Randomized clinical trial of antibiotic and prostaglandin treatments for uterine health and reproductive performance in dairy cows

J. Dubuc,^{*1,2} T. F. Duffield,^{*} K. E. Leslie,^{*} J. S. Walton,[†] and S. J. LeBlanc^{*}

^{*}Department of Population Medicine, and

[†]Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

ABSTRACT

The objectives of this study were to assess the efficacy of antibiotic treatment for preventing postpartum uterine disease among cows at high risk of uterine disease, and to assess the efficacy of PGF_{2α} for treating cytological endometritis (CYTO) and purulent vaginal discharge (PVD). A total of 2,178 Holstein cows in 6 herds were enrolled in a randomized clinical trial. Within 24 h after parturition, cows were classified at being at high risk of uterine disease (HRUD; *n* = 1,017) if they had twins, dystocia, or retained placenta. All remaining cows were classified as being at low risk of uterine disease (LRUD; *n* = 1,161). Cows in the HRUD group were randomly allocated in a factorial design to receive ceftiofur crystalline free acid (CCFA) at 24 h after parturition or be untreated, and to receive dinoprost (PGF_{2α}) at 35 and 49 (±3) days in milk (DIM) or to be untreated. Cows in LRUD were randomly allocated to receive PGF_{2α} at 35 and 49 (±3) DIM or to be untreated. Serum progesterone was measured at 21, 35, 49, and 63 (±3) DIM. Cows were examined at 35 (±3; exam 1) and 56 (±3; exam 2) DIM for CYTO (by cytobrush device; ≥6% polymorphonuclear cells in endometrial cytology) and for PVD (by Metricheck device; mucopurulent or purulent vaginal discharge). Statistical analyses were performed using multivariable logistic regression models accounting for herd clustering. Treatment with CCFA in HRUD cows was not associated with the probability of metritis overall, but interactions occurred such that CCFA decreased the incidence of metritis among HRUD cows that did not have retained placenta and among cows of parity ≥2. Treatment with CCFA in HRUD cows decreased the probability of PVD at exam 1. Treatment with PGF_{2α} did not affect the probability of cure of CYTO or PVD irrespective of progesterone concentration at the time

of treatment. Among cows affected by CYTO or PVD at exam 1, 66 and 63%, respectively, had spontaneously cured at exam 2. Cows persistently affected at exam 2 had an increased time to pregnancy and were more likely to have both CYTO and PVD at exam 1. Administration of PGF_{2α} at both 5 and 7 wk postpartum did not mitigate the effects of CYTO or PVD on reproductive performance. Clinical approaches to treatment of chronic postpartum reproductive tract infection and inflammation should be reassessed.

Key words: metritis, endometritis, prostaglandin, ceftiofur

INTRODUCTION

The postpartum uterine diseases metritis and endometritis are common in dairy cows (LeBlanc et al., 2002a; Gilbert et al., 2005; Overton and Fetrow, 2008). Metritis is a condition causing systemic signs of illness (fever, anorexia, decreased milk production) characterized by a foul-smelling, brown-red, watery vaginal discharge occurring within the first 21 DIM (Sheldon et al., 2006). Endometritis is based on examination >21 DIM using diagnostic criteria associated with detrimental effects on subsequent reproductive performance (LeBlanc et al., 2002a). Endometritis is diagnosed cytologically as endometrial inflammation, or clinically as mucopurulent or purulent vaginal discharge (LeBlanc et al., 2002a; Kasimanickam et al., 2004; Gilbert et al., 2005). Cytological endometritis (**CYTO**) and purulent vaginal discharge (**PVD**) were shown to be distinct manifestations of reproductive tract disease, with their own sets of risk factors (Dubuc et al., 2010b). It was proposed that the term PVD be used instead of clinical endometritis, because PVD was not necessarily indicative of endometrial inflammation (Dubuc et al., 2010a).

During pregnancy, the uterine lumen is sterile (Földi et al., 2006). At parturition, the cervix opens and allows bacterial contamination from the vagina and the environment into the uterus (Elliott et al., 1968; Griffin et al., 1974a; Földi et al., 2006). This contamination affects over 90% of cows (Elliott et al., 1968; Griffin

Received August 25, 2010.

Accepted November 30, 2010.

¹Corresponding author: jocelyn.dubuc@umontreal.ca

²Current address: Faculté de médecine vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec, J2S 7C6, Canada.

et al., 1974a; Sheldon et al., 2002b). Many species of bacteria can be found in the uterus in the early postpartum period (Elliott et al., 1968; Griffin et al., 1974a; Sheldon et al., 2002b). In the first 10 d after calving, bacteria such as *Streptococcus* spp. and *Staphylococcus* spp. are frequently isolated in animals without signs of metritis, whereas *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, and *Prevotella melaninogenica* are commonly isolated from animals with metritis (Elliott et al., 1968; Sheldon et al., 2002a; Williams et al., 2005). The presence of *E. coli* in the uterus during the first week postpartum can affect the function of polymorphonuclear (PMN) cells (neutrophils) and leads to PVD (Dohmen et al., 2000; Zerbe et al., 2001). Infection with *E. coli* in the first week postpartum is associated with infection with *A. pyogenes* in wk 3, and in turn with the prevalence of CYTO and impaired reproductive performance (Santos et al., 2008). Risk factors such as retained placenta (RP), twins, and dystocia can increase uterine contamination early postpartum (Gröhn et al., 1990; Noakes et al., 1991; Risco and Hernandez, 2003) and lead to a higher risk of uterine diseases (Dubuc et al., 2010b).

Parenteral administration of ceftiofur sodium (Drillich et al., 2001) or ceftiofur hydrochloride (Chenault et al., 2004) has been shown to be effective for treatment of metritis. Ceftiofur reduced the incidence of metritis in cows with retained fetal membranes (Drillich et al., 2003, 2006b; Risco and Hernandez, 2003). This effect was proposed to be linked with a decrease in uterine pathogen load in the early postpartum period (Risco and Hernandez, 2003). Ceftiofur crystalline free acid (CCFA) is a long-acting antibiotic for treatment of respiratory disease and footrot in cattle (Hibbard et al., 2002a,b; Van Donkersgoed et al., 2008). However, little is known regarding its efficacy for preventing metritis, CYTO, or PVD. Administered early in the postpartum period, CCFA could decrease uterine pathogen load and so reduce the incidence of subsequent uterine disease (McLaughlin et al., 2010; Stanisiewski et al., 2010).

A large body of conflicting data exists regarding the benefit of PGF_{2α} administration for improving uterine health and reproductive performance (Burton and Lean, 1995; LeBlanc, 2008; Galvão et al., 2009). The proposed benefits of PGF_{2α} would be from induction of estrus in cows having a responsive corpus luteum, causing evacuation of uterine contaminants (Kasimanickam et al., 2005b), or by improvement of uterine defenses by temporarily increasing estrogen and decreasing progesterone concentrations in plasma (Kasimanickam et al., 2005b).

Uterine contamination decreases over time (Elliott et al., 1968; Griffin et al., 1974a; Sheldon et al., 2002a)

and diagnostic criteria for uterine diseases vary during the postpartum period (LeBlanc et al., 2002a; Gilbert et al., 2005; Galvão et al., 2009). However, limited information exists on the proportions of cows that spontaneously recover from CYTO and PVD or on the risk factors associated with persistent uterine infection and inflammation during the postpartum period (Gautam et al., 2010).

