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Efficacy of an injection of dinoprost tromethamine when given subcutaneously on luteal regression in lactating Holstein cows

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Abstract

The objectives of these studies were to evaluate the efficacy of a $PGF_{2\alpha}$ (PGF) analog given through different routes on causing luteal regression in lactating dairy cows. In Experiment 1, lactating Holstein cows ($n = 118$) at random stages of lactation were blocked by parity and days in milk (DIM) and, within each block, randomly assigned to receive PGF as an intra-muscular (IM) injection in the semimembranous/semitendinous muscle (CON), subcutaneous (SC) injection in the cervical area (SCN), or SC injection in the ischio-rectal fossa (IRF). Blood was sampled at 0, 12, 24, 36, and 48 h after treatment for assessment of progesterone concentration. In Experiment 2, a total of 379 lactating Holstein cows, 46 ± 7 DIM, were blocked by DIM and, within each block, randomly assigned to receive treatment similar to CON or IRF groups from Experiment 1. Blood was sampled 0 and 48 h after treatment for assessment of progesterone concentration. Cows were classified as experiencing luteal regression when progesterone concentration was <1.0 ng/mL or <40% of initial concentration (0 h = 100%). In Experiment 1, there was no effect of route of PGF treatment on decline in progesterone concentration and on the proportion of cows experiencing luteal regression by 12, 24, 36, and 48 h after treatment. Similarly, in Experiment 2, route of treatment did not affect either the decline in progesterone concentration or the proportion of cows that had luteal regression by 48 h after treatment. Treatment of lactating dairy cows with 25 mg of PGF given SC in the ischio-rectal fossa did not affect either the decline in progesterone concentration or the proportion of cows that experienced luteal regression by 12, 24, 36, and 48 h after PGF treatment.

Keywords: Prostaglandin; Luteolysis; Lactating dairy cows

1. Introduction

Although milk is the primary product from lactating dairy cows, 20% of the beef produced in the U.S. is originated from non-fed beef, of which 75% is estimated to be derived from the slaughter of market lactating dairy cows [1]. According to the 1994 National Non-Fed Beef Quality Audit (NNFBQA), beef quality shortfalls and inconsistencies cost the industry $ 69.90 for every non-fed animal marketed, and in 1999 the cost of beef quality inconsistencies was estimated to be $ 68.82 [1]. Furthermore, approximately 35% of samples collected from outside rounds of dairy cows had lesions, in comparison to only 20% from beef cows [1].

Modern dairy operations rely on hormonal treatments to maximize reproductive efficiency [2], and the

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fixed time artificial insemination protocols that are widely implemented in dairy operations utilize at least one treatment with PGF [3–5]. Although, the recommended administration route for PGF is intramuscular (IM), in order to minimize injection-site lesions, this route should be avoided [6].

Intramuscular treatment with PGF results in delayed peak and smaller plasma concentration of PGF compared to intravenous treatment [7]. Atlhough dairy heifers treated with dinoprost thromethamine IM had a similar interval from treatment to estrus compared to those treated SC [8], SC treatment of beef heifers with cloprostenol resulted in longer interval between treatment and estrus than heifers treated IM [9]. In spite of possible differences in absorption rate and peak concentration of PGF after treatment through different routes, similar proportion of non-lactating dairy cows and dairy heifers treated with dinoprost tromethamine sterile solution SC in the ischio-rectal fossa experienced luteal regression compared to those treated IM in the glutei [10].

The hypotheses of the present study were that the decline in progesterone concentration and the proportion of lactating dairy cows experiencing luteolysis after dinoprost tromethamine treatment would not be different between IM and SC routes. Therefore, the objectives of the present study were to compare the decline in progesterone concentration and the proportion of lactating dairy cows experiencing luteolysis after IM and SC dinoprost tromethamine treatment.

2. Materials and methods

2.1. Animals, housing, and diets

Lactating Holstein cows from a commercial dairy farm with 3200 lactating dairy cows and a rolling herd average milk production of 12,035 kg of 3.5% FCM located in the San Joaquin Valley of California were used in these experiments. Cows were housed in free-stall barns and individual pens were virtually identical in design, size, and number of animals housed.

