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Effect of Pegbovigrastim on the leukocyte response of postpartum Holstein cows. Efeito do Pegbovigrastim na resposta leucocitária no pós-parto de vacas Holandesas.

Rafael Artur da Silva Júnior[1*], André Mariano Batista[2], Andrea Alice da Fonseca Oliveira[2], Antonio Santana dos Santos Filho[3], Pierre Castro Soares[2], Sebastião Inocêncio Guido[3], Cláudio Coutinho Bartolomeu[2]

[1-] Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, DMV-UFRPE, Recife/PE, Brasil.

[2-] Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Recife/PE, Brasil.

[3-] Instituto Agronômico de Pernambuco – IPA, São Bento do Una/PE, Brasil

*Corresponding author. E-mail: artur_rjs@hotmail.com

Abstract

In order to evaluate the leukocytic response to Pegbovigrastim in Holstein cows in the puerperium, 20 females were divided into two groups: the control group, receiving a placebo, and the treatment group, receiving 15 mg of Pegbovigrastim. There was variation in the number of leukocytes ($p<0.0001$) between groups, mainly due to an increase in the number of segmented neutrophils: on day 7 postpartum, the treatment group presented 19,935.5 ± 11,453.38 cells/µL of blood, while the control group 3,395.75 ± 1,518.87 cells/µL of blood ($p<0.0004$). There were no significant differences in uterine cytology. It can be concluded that Pegbovigrastim causes an increase in circulating of leukocytes, especially postpartum segmented neutrophils.

Keywords: G-CSF. Neutrophils. Puerperium.

Resumo

Para avaliar a resposta leucocitária ao Pegbovigrastim em vacas Holandesas no puerpério, 20 fêmeas foram divididas em dois grupos: o grupo controle, que recebeu placebo e o grupo de tratamento, que recebeu 15 mg de Pegbovigrastim. Houve variação no número de leucócitos ($p <0,0001$) entre os grupos, principalmente devido a um aumento no número de neutrófilos segmentados: no dia 7 pós-parto, o grupo de tratamento apresentou 19.935,5 ± 11.453,38 células / µL de sangue, enquanto o grupo controle 3.395,75 ± 1.518,87 células / µL de sangue ($p <0,0004$). Não houve diferenças significativas na citologia uterina. Pode-se concluir que o Pegbovigrastim causa um aumento na circulação de leucócitos, especialmente neutrófilos segmentados no pós-parto.

Palavras-chave: G-CSF. Neutrófilos. Puerpério.

Introduction

The negative energetic balance (NEB) experienced by dairy cows at the beginning of lactation contributes to a reduction in immune function and a predisposition for some diseases. Uterine disorders are associated with decreased neutrophil and lymphocyte function, which in turn are associated with decreased dry matter intake and a higher degree of NEB (HAMMON et al., 2006). Uterine diseases have great negative impact on reproductive performance, making it necessary to adopt practices that improve animal's immunity.

To face reproductive problems and consequently increase animal productivity, it is necessary to establish therapeutic and management protocols to increase the animal's immunity during the critical transition period. For this, management strategies are used wishing improvement in herds' production. Among them, the administration of Pegbovigrastim, a modified form of bovine granulocyte colony stimulating factor (bG-CSF) conjugated with polyethylene glycol (PEG).

Colony stimulating factors (CSF) are a family of glycoprotein cytokines that play a central role in the regulation of hematopoiesis and inflammation, granulocyte colony stimulating factor (G-CSF) drives the differentiation and maturation of a myeloblast in the bone marrow to a neutrophil in the circulation. An injection of G-CSF stimulates proliferation and increases the number of mature neutrophils in circulation (KEHRLI et al., 1991).

The objective of this study was to evaluate the effect of Pegbovigrastim administration on systemic and uterine leukocyte response in postpartum Holstein cows.

Material and Methods

The experiment was carried out at the São Bento do Una Experimental Station, belonging to the Agronomic Institute of Pernambuco, located in the municipality of São Bento do Una (08º31'36"S; 36º27'35"W) in the semiarid region of Pernambuco, at an altitude of 614 meters above sea level, average annual temperature of 22.0 °C and average annual rainfall of 288 millimeters. This experiment was approved by the Ethics Committee for the Use of Animals of the Universidade Federal Rural de Pernambuco under license number 102/2018 CEUA/UFRPE.

