Uterine Involution and Fertility of Holstein Cows Subsequent to Early Postpartum PGF_{2α} Treatment for Acute Puerperal Metritis*

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ABSTRACT

The objective was to evaluate the effect of 2 doses of PGF_{2α} injected early postpartum on uterine involution, serum concentration of acute phase proteins at 12 d postpartum, and fertility in Holstein cows with acute puerperal metritis. Only cows diagnosed with retained fetal membranes and metritis and treated with ceftiofur hydrochloride for 5 d were used in the study. Two hundred cows were assigned randomly to be treated (n = 100) or to serve as controls (n = 100). Treatment consisted of 2 i.m. injections of PGF_{2α} 8 h apart on d 8 postpartum. A subsample of 90 cows was selected randomly (45 treated cows; 45 controls) to evaluate uterine diameter using ultrasonography, uterine score, and serum concentrations of acute phase proteins at 12 d postpartum. The outcome variable for all cows was conception rate at first service. Postpartum, primiparous, treated cows had smaller uterine diameters and lower uterine scores than controls. Cows with a uterine diameter <5.1 cm at 12 d postpartum were 5.5 times more likely to conceive at first service than cows with larger uterine horn diameter. Treatment significantly reduced the concentrations of serum α1-acid glycoprotein. Within primiparous cows, treatment also increased conception at first service by 17%. It was concluded that 2 doses of PGF_{2α} 8 h apart at 8 d postpartum in primiparous cows with acute puerperal metritis decreased the diameter of uterine horns and serum concentration of α1-acid glycoprotein at 12 d postpartum and increased the conception rate at first service.

(**Key words:** acute puerperal metritis, PGF_{2α}, fertility, uterine involution)

Abbreviation key: APM = acute puerperal metritis, CRFS = conception rate at first service, OR = odds ratio, RFM = retained fetal membranes.

INTRODUCTION

Acute puerperal metritis (APM), defined as an enlarged and flaccid uterus with a foul-smelling discharge without systemic compromise, is one of the most common disorders affecting dairy cattle (Lewis, 1997). Incidence of APM varies from 2.2 to 37.3% (Kelton et al., 1998). Economic losses include decreased milk yield, reduced fertility, increased culling, and treatment costs (Kelton et al., 1998). Some studies have estimated a cost of \$236 per case (Barlett et al., 1986). When prevention of APM fails, treatment that will improve reproductive performance is needed to mitigate the economic loss associated with the condition.

Use of PGF_{2α} is common during the early postpartum period to improve uterine involution (Lindell and Kindahl, 1983; Nakao et al., 1997) and fertility in dairy cattle (Archbald et al., 1993, 1994). Results, however, have been controversial because concentrations of PGF_{2α} are elevated in the first 7 d postpartum. These concentrations are even greater in cows experiencing retained fetal membranes (RFM), metritis, or both than in normal cows (Lindell et al., 1982; Risco et al., 1994). In contrast, by d 8 to 10 postpartum, concentrations of PGF_{2α} are basal for both normal cows and those that have experienced RFM, metritis, or both (Kindahl et al., 1992; Risco et al., 1994; Kindahl et al., 1999).

Dose, frequency, and postpartum timing of treatment are factors to be considered when evaluating and comparing clinical trials (Archbald et al., 1994). Our clinical experience indicated that PGF_{2α} at d 8 postpartum might mitigate the effects of APM. Indeed, PGF_{2α} given twice daily for 10 d starting on d 3 postpartum decreased the time of uterine involution by about 1 wk (Lindell and Kindahl, 1983). This result might be

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explained because early postpartum application of $PGF_{2\alpha}$ increased myoelectrical activity and contraction of the uterus (Patil et al., 1980; Gajewski et al., 1999).

The hypothesis tested in this study was that cows with APM treated with 2 doses of $PGF_{2\alpha}$ at 8 d postpartum would have a smaller uterine size, which may reflect an improvement of uterine involution. Accordingly, the objectives of this study were to evaluate the effect of 2 doses of $PGF_{2\alpha}$ injected 8 h apart at 8 d postpartum on uterine diameters and uterine scores, serum concentration of acute phase proteins at 12 d postpartum, and fertility in Holstein cows with RFM that subsequently produced APM and were treated with ceftiofur hydrochloride.

MATERIALS AND METHODS

Dairy Farm and Management

This study was conducted in a Holstein dairy farm located in north central Florida (Bell, FL). The herd consisted of 3000 milking cows milked 3 times daily with a 305-d mature equivalent milk yield of 10,725 kg. Transition dairy cows (between 21 d before expected parturition and 21 d postpartum) were housed in pre- and postpartum dry lot pens. They were fed a TMR to meet or exceed nutrient requirements for lactating cows (NRC, 2001). The prepartum diet contained anionic salts to prevent hypocalcemia and other calving-related disorders.

