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Purulent vaginal discharge diagnosed in pasture-based Holstein–Friesian cows at 21 days postpartum is influenced by previous lactation milk yield and results in diminished fertility

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ABSTRACT

In a subset of dairy cows, prolonged pathological uterine inflammation results in purulent vaginal discharge (PVD), which can have negative consequences for both fertility and milk production. However, unlike for intensive systems, analysis of the effects of PVD in predominantly pasture-based herds is limited. The objective of this study was to assess the effect of PVD in spring-calving, pasture-based dairy cows on production and reproduction indices, stratified according to previous full-lactation milk yield. We assessed clinical disease as defined by vaginal mucus score (VMS) in 440 Holstein-Friesian cows from 5 farms. Cows were categorized as healthy (VMS 0) or having PVD (VMS 1–3) at 21 d postpartum. We recorded 305-d milk, milk protein, and milk fat yields (kg) before and after disease diagnosis, as well as fertility data, such as services per conception and the calving–conception period (CCP). Using SAS 9.4 (SAS Institute Inc., Cary, NC), we analyzed data using PROC MIXED, PROC PHREG, and PROC LOGISTIC to determine the least squares means differences and hazard and odds ratios between the groups, respectively. Overall, a 60% prevalence of PVD was recorded at 21 d postpartum. Milk yield and milk constituents were similar between all VMS categories and between healthy cows and cows with PVD. Although cows in the 4 VMS categories had statistically similar CCP, cows with PVD had a significantly longer CCP than healthy cows on average (9 d). The hazard ratio for cows with PVD was 0.66, indicating a 34% higher risk of a prolonged CCP than healthy cows. Odds ratio analysis determined that cows with PVD were 3 times

more likely not to conceive at all, twice as likely not to conceive at first service, twice as likely not to conceive by 100 d postpartum, and 3 times more likely to fail to conceive before 150 d postpartum compared with healthy cows. Cows were retrospectively categorized as having low or high milk yield, based on whether they were above or below the median 305-d milk yield of the study population (6,571 kg) in the lactation before vaginal mucus scoring. Based on a univariate odds ratio, high-yield cows were 1.6 times more likely to present with PVD in the subsequent lactation. The number of services per conception did not differ between healthy and PVD cows in the low- and high-yield groups. In the high-yield group, cows with PVD were 4.9 times more likely not to conceive, 2.7 times more likely to require multiple services to conceive, 2.1 times more likely to remain not pregnant by 100 d postpartum, and 4.4 times more likely to remain not pregnant by 150 d postpartum. The CCP was also significantly longer in cows with PVD than their healthy counterparts (115.9 ± 4.9 and 104 ± 7.4 d, respectively). In conclusion, PVD significantly increased the CCP in all cows, but to a greater extent in cows with a high milk yield in the lactation before disease diagnosis.

Key words: purulent vaginal discharge, milk yield, fertility, pasture-based cows, vaginal mucus score

INTRODUCTION

Uterine inflammation is known to play a physiological role in the uterus of the postpartum cow and is a key precursor to timely involution and tissue repair. However, the coincident combination of several factors can contribute to exacerbation of inflammation, potentially leading to uterine disease (Sheldon et al., 2014). The development of uterine disease can be influenced by predisposing factors such as negative energy

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balance, calving difficulties (dystocia, twin birth, still-birth, retained placenta, or an inclined vulval angle) and early postpartum uterine infection, known as metritis (Potter et al., 2010; Adnane M. et al., 2016; Daros et al., 2017). More recent analyses have assessed the relative contributions of the host immune response and pathogen presence on disease occurrence (Chapwanya et al., 2012). Cows with prolonged and excessive inflammation experience a significant shift in the reproductive tract microbiome in the days after calving, to a less diverse uterine microbiome population than their healthy counterparts (Santos and Bicalho, 2012; Miranda-CasoLuengo et al., 2019).

Uterine disease is categorized based on clinical symptoms at the specified time of diagnosis. Clinical endometritis is diagnosed by the presence of purulent (or mucopurulent) vaginal discharge (**PVD**) at 21 d postpartum (Sheldon and Noakes, 1998). Clinical endometritis was once believed to represent overall uterine health as a result of the drainage of purulent material from the uterus into the vagina. It is now apparent that PVD may instead reflect inflammation of the lower reproductive tract, including the cervix or vagina (Dubuc et al., 2010). The recommendation based on this finding was that the use of the term PVD—instead of clinical endometritis—provides a more accurate description of reproductive tract disease. Diagnosis of PVD is made by examining the contents (largely mucus) of the vagina by using a Metrichick device (Simcro, Hamilton, New Zealand), a vaginoscope, or a gloved hand. A visual scoring system (0–3 points) based on the degree of purulence of vaginal mucus is widely used (Williams et al., 2005). Initially, the prevalence of PVD was estimated to be 20% at 21 d postpartum (Sheldon et al., 2006). However, recent studies have recorded higher prevalence rates of up to 54% (Giuliodori et al., 2013a). Variances in prevalence rates may be due to several factors, including diagnostic methods, time of diagnosis, nutritional status, and production system (LeBlanc et al., 2002; Esposito et al., 2014).

