MHC IMMUNOGENETICS INFLUENCES THE REPRODUCTION IN SHEEP

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ABSTRACT

Experiment was conducted in four consecutive lambings to establish feto-maternal immune response in a sheep flock of 639 animals. A total of 16 DRB_1 microsatellite alleles[3], ranging between 353 and 857 bp were detected which were associated with variable reproductive performances among males and females. Ewes carrying 411 bp allele exhibited higher values for all recorded fertility traits ($p<0.001$), followed by 386 bp allele whereas 389 bp allele had negative values in all fertility traits. The associations of different alleles with variable reproductive traits in sheep could be individual variability to humoral immune response, cell recognition or tissue differentiation between carriers of various MHC genotypes. The observed associations within DRB_1 microsatellite locus of Ovine MHC in German Merino sheep may be used as a molecular marker for identifying QTL in genetic improvement of the sheep and also quantifying the MHC Immunogenetic influences in the reproduction in sheep.

Key words: Sheep, DRB_1, Microsatellite, Fertility, QTL

INTRODUCTION

The highly polymorphic genes of ovine Major Histocompatibility Complex (Ovar) have an important adaptive significance with respect to conferring parasite resistance (Coltman *et al.,* 2001), general vitality (Rupp *et al.,* 2007), mate choice and preference (Penn *et al.,* 2002, Milinski 2003) and maternal-fetal interactions (Ober *et al.,* 1998). MHC genes are identified as class I, class II and class III, with genes of class II being the most variable genes among vertebrates (Stear *et al.,* 2005). Within intron 2 of DRB_1 gene, a microsatellite is found, which is used to investigate the genetic variation of the DRB gene within class II MHC gene group (Ammer *et al.,* 1992). Since different MHC molecules bind diverse peptides, MHC heterozygous hosts can present a greater variety of peptides, and hence defend themselves against a larger variety of pathogens compared with MHC homozygous individuals (Penn *et al.,* 2002). It has been shown that MHC heterozygosity correlates positively with reproduction and diseases (Jeffery *et al.,* 2000, Moore *et al.,* 2002) as various immunological processes are involved in mammalian reproduction (Jin *et al.,* 1995, Ober *et al.,* 1998, Van *et al.,* 2000).

The immunogenetic fundamentals of this phenomenon remain unclear but hypothesis based on the influences of genes linked to the MHC like pre-implantation embryo development gene (PED) (Warner *et al.,* 1987, Warner *et al.,* 1991), T/t associated genes (Ho *et al.,* 1994) or varied maternal fetal interactions caused by MHC antigens (Beer *et al.,* 1985, Roy *et al.,* 1999) have been proposed.

Very little information is presently available with respect to the immunogenetic influence of MHC on the reproduction in sheep. With this experiment, attempt has been made to identify immunogenetic factors for improved fertility in sheep.

MATERIALS AND METHODS

A total of 07 rams, 249 ewes and 381 lambs maintained at research station, University of Hohenheim, Stuttgart Germany, were used in this study. Young, virgin female sheep were mated with the same ram for two consecutive periods in order to establish feto-maternal immune response. After two pregnancies ewes were taken out of the experiment. 10 ml blood or spleen samples were collected from live and dead

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animals and the respective DNA was extracted. The microsatellite in intron 2 of the DRB_1 gene was amplified as per the standard protocol (Griesinger *et al.,* 1999) by the polymerase chain reaction (PCR) using the following primers:

5'GGGGGATCCGCTTCGACAGCGACTGGGGCG3' and 5'CTGACCCAGAKTGAGTG AAAGTATC3' (K= G or T). Separation of the PCR product for fragment length analysis was carried on Automated Laser Fluorescent (ALF) sequencer (Pharmacia, Germany) and analysis of the data for fragment lengths was carried with the ALF-Fragment Manager Evaluation software in relation to external standards. During the research period, fertility parameters were recorded for rams and ewes and fed to a data base. Complete particulars with respect to fertility trait and genotype information of DRB_1 microsatellite from all animals were recorded and were analyzed for association between the DRB_1 microsatellite alleles of rams and ewe with fertility trait. ANOVA analysis was done with General Linear Models (GLM) of the statistical package SAS (1994).

