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# No associations between PrP genotypes and reproduction traits in INRA 401 sheep<sup>1</sup>

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**ABSTRACT:** In this study, the potential association of PrP genotypes with ewe reproductive traits and lamb growth traits was investigated. Data were included on ewes and lambs of the INRA 401 breed from the Bourges-La Sapinière INRA experimental farm. This breed was chosen because of good breeding (prolificacy and maternal abilities) and carcass qualities, and the large number of available animals with performance records and PrP genotypes. Ewes were categorized into 3 PrP genotype classes: ARR homozygous, ARR heterozygous, and animals without any ARR haplotype. Two analyses differing in the traits considered were carried out. Firstly, the potential association of the PrP genotype of ewes with their reproduction traits (fertility, litter size, and ovulation rate) was studied. The data

included 801, 779, and 587 genotyped ewes for fertility, litter size, and ovulation rate, respectively. Secondly, the association of the PrP genotype of the ewes with growth traits of their lambs (birth weight, ADG between 10 and 30 d of age) was investigated. The data included 3,749 and 3,512 lambs for birth weight and ADG, respectively. The different traits were analyzed using an animal model, where the PrP genotype effect was included as a fixed effect. The reproductive traits were modeled under a probit or a threshold approach. The results of this study indicate no evidence of an association between PrP genotypes and reproductive and growth traits. It is unlikely that selection for scrapie resistance will have an effect on the reproductive or performance traits studied in the INRA 401 breed.

**Key words:** association analysis, meat trait, PrP genotype, reproduction trait, sheep

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## INTRODUCTION

Scrapie susceptibility is mainly controlled by polymorphisms at the PRNP gene (Hunter et al., 1996; Elsen et al., 1999), although recent results have shown that the susceptibility of a given genotype also depends on the prion strain (Moum et al., 2005). Several punctual mutations exist at codons 136 (T, A, V), 154 (R, H), and 171 (R, Q, H, K) of the gene. Recently, other codons were detected, and some of them could be associated with scrapie resistance (Goldmann et al., 2005). The ARR haplotype (A at codon 136, R at codons 154 and 171) is mainly associated with resistance, and VRQ is associated with susceptibility (Hunter et al., 1996; Elsen et al., 1999; Díaz et al., 2005).

Production of lambs for meat has many components: ewe fertility, prolificacy or litter size, and lamb growth during suckling, which are well-known production traits. Recently, selection of the ARR/ARR genotype has been implemented in several European breeds to improve resistance to scrapie. Including PrP genotype in breeding objectives might cause undesirable changes in reproduction, growth traits, or both. Thus, the relationships between PrP genotypes and production traits need to be studied.

To date, studies on associations of PrP genotypes with reproduction traits support different conclusions (Brandsma et al., 2004, 2005; Casellas et al., 2005; De Vries et al., 2005). No analyses for PrP genotype and fertility or ovulation rate have been done until now.

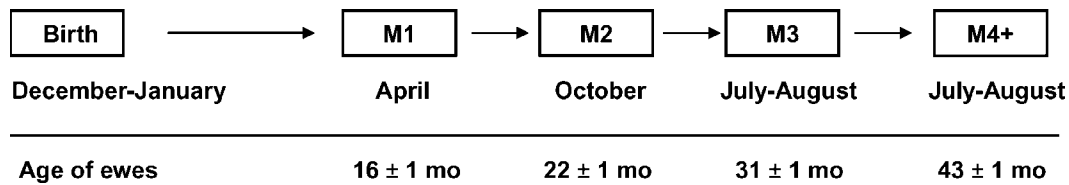
The aim of this work was to investigate the potential associations of PrP genotypes of ewes with their reproduction traits (fertility, litter size, ovulation rate). In addition, associations of ewes' PrP genotypes with their lambs' growth traits (birth weight, ADG between 10 and 30 d of age) were studied.

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**Figure 1.** Breeding scheme representing season and age of ewes at first mating (M1), second mating (M2), and their subsequent matings (M3 and M4+).

## MATERIALS AND METHODS

### Data and Management

Data were obtained from the Bourges-La Sapinière INRA experimental farm. This study included animals belonging to the INRA 401 breed. This breed was developed in France as a composite breed that is well suited to lamb production, with good breeding and carcass qualities (Moreno et al., 2001). The INRA 401 sheep were derived from crossing Berrichon du Cher (good carcass characteristics, but low prolificacy and maternal ability) and Romanov (good maternal ability, high prolificacy, but poor carcass characteristics) breeds.