Seasonal, pasture-based milk production requires dairy cows to conceive every 365 d to capitalize on the onset of fresh grass growth as a cheap source of nutrition (Roche, 2006). The subtle nutritional perturbations experienced by cows in pasture-based dairy systems leaves them more vulnerable to entering negative energy balance than cows in intensive systems, potentially resulting in suboptimal fertility and diminished farm profitability (Roche et al., 2007; Shalloo et al., 2014; Šavc et al., 2016).

The physiological demand on a dairy cow of the transition to high milk yield is known to be a principal risk factor in heightening subsequent susceptibility to disease and hindering reproductive efficiency (Dobson et

al., 2007). A study by Fleischer et al. (2001) found that cows with a high milk yield have an increased probability of developing milk fever (3.3 times), mastitis (2.5 times), and ovarian cysts (3.1 times); however, the majority of herds in that study were not pasture-based, and the incidence of PVD was not investigated. To our knowledge, no study has determined the susceptibility of pasture-based cows with a previously high milk yield to PVD, or how PVD may affect their fertility. To ensure that future herd health and the fertility rates of pasture-based cows do not deteriorate, the relationship between milk yield and PVD must be elucidated to identify subset-specific risk factors for reduced production and reproductive inefficiency. The objectives of this study were to evaluate the consequences of PVD on milk production and fertility, and to evaluate whether a high milk yield can increase susceptibility to PVD and hinder fertility in the next lactation. We assessed the effect of PVD on production and reproduction indices in spring-calving, pasture-based dairy cows and stratified findings according to previous full-lactation milk yield

MATERIALS AND METHODS

Study Design

A convenience sample of 440 cows from 5 commercial farms from the Leinster region of Ireland was enrolled in this study. Calving took place during the spring of 2014 and 2015, and a proportion of cows on each farm were sampled in both years. Average herd size was 73, but ranged from 30 to 188 cows. All farms employed a spring-calving production system, in which cows calved between January and April. Cows were maintained outdoors in a pasture-based grazing system from calving until November of the same year. Nutritional management on all farms consisted of grazed grass, with concentrate supplementation during milking. A grass silage-based diet was provided to lactating and dry cows when they were housed indoors. Drying off occurred in November/December, and cows were housed indoors from then until parturition. Cows in all herds were milked twice a day during the lactation period.

Uterine Disease

All cows were assessed for the presence of PVD at 3 wk postpartum (ranging from 13 to 32 d postpartum) based on the scoring system developed by (Williams et al., 2005). Vaginal mucus scores (**VMS**) were 0 (clear mucus), 1 (flecks of mucopurulent material), 2 (<50% purulent material in 50 mL of exudate), or 3 (≥50% purulent material in 50 mL of exudate). The cow's vulva

was wiped using a paper towel before a clean, lubricated gloved hand was placed through the vulva. Then, the mucosal contents of the ventral, lateral, and dorsal walls of the vagina and exterior cervix were extracted for clinical examination. Sampling was carried out on each farm once a week from January to April. To avoid intra-observer variation, all members of the sampling team were initiated with the same sampling protocol before sampling, and all members were involved across the 2 years of sampling. The vaginal mucus of all cows was visually assessed for color, the proportion and volume of purulent material, and the presence of a fetid odor. Cows without vaginal discharge or that were deemed to have a VMS of 0 were classified as healthy. Cows with a VMS of 1 to 3 were classified as having PVD.

Milk Production and Reproduction

The 305-d milk, fat, and protein yields (kg) for the lactational period before ($n = 322$) and after ($n = 424$) disease diagnosis were recorded by milk recording on a monthly basis during lactation. Milk recording data could not be obtained for 16 cows. Seven cows left their respective herds during lactation, and because their levels of production may not have been an accurate evaluation of their milk yield potential, they were omitted from analysis. Once each milking was completed, all milk-production data were uploaded to the Irish Cattle Breeding Federation database (www.icbf.com) and extracted for further analysis.

We collated reproductive data in the form of failure to conceive ($n = 305$), the number of services per conception ($n = 305$), and the length of the calving-conception period (CCP; $n = 379$). We also recorded the presence of each cow in the herd at the start of the year after sampling ($n = 434$). The presence of 6 cows in the herd at the start of the following year was not adequately defined, and they were omitted from analysis. We called this parameter “persistence,” instead of using other terminology (e.g., culling rate), because the available records did not specify the reasons for the removal of cows from the herd.