Cows were fed a total mixed ration twice a day and diets were based on corn silage, alfalfa hay, soybean meal, steam-rolled corn, whole cottonseed, calcium salts of palm oil, and a mineral and vitamin and protein supplement. The diet was designed to meet or exceed the requirements set forth by the NRC [11] for lactating Holstein cows weighing 650 kg and producing 45 kg/day of milk containing 3.5% fat.

2.2. Treatments

2.2.1. Experiment 1

One hundred and eighteen ($n = 118$) lactating Holstein cows at random stages of lactation were enrolled in this experiment. Cows were blocked by parity and DIM and, within each block, were assigned to receive one of three treatments. All cows received one injection of 25 mg of PGF (dinoprost tromethamine, Lutalyse Sterile Solution, Pfizer Animal Health, New York, NY, USA) 14 d prior to enrollment (study day 0 = enrollment day) for synchronization of the estrous cycle. On the day of enrollment, cows assigned to the control (CON, $n = 39$) group received one IM injection of 25 mg of PGF in the semimembranous/semitendinous muscle, cows assigned to the subcutaneous neck (SCN, $n = 40$) group received one SC injection of 25 mg of PGF in the cervical part of the trapezius muscle, and cows enrolled in the subcutaneous ischio-rectal fossa (IRF, $n = 39$) group received one SC injection of 25 mg of PGF in the ischio-rectal fossa. The needles used for treatment of cows in the CON and IRF groups were 18 gauge in diameter and 38 mm long, whereas the needles used for treatment of cows in the SCN group were 18 gauge in diameter and 25 mm long.

2.2.2. Experiment 2

Multiparous lactating Holstein cows ($n = 379$), between 39 and 53 DIM, were enrolled in this experiment. Fourteen days prior to enrollment, all cows received an injection of 25 mg of PGF IM for presynchronization. At enrollment cows were blocked by DIM and assigned to one of two treatments. Cows enrolled in the control (CON, $n = 186$) group received the same treatment as the CON group from Experiment 1 and cows enrolled in the subcutaneous ischio-rectal fossa (IRF, $n = 193$) group received the same treatment as the IRF group from Experiment 1. Needles used in this experiment were 18 gauge in diameter and 38 mm long.

2.3. Blood sample collection and characterization of CL regression

Evacuated tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing Na EDTA were used to collect blood (7 mL) from the median coccygeal vein or artery. Samples were immediately placed in ice and transported to the laboratory within 5 h of collection. Blood tubes were centrifuged at $2000 \times g$ for 15 min for plasma separation. Plasma samples were frozen at $-25\ ^{\circ}C$ and later analyzed for progesterone by ELISA [12]. Intra-assay CV was determined for each 96-well

plate, and a plasma sample with 2.5 ng/mL of progesterone was used in each plate to estimate the inter-assay coefficient of variation.

In Experiment 1, blood samples were collected on study day 0 (0 h) immediately prior to the injection of PGF and at 12, 24, 36, and 48 h after treatment. The sensitivity of the assay was 0.02 ng/mL and the intra and inter-assay CV were 7.4 and 8.7%, respectively. In Experiment 2, blood was sampled on study day 0 (0 h) immediately prior to the injection of PGF and 48 h after treatment. The sensitivity of the assay was 0.01 ng/mL and the intra and inter-assay CV were 6.1 and 8.9%, respectively.

Retrospectively, cows with progesterone concentration <1.0 ng/mL at 0 h were considered not to have a functional CL and were removed from the study. Cows were classified as having experienced luteal regression when progesterone concentration was <1.0 ng/mL or when relative progesterone concentration was $<40\%$. This second parameter was used because it has been previously demonstrated that cows and heifers that have a decline in progesterone concentration of this magnitude have progesterone concentration <1.0 ng/mL within 24 h later [13]. Relative progesterone concentration was calculated by dividing the progesterone concentration of the sampling time specified by the progesterone concentration at 0 h.

For evaluation of the effects of progesterone concentration at 0 h on occurrence of luteolysis, cows were classified as having progesterone concentration ≥ 2.5 or <2.5 ng/mL. Furthermore, when an effect of progesterone concentration at 0 h on the proportion of cows experiencing luteolysis was observed, progesterone concentration at 0 h was also categorized in increments of 1.0 ng/mL.

