



## Effect of recombinant bovine granulocyte colony-stimulating factor treatment during the peripartum period on postpartum diseases, reproductive performance, and milk production in Holstein cattle

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### ABSTRACT

To boost the immune function around parturition, recombinant bovine granulocyte colony-stimulating factor (rbG-CSF) has been used to increase the number of neutrophils. Therefore, the aim of this study was to quantify the effect of rbG-CSF administration on the incidence of postpartum pathologies, reproductive performance, and milk production during the first three months of lactation. A total of 199 Holstein cows from one herd were included and were randomly allocated into two groups: Control ( $n = 103$ ) and rbG-CSF ( $n = 96$ ). Cows in the rbG-CSF group received 2 doses of a rbG-CSF injectable formulation, one 7 days before the expected date of calving and the other within 24 h after calving. For 6 weeks following calving, animals were examined weekly to assess the presence of postpartum pathologies. Milk production, protein and fat content, and somatic cell count were determined monthly by the regional dairy herd improvement association. Data about the reproductive performance were collected from on-farm software. To analyse the effect of treatment on the incidence of postpartum pathologies, Pearson's  $\chi^2$  test and multivariable logistic regressions were performed. The effect on reproductive performance was analysed using Cox proportional hazard regression analysis for days open, binary logistic regression for first service conception rate and Oneway ANOVA test for the number of artificial inseminations. The effects of treatment on milk yield and milk composition were checked using GLM repeated measures analysis. No statistically significant differences were observed between treatment groups for any of the parameters evaluated. Only parity had a significant effect on days open and milk production ( $p < 0.05$ ). In conclusion, in the present study no evidence was found that rbG-CSF could have an effect on the reproductive and productive parameters evaluated.

### 1. Introduction

The peripartum period is critical for dairy cattle and dairy farms management. In this regard, postpartum pathologies not only impair the reproductive performance of the animals, but also their aptitude for milk production, both fundamental pillars for adequate economic performance. It is well documented that an impairment of immune function around calving, due to the negative energy balance and the subsequent

metabolic changes experienced by cows during this period, increases the risk of diseases such as mastitis, retained placenta, metritis, and endometritis (Dubuc et al., 2010; LeBlanc, 2014; Yáñez et al., 2022). In this way, innate immunity from polymorphonuclear neutrophils (PMN) has been reported as the predominant mechanism of early immune defence in the udder and the uterus (LeBlanc, 2014). However, there is evidence that concentrations of non-esterified fatty acids (NEFAs) around calving are associated with impaired immune function (Scalia et al., 2006; Ster

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et al., 2012).

Concerning immunity, the granulocyte colony-stimulating factor (G-CSF) has been established as an important factor for differentiation and maturation from myeloblasts in the bone marrow to PMN in circulation (Mehta et al., 2015). Therefore, aiming to boost the immune function around parturition, recombinant bovine G-CSF (rbG-CSF) has been used to increase the number of circulating PMN in dairy cows. In fact, some researchers have stated that, in grazing dairy farms, rbG-CSF treatment can lead to a total economic gain, boosting the annual partial net return per cow by US\$210 ± \$100 (Barca et al., 2023). However, literature shows that there is controversy about its efficacy regarding the reduction of the incidence or severity of peripartum diseases and milk production. It has been reported that rbG-CSF reduced the incidence of mastitis and retained placenta (RP) (Canning et al., 2017; Ruiz et al., 2017). However, others did not find any effect (Van Schyndel et al., 2021; Zinicola et al., 2018) or even observed an increasing incidence of postpartum diseases (Oliveira et al., 2020).

In terms of reproductive efficiency, Ruiz et al. (2017) found that cattle treated with rbG-CSF had greater odds of being inseminated during the first 100 days in milk (DIM). Similarly, Barca et al. (2022) observed that treatment with rbG-CSF in cows with high prepartum levels of NEFA, that also underwent clinical mastitis and uterine diseases, was associated with increased rates of pregnancy within 150 DIM. On the contrary, other researchers found no statistically significant differences between groups (Canning et al., 2017; Van Schyndel et al., 2021; Zinicola et al., 2018). Concerning milk production, it has been stated that rbG-CSF increased the productive performance of cows that had mastitis and metritis (Powell et al., 2018; Ruiz et al., 2017). Nevertheless, other studies showed no differences in milk production and composition between treated and non-treated cows (Kimura et al., 2014; Lopreiato et al., 2019; Oliveira et al., 2020; Zinicola et al., 2018). In addition, some researchers even observed a reduction in milk yield in cows treated with rbG-CSF (Van Schyndel et al., 2021).

