pulses were analyzed with a two-sided test, while analysis of peak amplitude, concentration, and pulse frequency were done with a one-sided test since it was anticipated that oxytocin treatment would lead to increases in these measurements.

Values were considered significantly different for $P < 0.05$ and tended to be different when $P < 0.10$ and $P > 0.05$. Data are presented as mean (±SEM).

Results

Experiment 1: Characterization of PGFM during CL regression and the first month of pregnancy

Of 16 bred animals, 5 were confirmed pregnant by ultrasound on day 28. Of 14 enrolled in the experiment as nonbred animals, 5 cows underwent luteal regression during the sampling period, 3 underwent luteal regression prior to sampling, and 6 regressed normally after sampling ended. Two animals that regressed after the sampling period were recycled through the protocol resulting in samples from two cycles for these animals. Animals which underwent luteal regression prior to the beginning of sampling were dropped from the trial.

Figure 1 shows the profiles of P4 and PGFM in nonbred cows that underwent CL regression after the sampling period ended but not during the 3-day sampling period. These cows are representative of the late luteal phase of the cycle (LLP) without CL regression. The patterns for the two cows that were repeated in separate luteal phases are shown on the bottom (6788 #1 and #2; 7443 #1 and #2). All cows showed at least one PGFM pulse during the 72-h sampling period but most of the pulses were small (peak amplitude < 50 pg/mL in 12/15 detected peaks). Reanalysis of PGFM peak concentrations and reanalysis of a random subset of basal samples produced results similar to the first assay. Circulating P4 remained elevated in all cows with high variability. Reanalysis of P4 showed fluctuations were repeatable, and not due to assay variation. No LLP cows underwent CL regression, based on circulating P4, during the sampling period, although cow 7346 had decreased P4 ($P < 0.05$) after the large PGFM pulse at hour 60. For all subsequent analyses of LLP animals, only the first 48 h of sampling were used to calculate basal concentrations and pulses to allow valid comparison to pregnant and regressed groups. This ensured that all cows in LLP were more than 24 h from beginning of functional CL regression, as measured by P4.

Figure 2 shows PGFM and P4 profiles for five cows that were not bred and underwent CL regression during sampling (left side, A–E) and for five bred cows confirmed pregnant at day 28 (right side, F–J). One cow, 7613, was first evaluated in the nonbred group and subsequently was bred and became pregnant. All cows in the CL regressed group had a decrease in circulating P4 from > 5 ng/mL to less than 0.5 ng/mL during the 72-h period. The decrease in circulating P4 was accompanied by a distinct PGFM pattern. One or more small PGFM pulses were observed, accompanied by no change in circulating P4. These small pulses were followed in ~24 h by a large PGFM pulse (>100 pg/mL) that was accompanied by a clear decrease in circulating P4, generally followed by other large PGFM pulses in the presence of low P4. In contrast, only three of five pregnant cows had PGFM pulses and these pulses were very small, <50 pg/mL, with no decrease in circulating P4 (Figure 2F–J).

Data were normalized to the peak of a PGFM pulse (hour 0), and concentrations of P4 were analyzed 4 h before and after the peak (Figure 3). Pulses of PGFM during regression were split into large and small pulses and evaluated separately, with a cut off between the two groups at 100 pg/mL. This cutoff was selected by looking at the largest pulse in each animal that underwent CL regression, where it was observed that the lowest PGFM concentration associated with a PGFM pulse associated with CL regression was 100.9 pg/mL (in 7076 at hour 64, Figure 2C). Furthermore, it was observed that pulses of PGFM that were <100 pg/mL did not lead to significant changes in circulating P4 ($P > 0.10$) in the regressing, pregnant, and late luteal phase groups. On average, there was a decrease from 78 ± 6% circulating P4 at 4 h before the peak of the PGFM pulse to 19 ± 5% of baseline P4 at 4 h after the peak of the pulse. Thus, circulating P4 was decreased at hours 0, 2, and 4 compared to hours –2 and –4 ($P < 0.05$).

Table 1 shows a comparison between the three groups in experiment 1: pregnant (day 18–21), CL regression (day 18–21), and LLP (day 18–21) with no CL regression. Basal PGFM was similar between the three groups ($P > 0.10$) and close to the sensitivity of the PGFM assay (9.9 pg/mL). Although all cows in the LLP and CL regression group had detectable PGFM pulses during the sampling period, two of the LLP cows had no pulses in the first 48 h, when the evaluation was performed. The characteristics of PGFM were similar for first month pregnant and LLP cows including maximum and average peak concentrations, and maximum and average pulse amplitude (Table 1). In contrast, both pregnant and LLP cows had reduced PGFM pulses compared to animals that underwent regression, as determined by any of the measures of peak concentrations or pulse amplitude. The maximum pulse amplitude was only ~20 pg/mL in pregnant or LLP cows, which is about 15% of the pulse amplitude in cows that had CL regression (129.5 pg/mL). Similarly, pregnant and LLP cows were much lower than cows with CL regression in maximum PGFM concentration (~19%), maximum pulse concentrations (~23%), average pulse concentrations (~30%), and average pulse amplitude (~38%) (Table 1; Figures 1 and 2). Thus, although basal PGFM was similar in all groups and there were some PGFM pulses detected in pregnant and LLP cows, it was clear that PGFM pulses were much larger and distinct in cows that underwent CL regression using any of these various classification methods.

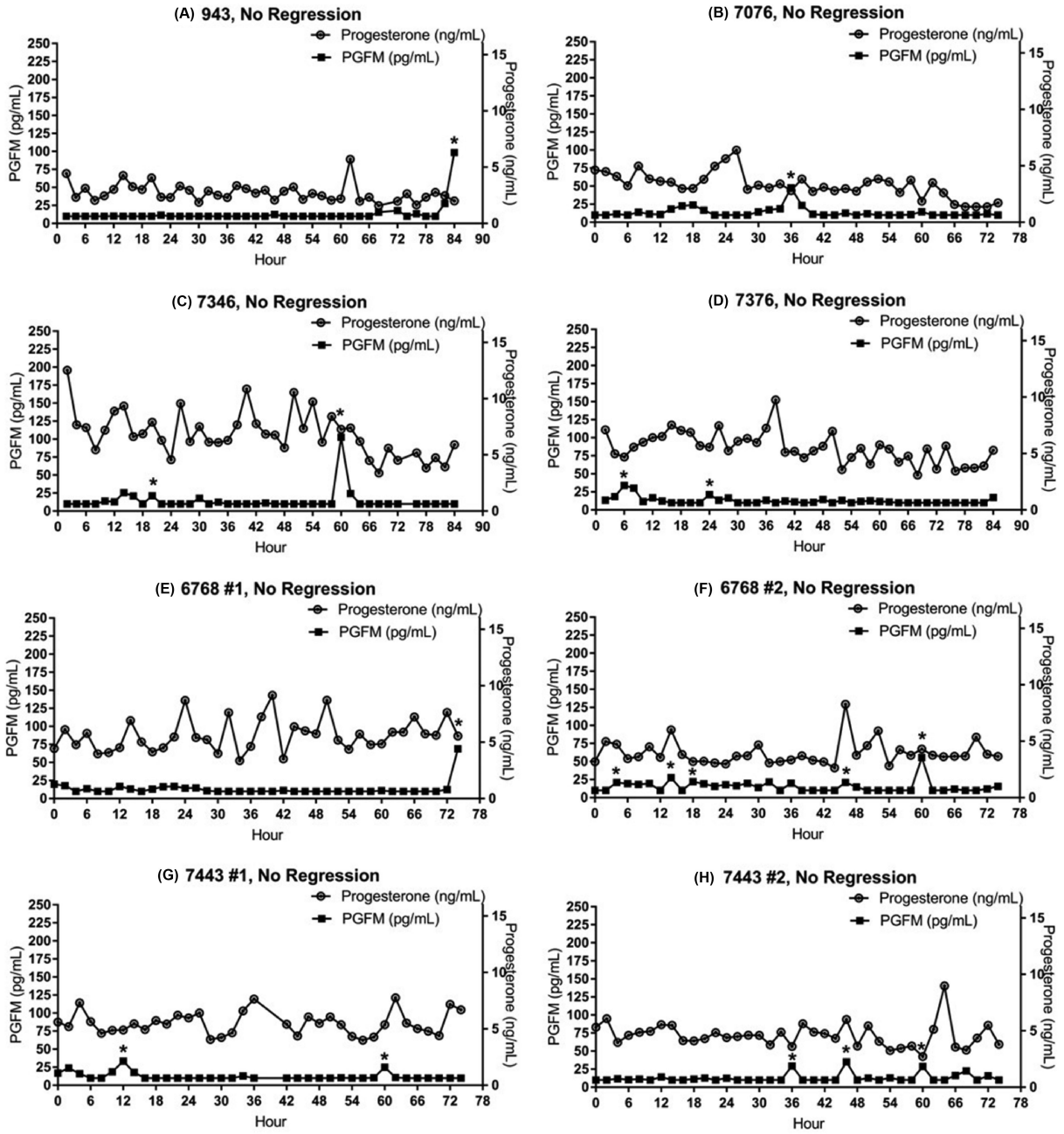

Figure 1. Cows that were not inseminated and failed to regress the CL during sampling period. Hour zero corresponds to 7:00 AM on day 18 of the cycle. Two animals were re-synchronized and recycled through the protocol due to lack of luteolysis in the sampling window. Two different cycles are shown for those cows (6768: E and F, 7443: G and H). Asterisks denote identified PGFM pulses.

