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

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9

10 Short title: Prolific mutations in two French sheep breeds

## 11 **Abstract**

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

30

31 **Keywords:** ovine; major gene; prolificacy; BMP15; B4GALNT2

32 In ovine breeds raised for meat purposes, numerical productivity represents an  
33 important technical and economic lever. The objective is to reach an optimum for the  
34 economic profitability of breeding. Improvement of this numerical productivity is  
35 achieved by increasing the number of lambs born per ewe at each lambing, i.e. the  
36 prolificacy, associated with the improvement of lamb viability as well as the maternal  
37 quality. This leads to increased post-natal survival and growth rate of the lambs. For  
38 decades, genetic selection efforts have been made particularly on improving prolificacy  
39 of sheep breeds. However, prolificacy is a weakly heritable polygenic trait ( $h^2= 0.05 -$   
40  $0.2$ ) (see the review by Bradford (Bradford, 1985)), allowing limited genetic gain.  
41 Nevertheless in some breeds, a very large effect on ovulation rate (OR) and litter size  
42 (LS) due to single mutation in fecundity major genes (called *Fec* genes, reviewed in  
43 (Fabre *et al.*, 2006)) has been demonstrated. The first evidence of the segregation of  
44 a prolificacy major gene was established in the early 1980's in Australian Booroola  
45 Merino. This was implicated by the observation of a large variability of LS and OR in  
46 this population and the presence of extremely prolific ewes in this low prolific breed  
47 (Piper and Bindon, 1982; Davis *et al.*, 1982). The causal mutation named *FecB<sup>B</sup>* was  
48 discovered 20 years later in the *BMPR1B* gene (Bone Morphogenetic Protein Receptor  
49 1B) on the ovine chromosome 6 by several independent research groups (Wilson *et*  
50 *al.*, 2001; Mulsant *et al.*, 2001; Souza *et al.*, 2001). This mutation was thereafter  
51 introgressed in several ovine breeds around the world for research purposes or to  
52 improve their prolificacy although these latter programmes resulted in mixed outcomes  
53 (Walkden-Brown *et al.*, 2009).

54 Up to now, many mutations were discovered worldwide in three other major genes  
55 namely *BMP15* (known as *FecX* (Galloway *et al.*, 2000)), *GDF9* (known as *FecG*  
56 (Hanrahan *et al.*, 2004)) and *B4GALNT2* (known as *FecL* (Drouilhet *et al.*, 2013)). In

57 France particularly, two genetic programmes were implemented to discover and to  
58 manage mutations with major effect in order to improve the prolificacy of commercial  
59 sheep populations (Mulsant *et al.*, 2003; Bodin *et al.*, 2011; Martin *et al.*, 2014) . The  
60 introgression of the Booroola *FecB<sup>B</sup>* mutation was started in Mérinos d'Arles in the  
61 1980's. Experimental testing by the French agricultural institute INRA has estimated  
62 the effect of the mutated allele on prolificacy at one extra lamb per lambing (Teyssier  
63 *et al.*, 1997). A controlled diffusion of genotyped animals in commercial flocks is now  
64 implemented in the Mérinos d'Arles population (Teyssier *et al.*, 2009). In the Lacaune  
65 breed, two different mutations affecting prolificacy were discovered in the selection  
66 nucleus of the OVI-TEST cooperative, *FecX<sup>L</sup>* in the *BMP15* gene on the chromosome  
67 X (Bodin *et al.*, 2007), and *FecL<sup>L</sup>* at the *B4GALNT2* locus on the chromosome 11  
68 (Drouilhet *et al.*, 2009, 2013). As soon as 2005, it was decided to eradicate the *FecX<sup>L</sup>*  
69 mutation inducing sterility at the homozygous state and to manage the *FecL<sup>L</sup>* mutation  
70 which increases LS by +0.5 lamb per lambing. The selection objective is to achieve  
71 50% of heterozygous *FecL<sup>L</sup>* carrier ewes in the Lacaune OVI-TEST selection nucleus  
72 flocks (Martin *et al.*, 2014; Raoul *et al.*, 2017).

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

80 In the present paper, we present an analysis of LS data from 34 French and one  
81 Spanish meat sheep breeds highlighting the suspicion of a mutation in a major gene

82 in two of them, the Noire du Velay and the Mouton Vendéen breeds. Through molecular  
83 genotyping we evidenced the segregation of mutations already known to control OR  
84 and LS in ovine breeds. Moreover, we give an early analysis of frequency and effects  
85 of these two mutations in commercial populations.

