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Effect of dose and timing of prostaglandin $F_{2\alpha}$ treatments during a Resynch protocol on luteal regression and fertility to timed artificial insemination in lactating Holstein cows

R. V. Barletta,*† P. D. Carvalho,* V. G. Santos,* L. F. Melo,* C. E. Consentini,* A. S. Netto,† and P. M. Fricke*[1]
*Department of Dairy Science, University of Wisconsin, Madison 53706
†Department of Animal Science, University of São Paulo, SP 13418-900, Brazil

ABSTRACT

Our objective was to evaluate the effect of a second $PGF_{2\alpha}$ treatment (25 mg of dinoprost) or a double dose of $PGF_{2\alpha}$ (50 mg of dinoprost) during a Resynch protocol on luteal regression and pregnancies per artificial insemination (P/AI) in lactating dairy cows. Lactating Holstein cows (n = 1,100) were randomly assigned at a nonpregnancy diagnosis to receive (1) Ovsynch (control: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH), (2) Ovsynch with a second $PGF_{2\alpha}$ treatment (GPPG: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 24 h, 25 mg of $PGF_{2\alpha}$; 32 h, 100 μg of GnRH), or (3) Ovsynch with a double dose of $PGF_{2\alpha}$ (GDDP: 100 μg of GnRH; 7 d, 50 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH). All cows received timed artificial insemination (TAI) approximately 16 h after the second GnRH treatment (G2). Pregnancy diagnosis was performed by transrectal palpation 39 ± 3 d after TAI, and pregnancy status was reconfirmed 66 d after TAI. Blood samples collected from a subset of cows in each treatment at the first $PGF_{2\alpha}$ treatment (n = 394) and at G2 (n = 367) were assayed for progesterone (P4). Data were analyzed by logistic regression using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). At 39 d after TAI, GPPG cows tended to have more P/AI than control cows [35% (137/387) vs. 31% (107/349)], whereas P/AI for GDDP cows [32% (118/364)] did not differ from that for control cows. Pregnancy loss from 38 to 66 d did not differ among treatments and was 8% (30/362). The percentage of cows with complete luteal regression (P4 <0.4 ng/mL at G2) tended to differ among treatments and was greater for GPPG cows than for GDDP and control cows (94% vs. 88% vs. 88%, respectively). Overall, cows with P4 <1 ng/mL at the first $PGF_{2\alpha}$ treatment had fewer P/AI than cows with P4 ≥1 ng/mL (27% vs. 38%), whereas cows with P4 ≥0.4 ng/mL at G2 had fewer P/AI than cows with P4 <0.4 ng/mL (15% vs. 38%). We conclude that adding a second $PGF_{2\alpha}$ treatment 24 h after the first within a Resynch protocol tended to increase the proportion of cows undergoing complete luteal regression and P/AI, whereas treatment with a double dose of $PGF_{2\alpha}$ at a single time did not.

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

INTRODUCTION

Recent research on fertility to timed AI (**TAI**) after an Ovsynch protocol has focused on incomplete luteal regression after the $PGF_{2\alpha}$ treatment during the protocol based on progesterone (**P4**) concentrations at the second GnRH treatment (**G2**) of the protocol. In these experiments, there is a subset of cows that have slightly elevated P4 (≥0.4 ng/mL) at G2, which is associated with a dramatic decrease in pregnancies per AI (**P/AI**; Giordano et al., 2012b; Wiltbank et al., 2014; Carvalho et al., 2015a). This observation has led to a modification of the Ovsynch protocol in which a second $PGF_{2\alpha}$ treatment is administered 24 h after the first. Although this strategy increases P/AI by increasing the percentage of cows with complete luteal regression (Carvalho et al., 2015a; Wiltbank et al., 2015; Santos et al., 2016), from a practical standpoint it also increases the number of times that cows need to be handled during the protocol.

