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Effect of prostaglandin $F_{2\alpha}$ on subclinical endometritis and fertility in dairy cows

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ABSTRACT

The objectives were to determine the effects of $PGF_{2\alpha}$ treatment on the prevalence of subclinical endometritis (SCE) and fertility of dairy cows. A total of 406 Holstein cows (167 primiparous and 239 multiparous) from 5 herds were used. Uterine lavage for diagnosis of SCE, $PGF_{2\alpha}$ treatment, evaluation of body condition scores (BCS), and collection of blood samples for estrous cyclicity determination were performed at 21, 35, and 49 d in milk (DIM). Polymorphonuclear cells (PMN) were quantified and thresholds for diagnosing SCE were selected by receiver operating characteristics analysis. Cows classified as having SCE at 35 DIM (≥6.5% PMN) and 49 DIM (≥4.0% PMN) had increased time to pregnancy; however, cows classified as having SCE at 21 DIM (≥8.5% PMN) did not. Median days to pregnancy were delayed by 30 (151 vs. 121 d) and 40 (169 vs. 129) d for cows classified as having SCE at 35 and 49 DIM, respectively. Treatment with $PGF_{2\alpha}$ did not affect the prevalence of SCE either at 35 (37.9 vs. 38.4%) or at 49 DIM (34.0 vs. 40.4%). Treatment with $PGF_{2\alpha}$ did not affect time to first insemination (AI; median 76 DIM for cows treated with $PGF_{2\alpha}$; 79 DIM for control. Nonetheless, $PGF_{2\alpha}$ treatment increased pregnancy to first AI in all the cows (35.5 vs. 24.1%) and hazard ratio (HR) of pregnancy in cows with BCS ≤2.5 when all of the cows were evaluated (HR = 1.5; 95% confidence interval; CI = 1.1 to 2.0) and when only cows without SCE were evaluated (HR = 1.8; 95% CI = 1.2 to 2.7). Treatment with $PGF_{2\alpha}$ did not affect the hazard of pregnancy in cows with SCE at 49 DIM (HR = 0.9; 95% CI = 0.6 to 1.3). In these farms, treatment with $PGF_{2\alpha}$ did not affect SCE or time to first insemination, but did increase first-service pregnancy per AI and decreased time to pregnancy in cows with low BCS.

Key words: subclinical endometritis, prostaglandin treatment, dairy cow

INTRODUCTION

Subclinical endometritis (**SCE**), characterized by increased proportion of polymorphonuclear cells (**PMN**) in uterine cytology (Kasimanickam et al., 2004; Gilbert et al., 2005), is prevalent in high-producing dairy cows and is associated with both decreased pregnancy per AI and extended interval to pregnancy (Kasimanickam et al., 2004; Gilbert et al., 2005). Administration of a $PGF_{2\alpha}$ analog to cows with SCE was as efficacious as intrauterine infusion of cephapirin benzathine in improving reproductive performance (Kasimanickam et al., 2005).

The benefit from $PGF_{2\alpha}$ administration is believed to arise from induction of estrus in cows having a $PGF_{2\alpha}$-responsive corpus luteum; the estrus leads to physical expulsion of bacterial contaminants and inflammatory products as well as a possible improvement in the uterine defenses under low progesterone (Kasimanickam et al., 2005). Nonetheless, although a positive effect of $PGF_{2\alpha}$ on reproductive performance was observed, it was not determined whether the prevalence of SCE was reduced or whether the effect was in cows with an active corpus luteum.

We hypothesized that SCE would be detrimental to fertility, but treatment with $PGF_{2\alpha}$ would reduce the prevalence of SCE in cows with an active corpus luteum and would improve reproductive performance. Therefore, our objectives were to determine the effects of $PGF_{2\alpha}$ on uterine health, pregnancy to first AI, and intervals from calving to AI and to pregnancy in lactating dairy cows.

MATERIALS AND METHODS

Animals, Housing, Feeding, and Reproductive Management

A total of 445 cows (185 primiparous and 260 multiparous) from 5 commercial Holstein dairy farms located in Cayuga County, New York State, were enrolled. The herds ranged from 70 to 1,500 milking cows and the rolling herd averages were about 11,500 kg of milk/cow

