Intravaginal instillation of prostaglandin $F_{2\alpha}$ was as effective as intramuscular injection for induction of luteal regression in lactating dairy cows

M. Masello, M. Scarbolo, M. V. Schneck, M. M. Perez, E. M. Schillkowsky, E. M. Sitko, F. Sosa Hernandez, E. M. Cabrera, R. M. Rossi, and J. O. Giordano*
Department of Animal Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Our objectives were to test the efficacy of intravaginal (IVG) administration of $PGF_{2\alpha}$ to induce corpus luteum (CL) regression, compare circulating progesterone (P4) profiles in cows receiving IVG versus intramuscular (IM) treatment with $PGF_{2\alpha}$, and evaluate reproductive outcomes. Lactating Holstein cows were synchronized using a Double-Ovsynch protocol [GnRH, 7 d later $PGF_{2\alpha}$, 3 d later GnRH, 7 d later GnRH, 7 d later $PGF_{2\alpha}$, 1 d later $PGF_{2\alpha}$, 32 h later GnRH, 16 to 20 h timed artificial insemination (TAI)] to receive TAI at 67 ± 3 d in milk. Seven days after the first GnRH treatment (time 0), cows with at least 1 visible CL ≥ 15 mm were blocked by parity and randomly assigned to a treatment that consisted of IM injection (IM-PGF; $n = 201$) or IVG instillation (IVG-PGF; $n = 201$) of $PGF_{2\alpha}$. Cows in IM-PGF received a single 25-mg dose of $PGF_{2\alpha}$ (dinoprost tromethamine) intramuscularly. Cows in IVG-PGF received two 25-mg doses of $PGF_{2\alpha}$ 12 h apart delivered through a catheter in the cranial portion of the vagina. Blood samples were collected at 0, 12, 48, and 72 h after treatment. Ovulation to the first GnRH of Double-Ovsynch was determined through transrectal ultrasonography. Only cows with P4 ≥ 1 ng/mL (functional CL) at time 0 (IM-PGF = 169; IVG-PGF = 179) were included in the analyses. Binary and quantitative data were analyzed by logistic regression and ANOVA with repeated measures, respectively. Results are presented as least squares means. Concentrations of P4 and the proportion of cows with a new CL at time 0 did not differ. Overall, the proportion of cows with CL regression using 1 ng of P4/mL (IM-PGF = 89.0%; IVG-PGF = 86.7%) or 0.5 ng of P4/mL (IM-PGF = 82.2%; IVG-PGF = 82.1%) as the cutoff did not differ. Concentrations of P4 were affected by treat-

ment, time, and treatment $\times$ time interaction. Cows in IVG-PGF had greater mean P4 at 12 h than cows in IM-PGF. Mean P4 did not differ at 48 or 72 h after treatment. The proportion of cows with estrus recorded within 3 d of treatment (IM-PGF = 45.4%; IVG-PGF = 48.9%), ovulation risk after treatment (IM-PGF = 88.5%; IVG-PGF = 85.1%), and pregnancies per artificial insemination after TAI (IM-PGF = 51.5%; IVG-PGF = 57.8%) did not differ. We concluded that 2 IVG doses of 25 mg of $PGF_{2\alpha}$ 12 h apart were as effective as a single 25-mg IM dose of $PGF_{2\alpha}$ for inducing luteal regression in lactating dairy cattle.

Key words: intravaginal, prostaglandin $F_{2\alpha}$, luteal regression, cattle

INTRODUCTION

Effective reproductive management programs for cattle optimize reproductive performance through timely and successful insemination of cows after they become eligible for pregnancy. In this regard, timed AI (TAI) after synchronization of ovulation is a technology that is essential for reproductive success in many dairy and beef cattle operations in North America and around the world (Lamb et al., 2010; Wiltbank and Pursley, 2014). Synchronization of ovulation protocols consists of administering a combination of 2 or more hormonal treatments in a sequence to manipulate follicular wave dynamics, corpus luteum (CL) regression, and ovulation (Pursley et al., 1995, 1997). Ultimately, timing of ovulation is synchronized within a narrow time frame, facilitating insemination of groups of cows by appointment regardless of estrus expression and detection (Pursley et al., 1995; Vasconcelos et al., 1999; Cartmill et al., 2001).

A wide variety of simple protocols ranging in type, number, and sequence of hormonal treatments are available and used by cattle operations with the main purpose of ensuring immediate insemination after the end of the voluntary waiting period or after a failed AI service (Chebel et al., 2006; Wijma et al., 2018).

Received September 15, 2019.

Accepted November 7, 2019.

*Corresponding author: jog25@cornell.edu

More sophisticated protocols including up to 6 or 7 hormonal treatments at inconvenient intervals, and some including sustained hormone release implants, can also be used to increase the fertility of dairy (Souza et al., 2008; Bartolome et al., 2009) and beef (Bridges et al., 2008; Kasimanickam et al., 2012) females. Regardless of their purpose and complexity, a common drawback of all synchronization of ovulation protocols is the need to administer multiple hormonal treatments. Such treatments are most commonly given in the form of intramuscular (IM) or subcutaneous injections and in some cases through implantation of sustained hormone-release intravaginal (IVG) devices. In all cases, cows that are synchronized need to be located or sorted in a pen and restrained in self-locking head gates, stalls, or palpation rails to receive injections or implants. These interventions can be labor intensive and costly and can affect cow natural behaviors. In addition, human and technical errors can reduce compliance, impairing protocol efficacy (Stevenson and Phatak, 2005; Galvão et al., 2013). Automation of synchronization of ovulation treatments may help reduce the burden of implementing synchronization of ovulation protocols, thus reducing labor needs, improving cow welfare by decreasing cow manipulation, and potentially improving reproductive performance by improving compliance and facilitating the development of novel protocols that optimize the physiological response to hormonal treatments.

