Original article

# *OLA-DRB1* microsatellite variants are associated with ovine growth and reproduction traits

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**Abstract** – The *DRB1* intron 2 $(GT)_n(GA)_m$ microsatellite was genotyped in experimental flocks of seven Merinoland rams and 249 ewes as well as their offspring (381 lambs) from consecutive lambings. A total of 16 *DRB1* alleles were detected, ranging between 353 and 857 bp. In comparison with carriers of other alleles, the ewes carrying the predominant 411 bp allele had higher values of all the recorded fertility traits. For ewes carrying the 394 and 857 bp alleles, the birth weight of lambs was about 400 g higher as compared to the residual group of ewes. The observed associations could be due to differences in disease resistance, cell recognition or tissue differentiation between carriers of various MHC haplotypes which can in turn affect individual fertility and growth performance.

**DRB1 / microsatellites / growth / reproduction / sheep**

## 1. INTRODUCTION

Vertebrate Major Histocompatibility Complex (MHC) comprises a series of highly polymorphic genes whose products belong to three classes (I, II and III) of molecules. Products of the MHC class I and II genes are the main contributors to the ability of discrimination between self and non self with class I proteins being expressed on the surface of all cell types while the products of the class II genes are restricted to antigen presenting cells. Class III genes encode components of the complement system (reviewed for example by Hauptmann and Bahram [12]). Individual MHC genes show high degrees of polymorphism, whereas the overall structure of this gene region is largely conserved among vertebrate species and it contains a high density of coding

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genes [18]. In addition to the central role of the MHC in host defence, MHC genotypes have been observed to be associated with *e.g.* spermatogenesis, mating preference or embryo development in humans and rodents [13, 20, 28].

In sheep however, the knowledge concerning the influence of the MHC on reproduction and growth related trait values of economic relevance is still limited. The sheep MHC (ovine leukocyte antigen system, OLA) is located on chromosome 20 [25] and covers a region of more than 10 cM [22]. Among OLA class II genes, the expressed DR beta 1 (*DRB1*) gene has been found to be highly polymorphic. To date, at least 106 *OLA-DRB1* alleles have been identified by DNA sequencing of exon 2 from various sheep breeds, and several reports are available which describe associations between carriers of distinct alleles and their disease resistance [14, 15, 24].

In our experiments, we used the polymorphic microsatellite $(GT)_n(GA)_m$ (GenBank accession number: U00222) within intron 2 of the *DRB1* gene, first described by Riess *et al.* [21]. This repeat is supposed to have been conserved between humans and artiodactyls for more than 70 million years [2]. *In vitro* experiments have revealed protein binding to $(GT)_n(GA)_m$ sequences, which points towards a biological function of this microsatellite in gene regulation [17]. In Merinoland sheep [7], a total of at least 36 $(GT)_n(GA)_m$ alleles have been described by Griesinger [8], and six alleles appear to be specific for this breed. The *DRB1* genotyping was performed in addition to the recording of a range of reproduction and growth related traits in Merinoland ewes and their offspring from consecutive lambings for subsequent association analyses.

## 2. MATERIALS AND METHODS

### 2.1. Animals and experimental design

Seven rams and 249 ewes of the Merinoland breed [7] were kept at an experimental station of the University of Hohenheim (Stuttgart, Germany) under a standardised management system. For the experiment, young and virgin ewes were chosen from flocks of different holders. Besides, the seven rams were unrelated with each other as well as with the ewes and purchased from different sheep breeders in Baden-Württemberg, Germany, by considering the available pedigree data of the sheep breeders association (Landesschafzuchtverband Baden-Württemberg, Germany). The study was performed over three years with several mating periods as shown in Table I. Each ewe was mated with a distinct ram in order to produce two consecutive pregnancies. During each mating period of 6 to 8 weeks, each ram had free access to a group of ewes.