2.4. Body condition score and milk yield

Cows were scored for body condition (1 = emaciated, 5 = obese) on study day 0, as described by Ferguson et al. [14]. For purpose of analyses of the effects of body condition score (BCS) on progesterone concentration, luteal regression, and decline in progesterone concentration cows were classified according to BCS as low if BCS ≤ 2.75 or moderate if BCS > 2.75.

Monthly milk yield was recorded for individual cows during official Dairy Herd Improvement Association test. In Experiment 1, milk yield in the test immediately prior to enrollment was utilized to evaluate the effect of milk production on progesterone concentration, decline in progesterone concentration, and luteal regression. In Experiment 2, average milk yield during the first 3 months of lactation was used because cows were between 39 and 53 DIM at enrollment. When data from Experiments 1 and 2 were combined, the milk yield in the test immediately prior to enrollment was utilized for all cows.

2.5. Experimental design and statistical analyses

The experimental designs of Experiments 1 and 2 were complete randomized with blocks. In Experiment 1, cows ($n = 118$) were blocked by parity and DIM and, within each block, randomly assigned to one of three treatments. In Experiment 2, a total of 379 multiparous cows were blocked by DIM and, within each block, randomly assigned to one of two treatments. After initial statistical analyses of Experiments 1 and 2 independently, data from CON and IRF cows that had progesterone concentration ≥ 1.0 ng/mL at 0 h from Experiments 1 and 2 were combined.

Concentration of progesterone at 0 h was analyzed by ANOVA using the GLM procedure of SAS [15] with a model that included treatment, parity, BCS, milk yield, and days in milk. Furthermore, average lactation number, BCS, milk yield, and days in milk of cows that did or did not experience luteolysis at different intervals after PGF treatment were analyzed by ANOVA using the GLM procedure of SAS [15].

The decline in progesterone concentration and change in relative concentration of progesterone over time were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS [15]. The model included treatment, the time after PGF treatment, and the interaction between treatment and time after PGF treatment. For evaluation of the effects of occurrence of luteolysis at 48 h after PGF treatment on progesterone concentration the model include of treatment, the time after PGF treatment, the occurrence of luteolysis, and the interactions between treatment and time and between occurrence of luteolysis and time. The covariance structure (unstructured, compound symmetry, toeplitz, and autoregressive order 1) for repeated measures model was tested [16] and chosen based on the Schawrz's Bayesian criterion.

Dichotomous data such as occurrence of luteolysis was analyzed by logistic regression using the LOGISTIC procedure of SAS [15]. The model included treatment, and other covariates such as parity, BCS, milk yield, and days in milk were only included in the model when univariate analyses demonstrated level of significance ≤ 0.20. To evaluate the effect of progesterone concentration at 0 h on the occurrence of luteolysis the model included treatment, progesterone concentration at 0 h,

and the interaction between treatment and progesterone concentration at 0 h. The final logistic regression models removed variables by backward elimination based on the Wald's statistics criterion if the significance was greater than 0.20.

The odds ratio (OR) and the 95% confidence interval (CI) from the logistic regression were obtained for each variable included in the statistical model. Regression analyses between the odds ratio for occurrence of luteolysis and progesterone concentration at 0 h categorized in increments of 1.0 ng/mL were evaluated using the regression procedure of MINITAB [17] to determine the fitted line plot that best described these relationships. Orthogonal polynomials with linear, quadratic, and cubic relationships were evaluated.

3. Results

3.1. Experiment 1

Seventeen cows (14.4%) had progesterone concentration <1.0 ng/mL at 0 h and were not used in the statistical analyses. Therefore, results presented for Experiment 1 represent data from 33, 36, and 32 cows from CON, SCN, and IRF groups, respectively. The average milk yield was not different ($P = 0.39$) between treatment groups and averaged (±S.E.M.) 34.70 ± 0.77 and 37.70 ± 0.67 kg/day for primiparous and multiparous cows, respectively. Furthermore, there was no difference ($P = 0.99$) in BCS at enrollment among treatment groups and it averaged (±S.E.M.) 3.1 ± 0.06 for all cows. Similarly, the average DIM at enrollment was not different ($P = 0.64$) among treatment groups (147.90 ± 8.13 d).

