Screening for Belclare (FecXB) mutation of the BMP15 gene in Tunisian Barbarine sheep

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Introduction

The establishment of selected prolific flocks, by screening prolific ewes, has proven to be an effective way to detect major genes for prolificacy. These genes, with differing sizes of effect on ovulation rate and litter size, have become a new option for sheep farmer aiming to significantly increase lambing percentages. Three types of fecundity gene (Davis, 2005) have been reported in sheep, namely bone morphogenetic protein receptor *IB* (*BMPRIB*) also known as Booroola fecundity gene (*FecB*) (Mulsant *et al*., 2001; Souza *et al*., 2001; Wilson *et al.*, 2001), growth differentiation factor 9 (*GDF9*) also known as *FecG* (Harnahan *et al*., 2004) and bone morphogenetic protein 15 (*BMP15* or *GDF9B*) also known as *FecX* (Galloway *et al.*, 2000; Hanrahan *et al*., 2004). All three fecundity genes belong to the transforming growth factor-β(*TGF-β*) superfamily (Fabre *et al*., 2006). *BMP15* is located in the X chromosome. Five mutations in this gene affecting prolificacy have been described. Ovulation rates are highly increased in the heterozygotes, the homozygotes show a primary ovarian failure resulting in complete sterility (Galloway *et al*., 2000; Montgomery *et al*., 2001; Hanrahan *et al*., 2004; Davis, 2005; Bodin *et al*., 2007). These mutations, named $FecX^G$ (Galway), $FecX^H$ (Hanna), $FecX^I$ (Inverdalle), $FecX^L$ (Lacaune) and $FceX^B$ (belclare), exhibit one to two additional ovulation, compared with noncarriers ewes. In particular, the $FecX^B$ mutation, was found in Belclare sheep breedin Irland and was showed very high ovulation rates (Davis, 2005). It's a non conservative amino acid position 99.

The Barbarine sheep, and specially the ewe, is well adapted to the local condition mainly because of its ability to deposit and mobiles body reserve not only from the tail (fat) but also from the rest of the body (Djemali *et al*., 1994, 1997; Atti *et al.*, 2004; Bedhiaf-romdhani and Djemali, 2006). The Barbarine sheep is a middle sized animal with a height raring between 60 and 80 cm in male and 55 and 70 cm in females. The body eight varies considerably according to nutritional conditions, it ranges between 45 and 85 kg in rams and 25 and 65 kg in ewes (Khaldi, 1989). Selection for prolificacy is of major economic interest in most husbandry situations. However, there is an optimum litter size for each environment which maximizes the return profit per ewe. Uniform optimum litter sizes result in the highest profit, and when mean prolificacy of a breed is close to this optimum, and then uniformity of litter sizes is a new additional objective. This economic interest of prolificacy has clearly been highlighted by several authors (Davis *et al*., 2001; Galloway *et al*., 2000; Wilson *et al.*, 2001; Mulsant *et al.*,2001; Hanrahan *et al.*, 2004; Gootwine *et al*., 2006).

The main objective of the present research was to apply PCR-RFLP technique for determining BMP15 gene polymorphism in Tunisian Barbarine sheep breed.

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Material and methods

Materials. A total of 334 individuals from Barbarine sheep breed were examined in this study from three various productions systems. Approximately 5 ml blood was collected aseptically from the jugular vein in EDTA. All samples were taken back to laboratory under low temperature. The genomic DNA was extracted from white blood cells using the iPrep purification instrument (iPrep PureLink gDNA Blood Kit). The DNA samples were storaged at + 4 °C.

Primer synthesizing and PCR reaction. Primers were synthesized by Invitrogen based on the sequences described by Hanrahan *et al.* (2004). Forced PCR-RFLP was used to detect the *FecX*^{B} mutation (G to T nucleotide change) that was a point mutation deliberately introduced into one of a pair of primers resulting in PCR products with exact restriction sites in *FecX*^{B} wild type individual, otherwise lacking the sites. The primers were designed as follows: B4-Dde1F: 5'-GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA-3' and B4: 5'-TTCTTGGGAAACCTGAGCTAGC-3'. Genomic DNA (50 ng) was used in a 20-µl of reaction volume. The amplification was carried out using 35 cycles at 94 °C for 30 s, 64 °C for 40 s and 72 °C for 30 s followed by 72 °C for 5 min. the PCR product were digested with DdeI over 4 h at 37 °C, and the resulting products were separated by 3 % agarose gel electrophoresis and visualized with ethidium bromide, photographed and analyzed.