An alternative strategy to increase luteal regression might be to increase the dose of $PGF_{2\alpha}$ administered as a single treatment at the scheduled time during the protocol (Ribeiro et al., 2012; Giordano et al., 2013). Administration of a double dose of $PGF_{2\alpha}$ (either cloprostenol sodium or dinoprost tromethamine) during a 5-d Ovsynch protocol resulted in fewer P/AI and failed to achieve similar rates of luteal regression compared with cows receiving 2 doses of $PGF_{2\alpha}$ 24 h apart (Ribeiro et al., 2012). In another study, increasing the dose of cloprostenol from 500 μg to 750 μg for cows submitted

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[1]Corresponding author: pmfricke@wisc.edu

to a 7-d Ovsynch protocol increased luteal regression in multiparous but not in primiparous cows and tended to increase P/AI (Giordano et al., 2012b). The results by Giordano et al. (2012b) support the idea that for cows submitted to a 7-d Ovsynch protocol, an increased dose of $PGF_{2\alpha}$ might increase the rate of luteal regression. In addition, simplification of hormonal protocols for TAI may increase on-farm protocol compliance and decrease labor associated with reproductive management.

Therefore, our objective was to evaluate the effect of the addition of a second $PGF_{2\alpha}$ treatment (25 mg/dose of dinoprost tromethamine) 24 h after the first, or treatment with a double dose of $PGF_{2\alpha}$ at a single time (50 mg of dinoprost tromethamine) during a Resynch protocol, on luteal regression and P/AI in lactating Holstein cows. Our hypothesis was that cows receiving a second $PGF_{2\alpha}$ treatment administered 24 h after the first or treated with a double dose of $PGF_{2\alpha}$ at a single time will increase the percentage of cows undergoing complete luteal regression by G2, thereby increasing P/AI compared with control cows receiving a single $PGF_{2\alpha}$ treatment during a protocol for resynchronization of ovulation.

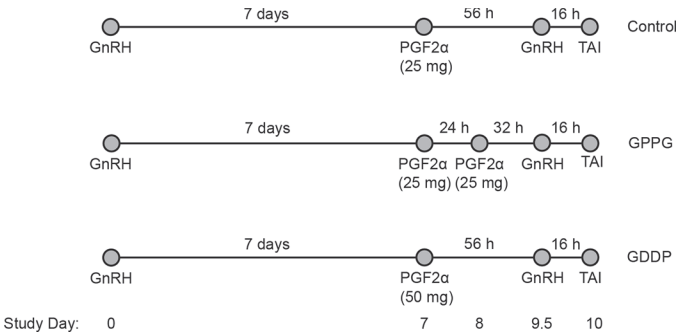

Figure 1. Schematic representation of the Resynch protocols. Lactating Holstein cows (n = 1,100; 417 primiparous and 683 multiparous cows) were randomly assigned after a nonpregnancy diagnosis to receive (1) Ovsynch (control: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH), (2) Ovsynch with a second $PGF_{2\alpha}$ treatment (GPPG: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 24 h, 25 mg of $PGF_{2\alpha}$; 32 h, 100 μg of GnRH), or (3) Ovsynch with a double dose of $PGF_{2\alpha}$ (GDDP: 100 μg of GnRH; 7 d, 50 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH). All cows received timed AI (TAI) approximately 16 h after the last GnRH treatment.

MATERIALS AND METHODS

All animal handling procedures were approved by the Animal Care and Use Committee of the College of Agriculture and Life Sciences at the University of Wisconsin–Madison.

Cows, Housing, and Feeding

This experiment was conducted in collaboration with a commercial dairy farm in Wisconsin milking approximately 8,000 Holstein cows. Cows were housed in cross-ventilated freestall barns bedded with sand and had ad libitum access to feed and water. Cows were fed twice daily a TMR diet consisting of corn silage and alfalfa hay as forage with corn and soybean meal-based concentrate formulated to meet or exceed the dietary requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% FCM (NRC, 2001). Cows were milked 3 times daily at approximately 8-h intervals and received bST (Posilac, 500 mg; Elanco Animal Health, Indianapolis, IN) starting at approximately 63 d postpartum and continuing every 14 d until cows were dried off.

