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Research Article

Oxytocin-induced prostaglandin F2-alpha release is low in early bovine pregnancy but increases during the second month of pregnancy†

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Abstract

Circulating prostaglandin F2α metabolite (PGFM) after an oxytocin challenge was evaluated throughout the first 2 months of pregnancy in lactating Holstein cows. On day 11, 18, and 25 after artificial insemination (AI), and on days 32, 39, 46, 53, and 60 of pregnancy, cows were challenged with 50 IU oxytocin, i.m. Blood was collected before (0 min), 30, 60, 90, and 120 min after oxytocin for plasma PGFM concentrations. Ultrasound evaluations were performed for pregnancy diagnosis on day 32–60 post-AI. Nonpregnant (NP) cows on day 18 were designated by a lack of interferon-stimulated genes in peripheral blood leukocytes and Pregnant (P) based on day 32 ultrasound. On day 11, P and NP were similar with low PGFM and no effect of oxytocin on PGFM. On day 18, oxytocin increased PGFM (3-fold) in NP with little change in P cows. Comparing only P cows from day 11 to 60, basal circulating PGFM increased as pregnancy progressed, with day 11 and 18, lower than all days from day 25 to 60 of pregnancy. Oxytocin-induced PGFM in P cows on day 25 was greater than P cows on day 18 (2.9-fold). However, oxytocin-induced PGFM was lower on day 25 compared to day 53 and 60, with intermediate values on day 32, 39, and 46 of pregnancy. Thus, the corpus luteum (CL) of early pregnancy (day 11, 18) is maintained by suppression of PGF, as reflected by suppressed PGFM in this study. However, during the second month of pregnancy, uterine PGF secretion was not suppressed since basal PGFM and oxytocin-induced PGFM secretion were elevated. Apparently, mechanisms other than suppression of oxytocin receptors maintain CL after day 25 of pregnancy.

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Summary Sentence

On day 18 of pregnancy, there is suppressed PGF production in response to oxytocin; however, at day 25–60 of pregnancy, there is increased basal PGF and increased oxytocin-induced PGF indicating the presence of oxytocin receptors and different mechanisms maintaining CL of pregnancy after day 25.

Key words: bovine, conceptus, pregnancy, oxytocin, prostaglandins

Introduction

Luteolysis involves dynamic functional and structural changes in the corpus luteum (CL) that result in the elimination of luteal progesterone (P4) production in about a 24 h period and a complete breakdown of CL integrity, blood flow, and tissue volume [1–3]. In ruminants, the secretion of prostaglandin $F_{2\alpha}$ (PGF) from the uterus clearly underlies the luteolytic process, with multiple distinct pulses of uterine PGF observed during luteolysis [1, 3–7]. These PGF pulses are initiated by oxytocin pulses secreted from the posterior pituitary gland [3, 8–10]. The acquisition of uterine oxytocin responsiveness is key to the timing of luteolysis in ruminants. The expression of oxytocin receptors in the endometrium occurs just before the time of normal luteolysis, initiated by follicular estradiol-17β binding to ESR1 (estrogen receptor alpha) in the endometrial cells [11–14]. Expression of endometrial oxytocin receptors allows oxytocin to activate a cascade of enzymes that release arachidonic acid from membrane phospholipids and convert it into PGF that is secreted in pulses [3, 15, 16]. Some of the PGF from the oxytocin-induced pulses is transported locally from the utero-ovarian vein to the ipsilateral ovarian artery and these PGF pulses induce the luteolytic process [3, 7, 17, 18]. Thus, oxytocin pulses, endometrial oxytocin responsiveness, and oxytocin-induced pulses of PGF are central to the process that eliminates the CL in a nonpregnant (NP) ruminant and leads to initiation of a new estrous cycle.

During early pregnancy, there is a blockade of the luteolytic process due to the secretion of interferon-tau (IFNT) by the elongating embryo. The IFNT is an antiviral, antiproliferative, and immunomodulatory molecule that is secreted by the trophectoderm of the ruminant conceptus, but only during a limited stage of pregnancy, with maximal secretion between day 17 and 20 after breeding [19–23]. Early reports showed that early pregnancy or intrauterine infusion of IFNT inhibited the normal induction of endometrial oxytocin receptors, probably by inhibiting expression of endometrial estrogen receptors [14, 24, 25]. In addition, IFNT stimulates the expression of specific genes, including a group of characteristic genes, termed the interferon-stimulated genes (ISGs). The ISGs have been detected during early ruminant pregnancy in many tissues including endometrium, CL, and peripheral blood leukocytes (PBL) [26]. The detection of ISGs in PBL has been used as a marker of early pregnancy [27–32]. Nevertheless, there is a dramatic decrease in ISGs that are detected in PBL by day 25 of pregnancy [27, 32], reflecting the loss of IFNT expression in the embryo by day 25 [19, 33]. Consistent with this idea, intrauterine infusion of ovine embryonic homogenates from day 14–15 extended CL lifespan, while homogenates of day 21–25 embryos did not alter CL lifespan [34, 35]. Thus, IFNT is critical for blocking luteolysis and maintaining the CL of early pregnancy but is not present and is likely not responsible for maintaining the CL in the second month of pregnancy.

The mechanisms that maintain the ruminant CL during the second month of pregnancy have not been adequately investigated. Recently, pulsatile PGF patterns have been found during the second month of pregnancy in dairy cattle suggesting that inhibition of uterine PGF secretion may not be the mechanism responsible for maintaining the CL after the first month of pregnancy [36]. The measurement of secreted PGF or PGFM after an oxytocin challenge has been an important method for evaluating uterine oxytocin responsiveness in P and NP ruminants [11, 25, 37–39]. Treatment with oxytocin on day 18–20 of the cycle or pregnancy increased circulating PGFM in NP heifers, however, in P heifers this response was suppressed [37, 40, 41]. A similar suppression of oxytocin-induced PGFM has been observed when IFNT is infused into the uterus [42, 43]. Few studies have evaluated oxytocin action or oxytocin receptor expression after the first month of bovine pregnancy [44, 45]. One study reported that 100 IU of oxytocin, i.v. increased circulating PGFM on day 50 of pregnancy but the response was much greater in cows treated with oxytocin on day 150, 250, and 280 of pregnancy [44]. Similarly, the oxytocin receptor was detectable but low on day 50 of pregnancy and increased 6-fold by day 280 of pregnancy [44]. In addition, endometrial oxytocin receptors were reported to be low on day 20 (165 fmol/mg protein) and day 50 (344 fmol/mg) of pregnancy but increased during later pregnancy [45]. No previous studies have systematically evaluated the changes in oxytocin responsiveness of the uterus during the first and second month of pregnancy.

Therefore, we hypothesized that oxytocin responsiveness, in terms of circulating PGFM profile after an oxytocin challenge, changes during the first 2 months of pregnancy in cattle. Our first specific hypothesis was that an oxytocin challenge would induce a much smaller increase in circulating PGFM on day 18 of pregnancy compared to day-18 NP cows. Our second hypothesis was that the PGFM response to an oxytocin challenge would increase as the pregnancy progressed with day 18 being lower than day 25 and subsequent increases in PGFM response to oxytocin on day 32 until day 60 of pregnancy. Thus, the main objective of this study was to evaluate and characterize the profile of PGFM before and during an oxytocin challenge, using a week-by-week systematic approach, throughout the first 60 days of pregnancy in lactating dairy cows.

