Evaluation of mammary gland health in dairy cows treated by pegylated granulocyte colony-stimulating factor

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Abstract

The study aimed to determine the effect of the treatment by pegbovigrastim on the health of the mammary gland in dairy cows. Experimental animals were successively treated with 15 mg of pegylated bovine granulocyte colony-stimulating factor (bG-CSF, pegbovigrastim, PEG, Imrestor, Elanco) 10 ± 3 days before expected parturition and 1 day after actual parturition. Cows in the control group remained without treatment. The occurrence of clinical and subclinical mastitis in dairy cows during the first three months postpartum, and bacteriological findings in milk and milk yield were evaluated after preventive pegbovigrastim treatment. The influence of pegbovigrastim, as an additional treatment of mastitis caused by *Streptococcus uberis* in dairy cows on the standard course of treatment, was evaluated. The average number of antimicrobial (AML) interventions necessary for healing, the proportion of cows with 1, 2, 3 and more AML interventions, milk somatic cell count before treatment, 1 and 2 months after treatment and average milk withdrawal time were evaluated between groups. The results of the study did not find positive effects of pegbovigrastim on any evaluated variables.

Cattle, mastitis, immune response, pegbovigrastim

Several metabolic disorders and infections including milk fever, ketosis, postpartum reproductive disorders and mastitis occur during the early postpartum period in dairy cows (Dubuc et al. 2011; Ribeiro et al. 2013; Heikkilä et al. 2018; Hisira et al. 2020). Mastitis is the most frequent disease of dairy cows and has serious detrimental effects on animal welfare and dairy farm profitability (Ruegg 2017). Bovine mastitis is an important cause of premature culling of dairy cows (Fetrow et al. 2006) and of substantial financial loss caused by discarded milk, treatment costs, lower milk yields and reduced reproduction (Rollin et al. 2015; Ryman et al. 2015; Ceniti et al. 2017; Liang et al. 2017; Heikkilä et al. 2018). The major cause of mastitis is intramammary infection (IMI) by a wide variety of species of bacteria (Keane 2018), including *Staphylococcus aureus,* non-*aureus* staphylococci (NAS), *Escherichia coli, Streptococcus uberis, Streptococcus dysgalactiae, Corynebacterium bovis* and many others (Heikkilä et al. 2018). The clinical outcome of mastitis depends on important host factors such as parity, lactational phase nutritional status, immune status and genetic make-up, and on pathogen-specific factors (Keane 2018). Milk producers have long sought effective methods to minimize the occurrence of mastitis in their herds. Management of IMI includes antimicrobial therapy, milking management, genetic selection, food supplementation and immunization (Ruegg 2017).

During the transition period, however, an animal's immune function is reduced due to a negative energy balance and increased concentration of some blood metabolites

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(Kehrli et al. 1989; Ingvartsen and Moyes 2013). Functions of the white blood cells decline during 2 weeks before parturition to their lowest efficiency on days 0–2 postpartum (Kehrli et al. 1989; Batistel et al. 2018). Dairy cows show a reduction in neutrophil phagocytosis, a decrease of oxidative burst and of myeloperoxidase activity which are predisposing factors to infectious diseases such as mastitis and metritis (Kehrli et al. 1989; Goff and Horst 1997). To improve postpartum immunocompetence of dairy cows, bovine granulocyte colony-stimulating factor (bG-CSF) has been used (Hassfurther et al. 2015; Ruiz et al. 2017). Bovine granulocyte colony-stimulating factor (bG-CSF) is an endogenous protein which enhances neutrophil functions and increases the production of neutrophils from bone marrow precursors. Pegbovigrastim (recombinant bG-CSF bound to polyethyleneglycol, PEG-gCSF) administered approximately 7 days before parturition and within 24 h after parturition has been shown to significantly increase numbers of circulating neutrophils, levels of phagocytosis, and myeloperoxidase release as well as changing the expression of several genes involved in neutrophil functions (Powell et al. 2018; Lopreiato et al. 2019; Lopreiato et al. 2020; Crookenden et al. 2021). The clinical effect of pegbovigrastim on the occurrence of clinical mastitis during the first month postpartum as well as on disease severity has been reported (Hassfurther et al. 2015; Ruiz et al. 2017; Canning et al. 2017). The clinical studies available, however, are not numerous and, consequently, confirmation of published results is necessary. Furthermore, some studies did not confirm previous results (Zinicola et al. 2018; Oliveira et al. 2020; Van Schyndel et al. 2021).