Ewes were managed under the following scheme (Figure 1). First, ewes were exposed to rams for the first time in April (out of the natural breeding season) at  $16 \pm 1$  mo of age. Later, those ewes that lambed in September were mated in October (natural breeding period) at  $22 \pm 1$  mo of age for their second lambing. Ewe selection on fertility was based on fall and spring lambing performance (September and March). Then, for their subsequent lambings, ewes were mated once per year in July-August (beginning of the breeding season) and lambed in December-January ( $31 \pm 1$ ,  $43 \pm 1$ , and  $55 \pm 1$  mo of age, and subsequently). No lambs were retained for replacement from the 2 first lambings of a ewe (spring and fall lambings). Replacement animals in the flock were born in December and January.

The reproduction traits analyzed were fertility, ovulation rate, and litter size. The fertility in each lambing season was coded as 1 or 0 for ewes that did or did not lamb, respectively. Ovulation rate was defined as the total number of corpora lutea observed by laparoscopy of both ovaries (1, 2, or 3+). To measure ovulation rate at each mating season, fertile, harnessed rams were joined with the females. Harness color was changed every week, and at the same time, ewes were examined for marks indicative of estrus. Ewes that were marked one week were examined by laparoscopy the next week. For those ewes returning to estrus, a second ovulation could also be recorded, but only ovulation of the fertile cycle was analyzed. Litter size was defined as the number of lambs (dead or alive) at birth (1, 2, or 3+). Lambs were weighed at birth and subsequently at 10 and 30 d of age to obtain the ADG (**ADG10-30**).

Ewes were genotyped using the Taqman method (Lee et al., 1993). Only 4 haplotypes were described in this

breed: ARR, AHQ, VRQ, and AR-. The AR- haplotype included ARQ and ARH haplotypes, which were confounded by the genotyping technique used.

The data consisted of 3,795 records from 801 ewes lambing between 1993 and 2004. Ewes were born between 1992 and 1998. Thus, the data set used did not include animals affected by selection on PrP genotypes, which began in 1999 in this flock. Summaries of the data for different traits are presented in Table 1.

Although the data set came from an experimental population, only 15% of the animals not selected on performance were genotyped for the PRNP gene. This could lead to biased results (genotyping bias). To test for the hypothesis that there was no genotyping bias, the distribution of genotypes in the sample of animals used in this study was compared with the frequencies in the same breed reported by Palhière et al. (2002), using a  $\chi^2$  goodness-of-fit test.

### Association Studies

To estimate the consequences of selection for the ARR/ARR genotype, ewes were categorized into 3 classes: animals homozygous for the ARR haplotype (ARR/ARR), animals heterozygous for the ARR haplotype (ARR/—), and animals without any ARR haplotype (—/—). The data included 801, 779, and 587 genotyped ewes for fertility, litter size, and ovulation rate, respectively. Lamb performance records came from animals born between 1995 and 2003 and included 3,749 and 3,512 lambs for birth weight and ADG10-30, respectively. Performance records were only collected on lambs retained for replacement (parities 3+).

**Table 1.** Data structure for reproductive and growth traits

Trait	No. of animals	No. of records	Mean (SD)
<b>Ewe</b>			
Fertility	801	3,795	0.93 (0.25)
Litter size	779	3,535	2.04 (0.65)
Ovulation rate	587	828	2.04 (0.67)
<b>Lamb</b>			
Birth weight, kg	3,749	3,749	4.10 (0.81)
ADG10-30, <sup>1</sup> kg/d	3,512	3,512	0.272 (0.058)

<sup>1</sup>ADG10-30 = ADG between 10 and 30 d of age.

