Association of bovine uterine involution disturbances with serum neuropeptide concentrations

Aida Abultdinova
Faculty of Veterinary Medicine and Technology of Animal Husbandry Kazakhstan
Issatay Jakupov
Faculty of Veterinary Medicine and Technology of Animal Husbandry Kazakhstan
Joachim Roth
University of Giessen
Klaus Failing
Biomathematics, University of Giessen
Axel Wehrend
Clinic for Gynecology, University of Giessen
Marlene Sickinger (Marlene.Sickinger@vetmed.uni-giessen.de)
Justus Liebig Universitat Giessen https://orcid.org/0000-0002-2231-1622

Research article

Keywords: Dairy cow, uterus involution, diagnostics, neuropeptides, inflammation

Posted Date: November 13th, 2019

DOI: https://doi.org/10.21203/rs.2.17210/v1

Version of Record: A version of this preprint was published at Veterinary World on January 1st, 2020. See the published version at https://doi.org/10.14202/vetworld.2020.1854-1857.

Abstract

Background: Puerperal diseases influence fertility and should be diagnosed as soon as possible. The present study aimed to evaluate the applicability of serum concentrations of substance P (SP), vasoactive intestinal polypeptide (VIP) and IL-1β in the early diagnosis of uterine involution disturbances. Blood serum samples of 86 dairy cows from 6 different farms were harvested within the first 20 days after calving from cows with uterine involution disturbances and healthy controls, respectively. Serum concentrations for SP, VIP and IL-1β were determined using commercially available ELISA test kits. Statistical analyses included timely changes in blood serum levels and group comparisons of healthy cows and cows with uterine disease.

Results: SP-concentrations increased significantly within 20 days after calving ($P < 0.04$) with no significant difference between the groups. No significant differences could be shown for VIP and logIL-1β.

Conclusion: None of the examined serum parameters seems suitable as indicator of uterine involution disorders. Due to the timely changes in serum concentrations of SP after calving, a correlation to diseases might not be precluded though. Further research is needed with regard to the establishment of normative values concerning this parameter.

Background

Postpartum uterine diseases result in infertility or subfertility and have high economic significance [14]. A literature-based calculation of the direct costs of treatment, reduced milk yield, and subfertility associated with uterine disease revealed economic losses of up to £ 16 million per year in the UK [14]. The incidence of metritis (pyrexia up to 10 days postpartum, purulent vaginal discharge, and delayed uterus involution) and clinical endometritis (purulent vaginal discharge for 21 days or more postpartum and delayed uterus involution) are reported to be 18.5–40% and 10–20%, respectively [14]. In the last years, several diagnostic possibilities have been developed and validated. Among these diagnostic measures are the transrectal palpation of the uterus, ultrasonography, and vaginal discharge evaluation [6,8,11]. Currently, studies have concentrated on pro-inflammatory factors associated with puerperal diseases [7,13]. These studies have focused on the local situation within the endometrium, whereas another study determined a possible association between serum pro-inflammatory and anti-inflammatory cytokines and uterine diseases in postpartum dairy cows [9]. In addition to inflammation that is associated with the release of fetal membranes [3], contractility of the myometrium is essential for a physiological involution of the uterus [5,16]. Therefore, the present study examined serum concentrations of the motility-influencing neuropeptides substance P (SP) and vasoactive intestinal polypeptide (VIP) as well as the cytokine concentrations of interleukin (IL)–1β. These examinations aimed to evaluate the possible applicability of these serum parameters in early diagnostics of uterine involution disturbances.

Results

Blood serum concentrations of SP showed a statistically significant increase within the first 20 days of calving (P < 0.036), with no significant slope difference between the groups (P = 0.564; Fig. 1). The difference between the adjusted means of the groups was barely nonsignificant (P = 0.084).

With respect to VIP, neither for the timely course (P = 0.504) nor for the adjusted group means (P = 0.110), significant differences were observed (Fig. 2).

Equivalent findings were obtained for logIL-1β values, with no statistically significant differences in the timely course (P = 0.166) nor within the group comparison (P = 0.296; Fig. 3). A detailed overview of the results of the one-way analysis of variance is provided in Table 1.
The exact Wilcoxon-Mann-Whitney test also yielded no statistically significant differences concerning the timely changes or group comparison for the parameter IL-1β.