Cows calved in the prepartum transition lot and were moved immediately to a processing pen where they were scored for body condition (1 = thin to 5 = fat; Ferguson et al., 1994), udder edema, reproductive tract status for lacerations, or the presence of RFM. If cows were suspected of having RFM, they stayed 24 h in a hospital barn until RFM was confirmed. After that, cows were moved to a postpartum transition pen and were monitored daily for development of metritis after the first milking within the first 7 d postpartum. When cows developed APM, they were treated with ceftiofur hydrochloride (Excenel; Pharmacia Animal Health, Kalamazoo, MI) for 5 consecutive days and were monitored for the next 5 d. Severe cases of toxic puerperal metritis, characterized by fever, dehydration, and depression (Smith et al., 1998), were sent to a hospital barn and treated accordingly.

Study Design

The experiment was conducted between October 2002 and March 2003. For a cow to be included in the study, it must have had RFM, subsequently developed APM, and treatment with ceftiofur hydrochloride.

Cows with RFM were defined as those with fetal membranes not expelled after 24 h postpartum; such cows were observed visually and by vaginal examination the day after parturition. Acute puerperal metritis was diagnosed by per rectum palpation of the uterus and its contents at 8 d postpartum. Cows with APM had an enlarged and flaccid uterus with a foul-smelling uterine discharge, without fever ($>39.5^{\circ}\text{C}$), clinical mastitis, diarrhea, or displaced abomasum. All study cows were treated i.m. with ceftiofur hydrochloride starting from 3 to 7 d postpartum at a dose of 2.2 mg/kg BW for 5 d. Therefore, at assignment to the study, cows had APM and were under the influence of antimicrobial treatment for 1 to 5 d. Cows developing toxic puerperal metritis were excluded from the study.

To find an increment in conception rate at first service (CRFS) of 15% (from 15 to 30%), a sample size of 94 cows per group was required (95% confidence, 80% power; Winepiscope, 2001). Two hundred cows with APM were assigned randomly to be treated ($n = 100$) or to serve as controls ($n = 100$). Treated cows received 2 25-mg, i.m. injections of $PGF_{2\alpha}$ (Lutalyse; Pharmacia Animal Health) at 8 d postpartum, 8 h apart. The first injection was applied during the first milking in the morning (0700 h), and the next was applied during the second milking (1500 h).

Treated and control cows were exposed to the same environment and management conditions. After the voluntary waiting period (70 d), all cows were subjected to a synchronization of ovulation and timed insemination protocol (Ovsynch) for the first service. This synchronization program consisted of one i.m. dose of GnRH (100 μg Cystorelin; Merial, Iselin, NJ) administered on d 0, one 25-mg dose of $PGF_{2\alpha}$ (Lutalyse) 7 d later, and a second dose of GnRH 2 d later with timed insemination 16 to 24 h after the second dose of GnRH. Pregnancy diagnosis was conducted by palpation per rectum of the uterus and its contents at 42 to 49 d after insemination.

To find a difference in the diameter of the previously gravid uterine horn of 4 ± 4 mm between cows in the treated and control group, a random subsample ($n = 45$ per group) from the 200 cows was subjected to a reproductive tract evaluation (95% confidence, 80% power; Winepiscope, 2001). Outcomes for all cows ($n = 200$) included CRFS, and for the subset ($n = 90$), ultrasonographic uterine measurements, reproductive tract evaluation by palpation per rectum, and serum acute phase proteins were indicators of uterine inflammation. Evaluations of reproductive characteristics were conducted at 12 d postpartum by a faculty veterinarian of the University of Florida. The evaluator was blind to treatment.

Figure 1. Cross-sectional ultrasonographic image of a postpartum uterine horn in a primiparous cow with acute puerperal metritis at 12 d postpartum 4 d after treatment with 2 doses of PGF_{2α}. a) Serosa to serosa diameter; b) submucosa to submucosa diameter.

Ultrasonography was conducted using a 5-MHz transrectal linear transducer (Aloka, 500, Wallingford, CT) to assess uterine characteristics of the previous gravid horn according to validated methodology (Okano and Tomizuka, 1987; Kamimura et al., 1993; Sheldon et al., 2003). Two diameters of the previously gravid uterine horn were evaluated approximately 10 cm from the bifurcation of the uterus. The first measurement was from serosa to serosa to obtain the gross diameter of the uterine horn (Sheldon and Dobson, 2000). The second measurement was from submucosa to submucosa to obtain lumen diameter (Figure 1). The difference between the first and the second measurements estimated the thickness of the myometrium. Because of the enlarged size of the uterus at 8 d postpartum, it was not feasible to determine accurately the uterine diameter and use it as a covariate in the statistical analysis.

