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Reducing the incidence of acute puerperal metritis in primiparous cows by application of pegbovigrastim in a Holstein dairy herd

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ABSTRACT: In dairy cows, immunosuppression occurs frequently during the periparturient period and is characterised by transient neutropenia and impaired polymorphonuclear neutrophil function. As a consequence, postpartum cows are more susceptible to production-related diseases such as mastitis, retained foetal membranes and metritis. Recently, it has been shown that a double injection with recombinant bovine granulocyte colony-stimulating factor covalently bound to polyethylene glycol (PEG rbG-CSF) increased polymorphonuclear neutrophil number and the exocytosis of myeloperoxidase by stimulating polymorphonuclear neutrophil, which also played a role in innate uterine immune defence. The aim of this randomised clinical study was to investigate the effects of two periparturient PEG rbG-CSF injections (IMR; Imrestor™, Elanco Animal Health, Bad Homburg/Germany) on the incidence of acute puerperal metritis, number of antibiotic doses required for treatment of acute puerperal metritis as well as parameters of milking performance (i.e., milk yield, milk fat, milk protein, and somatic cell count on the first dairy herd improvement test day) in primiparous cows within a dairy herd with an elevated incidence of acute puerperal metritis. In total, 169 heavily pregnant heifers were randomly assigned to the treatment group (IMR: n = 82) who received 15 mg PEG rbG-CSF subcutaneously 10 ± 3 days before the anticipated calving date and within 24 hours after calving, or to the untreated control group (Co: n = 87). In total, data from 157 animals (IMR: n = 75, Co: n = 82) were analysed. Administration of PEG rbG-CSF reduced the incidence of acute puerperal metritis in primiparous cows significantly (IMR: 22.7%, Co: 43.9%, P = 0.003; relative reduction: 48.3%). Moreover, the number of antibiotic doses per calving required for treatment of acute puerperal metritis was significantly lower in the pegbovigastrim group (IMR: 0.32 ± 0.66, Co: 0.59 ± 0.75, P = 0.005). No significant differences regarding incidence of clinical mastitis, milk production or milk composition were observed. These results suggest that further research should be performed to identify herd- and animal-specific factors that can be predictors of the beneficial effects of pegbovigrastim in preventing uterine diseases.

Keywords: cattle; postpartum; uterine diseases; prevention; bovine granulocyte colony-stimulating factor; G-CSF

List of abbreviations

APM = acute puerperal metritis, **DIM** = days in milk, **G-CSF** = granulocyte colony-stimulating factor, **MPO** = myeloperoxidase, **PMN** = polymorphonuclear neutrophil

In cattle, acute puerperal metritis (APM) occurs within 21 days post partum and is defined by an abnormally enlarged uterus, a fetid, watery, red-brown uterine discharge, a rectal temperature > 39.5 °C and signs of systemic illness (e.g., dullness and decreased milk yield) (Sheldon et al. 2006). The

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incidence of APM in dairy cattle ranges between 4 and 40% and is elevated in primiparous cows in comparison to multiparous animals (Wilson et al. 2004; Walsh et al. 2010). The disease compromises animal welfare as it causes visceral pain (Stojkov et al. 2015) and has an economical impact due to detrimental effects on reproductive performance, milk production and survivability (Overton and Fetrow 2008).

