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2 **The high prolificacy of D'man sheep is associated with the segregation**  
3 **of the *FecL<sup>L</sup>* mutation in the *B4GALNT2* gene**

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## 28 Contents

30 Mutations in the *FecL* locus are associated with large variation in ovulation rate and litter size in the French Lacaune sheep breed. It has been shown that the *B4GALNT2* gene within the *FecL* locus is most likely responsible for the high fecundity in the French breed. In this study, we have highlighted the segregation of the *FecL<sup>L</sup>* mutation within the *B4GALNT2* gene in North African sheep breeds and notably in the highly prolific D'man breed. Genotyping of a sample of 183 Tunisian D'man individuals revealed a high frequency (0.65) of the prolific allele *FecL<sup>L</sup>* which was attributed to the adoption of a decades-old breeding strategy based on the selection of ewe lambs born from large litter size. Homozygous *LL* ewes showed a significantly increased litter size compared to heterozygous and non-carrier ewes ( $FecL^L/FecL^L = 2.47 \pm 0.09$  versus  $FecL^L/FecL^+ = 2.23 \pm 0.09$ ,  $P < 0.05$  and  $FecL^+/FecL^+ = 1.93 \pm 0.18$ ,  $P < 0.01$ ). The presence of the *FecL<sup>L</sup>* polymorphism in both D'man and Lacaune breeds argues for an ancestral origin of this mutation and brings an answer to the old question of the genetic determinism of the extreme prolificacy of the D'man ewes. The results of this study can help to establish planned genotype-based mating allowing both higher profit for the breeders and an optimal management of the *FecL<sup>L</sup>* mutation in D'man sheep populations.

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**Keywords :** D'man sheep - Prolificacy - Major gene - B4GALNT2

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## 48 1 INTRODUCTION

The D'man sheep is a highly prolific breed that spreads in southwestern oases of Algeria (Boubekeur  
50 et al., 2015) and south of Morocco (Boujenane, 1999). This breed was established in Tunisia in 1994  
from a Moroccan founder flock and expanded widely in the country, particularly in the Tunisian oases  
52 (Rekik et al., 2005). A limited number of studies tried to shed light on the genetic determinism  
underlying the high prolificacy within the D'man breed. The conclusions were never in favor of the  
54 segregation of a major gene controlling this trait as it was seen in Merinos Booroola (Bradford et al.,  
1989; Boujenane et al., 1991; Piper and Bindon, 1985). Moreover, none of the studies made at the  
56 molecular level succeeded in identifying genes associated with ovulation rate or litter size (LS). For  
instance, Vacca et al., (2010) did not find any polymorphisms in seven genotyped mutations located  
58 in three previously described prolificacy major genes (*BMPRI1B*, *BMP15* and *GDF9*) within a sample  
of 47 D'man individuals. Another study carried out by Aherrahrou et al., (2015) has found difference  
60 in expression levels of the luteinizing hormone  $\beta$  subunit gene (*LHB*) between D'man and other  
normal prolific local breeds in Morocco. Knowing the role of this gene in the ovulation process, they  
62 suggested that the *LHB* gene might be a good candidate for prolificacy variation in D'man. However,  
these authors did not specify whether they found or not differences in expression levels or  
64 polymorphisms between highly and normal prolific D'man ewes.

Recently, we have performed a genetic variant data mining focused on the four prolificacy major gene  
66 loci (*B4GALNT2*, *BMP15*, *BMPRI1B* and *GDF9*; Vinet et al., 2012; Abdoli et al., 2016) from the  
comparative analysis of whole ovine genome sequence data publicly available from the European  
68 project NextGen (<http://projects.ensembl.org/nextgen/>). Notably, we have highlighted the presence of  
a previously described polymorphism in the intron 7 of the *B4GALNT2* gene at the prolific *FecL* locus  
70 (OAR 11; Drouilhet et al., 2013) in a subset of Moroccan sheep individuals within the D'man, Sardi,  
Beni Guil and some other local breeds. The prolific allele, named *FecL<sup>L</sup>* was originally shown to have  
72 large effects on ovulation rate and LS in the French Lacaune meat breed (Bodin et al., 2002, Drouilhet  
et al., 2009; Martin et al., 2014). At the molecular level, the *FecL<sup>L</sup>* mutation leads to a huge increase