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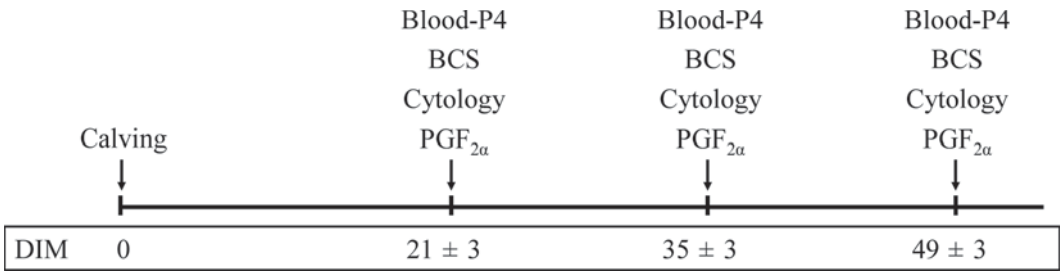

Figure 1. Timeline of activities during the study. Blood-P4 = blood sampling for progesterone measurement; BCS = body condition scoring; Cytology = low-volume uterine lavage for cytological examination; $PGF_{2\alpha}$ = treatment administration; cows with even-numbered identification ear tags received 25 mg of $PGF_{2\alpha}$ (Lutalyse, 5 mg of dinoprost tromethamine/mL, Pfizer Animal Health, New York, NY) and cows with odd-numbered identification ear tags received sterile saline solution.

per year. Lactating dairy cows were housed in free stall facilities and milked 3 times daily. Within herd, cows were fed the same TMR, formulated to meet or exceed the NRC (2001) nutrient requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% FCM.

Reproductive management varied among farms; however, all dairies relied on estrus detection for most of their breedings and their voluntary waiting periods were set to 49 DIM. All the cows were examined for pregnancy by one of the investigators (C. L. Guard) by palpation per rectum at 38 ± 3 d after AI. Cows not conceiving on the first AI were reexamined for pregnancy after each subsequent AI until 300 DIM. Pregnancy per AI was defined as the number of pregnant cows divided by the number of cows receiving AI in each treatment at 38 d after AI.

Treatments and Sample Collection

The timeline of treatments and sample collection are in Figure 1. During the routine weekly herd visits, cows at 21 ± 3 DIM were systematically assigned to 1 of 2 treatments. In a blind design, cows with even-numbered identification ear tags received 5 mL of solution B (25 mg of $PGF_{2\alpha}$, Lutalyse, 5 mg of dinoprost tromethamine/mL, Pfizer Animal Health, New York, NY) at 21, 35, and 49 ± 3 DIM ($PGF_{2\alpha}$ treatment; n = 218) and cows with odd-numbered identification ear tags received 5 mL of solution A (sterile saline solution) at 21, 35, and 49 DIM (control; n = 227). Solutions A and B were bottled at the Pharmacy of the College of Veterinary Medicine at Cornell University and were indistinguishable. Their identities were revealed to investigators only after completion of the trial.

A low-volume (20 mL) uterine lavage for diagnosis of SCE was performed immediately before treatment administration at 21, 35, and 49 ± 3 DIM. Cows with reproductive tract disorders such as pyometra and uterine adhesions or abscesses that could compromise sampling for SCE evaluation or future AI were not enrolled. Cows that could not be sampled at 21 DIM were not enrolled. Of the 445 cows that were initially sampled at 21 DIM, 39 cows were either missed at 35 or at 49 DIM or at both samplings; therefore, only 406 cows ($PGF_{2\alpha}$ treatment = 203; control = 203) were sampled at all time points and included in the final analysis. The diagnosis of SCE was based on the proportion of PMN out of a total of 200 cells, including all leukocyte types and epithelial cells, but excluding erythrocytes, as described previously (Gilbert et al., 2005). A single investigator read all the slides. This observer was unaware of treatments, and slides were ordered by sampling date within each farm and not by sampling date for each cow, which avoided any bias of knowing the counts of a previous slide for each cow.

All cows used had a blood sample collected at 21, 35, and 49 ± 3 DIM. Blood was collected by puncture of coccygeal vessels into Vacutainer tubes without anticoagulant (Becton, Dickinson and Company, Franklin Lakes, NJ). The samples were immediately placed in ice and transported to the laboratory within 4 h, where serum was separated by centrifugation at 2000 × *g* for 15 min and frozen at −25°C and later analyzed for progesterone by RIA (Beam and Butler, 1997). Interassay and intraassay coefficients of variation were 7.5 and 6.0%, respectively. Cows having serum progesterone concentration ≥1 ng/mL were assumed to have a functional corpus luteum (**CL**) at the time of sampling and therefore, were considered cyclic (Galvão et al., 2004). Body condition score was evaluated in all cows using a 5-point (1 = thin to 5 = fat) system (Ferguson et al., 1994) at each sampling.