In high-yielding dairy cows, immunosuppression occurs frequently during the periparturient period due to negative energy balance and is characterised, among other things, by transient neutropenia and impaired polymorphonuclear neutrophil (PMN) and lymphocyte function (Kehrli and Goff 1989; Kimura et al. 1999; Kimura et al. 2002; Hammon et al. 2006). Also, hypoglobulinaemia due to colostrogenesis as well as hormonal and metabolic fluctuations resulting in an increase of plasma corticosteroids might contribute to a weakened immune response in cattle which, however, can already arise during pregnancy because of nutritional requirements of the developing foetus for minerals and vitamins (Mordak and Nicpon 2006; Mordak and Stewart 2015; Mordak et al. 2017; Walraph et al. 2018). Deficiencies of micronutrients such as selenium, iron, copper, zinc, vitamins A, C and E and folic acid can therefore influence several different cell types of the innate immune system. A sufficient supply of iron and copper is for instance necessary for an adequate function of PMN as they are cofactors of enzymes catalysing the formation of reactive oxygen and nitrogen species within the respiratory burst reaction of neutrophils and macrophages (Erickson et al. 2000; Djoko et al. 2015). Thus, cows are more susceptible to production-related diseases such as mastitis, retained foetal membranes and metritis (Waller 2000; Kimura et al. 2002; Sordillo 2005; Hammon et al. 2006).

Granulocyte colony-stimulating factor (G-CSF), a glycoprotein cytokine, stimulates the production and differentiation of neutrophils by progenitor cells located in the bone marrow (Nomura et al. 1986; Nagata 1989). To directly improve PMN function, Kehrli et al. (1991) injected recombinant bovine granulocyte colony-stimulating factor (rbG-CSF) daily and examined PMN number and function in cows with and without intramammary *Staphylococcus aureus* infection. Recently, Kimura et al. (2014) demonstrated that a double injection (about six days before calving and within 24 hours after calving) with recombinant bovine granulocyte colony-stimulating factor covalently bound to polyethylene glycol (PEG rbG-CSF) increased the PMN number in blood (predominantly mature cells) as well as the exocytosis of myeloperoxidase (MPO) by stimulating PMN. An increased number of PMN that are ready to move to a site of infection may improve the ability of the cow to ward off clinical disease (Kimura et al. 2014).

We hypothesised that a double application of PEG rbG-CSF in the periparturient period can reduce the incidence of APM. The aim of this study was to investigate the effects of two periparturient PEG rbG-CSF injections on the incidence of APM and number of antibiotic doses required to treat APM (primary study outcomes) in primiparous cows within a dairy herd with an elevated incidence of APM. As secondary study outcomes, the effects of PEG rbG-CSF treatment on performance parameters (i.e., milk yield, milk fat, milk protein, and somatic cell count on the first dairy herd improvement test day) were analysed.

MATERIAL AND METHODS

Farm and management. The motivation for this field trial was an elevated incidence of APM in primiparous cows (annual mean 2015: 48%) in a commercial dairy herd. In pluriparous cows, APM incidence was considerably lower (annual mean 2015: 17%). Taking into consideration the varying number of calvings, there were no relevant differences between monthly APM incidences (data not shown). The study was conducted on a dairy farm in Saxony, Germany (latitude: 51° 4' N; longitude: 12° 50' E; altitude: 249 m) with an average livestock of 1800 German Holstein cows and a mean 305-day milk yield per cow of 10 300 kg. The herd was free of brucellosis, leukosis, bovine herpesvirus 1 (BoHV1) and was not suspected to be positive for bovine viral diarrhoea virus (BVDV) and *Mycobacterium avium* subspecies *paratuberculosis* (Map) infections.

The animals were housed in a freestall barn with cubicles equipped with rubber mats; the space between the mats was interspersed with a straw-chalk-mixture once a day. Cows were kept in groups of 60–90 animals each, separated into primipara and pluripara, respectively. In contrast to this hous-

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ing system, dry cows and heifers approaching their calving were kept in a separate calving stable from three weeks ante partum to one day postpartum on straw which was changed every two days. No heifers were introduced from other farms. At day six, female calves were transported to a specialised rearing stable of the farm (distance to the stable of the cows of approx. 4 km; average livestock of 800 calves/heifers) and housed in single calf igloos until week five. After that, they were reared in a freestall barn on straw in groups of 15 animals each until week 11 and 50 animals each until 11 months, respectively. Subsequently, heifers were transported back to the dairy plant and housed in a freestall barn in cubicles separated from the lactating cows and inseminated artificially at the age of 13–15 months.

