Randomized clinical trial to evaluate the efficacy of prostaglandin $F_{2\alpha}$ to treat purulent vaginal discharge in lactating dairy cows

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ABSTRACT

The objectives of this study were (1) to assess the effect of purulent vaginal discharge (PVD) on reproductive performance using a 4-point vaginal discharge score (VDS) and (2) to evaluate the effect of $PGF_{2\alpha}$ treatment in cows with PVD on reproductive performance. Holstein cows ($n = 2,473$) from 1 commercial dairy farm had their vaginal discharge scored at 32 ± 3 d in milk (DIM) using a 4-point scale. Cows were also scored for body condition score and had their ovaries examined using transrectal ultrasound. A VDS-2 or greater was used to define PVD. Cows with PVD were assigned to receive 2 doses of 500 μ g of $PGF_{2\alpha}$ analogue ($n = 277$; Cloprostenol; PGF Veyx forte, Veyx Pharma Ltd., Schwarzenborn, Germany) 14 d apart (odd ear tag number) or remained untreated ($n = 272$; even ear tag number). All cows not detected in estrus at 90 ± 3 DIM were enrolled into a timed artificial insemination (AI) protocol. The effect of VDS and the effect of $PGF_{2\alpha}$ in cows with PVD on reproductive performance were evaluated separately. First service conception risk (FSCR), time to first AI, and time to pregnancy were used as indicators for reproductive performance. Data were analyzed with Cox's regression and mixed logistic models. The prevalence of PVD was 22.2% (548/2,473). We detected an interaction of parity and VDS for FSCR and time to pregnancy. In primiparous cows, VDS had no effect on FSCR and time to pregnancy. In multiparous cows, VDS had an effect on FSCR and time to pregnancy. Multiparous cows having a VDS-2 or a VDS-3 had decreased odds of pregnancy at first AI compared with cows having a VDS-0. Multiparous cows with VDS-1 or VDS-3 had a decreased hazard of conceiving within 200 DIM. The treatment effect of $PGF_{2\alpha}$ was conditional on parity. In primiparous animals with PVD without a corpus luteum present at 32 DIM, treatment with $PGF_{2\alpha}$ reduced FSCR. Irrespective of

the presence of a corpus luteum, treatment with $PGF_{2\alpha}$ decreased the hazard of conceiving within 200 DIM in primiparous cows. Although multiparous cows with PVD treated with $PGF_{2\alpha}$ had increased odds of pregnancy at first AI, no treatment effect was observed on the hazard of conceiving within 200 DIM. Our results do not support the blanket use of $PGF_{2\alpha}$ as a treatment option in postpartum dairy cows having PVD.

Key words: dairy cow, prostaglandin $F_{2\alpha}$, purulent vaginal discharge, treatment

INTRODUCTION

Excessive inflammation of the reproductive tract is a common problem in modern dairy cows leading to reduced reproductive performance, increased culling risk, and ultimately decreased herd profitability (Gilbert et al., 2005; LeBlanc, 2008; Overton and Fetrow, 2008; Mahnani et al., 2015). Two distinct clinical conditions can be identified in association with excessive inflammation of the reproductive tract in early lactation (i.e., metritis and clinical endometritis). Metritis is characterized by a foul-smelling, brown-red, watery vaginal discharge with systemic signs of illness (fever, anorexia, decreased milk production) occurring within the first 21 DIM (Sheldon et al., 2006). Clinical endometritis is defined as the inflammation of the endometrium in a normal-sized uterus associated with purulent (>50% pus) vaginal discharge (**PVD**) after 21 DIM in the absence of systemic clinical disease (Sheldon et al., 2006). Although PVD does not have to be directly associated with an inflammation of the uterus (Deguillaume et al., 2012), it is consistently associated with reduced fertility in dairy cows and will be used further in this article (LeBlanc et al., 2002; Kasimanickam et al., 2005; Dubuc et al., 2011). An effective treatment strategy for PVD has been the objective of numerous studies (e.g., Hendricks et al., 2006; Galvão et al., 2009; Dubuc et al., 2011) and reviews (Lefebvre and Stock, 2012; Haimenl et al., 2013). Currently, 2 major therapeutic approaches are used for the treatment of PVD (i.e., the use of antibiotics or prostaglandins; Lefebvre and Stock, 2012).