Statistical Analyses

The PMN thresholds for classification of cows as having SCE were selected using receiver-operating characteristics analysis using MedCalc version 9.2 for Windows (MedCalc Software bvba, Mariakerke,

Belgium). Only the control cows were used for selection of the threshold because $PGF_{2\alpha}$ could affect the proportion of PMN at 35 and 49 DIM and bias our estimate. The dichotomous outcome used for selection of the thresholds was nonpregnancy by 150 DIM that was one-half of the observation period. In MedCalc, a threshold was selected automatically based on the best combined sensitivity and specificity. It is important to note that one could use a different approach such as choosing a threshold based on high sensitivity or high specificity depending on the goals of diagnosing cows with SCE. After selection of a threshold, cows were classified as above or below the PMN threshold and Kaplan-Meyer survival curves were used to evaluate whether cows above the threshold had increased time to conception. The results from the Kaplan-Meier survival analyses were confirmed by a Cox proportional hazard model controlling for the effect of herd (model was stratified by herd using the Strata option in SAS, SAS Institute Inc., Cary, NC). If cows classified as having SCE had increased time to conception (Kaplan-Meier) and decreased hazard of pregnancy (Cox model), the PMN threshold for SCE was considered as validated for indicating negative effects on fertility.

The outcomes SCE at 35 and 49 DIM, and first service pregnancy per AI were analyzed by mixed logistic regression using the Glimmix procedure of SAS (SAS Inst. Inc., Cary, NC). The models included the fixed effects of $PGF_{2\alpha}$ treatment at 21 DIM for SCE at 35 DIM and $PGF_{2\alpha}$ treatment at 21 and 35 DIM for SCE at 49 DIM (yes or no), cyclic measure by the time of $PGF_{2\alpha}$ administration (yes or no), parity (primiparous vs. multiparous), season of calving [winter (Dec, Jan, Feb), spring (Mar, Apr, May), summer (Jun, Jul, Aug), fall (Sep, Oct, Nov)], mean BCS for the 3 observations (<2.5 vs. ≥ 2.5), and the interactions between $PGF_{2\alpha}$ treatment and other covariates. Herd was included as a random effect. For SCE at 49 DIM, the Glimmix model included the effect of SCE presence at 35 DIM and the interaction between $PGF_{2\alpha}$ treatment and SCE at 35 DIM. Effect of SCE presence at 49 DIM on first service pregnancy per AI was first evaluated in a univariable analysis using the Glimmix procedure and then SCE at 49 DIM was included in the multivariable analysis.

The hazards of insemination up to 150 DIM and of pregnancy up to 300 DIM were analyzed by Cox proportional hazard model using the Phreg procedure of SAS (SAS Inst. Inc.). The effect of $PGF_{2\alpha}$ on the hazard of pregnancy was first evaluated in all cows, then in cows with SCE, and then in cows without SCE at 49 DIM (Kasimanickam et al., 2005). The hazard ratio (**HR**) was the conditional daily probability of a given event (AI or pregnancy) and may be interpreted as the AI rate or pregnancy rate (speed at which cows are submitted for AI or become pregnant). The time variable for the hazard of AI was the days between calving and first AI and for hazard of pregnancy was the days between calving and pregnancy, which was detected 38 ± 3 d after AI. Cows that were not AI by 150 DIM or were sold or died were censored in the analysis of time to first AI. Cows that were not AI or not pregnant by 300 DIM or were sold or died were censored in the analysis of time to pregnancy. The Cox models included the same variables cited for the Glimmix model; however, cyclic by 49 DIM was used instead of cyclic at the time of $PGF_{2\alpha}$ administration, and data were stratified by herd, using the Strata option, to control for clustering as previously reported (Bicalho et al., 2007). For the Strata option in the Phreg procedure, each stratum had its likelihood function derived from its hazard function, but partial likelihoods were multiplied to generate a single likelihood function to allow estimation of a single coefficient for each variable in the model. Proportionality of hazard rate between groups was assessed by inclusion of interaction of $PGF_{2\alpha}$ treatment with time and by evaluation of Kaplan-Meier curves. The median days to first AI or to pregnancy were obtained by survival analysis from the Kaplan-Meier model using the Lifetest procedure of SAS (SAS Inst. Inc.). The survival plot was generated using the survival option of MedCalc.

For all statistical tests, a 2-sided hypothesis was considered. Differences with $P \leq 0.05$ were significant. When an interaction between 2 dichotomous variables was observed, a new variable containing the 4 possible combinations (dummy variables: {0,0}; {0,1}; {1,0}; {1,1}) was created and the model was reanalyzed including only the new variable. For data presentation, only biologically relevant comparisons are shown. For both the logistic (Glimmix) and Cox proportional hazard models (Phreg), a hierarchical backward elimination was performed; variables were manually removed when $P > 0.05$. Treatment was forced into the final model in both types of models.