A potential strategy to automate synchronization of ovulation is through electronically controlled hormone delivery devices inserted in body cavities such as the vagina. Successful synchronization of ovulation by automated IVG hormone delivery devices is only feasible if hormones used in synchronization protocols elicit the physiological response of interest when given intravaginally. In this regard, Wijma et al. (2016) reported that IVG delivery of 2 doses of 25 mg of dinoprost (i.e., natural form of $PGF_{2\alpha}$) 12 h apart successfully induced complete luteal regression and resulted in a circulating progesterone (P4) profile similar to that of lactating dairy cows that received a single 25-mg IM dose of $PGF_{2\alpha}$. Circulating concentrations of P4 at 48, 60, and 72 h after treatment did not differ for cows that received IVG or IM $PGF_{2\alpha}$, and a similar proportion of cows in both groups had complete CL regression by 60 and 72 h after treatment. Wijma et al. (2016) also reported that delivering two 25-mg doses of dinoprost 12 h apart was the most effective regimen to induce complete CL regression compared with smaller or larger doses (i.e., 25 and 125 mg) or a similar dose (i.e., 50 mg) delivered as a single treatment. Two 25-mg doses of $PGF_{2\alpha}$ 12 h apart resulted in P4 concentration dynamics similar to those of cows that received a single 25-mg IM dose of

dinoprost, making this dose and treatment frequency for IVG delivery a reasonable choice for future experiments to evaluate CL regression and synchronization of ovulation. A caveat of the experiments reported in Wijma et al. (2016) was the limited number of cows used because the objectives were to provide proof of concept for IVG delivery of $PGF_{2\alpha}$ and to identify a feasible $PGF_{2\alpha}$ dose and treatment frequency for successful induction of CL regression. Thus, it was not possible to determine whether IVG delivery of $PGF_{2\alpha}$ would be as effective as IM treatment for induction of complete CL regression and whether it could be used as an alternative route of $PGF_{2\alpha}$ administration in cattle.

Therefore, the primary objective of the current experiment was to evaluate CL regression and the P4 concentration profile of dairy cows treated with $PGF_{2\alpha}$ by IVG instillation or IM injection. A secondary objective was to evaluate reproductive outcomes that depend on completion of CL regression. We hypothesized that the proportion of cows with complete CL regression and the P4 concentration profile would be similar for cows that received IVG and IM treatment with $PGF_{2\alpha}$. We also hypothesized that the proportion of cows detected in estrus and ovulation after treatment with GnRH would be similar for cows that received IVG or IM treatment with $PGF_{2\alpha}$ during the presynchronization portion of a fertility protocol for TAI.

MATERIALS AND METHODS

All procedures performed with cows were approved by the Animal Care and Use Committee of Cornell University (Ithaca, NY).

Cows and General Management

Lactating Holstein cows ($n = 402$) from the dairy unit of the Cornell University Ruminant Center (Harford, NY) were enrolled in this experiment conducted from September 2017 to November 2018. Cows were housed in freestall barns with concrete flooring, self-locking head gates, and fans and sprinklers in the feedline. During the experimental period, the average number of milking cows was 594 (range: 538–629) and the average milk yield per cow was 42 kg/d (range: 39–50). Data for cow numbers and milk yield were retrieved from the dairy herd management software (DairyComp305; ValleyAg Software, Tulare, CA) using the MONITOR command. Cows were milked thrice daily at approximately 8-h intervals and were fed a TMR once a day with ad libitum access to feed and water. The diet was formulated to meet or exceed the nutrient requirements of lactating dairy cows producing 48 kg/d with 3.8% fat

and 3.1% true protein as determined by the Cornell Net Carbohydrate and Protein System (Higgs et al., 2015).

Experimental Treatments

This experiment was conducted as a randomized complete block design using parity group (i.e., primiparous vs. multiparous) as blocking factor. Every week, lactating nonpregnant cows at 40 ± 3 DIM were enrolled in the Double-Ovsynch (DO) protocol (GnRH, 7 d later $PGF_{2\alpha}$, 3 d later GnRH, 7 d later GnRH, 7 d later $PGF_{2\alpha}$, 1 d later $PGF_{2\alpha}$, 32 h later GnRH, 16 to 20 h TAI) to receive first AI service at 67 ± 3 DIM. At the time of first $PGF_{2\alpha}$ treatment of the DO protocol, transrectal ultrasonography (TUS) of the ovaries was performed using a portable ultrasound machine with a 7.5-MHz attached linear probe (Ibex Pro; E.I. Medical Imaging, Loveland, CO). Cows with at least 1 visible CL ≥ 15 mm in diameter were blocked by parity and randomly allocated to 1 of 2 treatment groups: IM-PGF ($n = 201$) or IVG-PGF ($n = 201$). Cows without a CL ≥ 15 mm in diameter were excluded from the experiment. Immediately after randomization, cows in the IM-PGF treatment received 25 mg of $PGF_{2\alpha}$ (dinoprost tromethamine; Lutalyse HighCon, Zoetis, New York, NY) as a 2-mL IM injection in the semimembranosus or semitendinosus muscle. The number of injections and dose used for the IM-PGF treatment correspond to the treatment regimen recommended and approved for synchronization of estrus and ovulation in cattle. Cows in the IVG-PGF treatment received 2 IVG treatments of 25 mg of $PGF_{2\alpha}$ (2 mL of Lutalyse HighCon) 12 h apart. Cows from both groups received treatments while restrained in self-locking head gates. Before application of the IVG treatments, the vulva

and perineal area were manually cleaned using paper towels. Thereafter, the vulvar labia were manually opened and a uterine infusion catheter (44.5 cm long $\times$ 0.5 cm external diameter) attached to a plastic syringe was carefully inserted into the vagina until the vaginal fornix was reached. Once in the cranial portion of the vagina, the catheter was pulled backward 1 to 2 cm and 2 mL of $PGF_{2\alpha}$ was released at a similar rate as the IM-PGF treatment (i.e., as a bolus). After application of treatments, cows remained standing for a minimum of 30 min. Cows in both experimental groups completed the DO protocol to receive first AI service.

Evaluation of Ovarian Structures and Responses

Transrectal ultrasonography of the ovaries was performed in all cows at the time of the first GnRH treatment (G1) of the DO protocol, 7 d later at the time of application of experimental treatments (i.e., first $PGF_{2\alpha}$ treatment of DO), and at the second (G2) and third (G3) GnRH treatments of the DO protocol (Figure 1). At each TUS examination, the position and diameter of all ovarian structures (i.e., follicles and CL) ≥ 8 mm in diameter were recorded on an ovarian map. The diameter of the visualized structures was estimated using the average of 2 perpendicular measurements recorded on a single image of the apparent maximal diameter of the structure. All measurements were performed using the internal digital calipers of the ultrasound machine. Luteal volume was calculated as $V = 4/3 \times \pi \times r^3$, where the radius (r) was calculated as $0.5 \times$ CL diameter. For a CL with a cavity (anechoic area surrounded by echoic tissue), the volume of the cavity was calculated and subtracted from the total CL volume. A cow was considered to have ovulated in response to GnRH

Figure 1. Graphical depiction of experimental procedures. Lactating Holstein cows were synchronized using a Double-Ovsynch (DO) protocol [GnRH, 7 d later $PGF_{2\alpha}$, 3 d later GnRH, 7 d later GnRH, 7 d later $PGF_{2\alpha}$, 1 d later $PGF_{2\alpha}$, 32 h later GnRH, 16 to 20 h timed AI (TAI)] to receive TAI at 67 ± 3 DIM. Seven days after the first GnRH (G1) treatment, cows with at least 1 visible corpus luteum ≥ 15 mm were blocked by parity and randomly assigned to 1 of 2 treatments. Cows in the IM-PGF treatment ($n = 201$) received 25 mg of $PGF_{2\alpha}$ (dinoprost tromethamine) intramuscularly. Cows in the IVG-PGF treatment ($n = 201$) received two 25-mg doses of $PGF_{2\alpha}$ 12 h apart through a catheter in the cranial portion of the vagina. After application of treatments, cows in both experimental groups completed the DO protocol to receive first AI service. PGF = $PGF_{2\alpha}$; TUS = transrectal ultrasonography; BC = blood collection.

when 1 or more follicles ≥ 10 mm were recorded at the time of GnRH treatment and the presence of a new CL was confirmed in the same location 7 d later.