**Table I.** Experimental design.

| Year | 1 | 2 | | | | 3 | | | | 4 | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month | 12 | 3 | 6 | 9 | 12 | 3 | 6 | 9 | 12 | 3 | | |
| Ram | nME nLE | | | | nME nLE | nME nLE | | | nME nLE | | nME | nLE |
| 1 | 22 p1 9 | | | | 32 p2 25 | 18 p3 3 | | | 25 p4 13 | | 97 | 50 |
| 2 | 22 p1 13 | | | | 32 p2 26 | | | | | | 54 | 39 |
| 3 | 21 p1 6 | | | | 31 p2 23 | 23 p3 10 | | | 26 p4 16 | | 101 | 55 |
| 4 | 22 p1 13 | | | | 33 p2 27 | 18 p3 6 | | | 20 p4 15 | | 93 | 61 |
| 5 | | | | | 19 p2 9 | 21 p3 7 | | | 19 p4 13 | | 59 | 29 |
| 6 | | | | | 18 p2 14 | 15 p3 6 | | | 18 p4 12 | | 51 | 32 |
| 7 | | | | | | 21 p3 3 | | | 19 p4 13 | | 40 | 16 |
| Total | 87 41 | | | | 165 124 | 116 35 | | | 127 82 | | 495 | 282 |

p1, …, p4: Lambing period 1, …, 4; nME: number of mated ewes; nLE: number of lambing ewes.

The ewes that did not become pregnant in a mating season were mated again with the same ram in the next season. After two pregnancies, the ewes were replaced by again virgin, young ewes. Altogether, 381 lambs were born during the different lambing periods, 58 of which were stillborn or did not reach the age of three months.

## 2.2. Isolation of DNA

At least two EDTA stabilised blood samples (10 mL) or one spleen sample (from stillborn lambs) were collected per animal and stored at −20 °C. Isolation of genomic DNA was performed according to standard procedures. DNA preparation from spleen samples was based on protocols of Chapdelaine *et al.* [6].

## 2.3. Primers and PCR conditions

A DNA fragment which contained approximately 180 bp of exon 2 and intron 2 of the ovine *DRB1* including the $(GT)_n(GA)_m$

microsatellite locus was amplified by PCR, using the primers 5'-GGGGGATCCGCTTCGACAGCGACTGGGGCG-3' and 5'-CGTACCCAGAKTGAGTGAAGTATC-3' (K: G or T) according to Griesinger *et al.* [9]. Approximately 200 ng of DNA were used in a 25 $\mu$L reaction volume with 0.5 U Taq polymerase, 0.8 $\mu$M primers, 0.4 mM dNTP and 1.5 mM $MgCl_2$. Denaturation at 94 °C for 3 min was followed by 32 cycles with a denaturation time of 30 s and annealing (60 °C) as well as extension (72 °C) times of 60 s. The final cycle was concluded by an extension period of 5 min.

## 2.4. Fragment length analysis

Fragment length analysis was performed on an Automated Laser Fluorescent Sequencer (A.L.F., Pharmacia, Freiburg, Germany) using 5% Hydrolink gels. The lengths of allelic fragments were measured based on internal and external length standards. The A.L.F. software of Pharmacia (Freiburg, Germany) was used for electrophoresis and genotyping. Each genotyping was repeated at least twice.

## 2.5. Recording of reproduction and growth traits

The following traits or effects were scored corresponding to each mating season: (i) For each ewe: mating period; body weight at mating time; date of lambing; ages at mating and lambing; pregnancy status; number of lambs born and weaned per lambing. (ii) For each lamb: parents; date of birth; number of siblings; date of weaning or death; sex; birth and weaning weight. Other traits measured on the lambs were as follows: age at weaning; total weight gain (weaning weight - birth weight); daily weight gain (total weight gain / age at weaning).