Statistical Analyses

Statistical analyses were carried out using SAS 9.4 (SAS Institute Inc., Cary, NC). First, we calculated the prevalence of PVD using PROC FREQ and the aforementioned diagnostic criteria. Mixed general linear regression models were fitted using the MIXED procedure to evaluate the differences between healthy cows and cows with PVD, as well as the differences between the VMS categories for the dependent milk-production

variables (305-d milk, 305-d fat, and 305-d protein yields, in kg, of enrolled cows from the lactation before sampling and after sampling). Reproductive dependent variables that were brought forward for mixed general linear regression analysis were services per conception and CCP. Six CCP values were omitted from further analysis, because their excessive values were the result of management decisions unaffected by disease-related infertility. Statistical significance was set at $P < 0.05$.

Before analyzing the differences in the dependent variables, we tested residuals from the fitted models for normality. This was carried out by graphical checks using residuals plotted against fitted values to assess the homogeneity of variance, and the residual distribution was checked by plotting the quantiles of the residuals against the expected quantiles from a normal distribution. Where appropriate, we used a log-transformation to correct these issues. Each dependent variable was analyzed in a separate model with the inclusion of disease diagnosis, cow tag number, lactation, and farm as independent variables. The farm variable was included as a random effect in each individual model. Because a proportion of cows from each farm was sampled in both years, we included cow tag number in each model as a repeated effect. Explanatory variables were removed from the model in a backward elimination based on likelihood ratio test comparison, using $P > 0.05$ as the reason for removal. Services per conception were analyzed using the GLIMMIX procedure specifying a Poisson distribution because the values were in integer form. A Tukey post-hoc test was included for multiple comparisons when the 4 VMS categories were compared. The statistical model we used for analyses was as follows:

$$\gamma = \text{diagnosis} + \text{lactation} + \text{farm} (\mu) \\ + \text{cow tag number} (r),$$

where γ was the dependent variable (e.g., 305-d milk yield; kg); diagnosis was based on the VMS category or health status (healthy or PVD); μ was the random effect; and r was the repeated measure. Results are presented as least squares means with standard errors of the mean (SEM). Cows were categorized into 2 groups based on being above or equal to ($n = 161$) or below ($n = 160$) the sample population's median value of the 305-d milk yield for the lactation before sampling (6,571 kg of milk). We analyzed the differences in CCP and services per conception for healthy cows and cows with PVD in each median group using the same method.

The proportional hazard ratio of each category having a lengthened CCP compared with healthy cows were calculated by using a univariate Cox proportional haz-

ard model with the PHREG procedure. Kaplan-Meier survival curves were generated using the LIFETEST procedure by plotting the CCP values against the proportion of cows in each group left to complete the CCP at each time point. Curves were constructed to compare VMS categories and compare healthy cows and cows with PVD. CCPs over 150 d were censored. Univariate odds ratios were determined using the LOGISTIC procedure to establish the likelihood of cows with PVD to fail to conceive; to require multiple services to conceive; to fail to conceive before 100 d postpartum; and to fail to persist in their herd at the start of the year after sampling. These findings are presented as odds ratios (OR) with associated 95% confidence intervals (95% CI) and *P*-values.

RESULTS

Prevalence of Uterine Disease

The frequency of animals diagnosed with each VMS was as follows: VMS 0: 40% (*n* = 175); VMS 1: 27% (*n* = 117); VMS 2: 15% (*n* = 67); and VMS 3: 18% (*n* = 81); Figure 1A. Overall, the prevalence of PVD in this study was 60% (*n* = 265); 40% (*n* = 175) were healthy at 21 d postpartum (Figure 1B). The prevalence of PVD ranged from 38 to 87% in the 5 farms involved in this study.

Calving–Conception Period

Survival analysis demonstrated that the rate at which healthy cows became pregnant after service was higher than that of cows with PVD. The hazard ratios for VMS 0 (*n* = 159), 1 (*n* = 96), 2 (*n* = 51), and 3 (*n* = 67) were 1, 0.7 (*P* < 0.01), 0.71 (*P* = 0.05), and 0.59 (*P* < 0.01), respectively (Figure 2A). When cows were grouped as healthy and PVD, the hazard ratios were 1 and 0.66, respectively (*P* < 0.001; Figure 2B).

Despite this difference in survival analysis, we found no significant difference in mean length of CCP between VMS categories (VMS 0: 100.2 ± 10.2; VMS 1: 108.5 ± 10.2; VMS 2: 106.9 ± 11; and VMS 3: 111.4 ± 10.7; *P* > 0.05; Table 1). Cows with PVD (389.6 ± 8.7; *n* = 214) had a significantly longer CCP (9 d longer) than healthy cows (100.2 ± 10.2; *n* = 159; *P* < 0.01, Table 1).