Experimental Treatments

Lactating Holstein cows (n = 1,100; 417 primiparous and 683 multiparous cows) diagnosed not pregnant were randomly assigned to 1 of 3 protocols for resynchronization of ovulation and TAI (Figure 1). Cows randomized to the first treatment received an Ovsynch protocol with a single $PGF_{2\alpha}$ treatment (control: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH); cows randomized to the second treatment received an Ovsynch protocol with 2 $PGF_{2\alpha}$ treatments administered 24 h apart (**GPPG**: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 24 h, 25 mg of $PGF_{2\alpha}$; 32 h, 100 μg of GnRH); and cows randomized to the third treatment received an Ovsynch protocol with a double dose of $PGF_{2\alpha}$ (**GDDP**: 100 μg of GnRH; 7 d, 50 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH). All cows received TAI approximately 16 h after G2. The GnRH (100 μg/dose of gonadorelin hydrochloride; Factrel) and the $PGF_{2\alpha}$ (25 mg/dose of dinoprost tromethamine; Lutalyse) were from Zoetis (Madison, NJ).

Blood Sampling and P4 Assay

Blood samples were collected from a subgroup of cows in each treatment at the first $PGF_{2\alpha}$ treatment and at G2 of the Ovsynch protocols via venipuncture of the median coccygeal vein or artery into 10-mL evacuated serum collection tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). After collection, blood samples were allowed to clot in a refrigerator for 24 h and were subsequently centrifuged (2,000 × *g* for 20 min at 4°C), and serum was decanted into 2-mL Safe-Lock tubes (Eppendorf AG, Hamburg, Germany) and stored at −20°C until assayed for P4. Concentrations of P4 were determined using a solid-phase, no-extraction RIA (Coat-A-Count; Diagnostic Products Corp., Los Angeles, CA). The average sensitivity for the 6 P4 as-

says was 0.018 ng/mL. The intra- and interassay coefficients of variation were 2.16 and 2.58%, respectively. Cows were considered to have undergone complete luteal regression when P4 at G2 was <0.4 ng/mL as described elsewhere (Carvalho et al., 2015b; Fricke et al., 2015). The cut-off value of 0.4 ng/mL was chosen based on studies evaluating the relationship between P4 concentration at G2 and fertility in which P/AI is significantly decreased when P4 concentrations at G2 exceed 0.4 ng/mL (Carvalho et al., 2015b; Fricke et al., 2015).

Ovarian Ultrasonography and Pregnancy Diagnosis

Presence or absence of a corpus luteum (**CL**) based on a cutoff diameter of 15 mm (Giordano et al., 2016) was determined in a subgroup of cows at the first GnRH treatment of the Ovsynch protocols by transrectal ultrasonography using a portable scanner (Ibex Pro, E. I. Medical Imaging, Loveland, CO) equipped with a 7.5-MHz linear-array transducer. Pregnancy diagnosis was performed by palpation per rectum of the uterus at 39 d after TAI. Pregnant cows were re-examined by palpation per rectum at 66 d after TAI. Cows diagnosed pregnant 39 d after TAI and subsequently diagnosed not pregnant at the pregnancy reconfirmation 66 d after TAI were considered to have undergone pregnancy loss.

Statistical Analyses

The experimental design was a complete randomized block design with parity (primiparous vs. multiparous) as the blocking factor. Based on a statistical power calculation (Thrusfield et al., 2001), enrollment of 350 cows per treatment allowed for detection of an 8 percentage point treatment difference in P/AI (90% confidence; 80% power) based on a 1-tailed test.