86

## 87 **Material and methods**

### 88 *Data and statistical analysis*

89 *Relationship between mean and variance of LS.* Data come from the OVALL French  
90 national database for meat sheep genetic evaluation and research managed by the  
91 Institut de l'Élevage (French Livestock Institute) and the Centre de Traitement de  
92 l'Information Génétique (Genetic Information Processing Center, Jouy-en-Josas,  
93 France) gathering about 12 million lambings from 1986 to 2016. We have extracted  
94 the lambing career of purebred females alive in 2005 from 34 different breeds,  
95 representing 2 353 324 natural LS obtained without hormonal synchronisation  
96 treatment of oestrus. Moreover, we have added LS data from the Spanish database  
97 for genetic evaluation of Rasa Aragonesa – UPRA-Grupo Pastores (Fathallah *et al.*,  
98 2016). Basic statistical analysis (mean and variance of the observed LS) were used to  
99 characterize each breed as well as to select the animals entering in the genotyping  
100 programme.

101 *Expected frequencies of LS and variance - expected career.* A subsample of the 25  
102 most numerically important French breeds gathering 88 428 ewes with at least 5 LS  
103 records each was considered to estimate the parameters of the LS distribution. As in  
104 Bodin and Elsen (Bodin *et al.*, 1989), the second order regression coefficients of each  
105 LS frequency on the mean prolificacy of the breed were estimated on the subsample

106 of all ewes with 5 records each, excluding the Noire du Velay and Mouton Vendéen  
107 breeds as well as those known to carry a major gene for OR. These coefficients ( $\alpha_i$ ,  
108  $\beta_{1i}$ ,  $\beta_{2i}$ ) permitted estimation of the expected frequencies of each  $LS_i$  for a population  
109 of a given prolificacy (prol):  $LS_i = \alpha_i + \beta_{1i} \text{ prol} + \beta_{2i} \text{ prol}^2$  and consequently the expected  
110 variance which could be compared to the observed frequencies and variance. They  
111 were applied to a sample including 3 breeds without obvious major genes (Rava,  
112 Rouge de l'Ouest, Charollais), 2 breeds known to carry major genes (Lacaune and  
113 Grivette) and the two "suspected breeds" of the present study (Noire du Velay and  
114 Mouton Vendéen). According to the threshold model of LS (Gianola, 1982), and using  
115 the expected frequencies of these 7 populations, it was also possible to simulate  
116 lifetime LS data of females with 5 records each. Thus, 200 000 careers were simulated  
117 with a repeatability on the underlying variate equal to 0.20 (i.e.  $\sim 0.15$  on the observed  
118 scale). These simulations provided the expected percentage of animals with 5  
119 lambings which exceed a given mean prolificacy (i.e. 3.0). As before, this gives the  
120 expected value in the absence of a major gene in the population was compared to the  
121 observed value.

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

128 The heritability [ $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_p^2 + \sigma_e^2)$ ] and the repeatability [ $r = (\sigma_g^2 + \sigma_p^2) / (\sigma_g^2 + \sigma_p^2 + \sigma_e^2)$ ]  
129 were calculated through the estimation of the additive genetic variance  $\sigma_g^2$ , the

130 permanent environmental variance  $\sigma_p^2$  and the residual variance  $\sigma_e^2$ . These variances  
131 were estimated by a linear mixed model run with the ASReml software (Gilmour *et al.*,  
132 2014). This model included the flock, the year, the season of lambing, the age and the  
133 genotype as fixed effects as well as random effects which were a permanent  
134 environmental effect, an animal effect whose terms were linked by a pedigree, and a  
135 residual effect. The estimation of the genotype effects were obtained from the same  
136 model by the predicted values of the genotype fixed effect.

### 137 *Animals*

#### 138 *Initial sampling of Noire du Velay and Mouton Vendéen ewes based on extreme LS.*

139 The first suspicions led to selecting very small samples of relevant animals which were  
140 genotyped for the 3 known mutations naturally segregating in the French populations  
141 (*FecL<sup>L</sup>*, *FecX<sup>L</sup>* in Lacaune and *FecX<sup>GR</sup>* in Grivette). The lists of extreme highly and  
142 lowly prolific animals regarding their natural mean LS (without hormonal treatments)  
143 over at least 3 lambings were first extracted from the OVAL national database to  
144 establish the prolificacy groups. Flocks with at least 5 extreme ewes still alive at that  
145 time were selected and blood samples were collected. For the Noire du Velay breed,  
146 the final list gathered 56 females in 8 different flocks, 35 high-prolific ewes (LS mean  
147  $\geq 2.0$ ) and 21 low-prolific ewes used as control (LS mean  $\leq 1.6$ ). In the Mouton  
148 Vendéen breed, there were 114 samples from adult ewes with 87 high-prolific (LS  
149 mean  $\geq 2.20$ ) and 27 low-prolific (LS mean  $\leq 1.20$ ).