Per rectum palpation was conducted to establish a uterine score (1 to 3). Uterine score considered the size and tonicity of the uterus at the greater curvature of the uterine horn (3 = flaccid uterus larger than one hand, 2 = uterus with moderate tonicity and smaller than one hand, and 1 = high tonicity and less than 3 fingers width; Zemjanis, 1970). Conception rate at first service was defined as the proportion of cows diagnosed pregnant 42 to 49 d after the timed AI.

Blood was collected from the coccygeal vessels at treatment assignment (8 d postpartum) and 4 d later

(12 d postpartum) for the analysis of haptoglobin, a major acute phase protein in ruminants, and α 1-acid glycoprotein, a less specific acute phase protein in cattle (Tizard, 2000). Acute phase proteins were analyzed by a radial immunodiffusion test (Cardiotech, Inc., Louisville, KY).

Statistical Analyses

Ultrasonographic findings and acute phase protein concentrations were analyzed by ANOVA mixed models (Littell et al., 1996). Uterine score was analyzed using the Median-Rank Test because distribution of uterine score was not normal (Wilk-Shapiro Statistic = 0.78; Kolmogorov-Smirnov Statistic = 0.25). Models considered parity as a covariate. Correlation coefficients between acute phase proteins and uterine diameter were calculated. Statistical analysis was conducted using SAS for Windows 8.0 (SAS Inst., Inc, Cary, NC).

The ANOVA mixed models were defined as

$$y_{ijk} = \mu + T_i + \text{Cow } (T_i)_j + \text{Par}_k + (\text{Par} * T)_{ik} + e_{ijk}$$

where y_{ijk} = uterine diameter, μ = population mean, T_i = fixed effect of treatment, $\text{Cow } (T_i)_j$ = random effect of cow nested in treatment, Par_k = fixed effect of parity, $(\text{Par} * T)_{ik}$ = interaction parity and treatment, and e_{ijk} = random error term.

For acute phase proteins, the pretreatment concentrations were considered as covariates. Data for CRFS were analyzed by logistic regression, adjusting for parity, milk yield, and previous pathological conditions before first service. Previous pathological conditions were defined as any combination of clinical mastitis, lameness, and digestive disorder. Adjusted odds ratios (OR) and confidence intervals are reported. For cows with ultrasonographic evaluations, uterine diameter was used as a predictor of CRFS by using the median value of the distribution as a cut-off value, correcting for parity and pathological conditions prior to first service.

The logistic regression model was defined as

$$\text{Logit } (\pi/1 - \pi) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$$

where $(\pi/1 - \pi) = \log$ of the odds of the probability of the event (pregnant: yes, no), α = intercept, X_1 = treatment effect, X_2 = parity effect, and X_3 = previous pathological condition effect.

RESULTS

During the study period, 1536 cows calved. From this total, 15.3% (235 cows) developed RFM, APM

Table 1. Ultrasonographic uterine findings and uterine scores of the previous gravid horn of cows with acute puerperal metritis subsequently treated with 2 doses of PGF_{2α} at 8 d postpartum (LSM ± SEM).

Item	Primiparous		Multiparous	
	Treated	Controls	Treated	Controls
Cows (no.)	20	22	25	23
Uterine diameter (mm)	49.2 ± 2.6 ^a	56.5 ± 2.4 ^b	52.3 ± 2.2	47.5 ± 2.3
Uterine wall (mm)	24.7 ± 2.2	25.2 ± 2.0	21.7 ± 1.9	21.0 ± 1.9
Rank sum uterine scores	297 ± 32.5 ^a median = 2	483 ± 32.5 ^b median = 2	576 ± 42.4 median = 2	552 ± 42.4 median = 2

^{a,b}Means with different superscript letters within parity differ ($P \leq 0.05$).

without systemic disease, and were treated with ceftiofur hydrochloride. Two hundred cases selected at random were assigned to the study. Only 8.5% of the total calvings (130 cows) developed into toxic puerperal metritis. None of these cows was assigned to the study.

An interaction of treatment × parity influenced diameter of the previously gravid uterine horn. Treated primiparous cows had smaller ($P \leq 0.05$) serosa to serosa and submucosa to submucosa diameters than control primiparous cows (49.2 vs. 56.5 mm and 24.7 vs. 25.2 mm, respectively; Table 1). Among multiparous cows, no treatment effect was detected (Table 1). Thickness of the myometrium did not differ between treatments or between parities.

With regard to uterine scores in primiparous cows, although the median value was similar between treatments, the sum of the ranks for treated cows was less than that for control cows ($P \leq 0.05$; Table 1). Fewer rank scores meant that a greater proportion of treated cows had a score of 1 (high tonicity) and a higher proportion of control cows had a score of 3 (right skewed distribution; Figures 2 and 3).

For acute phase proteins, pretreatment concentrations were used as covariates in the analysis. Treatment with PGF_{2α} reduced ($P \leq 0.05$) concentrations of α1-acid glycoprotein concentration by d 12 postpartum (Table 2). For haptoglobin, no significant differences between treatments were detected. There was no association between parity and acute phase protein concentrations. The diameter of the previously gravid uterine horns was moderately correlated with concentrations of α1-acid glycoprotein ($r = 0.25$; $P < 0.05$) and haptoglobin ($r = 0.24$; $P < 0.05$).