74 of the *B4GALNT2* expression and glycosylation activity within the ovarian granulosa cells targeting  
the inhibin/activin system. This glycosylation mechanism was proposed as the physiological  
76 determinant of the regulation of ovulation rate and LS in the Lacaune breed (Drouilhet et al., 2013).  
The main purpose of the present study was to validate the presence of the polymorphism detected  
78 within the *B4GALNT2* gene at the *FecL* locus in a larger number of D'man animals originated from  
Tunisia and to investigate its association with prolificacy (measured through LS) in order to check  
80 that the mutation has also an effect on the D'man's genetic background.

## 2 MATERIALS AND METHODS

### 82 2.1 Ensembl/NextGen data mining

We have looked for previously identified SNP and small insertions/deletions in the sheep genome  
84 v3.1 using the Ensembl genome browser ([www.ensembl.org](http://www.ensembl.org), accessed January 2016) at the four  
known prolificacy major loci, *B4GALNT2* (11: 36,929,322-36,992,982), *BMP15* (X: 50,970,938-  
86 50,977,454), *BMP1B* (6: 29,361,947-29,448,079) and *GDF9* (5: 41,841,034-41,843,517). We have  
focused on the alleles named *FecL<sup>L</sup>* in *B4GALNT2*, *FecX<sup>L, H, B, G, L, R, Gr, O, Bar</sup>* in *BMP15*, *FecB<sup>B</sup>* in  
88 *BMP1B* and *FecG<sup>H, T, V, E, WN</sup>* in *GDF9* that were shown to affect prolificacy in various sheep breeds  
(reviewed in Vinet et al., 2012; Abdoli et al., 2016, genomic coordinates in Table 1). At each position  
90 we have extracted the population information data coming from the whole genome sequencing  
publicly available from the NextGen project (<http://projects.ensembl.org/nextgen/>).

### 92 2.2 Animal sampling

Jugular vein blood samples (5 ml per animal) were obtained from 152 D'man ewes and 31 rams in  
94 eleven different flocks spread across Tunisia by persons qualified in animal experimentation  
(European standards) and under the Tunisian Veterinary Authorities' rules. The flocks were ranked  
96 into three rearing mode (RM) categories corresponding to commercial private (RM1; 50% of the  
animals), state (RM2; 15% of the animals) and small familiar farms (RM3; 35% of the animals). Most  
98 of the individuals (91%) have at least 3 consecutive lambing records (Figure 1).

### 2.3 Polymorphism genotyping assays

100 At the *B4GALNT2* locus, the genotyping of *FecL<sup>L</sup>* (*B4GALNT2* intron 7, OAR11:36938224T>A,  
NC\_019468.1, Drouilhet et al., 2013) was obtained by a first step of KAPA Blood PCR amplification  
102 of a specific fragment encompassing the mutation position (KAPA Biosystems) and a second step of  
fluorescent Kompetitive Allele Specific PCR via the KASP V4.0 2x Master mix (LGC genomics) as  
104 already described (Chantepie et al., 2018). KAPA Blood PCR amplification was also used for  
subsequent DNA sequencing as described (Talebi et al., 2018) of exons 1 and 2 of *BMP15* and *GDF9*,  
106 and exon 7 of *BMPR1B*. Obtained reads were aligned against the ovine reference sequence for each  
gene of interest (ovine reference genome v3.1; *BMP15*: NC\_019484.1; *GDF9*: NC\_019462.1 and  
108 *BMPR1B*: NC\_019463.1) using CLC Main Workbench Version 7.6.4 (www.clcbio.com) in order to  
assess for polymorphisms. All primers used for genotyping are shown in Table 2.