The plasma progesterone concentration at 0 h tended ($P = 0.14$) to be different among treatment groups (CON = 2.84 ± 0.36, SCN = 3.31 ± 0.33, and IRF = 2.42 ± 0.36 ng/mL). Although, treatment group did not ($P = 0.40$) affect the decline in progesterone concentration after PGF treatment (Fig. 1), the average progesterone concentration throughout the study was greater ($P = 0.01$) for SCN cows (CON = 0.96 ± 0.12, SCN = 1.21 ± 0.08, IRF = 0.90 ± 0.09 ng/mL). The change in relative progesterone concentration over time was not affected ($P = 0.47$) by treatment group (Fig. 2).

The proportion of cows that experienced luteolysis by 12 h after PGF treatment was not affected ($P = 0.46$) by treatment group (CON = 81.8%, SCN = 66.7%, and IRF = 71.9%), but cows with moderate BCS were more likely ($P = 0.04$) to experience luteal regression (low = 55.5% and moderate = 78.2%). Similarly, treatment group did not affect ($P = 0.30$) the proportion of cows that experienced luteolysis by 24 h after PGF treatment (CON = 90.9%, SCN = 77.8%, and IRF = 84.4%). Although treatment group did not affect ($P = 0.20$) the proportion of cows that experienced luteolysis at 36 h, cows that were <79 DIM tended ($P = 0.06$) to be less likely to experience it (<79 DIM = 77.8% and ≥80 DIM = 92.3%). Finally, the treatment group did not affect ($P = 0.48$) the proportion of cows experiencing luteolysis by 48 h after PGF treatment (CON = 97.0%, SCN = 88.9%, and IRF = 90.6%). Similarly, no other covariates affected the proportion of cows experiencing luteolysis at 48 h after PGF treatment.

Among the cows classified as experiencing luteolysis at 48 h after PGF treatment ($n = 93$), 89 (95.7%) had progesterone concentration <1.0 ng/mL and four (4.3%) had relative progesterone concentration <40%. The average lactation number ($P = 0.06$) and the average DIM ($P = 0.07$) of cows that experienced

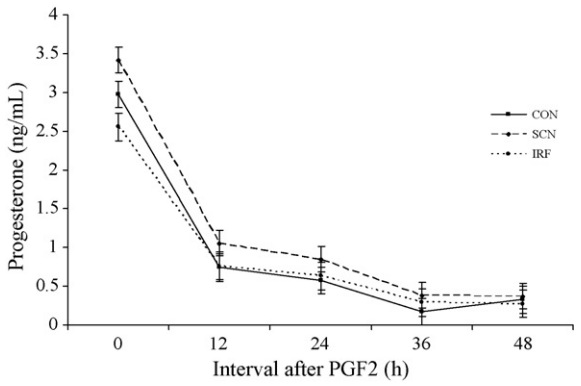

Fig. 1. Change in plasma progesterone concentration after PGF treatment (Experiment 1). Effects of treatment ($P = 0.01$), time ($P < 0.001$), and treatment by time interaction ($P = 0.40$).

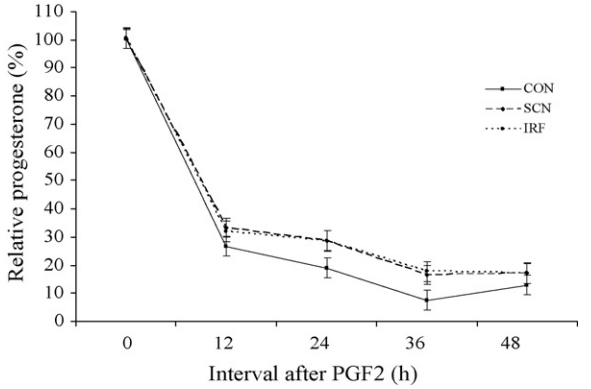

Fig. 2. Change in relative concentration of progesterone over time in Experiment 1. Effects of treatment ($P = 0.19$), time ($P < 0.001$), and treatment by time interaction ($P = 0.48$).