Material and methods

Experimental procedures

The experiment was conducted at Arlington Agricultural Research Station–University of Wisconsin–Madison, Arlington, WI. Animals were handled in accordance with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research, under an animal protocol that was approved by the Animal Care and Use Committee of the University of Wisconsin–Madison (RARC# A05712-A01). A total of 171 lactating Holstein cows ($n = 14$ primiparous and $n = 157$ multiparous), at 170.9 ± 4.1 DIM (ranging from 85 to 289), were enrolled in the experiment. Cows received the same TMR to meet or exceed the nutrient requirements for a lactating Holstein cow producing 50 kg of milk/d with

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Timeline								
Days after AI (N)	11 (n = 23)	18 (n = 23)	25 (n = 30)	32 (n = 13)	39 (n = 13)	46 (n = 12)	53 (n = 13)	60 (n = 12)
Non-Pregnant	Yes	Yes	No	No	No	No	No	No
Pregnant	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Oxytocin Challenge	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
ISGs blood sampling	Yes	Yes	No	No	No	No	No	No
Pregnancy Diagnosis	No	No	No	Yes	Yes	Yes	Yes	Yes
PSPB blood sampling	No	No	Yes	Yes	Yes	Yes	Yes	Yes

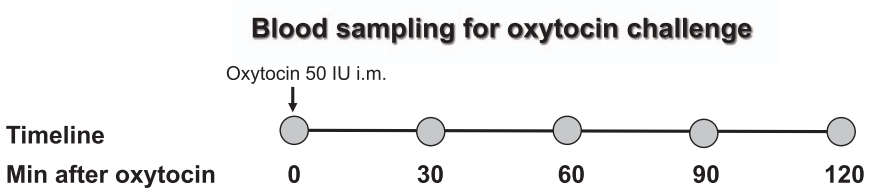

Figure 1. Schematic experimental design showing the measurements that were performed on different days and the timing of oxytocin treatment and blood sample collection.

3.5% fat and 3.1% true protein when DM intake was 24 kg/day [46]. Cows were submitted to an Ovsynch protocol (GnRH—7 days—PGF—1 day—PGF—32 h—GnRH—16 h—artificial insemination (AI) [47]) and all cows received AI at a fixed time. On specific days after AI, day 11 ($n = 23$), day 18 ($n = 23$), day 25 ($n = 30$), day 32 ($n = 13$), day 39 ($n = 13$), day 46 ($n = 12$), day 53 ($n = 13$), and day 60 ($n = 12$), cows were enrolled in the experiment.

Independent of the day after AI, all cows were submitted to an oxytocin challenge as described in Figure 1. A blood sample was collected by puncture of the coccygeal vein or artery, at time 0, before the challenge, in order to obtain basal circulating concentration of PGFM, P4, and pregnancy-specific protein B (PSPB). After collection of the first blood sample, cows received 50 IU i.m. of oxytocin (Agrilab, St. Joseph, MO) as described by Macuhová et al. [48] and had serial blood samples collected at times 30, 60, 90, and 120 min after challenge, respectively. This treatment was chosen in order to provide an oxytocin challenge greater than what normally occurs during milking, however not as acute and high as if oxytocin were given i.v. and in higher doses.

Blood samples were collected into heparinized tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) that were placed on ice and kept refrigerated until transported to the laboratory within 4 to 5 h for processing. Blood tubes were centrifuged at 1700 × *g* for 15 min at 4°C for plasma separation. Aliquots of plasma were frozen at −20°C until assayed.

On day 18 for cows from groups, day 11 and 18, blood samples were collected into a Tempus^{TM} blood RNA tube (Applied Biosystems, Foster City, CA) following manufacturer's instructions for ISGs analysis. On days 32, 39, 46, 53, and 60 after AI, a transrectal ultrasound exam using a real-time B-mode ultrasound scanner (Ibex Pro+Lite, E. I. Medical Imaging, Loveland, CO) equipped with a 7.5 MHz linear-array transducer was performed in all cows by the same technician, in order to identify the amniotic vesicle and heartbeat of the embryo/fetus as pregnancy diagnosis. For day 25, analyses were only done with cows that were diagnosed pregnant on day 32 and day 60. Information for cows that were collected on day 25 but were NP was not used.

Hormone assays

Plasma samples were assayed for PGFM by an ELISA assay that was previously validated for use in bovine plasma [49] with some modifications as described by Ochoa et al. [50]. The intra- and inter-assay CVs were 4.7% and 15.3%, respectively, and the sensitivity of the assay was 5.5 pg/mL.

Concentrations of P4 were determined using a solid-phase RIA kit containing antibody-coated tubes and 125I-labeled P4 (ImmuChem Coated Tube P4 125 RIA Kit, MP Biomedicals, Costa Mesa, CA) as described previously for mares [51] and validated for bovine plasma in our laboratory [52]. The intra- and inter-assay CVs and the sensitivity were 4.4%, 8.1%, and 0.08 ng/mL, respectively.

The PSPB concentrations were analyzed on day 25 to day 60 of pregnancy at time 0 (before the challenge) and on day 53 for all samples during the oxytocin challenge. A commercially available quantitative ELISA assay was used (Biopryn, BioTracking LLC, Moscow, ID) as previously described [53]. The inter- and intra-assay CVs were 2.3% and 2.2%, respectively for the two plates.

All cows from day 11 and 18 had whole blood collected into evacuated tubes for evaluation of ISGs in PBL (Tempus Blood RNA tubes, Applied Biosystems, Foster City, CA). After collection, samples were stored at −20°C until RNA extraction and DNAse treatment was performed using a commercial kit (Tempus, Spin RNA isolation kit, Cat. No. 4380204, Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Isolated RNA was evaluated for concentration and purity using a NanoDrop 2000

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Table 1. Gene, primer orientation, primer sequence (5′ → 3′), and National Center for Biotechnology Information (NCBI) access number and sequence for primers used in RT-qPCR assay.

Gene	Primer	Sequence (5′ → 3′)	NCBI sequence
ACTB	Forward	CTGGACTTCGAGCAGGAGAT	AY141970
	Reverse	GATGTCGACGTCACACTTC	
ISG15	Forward	GGTATCCGAGCTGAAGCAGTT	NM_174366
	Reverse	ACCTCCCTGCTGTCAAGGT	
RPL19	Forward	ATTGACCGCCACATGTATCA	NM_001040516
	Reverse	GCGTGCTTCCTTGGTCTTAG	
MX2	Forward	CTTCAGAGACGCCTCAGTCG	NM_173941
	Reverse	TGAAGCAGCCAGGAATAGTG	

spectrophotometer (Thermo Scientific, Rockford, IL). A total of 250 ng of RNA were reverse transcribed to complementary DNA using a commercial kit (iScript reverse transcription supermix for RT-qPCR, Cat. No. 1708841, BioRad, Hercules, CA) following manufacturer's instructions. After an initial activation at 60°C for 2 min followed by denaturation at 95°C for 10 min, the amplification protocol followed 40 cycles of 95°C for 15 s and 60°C for 1 min. Each sample was evaluated in triplicate, and the specificity for amplification was verified by melting curve analysis. Four genes were investigated (Table 1), including the two reference genes, beta-actin (*ACTB*) and ribosomal protein L19 (*RPL19*), and two target genes, *ISG15* and MX2.

Data handling and statistical analysis

The ISGs data were analyzed using the $\Delta\Delta C_t$ method and blood samples from cows on day 11 and 18 were used to confirm NP cows. Data from day 11 were used as a negative control for ISGs. On day 18, the expression of ISGs from cows confirmed pregnant by ultrasound on day 32 was used as a positive control. The highest value of ISG15 and MX2 expression in fold change on day 11 was determined as a cutoff value (2.5 and 2.3, respectively). Cows on day 11 with confirmed absence of any sign of an embryo on day 32 were considered NP, although because all cows had been bred, a conceptus could have been present on day 11 but died prior to pregnancy diagnosis. Cows on day 18 that were diagnosed NP on day 32 and that had higher expression of ISG15 and MX2 mRNA than the cutoff value were excluded from subsequent analyses. Cows were only considered NP if they had low expression of ISGs on day 18 with confirmed absence of any sign of an embryo on day 32. For groups day 25, 32, 39, 46, 53, and 60, cows were diagnosed for pregnancy on day 32 and had pregnancy confirmed weekly.

Continuous data were tested for normality of residuals using the UNIVARIATE procedure according to Shapiro–Wilk test. Data with residuals not normally distributed were transformed to logarithm, square root, or inverse scale before analysis if the residual distribution was improved. In addition, outliers were removed when necessary, and then data were analyzed using the MIXED procedure of Statistical Analysis System (SAS, Version 9.4 for Windows, SAS Institute Inc., Cary, NC). *Tukey* honest significant difference test was performed to determine differences.

For P4, data were analyzed before oxytocin challenge (time 0) to obtain the basal concentrations. The P4 data on day 11 and 18 at time 0 were analyzed separately to compare potential differences between P and NP cows. The model included the effect of pregnancy and day after AI and their interaction. Another comparison was made for P cows from day 25 to 60 and included the effect of day of pregnancy.