Given the noticeable differences among studies and the rather limited literature about this topic, it is important to conduct additional research to clarify if the administration of rbG-CSF is an appropriate method to prevent postpartum diseases and maximise milk production and reproductive efficiency in dairy farms. Consequently, the aim of this study was to quantify the effect of rbG-CSF on the incidence of postpartum pathologies, reproductive parameters, and milk production during the first three months of lactation.

## 2. Materials and methods

### 2.1. Animals

This randomized controlled trial was carried out in accordance with the European Union Legislation (2010/63/EU) as transposed in Spanish Regulations for the protection of animals used for scientific purposes (RD 53/2013). Additionally, the REFLECT Statement (Sargeant et al., 2010) was followed to prepare the manuscript. The experiment was conducted from September 2016 to June 2017 in one commercial Holstein dairy farm located in the Northwest of Spain. This was a free-stall farm, and it housed a total of 276 milking cows. Cows were milked twice a day in a conventional milking parlour, and the mean 305-day milk production was 12,417 kg/cow. Cows were fed a total mixed ration, and reproductive examinations were carried out every week by an experienced veterinarian. The voluntary waiting period was 60 and 70 days for multiparous and primiparous cows, respectively. Artificial insemination (AI) was performed following heat detection. Heat detection was performed by farm workers by visual observation of the animals and identification of oestrus signs like increase of activity, mounting and resting the chin on other cows. For those cows in which heat was not observed, an Ovsynch protocol was applied as follows: GnRH (0 d) – PGF2 $\alpha$  (7 d) – GnRH (56 h) – AI (16 h).

### 2.2. Study design

All cows that calved during the period above-mentioned ( $n = 199$ ) were randomly allocated into two groups: Control ( $n = 103$ ) and rbG-CSF ( $n = 96$ ). First, it was decided that the first animal to calve would be allocated in the rbG-CSF group; thereafter, every second cow was assigned the same allocation, and the remaining cows were assigned to the Control group. Cows in the rbG-CSF group received two subcutaneous administrations (15 mg each) of rbG-CSF injectable formulation (Imrestor®, Elanco AH, IN, USA), the first one 7 d before the expected date of calving, and the second one within 24 h after calving. Cows in the Control group were not administered a placebo to avoid unnecessary disturbance of the animals.

### 2.3. Data collection

For 6 weeks following calving, animals were examined weekly by an experienced veterinarian to assess the presence of postpartum pathologies: retained placenta (RP, presence of the foetal membranes in the genital tract  $\geq 24$  h after calving), metritis (MET, abnormally enlarged uterus, with purulent or mucopurulent content in the uterus and/or the vagina,  $< 21$  days after calving, with or without systemic signs), endometritis (EM, purulent or mucopurulent uterine exudate in the uterus and/or the vagina,  $\geq 21$  days after calving, in absence of systemic signs), anoestrus (absence of cyclic ovarian activity, checked by the absence of a functional corpus luteum (CL), 45 days after calving), left displaced abomasum (LDA, dislocation and gas distention of the abomasum, diagnosed by auscultation) and subclinical mastitis (somatic cell count, SCC,  $> 200,000$  cell/mL).

In addition, the presence of cytological endometritis (CE,  $\geq 5\%$  polymorphonuclear neutrophils (PMN) on the endometrium (Madoz et al., 2013), and none or little exudate inside the uterus,  $\geq 21$  days after calving) was also assessed. Endometrial cytologies were collected between 35 and 42 d postpartum in all animals. For that purpose, a small cytobrush (length 20 mm and diameter 0.6 mm) was located inside an insemination rod (Quicklock 2000, Minitube Iberica, Barcelona, Spain), and both the rod and the cytobrush were protected by an insemination sheath and covered with a plastic sanitary sleeve (Chemise Sanitaire, IMV Technologies, L'Aigle, France). The rod was then introduced through the vagina to the external cervical opening. Once inside the cervix, the sanitary sleeve was perforated by the insemination rod, which was passed into the uterine body. There, the cytobrush was externalized and a sample was collected by rotating it against the uterine wall. Before removing the rod, the cytobrush was replaced inside it to prevent cellular contamination from the cervix or vagina. Then, the sample was extended on a glass slide and was allowed to air-dry. Slides were stained with Diff Quick (Quick Panoptic kit, Quimica Clínica Aplicada S.A., Tarragona, Spain). Each slide was examined by the same person using an optical microscope at  $100\times$  magnification (CHT, Olympus Iberia S.A.U., Barcelona, Spain), by the count of at least 150 cells, excluding erythrocytes.