Twenty pluriparous Holstein cows were monitored between August and December 2018, from the seventh day before delivery until the 21st day postpartum. The cows had, before delivery, a mean weight of 500.0 ± 15.0 kg and a mean milk yield of 20.0 ± 0.5 kg, handled in a free stall confinement system, with water supply *ad libitum* and total diet, formulated according to the nutritional requirements of production for the studied animal category. The animals were previously synchronized and inseminated, and it was possible to establish the expected date of delivery, only females that had eutocic delivery were included in the study.

The cows were randomized into two groups of 10 animals: treatment group (TG), which received two subcutaneous applications in the lateral cervical region, containing 15 mg of Pegbovigrastim (Imrestor™, Elanco Animal Health, Greenfield, USA), following the recommendations of the manufacturer, the first being approximately seven days before the expected date of delivery and the second until 24 hours postpartum; the Control group (CG), received two subcutaneous applications in the lateral cervical region, containing 2,7 ml of 0.09% NaCl solution following the same protocol as the treated group.

Blood samples were collected for realization the leucogram, at five different times: seven days before delivery, up to 24 hours after delivery, seven days postpartum, fourteen days postpartum and 21 days postpartum. Blood samples were collected by puncture of the external jugular vein using 25x8 mm (21G) needles coupled to siliconized tubes containing an aqueous solution of tripotassium ethylene diamine tetra acetate (EDTA-K3) at 15%, and with sufficient vacuum to aspirate 4 mL of blood.

Leukocyte counting was performed in Neubauer's chamber. Two blood smears were readymade using *in natura* blood destined to differential count of leucocytes. These smears, after drying, were stained using the rapid panoptic staining according to the protocol recommended by the manufacturer. In each blood smear, 100 leukocytes were identified and classified according to their morphological characteristics, neutrophils with rod nucleus, neutrophils with segmented nucleus, eosinophils, basophils, lymphocytes and monocytes.

Uterine material was collected through cytobrush according to Cardoso et al. (2017) at three different times: seven days postpartum, fourteen days postpartum and 21 days postpartum. After the collected the applicator was carefully removed from the genital tract and immediately, the material was transferred to slides by imprint. The slides were immediately fixed in methyl alcohol and sent to the Histopathology Laboratory of the Department of Veterinary Medicine of UFRPE, where they were stained. The staining was performed using the rapid panoptic kit following the same technique performed for blood smears. After staining, the slides were visualized in an optical microscope, with a 40X increase, in which 100 cells were counted and differentiated into segmented neutrophils and other cell types, in order to evaluate the neutrophil infiltrate in the tissue.

The experimental model used was completely randomized with two groups. The data were tested for their normal distribution using the Kolmogorov-Smirnov test, the leukocyte, neutrophil, monocyte, eosinophil and basophil counts were not normally distributed, so they were transformed by Log10, and were expressed in central trend measures and processed using the GLM procedure of SAS - Statistical Analysis System for analysis of variance (ANOVA) as a measure repeated over time. In the case of significance in ANOVA, the means contrast was performed by the least significant difference of the Student-Newman-Keuls test. A 5% probability was considered for data analysis.

Results

The absolute values and standard deviation of the leukogram hematological variables are shown in Table 1. The total leukocyte count was globally higher in cows treated with Pegbovigrastim than in cows in the control group ($p < 0.0001$), however, no statistical difference was observed between the evaluated moments ($p = 0.1398$). There was a significant variation between groups for total lymphocyte count ($p = 0.0099$), eosinophils ($p = 0.0003$) and monocytes ($p = 0.0005$), with the exception of rods ($p = 0.1779$).

In the absolute count of segmented neutrophils, significant differences were found between the groups ($p = 0.0004$) and between the evaluated moments ($p = 0.0286$). In addition, there was a significant interaction between time and group ($p = 0.0190$), with a greater number of segmented neutrophils being observed on the seventh day postpartum in females treated with Pegbovigrastim.

There was no significant difference in the uterine neutrophilic infiltrate between the groups ($p = 0.5878$) or between the moments ($p = 0.4835$) evaluated.