RESULTS

The PMN thresholds selected by the receiver-operating characteristics analysis for classifying cows as having SCE were ≥8.5, 6.5, and 4.0% at 21, 35, and 49 DIM, respectively. Using these thresholds, 66.7% (n = 271), 38.2% (n = 155), and 37.2% (n = 151) of the 406 cows were classified as having SCE at the 3 examination days. The PMN threshold at 21 DIM was not associated with time to pregnancy by Kaplan-Meier survival analysis. Higher thresholds were tested (≥15, 20, and 30% PMN); however, none was associated with time to pregnancy (all $P > 0.50$). On the other hand,

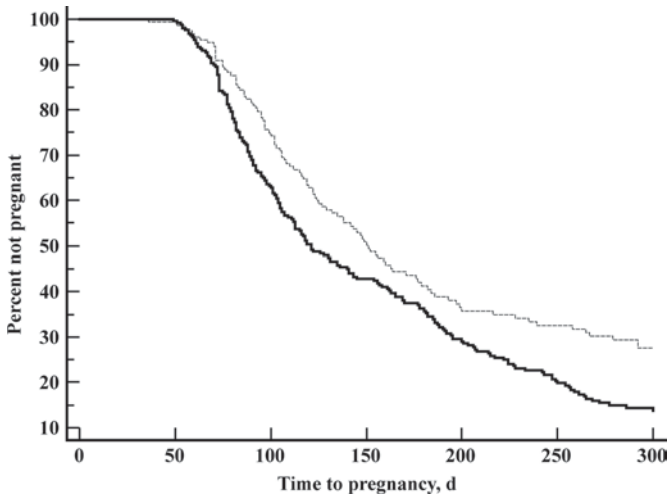

Figure 2. Time to pregnancy in dairy cows that were classified as having (n = 155; dashed line) or not having (n = 251; solid line) subclinical endometritis (SCE) at 35 DIM. Cows with SCE had increased ($P = 0.003$) median days to pregnancy compared with cows without SCE (151 vs. 121 d).

the thresholds used for classifying cows as having SCE at 35 and 49 DIM led to increased ($P < 0.05$) time to pregnancy. Median days to pregnancy were delayed by 30 (151 vs. 121 d), and 40 (169 vs. 129) days for cows classified with SCE at 35 and 49 DIM, respectively. Figures 2 and 3 compare the survival curves for cows having or not having SCE at 35 and 49 DIM, respectively. A univariable Cox's model confirmed the findings in the Kaplan-Meier graph and showed that the hazard of pregnancy was reduced ($P < 0.05$) for cows having SCE at 35 (HR = 0.7; 95% CI = 0.6 to 0.9) and 49 DIM (HR = 0.8; 95% CI = 0.6 to 1.0).

Table 1 shows the effect of $PGF_{2\alpha}$ treatment on SCE at 35 and 49 DIM. Treatment with $PGF_{2\alpha}$ did not affect the prevalence of SCE either at 35 or at 49 DIM. The only variable affecting prevalence of SCE at 35 and 49 DIM was presence of an active CL at 21 DIM; cows with an active CL (n = 106) had lower ($P < 0.05$) prevalence of SCE at 35 DIM (26.4 vs. 42.3%) and 49 DIM (25.5 vs. 41.3%). Neither presence of an active CL by 35 DIM nor any other measured variable affected prevalence of SCE at 49 DIM. There was no interaction ($P = 0.57$) between presence of an active CL at the time of $PGF_{2\alpha}$ administration and $PGF_{2\alpha}$ treatment on the prevalence of SCE at 35 or 49 DIM. Furthermore, there was no interaction ($P = 0.20$) between $PGF_{2\alpha}$ treatment and presence of SCE at 35 DIM on the prevalence of SCE at 49 DIM.

Hazard of first AI was not affected by either SCE at 49 DIM (HR = 0.9; 95% CI = 0.7 to 1.1; $P = 0.40$) or $PGF_{2\alpha}$ treatment at 21, 35, and 49 DIM (HR = 1.1; 95% CI = 0.9 to 1.3; $P = 0.63$). Median days to first AI was 78 d for cows with or without SCE and 76 and 79 d for cows that did or did not receive $PGF_{2\alpha}$ treatment, respectively. The only variable affecting the hazard of first AI was cyclicity by 49 DIM; cows that were cyclic (n = 315) had increased hazard of AI (HR = 1.5; 95% CI = 1.2 to 2.0; $P = 0.003$) leading to a 21-d shorter median time to first AI compared with noncyclic cows (74 vs. 95 d; Figure 4). There was no interaction ($P > 0.05$) between $PGF_{2\alpha}$ treatment and SCE, cyclicity by