**Table 2.** Number of observations for each level of the fixed effects in ewes

Effect	Ewe		
	Fertility	Litter size	Ovulation rate
Year of lambing			
1993	48	48	
1994	90	90	57
1995	166	166	55
1996	221	219	51
1997	274	269	27
1998	484	481	145
1999	780	752	177
2000	447	416	146
2001	427	410	170
2002	432	303	
2003	275	245	
2004	151	136	
Type of birth – type of rearing			
Single – single	255	219	49
Multiple – single	100	98	25
Twin – twin	2,149	2,003	446
Multiple – twin	766	717	173
Artificial rearing	525	498	135
Ewe parity			
1	801	778	483
2	681	671	175
3	620	595	170
4	614	525	
5	502	441	
6	344	318	
7	184	164	
8	49	43	
PrP genotype			
ARR/ARR	569	523	126
ARR/—	1,876	1,750	415
—/—	1,350	1,262	287

## Analyses

Two different models were used in the association analyses. Model I was for fertility, litter size, and ovulation rate. Due to the categorical nature of the fertility traits, a threshold (also called probit) model was used (e.g., Gianola, 1982; Foulley and Manfredi, 1991). According to this model, the observed phenotype is determined by a continuous, nonobserved liability, which determines the observed phenotype according to a set of thresholds. The threshold model therefore considers the categorical nature of the trait and the nonnormal distribution of the phenotypes.

The liability was modeled as follows:

$$y_{ijklmn} = \mu + yl_i + te_j + g_k + PrP_l + a_m + p_m + e_{ijklmn},$$

where  $y_{ijklmn}$  is the liability for the trait (fertility, litter size, and ovulation rate),  $\mu$  is a constant,  $yl_i$  is the year of lambing ( $i = 1, 12$ ),  $te_j$  is the type of birth and rearing of ewes ( $j = 1, 5$ ),  $g_k$  is the ewe parity ( $k = 1, 8$ ),  $PrP_l$  is the PrP effect (genotype of ewe;  $l = 1, 3$ ),  $a_m$  is the random additive effect of the ewe  $m$ ,  $p_m$  is the random permanent environmental effect of the ewe, and  $e_{ijklmn}$  is the

**Table 3.** Number of observations for each level of fixed effects in lambs

Effect	Lamb	
	Birth weight	ADG10-30 <sup>1</sup>
Sex		
Male	1,828	1,719
Female	1,921	1,793
Year of birth		
1995	102	100
1996	179	171
1997	339	318
1998	464	436
1999	554	505
2000	424	406
2001	784	738
2002	594	548
2003	309	290
Type of birth – type of rearing		
Single – single	232	229
Multiple – single	152	142
Twin – twin	1,916	1,850
Multiple – twin	868	840
Artificial rearing	581	451
Ewe parity		
3	511	494
4	1,143	1,083
5	975	916
6	669	609
7	369	337
8	82	73
Ewe PrP genotype		
ARR/ARR	567	529
ARR/—	1,849	1,737
—/—	1,333	1,246

<sup>1</sup>ADG10-30 = ADG between 10 and 30 d of age.

random residual effect. Due to the management procedure (Figure 1), there was confounding between age at mating and season, and this effect was not considered in the model. The number of observations in each level of fixed effect for each trait is presented in Table 2.

Model II was used for the analysis of birth weight and growth rate. These traits were modeled using an animal model with maternal effects:

$$y_{ijklmnop} = \mu + sx_i + yb_j + tl_k + g_l + PrP_m + a_n + m_o + p_o + e_{ijklmnop},$$

where  $y_{ijklmnop}$  is the performance trait of lambs (birth weight, ADG10-30),  $\mu$  is a constant,  $sx_i$  is the sex effect ( $i = 1, 2$ ),  $yb_j$  is the year of birth ( $j = 1, 9$ ),  $tl_k$  is the type of birth and rearing of lambs ( $k = 1, 5$ ),  $g_l$  is the ewe parity ( $l = 1, 6$ ),  $PrP_m$  is the PrP effect (genotypes of the dam;  $m = 1, 3$ ),  $a_n$  is the random direct additive genetic effect of the lamb  $n$ ,  $m_o$  is the random maternal genetic effect of the ewe  $o$  (the latter 2 effects are assumed to be genetically correlated, as usual),  $p_o$  is the random maternal permanent environmental effect, and  $e_{ijklmnop}$  is the random residual effect. The number of observations in each level of fixed effect for each trait is presented in Table 3. In both analyses, to take into account