## 110 2.4 Statistical analyses

The genotypic frequencies at the *FecL* locus were tested for deviation from Hardy-Weinberg  
112 equilibrium according to a Chi-square ( $\chi^2$ ) test and level of significance at 1 degree of freedom. The  
association between *FecL* genotypes and LS was analyzed using a two-way analysis of variance  
114 (ANOVA) followed by post hoc Tukey's multiple comparisons test. The ANOVA model used was:  
 $LS_{ij} = \mu + RM_i + G_j + e_{ij}$ , where LS denotes the mean litter size,  $\mu$  the overall sample mean,  $RM_i$  the  
116 fixed effect of the rearing mode ( $i=1, 2, 3$ ),  $G_j$  the fixed effect of the genotype ( $j=++, L+, LL$ ) and  $e_{ij}$   
is the random error. The association between genotypes at *FecX*, *FecG* and *FecB* loci and LS of *FecL*  
118  $+/+$  ewes was analyzed using a one-way ANOVA in order to test only for the genotype effect. A  
significant difference was considered when the P-value  $P < 0.05$ .

## 120 3 RESULTS

A data mining approach aimed at identifying genomic variants (SNP and small Indels) recorded in  
122 the Ensembl database (www.ensembl.org) was performed at the four loci presently known to affect  
ovulation rate in sheep. We have particularly focused on the positions of the sixteen proven prolific  
124 alleles within the four following genes: *B4GALNT2*, *BMP15*, *BMPR1B* and *GDF9*. As shown in  
Table 1, only the *FecL<sup>L</sup>* prolific allele (OAR11: 36938224A) within the *B4GALNT2* gene was detected

126 (0.18 in frequency among 160 individuals) in a subset of Moroccan sheep (MOOA) from several  
breeds whose genome was sequenced in the frame of the NextGen project  
128 (<http://projects.ensembl.org/nextgen/>). When looking more specifically at the Moroccan breeds  
affected, we have highlighted the segregation of the *FecL<sup>L</sup>* allele in D'man, Sardi, Beni Guil and some  
130 other local breeds (supplementary Table S1). Notably, the well-known prolific D'man breed exhibited  
a very high frequency (0.58) of the *FecL<sup>L</sup>* allele (23 carriers among the 30 randomly chosen animals).  
132 The apparent frequency appeared lower in Sardi (4 heterozygous among 27 animals), in Beni Guil (2  
heterozygous among 6 animals) and in a mix of local populations (15 carriers, including 2  
134 homozygous, among 72 animals).

In order to specifically confirm the segregation of the *FecL<sup>L</sup>* allele and its effect on prolificacy in the  
136 D'man breed, we have genotyped 183 individuals (31 males and 152 females with litter size records)  
at the SNP present in the intron 7 of *B4GALNT2* (OAR11:36938224T>A) in the Tunisian D'man  
138 sheep population. As shown in Table 3, we have confirmed the segregation of the *FecL<sup>L</sup>* prolific allele  
in this D'man sheep population. Observed genotype frequencies in the whole sampled animals were  
140 43.2 %, 44.3 % and 12.5 %, for *LL*, *L+* and *++* genotypes, respectively (Table 3), and in accordance  
with the Hardy-Weinberg equilibrium ( $\chi^2=0.10$ ,  $P = 0.75$ ). Genotype frequencies of homozygous  
142 individuals varied widely between RM1/RM3 and RM2 flocks with values ranging from 5.5 % (RM1)  
to 41 % (RM2) for *++* individuals and from 18 % (RM2) to 48.5% (RM1) for *LL* animals (Table 3).  
144 Genotype of the ewe ( $P = 0.0023$ ) and rearing mode ( $P = 0.044$ ) had a significant effect on litter size  
(LS) with no interaction between the two parameters. Average LS was  $1.93 \pm 0.18$ ,  $2.23 \pm 0.09$  and  
146  $2.47 \pm 0.09$  for *++*, *L+* and *LL* ewes, respectively (Table 4). Tukey test showed that mean LS was  
significantly higher in ewes with *LL* genotype than in those with *++* ( $P < 0.01$ ) and *L+* genotypes ( $P$   
148  $< 0.05$ ). However, the increased LS of *L+* ewes compared to *++* was not significant ( $P = 0.21$ ) surely  
due to the low number of *++* individuals ( $n=15$ ) and a high variance observed with some *++* ewes  
150 having high LS ( $\geq 3$ ) during their reproductive career (Figure 2). This may indicate either a second  
major prolificacy gene segregating within the Tunisian D'man population or a polygenic effect. In  
152 order to test the first hypothesis at the molecular level, we have checked whether mutations in other