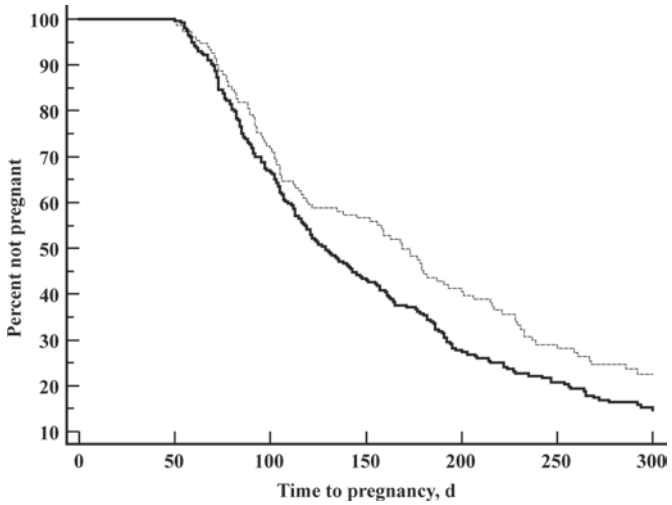

Figure 3. Time to pregnancy in dairy cows that were classified as having (n = 151; dashed line) or not having (n = 255; solid line) subclinical endometritis (SCE) at 49 DIM. Cows with SCE had increased ($P = 0.03$) median days to pregnancy compared with cows without SCE (169 vs. 129 d).

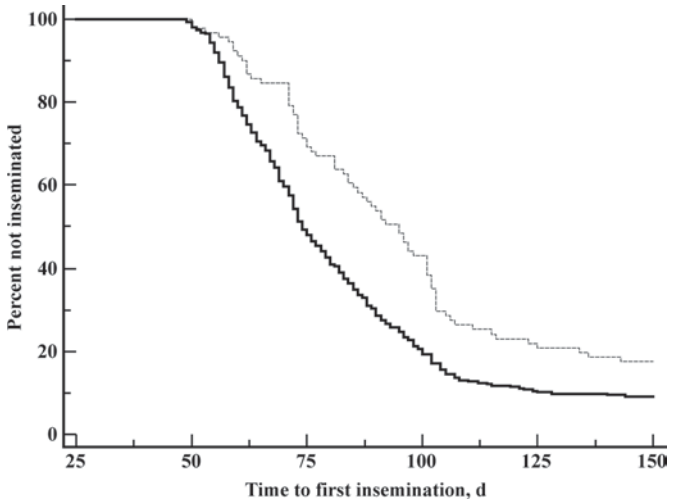

Figure 4. Time to first AI in dairy cows that were cyclic (n = 315; solid line) or noncyclic (n = 91; dashed line) by 49 DIM. Cyclic cows had decreased ($P = 0.003$) median days to first AI compared with noncyclic cows (74 vs. 95 d).

Table 1. Effect of $PGF_{2\alpha}$ treatment on prevalence of subclinical endometritis (SCE) at 35 and 49 DIM

Outcome variable	Level	Cows, n	SCE, %	*P*-value	OR[1]	95% CI[2]
SCE 35 DIM[3]						
Treatment[4]	$PGF_{2\alpha}$	203	37.9	0.76	0.9	0.6 to 1.5
	Control	203	38.4	—	—	—
SCE 49 DIM						
Treatment	$PGF_{2\alpha}$	203	34.0	0.18	0.8	0.5 to 1.1
	Control	203	40.4	—	—	—

[1]OR = odds ratio.

[2]CI = confidence interval.

[3]Subclinical endometritis was characterized by the presence of ≥6.5 and 4.0% PMNL in the uterine cytology at 35 and 49 DIM, respectively.

[4]Cows in the $PGF_{2\alpha}$ treatment group received an i.m. injection of 25 mg of $PGF_{2\alpha}$ at 21, 35, and 49 ± 3 DIM; control cows received sterile saline solution. Only $PGF_{2\alpha}$ treatment at 21 DIM was considered in the model for SCE at 35 DIM and only $PGF_{2\alpha}$ treatment at 21 and 35 DIM was considered in the model for SCE at 49 DIM.

49 DIM, or any other variable on the hazard of first insemination.

Table 2 shows the effect of $PGF_{2\alpha}$ treatment on first-service pregnancy per AI. Treatment with $PGF_{2\alpha}$ increased ($P = 0.01$) first-service pregnancy per AI. No other variable affected first-service pregnancy per AI. There was no effect of SCE or interaction ($P > 0.05$) between $PGF_{2\alpha}$ treatment and SCE, cyclicity by 49 DIM, or any other variable on first-service pregnancy per AI.

