XIII MIDDLE EUROPEAN BUIATRIC'S CONGRESS
Best Western Hotel M, Belgrade, Serbia, June 5 - 8, 2013

CONGRESS PROCEEDINGS

ORANIZERS:
Serbian Buiatric's Association
Faculty of Veterinary Medicine

ENDORSEMENT:
Ministry of Education, Science and Technological Development

Congress Proceedings
XIII Middle European Buiatric's Congress
Best Western Hotel M, Belgrade, Serbia, June 5 - 8, 2013

All papers have been reviewed by the members of the scientific committee.

Publisher:	Serbian Buiatric's Association, Faculty of Veterinary Medicine, University of Belgrade
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Prepres and technical editor:	BBN Congress Management d.o.o., 9 Deligradska, Belgrade
Cover Design:	Marija Markovic
Printing Office:	Radunic, Belgrade
Circulation:	230

OVDE IDE ISBN.

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UDC: 636.234+618.56:611.018.52

PREPARTAL PLATELET COUNT AND FIBRINOGEN CONCENTRATION IN DAIRY COWS WITH AND WITHOUT RETAINED PLACENTA

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ABSTRACT

Although extensive data are available on leukocyte changes in prepartal period in cows with and without retained placenta, there is no data describing changes in platelet count. The aim of this study was to define changes in platelet count in cows, with and without retained placenta, three weeks before and one day after calving. Using fibrinogen level as a general reference to cow's health, we also tested the hypothesis that cows, that develop placenta retention in prepartal period, have a subclinical systemic inflammatory reaction. Blood samples taken from 14 Holstein cows were analyzed, three times in a week interval before and 24 hours after calving. After calving, cows were retrospectively divided, in the group without (n=9) and the group with retained placenta (n=5). Clauss method was used for determination of fibrinogen concentration and blood smear examination for assessment of platelet count. The results have shown that retained placenta is associated with increase in platelet count seven days before calving and decrease in platelet count 24 hours after calving comparing to precalving values. The week before calving group of cows, that developed retained placenta had platelet count significantly higher, comparing to control group of cows. Fibrinogen level was not significantly different between groups. The recorded increase in platelet count, one week before calving can be related to unidentified stress factors, and decrease of platelet count 24 hours after calving, can be explained by their increased consumption, due to activation of haemostatic mechanisms after placenta abruption.

KEY WORDS: cattle, fibrinogen, platelets, prepartal period, stress

INTRODUCTION

Retained placenta is a common problem in dairy cow farms and leads to major economic losses. Numerous studies have shown an association between retained placenta and twin pregnancy, cow's age, cow's body mass index and size of the calves (Laven and Peters, 1996; Gaafar et al., 2011). The prepartal risk factors that lead to disorders associated with retained placenta, also include some infective diseases and disorders of the immune system caused by deficiency of different nutritional factors (rev: Bealey et al., 2010). Unfortunately, in clinical conditions, individuals that will develop retained placenta cannot be recognized in advance. Our previous results showed that, total and differential leukocyte count, three weeks before calving and 24 hours after calving, were the same in cows with and without retained placenta (Lužajić et al., 2013, unpublished results). The only difference was a slight prepartal increase in segmented

neutrophil count in the group of cows, that developed retained placenta, comparing with cows without this disorder (Lužajić et al., 2013, unpublished results). However, in cattle, total leukocyte and differential counts are not good indicators of systemic reaction to tissue damage or stress (Nemi, 1993).

Deviation of platelet count is the most common hematological abnormality in veterinary medicine, yet it has never been assessed in dairy cows during peripartal period. In this study, we described changes in platelet count in cows, with and without retained placenta, three weeks before and 24 hours after calving. Using fibrinogen level, as a general reference to cow's health, we also tested the hypothesis that cows, that develop placenta retention in prepartal period, have a subclinical systemic inflammatory reaction.

MATERIALS AND METHODS

The study was performed on 14 Holstein dairy cows during the summer period. The animals were kept outdoors until the calving. They were 3 to 9 years old, with a uniform average annual milk yield. All animals were clinically examined and had no signs of disease a month before parturition. After calving, retained placenta was diagnosed in five cows (experimental group) and nine cows had puerperium without visible problems (control group). Blood was sampled three times before parturition, in a week interval, before the expected date of calving, and once again 24 hours after calving. To determine the concentration of fibrinogen, blood samples were obtained by puncture of *v.coccigea* and collected in tubes containing 3.2% Na-citrate (Vacuette, Greiner Bio-One). Blood smears were made immediately after sampling and stained with Hemacolor® (Merck). Fibrinogen concentration was determined by Clauss method (Clauss, 1957) and the platelet count was estimated from blood smear (Harvey, 2001). Statistical analysis was conducted using Student's t-test and analysis of variance for repeated measures (Repeated Measures ANOVA) with application of the Greenhouse-Geisser test.

RESULTS

Control group of cows had no variation in platelet count before and after calving (Table 1, Figure 1A). In the experimental group, the platelet count increased during prepartal period reaching a nadir one week before calving and significantly decreased after calving (Table 1, Figure 1A). Change in the platelet count in time, within the cows in the experimental group, was statistically significant (Table 2). Also, change between groups occurred in significantly different manner (Table 2). Platelet count was significantly lower in experimental group of cows three weeks and significantly higher one week before calving (Table 1).