known major prolificacy genes (i.e. *FecX*, *FecG* and *FecB*) were involved in the variability of D'man  
154 LS. For this purpose, exons 1 and 2 of *BMP15* (*FecX*) and *GDF9* (*FecG*), and exon 7 of *BMPR1B*  
(*FecB*) genes were sequenced. Apart the already known polymorphisms  $\Delta$ CTT/B1 in *BMP15* and G3,  
156 G4, G5 and G6 in *GDF9* (Hanrahan et al., 2004; Demars et al., 2013, Table 5) we did not identify  
any other mutations in these genes associated with prolificacy confirming the results from Vacca et  
158 al. (2010). Additionally, we also sequenced the exon 7 of *BMPR1B* to genotype for the Booroola  
mutation (*FecB<sup>B</sup>*, OAR6:29382188A>G, NC\_019463.1; Mulsant et al., 2001). The *FecB<sup>B</sup>* prolific  
160 allele was absent, but we have observed the recently evidenced SNP (OAR6:29382337G>A) shown  
to be associated with prolificacy in the Iranian Mehraban breed (Talebi et al., 2018). As shown in the  
162 Table 4, even if A/A ewes exhibited a lower mean LS ( $1.68 \pm 0.49$ , n=6) than G/A ( $1.97 \pm 0.45$ , n=5)  
or G/G ewes ( $2.22 \pm 0.19$ , n=3), no significant association was evidenced with the prolificacy of these  
164 D'man ++ ewes. Consequently, we failed to validate the existence of a second major gene segregating  
in our D'man samples.

#### 166 **4 DISCUSSION**

Based on a first public genomic data extraction from the NextGen project and subsequent molecular  
168 genotyping, we have evidenced the segregation of the prolific *FecL<sup>L</sup>* allele in the D'man sheep breed  
from Moroccan and Tunisian populations. Even if quite imprecise due to the relatively low number  
170 of animals studied, we have determined a high frequency (0.65) and a rather strong effect (+0.25  
lamb/lambing) of the *FecL<sup>L</sup>* allele in the Tunisian D'man sheep, highlighting the presence of a first  
172 major gene involved in the genetic determinism of LS variability within this breed known for its high  
prolificacy. The *FecL<sup>L</sup>* mutation was firstly discovered in Lacaune breed (Drouilhet et al., 2013) and  
174 more recently in Noire du Velay (Chantepie et al., 2018), thus raising the question of the origin of this  
mutation. It is more likely that the *FecL<sup>L</sup>* mutation has an ancestral origin which occurred before the  
176 establishment of Lacaune, Noire du Velay and D'man breeds. Previous studies suggested that modern  
breeds (those with improved traits) were first selected in South-West Asia and then spread into  
178 Europe, Africa and the rest of Asia (Chessa et al., 2009). The search and detection of the *FecL<sup>L</sup>*



180 mutation in other ovine breeds worldwide could give more accurate information about its origin. As  
a first element, other Moroccan sheep breeds (as Beni Guil, Sardi and undetermined local  
populations) are carriers of the *FecL<sup>L</sup>* allele as evidenced in the NextGen genomics data  
182 (supplementary Table S1), and will require further investigations.

We have observed a large difference in the *FecL<sup>L</sup>* frequency in the various D'man flocks studied that  
184 may result from different selection strategies used by the breeders in these different types of flock.  
Indeed, the low proportion in ++ individuals found in the commercial RM1 flock is more likely due  
186 to an indirect counter-selection of ++ individual carriers through a systematic selection of lambs born  
as triplets which are most likely born from either *L+* or *LL* parents (Figure 2), as explained by the  
188 breeder itself. This breeding strategy resulted in 94 % of individuals possessing either *LL* or *L+*  
genotype (Table 3). Similar proportions of *LL* ewes were found in the RM3 indicating that breeders  
190 of the small familiar flocks most probably select for prolific ewes using the same strategy. This  
decades-old practice used by some D'man breeders could also explain the gap with the only 0.14  
192 allele frequency of *FecL<sup>L</sup>* observed in the French Lacaune breed (vs. 0.65 in Tunisian D'man) while  
it is selected on prolificacy since the 80s but with the notable specific exclusion of very prolific  
194 animals between 1996 and 2010 (Martin et al., 2014). Interestingly, when looking at the NextGen  
data (supplementary Table S1), the Moroccan D'man individuals also exhibit a very high frequency  
196 (0.58) of the *FecL<sup>L</sup>* allele with 12 homozygous carriers among the 30 randomly chosen animals.