## 2.6. Statistical analysis

Allele frequencies, heterozygosity and deviations from Hardy Weinberg equilibrium were calculated using BIOSYS-2 [27]. Associations between parameters of the *DRB1* microsatellite and trait values were analysed using the GLM-procedure of SAS®, version 8 (SAS® Institute Inc., Cary, NC, USA). Each ewe was mated with only one sire and so the ewe factors and ram used for different flocks were disconnected. The unrelated rams used for the experiment had already been used for breeding and had shown their successful

fertility. Considering these conditions, the statistical models used for the calculations were the following:

– for reproduction traits of ewes (pregnancy status; number of lambs born; number of lambs weaned):

$$y_{ijk} = \mu + AE_i + P_j + b_1(MAE_{ijk} - \overline{MAE}) + b_2(MAE_{ijk}^2 - \overline{MAE^2}) + e_{ijk} \quad (1)$$

– for growth traits of lambs (birth and weaning weight; daily weight gain):

$$y_{ijklm} = \mu + AE_i + P_j + S_k + LB_l + b_1(LAE_{ijklm} - \overline{LAE})$$
$$+ b_2(LAE_{ijklm}^2 - \overline{LAE}^2) + \{b_{j3}(WAL_{ijklm} - \overline{WAL}_j)\} * + e_{ijklm}, \quad (2)$$

with $y$: observed trait value of an animal; $\mu$: mean value of the population; $AE$: fixed effect of the allele (of ewe or lamb) in two allele classes (first class: animals with the specific allele; second class: animals without the specific allele) or genotype classes (animals homozygous for the specific allele, heterozygous for the specific allele, or without the specific allele, and up to two specific alleles regarded) or combination of ewe and ram alleles; $P$: fixed effect of mating period; $b_1, b_2$: linear and squared regression values on the age of the ewe at mating; *MAE, MAE²*: age of the ewe at mating and its squared value; $\overline{MAE}$, $\overline{MAE}^2$: mean of the age of the ewe at mating and its squared value; $S$: fixed effect of lamb sex; *LB*: fixed effect of the number of lambs born; $b_{j3}$: linear regression of weaning weight/gain on the weaning age of lambs nested within the period; *LAE, LAE²*: fixed effect of lambing age of the ewe and its squared value; $\overline{LAE}$, $\overline{LAE}^2$: mean age of the ewe at lambing and its squared value; *WAL:* fixed effect of the lamb weaning age; $\overline{WAL}$: mean weaning age of the lambs; $\{b_{j3}(WAL_{ijklm} - \overline{WAL}_j)\}$: continues independent variable included for analysing the dependent traits of the lambs; *: not included in the model for the birth weight of lambs; $e$: residual error.

The ram effect was considered in alternative models (not shown).

## 3. RESULTS

### 3.1. Allelic diversity

A total of 16 *DRB1* alleles were detected, ranging between 353 and 857 bp. The fragment lengths and their distribution in the flocks are shown in Figure 1. The 411 bp allele was the most frequent with 22.5% (n: 232) followed by the 405 (14.5%; n: 150), 394 (14.1%; n: 146) and 383 bp (12.2%; n: 126) alleles.
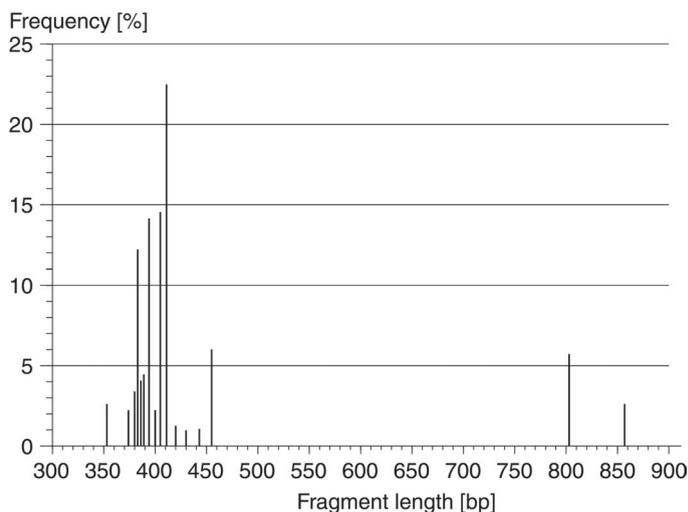
**Figure 1.** Distribution of *DRB1* microsatellite fragment lengths in the experimental flock.

The predominant genotypes 394/411 (7.0%; n: 36), 411/411 (6.0%; n: 31), 394/405 (5.6%; n: 29) and 405/411 (5.4%; n: 28) were observed among a total of 91 different genotypes.