In order to detect the effect of oxytocin on circulating P4, data were analyzed as repeated measures over time. The same comparisons and models used for basal P4 were performed, but included time (before and after oxytocin) as the repeated statement in which before was the circulating P4 at time 0 and after was the average of P4 concentration on 30, 60, 90, and 120 min after challenge.

In order to evaluate week-to-week differences in circulating PGFM in response to oxytocin challenge, comparisons were performed between specific days of pregnancy, in which challenge time (0, 30, 60, 90, and 120 min) was considered the repeated statement, and the effects of the day, pregnancy, and their interaction were evaluated. Comparisons that were made included: day 18 and 11 for P vs NP cows, day 18-P vs 25, day 25 vs 32, day 32 vs 39, day 39 vs 46 vs 53 vs 60.

To characterize the variation in circulating PGFM for day 18 NP cows, the distribution of PGFM before (time 0) and after challenge (average of 30, 60, 90, and 120 min) was presented with data organized for each cow numbered from 1 to 8.

The basal PGFM, considering the circulating concentration at challenge time 0, was compared among groups. The model was composed of fixed effects of pregnancy, day and their interaction.

Another analysis evaluated circulating PGFM concentration in response to oxytocin, comparing the groups (day 11 [P or NP], day 18 [P or NP], day 25, 32, 39, 46, 53, and 60 [P]). In order to obtain the isolated effect of oxytocin challenge, the baseline concentration of PGFM at time 0 (before challenge) was subtracted from averaged results of 60 to 120 min after oxytocin. Mean responses were compared among groups. In this analysis, the effects of day and pregnancy status, as well as their interaction, were included in the model.

The basal PSPB before the challenge (time 0) was compared among days of pregnancy, from day 25 to 60. For this analysis, the effect of day was included.

To demonstrate the potential effect of the oxytocin challenge on circulating PGFM, P4, and PSPB, the circulating concentrations of all three variables from cows on day 53 of pregnancy were analyzed during challenge times (0, 30, 60, 90, and 120 min). In this case, data were analyzed as repeated measures, using time as the repeated statement. However, since the analysis was performed using a single day, only the effect of challenge time was considered in the model.

Differences were considered significant for $P \leq 0.05$, whereas a tendency was designated when $P < 0.10$ and $P > 0.05$. Data are presented as least squares means ± SEM.

Results

Three cows from day 18 that were diagnosed as NP on day 32, had elevated ISGs on day 18 and, therefore, were excluded from the analyses. One cow from the NP group, had low P4 on day

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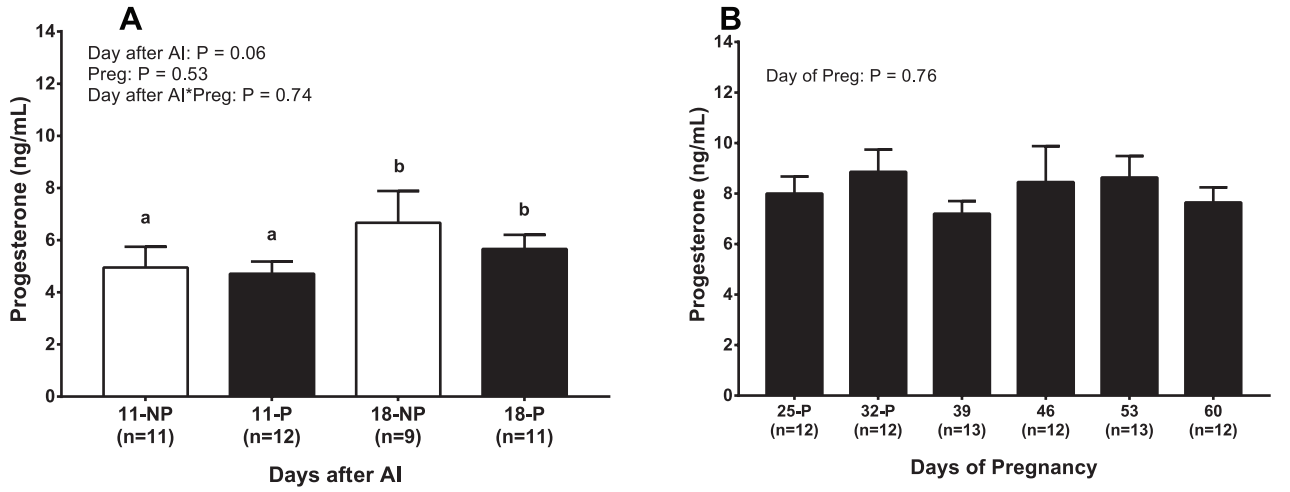

Figure 2. Basal concentrations of P4 for the groups. (A) Comparison between day 18 and 11, pregnant (P) and nonpregnant (NP). (B) Comparisons among groups on days of pregnancy from day 25 to 60. Data are shown as least squares means ± SEM. $^{a,b}P \leq 0.05$.

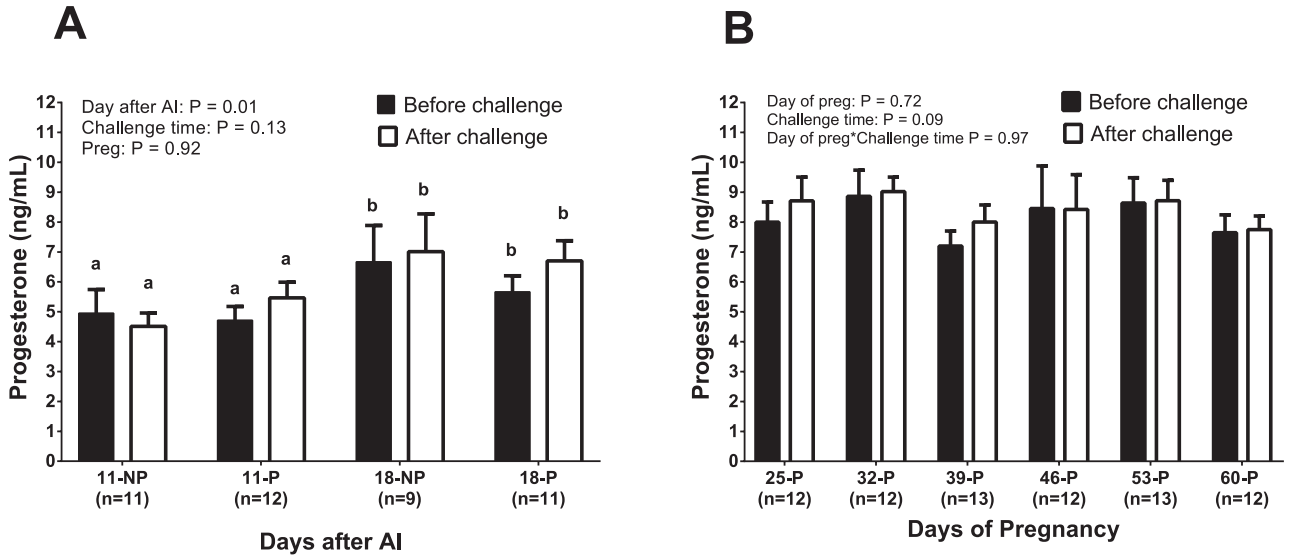

Figure 3. Circulating P4 concentrations before (time 0) and after (average of 30, 60, 90, and 120 min) oxytocin challenge. Interactions not shown in the figure are not significant: day after AI* challenge time: $P = 0.56$; day after AI*Preg: $P = 0.44$; day after AI*challenge time*Preg: $P = 0.37$. Data are shown as least squares means ± SEM. $^{a,b}P \leq 0.05$.

18 (≤0.15 ng/mL), indicating that this cow had already undergone luteolysis and this cow was not used in the analyses of PGFM.