All statistical analyses were performed using SAS computational software (version 9.4 for Microsoft Windows; SAS Institute Inc., Cary, NC). Analysis of binary response data (P/AI, pregnancy loss, percentage of cows with P4 ≥1.0 ng/mL at $PGF_{2\alpha}$, and percentage of cows with P4 <0.4 ng/mL at G2) was performed by logistic regression using the GLIMMIX procedure of SAS. Fixed effects included in the models were treatment, parity (primiparous vs. multiparous), and their interaction, whereas cow was included as a random effect. The treatment × parity interaction was removed from all models because the *P*-value for this interaction was >0.10 in the models. Differences in P4 concentrations at the $PGF_{2\alpha}$ treatment and at G2 were determined by ANOVA using the MIXED procedure of SAS. The model contained the fixed effects of treatment and parity (primiparous vs. multiparous). Two preplanned orthogonal contrasts (C1: control vs. GPPG cows; C2: control vs. GDDP cows) were used to assess treatment effects on luteal regression and P/AI. A significant difference between the levels of a classification variable was considered when $P \leq 0.05$, whereas differences between $P > 0.05$ and $P \leq 0.10$ were considered a statistical tendency. Data are presented as means ± standard error of the mean, obtained using the MEANS procedure of SAS.

RESULTS AND DISCUSSION

Effect of Treatment on P4 Concentrations

At the time of the $PGF_{2\alpha}$ treatment, P4 concentrations did not differ ($P = 0.13$) among treatments (Table 1); however, primiparous cows had greater ($P < 0.01$) P4 concentrations than did multiparous cows (3.7 ± 0.2 vs. 2.9 ± 0.2 ng/mL, respectively). In addition, the percentage of cows with P4 ≥1.0 ng/mL did not differ ($P = 0.32$) among treatments (Table 1); however, it tended to be greater ($P = 0.10$) for primiparous cows than for multiparous cows [78% (134/171) vs. 71% (159/223), respectively]. At G2, P4 concentrations did not differ ($P = 0.14$) among treatments (Table 1). Cows receiving 2 $PGF_{2\alpha}$ treatments (GPPG) tended ($P = 0.06$) to have lower P4 concentrations than did control cows (Table 1); however, P4 concentrations did not differ ($P = 0.37$) between control and GDDP cows (Table 1). Concentrations of P4 did not differ ($P = 0.91$) between parities (0.25 ± 0.05 vs. 0.25 ± 0.06 ng/mL for primiparous and multiparous cows, respectively). The percentage of cows with complete luteal regression (P4 <0.4 ng/mL at G2) tended to differ ($P = 0.08$) among treatments (Table 1) and was greater for GPPG cows than for control cows ($P = 0.03$), but it did not differ ($P = 0.89$) between control and GDDP cows (Table 1). In addition, the percentage of cows undergoing complete luteal regression did not differ ($P = 0.88$) between parities [90% (145/162) vs. 90% (185/205) for primiparous and multiparous cows, respectively].

The bovine CL acquires luteolytic capacity around d 6 or 7 of the estrous cycle (Rowson et al., 1972; Momont and Sequin, 1984; Nascimento et al., 2014). The lack of regression of the early CL is an intriguing biological phenomenon. Considering that ovulation occurs 28 to 32 h after G2 of an Ovsynch protocol (Pursley et al., 1995), cows ovulating in response to the first GnRH treatment will have a d-6 CL at the time of the $PGF_{2\alpha}$ treatment during the protocol. The $PGF_{2\alpha}$ receptor (**FP**) belongs to a large receptor family containing 7 transmembrane domains coupled to GTP-binding proteins (Sakamoto et al., 1994). In the CL, FP are

Table 1. Effect of treatment on mean (± SEM) progesterone (P4) concentrations at the $PGF_{2\alpha}$ and the second GnRH treatment (G2) of the Resynch protocols, and percentage of cows [% (no./no.)] with P4 ≥1.0 ng/mL at $PGF_{2\alpha}$ and P4 <0.4 ng/mL at G2 in lactating Holstein cows