Blood Sample Collection and Determination of P4 Concentrations

Blood samples (~ 8 to 9 mL) were collected by puncture of the caudal vein or artery using evacuated tubes (Vacutainer; BD, Franklin Lakes, NJ) with EDTA. Samples were placed in crushed ice within 5 min of collection and transported to the laboratory for further processing. Samples were centrifuged at $2,000 \times g$ for 20 min at 4°C. Plasma aliquots were harvested and transferred to Eppendorf vials for storage at -20°C until assayed.

A schematic representation of the blood sample collection schedule is presented in Figure 1. The first blood sample was collected from all cows enrolled at G1 and 7 d later coincident with the experimental treatments (time 0). At this point, plasma concentrations of P4 were used to confirm the presence of a functional CL (i.e., $\text{P4} \geq 1$ ng/mL) in all cows that received the experimental treatments. After application of treatments, blood samples were collected at 12, 48, and 72 h to evaluate circulating P4 concentration dynamics. The 72-h time point coincided with the G2 treatment of the DO protocol. At G1 and G3, dichotomized P4 concentrations were used to determine the percentage of cows with a functional CL, whereas P4 concentrations at G2 (i.e., 72 h after application of treatments) were used to assess luteal regression after the $\text{PGF}_{2\alpha}$ treatments. A cow was considered to have complete CL regression if P4 was ≥ 1 ng/mL at time 0 and P4 was < 1 ng/mL 72 h after application of treatments. We further examined luteal regression outcomes using 0.5 ng of P4/mL as the cutoff value because P4 levels above this threshold at the time of TAI have been associated with negative reproductive outcomes in lactating dairy cows (Bisinotto et al., 2010; Giordano et al., 2013).

All plasma samples were assayed for P4 using a commercial solid-phase, no-extraction RIA (ImmuChem Coated Tube; MP Biomedicals, Costa Mesa, CA) previously validated for use in bovines (Garbarino et al., 2004; Skenandore et al., 2017). To assess the precision of the assay, control samples with high (5.1 ng/mL) and low (0.3 ng/mL) concentrations of P4 were included at the beginning and end of each assay ($n = 19$). The average detection limit of the assay was 0.1 ng/mL. For the high-concentration sample, the average intra-assay coefficient of variation (CV) was 8.2% and the interassay CV was 16.5%. For the low-concentration sample, the average intra-assay CV was 21.4% and the interassay CV was 36.7%.

BCS, BW, and Evaluation of Reproductive Tract Health

For all cows, BCS and reproductive tract health status were evaluated and recorded at the time of enrollment (G1). For BCS, a scale of 1 (emaciated) to 5 (fat) with increments of 0.25 was used (Edmonson et al., 1989). Data for BCS were dichotomized using a threshold BCS of 2.75 units (high ≥ 2.75 , low < 2.75). To determine reproductive tract health, vaginal discharge was examined using a Metrichex device (Simcro, Hamilton, New Zealand) and scored on a 0-to-5 scale (0 = no discharge, 1 = clear mucus, 2 = clear mucus with flecks of pus, 3 = mucopurulent but $< 50\%$ pus, 4 = mucopurulent with $> 50\%$ pus, and 5 = foul-smelling discharge) as previously described by McDougall et al. (2007). Based on previous work from our group (Stangaferro et al., 2018), purulent vaginal discharge (**PVD**) was defined as a Metrichex score ≥ 2 . In addition, BW was recorded at G1 from a subgroup of cows in both treatment groups (IM-PGF = 101; IVG-PGF = 106).

Detection of Estrus

All cows were fitted with a neck-mounted electronic rumination and activity monitoring tag (Heat and Rumination System, SCR Dairy by Allflex, Netanya, Israel) approximately 10 d before application of experimental treatments to provide enough time (i.e., at least 5 d) for individual cow baseline data collection. Data generated from individual tags were transferred every 20 min via antennas located in the barn to the system software (Dataflow; SCR Dairy) installed on the on-farm computer. The number of cows reaching the heat index score (**HI**; 0 to 100 arbitrary units) threshold set by the activity-monitoring system software (i.e., $\text{HI} > 35$) was recorded for up to 3 d after application of treatments. For each cow, the HI was generated based on activity, rumination time, and estrus length.

Pregnancy Diagnosis

In cows from both treatment groups, pregnancy diagnosis was conducted by TUS 32 ± 3 d after first service if the cow was not previously reinseminated at detected estrus. A positive pregnancy outcome was based on the presence of an amount of anechoic uterine fluid and an embryo size consistent with the expected stage of pregnancy. The presence of a heartbeat in the embryo was also used as confirmation of embryo viability. Reconfirmation of pregnancy was performed 60 ± 3 d after AI by TUS in all cows confirmed pregnant at the initial examination unless the cow was reinseminated at detected estrus or left the herd. A cow was consid-

ered to have undergone pregnancy loss when confirmed pregnant at the initial examination and nonpregnant at the time of reconfirmation or if reinseminated at detected estrus after confirmation of pregnancy.

Statistical Analysis

A sample size calculation was performed using WinPepi software (Abramson, 2011) to assess the noninferiority of the IVG-PGF treatment compared with the IVG-IM treatment. Based on an expected proportion of cows undergoing luteal regression of 80% and a noninferiority margin of 10%, a total of 167 cows per treatment were needed for a 1-sided noninferiority test with a probability of type I error (α) of 5% and a probability of type II error (β) of 20%.