RESULTS AND DISCUSSION

Based on the length of amplified fragment, 16 alleles and 92 genotypes were detected in the experimental flock at this locus (Table). Significant difference ($p<0.05$) was found in the pregnancy status for ram 1 (389/411) {low value} in comparison to ram 6 (405/420) {high value}. However, for data on pregnant ewes, ram 1 differed positively ($p<0.01$) in lambs born comparing to all other rams except the ram 7. DRB_1 microsatellite genotypes of the ewes were highly associated with the fertility traits of the ewes ($p<0.01$ to $p<0.08$). The genotype classes of 380/411 and 386/405 were associated with high numbers of lambs born and the genotype classes 383/383, 394/405 and 405/455 were negatively associated with pregnancy status and lambs weaned. In pregnant ewes, genotype classes 374/411 and 380/411 of ewes showed positive effects (i.e. increased) on number of lambs weaned, whereas genotype classes 383/383 and 389/411 were observed with negative effects. The genotype class 383/383 of ewes had considerable negative effects (i.e. decreased) in all fertility traits. The effects of genotypes with allele 394, allele 411 and the residual allele have been analyzed in ewes and it was shown that genotype with above mentioned alleles have strong positive effects on the fertility traits in ewes as well as in rams, with slight superior fertility associated with allele 411 than allele 394. Within mated ewes, the classes of genotypes 411/411 and 411/rest were superior to the residual class in pregnancy status, lambs weaned and lambs born. The genotype classes containing the allele 411 (394/411, 411/rest, 411/411) were associated with higher values of lambs born and lambs weaned than the class 394/rest and the residual genotype class (Figure). Significant association between some DRB_1 microsatellite alleles and fertility traits in German Merino sheep has been observed in this study, as reproduction is governed by heterogeneous immunological signaling between dam and fetuses including the synergistic effects from disease resistance, partly controlled by MHC genes. It was found in our results that heterozygotes of DRB_1 microsatellite had better fertility values than homozygotes although the difference was not at significant level. In contrast to clinical organ transplantation where compatibility is beneficial, an intense selection pressure against fetuses that are compatible with their mothers at Major Histocompatible Complex loci have been observed in inbred animals. However, Melnick *et al.,* (1981) did not found any evidence of hybrid vigor or histoincompatibility on the outcome of fetal loss, though, allelic variants at or near H2 complex confer some selective advantage as measured by fetal survival and fetal growth in mice. Furthermore, concept of immunotrophism is advocated for increase of pregnancy successes in case of heterozygous matings (Aguilar *et al.,* 1997). At the fetal membranes, MHC-G is the only antigen found in most of the animals which is recognized by the mother and is important for the production of blocking antibodies (Ober and Van Der Ven 1997). Excess of "blocking antibodies" are produced in response to heterozygous state at MHC, which in turn leads to increased production of cytokines responsible for successful pregnancy. Improved picture of fertility in heterozygotes in this study may be due to less sharing of DR and DQ loci within MHC, which otherwise cause adverse immunological sensitization if male and female have similarity at these loci (Kostyu 1994).

The results of the present study clearly demonstrate that the survival of fetal allograft in mammals is influenced by genes of MHC and maternal tolerance of an allogenic fetus is a paradox and remains a central theme in current reproductive immunological research.

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Table : Allele ([1]) / Genotype ([2]) frequencies of DRB$_1$ microsatellite locus