The first objective of this study was to determine whether antimicrobial therapy against putative uterine pathogens, targeted at cows with known risk factors for metritis, could reduce the incidence of metritis, PVD, and CYTO. The hypothesis was that selective antibiotic therapy soon after parturition would reduce the challenge from uterine bacterial pathogens (*E. coli* and anaerobes) and thereby reduce the incidence of uterine diseases. Ceftiofur was chosen because data were available (Drillich et al., 2006a) that support it being able to achieve therapeutic concentrations in the uterus and thereby test the hypothesis. The second objective was to determine the effect of PGF_{2α} treatment for improving uterine health and reproductive performance. The hypothesis was that PGF_{2α} would improve the probability of cure of CYTO and PVD, and would improve pregnancy rate. The third objective was to determine the proportions of cows that spontaneously recover from CYTO or PVD.

MATERIALS AND METHODS

A total of 2,178 Holstein cows from 6 commercial herds (3 in southwestern Ontario, Canada, and 3 in western New York State) were enrolled in a randomized clinical trial. Cows were housed in freestall barns, and herd size ranged from 300 to 1,000 cows. The study was conducted from September 2007 to November 2008. Participating herds were recruited based on convenience, but were required to use monthly DHIA milk recording service. Cows were enrolled only once during the study.

In 3 herds, all cows were enrolled in the study. At parturition, cows experiencing dystocia (pull necessitating ≥2 people or a calf-jack; assistance for longer than 15 min; veterinary-assisted), delivering twins, or having RP (≥24 h after parturition) were enrolled in the high risk of uterine disease group (HRUD; n = 490). All remaining cows in these 3 herds that were unaffected by the preceding conditions were enrolled in the low risk of uterine disease group (LRUD; n = 1,161). In 3 other herds, only cows classified as HRUD were enrolled in the study (n = 527). Enrolling all cows (HRUD and LRUD) in 3 herds and only HRUD cows in 3 other herds enabled us to obtain sufficient sample sizes for each group (HRUD and LRUD).

HRUD Group

Within 24 h after parturition, cows were randomly assigned within herd and in balanced blocks of 4 to receive a single treatment of CCFA (Excede, Pfizer Animal Health, Kirkland, Québec, Canada) at a dose of 6.6 mg/kg subcutaneously at the base of the ear, or to be untreated controls (no placebo was used). To detect a main effect of CCFA reducing the incidence of metritis or PVD from 30 to 20%, allowing for 3 covariates, with 95% confidence and 80% power, required 380 cows per group (Dohoo et al., 2003). Cows were also randomly assigned, within the CCFA allocation block, to receive 25 mg of dinoprost intramuscularly (PGF_{2α}; Lutalyse, Pfizer Animal Health) twice, at 35 (±3) and 49 (±3) DIM, or to receive no PGF_{2α} (no placebo was used). Therefore, cows were randomly allocated in this factorial design clinical trial to 1 of 4 treatment groups: negative controls, CCFA treatment only, PGF_{2α} treatment only, or both CCFA and PGF_{2α} treatments. Individuals involved in the diagnosis of uterine diseases were blinded to treatment allocation.

LRUD Group

Within 24 h after parturition, cows were randomly assigned to receive 25 mg of dinoprost intramuscularly (PGF_{2α}; Lutalyse, Pfizer Animal Health) twice, at 35 (±3) and 49 (±3) DIM, or to be untreated controls (no placebo was used). No cows in the LRUD group received CCFA. To measure a treatment (PGF_{2α}) effect that would improve first-service conception risk from 30 to 40%, allowing for 3 covariates with low correlation with treatment, with 95% confidence and 80% power, required approximately 470 cows per treatment group (Dohoo et al., 2003). Individuals involved in the diagnosis of uterine diseases were blinded to treatment allocation.

Sample and Data Collection

Although experimental treatment allocation was different between the LRUD and HRUD groups, sample and data collection were the same. Farms were visited weekly by a technician and a veterinarian. Body condition score was measured at enrollment in the study and at 63 (±3) DIM (Ferguson et al., 1994). A blood sample from the coccygeal blood vessels was collected into an evacuated sterile tube without anticoagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) from each cow at 21, 35, 49, and 63 (±3) DIM. Samples were kept chilled and allowed to clot. Within 5 h of blood collection, samples were centrifuged to harvest serum,

which was frozen at −20°C. Serum progesterone (P4) analysis was conducted at the Animal Health Laboratory of the University of Guelph with a sequential competitive ELISA (Immulite, Siemens, Mississauga, Ontario, Canada) validated for use in cattle (Martin et al., 2007). The inter- and intraassay CV for P4 were 9.6 and 7.2%, respectively.

Cows were examined for vaginal discharge, cervical diameter, and endometrial inflammation at 35 (±3; exam 1) and 56 (±3; exam 2) DIM. Endometrial cytology samples were collected in the dorsal aspect of the uterine body using the cytobrush technique (Kasimanickam et al., 2005a) and were smeared on glass microscope slides. Slides were stained with modified Wright-Giemsa stain (Protocol Hema-3; Biochemical Sciences, Swedesboro, NJ) and were examined under a microscope to count the number of PMN within 100 cells (% PMN). The clinical examination of cows was performed using the Metrichick device (Simcro, Waikato, New Zealand) and scoring scale (McDougall et al., 2007) and by measuring cervical diameter by transrectal palpation.

Individual cow data were recorded on farm using DairyComp 305 (Valley Agricultural Software, Tulare, CA). Individual cow parity group (first; second or greater) and season of calving (winter: December to February; spring: March to May; summer: June to August; fall: September to November) were recorded. Metritis was diagnosed by herd managers using a standardized definition: the presence of a foul-smelling, red-brown, watery vaginal discharge with rectal temperature >39.5°C and systemic signs of illness (dullness, decreased appetite, decreased milk production) <21 DIM (Sheldon et al., 2006). Cows diagnosed with metritis were treated according to farm protocol, typically with ceftiofur hydrochloride or procaine penicillin, according to product manufacturer's label.

All herds used AI exclusively after a voluntary waiting period of 63 d. All herds relied on estrus detection for their first breeding at the end of the voluntary waiting period. If cows were not first bred by 70 to 80 DIM, and for subsequent breedings after diagnosis of nonpregnancy, cows were enrolled on an ovulation synchronization program. The objective of the experimental treatments with PGF_{2α} was to measure their effects on reproductive health, separate from effects on synchronization of estrus for breeding management. Therefore, enrollment on an ovulation synchronization program could only occur >21 d after the final administration of PGF_{2α}. Reproductive data were collected until 300 DIM or until culling if earlier. Pregnancy diagnosis was performed by transrectal palpation between 35 and 49 d after the last insemination.

Statistical Analyses

Individual cow data were exported from DairyComp 305 to Microsoft Excel (Microsoft Corp., Richmond, WA). Statistical analyses were performed with SAS (version 9.2, SAS Institute Inc., Cary, NC), considering the cow as the unit of interest.

Body condition scores at parturition and 63 d after were classified as fat (≥ 3.75), moderate (3.0 to 3.5), or thin (≤ 2.75). Progesterone data were dichotomized, using a threshold of 1 ng/mL to indicate the presence of an active corpus luteum. Diagnostic criteria for uterine diseases (CYTO and PVD) were established based on detrimental effects on subsequent reproductive performance, using a subsample of the cows enrolled in the current study that were not treated with PGF_{2 α} (Dubuc et al., 2010a). Thus, CYTO was defined as the presence of $\geq 6\%$ PMN and $\geq 4\%$ PMN in endometrial cytology at exam 1 and exam 2, respectively. Diagnostic criteria for PVD were defined as the presence of mucopurulent or purulent vaginal discharge at exam 1 and exam 2. Cervical diameter was not associated with a detrimental effect on reproduction. Cytological endometritis and PVD had additive detrimental effects on reproductive performance. Uterine health status was classified as CYTO only, PVD only, both CYTO and PVD, or unaffected.