Table 1 – Means and standard deviation of leukogram in Holstein cows in transition period treated or not with Pegbovigrastim (IPA, São Bento do Una - PE)

Groups	Collect days (pre and postpartum)					GA
	D -7	D 1	D 7	D 14	D 21	
Total Leucocytes						
Control	14,385 ± 2,7934	14,910 ± 6,658	12,442 ± 5,038	11,760 ± 5,550	13,380 ± 3,506	**13,376** [b*]
Treatment	16,170 ± 4,828	29,347 ± 7,701	35,332 ± 11,933	23,887 ± 12,192	22,010 ± 6,091	**25,350** [a]
GA	**15,278** [A**]	**17,695** [A]	**17,824** [A]	**22,129** [A]	**23,888** [A]	
Lymphocytes						
Control	7,833 ± 2,399	7,752 ± 2,698	7,863 ± 3,973	6,601 ± 2,576	7,129 ± 2,757	**7,452** [b]
Treatment	8,823 ± 3,069	12,778 ± 4,899	12,108 ± 4,144	12,682 ± 10,554	13,167 ± 5,648	**11,846** [a]
GA	**8,328** [A]	**9,642** [A]	**9,986** [A]	**10,148** [A]	**10,266** [A]	
Segmental Neutrophils						
Control	4,349 ± 2,053 [Aa]	5,708 ± 4,579 [Aa]	3,395 ± 1,518 [Ba]	4,017 ± 3,399 [Aa]	3,799 ± 1,031 [Aa]	**4,278** [b]
Treatment	5,014 ± 2,879 [Ab]	12,289 ± 4,685 [Aab]	19,935 ± 11,453 [Aa]	8,183 ± 1,462 [Ab]	5,726 ± 2,071 [Ab]	**10,467** [a]
GA	**4,682**	**4,763**	**6,101**	**8,999**	**11,666**	
Rods						
Control	82 ± 164	233 ± 466	15 ± 29	67 ± 81	57.33 ± 100	**92** [a]
Treatment	37 ± 74	515 ± 1,030	674 ± 1,140	242 ± 398	201 ± 347	**341** [a]
GA	**59** [A]	**374** [A]	**344** [A]	**154** [A]	**129** [A]	
Eosinophils						
Control	398 ± 284	383 ± 441	377 ± 584	126 ± 154	41 ± 392	**335** [b]
Treatment	473 ± 152	1,104 ± 920	765 ± 380	1,098 ± 850	2,149 ± 628	**1,063** [a]
GA	**435** [B]	**743** [AB]	**571** [AB]	**612** [AB]	**1,281** [A]	
Monocytes						
Control	1,038 ± 857	833 ± 282	791 ± 424	948 ± 466	910 ± 126	**904** [b]
Treatment	1,821 ± 1,116	2,661 ± 1,505	1,849 ± 597	1,731 ± 751	1,274 ± 210	**1,898** [a]
GA	**1,430** [A]	**1,747** [A]	**1,320** [A]	**1,339** [A]	**1,092** [A]	

GA = General Average, * Different lowercase letters in the columns indicate difference at 5% probability level, ** Different capital letters on the lines indicate differences at 5% probability level.

Discussion

The significant increase in the number of leukocytes in the animals in the treated group when compared to control animals in the period between seven days before delivery and on the day of delivery shows the action of Pegbovigrastim in stimulating the increase of circulating leukocytes in the animal. As expected, transient leukocytosis occurred on the day of parturition in all animals,

probably associated with inflammatory processes that occur during this period (BURTON et al., 2005).

Pegbovigrastim treatment has been shown to be associated with a significant increase in circulating leukocytes, at least until the 2st day after delivery, corroborating the data obtained by McDougall et al. (2017) and expanding the findings of Kimura et al. (2014), who certified the increase in the amount of leukocytes until the 13th day after delivery. This leukocytosis was due to neutrophilia, confirming the data presented by Van Schyndel et al. (2018), who observed that cows treated with Pegbovigrastim obtained substantial additive increases in the circulating neutrophil count after each application of the product.

The significant increase in neutrophils in animals treated with Pegbovigrastim can improve the immune response and mitigate disorders caused by uterine infections, common in the puerperium. According to Azawi (2008), the invasion of the uterine environment by neutrophils in response to challenges caused by the presence of bacteria is considered the most important phagocytic response. In the presence of pathogens, neutrophils are the defense cells most quickly recruited from the bloodstream into the uterine environment (SHELDON AND DOBSON, 2004).

