Polymorphisms In The Leptin Receptor Gene Are Associated To Change In Immunological Parameters In Dairy Cows During Peripartum

F. Napolitano[*], G. De Matteis[*], G. Catillo[*], B. Moioli[*] and F. Abeni†

Introduction

In the dairy cow, the transition from the pregnant, nonlactating state to the nonpregnant, lactating state is often a critical experience. In particular parturition and the onset of lactation impose physiological challenges to the homeostatic mechanisms.

To reduce disease incidence, the maintenance of a strong immune system is required (Goff and Horst, 1997). It is well known that the incidence of bovine infectious diseases such as mastitis or puerperal infections is high during the peripartum period, which is characterized by a reduction in immunological defenses, mainly due to impairment in neutrophil cells function, by impairing the ability to migrate into the infected tissue and increasing neutrophil count in the peripheral blood (Sordillo and Streicher (2002); Tempelman, R.J., Saama, P.M., Freeman, A.E. *et al*. (2002)). Beside neutrophil function, also lymphocytes blastogenesis appears to be affected during peripartum in dairy cows (Ishikawa, 1987).

Leptin is primarily known for its role in the regulation of whole-body energy balance, and it exerts effects also on reproductive and immune system. In Holstein cows, Liefers, S.C., Veerkamp, R.F., te Pas, M.F.W. *et al*. (2004) associated polymorphisms in the leptin gene receptor (***LEPR***) to leptin concentration during late pregnancy, while Brickell, J.S., Pollott, G.E., Clempson, A.M. *et al*. (2010) associated polymorphisms in the leptin gene (***LEPT***) to perinatal mortality.

In the current study, 67 Holstein cows were submitted to analysis of white blood cells (WBC), neutrophil (NEU) and lymphocytes (LYM), during six weeks around calving, and were genotyped at six markers within the leptin receptor gene.

Material and methods

Sampling. Whole blood samples from 67 Holstein cows were collected 6 times during peripartum and submitted to haematological parameter analysis. In detail, blood collection was performed every week starting from the third week before calving to the third week after calving. The haematological profile was determined on blood samples collected in K_3EDTA treated tube, using a Cell-Dyn 3700 haematology analyzer (Abbott Diagnostici, Roma, Italy). Measurements were: total white blood cells number (WBC; k/µl); neutrophils (NEU; k/µl) and lymphocytes (LYM; k/µl).

PCR amplification and detection. DNA of the 67 cows, extracted from 5 ml whole blood with the Genomix kit (Talent), was PCR amplified using 5 primers pairs (table 1), encompassing part of the coding sequence of ***LEPR*** (Accession NW_001494806).
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[*] CRA-PCM via Salaria 31, 00015 Monterotondo, Italy
† CRA-FLC via Porcellasco 7, 26100 Cremona, Italy

Table 1. Primers used for PCR amplification of the *LEPR*.

Primers (Forward and Reverse)	T.a.	bp	Covered region
5' GGGATTGTAGGGATGGTCCT 3' 5' TTTTGCAACATGGTTTTGGA 3'	55°C	499	Promoter
5' AACTTCCATCTCCGGCTTG 3' 5' GGAGGATGTATTTTTATGCCAGT 3'	58°C	407	Exon 10
5' CGATGAAATCAGAAAAGGGTATG 3' 5' TGCAGTGTGGATTCCTGAAA 3'	58°C	353	Intron 15 Exon 16
5' CACACTTCCATTTCTTCCATGA 3' 5' TGGTGGAGAATTGTTGCTCA 3'	58°C	485	Exon 18
5' GGTGAAACTGAGGAGGAGCA 3' 5' TGTTTTCTTTTCTACTCTCCTAACCA 3'	63°C	607	Exon 18

Amplicons were submitted to direct sequencing on a PerkinElmer ABI Prism 310 DNA sequencer.

Statistical analyses. SNPs substitution effect were estimated for each time of peripartum by regressing the value of haematological parameters on the number of copies of each allele using the following general linear model (GLM procedure; SAS Institute, Inc. (2006)):

$Y_{ijklmn} = \mu + H_i + S_j + OP_k + DD_l + G_m(DD_l) + E_{ijklmn}$

in which Y_{ijklmn} is the haematological trait; μ is the overall mean; H_i is the fixed effect of herd (i= 1...3); S_j is the fixed effect of the calving season: autumn, winter, spring (j= 1...3); OP_k is the fixed effect of the calving group: primiparous and multiparous (k=1,2); DD_l is the fixed effect of the peripartum week, where the blood sample was collected (l= 1.....6); G_m is the allele substitution effect of either each single SNP; E_{ijklmn} is the residual random error associated with the individual observation.