HRUD Group. The effect of CCFA on the probability of metritis was evaluated in 2 steps. First, a logistic regression model (GLIMMIX procedure) containing only CCFA as a predictor and herd as a random effect was used. Then, a multivariable logistic regression model (GLIMMIX procedure), considering herd as a random effect, was built using a backward elimination procedure until only significant variables or interactions were retained ($P < 0.05$). Predictors offered to this model were CCFA treatment, parity group, dystocia, twins, RP, season, BCS at parturition, and interactions with the main effect of CCFA treatment. Least squares means of adjusted disease prevalence were obtained for variables retained in the final model.

Uterine health classifications (CYTO only, PVD only, or both) at exam 1 were used as outcomes in multivariable logistic regression models, with herd as a random effect (GLIMMIX procedure). Experimental treatment (CCFA) was forced into all models. Predictors offered to the models were parity group, dystocia, RP, twins, BCS at parturition, metritis, P4 > 1 ng/mL at 21 or 35 DIM or both, season, first test-day milk production data, DIM at first test-day, and interactions with CCFA treatment. A manual backward elimination procedure was used until only significant variables or interactions were retained ($P < 0.05$).

Similarly, uterine health classifications (CYTO only, PVD only, or both) at exam 2 were used as outcomes in multivariable logistic regression models, with herd as a random effect (GLIMMIX procedure). Experimental treatments (CCFA and PGF_{2 α}) were forced into all models. Predictors offered to the models included those offered for the exam 1 models, the interaction of CCFA and PGF_{2 α} , P4 > 1 ng/mL at 49 DIM, and uterine health status at exam 1.

LRUD Group. A similar approach to the HRUD group was used, except that no models were built for metritis or for uterine health status at exam 1, because CCFA was not used as an experimental treatment. Model building for uterine health status at exam 2 (CYTO only, PVD only, or both) was performed as for the HRUD group. Experimental PGF_{2 α} treatment was forced into all models. Predictors offered to the models were parity, BCS at parturition, metritis, season, P4 > 1 ng/mL at 21, 35, or 49 DIM, first test-day milk production data, DIM at first test-day, uterine health status at exam 1, and interactions with the main effect of PGF_{2 α} treatment. A manual backward elimination procedure was used to build the final model until only significant variables or interactions were retained ($P < 0.05$).

Both HRUD and LRUD Groups. First-service pregnancy risk (FSPR; probability of pregnancy at first service) was considered as one outcome measure of reproductive performance in a multivariable logistic regression model (GLIMMIX procedure) with herd as a random effect. Predictors offered to the model included parity group, dystocia, retained placenta, twins, BCS at parturition, metritis, season, P4 > 1 ng/mL at 21, 35, 49, or 63 DIM, first test-day milk production data, DIM at first test-day, as well as PGF_{2 α} experimental treatment (forced into the model), its interactions with other predictors, and the risk group for uterine disease (HRUD or LRUD). A backward elimination procedure was used until only significant variables, or their interactions, were retained ($P < 0.05$). A Cox proportional hazard model (PHREG procedure) of time to first service, up to 100 DIM, was used to evaluate the effect of PGF_{2 α} treatment on interval to first service. Herds were treated as strata to account for clustering (Gröhn et al., 1998). The assumption of proportionality of hazards in the model was assessed graphically by plotting the logarithm of the hazard function by the logarithm of the time. If predictors did not have proportional hazards over time, an interaction term of the log time at risk and the predictor was forced into the model (Dohoo et al., 2003). A similar Cox proportional hazard model (PHREG procedure) was used for evaluating the effect of CCFA treatment (HRUD only), PGF_{2 α} treat-

Table 1. Description of 2,178 Holstein cows (% of total cows) enrolled in a clinical trial investigating the effect of ceftiofur crystalline free acid and PGF_{2α} on uterine health and reproductive performance

Variable	Level	Classification of risk for uterine disease ¹	
		LRUD (n = 1,161)	HRUD (n = 1,017)
Parity	1	30	39
	≥2	70	61
Twins	Yes	0	19
Dystocia	Yes	0	55
Retained placenta	Yes	0	38
Metritis	Yes	12	28
BCS at parturition	≤2.75	2	26
	3.0 to 3.5	74	57
	≥3.75	24	17
CL ² ≤35 DIM	Yes	59	53
CL ³ ≤63 DIM	Yes	83	81
First-service pregnancy risk	Yes	42	41
Culling ⁴	Yes	21	20

¹LRUD = low risk of uterine disease group (cows without retained placenta, twins, or dystocia); HRUD = high risk of uterine disease group (cows with retained placenta, twins, or dystocia).

²Corpus luteum (serum progesterone >1 ng/mL) at 21 or 35 DIM, or both.

³Corpus luteum (serum progesterone >1 ng/mL) at least once at 21, 35, 49, or 63 DIM.

⁴Culling ≤300 d after parturition.

ments (HRUD and LRUD), and its interaction on time to pregnancy, up to 300 d after parturition.

A comparison of uterine health status at exam 1 and exam 2 was performed to classify uterine disease over time. Cows were categorized as unaffected at both times, persistent condition, cure, or newly affected. Mixed logistic regression models, with herd as a random effect (GLIMMIX procedure), were used to identify risk factors for each pattern of disease. Predictors offered to the models included PGF_{2α}, season, parity group, twins, dystocia, retained placenta, metritis, BCS at parturition, risk group of uterine disease (LRUD or HRUD), test-day milk production at first test, DIM at first test-day, and P4 >1 ng/mL at 35 or 49 DIM or both. A backward elimination procedure was used until only significant variables or their interactions were retained ($P < 0.05$). Least squares means of adjusted prevalence for variables retained in the final model were obtained.

RESULTS

In total, 2,178 cows were enrolled in this study, with 2,072 and 2,022 cows examined at exam 1 and exam 2, respectively (cows had been culled between exams

1 and 2). Frequencies of postpartum events and the prevalence of uterine health status at exam 1 and exam 2 are presented in Tables 1 and 2.

HRUD Group

In total, 1,017 cows from 6 herds were enrolled in the HRUD group. Overall, treatment of cows with risk factors for uterine disease with CCFA was not associated with the incidence of metritis (28.9% in cows that received CCFA vs. 30.7% in controls; univariable analysis $P = 0.12$). However, 2 CCFA interaction terms were significant (Table 3) in the multivariable model. Least squares means of these interactions are presented in Figure 1. Administration of CCFA in the HRUD group decreased the incidence of metritis among cows that did not have retained placenta and among multiparous cows.

Treatment with CCFA had no effect on CYTO only ($P = 0.98$), but resulted in a substantial decrease in PVD only ($P < 0.01$; Table 4), and therefore a decrease in the prevalence of both ($P = 0.03$) at exam 1. Cows treated with CCFA were 34 and 32% less likely to have PVD only or both PVD and CYTO at exam 1, respectively, than untreated cows. Treating cows with CCFA

Table 2. Prevalence of uterine diseases at 35 (± 3 ; exam 1) and 56 (± 3 ; exam 2) DIM in 2,072 Holstein cows enrolled in a clinical trial investigating the effect of ceftiofur crystalline free acid and PGF_{2 α} on uterine health

Item ¹	Classification of risk for uterine disease ²		
	LRUD	HRUD	Overall
Exam 1	(n = 1,116)	(n = 956)	(n = 2,072)
CYTO only (%)	13.3	13.1	13.2
PVD only (%)	4.9	14.7	9.4
Both (%)	3.6	10.4	6.7
Exam 2	(n = 1,095)	(n = 927)	(n = 2,022)
CYTO only (%)	9.5	10.0	9.8
PVD only (%)	4.2	10.8	7.2
Both (%)	2.6	5.7	4.0

¹CYTO = cows diagnosed with cytological endometritis only; PVD = cows diagnosed with purulent vaginal discharge only; Both = cows diagnosed with both cytological endometritis and purulent vaginal discharge.