The aim of the study was, therefore, to evaluate the effect of PEG-gCSF on mammary gland health postpartum in dairy cattle along with two sub-goals: 1) the influence of pegbovigrastim on the occurrence of clinical and subclinical mastitis during the first three months postpartum in dairy cows; 2) the influence of pegbovigrastim, as an additional treatment of mastitis caused by *Streptococcus uberis* in dairy cows, on the course of the standard treatment.

The use of PEG as a part of mastitis treatment has not been previously described.

Materials and Methods

The experiments were carried out on two commercial dairy farms cooperating with the University of Veterinary Sciences Brno (VETUNI Brno). Newly built stables using modern technology housed cows kept in free stalls with bedding boxes. The total number of animals was 700–800 Holstein dairy cows reaching a milk yield of about 10,500 l annually on each farm. Total mixed ration (TMR) was based on corn silage, alfalfa hay and concentrates. The ratio of individual components of TMR differed for individual stages of lactation and fibre was provided as needed with cut straw. The animals were handled in accordance with the Experimental Animal Welfare Committee of VETUNI Brno (protocol number 14/2018), which complies with ethical principles in animal research. Farm veterinarians directly participated in the experiments.

Study design

Experiment 1: The influence of pegbovigrastim on the occurrence of clinical and subclinical mastitis during the first three months postpartum in dairy cows

Animals

Late pregnant cows and heifers were selected for the experiment based on the expected date of parturition. The experimental and control groups were created in such a way as to achieve a similar representation of parity of the animals. The animals were listed according to the expected birth date. Subgroups by parity were created according to the order of expected parturition: 1, 2, 3, 4, 5 and more. Odd-numbered animals on the list in each subgroup fell into the experimental group, even-numbered animals into the control group. From the original group of animals included in the monitoring (252 cows), some cows were later excluded from the experiment (necessary slaughter, deaths). The final number of animals in the experimental group was 116, the number of animals in the control group was 125.

Treatment

Experimental animals were successively treated with two doses of 15 mg of pegylated bovine granulocyte colony-stimulating factor (pegbovigrastim, PEG bG-CSF, Imrestor, Elanco Animal Health, Bad Homburg, Germany. Injections were given subcutaneously behind the shoulder, the first dose 10 ± 3 days before the expected parturition and the second dose one day after the actual parturition (PEG). Cows in the control group remained without treatment (CON).

Clinical mastitis

Incidence of clinical mastitis was observed during the first three months postpartum. The foremilk and udder of every cow were examined twice daily and any quarter identified with abnormal milk was adjudged mastitic. After an on-farm bacteriological examination of milk samples, the cows received a course of antibiotics administered via the intramammary or intramuscular route, or non-antibiotic treatment. Additional adjunct therapies given were based on an assessment of the degree of udder inflammation as assessed by palpation of heat, swelling and degree of pain of the affected quarters. The degree of mastitis was not taken into account in the evaluation of the experiment. The total incidence of clinical mastitis was evaluated between groups.

Subclinical mastitis

The occurrence of subclinical mastitis was evaluated using the Milkprofitdata software available online. This software provides an evaluation of selected milk parameters taken as part of the monthly performance check. The values were determined in the central laboratory of the Czech-Moravian Society of Breeders. Somatic cell count (SCC) was determined in all cows in the experiment. Cows that showed clinical mastitis were excluded from the list. Cows with a detected SCC greater than 200,000 in 1 ml of milk were scored as showing subclinical mastitis. The incidence of subclinical mastitis detected during the first three months postpartum was compared between groups.

Presence of serious pathogens in the mammary gland

Bacteriological examination of milk was performed in the 3rd and 8th week (1st sampling, 2nd sampling) postpartum in 56 cows from each group. The aseptically retrieved milk samples were subjected to bacteriological examination according to standardized protocols established in the laboratory. Milk samples were cultured on blood agar and MacConkey agar (Oxoid CZ, Brno, Czech Republic) at 37 °C for 24 h. The bacterial strains grown were identified using MALDI-TOF system (Bruker Daltonik, Hamburg, Germany).

Presence of the *Streptococcus agalactiae, S. dysgalactiae, S. uberis, Staphylococcus aureus, S. chromogenes, S. haemolyticus, S. xylosus, Escherichia coli, Trueperella pyogenes* and *Corynebacterium bovis* was evaluated as a positive finding.

