Effects of 1 or 2 treatments with prostaglandin $F_{2\alpha}$ on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination

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ABSTRACT

The objectives of the current study were to investigate the efficacy of $PGF_{2\alpha}$ as a therapy to reduce the prevalence of subclinical endometritis and improve pregnancy per artificial insemination (P/AI) in cows subjected to a timed artificial insemination (AI) program. A total of 1,342 lactating Holstein dairy cows were allocated randomly at 25 ± 3 d in milk (DIM) to remain as untreated controls (control, $n = 454$) or to receive a single $PGF_{2\alpha}$ treatment at 39 ± 3 DIM (1PGF, $n = 474$) or 2 treatments with $PGF_{2\alpha}$ at 25 ± 3 and 39 ± 3 DIM (2PGF, $n = 414$). All cows were enrolled in the double Ovsynch program at 48 ± 3 DIM and were inseminated at 75 ± 3 DIM. A subset of 357 cows had uterine samples collected for cytological examination at 25 ± 3 , 32 ± 3 , and 46 ± 3 DIM to determine the percentage of polymorphonuclear leukocytes (PMNL). Subclinical endometritis was defined by the presence of $\geq 5\%$ PMNL. Vaginal discharge score was evaluated at 25 ± 3 DIM and used to define the prevalence of purulent vaginal discharge. Body condition score was assessed at 25 ± 3 DIM. Pregnancy was diagnosed 32 d after AI and reconfirmed 28 d later. At 32 ± 3 DIM, the prevalence of subclinical endometritis was reduced by treatment with $PGF_{2\alpha}$ at 25 ± 3 DIM in 2PGF (control = 23.5% vs. 1PGF = 28.3% vs. 2PGF = 16.7%); however, this benefit disappeared at 46 ± 3 DIM, and 14% of the cows remained with subclinical endometritis. One or 2 treatments with $PGF_{2\alpha}$ did not influence P/AI on d 32 or 60 after timed AI, which averaged 39.9 and 35.2%. Similarly, treatment with $PGF_{2\alpha}$ had no effect on pregnancy loss between 32 and 60 d of gestation (11.9%). Cows diagnosed with both purulent vaginal discharge and subclinical endometritis

had the lowest P/AI and the highest pregnancy loss compared with those diagnosed with only 1 of the 2 diseases or compared with cows having no diagnosis of uterine diseases. Interestingly, subclinical endometritis depressed P/AI and increased pregnancy loss only when it persisted until 46 DIM. On d 32 after AI, cows not diagnosed with subclinical endometritis and those that resolved subclinical endometritis by 46 DIM had greater P/AI than those that remained with subclinical endometritis at 46 DIM (45.4 and 40.0 vs. 25.0%, respectively). Similar to P/AI, cows not diagnosed with subclinical endometritis and those that resolved subclinical endometritis by 46 DIM had less pregnancy loss than those with subclinical endometritis at 46 DIM (9.6 and 13.5 vs. 43.9%, respectively). One or 2 treatments with $PGF_{2\alpha}$ before initiation of the timed AI program were unable to improve uterine health, P/AI, and maintenance of pregnancy in lactating dairy cows. Cows diagnosed with both purulent vaginal discharge and subclinical endometritis had the greatest depressions in measures of fertility at first AI, particularly when subclinical endometritis persisted in the early postpartum period.

Key words: dairy cow, endometritis, fertility, prostaglandin

INTRODUCTION

Uterine diseases are prevalent in dairy cows and they have been associated with reduced reproductive performance, which ultimately affects herd profitability (Gilbert et al., 2005; LeBlanc, 2008). Uterine diseases are often classified according to clinical presentation and defined based on their effects on pregnancy per AI (P/AI) or time to pregnancy (Sheldon et al., 2006). Among them, clinical endometritis is defined as presence of inflammation in the reproductive tract visible by the type of vaginal discharge that typically contains pus and persists after 21 DIM (LeBlanc et al., 2002b; Sheldon et al., 2006). More recently, clinical endometritis, as diagnosed by presence of pus in the vagina, was

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classified as purulent vaginal discharge (**PVD**) because of the large proportion of cows without concurrent neutrophil infiltration in the endometrium (Dubuc et al., 2010). On the other hand, a large proportion of cows not diagnosed with any clinical signs of uterine disease have presence of inflammatory cells in the endometrium; usually, more than 5% PMNL in endometrial cytology reduces P/AI and extends the interval postpartum to pregnancy (Gilbert et al., 2005; Galvão et al., 2009a).