²LRUD = low risk of uterine disease group (cows without retained placenta, twins, or dystocia); HRUD = high risk of uterine disease group (cows with retained placenta, twins, or dystocia).

or PGF_{2 α} had no effect ($P > 0.10$) on the prevalence of CYTO only, PVD only, or both at exam 2, accounting for uterine health status at exam 1. No interaction ($P > 0.20$) between the effects of CCFA and PGF_{2 α} was observed in these models. Serum P4 > 1 ng/mL at 35 or 49 DIM (53 and 70% of HRUD cows, respectively) and its interaction with PGF_{2 α} administration at these times were not significant ($P > 0.20$) in these models.

LRUD Group

A total of 1,161 cows from 3 herds were enrolled in the LRUD group. Treatment with PGF_{2 α} had no effect on the prevalence of CYTO only, PVD only, or both

at exam 2. Serum P4 > 1 ng/mL at 35 or 49 DIM (59 and 74% of LRUD cows, respectively) and its interaction with PGF_{2 α} were not significant ($P > 0.20$) in these models, consistent with the findings in the HRUD group.

Both HRUD and LRUD Groups

Treatment of cows with CCFA or PGF_{2 α} had no effect on time to first service, FSPR, or time to pregnancy ($P > 0.20$), irrespective of P4 status (Table 5). At PGF_{2 α} administration at 35 and 49 (± 3) DIM, 26% of cows had low P4 (< 1 ng/mL) at both times, 20% had P4 > 1 ng/mL at one injection, and 54% had P4 consistent with the presence of a corpus luteum at both times. The effect of uterine health status on reproductive performance was variable. The effect of uterine health status on FSPR, accounting for PGF_{2 α} treatment and retained placenta, is presented in Figure 2. Cows classified as PVD only at exam 1 were 0.6 times as likely as cows having no uterine disease at exam 1 to become pregnant at first insemination ($P < 0.01$). No significant association occurred between CYTO only at exam 1 and FSPR ($P = 0.80$). Median time to first service was not different ($P = 0.79$) among unaffected cows, CYTO only, PVD only (74 d; 95% CI: 74–75) or both (75 d; 95% CI: 74–76). Uterine health status had a detrimental effect on time to pregnancy until 300 d after parturition (Table 6; Figure 3).

Uterine health status at exam 1 and exam 2 was used to classify cows into categories regarding disease progression: unaffected at both examinations, persistent condition, spontaneous recovery (given no effects of PGF_{2 α} on recovery), or newly affected at exam 2. For

Table 3. Final multivariable logistic regression model for metritis, accounting for the random effect of herd, in 1,017 Holstein cows with dystocia, twins, or retained placenta enrolled in a clinical trial investigating the effect of ceftiofur crystalline free acid (CCFA) and PGF_{2 α} on uterine health

Predictor	Class	Cows ¹ (%)	Coefficient	SE	P-value
Intercept					
CCFA	No	49.9	−0.95	0.28	0.01
	Yes	50.1	Referent		
Parity	1	39.3	−0.04	0.24	0.86
	≥ 2	60.7	Referent		
Retained placenta	No	61.8	−0.97	0.23	< 0.01
	Yes	38.2	Referent		
CCFA $\times$ parity	No $\times$ 1	19.2	1.33	0.23	< 0.01
	No $\times$ ≥ 2	30.7	−0.64	0.30	0.03
	Yes $\times$ 1	20.1			
	Yes $\times$ ≥ 2	30.0			
CCFA $\times$ retained placenta	No $\times$ No	30.9	0.95	0.30	< 0.01
	No $\times$ Yes	19.0			
	Yes $\times$ No	30.9			
	Yes $\times$ Yes	19.2			

¹Proportion of cows in each category of each predictor; within each predictor the sum is 100%.

Table 4. Final multivariable logistic regression model, accounting for the random effect of herd, for presence of purulent vaginal discharge only (PVD only; no simultaneous cytological endometritis) at 35 (± 3) DIM in 956 Holstein cows, classified at high risk of uterine disease (HRUD; having dystocia, twins, or retained placenta), enrolled in a clinical trial investigating the effect of ceftiofur crystalline free acid (CCFA) and PGF_{2 α} on uterine health

Predictor	Class	Cows ¹ (%)	Odds ratio	95% CI	P-value
CCFA	No	50.2	Referent		
	Yes	49.8	0.66	0.48–0.89	<0.01
Metritis	No	62.9	Referent		
	Yes	37.1	3.03	2.17–4.17	0.02
Retained placenta	No	71.6	Referent		
	Yes	28.4	1.85	1.33–2.56	<0.01

¹Proportion of cows in each category of each predictor; within each predictor the sum is 100%.

CYTO, 73% (1,481/2,022) of cows were unaffected at both examinations. Among cows ($n = 400$) that were classified positive at exam 1, 66% ($n = 262$) had spontaneously cured and 34% ($n = 138$) were persistently affected at exam 2. Among cows ($n = 1,622$) unaffected at exam 1, 9% ($n = 141$) were diagnosed positive at exam 2. For PVD, 79% (1,594/2,022) of cows were unaffected at both examinations. Among cows ($n = 323$) that were classified positive at exam 1, 63% ($n = 202$) had spontaneously cured and 37% ($n = 121$) were persistently affected at exam 2. Among cows ($n = 1,699$) unaffected at exam 1, 6% ($n = 105$) were diagnosed positive at exam 2.

Among cows diagnosed with uterine disease at exam 1, those having both CYTO and PVD had the greatest proportion (60%) that were persistently affected at exam 2 (CYTO only = 34%, PVD only = 42%). The

risk factors associated with persistent uterine disease were metritis ($P < 0.01$) and being classified as both ($P < 0.01$) at exam 1. Disease status from exam 1 to exam 2 had no effect on time to first service or FSPR ($P > 0.20$) but was associated with an increase in time to pregnancy (Figure 4).

DISCUSSION

This is the first large-scale study to simultaneously consider metritis, CYTO, and PVD as outcomes for evaluating the efficacy of treatments and their effects on reproductive performance. It is also one of the largest investigations of risk factors during the postpartum period and of the progression of uterine disease over that period. In herds ($n = 3$) where all cows (LRUD and HRUD) were enrolled, the proportion of cows in

Figure 1. Conditional effects, stratified by retained placenta and parity, of 1 dose of ceftiofur crystalline free acid (CCFA) within 1 d after calving on the incidence of metritis in 1,017 Holstein cows with dystocia, twins, or retained placenta enrolled in a randomized clinical trial.