#### 150 *Samples for studies of the frequency and the effects of the encountered mutations.* In

151 order to avoid any bias due to selection, large cohorts of unselected animals were  
152 collected in both breeds. For the Noire du Velay breed, the estimation of allele  
153 frequencies was made on unselected adult ewes (n=2728) collected in 22 different



154 flock. After genotyping, the allele frequencies were calculated on this sample. The  
155 gene effect was estimated by a linear mixed model on the whole natural LS dataset of  
156 all ewes born after year 2000. These data (111654 records from 26398 females) as  
157 well as the pedigree of the animals were extracted from the OVALL national database.  
158 Genotypes were either unknown or determined by genotyping. The model included the  
159 flock (67 levels), the year of birth (17 levels), the age at lambing (10 levels), the season  
160 of lambing (3 levels) and the genotype (4 levels: ++, L+, LL or unknown) as fixed  
161 effects, and two random effects: a permanent environmental effect and the animal  
162 additive genetic effect.

163 In the Mouton Vendéen breed, blood sampling of the whole cohort of replacement ewe  
164 lambs (n=1200) belonging to 19 flocks of the selection nucleus was undertaken in  
165 2016. A few months after sampling, these ewe lambs had their first lambing allowing  
166 estimation of the gene effect at this young age. A few adult sires were also genotyped  
167 (n=6) and the production of their daughters extracted from the national database. As  
168 for the Noire du Velay breed, the gene effect was estimated by a linear mixed model  
169 on the whole natural LS dataset of all ewes born after year 2000. These data (41269  
170 records from 14550 females) as well as the pedigree of the animals were also extracted  
171 from the OVALL national database and the same model was applied. Levels for the  
172 fixed effects were 87 for the flock and 18 for the year of birth.

173 *Blood sampling and KAPA-KASP genotyping.* Blood samples (5 ml per animal) were  
174 collected from jugular vein by Venoject system containing EDTA in commercial flocks  
175 and directly stored at -20°C for further use. Genotyping was obtained by a first step of  
176 KAPA Blood PCR amplification of a specific fragment encompassing the mutation  
177 position (KAPA Biosystems). Primers used for PCR amplification were designed using  
178 Primer 3 software (Table 1). A one µl sample of total blood was run for PCR with a

179 mixture containing 5µl of KAPA Blood kit solution and 0.25µl of each specific primer at  
180 10nM in a final volume of 20 µl. PCR amplifications were conducted on an ABI 2400  
181 thermocycler (Applied Biosystems) with the following conditions: 5 min initial  
182 denaturation at 94 °C, 32 cycles of 30 s at the melting temperature, 30 s extension at  
183 72 °C and 30 s at 94 °C, followed by 5 min final extension at 72 °C.

184 In the second step, the specific resulting KAPA Blood PCR fragments were used as  
185 template for the genotyping of *FecL<sup>L</sup>* (*B4GALNT2* intron 7, OAR11:36938224T>A,  
186 NC\_019468, (Drouilhet *et al.*, 2013)), *FecX<sup>L</sup>* (*BMP15* exon 2, OARX: 50980449G>A,  
187 NC\_019484, (Bodin *et al.*, 2007)) or *FecX<sup>GR</sup>* (*BMP15* exon 2, OARX: 50980461C>T,  
188 NC\_019484, (Demars *et al.*, 2013)). The genotyping was done by fluorescent  
189 Kompetitive Allele Specific PCR via the KASP V4.0 2x Master mix (LGC genomics) as  
190 follow: reaction of 1.2µl of the KAPA Blood PCR product, 0.07µl primers premix and  
191 2.5µl of the 2x KASP Master mix. The primers premix is prepared as follow: 1.2µl of  
192 each forward fluorescent allele specific primers at 100µM, 3µl of the common reverse  
193 primer at 100µM in a final volume of 10µl. Primers used for KASP PCR amplification  
194 are indicated in the Table 1. The PCR amplification condition was 15 min at 94°C for  
195 the hot-start activation, 10 cycles of 20 s at 94°C, 61- 55°C for 60 s (dropping 0.6°C  
196 per cycle), then 26 cycles of 20 s at 94°C and 60 s at 55°C. KASP genotyping was  
197 analysed by a final point read of the fluorescence on an ABI 7900HT Real-Time PCR  
198 System and using the SDS Software 2.4 (Applied Biosystems).

