

## THE EFFECT OF BOVINE GRANULOCYTE COLONY STIMULATING FACTOR ON HIGH YIELDING COWS WHITE BLOOD CELLS AND INCIDENCE OF MASTITIS AND METRITIS

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**Abstract.** The objective of this study was to evaluate the response of dairy cows to granulocyte colony stimulating factor (GCSF) according to white blood cells and incidence of mastitis and metritis. Experimental cows (n = 15) received GCSF 7 days before expected calving and again within 24 hours after calving. Control cows (n = 15) injected with saline. Blood samples collected 3 times (-7;0;7 days relative to parturition) from each cow immediately before GCSF and saline injection. Total leucocyte and granulocyte in the whole blood counted using hematology analyzer. To determine the presence of polymorphonuclear leucocyte (PMN), blood smear slides were prepared and stained. In experimental group, significant increase observed on white blood cells (WBC), lymphocyte (LYM) and granulocyte (GRA) counts, especially in band and segmented groups of neutrophils. In GCSF treated group GRA count were elevated from 67.7 % to 74.5% compared to the control group. Band neutrophil cells was higher by 40.5% compared to the control group. Segmented neutrophil cells was 22 % higher compared to the control group. Lymphocyte (LYM) count in experimental group was 25.5% to 39.8% higher compared to the control group. Clinical mastitis diagnosed in both groups equally by 6.7 %. Clinical metritis with 20% occurrence ratio diagnosed only for experimental cows. In our study GCSF did not reduced the incidence of clinical mastitis in cows during the periparturient period and increased clinical metritis incidence ratio, but represents an innovative way to reduce periparturient diseases incidence by affecting immune system.

**Keywords:** Immunosuppression Pegbovigrastim, Peripartum, Granulocyte, Mastitis, Metritis

**Introduction.** The transition period is crucial dairy cow's time from 3 weeks prior to calving to 3 weeks post-calving (Grummer, 1995). This period is termed due to naturally occurring metabolic, physiological, nutrition and management changes associated with stress of parturition (Drackley et al., 2001). The animal that fails to adapt to the changes may easily suffer from non-specific or innate immunosuppression and bacterial infections induction, resulting in increased dairy cow reproduction disorders incidence and metabolic diseases during this perinatal period (LeBlanc et al., 2006). Optimal regulation of the immune system is necessary to allow an appropriate immune response during parturition. However, the immune system affected in transition period. Several studies have demonstrated that neutrophil functions of dairy cows reduced significantly at peripartum, additionally risk of mastitis and metritis increases (Sordillo, 2016). Neutrophils are the main component of the innate immune system accounting for the majority of innate immune function in body tissues such as the udder (Benedictus et al., 2011). Neutrophils are crucial for the successful expulsion of the placenta within 24 hours of parturition, later (>24h) placenta retention associated with poor placentome degradation mediated by the immune system (LeBlanc, 2008). Compromised neutrophil functions also related with reproductive tract pathologies post-calving: purulent vaginal discharge, endometritis and/or cervicitis (LeBlanc, 2014). The innate immunity is responsible for tackling bacterial contamination that occurs within the reproductive tract after calving. There are many causes of periparturient immunosuppression: negative energy balance, hypocalcaemia, sub-clinical and clinical milk fever can also have an indirect effect on immunity via significantly increasing the risk of ketosis and fatty liver

disease (Kimura et al., 2006). Prolonged high levels of non-esterified fatty acids (NEFAs) and ketone bodies- beta-hydroxy butyrate (BHB) also affect neutrophil function and exacerbate the disease state for cows and heifers where feeding issues poorly managed. The production of neutrophils regulated by granulocyte colony-stimulating factor (GCSF) (Bendall, Bradstock, 2014). GCSF produced by a variety of cells including monocytes, macrophages, and cells of mesodermal origin (Demetri, Griffin, 1991). GCSF induces differentiation of progenitor cells into mature neutrophils, shortens maturation time within the bone marrow and increase functional activity (Avalos, 1996). Studies shown that Pegbovigrastim (PG) can increase neutrophil count in peripheral circulation and promote neutrophil functionality in dairy cattle (Hassfurther et al., 2015). Available scientific data have demonstrated a lower clinical mastitis incidence in early lactation following PG treatment (Canning et al., 2017; Hassfurther et al., 2015; McDougall et al., 2017). Effect of PG on cytoimmune response is various. However, the reduction in case of mastitis is unequal between farms. Moreover, information regarding the effect of PG on the mastitis and metritis is still limited.