For different reasons, 15 control (3 dead, 8 culled, and 4 not synchronized) and 8 treated cows (1 dead, 4 culled, and 3 not synchronized) did not complete the timed insemination protocol; therefore, they were excluded from the statistical analysis of fertility data. Days to first service did not differ between treatments (109.3 vs. 108.2 d for treated and control groups, respectively). The overall CRFS was not different between treatments. However, there was a tendency ($P = 0.07$) for a treatment × parity interaction. When analyzing the data within parity, treatment increased CRFS by 17 percentage points in primiparous cows (P

Figure 2. Frequency distribution of uterine scores in primiparous treated and control cows at 12 d postpartum. Score 1 (open bars) = high tonicity and <3 fingers width, Score 2 (stippled bars) = uterus with moderate tonicity and smaller than one hand, and Score 3 (filled bars) = flaccid uterus and larger than one hand (Zemjanis, 1970).

Figure 3. Frequency distribution of uterine scores in multiparous treated and control cows at 12 d postpartum. Score 1 (open bars) = high tonicity and <3 fingers width, Score 2 (stippled bars) = uterus with moderate tonicity and smaller than one hand, and Score 3 (filled bars) = flaccid uterus and larger than one hand (Zemjanis, 1970).

Table 2. Plasma acute phase protein concentrations (LSM $\pm$ SEM) before (8 d postpartum) and 4 d after treating cows with acute puerperal metritis with 2 doses of PGF_{2 α} .

	Haptoglobin		α 1-acid glycoprotein	
	PGF _{2α}	Control	PGF _{2α}	Control
Cows (no.)	45	45	45	45
Before treatment	450.2 $\pm$ 30.1 ^a	470.1 $\pm$ 30.3 ^a	1520.1 $\pm$ 60.1 ^a	1480.9 $\pm$ 60.0 ^a
After treatment	210.4 $\pm$ 30.3 ^b	240.4 $\pm$ 30.2 ^b	1320.0 $\pm$ 40.4 ^b	1330.5 $\pm$ 40.3 ^c

^{a,b,c}Means with different superscript letters within row differ ($P \leq 0.05$).

≤ 0.05 ; Table 3), which indicated that when correcting for days to first service and pathological conditions prior to first breeding, treated primiparous cows were 4.2 times more likely to conceive at first service than control primiparous cows (95% CI OR = 1.05 to 16.5). Within multiparous cows, CRFS did not differ between treatments (Table 4).

Median value for uterine diameter was 5.1 cm. Cows with a uterine diameter <5.1 cm had a CRFS of 30.8%, whereas cows with uterine diameters ≥ 5.1 cm had a CRFS of 7.9% ($P \leq 0.05$). Adjusting for parity and pathological condition before first service, cows with uterine diameters <5.1 cm were 5.5 times more likely (95% CI OR = 1.37 to 22.1) to conceive at first service than were cows with uterine diameters ≥ 5.1 cm (Table 5).

DISCUSSION

Uterine involution and diameter of uterine horns can be monitored directly using transrectal ultrasonography (Okano and Tomizuka, 1987; Kamimura et al., 1993; Sheldon et al., 2003), palpation per rectum (Kindahl et al., 1999), or indirectly by estimating the concentration of PGF_{2 α} metabolite or acute phase proteins in serum (Hirvonen et al., 1999; Sheldon et al., 2001). Transrectal ultrasonography for uterine cross-

sectional images had high coefficients of determination (Okano and Tomizuka, 1987). Unfortunately, it was not technically feasible to measure uterine diameter at 8 d postpartum to conduct a covariance analysis when comparing size of the uterus at 12 d postpartum. This technicality may be a source of potential bias on the results and conclusions of our study. However, randomization, a consistent case definition, and blindness of the study play an important role in the reduction of this bias. Therefore, the results of the present study demonstrated that 2 doses of PGF_{2 α} 8 h apart at 8 d postpartum in cows that developed APM and were treated with ceftiofur hydrochloride reduced the size of the previously gravid uterine horn, increased the tonicity of uterus, decreased concentrations of the α 1-acid glycoprotein, and increased CRFS in primiparous lactating dairy cows. These findings indicated that PGF_{2 α} had an effect on uterine involution in primiparous cows. Uterine involution was defined as the process associated with the return of the postpartum uterus to the state of initiating and supporting another pregnancy (Zemjanis, 1970).

Uterine involution is concluded around 45 d postpartum and consists of the reduction in size, loss of tissue, and tissue regeneration of the uterus (Kiracofe, 1980). Reduction in size is, in general, a response of myometrial contractility that plays a major role in clearing

Table 3. Logistic regression model for conception rate at first service (CRFS) in primiparous cows with acute puerperal metritis treated with 2 doses of PGF_{2 α} at 8 d postpartum.