In the United States, no particular treatment is labeled for use in cows that have either PVD or subclinical endometritis (**SCE**), although intrauterine infusion of 500 mg of cephapirin as benzathine has demonstrated efficacy in reducing the interval to pregnancy in cows with PVD (LeBlanc et al., 2002a) or improving pregnancy at first AI in cows with SCE (Kasimanickam et al., 2005). In those studies, cows were not subjected to standardized programs for first postpartum AI and many were inseminated following detection of estrus. When cows were subjected to a presynchronized timed AI program, use of intrauterine antibiotics did not benefit P/AI of dairy cows (Galvão et al., 2009b), even in those with previous diagnosis of PVD. An alternative therapy is the use of PGF_{2α} in an attempt to induce estrus and eliminate bacterial contamination that might be causing the inflammatory process in the endometrium. Use of PGF_{2α} in cows during diestrus results in luteolysis and induces cows to return to estrus, which has been suggested to enhance uterine immunity by removal of immunosuppressive effects of progesterone (Lewis, 2004). Kasimanickam et al. (2005) suggested that the improvements in P/AI caused by PGF_{2α} in postpartum cows were caused by inducing estrus and the concurrent opening of the cervix and myometrium contractions that might enhance mechanical cleansing of the endometrium.

When PGF_{2α} is administered in early lactation, the benefits to fertility might not be related to enhancing uterine health but confounded with effects of presynchronizing the estrous cycle before timed AI programs (Moreira et al., 2001; Galvão et al., 2007). It is known that the stage of the estrous cycle when cows initiate timed AI protocols based on GnRH is critical for fertility (Vasconcelos et al., 1999), and treatment with PGF_{2α} 11 to 12 d before the initiation of the timed AI increases P/AI (Moreira et al., 2001; Galvão et al., 2007). In fact, in most studies evaluating PGF_{2α} as therapy for treatment of uterine diseases and subsequent effects on fertility, uterine health was not evaluated after treatment to justify the increase in P/AI (LeBlanc et al., 2002a; Kasimanickam et al., 2005). In some cases, when uterine health was evaluated after PGF_{2α} treatment, P/AI at first AI improved, but the benefits were

not linked to a reduction in the prevalence of SCE in treated cows (Galvão et al., 2009a).

Timed AI programs are commonly used for reproductive management of dairy herds for first and re-synchronized inseminations to mitigate the negative effects of poor estrous detection in lactating dairy cows (Caraviello et al., 2006). An alternative presynchronization treatment, in which stage of the estrous cycle is synchronized in cyclic and anovular cows, is called double Ovsynch (Souza et al., 2008). When PGF_{2α} is administered before the double Ovsynch protocol, the effects on uterine health or measures of fertility are not expected to be mediated by altering the stage of the estrous cycle when cows are subjected to the timed AI protocol. The goal of the current study was to demonstrate an improvement in P/AI in dairy cows with the systematic use of PGF_{2α} by enhancing uterine health based on the reduction in the prevalence of SCE.

The hypothesis of the current study was that treatment with PGF_{2α} would reduce the prevalence of SCE and improve first-service P/AI in cows subjected to a presynchronized timed AI program. Therefore, the objectives were to investigate the efficacy of systematic use of 1 or 2 treatments with PGF_{2α} preceding a presynchronized timed AI protocol on the prevalence of SCE and P/AI in lactating dairy cows.

MATERIALS AND METHODS

The University of Florida Institute of Food and Agricultural Sciences Animal Research Committee approved all procedures in this study.

Animals, Housing, and Feeding

A total of 1,342 lactating Holstein cows from a commercial dairy farm located in north central Florida were used in this study. Cows enrolled in the study calved from August 2009 to July 2010. Cows were housed in freestall barns equipped with fans and sprinklers for forced evaporative cooling. Cows from all treatments were kept together in the same pens throughout the entire period of the study. Lactating cow diets were formulated using the CPM Dairy cattle ration analyzer (ver. 3.0.8; Cornell University, Ithaca, NY; Penn State University, University Park; The Miner Institute, Chazy, NY) to meet or exceed the nutrient requirements established by NRC (2001) for lactating Holstein cows weighing 650 kg, consuming 24 kg of DM, and producing 45 kg/d of milk containing 3.5% fat and 3.1% true protein during the first 80 d of lactation. The first insemination for cows in the study occurred between November 2009 and October 2010.