Binary outcomes [i.e., proportion of cows with P4 ≥ 1 ng/mL, luteal regression after application of experimental treatments, proportion of cows detected in estrus, ovulatory response to G1 and G2, pregnancies per AI (P/AI), and pregnancy loss] were analyzed using logistic regression with the GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC) fitting a binomial distribution. Continuous quantitative outcomes were analyzed by ANOVA with (P4 concentration dynamics) or without (follicle size, CL volume, and HI) repeated measurements using PROC MIXED of SAS. For all analyses, only cows with a functional CL present at time 0 (i.e., at the time of application of experimental treatments) were included (IM-PGF = 169, IVG-PGF = 179).

Because of the known effect of CL maturity on PGF $_{2\alpha}$ -induced CL regression in lactating dairy cows (Momont and Seguin, 1984; Giordano et al., 2012; Stevenson, 2016), the effect of experimental treatments on luteal regression and P4 concentration dynamics was evaluated for all cows regardless of the type of CL present at treatment and within each of 3 groups of cows created based on the expected level of CL maturity at the time of application of experimental treatments. Briefly, the CL maturity groups were created as follows: (1) new CL only (NCL; no visible CL at G1 with newly formed CL 7 d later), (2) old CL only (OCL; same CL visualized on ovarian maps at G1 and 7 d later), and (3) old and new CL (O+NCL; at least 1 CL visualized on ovarian maps at both the time of G1 and 7 d later, and at least 1 newly formed CL at the time of treatment).

The effects of experimental treatment (IM-PGF vs. IVG-PGF), parity (primiparous vs. multiparous), and the treatment $\times$ parity interaction were offered as explanatory variables to all models, whereas logistic regression models for luteal regression, estrus expres-

sion, and P/AI also included the effect of BCS, BW, and PVD at enrollment (G1). In addition, for outcomes related to luteal regression, estrus expression, and ovulatory response, the effects of total CL volume and P4 at time 0 were offered to models as categorical covariates. In both cases, variables were dichotomized using a threshold corresponding to the median value within parity group generated with PROC UNIVARIATE of SAS (CL volume: primiparous = 7,821 mm³, multiparous = 9,278 mm³; P4 at time 0: primiparous = 4.3 ng/mL, multiparous = 3.7 ng/mL). Further, the effect of season (cool vs. warm) was offered to the logistic regression models for proportion of cows detected in estrus (season of treatment) and P/AI (season of AI). The cold season was defined as the period from September 21 to June 20, and the warm season was defined as the period from June 21 to September 20.

The effect of experimental treatments on P4 concentration profiles (i.e., P4 at 0, 12, 48, and 72 h after treatment) was evaluated with models that included treatment, time, parity, and the treatment $\times$ time interaction as fixed effects, whereas cow within treatment was included as a random effect. In addition, trial $\times$ parity and time $\times$ parity interactions were offered to the models. In all cases, cow within treatment was the subject of repeated-measures analysis using a spatial power covariance structure to adjust for the varying time intervals at which blood was collected.

In all cases, the final model for outcomes of interest was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Akaike information criterion. Treatment and parity were forced in all models. Body weight, BCS, PVD, CL volume, and P4 at time 0 were removed from the luteal regression models and season, BW, and PVD were removed from the estrus expression and P/AI models because $P > 0.10$.

Assumptions of normality of residuals and homoscedasticity for linear regression models were tested by evaluating normal probability plots (quantile–quantile plot) and plotting residuals versus predicted values. For logistic regression models, goodness of fit was evaluated using the ratio between the Pearson statistic and its degrees of freedom. In all cases, model fit was deemed acceptable based on a ratio of approximately 1.

All proportions reported are least squares means generated using the LSMEANS statement of PROC GLIMMIX of SAS. Quantitative outcomes are reported as least squares means $\pm$ standard error of the mean obtained with the LSMEANS option of PROC MIXED of SAS. When appropriate, the least significant difference post hoc mean separation test was used to determine differences between least squares means. All explana-

Table 1. Physiological parameters and ovarian status before application of experimental treatments

Item	Treatment ¹		P-value
	IM-PGF	IVG-PGF	
Cows with P4 ≥1 ng/mL at G1, ² % (no.)	49.5 (168)	54.6 (178)	0.34
Ovulation to G1, % (no.)	74.0 (168)	75.1 (179)	0.81
CL volume at time 0, mm ³	10,032 ± 422	9,902 ± 409	0.81
CL maturity ³ at time 0, % (no.)			
NCL	44.7 (168)	43.1 (179)	0.77
OCL	26.0 (168)	24.9 (179)	0.81
O+NCL	29.0 (168)	31.7 (179)	0.58

¹Lactating Holstein cows were synchronized using a Double-Ovsynch protocol to receive timed AI at 67 ± 3 DIM. Seven days after the first GnRH (G1) treatment, cows with at least 1 visible corpus luteum (CL) ≥15 mm were blocked by parity and randomly assigned to 1 of 2 treatments. Cows in IM-PGF received 25 mg of PGF_{2α} intramuscularly. Cows in IVG-PGF received two 25-mg doses of PGF_{2α} 12 h apart through a catheter in the cranial portion of the vagina. All proportions and quantitative outcomes are reported as least squares means.

²P4 = progesterone; G1 = first GnRH of Double-Ovsynch protocol. Two cows were eliminated from the analysis because of missing data for P4 concentration at G1.

³NCL = new CL only (no visible CL at G1 with a newly formed CL 7 d later); OCL = old CL only (same CL visualized at G1 and 7 d later); O+NCL = old and new CL (at least 1 CL visualized at both G1 and 7 d later, and at least 1 newly formed CL at time 0). One cow from IM-PGF was eliminated from the analysis because of missing data for CL maturity at time 0.

tory variables and their interactions were considered significant if $P \leq 0.05$, whereas $P > 0.05$ and ≤ 0.10 was considered a tendency.

RESULTS

Physiological Parameters and Ovarian Status Before Application of Experimental Treatments

At G1, the proportion of cows with BCS <2.75 was similar ($P = 0.64$) for cows in both treatments (IM-PGF = 20.0%, $n = 168$; IVG-PGF = 18.2%, $n = 179$) but was affected ($P < 0.001$) by parity because more multiparous than primiparous cows had BCS <2.75 (primiparous = 9.8%; multiparous = 33.9%). Likewise, BW was similar ($P = 0.73$) for both treatment groups (IM-PGF = 654 ± 7 kg; IVG-PGF = 651 ± 7 kg) but was greater ($P < 0.001$) for multiparous cows (710 ± 6 kg) than for primiparous cows (594 ± 9 kg). In addition, the proportion of cows with PVD was similar ($P = 0.20$) for both treatments (IM-PGF = 43.0%, $n = 167$; IVG-PGF = 36.2%, $n = 179$) and for primiparous and multiparous cows ($P = 0.37$). In all cases, no interaction was observed between treatment and parity ($P > 0.10$).