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The benefit from injection of PGF_{2α} is believed to arise from induction of estrus. The estrus leads to physical expulsion of bacterial contaminants and inflammatory products as well as a possible improvement in the uterine defense mechanisms (Kasimanickam et al., 2005). A frequently implemented protocol for PGF_{2α} as treatment for PVD consisted of 2 injections 14 d apart starting earliest with 20 DIM (LeBlanc et al., 2002; Dubuc et al., 2011). Although the effects of this procedure on reproductive performance were equivocal (LeBlanc et al., 2002; Hendricks et al., 2006; Dubuc et al., 2011; Giuliodori et al., 2017), it is still a common procedure in German dairy farms. The controversy regarding the evidence of the efficacy of PGF_{2α} to treat PVD arises from the fact that comparison among studies is difficult because of different case definitions, low numbers of cows per treatment, lack of negative controls, and treatment success based on clinical cure instead of on reproductive performance (Lefebvre and Stock, 2012).

Therefore, the objectives of our study were (1) to assess the effect of PVD on reproductive performance using a 4-point vaginal discharge scoring (VDS) system, and (2) to evaluate the effect of PGF_{2α} treatment in cows with PVD on reproductive performance. We hypothesized that an increase in VDS is associated with deleterious effects on reproductive performance and treatment with PGF_{2α} is beneficial in cows with PVD.

MATERIALS AND METHODS

The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee of the Freie Universität Berlin.

Farm and Animal Management

All cows calving between October 2015 and February 2017 on a commercial dairy farm (Brandenburg, Germany) were enrolled weekly in a randomized clinical trial on d 32 ± 3 after parturition. Overall, 2,876 Holstein cows were enrolled. In total, 403 animals were not considered for the analyses (14.0%) because they were missing at the first examination ($n = 373$) or were declared do not breed due to multiple reasons (e.g., severe lameness, chronic mastitis) before the first examination ($n = 8$). In addition, cows with cesarean section ($n = 8$) or adhesions within the reproductive tract ($n = 14$) were excluded from the analysis.

Pre-fresh cows and heifers were kept together on deep-bedded pack and moved to a maternity pen when imminent signs of calving (e.g., appearance of the water bag outside the vulva) were visible. Fresh cows (primip-

arous and multiparous) were housed in a freestall barn with slatted floors and cubicles equipped with rubber mats for 10 d after parturition. Subsequently, cows were housed until confirmed pregnant in a freestall barn with a solid concrete floor and cubicles equipped with manure solids as bedding. Primiparous cows were grouped separately from multiparous cows after 10 DIM until the end of lactation using a similar pen design. Cows were milked 3 times a day in a rotary milking parlor and annual milk production (ECM: 4.0% fat and 3.4% protein) was 9,500 kg per cow. Cows were fed a TMR and feed was delivered over a conveyor belt system 2 to 3 times per day. The TMR for lactating cows consisted of corn silage and grass silage as forage with a corn-, soybean meal-, and canola-meal-based concentrate. The TMR was balanced to meet or exceed minimum nutritional requirements for dairy cows (NRC, 2001).

Vaginal Discharge Evaluation and Assignment to Treatment

All cows were examined at 32 ± 3 DIM by a single investigator (S. Borchardt) to evaluate vaginal discharge and BCS, and to scan their ovaries (Figure 1).

Vaginal discharge was evaluated using a vaginal device (Metricheck, Simcro Ltd., Hamilton, New Zealand) as described elsewhere (Pleticha et al., 2009). Discharge adhering to the diagnostic device was classified on a scale from 0 to 3 (0 = clear or translucent mucus, 1 = mucus containing flecks of white or off-white pus, 2 = mucus containing less than 50% white or off-white mucopurulent material, 3 = discharge containing more than 50% white or yellow pus or serosanguineous). Purulent vaginal discharge was defined as scores of 2 and 3 (LeBlanc et al., 2002; Williams et al., 2005; Dubuc et al., 2010). Weekly cohorts of cows with PVD (VDS of 2 and 3) were randomly assigned to either treatment or control group based on the last digit of their unique 10-digit animal identification number. Odd-numbered cows were assigned to the treatment group and even-numbered cows were assigned to the control group. Treatment consisted of 2 applications of PGF_{2α} (500 µg of Cloprostenol, PGF Veyx forte, Veyx Pharma Ltd., Schwarzenborn, Germany) 14 d apart (32 ± 3 DIM and 46 ± 3 DIM). Cows in the control group did not receive any treatment.

