Livestock Science 216 (2018) 44–50

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Presence of causative mutations affecting prolificacy in the Noire du Velay and Mouton Vendéen sheep breeds

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ARTICLE INFO

Keywords:
Ovine
Major gene
Prolificacy
BMP15
B4GALNT2

ABSTRACT

For many decades, prolificacy has been selected in meat sheep breeds as a polygenic trait but with limited genetic gain. However, the discovery of major genes affecting prolificacy has changed the way of selection for some ovine breeds implementing gene-assisted selection as in the French Lacaune and Grivette meat breeds, or in the Spanish Rasa Aragonesa breed. Based on statistical analysis of litter size parameters from 34 French meat sheep populations, we suspected the segregation of a mutation in a major gene affecting prolificacy in the Noire du Velay and in the Mouton Vendéen breeds exhibiting a very high variability of the litter size. After the genotyping of mutations known to be present in French sheep breeds, we discovered the segregation of the $FecL^L$ mutation at the *B4GALNT2* locus and the $FecX^{Gr}$ mutation at the *BMP15* locus in Noire du Velay and Mouton Vendéen, respectively. The frequency of ewes carrying $FecL^L$ in the Noire du Velay population was estimated at 21.2% and the Mouton Vendéen ewes carrying $FecX^{Gr}$ at 10.3%. The estimated mutated allele effect of $FecL^L$ and $FecX^{Gr}$ on litter size at +0.4 and +0.3 lamb per lambing in Noire du Velay and Mouton Vendéen, respectively. Due to the fairly high frequency and the rather strong effect of the $FecL^L$ and $FecX^{Gr}$ prolific alleles, specific management programmes including genotyping should be implemented for a breeding objective of prolificacy adapted to each of these breeds.

In ovine breeds raised for meat purposes, numerical productivity represents an important technical and economic lever. The objective is to reach an optimum for the economic profitability of breeding. Improvement of this numerical productivity is achieved by increasing the number of lambs born per ewe at each lambing, i.e. the prolificacy, associated with the improvement of lamb viability as well as the maternal quality. This leads to increased post-natal survival and growth rate of the lambs. For decades, genetic selection efforts have been made particularly on improving prolificacy of sheep breeds. However, prolificacy is a weakly heritable polygenic trait ($h^2 = 0.05 - 0.2$) (see the review by Bradford (Bradford, 1985)), allowing limited genetic gain. Nevertheless in some breeds, a very large effect on ovulation rate (OR) and litter size (LS) due to single mutation in fecundity major genes (called *Fec* genes, reviewed in Fabre et al. (2006)) has been demonstrated. The first evidence of the segregation of a prolificacy major gene was established in the early 1980's in Australian Booroola Merino. This was implicated by the observation of a large variability of LS and OR in this population and the presence of extremely prolific ewes in this low prolific breed (Piper and Bindon, 1982; Davis et al., 1982). The causal mutation named $FecB^B$ was discovered 20 years later in the *BMPR1B* gene (Bone Morphogenetic Protein Receptor 1B) on the ovine chromosome 6 by several independent research groups (Wilson et al., 2001; Mulsant et al., 2001; Souza et al., 2001). This mutation was thereafter introgressed in several ovine breeds around the world for research purposes or to improve their prolificacy although these latter programmes resulted in mixed outcomes (Walkden-Brown et al., 2009).

Up to now, many mutations were discovered worldwide in three other major genes namely *BMP15* (known as *FecX* (Galloway et al., 2000)), *GDF9* (known as *FecG* (Hanrahan et al., 2004)) and *B4GALNT2* (known as *FecL* (Drouilhet et al., 2013)). In France particularly, two genetic programmes were implemented to discover and to manage mutations with major effect in order to improve the prolificacy of commercial sheep populations (Mulsant et al., 2003; Bodin et al., 2011; Martin et al., 2014) . The introgression of the Booroola $FecB^B$ mutation was started in Mérinos d'Arles in the 1980's. Experimental testing by the French agricultural institute INRA has estimated the effect of the mutated allele on prolificacy at one extra lamb per lambing (Teyssier et al., 1997). A controlled diffusion of genotyped animals in commercial flocks is now implemented in the Mérinos d'Arles population (Teyssier et al., 2009). In the Lacaune breed, two different mutations affecting prolificacy were discovered in the selection nucleus of the OVI-TEST cooperative, $FecX^L$ in the *BMP15* gene on the