The proportion of cows with P4 ≥1 ng/mL at G1 was similar for cows in the IM-PGF and IVG-PGF treatments ($P = 0.34$; Table 1) and for primiparous and multiparous cows ($P = 0.91$; primiparous = 52.4%, multiparous = 51.8%). Similarly, the proportion of cows ovulating in response to G1 did not differ by treatment ($P = 0.81$; Table 1) or parity ($P = 0.12$; primiparous =

78.4%, multiparous = 70.2%), and there was no treatment × parity interaction ($P = 0.51$). Total CL volume tissue present at time 0 was similar ($P = 0.81$; Table 1) for the IM-PGF and IVG-PGF treatments but was greater ($P < 0.01$) for multiparous cows (11,051 ± 332 mm³) than for primiparous cows (8,883 ± 516 mm³) and was greater for O+NCL cows (13,336 ± 518 mm³) than for OCL (8,399 ± 552 mm³) and NCL (8,167 ± 437 mm³) cows. Similarly, circulating concentration of P4 at time 0 was similar ($P = 0.71$) for cows in the IM-PGF (4.7 ± 0.2 ng/mL) and IVG-PGF (4.8 ± 0.2 ng/mL) treatments but was greater ($P = 0.003$) for primiparous cows (5.1 ± 0.2 ng/mL) than for multiparous cows (4.3 ± 0.1 ng/mL). In addition, CL maturity group affected ($P < 0.001$) P4 concentration at time 0 because it was greater for N+OCL cows than for NCL and OCL cows and for OCL cows than for NCL cows (NCL = 3.1 ± 0.2 ng/mL; OCL = 5.2 ± 0.2 ng/mL; O+NCL = 5.8 ± 0.2 ng/mL).

The distribution of cows based on CL maturity group at time 0 for each treatment group is presented in Table 1. At time 0, there was a similar proportion of cows in the NCL ($P = 0.77$), OCL ($P = 0.81$), and O+NCL ($P = 0.58$) groups in both treatments. For all CL maturity groups, no effect of parity ($P > 0.10$) or treatment × parity interaction ($P > 0.10$) was observed.

Luteal Regression

Overall, the proportion of cows with complete luteal regression at 72 h after application of treatments using the P4 <1.0 ng/mL cutoff was similar ($P = 0.48$; Fig-

ure 2A) for the IM-PGF and IVG-PGF treatments but tended ($P = 0.06$) to be greater for primiparous cows (91.3%) than for multiparous cows (83.4%). Similarly, the proportion of cows with $P_4 < 0.5$ ng/mL at 72 h was similar ($P = 0.98$; Figure 2A) for the IM-PGF and IVG-PGF treatments and was not affected by parity ($P = 0.32$; primiparous = 84.3%; multiparous = 79.7%). In both cases, no treatment $\times$ parity interaction was observed ($P > 0.10$). For cows in the IVG-PGF treatment, PVD did not affect luteal regression risk when using the circulating P_4 level cutoff of 1.0 ng/mL ($P = 0.32$; PVD = 84.8% vs. no PVD = 89.5%) or 0.5 ng/mL ($P = 0.39$; PVD = 80.0% vs. no PVD = 84.8%).

For cows in the IM-PGF treatment, CL maturity group affected ($P = 0.04$) the proportion of cows with $P_4 < 1$ ng/mL 72 h after treatment, whereby the proportion was greater for cows in the O+NCL group than for cows in the NCL group (NCL = 81.0%, $n = 73$; OCL = 90.6%, $n = 47$; O+NCL = 96.3%, $n = 48$). In contrast, for the IVG-PGF treatment, the proportion of cows with $P_4 < 1$ ng/mL did not differ ($P = 0.28$) by CL maturity group (NCL = 89.7%, $n = 75$; OCL = 91.3%, $n = 48$; O+NCL = 82.3%, $n = 56$). For cows in the NCL group, there was no effect of treatment ($P = 0.23$; Figure 2B) for CL regression using $P_4 < 1.0$ ng/mL as the cutoff, but more ($P = 0.03$) primiparous cows (93.9%) than multiparous cows (78.3%) had complete CL regression. For the OCL group, the proportion of cows with $P_4 < 1$ ng/mL was not affected by treatment ($P = 0.76$; Figure 2B) or parity ($P = 0.72$; primiparous = 86.3%, multiparous = 89.1%). In contrast, for the O+NCL group, treatment affected ($P = 0.02$; Figure 2B) the proportion of cows with $P_4 < 1$ ng/mL because it was greater for the IM-PGF treatment than for the IVG-PGF treatment but was similar ($P = 0.32$) for primiparous (94.0%) and multiparous (88.5%) cows. In all cases, no interaction between treatment and parity was observed ($P > 0.10$).

Circulating P₄ Concentration Dynamics

The effect of treatment on circulating P_4 concentration profiles is presented in Figures 3 and 4. For all cows combined (Figure 3A), there was an effect of treatment ($P = 0.01$), time ($P < 0.001$), and a treatment $\times$ time interaction ($P < 0.001$) on circulating concentrations of P_4 for up to 72 h after treatment. At 12 h, cows in the IM-PGF treatment had lesser P_4 concentrations than cows in the IVG-PGF treatment. No differences were observed at 48 and 72 h after treatment. In addition, parity tended ($P = 0.09$) to affect P_4 concentrations and an interaction between parity and time ($P = 0.01$) was observed, whereby primiparous cows had greater circulating P_4 concentrations at 0 and 12 h after treat-

Figure 2. (A) Proportion of cows with <1 ng/mL and <0.5 ng/mL of progesterone (P_4) at 72 h after application of experimental treatments. Cows in IVG-PGF received two 25-mg doses of PGF_{2α} 12 h apart through a catheter in the cranial portion of the vagina. Cows in IM-PGF received 25 mg of PGF_{2α} intramuscularly. (B) Proportion of cows with $P_4 < 1$ ng/mL 72 h after treatment based on corpus luteum (CL) maturity group at time 0. The CL maturity groups were as follows: new CL [no visible CL at first GnRH (G1) with a newly formed CL 7 d later], old CL (same CL visualized at G1 and 7 d later), and old and new CL (at least 1 CL visualized at both G1 and 7 d later, and at least 1 newly formed CL at time 0). Only cows with a functional CL at time 0 were included in the analyses (IM-PGF = 169; IVG-PGF = 179).

ment than multiparous cows but similar concentrations at 48 and 72 h after treatment. No interaction was observed between treatment and parity.