Results and discussion

In table 2 we report the position of the detected SNP on the corresponding GenBank accession, the position in the gene and the frequency of the mutated allele in our sample. Three of the SNP (*g.183395 A>G; g.195382 C>T; g.209779 C>T*) were already described in GenBank. The remaining SNP (*g.134260 C>T; g.134261 G>C ; g.195295 C>A; g.210413 A>C*) were detected during the present research. WBC and NEU have a similar trend during the whole considered period (figure 1) and show a decrease in number in the two weeks after calving, where a significant allelic substitution effect was evident for SNP *g.210413 A>C*, with cows carriers of allele *C* having a lower number of both WBC (-1.83 and -1.76; $P<0.01$) and NEU (-1.09 and -1.17; $P<0.05$) respectively at the two times. This is a novel here detected SNP, located in exon 18 of ***LEPR***, but giving a synonymous mutation. WBC and NEU count, during peripartum, can be used as indicators of mastitis incidence. In fact, Kulberg, S., Storset, A.K., Heringstad, B., *et al.* (2002) studied two groups of Norwegian

cows, obtained through a selection programme for mastitis resistance, and observed that the low clinical mastitis group showed also a significant decrease in WBC and NEU in blood.

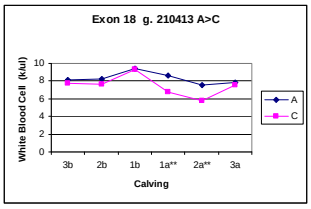
Table 2. Genotyped SNP in the *LEPR*

SNP	Accession	Gene position	Allele frequency, %
g.134260 C>T	NW_001494806	Promoter	C 84.9 T 15.1
g.134261 G>C	NW_001494806	Promoter	G 89.5 C 10.5
g.183395 A>G	rs43349293	Exon 10	G 94.9 A 5.1
g.195295 C>A	NW_001494806	Intron 15	A 83.6 C 16.4
g.195382 C>T	rs43349279	Exon 16	T 65.1 C 34.9
g.209779 C>T	AJ580801	Exon 18	C 89.9 T 10.1
g.210413 A>C	NW_001494806	Exon 18	A 92.4 C 7.6

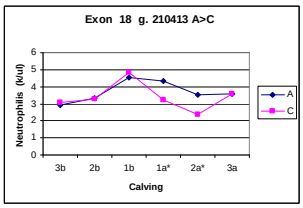
SNP position was assigned in the current bovine genome assembly (Btau 3.0, GI:194665724)

Allelic substitution effect of SNP *g.183395 A>G* and *g.195295 C>A* on LYM is similar (figure 1), with a lower LYM count (P<0.05-P<0.01) in the cows carriers of allele g.183395 G (-0.78; -0.70 and -0.91) and g.195295 A (-0.49; -0.34 and -0.26) during three weeks before calving, respectively . Allelic substitution effect of SNP *g.195382 C>T* is significant from the third to the second weeks before calving, with cows carriers of allele C showing a decrease in LYM by -0.29 (P<0.05) and -0.35 (P<0.01) respectively at the two times. This finding is interesting because the bovine endometrium is infiltrated with lymphocytes and antigen-presenting macrophages, which may be important for the recognition and processing of foreign antigens, including pathogenic bacteria entering the uterus (Singh, J., Murray, R.D., Mshelia, G. *et al*. (2008)). Moreover, Mallard, B.A., Dekkers, J.C., Ireland, M.J. *et al*. (1998) report that altered lymphocyte responsiveness around calving is linked to increased mastitis susceptibility.

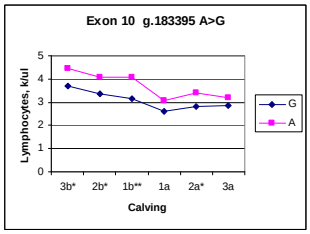
a – Trend of WBC

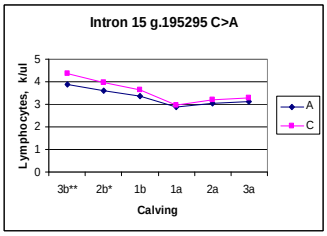


b - Trend of NEU



c - Trend of LYM





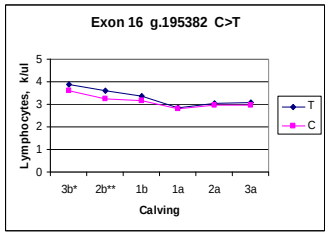


Figure 1. Allelic substitution effect of the analysed SNP on the trend of the haematological parameters during peripartum

Conclusion

In this study, we used WBC, NEU and LYM count as measure of the immunological state of dairy cows during peripartum. The novel detected SNP (*g.210413 A>C*) in exon 18 of ***LEPR***, because associated to a lower WBC and NEU count, might be proposed as potential marker for infectious disease resistance. Moreover, we could associate three further SNP (*g.183395 A>G*; *g.195295 C>A* and *g.195382 C>T*) with LYM count, so to allow to assessing the ability of the cow to respond to pregnancy and lactation stressors, and prevent disease incidence.

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