Item	CRFS, %	OR ¹	95% CI ² OR	P
Primiparous treated (n = 39)	28.2 ^a	4.15	1.05–16.5	≤ 0.05
Primiparous control (n = 35)	11.4 ^b	Referent	—	
Days to first breeding < 108 d	19.2	3.32	0.79–13.9	> 0.05
Days to first breeding ≥ 108 d	24.4	Referent	—	
No pathological condition prior to first service	23.1	2.84	0.32–24.7	> 0.05
Pathological condition detected before first service	17.3	Referent	—	

^{a,b}Means with different superscript letters within item differ ($P \leq 0.05$).

¹OR = Odds ratio.

²CI = Confidence interval.

lochia debris from the uterus after calving (Slama et al., 1991; Hirsbrunner et al., 2002). Therefore, our results are consistent with studies that have investigated myometrial physiology in dairy cows. Normal myoelectrical activity of the uterus is greater at calving and decreases drastically around 7 to 9 d postpartum (Gajewski and Faundez, 1992; Gajewski et al., 1999). However, when exogenous uterotonic products are administered (oxytocin, depotocin, or prostaglandins), the myometrium responded with strong contractions (Patil et al., 1980; Gajewski et al., 1999). In the case of $PGF_{2\alpha}$, uterine myoelectrical activity was increased during the early postpartum period (14 d postpartum; Gajewski et al., 1999). Interestingly, although an inflamed uterus (metritis) produces additional prostaglandins, the uterine musculature does not respond to these endogenous prostaglandins, and the involution process is delayed (Kindahl et al., 1999). Conversely, other mechanisms such as leukocyte function, cytokines, endotoxin response, and antibody production might play additional roles in uterine involution (Mateus et al., 2002, 2003) that we were not able to determine in the present study. In contrast, other studies (Eiler et al., 1984; Burton et al., 1987) did not find a positive effect of $PGF_{2\alpha}$ on myometrial activity in dairy cows during the first 4 d postpartum.

Multiparous cows with metritis were not affected by treatment. We can speculate that older cows with metritis were not affected by treatment because of physiological differences between primiparous and multiparous cows. Although many important mechanisms might explain differences between parities, we focus our discussion on one of these processes, which is related to uterine size in the short term, a key indicator of uterine involution (Zemjanis, 1970). Multiparous cows are consistently more likely to develop hypocalcemia (plasma Ca < 7.5 mg/dL) than primiparous cows within the first 10 d postpartum (Goff and Horst, 1997; Horst et al., 1997; Goff, 1999). Concentrations of plasma Ca are less in cows with retained fetal membranes within 24 h after parturition and during the first week postpartum compared with control cows without RFM (6.3 ± 0.2 vs. 7.4 ± 0.2 mg/100 mL; Risco et al., 1994). By d 8 postpartum, concentrations of Ca are >7.5 mg/dL (Risco et al., 1994). Unfortunately, in the present study, the dynamics of Ca concentration were not evaluated. Concentration of Ca at 8 d postpartum in cows with metritis has not been reported.

Calcium is a key mediator for muscle contraction (Nelson and Cox, 2000). Indeed, Coruzzi et al. (1989) demonstrated that Ca channel blockers inhibited spontaneous and electrically induced contractions in the uterus of the mare. Those researchers concluded that Ca channel blockers are potent inhibitors of mare

uterine motility in vitro and emphasized the importance of Ca-related mechanisms in the control of uterine smooth muscle contractility. Consequently, uterotonic compounds, such as $PGF_{2\alpha}$, might be less effective in triggering uterine motility and tonicity in hypocalcemic cows. Indeed, mean diameter of the uterine horns in cows with milk fever was greater than that of the controls between d 15 and 32 postpartum (Risco et al., 1994). In addition, cows that developed hypocalcemia within the first 10 d postpartum had more prolonged intervals to complete uterine and cervical involution than normocalcemic cows (Kamgarpour et al., 1999). Calcium is also important for the activation of many enzymatic processes at the cellular level through phosphorylation of key enzymes. Therefore, other mechanisms involved in uterine involution, such as tissue degeneration and regeneration, might be Ca-dependent mechanisms as well (Nelson and Cox, 2000).