For those cows with complete luteal regression only (Figure 3B), an interaction was observed between treatment and time ($P < 0.001$), whereby cows in the IM-PGF treatment had lesser ($P < 0.001$) P_4 concentrations at 12 h after treatment, but no differences were observed at 48 and 72 h. A parity $\times$ time interaction ($P = 0.02$) was also observed because primiparous cows

Figure 3. Circulating concentrations of progesterone (P4) from 0 to 72 h after application of treatments for (A) all cows, (B) cows with complete luteal regression ($P4 < 1$ ng/mL at 72 h) only, and (C) cows with incomplete luteal regression ($P4 \geq 1$ ng/mL at 72 h) only. Cows in IVG-PGF received two 25-mg doses of PGF_{2α} 12 h apart through a catheter in the cranial portion of the vagina. Cows in IM-PGF received 25 mg of PGF_{2α} intramuscularly. Only cows with a functional corpus luteum at time 0 were included in the analyses (IM-PGF = 169; IVG-PGF = 179). Values are presented as LSM $\pm$ SEM. Within each time point, an asterisk (*) represents differences ($P \leq 0.05$) for pairwise comparisons between treatments.

Figure 4. Circulating concentrations of progesterone (P4) from 0 to 72 h after application of treatments for cows with (A) an old corpus luteum (CL) only, (B) a new CL only, and (C) an old and a new CL at time 0. Cows in IVG-PGF received two 25-mg doses of PGF_{2α} 12 h apart through a catheter in the cranial portion of the vagina. Only cows with a functional CL at time 0 were included in the analyses (IM-PGF = 169; IVG-PGF = 179). Values are presented as LSM $\pm$ SEM. Within each time point, an asterisk (*) represents differences ($P \leq 0.05$) for pairwise comparisons between treatment.

Table 2. Estrus expression and ovarian responses after application of experimental treatments

Item	Treatment ¹		P-value
	IM-PGF	IVG-PGF	
Cows with detected estrus, ² % (no.)	45.4 (166)	48.9 (174)	0.54
Ovulation after G2, ³ % (no.)	88.5 (166)	85.1 (176)	0.35
Size of the ovulatory follicle (mm)	16.5 ± 0.3	16.5 ± 0.3	0.99
Cows with P4 ≥ 1 ng/mL at G3, ⁴ % (no.)	92.7 (167)	91.5 (176)	0.66
Corpus luteum volume at G3, mm ³	7,625 ± 324	7,624 ± 317	0.99

¹Lactating Holstein cows were synchronized using a Double-Ovsynch protocol to receive timed AI at 67 ± 3 DIM. Seven days after the first GnRH (G1) treatment, cows with at least 1 visible corpus luteum (CL) ≥ 15 mm were blocked by parity and randomly assigned to 1 of 2 treatments. Cows in IM-PGF received 25 mg of PGF_{2α} intramuscularly. Cows in IVG-PGF received two 25-mg doses of PGF_{2α} 12 h apart through a catheter in the cranial portion of the vagina. All proportions and quantitative outcomes are reported as least squares means.

²Three cows from the IM-PGF treatment and 5 cows from the IVG-PGF treatment were not included in the analysis because of collar malfunction or misplacement during data collection.

³G2 = second GnRH treatment of the Double-Ovsynch protocol. Three cows from the IM-PGF treatment and 3 cows from the IVG-PGF treatment exited the herd before confirmation of ovulation.

⁴G3 = third GnRH treatment of the Double-Ovsynch protocol.

had greater P4 concentrations at 0 and 12 h after treatment than multiparous cows, whereas no differences were observed at 48 and 72 h after treatment. When only cows without complete luteal regression were analyzed (Figure 3C), an interaction ($P < 0.001$) between treatment and time was also observed, whereby cows in the IM-PGF treatment had lesser P4 concentrations at 0 and 12 h after treatment compared with cows in the IM-IVG treatment, whereas no differences were observed 48 and 72 h after treatment. For cows without complete luteal regression, no effects of parity or parity × time interaction were observed.

The effect of treatments on circulating concentrations of P4 within each CL maturity group are presented in Figure 4. For cows in the OCL group (Figure 4A), we observed a tendency ($P = 0.08$) for a treatment effect; however, we did not observe a treatment × time interaction ($P = 0.32$). Similarly, we observed an effect of parity ($P = 0.02$) but no effects of the parity × time ($P = 0.72$) or parity × treatment ($P = 0.76$) interactions. In contrast, for the NCL group (Figure 4B), there was a treatment × time interaction ($P < 0.001$) for circulating concentrations of P4, whereby cows in the IM-PGF treatment had lesser P4 concentrations at 12 h than cows in the IVG-PGF treatment, but no difference was observed at 48 and 72 h after treatment. For this group of cows, no effects of parity ($P = 0.81$) or treatment × parity interaction ($P = 0.66$) were observed. Likewise, for the O+NCL group, we observed an interaction ($P < 0.001$) between treatment and time. In this group of cows, P4 concentrations were lesser for the IM-PGF treatment than for the IVG-PGF treatment at 12 h but were similar at 48 and 72 h after treatment. No effect of parity ($P = 0.15$) or treatment × parity interaction ($P = 0.26$) was observed.

Estrus Expression and Ovarian Responses After Treatment

Within 3 d of the application of treatments, the proportion of cows that displayed estrus as determined by increased physical activity was similar ($P = 0.54$; Table 2) for the IM-PGF and IVG-PGF treatments (45.4% vs. 48.9%, respectively) but tended ($P = 0.06$) to be greater for primiparous cows (52.3%) than for multiparous cows (41.1%). In addition, BCS ($P < 0.01$) and P4 concentration at the time of treatment ($P < 0.001$) affected the proportion of cows displaying estrus, which was greater for cows with BCS ≥ 2.75 (57.3%) than for cows with BCS < 2.75 (37.3%) and lesser for cows with high P4 (34.8%) than for cows with low P4 (59.9%) at the time of treatment.

Among cows that displayed estrus within 3 d after treatment (IM-PGF = 78, IVG-PGF = 89), the proportion of cows detected in estrus on d 1 and 2 combined was 40.1% (67/167; arithmetic mean presented) and was not affected by treatment ($P = 0.92$; IM-PGF = 31.8% vs. IVG-PGF = 32.7%) but was greater ($P < 0.001$) for cows with low P4 (58.7%) than for cows with high P4 (13.8%) at the time of treatment. The rest of the cows were detected in estrus on d 3. Further, for cows that displayed estrus, the average HI was greater ($P < 0.001$) for those in the IVG-PGF treatment (86.5 ± 1.0) than for those in the IM-PGF treatment (81.1 ± 1.0). In addition, average HI was affected by parity ($P < 0.0001$; primiparous = 86.5 ± 1.2; multiparous = 81.1 ± 0.9) and CL volume at the time of treatment ($P = 0.03$; low = 85.3 ± 1.0; high = 82.3 ± 1.0). Similarly, treatment ($P = 0.001$) and parity ($P = 0.004$) affected the proportion of cows with HI > 80, which was greater for the IVG-PGF treatment (86%; $n = 89$) than for the

IM-PGF treatment (64.1%; $n = 78$) and for primiparous cows (86%) than for multiparous cows (64.0%).