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https://doi.org/10.1016/j.livsci.2018.07.007
Received 16 March 2018; Received in revised form 6 July 2018; Accepted 9 July 2018

chromosome X (Bodin et al., 2007), and *FecL*^{L} at the *B4GALNT2* locus on the chromosome 11 (Drouilhet et al., 2009, 2013). As soon as 2005, it was decided to eradicate the *FecX*^{L} mutation inducing sterility at the homozygous state and to manage the *FecL*^{L} mutation which increases LS by +0.5 lamb per lambing. The selection objective is to achieve 50% of heterozygous *FecL*^{L} carrier ewes in the Lacaune OVI-TEST selection nucleus flocks (Martin et al., 2014; Raoul et al., 2017).

Beyond these genetic programmes, research of putative mutations affecting LS was undertaken in several French and foreign sheep populations, leading to the discovery of three original causal mutations affecting the *BMP15* gene in the French Grivette, the Polish Olkuska and the Tunisian Barbarine breeds (Demars et al., 2013; Lassoued et al., 2017). In contrast with the seven other known mutations in the *BMP15* gene affecting prolificacy, the homozygous Grivette and Olkuska carrier ewes are not sterile but hyper-prolific (Demars et al., 2013).

In the present paper, we present an analysis of LS data from 34 French and one Spanish meat sheep breeds highlighting the suspicion of a mutation in a major gene in two of them, the Noire du Velay and the Mouton Vendéen breeds. Through molecular genotyping we evidenced the segregation of mutations already known to control OR and LS in ovine breeds. Moreover, we give an early analysis of frequency and effects of these two mutations in commercial populations.

1. Material and methods

1.1. Data and statistical analysis

Relationship between mean and variance of LS. Data come from the OVALL French national database for meat sheep genetic evaluation and research managed by the Institut de l'Elevage (French Livestock Institute) and the Centre de Traitement de l'Information Génétique (Genetic Information Processing Center, Jouy-en-Josas, France) gathering about 12 million lambings from 1986 to 2016. We have extracted the lambing career of purebred females alive in 2005 from 34 different breeds (supplementary Table S1), representing 2,353,324 natural LS obtained without hormonal synchronisation treatment of oestrus. Moreover, we have added LS data from the Spanish database for genetic evaluation of Rasa Aragonesa – UPRA-Grupo Pastores, a breed where the prolific *FecX*^{R} mutation is segregating (Martinez-Royo et al., 2008; Fathallah et al., 2016). Basic statistical analysis (mean and variance of the observed LS) were used to characterize each breed as well as to select the animals entering in the genotyping programme.

Expected frequencies of LS and variance - expected career. A subsample of the 25 most numerically important French breeds gathering 88,428 ewes with at least 5 LS records each was considered to estimate the parameters of the LS distribution (supplementary Table S1). As in Bodin and Elsen (Bodin et al., 1989), the second order regression coefficients of each LS frequency on the mean prolificacy of the breed were estimated on the subsample of all ewes with 5 records each, excluding the Noire du Velay and Mouton Vendéen breeds as well as those known to carry a major gene for OR. These coefficients (α_i, $\beta1_i$, $\beta2_i$) permitted estimation of the expected frequencies of each LS_i for a population of a given prolificacy (prol): $LS_i = \alpha_i + \beta1_i\ prol + \beta2_i\ prol^2$ and consequently the expected variance which could be compared to the observed frequencies and variance. They were applied to a sample including 3 breeds without obvious major genes (Rava, Rouge de l'Ouest, Charollais), 2 breeds known to carry major genes (Lacaune and Grivette) and the two "suspected breeds" of the present study (Noire du Velay and Mouton Vendéen). According to the threshold model of LS (Gianola, 1982), and using the expected frequencies of these 7 populations, it was also possible to simulate lifetime LS data of females with 5 records each. Thus, 200,000 careers were simulated with a repeatability on the underlying variate equal to 0.20 (i.e. ~0.15 on the observed scale). These simulations provided the expected percentage of animals with 5 lambings which exceed a given mean prolificacy (i.e. 3.0). As before, this gives the expected value in the absence of a major gene in the population was compared to the observed value.