Fodder was provided as a needs-based total mix ration (TMR) (GfE 2001) by an automated system. Cows were milked in a rotary parlour (TurnStyles® DeLaval, Fa. DeLaval GmbH, Glinde, Germany) twice daily. The farm participated monthly in a dairy herd improvement test (DHIT) provided by a local organisation (Sachsischer Landeskontrollverband e.V., Lichtenwalde, Germany).

Experimental design and case definitions. In Europe, Imrestor™ (IMR; containing PEG rbG-CSF designated as pegbovigrastim) is approved to reduce the risk of clinical mastitis in dairy cows and heifers during the 30 days following calving within herd management programs (EMA 2016). For reasons of cost, the executive board of the dairy farm decided to treat only half of the heifers to reduce the incidence of clinical mastitis (label use). Thus, we fortunately had the chance to analyse the effects of pegbovigrastim on the incidence of APM under field conditions. Therefore, the study was exempt from ethical approval according to the German regulations.

This field study was designed as a prospective, unicentric, randomised, controlled and partially blinded (herd manager, stockmen, milkers) parallel group comparison. The herd veterinarian and the statistician were not blinded. Sample size was calculated using a web-based calculator (http://imsieweb.uni-koeln.de/beratung/rechner/b2.html). On the basis of the mean incidence of APM in primiparous cows known from historical herd data, the effect size was planned. A sample size of at least 71 analysable animals in each group was necessary to allow a decrease of APM incidence from 48% to 24% to be detected with a statistical power of 0.8 and $\alpha = 0.05$ following Machado et al. (2014) who also assumed an APM incidence reduced by half for effect size calculation. In consideration of a removal rate of 10% from the herd during the first 30 days in milk (DIM), the enrolment of 80 heifers in each group was considered sufficient.

Heavily pregnant heifers were enrolled once weekly from August 30, 2016 to November 1, 2016. The follow-up period lasted until December 18, 2016 (30 DIM). Inclusion criteria were the following: heifers 10 ± 3 days before the expected calving date (calculation: date of successful artificial insemination + 280 days) and 22–29 months of age. Disqualifying criteria were non-fulfilment of one of the inclusion factors, a disturbed general condition, a visual lameness as well as a systemic or local treatment using antibiotics or antiphlogistics within a period of 28 days before starting the trial. Heifers enrolled in this trial were randomised on the basis of the final numeral of the ear tag number (FNET). Pegbovigrastim (Imrestor™; Elanco Animal Health, Bad Homburg, Germany) was administered subcutaneously (*s.c.*) at a dose of 15 mg to animals assigned to the treated group (IMR; FNET 0–4) at the time of enrolment and within 24 hours after calving according to the manufacturer's instructions. Injections were carried out at the middle third of the neck. Heifers allocated to the control group (Co; FNET 5–9) did not receive any treatment or placebo.

Information regarding the calving ease was provided by the farm workers using a 5-point scale according to the German Cattle Breeders Federation (ADR 2006): 0, calving not monitored; 1, no assistance; 2, slight assistance by one person; 3, support by more than one person or mechanical pull; 4, caesarean section surgery or foetotomy. A calf was considered as stillbirth if heart beat or breathing were absent after delivery, or if the animal died within 24 hours postnatum.

After calving, primiparous cows were kept within the same pen for three weeks. This pen was monitored by the herd veterinarian and the herd manager. If animals showed signs of depression and dullness or a decrease in daily milk yield, a physical exam was performed (Dirksen et al. 2012). Retained foetal membranes were diagnosed if the foetal part of the placenta was not expulsed within 24 hours postpartum (LeBlanc 2008), and, then, removal was tried manually for three minutes

