The effect of pegbovigrastim on early-lactation disease, production, and reproduction in dairy cows

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ABSTRACT

The objective of this randomized, double-blind, controlled trial was to evaluate the effect of pegbovigrastim (pegylated recombinant bovine granulocyte colony-stimulating factor) on early-lactation disease, milk yield, and reproduction on commercial dairy farms. A total of 1,607 Holstein cows from 6 farms in Ontario and Québec, Canada, were randomly assigned to receive two 2.7-mL subcutaneous injections of either 15 mg of pegbovigrastim (n = 798; Imrestor, Elanco) or sterile physiological saline (placebo; n = 809). The first injection was administered by investigators 1 wk before expected calving, and the second by farm personnel within 24 h after calving, according to the product label. Producers inspected cows daily and using standardized disease definitions, recorded cases of retained placenta, metritis, displaced abomasum, and clinical mastitis until 63 d in milk. Progesterone concentration was measured in serum at wk 3, 5, 7, and 9 postpartum. Cows were examined for purulent vaginal discharge using the Metricheck (Simcro) device and endometritis using the cytobrush method at wk 5 postpartum. Milk production and reproduction data were obtained from farm management software and the national milk recording database. Disease and culling outcomes were assessed with logistic regression, milk production with linear regression, and time-to-event outcomes with proportional hazards regression. All analyses considered parity and pre-treatment body condition score and their interaction with treatment, and accounted for clustering of cows within farm. In a subset of 246 cows, the effect of treatment on metabolic markers (serum concentrations of glucose, β-hydroxybutyrate, nonesterified fatty acids, cholesterol, haptoglobin, albumin, and calcium) was assessed in wk 1 and 2 postpartum. Pegbovigrastim had no significant effects on the incidence of retained placenta, metritis, displaced abomasum, clinical or subclinical mastitis, purulent vaginal discharge, or endometritis. Treatment reduced the serum concentration of glucose, slightly reduced the concentration of albumin, and slightly increased concentrations of β-hydroxybutyrate and nonesterified fatty acids, with no effect on the other markers. There were no differences between treatments in culling risk, time to first insemination, pregnancy at first insemination, or time to pregnancy. Milk yield over the first 3 test days of lactation was 1.0 kg per day lower in the pegbovigrastim group, although a mechanism for that effect could not be explained through analysis of our data. Key words: transition, health, immunity, granulocyte colony-stimulating factor, randomized controlled trial

INTRODUCTION

During the periparturient period, it is well documented that dairy cattle experience alterations in immune function that can negatively affect health in early lactation. Specifically, around parturition, there is a decrease in the number and function of circulating neutrophils, which are considered the primary immune cells involved in inflammation and the elimination of infection in the uterus and the mammary gland (Kehrli et al., 1989; Moyes et al., 2014). Changes in the metabolic status of the cow contribute to impairments of gene expression (Moyes et al., 2014; Crookenden et al., 2016), superoxide anion production, random migration, phagocytosis, oxidative burst, and myeloperoxidase activity by neutrophils (Dosogne et al., 1999; Rinaldi et al., 2008).

Altered innate immune function, primarily characterized by reduced functional capacity of neutrophils, con-
tributes to increased risk of disease including retained placenta (RP), metritis, endometritis, and mastitis (Cai et al., 1994; Hammon et al., 2006). Gunnink (1984) determined that the occurrence of RP was correlated with decreased leukocyte activity in the days before parturition. Similarly, Kimura et al. (2002) showed that cows that developed RP had lesser neutrophil function from 2 wk before calving. Moretti et al. (2016) found lower circulating neutrophil counts at the time of parturition in cows that developed RP. The killing ability of neutrophils in cows with metritis or mastitis was significantly impaired before parturition (Cai et al., 1994). Blood neutrophil function, as measured by myeloperoxidase activity and cytochrome c reduction activity, was lesser in cows that were subsequently diagnosed with metritis or endometritis, compared with healthy cows (Hammon et al., 2006). Taken together, these studies suggest that enhancement of neutrophil capacity (the product of circulating neutrophil count and function per cell) should reduce the incidence or severity of uterine disease and mastitis.