199

## 200 **Results**

201 *Suspicion of mutation in major genes affecting prolificacy*

202 As previously described, as long as there are less than 1% triplets in a sheep  
203 population, the distribution of LS approximately follows a binomial distribution for which  
204 the variance is directly linked to the mean (Bodin *et al.*, 1989). For the breeds with a  
205 higher percent of triplets, the mean-variance relationship remains very strong. A plot  
206 of the relationship between mean and variance of LS following natural oestrus in 34  
207 different French breeds and one Spanish breed is shown in Fig.1. The breeds in which  
208 a mutation in a major gene is segregating were distinctly marked (Lacaune, Grivette  
209 and Rasa Aragonesa, black squares) and clearly stood out from the quadratic trendline  
210 (dashed line) which had a high  $r^2$  (0.93). Excluding the three breeds with known  
211 mutations in major genes gave a quadratic trendline (plain line) with a higher  $r^2$  (0.97).  
212 Based on this second trendline, the figure 1 shows that the Noire du Velay and Mouton  
213 Vendéen breeds (circles) also clearly deviated from their expected place, which could  
214 suggest the segregation of a mutation in a major gene in these two populations.

215 Following this first hint, we analysed the evolution of the mean prolificacy of the 34  
216 French breeds between 1986 and 2016. In the figure 2, we have plotted the overall  
217 annual mean LS weighted by the number of individuals in each population. We  
218 observed a regular increase of the mean LS of these breeds during the last three  
219 decades corresponding to +0.70 lamb/100 ewes/year. In contrast to the increase  
220 observed for the Lacaune, Grivette and Noire du Velay breeds, the Mouton Vendéen  
221 breed showed a slight regular decrease of its mean LS. Remarkably, the Noire du  
222 Velay breed had a strong increase of the mean LS with +1.40 lambs/100 ewes/year,  
223 twice as fast as what was observed for the overall mean and even faster than the  
224 Lacaune and Grivette breeds (respectively +0.68 and +0.75 lambs/100 ewes/year),  
225 known to carry a mutation in a major gene increasing prolificacy. Even if we could  
226 suppose that a strong improvement of the environment had occurred for improving the

227 prolificacy of the Noire du Velay breed during the last decades, we can also speculate  
228 on the segregation of mutation in major genes influencing this trait.

229 The ratio of the observed distribution of each LS class to its expectation (provided by  
230 regression coefficients estimated on a large dataset) is given by  $\rho$  in Table 2.  
231 Estimations of LS frequencies were very close to the observed values for the Rava,  
232 the Rouge de l'Ouest and the Charollais breeds, as  $\rho$  ranged from 0.98 to 1.03. In  
233 contrast,  $\rho$  for the Noire du Velay and the Mouton Vendéen breeds ranged further apart  
234 from 1 (0.91 to 1.36). Similar results were obtained for the two breeds carrying a  
235 mutation in a major gene, Lacaune and Grivette ( $\rho$  ratio from 0.89 to 1.24).  
236 Consequently,  $\rho$  ratios for LS variance were remarkably close to 1 for the non-carrier  
237 breeds in contrast to those of the Lacaune and Grivette as well as the Noire du Velay  
238 and Mouton Vendéen breeds (ranging from 1.08 to 1.32). Thus, the LS distributions  
239 observed in Noire du Velay and Mouton Vendéen breeds break the rules of  
240 homogenous populations and suggested for each breed a mixture of females with  
241 different prolificacy level.

242 The final hint was the excess of highly prolific animals in these breeds (Table 2). The  
243 observed number of females having a mean prolificacy higher than 3 on five records  
244 were generally low for all the main French breeds with mean LS under 2.0. Obviously,  
245 this parameter increased regularly when the mean prolificacy of the population  
246 increased, however it was higher for the breeds known to carry or suspected to carry  
247 a mutation in a major gene reaching 28‰ in Grivette, for example (Table 2).  
248 Furthermore,  $\rho$  was close to 1 for the non-carrier breeds and higher for the other  
249 breeds. For the Noire du Velay breed, the number of females with a prolificacy higher  
250 than 3 was 16 times higher than expected for a comparable population without

251 mutation in a major gene. Although this parameter was lower for the Mouton Vendéen  
252 breed, it was higher than for the non-carrier breeds.

253 *Genotyping of known mutations affecting prolificacy in French ovine populations:*

254 *FecL<sup>L</sup>, FecX<sup>L</sup> and FecX<sup>Gr</sup>*

255 Extremely low and high-prolific ewes from the Noire du Velay (n=56) and Mouton  
256 Vendéen (n=114) populations were genotyped for the 3 known mutations naturally  
257 segregating in the French populations i.e. *FecL<sup>L</sup>*, *FecX<sup>L</sup>* in Lacaune and *FecX<sup>Gr</sup>* in  
258 Grivette (Table 3). The *FecX<sup>L</sup>* mutation was not found but the *FecL<sup>L</sup>* allele was found  
259 in the Noire du Velay high-prolific group and the *FecX<sup>Gr</sup>* allele was found in the high-  
260 prolific group of Mouton Vendéen ewes (Table 3). All the low-prolific ewes from both  
261 breeds were wild-type at the genotyped loci. For both breeds, the exact Fisher test was  
262 highly significant ( $P < 0.001$ ) showing a clear disequilibrium between prolificacy groups  
263 and genotypes of these mutations.