Table 3 shows the effect of $PGF_{2\alpha}$ treatment on hazard of pregnancy up to 300 DIM when all cows were evaluated. There was an interaction ($P = 0.02$) between $PGF_{2\alpha}$ treatment and BCS; treatment with $PGF_{2\alpha}$ increased ($P = 0.02$) the hazard of pregnancy in cows with BCS ≤2.5, which led to a decrease of 45 d in median time to pregnancy (116 vs. 161 d; Figure 5); however, $PGF_{2\alpha}$ treatment did not affect ($P = 0.34$) the hazard of pregnancy in cows with BCS >2.5. No other variable or interaction affected the hazard of pregnancy up to 300 DIM.

Table 4 shows the effect of $PGF_{2\alpha}$ treatment on hazard of pregnancy up to 300 DIM when evaluating only cows with SCE or when evaluating only cows without SCE. Treatment with $PGF_{2\alpha}$ had no effect ($P = 0.45$) on the hazard of pregnancy up to 300 DIM when only cows with SCE were evaluated. Median days open was the same (169 d) for $PGF_{2\alpha}$-treated and control cows. No variable or interaction affected the hazard of conception in cows with SCE. Nevertheless, when only cows without SCE were evaluated, there was an interaction between $PGF_{2\alpha}$ treatment and BCS ($P = 0.01$) on the hazard of pregnancy. Similar to when all cows were evaluated, treatment with $PGF_{2\alpha}$ increased ($P = 0.006$) the hazard of pregnancy in cows with BCS ≤2.5, which led to a decrease of 49 d in median time to pregnancy (112 vs. 161 d); however, $PGF_{2\alpha}$ treatment did not affect ($P = 0.48$) the hazard of pregnancy in cows with BCS >2.5. No other variable or interaction affected the hazard of pregnancy up to 300 DIM in cows without SCE.

DISCUSSION

This study was designed to evaluate the effect of a treatment regimen with $PGF_{2\alpha}$ at 21, 35, and 49 DIM on the prevalence of SCE at 35 and 49 DIM and on subsequent fertility. Herein, PMN threshold (8.5%) at 21 DIM was not diagnostic for SCE based on negative effects on time to pregnancy. But, because previous reports indicated that 18% PMN at cytological examination between 20 and 33 DIM resulted in increased time to pregnancy (Kasimanickam et al., 2004), we evaluated higher thresholds (15, 20, and 30% PMN), albeit with

Table 2. Effect of $PGF_{2\alpha}$ treatment on first-service pregnancy per AI (PRAI)

Level	Treatment[1]	Cows, n	PRAI, %	*P*-value	OR[2]	95% CI[3]
Treatment	$PGF_{2\alpha}$	203	35.5	0.01	1.7	1.1 to 2.7
	Control	203	24.1	—	—	—

[1]Cows in the $PGF_{2\alpha}$ treatment group received an i.m. injection of 25 mg of $PGF_{2\alpha}$ at 21, 35, and 49 ± 3 DIM; control cows received sterile saline solution.

[2]OR = odds ratio.

[3]CI = confidence interval.

no effect. It is noteworthy that only cows with no abnormal uterine discharge were enrolled in the study by Kasimanickam et al. (2004). Such an exclusion criterion was not employed in the current study. In defining clinical endometritis from 20 to 33 DIM based on hazard of pregnancy, LeBlanc et al. (2002) concluded that cows with more severe forms of uterine discharge such as foul and purulent discharge should be included in the case definition from 20 to 33 DIM; however, less severe forms such as mucopurulent discharge should only be included from 27 to 33 DIM. Another difference in the current study is that cows were examined at 21 ± 3 d, rather than 20 to 33 as in Kasimanickam et al. (2004). When no cows were excluded from evaluation for SCE, it is likely that presence of PMN in the uterus around 21 DIM is part of the physiological process of uterine involution; and therefore, not a pathological finding. In the early postpartum period, neutrophils are active in phagocytosing bacteria and debris to prevent excessive bacterial colonization and disease and to restore normal uterine anatomy and histology (Földi et al., 2006). Nonetheless, the thresholds at 35 and 49 DIM, although slightly lower than previous reports (Kasimanickam et al., 2004; Gilbert et al., 2005), resulted in reduced fertility; the presence of PMN above a certain threshold at those times may be considered pathological.

Herein, cows that were cyclic at 21 DIM had decreased prevalence of SCE at 35 and 49 DIM; however, cyclicity by 35 DIM (cycling at 21 or 35 DIM) did not affect SCE at 49 DIM, which indicates that early cyclicity (21 DIM) is the main determinant of later uterine health. Early cyclicity (Beam and Butler, 1997) and uterine diseases (Hammon et al., 2006) were associated with the energy status of dairy cows; therefore, cyclicity at 21 DIM might be an indicator of overall good health and uncomplicated transition.