Figure 2. Effect of uterine health status [unaffected; cytological endometritis (CYTO) only; purulent vaginal discharge (PVD) only; or both cytological endometritis and purulent vaginal discharge (both)] at exam 1 (35 ± 3 DIM) on first-service pregnancy risk (FSPR) in 2,072 Holstein cows enrolled in a clinical trial. Bars with different letters differ (*P* < 0.05).

the HRUD group was 27 to 31%, whereas the proportion of HRUD cows in the entire study was 47%. It is important to consider this when comparing the incidence of diseases and conditions in Tables 1 and 2 with other studies. Although the classification of cows in the HRUD group was based on factors (twins, dystocia,

Table 5. Summary of the effects of random assignment to 2 administrations of PGF_{2α} at 35 and 49 (±3) DIM on reproductive performance of 2,022 Holstein cows from 6 herds¹

Serum progesterone (ng/mL)	PGF _{2α}	Outcome measure		95% CI	<i>P</i> -value ²
Time to first insemination					
		Median (d)	HR ³		
≤1 ⁴	No	74	Referent	0.83–1.02	0.07
	Yes	74	0.90		
>1 ⁵	No	74	Referent	0.84–1.05	0.07
	Yes	74	0.92		
Pregnancy at first insemination					
		Pregnant (%)	Odds ratio		
≤1 ⁴	No	33.9	Referent	0.54–1.03	0.08
	Yes	27.7	0.75		
>1 ⁵	No	27.5	Referent	0.86–1.49	0.38
	Yes	30.0	1.13		
Time to pregnancy					
		Median (d)	HR ⁶		
≤1 ⁴	No	146	Referent	0.94–1.15	0.44
	Yes	156	1.04		
>1 ⁵	No	135	Referent	0.91–1.03	0.30
	Yes	133	0.97		

¹No interaction of the effects of PGF_{2α} with the experimental antibiotic treatment in the block of cows with dystocia, twins, or retained placenta (HRUD) that had a factorial treatment assignment of a single dose of long-acting ceftiofur <24 h after calving and PGF_{2α} at 35 and 49 DIM.

²P-value for hazard or odds ratio.

³Hazard ratio from a Cox proportional hazard model of time to first insemination, to 100 DIM, accounting for herd clustering, HR <1 indicates a reduced instantaneous relative risk of insemination; HR >1 indicates an increased instantaneous relative risk of insemination.

⁴At both d 35 and 49 after parturition.

⁵At least once at d 35 or 49 after parturition.

⁶Hazard ratio from a Cox proportional hazard model of time to pregnancy, to 300 DIM, accounting for herd clustering, HR <1 indicates a reduced instantaneous relative risk of pregnancy; HR >1 indicates an increased instantaneous relative risk of pregnancy.

Table 6. Final Cox proportional hazard model of time to pregnancy (by 300 d after parturition) in 2,072 Holstein cows enrolled in a clinical trial investigating the effect of PGF_{2α} on uterine health, and examined for uterine health status 35 DIM (± 3 ; exam 1)

Predictor	Class	Coefficient	SE	HR ¹	95% CI	P-value
PGF _{2α}	No	Referent				
	Yes	-0.01	0.01	0.99	0.96–1.02	0.50
Uterine health status ²	Unaffected	Referent				
	CYTO only	-0.21	0.03	0.81	0.76–0.86	<0.01
	PVD only	-0.32	0.08	0.72	0.62–0.84	<0.01
	Both CYTO and PVD	-0.54	0.08	0.59	0.50–0.68	<0.01
Parity	1	Referent				
	≥ 2	-0.26	0.01	0.77	0.75–0.79	<0.01
CL ³ ≤ 63 d	No	Referent				
	Yes	0.21	0.09	1.23	1.03–1.47	0.02

¹Hazard ratio; HR <1 indicates a reduced instantaneous relative risk of pregnancy; HR >1 indicates an increased instantaneous relative risk of pregnancy.

²CYTO = cytological endometritis; PVD = purulent vaginal discharge.

³Corpus luteum (serum progesterone >1 ng/mL) at least once before 63 DIM.

and retained placenta) previously reported to increase the risk of developing uterine disease (Gröhn et al., 1990; Correa et al., 1993; LeBlanc et al., 2002a), these are not the only determinants of uterine disease. It is noteworthy that the incidence of metritis in the LRUD

group was 12.0%, not zero. In the HRUD group, the incidence of metritis was 28%. The rationale for blocking cows (LRUD or HRUD) on these risk factors was to test the benefit of using antibiotic therapy in a targeted population of cows affected by factors increasing uterine

Figure 3. Survival curves for time to pregnancy up to 300 d after parturition in 2,072 Holstein cows in 6 herds, according to their uterine health status at 35 DIM (± 3 ; exam 1): unaffected (UNAF; $n = 1493$); cytological endometritis (CYTO) only ($\geq 6\%$ polymorphonuclear cells; $n = 260$); purulent vaginal discharge (PVD) only (mucopurulent or purulent vaginal discharge; $n = 188$); or both CYTO and PVD (both; $n = 131$). Median time to pregnancy (95% CI) was 132 d (127–137), 148 d (139–157), 156 d (144–168), and 195 d (174–216) for UNAF, CYTO only, PVD only, and both, respectively.

Figure 4. Survival curves for time to pregnancy up to 300 d after parturition in 2,022 Holstein cows, according to uterine health status at 35 (± 3 ; exam 1) and 56 DIM (± 3 ; exam 2): unaffected at exam 1 and exam 2 (UNAF; $n = 1274$); newly affected at exam 2 (new; $n = 173$); persistently affected at exam 1 and exam 2 (PERS; $n = 240$); or spontaneously cured at exam 2 (cure; $n = 335$). Median time to pregnancy (95% confidence interval) was 132 d (126–138), 144 d (125–163), 180 d (171–189), and 150 d (142–158) for UNAF, new, PERS, and cure, respectively.

contamination. The hypothesis was that such cows were likely to be infected with *E. coli*, that early treatment of this infection would reduce the risk of progression to metritis, and that ceftiofur would be a suitable antibiotic to reduce the load of uterine pathogens (Drillich et al., 2006a).

Interestingly, the proportion of cows classified as having a BCS ≤ 2.75 was much greater in the HRUD group than in the LRUD group. Although these groups are not directly comparable because of the study design, this finding suggests that cows in HRUD group were generally thinner, which may be indicative of a poor dry period and reduced feed intake.

The effect of CCFA therapy for preventing metritis was evaluated in 2 steps to evaluate its overall effect (model without 2-way interaction) and the potential for conditional effects (model with 2-way interactions). As used in the present experiment, CCFA did not decrease the incidence of metritis overall. Although the design was to examine the effect of antibiotic treatment on metritis or endometritis among cows with any of RP, twins, or dystocia, interactions in the final multivariable model indicated conditional effects. Cows with dystocia or twins that did not have RP, and cows that

had 1 of these 3 conditions and were in parity ≥ 2 that received CCFA had a reduced incidence of metritis. These effects suggest that the experimental hypothesis should not be discarded but explored further in future randomized clinical trials designed to test the effects of antimicrobial therapy for prevention of metritis in these sub-populations. The lack of a significant overall effect of metaphylactic antibiotic treatment despite achieving the planned sample size for detection of this main effect might be related to the dose or pharmacology of the product used. It is unknown if the dose and single administration of CCFA used in this study were sufficient to reach and maintain antimicrobial concentration in the endometrium or uterine lumen to suppress bacterial pathogens. Evidence from other studies suggests that ceftiofur sodium and ceftiofur hydrochloride have a spectrum of activity that covers the common and important uterine contaminants soon after parturition, such as *E. coli*, *F. necrophorum*, *P. melaninogenicus*, and *A. pyogenes* (Bondurant, 1999; Okker et al., 2002; Drillich et al., 2006a). Recent studies report conflicting evidence about whether certain genomic or phenotypic groups of *E. coli* may be associated with uterine disease in cattle (Silva et al., 2009; Sheldon et al., 2010), al-

though no data indicate that the minimum inhibitory concentration of antibiotic differs among these strains. Alternatively, other variables such as innate immune function in the uterus may be more important than the level of bacterial contamination in the early postpartum period (Sheldon et al., 2009a). Cows in the HRUD group that did not have RP may have experienced a lower burden of uterine contamination than cows that had RP; if so, the dose of CCFA may have been sufficient to reduce the uterine pathogen load, and thereby decrease the incidence of metritis. The interaction of CCFA with parity is more difficult to explain. Multiparous cows have a larger uterus, which might interfere with the draining of lochia (Marion et al., 1968). In some multiparous cows, antibiotic treatment may help to shift the balance between infection and host response.