264 *FecL<sup>L</sup> and FecX<sup>Gr</sup> genotype frequency and effect on prolificacy*

265 Large cohorts of unselected animals were genotyped in order to accurately estimate  
266 the allele frequencies in the Noire du Velay and the Mouton Vendéen populations  
267 (Table 4). In Noire du Velay, the frequency of the *L* prolific allele at the *FecL<sup>L</sup>* locus  
268 was 0.11 with 20.1% heterozygous and 1.1% homozygous carriers. These frequencies  
269 are in Hardy Weinberg equilibrium ( $P = 0.36$ ). Among the Mouton Vendéen  
270 replacement ewe lambs, the frequency of the *Gr* prolific allele at the *FecX<sup>Gr</sup>* locus was  
271 0.05 and we observed 10.3% carrier ewes (+/*Gr* and *Gr/Gr*), only 3 animals being  
272 homozygous *Gr/Gr*. These frequencies were also in Hardy Weinberg equilibrium ( $P =$   
273 0.84).

274 The estimated genetic effects are presented in the Table 5. Genetic parameters were  
275 very similar in both breeds. Heritability ( $h^2$ : 0.09) and repeatability ( $r$ ; 0.10 to 0.14) were  
276 low and in full agreement with the classical values of these parameters for this species  
277 (Janssens *et al.*, 2004). A single copy of the *FecL<sup>L</sup>* in Noire du Velay increased the  
278 mean prolificacy by 0.42 lamb per lambing ( $P < 0.001$ ). The additional increase due to  
279 a second copy of the mutation in homozygous carriers was lower (0.13 ;  $P = 0.096$ ). In  
280 the Mouton Vendéen breed, the effect of a single copy of the *FecX<sup>Gr</sup>* allele increased  
281 the prolificacy by 0.30 lamb per lambing ( $P < 0.001$ ), while the effect of a second copy  
282 leading to a homozygous carrier, does not further increase the prolificacy ( $P = 0.485$ ).  
283 In both breeds, as expected, females of the unknown genotype group were slightly,  
284 although not significantly, more prolific than the corresponding females known as wild-  
285 type.

286

## 287 **Discussion**

288 Simple analyses of livestock industry data suggested the segregation of a mutation in  
289 a major gene affecting prolificacy of the Noire du Velay and Mouton Vendéen breeds.  
290 Small samples of extremely high and low prolific ewes were then genotyped for known  
291 mutations and revealed the presence of causal mutations. Among the different strands  
292 of evidence, the deviation from the relationship between mean and variance of  
293 prolificacy for breeds with a mutation in a major gene comparing to other breeds was  
294 quite remarkable. Even if these parameters were calculated without any correction for  
295 variation factors, the very high number of observations for each breed, smoothed all  
296 the effects and led to a very strong relationship among non-carrying populations. Only  
297 LS after natural oestrus were extracted from the national database as using hormonal  
298 treatments to induce ovulations modifies the mean-variance relationship (Bodin *et al.*,

299 1989). Moreover, the within breed variability of LS variance due to polygenic effects is  
300 generally very small (Bodin *et al.*, 1989; Amer and Bodin, 2006; Cottle *et al.*, 2016) and  
301 could not affect the mean-variance relationship at a broad scale. Finally, the observed  
302 deviation was due to the mixture into the populations of two groups of animals widely  
303 differing in prolificacy as it had been already viewed in Lacaune (Martin *et al.*, 2014)  
304 and in Rasa Aragonesa breed (Fathallah *et al.*, 2016).

305 The procedure used in the present study is relevant only for genes having large effect  
306 (about 0.5 standard deviation of the prolificacy mean). If the presence of known  
307 mutations was not found, the process would have been followed by a GWAS to  
308 compare allele frequencies between the few selected high (cases) and low (controls)  
309 prolific ewes. The selection of two small samples of very extreme animals and their  
310 analysis considering they are two states of a qualitative trait has been already  
311 successful and allowed the discovery of two new mutations for ovulation rate (Demars  
312 *et al.*, 2013). However, as shown in the Table 3, some highly prolific Noire du Velay  
313 and Mouton Vendéen ewes were non-carriers of known prolific alleles. Their extreme  
314 prolificacy could be either explained by the polygenic determinism of this trait or by the  
315 segregation of another major mutation as already described in the Lacaune population  
316 carrying both *FecX<sup>L</sup>* and *FecL<sup>L</sup>* (Bodin *et al.*, 2007; Drouilhet *et al.*, 2009).