Table 1: Detailed overview concerning the results of the one-way analysis of covariance

Parameter	Equality of slopes (P-values)	Common slope		Adjusted group means		
		Estimate	P-value	diseased	healthy	P-value
Substance P	0.564	0.51 pg/ml x day	0.036	39.7	44.9	0.084
VIP	0.379	0.19 pg/ml x day	0.504	52.4	46.6	0.110
IL 1β (explorative)	0.205	0.02 pg/ml x day	0.166	1.13	1.31	0.296

Discussion

Uterine involution disturbances with concurrent fertility problems because of subclinical endometritis are often a reason for therapeutic measures or, even worse, for culling [14]. Therefore, there is an urgency to develop reliable and comfortable early diagnostic tools. Previous studies have reported lower concentrations of tumor necrosis factor (TNF)-α, IL−1β, IL−6, and IL−8 before calving in cows that developed retained fetal membranes after parturition [3]. In contrast, elevated serum and tissue concentrations for pro-inflammatory cytokines, including TNF-α, IL−1β, and IL−6, were observed in cows with metritis, endometritis, or subclinical endometritis [9]. In cystic ovarian disease, another reason for infertility, cytokine expression was observed to be altered within the follicular structures [15].

The present study aimed to clarify whether serum concentrations of IL−1β, SP, and VIP change over time after calving and if these concentrations are correlated to puerperal diseases.

With respect to the concentrations of pro-inflammatory cytokines, our results could not show statistically significant changes within the first 20 days of calving. In contrast to previous studies, no statistically significant differences were observed between healthy and diseased cows. Nevertheless, when interpreting the results of related to IL−1β, we must consider that the IL−1β concentration in a considerable number of cows was under the detection limit (6.4 pg/ml). To our knowledge, this finding has not been reported previously. Additionally, there might be breed differences concerning the serum concentrations of cytokines. The aforementioned study in cows that were at a risk of fetal membrane retention involved Zebu breeds and other Indian crossbreds, in which retention of the fetal membranes is reported to be more common than in other breeds, such as Holstein Friesians (HFs) [3]. Nevertheless, the central role of inflammatory processes and placental maturing in the physiology of release of fetal membranes have also been shown for HFs [1,2].

However, according to our results, IL−1β is not suitable as a diagnostic marker for uterine diseases within the first 20 days of calving.

VIP also seems to be of no significance either for uterine involution or for uterine health. Our results revealed no significant differences, according to time after calving or healthy or diseased condition. A study on VIP within the reproductive tract only refers to tissue distribution, not serum concentrations [10].

In contrast, with respect to SP, statistical analyses revealed significant changes over time for the first three puerperal weeks. SP and VIP typically are present within the vaginal and uterine tissues of cows [10]; in particular, the role of SP is postulated in cervical softening and birth [12]. To our knowledge, the present study is the first to describe a time-related change in SP serum concentrations after calving. Increasing concentrations of this neuropeptide might be associated with uterine involution. Although the group comparison between healthy and diseased animals showed a barely significant difference, the difference may be significant if the number of examined animals could be increased. Future studies should therefore concentrate on the establishment of normative values in relation to breed, age, lactation status, and pregnancy, because previously published literature only refers to SP concentrations in calves [4].

Conclusion

In conclusion, neither the pro-inflammatory cytokine IL−1β nor the neuropeptide VIP is proposed as a suitable indicator of uterine involution disturbances or uterine diseases within the first 3 weeks of calving. Concerning the neuropeptide SP, timely changes were observed in serum concentrations after calving and a correlation to diseases might not be precluded. Further research is needed with regard to the establishment of normative values concerning this parameter. Due to its involvement as a transmitter of pain and inflammation via sensible neurons, SP concentrations also might serve as an indicator for the necessity of anti-inflammatory therapy.

Methods

Animals and blood sampling

Blood samples were collected from 86 dairy cows (age 2.5–9 years) at 6 local farms. Animals had calved between 1 and 20 days before sampling and were grouped according to the results of a clinical and gynecological examination into healthy (n = 62) or diseased (n = 24) groups; Diseased cows were characterized by clinical signs for endometritis, metritis, or involution disturbances because of another cause.

The samples were allowed to clot overnight at 4 °C. They were subsequently centrifuged for 20 min at approximately 1000 ×g, and stored at –80 °C until evaluation. The serum concentrations of SP, VIP, and IL–1β were determined using commercially available bovine-specific ELISA kits (USCN Cloud-Clone Corp., Houston TX, USA).