Experiment 2: Characterization of PGFM in the second month of pregnancy

Cows were between day 47 and 61 of pregnancy at the start of blood sampling in experiment 2 (Figure 4: control and Figure 5: oxytocin-treated). All control and all but one treated cow remained pregnant through 2 weeks after the end of the experiment. One treated cow lost the pregnancy (Figure 5H) during the oxytocin treatments with placental membranes observed protruding from the vulva at hour 42 (3:00 PM, day 3). Prior to the first oxytocin administration, this cow already had a PGFM concentration of 239 pg/mL, a concentration greater than that observed in any other pregnant cow at any time during this study, including other treated cows, and continued to have high PGFM pulse peaks throughout the sampling period (average peak concentration 275.8 ± 41.9, which was greater than the average maximum PGFM peak concentration for all other treated cows; $P < 0.01$). It seems likely that this cow had already begun to lose the pregnancy prior to the start of the experiment. This animal was not used in any further analyses. The number of cows that maintained pregnancies did not differ between groups ($P = 0.37$).

Basal PGFM did not differ between groups ($P = 0.15$, control: 29.5 ± 5.1, oxytocin-treated: 24.8 ± 5.4 pg/mL). A total of 87.5%

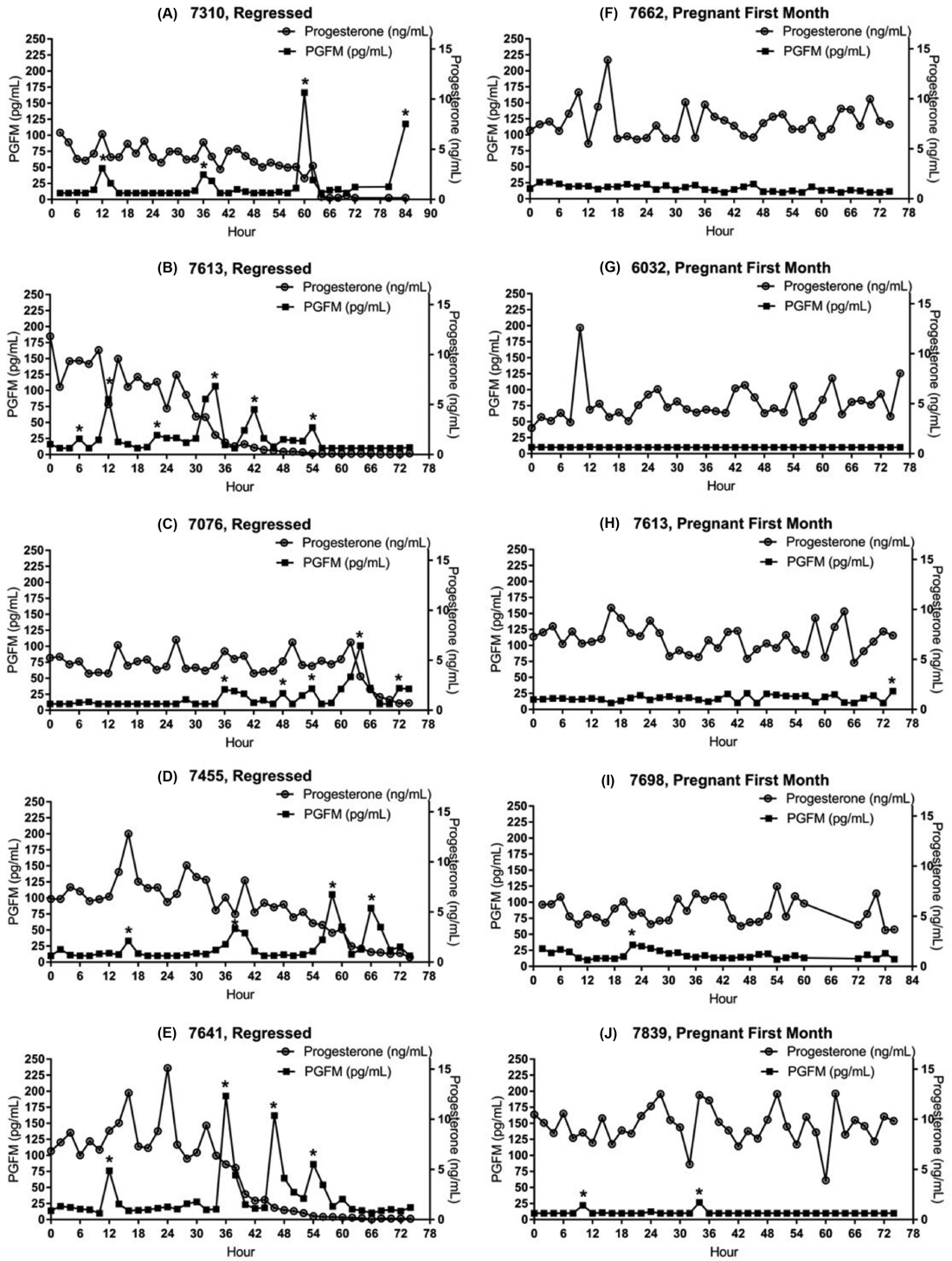

Figure 2. On the left: PGFM and P4 concentration in cows that were not inseminated and underwent luteolysis in the sampling period. On the right: cows confirmed pregnant at day 28 via ultrasound. Hour zero corresponds to 7:00 AM on day 18 of the cycle. Asterisks denote identified PGFM pulses.

(7/8) of control cows displayed at least one pulse of PGFM (Figure 4). The only control cow, 7884, without a detected PGFM pulse had elevated basal PGFM (57.7 pg/mL, 1.9 times greater than average basal concentration for the control group), and this may have reduced statistical power to detect pulses using our criteria. All oxytocin-treated cows had detectable PGFM pulses during sampling period (Figure 5). Maximum PGFM concentration tended to be greater for oxytocin-treated animals than controls ($P = 0.09$; 123%) as did average pulse concentration ($P = 0.09$; 124%). Oxytocin-treated cows were greater than controls for maximum pulse amplitude ($P = 0.02$;

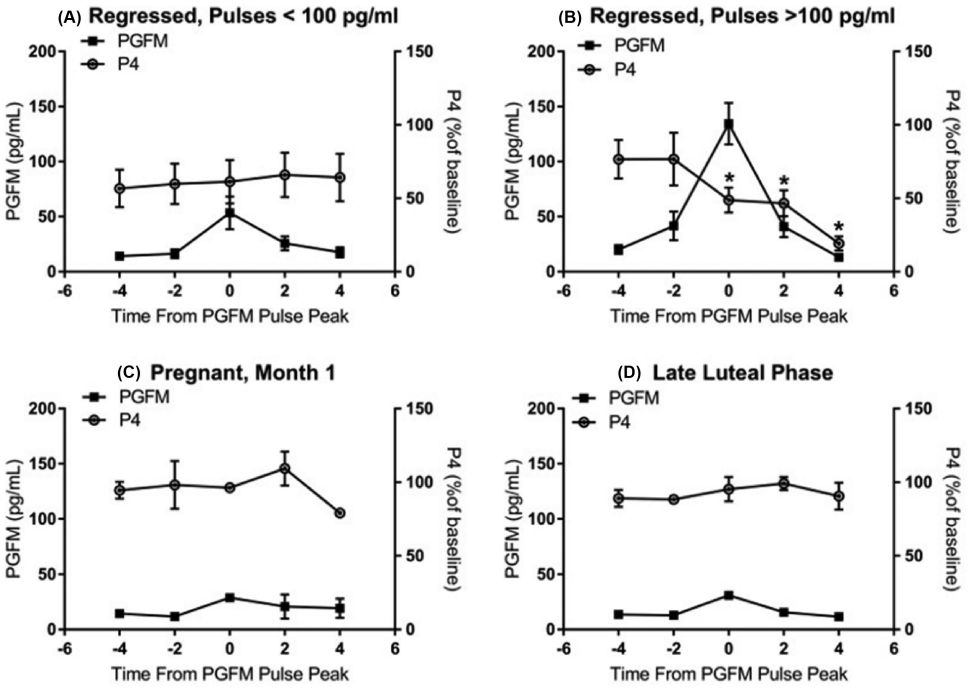

Figure 3. PGFM concentrations and P4 as percentage of baseline. Hour zero corresponds to the peak of an identified pulse. Asterisks denote P4 values that are significantly different than values at hour –4 ($P < 0.05$). (A) All pulses less than 100 pg/mL in cows that underwent regression, n = 5, total of 11 pulses. (B) First pulse over 100 pg/mL in cows that underwent regression n = 5, total of 5 pulses. (C) All pulses in cows in the first month of pregnancy, n = 3, total of 4 pulses. (D) Pulses in the late luteal phase, n = 6, total of 11 pulses (LLP only includes pulses from the first 48 h of sampling).