Milk production, protein and fat content, and SCC were determined monthly by the regional dairy herd improvement association (DHI) in LIGAL (Laboratorio Interprofesional Galego de Análise do Leite). On test day, milk samples were collected from each lactating cow in 50 mL plastic containers with the preservative bronopol (2-bromo-2-nitro-1,3-propanediol) previously added, and transported to LIGAL. Somatic cell count values were analysed using flow cytometry (Fossomatic™, Foss, Hillerød, Denmark) according to the manufacturer's instructions. Protein and fat content were determined by infrared spectroscopy (Milko-Scan™, Foss, Hillerød, Denmark). Data from a total of three-monthly reports were collected for this study, including milk yield, protein and fat content, SCC, and days in milk (DIM). Additionally, the SCC was transformed to linear score (LS) using the following formula (Schukken et al., 2003):

**Table 1**

Data from 199 cows enrolled in a randomized controlled trial (RCT) investigating the effect of recombinant bovine granulocyte colony-stimulating factor (rbG-CSF) on reproductive health. Descriptive statistics and results for the multivariable logistic regression analysis including mastitis, retained placenta (RP), metritis, endometritis (EM), cytological endometritis (EC), and culling rate regarding treatment (Control or rbG-CSF). Treatment group, number of animals (N), percentage (%), Odds Ratio (OR), 95% confidence interval (CI) and significance (P-value) are displayed.

	Treatment group	N	%	OR	95% CI		P-value
					Lower	Upper	
Mastitis	Control	38	36.9	–	–	–	–
	rbG-CSF	26	26.8	0.719	0.368	1.405	0.335
RP	Control	8	7.8	–	–	–	–
	rbG-CSF	10	10.3	0.968	0.516	7.502	0.322
Metritis	Control	33	32.0	–	–	–	–
	rbG-CSF	27	27.8	0.873	0.402	1.745	0.636
EM	Control	28	27.2	–	–	–	–
	rbG-CSF	18	18.6	0.928	0.437	1.968	0.845
EC	Control	26	25.2	–	–	–	–
	rbG-CSF	18	18.8	0.112	0.512	2.415	0.788
Culling rate	Control	13	12.6	–	–	–	–
	rbG-CSF	11	11.3	0.793	0.298	2.110	0.642

$$LS = \log_2 \left( \frac{SCC}{100} \right) + 3$$

Finally, data about the reproductive performance were collected from on-farm software (Gando Nuevas Tecnologías, Spain). Information about the days in milk at the first AI, days open, and number of AI needed to become pregnant was collected. Pregnancy diagnoses were performed ~30 d after AI by transrectal ultrasonography, and data about the pregnancy outcome was also collected. Finally, date of culling, if appropriate, was recorded for each animal.

$$ECM \left( \frac{kg}{d} \right) = 12.55 \times fat \left( \frac{kg}{d} \right) + 7.39 \times protein \left( \frac{kg}{d} \right) + 0.2595 \times milk\ yield \left( \frac{kg}{d} \right)$$

#### 2.4. Statistical analyses

The categorical variables measured for this study, divided into two groups according to the presence or absence of the disease/treatment, were: treatment group, RP, MET, EM, anoestrus, LDA, CE, subclinical mastitis, and culling. Parity (1, 2, 3, 4, and ≥ 5), and season (winter, spring, summer, autumn) were also included as categorical variables. Days open, number of AI, DIM at first AI, DIM, LS, and ECM were considered continuous variables.

First, a preliminary Pearson’s  $\chi^2$  test was performed to assess the effect of treatment on the incidence of postpartum pathologies, including subclinical mastitis, RP, MET, EM, anoestrus, LDA, and CE. Thereafter, separate multivariable logistic regressions for each of these factors as dependent variable were performed to check the influence of treatment, season, parity, culling rate, and the remaining diseases. Anoestrus and LDA were not included in the multivariable logistic regression due to the low number of cases.