Overall, the total proportion of cows that ovulated after G2 was similar ($P = 0.35$; Table 2) for the IM-PGF (88.5%) and IVG-PGF (85.1%) treatments and for primiparous (87.1%) and multiparous (86.6%) cows. In contrast, CL volume at the time of treatment with PGF_{2α} affected ($P < 0.01$) the proportion of cows ovulating because it was lesser for cows with high CL volume (81.6%) than for cows with low CL volume (90.8%). Among cows that ovulated in response to G2 (IM-PGF = 146, IVG-PGF = 148), ovulatory follicle size was similar ($P = 0.99$; Table 2) for cows in both treatments but was greater ($P < 0.001$) for multiparous cows (17.0 ± 0.2 mm) than for primiparous cows (15.9 ± 0.3 mm). In addition, P4 concentration at the time of PGF_{2α} treatment affected ovulatory follicle size because it was greater ($P < 0.001$) for cows with low P4 levels (17.0 ± 0.3 mm) than for cows with high P4 levels (16.0 ± 0.3 mm).

The proportion of cows with P4 ≥ 1 ng/mL at G3 (i.e., 7 d after treatment with GnRH) was not affected by treatment ($P = 0.66$; Table 2) and was similar for primiparous (93.2%) and multiparous (90.9%) cows. Further, CL volume at G3 (cows with CL only) was similar for cows in both treatments ($P = 0.99$; Table 2) but was greater ($P = 0.004$) for multiparous cows ($8,318 \pm 258$ mm³) than for primiparous cows ($6,931 \pm 397$ mm³). In addition, P4 at the time of PGF_{2α} treatment affected CL volume at G3, which was greater ($P = 0.01$) for cows with low P4 levels ($8,164 \pm 325$ mm³) than for cows with high P4 levels ($7,084 \pm 316$ mm³).

P/AI and Pregnancy Loss

Forty-one treated cows (IM-PGF = 22, IVG-PGF = 19) were not included in the P/AI analyses because they either did not complete the DO protocol (due to farm management decisions beyond the control of the researchers) or left the herd before pregnancy diagnosis. Overall, P/AI at 32 ± 3 d after AI was not affected ($P = 0.29$) by treatment (IM-PGF = 51.5%, $n = 147$; IVG-PGF = 57.8%, $n = 160$) but was greater ($P = 0.01$) for primiparous cows (64.1%, $n = 86$) than for multiparous cows (44.9%, $n = 221$) and tended ($P = 0.08$) to be greater for cows with BCS ≥ 2.75 (60.5%, $n = 226$) than for cows with BCS < 2.75 (48.7%, $n = 81$). Likewise, P/AI at 60 ± 3 d was similar ($P = 0.28$) for cows in the IM-PGF (47.2%, $n = 145$) and IVG-PGF (53.1%, $n = 160$) treatments but greater ($P = 0.01$) for primiparous cows (59.5%, $n = 86$) than for multiparous cows (40.9%, $n = 219$) and greater for cows with BCS ≥ 2.75 (57.2%; $n = 224$) than for cows with BCS < 2.75 (43.1%; $n = 81$). Pregnancy loss for cows diagnosed

pregnant at initial examination was similar ($P = 0.80$) for both treatments (IM-PGF = 5.4%, $n = 71$; IVG-PGF = 6.3%, $n = 90$) and was not affected ($P = 0.66$) by parity (primiparous = 5.0%, $n = 59$; multiparous = 6.8%, $n = 102$).

DISCUSSION

In support of our main hypothesis, the proportion of cows with complete CL regression was similar for cows that received the IVG or IM PGF_{2α} treatment. Our data supported the hypothesis when using the traditional P4 concentration cutoff for complete CL regression of 1 ng/mL as well as when using a more stringent cutoff of 0.5 ng/mL. Conversely, our hypothesis that the circulating P4 profile would be similar for both treatments was partially supported because of the greater circulating levels of P4 for the IVG-treated group at 12 h and similar levels at 48 and 72 h after treatment. Moreover, the lack of difference for proportion of cows detected in estrus and with ovulation after treatment with GnRH supported our secondary hypothesis. The similar P/AI of cows in both treatments also supported our secondary hypothesis. Nevertheless, the results should be interpreted with caution because the effect of lack of luteal regression or a lesser reduction in circulating P4 after induction of luteolysis during the presynchronization part of a fertility program such as DO has not been documented. Indeed, based on the limited number of cows with incomplete luteal regression in our experiment, it seems that this may not be as relevant because P/AI was similar for cows with complete and incomplete luteal regression after treatment (data not shown). Collectively, our current results provide additional evidence of the efficacy of 2 IVG doses of 25 mg of the natural form of PGF_{2α} (i.e., dinoprost) to cause complete CL regression in lactating dairy cows. In addition, the results indicated that no differences in important reproductive outcomes (i.e., estrus expression and ovulation in response to GnRH) dependent on completion of CL regression should be expected for cows that receive PGF_{2α} through the IVG or IM route of administration. Overall, our current results are in agreement with the few experiments conducted with a limited number of cows to evaluate the effect of IVG instillation of PGF_{2α} on CL regression and other reproductive outcomes (Zdunczyk et al., 1994; Heinonen et al., 1996; Wijma et al., 2016).