317 The frequency of carrier ewes is much higher in the Noire du Velay breed (~20%) than  
318 in the Mouton Vendéen breed (~10%) which can explain that the deviation from the  
319 mean-variance relationship is also higher in this breed. However, the Hardy Weinberg  
320 equilibrium is still very well conserved. This means that in both populations prolific  
321 allele frequencies are not strongly affected by selection, particularly in the Mouton  
322 Vendéen breed as shown by the mean LS evolution during the last three decades. In  
323 both breeds it seems that carrier ewes do not produce more replacement ewe lambs



324 in spite of their higher prolificacy and that there is no preferential culling according to  
325 the genotype. In Lacaune, Hardy Weinberg equilibrium did not hold for a long time  
326 because between 1996 and 2010 the cooperative excluded animals that were too  
327 prolific and since 2011 the cooperative's aim has been to achieve 50% of L+ ewes  
328 (Martin *et al.*, 2014).

329 The effects of one copy of the *FecL<sup>L</sup>* mutation on LS is similar in the Noire du Velay  
330 (+0.42 lamb per lambing) and in the Lacaune breed (+0.47, (Martin *et al.*, 2014)), and  
331 is of the same order of magnitude as the effect of most of other known major genes for  
332 prolificacy (Bodin *et al.*, 2011; Jansson, 2014). However, as it has been already noted,  
333 the effect of the *FecL<sup>L</sup>* mutation is much higher than the effect of the *FecX<sup>Gr</sup>* mutation  
334 observed in the Grivette population (+0.10 lamb per lambing, (Demars *et al.*, 2013)).  
335 In Mouton Vendéen, the effect of one copy of the *FecX<sup>Gr</sup>* mutation (+0.30 ± 0.04 lamb  
336 per lambing) seems higher than the effect of the same mutation in the Grivette  
337 population, although in this latter population, the analysis of the allele effect was not  
338 conducted on a large sample. *FecX<sup>Gr</sup>* homozygous carrier ewes in the Grivette or the  
339 Mouton Vendéen population are as prolific, if not more, than the heterozygous ones  
340 (present work and (Demars *et al.*, 2013)), in contrast to most mutations of the *BMP15*  
341 gene which induce sterility at the homozygous state (Bodin *et al.*, 2007; Demars *et al.*,  
342 2013).

343

## 344 **Conclusion**

345 Based on an analysis of a very large LS dataset from 34 French meat sheep breeds  
346 and molecular genotyping, we have highlighted and evidenced the segregation of two  
347 mutations in the *FecL* and *FecX* major genes in the Noire du Velay and the Mouton  
348 Vendéen breeds. We have determined a fairly high frequency (0.05 to 0.11) and a



349 rather strong effect (+0.3 to +0.4 lamb/lambing) of the *FecL<sup>L</sup>* and *FecX<sup>Gr</sup>* prolific alleles.  
350 This discovery should serve as a basis for implementing specific management  
351 programmes, including genotyping of reproducers, in relation with the Noire du Velay  
352 and Mouton Vendéen selection organizations in line with their breeding objective of  
353 prolificacy.

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365

## 366 **Declaration of interest**

367 The authors declare that they have no competing interests

368

## 369 **Ethics statement**

370 The blood sampling procedure was approved (approval number 01171.02) by the  
371 French Ministry of Teaching and Scientific Research and local ethical committee  
372 C2EA-115 (Science and Animal Health) in accordance with the European Union  
373 Directive 2010/63/EU on the protection of animals used for scientific purposes.

374

## 375 **Data repository resources**

376 Raw data from the OVALL database were managed by the Institut de l'Élevage (French  
377 Livestock Institute) and the Centre de Traitement de l'Information Génétique (Genetic  
378 Information Processing Center, Jouy-en-Josas, France). Derived data supporting the  
379 findings of this study are available from the corresponding author upon reasonable  
380 request.

381

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482

### 483 **Figure captions**

484

485 **Figure 1** Plot of mean and variance of litter size for 35 sheep breeds

486 Data are from the French national OVAL database for genetic evaluation and  
487 research – Institut de l'Élevage, France and the database for genetic evaluation of  
488 Rasa Aragonesa – UPRA-Grupo Pastores, Spain (2 353 324 natural LS of purebred  
489 ewes alive in 2005). Each spot corresponds to a given population. The black squares  
490 correspond to breeds with an identified mutation in a major gene affecting prolificacy  
491 (1= Grivette, *FecX<sup>Gr</sup>*; 2=Lacaune, *FecX<sup>L</sup>* and *FecL<sup>L</sup>*; 3=Rasa Aragonesa, *FecX<sup>R</sup>*). The  
492 dashed line is the quadratic regression curve modelling all points ( $R^2=0.93$ ). The plain  
493 line is the quadratic regression modelling points without black squares ( $R^2=0.97$ ). The  
494 open circles are breeds suspected to carry a mutation in a major gene affecting  
495 prolificacy (4=Noire du Velay; 5=Mouton Vendéen).