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by the herd veterinarian. Until day 10 postpartum, the rectal body temperature of the cows was measured (Burfeind et al. 2013) and documented once daily (morning measurement) using a digital thermometer (Microlife® VT 1831; Microlife AG Swiss Corporation, Widnau, Switzerland) by the herd manager. APM occurred within 21 days after parturition and was characterised by an enlarged uterus, a watery, red-brown, fetid vaginal discharge and a rectal temperature > 39.5 °C (Sheldon et al. 2006). APM was diagnosed and treated by the herd veterinarian. Treatment of these animals comprised single subcutaneous doses of 6.6 mg/kg ceftiofur crystalline-free acid (Naxcel® 200 mg/ml; Zoetis Deutschland GmbH, Berlin, Germany) and 1.4 mg/kg carprofen (Rimadyl® 50 mg/ml; Zoetis Deutschland GmbH, Berlin, Germany). If a rectal temperature > 39.5 °C persisted after that therapy, application of ceftiofur and carprofen was repeated after 48 hours (at most three treatments).

All measures within this study were part of routine management procedures within the farm and were performed with informed owner consent.

Data collection and statistical analysis. For collection and processing of data, a standard software package was used (Microsoft Office®, Microsoft Corporation, Redmond, USA). Individual animal data (age, pregnancy stage, diagnosis of clinical disorders, data on reproductive performance and DHIT) were extracted from the herd management software of the farm (HERDE®, dsp-Agrosoft GmbH, Ketzin, Germany).

For statistical data analysis, the software JMP® 12 (SAS, Marlow, Great Britain) and WinSTAT® (R. Fitch Software, Bad Krozingen, Germany) were used. As descriptive measures for categorical data, frequency tables and cross tabulations were used. Continuous data were described by count, mean, standard deviation, median, minimum and maximum. For survival data (time to an event), the number of events and the median time with 95% confidence interval were reported. For visualising survival data, Kaplan-Meier plots were drawn.

For binary and ordinal outcomes (incidence of APM, number of antibiotic treatments), multiple logistic regression models were used. The effect of parameters on survival data (time from calving to diagnosis of APM) was investigated with multiple Cox regression models. Age in days at calving, interval (days) from first pegbovigrastim injection to calving, calving ease, stillbirth, and retained foetal membranes were offered to the models. The Mann-Whitney *U*-test was used to compare the DHIT data (milk yield, milk fat, milk protein, common logarithmised somatic cell count) between the IMR and Co groups. The chi-square test was used to compare the proportions of cows removed from the herd before 30 DIM. *P*-values ≤ 0.05 were considered as statistically significant.

RESULTS

In total, 169 heifers (IMR: n = 82, Co: n = 87) were enrolled in the study. One animal from the IMR group was excluded at the time of expected calving because it was found to be non-pregnant. Furthermore, 11 cows (IMR: n = 6, Co: n = 5) were excluded due to a lack of compliance with the treatment protocol stipulated by the manufacturer (interval from first pegbovigrastim application to calving < 3 or > 17 days). Therefore, data of 157 animals (IMR: n = 75, Co: n = 82) were analysed. On average, the first pegbovigrastim injection was performed 10.6 ± 3.6 days (mean ± standard deviation) before parturition. None of the enrolled animals exhibited a disturbed general condition at this time. The *s.c.* injections caused no local swellings or general symptoms of intolerance. On the 30th DIM, 143 primiparous cows (IMR: n = 68, Co: n = 75) remained in the herd. Removal rates up until 30 DIM (IMR: 9.3%, Co: 8.5%) did not differ statistically (P = 0.861).

Descriptive statistics of the IMR and Co groups regarding age, gestation length, calving ease, incidence of stillbirth, retained foetal membranes, and twin calvings as well as DIM at first DHIT day are presented in Table 1.