Transrectal ultrasonography was performed using an ultrasound scanner fitted with a 7.5-MHz linear-array transducer (Easi-Scan portable; BCF Technology Ltd., Livingston, UK) to determine the presence or absence of a CL. Corpora lutea were counted and recorded for each ovary. The diameter of the visualized CL was estimated by using an image of the apparent maximal

diameter of the structure, using on-screen background gridlines of 5-mm squares. Luteal volume was calculated as $V = 4/3 \times \pi \times r^3$, where the radius (r) was calculated as $0.5 \times \text{CL diameter}$. For CL with a fluid-filled cavity, the volume of the cavity was calculated and subtracted from the total CL volume.

Cows were evaluated for BCS on a 1 to 5 scale (1 = emaciated and 5 = extremely fat) in increments of 0.25, as outlined by Edmonson et al. (1989). Cows were classified according to their BCS at 32 ± 3 DIM as BCS ≤ 2.50 (low) or BCS > 2.50 (high).

Reproductive Management

Voluntary waiting period (VWP) was set at 50 DIM (Figure 1). Visual observation for estrus detection was conducted 3 times per day for 30 min in the barn by a single professional AI technician. All cows not detected in estrus ($n = 961$) at 90 ± 3 DIM were enrolled into an Ovsynch-56 TAI protocol: GnRH (100 μg of Gonadorelin, Gonavet Veyx, Veyx Pharma Ltd.) at 90 ± 3 DIM, PGF_{2 α} (500 μg of Cloprostenol, PGF Veyx forte, Veyx Pharma Ltd.) 7 d later, GnRH 56 h after PGF_{2 α} , and TAI 12 to 16 h later at 100 ± 3 DIM.

Pregnancy diagnosis was performed using transrectal palpation. Pregnancy status was evaluated using transrectal palpation by the AI technician 39 ± 3 d after AI. Nonpregnant cows with a CL present at nonpregnancy diagnosis were administered PGF_{2 α} (500 μg of Cloprostenol) followed 2 d later by GnRH (100 μg of Gonadorelin). Artificial insemination was performed 12 to 16 h after the GnRH injection. Nonpregnant cows without a CL were enrolled into an Ovsynch-56 TAI protocol as described above.

A single professional AI technician performed all inseminations.

Breeding dates and results of pregnancy diagnosis were obtained from the on-farm computer system (HerdeW, version 5.8, dsp-Agrosoft Ltd., Ketzin, Germany).

Statistical Analyses

Breeding information (i.e., cow ID, calving date, lactation number, breeding date) and results of pregnancy diagnoses were obtained from the on farm computer system, exported to Excel (Office 2010, Microsoft Deutschland Ltd., Munich, Germany) spreadsheets and analyzed using SPSS for Windows (version 22.0, SPSS Inc., IBM, Ehningen, Germany).

We evaluated the effect of vaginal discharge score (approach 1) and PGF_{2 α} treatment in cows with PVD (approach 2) on reproductive performance separately. In approach 1, cows with PVD (VDS-2 and VDS-3) that were treated with PGF_{2 α} were excluded from these analyses. Only cows without PVD (VDS-0 and VDS-1) and untreated cows with PVD were considered here. In approach 2, cows without PVD (VDS-0 and VDS-1) were excluded from these analyses. Only cows with PVD (treated and untreated) were considered here. We used first service conception risk (FSCR), time to first AI, and time to pregnancy as an indicator for reproductive performance.

The analysis of FSCR was performed by logistic regression using the GENLINUX procedure of SPSS. Cow was the experimental unit. According to the model-building strategies described by Dohoo et al. (2009), each parameter considered for the mixed

Figure 1. Scheme of the experimental design. Lactating dairy cows ($n = 2,473$) were screened for purulent vaginal discharge (PVD) at 32 ± 3 DIM using the Metrichick (Simcro Ltd., Hamilton, New Zealand) device (exam 1). Cows diagnosed with PVD (score 2 or 3) were randomly assigned to 1 of 2 groups: (1) treatment ($n = 277$) consisted of 2 applications of PGF_{2 α} 14 d apart (32 ± 3 DIM and 46 ± 3 DIM), and (2) cows in the control group ($n = 272$) did not receive any treatment. All cows were subjected to the same reproductive program [voluntary waiting period (VWP) was 50 DIM; first AI was facilitated using either estrus detection (DIM 50 to 90) or timed AI].

model should be separately analyzed in an univariable model, including the parameter as a fixed factor (i.e., categorical parameter) or covariate (i.e., continuous parameter). Only parameters resulting in univariate models with $P \leq 0.2$ should be included in the final mixed model. Selection of the model that best fit the data was performed by testing each effect separately in an univariate model and finding the model with the lowest value for the Akaike information criterion using a backward elimination procedure that removed all variables with $P > 0.10$ from the model. The initial model contained the following explanatory variables as fixed effects: parity (primiparous vs. multiparous), calving season (January to March; April to June; July to September; October to December), BCS (low vs. high), presence of a CL (yes vs. no), time to first AI (continuous), first test day milk yield (continuous), and all biologically plausible 2-way interactions.