Basal circulating P4 (Time 0) tended to be greater for day 18 compared to day 11 (Figure 2A; $P = 0.06$) but there was no effect of pregnancy ($P = 0.53$). After day 25 there was no effect of day ($P = 0.76$) on basal circulating P4 (Figure 2B). Challenge time 30 to 120 was averaged and compared to 0 min (basal concentration) to evaluate the effect of oxytocin challenge on circulating P4. As shown in Figure 3A, on day 18 (before and after challenge), the cows had greater P4 than on day 11 ($P = 0.01$); however, there was no effect of oxytocin challenge (challenge time; $P = 0.13$) or pregnancy ($P = 0.92$) on circulating P4. Interactions among factors were not significant (see legend; $P > 0.10$). A similar comparison of circulating P4 after day 25 of pregnancy (Figure 3B) detected no effect of day of pregnancy ($P = 0.72$) and no interaction between challenge time and day of pregnancy ($P = 0.97$). Curiously circulating P4 tended to be slightly greater after oxytocin challenge ($P = 0.09$), although there were no detectable differences before and after challenge for any day of pregnancy (analyzed within a day).

In Figure 4A, on day 11 (P or NP), pregnancy ($P = 0.12$) and time had no effect ($P = 0.93$) on circulating PGFM, and there was no interaction of challenge time and pregnancy ($P = 0.84$), indicating that PGFM concentrations were constant before and after the oxytocin challenge. In contrast, on day 18, there was an effect of challenge time ($P < 0.0001$), pregnancy ($P < 0.001$), and interaction of pregnancy and challenge time (Figure 4B, $P = 0.05$) on circulating PGFM. For example, at 30 min after oxytocin treatment on day 18, NP cows had a 3.6-fold increase in circulating PGFM concentration compared to basal concentrations (50.2 ± 13.5 vs 13.8 ± 2.3, $P = 0.0001$), and continued to be greater than basal concentrations at all other times ($P \leq 0.001$): 3.2-fold greater at 60 min (44.7 ± 12.2), 5.7-fold at 90 min (79.4 ± 34.4), and 7.6-fold greater at 120 min after oxytocin (105.7 ± 35.3). On the other hand, P cows on day 18 had a minimal increase in PGFM concentrations after oxytocin

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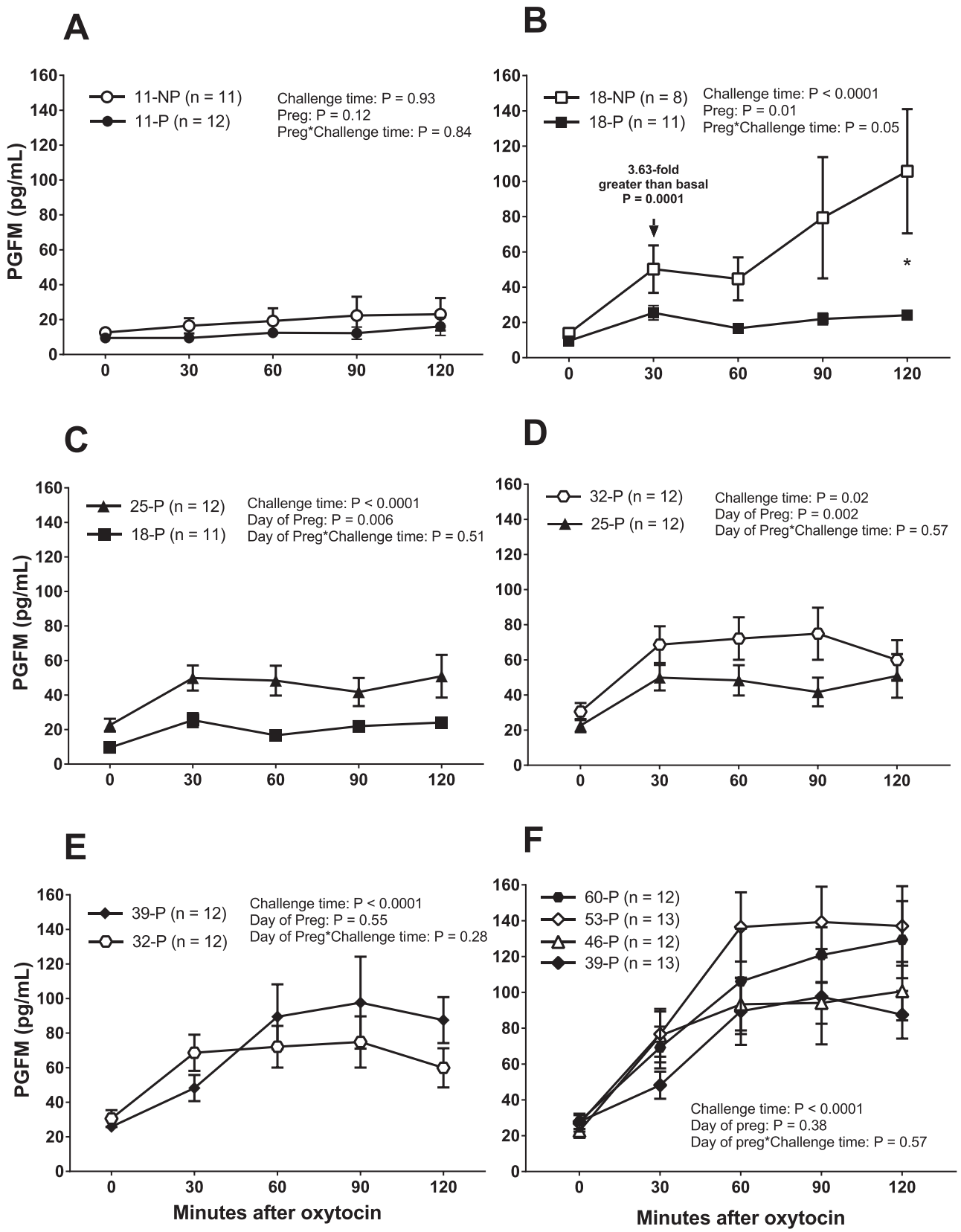

Figure 4. Response to the oxytocin challenge on circulating concentration of PGFM for cows on day 11 to 60 after AI. (A) Comparison of day 11 P vs NP cows; (B) Comparison of day 18 P vs NP cows; (C) Comparison of day 18-P vs 25-P; (D) Comparison of day 25-P vs 32-P; (E) Comparison of day 32-P vs 39-P, and (F) Comparison of day 39-P, 46-P, 53-P, and 60-P. Data are shown as least squares means ± SEM. $^{*}P \leq 0.05$.

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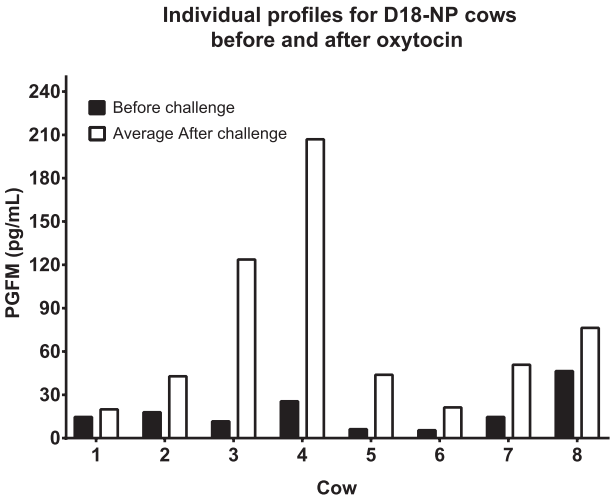

Figure 5. Individual profiles for NP cows from day 18. Data demonstrate the variation in circulating PGFM in response to oxytocin for individual cows. Cows that were pregnant on day 32 or that had high Interferon-stimulated gene (ISG) expression on day 18 are not included.

challenge with only the 30 min challenge time being different from basal concentrations (25.4 ± 4.1 vs 9.6 ± 1.2, $P < 0.01$). Concentrations of PGFM at all times after oxytocin challenge were greater in day 18-NP than day 18-P from 30 min until 120 min after oxytocin.

A comparison of P cows on day 18 vs 25 (Figure 4C) showed that there was an effect of challenge time ($P < 0.0001$) and day of pregnancy ($P = 0.006$) but no interaction ($P = 0.51$). The oxytocin-induced PGFM in P cows on day 25 (Figure 4C; 48.3 ± 8.6) was greater than in P cows on day 18 (16.7 ± 2.9; $P = 0.006$), especially at 60 min after challenge ($P = 0.01$) when values were 2.9-fold greater on day 25 than day 18. Similarly, day 32-P cows had greater circulating PGFM than day 25-P cows (Figure 4D), as indicated by an effect of day of pregnancy ($P = 0.002$), with both groups having an increase in PGFM after oxytocin ($P = 0.002$). For example, there was more than a 2-fold increase in PGFM by 30 min after oxytocin compared to basal (day 25 = 49.9 ± 7.3 vs 22.6 ± 3.7; day 32 = 68.7 ± 10.5 vs 30.6 ± 4.8; $P = 0.02$). In contrast, there was no effect of day of pregnancy when comparing day 32 vs 39-P cows ($P = 0.55$; Figure 4E) or day 39, 46, 53, and 60 of pregnancy ($P = 0.38$; Figure 4F), although there was an effect of time after oxytocin challenge in all groups.