FecX^{B} genotyping. The *FecX*^{B} variant was detected using the method improved by Hanrahan *et al*. (2004). A point mutation was introduced in F5 to generate a restriction site DdeI (C/TTAG) in the PCR products. The wild-type strand was cleaved with DdeI, restriction digestion of PCR products produce a 123 and 30 bp. The mutation type individuals with *FecX*^{B} variant standed against the digestion of DdeI at 153 bp. Animals heterozygous for this mutation have fragments of 153, 123 and 30 bp.

Results and discussion

In the present study, the *FecX*^{B} mutation was investigated in Tunisian Barbarine sheep breed. To improve that *FecX*^{B} allele exists, a DNA fragment with the size of 153 bp was amplified from exon 2 of BMP15 using B4-Dde1F and B4 primers. All the individuals were generated one strand with a weight is 153 bp. The resulted PCR products were digested with DdeI. The electrophoregrams of forced PCR-RFLP are showed in Figure 2. Two genotypes were detected, individuals heterozygous (123 bp/ 153 bp) and individuals homozygotes (123 bp/ 123 bp). A total of 334 individuals of Barbarine sheep were analyzed for the *FecX*^{B} mutation. Out of which 142 were heterozygous. All the rest of individuals were homozygous wild type. About 57 % of Barbarine sheep were found carrier for the *FecX*^{B} mutation and allele frequency was about 21,25 %. The results can be indicated that the twining lambing in these breeds is linked with the *FecX*^{B} mutation. All sheep were non carried homozygous for the Belclare mutation.

The establishment of prolific Barbarine strain, from Tunisian national prolific ewes, indicates the possibility for prolificacy selection. This program speared over 10 years (1990-1999) and founded by Tunisian National Institute for Agronomic Research (INRAT) in experimental

center of Oueslatia Kairouan. The means litter size varies from 140 % to 180 % with an average of 160 % (khaldi, 1989; Atti *et al.*, 2001). In order to obtain meaningful results, breed evaluation studies must sample many individuals of the breeds to be evaluated. If few individuals are sampled, there is a chance to selected individuals may be much better or far worse than the average of the breed, and subsequent results would not be indicative of the true performance of the breed. Ideally, breed evaluations would be repeated at several locations under different conditions to determine if breeds ranked similarly in different environments. The molecular selection constitute a shortcut of the realization of genetic improvement programs. The identification of Barbarine individuals carrying natural mutation in *BMP15* gene signaling pathway illustrate the way in which discoveries can contribute to genetic amelioration and reproductive process in Tunisian breed , as well as providing benefits to agriculture.

Table 1: Allelic and genotypic frequencies of the $FecX^B$ mutatiuon of *BMP15* gene

Gene	Nbre. of ewes	Allelic frequency	Genotypic frequency
BMP15	334	0,22	0,57

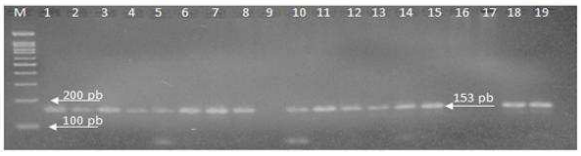

Figure 1: PCR amplification of the $FecX^B$ allele of *BMP15* using B4-Dde1F and B4 primers (3 % agarose gel stained with ethidium bromide). Line M: Molecular weight marker. Line 1-19: individuals of Barbarine sheep. 153 bp: PCR products of *BMP15* exon 2.

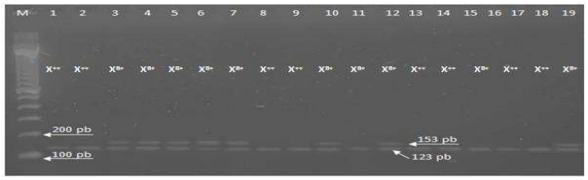

Figure 2: Forced RFLP-PCR products of the $FecX^B$ allele of *BMP15* digested with DdeI (3 % agarose gel stained with ethidium bromide). Line M: Molecular weight marker. Line 3, 4, 5, 6, 7, 10, 12 and 19: individuals of Barbarine sheep heterozygous (123 bp/153 bp). Line 1, 2, 8, 9, 11, 13, 14, 15, 16, 17and 18 individuals of Barbarine sheep homozygous (123 bp/ 123bp).

Conclusion

The identification of Barbarine individuals carrying natural mutation in *BMP15* gene ($FceX^B$ allele) signaling pathway illustrate the way in which discoveries can contribute to genetic amelioration and reproductive process in Tunisian breed , as well as providing benefits to agriculture.

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