### **Objective of this study**

To examine and evaluate the response of dairy cows to granulocyte colony stimulating factor according to white blood cells and incidence of mastitis and metritis.

### **Materials and methods**

#### **Experimental design**

The experiment carried out in 2016 at the Veterinary Academy of Lithuanian University of Health Science. The research was conducted in accordance with the provisions of the Law of the Republic of Lithuania No. 1-2271 on Protection, Keeping and Use of Animals, dated 03/10/2012

(Valstybės žinios (Official Gazette) No. 122-6126 dated 20/10/2012) and of the by-laws, Education and training purposes of animals used in storage, maintenance and conditions of use No. B1-866, dated 31/10/2012 (Valstybės žinios (Official Gazette) No. 130-6595, dated 10/11/2012). The research performed on 30 Lithuanian Black and White dairy cows: four to six years old. The herd consisted of 600 dairy cows in total. The average cow yield – 10000 kg of milk per lactation. Cows kept in pens on deep litter floor free in cold storage barns, feed with total mixed ration (TMR) and managed following ration: a high- straw, low energy close – up diet applied the last 3 weeks of pregnancy and corn with grass silage – based lactation diet balanced for 34 kg of milk production.

#### Cow treatment and sampling

The selected cows were clinical healthy, body condition score was among 3.0 to 3.5 and they showed no signs of lameness or other systemic disorders and were in drying period. Cows divided in 2 groups: 1<sup>st</sup> control group (n=15) received saline, 2<sup>nd</sup> experimental group (n=15) treated with granulocyte colony stimulating factor (GCSF (Pegbovigrastim) - Imrestor, Elanco Animal Health, Basingstoke, UK). The GCSF – 3 ml per cow or saline 3 ml/ cow administered subcutaneously in the neck region twice. First dose injected 7 days before parturition depending on expected calving time and physical changes close-up parturition. Second dose administered within 24 hours after calving. Blood samples collected 3 times from each cow: first time - 7 days prepartum, second time - at parturition immediately before GCSF injection and third time - 7 days postpartum. Blood collected by coccygeal venipuncture. Samples intended for morphology assay was collected using EDTA anticoagulant in tubes (Venosafe; Terumo Europe n.v. Belgium).

#### Total and differential white blood cell count

Total leucocyte, granulocyte and lymphocyte in the whole blood counted using hematology analyzer (Abacus Junior Vet, Hungary) at the large animal clinics laboratory of Lithuanian University of Health Sciences. To determine the presence of polymorphonuclear leucocyte (PMN), blood smear slides were prepared and stained. Smears were examined under the microscope to determinate the percentage of each PMN in 100 leucocyte count.

#### Diagnosis and treatment of clinical mastitis and metritis

Mastitis and metritis incidence observed from 0 to 30 days in milk (DIM). Cows with clinical metritis diagnosed based on rectal temperature increase above 39,3 °C, and purulent fetid vaginal discharge expression. Metritis treated with subcutaneous injection of ceftiofur and intrauterine injection of rifaksimin. Cows with clinical mastitis were diagnosed based on swelling udder quarter and milk abnormalities (clots, flakes, pus) and treated with local (intramammary suspension mix from Tetracycline, Neomycine, Bacitracine and Prednisolone) and systemic (Enrofloxacin) antibiotics therapy.

#### Statistical analysis

Statistical analysis performed using SPSS for Windows 15 (SPSS Inc., Chicago, IL, USA). The analysis accomplished using descriptive (ANOVA) model and

Pearson correlation coefficient. The differences determined by Student's t-test. The data was statistically significant when  $P \leq 0.05$ .

#### Results

##### White blood cell count

In experimental group, we observed significant increase on white blood cells (WBC) (Fig. 1) and granulocyte (GRA) counts (Fig. 2), especially in band and segmented groups of neutrophils (Fig. 3). Difference in total granulocyte number were significant at parturition ( $P < 0.005$ ) and 7 days postpartum (PP) ( $P < 0.005$ ). In experimental group, GRA at parturition was 67.7 % and after 7 days was 74.5% higher, compared to the control group. Difference in band neutrophil number were significant only at parturition ( $P < 0.05$ ). In experimental group band cells at parturition was 40.5 % higher compared to the control group. At day 7 the band's level of experimental cows were still elevated and 23.3% higher than in control group, but this difference were not significant.