### 3.2. Fertility traits

Associations between the *DRB1* alleles of the mated ewes and their fertility traits were examined using model (1), checking ewes carrying individual alleles against a residual class containing carriers of all other alleles. Pregnant ewes carrying the 386 bp allele had a higher ($P < 0.01$) number of lambs born and carriers of the 389 bp allele had a lower ($P < 0.01$) number of lambs weaned (not shown). Table II elucidates the superior fertility of ewes carrying the predominant 411 bp allele. Mated ewes with the 411 bp allele were superior to the residual class in pregnancy status ($P < 0.01$), lambs born ($P < 0.001$) and lambs weaned ($P < 0.001$) (Fig. 2). Regarding environmental factors, we found that the period of mating had an influence ($P < 0.001$) on all fertility traits of the mated ewes. The ewes were mated with the same ram for two consecutive pregnancies, showing that the number of lambs born in the first lambing (1.34 ± 0.11) was slightly higher (n.s.) than in the second lambing (1.17 ± 0.18). Calculation of results using a model with a ram effect revealed no influence on estimates for fertility traits concerning the allele classes (not shown).

**Table II.** Associations between genotype or allele classes and trait values[1].
a) Fertility traits [2].

| Source of variance | n [3] | Pregnancy status [4] | | Number of lambs born | | | Number of lambs weaned | | |
|---|---|---|---|---|---|---|---|---|---|
| **Genotype of ewe [5]:** | | | | | | | | | |
| 394/rest | 101 | .572 | .044 | .665 | .067 | b | .508 | .060 | b |
| 394/411 | 31 | .645 | .080 | .914 | .120 | | .699 | .109 | |
| 411/rest | 102 | .607 | .044 a | .899 | .066 | a | .675 | .060 | a |
| 411/411 | 32 | .710 | .078 a | .942 | .118 | a | .773 | .107 | a |
| Rest/rest | 223 | .500 | .030 b | .675 | .045 | b | .488 | .041 | b |
| Signif. [6] | | a > b: *P* < 0.05 | | a > b: *P* < 0.01 | | | a > b: *P* < 0.05 | | |
| **Allele of ewe:** | | | | | | | | | |
| 394 | 135 | .591 | .039 | .724 | .059 | | .556 | .053 | |
| Rest | 357 | .550 | .024 | .763 | .036 | | .567 | .033 | |
| Signif. | | n.s. | | n.s. | | | n.s. | | |
| 411 | 165 | .633 | .034 | .909 | .052 | | .698 | .047 | |
| Rest | 327 | .523 | .025 | .672 | .037 | | .495 | .034 | |
| Signif. [6] | | *P* < 0.01 | | *P* < 0.001 | | | *P* < 0.001 | | |
| **Allele of ewe resp. allele of ram:** | | | | | | | | | |
| 411 resp. 411 | 54 | .627 | .060 a | .968 | .090 | a | .672 | .082 | ac |
| 411 resp. rest | 111 | .636 | .042 a | .881 | .063 | a | .711 | .057 | a |
| Rest resp. 411 | 144 | .487 | .037 b | .675 | .055 | b | .471 | .050 | b |
| Rest resp. rest | 183 | .553 | .033 | .669 | .050 | b | .515 | .045 | bc |
| Signif. [6] | | a > b: *P* < 0.05 | | a > b: *P* < 0.01 | | | a > b: *P* < 0.01 | | |

### 3.3. Growth traits

Figure 3 illustrates the associations between alleles of ewes and birth weight of the lambs, analysed by model (2). For carriers of the 394 and 857 bp alleles the weight of the lambs was about 400 g higher (*P* < 0.001 and *P* < 0.05 respectively) as compared to the residual group whereas the 443 bp allele was negatively associated (*P* < 0.05). Significant effects on growth traits other than birth weight in combination with *DRB1* alleles was also observed for the 400 bp allele which was positively associated (*P* < 0.05) with weight up to weaning (not shown). As shown in Table II for the predominant 411 and 394 bp alleles, the ewes carrying the 394 bp allele were superior in their offspring not only for birth weight but also for the other growth traits. Calculation of the results using a model with a ram effect revealed a significant effect (*P* < 0.001) on estimates for growth traits of the lambs, but with marginal influence on the estimates of the ewe allele classes.