Individual profiles for NP cows on day 18 before and after challenge (average of 30, 60, 90, and 120 min combined) are shown in Figure 5. The variation in oxytocin-induced PGFM in individual cows is evident as shown by three cows having more than a 7-fold increase in PGFM after oxytocin compared to basal PGFM (#3 = 10.6X, #4 = 8.1X, and #5 = 7.1X), three cows having intermediate values (#2 = 2.4X, #6 = 3.9X, and #7 = 3.5X), and two cows having a low response with less than a 2-fold increase after oxytocin challenge (#1 = 1.4X and #8 = 1.6X). Moreover, even before the challenge, substantial variation in basal PGFM is apparent, ranging from 5.5 to 46.4 pg/mL.

An analysis of circulating PGFM prior to the oxytocin challenge (0 min) indicated that baseline PGFM (Figure 6A) was affected by pregnancy ($P = 0.05$) and day of pregnancy ($P < 0.0001$), but there was no interaction ($P = 0.94$). Pregnant cows from day 11 and day 18, had lower basal PGFM (day 11-P = 9.5 ± 2.3 and 18-P = 9.6 ± 1.2) than all groups of P cows after day 25. In addition, NP cows on day 11 and 18 were lower (day 11-NP = 13.7 ± 2.8 and day 18-NP = 13.8 ± 2.3) than P cows on all days of pregnancy after day 25, except day 46 which had similar basal PGFM concentrations (22.7 ± 3.9) as found in NP cows on day 11 and 18. From day 25 to 60 of pregnancy (day 25 = 22.6 ± 3.7; day 32 = 30.6 ± 4.9; day 39 = 27.9 ± 2.7; day 46 = 22.7 ± 3.9; day 53 = 29.0 ± 4.5; day 60 = 28.0 ± 4.2) there was no difference among days of pregnancy ($P > 0.05$) for basal PGFM concentrations (Figure 6A).

Overall PGFM response to oxytocin increased throughout gestation and there was an interaction between the day of pregnancy and challenge time ($P < 0.001$). To determine the increase in PGFM after the oxytocin challenge, the PGFM concentrations at 60, 90, and 120 min after oxytocin challenge were averaged for a given d after AI and the baseline PGFM was subtracted to provide an average oxytocin-induced response (Figure 6B). Minimal responses to oxytocin were observed for P and NP cows on day 11 and for P cows on day 18. In addition, the PGFM response in P cows on day 25 P was intermediate and not different from day 11-P, 11-NP, and 18-P, but it was also not different from day 18-NP and 32-P. The greatest PGFM response to oxytocin was observed on day 53 and 60 of pregnancy. The PGFM response to oxytocin was similar for day 18-NP and P cows on day 25, 32, 39, and 46 (Figure 6B).

The PSPB concentrations were evaluated throughout pregnancy (Figure 7). On day 25, PSPB was at the lowest concentrations, although day 46 and 60 were not different from day 25. The PSPB on day 32 and 39 were greater than day 25, 46, and 60 and tended to be greater than day 53 ($P \leq 0.08$).

Since day 53 had the most impressive response in PGFM after the oxytocin challenge (Figure 6B), the profiles for PGFM, P4, and PSPB were evaluated before and after the oxytocin challenge (Figure 8). Circulating PGFM was greater than 0 min (26.9 ± 4.6) at 30 min (76.7 ± 12.6; $P < 0.0001$), 60 min (136.5 ± 19.3), and 120 min (137.1 ± 22.2) after oxytocin (Figure 8A). In contrast, there was no effect of the oxytocin challenge on P4 ($P = 0.55$; Figure 8B) or PSPB ($P = 0.40$; Figure 8C) concentrations.

Discussion

The uniqueness of this study was that we could determine the precise timing during pregnancy when the uterus ends refractoriness and initiates responsiveness to oxytocin, as measured by increases in circulating PGFM in response to an exogenous oxytocin challenge. As expected, the uterus was unresponsive to oxytocin during the time period of early pregnancy when normal luteolysis occurs in NP cows, consistent with previous studies [37, 39–41, 54]. These data are also somewhat consistent with results in heifers without oxytocin stimulation in which pregnant heifers had lower average PGFM concentration and less prominent peaks of PGFM compared to NP heifers at the expected time of luteolysis (day 16 to 18) [55]. However, as production of embryonic IFNT wanes by day 25 of pregnancy [19], there was an increase in basal circulating PGFM and responsiveness to oxytocin began to increase. Indeed, responsiveness to oxytocin was similar in P cows after day 25 as observed in NP cows during the normal luteolytic period (day 18). After day 50 of pregnancy, cows had even greater oxytocin responsiveness, as measured by circulating PGFM, compared to NP cows on day 18. These results provide critical insights that will help in designing future experiments on the physiological mechanisms regulating CL function during ruminant pregnancy and are likely to be of considerable practical value in providing physiological targets

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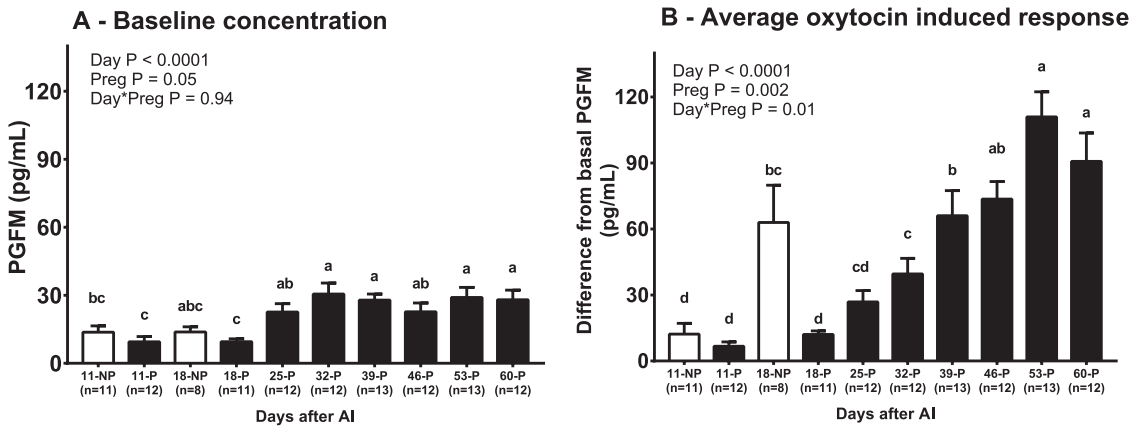

Figure 6. Basal circulating concentration of PGFM, before oxytocin challenge (0 min) during the first 60 days of pregnancy (A) and the oxytocin-induced response in PGFM concentrations 60 to 120 min after oxytocin challenge (B). The PGFM concentrations at 60, 90, and 120 min after oxytocin were averaged and the basal concentration was subtracted to obtain the induced PGFM response as the pregnancy progresses. Data are shown as least squares means ± SEM. $^{a–d}P \leq 0.05$.