The present study highlights the segregation of a single mutation with a major effect on prolificacy  
198 in the D'man breed thus bringing an important element of answer to the old debate about its existence.  
Indeed, the high LS, the high repeatability of ovulation rate and LS observed in D'man ewes (and F1  
200 crosses) were possible indicators of the segregation of a prolificacy major gene. However, these and  
other estimates were not clearly different from Romanov and its crosses in which the inheritance  
202 pattern of LS is polygenic (Bradford et al., 1989; Ricordeau et al., 1990; Boujenane et al., 1991). The  
difficulties experienced in the previous studies to conclude or not for the presence of a prolificacy  
204 major gene in the D'man breed may be due to the relatively high frequency of the *FecL<sup>L</sup>* prolific allele  
with 80-90% of carrier animals.

206 From this study, we have established the *FecL<sup>L</sup>* allele effect on prolificacy of D'man ewe around  
+0.25 lambs per lambing. This is lower than the +0.4-0.5 lambs per lambing observed in Lacaune  
208 (Martin et al., 2014) or in Noire du Velay breeds (Chantepie et al., 2018). The model used in the  
present study did not consider several factors that were shown to affect LS such as lambing rank, age  
210 at first lambing, interval since last lambing and season of lambing. The failure to control for these  
factors may cause allelic effects to become confounded with variation at these factors, leading to  
212 underestimation of the true allelic effects. In its existing state, the Tunisian sheep recording system  
lacks complete information, in particular for the minority breeds among which the D'man breed.  
214 Anyway, we have demonstrated that the *FecL<sup>L</sup>* prolific mutation firstly discovered in the Lacaune  
sheep has also an effect on the D'man's genetic background. The present results do not provide direct  
216 information about the mechanism of action of the *FecL<sup>L</sup>* mutation, but it seems robust across the  
different genetic backgrounds in which it expresses itself to control OR and LS (Lacaune, Noire du  
218 Velay, D'man). The precise mechanism of action of the intronic *FecL<sup>L</sup>* on the *B4GALNT2* gene  
regulation and over expression within the granulosa cells is still unknown. Several hypotheses such  
220 as the impairment of transcription factor or miRNA binding, and mRNA alternative splicing were  
tested *in vitro* or *in silico* but without any success (Drouilhet et al., 2013). The use of high-throughput  
222 sequencing technologies dedicated to chromatin accessibility and detection of all kind of RNA (small  
or long non-coding RNA, mRNA) in the presence or absence of the mutation could contribute to  
224 understand the mechanism in the future. Interestingly and independently of the presence of the *FecL<sup>L</sup>*  
mutation, some other polymorphisms of the *B4GALNT2* gene have been recently showed to be  
226 associated with LS variability in Small Tail Han sheep (Guo et al., 2018), reinforcing the important  
role of this gene in the control of sheep prolificacy.  
228 From a more practical point of view, the discovery of the segregation of the *FecL<sup>L</sup>* mutation in the  
D'man breed can help to establish planned genotype-based mating allowing both higher profit for the  
230 breeders and an optimal management of prolificacy in D'man sheep populations.

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of the D'man flocks and the farmers who participated in this study.

## 238 CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

## 240 AUTHOR CONTRIBUTIONS

242 SBJ, NL and SF designed the study. SBJ, JR and SF performed the blood sampling. FW, JS and SF  
performed the genotyping. SBJ and SF performed the data analyses and wrote the manuscript.