**Table II.** Continued.

b) Growth traits.

| Source of variance | n [3] | Birth weight of lamb (kg) | | | Weaning weight (kg) | | | Daily gain up to weaning (kg) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Genotype of ewe[5]: | | | | | | | | | | |
| 394/rest | 71/55 | 5.203 | .106 | a | 25.10 | .79 | | 214.0 | 8.5 | |
| 394/411 | 30/24 | 5.276 | .157 | a | 24.63 | .96 | | 212.9 | 10.3 | |
| 411/rest | 90/69 | 4.838 | .091 | bc | 24.55 | .68 | | 213.5 | 7.3 | |
| 411/411 | 33/28 | 4.555 | .146 | c | 24.30 | .86 | | 214.6 | 9.3 | |
| Rest/rest | 151/111 | 4.890 | .073 | b | 24.25 | .64 | | 208.6 | 6.9 | |
| Signif. [6] | | a > b: | $P < 0.001$ | | | n.s. | | | n.s. | |
| Allele of ewe: | | | | | | | | | | |
| 394 | 101/79 | 5.229 | .093 | | 24.98 | .72 | | 214.3 | 7.7 | |
| Rest | 274/208 | 4.834 | .058 | | 24.36 | .57 | | 211.5 | 6.1 | |
| Signif. | | $P < 0.001$ | | | | n.s. | | | n.s. | |
| 411 | 153/121 | 4.849 | .076 | | 24.47 | .60 | | 213.5 | 6.5 | |
| Rest | 222/166 | 4.972 | .065 | | 24.45 | .62 | | 210.0 | 6.6 | |
| Signif. [6] | | n.s. | | | n.s. | | | n.s. | | |
| Allele of lamb: | | | | | | | | | | |
| 394 | 67/60 | 5.185 | .109 | | 24.64 | .76 | | 210.1 | 8.1 | |
| Rest | 200/173 | 4.886 | .067 | | 24.25 | .59 | | 210.0 | 6.3 | |
| Signif. | | $P < 0.01$ | | | | n.s. | | | n.s. | |
| 411 | 116/92 | 4.860 | .083 | | 24.52 | .66 | | 213.4 | 7.0 | |
| Rest | 151/141 | 5.023 | .077 | | 24.18 | .62 | | 207.8 | 6.5 | |
| Signif. [6] | | n.s. | | | n.s. | | | n.s. | | |
| Allele of ewe resp. allele of ram: | | | | | | | | | | |
| 394 resp. 394 | 19/14 | 5.348 | .193 | ac | 27.58 | 1.16 | a | 241.6 | 12.5 | a |
| 394 resp. rest | 82/65 | 5.201 | .101 | a | 24.43 | .74 | b | 208.7 | 7.9 | b |
| Rest resp. 394 | 71/53 | 4.933 | .101 | bc | 24.29 | .70 | b | 209.5 | 7.6 | b |
| Rest resp. rest | 203/155 | 4.798 | .066 | b | 24.39 | .60 | b | 212.4 | 6.4 | b |
| Signif. [6] | | a > b: | $P < 0.001$ | | a > b: | $P < 0.05$ | | a > b: | $P < 0.05$ | |

[1] LS Means and Standard Errors. The letters indicate groups which are significantly ($P < 0.05$) different.

[2] Traits were defined for the mated ewes, and therefore the genotypes of lambs were not considered.

[3] Number of ewes (for fertility traits) or lambs (for growth traits). First number: lambs born; second number: lambs weaned.

[4] Pregnancy status: Number of lambing ewes in relation to the number of mated ewes, *i.e.* ratio of ewes that became pregnant after the mating period.

[5] Only 2 carriers of the 394/394 genotype were observed and therefore data are not shown.