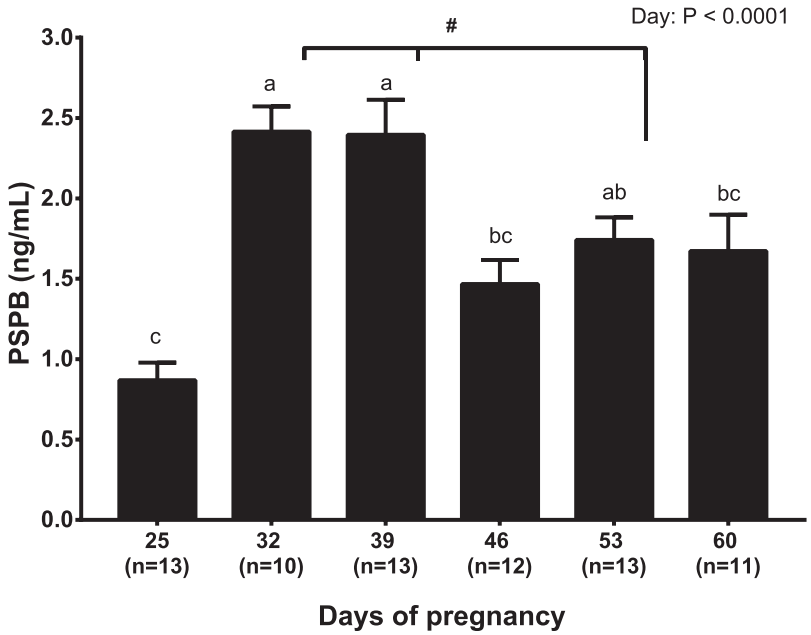

Figure 7. Circulating PSPB concentrations (ng/mL) at time 0 (before challenge) for pregnant cows from day 25 until day 60. Data are shown as least squares means ± SEM. $^{a–c}P \leq 0.05$; $^{\#}P < 0.10$.

for overcoming the substantial pregnancy losses that occur during this period in dairy cattle [56], beef cattle [57], and recipients of embryos [58].

The first hypothesis, that oxytocin responsiveness would be suppressed during early pregnancy (day 18), was clearly supported by our results and by previous studies [37, 38, 40, 41]. The underlying physiological basis for these results depends on embryonic IFNT [34, 59, 60] suppressing PGFM response to oxytocin [40–43] due to the suppression of endometrial expression of oxytocin receptor [61–63]. In contrast, NP cows have detectable endometrial oxytocin receptor [41] and a 3-fold greater response to oxytocin compared to pregnant cows on day 16 after AI [37]. Nevertheless, there was substantial variation among animals in the magnitude of the oxytocin-induced PGFM increase perhaps indicating differences in endometrial oxytocin receptor between individuals. Previous studies have shown substantial variation in timing of luteolysis in individual cows, particularly in cows with two vs three follicular waves [64, 65]. Induction of endometrial oxytocin receptors likely occurs in response to activation of ESR1 by estradiol secreted by the dominant follicle of either the second or third follicular wave [13, 65, 66]. Thus, in our study NP cows that were likely to have earlier luteolysis, associated with two follicular waves, probably had much greater uterine oxytocin responsiveness than cows that had not yet obtained sufficient oxytocin responsiveness, that is, cows that were likely to have three follicular waves.

Our second hypothesis, that oxytocin responsiveness would increase as the pregnancy progressed, was clearly supported by our results. There was a minimal increase in circulating PGFM after oxytocin treatment on day 18 but increased basal and oxytocin-induced PGFM secretion by day 25 with further increases by day 53 of pregnancy. A previous study [44] reported increased PGFM response to oxytocin challenge as pregnancy progressed (50, 150, 250, or 280 days) with a 7-fold greater increase in circulating PGFM on day 280 than on day 50. In addition to the oxytocin challenge,

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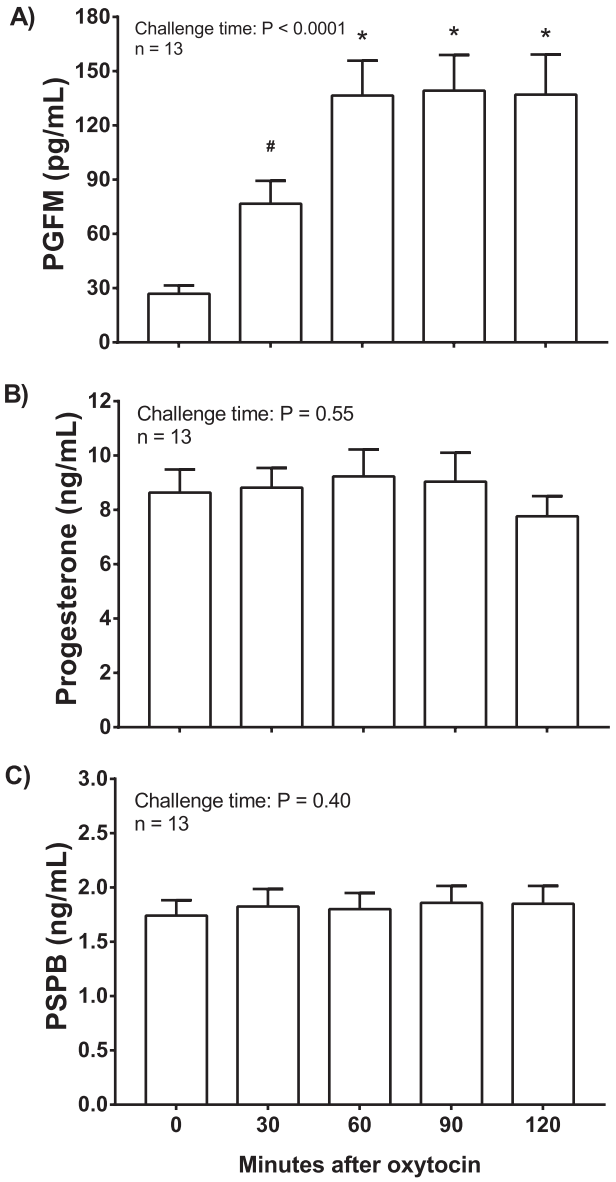

Figure 8. Comparison of changes in PGFM, P4, and PSPB in pregnant cows on day 53 before and after the oxytocin challenge. Data are shown as least squares means ± SEM. $^{*}P \leq 0.05$; $^{\#}P < 0.10$.

presence of intercaruncular endometrial oxytocin receptors was found on day 50 of pregnancy [44]. Our results extend these previous findings by demonstrating that there is an increase in basal PGFM and oxytocin-induced PGFM within the first few days after loss of IFNT secretion by the embryo, day 25 of pregnancy in our study. The previous study focused on the elevation in PGFM and oxytocin responsiveness as parturition approached, whereas our study focused on the timing of oxytocin-induced PGFM during the first 2 months of pregnancy in order to understand how the CL is maintained after IFNT secretion declines.

A previous study also detected PGFM in the posterior vena cava in pregnancies maintained by exogenous progestins or by an accessory CL that was induced during the second month of pregnancy [67]. The animals in our study all had pregnancy maintenance after the oxytocin challenge and did not have an accessory CL or treatment with exogenous progestin. Hence, our results, as well as some previous results are consistent with an increase in basal circulating PGFM during the second month of pregnancy, although they do not provide information on the source of PGF that leads to this increased basal PGFM during pregnancy.

The effects of exogenous oxytocin on circulating PGFM were dramatic during the second month of pregnancy with oxytocin-induced PGFM reaching concentrations that were as high or higher than those observed near the time of normal luteolysis in NP cows. Remarkably, in spite of the striking effect of oxytocin on circulating PGFM, there was no detectable effect on circulating P4 and none of the pregnancies were lost after the oxytocin challenge. Previous studies have shown clear decreases in circulating P4 during a similar timeframe after the administration of exogenous PGF [1, 4]. Thus, the pregnant uterus acquires clear oxytocin responsiveness during the late stages of the first month of bovine pregnancy with clear increases in this oxytocin responsiveness during the second month of pregnancy. The lack of CL regression in the face of PGFM pulses that are of a magnitude that would be expected to be luteolytic in NP cows indicate that the CL during the second month of pregnancy is maintained by mechanisms other than suppression of uterine oxytocin receptors and corresponding PGF secretion, as occurs during early bovine pregnancy. Other data have previously suggested that a parsimonious explanation for the lack of CL regression in spite of uterine PGF secretion is that PGF does not reach the CL through local mechanisms due to the elevated blood flow in the uterine horn ipsilateral to the pregnancy during the second month of pregnancy [58]. This physiologic model may also explain the regression of the contralateral accessory CL during 33–60 days of pregnancy, but continued maintenance of the pregnancy and ipsilateral CL [68] because blood flow in the uterine horn contralateral to the pregnancy increases at a slower rate than in the ipsilateral horn [69]. Thus, a local mechanism exists during the second month of pregnancy that protects the CL that is ipsilateral but not contralateral to the pregnancy.