Table 1
Averages for lactation number, BCS, milk yield, and days in milk (DIM) for cows that did or did not experience luteolysis at various intervals after PGF treatment (Experiment 1)

Items (mean ± S.E.M.)	No luteal regression	Luteal regression	*P*-value
12 h after PGF			
Lactation number	2.70 ± 0.29	2.07 ± 0.17	0.06
BCS	3.07 ± 0.07	3.14 ± 0.04	0.37
Milk yield (kg/day)	44.90 ± 1.66	44.49 ± 1.01	0.83
DIM	123.30 ± 15.53	156.88 ± 9.38	0.07
24 h after PGF			
Lactation number	2.81 ± 0.37	2.13 ± 0.16	0.10
BCS	3.13 ± 0.09	3.12 ± 0.04	0.91
Milk yield (kg/day)	43.21 ± 2.16	44.86 ± 0.94	0.48
DIM	145.75 ± 20.52	148.31 ± 8.90	0.91
36 h after PGF			
Lactation number	2.39 ± 0.42	2.22 ± 0.16	0.71
BCS	3.10 ± 0.10	3.12 ± 0.04	0.82
Milk yield (kg/day)	46.12 ± 2.39	44.38 ± 0.92	0.50
DIM	93.69 ± 22.00	155.91 ± 8.46	0.01
48 h after PGF			
Lactation number	1.75 ± 0.53	2.28 ± 0.16	0.34
BCS	3.25 ± 0.12	3.11 ± 0.04	0.26
Milk yield (kg/day)	44.43 ± 3.06	44.61 ± 0.90	0.96
DIM	120.0 ± 28.87	150.30 ± 8.47	0.32

luteolysis within 12 h after PGF treatment tended to be different compared to cows that did not experience luteolysis (Table 1). Similarly, there was a tendency ($P = 0.10$) for the average lactation number of cows that did and did not experience luteolysis at 24 h after PGF treatment to differ (Table 1). Cows that experienced luteolysis at 36 h had greater ($P = 0.01$) average DIM than those that did not experience it (Table 1). However, there was no difference in average lactation number, average BCS, average milk yield, and average DIM for cows that did or did not experience luteolysis within 48 h after PGF treatment (Table 1).

There was no difference ($P = 0.25$) in the progesterone concentration at 0 h for cows that experienced or did not experience luteolysis by 48 h after PGF treatment (3.13 ± 0.19 and 2.36 ± 0.65 ng/mL, respectively). Similarly, the proportion of cows with progesterone concentration ≥2.5 ng/mL at 0 h that experienced luteolysis by 48 h after PGF treatment was not different ($P = 0.15$) compared to cows with progesterone concentration <2.5 ng/mL at 0 h (96.1 and 88.0%, respectively).

3.2. Experiment 2

Sixty-nine cows (18.2%) had a progesterone concentration <1.0 ng/mL on study day 0 and were not used in the statistical analysis, resulting in 153 cows in the CON group and 157 cows in the IRF group. The average milk yield during the first 3 months of lactation was not different ($P = 0.99$) between treatment groups and averaged (±S.E.M.) 47.72 ± 0.46 kg/day. Furthermore, there was no difference ($P = 0.77$) in BCS at enrollment among treatment groups and it averaged (±S.E.M.) 2.9 ± 0.1 for all cows. Similarly, the average days in milk at enrollment was not different ($P = 0.73$) among treatment groups (46.09 ± 0.24 d).

At enrollment (0 h) the progesterone concentration did not differ ($P = 0.59$) among treatment groups (CON = 3.42 ± 0.14 and IRF = 3.53 ± 0.13 ng/mL). Treatment group did not affect ($P = 0.43$) the decline in progesterone concentration from 0 to 48 h after PGF treatment, and the average progesterone concentration at 48 h after PGF treatment was 0.90 ± 0.12 and 0.83 ± 0.11 ng/mL for cows in the CON and IRF groups, respectively. Similarly, the change in relative progesterone concentration was not affected ($P = 0.43$) by treatment group and averaged 34.10 ± 3.10 and 29.26 ± 3.06% at 48 h after PGF treatment for cows in the CON and IRF groups, respectively.