Services per Conception

The number of services per conception were recorded for 417 cows. By category, the number of services per conception was as follows: VMS 0: 1.54 ± 0.13 services (*n* = 170); VMS 1: 1.86 ± 0.14 services (*n* = 113); VMS

2: 1.84 ± 0.21 services (*n* = 57); and VMS 3: 1.96 ± 0.2 services (*n* = 77); *P* > 0.05. Cows with PVD required more services to conceive (1.87 ± 0.11 services, *n* = 247) than healthy cows (1.55 ± 0.13 services, *n* = 170; *P* = 0.04). In both models, the farm effect was significant (*P* = 0.04; Table 1).

In total, 31 cows did not conceive after VMS was evaluated. Of these, 25 were diagnosed with PVD (80.7%), and 6 were healthy (19.3%). Cows that did not conceive were 3 times more likely to have PVD than to be healthy (95% CI: 1.8–7.4; *P* = 0.02). The

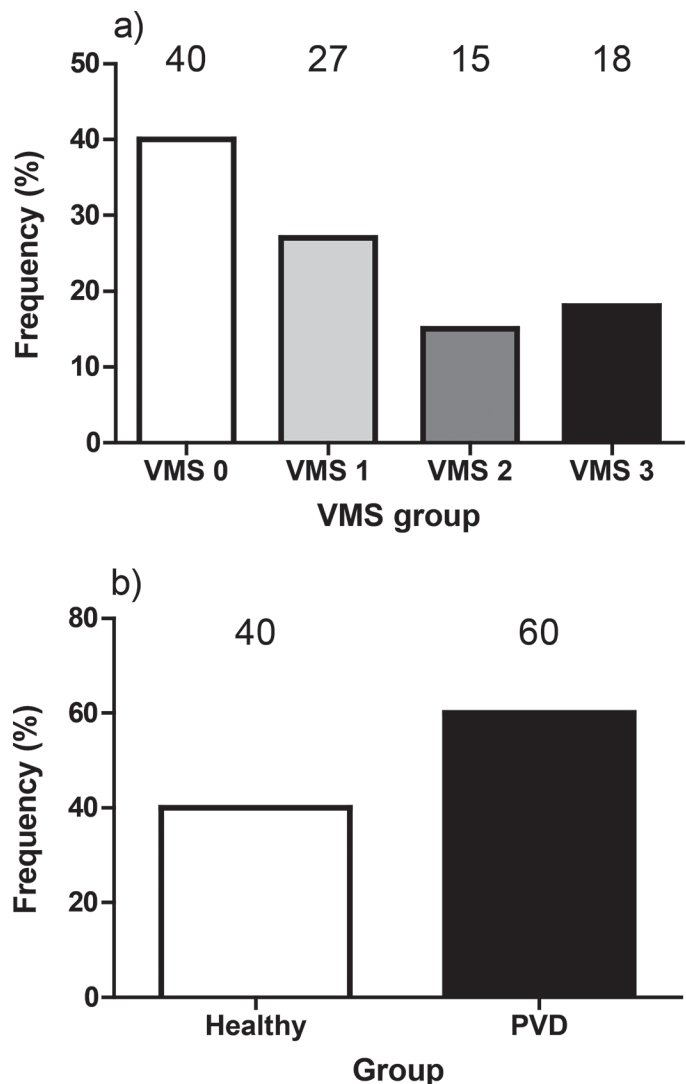


Figure 1. Vaginal mucus scores (VMS) for 440 dairy cows on day 21 postpartum: (a) number of animals with each score, and (b) prevalence rate of healthy cows (VMS 0) or those with purulent vaginal discharge (PVD; VMS 1, 2, and 3 combined). We assigned VMS following assessment of vaginal mucus based on the protocol developed by Williams et al. (2005) as 0 (clear mucus), 1 (flecks of mucopurulent material), 2 (<50% purulent material in 50 mL of exudate) or 3 (≥50% purulent material in 50 mL of exudate).