The primary outcomes of the study (incidence of APM and number of antibiotic doses required for the treatment of APM) are summarised in Table 2. Kaplan-Meier survival curves illustrate the impact of pegbovigrastim injections on the occurrence of APM over time (Figure 1). Application of pegbovigrastim reduced the incidence of APM (relative change: 48.3%) significantly in comparison to the untreated control group. Furthermore, the number of antibiotic doses per parturition used to treat APM was significantly lower in the IMR group. This was the result of a reduction of APM incidence within this group, but not due to fewer antibiotic doses in cows suffering from APM (one,

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Table 1. Descriptive statistics of treatment groups

	Pegbovigrastim group[a]	Control group[b]
Age at calving (days; mean ± SD)	715.8 ± 31.3	721.9 ± 41.9
Days of gestation at calving (days; mean ± SD)	279.5 ± 3.3	279.5 ± 3.2
Calving ease (*n* (%))		
No assistance	49/75 (65.3)	58/82 (70.7)
Slight assistance by one person	20/75 (26.7)	19/82 (23.2)
Support by more than one person or mechanical pull	6/75 (8.0)	5/82 (6.1)
Stillbirth (*n* (%))	8/75 (10.7)	12/82 (14.6)
Twin calvings (*n* (%))	0/75 (0.0)	0/82 (0.0)
Retentio secundinarum (*n* (%))	4/75 (5.3)	2/82 (2.4)
DIM on first DHIT day (mean ± SD)	17.9 ± 8.9	20.4 ± 8.9

DHIT = dairy herd improvement test, DIM = days in milk

[a]Animals received two doses of 15 mg pegbovigrastim each (10 days before the calculated calving date and within 24 hours after calving)

[b]Control group remained untreated

two or three antibiotic doses – IMR: 64.7, 29.4 and 5.9%; Co: 69.4, 27.8 and 2.8%). In the final models, covariates with significant effects were retained foetal membranes (logistic regression: $P = 0.007$; Cox regression: $P = 0.002$) in the APM incidence models and retained foetal membranes ($P < 0.001$) and calving ease ($P = 0.001$) in the antibiotic treatment model, respectively.

Significant treatment effects in the secondary outcomes of the study (results of first DHIT day) were not observed (Figure 2).

DISCUSSION

In cows, bacterial contamination of the uterine lumen is almost ubiquitous after parturition (Sheldon 2004), and a plethora of possible pathogens can be involved (Williams et al. 2005, Williams et al. 2007). Although many risk factors for uterine disease have been described, preventive strategies are not widely adopted (Sheldon 2004). As one preventive approach, Machado et al. (2014) successfully vaccinated dairy heifers

Table 2. Incidence of acute puerperal metritis and number of antibiotic doses for treatment of acute puerperal metritis in the study groups

	Pegbovigrastim group[a]	Control group[b]	*P*
Incidence of acute puerperal metritis (*n* (%))	17/75 (22.7)	36/82 (43.9)	0.003
Model 1: logistic regression[a] (OR (95% CI))	baseline	2.67 (1.33–5.34)	
Incidence of acute puerperal metritis (HR (95% CI))	baseline	2.32 (1.71–2.92)	0.007
Model 2: Cox regression*, time of acute puerperal metritis diagnosis (median DIM (95% CI))	3 (2–7.8)	3 (2–5.6)	
Number of antibiotic doses per calving (treatment of acute puerperal metritis)*,**, (mean ± SD)	0.32 ± 0.66	0.59 ± 0.75	0.005

CI = confidence interval, DIM = days in milk, HR = hazard ratio, OR = odds ratio

[a]Animals received two doses of 15 mg pegbovigrastim each (10 days before the calculated calving date and within 24 hours after calving)

[b]Control group remained untreated

*Age in days at calving, interval (days) from first pegbovigrastim injection to calving, calving ease, stillbirth and retained foetal membranes were offered to the models

**Treatment of these animals comprised single subcutaneous doses of 6.6 mg/kg ceftiofur crystalline-free acid and 1.4 mg/kg carprofen. If a rectal temperature > 39.5 °C persisted after that therapy, application of ceftiofur and carprofen was repeated after 48 hours (at most three treatments)