(bp)	Parents (n)	%	Offspring (n)	%	Total (n)	%
Allele frequencies						
353	15	3.0	12	2.3	27	2.6
374	16	3.2	7	1.3	23	2.2
380	21	4.2	14	2.7	35	3.4
383	69	13.7	57	10.8	126	12.2
386	28	5.6	14	2.7	42	4.1
389	26	5.2	20	3.8	46	4.5
394	72	14.3	74	14.0	146	14.1
400	18	3.6	5	0.9	23	2.2
405	65	12.9	85	16.1	150	14.5
411	102	20.2	130	24.6	232	22.5
420	2	0.4	11	2.1	13	1.3
430	3	0.6	7	1.3	10	1.0
443	7	1.4	4	0.8	11	1.1
455	26	5.2	36	6.8	62	6.0
803	13	2.6	46	8.7	59	5.7
857	21	4.2	6	1.1	27	2.6
Genotype frequencies						
383/383	10	4.0	6	2.3	16	3.1
383/394	3	1.2	8	3.0	11	2.1
383/405	15	6.0	10	3.8	25	4.8
383/411	15	6.0	8	3.0	23	4.5
389/411	8	3.2	5	1.9	13	2.5
394/405	11	4.4	18	6.8	29	5.6
394/411	18	7.1	18	6.8	36	7.0
405/411	2	0.8	26	9.8	28	5.4
405/803	3	1.2	8	3.0	11	2.1
411/411	15	6.0	16	6.1	31	6.0
411/455	3	1.2	12	4.5	15	2.9
411/803	2	0.8	12	4.5	14	2.7

1. Allele number less than 2 not mentioned in the table
2. Genotype number less than 2 not mentioned in the table

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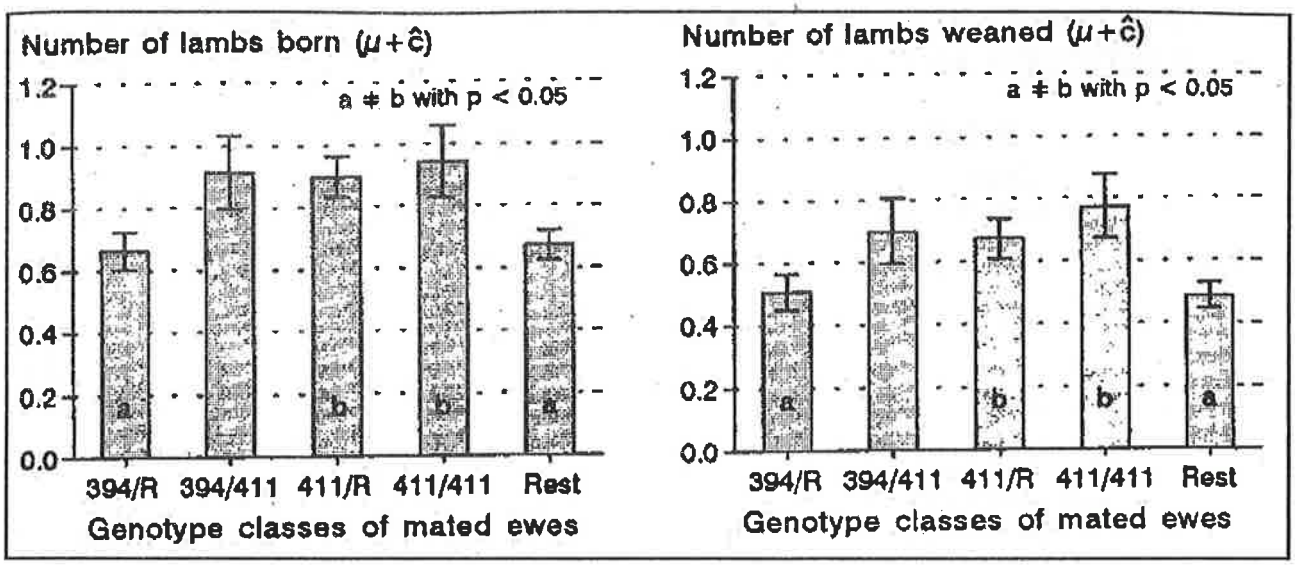

