Histocompatibility genes and Somatic Cell Count (SCC) in Italian Holstein Friesian

M. Zanotti[1], M.G. Strillacci[1], I. Taboni[1], A.B. Samorè[2], M. Longeri[1]

[1] Istituto di Zootecnica – Facoltà di Medicina Veterinaria – Università di Milano, Italy.
[2] Associazione Nazionale Allevatori Frisona Italiana – Cremona, Italy.

RIASSUNTO – Geni di istocompatibilità e conta delle cellule somatiche (SCC) nella Frisona Italiana – *Lo studio ha considerato l'effetto del Complesso Maggiore di Istocompatibilità (MHC) sulla mastite, clinica e subclinica, utilizzando l'Indice Genetico (I.G.) per la conta delle cellule somatiche: SCC (Somatic Cell Count). Su un totale di 302 tori di razza Frisona Italiana, valutati geneticamente per le cellule somatiche, sono stati analizzati il polimorfismo degli antigeni di istocompatibilità di classe I (test di microlinfocitotossicità* locus *BoLA-A) e di classe II (PCR/RFLP del* locus *DRB3 esone 2). L'effetto degli antigeni di istocompatibilità sugli indici genetici è stato valutato con un modello di sostituzione genica.*

KEY WORDS: mastitis, SCC, MHC, cattle.

INTRODUCTION – Mastitis is a dairy cattle disease leading to great economic losses in milk production, management costs and veterinary treatments. This character generally shows an unfavourable genetic correlation with milk production. However, genetic factors influencing mastitis susceptibility independent from those influencing milk production could exist. Therefore, it should be possible to select at the same time against mastitis and for high milk production. The "somatic cell count" (SCC) can be used for selection against mastitis due to high genetic correlation with mastitis (Weller *et al.*, 1992), higher heritability than clinical mastitis, lower costs for data collection and data availability at national level with an objective measurement. Genetic evaluation for "somatic cell count" has been recently officially introduced also in Italy for Holstein Friesian (Samorè *et al.*, 2001) and can be also used to find candidate genes associated with mastitis resistance to be applied in a maker assisted selection (MAS) or in the choice of bulls to be progeny tested. In the recent period, several trials focused on Major Histocompatibility Complex (MHC) as candidate gene. In the last 20 years, several authors found some associations with class I antigens, in discrepancy among themselves. Similarly, genes for resistance/susceptibility to mastitis within MHC class II genes have been recorded. Recently Dietz *et al.* (1997), Kelm *et al.* (1997) and Sharif *et al.* (1998), correlating SCC and polymorphism of DRB3 exon 2 (which corresponds to the most expressed and polymorphic class II molecule in the immune system cell membrane), found a statistically significant association, but in clear contrast between them, for the same allele. Results so far obtained suggested that histocompatibility antigens really affect the disease. The present work deals for the first time in Italy with the identification of a class I and class II antigen polymorphisms inside Major Histocompatibility Complex (MHC) which could affect bull genetic indexes for milk somatic cell count (SCC).

MATERIAL AND METHODS – Sample consisted of DNA extracted, according to the standard procedures, by peripheral blood or semen of 302 Italian Holstein Friesian bulls with genetic index for SCC calculated by A.N.A.F.I. according to a "Test Day Repeatability Animal Model". Class II DRB3.2 typing was carried out by PCR-RFLP technique (Van Eijk *et al.* 1992) on 302 bulls. HYPERLINKClass I antigen typing was carried out by microlymphocytotoxicity test (Spooner *et al.* 1979) using a panel of 95 reagents detecting 25 BoLA-A specificities on 196 animals. BoLA polymorphism analyses were based on methods internationally accepted by the BoLA Nomenclature Committee (http://www.ri.bbsrc.ac.uk/bola/classii.htm).

Statistical analysis to verify the effect of MHC alleles on SCC genetic indexes has been carried out using the Proc Reg of SAS software package. The effect of each haplotype has been estimated as multiple regression coefficient referred to the mean. Alleles showing <5% frequencies have been grouped in the class "others" (Zanotti *et al.*, 1995).

RESULTS AND CONCLUSIONS – By regression analysis, considering DRB3.2 polymorphism, alleles 8 and 22 showed a significant negative effect and allele 16 a significant positive effect on the SCC parameter (Tab.1).