146%) and maximum peak concentration ($P = 0.01$; 133%) when considering cows with pulses (Table 2).

There were three treatments with oxytocin, two of which occurred within an hour after milking. Number of PGFM pulses per day tended to be greater in oxytocin-treated cows than controls ($P = 0.07$). However, number of PGFM pulses detected within 3 h after treatment was much greater ($P < 0.001$) in oxytocin-treated compared to control cows. A total of 76% of oxytocin treatments induced a detectable PGFM pulse in oxytocin-treated animals (16/21), whereas only 8.3% (2/24) of treatment times were followed by a PGFM pulse in control cows ($P < 0.001$).

When results were normalized to basal PGFM and baseline P4, there was a greater concentration of PGFM in treated animals at 1 and 2 h after treatment, though this was not accompanied by any difference or change in P4 between groups or within groups (Figure 6).

In cows with CL regression in experiment 1, pulses > 100 pg/mL were associated with a decline in P4. In second-month, control animals there were no pulses > 100 pg/mL, and only five pulses in three animals in the oxytocin-treated animals. Three of five pulses over 100 pg/mL occurred during hourly sampling, and two occurred during bihourly sampling. Therefore, PGFM and P4 concentrations in samples before and after the pulse peak were combined to obtain a composite PGFM/P4 profile for these pulses, regardless of whether this sample was 1 or 2 h away from pulse peak. There was no change in P4, measured as percentage of baseline, in control second-month cows (pulse peaks < 100 pg/mL) or associated with PGFM pulses > 100 pg/mL in oxytocin-treated cows (Figure 7).

Table 1. Quantification and comparison of PGFM (pg/mL) pulse characteristics during CL regression, first month of pregnancy, or late luteal phase without CL regression.

Characteristic measured	Pregnant	CL regressed	Late luteal phase*	*P*-value
Basal PGFM	11.6 ± 0.7	11.6 ± 1.0	9.9 ± 0.0	0.17
Number of cows displaying pulses	3/5	5/5	5/6	
Pulses/day	0.26 ± 0.12[a]	1.44 ± 0.14[b]	0.63 ± 0.15[a]	0.0002
Maximum concentration, all cows	25.1 ± 3.8[a]	134.5 ± 18.9[b]	25.6 ± 3.3[a]	<0.0001
Maximum peak amplitude, those with pulses	18.2 ± 2.1[a]	129.5 ± 19.8[b]	23.0 ± 4.0[a]	0.0003
Maximum peak concentration, those with pulses	28.8 ± 3.1[a]	134.5 ± 18.9[b]	32.9 ± 4.0[a]	0.0002
Average peak amplitude, those with pulses	18.22 ± 2.1[a]	66.6 ± 13.5[b]	20.5 ± 4.3[a]	0.0076
Average peak concentration, those with pulses	28.8 ± 3.1[a]	79.3 ± 14.7[b]	30.4 ± 4.3[a]	0.0089

*Late luteal phase cows did not undergo CL regression between days 18 and 21, values are from first 48 h to ensure cows were at least 24 h away from functional regression

[a, b]When superscripts are different across a row values are different ($P < 0.05$).

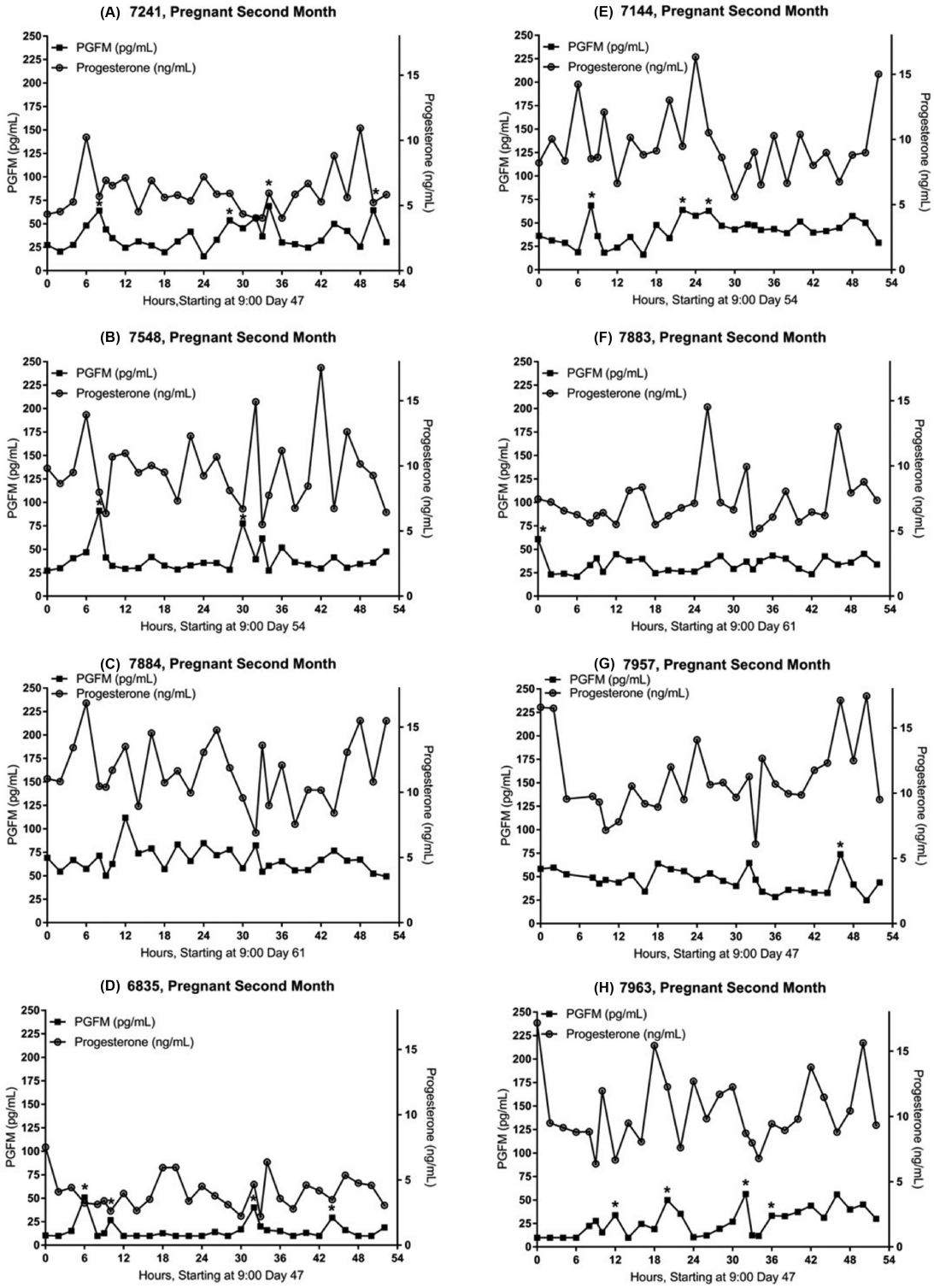

Figure 4. PGFM and P4 concentrations in individual cows in the second month of pregnancy in the control group. Asterisks denote identified pulses.

Average P4 concentrations during the sampling period tended to be greater in control than oxytocin-treated groups (8.7 vs 6.0 ng/mL; $P = 0.08$). However, this difference was also observed in samples taken prior to oxytocin treatment (8.8 vs 6.5 ng/mL; $P = 0.19$), with no difference in the change in P4 concentration in the samples prior to treatment compared to the samples taken after treatment (control: difference of -0.2 ± 0.7 ng/mL, treated: difference of -0.7 ± 0.9 ng/ml, $P = 0.44$).