Although the use of CCFA in HRUD cows did not reduce the incidence of CYTO, it did significantly decrease the probability of PVD at exam 1. Uterine diseases are associated with infection with *E. coli* in the first week after calving (Dohmen et al., 2000; Zerbe et al., 2001), and PVD is associated with *A. pyogenes* (Bondurant, 1999; Williams et al., 2005). As used, CCFA may have decreased the uterine load of bacteria such as *A. pyogenes*, but might not have sufficiently reduced numbers of other bacteria that are associated with metritis earlier in the postpartum period, such as *E. coli*. This may be explained by the higher minimum inhibitory concentration of *E. coli* compared with that of *A. pyogenes* (Sheldon et al., 2004). Because the risk factors associated with CYTO are linked to energy balance and immune function (Sheldon et al., 2009a; Dubuc et al., 2010b), it is not surprising that antimicrobial therapy had no direct effect on it, in contrast to PVD. The relationship between uterine pathogen load and endometrial inflammation has been described (Santos et al., 2008) but is not well understood, and it has been suggested that endometrial inflammation may persist after bacterial pathogens are no longer detected (Sheldon et al., 2009a,b).

A greater proportion of cows in the HRUD group than the LRUD group had metritis and PVD but not CYTO. Cytological endometritis and PVD have been shown to be distinct because they have separate risk factors and effects on reproduction (Dubuc et al., 2010a). It was expected that cows with RP, twins, or dystocia would have higher incidences of metritis and PVD because these diseases are associated with reproductive tract trauma (Dubuc et al., 2010b; Potter et al., 2010). It was surprising that the prevalence of CYTO was very similar between LRUD and HRUD groups, for reasons that remain unclear. Endometrial inflammation may not necessarily be linked to bacterial pathogen load but to factors such as regulation of the immune response

(Herath et al., 2009), which may not be captured in classifications based on crude risk factors at calving (HRUD and LRUD).

Data on the effect of PGF_{2α} on uterine health and reproduction are conflicted (Burton and Lean, 1995; LeBlanc et al., 2002b; Galvão et al., 2009). Reported benefits of PGF_{2α} are probably linked to an effect of estrus synchronization, because most of these studies did not consider uterine health status as a predictor or an outcome (Burton and Lean, 1995). Among investigations of uterine health, none until now considered CYTO and PVD simultaneously. In the present trial, PGF_{2α} treatment at 35 d and 49 DIM did not influence uterine health at exam 2 in either the LRUD or HRUD groups, accounting for uterine health status at exam 1. Surprisingly, this lack of effect was not modulated by the presence of a corpus luteum (serum P4 >1 ng/mL) at PGF_{2α} administration. This finding is consistent with a recent study that investigated the effect of PGF_{2α} for treatment of CYTO (Galvão et al., 2009). In the present data, PGF_{2α} treatment had no effect on time to first service, FSPR, or time to pregnancy, again independent of whether cows were at high or low risk of uterine disease. None of the variables considered (CCFA, RP, dystocia, twins, metritis, parity, progesterone status at 21, 35, 49 or 63 DIM, BCS at parturition and 63 DIM, and uterine health status at 35 and 56 DIM) identified a sub-population in which PGF_{2α} was associated with an improvement of FSPR or pregnancy rate (hazard ratio). This is surprising and different from another study, which found an increase in FSPR in all cows treated with PGF_{2α}, and an increased pregnancy rate in cows with a body condition score ≤2.5 treated with PGF_{2α} (Galvão et al., 2009). In that study, the PGF_{2α} treatment group received PGF_{2α} at 21, 35, and 49 DIM and the voluntary waiting period was 49 d, confounding possible uterine health and estrus synchronization effects on FSPR and pregnancy rate (Galvão et al., 2009). In the present study, to assess putative effects of PGF_{2α} on reproductive tract health separately from effects of estrus synchronization, cows in the PGF_{2α} group were not bred using PGF_{2α} treatments as estrus or ovulation synchronization tools, which could confound the association of PGF_{2α} treatment with reproductive performance.

The hypothesis was that inducing estrus with PGF_{2α} should lead to evacuation of uterine contaminants and improvement of uterine health (Kasimanickam et al., 2005b). The present study was not designed to document luteolysis after treatment with PGF_{2α}, and does not offer a mechanistic explanation of the lack of observed PGF_{2α} of PGF_{2α} on uterine health. Nevertheless, in this very large scale field study in multiple herds, PGF_{2α} treatment was not associated with the

prevalence of PVD or CYTO 1 wk after 2 injections of PGF_{2α}, or with the economically relevant outcome of time to pregnancy in cows with or without uterine disease at 5 wk postpartum. Other studies found similar results for CYTO following 3 injections of PGF_{2α} given every other week (Galvão et al., 2009) or for PVD after a single injection of PGF_{2α} (LeBlanc et al., 2002b). It is likely that PGF_{2α} treatment induces estrus in cows with a responsive corpus luteum, but under the conditions of this study, PGF_{2α} treatment did not result in an observed cytological, clinical, or reproductive performance benefit.

Surprisingly, a statistical trend of detrimental effect of PGF_{2α} treatment on risk of pregnancy at first insemination was noted (Table 5). Although a similar finding has been reported previously (LeBlanc et al., 2002b), it remains unclear why cows treated with PGF_{2α} during the postpartum period would have decreased fertility at first insemination. A detrimental effect on ovarian and immune functions may be implied but further research is needed to better understand this phenomenon.

As expected, uterine health status was associated with reproductive performance, which is consistent with similar studies (LeBlanc et al., 2002a; Gilbert et al., 2005; Barlund et al., 2008). Endometrial inflammation is detrimental to embryo survival (Hill and Gilbert, 2008), and previous studies have reported that cows with CYTO had reduced FSPR (Gilbert et al., 2005; Barlund et al., 2008). The lack of a negative effect of CYTO on FSPR in the present trial could be explained by the differentiation between CYTO only and the “both” categories, whereas only cows with CYTO were considered by Barlund et al. (2008). Moreover, little information on reproductive management was presented in other reports (Gilbert et al., 2005; Barlund et al., 2008), which makes comparison with the present study difficult. Nonetheless, it was surprising that CYTO had no effect on FSPR, but was detrimental to pregnancy rate in the longer term, with an increase of 16 d in median time to pregnancy compared with cows with neither CYTO nor PVD. Similarly, other studies found that cows with CYTO had an increased time to pregnancy of 30 to 88 d (Gilbert et al., 2005; Barlund et al., 2008). In the present study, cows having PVD only and both PVD and CYTO had an increased time to pregnancy of 24 and 63 d, respectively, compared with cows with neither CYTO nor PVD. The effect of PVD was similar to an increase of 34 d reported by LeBlanc et al. (2002a). Unfortunately, in all these previous studies, it was impossible to determine which cows were affected by both PVD and CYTO, because they did not use CYTO and PVD diagnostic techniques simultaneously (LeBlanc et al., 2002a; Gilbert et al., 2005; Barlund et al., 2008).