## REFERENCES

- Abdoli R, Zamani P, Mirhoseini S Z, Ghavi Hossein-Zadeh N, Nadri S, 2016: A review on  
prolificacy genes in sheep. *Reprod Domest Anim* **51**, 631–637.
- Aherrahrou R, Aherrahrou Z, Kaiser F J, Braunholz D, Erdmann J, Moumni M, 2015: Identification  
of a single SNP that affects the LH-beta promoter activity in the Moroccan prolific D'man breed.  
*J Anim Sci* **93**, 2064–2073.
- Boujenane I, 1999: Les ressources génétiques ovines au Maroc. Actes Editions, Rabat, ISBN : 9981-  
801-41-0.
- Boujenane I, Bradford G E, Famula T R, 1991: Inheritance of litter size and its components in crosses  
between the D'man and Sardi breeds of sheep. *J Anim Sci* **69**, 517–524.
- Boubekeur A, Benyoucef M T, Lounassi M, Slimani A, Amiali M, 2015: Phenotypic characteristics  
of Algerian D'man sheep breed in Adrar oases. *Livestock Res Rural Dev* **27**, 7.
- Bodin L, SanCristobal M, Lecerf F, Mulsant P, Bibé B, Lajous D, Belloc J-P, Eychenne F, Amigues  
Y, Elsen J-M, 2002: Segregation of a major gene influencing ovulation in progeny of Lacaune  
meat sheep. *Genet Sel Evol* **34**, 447–464.
- Bradford G E, Lahlou-Kassi A, Berger Y M, Boujenane I, Derqaoui L, 1989: Performance of  
D'man and Sardi sheep on accelerated lambing II. Ovulation rate and embryo survival. *Small  
Rumin Res* **2**, 241–252.

- Chantepie L, Bodin L, Sarry J, Woloszyn F, Ruesche J, Drouilhet L, Fabre S, 2018: Presence of causative mutations affecting prolificacy in the Noire du Velay and Mouton Vendéen sheep breeds. *Livest Sci* **216**, 44–50.
- Chessa B, Pereira F, Arnaud F, Amorim A, Goyache F, Mainland I, Kao R R, Pemberton J M, Beraldi D, Stear M, Alberti A, Pittau M, Iannuzzi L, Banabazi M H, Kazwala R, Zhang Y-P, Arranz J J, Ali B A, Wang Z, Uzun M, et al., 2009: Revealing the history of sheep domestication using retrovirus integrations. *Science* **324**, 532–536.
- Demars J, Fabre S, Sarry J, Rossetti R, Gilbert H, Persani L, Tosser-Klopp G, Mulsant P, Nowak Z, Drobik W, Martyniuk E, Bodin L, 2013: Genome-Wide Association Studies Identify Two Novel BMP15 Mutations Responsible for an Atypical Hyperprolificacy Phenotype in Sheep. *PLoS Genet* **9**, e1003482.
- Drouilhet L, Lecerf F, Bodin L, Fabre S, Mulsant P, 2009: Fine mapping of the FecL locus influencing prolificacy in Lacaune sheep. *Anim Genet* **40**, 804–812.
- Drouilhet L, Mansanet C, Sarry J, Tabet K, Bardou P, Woloszyn F, Lluch J, Harichaux G, Vigié C, Monniaux D, Bodin L, Mulsant P, Fabre S, 2013: The Highly Prolific Phenotype of Lacaune Sheep Is Associated with an Ectopic Expression of the B4GALNT2 Gene within the Ovary. *PLOS Genet* **9**, e1003809.
- Guo X, Wang X, Liang B, Di R, Liu Q, Hu W, He X, Zhang J, Zhang X, Chu M, 2018: Molecular Cloning of the B4GALNT2 Gene and Its Single Nucleotide Polymorphisms Association with Litter Size in Small Tail Han Sheep. *Animals* **8**, 160.
- Hanrahan J P, Gregan S M, Mulsant P, Mullen M, Davis G H, Powell R, Galloway S M, 2004: Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biol Reprod* **70**, 900–909.
- Martin P, Raoul J, Bodin L, 2014: Effects of the FecL major gene in the Lacaune meat sheep population. *Genet Sel Evol* **46**, 48.
- Mulsant P, Lecerf F, Fabre S, Schibler L, Monget P, Lanneluc I, Pisselet C, Riquet J, Monniaux D, Callebaut I, Cribiu E, Thimonier J, Teyssier J, Bodin L, Cognié Y, Chitour N, Elsen J-M, 2001: Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Mérino ewes. *Proc Natl Acad Sci U S A* **98**, 5104–5109.
- Piper L R, Bindon B M, Davis G H, 1985: The single gene inheritance of the high litter size of the Booroola Merino. in *Genetics of reproduction in sheep* (Butterworths).
- Rekik M, Ben Salem I, Ben Hamouda M, Diallo H, Ammar H, Aloulou R, 2005: Numerical and weight productivities of crossbred D'man by local Queue Fine de l'Ouest ewes. *Rev Elev Méd Vét Pays Trop* **58**, 81–88.
- Ricordeau G, Thimonier J, Poivey J P, Driancourt M A, Hochereau-De-Reviers M T, Tchamitchian L, 1990: I.N.R.A. research on the Romanov sheep breed in France: A review. *Livest Prod Sci* **24**, 305–332.
- Talebi R, Ahmadi A, Afraz F, Sarry J, Woloszyn F, Fabre S, 2018: Detection of single nucleotide polymorphisms at major prolificacy genes in the Mehraban sheep and association with litter size. *Ann Anim Sci* **18**, 685–698.