Administration of pegylated recombinant bovine granulocyte colony-stimulating factor (pegbovigrastim; PBG) in periparturient dairy cattle elicits a 6- to 10-fold increase in the circulating count of neutrophils (Hassfurther et al., 2015; Canning et al., 2017; Van Schyndel et al., 2018; Zinicola et al., 2018). It has also been shown to increase the exocytosis of myeloperoxidase by approximately 20% (McDouggall et al., 2017), but no effects of PBG have been reported on neutrophil superoxide release, oxidative burst, or phagocytic activity (Kimura et al., 2014; McDougall et al., 2017). Pegbovigrastim decreased the incidence of clinical mastitis in early lactation by approximately 25% in several studies (Hassfurther et al., 2015; Canning et al., 2017; Ruiz et al., 2017). The hypothesis is that the elevated number of mature neutrophils in peripheral circulation leads to an increased capacity to respond effectively to bacterial pathogens in the udder. The effectiveness and regulation of neutrophil response are understood to be important in the development of each of RP, metritis, purulent vaginal discharge (PVD), and endometritis (Sheldon et al., 2019; Pascottini and LeBlanc, 2020). Therefore, we hypothesized that administration of PBG may also reduce the incidence of each of these reproductive tract diseases, and so potentially improve reproductive performance.

The objective of this randomized, double-blind controlled trial was to evaluate the effect of PBG given 1 wk before and within 1 d after calving on early-lactation disease, milk yield, and reproduction on commercial dairy farms.

MATERIALS AND METHODS

Study Herds

This study was a randomized, double-blind controlled trial conducted in 2 commercial herds in Southwestern Ontario and 4 commercial herds in Québec from January 2017 to March 2018. Animal use was approved by the Animal Care Committee of the University of Guelph, Animal Utilization Protocol #3642. All animals were bred exclusively by AI and fed TMR according to the owners’ standard practices, which did not change during the study. Herds 1 and 2 were in Ontario and had 425 and 400 milking cows, respectively, milked thrice daily and housed in freestall barns. The owners or managers did inseminations. Both herds used automated activity monitors worn by cows, with data captured in the milking parlor to detect estrus. This was the primary means of first insemination, but if cows were not detected in estrus by ~70 DIM, they were enrolled in the Ovsynch program for timed AI. Cows detected in estrus after AI were re-inseminated. Pregnancy was diagnosed with ultrasound at the first weekly veterinary visit >28 d after insemination. If diagnosed not pregnant, cows were enrolled in the Ovsynch program for timed AI. Herds 3 to 6 were in Québec. Herds 3 and 6 milked 120 and 100 cows, respectively, twice daily in tiestall housing, whereas herds 4 and 5 milked 225 and 200 cows, respectively, with freestall housing. Herds 3 and 6 used professional insemination services, whereas the owners or managers did inseminations in herds 4 and 5. Herds 3 to 6 primarily used synchronization for timed AI for first insemination, with detection of estrus for repeat services. Pregnancy was diagnosed with ultrasound at the first biweekly veterinary visit >28 d after insemination. If diagnosed not pregnant, cows were enrolled in the Ovsynch program for timed AI. The sample size was determined based on the main outcomes of RP, metritis, and reproductive performance, using a significance level of α = 0.05 and 80% power. To detect a treatment effect to change the incidence risk of RP or metritis from 10% to 6% required a total of 1,442 cows, and to detect a change in the median time to pregnancy from 130 to 110 d with a 250-d follow-up period required 1,472 cows (Abramson, 2011). Therefore, the target sample size was 1,472 cows, and to account for an expected 8% loss to follow-up due to culling we aimed to enroll 1,600 cows. Herds were selected based on previous research cooperation with the Ontario Veterinary College of the University of Guelph, or the Faculté de médecine vétérinaire de l’Université de Montréal. Each farm was also required.
to participate in DHI milk recording and to maintain consistent disease recording, using standardized definitions provided by the investigators.

**Study Design**

All cows and heifers expected to calve within 4 to 10 d of each weekly enrollment farm visit were included, provided that they were apparently healthy and expected to remain in the herd until at least 63 DIM. All cows were randomly assigned to receive two 2.7-mL subcutaneous injections of either 15 mg of pegbovigrastim (Inrestor; Elianco) or sterile physiological saline (0.9% sodium chloride). Randomization was done formally with lists for each farm, blocked into muted blocks of 4 animals. Prelabeled, prefilled study syringes were refrigerated (2 to 8°C) until use. Empty syringes identical to those containing the treatment were provided by the manufacturer of the PBG for the placebo, and the barrels of all syringes were covered with an opaque label identified only with a syringe number. Both the investigators and the producers were blinded to treatment assignment. All cows received 2 injections of their assigned treatment, the first administered by the investigators 1 wk before expected calving (272 to 279 d of gestation), and the second by farm personnel within 24 h after calving, according to the product label. Both the investigators and the producers were blinded to treatment assignment. Cows were classified as having PVD if the score was ≥2 (% PMN >5%) or without PVD were classified as having subclinical endometritis, and cows with both endometritis and PVD were referred to as **PVD+ENDO**.