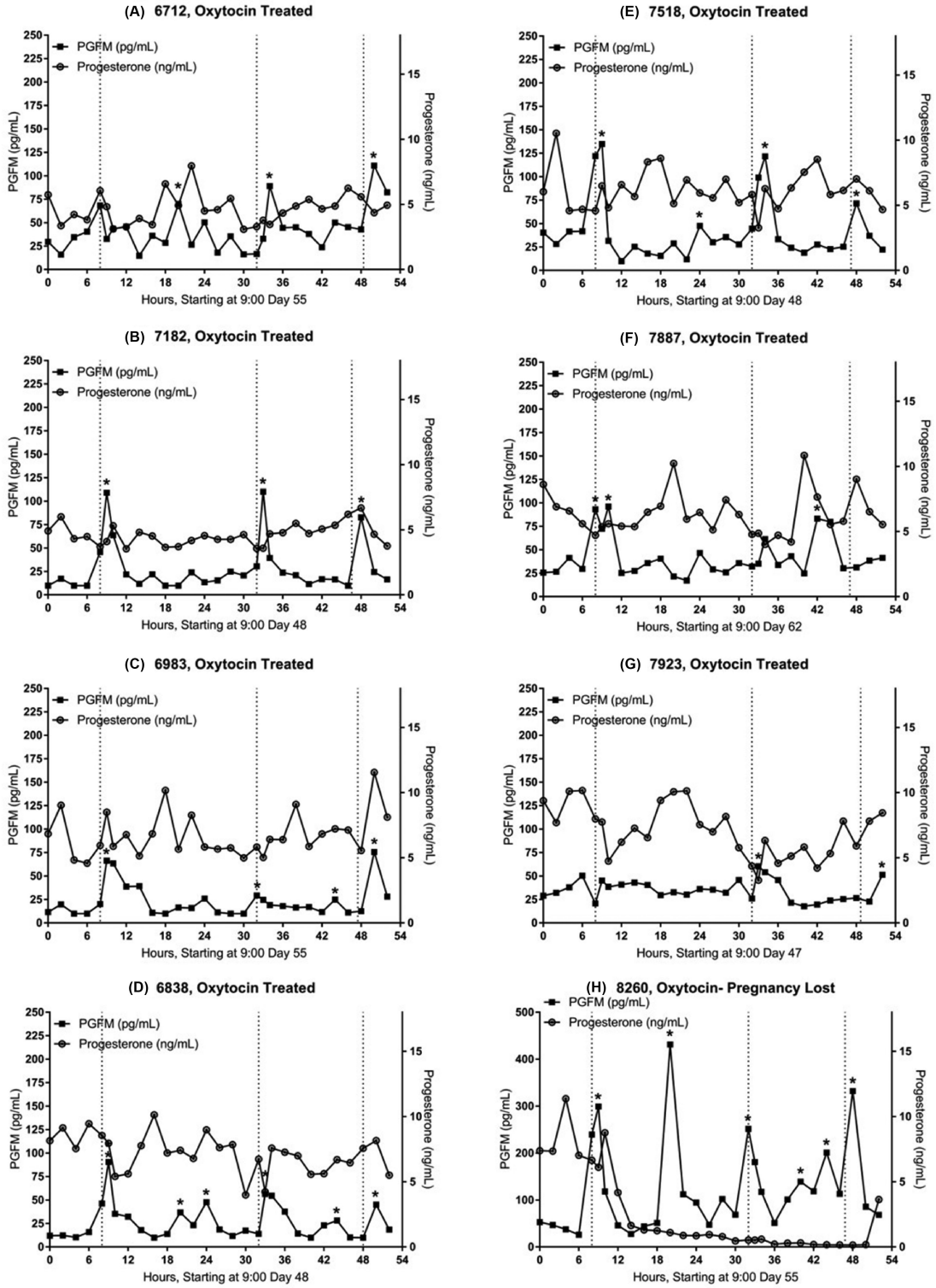

Figure 5. PGFM and P4 concentrations in individual cows in the second month of pregnancy treated with oxytocin. Vertical dotted lines display when oxytocin was administered. Asterisks denote identified PGFM pulses. Panel H shows a pregnant cow that underwent CL regression during the sampling period. The data from this cow were not included in subsequent analyses.

Table 2. Quantification and comparison of PGFM (pg/mL) pulse characteristics in control and oxytocin-treated cows in the second month of pregnancy.

Characteristic measured	Control	Oxytocin treated	*P*-value
Basal PGFM	29.5 ± 5.1	24.8 ± 5.4	0.15
Number of cows displaying pulses	7/8	7/7	
Pulses/day	1.10 ± 0.26	1.65 ± 0.22	0.07
Number of pulses after treatment time	0.25 ± 0.25	2.29 ± 0.29	0.0001
Maximum concentration, all cow	72.8 ± 7.1	89.7 ± 10.1	0.09
Maximum peak amplitude, those with pulses	50.8 ± 5.0	74.4 ± 9.1	0.02
Maximum peak concentration, those with pulses	67.2 ± 5.0	89.7 ± 10.1	0.01
Average peak amplitude, those with pulses	41.5 ± 6.6	54.3 ± 7.8	0.12
Average peak concentration, those with pulses	61.0 ± 6.2	75.8 ± 8.6	0.09
% Baseline P4 post-treatment	99 ± 3	92 ± 4	0.16

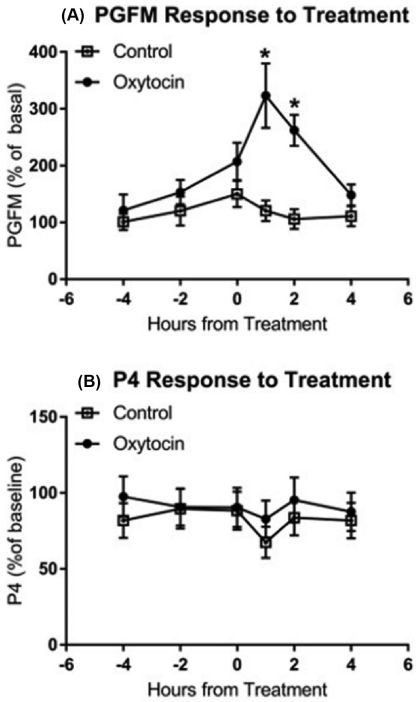

Figure 6. Average normalized PGFM (A) and P4 (B) concentrations before and after oxytocin treatment during three oxytocin challenges. Asterisks indicate differences between treated and control groups ($P < 0.05$). Oxytocin was administered in treated cows immediately after the sample was taken at hour zero.

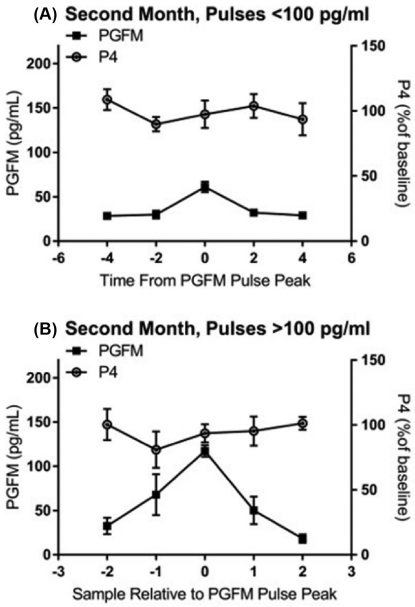

Figure 7. Response of P4 (as percentage of baseline) to PGFM pulses in the second month, with time zero set to the peak of the observed PGFM pulse. There were no differences in P4 in the hours prior to and after the PGFM pulse peak. (A) All pulses in cows in the second month of pregnancy control group. All observed PGFM pulses were < 100 pg/mL, n = 7, total of 18 pulses. (B) PGFM pulses > 100 pg/mL in the second month of pregnancy. All observed peaks over 100 pg/mL were in oxytocin-treated cows, n = 3, total of 5 pulses (Three of these pulses occurred during the extra sample taken after oxytocin administration, two were observed during bihourly sampling). Thus, samples were normalized to pulse peak and an average taken of samples directly before or after the peak (sample –1 and 1 on the graph) or two samples before and after the peak (–2 and 2).

Dynamics of PGFM, month 1 vs month 2 of pregnancy

Control second month pregnant cows were compared with first-month pregnant cows and those which had undergone CL regression during day 18–21 (Table 3). Basal PGFM concentrations were ~2.5-fold greater ($P < 0.01$) in cows during the second month of pregnancy than during the first month of pregnancy or in cows that underwent CL regression. More PGFM pulses were observed in cows during the second month of pregnancy (1.10 pulses/day) than during the first month of pregnancy (0.26 pulses/day), and cows in the second month were similar in pulse frequency to cows undergoing CL regression (1.44 pulses/day). There were similar average pulse amplitudes and concentration in second-month pregnant animals and animals undergoing regression, though the maximal concentration and amplitude were greater in animals undergoing regression than second-month pregnant animals. Second-month pregnant cows had greater PGFM pulses (both amplitude and peak concentration, both maximal and average) than first-month pregnant cows, although no second-month control cows aborted (Table 3).