Reproductive Management

All cows in the study had ovulation synchronized for first postpartum AI, with the double Ovsynch program starting on 48 ± 3 DIM, as depicted in Figure 1 (Souza et al., 2008). A total of 4 technicians and 18 sires were distributed randomly for all treatments. At 32 d after the first postpartum timed AI, cows were diagnosed for pregnancy by ultrasonographic examination of the uterus and its contents. The presence of an embryo with a heartbeat was the criterion used to determine pregnancy. Cows diagnosed pregnant were re-examined by palpation per rectum of the uterus and its contents 28 d later, at 60 d of gestation, to reconfirm pregnancy status and to identify pregnancy loss.

Treatments and Body Condition Scoring

Weekly cohorts of cows at 25 ± 3 DIM were blocked by parity and, within each block, allocated randomly to remain untreated (control, $n = 454$), or to receive a single i.m. injection of 25 mg $\text{PGF}_{2\alpha}$ (dinoprost tromethamine; Lutalyse sterile solution, Zoetis, Madison, NJ) treatment at 39 ± 3 DIM (**1PGF**, $n = 474$) or 2 treatments with $\text{PGF}_{2\alpha}$ at 25 ± 3 and 39 ± 3 DIM (**2PGF**, $n = 414$), as depicted in Figure 1.

The body condition of all cows was assessed at 25 ± 3 DIM using a 1 (emaciated) to 5 (obese) scale according to Ferguson et al. (1994) as depicted in the Elanco BCS chart (Elanco Animal Health, 2009).

Evaluation of Uterine Health

Samples of vaginal discharge and uterine endometrial cytology were collected from a subset of 357 cows (control = 115; 1PGF = 125; and 2PGF = 117). All samples were collected by the investigators, who were blinded to treatments. Vaginal discharge retrieved using the Metrichick device (Simcro, New Zealand) at 25 ± 3 DIM was used as a criterion to determine PVD (Dubuc et al., 2010), formerly known and classified as clinical endometritis (Sheldon et al., 2006). Briefly, vaginal discharge was scored as follows: 1 = clear or translucent mucus; 2 = mucus containing flecks of white or off-white pus; 3 = discharge containing 50% or less white or off-white mucopurulent material; 4 = discharge containing more than 50% purulent material, usually white or yellow; and 5 = bloody, purulent, and fetid discharge. Cows with score >2 were classified as having PVD.

Uterine cytology samples were collected on d 25 ± 3 , 32 ± 3 , and 46 ± 3 postpartum using the cytobrush technique (Kasimanickam et al., 2005) with the stainless steel gun protected by a one-way plastic tube protector (Continental Plastics, Delavan, WI). The DIM at sampling were selected to be able to evaluate the effects of treatments with $\text{PGF}_{2\alpha}$ before cows were enrolled in the double Ovsynch protocol at 48 DIM. The evaluations on d 32 and 46 postpartum were to maintain the same interval of 7 d between each $\text{PGF}_{2\alpha}$ treatment and the endometrial cytology.

Figure 1. Diagram of treatments according to DIM (± 3). Treatments were control, with no administration of $\text{PGF}_{2\alpha}$, 1PGF, in which cows received a single injection of $\text{PGF}_{2\alpha}$ on d 39 postpartum, and 2PGF, in which cows received an injection of $\text{PGF}_{2\alpha}$ on d 25 and another on d 39 postpartum. All cows were inseminated at fixed time following the double Ovsynch protocol. MS = mucus score; UC = uterine cytology.

After collecting the endometrial cytology, the cytobrush was rolled onto a slide and air-dried immediately. The slides were transported to the laboratory and stained using the Diff-Quick differential stain kit (IMEB, San Marcos, CA). Three technicians unaware of treatments read the slides. On each slide, 200 cells were counted using a microscope at $400\times$ magnification to determine the proportion of PMNL relative to the total of PMNL, mononuclear, and endometrial cells counted. Cows with $\geq 5\%$ PMNL were classified as having SCE (Gilbert et al., 2005).