Regardless of the significance level, VDS (VDS-0, VDS-1, VDS-2, and VDS-3) or treatment group (PGF_{2α} vs. control) was forced to remain in the model. To account for multiple comparisons (e.g., VDS), a Bonferroni adjustment was used.

Survival analysis was conducted to evaluate the effect of treatment group on the hazard of receiving AI within 150 DIM and becoming pregnant within 200 DIM by creating Cox semiparametric proportional hazard model using SPSS.

The outcome variable for the Cox model for receiving AI within 150 DIM was the DIM at which cows received their first AI. Right censoring occurred for cows that left the herd due to live culling or death within 150 DIM. The model for time to first AI included parity (primiparous vs. multiparous), calving season (January to March; April to June; July to September; October to December), BCS (low vs. high), presence of a CL (yes vs. no) as a categorical variable, and first test day milk yield as a continuous explanatory variable.

The outcome variable for the Cox model for conceiving within 200 DIM was the DIM at which cows conceived. Right censoring occurred for cows that left the herd due to live culling or death within 200 DIM. The model for time to pregnancy included parity (primiparous vs. multiparous), calving season (January to March; April to June; July to September; October to December), BCS (low vs. high), presence of a CL (yes vs. no) as a categorical variable, and first test day milk yield as a continuous explanatory variable.

Selection of the model that best fit the data was performed by using a backward elimination procedure that removed all variables with $P > 0.10$ from the model. Regardless of the significance level, VDS or treatment group was forced to remain in the model. Survival

analysis curves were constructed to illustrate the rate at which cows received first AI and conceived until 200 DIM using the Cox proportional hazards model. Proportional hazards assumption was graphically assessed by plotting the $-\ln[-\ln(\text{survival})]$ curves against the $\ln(\text{survival time})$. Proportional hazards assumption was assumed to be met when the lines were approximately parallel.

RESULTS

In total, 2,473 cows (843 primiparous and 1,630 multiparous) were considered for the analyses (Figure 2; Table 1). The percentages of cows having VDS-0, VDS-1, VDS-2, and VDS-3 were 50.2% (1,241/2,473), 27.7% (684/2,473), 13.3% (330/2,473), and 8.8% (218/2,473), respectively. The prevalence of PVD (VDS-2 and VDS-3) was 22.2% (548/2,473). Parity had an effect ($P = 0.001$) on the prevalence of PVD with primiparous cows having a higher prevalence (26.2%; 221/843) of PVD compared with multiparous cows (20.1%; 327/1,630).

Effect of Vaginal Discharge Score on Reproductive Performance

Cows with PVD that were treated with PGF_{2α} were excluded from these analyses. We evaluated the effect of VDS on FSCR and detected an interaction of parity and VDS ($P = 0.015$). Therefore, we analyzed the effects of VDS on FSCR separately for primiparous and multiparous cows. In primiparous cows ($n = 655$), VDS had no effect on FSCR ($P = 0.810$; Figure 3). Time to first AI ($P = 0.022$) was the only explanatory variable that remained in the final model for primiparous cows. In multiparous cows ($n = 1,283$), VDS had an effect on FSCR ($P = 0.003$; Figure 3). Multiparous cows having a VDS-2 (odds ratio = 0.59; 95% CI 0.36 to 0.96; $P = 0.035$) or a VDS-3 (odds ratio = 0.18; 95% CI 0.06 to 0.52; $P = 0.002$) had decreased odds of pregnancy at first AI compared with cows having a VDS-0. No difference was observed in the odds of pregnancy at first AI (odds ratio = 0.86; 95% CI 0.66 to 1.12; $P = 0.272$) between multiparous cows having a VDS-1 compared with multiparous cows having a VDS-0. Time to first AI ($P = 0.001$) was the other explanatory variable that remained in the final model for multiparous cows.