A total of 268 cows were classified as experiencing luteolysis at 48 h after PGF treatment, and 230 (85.8%) of these cows had progesterone concentration <1.0 ng/mL, whereas 38 (14.2%) had relative progesterone concentration <40%. The proportion of cows experiencing luteolysis at 48 h after PGF treatment was not affected ($P = 0.93$) by treatment group (CON = 86.3% and IRF = 86.6%).

There was no difference in the average lactation number ($P = 0.34$), average BCS ($P = 0.25$), average milk yield during the first 3 months of lactation ($P = 0.30$), and average DIM ($P = 0.54$) between cows that did or did not experience luteolysis within 48 h after PGF treatment (Table 2). However, cows that did

Table 2
Average lactation number, average BCS, average milk yield, and average days in milk (DIM) for cows that did or did not experience luteolysis at 48 h after PGF treatment

Items (mean ± S.E.M.)	No luteal regression	Luteal regression	*P*-value
Experiment 2			
Lactation number	3.07 ± 0.16	3.24 ± 0.06	0.34
BCS	2.90 ± 0.05	2.84 ± 0.02	0.25
Milk yield (kg/day)	46.51 ± 1.25	47.91 ± 0.50	0.30
DIM	46.45 ± 0.64	46.03 ± 0.25	0.54
Experiments 1 and 2 combined			
Lactation number	2.96 ± 0.17	3.04 ± 0.07	0.67
BCS	2.94 ± 0.05	2.89 ± 0.02	0.31
Milk yield (kg/day)	48.26 ± 1.37	49.71 ± 0.51	0.32
DIM	54.24 ± 7.83	66.00 ± 2.93	0.16

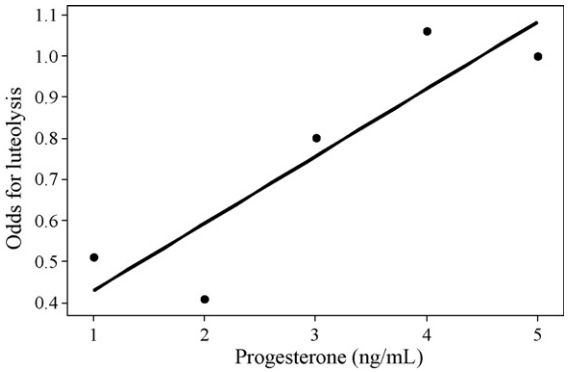

Fig. 3. Relationship between the odds ratio for luteolysis and progesterone concentration at 0 h. Odds = 0.2663 + 0.1633 × progesterone concentration; $r^2 = 72.4\%$. Effect of progesterone concentration according to the multivariate logistic regression model: $P = 0.05$ (Experiment 2).

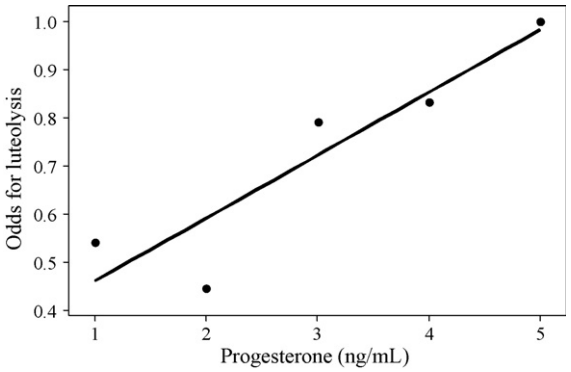

Fig. 4. Relationship between the odds ratio for luteolysis and progesterone concentration at 0 h. Odds = 0.3315 + 0.1303 × progesterone concentration; $r^2 = 78.2\%$. Effect of progesterone concentration according to the multivariate logistic regression model: $P = 0.02$ (Experiments 1 and 2 combined).

not experience luteolysis had smaller ($P = 0.02$) concentration of progesterone at 0 h compared to those that did experience it (2.93 ± 0.26 and 3.56 ± 0.10 ng/mL, respectively). Actually, greater proportion of cows with progesterone concentration ≥2.5 ng/mL at 0 h experienced luteal regression compared to those that had progesterone concentration <2.5 ng/mL (89.3 and 81.0%, respectively), and an increase in 1.0 ng/mL in progesterone concentration at 0 h was associated with an increase in the odds ratio for luteolysis at 48 h after PGF treatment of approximately 16% (Fig. 3).