**Table 4.** Estimated differences between PrP genotypic classes for ewe traits

Contrast	No. of animals	PM <sup>1</sup>	PSD <sup>2</sup>	M <sup>3</sup>	<i>P</i> > 0 <sup>4</sup>
<b>Fertility</b>					
ARR/ARR to ARR/—	100 to 393	0.046	0.194	0.004	0.58
ARR/ARR to —/—	100 to 308	0.236	0.209	0.019	0.87
ARR/— to —/—	393 to 308	0.189	0.147	0.016	0.90
<b>Litter size</b>					
ARR/ARR to ARR/—	100 to 385	0.040	0.040	0.038	0.84
ARR/ARR to —/—	100 to 294	0.009	0.045	0.008	0.58
ARR/— to —/—	385 to 294	-0.031	0.030	-0.029	0.15
<b>Ovulation rate</b>					
ARR/ARR to ARR/—	81 to 288	-0.052	0.069	-0.049	0.23
ARR/ARR to —/—	81 to 218	-0.088	0.075	-0.084	0.12
ARR/— to —/—	288 to 218	-0.036	0.052	-0.034	0.24

<sup>1</sup>Posterior mean.<sup>2</sup>Posterior SD.<sup>3</sup>Mean in the phenotypic scale.<sup>4</sup>Posterior probability of a positive contrast.

the performance selection process, pedigrees included all available ancestors of the animals in the data set.

Marginal posterior distributions of contrasts between PrP-effect levels were estimated using a Gibbs Sampling procedure, applying the specificities of a threshold model for the fertility traits (Sorensen et al., 1995). Bounded flat priors were used for variance components and fixed effects. For the analyses, 250,000 iterations of the sampler were run, 50,000 iterations were discarded as burn-in, and 1 of every 10 iterations was retained. Thus, 20,000 samples were used to compute the features of the marginal posterior distributions. The length of the burn-in period was assessed by visual examination of trace plots of the variance components. Convergence diagnostics were assessed using the methods implemented in the BOA (Bayesian Output Analysis) software (Smith, 2005).

## RESULTS

The allelic frequencies of genotyped ewes of the sample studied were compared with the population frequencies reported by Palhière et al. (2002). In the INRA 401 breed, these authors estimated a frequency of 0.36, 0.07, 0.46, and 0.11 for ARR, AHQ, AR-, and VRQ haplotypes,

respectively. In our sample, the frequencies were 0.38, 0.07, 0.44, and 0.11 for ARR, AHQ, AR-, and VRQ haplotypes, respectively. The  $\chi^2$  test showed that the haplotype frequencies in the sample were not statistically different from the population frequencies. Thus, the results confirmed that there was no bias due to incomplete genotyping.

### Reproductive Traits

The estimated differences for the contrasts between levels of PrP-genotype effect for fertility, litter size, and ovulation rate are shown in Table 4. The results are also presented in the phenotypic scale (Van Tassell et al., 2003). No association of PrP genotypes with reproduction traits was observed (Table 4).

### Growth Traits

For lambs, the effect of ewes' PrP-genotype on birth weight and ADG10-30 was studied, and results are presented in Table 5. The genotype of ewes did not have an effect on growth traits.

**Table 5.** Estimated differences between PrP genotypic classes for lamb traits

Contrast	No. of animals	PM <sup>1</sup>	PSD <sup>2</sup>	<i>P</i> > 0 <sup>3</sup>
<b>Birth weight (g)</b>				
ARR/ARR to ARR/—	567 to 1,849	-0.047	0.049	0.17
ARR/ARR to —/—	567 to 1,333	-0.014	0.058	0.40
ARR/— to —/—	1,849 to 1,333	0.033	0.039	0.80
<b>ADG10-30<sup>4</sup> (g/d)</b>				
ARR/ARR to ARR/—	529 to 1,737	-1.5	3.6	0.34
ARR/ARR to —/—	529 to 1,246	-0.1	4.1	0.49
ARR/— to —/—	1,737 to 1,246	1.4	2.8	0.69

<sup>1</sup>Posterior mean.<sup>2</sup>Posterior SD.<sup>3</sup>Posterior probability of a positive contrast.<sup>4</sup>ADG10-30 = ADG between 10 and 30 d of age.