Genetic parameter analyses. The test of deviation from the Hardy Weinberg equilibrium was performed using a Pearson chi square test, while the association between genotypes and prolificacy groups (defined bellow) was analysed using an exact Fisher test which can take into account the very small sample size in some categories of the contingency tables. Both tests were performed using specific functions of the R package (R Development Core Team, 2008).

The heritability $[h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_p^2 + \sigma_e^2)]$ and the repeatability $[r = (\sigma_g^2 + \sigma_p^2)/(\sigma_g^2 + \sigma_p^2 + \sigma_e^2)]$ were calculated through the estimation of the additive genetic variance σ_g^2, the permanent environmental variance σ_p^2 and the residual variance σ_e^2. These variances were estimated by a linear mixed model run with the ASReml software (Gilmour et al., 2014). This model included the flock, the year, the season of lambing, the age and the genotype as fixed effects as well as random effects which were a permanent environmental effect, an animal effect whose terms were linked by a pedigree, and a residual effect. The estimation of the genotype effects were obtained from the same model by the predicted values of the genotype fixed effect.

1.2. Animals

Initial sampling of Noire du Velay and Mouton Vendéen ewes based on extreme LS. The first suspicions led to selecting very small samples of relevant animals which were genotyped for the 3 known mutations naturally segregating in the French populations (*FecL*^{L}, *FecX*^{L} in Lacaune and *FecX*^{GR} in Grivette). The lists of extreme highly and lowly prolific animals regarding their natural mean LS (without hormonal treatments) over at least 3 lambings were first extracted from the OVALL national database to establish the prolificacy groups. Flocks with at least 5 extreme ewes still alive at that time were selected and blood samples were collected. For the Noire du Velay breed, the final list gathered 56 females in 8 different flocks, 35 high-prolific ewes (LS mean ≥ 2.0) and 21 low-prolific ewes used as control (LS mean ≤ 1.6). In the Mouton Vendéen breed, there were 114 samples from adult ewes with 87 high-prolific (LS mean ≥ 2.20) and 27 low-prolific (LS mean ≤ 1.20).

Samples for studies of the frequency and the effects of the encountered mutations. In order to avoid any bias due to selection, large cohorts of unselected animals were collected in both breeds. For the Noire du Velay breed, the estimation of allele frequencies was made on unselected adult ewes (n = 2728) collected in 22 different flock. After genotyping, the allele frequencies were calculated on this sample. The gene effect was estimated by a linear mixed model on the whole natural LS dataset of all ewes born after year 2000. These data (111,654 records from 26,398 females) as well as the pedigree of the animals were extracted from the OVALL national database. Genotypes were either unknown or determined by genotyping. The model included the flock (67 levels), the year of birth (17 levels), the age at lambing (10 levels), the season of lambing (3 levels) and the genotype (4 levels: + +, L+, LL or unknown) as fixed effects, and two random effects: a permanent environmental effect and the animal additive genetic effect.

In the Mouton Vendéen breed, blood sampling of the whole cohort of replacement ewe lambs (n = 1200) belonging to 19 flocks of the selection nucleus was undertaken in 2016. A few months after sampling, these ewe lambs had their first lambing allowing estimation of the gene effect at this young age. A few adult sires were also genotyped (n = 6) and the production of their daughters extracted from the national database. As for the Noire du Velay breed, the gene effect was estimated by a linear mixed model on the whole natural LS dataset of all ewes born after year 2000. These data (41,269 records from 14,550 females) as well as the pedigree of the animals were also extracted from the OVALL national database and the same model was applied. Levels for the fixed effects were 87 for the flock and 18 for the year of birth.

Blood sampling and KAPA-KASP genotyping. Blood samples (5 ml per