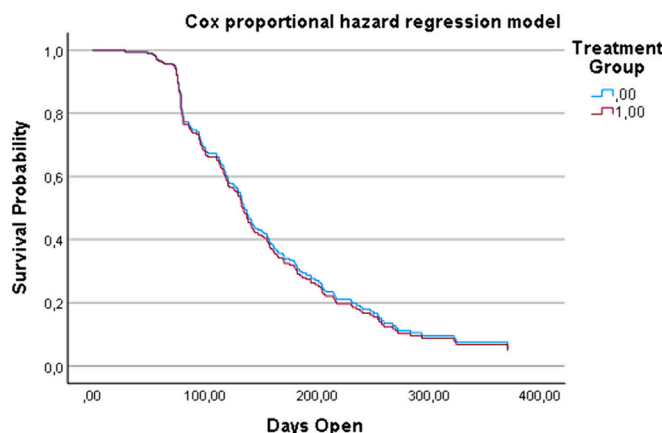
Additionally, treatment group, calving season, and parity were included in a Cox proportional hazard regression model for days open. All the independent variables were confirmed to comply the proportional hazards assumption. Models were built by forcing all the variables in, with threshold *p* values < 0.05 for inclusion and > 0.1 for exclusion.

To assess the influence of treatment on the first service conception rate, a binary logistic regression was performed including first service conception rate as dependent variable and treatment, season, parity, DIM, milk production at the last control before AI, MET, CE, and subclinical mastitis as independent variables. Moreover, the effects of treatment, parity, and season on the number of AI needed to become pregnant was analysed using a Oneway ANOVA test, including the

number of AI as dependent variable and the remaining data as factors.

Last, the effects of treatment, parity, and calving season on milk yield, protein and fat content, and LS were analysed using GLM repeated measures test. Interactions between these factors were also analysed, and the influence of DIM was also considered in the final model. Milk yield, protein and fat content were encompassed in a single variable by calculating the energy corrected milk production (ECM) according to the following formula (Santschi et al., 2016):

All analyses were conducted in SPSS version 28.0 for Windows (IBM Corp., Armonk, NY, USA). Differences were considered significant at *p* ≤ 0.05.



**Fig. 1.** Data from 199 cows enrolled in a randomized controlled trial (RCT) investigating the effect of recombinant bovine granulocyte colony-stimulating factor (rbG-CSF) on reproductive performance. Results for the Cox proportional hazard regression analysis, including days open as event, regarding treatment group (rbG-CSF in red and Control in blue). No significant differences were observed between groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**

Data from 199 cows enrolled in a randomized controlled trial (RCT) investigating the effect of recombinant bovine granulocyte colony-stimulating factor (rbG-CSF) on reproductive performance. Results for the Cox proportional hazard regression analysis including days open as event and calving season, treatment group and parity as variables.

Variable		P-value	HR	95% CI for HR	
Calving season	Winter	0.236	–	–	–
	Spring	0.511	0.862	0.554	1.342
	Summer	0.081	2.085	0.912	4.767
	Autumn	0.674	0.925	0.642	1.333
Treatment	Control	–	–	–	–
	rbG-CSF	0.812	1.041	0.750	1.445
Parity	1	0.098	–	–	–
	2	0.526	0.882	0.599	1.299
	3	0.238	0.763	0.486	1.197
	4	0.043*	0.568	0.329	0.983
	≥5	0.029*	0.455	0.224	0.924

\* p < 0.05 HR: hazard ratio. 95% CI for HR: 95% confidence interval for hazard ratio.

**3. Results**

The overall incidence of the different diseases is displayed in Table 1. Moreover, cows were grouped by parity as follows: 1 (n = 74), 2 (n = 52), 3 (n = 34), 4 (n = 25), ≥5 (n = 15). Additionally, the mean DIM was 17 (5–35), 48 (31–66), and 81 (61–128) for DHI controls 1, 2, and 3, respectively.

The results for Pearson’s  $\chi^2$  test did not show any significant differences between treatment groups for RP, MET, EM, CE, subclinical mastitis, LDA, anoestrus and culling (p > 0.05). However, the prevalence of CE in cows that underwent MET was lower in the rbG-CSF group than in the Control group (23.1% and 48.5%, respectively, p = 0.045). According to the multivariable logistic regression analyses, there are no significant differences between treatment groups for any of the above-mentioned diseases and culling rate (Table 1). Besides, the odds of MET were 31.9 times higher in cows with RP than in cows without RP (95% CI: 6.8–149.9, p < 0.001), and the odds of EM and CE were 2.5 (95% CI: 1.2–5.4, p = 0.018) and 2.8 (95% CI: 1.3–6.3, p = 0.009) times higher in cows with MET than in cows without MET, respectively. Regarding season, the odds of mastitis, EM and CE were decreased by 65%, 80%, and 82% during spring compared to winter (OR = 0.35 p = 0.038, OR = 0.2 p = 0.042, and OR = 0.18 p = 0.003, respectively). Moreover, the odds of RP are decreased by 87% in autumn compared to winter (OR = 0.13, p = 0.006).