Potential practical benefits could result from the future application of this fundamental research. Pregnancy loss prior to day 35 appears to be initiated by the death of the embryo [70]; however, during the second month of pregnancy, little is known how much is primarily related to embryonic death and what percentage is caused by inappropriate regression of the CL. It seems possible that the increase in uterine oxytocin responsiveness and PGFM secretion during the second month of pregnancy may be excessive in certain circumstances and that animals in these conditions may benefit from treatments directed at reducing PGF secretion. For example, it is well-established that recipients of cloned embryos have extremely high pregnancy loss during the second month of pregnancy with placental abnormalities and vascular problems being implicated as causative factors [71, 72]. In addition, pregnancy loss is substantial during the second month of pregnancy in lactating cattle and in recipients of *in vitro*-produced embryos [56, 73]. It seems likely that some of the pregnancy loss is due to inappropriate CL regression attributable to inadequacies in mechanisms maintaining the CL during this second month of pregnancy [74]. A delay in the mechanisms protecting the CL, such as a delayed increase in uterine blood flow, may result in inadequate inhibition of the increasing PGFM secretion that occurs at day 25 of pregnancy and beyond and untimely CL regression. The development of physiologically rational methods to overcome this pregnancy loss could lead to substantial increases in reproductive efficiency [75]. Unfortunately, no studies have clearly differentiated if a defective embryo or inappropriate CL regression underlies pregnancy loss in the second month of pregnancy, which

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could more effectively focus future research on the root cause of pregnancy losses in cattle.

In conclusion, consistent with the hypothesis in this study and previous reports, the CL of early pregnancy is maintained due to the suppression of uterine oxytocin receptors and PGF secretion likely resulting from actions of embryonic IFNT. However, during the second month of pregnancy, uterine PGF secretion was not suppressed since basal PGFM and oxytocin-induced PGF secretion was greatly elevated (equal or greater than in day 18-NP cows). These results indicate that there are alternative mechanisms for maintenance of the CL during the second month of pregnancy that do not involve suppression of uterine PGF secretion.

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References

1. Ginther OJ, Silva LA, Araujo RR, Beg MA. Temporal associations among pulses of 13,14-dihydro-15-keto-PGF(2alpha), luteal blood flow, and luteolysis in cattle. *Biol Reprod* 2007; **76**:506–513.
2. 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**:130:1–13.
3. McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. *Physiol Rev* 1999; **79**:263–323.
4. 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.
5. Kindahl H, Edqvist L-E, Bane A, Granstrom E. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin F 2a during the normal oestrous cycle and early pregnancy in heifers. *Acta Endocrinol* 1976; **82**:134–149.
6. Silvia WJ, Lewis GS, McCracken JA, Thatcher WW, Wilson L. Hormonal regulation of uterine secretion of prostaglandin F2alpha during luteolysis in ruminants. *Biol Reprod* 1991; **45**:655–663.
7. Ginther OJ, Araujo RR, Palhao MP, Rodrigues BL, Beg MA. Necessity of sequential pulses of prostaglandin F2alpha for complete physiologic luteolysis in cattle. *Biol Reprod* 2009; **80**:641–648.
8. Ginther OJ, Khan FA, Hannan MA, Beg MA. Temporal interrelationships at 15-min intervals among oxytocin, LH, and progesterone during a pulse of a prostaglandin F2 alpha metabolite in heifers. *Anim Reprod Sci* 2012; **133**:63–70.
9. Vanderwall DK, Silvia WJ, Fitzgerald BP. Concentrations of oxytocin in the intercavernous sinus of mares during luteolysis: temporal relationship with concentrations of 13,14-dihydro-15-keto-prostaglandin F2 alpha. *J Reprod Fertil* 1998; **112**:337–346.
10. Lukaszewska J, Hansel W. Corpus luteum maintenance during early pregnancy in the cow. *J Reprod Fertil* 1980; **59**:485–493.
11. Parkinson TJ, Lamming GE. Interrelationships between progesterone, 13,14-dihydro-15-keto PGF-2 alpha (PGFM) and LH in cyclic and early pregnant cows. *J Reprod Fertil* 1990; **90**:221–233.
12. Robinson RS, Mann GE, Lamming GE, Wathes DC. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. *Reproduction* 2001; **122**:965–979.
13. Araujo RR, Ginther OJ, Ferreira JC, Palhao MM, Beg MA, Wiltbank MC. Role of follicular estradiol-17beta in timing of luteolysis in heifers. *Biol Reprod* 2009; **81**:426–437.
14. Romero JJ, Antoniazzi AQ, Nett TM, Ashley RL, Webb BT, Smirnova NP, Bott RC, Bruemmer JE, Bazer FW, Anthony RV, Hansen TR. Temporal release, paracrine and endocrine actions of ovine conceptus-derived interferon-tau during early pregnancy. *Biol Reprod* 2015; **93**:146:1–10.
15. Madore E, Harvey N, Parent J, Chapdelaine P, Arosh JA, Fortier MA. An aldose reductase with 20 alpha-hydroxysteroid dehydrogenase activity is most likely the enzyme responsible for the production of prostaglandin f2 alpha in the bovine endometrium. *J Biol Chem* 2003; **278**:11205–11212.
16. Niswender GD, Juengel JL, Silva PJ, Rollyson MK, McIntush EW. Mechanisms controlling the function and life span of the corpus luteum. *Physiol Rev* 2000; **80**:1–29.
17. Moor RM. Effect of embryo on corpus luteum function. *J Anim Sci* 1968; **27** Suppl 1:97–118.
18. Moor RM, Rowson LE. The corpus luteum of the sheep: effect of the removal of embryos on luteal function. *J Endocrinol* 1966; **34**: 497–502.
19. 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.
20. Godkin JD, Bazer FW, Moffatt J, Sessions F, Roberts RM. Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at day 13-21. *J Reprod Fertil* 1982; **65**:141–150.
21. Godkin JD, Bazer FW, Roberts RM. Ovine trophoblast protein-1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein-synthesis. *Endocrinology* 1984; **114**:120–130.
22. Bazer FW, Spencer TE, Ott TL. Interferon tau: a novel pregnancy recognition signal. *Am J Reprod Immunol* 1997; **37**:412–420.
23. Roberts RM, Ealy AD, Alexenko AP, Han CS, Ezashi T. Trophoblast interferons. *Placenta* 1999; **20**:259–264.
24. Jenner LJ, Parkinson TJ, Lamming GE. Uterine oxytocin receptors in cyclic and pregnant cows. *J Reprod Fertil* 1991; **91**:49–58.
25. Wathes DC, Lamming GE. The oxytocin receptor, luteolysis and the maintenance of pregnancy. *J Reprod Fertil Suppl* 1995; **49**:53–67.
26. Oliveira JF, Henkes LE, Ashley RL, Purcell SH, Smirnova NP, Veeramachaneni DN, Anthony RV, Hansen TR. Expression of interferon (IFN)-stimulated genes in extrauterine tissues during early pregnancy in sheep is the consequence of endocrine IFN-tau release from the uterine vein. *Endocrinology* 2008; **149**:1252–1259.
27. Pugliesi G, Miagawa BT, Paiva YN, Franca MR, Silva LA, Binelli M. Conceptus-induced changes in the gene expression of blood immune cells and the ultrasound-accessed luteal function in beef cattle: how early can we detect pregnancy? *Biol Reprod* 2014; **91**:95.
28. Carvalho PD, Consentini CC, Weaver SR, Barleta RV, Hernandez LL, Fricke PM. Temporarily decreasing progesterone after timed artificial insemination decreased expression of interferon-tau stimulated gene 15 (ISG15) in blood leukocytes, serum pregnancy-specific protein B concentrations, and embryo size in lactating Holstein cows. *J Dairy Sci* 2017; **100**:3233–3242.
29. 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.
30. 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.
31. Gifford CA, Racicot K, Clark DS, Austin KJ, Hansen TR, Lucy MC, Davies CJ, Ott TL. Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. *J Dairy Sci* 2007; **90**:274–280.