All blood samples were harvested from cows that underwent blood work due to herd management and herd health diagnostics. The samples were collected with a notification of the local ethics authority and conformed to national ethic guidelines and legislation (internal number of correspondence, IRB number: kTV 11–2018).

Statistical analysis

Data analysis involved the testing of the timely changes in blood serum levels within the first 20 days of calving as well as the group comparison between healthy and diseased cows. To evaluate the timely course of the neuropeptide concentrations, a regression analysis was performed. For the group comparison with the covariate time, a one-way analysis of covariance was conducted using the statistical software package BMDP, particularly the program BMDP1V. $P < 0.05$ was considered statistically significant.

Because of a biased distribution of IL–1β, IL–1β values were logarithmically transformed in the analysis and data description is provided by box plots.

As a considerable percentage of values for IL–1β was below the detection limit, timely changes and group comparisons for IL–1β were exploratively calculated using one-way analysis of covariance. Additionally, IL–1β values within the first 10 days of calving and within days 11–20 were compared by performing the non-parametric Wilcoxon-Mann-Whitney test in the exact version (program StatXact®, version 9.0.0; Cytel studio, Cambridge, MA, USA).

Abbreviations

HF: Holstein Friesians; IL: Interleukin; SP: Substance P; TNF: Tumor necrosis factor; VIP: Vasoactive intestinal polypeptide.

Declarations

Acknowledgments

We thank Jolanta Murgot (Technical Assistant at the Institute for Veterinary Physiology) for her assistance with the ELISA tests. We would like to thank Editage (www.editage.com) for English language editing.

Authors´ contributions

AW, IJ and MS designed the experiment. AA and MS performed the experiment and analyzed data. MS drafted the manuscript. KF did the statistical analysis. JR, KF and AW contributed with the manuscript corrections. All authors read and approved the final manuscript.

Funding

The Ewald and Hilde Berge Foundation (grant number 5132100501, Germany) supported this research. The foundation supported the study financially, but had no influence on data preparation or publication of data.

Availability of data and materials

The data analyzed during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The current study was evaluated by the local authority for ethics approval (Regional Board Giessen) and was determined not to require official or institutional ethical approval. Concerning sampling, a notification of the local ethics authority was performed via the animal welfare office of the Justus-Liebig-University Giessen (internal number of correspondence, IRB number: kTV 11-2018).

Blood sampling was performed on request of the owner, consent to participate in this study was given verbally.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Beagley JC, Whitman KJ, Baptiste KE, Scherzer J. Physiology and treatment of retained fetal membranes in cattle. J Vet Internal Med. 2010;24:261–268.
2. Boos A, Janssen V, Mülling C. Proliferation and apoptosis in bovine placentomes during pregnancy and around induced and spontaneous parturition as well as in cows retaining the fetal membranes. Reproduct. 2003;126:469480.
3. Boro P, Kumaresan A, Pathak R, Patbandha TK, Kumari S, Yadav A, Manimaran A, Baithalu RK, Attupuram NM, Mohanty TK. Alteration in peripheral blood concentration of certain pro-inflammatory cytokines in cows developing retention of fetal membranes. Anim Reproduct Sci. 2015;157:1116.
4. Coetzee JF, Lubbers BV, Toerber SE, Gehring R, Thomson DU, White BJ, Apley MD. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. Am J Vet Res. 2008;69:751762.
5. Gajewski Z, Thun R, Faundez R, Boryczko Z. Uterine motility in the cow during puerperium. Reproduct Dom Animal. 1999;34:185191.
6. Grunert Die gynäkologische Untersuchung. In: Ahlers D, Berchtold M, Grunert E, Kruif Ad (eds) Fertilitätsstörungen beim weiblichen Rind. 3rd edn. Parey, Berlin, 1999:29–61.
7. Heppelmann M, Weinert M, Ulbrich SE, Brömmling A, Piechotta M, Merbach S, Schoon HA, Hoedemaker M, Bollwein H. The effect of puerperal uterine disease on histopathologic findings and mRNA expression of proinflammatory cytokines of the endometrium in dairy cows. Theriogenology. 2016;85:13481356.
8. Jaureguiberry M, Giuliodori MJ, Mang AV, Madoz LV, Pothmann H, Drillich M, de La Sota RL. Short communication. Repeat breeder cows with fluid in the uterine lumen had poorer fertility. J Dairy Sci. 2017;100:30833085.
9. Kasimanickam RK, Kasimanickam VR, Olsen JR, Jeffress EJ, Moore DA, Kastelic JP. Associations among serum pro- and anti-inflammatory cytokines, metabolic mediators, body condition, and uterine disease in postpartum dairy cows. Reproduct Biol Endocrinol. 2013;11:103.
10. Lakomy M, Kaleczyc J, Majewski M, Sienkiewicz W. Peptidergic innervation of the bovine vagina and uterus. Acta Histochem. 1995;97:5366.
11. LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, Johnson WH. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy

cows. J Dairy Sci. 2002;85:22232236.

12. Mowa CN, Usip S, Storey-Workley M, Amann R, Papka R. Substance P in the uterine cervix, dorsal root ganglia and spinal cord during pregnancy and the effect of estrogen on SP synthesis. Peptides. 2003;24:761771.
13. Peter S, Michel G, Hahn A, Ibrahim M, Lubke-Becker A, Jung M, Einspanier R, Gabler C. Puerperal influence of bovine uterine health status on the mRNA expression of pro-inflammatory factors. J Physiol Pharmacol. 2015;66:449462.
14. Sheldon IM, Dobson H. Postpartum uterine health in cattle. Animal Reproduct Sci. 2004;82-83:295-306.
15. Stassi AF, Baravalle ME, Belotti EM, Rey F, Gareis NC, Diaz PU, Rodriguez FM, Leiva CJ, Ortega HH, Salvetti NR. Altered expression of cytokines IL-1 alpha, IL-6, IL-8 and TNF-alpha in bovine follicular persistence. Theriogenology. 2017;97:104112.
16. Thun R, Kundig H, Zerobin K, Kindahl H, Gustafsson BK, Ziegler W. Die Uterusmotorik des Rindes während Spätgravidität, Geburt und Puerperium. III. Anwendung von Flunixin Meglumin und endokrine Veränderungen. Schweiz Arch Tierheilk. 1993;135:333344.