Transient fluctuations in P4 were noted in both the first and second month of pregnancy. However, there was no difference in amount of variation when P4 was normalized as a percentage of baseline for each cow ($P = 0.56$). Additionally, there were no detectable changes in P4 concentrations correlated with PGFM pulses from 4 h before or 4 h after a pulse in LLP, first-month pregnant, and second-month pregnant cows (both PGFM pulses less than or greater than 100 pg/mL), as well as in cows which would eventually undergo CL regression when only evaluating PGFM pulses that were

Table 3. Quantification and comparison of PGFM (pg/mL) pulse characteristics during CL regression and the first and second month of pregnancy.

Characteristic measured	First month pregnant	Second month pregnant	First month regressed	*P*-value
Basal PGFM	11.6 ± 0.7[a]	29.5 ± 5.1[b]	11.6 ± 1.0[a]	0.0062
Number of cows displaying pulses	3/5	7/8	5/5	
Pulses/day	0.26 ± 0.12[a]	1.10 ± 0.26[b]	1.44 ± 0.14[b]	0.01
Maximum concentration, all cows	25.1 ± 3.8[a]	72.8 ± 7.1[b]	134.5 ± 18.9[c]	<0.0001
Maximum peak amplitude, those with pulses	18.2 ± 2.1[a]	50.8 ± 5.0[b]	129.5 ± 19.8[c]	0.0003
Maximum peak concentration, those with pulses	28.8 ± 3.1[a]	67.2 ± 5.0[b]	134.5 ± 18.9[c]	0.0003
Average peak amplitude, those with pulses	18.2 ± 2.1[a]	41.5 ± 6.6[b]	66.6 ± 13.5[b]	0.0265
Average peak concentration, those with pulses	28.8 ± 3.1[a]	61.0 ± 6.2[b]	79.3 ± 14.7[b]	0.0293

[a, b, c]When superscripts are different across a row values are different ($P < 0.05$).

less than 100 pg/mL. The only situation in which P4 concentrations changed, when evaluated relative to a PGFM pulse, was in animals that underwent CL regression during the first pulse over 100 pg/mL (Figures 3 and 7).

Discussion

The mechanisms involved in maintaining pregnancy entail intriguing biology and are of prime economic significance for dairy and beef cattle operations and in embryo recipients [45]. Therefore, it was surprising that PGF, the key hormone involved in CL regression, had not been well characterized in cattle during the second month of pregnancy. Reports in the literature were also surprisingly inconsistent in ruminants for PGF production during the first month of pregnancy. However, in the second month of pregnancy, while there were no published reports of PGFM profiles during multiple days there was an intriguing study which showed exogenous oxytocin led to PGFM production on day 50 of pregnancy [36]. Thus, this study was designed to characterize PGFM profiles during the first month of bovine pregnancy, compared directly to PGFM profiles in nonbred cattle undergoing CL regression during this same time period. In addition, our recent results on contralateral CL regression during the second month of pregnancy [39] provided an intriguing rationale for investigating the dynamics of PGFM secretion during the second month. To our knowledge, this is the first published report of PGFM profiles during the second month of pregnancy. This characterization, along with the oxytocin challenge that was done in this study, has led to proposal of a novel physiologic model for PGF secretion throughout pregnancy, based on the absence and later presence of oxytocin receptors in the uterus of pregnant cows (Figure 8).

Unsurprisingly, we accepted our first hypothesis, that PGFM pulses are present during CL regression, consistent with the bulk of the literature on CL regression in ruminants [22, 23, 27, 29, 46, 47]. Basal PGFM concentrations were low in all cows (nonbred with or without CL regression, pregnant) during this time period (day 18–21) and were not elevated in cows that ultimately underwent CL regression during our sampling interval. In all cows that underwent CL regression during our sampling period, there was at least one, and often multiple, small PGFM pulses (<100 pg/mL) that preceded the larger luteolytic PGFM pulses. These pulses clearly preceded regression of the CL and were found to have no effect on circulating P4 concentrations (Figure 3A). Most previous studies have reported that the decrease in circulating P4 was associated with multiple PGFM pulses in ruminants, some of smaller amplitude [27, 29, 46, 48]. In contrast, all five cows that underwent CL regression during the sampling period in this study had a single, larger (≥100 pg/mL) PGFM pulse associated with almost all of the decrease in circulating P4 associated with CL regression (Figure 2).

Previous animal models with intrauterine infusions of multiple, low-amplitude pulses of PGF also suggest that multiple PGF pulses are required for complete CL regression [49–51]. Nevertheless, as best illustrated in Figure 3B, the cows that underwent CL regression in our study experienced a large drop in P4, to an average of 19% of initial P4 concentrations within 4 h after the first PGFM pulse greater than 100 pg/mL. This all-or-nothing response, at least in terms of circulating P4, may be related to distinctive PGFM profiles in lactating dairy cows or the timing of sampling in our study. Still, it is striking how rapidly the P4 decline occurs in our study and how closely the P4 decline was associated with the larger PGFM pulse. This result emphasizes the need for future studies to determine the

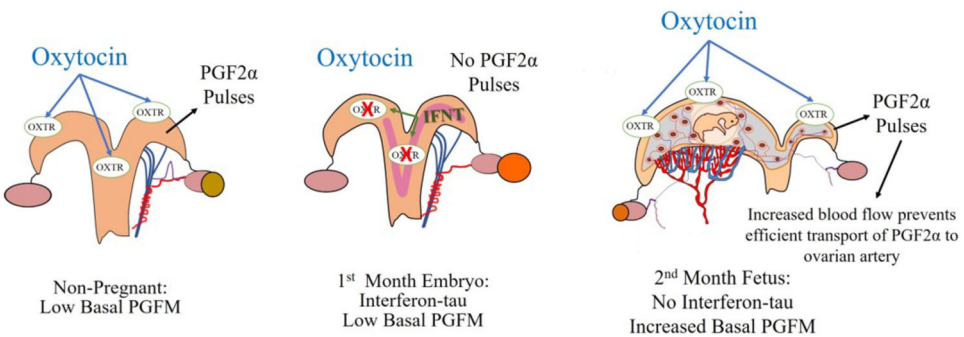

Figure 8. Proposed model for CL maintenance during pregnancy. In the first month of pregnancy, IFNT prevents PGF secretion and therefore the CL does not regress. In the second month of pregnancy, oxytocin receptors become active, and PGF pulses and basal PGFM concentrations increase. However, increased blood flow to the uterus prevents efficient transfer of PGF from the uterine vein to the ovarian artery, resulting in insufficient PGF exposure to induce luteolysis.

role, or lack of role, for smaller PGF pulses to prime responsiveness of the CL to subsequent larger PGF pulses. However, it is important to note that even without a decrease in circulating P4, smaller intrauterine PGF pulses can induce changes in luteal gene expression, including transcription factors such as the early response genes FOS, JUN, NR4A1, and EGR1, consistent with these small pulses beginning the luteolytic process prior to dramatic decreases in circulating P4 [50].

The average interovulatory interval in dairy cattle is 22.9 ± 0.7 days, with luteolysis preceding ovulation by 5.2 ± 0.2 days [52]. Thus, we chose to sample from days 18–21. However, fewer animals than anticipated underwent CL regression during the sampling period, resulting in nonbred cows that did not undergo luteolysis during the sampling period (LLP cows). These cows either had no or low PGFM pulses during the first 48 h, when luteolysis clearly was not yet occurring. It seems likely that cows with delayed luteolysis were cows with three follicular waves and that cows that underwent CL regression within our sampling period had two follicular waves, although this was not explicitly tested in our experiment. This observation highlights the importance of considering individual cows rather than average values in studies of CL regression [53].

Our second hypothesis that PGFM pulses would be absent during the first month of pregnancy accompanied by low basal circulating PGFM was generally supported, consistent with (1) other studies evaluating PGFM profiles in early bovine pregnancy using continual sampling [22, 23], (2) the decrease in PGF secretion associated with intrauterine recombinant IFNT administration in cattle [19], and (3) lack of oxytocin responsiveness and oxytocin receptors during early pregnancy, potentially preventing PGFM pulses [18, 54]. Our results contrast with the report in ewes of prominent PGFM pulses during early pregnancy [29] and a recent report of similar frequency of PGFM pulses with reduced amplitude (65% concentration) in early pregnant compared to nonpregnant heifers [30]. Thus, our study provides additional evidence consistent with the hypothesis that PGFM pulses are more infrequent and of much lower amplitude in early pregnant cows compared to nonpregnant cows. However, future studies may need to sample more frequently (hourly) and for a longer time interval to assure that any PGFM pulses are not overlooked during early pregnancy, particularly considering the wide window in which CL regression occurs in nonpregnant cows.