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32. Kizaki K, Shichijo-Kizaki A, Furusawa T, Takahashi T, Hosoe M, Hashizume K. Differential neutrophil gene expression in early bovine pregnancy. *Repro Biol Endocrino* 2013; **11**:6.
33. 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.
34. 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. *J Reprod Fertil* 1967; **13**:511–516.
35. Godkin JD, Bazer FW, Thatcher WW, Roberts RM. Proteins released by cultured day 15-16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. *J Reprod Fertil* 1984; **71**:57–64.
36. Mezera MA, Hamm CS, Gamarra CA, Gennari RS, Prata AB, Sartori R, Wiltbank MC. Profiles of prostaglandin F2α metabolite (PGFM) in dairy cattle during luteal regression and pregnancy: implications for corpus luteum maintenance. *Biol Reprod* 2019; **101**:76–90.
37. 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.
38. Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 2001; **121**:175–180.
39. Wolfenson D, Bartol F, Badinga L, Barros C, Marple D, Cummins K, Wolfe D, Lucy M, Spencer T, Thatcher W. Secretion of PGF2α and oxytocin during hyperthermia in cyclic and pregnant heifers. *Theriogenology* 1993; **39**:1129–1141.
40. Parkinson TJ, Jenner LJ, Lamming GE. Comparison of oxytocin/prostaglandin F-2 alpha interrelationships in cyclic and pregnant cows. *J Reprod Fertil* 1990; **90**:337–345.
41. Robinson RS, Mann GE, Lamming GE, Wathes DC. The effect of pregnancy on the expression of uterine oxytocin, oestrogen and progesterone receptors during early pregnancy in the cow. *J Endocrinol* 1999; **160**:21–33.
42. Plante C, Thatcher WW, Hansen PJ. Alteration of oestrous cycle length, ovarian function and oxytocin-induced release of prostaglandin F-2 alpha by intrauterine and intramuscular administration of recombinant bovine interferon-alpha to cows. *J Reprod Fertil* 1991; **93**:375–384.
43. Ott T, Fleming J, Spencer T, Joyce M, Chen P, Green C, Zhu D, Welsh T Jr, Harms P, Bazer F. Effects of exogenous recombinant ovine interferon tau on circulating concentrations of progesterone, cortisol, luteinizing hormone, and antiviral activity; interestrous interval; rectal temperature; and uterine response to oxytocin in cyclic ewes. *Biol Reprod* 1997; **57**:621–629.
44. Fuchs AR, Rollyson MK, Meyer M, Fields MJ, Minix JM, Randel RD. Oxytocin induces prostaglandin F-2 alpha release in pregnant cows: Influence of gestational age and oxytocin receptor concentrations. *Biol Reprod* 1996; **54**:647–653.
45. Fuchs AR, Helmer H, Behrens O, Liu HC, Antonian L, Chang SM, Fields MJ. Oxytocin and bovine parturition: a steep rise in endometrial oxytocin receptors precedes onset of labor. *Biol Reprod* 1992; **47**:937–944.
46. *NRC. Nutrient Requirements of Dairy Cattle*, 7th rev. ed. Washington, DC: National Academies Press; 2001.
47. Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. *Theriogenology* 1995; **44**:915–923.
48. Mačuhová J, Tančin V, Bruckmaier R. Effects of oxytocin administration on oxytocin release and milk ejection. *J Dairy Sci* 2004; **87**: 1236–1244.
49. 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.
50. 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.
51. Ginther OJ, Beg MA, Gastal EL, Gastal MO, Baerwald AR, Pierson RA. Systemic concentrations of hormones during the development of follicular waves in mares and women: a comparative study. *Reproduction* 2005; **130**:379–388.
52. Melo LF, Monteiro PLJ Jr, Nascimento AB, Drum JN, Spies C, Prata AB, Wiltbank MC, Sartori R. Follicular dynamics, circulating progesterone, and fertility in Holstein cows synchronized with reused intravaginal progesterone implants that were sanitized by autoclave or chemical disinfection. *J Dairy Sci* 2018; **101**:3554–3567.
53. Toledo MZ, Baez GM, Garcia-Guerra A, Lobos NE, Guenther JN, Trevisol E, Luchini D, Shaver RD, Wiltbank MC. Effect of feeding rumen-protected methionine on productive and reproductive performance of dairy cows. *PLoS ONE* 2017; **12**.
54. Mann GE, Payne JH, Lamming GE. Hormonal regulation of oxytocin-induced prostaglandin F-2 alpha secretion by the bovine and ovine uterus in vivo. *Domest Anim Endocrinol* 2001; **21**:127–141.
55. Pinaffi F, Araujo E, Ginther O. Concentrations of a PGF2α metabolite during pregnancy on the days that luteolysis occurs in nonbred heifers. *Domest Anim Endocrinol* 2018; **62**:76–82.
56. 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.
57. Pohler KG, Peres RFG, Green JA, Graff H, Martins T, Vasconcelos JLM, Smith MF. Use of bovine pregnancy-associated glycoproteins to predict late embryonic mortality in postpartum Nelore beef cows. *Theriogenology* 2016; **85**:1652–1659.
58. 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 Sci* 2018; **15**:805–821.
59. Knickerbocker JJ, Thatcher WW, Bazer FW, Drost M, Barron DH, Fincher KB, Roberts RM. Proteins secreted by day-16 to -18 bovine conceptuses extend corpus luteum function in cows. *J Reprod Fertil* 1986; **77**:381–391.
60. Martal J, Lacroix MC, Loudes C, Saunier M, Wintenberger-Torres S. Trophoblastin, an antiluteolytic protein present in early pregnancy in sheep. *J Reprod Fertil* 1979; **56**:63–73.
61. Spencer TE, Ing NH, Ott TL, Mayes JS, Becker WC, Watson GH, Mirando MA, Bazer FW. Intrauterine injection of ovine interferon-τ alters oestrogen receptor and oxytocin receptor expression in the endometrium of cyclic ewes. *Mol Endocrinol* 1995; **15**:203–220.
62. 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.
63. Spencer TE, Bazer FW. Ovine interferon tau suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. *Endocrinology* 1996; **137**:1144–1147.
64. Sartori R, Rosa GJ, Wiltbank MC. Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. *J Dairy Sci* 2002; **85**:2813–2822.
65. 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.
66. Jaiswal R, Singh J, Marshall L, Adams G. Repeatability of 2-wave and 3-wave patterns of ovarian follicular development during the bovine estrous cycle. *Theriogenology* 2009; **72**:81–90.
67. Bridges PJ, Wright DJ, Buford WI, Ahmad N, Hernandez-Fonseca H, 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.

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68. 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.
69. Pinaffi FLV, Araujo ER, Silva LA, Ginther OJ. Color-Doppler signals of blood flow in the corpus luteum and vascular perfusion index for ovarian and uterine arteries during expansion of the allantochorion in Bos taurus heifers. *Theriogenology* 2017; **102**:35–43.
70. Wiltbank J, Rothlisberger J, Zimmerman D. Effect of human chorionic gonadotrophin on maintenance of the corpus luteum and embryonic survival in the cow. *J Anim Sci* 1961; **20**:827–829.
71. Hill JR, Burghardt RC, Jones K, Long CR, Looney CR, Shin T, Spencer TE, Thompson JA, Winger QA, Westhusin ME. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol Reprod* 2000; **63**:1787–1794.
72. Lee RSF, Peterson AJ, Donnison MJ, Ravelich S, Ledgard AM, Li N, Oliver JE, Miller AL, Tucker FC, Breier B, Wells DN. Cloned cattle fetuses with the same nuclear genetics are more variable than contemporary half-siblings resulting from artificial insemination and exhibit fetal and placental growth deregulation even in the first trimester. *Biol Reprod* 2004; **70**:1–11.
73. Santos JE, Thatcher WW, Chebel RC, Cerri RL, Galvao KN. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim Reprod Sci* 2004; **82-83**:513–535.
74. Wiltbank M, Median R, Ochoa J, Baez G, Giordano J, Ferreira J, Sartori R. Maintenance or regression of the corpus luteum during multiple decisive periods of bovine pregnancy. *Anim Reprod Sci* 2016; **13**:217–233.
75. Giordano JO, Guenther JN, Lopes G Jr, Fricke PM. Changes in serum pregnancy-associated glycoprotein, pregnancy-specific protein B, and progesterone concentrations before and after induction of pregnancy loss in lactating dairy cows. *J Dairy Sci* 2012; **95**:683–697.

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