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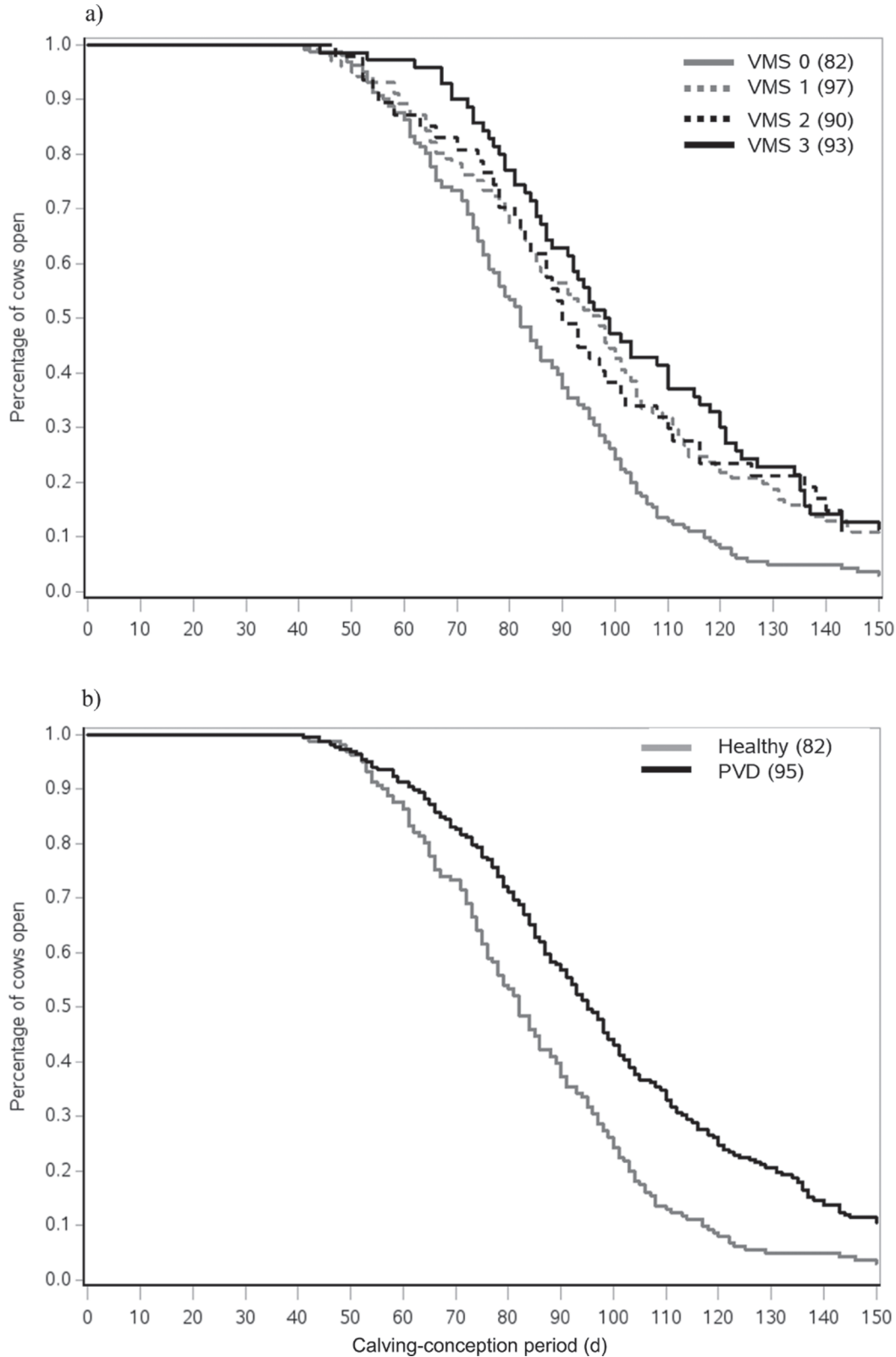


Figure 2. Survival curves for 377 cows illustrating the duration to completing the calving–conception period for (a) each vaginal mucus score (VMS) category and (b) for healthy cows and cows with purulent vaginal discharge (PVD). The hazard ratios for VMS 0, 1, 2, and 3 were 1, 0.7, 0.71, and 0.59, respectively ($P < 0.05$). The “healthy” and “PVD” groups’ hazard ratios were 1 and 0.66, respectively ($P < 0.001$). The median calving–conception period values for each group are in parentheses in the legend.

Table 1. Prevalence rate of purulent vaginal discharge (PVD), and means \pm SEM for calving-conception period, 305-d milk, milk protein, and milk fat yields for each vaginal mucus score (VMS) category, and for healthy cows and cows with PVD¹

Trait	VMS 0	VMS 1	VMS 2	VMS 3	P-value ²	Healthy	PVD	P-value ³
Prevalence (%)	40	27	15	18	—	40	60	—
Calving-conception period (d)	100.2 \pm 10.2	108.5 \pm 10.2	106.9 \pm 11	111.4 \pm 10.7	NS	100.2 \pm 10.2	109 \pm 9.8	**
Services per conception	1.54 \pm 0.2	1.79 \pm 0.2	1.65 \pm 0.2	1.89 \pm 0.2	*	1.54 \pm 0.2	1.77 \pm 0.1	*
305-d milk (kg)	7,704.8 \pm 103.7	7,761.7 \pm 485.2	7,822.3 \pm 494.1	7,794.8 \pm 493	NS	7,704.8 \pm 103.7	7,791.8 \pm 75.1	NS
305-d milk fat (kg)	321.7 \pm 4.9	319.6 \pm 20.9	328.1 \pm 21.4	324.8 \pm 21.3	NS	321.7 \pm 4.9	323 \pm 3.6	NS
305-d milk protein (kg)	266.8 \pm 3.7	270.4 \pm 14.6	271.2 \pm 14.9	269.1 \pm 14.9	NS	266.8 \pm 3.7	269.6 \pm 2.7	NS

¹VMS groups were assigned based on visual assessment of vaginal mucus, as recommended by Williams et al. (2005). Mucus was scored as 0 (clear mucus), 1 (flecks of mucopurulent material), 2 (<50% purulent material in 50 mL of exudate), or 3 (\geq 50% purulent material in 50 mL of exudate). Cows with VMS 0 were classified as "healthy," and cows with VMS 1 to 3 were classified as "PVD."²

²Comparisons between VMS groups.