When evaluating the neutrophilic uterine infiltrate by exfoliative cytology, values above 43% ± 0.19 of the neutrophils were observed in the two groups in the three evaluated moments, a very high amount when compared to the results reported by Ahmadi et al. (2006), who underwent endometrial cytology in the postpartum period of Holstein cows, verifying that, between 25 and 30 days postpartum, the percentage of neutrophils was 8.44% ± 0.45.

The increase in the number of circulating neutrophils due to the administration of Pegbovigrastim is likely to influence the increase in the amount of this cell type in the uterus. However, the regulation of the postpartum uterine inflammatory response in cattle is quite complex and is not necessarily correlated to the presence of a pathogen (ESPOSITO et al., 2014). In this context, the benefits in the control of uterine diseases in the postpartum period with the administration of Pegbovigrastim need to be further investigated.

Conclusion

For the conditions under which this study was performed, treatment with Pegbovigrastim caused an increase in the total amount of circulating leukocytes, mainly segmented neutrophils.

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References

AHMADI, M. R.; NAZIFI, S.; GHAISARI, H. R. Comparison of hormonal changes of estrous cycle with cytology of cervical mucosa and hematological parameters in dairy heifers. **Comparative Clinical Pathology**, v. 15, n. 2, p. 94-97, 2006.

AZAWI, O. I. Postpartum uterine infection in cattle. **Animal Reproduction Science**, v. 105, n. 3-4, p. 187-208, 2008.

BURTON, J. L.; MADSEN, S. A.; CHANG, L.; WEBER, P. S. D.; BUCKHAM, K. R.; DORP, R. V.; HICKEY, M.; EARLEY, B. Gene expression signatures in neutrophils exposed to glucocorticoids : A new paradigm to help explain '" neutrophil dysfunction "' in parturient dairy cows. **Veterinary Immunology and Immunopathology**, v. 105, p. 197-219, 2005.

CARDOSO, B.; OLIVEIRA, M. L.; PUGLIESI, G.; BATISTA, E. O. S.; BINELLI, M. Cytobrush: A tool for sequential evaluation of gene expression in bovine endometrium. **Reproduction in Domestic Animals**, v. 53, p. 1153-1157, 2017.

ESPOSITO, G.; IRONS, P. C.; WEBB, E. C.; CHAPWANYA, A. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. **Animal Reproduction Science**, v. 144, p. 60-71, 2014.

HAMMON, D. S.; EVJEN, I. M.; DHIMAN, T. R.; GOFF, J. P.; WALTERS, J. L. Neutrophil function and energy status in Holstein cows with uterine health disorders. **Veterinary Immunology and Immunopathology**, v. 113, n. 1-2, p. 21-29, 2006.

KEHRLI, M. E.; GOFF, J. P.; STEVENS, M. G. Effects of granulocyte colony-stimulating factor administration to periparturient cows on neutrophils and bacterial shedding. **Journal of Dairy Science**, v. 74, p. 2448-2458, 1991.

KIMURA, K.; GOFF, J. P.; CANNING, P.; WANG, C.; ROTH, J. A. Effect of recombinant bovine granulocyte colony-stimulating factor covalently bound to polyethylene glycol injection on neutrophil number and function in periparturient dairy cows. **Journal of Dairy Science**, v. 97, p. 4842-4851, 2014.

MCDOUGALL, S.; LEBLANC, S. J.; HEISER, A. Effect of prepartum energy balance on neutrophil function following Pegbovigrastim treatment in periparturient cows. **Journal of Dairy Science**, v. 100, n. 9, p. 7478-7492, 2017.

SHELDON, I. M.; DOBSON, H. Postpartum uterine health in cattle. **Animal Reproduction Science**, v. 82-83, p. 295-306, 2004.

VAN SCHYNDEL, S. J.; CARRIER, J.; BOGADO PASCOTTINI, O.; LEBLANC, S. J. The effect of Pegbovigrastim on circulating neutrophil count in dairy cattle: A randomized controlled trial. **PLoS One**, v. 13, p. 1-8, 2018.

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