[6] Significance between groups.
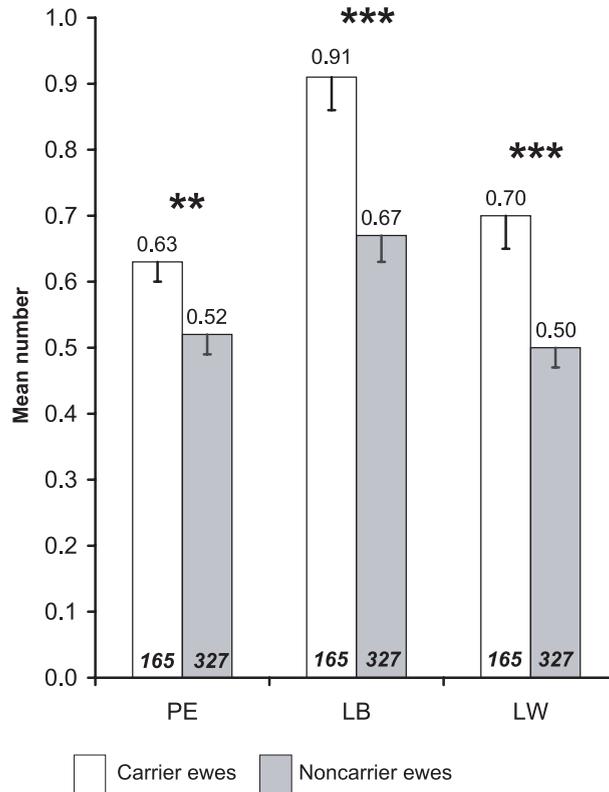
**Figure 2.** Pregnancy status (PE), number of lambs born (LB) and number of lambs weaned (LW) from mated ewes with or without the *DRB1* microsatellite 411 bp allele. The values and levels of significance ($_{***}$: $P < 0.001$; $_{**}$: $P < 0.01$) are presented at the head of each column; observation numbers are indicated at the bottom. The error bars represent the standard error of the mean.

## 4. DISCUSSION

The 16 observed *DRB1* alleles in this study ranged between 353 and 857 bp which agrees with the findings of previous studies in the same breed [9] as well as for other sheep breeds [11, 23]. The three most frequent alleles (411, 405, and 394 bp) were each found in two of the seven sires, which is partly responsible for the predominant occurrence of these alleles in the lambs.

### 4.1. MHC variants and fertility traits

Two major concepts about MHC involvement in reproduction are currently being discussed. The first assumes that immunological interactions between
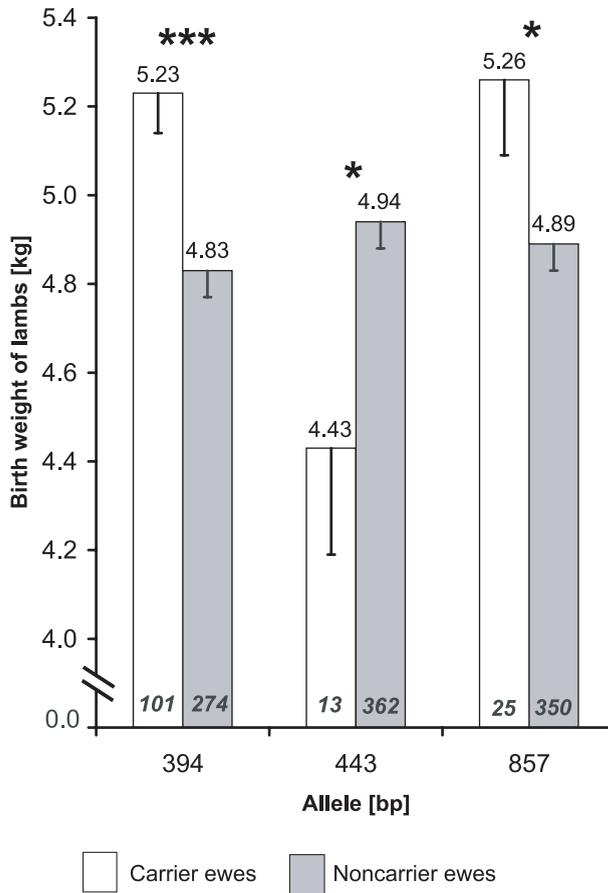
**Figure 3.** Associations between birth weight of lambs and maternal *DRB1* microsatellite alleles. The values and levels of significance ($_{***}$: $P < 0.001$; $_{*}$: $P < 0.05$) are presented at the head of each column; observation numbers are indicated at the bottom. The error bars represent the standard error of the mean.