Isolation of the *Bacillus, Proteus, Aerococcus, Arthrobacter* and *Acinetobacter* spp. was evaluated as contamination. The presence of the important bacterial strains causing mastitis at week 3, at week 8 and at both weeks 3 and 8 was evaluated between groups.

Milk yield by 100 days in milk

Milk yield was assessed based on results of the monthly performance check made by the Czech-Moravian Society of Breeders. Milk yield by 100 days in milk (100 DIM) was compared between groups.

Experiment 2: The influence of pegbovigrastim, as an additional treatment of mastitis caused by *Streptococcus uberis* in dairy cows, on the course of the standard treatment

Animals

Cows showing clinical signs of mastitis were subjected to the bacteriological examination of milk using an on-farm Pure Milk Test (PM test, LabMediaServis, Jaromer, Czech Republic). Cows infected by *Streptococcus uberis* were included in the experiment.

Treatment

Mastitic cows infected by *Streptococcus uberis* were treated with antimicrobials (AML) and pegbovigrastim (two injections at a 14 days interval) (PEG group, n = 36) or only by AML (control group, n = 83) according to the usual schedule at dairy farms. Absence of clinical signs of inflammation, normal milk and a negative California mastitis test after the AML intervention was assessed as a successful treatment. If signs of mastitis were still present, another AML intervention was started. The average number of AML interventions necessary for healing, the proportion of cows with 1, 2, 3 and more AML interventions, milk somatic cell count (SCC) before treatment and 1 and 2 months after treatment, and average milk withdrawal time were evaluated between groups.

Statistical analysis

For the comparison of proportions in treatment and control groups the Pearson independence test was used (all expected counts were sufficient). The group mean values for the variables of milk yield, number of interventions, milk withdrawal time, and somatic cell count were compared using the Welch's variant of *t*-test (equality of variance was not fulfilled) with a two-sided alternative hypothesis. All computations were performed using R software (R Core Team 2022).

Results

Experiment 1

The results of all examined variables were similar between groups. Preventive administration of pegbovigrastim did not influence the occurrence of clinical and subclinical mastitis, positive milk culture test and milk yield by 100 DIM in experimental animals. The results are summarized in Table 1.

Table 1. Occurrence of clinical and subclinical mastitis, positive milk culture test, and milk yield by 100 DIM in cows after preventive treatment with pegbovigrastim (PEG) and in the control group (CON).

Variable	PEG (%)	CON (%)	*P* value
Clinical mastitis by 60 DIM	33.62	26.40	0.2211
Clinical mastitis by 90 DIM	35.34	30.40	0.4139
Subclinical mastitis at milk test 1	16.81	17.95	0.8205
Subclinical mastitis at milk test 2	10.62	11.11	0.9047
Subclinical mastitis at milk test 3	10.62	10.26	0.9283
Positive milk culture at week 3	14.28	16.07	0.7923
Positive milk culture at week 8	10.71	10.71	1.0000
Positive milk culture at week 3 and 8	23.21	19.64	0.6451
Milk yield by 100 DIM	3799.00 ± 63.85	3786.00 ± 83.83	0.9008

Experiment 2

The number of interventions necessary for the healing of clinical mastitis caused by *Streptococcus uberis*, the average number of interventions and average milk withdrawal time are summarized in Table 2.

Table 2. Course of mastitis treatment in cows treated either by pegbovigrastim and antimicrobials (PEG+AML) or only by antimicrobials (AML).

Variable		PEG + AML	AML	*P* value
1 intervention	%	58.33	55.42	0.7686
2 interventions	%	30.56	24.10	0.4609
3 and more interventions	%	11.11	20.48	0.2180
Average number of interventions	count	1.68 ± 0.12	1.73 ± 0.19	0.7965
Average milk withdrawal time	days	15.62 ± 1.03	15.93 ± 1.30	0.8530

The results of all examined variables were similar between groups. Administration of pegbovigrastim as a treatment of clinical mastitis caused by *Streptococcus uberis* together with the usual antibiotic cure did not influence the number of interventions necessary for healing and average milk withdrawal time in experimental animals.

The somatic cell count during mastitis treatment is summarized in Table 3.

Table 3. Somatic cell count in cows treated either by pegbovigrastim and antimicrobials (PEG + AML) or only by antimicrobials (AML).