Cows were classified according to uterine disease status at both exam 1 and exam 2. At exam 2, the proportion of cows that experienced spontaneous cure of the form of uterine disease with which they were affected at exam 1, was 66% and 63% for CYTO and PVD, respectively. This is consistent with another study investigating cows affected by PVD, which reported 75% spontaneous cure (Gautam et al., 2010), albeit over an interval of 1 mo. This proportion of cure for PVD in both studies is high and may be reflective of the natural process of uterine involution in the postpartum period. However, the study by Gautam et al. (2010) did not find any detrimental effect on subsequent reproductive performance for cows that spontaneously cured. This is different from the present study, which found an increase of 18 d in median time to pregnancy in cows that cured between exam 1 and exam 2 relative to those unaffected by any form of uterine disease at both examinations. In fact, cows that spontaneously cured between exam 1 and exam 2 had similarly increased median time to pregnancy as cows found negative at exam 1 and newly diagnosed positive at exam 2. These data suggest that although spontaneous cure happens in a large proportion of cows, these cows still experience reduced subsequent reproductive performance. Thus, if the definition of a healthy cow is based on subsequent time to pregnancy, cows should resolve uterine inflammation and vaginal discharge before 35 DIM.

Unfortunately, not all cows with uterine disease at exam 1 spontaneously cured between exam 1 and exam 2. The proportion of cows persistently affected was 35 and 37% for CYTO and PVD, respectively. For PVD, this is greater than the 25% persistence in a comparable study, but with a 2-wk longer interval between examinations (Gautam et al., 2010). These persistently affected cows had an increase in median time to pregnancy of 48 d compared with cows unaffected by CYTO or PVD. The group with both PVD and CYTO had the greatest proportion (60%) of cows that were found positive at exam 1 and that remained positive at exam 2. Future research should focus on finding effective therapies for cows diagnosed with both CYTO and PVD; this group may benefit the most from these therapies, because their reproductive performance is most affected.

Among cows found negative at exam 1, 9 and 6% were found positive at exam 2 for CYTO and PVD, respectively. This is similar to 11% for apparent new PVD reported by Gautam et al. (2010). Intuitively, cows should cure from CYTO and PVD over time and should not become newly affected or relapse. However, it is unknown what induces these apparently newly affected cows. It could be speculated that the procedures at exam 1 may have caused iatrogenic uterine disease in some cows. Other hypotheses are that these cows were

false negative at exam 1 or that spontaneous occurrence of uterine disease occurs later in the postpartum period. It has been reported that bacterial infection can re-occur after the uterus has become sterile in the postpartum period (Griffin et al., 1974a,b), which may lead to endometrial inflammation or PVD. This may suggest that this is a naturally occurring process, but further research is needed.

CONCLUSIONS

The administration of CCFA soon after parturition in cows at high risk of uterine disease conditionally reduced the incidence of metritis, particularly among cows with dystocia or twins but without RP, and reduced the prevalence of PVD at 35 DIM. These findings support the concept of decreasing the uterine bacterial contamination load in the early postpartum period to prevent subsequent uterine disease. Treatment of cows with PGF_{2α} at 35 and 49 d after parturition had no effect on uterine health or subsequent reproductive performance when not used for estrus synchronization. Herd-specific reassessment of the prevalence, effect, and approach to treatment of reproductive tract disease before breeding is warranted. Cytological endometritis and PVD had additive detrimental effects on subsequent reproduction. Spontaneous cure of both conditions between 5 and 8 wk postpartum was common but the conditions were still detrimental, and failure to cure was associated with a substantial reduction in pregnancy rate in affected individuals.

ACKNOWLEDGMENTS

This project was financially supported by Pfizer Animal Health (Kirkland, Québec, Canada). The authors thank the farmers, veterinarians, and technical staff for their participation.

REFERENCES

- Barlund, C. S., T. D. Carruthers, C. L. Waldner, and C. W. Palmer. 2008. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology* 69:714–723.
- Bondurant, R. H. 1999. Inflammation in the bovine female reproductive tract. *J. Anim. Sci.* 77:101–110.
- Burton, N. R., and I. J. Lean. 1995. Investigation by meta-analysis of the effect of prostaglandin F_{2α} administered post partum on the reproductive performance of dairy cattle. *Vet. Rec.* 136:90–94.
- Chenault, J. R., J. F. McAllister, S. T. Chester Jr., K. J. Dame, F. M. Kausche, and E. J. Robb. 2004. Efficacy of ceftiofur hydrochloride sterile suspension administered parenterally for the treatment of acute postpartum metritis in dairy cows. *J. Am. Vet. Med. Assoc.* 224:1634–1639.
- Correa, M. T., H. Erb, and J. Scarlett. 1993. Path analysis for seven postpartum disorders of Holstein cows. *J. Dairy Sci.* 76:1305–1312.
- Dohmen, M. J. W., K. Joop, A. Sturk, P. E. J. Bols, and J. A. C. M. Lohuis. 2000. Relationship between intra-uterine bacterial contamination, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained placenta. *Theriogenology* 54:1019–1032.
- Dohoo, I., W. Martin, and H. Stryhn. 2003. *Veterinary Epidemiologic Research*. 1st ed. AVC Inc., Charlottetown, Prince Edward Island, Canada.
- Drillich, M., S. Arlt, S. Kersting, A. A. Bergwerff, P. Scherpenisse, and W. Heuwieser. 2006a. Ceftiofur derivatives in serum, uterine tissues, cotyledons, and lochia after fetal membrane retention. *J. Dairy Sci.* 89:3431–3438.
- Drillich, M., O. Beetz, A. Pfützner, M. Sabin, H. J. Sabin, P. Kutzer, H. Nattermann, and W. Heuwieser. 2001. Evaluation of a systemic antibiotic treatment of toxic puerperal metritis in dairy cows. *J. Dairy Sci.* 84:2010–2017.
- Drillich, M., A. Pfützner, H. J. Sabin, M. Sabin, and W. Heuwieser. 2003. Comparison of two protocols for the treatment of retained fetal membranes in dairy cattle. *Theriogenology* 59:951–960.
- Drillich, M., U. Reichert, M. Mahlstadt, and W. Heuwieser. 2006b. Comparison of two strategies for systemic antibiotic treatment of dairy cows with retained fetal membranes: Preventive vs. selective treatment. *J. Dairy Sci.* 89:1502–1508.
- Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010a. Definitions and diagnosis of postpartum endometritis in dairy cows. *J. Dairy Sci.* 93:5225–5234. doi:10.3168/jds.2010-3428.
- Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010b. Risk factors for postpartum uterine diseases in dairy cows. *J. Dairy Sci.* 93:5764–5771. doi:10.3168/jds.2010-3429.
- Elliott, L., K. J. McMahon, H. T. Gier, and G. B. Marion. 1968. Uterus of the cow after parturition: Bacterial content. *Am. J. Vet. Res.* 29:77–81.
- Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703.
- Földi, J., M. Kulcsár, A. Pécsi, B. Huyghe, C. d. Sa, J. A. C. M. Lohuis, P. Cox, and G. Huszenicza. 2006. Bacterial complications of postpartum uterine involution in cattle. *Anim. Reprod. Sci.* 96:265–281.
- Galvão, K. N., M. Frajblat, S. B. Brittin, W. R. Butler, C. L. Guard, and R. O. Gilbert. 2009. Effect of prostaglandin F_{2α} on subclinical endometritis and fertility in dairy cow. *J. Dairy Sci.* 92:4906–4913.
- Gautam, G., T. Nakao, K. Koike, S. T. Long, M. Yusuf, R. M. S. B. K. Ranasinghe, and A. Hayashi. 2010. Spontaneous recovery or persistence of postpartum endometritis and risk factors for its persistence in Holstein cows. *Theriogenology* 73:168–179.
- Gilbert, R. O., S. T. Shin, C. L. Guard, H. N. Erb, and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology* 64:1879–1888.
- Griffin, J. F. T., P. J. Hartigan, and W. R. Nunn. 1974a. Non-specific uterine infection and bovine fertility: I. Infection patterns and endometritis during the first seven weeks post-partum. *Theriogenology* 1:91–106.
- Griffin, J. F. T., P. J. Hartigan, and W. R. Nunn. 1974b. Non-specific uterine infection and bovine fertility: II. Infection patterns and endometritis before and after service. *Theriogenology* 1:107–114.
- Gröhn, Y. T., S. W. Eicker, V. Ducrocq, and J. A. Hertl. 1998. Effect of diseases on the culling of Holstein dairy cows in New York State. *J. Dairy Sci.* 81:966–978.
- Gröhn, Y. T., H. N. Erb, C. E. McCulloch, and H. S. Saloniemi. 1990. Epidemiology of reproductive disorders in dairy cattle: Associations among host characteristics, disease and production. *Prev. Vet. Med.* 8:25–39.
- Herath, S., S. T. Lilly, N. R. Santos, R. O. Gilbert, L. Goetze, C. E. Bryant, J. O. White, J. Cronin, and I. M. Sheldon. 2009. Expression of genes associated with immunity in the endometrium of cattle with disparate postpartum uterine disease and fertility. *Reprod. Biol. Endocrinol.* 7:55–68.