³Comparisons between healthy cows and cows with PVD.

* $P \leq 0.05$; ** $P \leq 0.01$.

number of cows that did not conceive at first service was 158; 110 of these had PVD (69.6%), and 48 were healthy (30.4%). A univariate odds ratio analysis found that cows with PVD were 1.9 times more likely to fail to conceive at first service than healthy cows (95% CI: 1.3–2.9; $P < 0.01$). In total, 71 and 77% of cows that conceived after 100 d postpartum ($n = 97$) and 150 d postpartum ($n = 22$), respectively, were diagnosed with PVD. Univariate odds ratio analysis determined that cows with PVD were 2 and 3 times more likely not to conceive within 100 d postpartum (95% CI: 1.3–3.1; $P < 0.01$) or 150 d postpartum (95% CI: 1.2–7.47; $P = 0.02$), respectively. Cows that did not conceive by 150 d postpartum ($n = 15$) were 3 times more likely (95% CI: 1.21–7.47) to present with PVD ($n = 13$, 87%).

Milk Components

Cows with PVD had a numerically higher 305-d milk yield ($n = 253$; 7,704.8 \pm 103.7 kg) than healthy cows ($n = 170$; 7,791.4 \pm 75.1 kg). We found no significant differences between the 2 groups ($P > 0.05$), but we did find significant inter-farm and lactation effects ($P < 0.0001$). This finding was replicated in the VMS categories. We found no differences between VMS categories or between healthy cows and cows with PVD for 305-d milk fat or milk protein yield ($P > 0.05$, Table 1).

Persistence

Of the 19 cows that did not persist in the following year's herd, 12 presented with PVD (63.2%), and 7 were healthy (36.8%). A univariate odds ratio showed that cows with PVD were less likely to persist into the following year's herd than healthy cows, although the difference was not statistically significant (OR 1.1; 95% CI: 0.41–2.83; $P = 0.89$).

Relationship Between Previous Lactation Milk Yield and PVD Susceptibility

We calculated a univariate odds ratio to determine the likelihood of cows presenting with PVD after an above-median 305-d milk yield in the previous lactation. We found that 160 cows were below the median value (low-yield cows; range 3,568–6,567 kg) and 161 cows were equal to or above the median (high-yield cows; range 6,571–11,774 kg). The proportions of cows with PVD in the low- and high-yield groups were 46.9% ($n = 75$) and 63.4% ($n = 102$), respectively. Low-yield cows were 37% less likely to present with PVD (OR 0.63; 95% CI 0.4–0.98; $P = 0.04$). High-yield cows were 1.6 times more likely to develop PVD in the next lacta-

tion than to be diagnosed as healthy (95% CI 1.03–2.49; $P = 0.04$).

Relationship Between Previous Lactation Milk Yield and Reproductive Inefficiency

We found no differences in services per conception between healthy cows and cows with PVD in the low-yield (1.3 ± 0.13 services and 1.69 ± 0.15 services respectively; $P = 0.08$) or high-yield groups (1.4 ± 0.15 services and 1.69 ± 0.13 services, respectively; $P = 0.18$; Figure 3A). After a low milk yield, cows with PVD ($n = 81$) and healthy cows ($n = 79$) had similar CCP lengths in the following lactation (87.9 ± 7.4 d and 82.8 ± 6.9 d, respectively). Cows with PVD after a high milk yield ($n = 62$) had a longer CCP (115.9 ± 4.9 d) than healthy cows ($n = 89$; 104 ± 7.4 d; $P = 0.04$; Figure 3B). Focusing on the high-yield group, cows with PVD were 4.9 times (95% CI 1.1–22.2; $P = 0.04$) more likely not to conceive at all. In total, 16 high-yield cows did not conceive, and 88% of those had PVD. Of the 57 cows that did not conceive by 100 d postpartum, 72% presented with PVD. A univariate odds ratio determined that cows with PVD were 2.1 times (95% CI 1.1–4.3; $P = 0.04$) more likely not to conceive before 100 d postpartum than healthy cows. Cows with PVD were 4.4 times (95% CI 0.96–20.26; $P = 0.06$) more likely not to conceive before 150 d postpartum than healthy cows. Fifteen cows were in this category, and 87% of those had PVD.