Interestingly, we observed a delay in P4 decline after IVG treatment with PGF_{2α} in both cows that had complete CL regression and cows in which regression was induced but the CL did not fully regress (i.e., presented a rebound in circulating concentrations of P4 after 12 h of treatment). This phenomenon, which would likely

be more relevant for cows that have complete CL regression and if cows receive IVG PGF_{2α} for induction of luteal regression before TAI (Martins et al., 2011), was not observed by Wijma et al. (2016) for cows that received the same treatment. Multiple factors could explain the observed delay in P4 decline; however, delayed or reduced absorption of PGF_{2α} from the vagina and potentially both factors combined are the most reasonable explanations. Regardless of the cause for the delay in P4 decline, it is plausible to suggest that the success of the IVG treatment was due to the second PGF_{2α} application, which likely provided the additional PGF_{2α} required to complete luteal regression and result in a similar rate of P4 decline for both treatments after 12 h. Indeed, Wijma et al. (2016) reported that most cows with incomplete CL regression after a single IVG treatment with PGF_{2α} had a decline in circulating P4 within 6 h of treatment followed by a rebound in P4 levels between 12 and 18 h after treatment. These observations led to the addition of the second 25-mg dose of PGF_{2α} within 12 h of the initial treatment, which, in turn, resulted in complete CL regression in all cows. In the current experiment using the same treatment regimen but with a much larger number of cows, we still observed lack of complete CL regression in some cows despite an initial decline in circulating P4 levels within 12 h of treatment. This was not surprising because lack of complete CL regression in cows that receive PGF_{2α} 7 d after treatment with GnRH is common and has been extensively reported for cows that received IM PGF_{2α} (Brusveen et al., 2009; Martins et al., 2011; Giordano et al., 2012). In addition, each 25-mg dose of PGF_{2α} was given in a volume of 5 mL in Wijma et al. (2016) and 2 mL in the current experiment. Different volumes of PGF_{2α} solution used to provide the same dose of active compound may explain the discrepancy between experiments. Future research should explore the use of more than 2 sequential IVG treatments with PGF_{2α}, different volumes of administration, or both to further improve luteal regression rates and mimic the P4 profiles observed after IM treatment with PGF_{2α} in cattle. In the event that automated IVG hormone delivery devices are developed, the frequency, rate, and volume of PGF_{2α} delivery could be easily adjusted to optimize CL regression rates and P4 profiles.

The relevance and practical implications of the apparent delay in circulating P4 decline for cows in the IVG-treated group are currently unclear. Based on the results for estrus expression, ovulation after GnRH treatment, and P/AI it does not seem that this delay in P4 reduction in circulation could have major negative consequences when treatment is applied at the first PGF_{2α} of the DO protocol. Our results for estrus expression are in agreement with Zdunczyk et al. (1994),

who reported a similar proportion of *Bos indicus* cows detected in estrus for up to 10 d after IM or IVG treatment with the synthetic analog of PGF_{2α} cloprostenol. A caveat of the current experiment is that treatment with GnRH 72 h after induction of CL regression may have precluded expression of estrus in some cows despite having had complete CL regression. In addition, P/AI was not evaluated for the ovulation immediately after induction of luteal regression with the IVG treatment but rather after a synchronized ovulation with the Ovsynch-56 protocol after presynchronization of the estrous cycle. Thus, even though our current results for estrus expression, ovulation after GnRH treatment, and P/AI are encouraging, they should be interpreted with caution and further research should be conducted to evaluate these important reproductive outcomes. More time should be provided for cows to display estrus after IVG treatment with PGF_{2α}, and AI should be performed after a synchronized ovulation in cows in which CL regression was induced by IVG delivery of PGF_{2α}.

In an attempt to elucidate potential reasons for CL regression failures in IVG-treated cows and better explain differences in circulating P4 profiles between treatments, we evaluated CL regression risk and explored P4 profiles of cows grouped based on the level of maturity of their CL. Cows were grouped based on CL maturity levels as determined by timing of their formation in relationship to the PGF_{2α} treatment. Based on previous observations for CL regression and P4 profiles in cows with CL of different levels of maturity treated with IM PGF_{2α} (Giordano et al., 2012; Stevenson, 2016; Stevenson et al., 2018), we expected that cows with CL formed after the G1 of DO (i.e., <7 d old before PGF_{2α} treatment) and treated with PGF_{2α} would be less likely to present complete luteal regression and that P4 levels would not decline to the same levels as for cows with CL formed before the G1 of DO (i.e., >7 d old at time of PGF_{2α} treatment). Moreover, for cows with a combination of CL formed before and after the G1 of DO (i.e., at least 1 CL >7 d old and at least 1 CL <7 d old), we expected similar luteal regression rates and P4 profiles as for cows with CL formed before G1 of DO. If the level of CL maturity affected the response to PGF_{2α}, strategies to improve the response to IVG treatment could be identified. Results were as expected for the IM-PGF treatment, providing additional evidence of compromised luteal regression in cows that receive an IM luteolytic dose of PGF_{2α} 7 d after ovulating in response to a GnRH treatment. Contrary to our expectations, the similar CL regression risk for cows in the IVG-PGF treatment with different types of CL suggested that the relationship between level of CL maturity and CL regression success may not be the same as that for IM-treated cows. Based on the P4 profiles

observed, which were characterized by greater P4 levels in the O+NCL group, a potential explanation for our findings may be the interplay between the circulating concentrations of P4 at the time of treatment (Figure 4) and the slower rate of circulating P4 decline in cows that received the IVG PGF treatment. We speculate that some cows in the OCL and O+NCL groups with circulating concentrations of P4 in the upper range for this experiment may not have had sufficient time for circulating P4 to decline below the CL regression cutoff. Even though this could explain our observations for cows in the IVG-PGF treatment, the relatively high CL regression rate for the NCL group in the IVG-PGF treatment compared with other CL groups and the IM-treated group suggests that other mechanisms may affect the success of the IVG treatment. Our findings for the differences in CL regression risk within and across treatments should be interpreted with caution because the relatively limited number of cows in each CL group may have affected the results of the statistical comparisons. Additional research should be conducted with the proper number of IVG-treated cows to test hypotheses related to luteal responses in cows with CL of different levels of maturity.

CONCLUSIONS

Intravaginal instillation of two 25-mg doses of the natural form of $PGF_{2\alpha}$, dinoprost, was as effective as a single IM 25-mg treatment with $PGF_{2\alpha}$ for induction of complete CL regression when used in the presynchronization of the estrous cycle portion of a fertility protocol such as DO for lactating dairy cows. A similar risk of CL regression was observed despite an apparent temporary delay in P4 decline after the IVG treatment. The implications of the delay in rate of P4 decline after the first $PGF_{2\alpha}$ of the DO protocol were likely minor because there were no differences in the proportion of cows detected in estrus and cows with ovulation after GnRH treatment. Although further research is required to evaluate the success of synchronization of ovulation protocols including IVG treatment with $PGF_{2\alpha}$, the results of this experiment indicated that two 25-mg IVG treatments with dinoprost 12 h apart can be as effective as the regular treatment and route of administration (i.e., 25 mg IM) of dinoprost for induction of complete luteal regression in lactating dairy cows.

ACKNOWLEDGMENTS

This research was supported by Hatch funds under project NYC-127434 from the USDA National Institute of Food and Agriculture (Washington, DC) and a grant

from the Research Innovation Fund from the Cornell Initiative for Digital Agriculture. We thank personnel from the Dairy Unit of the Cornell University Ruminant Center (Harford, NY) for their help conducting this experiment. The authors have not stated any conflicts of interest.

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