- Vacca G M, Dhaouadi A, Rekik M, Carcangiu V, Pazzola M, Dettori M L, 2010: Prolificacy genotypes at BMP1B, BMP15 and GDF9 genes in North African sheep breeds. *Small Rumin Res* **88**, 67–71.
- Vinet A, Drouilhet L, Bodin L, Mulsant P, Fabre S, Phocas F, 2012: Genetic control of multiple births in low ovulating mammalian species. *Mamm Genome* **23**, 727–740.

Table 1. Ovine prolific alleles

Gene/Locus/ Prolific allele	Chr	Position (v3.1)	Reference allele	Variant allele	Variant ID	NextGen Pop./ Allele freq.
<i>B4GALNT2</i>						
<i>FecL/L</i>	11	36938224	T	A	rs588626728	MOOA T: 0.82; A: 0.18
<i>BMP15</i>	X	50971020	C	A	NA	ND
<i>FecX/B</i>						
<i>FecX/O</i>	X	50971111	T	G	NA	ND
<i>FecX/L</i>	X	50971158	C	T	NA	ND
<i>FecX/Gr</i>	X	50971170	G	A	NA	ND
<i>FecX/I</i>	X	50971224	T	A	rs398521635	ND
<i>FecX/H</i>	X	50971249	G	A	rs413916687	ND
<i>FecX/G</i>	X	50971402	G	A	rs425019156	ND
<i>FecX/R</i>	X	50971643	TGGGTCCAGAAAAGCCCA	T	rs421419167	ND
<i>FecX/Bar</i>	X	50977117	GCTACACTAGC	GGCTACACA	NA	ND
<i>BMPIB</i>						
<i>FecB/B</i>	6	29382188	T	C	rs418841713	ND
<i>GDF9</i>						
<i>FecG/T</i>	5	41841117	T	G	NA	ND
<i>FecG/H</i>	5	41841212	G	A	NA	ND
<i>FecG/WN</i>	5	41841285	C	T	rs403536877	ND
<i>FecG/E</i>	5	41841362	A	C	rs1092755620	ND
<i>FecG/V</i>	5	41841453	G	A	NA	ND

246 Chr: Chromosome; MOOA : MOroccan Ovis Aries; NA: not available; ND: not detected.

Table 2. PCR primer sequences

Gene (Chr. NCBI RefSeq)	Amplified region	<sup>a</sup> Primer sequence (5'3')	Application
<i>B4GALNT2</i> (NC_019468.1)	SNP intron 7	Fwd: TCTCAAGGCATTTTGGAGGA Rev: TATGCATGGCATGTGATAGG	KAPA PCR
		Fwd allele 1: GCAAGAAGCTGCGTGTGT Fwd allele 2: GCAAGAAGCTGCGTGTGA Rev: TATGCATGGCATGTGATAGG	KASP genotyping
<i>GDF9</i> (NC_019462.1)	exon 1	Fwd: GAAGACTGGTATGGGGAAATG Rev: CCAATCTGCTCCTACACACCT	Sanger sequencing
	exon 2	Fwd: TGGCATTACTGTTGGATTGTTTT Rev: GCTCCTCCTTACACAACACACAG	Sanger sequencing
<i>BMP15</i> (NC_019484.1)	exon 1	Fwd: CACAAAGGATAGGGCAAGGA Rev: ACTTTTCTTCCCCATTTTCTCCC	Sanger sequencing
	exon 2	Fwd: CGCTTTGCTCTTGTTCCTC Rev: GGCAATCATACCCTCATACTCC	Sanger sequencing
<i>BMPIB</i> (NC_019463.1)	exon 7	Fwd: AGTGTGTTGGGGGATTTA Rev: AAAGAGAGGAAAGCTAGGAA	Sanger sequencing