Blood was collected for measurement of progesterone concentration at wk 3 (15 to 21 DIM), wk 5 (29 to 35 DIM), wk 7 (43 to 49 DIM), and wk 9 (57 to 63 DIM) postpartum. Approximately 10 mL of blood was collected from the coccygeal vessels into evacuated tubes without anticoagulant (Becton Dickinson), allowed to clot, and then centrifuged at 3,000 × g for 15 min at 20°C, within 2 h of collection. Serum was separated into clean tubes (Sarstedt Inc.) and frozen at −20°C until analysis. Progesterone was measured using a validated (Broes and LeBlanc, 2014) ELISA kit (Ovucheck Plasma, Biovet), with a range of quantification from 0.891 to 11.22 ng/mL. The mean intraassay coefficient of variation (CV) was 7.9% (n = 38 plates) and the mean interassay CV was 11.8% (n = 21 plates). Cows were considered to have ovulated if the progesterone concentration was ≥1.0 ng/mL at a given time point (Stevenson et al., 2006).

**Disease Definitions**

The clinical health outcomes recorded by farm personnel were the occurrence of RP, metritis, clinical mastitis, or displaced abomasum (DA) in the first 63 DIM. All producers were given explicit instructions with respect to disease definitions for RP, metritis, and clinical mastitis at the start of the study and were directed to record cases of disease throughout the study period. Disease events were recorded either in DairyComp 305 (CanWest DHI) or on paper forms supplied by the investigators. Dystocia was defined as difficult parturition requiring >15 min of human assistance, mechanical assistance, or surgery. Retained placenta was defined as fetal membranes still visible 24 h after the first observation of calving (Sheldon et al., 2006). Cows were inspected daily by producers for metritis and those with foul-smelling vaginal discharge had their temperature taken and recorded. Metritis...
was defined as the presence of fetid or foul-smelling vulvar discharge, with or without fever (temperature >39.5°C). Displaced abomasum was confirmed by a veterinarian, with dislocation and gas distention of the abomasum and auscultation of a characteristic tympanic resonance (“ping”). Clinical mastitis was scored according to severity: 1 = visibly abnormal milk only; 2 = visibly abnormal milk with swelling or redness of the udder; 3 = visibly abnormal milk with systemic signs of illness. Somatic cell counts were done at routine milk recording in certified laboratories using Fossomatic automated machines (Foss A/S). Cows were considered to have subclinical mastitis at milk recording test d 1, 2, or 3 if SCC >200,000 cells/mL.

**Metabolic Markers**

Blood samples collected from a subset of cows in herds 1 and 2 between August and October 2017 were used to assess the effect of PBG on metabolic markers of health. All cows enrolled from the 2 herds were included until the target sample size was reached, so no selection of cows occurred and study personnel remained blinded. Ten milliliters of blood was collected from the coccygeal vessels into evacuated tubes without anticoagulant at wk −1 (immediately before the first treatment), 1, and 2 relative to calving. Samples were allowed to clot for 30 to 60 min, then stored at 4°C for up to 4 h before centrifugation to separate the serum, which was stored at −20°C until analysis. Samples were analyzed at the Animal Health Laboratory of the University of Guelph with a Roche autoanalyzer to measure the concentrations of albumin, BHB, calcium, cholesterol, glucose, haptoglobin, and nonesterified fatty acids (NEFA). Details of the test kits and their intraassay coefficients of variation are in Supplemental Table S1 (http://dx.doi.org/10.17632/v4cvdlbnk5.1, LeBlanc, 2021). The planned sample size was 284 cows, based on detection of a 0.2 mmol/L difference in BHB or NEFA with an expected standard deviation of 0.6. The effect size was based on the smallest mean difference that we considered to be of interest.

**Statistical Analyses**

Data were compiled in Excel (Microsoft Corp.). Cow demographic variables, recorded cases of disease, and reproductive variables were exported from DairyComp 305 or transcribed from farm records. Test-day milk, fat, and protein yield, and SCC from the first 3 test days were exported from the national milk recording database. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc.). Parity was dichotomized as primiparous (calving to start the first lactation) and multiparous (lactation ≥2). Baseline variables are described in Table 1 and were comparable between treatment groups. Body condition score at enrollment was categorized using the median value as ≥3.75 or ≤3.5. The interval between the first and second treatment was categorized as short (0 to 3 d), on target (4 to 10 d), or long (≥11 d), according to the target period of 4 to 10 d, as per the product label.