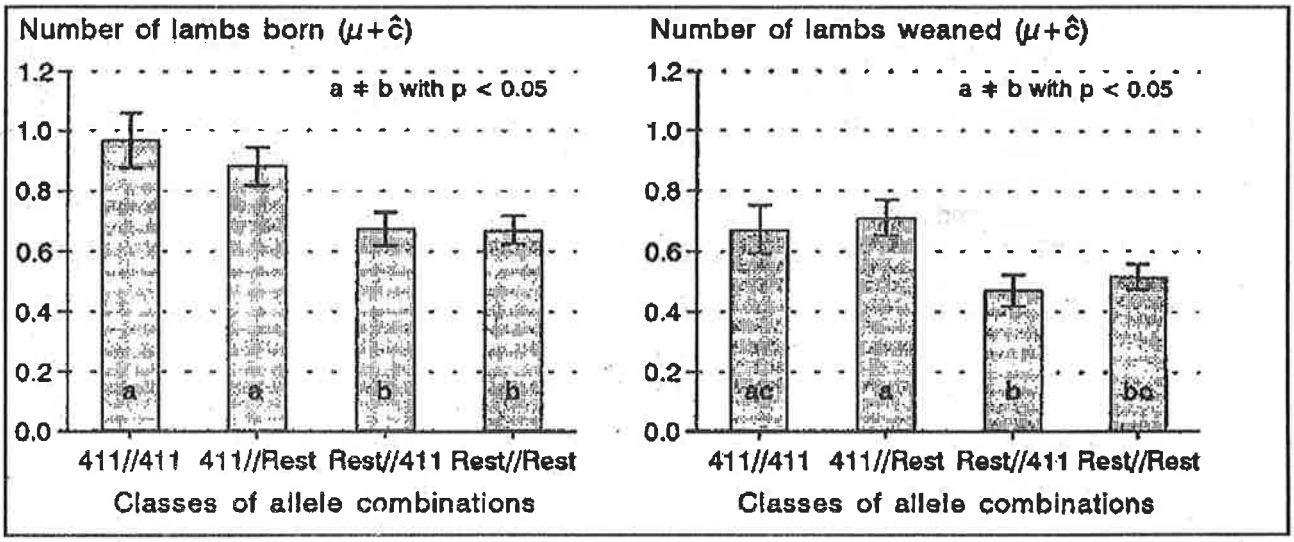

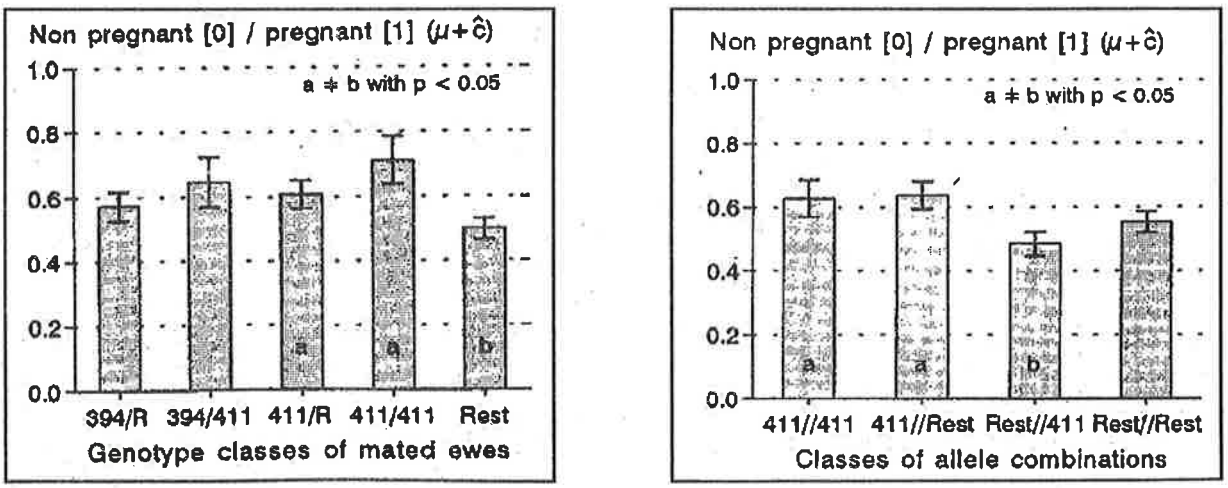

Figure : Fertility trait values within ewes grouped for specific genotypes