Figures

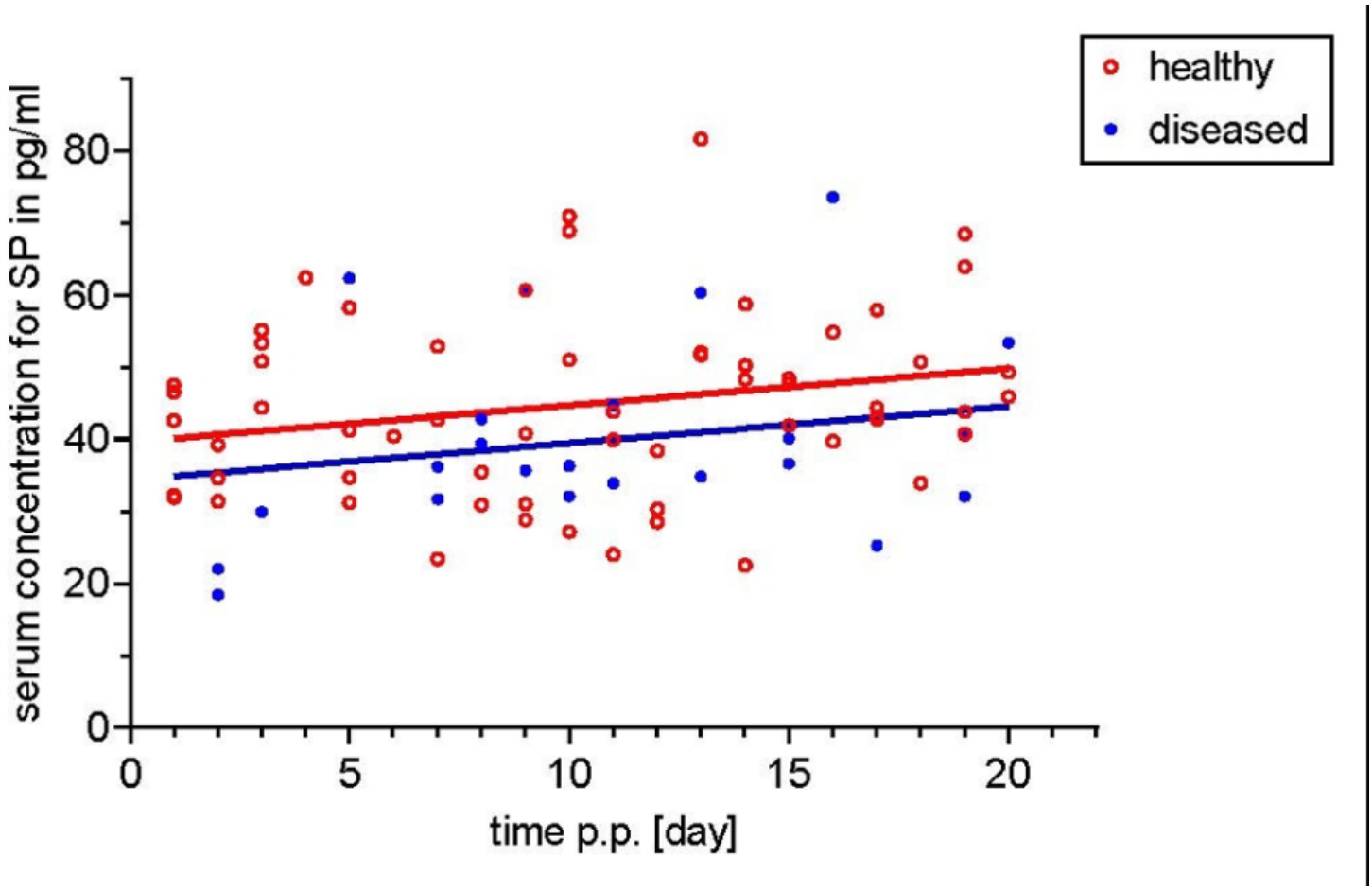

Figure 1

Comparison of serum concentrations of SP within the first 20 days after calving Regression lines for healthy (red) and diseased (blue) cows are given. Significant increase of SP concentrations over time for both groups is shown (P < 0.04).