<sup>a</sup>Fwd and Rev: Forward and Reverse primers

Table 3 : Genotype frequencies for the 183 genotyped D'man individuals. The numbers in brackets refer to the genotype frequencies in rams.

Genotype	Number	Overall frequency	Frequency per rearing mode		
			RM1	RM2	RM3
++	23	12.5 %	5.5% (6%)	41% (80%)	11% (30%)
L+	81	44.3%	46% (44%)	41% (20%)	42% (70%)
LL	79	43.2%	48.5% (50%)	18% (0%)	47% (0%)

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Table 4. Effects of *FecL* genotypes on litter size in the D'man population (mean  $\pm$  sd and 95 % confidence interval (CI)). Results are averaged over the levels of the three rearing modes (RM1, RM2 and RM3).

Genotype	Number	Mean $\pm$ sd	95 % CI
++	15	1.93 <sup>a</sup> $\pm$ 0.18	[1.57-2.29]
L+	66	2.23 <sup>a</sup> $\pm$ 0.09	[2.05-2.41]
LL	71	2.47 <sup>b</sup> $\pm$ 0.09	[2.29-2.66]

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<sup>a, b</sup> means with different superscripts differ significantly (P < 0.05).

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Table 5. Polymorphisms detected in *FecL* ++ ewes at various prolificacy major gene loci and association with litter size (LS).

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Gene (Chr. NCBI RefSeq)	Polymorphism	Genotype	n=	LS (mean $\pm$ sd)
FecX/BMP15 (NC_019484.1)	X:50977457delinsCTT (B1) <sup>a</sup>	Ins/Ins	7	1.69 $\pm$ 0.45
		Ins/Del	6	2.05 $\pm$ 0.45
		Del/Del	2	2.00 $\pm$ 0.47
FecG/GDF9 (NC_019462.1)	5:41841919A>G (G3) <sup>a</sup>	A/A	2	2.17 $\pm$ 0.24
		A/G	8	1.82 $\pm$ 0.58
		G/G	5	1.90 $\pm$ 0.32
	5:41841675A>G (G4) <sup>a</sup>	A/G	1	2.33 $\pm$ ND
		G/G	14	1.86 $\pm$ 0.46
	5:41841418A>G (G5) <sup>a</sup>	A/A	11	1.89 $\pm$ 0.38
A/G		3	1.89 $\pm$ 0.84	
G/G		1	2.00 $\pm$ ND	
5:41841402G>A (G6) <sup>a</sup>	G/G	11	1.89 $\pm$ 0.38	
	G/A	3	1.89 $\pm$ 0.84	
	A/A	1	2.00 $\pm$ ND	
FecB/BMP1B (NC_019463.1)	6: 29382337G>A <sup>a</sup>	G/G	4	2.22 $\pm$ 0.19
		G/A	5	1.97 $\pm$ 0.45
		A/A	6	1.68 $\pm$ 0.49

<sup>a</sup>no significant association between genotypes and LS was detected by one-way ANOVA

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## FIGURE LEGENDS

268 Figure 1. Proportions of the number of litter size records for D'man ewes sampled for the study (n =  
152).

270 Figure 2. Proportion of litter size depending on the *FecL* genotypes.

The histograms show the distribution (percentage of the whole dataset, n=488 lambing records) of  
272 the lambing mode, simple (LS=1), twin (LS=2), triplet (LS=3) and quadruplet and more (LS=4+)  
depending on the 3 genotypes at the *FecL* locus (++ , L+ and LL).

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