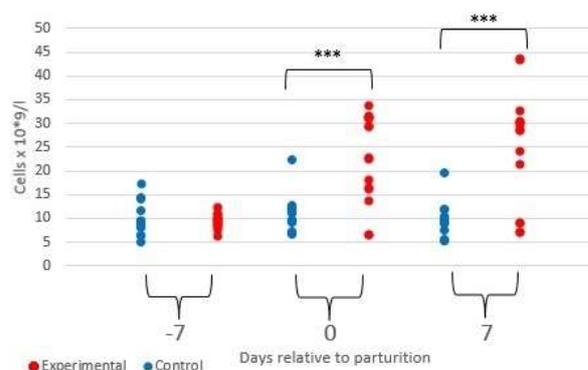


Figure 1. Effect of granulocyte colony stimulating factor on white blood cells count at days (-7;0;7) according to parturition. Asterisks \*\*\* represents ( $P < 0.005$ ) in comparison between groups at the same sampling point.

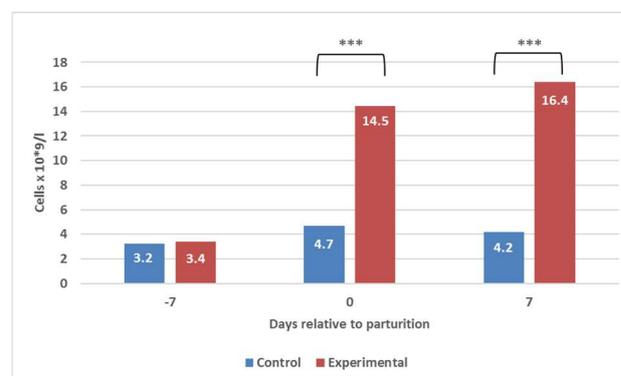
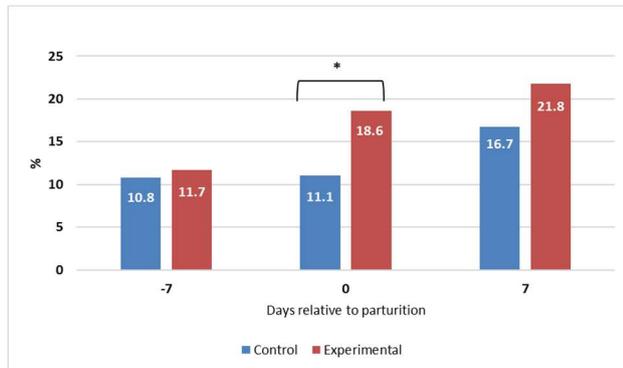


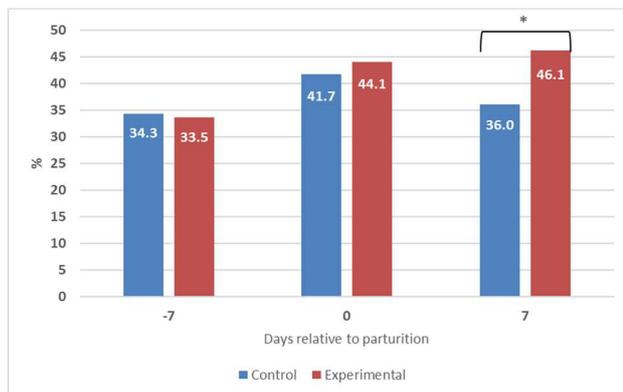
Figure 2. Effect of granulocyte colony stimulating factor on granulocyte count at days (-7;0;7) according to parturition. Asterisks \*\*\* represents ( $P < 0.005$ ) in comparison between groups at the same sampling point

Difference in segmented neutrophil number were significant only at day 7 ( $P < 0.05$ ). In experimental cows

segmented cells at day 7 was 22 % higher than in control group. We have noticed that difference in total lymphocyte (LYM) number were significant at parturition ( $P < 0.05$ ) and 7 days postpartum (PP) ( $P < 0.05$ ) (Fig.4). In experimental cows, LYM at parturition was by 25.5 % and after 7 days was by 39.8% higher compared to the control group.



A



B

Figure 3. Effect of granulocyte colony stimulating factor on band neutrophil count (A) and segmented neutrophil count (B) at days (-7;0;7) according to parturition. Asterisk \* represents ( $P < 0.05$ ) in comparison between groups at the same sampling point.