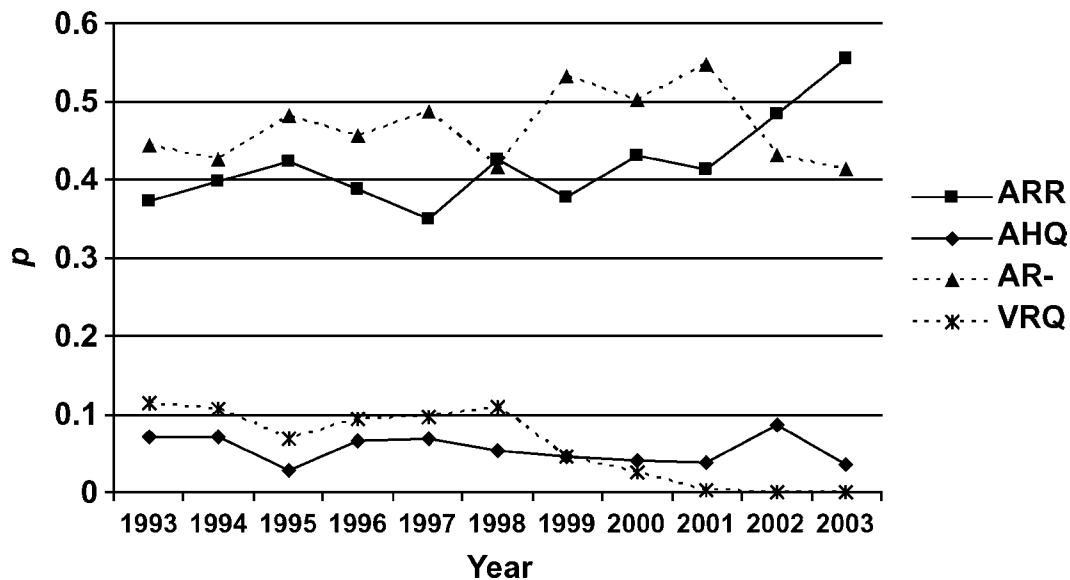


Figure 2. Evolution of the PrP haplotype frequencies ( $p$ ) in INRA 401 breed.

## DISCUSSION

The results of association studies suggest no evidence of an effect of the PrP genotype on fertility, litter size, or ovulation rate. Similar results were found on birth weight and ADG during the first month of suckling. Therefore, it is unlikely that selection for scrapie resistance will have an effect on these traits in this breed.

Potential sources of bias need to be considered in association analyses. The implementation of the eradication program changed the PrP haplotype frequencies. For INRA 401 ewes, Figure 2 shows the evolution in PrP haplotype frequencies on the Bourges-La Sapinière INRA experimental farm. A decrease in the frequency of the VRQ haplotype was observed from 1999, and hence, it was considered that selection began in 1998. The records of animals born after that date were discarded to eliminate bias due to PrP selection. In addition, haplotype frequencies on the sample of animals used in this study were consistent with the population frequencies reported by Palhière et al. (2002). These results provide evidence that genotyping bias did not affect the results.

This study, as other studies (Brandsma et al., 2005; De Vries et al., 2005), indicates no association between PrP genotypes and litter size. However, evidence of an association between the ARR/ARR genotype and prolificacy was found by Brandsma et al. (2004), Casellas et al. (2005), and Alexander et al. (2005). The results of these studies are not consistent. In Brandsma et al. (2004) a small positive effect of ARR on litter size was found. However, in the Ripollesa breed, with only 3 observed haplotypes (ARR, ARQ, ARH), Casellas et al. (2005) found differences between ARH and ARR haplotypes, suggesting that the ARH haplotype was superior for litter size. In the Suffolk breed, Alexander et al. (2005) found that ewes without the R variant at codon

171 gave birth to more twins and triplets than animals heterozygous at codon 171. These different reported associations of PrP-genotypes with performance traits might have 2 explanations. Either the differences are due to inherent differences among breeds or populations studied or are due to the effect of a gene closely linked to the PrP locus. To provide more evidence about the second point, a QTL approach is needed to confirm the association or lack thereof with this hypothetical gene.

Selection of animals with the ARR haplotype is an effective tool for breeders in improving genetic resistance to scrapie. However, in some breeds, undesirable associations with performance traits were reported for animals carrying the ARR haplotype. The potential repercussions of selecting in favor of the ARR haplotype point out the importance of a careful design of the breeding program to achieve resistance. In France, susceptible haplotypes (i.e., ARQ, ARH, or VRQ haplotypes) were preserved (for example, in semen banks) before the implementation of the national scrapie resistance program to avoid potential future problems, such as associations with important traits, reduction of genetic variability, or an increase in inbreeding.

## IMPLICATIONS

Selection for the PrP genotype does not affect ewe reproductive traits or lamb growth traits in the INRA 401 breed. Therefore, it is unlikely that selection for scrapie resistance using this genotype will have an effect on the traits studied.

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