	Treatment[1]			*P*-value		
Point of Resynch protocol	Control	GPPG	GDDP	Treatment	C1[2]	C2[3]
$PGF_{2\alpha}$						
P4, ng/mL	3.2 ± 0.2	2.9 ± 0.2	3.4 ± 0.2	0.13	0.16	0.54
P4 ≥1.0 ng/mL, % (no./no.)	75 (102/136)	77 (116/150)	69 (75/108)	0.32	0.60	0.32
G2						
P4, ng/mL	0.31 ± 0.05	0.18 ± 0.05	0.28 ± 0.05	0.14	0.06	0.37
P4 <0.4 ng/mL, % (no./no.)	88 (112/128)	94 (133/142)	88 (85/97)	0.08	0.03	0.89

[1]Lactating Holstein cows (n = 1,100; 417 primiparous and 683 multiparous) were randomly assigned after a nonpregnancy diagnosis to receive (1) Ovsynch (control: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH), (2) Ovsynch with a second $PGF_{2\alpha}$ treatment (GPPG: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 24 h, 25 mg of $PGF_{2\alpha}$; 32 h, 100 μg of GnRH), or (3) Ovsynch with a double dose of $PGF_{2\alpha}$ (GDDP: 100 μg of GnRH; 7 d, 50 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH).

[2]C1 = preplanned contrast comparing control and GPPG treatments.

[3]C2 = preplanned contrast comparing control and GDDP treatments.

located in the plasma membranes of large luteal cells (Wiltbank et al., 1995; Anderson et al., 2001), which arise from luteinization of granulosa cells of the ovulatory follicle (Alila and Hansel, 1984). Interestingly, although FP were not detected in granulosa cells, they were detected in large luteal cells, and no differences in FP concentrations or binding affinity were detected on various days of the estrous cycle (d 2, 4, 6, or 10; Wiltbank et al., 1995). Consequently, the FP appears to be expressed quickly after luteinization of granulosa cells that differentiate into large luteal cells, and therefore the lack of responsiveness of the early CL to exogenous $PGF_{2\alpha}$ is not attributable to a lack of FP.

Several sequential $PGF_{2\alpha}$ pulses are required to induce luteolysis (McCracken et al., 1999; Ginther et al., 2009), whereas inhibition of $PGF_{2\alpha}$ secretion delayed luteolysis (Pugliesi et al., 2011). Interestingly, treatment with $PGF_{2\alpha}$ increased intraluteal $PGF_{2\alpha}$ production in cows that had acquired luteolytic capacity (Tsai and Wiltbank, 1997, 1998). In addition, treatment with Epostane (a hydroxy-delta-5-steroid dehydrogenase, 3 β- and steroid delta-isomerase 1 inhibitor) decreased intraluteal P4 production in the early CL. Although Epostane alone did not increase intraluteal $PGF_{2\alpha}$ secretion, the combined treatment using Epostane with $PGF_{2\alpha}$ increased intraluteal $PGF_{2\alpha}$ secretion and induced luteal regression of CL that are normally refractory to $PGF_{2\alpha}$-induced luteolysis (Diaz et al., 2011). Furthermore, CL failing to undergo luteal regression after 2 intrauterine $PGF_{2\alpha}$ infusions had no reduction in steroidogenic acute regulatory protein, 15-hydroxyprostaglandin dehydrogenase, and vascular endothelial growth factor A mRNA compared with CL that underwent complete luteal regression (Atli et al., 2012). Inhibition of mRNA for steroidogenic acute regulatory protein begins after the second pulse of $PGF_{2\alpha}$; however, a decrease in mRNA for cytochrome P450 family 11 subfamily A member 1 begins after the fourth $PGF_{2\alpha}$ pulse (Wiltbank et al., 2012). Therefore, it is possible that the lack of luteal regression of the early CL might be caused by an inability of $PGF_{2\alpha}$ to decrease intraluteal P4 production and stimulate intraluteal $PGF_{2\alpha}$ secretion.