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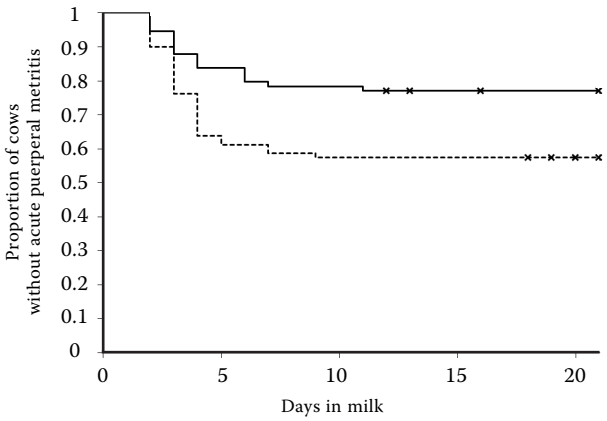

Figure 1. Kaplan-Meier survival plots demonstrating the effects of pegbovigrastim application on the occurrence of acute puerperal metritis (APM) over time (log-rank test, $P = 0.009$)

Solid line = animals of the IMR group (Imrestor^{TM}) received two doses of 15 mg pegbovigrastim each (10 days before the calculated calving date and within 24 hours after calving); broken line = control group remained untreated; × = cows which left the herd before the end of the study were treated as censored data

with multivalent vaccines containing inactivated bacterial components and/or protein subunits of *Escherichia coli*, *Fusobacterium necrophorum* and *Trueperella pyogenes* to prevent puerperal metritis during the first lactation. However, we previously observed that vaccinating dairy heifers using an inactivated multivalent herd-specific metritis vaccine did not show beneficial effects on uterine health and fertility of cows in their first lactation (Freick et al. 2017).

Hammon et al. (2006) demonstrated that blood PMN functions are significantly impaired during