The Cox proportional hazard regression model showed no significant differences for days open concerning treatment group and calving season. As for parity, cows in their fourth and fifth or subsequent lactations take longer to become pregnant (Fig. 1, Table 2).

According to the binary logistic regression, none of the variables evaluated had a significant influence on first service conception rate (p > 0.05). In addition, there were no significant differences in the number

**Table 3**

Data from 199 cows enrolled in a randomized controlled trial (RCT) investigating the effect of recombinant bovine granulocyte colony-stimulating factor (rbG-CSF) on productive performance. Results for the General Linear Model repeated measures analysis regarding Linear Score and Energy Corrected Milk in the first 3 dairy herd improvement controls (DHI) including treatment group (rbG-CSF and Control). No significant differences were observed between groups.

DHI (n)	Linear Score		Energy corrected milk (kg)	
	rbG-CSF	Control	rbG-CSF	Control
1	3.10 ± 1.92	3.17 ± 1.61	39.88 ± 12.26	43.87 ± 12.22
2	2.85 ± 1.95	2.73 ± 1.61	44.46 ± 10.36	49.08 ± 11.46
3	3.00 ± 1.90	2.91 ± 1.61	44.00 ± 10.06	49.91 ± 11.71

of AI regarding treatment group, calving season, and parity (p > 0.05).

Concerning milk production, descriptive statistics are displayed in Table 3. The GLM repeated measures test showed no significant differences for LS and EMC regarding treatment group (p > 0.05). Only parity had a significant influence in both measurements (p = 0.01 and p < 0.001, respectively).

**4. Discussion**

Although the aim of rbG-CSF is to boost immune function around calving, our results showed that treatment of cows with rbG-CSF did not influence the occurrence of postpartum diseases. There were not any statistically significant differences between treatment groups concerning the incidence of subclinical mastitis, RP, MET, EM, and EC. In this regard, our Pearson’s  $\chi^2$  test indicated a preventive effect concerning CE in cows that had been previously affected by MET, as Barca et al. (2022) have previously stated. However, our final model for the multivariable logistic regression showed no significant results. Similarly, other researchers did not find any difference between treated and non-treated cows in the incidence of RP, MET, clinical or subclinical mastitis, EM and culling rate (Lopreato et al., 2019; Van Schyndel et al., 2021; Zinicola et al., 2018). As it was previously mentioned, impairment of immune function around calving is well documented, and its incidence and severity are influenced by multiple risk factors, such as calving intervention, uterine injuries, hygiene, body condition score, negative energy balance, stress, and herd management, among others (Dubuc et al., 2010; Pascottini and LeBlanc, 2020; Van Schyndel et al., 2021). For instance, Barca et al. (2022) reported different health, productive and reproductive outcomes in cows with high prepartum concentration of NEFAs than in those who did not. Therefore, all those factors could affect not only immune function, but also the impact of rbG-CSF.

On the other hand, it has been reported a negative association of treatment regarding the occurrence of metritis (Oliveira et al., 2020; Ruiz et al., 2017). On the contrary, there are some researchers that found a beneficial effect of rbG-CSF in terms of prevention of peripartum pathologies. Ruiz et al. (2017) and Barca et al. (2021) claimed that the treatment reduced the occurrence of a first case of clinical mastitis up to 30 d after calving, and the latter also found a reduced rate of total mastitis cases during the full lactation. It has also been reported a beneficial effect of rbG-CSF treatment as an adjunct therapy in cases of naturally occurring severe clinical mastitis, as it resulted in better survival 30 d after enrolment (Denis-Robichaud et al., 2021). Similarly, Barca et al. (2023) observed statistically significant differences regarding the cost of clinical mastitis between treated and control cows, as well as a reduction in the cost of culling per year in the first group. Given this circumstance, these researchers have hypothesized that rbG-CSF treatment may reduce early culling as a consequence of disease.