Statistical Analyses

The sample size was calculated using Minitab 15 (Minitab Inc., State College, PA) for a 2-tailed test ($\alpha = 0.05$; $\beta = 0.80$). It was assumed that SCE would affect 30% of the cows and that treatment with $PGF_{2\alpha}$ would reduce the prevalence of SCE by 12 percentage units. Under those assumptions, 120 cows/treatment were needed to evaluate the effects of treatment on the prevalence of SCE. For P/AI in all cows, the sample size was calculated based on an increase of at least 6 percentage units. Others have demonstrated that administration of $PGF_{2\alpha}$ to postpartum cows inseminated on estrus or following a combination of detected estrus and timed AI had increments in pregnancy at first postpartum AI of 12 to 15 percentage units (Kasimanickam et al., 2005; Galvão et al., 2009a). A maximum of 433 cows/treatment was calculated to allow for detection of statistical effect when the difference between treatments was at least 6 percentage units in P/AI.

Binary data such as prevalence of SCE and P/AI were analyzed by logistic regression using the GLIMMIX procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) and fitting a binary distribution. The models included the effects of treatment (control vs. 1PGF vs. 2PGF), parity (primiparous vs. multiparous), BCS categorized as ≤ 2.75 or > 2.75 , and season of breeding classified as cool when AI occurred from October 1 to May 14, or hot when AI occurred from May 15 to September 30. For SCE, the prevalence on d 25 postpartum was used as covariate. For P/AI, technician and sire were also included in the statistical models. Treatment was forced in the final models. Covariates and interactions between treatment and covariates were maintained in the statistical models if $P < 0.10$. Orthogonal contrasts were performed to determine the effect of $PGF_{2\alpha}$ (control vs. 1PGF + 2PGF) and number of $PGF_{2\alpha}$ treatments (1PGF vs. 2PGF).

Two additional multivariable analyses were performed with the subset of cows in which uterine health was evaluated to model P/AI and pregnancy loss. In the first model, cows were classified based on uterine

health as no uterine disease, when no PVD or SCE was diagnosed at any time in the first 46 DIM, or as having PVD only, SCE only, or both PVD and SCE. A cow was considered positive for SCE if it was present at least once in the evaluations at 25, 32, and 46 DIM. The models included the effects of treatment (control vs. 1PGF vs. 2PGF) and uterine health (no uterine disease vs. PVD only vs. SCE only vs. PVD and SCE). Orthogonal comparisons were performed to evaluate the effect of uterine health (no uterine disease vs. all others), the differential effect of PVD compared with SCE (PVD only vs. SCE only), and the additive effect of PVD and SCE (PVD only + SCE only vs. both PVD and SCE). In the second model, cows were classified only based on SCE as never being diagnosed with SCE, having resolved SCE when diagnosed on d 25 or 32, or both, but negative on d 46 postpartum, and those that persisted with SCE based on diagnosis on d 46 postpartum. The models for P/AI and pregnancy loss included the effects of treatment (control vs. 1PGF vs. 2PGF) and SCE (no SCE vs. SCE that resolved by d 46 vs. SCE on d 46). Orthogonal comparisons were performed to evaluate the effect of SCE (no SCE vs. resolved + persistent), and the effect of persistent SCE (resolved vs. persistent).

Adjusted proportions for binary data were generated by back-transforming the estimates using the *ilink* function of SAS. Proportions are displayed for binary data, whereas LSM and SEM are displayed for continuous data. Differences with $P \leq 0.05$ were considered significant, whereas those with $0.05 < P \leq 0.10$ were considered tendency to differ.

RESULTS

Cows in the 3 treatments had similar lactation number (2.93 ± 0.05), DIM at enrollment in the study (24.7 ± 0.05), DIM at AI (81.0 ± 0.2), vaginal discharge score on d 25 postpartum (2.08 ± 0.06), and percentage of PMNL in endometrial cytology on d 25 postpartum (6.98 ± 0.67); however, BCS at enrollment was greater ($P = 0.01$) for control and 2PGF than for 1PGF cows (control = 2.96 ± 0.02 vs. 1PGF = 2.91 ± 0.02 vs. 2PGF = 2.98 ± 0.02).