- Hibbard, B., E. J. Robb, S. T. Chester Jr., K. J. Dame, J. F. Boucher, and G. R. Alaniz. 2002a. Dose determination and confirmation of a long-acting formulation of ceftiofur (ceftiofur crystalline free acid) administered subcutaneously for the treatment of bovine respiratory disease. *J. Vet. Pharmacol. Ther.* 25:175–180.
- Hibbard, B., E. J. Robb, S. T. Chester Jr., K. J. Dame, W. W. Moseley, and W. L. Bryson. 2002b. Dose determination and confirmation for ceftiofur crystalline-free acid administered in the posterior aspect of the ear for control and treatment of bovine respiratory disease. *Vet. Ther.* 3:22–30.
- Hill, J., and R. Gilbert. 2008. Reduced quality of bovine embryos cultured in media conditioned by exposure to an inflamed endometrium. *Aust. Vet. J.* 86:312–316.
- Kasimanickam, R., T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, and W. H. Johnson. 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology* 62:9–23.
- Kasimanickam, R., T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, and W. H. Johnson. 2005a. A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. *Can. Vet. J.* 46:255–259.
- Kasimanickam, R., T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, and W. H. Johnson. 2005b. The effect of a single administration of cephalixin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. *Theriogenology* 63:818–830.
- LeBlanc, S. J. 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. *Vet. J.* 176:102–114.
- LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, and W. H. Johnson. 2002a. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.* 85:2223–2236.
- LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, and W. H. Johnson. 2002b. The effect of treatment of clinical endometritis on reproductive performance in dairy cows. *J. Dairy Sci.* 85:2237–2249.
- Marion, G. B., J. S. Norwood, and H. T. Gier. 1968. Uterus of the cow after parturition: Factors affecting regression. *Am. J. Vet. Res.* 29:71–75.
- Martin, J. L., K. A. Vonnahme, D. C. Adams, G. P. Lardy, and R. N. Funston. 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. *J. Anim. Sci.* 85:841–847.
- McDougall, S., R. Macaulay, and C. Compton. 2007. Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. *Anim. Reprod. Sci.* 99:9–23.
- McLaughlin, C., C. LaGrow, C. Daugherty, E. Stanisiewski, and M. Lucas. 2010. Control of acute postpartum metritis in lactating dairy cows at high risk of developing metritis following dystocia, stillbirth, twinning and/or retained placenta/fetal membranes with ceftiofur crystalline free acid sterile suspension (CCFA-SS). *J. Dairy Sci.* 93(E-Suppl. 1):407. (Abstr.)
- Noakes, D. E., L. Wallace, and G. R. Smith. 1991. Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms. *Vet. Rec.* 128:440–442.
- Okker, H., E. J. Schmitt, P. L. A. M. Vos, P. Scherpenisse, A. A. Bergwerff, and F. H. Jonker. 2002. Pharmacokinetics of ceftiofur in plasma and uterine secretions and tissues after subcutaneous postpartum administration in lactating dairy cows. *J. Vet. Pharmacol. Ther.* 25:33–38.
- Overton, M., and J. Fetrow. 2008. Economics of postpartum uterine health. Pages 39–43 in *Proc. Dairy Cattle Reprod. Coun. Conv.*, Omaha, NE. Dairy Cattle Reproductive Council, Hartland, WI.
- Potter, T. J., J. Guitan, J. Fishwick, P. J. Gordon, and I. M. Sheldon. 2010. Risk factors for clinical endometritis in postpartum dairy cattle. *Theriogenology* 74:127–134.
- Risco, C. A., and J. Hernandez. 2003. Comparison of ceftiofur hydrochloride and estradiol cypionate for metritis prevention and reproductive performance in dairy cows affected with retained fetal membranes. *Theriogenology* 60:47–58.
- Santos, N.R., K.N. Galvão, S.B. Brittin, and R.O. Gilbert. 2008. The significance of uterine *E. coli* infection in the early postpartum period of dairy cows. *Reprod. Domest. Anim.* 43(Suppl.1):63. (Abstr.)
- Sheldon, I. M., M. Bushnell, J. Montgomery, and A. N. Rycroft. 2004. Minimum inhibitory concentrations of some antimicrobial drugs against bacteria causing uterine infections in cattle. *Vet. Rec.* 155:383–387.
- Sheldon, I. M., J. Cronin, L. Goetze, G. Donofrio, and H.-J. Schuberth. 2009a. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. *Biol. Reprod.* 81:1025–1032.
- Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. *Theriogenology* 65:1516–1530.
- Sheldon, I. M., D. E. Noakes, A. N. Rycroft, and H. Dobson. 2002a. Effect of postpartum manual examination of the vagina on uterine bacterial contamination in cows. *Vet. Rec.* 151:531–534.
- Sheldon, I. M., D. E. Noakes, A. N. Rycroft, D. U. Pfeiffer, and H. Dobson. 2002b. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction* 123:837–845.
- Sheldon, I. M., S. B. Price, J. Cronin, R. O. Gilbert, and J. E. Gadsby. 2009b. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. *Reprod. Domest. Anim.* 44:1–9.
- Sheldon, I. M., A. N. Rycroft, B. Dogan, M. Craven, J. J. Bromfield, A. Chandler, M. H. Roberts, S. B. Price, R. O. Gilbert, and K. W. Simpson. 2010. Specific strains of *Escherichia coli* are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease in cattle and mice. *PLoS ONE* 5:e9192.
- Silva, E., S. Leitão, T. Tenreiro, C. Pomba, T. Nunes, L. Lopes da Costa, and L. Mateus. 2009. Genomic and phenotypic characterization of *Escherichia coli* isolates recovered from the uterus of puerperal dairy cows. *J. Dairy Sci.* 92:6000–6010.
- Stanisiewski, E., C. Daugherty, J. Hallberg, and M. Lucas. 2010. Evaluation of ceftiofur crystalline free acid sterile suspension (CCFA-SS) administered to dairy cows exhibiting risk factors for acute postpartum metritis. *J. Dairy Sci.* 93(E-Suppl. 1):408. (Abstr.)
- Van Donkersgoed, J., M. Dussault, P. Knight, and L. Byers. 2008. Clinical efficacy of a single injection of ceftiofur crystalline free acid sterile injectable suspension versus three daily injections of ceftiofur sodium sterile powder for the treatment of footrot in feedlot cattle. *Vet. Ther.* 9:157–162.
- Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. W. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* 63:102–117.
- Zerbe, H., C. Oßadnik, W. Leibold, and H. J. Schuberth. 2001. Influence of *Escherichia coli* and *Arcanobacterium pyogenes* isolated from bovine puerperal uteri on phenotypic and functional properties of neutrophils. *Vet. Microbiol.* 79:351–365.