Additionally, Barca et al. (2021) found that treated cows with metritis showed a reduced occurrence of endometritis. This research group suggested in a subsequent study that targeting rbG-CSF treatment to cows at a higher risk of disease due to metabolic challenges and lower immune competence would be the most efficient option from an economic point of view (Barca et al., 2023). However, noticeable differences in both farm management and methodology between this study and our study should be taken into account to explain the disparity of the results: grazing vs freestall management, blanket antibiotic dry cow therapy vs selective antibiotic dry cow therapy, sample size, farm size, and considered factors (we did not include body condition score, which was reported to influence the effect of the rbG-CSF treatment).

It should be noted that most studies used a 2-dose protocol of 15 mg of rbG-CSF treatment, 7 d before and within 24 h after calving. However, there are some exceptions, mostly regarding the pre-calving administration. In this way, intervals from 10 to 4 d before the expected calving date have been established to administer the first dose (Oliveira et al., 2020; Barca et al., 2022). Nonetheless, it is rather unlikely that this small time difference is responsible for the difference in observed results,

especially since the timing of the first dose is calculated from an estimated and therefore variable date. In this way, other factors such as herd management should be considered.

In this regard, it has been stated that the dosage of this treatment could be adjusted depending on herd management. Crookenden et al. (2021) observed that neutrophil counts and cell function were improved in cows treated with rbG-CSF in a dose-dependent way. More specifically, these researchers concluded that one dose at calving might be enough to improve both neutrophil number and function in early postpartum in grazing dairy cows, as these animals have a different pathogen exposure, genetic base, and they tend to have lower milk production and body weight. Nevertheless, the effect was only noted the day after calving, thus not administering the treatment at parturition may lead to a reduction in the protective effect. Consequently, this approach would entail an additional workload of monitoring animals at pasture. Therefore, further research is required to verify its effectiveness and feasibility of implementation.

Concerning reproductive parameters, we did not find any difference between treatment groups for days open, number of AI, first service conception rate, and days in milk at first AI. In this way, it has been previously stated that treatment with rbG-CSF did not affect reproductive performance of cows (Van Schyndel et al., 2021; Zinicola et al., 2018). As postpartum diseases are one of the main causes that influence reproductive performance, the absence of differences between groups regarding the incidence of these diseases may explain the lack of variation of reproductive parameters between treatment groups. However, Ruiz et al. (2017) found that cows treated with rbG-CSF had better chance of being inseminated within the first 100 DIM, which may be due to the protective effect these authors found for the occurrence of mastitis.

Last, in our study milk production and milk components for the first three controls did not differ between treatment groups. In the same way, other researchers observed that treatment with rbG-CSF did not alter milk yield or milk composition (Canning et al., 2017; Lopreiato et al., 2019; Oliveira et al., 2020). Nevertheless, an increased milk yield has been reported in cows that developed mastitis during the first 30 DIM and that had received rbG-CSF treatment compared to non-treated cows (Ruiz et al., 2017). Additionally, an interaction between treatment and parity has been reported by Zinicola et al. (2018), who observed increased milk production in primiparous, treated cows. On the contrary, a drop in milk production has first been related to rbG-CSF treatment by Van Schyndel et al. (2021), who observed that cows that had received the treatment produced 1 kg/day less than non-treated animals. However, the mechanism of this finding is yet to be determined.

## 5. Conclusions

In conclusion, according to our study design, any effect that treatment with rbG-CSF had on reproductive health or reproductive and productive performance of Holstein cattle was too small to be detected in our study design. Thus, certain limitations should be considered, such as the restricted number of animals enrolled and the fact that only one farm was included. Therefore, further investigation is required to clarify whether this treatment could provide some beneficial effects, especially in different herd managements and in farms that struggle with postpartum health and impairment of immune function around calving.

## Ethics approval

The experiment was conducted according to the European Union Legislation (2010/63/EU) and the Spanish Regulations for the protection of animals used for scientific purposes (RD 53/2013).

## CRedit authorship contribution statement

**Uxía Yáñez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jacobo Álvarez:** Writing – review & editing, Methodology, Investigation, Data curation. **Guillermo Lorenzo:** Resources, Methodology, Conceptualization. **Juan Caínzos:** Writing – review & editing, Visualization, Resources, Conceptualization. **Jocelyn Dubuc:** Writing – review & editing, Visualization, Formal analysis. **Juan J. Becerra:** Writing – review & editing, Visualization, Validation, Supervision. **Pedro G. Herradón:** Writing – review & editing, Validation, Supervision, Resources. **Ana I. Peña:** Writing – review & editing, Validation, Supervision. **Luis A. Quintela:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data can be provided by the correspondence author by request.

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