**Disease and Ovulation Outcomes.** The effect of treatment on the following categorical disease outcomes was evaluated in separate multivariable logistic regression models: RP, metritis, clinical mastitis, DA, PVD, endometritis, subclinical endometritis, PVD+ENDO, and ovulation at wk 3, 5, 7, and cumulatively to wk 9 postpartum. Cows were excluded from analysis if they did not have a recorded case of the disease of interest for the model and were culled before the end of the risk period for RP, metritis, DA, or mastitis (2, 14, 30, and 63 DIM, respectively). For all these outcomes, mixed logistic regression models were used (GLIMMIX procedure in SAS), including farm as a random effect with a compound symmetry covariance structure. For subclinical mastitis (SCC >200,000 cells/mL), repeated measures of the outcome at test d 1, 2, and 3 were accounted for in a mixed logistic regression model, using the GLIMMIX procedure in SAS with farm as a random effect with compound symmetry covariance, and an autoregressive type 1 covariance structure to account for repeated measures on cow nested in herd. The baseline covariates parity group, BCS at enrollment, and the interval between treatments, as well as their first-order interactions with treatment were offered to each model and retained if P < 0.05. Odds ratios (OR) and 95% CI were used to compare the effects of PBG to control, and absolute values are described as least squares means of incidence risks with their 95% CI.

**Time-to-Event Outcomes.** The unadjusted effect of treatment on time to culling, to first service, and to pregnancy was described using the product-limit method of survival analysis (the LIFETEST procedure in SAS). Periods of risk were 63, 150, and 250 DIM for culling, first insemination, and pregnancy, respectively. The time-at-risk variable was calculated as the interval from calving to the outcome of interest or to culling for cows culled before the end of the risk period, or censored at the end of the period. Cox proportional hazards models (the PHREG procedure in SAS) were used for final assessment of treatment effects, accounting for clustering of cows within farm [using the ‘coves(aggregate)’ option, analogous to including farm as a random effect], and considering the baseline covariates parity group and BCS at enrollment.
Test-Day Milk Yield and SCC. The effects of treatment on test-day milk yield, fat and protein percentages and yields, and SCC linear score \(\log_2(\text{SCC}/100) + 3\) were analyzed with separate mixed multivariable linear regression models (the MIXED procedure of SAS), with farm as a random effect and accounting for repeated measures with an autoregressive type 1 covariance structure for cow nested within farm. Covariates were parity group, BCS at enrollment, test day, and their first-order interactions with treatment.

Metabolic Markers. The effect of treatment on metabolic markers was analyzed using multilevel mixed linear regression models (the MIXED procedure in SAS). The sample taken at wk −1 was included as a pre-treatment covariate, with the samples at wk 1 and 2 as the outcome. Farm was included as a random effect with a compound symmetry covariance structure and time was included as a repeated effect, with cow as the subject and an ante-dependence covariance structure. If the distribution of model residuals was not normal, log10, ln (natural logarithm), square root, square, or cubic transformations of the outcome were performed until visual inspection of the histogram and quantile plot of the residuals indicated the most nearly normal distribution, and a scatter plot of model-predicted values versus residuals indicated homoscedasticity. Concentrations of BHB, NEFA, cholesterol, and haptoglobin were log10 transformed, albumin and calcium were cubic transformed, and glucose was not transformed for statistical analysis. Concentrations of BHB and NEFA were also categorized based on cut-points associated with increased disease risk: BHB ≥1.2 mmol/L, and NEFA ≥0.7 or ≥1.0 mmol/L postpartum (McArt et al., 2013). When BHB and NEFA were analyzed as binary outcome variables it was done with logistic regression models (GLIMMIX procedure in SAS), with farm as a random effect. For all models, the following variables were tested: treatment, time, parity (primiparous or multiparous), BCS at enrollment, test day, and their first-order interactions with treatment.

RESULTS

A total of 1,664 cows were enrolled in the study, with 1,607 cows receiving treatment according to the protocol and included in the analyses, which were on a per protocol basis. Figure 1 details the exclusions of animals from the analysis. A total of 798 cows (49.7%) were treated with PBG and 809 cows (50.3%) with placebo. The median interval between the first and second
injection was 6 d (interquartile range 3 to 9 d) for PBG, with a range of 0 to 30 d, and 6 d (interquartile range 4 to 9 d) for control cows, with a range of 0 to 23 d. There were no adverse events in either group.