DISCUSSION

In pasture-based dairy systems, aligning peak grass growth in spring with maximal animal requirements underpins economic sustainability. The short period of time (approximately 85 d) during which cows in seasonal grazing systems must conceive to maintain reproductive efficiency leaves little room for delay (Roche et al., 2017). Because this production system is predicted to underpin future expansion in milk production, studying the effect of postpartum diseases is warranted (Britt et al., 2018). Negative consequences of PVD for milk production and reproduction have been widely reported for cows in intensive and high-concentrate farming systems (LeBlanc et al., 2002; Sheldon et al., 2006), but pasture-based systems have not been studied to the same extent.

The reported prevalence of PVD is highly variable in the literature. In intensive systems, the prevalence of PVD ranges from 5 to 47.5% due to differences in time of diagnosis, disease categorization, and method used (Gautam et al., 2009; Pleticha et al., 2009; LeBlanc, 2014; Bicalho et al., 2016). In pasture-based herds, us-

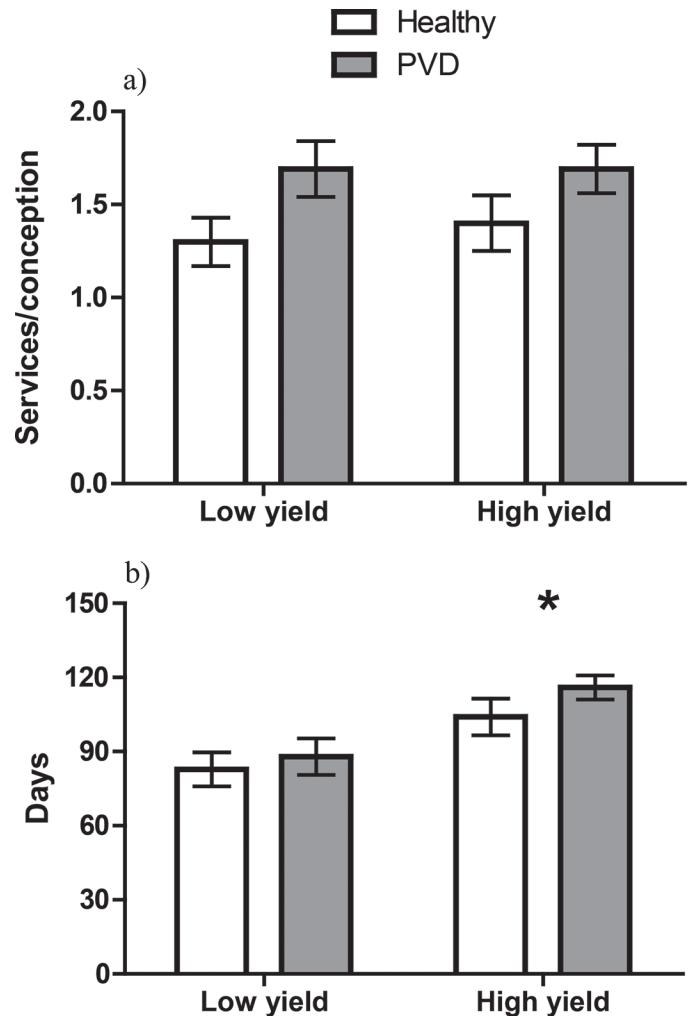


Figure 3. (a) Number of services per conception, and (b) length of the calving-conception period for low- and high-yield cows. Cows ($n = 322$) were divided into 2 groups depending on whether they were below the median 305-d milk yield (kg) of the previous lactation (low yield, $n = 161$) or equal to/above it (high yield, $n = 160$). The calving-conception period for low-yield healthy cows and cows with PVD were similar ($P > 0.05$). High-yield cows with purulent vaginal discharge (PVD) had a calving-conception period that was 12 d longer than high-yield healthy cows ($P = 0.04$). Results are presented as LSM with SEM. * $P < 0.05$.

ing a VMS scoring system as described here, PVD prevalence has been recorded at 27% (Potter et al., 2010), 35% (Plontzke et al., 2011), and 54% (Giuliodori et al., 2013a). The higher prevalence of 60% in our study may be attributable to differences in risk factors such as cow parity, breed, calving difficulties, and possibly the presence of metabolic disorders (Potter et al., 2010; Aungier et al., 2014). As expected with any disease diagnosis, a proportion of cows can clear infection without intervention or reproductive impairment owing to natural cleansing mechanisms in the uterus. Many studies have shown how time of diagnosis affects disease prevalence.