Uterine score was a combination of size and tonicity of the uterus assessed by palpation per rectum. Dynamics of the size of the uterus obtained by palpation per rectum during the first 14 d postpartum was conducted based on Zemjanis (1970). To our knowledge, uterine tone has been barely evaluated as an outcome variable in clinical trials conducted on dairy cattle. Reasons for this are the subjectivity of the method. However, using a well-defined scoring system, which is conducted consistently, usefulness of uterine tonicity might have practical implications (Loeffler et al., 1999). Indeed, in studies evaluating ovarian anovulatory conditions (ovarian cysts), uterine tonicity has been a key factor for diagnostic purposes (Bartolome et al., 2000). The difference in uterine tonicity and size might be a reflection of uterine contractility, reduction of uterine diameter, and shorter time until anatomical involution after $PGF_{2\alpha}$ treatment. Uterine tone and diameter of the uterine horns have been related in the early postpartum period. Cows with smaller uterine diameters had greater tonicity (Slama et al., 1991).

Acute phase proteins are typical pathological responses to tissue damage or inflammation produced by infections, trauma, neoplasia, or other causes. Haptoglobin is one of the most reactive proteins in cattle, and $\alpha 1$ -acid glycoprotein is less specific in the bovine (Gruys et al., 1994; Tizard, 2000). In the present study, concentrations of haptoglobin and $\alpha 1$ -acid glycoprotein were similar to those reported in other studies in cows with APM (Smith et al., 1998; Hirvonen et al., 1999; Sheldon et al., 2001). Furthermore, concentrations of both proteins, as in previously cited studies, decreased over time. Interestingly, $\alpha 1$ -acid glycoprotein was significantly less in treated than control cows, which was probably related to the reduction of uterine

Table 4. Logistic regression model for conception rate at first service (CRFS) in multiparous cows with acute puerperal metritis treated with 2 doses of PGF_{2α} at 8 d postpartum.

Item	CRFS, %	OR ¹	95% CI ² OR	P
Multiparous treated (n = 50)	20.8	1.03	0.39–2.72	>0.05
Multiparous control (n = 53)	26.0	Referent	—	
Days to first breeding < 108 d	21.6	1.05	0.38–2.89	>0.05
Days to first breeding ≥ 108 d	25.7	Referent	—	
No pathological condition prior to first service	29.1 ^a	4.18	1.27–13.74	≤0.05
Pathological condition detected before first service	13.8 ^b	Referent	—	

^{a,b}Means with different superscript letters within item differ ($P \leq 0.05$).

¹OR = Odds ratio.

²CI = Confidence interval.

horn diameter caused by the effect of PGF_{2α}. Haptoglobin was not different between treatments, and perhaps this resulted from its greater variability among animals (Hirvonen et al., 1999). A moderately strong positive correlation was detected for haptoglobin and α 1-acid glycoprotein concentrations with the diameter of the previously gravid horn. This weak association indicated that acute phase protein concentrations are related slightly to size of uterus. Similar findings were reported by Sheldon et al. (2003), who found a positive correlation between uterine diameter and acute phase protein concentrations ($r = 0.37$ and 0.46 for α 1-acid glycoprotein and haptoglobin, respectively). If PGF_{2α} reduced the diameter of uterine horns in cows that developed metritis, it is reasonable to suggest that the size of the uterus decreased, but does not necessarily indicate that the inflammation and infection were resolved.

Cows with smaller uterine diameters (<5.1 cm) had greater CRFS (30.8% vs. 7.9%, respectively) than cows with a uterine diameter ≥5.1 cm. This finding is consistent with other studies that reported uterine diameter during the postpartum period was associated with reduced fertility (LeBlanc et al., 2002).

Conception rate at first service was greater in treated primiparous cows than in control primiparous cows. At the same time, treatment reduced the size of the uterus at 12 d postpartum in primiparous cows. In addition, cows with smaller uterine size had a greater CRFS than cows with larger uterine size. Although, in the present study, improved fertility also might be related to other confounding variables that we were not able to identify, it is suggestive that 2 doses of PGF_{2α} as early as 8 d postpartum hastened uterine involution in primiparous cows that developed APM within the first week postpartum. Hastened uterine involution might have enhanced the uterine environment for establishment of a successful conception at first service. Indeed, PGF_{2α} given twice daily during the early postpartum period decreased the time of uterine involution in about 1 wk in healthy cows (Lindell and Kindahl, 1983) and improved CRFS and decreased the incidence of endometritis in cows with dystocia and RFM (Nakao et al., 1997).

CONCLUSIONS

It was concluded that 2 doses of PGF_{2α} 8 h apart at 8 d postpartum in primiparous cows with APM de-

Table 5. Logistic regression model for diameter of previous gravid uterine horn as a predictor of conception rate at first service (CRFS).

Independent variable	CRFS, %	OR ¹	95% CI ² OR	P
Diameter of uterine horn < 5.1 mm (n = 89)	30.8	5.5	1.37–22.1	≤0.05
Diameter uterine horn ≥ 5.1 mm (n = 88)	7.92	Referent	—	
Primiparous (n = 74)	20.0	0.69	0.18–2.65	>0.05
Multiparous (n = 103)	23.3	Referent	—	
No pathological condition prior to first service (n = 83)	23.1	2.04	0.50–8.60	>0.05
Pathological condition detected before first service (n = 94)	17.3	Referent	—	

¹OR = Odds ratio.