3.3. Experiments 1 and 2 combined

Results presented for Experiments 1 and 2 combined represent data from 375 cows (CON = 186 and IRF = 189). There was no difference ($P = 0.67$) in average milk yield immediately prior to enrollment among treatment groups and the average was 49.54 ± 0.48 kg/day. Similarly, the body condition score at enrollment did not differ ($P = 0.74$) among treatment groups (2.9 ± 0.1). The average DIM at enrollment was not different ($P = 0.59$) among treatment groups and it averaged 64.55 ± 2.75 day.

The progesterone concentration at 0 h was not different ($P = 0.98$) among treatment groups (CON = 3.08 ± 0.19 ng/mL versus IRF = 3.08 ± 0.18 ng/mL). Treatment group did not affect ($P = 0.73$) the decline in progesterone concentration and the progesterone concentrations of cows from the CON and IRF groups at 48 h after PGF treatment were 0.63 ± 0.14 and 0.55 ± 0.14 ng/mL, respectively. Similarly, the relative progesterone concentration at 48 h after PGF treatment was not affected ($P = 0.65$) by treatment group (CON = 24.74 ± 3.55% and IRF = 22.05 ± 3.55%).

Among the cows that experienced luteal regression at 48 h after PGF treatment ($n = 329$), 289 (87.8%) had progesterone concentration <1.0 ng/mL and 40 (12.2%) had relative progesterone concentration <40%. The proportion of cows that experienced luteolysis by 48 h after PGF treatment was not affected ($P = 0.81$) by treatment group (CON = 88.2 and IRF = 87.3%).

The average lactation number ($P = 0.67$), average BCS ($P = 0.31$), average milk yield immediately prior to enrollment ($P = 0.32$), and average DIM at enrollment ($P = 0.16$) were not different between cows that experience or did not experience luteolysis at 48 h after PGF treatment (Table 2). Cows that did not experience luteolysis, however, had a lower concentration ($P = 0.04$) of progesterone at 0 h than those that experienced luteal regression (2.88 ± 0.25 and 3.43 ± 0.09 ng/mL, respectively). Greater proportion of cows with progesterone concentration ≥2.5 ng/mL at 0 h experienced luteolysis compared to those with progesterone concentration <2.5 ng/mL (90.5 and 83.2%, respectively), and as the progesterone concentration at 0 h increased in 1.0 ng/mL the odds ratio for luteolysis increased in approximately 13% (Fig. 4).

4. Discussion

Large dairy operations are challenged by reduced fertility of lactating dairy cows due to reduced estrous expression and detection. Herd managers often adopt estrous/ovulation synchronization protocols that usually utilize at least one treatment with PGF. Although the label recommendation for route of administration of PGF is IM, it is highly recommended that such treatments are administered in areas of lower quality meat such as the

neck [6]. However, during daily management of lactating dairy cows, the treatment with injectable drugs in the posterior of the animals is more feasible because they are restrained by head stanchions and herdsmen and veterinarians usually work on them from behind.

Lactating dairy cows treated with 25 mg of PGF SC in the neck or ischio-rectal fossa had similar decline in progesterone concentration and change in relative progesterone concentration over time compared to cows receiving treatment in the semimembranous/semitendinous muscle. The exogenous PGF stimulates the release of oxytocin by the luteal cells [18], which, upon biding to oxytocin receptors in the endometrium, stimulates the endogenous pulsatile release of PGF by the endometrial cells [19,20]. The pulsatile release of PGF results in alteration of blood flow to the CL, change in morphology of the CL, stimulation of intracellular signaling, and stimulation of immune-mediated events that culminate with luteolysis [21]. Stellflug et al. [7] demonstrated that cows treated with 30 or 60 mg of PGF IM had a peak in PGF plasma concentration of 6.0 ng/mL within 10 min of treatment, returning to basal levels within 90 min, whereas cows that received IV treatment with 5 mg of PGF had a peak of 25 ng/mL within 5 min and basal levels were observed within 15 min of the initial treatment. To our knowledge there are no studies evaluating the change in PGF plasma concentration following SC treatment with PGF compared to IM treatment. However, in the present study, the lack of difference in the decline of progesterone concentration and change in relative progesterone concentration over time between cows receiving SC or IM treatment with PGF suggested that the plasma concentration of PGF achieved following SC treatment was adequate to trigger the luteolytic cascade in lactating dairy cows.