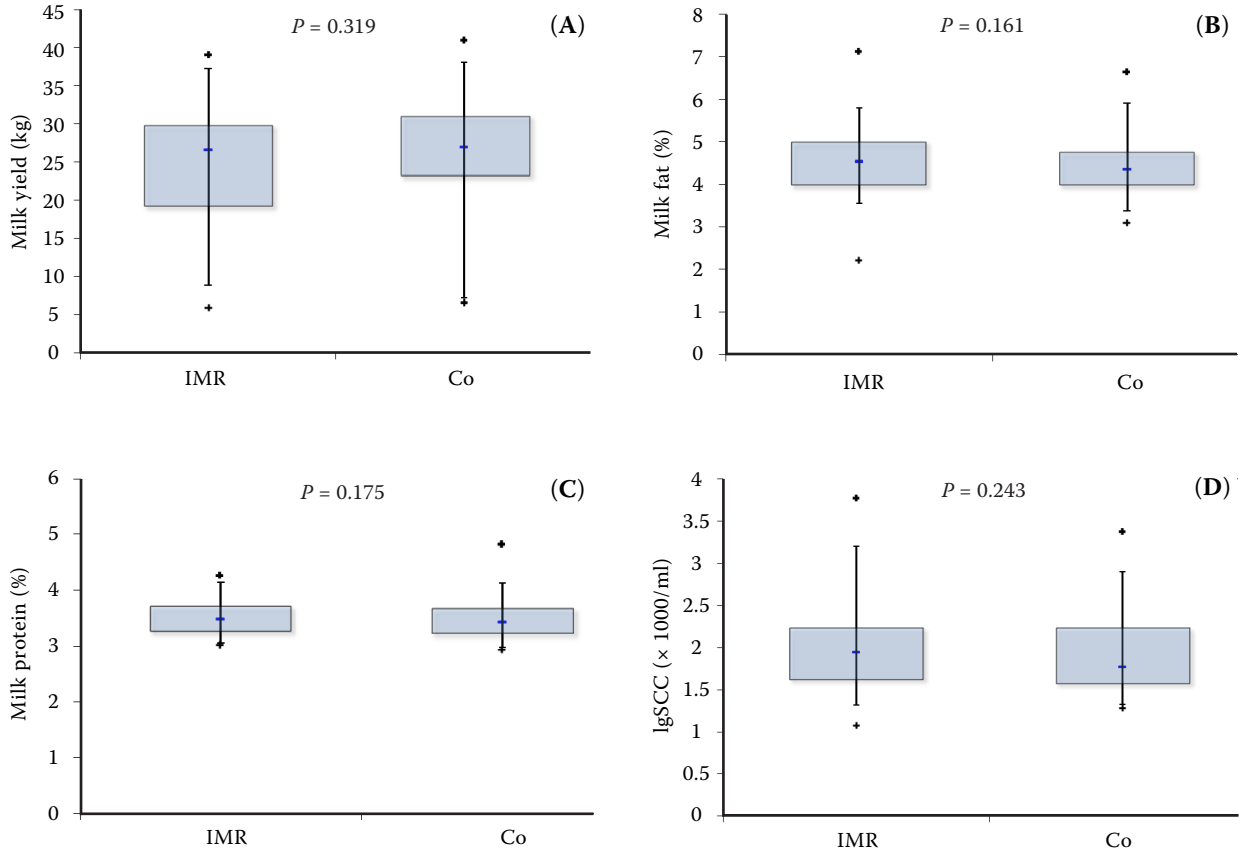

Figure 2. Box-Whisker plot illustrating the effects of pegbovigrastim application on milk yield ($P = 0.319$) (**A**), milk fat ($P = 0.161$) (**B**), milk protein ($P = 0.175$) (**C**), and somatic cell count ($P = 0.243$) (**D**) on the first dairy herd improvement test day

Animals of the IMR group (Imrestor^{TM}) received two doses of 15 mg pegbovigrastim each (10 days before the calculated calving date and within 24 hours after calving), control group (Co) remained untreated

lgSCC = common logarithmised somatic cell count

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the periparturient period in cows that develop postpartum uterine health disorders. Recently, it has been shown that administration of PEG rbG-CSF (pegbovigrastim) twice subcutaneously, about six days before calving and within 24 h after calving, increased PMN numbers quickly and these remained elevated in the blood for at least up to 13 days after calving (Kimura et al. 2014). Additionally, exocytosis of MPO by stimulated PMN, which is generally decreased in periparturient cows, was increased and persisted for at least 10 days after calving (Kimura et al. 2014). MPO generates the most powerful microbiocidal substances available to PMN (Klebanoff et al. 2013). Thus, we investigated pegbovigrastim as a promising novel approach to prevent APM.

In the study herd APM incidence in primiparous cows was elevated, although standard operating procedures regarding housing and management of periparturient heifers/cows as well as birth monitoring and delivery hygiene (including disinfection of the vulva before obstetric examinations, wearing disposable gloves and disinfection of tools after each use) were established. We could demonstrate a significant reduction in APM incidence by label use of pegbovigrastim. This was most likely achieved due to an increase in circulating numbers of PMN, improved PMN function supporting the innate immune system (Kimura et al. 2014) and possibly by induction of other cytokines as observed in humans (Xu et al. 2000). As neutrophils play a primary role in the defence of the uterus against infection (Singh et al. 2008), the increased numbers of PMN ready to move early to a site of infection, along with the increased ability to release MPO, might have improved the ability of the cows to ward off clinical disease (Kimura et al. 2014). Our results are in contrast to those of the study by Ruiz et al. (2017) who investigated 10 238 cows from 17 Mexican Holstein dairy herds and observed an increase in the incidence of metritis by 17.1% in the pegbovigrastim group. A reason for this discrepancy might be the considerably lower basic metritis incidence (IMR: 9.79%, Co: 8.36%) in the Mexican herds under investigation (Ruiz et al. 2017) in comparison to our study herd. Moreover, Ruiz et al. (2017) diagnosed metritis only by visual observation of vaginal discharges and transrectal palpation of the uterus. The rise of the rectal temperature, which can help to identify uterine inflammation (Benzaquen et al. 2007; Burfeind et al. 2013), was not included in their diagnostic regimen in contrast to our report. Also, a different supply of micronutrients should be considered as a further cause inducing distinct immune responses in the animals of these two studies.