### Disease and Culling

Treatment did not affect health or culling outcomes. The main effects for each disease outcome model are summarized in Table 2. There were no differences between PBG and control in the incidence of RP, metritis, DA, clinical or subclinical mastitis, PVD, endometritis, or culling. For the first case of clinical mastitis within 30 DIM, 20% (n = 11) cases were scored with a severity of 1, 52% (n = 28) with a severity of 2, 28% (n = 15) with a severity of 3, and 7 cases were not scored for severity. We did not analyze the effect of treatment on the severity of mastitis because there were not enough cases to assess it properly. Accounting for parity and clustering within farm in the proportional hazards analysis, treatment did not affect time to culling to 63 DIM (hazard ratio 1.2, 95% CI = 0.85 to 1.7, \( P = 0.30 \)).

### Reproduction

Treatment did not affect reproductive performance outcomes. Accounting for parity, the proportion of cows with serum progesterone >1 ng/mL at wk 3 postpartum was lower in PBG (28%) than control (34%); OR = 0.75, 95% CI = 0.60 to 0.93, \( P = 0.01 \). Subsequently, the cumulative proportion of cows with at least 1 sample with serum progesterone >1 ng/mL was not different between PBG and control cows up to wk 5 (38 and 43%, respectively, OR = 0.80, 95% CI = 0.64 to 1.0, \( P = 0.054 \)), wk 7 (66 and 68%, OR = 0.91, 95% CI = 0.72 to 1.2, \( P = 0.47 \)), and wk 9 (88 and 89%, OR = 0.9, 95% CI = 0.63 to 1.3, \( P = 0.58 \)). The median time to first insemination was not different (\( P = 0.68 \)) between PBG (65 d, 95% CI = 63 to 66 d) and the control group (65 d, 95% CI = 63 to 67 d). Accounting for parity, the odds of pregnancy at first insemination were

<table>
<thead>
<tr>
<th>Outcome</th>
<th>n</th>
<th>Pegbovigrastim</th>
<th>Control</th>
<th>Odds ratio (95% CI)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retained placenta(^1)</td>
<td>1,601</td>
<td>6.3 (4.1 to 9.7)</td>
<td>5.2 (3.3 to 8.2)</td>
<td>1.2 (0.82 to 1.8)</td>
<td>0.33</td>
</tr>
<tr>
<td>Metritis by 14 DIM(^2)</td>
<td>1,578</td>
<td>5.3 (2.7 to 10.2)</td>
<td>5.5 (2.8 to 10.5)</td>
<td>0.96 (0.65 to 1.4)</td>
<td>0.84</td>
</tr>
<tr>
<td>Displaced abomasum by 30 DIM(^3)</td>
<td>1,537</td>
<td>2.2 (1.0 to 4.8)</td>
<td>1.8 (1.0 to 4.1)</td>
<td>1.2 (0.64 to 2.3)</td>
<td>0.56</td>
</tr>
<tr>
<td>Clinical mastitis by 30 DIM(^4)</td>
<td>1,539</td>
<td>3.4 (1.9 to 6.2)</td>
<td>3.2 (1.8 to 5.9)</td>
<td>1.0 (0.64 to 1.8)</td>
<td>0.92</td>
</tr>
<tr>
<td>Subclinical mastitis at milk test d 1, 2, and 3(^5)</td>
<td>1,522</td>
<td>13.1 (11.1 to 15.3)</td>
<td>13.0 (11.0 to 15.1)</td>
<td>1.0 (0.82 to 1.2)</td>
<td>0.20</td>
</tr>
<tr>
<td>Purulent vaginal discharge (PVD) at wk 5(^6)</td>
<td>1,475</td>
<td>11.7 (7.0 to 19.0)</td>
<td>9.8 (5.8 to 16.1)</td>
<td>1.2 (0.9 to 1.7)</td>
<td>0.69</td>
</tr>
<tr>
<td>Endometritis at wk 5(^7)</td>
<td>1,375</td>
<td>33.5 (26.9 to 40.8)</td>
<td>34.5 (27.9 to 41.9)</td>
<td>0.96 (0.76 to 1.3)</td>
<td>0.22</td>
</tr>
<tr>
<td>Subclinical endometritis at wk 5(^8)</td>
<td>1,368</td>
<td>23.8 (18.7 to 29.7)</td>
<td>26.8 (21.3 to 33.0)</td>
<td>0.85 (0.66 to 1.1)</td>
<td>0.22</td>
</tr>
<tr>
<td>PVD and endometritis at wk 5(^9)</td>
<td>1,368</td>
<td>8.9 (5.7 to 13.6)</td>
<td>7.0 (4.4 to 11.0)</td>
<td>1.3 (0.87 to 1.9)</td>
<td>0.20</td>
</tr>
<tr>
<td>Culling by 63 DIM(^10)</td>
<td>1,607</td>
<td>6.2 (3.3 to 11.4)</td>
<td>5.2 (2.7 to 9.7)</td>
<td>1.2 (0.85 to 1.7)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