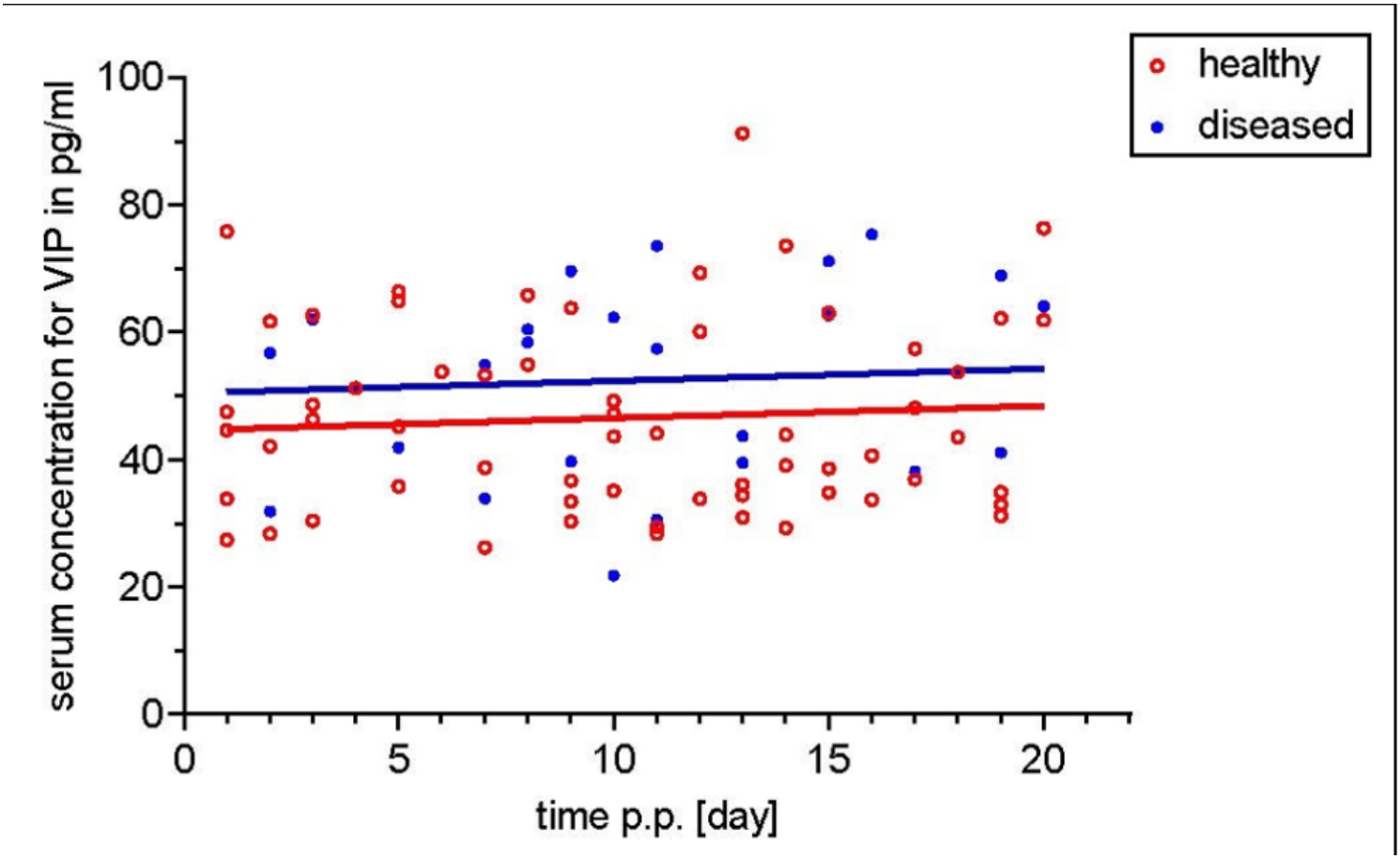

Figure 2

Comparison of serum concentrations of VIP within the first 20 days after calving No significant difference could be detected.

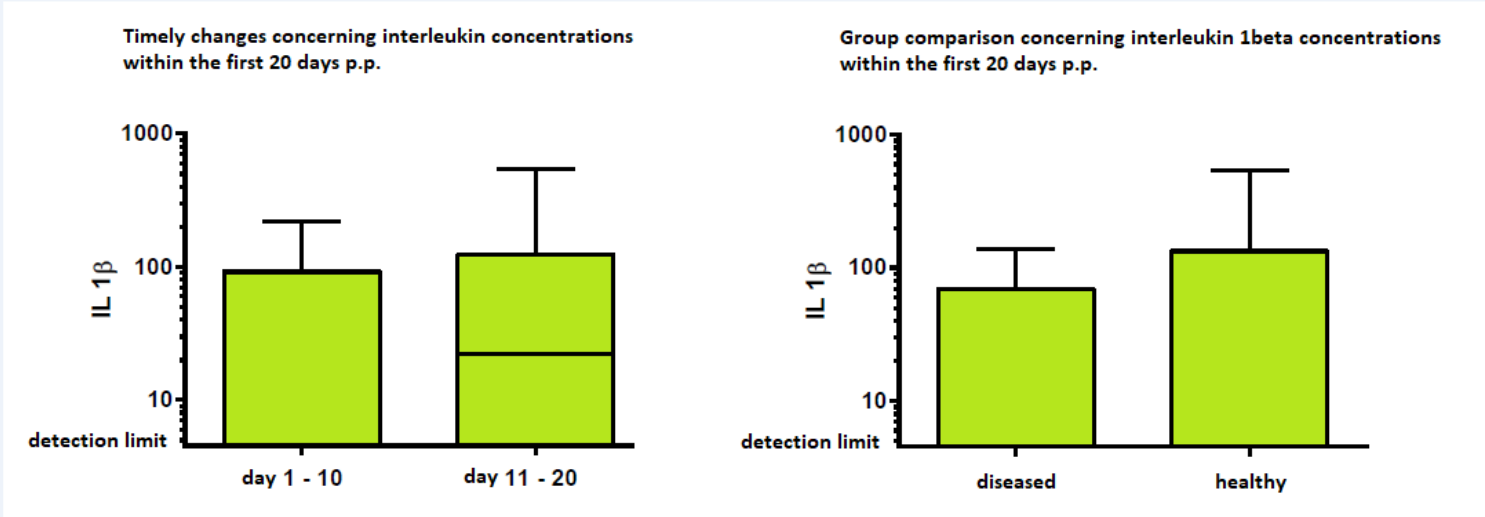

Figure 3

Il-1 β serum concentrations Left: IL-1β concentrations within the first 10 days after calving in comparison to days 11 to 20 after calving. Right: Group comparison between healthy and diseased cows within the first 20 days after calving. No statistically significant differences could be detected.