Furthermore, the number of antibiotic doses per parturition to treat APM was significantly lower in the IMR group as a result of the reduction of APM incidence within this group but was not due to fewer antibiotic doses in cows suffering from APM. Similarly, Hassfurther et al. (2015) found no effect of a pegbovigrastim treatment on the severity or duration of clinical mastitis cases, and Ruiz et al. (2017) observed no differences in the number of medical treatments for metritis. Hassfurther et al. (2015) hypothesised that neutrophils primed for improved microbicidal activity by exposure to PEG bG-CSF in the circulation might have an increased ability to eliminate infections before an inflammatory response is apparent, but, once the immune response is overwhelmed and an acute inflammatory response is triggered, the severity and amount of time required for resolution remain unchanged.

Milk production and milk composition on the first DHIT day was not significantly affected by pegbovigrastim administration. This is in accordance with the study by Hassfurther et al. (2015). Ruiz et al. (2017) reported that daily milk yield increased from 0 to 120 DIM in multiparous metritic cows previously treated with pegbovigrastim, but not in primiparous cows. Thus, this effect might be specific for parity. Incidences of mastitis (IMR: 5.3%, Co: 3.6%) and retained foetal membranes (IMR: 5.3%, Co: 2.4%) in the study herd during the observation period were low and differences between groups were not statistically significant. Furthermore, there were no significant differences in the incidence of digital dermatitis during the first 30 DIM between the study groups (IMR: 37.3%, Co: 29.3%) (data not shown).

Our field study was conducted on only one dairy farm. This unicentric study design is a disadvantage regarding the generalisability of the results, as herd effects can have a significant impact on the success of treatment and prevention strategies. In the case of postpartum uterine diseases, candidate herd-specific factors can be, e.g., the herd-specific basic incidence of retained foetal membranes and APM and treatment regimen of APM, but also the housing (including the extent and duration of heat stress periods) (Burfeind et al. 2012; Dash et al. 2016)

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and feeding conditions in the periparturient period (including extent and duration of negative energy and protein balance and alterations in mineral and vitamin status). Remarkably, neutrophil function is significantly impaired in cows with peripartum negative energy balance (Hammon et al. 2006). Parity, body condition score (BCS) and incidence of clinical/subclinical ketosis and hypocalcaemia should be considered as candidate cohort-specific factors within herds that could influence the effect of pegbovigrastim. Furthermore, it was not possible to blind the herd veterinarian under the given practical circumstances. This lack of blinding must be emphasised as a weakness of the study design as the herd veterinarian diagnosed and treated APM cases. However, rectal body temperature of the cows as a major criterion in the definition of APM was recorded by the blinded herd manager and, thus, bias was minimised. Another issue of the study was the lack of a placebo administration in cows of the control group. Thus, effects of the injection itself and/or additional ingredients of the pharmaceutical on study outcomes cannot be excluded completely.

In summary, administration of pegbovigrastim is a promising approach to control APM in dairy cattle. In making the decision whether to implement a strategic use of pegbovigrastim in herd management protocols, not only monetary effects, but also a possible reduction in antibiotic consumption and improved animal welfare should be taken into consideration.

Further research (preferably multicentric studies and subsequent meta-analyses) is necessary to identify herd- and cohort-specific factors that can be predictors of the beneficial effects of a pegbovigrastim treatment. Moreover, investigations on the number and function of PMN in the bovine endometrium and uterine lumen as well as changes in the postpartum uterine microbiome after pegbovigrastim administration are goals for further studies.

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