Effects of $PGF_{2\alpha}$ Treatments on the Prevalence of SCE

The prevalence of PVD on d 25 postpartum tended ($P = 0.10$) to be greater for cows in the 2PGF than those receiving 1PGF, whereas control cows had intermediate prevalence that did not differ from the other 2 groups (Figure 2). Nevertheless, the prevalence of SCE in cows on d 25 postpartum, before treatments were

applied, did not differ among treatments and averaged 29.5%. Treatment with PGF_{2α} at 25 ± 3 DIM in 2PGF reduced ($P < 0.05$) the prevalence of SCE on d 32 postpartum compared with control and 1PGF cows (Figure 2). However, the prevalence of SCE did not differ at 46 DIM. Cows with PVD on d 25 postpartum had greater ($P < 0.001$) prevalence of SCE than those without PVD on d 25 (47.3 vs. 17.8%) and d 32 (40.3 vs. 16.8%) postpartum, but the same association was not observed on d 46 postpartum (PVD = 17.4 vs. no PVD = 12.1%; $P = 0.20$). Additionally, no association was observed between parity, BCS, and season of AI with the prevalence of SCE.

Effects of PGF_{2α} on Pregnancy per AI and Pregnancy Loss

Treatments with 1PGF or 2PGF failed to increase P/AI on d 32 and 60 after insemination in cows synchronized and bred with the double Ovsynch timed AI program (Table 1). Overall, 39.9 and 35.2% of the cows were pregnant on d 32 and 60 after AI, respectively. Pregnancy loss between 32 and 60 d after AI affected 11.9% of the pregnant cows, and treatment with either

1PGF or 2PGF had no influence on maintenance of pregnancy in the first 60 d of gestation.

Parity and season of AI affected ($P < 0.01$) P/AI on d 32 and 60 after timed insemination. Primiparous cows had greater ($P = 0.01$) P/AI than multiparous cows on d 32 (42.5 vs. 35.0%) and d 60 after AI (37.1 vs. 30.5%). Cows inseminated during the cool season had greater ($P < 0.001$) P/AI than those inseminated during the hot season on d 32 (47.1 vs. 30.8%) and d 60 (41.7 vs. 26.6%) after insemination. None of the other covariates evaluated influenced pregnancy loss between 32 and 60 d of gestation.

Associations Among PVD and SCE with Measures of Fertility

The negative effect of uterine diseases on P/AI and maintenance of pregnancy were only observed when cows were diagnosed with both PVD and SCE (Table 2). On d 32 and 60 after insemination, cows not diagnosed with uterine diseases had greater ($P < 0.05$) P/AI than those diagnosed with both PVD and SCE. However, P/AI did not differ statistically among cows not diagnosed with uterine diseases and those diagnosed

Figure 2. Effect of 1 or 2 treatments of PGF_{2α} on the prevalence of subclinical endometritis on d 32 and 46 postpartum in dairy cows. The analysis included the effect of 1 or 2 treatments of PGF_{2α} on the prevalence of subclinical endometritis on d 32 and 46 postpartum in dairy cows. Control = no treatment with PGF_{2α} before enrollment in the timed AI protocol; 1PGF = a single treatment with PGF_{2α} on d 39 postpartum; 2PGF = treatment with PGF_{2α} on d 25 and 39 postpartum. Purulent vaginal discharge (PVD) and subclinical endometritis on d 25 postpartum were the baseline prevalence at enrollment in the study. For subclinical endometritis on d 32 and 46, the analysis included the effects of treatment ($P = 0.98$), day postpartum ($P = 0.001$), and interaction between treatment and day postpartum ($P = 0.04$). ^{a,b}Different lowercase letters among treatments denote statistical difference ($P < 0.05$); ^{A,B}different uppercase letters among treatments tended to differ ($P < 0.10$).

Table 1. Effect of 1 or 2 treatments of PGF_{2α} on pregnancy per AI and pregnancy loss of dairy cows subjected to a timed AI program

Item	Treatment ¹			P-value ²		
	Control	1PGF	2PGF	Trt	C1	C2
Pregnant, % (no./no.)						
d 32	38.1 (173/454)	40.7 (193/474)	41.1 (170/414)	0.58	0.32	0.72
d 60	33.7 (153/454)	36.7 (174/474)	35.0 (145/414)	0.70	0.43	0.78
Loss	11.6 (20/173)	9.8 (19/193)	14.7 (25/170)	0.36	0.87	0.16

¹Control = no treatment with PGF_{2α} before enrollment in the timed AI protocol; 1PGF = a single treatment with PGF_{2α} on d 39 postpartum; 2PGF = treatment with PGF_{2α} on d 25 and 39 postpartum.