²CI = Confidence interval.

creased the diameter of uterine horns and serum concentration of α 1-acid glycoprotein at 12 d postpartum and increased the CRFS. This response might be attributed to a positive effect of PGF_{2α} on the process of uterine involution.

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REFERENCES

- Archbald, L. F., C. Risco, P. Chavatte, S. Constant, T. Tran, E. Klapstein, and J. Elliot. 1993. Estrus and pregnancy rate of dairy cows given one or two doses of prostaglandin F₂ alpha 8 or 24 hours apart. *Theriogenology* 40:873–884.
- Archbald, L. F., S. Constant, T. Tran, C. Risco, E. Klapstein, and J. Elliot. 1994. Effect of sequential treatment with prostaglandin F₂ alpha and/or oxytocin on estrus and pregnancy rate of lactating dairy cows. *Theriogenology* 42:773–780.
- Bartlett, P. C., J. H. Kirk, M. A. Wilke, J. B. Kaneene, and E. C. Mather. 1986. Metritis complex in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. *Prev. Vet. Med.* 4:235–248.
- Bartolome, J. A., L. F. Archbald, P. Morresey, J. Hernandez, T. Tran, D. Kelbert, K. Long, C. A. Risco, and W. W. Thatcher. 2000. Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies for bovine ovarian cysts in the dairy cow. *Theriogenology* 53:815–825.
- Burton, M. J., R. C. Herschler, H. E. Dziuk, M. L. Fahning, and R. Zemjanis. 1987. Effect of fenprostalene on postpartum myometrial activity in dairy cows with normal or delayed placental expulsion. *Br. Vet. J.* 143:549–554.
- Coruzzi, G., E. Poli, and G. Bertaccini. 1989. Effect of calcium-channel blockers and salbutamol on the isolated mare uterus: interaction with the calcium agonist Bay K 8644. *J. Vet. Phar. Therp.* 12:404–410.
- Eiler, H., F. M. Hopkins, C. S. Armstrong-Backus, and W. A. Lyke. 1984. Uterotonic effect of prostaglandin F_{2α} and oxytocin on the postpartum cow. *Am. J. Vet. Res.* 45:1011–1014.
- Ferguson, J. M., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703.
- Gajewski, Z., and R. Faundez. 1992. Characteristics and analysis of the uterus electrical activity in the pregnant cow. *Theriogenology* 37:1133–1145.
- Gajewski, Z., R. Thun, R. Faundez, and Z. Boryezko. 1999. Uterine motility in the cow during puerperium. *Reprod. Domest. Anim.* 34:185–191.
- Goff, J. P. 1999. Treatment of calcium, phosphorus, and magnesium balance disorders. *Vet. Clin. North Amer. Food Anim. Pract.* 15:619–639.
- Goff, J. P., and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.* 80:1260–1268.
- Gruys, E., M. J. Obwolo, and M. J. M. Toussaint. 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry. *Vet. Bull.* 64:1009–1018.
- Hirsbrunner, G., B. Knutti, I. Liu, U. Küpfer, G. Scholtysik, and A. Steiner. 2002. An in vivo study on spontaneous myometrial contractility in the cow during estrus and diestrus. *Anim. Reprod. Sci.* 70:171–180.
- Hirvonen, J., G. Huszenicza, M. Kulcsár, and S. Pyörälä. 1999. Acute-phase response in dairy cows with acute postpartum metritis. *Theriogenology* 51:1071–1083.
- Horst, R. L., J. P. Goff, T. A. Reinhardt, and D. R. Buxton. 1997. Strategies for preventing milk fever in dairy cattle. *J. Dairy Sci.* 80:1269–1280.
- Kamgarpour, R., R. C. Daniel, D. C. Fenwick, K. McGuigan, and G. Murphy. 1999. Postpartum subclinical hypocalcemia and effects on ovarian function and uterine involution in a dairy herd. *Vet. J.* 158:59–67.
- Kamimura, S., T. Oui, M. Takahashi, and T. Tsukamoto. 1993. Postpartum resumption of ovarian activity and uterine involution monitored by ultrasonography in Holstein cows. *J. Vet. Med. Sci.* 55:643–647.
- Kelton, D. F., K. D. Lissemore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. *J. Dairy Sci.* 81:2502–2509.
- Kindahl, H., M. Bekana, K. Kask, K. Königsson, H. Gustafsson, and K. Odensvik. 1999. Endocrine aspects of uterine involution in the cow. *Reprod. Domest. Anim.* 34:261–268.
- Kindahl, H., K. Odensvik, S. Aiumlamai, and G. Fredriksson. 1992. Utero-ovarian relationship during the bovine postpartum period. *Anim. Reprod. Sci.* 28:363–369.
- Kiracofe, G. H. 1980. Uterine involution: Its role in regulating postpartum intervals. *J. Anim. Sci.* 51(Suppl. 2):16–28.
- LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, and W. H. Johnson. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.* 85:2223–2236.
- Lewis, G. S. 1997. Uterine health and disorders. *J. Dairy Sci.* 80:984–994.
- Lindell, J. O., and H. Kindahl. 1983. Exogenous prostaglandin F₂ alpha promotes uterine involution in the cow. *Acta Vet. Scand.* 24:269–274.
- Lindell, J. O., H. Kindahl, L. Jansson, and L. E. Edqvist. 1982. Postpartum release of prostaglandin F₂ alpha and uterine involution in the cow. *Theriogenology* 17:237–245.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. *SAS System for Mixed Models*. Books by User's Press, Cary, NC.
- Loeffler, S. H., M. J. de Vries, Y. H. Schukken, A. C. de Zeeuw, A. A. Dijkhuizen, F. M. de Graaf, and A. Brand. 1999. Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. *Theriogenology* 51:1267–1284.
- Mateus, L., L. Lopes da Costa, H. Carvalho, P. Serra, and J. Robalo Silva. 2002. Blood and intrauterine leukocyte profile and function in dairy cows that spontaneously recovered from postpartum endometritis. *Reprod. Domest. Anim.* 37:176–180.
- Mateus, L., L. Lopes da Costa, P. Diniz, and A. J. Ziecik. 2003. Relationship between endotoxin and prostaglandin (PGE₂ and PGFM) concentrations and ovarian function in dairy cows with puerperal endometritis. *Anim. Reprod. Sci.* 76:143–154.
- Nakao, T., A. Gamal, T. Osawa, K. Nakada, M. Moriyoshi, and K. Kawata. 1997. Postpartum plasma PGF metabolite profile in cows with dystocia and/or retained placenta, and effect of fenprostalene on uterine involution and reproductive performance. *J. Vet. Med. Sci.* 59:791–794.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*, 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Nelson, D. L., and M. M. Cox. 2000. Protein interactions modulated by chemical energy: Actin, myosin, and molecular motors. Pages 233–239 in *Lehninger Principles of Biochemistry*. 3rd ed. Worth Publishers, New York, NY.
- Okano, A., and T. Tomizuka. 1987. Ultrasonic observation of postpartum uterine involution in the cow. *Theriogenology* 27:369–376.
- Patil, R. K., S. N. Sinha, S. Einarsson, and I. Settergren. 1980. The effect of prostaglandin F₂ alpha and oxytocin on bovine myometrium in vitro. *Nord. Vet. Med.* 32:474–479.
- Risco, C. A., M. Drost, W. W. Thatcher, J. Savio, and M. J. Thatcher. 1994. Effects of calving-related disorders on prostaglandin, cal-