\(^1\) Model covariate is parity (\( P < 0.001 \)).
\(^2\) Model covariate is parity (\( P = 0.002 \)). The median diagnosis of metritis was at 5 DIM.
\(^3\) Model covariate is parity (\( P = 0.005 \)). The median diagnosis of displaced abomasum was at 10 DIM.
\(^4\) Model covariate is parity (\( P < 0.001 \)). The median diagnosis of clinical mastitis by 30 DIM was at 8 DIM.
\(^5\) Model covariates are parity (\( P < 0.001 \)) and test day DIM (\( P = 0.002 \)). The median test day DIM were 23, 60, and 97.
\(^6\) Model covariate is parity (\( P = 0.08 \)). Purulent vaginal discharge was muco-purulent or purulent discharge based on one examination with a Metricheck device (Simcro).
\(^7\) Model includes treatment only. Endometritis was >5% polymorphonuclear cells in uterine cytology.
\(^8\) Model includes treatment only. Subclinical endometritis was >5% polymorphonuclear cells in uterine cytology without PVD.
\(^9\) Model covariate is parity (\( P = 0.06 \)).
\(^10\) Model covariate is parity (\( P = 0.001 \)). Median time of culling was 29 DIM.
pregnancy did not differ between treatment groups in the product-limit survival estimate (110 d, 95% CI = 101 to 115 d for PBG versus 101 d, 95% CI = 96 to 109 d in control, \( P = 0.34 \)). Similarly, in the proportional hazards model controlling for parity and pre-treatment BCS, there was no effect of treatment on time to pregnancy (hazard ratio 0.96, 95% CI = 0.85 to 1.07, \( P = 0.53 \)).

### Milk Yield

Treatment had an effect on milk yield in early lactation. Table 3 summarizes results from covariate-adjusted models for test-day milk production and milk components for the first 3 DHIA test days of lactation. There were no treatment by test-day interactions. In summary, cows treated with PBG produced approximately 1.0 kg/d less milk in early lactation than controls, with similar fat and protein percentages and SCC linear scores between groups. Energy-corrected milk yield was 1.3 kg/d lower in the PBG group. A model (n = 2,598 observations) of test-day milk yield accounting for previous lactation 305-d milk yield (\( P < 0.001 \)) and test day (\( P < 0.001 \)) similarly showed a difference between treatments (47.0 kg, 95% CI = 43.4 to 50.6 for PBG, and 47.9 kg, 95% CI = 44.2 to 51.5 for control, \( P = 0.04 \)); treatment difference −0.9 kg, 95% CI −1.7 to −0.1.

### Table 3. Least squares means estimates of test-day milk production, for the first 3 DHIA test days of lactation from multivariable mixed linear regression models in a randomized controlled trial involving a total of 1,607 cows from 6 farms treated with either pegbovigrastim or saline (control)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Observations in the model (n)</th>
<th>Pegbovigrastim, mean ± SE (95% CI)</th>
<th>Control, mean ± SE (95% CI)</th>
<th>Treatment difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test-day milk yield ( ^1 ) (kg)</td>
<td>4,360</td>
<td>42.2 ± 1.9 (37.3 to 47.0)</td>
<td>43.2 ± 1.9 (38.3 to 48.1)</td>
<td>−1.0 (−1.7 to −0.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>Fat ( ^2 ) (%)</td>
<td>4,328</td>
<td>4.07 ± 0.03 (3.98 to 4.15)</td>
<td>4.06 ± 0.03 (3.98 to 4.14)</td>
<td>0.0 (−0.05 to 0.05)</td>
<td>0.91</td>
</tr>
<tr>
<td>Fat yield ( ^3 ) (kg)</td>
<td>4,325</td>
<td>1.70 ± 0.08 (1.50 to 1.89)</td>
<td>1.74 ± 0.08 (1.54 to 1.94)</td>
<td>−0.05 (−0.08 to −0.02)</td>
<td>0.001</td>
</tr>
<tr>
<td>Protein ( ^4 ) (%)</td>
<td>4,328</td>
<td>3.11 ± 0.02 (3.07 to 3.16)</td>
<td>3.13 ± 0.02 (3.09 to 3.17)</td>
<td>−0.02 (−0.04 to −0.01)</td>
<td>0.16</td>
</tr>
<tr>
<td>Protein yield ( ^5 ) (kg)</td>
<td>4,325</td>
<td>1.30 ± 0.05 (1.16 to 1.44)</td>
<td>1.34 ± 0.05 (1.20 to 1.48)</td>
<td>−0.04 (−0.06 to −0.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ECM ( ^6 ) (kg)</td>
<td>4,325</td>
<td>45.7 ± 2.0 (40.5 to 51.0)</td>
<td>47.0 ± 2.0 (41.8 to 52.2)</td>
<td>−1.3 (−1.9 to −0.60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SCC linear score ( ^7 )</td>
<td>4,354</td>
<td>1.97 ± 0.16 (1.58 to 2.36)</td>
<td>1.96 ± 0.16 (1.57 to 2.35)</td>
<td>0.01 (−0.15 to 0.17)</td>
<td>0.88</td>
</tr>
</tbody>
</table>