the parents or between the mother and foetus are significant causes for the efficiency of conception and pregnancy. An increase in pregnancy success has been observed in heterozygous matings [1]. On fetal membranes, MHC-G is the only antigen found in most animals, and it is supposed that paternal MHC-G is recognised by the maternal organism [19] where an excess of "blocking antibodies" causes an increased production of cytokines. This leads to an advantage of the heterozygote in fertility traits which concurs with the results of our study where *DRB1* microsatellite heterozygous ewes had higher fertility values than homozygous ewes (not shown) although the difference was

not statistically significant in our study and not observed for the predominant 411 bp allele (Tab. II). The fertility of ewes in second mating was reduced which may also indicate an immune reaction.

The second notion focuses on lethal and semi-lethal genes in close proximity of the MHC such as for example the murine T/t-complex (reviewed for example by Willison and Lyon [29]). The T/*t* haplotypes are variants of the proximal third of chromosome 17 and have been described in most natural mouse populations [3, 4]. Depending on homo- or heterozygosity for these haplotypes, a range of symptoms from reduced male fertility to embryonic malformations or death can occur, and a number of genes that are expressed during spermatogenesis have been found to be associated with the murine T/t complex [5, 29]. The highly significant association which we found between the 411 bp allele and the pregnancy status, the effect of the ram allele as well as the numbers of lambs born and weaned (Tab. II) probably reflects the superior effect of an allelic haplotype which includes several closely linked loci. In humans for example more than 600 coding loci were mapped in close proximity of the MHC [18], and the low recombination probabilities hinder the analysis of causative polymorphic sites in population data. The results presented here justify a direct genotyping of DNA variants within the T/t complex together with further MHC linked marker loci in order to verify the association between the MHC chromosome region and fertility traits in independent groups of ewes.

## 4.2. MHC variants and growth traits

Associations between serologically defined class I polymorphisms and growth in cattle have been reported [10] which point to more or less indirect MHC effects on growth traits. Such associations could be due to differences in disease resistance between carriers of various MHC haplotypes that can in turn affect the individual growth performance. In sheep, associations of *DRB1* alleles with resistance to bovine leukaemia virus infection [16] or parasitic nematodes [26] are known. We found the birth weight of lambs to be significantly associated with *DRB1* microsatellite alleles (Tab. II) with – regarding the frequent alleles – a superiority of the 394 bp allele carriers. Figure 3 shows that the rare 443 and 857 bp alleles were also significantly associated with birth weight. Interestingly, the large 857 bp allele, which did not occur in the rams and was found to be breed specific  [9], was detected to be a second advantageous allele for birth weight.

These findings differ from earlier observations by Gruszynska *et al.* [11] who described associations with different alleles for birth weight in Polish Heath Sheep that could be caused by breed specific haplotypes; but the authors only worked with 115 lambs and their calculations were based on observation numbers between one and seven. Moreover, in our study we found associations with superior birth weight when regarding the ewe as well as the lamb 394 bp allele (Tab. II).

## 5. CONCLUSION

Our results suggest the presence of QTL for fertility and growth in close proximity of the *DRB1* microsatellite, and the association of this locus with both traits allows allele-/ genotype information to be applied – along with other markers – in sheep breeding. The observed associations could be due to differences in disease resistance between carriers of various MHC haplotypes which can in turn affect individual fertility and growth performance. Moreover, the T/t complex and interaction between parental or mother / foetus MHC haplotypes may be associated with cell recognition and tissue differentiation. Further studies with numerous markers and genes in the OLA region and regarding further breeds will be required in order to understand sheep MHC genetics and clarify the genetic background for immunological influences on fertility and growth.

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