Vaginal discharge score had an effect on time to first AI ($P = 0.001$) using Cox proportional hazard model ($n = 2,134$). Cows with VDS-3 had a decreased hazard of being inseminated within 150 DIM (hazard ratio = 0.66; 95% CI 0.53 to 0.82; $P = 0.002$). Having a VDS-1 ($P = 0.209$) or VDS-2 ($P = 0.848$) did not affect the hazard of being inseminated within 150 DIM. No

Figure 2. Flowchart of total number of animals enrolled and considered for final analyses. Vaginal discharge score (VDS; VDS-0 = clear or translucent mucus, VDS-1 = mucus containing flecks of white or off-white pus; VDS-2 = mucus containing less than 50% white or off-white mucopurulent material; VDS-3 = discharge containing more than 50% white or yellow pus or serosanguineous) was assessed at 32 ± 3 DIM. Purulent vaginal discharge (PVD) was defined as a vaginal discharge score of 2 or greater. The effect of vaginal discharge score and treatment with $\text{PGF}_{2\alpha}$ in cows with PVD on reproductive performance was analyzed separately. Number of observations included in each analysis might differ for several reasons (e.g., culling, do not breed decision, missing information of pregnancy test). Cows with PVD and treated with $\text{PGF}_{2\alpha}$ were excluded from the analysis of the effect of vaginal discharge on reproductive performance. FSCR = first service conception risk. Time to first AI within 150 DIM. Time to pregnancy within 200 DIM. Cows with VDS-0 and VDS-1 were excluded from the analysis of the effect of $\text{PGF}_{2\alpha}$ treatment on reproductive performance in lactating cows with PVD.

Table 1. Distribution of lactating dairy cows (no.) stratified by parity with respect to vaginal discharge score (VDS), BCS, calving season, and presence of a corpus luteum (CL) at the first exam

Variable	Cows total	Primiparous	Multiparous
VDS ¹	2,473	843	1,630
VDS-0	1,241	373	868
VDS-1	684	249	435
VDS-2	330	118	212
VDS-3	218	103	115
BCS			
BCS ≤ 2.50	1,336	303	1,033
BCS > 2.50	1,137	540	597
Calving season			
Spring	685	246	439
Summer	275	124	151
Fall	501	183	318
Winter	1,012	290	722
Presence of CL ²			
Yes	1,387	465	922
No	1,086	378	708

¹Vaginal discharge score (VDS-0 = clear or translucent mucus; VDS-1 = mucus containing flecks of white or off-white pus; VDS-2 = mucus containing less than 50% white or off-white mucopurulent material; VDS-3 = discharge containing more than 50% white or yellow pus or serosanguineous) was assessed at 32 ± 3 DIM using a Metrichick (Simcro Ltd., Hamilton, New Zealand) device.

²Presence of a CL was determined using transrectal ultrasound.

interaction was observed between parity and VDS ($P = 0.978$). Parity ($P = 0.037$), BCS ($P = 0.007$), calving season ($P = 0.033$), and first test-day milk yield ($P = 0.001$) were the other explanatory variables that remained in the final model.

Regarding time to pregnancy, an interaction was observed between VDS and parity ($P = 0.001$). Therefore, we analyzed the effects of VDS on time to pregnancy separately for primiparous and multiparous cows using 2 different Cox proportional hazard models. In primiparous cows ($n = 704$), VDS had no effect on time to pregnancy ($P = 0.200$; Figure 4). Body condition score ($P = 0.013$) and milk yield at first test-day ($P = 0.003$) were the other explanatory variables that remained in the final model for primiparous cows. In multiparous cows ($n = 1,430$), VDS had an effect on time to pregnancy ($P = 0.001$; Figure 4). Multiparous cows with VDS-1 (HR = 0.84; 95% CI 0.72 to 0.98; $P = 0.023$) or VDS-3 (HR = 0.23; 95% CI 0.23 to 0.60; $P = 0.001$) had a decreased hazard of conceiving within 200 DIM. Having a VDS-2 ($P = 0.302$) did not affect the hazard of conceiving within 200 DIM. Calving season ($P = 0.001$) and first test-day milk yield ($P = 0.035$)

were the other explanatory variables that remained in the final model.

Effect of PGF_{2α} Treatment on Reproductive Performance in Lactating Cows with PVD

Cows without PVD (VDS-0 and VDS-1) were excluded from these analyses. We evaluated the effect of treatment with PGF_{2α} in cows with PVD (VDS-2 and VDS-3) on FSCR and detected an interaction of parity and treatment ($P = 0.029$). Therefore, we analyzed the effects of treatment on FSCR separately for primiparous and multiparous cows. In primiparous cows with PVD ($n = 190$), there was an interaction of treatment with PGF_{2α} and presence of a CL ($P = 0.044$; Figure 5). There was no treatment effect in primiparous cows with PVD and a CL ($P = 0.888$). However, there was a treatment effect in primiparous cows with PVD and without a CL ($P = 0.010$). No other explanatory variable remained in the final model for primiparous cows. In multiparous cows with PVD ($n = 288$), there was a treatment effect on FSCR ($P = 0.050$; Figure 5). Multiparous cows with PVD treated with PGF_{2α} had increased odds (odds ratio = 1.66; 95% CI 1.00 to 2.76; $P = 0.050$) of pregnancy at first AI compared with control cows with PVD. No interaction was observed between treatment and presence of a CL ($P = 0.512$).