The prevalence of PVD decreased from 49% at 20 to 26 d postpartum to 22% at 27 to 33 d postpartum in dairy cows in intensive systems in North America (LeBlanc et al., 2002). In the previously mentioned studies with pasture-based herds, PVD prevalence decreased to 18% at 35 d postpartum (Plontzke et al., 2011) and 33% at 31 d postpartum (Giuliodori et al., 2013a). Complete involution of the bovine cervix and uterus occurs between 25 and 47 d postpartum (Gier and Marion, 1968) and as such the prevalence of PVD in our study may have been lower if we had sampled at a later time point. However, the accepted standard date for PVD diagnosis is 21 d postpartum (Sheldon and Dobson, 2004), and this date concurs with previous work performed by our group. However, we recognize that reassessment of the same herd at a later time point may improve the accuracy of disease diagnosis. Although it is not possible to compare all risk factors between studies, the weather conditions that the cows in our study withstood may have been a contributing factor in developing PVD. During the sampling period of our study, temperatures averaged 5.4°C and rainfall averaged 3.1 cm. This was colder and wetter than the temperatures during sampling in studies by Giuliodori et al. (2013a; 16°C, <0.5 cm rain) and Plontzke et al. (2011; 18°C, <0.2 cm rain; www.accuweather.com). Cows that endure cold and wet conditions have been shown to have increased circulating cortisol and fatty acids due to fat reserve mobilization in an effort to maintain homeostasis (Tucker et al., 2007). Interestingly, cows that experienced these conditions for a week had reduced circulating numbers of lymphocytes and basophils (Webster et al., 2008). These immune-cell perturbations could also contribute to the higher prevalence of PVD detected in this study.

The effect of PVD on milk yield in dairy cows has been reported across various studies with contrasting results. Giuliodori et al. (2013b) reported a higher milk yield of 2 kg/d in cows with PVD compared with healthy cows, but the majority of related studies did not find a significant effect (Fourichon et al., 1999; Dubuc et al., 2011a). Similarly, we detected no significant difference in milk production between VMS categories or between healthy cows and cows with PVD ($P > 0.05$).

Multiple previous studies have identified consistent negative effects of PVD on fertility, including a 205 d prolonged CCP in pregnancy rate (Gautam et al., 2009) and an average increased CCP of approximately 30 d (LeBlanc et al., 2002). Our finding that cows with PVD had lower conception rates at first service agreed with previous studies (LeBlanc et al., 2002; McDougall et al., 2007). Cows that presented with VMS 0 at first service had an OR of 2.99 to conceive (Aungier et al., 2014). Cows with PVD at 35 d postpartum were 40% less likely to conceive at first service (Dubuc et

al., 2011b). In our study, a diagnosis of PVD led to a 35% higher risk of a longer CCP (9 d) and more services per conception. Such inferior fertility may be due to delayed estrous, possibly as a result of uterine bacterial contamination. A potential mechanism has been recently identified, via the effects of the bacterial endotoxin lipopolysaccharide on endocrine and ovarian function (Fourichon et al., 2000; LeBlanc et al., 2002; Lopez et al., 2004). Related work found that the median CCP was extended by 57 and 70 d in cows with PVD (Giuliodori et al., 2013a; Bicalho et al., 2016). The concurrent presence of cytological endometritis has been shown to have an additive negative effect on fertility (Galvão et al., 2009; Dubuc et al., 2011a). The presence of this disease in healthy cows could have led to an underestimate of the true reproductive effect in the current study.

Our study has identified that the risk of presenting with PVD is heightened in cows with a higher milk yield in the lactation before PVD diagnosis, and that cows in this high milk yield subset who develop PVD incur more severe reproductive impediments than their healthy cow counterparts. Gröhn et al. (1995) assessed the effect of a high milk yield in the previous lactation has on susceptibility to retained placenta, metritis, ovarian cyst, milk fever, ketosis, abomasal displacement, and mastitis. They concluded that high milk yield was only a risk factor for mastitis. Purulent vaginal discharge is a multifactorial disease that can be brought on by the interactions of advanced age, inadequate peripartum nutrition, or excessively high or low BCS. Milk production is prioritized over other processes such as metabolic and reproductive pathways in the dairy cow as a result of homeorhetic processes in the postpartum period (Bauman and Currie, 1980), and the results of the present study suggest that a cumulative effect of high milk yield extends beyond a single lactation to have detrimental effects on reproduction in the subsequent production cycle.

CONCLUSIONS

This study investigated the effects of PVD on the milk production and fertility of dairy cows in a pasture-based grazing system. The occurrence of PVD impedes fertility; cows with PVD were 3 times more likely not to conceive, twice as likely to require multiple services to conceive, and twice as likely to fail to conceive before 100 d postpartum. They also had a CCP 9 d longer than that of healthy cows. The effect was exacerbated in cows with PVD that had a high milk yield in the previous lactation. Cows with these traits were 5 times more likely to fail to conceive and more than twice as likely to require more than 1 service to conceive and to

fail to conceive before 100 d postpartum. These cows also incurred a CCP 12 d longer than healthy cows. The significant effect of compromised uterine health on subsequent reproductive metrics and the importance of the accurate diagnosis of clinical uterine disease in pasture-based dairy systems have been clarified in this study. These findings are important for animal welfare and farm economic sustainability, particularly in the context of dairy sector expansion.

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