\(^1\) Model covariates are parity (\( P < 0.001 \)) and test day (\( P < 0.001 \)). No interaction of treatment with parity (\( P = 0.94 \)) or test day (\( P = 0.75 \)).

\(^2\) Model covariates are parity (\( P = 0.50 \)) and test day (\( P < 0.001 \)). No interaction of treatment with parity (\( P = 0.43 \)) or test day (\( P = 0.72 \)).

\(^3\) Model covariates are parity (\( P < 0.001 \)) and test day (\( P < 0.001 \)). No interaction of treatment with parity (\( P = 0.77 \)) or test day (\( P = 0.60 \)).

\(^4\) Model covariates are parity (\( P < 0.001 \)) and test day (\( P < 0.001 \)). No interaction of treatment with parity (\( P = 0.52 \)) or test day (\( P = 0.25 \)).

\(^5\) Model covariates are parity (\( P < 0.001 \)) and test day (\( P < 0.001 \)). No interaction of treatment with parity (\( P = 0.60 \)) or test day (\( P = 0.35 \)).

\(^6\) Energy-corrected milk (to 3.5% fat and 3.2% protein) = (0.327 × milk yield) + (12.95 × fat yield) + (7.65 × protein yield) (Tyrrell and Reid, 1965, adjusted for true protein). Model covariates are parity (\( P < 0.001 \)) and test day (\( P < 0.001 \)). No interaction of treatment with parity (\( P = 0.99 \)) or test day (\( P = 0.55 \)).

\(^7\) Model covariates are parity (\( P < 0.001 \)) and test day (\( P < 0.001 \)). No interaction of treatment with parity (\( P = 0.78 \)) or test day (\( P = 0.65 \)).
In the subset of 246 animals (128 from farm 1 and 118 from farm 2) in which metabolic markers were measured, there were differences between treatments in the serum concentrations of BHB, NEFA, glucose, and albumin, shown in Figure 2. The concentrations of BHB and NEFA were slightly greater following treatment with PBG. However, the proportion of cows with hyperketonemia (BHB ≥1.2 mmol/L) at wk 1 (12% vs. 5%, P = 0.05) or wk 2 (11% vs. 6%, P = 0.14) was not different between PBG and control, respectively. Similarly, the proportions of cows with elevated serum NEFA at wk 1 (≥0.7 mmol/L: 50% vs. 44%, P = 0.32; ≥1.0 mmol/L: 28% vs. 26%, P = 0.71) or wk 2 (≥0.7 mmol/L: 27% vs. 30%, P = 0.69; ≥1.0 mmol/L: 8% vs. 15%, P = 0.09) were not different between PBG and control, respectively. Serum concentrations of glucose and albumin were lesser in the PBG group (Figure 2). There were no treatment effects on serum concentrations of calcium, cholesterol, or haptoglobin (Supplemental Figure S1, http://dx.doi.org/10.17632/v4cdfbmk5.1, LeBlanc, 2021).

**DISCUSSION**

Contrary to our hypothesis, administration of PBG had no effects on the incidence of reproductive or other disease. There was no effect of treatment on reproductive performance. Unexpectedly, cows treated with PBG produced approximately 1 kg/d less milk through the first 3 mo of lactation. Our results are generalizable to well-managed, housed, TMR-fed dairy herds.