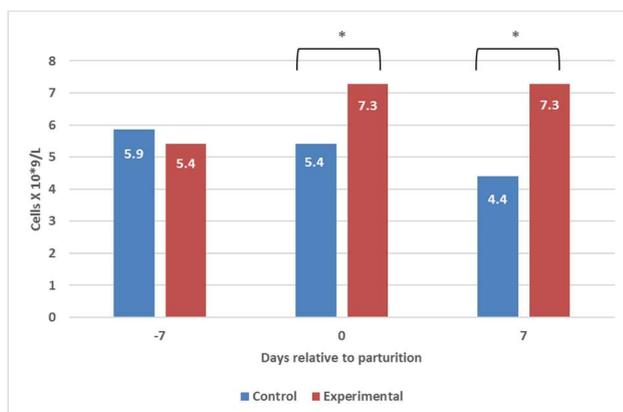


Figure 4. Effect of granulocyte colony stimulating factor on lymphocyte count at days (-7;0;7) according to parturition. Asterisk \* represents ( $P < 0.05$ ) in comparison between groups at the same sampling point.

### Disease incidence

Five cows (1 in control group and 4 in experimental group) had clinically diagnosed problems (Fig.5). Clinical mastitis diagnosed in both groups equally (one in experimental group and one in control group). Clinical metritis diagnosed only for experimental cows, in control group, no clinical signs observed. All cows with clinical metritis and mastitis were included in our data analysis.

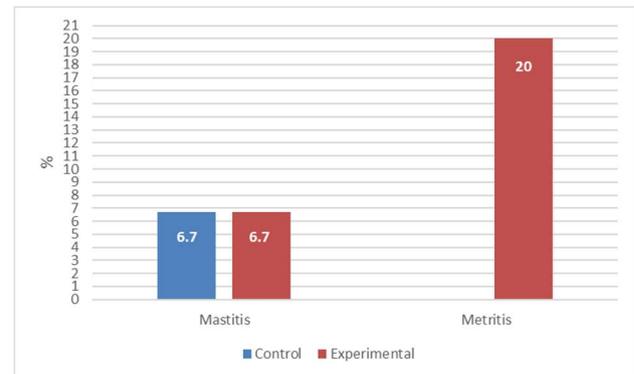


Figure 5. Clinical mastitis and metritis occurrence ratio in control and experimental groups

### Discussion

The transition period is physiologically determined and most critical time for any dairy cow. Most of researchers concentrated on important challenge during this time to avoid potential metabolic and reproductive disorders that animals may suffer and that may determine economical losses for entire dairy farm. In our study, we wanted to test new and innovative way to prevent immunosuppression and to ensure optimal fresh cow health status. After administration of PG to periparturient cows, circulating total white blood cells and granulocyte counts increased 3 to 4 times. These results were consistent with previous studies analyzing the effect of GCSF on hematology parameters (Canning et al., 2017; Kehrl et al., 1991). In our study PG treated animals maintained elevated level of granulocyte counts for 7 days postpartum. These results were similar with other study and confirms that elevated granulocyte count lasts more than a week after parturition and could be noticeable more than 14 days after calving (Canning et al., 2017). Prolonged effect of immunostimulator enables a reduction in consumption of daily administrable nonpegylated GCSF which used in past study (Cullor et al., 1992). The lymphocyte concentrations differed between treated and control cows at parturition and 7 days postpartum. These findings are consistent with previous study where most significant lymphocyte changes observed two weeks around parturition (McDougall et al., 2017). The mastitis incidence results in our study not differed significantly among control and experimental groups. These results might be due to small overall sample size (30 cows). Other studies shows that clinical mastitis (within 30 days of lactation) incidence ratio reduced by 20-30% (Hassfurther et al., 2015; Ruiz et al., 2017). Clinical metritis diagnosed only in GCSF treated group – 20% and could supplement other study results where PG treatment

resulted a greater incidence of metritis (17.6%) and greater odds (16.4%) to develop metritis in the first 21 days of lactation (Ruiz et al., 2017). The regulation of the inflammatory response in the periparturient cow is quite complex and does not necessarily correlate with the presence of a pathogen (Esposito et al., 2014). Based on action of GCSF, a more vigorous neutrophil response could lead to more animals exhibiting clinically observable inflammation (Ruiz et al., 2017). All periparturient dairy cattle have bacterial contamination of the uterus for 2 to 3 weeks after calving and that could be enough to develop metritis (LeBlanc et al., 2011).

#### Conclusion

Administration of pegbovigrastim increased total white blood cell count in periparturient cows, and this study demonstrated that increase is noticeable in neutrophil and lymphocyte populations. Pegbovigrastim did not reduce the incidence of clinical mastitis in cows during the periparturient period and increased clinical metritis incidence ratio for experimental cows. Pegbovigrastim represents an innovative way to reduce the incidence of clinical mastitis by affecting immune system, but further investigation is necessary.

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