Table 1. The platelet count and fibrinogen levels in cows from the control (n = 14) and experimental group (n = 5)

Variable and group	21 days before calving	14 days before calving	7 days before calving	24 hours after calving
Platelets ($x10^9$/L)				
Control group	452.89±79.39	440.89±135.60	448.00±69.66	431.56±97.91
Experimental group	288.00±82.66*	391.20±126.31	572.80±131.74*‡	365.60±145.54
Fibrinogen (g/L)				
Control group	3.43±1.07	3.93±0.88	3.28±0.57‡	4.87±1.63
Experimental group	3.24±0.56	4.60±1.17	3.82±1.89	4.54±2.54

NOTES: All values are given as mean ± standard deviation;

* - $p<0.01$ in comparison with control group.

‡ $p<0.01$ in comparison with mesurment after calving

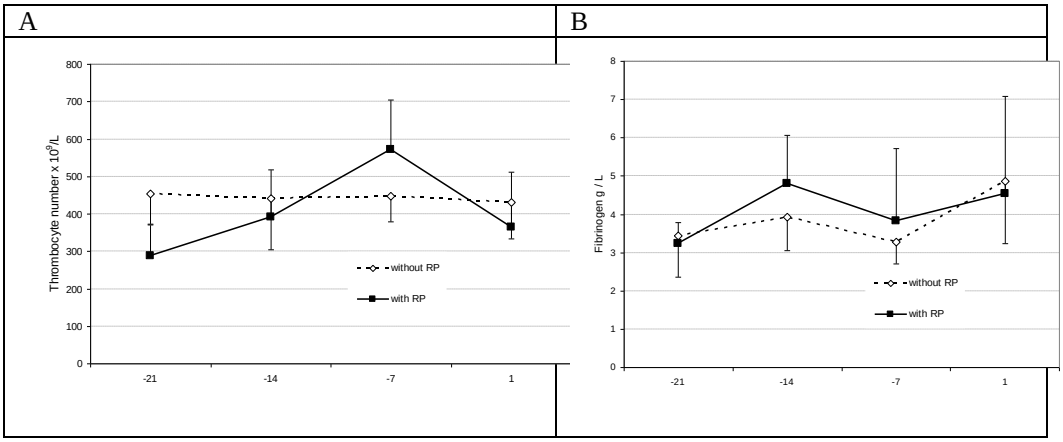

Figure1. A and B. Changes in the platelet count and fibrinogen level before and after calving

Fibrinogen levels, during prepartal period, were similar in both groups of cows, yet the values were slightly higher in the experimental group (Table 1, Figure 1B). Significant increase in fibrinogen level has been observed in the control group of cows one day after calving, in comparison with levels one week before calving (Table 1). Analysis of variance for repeated measures, confirmed that in the control group, these changes were statistically significant (Table 2).

Table 2. Assessment of the impact of time and group on the investigated characteristics

Variable and type of effect	F	P	Partial Eta Squared
Platelets			
Within subjects effects of time in control group	0.075	0.922	0.009
Within subjects effects of time in experimental group	4.336	**0.047**	0.520
Within subjects effects of time in all cows	3.808	**0.036**	0.241
Within subjects effects of interaction time*group	3.751	**0.038**	0.238
Between subjects effects of group	2.109	0.172	0.150
Fibrinogen			
Within subjects effects of time in control group	4.473	**0.023**	0.359
Within subjects effects of time in experimental group	0.758	0.433	0.174
Within subjects effects of time in all cows	3.411	**0.046**	0.221
Within subjects effects of interaction time*group	0.540	0.601	0.043
Between subjects effects of group	0.144	0.711	0.012

NOTE: * - $p<0.05$

DISCUSSION

Our results evidenced that retained placenta cows had an increase in platelet count a week before calving. It was previously shown that pro-inflammatory cytokines stimulate increase in platelet count in humans (rev: Klinger and Jelkmann, 2002) and some animal species (Sellon et al., 1997, Hammer, 1991; Neel et al., 2012). Corticosteroids also enhance platelet count (Mandell, 2000). In our experimental group, prepartal increase in platelet count, as well as slight increase in segmented neutrophils, (Lužajić et al., 2013, unpublished results) can be related to unidentified stress factors. Response to stress includes hypothalamic - pituitary - adrenal cortex axis activation and leads to rise in glucocorticosteroids and possible

enhancement of thrombopoesis. Simultaneously, glucocorticosteroids prevent the transendothelial migration of neutrophils (Vlahos et al., 2012; Mandell, 2000). As the prepartal fibrinogen concentration was in reference range and was not significantly different between two groups before calving, we can conclude that subclinical inflammation was not present in that period. However, inclusion of more sensitive biomarkers (Eckersall and Bell, 2010) could be more reliable method for subclinical inflammation diagnostics.

Our results also showed that 24 hours after calving, platelet count decreased in a significant manner, when animals develop retained placenta. These data suggest that, platelets are activated and rapidly consumed in cows with retained placenta. In addition, 24 hours after birth, in the control group, fibrinogen concentration increased significantly, and was equal to the concentration of fibrinogen in the group with retained placenta. These data, as well as, data showing significant increase in the number of band neutrophils 24 hours after calving, (Lužajić et al., 2013, unpublished results) as well as, increase in globulin concentrations, and a decrease in the total number of leukocytes, albumin and iron, (Katić, 2011) suggests that, tissue damage during calving, induce a systemic inflammatory response in all cows.

Based on all previous results, we consider, that the recorded increase in platelet count, one week before calving, in cows that develop retained placenta, can be related to unidentified stress factors, and decrease of platelet count 24 hours after calving, can be explained by their increased consumption, due to activation of haemostatic mechanisms after placenta abruption.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science, Republic of Serbia (grant No. 175061)

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