Variable	PEG + AML	AML	P value
SCC before treatment (10^3)	1613.00 ± 340.94	1271.00 ± 306.87	0.4589
SCC 1 month after treatment (10^3)	870.00 ± 252.30	557.00 ± 169.11	0.3065
% of the original value	53.94	43.82	
SCC 2 months after treatment (10^3)	821.00 ± 316.67	415.00 ± 103.24	0.2309
% of the original value	50.90	32.65	

The results of all examined variables were similar between groups. Administration of pegbovigrastim as an additional treatment of clinical mastitis caused by *Streptococcus uberis* together with the usual antibiotic cure did not influence the number of SCC before treatment and in two subsequent terms of SCC measurement (1 and 2 months after mastitis treatment).

Discussion

All available studies describe a stimulative effect of pegbovigrastim on the immune system of treated animals. Cows treated with pegbovigrastim exhibited 3 to 5-fold increase in circulating neutrophil numbers (Canning et al. 2017; Zinicola et al. 2018) or increased the numbers of neutrophils, band cells, lymphocytes, and monocytes (Crookenden et al. 2021). Treatment of cows with pegbovigrastim increased the mRNA abundance level of 34 genes involved in immune response, which suggests a thorough activation of the immune machinery during the critical postpartum period (Lopreiato et al. 2020).

Studies describing the final effect of pegbovigrastim on animal health, however, showed contradictory results. Several studies have shown a reduction in mastitis incidence following treatment with pegbovigrastim both in experimentally induced and naturally occurring mastitis (Nickerson et al. 1989; Hassfurther et al. 2015). Pegbovigrastim treated animals exhibited a 35% (Canning et al. 2017) or 25% (Ruiz et al. 2017) decrease in the incidence of clinical mastitis relative to the controls during the first 30 d of lactation. In contrast, no effect on the incidence of clinical mastitis and milk composition (Freick et al. 2018) or on the incidence of retained placenta, metritis, displaced abomasum, clinical or subclinical mastitis, purulent vaginal discharge or endometritis (Zinicola et al. 2018; Van Schyndel et al. 2021) was observed in cows after pegbovigrastim administration.

The occurrence of mastitis was higher in our study (cumulative occurrence by 90 DIM was 35.34% and 30.40% for PEG and CON group, respectively) than in other studies. This was probably caused by the use of separated manure as bedding at our farms. Such a high occurrence of mastitis could provide an opportunity for the influence of pegbovigrastim. The occurrence of clinical mastitis by DIM 60 and 90 and subclinical mastitis in all sampling was, however, similar in both the experimental and control groups. The bacteriological findings in our study were the same in both terms of sampling.

Data concerning milk yield are also contradictory, even if slightly different methods of calculation between studies are taken into account. There are articles describing no affected milk yield and milk composition (Hassfurther et al. 2015; Crookenden et al. 2021), a positive effect of pegbovigrastim on milk yield (Ruiz et al. 2017; Powell et al. 2018) and even lower milk yield over the first 3 test days of lactation in the pegbovigrastim group (Van Schyndel et al. 2021). In our study, milk yield was the same in both groups for the first 100 DIM.

Recently, a study comparing the total amount of antibiotic therapy has been published (Cook 2020). Pegbovigrastim treatment was associated with a reduced likelihood of receiving antibiotic therapy in the first 30 DIM, but results were not consistent across all farms. Pre-calving pegbovigrastim treatment may be useful in some herds for reducing the incidence of antimicrobial treatments during early lactation (Cook 2020); however, this study was carried out on a small number of herds with high standards of management. In another study, pegbovigrastim treatment reduced the number of medical treatments required for mastitis by 6%, and less milk was discarded due to medication for mastitis treatment (Ruiz et al. 2017). In our study, the number of interventions necessary for mastitis to heal, the average number of interventions, the average milk withdrawal time and SCC after mastitis treatment were similar for both the PEG and CON groups. In this part of the study, however, we used pegbovigrastim administration as an accessory treatment for mastitis caused by *Streptococcus uberis* when the first pegbovigratim administration was performed together with AML treatment, not before parturition.

The number of animals in our study was rather limited compared to others where groups of 10,238, 840, and 1,607 animals were examined (Ruiz et al. 2017; Zinicola et al. 2018; Van Schyndel et al. 2021). We are not able to assess whether a higher number of cows in our study would have led to significant differences in any variables because not even a one-sided numerical tendency was observed. Perhaps only specific conditions on some individual farms will allow the effect of pegbovigrastim to manifest.

In conclusion, the results of the study did not confirm any positive effects of pegbovigrastim on the occurrence of clinical and subclinical mastitis, on bacteriological findings in milk, milk yield for the first 100 DIM or on the course of the standard treatment of mastitis.

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