The low basal concentrations of PGFM were near the limit of detection for our assay (9.9 pg/mL) and similar during early pregnancy (11.6 ± 0.7 pg/mL) and in cows undergoing CL regression (11.6 ± 1.0 pg/mL) during similar days of the cycle. This is consistent with similar basal concentrations of PGFM observed in three previous studies that compared mean PGF concentrations in the uterine/utero-ovarian vein of ewes [25, 26, 55]. Analysis of only PGF and not PGFM, combined with the timing of sample collection (single sample on day 15 [55], samples every 3 h from day 14–17 [26], or samples every 30 min during a 4-h period on day 10–14 [25]), makes it likely that values represent basal concentrations since peaks of PGF would have been unlikely to be detected, given the short half-life of PGF [56]. Thus, our results in cattle support the view that uterine PGF secretion is low under basal conditions during early pregnancy and during a similar stage of nonpregnancy and that PGF pulses, induced by oxytocin in nonpregnant cows, are suppressed during early pregnancy, likely due to direct effects of IFNT on uterine endometrial cells [57].

Our third hypothesis that PGFM pulses would increase coincident with elevated basal concentrations of PGFM during the second month of pregnancy was supported. Basal PGFM during the second month was 2.5-fold greater than basal PGFM in the first month of pregnancy. Additionally, consistent with our fourth hypothesis, oxytocin treatments were capable of inducing PGFM pulses. This result suggests that endogenous oxytocin may be responsible for PGFM pulses observed during the second month of pregnancy.

These conclusions are consistent with a working physiologic model of PGF secretion during pregnancy (Figure 8). In the first month of pregnancy, embryonic IFNT inhibits an increase in uterine oxytocin receptors and thereby suppresses luteolytic pulses of PGF that would occur during the estrous cycle in nonpregnant cows. However, as pregnancy progresses into the second month, there is a cessation of IFNT production, and uterine oxytocin receptors increase, allowing endogenous oxytocin pulses to be a stimulus for uterine PGF production. There is substantial research that supports the mechanisms proposed for the first month of pregnancy, whereas the model of PGF secretion during the second month is more speculative. While there has been an analysis of oxytocin receptors in the second month, this study lacked cows from the first month to provide a true comparison [18]. Additionally, our study and the previous study using an oxytocin challenge on day 50 of pregnancy are unable to determine the origin of the PGF that is measured as circulating PGFM. Uterine endometrial cells are a likely source for PGF; however, PGF secretion by the embryo and placental tissues has also been reported in ruminants [58, 59]. Thus, the timing of induction, cellular origin, hormonal stimulator, and physiologic function, if any, for basal PGFM and PGFM pulses during the second month of pregnancy remain to be defined.

Regardless of the source of PGF, clearly there are pulses of PGFM during the second month, unlike our results during the first month of pregnancy. Since IFNT is no longer present during the second month, this raises the question of what maintains the CL after the first month [31]. Three potential mechanisms that maintain the CL after IFNT are (1) a circulating substance coming from the pregnancy protects the CL from regression; (2) there is insufficient PGF pulsatile activity, either in frequency, amplitude, and/or peak concentration to induce CL regression; or (3) uterine PGF does not reach the CL. These mechanisms, if present, are not necessarily mutually exclusive and could be simultaneously occurring to maintain the CL during the second month of pregnancy.

Data in ewes are not consistent with a circulating luteoprotective factor during the second month of pregnancy [3, 60, 61], although this idea has not been validly tested in cattle. The possibility that peak PGF concentrations or frequency of PGF pulses are insufficient to induce luteolysis during the second month cannot be discounted at this time. While the average pulse amplitude and concentration is similar during the second month as observed during the time of luteolysis, the maximal concentrations and amplitude of the largest PGFM pulses were more than 2-fold greater in cows undergoing regression compared to cows in the second month of pregnancy. In cows that underwent CL regression, PGFM pulses under 100 pg/mL were insufficient to cause any detectable change in circulating P4, and all pulses were under 100 pg/mL in control cows during the second month of pregnancy. Nevertheless, in oxytocin-treated cows there were PGFM pulses above 100 pg/mL (five pulses with average of 116.26 ± 6.05 pg/mL) and no detectable changes in P4. This suggests PGFM pulses that were sufficient to cause luteolysis in nonpregnant cows were not adequate to produce luteolysis during the second month of pregnancy. Thus, cows during the second month of pregnancy have similar number of PGFM pulses as cows undergoing CL regression and, in oxytocin-treated cows, the amplitude of PGFM pulses was in the luteolytic range, yet stable P4 during these

pulses suggests the CL is not being exposed to these pulses during the second month of pregnancy.

Considering the available evidence, a lack of efficient PGF transport from the uterus to the ovarian artery appears to be the most parsimonious explanation for maintenance of the CL ipsilateral to the pregnancy in the second month despite the uterine production of potentially luteolytic PGFM pulses, as previously suggested [3, 39]. Further support of this model comes from the dramatic increase in uterine blood flow, particularly in the horn with the pregnancy, after day 20 of pregnancy [62, 63], combined with evidence in ewes that intrauterine infusion of a nitric oxide donor to induce vasodilation (and thus, increase blood flow experimentally) during the normal time of CL regression prevents luteolysis, as evidenced by elevated P4 and maintenance of CL volume compared to nontreated controls [64]. This increase in blood flow may be related to the high concentrations of estrogens present in the allantoic fluid of the conceptus located in the uterine horn ipsilateral to the ovary bearing the CL [65]. This could provide a mechanism by which a local increase in vasodilation [66] is achieved in the horn with the pregnancy. Thus, our favored explanation continues to be that elevated blood flow in the uterine vein results in inefficient local transport of PGF into the ovarian artery on the ipsilateral side to the pregnancy, although contralateral accessory CL regress during the second month of pregnancy due to insufficient contralateral uterine blood flow [39]. During luteolysis, most PGF arrives at the CL via local mechanisms [67, 68], and therefore inefficient local transport of PGF could act as a luteal protective mechanism during pregnancy (Figure 8).

Another interesting observation was the dramatic fluctuation in P4 that was observed in all cows, regardless of pregnancy status, consistent with multiple previous reports of fluctuations in P4 [46, 69]. These fluctuations are clearly not entirely related to PGFM, as we saw no detectable effect of small PGFM pulses on P4, and these fluctuations in P4 are noted in cows without detectable PGFM pulses. Fluctuations in P4 are known to be correlated with LH pulses [69, 70], although treatment with a GnRH antagonist that eliminated LH pulses did not eliminate P4 fluctuations, suggesting other potential regulatory mechanisms [71], such as changes in P4 metabolism which could be associated with changes in liver blood flow related to feed intake [72, 73]. Understanding the physiologic origin and consequences of these large fluctuations in P4 could have implications for successful maintenance of pregnancy since nadir P4 concentrations were generally less than 50% of peak P4 and were below 5 ng/mL during some times in almost all pregnant cows.

In summary, in this study we determined there was a decrease in frequency and amplitude of PGFM pulses in pregnant cows during the normal time of CL regression in nonpregnant cows. In cycling cows, PGFM pulses were observed that coincide with luteolysis, yet basal PGFM concentrations were low and similar in pregnant cows as in cows that underwent CL regression during this period. In contrast, during the second month of pregnancy, there is an increase in basal PGFM (2.5-fold) and PGFM pulses are observed, likely due to stimulation of endometrial oxytocin receptors, as evidenced by increased PGFM in response to exogenous oxytocin. Despite the presence of PGFM pulses, no pregnancies were lost in the control group in the second month. Thus, our results indicate that suppression of PGF pulses is an important mechanism for maintaining the CL during the first month of pregnancy but that suppression of PGF secretion is unlikely to be the primary mechanism maintaining the CL during the second month of pregnancy, and perhaps during even later stages of pregnancy. Rather, it seems clear there is a different mechanism maintaining the CL after IFNT is no longer present. Further research to understand this mechanism of pregnancy maintenance could result in physiologically informed strategies to mitigate pregnancy losses during the second month, potentially increasing reproductive efficiency in ruminants and perhaps other species.