The proportion of cows that experienced luteolysis in Experiment 1, regardless of interval from PGF treatment, was similar between cows receiving IM treatment and those receiving SC treatment in different sites. Similarly, in Experiment 2 and Experiments 1 and 2 combined, there was no difference in the proportion of lactating dairy cows experiencing luteolysis 48 h after PGF treatment when they received SC or IM treatments. Previous studies have demonstrated that the proportion of non-lactating dairy cows and dairy heifers that experienced luteolysis following SC treatment with PGF was similar to that of animals receiving IM treatment [10]. The interval from cloprostenol treatment to expression of estrus was greater in beef heifers receiving SC treatment than those treated IM [9]; however, dairy heifers receiving SC treatment of dinoprost tromethamine had a similar interval from treatment to expression of estrus compared to those treated IM [8]. These differences could be accounted for by the different prostaglandin analogs used in the studies cited above. Furthermore, the interval between PGF treatment and expression of estrus was more dependent upon size of follicles present in the ovaries at the time of treatment than the nature of prostaglandin analogs or route of PGF treatment [22].

It is known that the rate of absorption and the peak concentration of plasma PGF following treatment with PGF may differ according to the route of treatment [7]. However, the similar proportion of cows experiencing luteolysis following treatment with PGF regardless of route and site of administration, indicates that SC treatment of lactating dairy cows with 25 mg of dinoprost tromethamine in the neck or ischio-rectal fossa was as efficacious as IM treatment in promoting luteolysis.

Although the proportion of cows that experienced luteolysis at 12 and 36 h after PGF treatment in Experiment 1 was affected by BCS and DIM at enrollment, respectively, the overall proportion of cows experiencing luteolysis at 48 h after PGF treatment was not affected by parity, BCS, milk yield, or DIM. Similarly, there was a tendency for cows that did or did not experience luteolysis at 12 and 24 h after PGF treatment to differ in average lactation number and average DIM. However, these differences were not observed when comparing cows that experienced luteolysis at 48 h to those that did not. Analyses of data from Experiment 2 and Experiments 1 and 2 combined did not reveal any effects of parity, BCS, milk yield, and DIM on the proportion of cows experiencing luteolysis at 48 h after PGF treatment. Furthermore, in Experiment 2 and Experiments 1 and 2 combined, there were no differences in average lactation number, average BCS, average milk yield, and average DIM between cows that did or did not experience luteolysis at 48 h after PGF treatment. Therefore, we concluded from these experiments that the luteolytic activity of PGF when given IM or SC was not affected by parity, BCS, milk yield, and DIM.

The progesterone concentration at 0 h was not correlated with the proportion of cows experiencing luteolysis according to Experiment 1. However, cows that had progesterone concentration $\geq$2.5 ng/mL at 0 h in Experiment 2 and Experiments 1 and 2 combined were more likely to experience luteolysis at 48 h. Furthermore, as progesterone concentration at 0 h increased in 1.0 ng/mL the odds ratio for cows to experience luteolysis at 48 h after PGF treatment

increased in approximately 15% according to Experiment 2 and Experiments 1 and 2 combined. Progesterone concentration in lactating dairy cows increases from ≤1.0 ng/mL on the day of estrus (estrous cycle day 0) to approximately 6.0 ng/mL around day 8 of the estrous cycle [23]. It has been demonstrated that exogenous PGF is unable to cause luteolysis when given in early stages of the estrous cycle [20]. Therefore, it is reasonable to suggest that cows with low progesterone concentration at the time of enrollment were in early stages of the estrous cycle and had CL that were unresponsive to PGF treatment. The lack of difference in Experiment 1 was likely due to the smaller sample size, as the proportion of cows with progesterone concentration ≥2.5 ng/mL at 0 h that experienced luteolysis was numerically greater than that of cows with progesterone concentration <2.5 ng/mL.

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