²Trt = effect of treatment; C1 = contrast for the effect of treatment with PGF_{2α} (control vs. 1PGF + 2PGF); C2 = contrast for the effect of number of treatments with PGF_{2α} (1PGF vs. 2PGF).

with only PVD or SCE. Interestingly, cows diagnosed with both diseases had lower ($P = 0.03$) P/AI on d 60 than those diagnosed with only either PVD or SCE. Similar to P/AI, pregnancy loss increased ($P = 0.05$) only when cows were diagnosed with both PVD and SCE.

Subclinical endometritis depressed P/AI and increased pregnancy loss in dairy cows, but these negative effects were only observed when the disease persisted until 46 DIM (Table 3). Of the 50 cows categorized as having persistent SCE (diagnosis at 46 DIM), 19 had the first diagnosis with $\geq 5\%$ PMNL at 46 DIM, and the remaining 31 had an earlier diagnosis. Cows with no diagnosis of SCE had similar P/AI on d 32 or 60 after insemination compared with cows diagnosed with SCE that resolved by 46 DIM. Nevertheless, those cows in which SCE persisted until 46 DIM, immediately before enrollment on the double Ovsynch protocol, tended ($P = 0.08$) to have lower P/AI on d 32 and had lower P/AI ($P = 0.01$) on d 60 after insemination because of greater ($P = 0.04$) pregnancy loss than cows that resolved SCE by 46 DIM.

DISCUSSION

Treatments with 1 or 2 doses of PGF_{2α} before enrollment in a timed AI protocol had minor effects on the prevalence of SCE and did not improve P/AI or reduce pregnancy loss in lactating Holstein cows. The design of the current experiment allowed us to evaluate the effects of 1 or 2 doses of PGF_{2α} on measures of fertility in dairy cows while excluding the confounding effect of presynchronization of the estrous cycle when cows are subjected to timed AI protocols (Moreira et al., 2001; Galvão et al., 2007) or inducing earlier insemination because of estrus. The current study was designed to evaluate the effects of systematic use of PGF_{2α} on the prevalence of SCE and P/AI, but it was not our aim to assess PGF_{2α} as a direct therapy for cows with PVD or only those diagnosed with SCE.

The strategic use of PGF_{2α} early postpartum, when PVD and SCE are prevalent (Gilbert et al., 2005), was initially thought to reduce the prevalence of SCE, which could improve P/AI in cows inseminated exclusively by timed AI. The suggested mechanism of PGF_{2α}

Table 2. Association between purulent vaginal discharge (PVD) or subclinical endometritis (SCE) with fertility of dairy cows at first postpartum insemination

Item	Category ¹				Contrast ²		
	No disease	PVD only	SCE only	PVD and SCE	C1	C2	C3
Cows, no.	156	22	105	74	—	—	—
Pregnant, %							
d 32	48.0 ^a	49.1 ^{ab}	39.8 ^{ab}	33.4 ^b	0.21	0.43	0.17
d 60	43.3 ^a	44.9 ^a	34.0 ^A	22.8 ^{b, B}	0.08	0.34	0.03
Loss, %	9.1 ^b	7.1 ^b	13.7 ^B	30.3 ^{a, A}	0.33	0.52	0.05

^{a, b}Different superscripts within a row differ ($P < 0.05$).

^{A, B}Different superscripts within a row tend to differ ($P < 0.10$).

¹No disease = no diagnosis of PVD or SCE; PVD = vaginal discharge score > 2 on d 25 ± 3 postpartum; SCE = cows with uterine cytology containing $\geq 5\%$ PMNL on one or more of the days on which diagnosis was performed (25 or 32 or 46 ± 3 d postpartum).

²C1 = effect of uterine disease (no uterine disease vs. PVD only + SCE only + PVD and SCE); C2 = effect of PVD compared with SCE (PVD only vs. SCE only); C3 = additive effect of PVD and SCE (PVD only + SCE only vs. PVD and SCE).

Table 3. Association between subclinical endometritis (SCE) with fertility of dairy cows at first postpartum insemination

Item	Category ¹			Contrast ²	
	No SCE	Resolved SCE	Persistent SCE	C1	C2
Cows, no.	178	129	50		
Pregnant, %					
d 32	45.4	40.0	25.0	0.03	0.08
d 60	40.5	34.3	13.7	<0.01	0.01
Loss, %	9.6	13.5	43.9	0.03	0.04

¹Diagnosis of SCE was based on uterine cytology containing $\geq 5\%$ PMNL; resolved SCE = cows diagnosed with SCE on d 25 or 32 postpartum, but negative on d 46 postpartum; persistent SCE = cows with presence of SCE on d 46 postpartum.