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References

1. Estergreen VL, Frost OL, Gomes WR, Erb RE, Bullard JF. Effect of ovariectomy on pregnancy maintenance and parturition in dairy cows. *J Dairy Sci* 1967; **50**:1293–1295.
2. Wiltbank MC, Baez GM, Garcia-Guerra A, Toledo MZ, Monteiro PL, Melo LF, Ochoa JC, Santos JE, Sartori R. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. *Theriogenology* 2016; **86**:239–253.
3. Wiltbank MC, Mezera MA, Toledo MZ, Drum JN, Baez GM, Garcia-Guerra A, Sartori R. Physiological mechanisms involved in maintaining the corpus luteum during the first two months of pregnancy. *Anim Reprod* 2018; **15**:805–821.
4. Burns PD, Graf GA, Hayes SH, Silvia WJ. Cellular mechanisms by which oxytocin stimulates uterine PGF2 alpha synthesis in bovine endometrium: roles of phospholipases C and A2. *Domest Anim Endocrinol* 1997; **14**:181–191.
5. McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. *Physiol Rev* 1999; **79**:263–323.
6. McCracken JA, Samuelss B., Goding JR, Glew ME, Green K, Carlson JC, Baird DT. Prostaglandin-F2-alpha identified as a luteolytic hormone in sheep. *Nat New Biol* 1972; **238**:129–134.
7. Bazer FW, Thatcher WW. Chronicling the discovery of interferon tau. *Reproduction* 2017; **154**:F11–F20.
8. Spencer TE, Hansen TR. Implantation and establishment of pregnancy in ruminants. *Adv Anat Embryol Cel* 2015; **216**:105–135.
9. Arosh JA, Banu SK, McCracken JA. Novel concepts on the role of prostaglandins on luteal maintenance and maternal recognition and establishment of pregnancy in ruminants. *J Dairy Sci* 2016; **99**:5926–5940.
10. Meyer MD, Hansen PJ, Thatcher WW, Drost M, Badinga L, Roberts RM, Li J, Ott TL, Bazer FW. Extension of corpus luteum lifespan and reduction of uterine secretion of prostaglandin F2 alpha of cows in response to recombinant interferon-tau. *J Dairy Sci* 1995; **78**:1921–1931.
11. Helmer SD, Hansen PJ, Thatcher WW, Johnson JW, Bazer FW. Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend corpus luteum lifespan in cyclic cattle. *Reproduction* 1989; **87**:89–101.
12. Ochoa JC, Penagaricano F, Baez GM, Melo LF, Motta JCL, Garcia-Guerra A, Meidan R, Pinheiro Ferreira JC, Sartori R, Wiltbank MC. Mechanisms for rescue of corpus luteum during pregnancy: gene expression in bovine corpus luteum following intrauterine pulses of prostaglandins E1 and F2alpha. *Biol Reprod* 2018; **98**:465–479.
13. Henderson KM, Scaramuzzi RJ, Baird DT. Simultaneous infusion of prostaglandin E2 antagonizes luteolytic action of prostaglandin F2alpha in vivo. *J Endocrinol* 1977; **72**:379–383.
14. Bott RC, Ashley RL, Henkes LE, Antoniazzi AQ, Bruemmer JE, Niswender GD, Bazer FW, Spencer TE, Smirnova NP, Anthony RV, Hansen TR. Uterine vein infusion of interferon Tau (IFNT) extends luteal life span in ewes. *Biol Reprod* 2010; **82**:725–735.

15. Antoniazzi AQ, Webb BT, Romero JJ, Ashley RL, Smirnova NP, Henkes LE, Bott RC, Oliveira JF, Niswender GD, Bazer FW, Hansen TR. Endocrine delivery of interferon tau protects the corpus luteum from prostaglandin F2 alpha-induced luteolysis in ewes. *Biol Reprod* 2013; **88**:144–144.
16. Wiltbank JN, Casida LE. Alteration of ovarian activity by hysterectomy. *J Animal Sci* 1956; **15**:134–140.
17. Spencer TE, Ing NH, Ott TL, Mayes JS, Becker WC, Watson GH, Mirando MA, Bazer FW. Intrauterine injection of ovine interferon-Tau alters estrogen receptor and oxytocin receptor expression in the endometrium of cyclic ewes. *J Mol Endocrinol* 1995; **15**:203–220.
18. Fuchs AR, Behrens O, Helmer H, Liu CH, Barros CM, Fields MJ. Oxytocin and vasopressin receptors in bovine endometrium and myometrium during the estrous cycle and early pregnancy. *Endocrinology* 1990; **127**:629–636.
19. Meyer MD, Hansen PJ, Thatcher WW, Drost M, Badinga L, Roberts RM, Li J, Ott TL, Bazer FW. Extension of corpus luteum lifespan and reduction of uterine secretion of prostaglandin F2α of cows in response to recombinant interferon-τ. *J Dairy Sci* 1995; **78**:1921–1931.
20. Schallenberger E, Schams D, Meyer HHD. Sequences of pituitary, ovarian and uterine hormone secretion during the first 5 weeks of pregnancy in dairy cattle. *J Reprod Fertil* 1989; **37**:277–286.
21. Wolfenson D, Thatcher WW, Drost M, Caton D, Foster DB, LeBlanc MM. Characteristics of prostaglandin F measurements in the ovarian circulation during the oestrous cycle and early pregnancy in the cow. *Reproduction* 1985; **75**:491–499.
22. Kindahl H, Edquist L, Bane A, Granstrom E. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin F 2 alpha during the normal oestrous cycle and early pregnancy in heifers. *Eur J Endocrinol* 1976; **82**:134–149.
23. Betteridge KJ, Randall GCB, Eaglesome MD, Sugden EA. The influence of pregnancy on PGF2-Alpha secretion in cattle. 1. Concentrations of 15-Keto-13,14-dihydroprostaglandin F2-Alpha and progesterone in peripheral blood of recipients of transferred embryos. *Anim Reprod Sci* 1984; **7**:195–216.
24. Lewis GS, Wilson L, Wilks JW, Pexton JE. PGF2α and its metabolites in uterine and jugular venous plasma and endometrium of ewes during early pregnancy. *J Anim Sci* 1977; **6**:320–327.
25. Silvia WJ, Ottobre JS, Inskeep EK. Concentrations of prostaglandins E2, F2α and 6-keto-prostaglandin F1α in the utero-ovarian venous plasma of nonpregnant and early pregnant ewes. *Biol Reprod* 1984; **30**:936–944.
26. Nett TM, Staigmiller RB, Diekman MA, Ellinwood WE, Niswender GD. Secretion of prostaglandin F2α in cycling and pregnant ewes. *J Anim Sci* 1976; **42**:876–880.
27. Peterson AJ, Tervit HR, Fairclough RJ, Havik PG, Smith JF, Zealand N. Jugular levels of 13,14-dihydro-15-keto-prostaglandin F and progesterone around luteolysis and early pregnancy in the ewe. *Prostaglandins* 1976; **12**:551–558.
28. Wilson L, Butcher RL, Inskeep EK. Prostaglandin F2α in the uterus of ewes during early pregnancy. *Prostaglandins* 1972; **1**:479–482.
29. Zarco L, Stabenfeldt GH, Basu S, Bradford GE, Kindahl H. Modification of prostaglandin F-2 alpha synthesis and release in the ewe during the initial establishment of pregnancy. *Reproduction* 1988; **83**:527–536.
30. Pinaffi FLV, Araujo ER, Ginther OJ. Concentrations of a PGF2α metabolite during pregnancy on the days that luteolysis occurs in nonbred heifers. *Domest Anim Endocrinol* 2018; **62**:76–82.
31. Ealy AD, Yang QE. Control of interferon-tau expression during early pregnancy in ruminants. *Am J Reprod Immunol* 2009; **61**:95–106.
32. Ezashi T, Imakawa K. Transcriptional control of IFNT expression. *Reproduction* 2017; **154**:F21–F31.
33. Rowson LE, Moor RM. The influence of embryonic tissue homogenate infused into the uterus, on the life-span of the corpus luteum in the sheep. *Reproduction* 1967; **13**:511–516.
34. Guillomot M, Michel C, Gaye P, Charlier N, Trojan J, Martal J. Cellular localization of an embryonic interferon, ovine trophoblastin and its mRNA in sheep embryos during early pregnancy. *Biol Cell* 1990; **68**:205–211.
35. Ealy AD, Larson SF, Liu L, Alexenko AP, Winkelman GL, Kubisch HM, Bixby JA, Roberts RM. Polymorphic forms of expressed bovine interferon-tau genes: relative transcript abundance during early placental development, promoter sequences of genes and biological activity of protein products. *Endocrinology* 2001; **142**:2906–2915.
36. Fuchs AR, Rollyson MK, Meyer M, Fields MJ, Minix JM, Randel RD. Oxytocin induces prostaglandin F2 alpha release in pregnant cows: influence of gestational age and oxytocin receptor concentrations. *Biol Reprod* 1996; **54**:647–653.
37. Kindahl H, Edqvist L-E, Granstrism E. The release of prostaglandin F2α as reflected by 15-Keto-13,14-dihydroprostaglandin F2α in the peripheral ciculation during normal luteolysis in heifers. *Prostaglandins* 1976; **11**:871–878.
38. Bridges PJ, Wright DJ, Buford WI, Ahmad N, McCormick ML, Schrick FN, Dailey RA, Lewis PE, Inskeep EK. Ability of induced corpora lutea to maintain pregnancy in beef cows. *J Anim Sci* 2000; **78**:2942–2949.
39. Baez GM, Trevisol E, Barletta RV, Cardoso BO, Ricci A, Guenther JN, Cummings NE, Wiltbank MC. Proposal of a new model for CL regression or maintenance during pregnancy on the basis of timing of regression of contralateral, accessory CL in pregnant cows. *Theriogenology* 2017; **89**:214–225.
40. Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. *Theriogenology* 1995; **44**:915–923.
41. Souza AH, Ayres H, Ferreira RM, Wiltbank MC. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology* 2008; **70**:208–215.
42. Herlihy MM, Giordano JO, Souza AH, Ayres H, Ferreira RM, Keskin A, Nascimento AB, Guenther JN, Gaska JM, Kacuba SJ, Crowe MA, Butler ST et al. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. *J Dairy Sci* 2012; **95**:7003–7014.
43. Ginther OJ, Shrestha HK, Fuenzalida MJ, Shahiduzzaman AKM, Beg MA. Characteristics of pulses of 13,14-dihydro-15-keto-Prostaglandin F2alpha before, during, and after spontaneous luteolysis and temporal intrapulse relationships with progesterone concentrations in cattle. *Biol Reprod* 2010; **82**:1049–1056.
44. Ochoa JC, Penagaricano F, Baez GM, Melo LF, Motta JCL, Garcia-Guerra A, Meidan R, Ferreira JCP, Sartori R, Wiltbank MC. Mechanisms for rescue of corpus luteum during pregnancy: gene expression in bovine corpus luteum following intrauterine pulses of prostaglandins E-1 and F-2 alpha. *Biol Reprod* 2018; **98**:465–479.
45. Wiltbank MC, Baez GM, Garcia-Guerra A, Toledo MZ, Monteiro PLJ, Melo LF, Ochoa JC, Santos JEP, Sartori R. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. *Theriogenology* 2016; **86**:239–253.
46. Greco LF, Neves Neto JT, Pedrico A, Lima FS, Bisinotto RS, Martinez N, Ribeiro ES, Thatcher WW, Staples CR, Santos JEP. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on spontaneous luteolysis in lactating dairy cows. *J Dairy Sci* 2018; **101**:10536–10556.
47. Harvey D, Basu S, Betteridge KJ, Goff AK, Kindahl H. The Influence of pregnancy on PGF2-Alpha secretion in cattle .2. Urinary levels of 11-Ketotetranor PGF metabolites and plasma progesterone concentrations during the estrous cycle and early pregnancy. *Anim Reprod Sci* 1984; **7**:217–234.
48. Ginther OJ, Shrestha HK, Fuenzalida MJ, Shahiduzzaman AKM, Hannan MA, Beg MA. Intrapulse temporality between pulses of a metabolite of prostaglandin F-2 alpha and circulating concentrations of progesterone before, during, and after spontaneous luteolysis in heifers. *Theriogenology* 2010; **74**:1179–1186.
49. Ginther OJ, Araujo RR, Palhão MP, Rodrigues BL, Beg MA. Necessity of sequential pulses of prostaglandin F2 alpha for complete physiologic luteolysis in cattle. *Biol Reprod* 2009; **80**:641–648.
50. Atli MO, Bender RW, Mehta V, Bastos MR, Luo W, Vezina CM, Wiltbank MC. Patterns of gene expression in the bovine corpus luteum following repeated intrauterine infusions of low doses of prostaglandin F2alpha. *Biol Reprod* 2012; **86**:130.