Time to first AI ($P = 0.023$) was the other explanatory variable that remained in the final model for multiparous cows.

Treatment with PGF_{2α} had no effect on time to first AI ($P = 0.907$) using the Cox proportional hazard model ($n = 531$). No interaction was observed between parity and VDS ($P = 0.697$). Body condition score ($P = 0.058$) and first test-day milk yield ($P = 0.051$) were the other explanatory variables that remained in the final model.

Regarding time to pregnancy, an interaction was present between treatment and parity ($P = 0.027$). Therefore, we analyzed the effects of treatment on time to pregnancy separately for primiparous and multiparous cows using 2 different Cox proportional hazard models. In primiparous cows ($n = 209$), treatment had an effect on time to pregnancy ($P = 0.033$; Figure 6). Primiparous cows with PVD that received treatment with PGF_{2α} had a decreased hazard (HR = 0.69; 95% CI 0.50 to 0.97; $P = 0.033$) of conceiving within 200 DIM. Body condition score ($P = 0.003$) and milk yield at first test-day ($P = 0.005$) were the other explanatory variables that remained in the final model for primiparous cows. In multiparous cows ($n = 322$), treatment with PGF_{2α} did not affect the hazard ($P = 0.322$; Figure 6) of conceiving within 200 DIM. Calving season ($P = 0.064$) and first test-day milk yield ($P = 0.007$)

Figure 3. Comparison of first service conception risk (FSCR) based on vaginal discharge score (VDS; VDS-0 = clear or translucent mucus; VDS-1 = mucus containing flecks of white or off-white pus; VDS-2 = mucus containing less than 50% white or off-white mucopurulent material; VDS-3 = discharge containing more than 50% white or yellow pus or serosanguineous) assessed at 32 ± 3 DIM for primiparous cows and multiparous cows, separately, using least square estimates (mean $\pm$ SEM) from the GENLIMIXED procedure of SPSS (SPSS Inc., IBM, Ehningen, Germany). Two different models were used for primiparous cows and multiparous cows, respectively, due to an interaction of parity and VDS. Bars with different letters (a-c) differ significantly from each other within parity.

were the other explanatory variables that remained in the final model.

DISCUSSION

The objectives of our study were to assess a 4-point scale of VDS for diagnosis of PVD and its association with reproductive performance, and the effect of $\text{PGF}_{2\alpha}$

treatment in cows with PVD. The results from this study indicate that the effect of PVD and treatment of PVD with prostaglandin was conditional on parity. Presence of PVD determined at 32 DIM had no effect on reproductive performance in primiparous cows. In multiparous cows, however, PVD had a negative effect. Treatment of PVD with $\text{PGF}_{2\alpha}$ had a negative effect on reproductive performance in primiparous cows, whereas no effect was observed in multiparous cows.

The overall prevalence of PVD was 22% in this study using the Metrichick device 32 DIM and a VDS of 2 or greater to define cows PVD. This is in agreement with other studies (LeBlanc et al., 2002; Barlund et al., 2008; Dubuc et al., 2010; Denis-Robichaud and Dubuc, 2015) representing dairy cows under an intensive confinement system and a similar range of DIM at examination. A comparison of the prevalence, however, is difficult because diagnostic techniques and criteria may vary among studies. In our study, primiparous cows (26.2%) had a greater prevalence of PVD compared with multiparous cows (20.1%). Such a difference would likely be caused by the presence of some risk factors for PVD

Figure 4. Kaplan-Meier survival analysis illustrating the effect of vaginal discharge score (VDS; VDS-0 = clear or translucent mucus, green line; VDS-1 = mucus containing flecks of white or off-white pus, light blue line; VDS-2 = mucus containing less than 50% white or off-white mucopurulent material, orange line; VDS-3 = discharge containing more than 50% white or yellow pus or serosanguineous, red line) assessed at 32 ± 3 DIM on time to pregnancy. Cox proportional analysis of time to pregnancy determined an interaction of VDS and parity ($P = 0.001$). In primiparous cows (panel A), VDS did not affect ($P = 0.274$) median days to pregnancy (118, 124, 104, and 118 d for cows with VDS-0, VDS-1, VDS-2, and VDS-3, respectively). In multiparous cows (panel B), VDS affected ($P = 0.001$) median days to pregnancy (130, 146, and 149 d for cows with VDS-0, VDS-1, and VDS-2, respectively). Median days to pregnancy for multiparous cows with VDS-3 could not be determined because less than 50% of these animals were not pregnant by 200 DIM. Color version available online.