496

497 **Figure 2** Annual evolution of mean prolificacy in French sheep breeds

498 Litter size (LS) data from 1986 to 2016 are from the French national OVAL database  
499 for genetic evaluation and research. The annual mean LS is plotted year by year for  
500 each breed (plain lines) and for the whole populations weighted by the number of  
501 individuals in each population (dashed line, weighted  $\mu$ ). NdV denotes the Noire du  
502 Velay breed.



503 **Table 1** List of PCR primers used in the study.

Locus/ Chromosome	Primer sequence (variant allele underlined)	Position <sup>1</sup> (start, bp)	Application
BMP15/ OARX	GGCACTTCATCATTGGACT	50971433	KAPA PCR <i>FecX<sup>Gr</sup>/FecX<sup>L</sup></i>
	GGCAATCATACCCTCATACTCC	50970959	
	TCTGATCCACCAGCTCACTG	50971066	KASP PCR <i>FecX<sup>Gr</sup></i>
CATTGCTCCCCATCTCTATAC	50971170		
CATTGCTCCCCATCTCTATA <u>T</u>	50971170		
	GATGGGCCTGAAAGTAACCA	50971248	KASP PCR <i>FecX<sup>L</sup></i>
	ACCCGAGGACATACTCCCTTAC	50971137	
	ACCCGAGGACATACTCCCTTA <u>T</u>	50971137	
B4GALNT2/ OAR11	TGGTTCAAACCTCCTACATGCAAGA	36938189	KAPA PCR <i>FecL<sup>L</sup></i>
	TATGCATGGCATGTGATAGG	36938314	
	TATGCATGGCATGTGATAGG	36938314	KASP PCR <i>FecL<sup>L</sup></i>
	GCAAGAAGCTGCGTGTGT	36938207	
	GCAAGAAGCTGCGTGTGA <u>A</u>	36938207	

504 <sup>1</sup>Start positions of primers (in base pair) are based on the OARv3.1 ovine genome assembly

505 **Table 2** Distributions of litter size in seven different breeds carrying - or not - mutation  
506 in major gene influencing prolificacy

Breed	Mut	Mean LS	LS variance		%LS=						%♀ with LS ≥ 3.0 over 5 lambings	
			Obs	ρ	1		2		3		Obs	ρ
Rava	no	1.50	0.31	1.01	52.9	1.00	44.4	1.00	2.6	0.98	0.0	E=0
Rouge de l'Ouest	no	1.78	0.41	1.00	33.1	1.00	56.2	1.00	10.7	1.02	2.5	0.9
Charollais	no	1.70	0.39	1.01	38.1	1.00	53.9	0.99	8.0	1.03	1.8	1.4
Noire du Velay	?	1.62	0.43	1.23	46.7	1.07	46.1	0.91	7.7	1.36	5.7	16.2
Mouton Vendéen	?	1.72	0.42	1.08	37.8	1.03	52.6	0.96	9.6	1.13	3.6	2.7
Lacaune	yes	1.75	0.53	1.32	39.0	1.11	49.4	0.89	11.6	1.24	18.2	10.5
Grivette	yes	1.92	0.56	1.24	28.7	1.12	54.3	0.92	17.8	1.09	28.3	3.07

507 Mut = Prolific mutation; LS = Litter size; Obs = observed distribution of the parameters (% or ‰); E =  
508 expected parameters estimated through the second order regression of the LS on the mean prolificacy;  
509 ρ = Obs / E

510 **Table 3** *FecL<sup>L</sup>, FecX<sup>L</sup> and FecX<sup>Gr</sup> genotypes of few extreme ewes for each breed and*  
 511 *prolificacy group*

Breed	Prolific group	Locus/ Genotype								
		<i>FecL</i>			<i>FecX</i>			<i>FecX</i>		
		+/+	+/L	L/L	+/+	+/L	L/L	+/+	+/Gr	Gr/Gr
Noire du Velay	Low (n=21)	21	0	0	21	0	0	21	0	0
	High (n=35)	10	23	2	35	0	0	35	0	0
Mouton Vendéen	Low (n=27)	27	0	0	27	0	0	27	0	0
	High (n=87)	87	0	0	87	0	0	56	29	2

512

513 **Table 4** *FecL<sup>L</sup> and FecX<sup>Gr</sup> genotyping in the Noire du Velay and Mouton Vendéen*  
 514 *populations*