51. Ochoa JC, Peñagaricano F, Baez GM, Melo LF, Motta JC, Guerra AG, Meidan R, Ferreira JCP, Sartori R, Wiltbank MC. Mechanisms for rescue of CL during pregnancy: Gene expression in bovine CL following intrauterine pulses of prostaglandins E1 and F2α. *Biol Reprod* 2018; **98**:465–479.
52. Sartori R, Haughian JM, Shaver RD, Rosa GJ, Wiltbank MC. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J Dairy Sci* 2004; **87**:905–920.
53. Ginther OJ, Knopf L, Kastelic JP. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *Reproduction* 1989; **87**:223–230.
54. Kubo T, Iga K, Fukuju N, Kizaki K, Osawa T, Izaike Y, Takahashi T. Different prostaglandin F2 alpha secretion in response to oxytocin injection between pregnant and non-pregnant cows: effect of the day of oxytocin challenge test for determining the difference. *Anim Sci J* 2018; **89**:332–339.
55. Pexton JE, Weems CW, Inskeep EK. Prostaglandins F in uterine venous plasma, ovarian arterial and venous plasma and in ovarian and luteal tissue of pregnant and nonpregnant ewes. *J Anim Sci* 1975; **41**:154–159.
56. Shrestha HK, Beg MA, Burnette RR, Ginther OJ. Plasma clearance and half-Life of prostaglandin F2alpha: a comparison between mares and heifers. *Biol Reprod* 2012;**87**:ARTN 18.
57. Imakawa K, Bai R, Nakamura K, Kusama K. Thirty years of interferon-tau research: past, present and future perspective. *Anim Sci J* 2017; **88**:927–936.
58. Rawlings NC, Hyland JH. Prostaglandin F and E levels in the conceptus, uterus and plasma during early pregnancy in the ewe. *Prostaglandins* 1985; **29**:933–951.
59. Shemesh M, Milaguir F, Ayalon N, Hansel W. Steroidogenesis and prostaglandin synthesis by cultured bovine blastocysts. *Reproduction* 1979; **56**:181–185.
60. Silvia WJ, Niswender GD. Maintenance of the corpus luteum of early pregnancy in the ewe. IV. Changes in luteal sensitivity to prostaglandin F2-alpha throughout early pregnancy. *J Anim Sci* 1986; **63**:1201–1207.
61. Silvia WJ, Niswender GD. Maintenance of the corpus luteum of early pregnancy in the ewe. III. Differences between pregnant and nonpregnant ewes in luteal responsiveness to prostaglandin F2-alpha. *J Anim Sci* 1984; **59**:746–753.
62. Ford SP, Chenault JR, Echternkamp SE. Uterine blood flow of cows during the oestrous cycle and early pregnancy: effect of the conceptus on the uterine blood supply. *Reproduction* 1979; **56**:53–62.
63. Panarace M, Garnil C, Marfil M, Jauregui G, Lagioia J, Luther E, Medina M. Transrectal Doppler sonography for evaluation of uterine blood flow throughout pregnancy in 13 cows. *Theriogenology* 2006; **66**: 2113–2119.
64. Weems YS, Lennon E, Uchima T, Raney A, Goto K, Ong A, Zaleski H, Weems CW. Mechanism whereby nitric oxide (NO) infused chronically intrauterine in ewes is antiluteolytic rather than being luteolytic. *Prostaglandins Other Lipid Mediat* 2008; **85**:33–41.
65. Eley RM, Thatcher WW, Bazer FW. Hormonal and physical changes associated with bovine conceptus development. *Reproduction* 1979; **55**:181–190.
66. Killam AP, Rosenfeld CR, Battaglia FC, Makowski EL, Meschia G. Effect of estrogens on the uterine blood flow of oophorectomized ewes. *Am J Obstet Gynecol* 1973; **115**:1045–1052.
67. Bonnin P, Huynh L, Haridon RL, Chene N, Martal J. Transport of uterine PGF2α to the ovaries by systemic circulation and local lymphovenous-arterial diffusion during luteolysis in sheep. *Reproduction* 1999; **166**:199–210.
68. Mapletoft RJ, Del Campo MR, Ginther OJ. Local venoarterial pathway for uterine-induced luteolysis in cows. *Exp Biol Med* 1976; **153**: 289–294.
69. Procknor M, Dachir S, Owens RE, Little DE, Harms PG. Temporal relationship of the pulsatile fluctuation of luteinizing hormone and progesterone in cattle: a time series cross-correlation analysis. *J Anim Sci* 1986; **62**:191–198.
70. Ginther OJ, Fuenzalida MJ, Shrestha HK, Beg MA. Concomitance of luteinizing hormone and progesterone oscillations during the transition from preluteolysis to luteolysis in cattle. *Domest Anim Endocrinol* 2011; **40**:77–86.
71. Ginther OJ, Fuenzalida MJ, Pugliesi G, Hannan MA, Beg MA. Effect of luteinizing hormone oscillations on progesterone concentrations based on treatment with a gonadotropin-releasing hormone antagonist in heifers. *Domest Anim Endocrinol* 2011; **40**:119–127.
72. Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17 beta in dairy cattle. *J Dairy Sci* 2002; **85**:2831–2842.
73. Endo N, Nagai K, Tanaka T, Kamomae H. Changes in plasma progesterone levels in the caudal vena cava and the jugular vein and luteinizing hormone secretion pattern after feeding in lactating and non-lactating dairy cows. *J Reprod Dev* 2013; **59**:107–114.