Figure 5. Comparison of first service conception risk (FSCR) for cows with purulent vaginal discharge (PVD) assessed at 32 ± 3 DIM either left untreated (control) or treated with $\text{PGF}_{2\alpha}$ (treatment) twice 14 d apart for primiparous cows and multiparous cows, separately, using least square estimates (mean $\pm$ SEM) from the GENLIMIXED procedure of SPSS (SPSS Inc., IBM, Ehningen, Germany). Purulent vaginal discharge was defined as a vaginal discharge score (VDS) of 2 or greater (VDS-0 = clear or translucent mucus; VDS-1 = mucus containing flecks of white or off-white pus; VDS-2 = mucus containing less than 50% white or off-white mucopurulent material; VDS-3 = discharge containing more than 50% white or yellow pus or serosanguineous). Two different models were used for primiparous cows and multiparous cows, respectively, due to an interaction of parity and treatment. In addition, there was an interaction of treatment and the presence of a corpus luteum (CL) at 32 ± 3 DIM in primiparous cows. Bars with different letters differ significantly from each other (a,b in primiparous cows; x,y in multiparous cows).

Figure 6. Kaplan-Meier survival analysis illustrating the effect of treatment with $\text{PGF}_{2\alpha}$ twice 14 d apart in lactating cows with purulent vaginal discharge (PVD) assessed at 32 ± 3 DIM on time to pregnancy (treatment group: green line; control group: light blue line). Cox proportional analysis of time to pregnancy determined an interaction of treatment and parity ($P = 0.027$). In primiparous cows with PVD (panel A), treatment with $\text{PGF}_{2\alpha}$ affected ($P = 0.002$) median days to pregnancy (144 and 113 d for primiparous cows either treated with $\text{PGF}_{2\alpha}$ or left untreated, respectively). In multiparous cows with PVD (panel B), treatment did not affect ($P = 0.173$) median days to pregnancy (158 and 177 d for multiparous cows either treated with $\text{PGF}_{2\alpha}$ or left untreated, respectively). Purulent vaginal discharge was defined as a vaginal discharge score (VDS) of 2 or greater (VDS-0 = clear or translucent mucus; VDS-1 = mucus containing flecks of white or off-white pus; VDS-2 = mucus containing less than 50% white or off-white mucopurulent material; VDS-3 = discharge containing more than 50% white or yellow pus or serosanguineous). Color version available online.

specific for primiparous cows in this herd. Some of these risk factors could include dystocia, retained placenta, and metritis (Dubuc et al., 2010).

In agreement with our hypothesis, VDS was associated with reproductive performance. This was, however, limited to multiparous cows. In these cows, a greater VDS was associated with decreased odds of pregnancy at first AI and longer time to pregnancy. Previous stud-

ies have reported the detrimental effects of PVD on reproduction (LeBlanc et al., 2002; Williams et al., 2005; McDougall et al., 2007; Runciman et al., 2008; Dubuc et al., 2010), and our data agree with the fact that reproductive performance varies with VDS. The reason why PVD had no effect on reproductive performance in primiparous cows remains speculative. Besides parity-specific differences related to the effect of PVD on reproductive performance, other contributors might be responsible for reduced fertility in multiparous cows as well, such as the greater uterine size (Baez et al., 2016), greater BCS loss in early lactation (Carvalho et al., 2014), or a greater disease incidence in the transition period, which was associated with reduced fertility later in lactation (Santos et al., 2010). Multiparous cows might have to cope with greater metabolic challenges (e.g., hypocalcemia, hyperketonemia), which might limit their immunocompetence to clear an intrauterine infection (Martinez et al., 2012).

Our definition of PVD (VDS-2 or greater) was based on the results of previous studies (Dubuc et al., 2010; Giuliodori et al., 2017). At least for multiparous cows, this definition was meaningful hence a VDS of 2 or greater was associated with decreased odds of pregnancy at first AI. Regarding time to pregnancy, a VDS of 1 and 3 was significantly associated with a decreased hazard. While a numerical decrease was also observed in the hazard of pregnancy for cows with VDS-2, the power of this study was limited to detect a significant difference.