Genotype	Breed / Locus					
	Noire du Velay / <i>FecL</i> (n=2728)			Mouton Vendéen / <i>FecX</i> (n=1200)		
	+/+	+/L	L/L	+/+	+/Gr	Gr/Gr
Number	2151	548	29	1076	121	3
Frequency ( % )	78.8	20.1	1.1	89.7		10.3
SE <sup>1</sup> of frequency (%)	0.8	0.8	0.2	0.9		0.9
Raw mean LS <sup>2</sup>	1.58	2.03	2.18	1.55		2.01

515 <sup>1</sup>SE = Standard error

516 <sup>2</sup>LS = Litter size

517

518

519 **Table 5** Genetic parameters and allelic estimated values of litter size in Noire du Velay  
 520 and Mouton Vendéen breeds

Breed	n LS	n ♀	Genetic parameters					Allelic estimated values			
			$\sigma^2_g$	$\sigma^2_p$	$\sigma^2_e$	$h^2$	$r$	zz	+/+	+/M <sup>1</sup>	M/M
Noire du Velay	111654	26398	0.036	0.017	0.334	0.09 (0.005)	0.14 (0.003)	1.60 <sup>a</sup>	1.57 <sup>a</sup>	1.99 <sup>b</sup>	2.12 <sup>b</sup>
Mouton Vendéen	41269	14550	0.034	0.005	0.344	0.09 (0.007)	0.10 (0.005)	1.71 <sup>a</sup>	1.68 <sup>a</sup>	1.98 <sup>b</sup>	1.99 <sup>b</sup>

521 LS = Litter size;  $\sigma^2_g$  = Additive genetic variance;  $\sigma^2_p$  = Permanent environmental variance;  $\sigma^2_e$  = Residual  
 522 variance;  $h^2$  = Heritability (standard errors are between brackets);  $r$  = Repeatability (standard errors are  
 523 between brackets); zz = Unknown genotype; ++ = Homozygous wild type, M+: heterozygous, MM:  
 524 homozygous mutated type.

525 <sup>1</sup> M denotes the mutated prolific allele within each breed (*FecL<sup>L</sup>* or *FecX<sup>Gr</sup>*)

526 <sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.001$ .

Figure 1

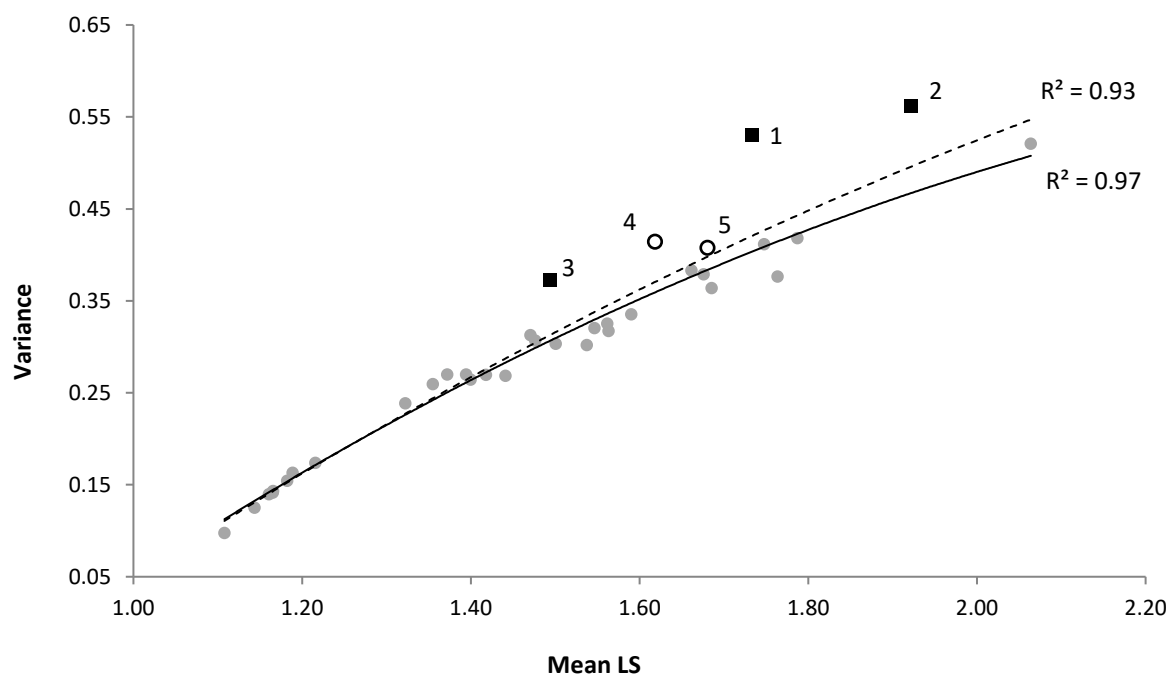


Figure 2