²C1 = effect of SCE (no SCE vs. resolved SCE + persistent SCE); C2 = effect of persistent SCE (resolved SCE vs. persistent SCE).

action in cyclic cows involves induction of luteolysis and return to estrus, leading to opening of the cervix and myometrium contractions that might improve mechanical cleansing of the uterus by eliminating bacteria and the products that attract PMNL. However, PGF_{2α} may have effects on the uterus beyond induction of luteolysis and estrus in cyclic cows. In human uterine tissue in vitro, PGF_{2α} has been shown to induce myometrium contractions (Senior et al., 1992). Ulug et al. (2001) demonstrated that PGF_{2α} stimulated the release of pro-matrix metalloproteinase-2 and pro-matrix metalloproteinase-9 from uterine tissue explants that are involved with breakdown of extracellular matrix mainly by degrading collagen type IV, which might aid myometrium contraction and uterine involution independent of cyclic status. Administration of exogenous PGF_{2α} early postpartum promoted uterine involution in cows (Lindell and Kindahl, 1983). In the current study, reduction in the prevalence of SCE only occurred at 32 DIM in cows receiving PGF_{2α} on d 25 postpartum, when the proportion of estrous cyclic cows and luteolytic response to PGF_{2α} are typically low (Galvão et al., 2010). The positive effect of PGF_{2α} on SCE was no longer apparent by 46 DIM, probably because of the observed high spontaneous cure documented in control cows. In fact, a single treatment with PGF_{2α} at 39 DIM resulted in no benefit in reducing the prevalence of SCE compared with control cows. Therefore, the initial benefits of PGF_{2α} on uterine health of earlier postpartum cows were offset by spontaneous resolution of uterine inflammatory process 2 wk later. Interestingly, PGF_{2α} as administered was unable to reduce the prevalence of SCE at 46 DIM, which was observed to have marked effects on P/AI and maintenance of pregnancy at first postpartum AI. One of the potential limitations of the study is that estrous cyclic status and presence of a corpus luteum on the days when PGF_{2α} was administered was unknown, so it is possible that a high prevalence of anovular cows would have limited the benefit of PGF_{2α}

on improving uterine health through induction of luteolysis. Nevertheless, Dubuc et al. (2011) observed that administration of PGF_{2α} on d 35 and 49 postpartum did not improve P/AI at first service or reduced time to pregnancy in estrous cyclic cows.

The effects of PGF_{2α} on uterine health and fertility have been evaluated by others in cows subjected to varying reproductive management at first AI (Kasimanickam et al., 2005; Galvão et al., 2009a; Dubuc et al., 2011). In agreement with our findings, administration of PGF_{2α} given 14 d apart with the last treatment at 49 DIM (Galvão et al., 2009a; Dubuc et al., 2011) did not reduce the prevalence of SCE in dairy cows.

Kasimanickam et al. (2005) observed that cows with SCE treated with PGF_{2α} had increased P/AI at first AI and pregnancy rate compared with untreated controls. The benefits of PGF_{2α} on fertility were similar between treatment with PGF_{2α} or with intrauterine administration of cephalixin suggesting an effect on the uterine microbiota and improved uterine health, although these responses were not verified. The lack of reduction in the prevalence of SCE at 46 DIM with PGF_{2α} treatments supports the fact that PGF_{2α} did not improve P/AI or reduce pregnancy loss in cows bred exclusively by timed AI. The double Ovsynch timed AI protocol presynchronizes the estrous cycle of dairy cows (Ribeiro et al., 2012), thereby eliminating a potential effect of prior presynchronization with PGF_{2α} at improving response to the timed AI program. On the other hand, one cannot completely exclude the possibility that, by imposing the double Ovsynch protocol for first AI, the benefits of PGF_{2α} might have been reduced as cows receive additional hormonal interventions for synchronization of ovulation that might have effects on the uterus through induction sequential estruses and ovulations.