It was postulated that estrus induction in cows with PVD using $\text{PGF}_{2\alpha}$ results in clearance of the uterus by opening of the cervix and estrus-related contractions (Kasimanickam et al., 2005). In addition, decreasing the progesterone level might have a favorable influence on uterine phagocytes, helping to improve uterine health (LeBlanc et al., 2002). In our study, treatment of cows with PVD using $\text{PGF}_{2\alpha}$ twice 14 d apart was conditional on parity. In multiparous cows, there was a slight increase in the odds of pregnancy at first AI but no effect on time to pregnancy. In primiparous cows with PVD, treatment with $\text{PGF}_{2\alpha}$ had a negative effect on time to pregnancy. In addition, in primiparous cows with PVD but without a CL at the first examination treatment with $\text{PGF}_{2\alpha}$ had a negative effect on FSCR. This was unexpected. Detrimental effects of $\text{PGF}_{2\alpha}$ treatment on FSCR in cows without a CL have been reported previously by other researchers in 2 studies (LeBlanc et al., 2002; Dubuc et al., 2011). The reason for this negative effect remains speculative. It is possible that during the re-establishment of ovarian cyclicity early in lactation treatment with exogenous $\text{PGF}_{2\alpha}$ may cause premature luteolysis, therefore depriving the hypothalamus, pituitary gland, and ovaries

of progesterone needed to re-establish normal endocrine signaling. Overall, our data support the conclusion of a recent meta-analysis failing to find any beneficial effect on reproductive performance of cows with PVD after treatment with PGF_{2α} (Haimerl et al., 2013).

A certain percentage (7.6%; n = 373) of cows with the intent to treat were missed because they could not be located in their specific groups. In most instances these cows were sick (e.g., mastitis, lameness) and moved to the hospital pen without changing the pen in the herd management software. We feel confident that this did not bias our results. The external validity of our study is limited because only 1 farm was included.

Random allocation of cows with PVD into the treatment group was based on the last digit of their unique 10-digit animal identification number. Ear tags had been attached at birth of the calves in a consecutive order without knowledge of this study being implemented years later. Other studies randomizing cows based on their animal identification number had not been performed on the participating farm.

All cows not detected in estrus at 90 DIM were enrolled into a timed AI protocol in this study. Therefore, the negative effect of VDS on reproductive performance might have been underestimated because cows were forced to receive an insemination. This is, however, common practice on modern dairy farms (Caraviello et al., 2006).

Furthermore, we did not examine the animals for a second time to evaluate clinical cure, BCS, luteal function, or a combination of these, as reported in other studies (Dubuc et al., 2010; Giuliadori et al., 2017) due to labor constraints. Evaluating luteal function would have been meaningful to determine cyclicity and whether treatment with PGF_{2α} was conditional on the presence of a CL at exam 1 or 2, or both. Clinical cure was evaluated in other studies (Dubuc et al., 2011) and self-cure rate was substantial (63%). The biological outcome (i.e., pregnancy, time to pregnancy), however, is more meaningful because it ultimately affects economic performance of a dairy farm.

A single assessment of BCS on d 32 had no effect on reproductive performance in our study. Multiple studies were able to show an effect of BCS near the time of AI on FSCR (Souza et al., 2008; Santos et al., 2009; Ribeiro et al., 2012) where thin cows had decreased FSCR. The reason for this discrepancy might be due to the different timing of BCS assessment. Previous studies have shown that as BCS near AI increased, the proportion of anovular cows (P4 concentrations less than 1 ng/mL) decreased (Gümen et al., 2003; Lopez et al., 2004; Santos et al., 2009). Reduced BW change and early resumption of cyclicity is not only important for the induction of estrus using PGF_{2α} but also a prerequi-

site for fertility to the first AI. Therefore, future studies should evaluate development of BCS rather than using a single assessment. Moreover, it was shown recently that BCS loss was more predictive for conception risk compared with a single assessment (Carvalho et al., 2014).

CONCLUSIONS

In conclusion, we used a vaginal device and a 4-point scale to assess discharge to identify cows with PVD. Purulent vaginal discharge was associated with reproductive performance. This was, however, limited to multiparous cows. In these cows, a greater VDS was associated with decreased odds of pregnancy at first AI and longer time to pregnancy. The treatment of PVD with 2 doses of PGF_{2α} 14 d apart had negligible effects on the reproductive performance in multiparous cows and slightly negative effects in primiparous cows. Therefore, we do not recommend the therapeutic use of 2 doses of PGF_{2α} in postpartum dairy cows having PVD.

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