Although $PGF_{2\alpha}$ administration did not affect the prevalence of SCE, it was effective in increasing first-service pregnancy per AI in all the cows and the hazard of pregnancy in cows with low BCS when all the cows were evaluated or when only cows without SCE at 49 DIM were evaluated. However, treatment with $PGF_{2\alpha}$ had no effect in cows with SCE at 49 DIM. In the present study, $PGF_{2\alpha}$ treatment appeared to be exerting a positive effect on fertility but not via attenuating incidence of SCE. Kasimanickam et al. (2005) evaluated the effect of a single treatment with $PGF_{2\alpha}$ on fertility and reported an overall increase in pregnancy to first AI and on the hazard of pregnancy; however, in contrast to our findings, the positive effect of $PGF_{2\alpha}$ treatment on fertility was attributed to an improvement in cows with SCE, not in cows without SCE at the time of $PGF_{2\alpha}$ treatment. Although treatment regimens dif-

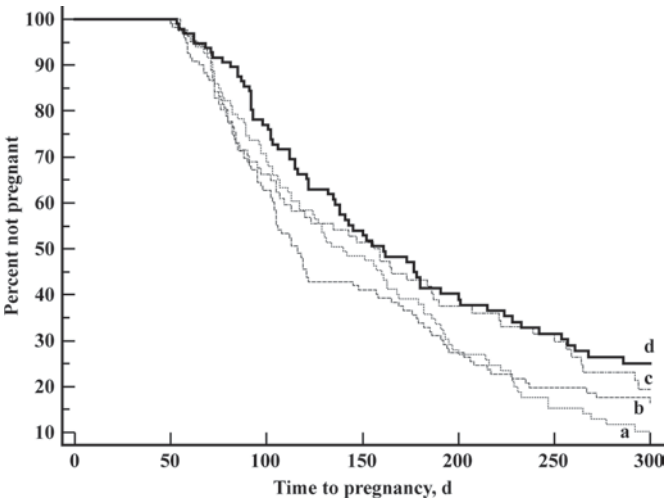

Figure 5. Time to pregnancy in dairy cows with BCS ≤2.5 that received $PGF_{2\alpha}$ treatment (n = 121; b) or sterile saline (n = 96; d) at 21, 35, and 49 DIM and in dairy cows with BCS >2.5 that received $PGF_{2\alpha}$ treatment (n = 82; c) or sterile saline (n = 107; a) at 21, 35, and 49 DIM. Median days to pregnancy was decreased by $PGF_{2\alpha}$ treatment in cows with BCS ≤2.5 (116 vs. 161 d), but not in cows with BCS >2.5 (159 vs. 140 d).

Table 3. Effect of $PGF_{2\alpha}$ treatment on hazard ratio (HR) of pregnancy up to 300 DIM

Variable[1]	Level[2]	Cows, n	*P*-value	HR	95% CI[3]
$PGF_{2\alpha}$ × BCS[4]	BCS (0), PGF (0)	96	—	—	—
	BCS (0), PGF (1)	121	0.02	1.5	1.1 to 2.0
$PGF_{2\alpha}$ × BCS[5]	BCS (1), PGF (0)	107	—	—	—
	BCS (1), PGF (1)	82	0.34	0.9	0.6 to 1.2

[1]PGF = Cows in the $PGF_{2\alpha}$ treatment group received an i.m. injection of 25 mg $PGF_{2\alpha}$ at 21, 35, and 49 ± 3 DIM; control cows received sterile saline solution.

[2]BCS (0) = BCS ≤2.5; BCS (1) = BCS >2.5; PGF (0) = control; PGF (1) = $PGF_{2\alpha}$ treatment.

[3]CI = confidence interval.

[4]An interaction between $PGF_{2\alpha}$ treatment and BCS was observed. Comparison between cows with BCS ≤2.5 that received $PGF_{2\alpha}$ [BCS (0), PGF (1)] and cows with BCS ≤2.5 that did not receive $PGF_{2\alpha}$ [BCS (0), PGF (0); reference group].

[5]Comparison between cows with BCS >2.5 that received $PGF_{2\alpha}$ [BCS (1), PGF (1)] and cows with BCS >2.5 that did not receive $PGF_{2\alpha}$ [BCS (1), PGF (0); reference group].