Cows with SCE had reduced P/AI, which corroborates previous reports (Kasimanickam et al., 2005; Galvão et al., 2009a; Dubuc et al., 2011) that identified a negative association between fertility and PVD,

SCE, or both. In the current study, cows that persisted with SCE at 46 DIM had a remarkable reduction in P/AI compared with healthy cows or cows that resolved SCE by 46 DIM. It is clear that the prevalence of SCE decreases with day postpartum, but cows with SCE that persisted until enrollment in the timed AI protocol suffered reductions in P/AI and had increased risk of pregnancy loss. It is unknown if the prevalence of SCE reduced even further after 46 DIM. With the exception of Galvão et al. (2009a), who observed a tendency for increased pregnancy loss in cows with PVD, none of the previous studies reported an association between PVD or SCE and increased risk of pregnancy loss (Gilbert et al., 2005; Kasimanickam et al., 2005; Dubuc et al., 2011). In some studies, pregnancy was evaluated only once, so data for pregnancy loss were not available (Gilbert et al., 2005; Kasimanickam et al., 2005). It is unclear the exact mechanism by which SCE decreases P/AI and increases pregnancy loss. Products of endometrial inflammation compromised early embryo development *in vitro* (Hill and Gilbert, 2008). Soto et al. (2003) suggested that mediators of the inflammatory cascade, including cytokines, can impair early embryo development and might be part of the mechanism by which fertility is depressed in cows suffering from inflammatory diseases in early lactation.

Evidence is growing that cows with SCE have altered embryo quality and endometrial function. Inflammation in the endometrium has been shown to reduce fertilization in single ovulating postpartum dairy cows (Cerri et al., 2009). Dairy cows with no detectable PMNL in endometrial cytology had an increased number of transferable embryos when subjected to superstimulation compared with cows with presence of PMNL in endometrial cytology (Drillich et al., 2012). Cows diagnosed with SCE have altered endometrial and embryonic gene expression that might explain the reduced fertility (Hoelker et al., 2012). Endometrium from cows diagnosed with SCE had an altered pattern of expression of genes involved in cell adhesion and immune modulation, which was then linked to changes in d 7 embryo gene expression. The changes in endometrial gene expression might be induced by an altered number of immune cells present in the tissue. Nevertheless, embryos from cows with SCE had altered pattern of gene expression involving pathways in cell cycle and apoptosis, which might explain a reduction in P/AI or even increased risk of pregnancy loss (Hoelker et al., 2012). Whether SCE *per se* is the causative agent of changes in endometrial and embryonic gene expression or that cows that develop SCE have underlying factors that also cause changes in the transcriptome remains to be elucidated.

It is noteworthy that the combination of PVD and SCE led to additive negative effects on P/AI and pregnancy loss in dairy cows compared with PVD or SCE alone. Dubuc et al. (2011) reported a decline in first-service P/AI in cows suffering from both PVD and SCE compared with cows diagnosed with only SCE. In the same study, cows with both PVD and SCE had longer interval to pregnancy compared with counterparts diagnosed with only 1 of the 2 problems. Similar to our findings, Dubuc et al. (2011) also showed that cows that persist with uterine disease before the end of the voluntary waiting period are those that suffer the greatest negative consequences in interval to pregnancy. In the current study, 14% of the cows had SCE by 46 DIM, and these cows suffered the most losses in fertility at first postpartum AI. Similar to the effect of SCE, Dubuc et al. (2011) observed that PVD can also persist in some cows. According to their data, 42% of the cows diagnosed with PVD and SCE on d 35 postpartum persisted with PVD at 56 DIM. The persistence of PVD was not evaluated in the current study. The mechanisms by which some cows are unable to eliminate inflammation from the uterus are not completely elucidated; however, previous studies suggest that endometrial inflammation is regulated by immune response rather than by pathogen load (Herath et al., 2009). It is possible that cows with inadequate immune function are those with longer duration of the endometrial inflammatory process that compromises fertility.

CONCLUSIONS

Treatment with 1 or 2 injections of PGF_{2α} in early lactation, before cows were subjected to a presynchronized timed AI protocol, was unable to improve uterine health and measures of fertility in lactating dairy cows. Subclinical endometritis impaired P/AI and maintenance of pregnancy in lactating dairy cows, particularly when associated with PVD, and the negative effect of SCE was observed when the inflammatory process persisted until 46 DIM. Interestingly, when both PVD and SCE were associated or when SCE persisted until 46 DIM, pregnancy loss increased.

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