- cium, ovarian activity and uterine involution in postpartum dairy cows. *Theriogenology* 42:183–203.
- Sheldon, I. M., and H. Dobson. 2000. Effect of administration of eCG to postpartum cows on folliculogenesis in the ovary ipsilateral to the previously gravid uterine horn and uterine involution. *J. Reprod. Fertil.* 119:157–163.
- Sheldon, I. M., D. E. Noakes, A. Rycroft, and H. Dobson. 2001. Acute phase protein responses to uterine bacterial contamination in cattle after calving. *Vet. Rec.* 148:172–175.
- Sheldon, I. M., D. E. Noakes, A. N. Rycroft, and H. Dobson. 2003. The effect of intrauterine administration of estradiol on postpartum uterine involution in cattle. *Theriogenology* 59:1357–1371.
- Slama, H., D. Vaillancourt, and A. K. Goff. 1991. Pathophysiology of the puerperal period: Relationship between prostaglandin E2 (PGE2) and uterine involution in the cow. *Theriogenology* 36:1071–1090.
- Smith, B. I., G. A. Donovan, C. A. Risco, C. R. Young, and L. H. Stanker. 1998. Serum haptoglobin concentrations in Holstein dairy cattle with toxic puerperal metritis. *Vet. Rec.* 142:83–85.
- Tizard, I. R. 2000. Innate immunity: Inflammation. Pages 36–46 in *Veterinary Immunology*. 6th ed. I. R. Tizard, ed. W. B. Saunders Company, Philadelphia, PA.
- WinEpiscope 2.0. 2001. Software for Quantitative Veterinary Epidemiology. Facultad de Veterinaria, Zaragoza, Spain, Agricultural University Wageningen, The Netherlands, University of Utrecht, The Netherlands, University of Edinburgh, United Kingdom. Borland Delphi 1.0.
- Zemjanis, R. 1970. Examination of the cow. Pages 3–87 in *Diagnostic and Therapeutic Techniques in Animal Reproduction*. 2nd ed. R. Zemjanis, ed. The Williams & Wilkins Company, Waverly Press, Inc., Baltimore, MD.