Table 1. Regression analysis (P>F=0.0003; R-square 0.098): effects of DRB3 exon 2 PCR-RFLP haplotypes (=Allele) on SCC genetic indexes.

Allele	Estimate	Standard Error	P>T
3	0.060	0.166	0.716
7	-0.034	0.235	0.884
8	**-0.484**	**0.159**	**0.002**
11	-0.003	0.221	0.987
16	**0.344**	**0.138**	**0.013**
22	**-0.352**	**0.104**	**0.0009**
23	-0.136	0.168	0.418
24	-0.041	0.115	0.723
27	0.370	0.217	0.089

Considering class I polymorphism alleles 15 and 20 showed significant negative effect (Tab.2). In addition, Dietz *et al.* 1997, Kelm *et al.* 1997, Simon *et al.* 1993 and Sharif *et al.* 1998 found a positive effect on SCC of DRB3.2 allele 16, while for class I our data do not confirm previous results. Nevertheless to evaluate the susceptibility/resistance to the disease, Ashwell *et al.* (1996) were the only authors considering bull genetic indexes. In most cases the presence vs. absence of clinical mastitis in cows or SCC phenotypic level have been utilised.

The use of Bull Genetic Indexes, when compared to cow phenotypic data, present the advantage of having higher reliability and of accounting for environmental and mating factors. Nevertheless, since whole male population standard deviation (S.D.=0.99) was greater than estimate values of significant alleles, it is not possible to state a major gene effect. The discrepancies observed among results of different authors and of the present study can be ascribed to the different populations studied, to the methods used for SCC genetic index determination and to the different species of pathogens causing the disease. So far no study on this matter has been carried on Italian Holstein and, considering the availability of bull genetic indexes for SCC, we plan to use them in linkage studies for candidate major genes, also with more informative models as Grand Daughter Design.

Table 2. Regression analysis (P>F= 0.001; R-square 0.136): effects of BoLA class I haplotypes (=Allele) on SCC genetic indexes.

Allele	Estimate	Standard Error	P>T
A6	0.058	0.125	0.639
A619	0.210	0.212	0.321
A10	-0.029	0.164	0.856
A11	0.009	0.166	0.956
A12	**0.241**	**0.127**	**0.059**
A13	-0.153	0.149	0.308
A14	0.095	0.135	0.484
A15	**-0.339**	**0.115**	**0.003**
A20	**-0.425**	**0.109**	**0.0001**
Others	0.332	0.363	0.362

ACKNOWLEDGEMENTS – This research was supported by Italian MURST 40% - Cofin 2001. We want to thank ANAFI, LGS and Lazzaro Spallanzani for sample collection.

REFERENCES – **Ashwell**, M.S., Rexroad, C.E., Miller, R.H., VanRaden, P.M., 1996. Animal Genetics 27:235-242. **Aarestrup**, F.M., Jensen, N.E., Ostergard, H., 1995. Journal of Dairy Science 78:1684-1692. **Dietz**, A.B., Cohen, N.D., Timms, L., Kehrli, M.E., 1997. Journal of Dairy Science 80:406-412. **Van Eijk**, M.J.T., Stewart-Haynes, J.A., Beever, J.E., Fernando, R.L., Lewin, H.A., 1992. Immunogenetics 37:64-68. **Kelm**, S.C., Detilleux, J.C., Freeman, A.E., Kehrly, M.E., Dietz, A.B., Fox, L.K., Butler, J.E., Kasckovis, I., Kelley, D.H., 1997. Journal of Dairy Science 80:1767-1775. **Samorè**, A.B., Bagnato, A., Canavesi, F., Biffani, S., Groen, A.F., 2001. Proc. 14th Nat. Cong. ASPA, 2:22-24. **Sharif**, S., Mallard, B.A., Wilkie, B.N., Sargeant, J.M., Scott, H.M., Dekkers, J.C.M., Lesile, E., 1998. Animal Genetics 29:185-193. **Simon**, M., Dushinski, R., Shtyavikova, R., Dusinsky, M., Stavikova, M., 1993. Genetika 29(10):1681-1684. **Weller**, J.I., Saran, A., Zeliger, Y., 1992. Journal of Dairy Science 75:2532-2540. **Zanotti Casati**, M., Longeri, M., Polli, M., Ceriotti, G., Poli, G., 1995. Journal of Animal Breeding and Genetics 112:391-400.