Although innate immune function is important in RP, metritis, PVD, and endometritis (Kimura et al., 2002; Hammon et al., 2006; Moretti et al., 2016), these diseases have other risk factors (Giuliodori et al., 2013; Dubuc et al., 2010b). Other variables such as the extent of trauma to the reproductive tract during calving, and...
the effectiveness and regulation of inflammation (Sheldon et al., 2019; Pascottini and LeBlanc, 2020) may be more influential in the development of these diseases and appear to outweigh the effect of PBG, which is primarily to increase the circulating numbers of mature neutrophils. In a randomized controlled trial with 860 cows, Zinicola et al. (2018) also found no effect of PBG on the incidence of RP, metritis, PVD, or neutrophil percentage in vaginal cytology. In a trial with 270 cows, Oliveira et al. (2020) reported increased odds of clinical metritis (fetid discharge) in cows that received PBG. Cook (2020) randomized 1,865 cows on 4 farms to PBG, with no overall reduction in the proportion of cows (12 to 13%) receiving antibiotic treatments, but an apparent benefit on one farm suggested possible between-farm differences.

We did not observe a difference in the incidence of clinical mastitis in PBG-treated cows, similar to Zinicola et al. (2018). Hassfurther et al. (2015) showed reductions in the incidence of mastitis, from 34% in the control group, to 16.7% in a group that received 10 µg/kg PBG and 9.4% in a 20 µg/kg group. However, the methods employed by Hassfurther et al. (2015) to obtain a high incidence of mastitis (ensuring fresh pens were kept wet to increase the risk of mammary infection) differ from the well-managed herds used here, with 3% incidence of clinical mastitis to 63 DIM. Canning et al. (2017) reported a reduction in mastitis with PBG from 23% to 15% in herds across the United States, which is also a greater overall incidence than in our sample. Ruiz et al. (2017) detected a difference in mastitis incidence (3.7% in PBG and 4.9% in control cows) but had a very large sample size (n = 10,238) and more power to detect a small difference between treatment groups. Given the low incidence of clinical mastitis in our study, we did not have the study power to detect such an effect of treatment.

With the possible exception of circulating glucose concentrations, PBG did not have biologically important effects on the metabolic status of dairy cows, as indicated by the widely used markers examined here. Treatment with PBG increased serum concentrations of BHB, and NEFA and decreased serum albumin, but the differences were small and probably not of practical importance. Kimura et al. (2014) reported no significant PBG effect on serum NEFA concentration, and although there was a small treatment effect on NEFA concentration in the present study, there were no differences in the proportion of cows above thresholds associated in other studies with increased risk of undesirable outcomes.

Circulating concentrations of glucose were significantly (P < 0.001 at wk 1 and 2; Figure 2) lower in the PBG group. We speculate that the observed decrease in blood glucose concentrations in treated cows might relate to the energetic cost of the massive production of neutrophils (which we reported separately in a subset of these cows; Van Schyndel et al., 2018), which is glucose dependent. An activated immune response results in hypoglycemia as the consumption and depletion of circulating glucose exceeds that of glucose-sparing mechanisms, therefore reducing the amount of glucose available for milk synthesis (Kvidera et al., 2017). We encourage further research to confirm and explore the effect of PBG on circulating glucose concentrations.

In smaller studies, Kimura et al. (2014), Hassfurther et al. (2015), and Canning et al. (2017) reported no effect of PBG on milk yield or components. Zinicola et al. (2018) found increased milk yield in primiparous cows that received PBG, and no effect in older cows. They found no effect of PBG on milk components or ECM. In a trial in 270 cows in one herd, Oliveira et al. (2020) did not detect an effect of PBG on milk yield. Ours is the first study to report a reduction in milk yield associated with PBG, which was present even when controlling for milk yield in the previous lactation, which might have been a confounder of the apparent effect of PBG. Although the acute response to an inflammatory challenge is energetically costly and reduces milk yield by redirecting resources to the immune system (Kvidera et al., 2017), the cost of increased production of neutrophils in response to PBG as given here is unlikely to explain the observed duration (>3 mo) of the difference in milk yield. The increase in circulating numbers of neutrophils in response to administration of PBG lasts for approximately 2 wk (Van Schyndel et al., 2018; data from a sample of the cows in the present trial). Similarly, here we observed a meaningful reduction in serum glucose concentrations in the 2 wk after administration of PBG, which might reflect the glucose cost of production of billions of additional neutrophils. However, that would not directly account for a modest reduction in milk yield that was present for more than 3 mo. Therefore, we cannot explain a mechanism by which administration of PBG might lower milk production.

CONCLUSIONS

Administration of PBG did not affect the incidence of disease or reproductive performance. Milk production was approximately 1 kg/d lower in the PBG group, although we could not elucidate a mechanism with our data. Further research should investigate why the documented increase in neutrophil count following PBG administration did not translate to lower incidence of reproductive disease, with the goal of identifying measures of immune function or other aspects of me-
tabolism that are the key determinants of reproductive tract infection and inflammatory disease.

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