Table 4. Effect of $PGF_{2\alpha}$ treatment and other significant covariates on the hazard ratio (HR) of pregnancy up to 300 DIM in cows with or without subclinical endometritis (SCE) at 49 DIM

Stratum variable	Level[1]	Cows, n	*P*-value	HR	95% CI[2]
Cows with SCE[3]					
Treatment	$PGF_{2\alpha}$	69	0.45	0.9	0.6 to 1.3
	Control	82	—	—	—
Cows without SCE					
$PGF_{2\alpha}$ × BCS[4]	BCS (0), PGF (0)	53	—	—	—
	BCS (0), PGF (1)	82	0.006	1.8	1.2 to 2.71
$PGF_{2\alpha}$ × BCS[5]	BCS (1), PGF (0)	68	—	—	—
	BCS (1), PGF (1)	52	0.48	0.9	0.6 to 1.3

[1]Cows in the $PGF_{2\alpha}$ treatment group received an i.m. injection of $PGF_{2\alpha}$ at 21, 35, and 49 ± 3 DIM; control cows received sterile saline solution. BCS (0) = BCS ≤2.5; BCS (1) = BCS >2.5; PGF (0) = control; PGF (1) = $PGF_{2\alpha}$ treatment.

[2]CI = confidence interval.

[3]Subclinical endometritis was characterized by the presence of ≥4.0% PMNL in the uterine cytology at 49 DIM.

[4]An interaction between $PGF_{2\alpha}$ treatment and BCS was observed. Comparison between cows with BCS ≤2.5 that received $PGF_{2\alpha}$ [BCS (0), PGF (1)] and cows with BCS ≤2.5 that did not receive $PGF_{2\alpha}$ [BCS (0), PGF (0); reference group].

[5]Comparison between cows with BCS >2.5 that received $PGF_{2\alpha}$ [BCS (1), PGF (1)] and cows with BCS >2.5 that did not receive $PGF_{2\alpha}$ [BCS (1), PGF (0); reference group].

fered considerably (a single dose vs. 3-dose regimen), the discrepancy in findings is not readily explained. In the present study, the improvement in hazard of pregnancy in cows with low BCS was not expected. Cows with low BCS are more likely to be noncyclic by 65 DIM and have decreased pregnancy per AI (Santos et al., 2009). However, a hypothesis to explain the differential effect in this particular group of cows could not be crafted at this point. Prostaglandin $F_{2\alpha}$ is thought to act mainly by inducing estrus in cows with a responsive CL and promoting physical clearance of the uterus and possibly promoting an improvement in uterine defenses under low progesterone (Kasimanickam et al., 2005). Nevertheless, the effect of $PGF_{2\alpha}$ did not depend on presence of an active CL at the time of treatment.

Although a decrease in time to first AI could be expected as one of the immediate benefits from $PGF_{2\alpha}$, no difference was observed in time to first AI. Time to first AI probably was not affected by $PGF_{2\alpha}$ treatment because $PGF_{2\alpha}$ did not appear to benefit cyclic cows; cyclic cows were inseminated at a faster rate than noncyclic cows independently of $PGF_{2\alpha}$ treatment. This response was expected because all dairies relied upon detection of estrus for most of their AI. Therefore, the benefit from $PGF_{2\alpha}$ on time to pregnancy was probably because of increased pregnancy per AI. Furthermore, SCE, which affected time to pregnancy, did not affect time to first AI, suggesting that detrimental effects from SCE are on pregnancy per AI and might be mediated by local effects on the uterus and or embryo. Subclinical endometritis was associated with pathogenic bacteria such as *Arcanobacterium pyogenes* (Gilbert et al., 2007) that can damage the uterus and lead to infertility (BonDurant, 1999). Furthermore, induction of inflammation in the absence of bacteria can lead to reduced embryo quality (Hill and Gilbert, 2008).

CONCLUSIONS

Proportion of PMN at cytological examination at 21 DIM was not diagnostic of SCE based on negative effect on time to pregnancy; however, 6.5% PMN at 35 DIM and 4.0% PMN at 49 DIM resulted in increased time to pregnancy and were considered diagnostic for SCE at those time points. Treatment with $PGF_{2\alpha}$ at 21, 35, and 49 DIM did not decrease prevalence of SCE evaluated at 35 or 49 DIM or increase hazard of pregnancy in cows diagnosed with SCE at 49 DIM. However, $PGF_{2\alpha}$ treatment increased first AI pregnancy per AI in all cows and hazard of pregnancy up to 300 DIM in cows with low BCS when all cows were evaluated or when only cows without SCE at 49 DIM were evaluated. Treatment with $PGF_{2\alpha}$ did not decrease SCE at the time points evaluated, but improved pregnancy per AI and hazard of pregnancy particularly in cows with low BCS.

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