With the exception of cows undergoing early luteal regression, cows with a CL or cows with high P4 (>1 ng/mL) concentrations at initiation of the Ovsynch protocol will have an older and more mature CL 7 d later (at the time of the $PGF_{2\alpha}$ treatment) that is fully responsive to a single $PGF_{2\alpha}$ treatment. By contrast, cows without a CL or cows with low (<1 ng/mL) P4 concentrations at initiation of the Ovsynch protocol have a greater amplitude of the GnRH-induced LH surge and an increased ovulatory response to GnRH treatment (Giordano et al., 2012a; Lima et al., 2013; Carvalho et al., 2015c), which increased the proportion of cows with a single young CL 7 d later that is not yet fully capable of responding to a single $PGF_{2\alpha}$ treatment. Interestingly, the rate of luteal regression increased for cows with low and high P4 concentrations at initiation of the Ovsynch protocol after administration of a second $PGF_{2\alpha}$ treatment (70% vs. 96% for cows with low P4 and 89% vs. 98% for cows with high P4; Carvalho et al., 2015a). In addition, for cows submitted to a 5-d Ovsynch protocol, treatment with 2 sequential doses of $PGF_{2\alpha}$ increased the rate of luteal regression compared with a single or double dose of $PGF_{2\alpha}$ (Santos et al., 2010; Ribeiro et al., 2012).

Effect of Treatment on P/AI

At 39 d after TAI, GPPG cows tended to have more ($P = 0.08$) P/AI than control cows, whereas P/AI for

Table 2. Effect of treatment on pregnancies per AI [P/AI, % (no./no.)] on d 38 and 66 after timed AI and on pregnancy loss [% (no./no.)] from d 39 to 66 after timed AI in lactating Holstein cows

	Treatment[1]			*P*-value		
Item	Control	GPPG	GDDP	Treatment	C1[2]	C2[3]
P/AI at 39 d, % (no./no.)	30.7 (107/349)	35.4 (137/387)	32.4 (118/364)	0.20	0.08	0.30
P/AI at 66 d, % (no./no.)	28.1 (98/349)	32.8 (127/387)	29.4 (107/364)	0.19	0.08	0.35
Pregnancy loss, % (no./no.)	8.4 (9/107)	7.3 (10/137)	9.3 (11/118)	0.84	0.75	0.81

[1]Lactating Holstein cows (n = 1,100; 417 primiparous and 683 multiparous) were randomly assigned after a nonpregnancy diagnosis to receive (1) Ovsynch (control: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH), (2) Ovsynch with a second $PGF_{2\alpha}$ treatment (GPPG: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 24 h, 25 mg of $PGF_{2\alpha}$; 32 h, 100 μg of GnRH), or (3) Ovsynch with a double dose of $PGF_{2\alpha}$ (GDDP: 100 μg of GnRH; 7 d, 50 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH).

[2]C1 = preplanned contrast comparing control and GPPG treatments.

[3]C2 = preplanned contrast comparing control and GDDP treatments.

GDDP cows did not differ ($P = 0.34$) from that of control cows (Table 2). Similarly, at 66 d after TAI, GPPG cows tended to have more ($P = 0.08$) P/AI than control cows, whereas P/AI for GDDP cows did not differ ($P = 0.30$) from that for control cows (Table 2). Pregnancy loss from 39 to 66 d did not differ ($P = 0.84$) among treatments (Table 2).

For cows without a CL at the first GnRH treatment of the Resynch protocols, P/AI did not differ ($P = 0.19$) among treatments (Table 3). However, GPPG cows tended ($P = 0.09$) to have more P/AI than control cows, whereas P/AI did not differ ($P = 0.37$) between control and GDDP cows (Table 3). For cows with a CL at the first GnRH treatment of the Resynch protocols, P/AI did not differ ($P = 0.46$) among treatments (Table 3). In addition, P/AI did not differ ($P = 0.40$) between control and GPPG cows or between ($P = 0.46$) control and GDDP cows (Table 3). The small sample for CL status at initiation of the Ovsynch protocol may explain why a 19 percentage point difference in P/AI did not reach a significant *P*-value when comparing treatment effects for cows without a CL.

At 39 d after AI, cows with P4 <1 ng/mL at the $PGF_{2\alpha}$ treatment had fewer ($P = 0.05$) P/AI than cows with P4 ≥1 ng/mL [27% (26/96) vs. 38% (105/276)]. In addition, cows with P4 <0.4 ng/mL at G2 had more ($P < 0.01$) P/AI than cows with P4 ≥0.4 ng/mL [38% (118/312) vs. 15% (5/34)].

To define the cutoff P4 level at which P/AI decreases, thereby defining lack of complete luteal regression, cows were stratified into P4 classes to assess the effect of P4 near TAI on P/AI. Using this methodology, the level of P4 near AI after which P/AI dramatically decreases has been reported to range from 0.3 to 0.5 ng/mL (Ribeiro et al., 2012; Wiltbank et al., 2014; Fricke et al., 2015). The effect of incomplete luteal regression, which is defined as slightly elevated P4 (≥0.4 ng/mL) near AI, on P/AI has been tested in several studies with consistent results (Giordano et al., 2012b; Carvalho et al., 2015a; Fricke et al., 2015). For instance, evaluating the relationship between P4 concentrations near AI and P/AI, cows with P4 concentrations ≥0.4 ng/mL near AI had dramatically fewer P/AI than cows with P4 concentrations <0.4 ng/mL. The exact mechanism by which a slight elevation in P4 near AI affects P/AI is not clearly understood; however, we speculate that slightly elevated P4 (≥0.4 ng/mL) might interfere with gamete transport based on a decrease in fertilization rate asso-

Table 3. Effect of the presence or absence of a corpus luteum (CL) at the first GnRH treatment of the Resynch protocols (G1) on pregnancies per AI [% (no./no.)] 39 d after timed AI in lactating Holstein cows

	Treatment[1]			*P*-value		
Item	Control	GPPG	GDDP	Treatment	C1[2]	C2[3]
No CL at G1, % (no./no.)	25.0 (6/24)	44.0 (8/18)	29.2 (7/24)	0.19	0.09	0.37
CL at G1, % (no./no.)	33.7 (30/89)	32.0 (31/97)	34.4 (32/93)	0.46	0.40	0.46

[1]Lactating Holstein cows (n = 1,100; 417 primiparous and 683 multiparous) were randomly assigned after a nonpregnancy diagnosis to receive (1) Ovsynch (control: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH), (2) Ovsynch with a second $PGF_{2\alpha}$ treatment (GPPG: 100 μg of GnRH; 7 d, 25 mg of $PGF_{2\alpha}$; 24 h, 25 mg of $PGF_{2\alpha}$; 32 h, 100 μg of GnRH), or (3) Ovsynch with a double dose of $PGF_{2\alpha}$ (GDDP: 100 μg of GnRH; 7 d, 50 mg of $PGF_{2\alpha}$; 56 h, 100 μg of GnRH).

[2]C1 = preplanned contrast comparing control and GPPG treatments.

[3]C2 = preplanned contrast comparing control and GDDP treatments.

ciated with a slight elevation in P4 concentrations near AI (Wiltbank et al., 2011). In addition, endometrial thickness increases after treatment with $PGF_{2\alpha}$ (Souza et al., 2011). Slight increases in P4 concentrations at G2 are associated with decreased endometrial thickness, and cows with a thinner endometrium at G2 had fewer P/AI (Souza et al., 2011). In conclusion, adding a second $PGF_{2\alpha}$ treatment 24 h after the first within a Resynch protocol tended to increase the proportion of cows undergoing complete luteal regression and P/AI, whereas treatment with a double dose of $PGF_{2\alpha}$ at a single time did not.

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