



Study for identification FecXI and FecXH mutations in Tunisian Barbarine sheep

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Abstract

A total of 334 ewes recorded with twinning births from the Tunisian fat tail Barbarine sheep were genotyped in the objective to identify the FecX^L and FecX^H mutations. The genotyping was carried out by forced restriction fragment length polymorphism PCR (RFLP-PCR). None of mutations was detected in Barbarine sheep. Results showed that all analyzed ewes were homozygous non carriers. These results suggest that fecundity of this breed is not linked to the same mutations. Therefore, it is necessary to seek for other mutations or fecundity genes in order to develop marker assistance selection techniques and study the prolific mechanism of the Barbarine breed.

Keywords: Barbarine, FecX, Mutation, Prolificacy, Tunisia

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 have been reported in sheep, namely bone morphogenetic protein receptor IB (BMPRII) also known as Booroola fecundity gene (FecB) (Souza et al., 2001; Wilson et al., 2001), growth differentiation factor 9 (GDF9) also known as FecG (Hanrahan 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; Hanrahan et al., 2004). These mutations, named FecX^G (Galloway), FecX^H (Hanna), FecX^L (Inverdalle), FecX^L (Lacaune) and FecX^B (belclare), exhibit one to two additional ovulation, compared with noncarriers ewes. In particular, the FecX^L and FecX^H mutations were found respectively in Inverdalle and Hanna sheep and have showed very high ovulation rates (Davis, 2005).

The Barbarine sheep, and specially the ewe, is well adapted to the local condition mainly because of its ability to deposit and mobilize 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 ranging between 60 and 80 cm in male and 55 and 70 cm in females. The body weight 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; Hanrahan et al., 2004).

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

Materials and Methods

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 stored at + 4 °C.

Primers were synthesized by Invitrogen based on the sequences described by Galloway et al. (2000). Forced PCR-RFLP was used to detect the FecX^L and FecX^H mutations. The reverse primer deliberately introduced by a point mutation would create a restriction site in mutated strand, wild type ewes lacking the sites. The primers were designed as follows:

Genomic DNA (50 ng) was used in a 25 µl of reaction volume. The amplification was carried out using 40 cycles at 95 °C for 30 s, 63 °C (FecX^L) and 64 °C (FecX^H) for 40 s and 72 °C for 45 s followed by 72 °C for 10 min. The PCR product was digested with SpeI (FecX^H) and XbaI (FecX^L). Digestion reaction contain 5 µl of PCR product, 5 U appropriate enzyme, 2.5 µl buffer 10× in 25 µl final volume. . FecXH (C to

T nucleotide change) was detected using SpeI (Galloway et al., 2000), the mutation-type strand was cleaved. FecXI (T to A nucleotide change) was detected using XbaI (Galloway et al., 2000); the mutation-type strand was cleaved. The resulting products were separated by 3 % agarose gel electrophoresis and visualized with ethidium bromide, photographed and analyzed.

Results

Quality and quantity of extracted DNA from analyzed samples was tested by electrophoresis on agarose gel (Figure 1) and spectrophotometer method. Electrophoresis showed a height DNA quality. The ratio of absorption amount resulted in 260 nm to the result of 280 nm is between 1.7 and 2. So purity of extracted DAN was acceptable.

As expected, the size of PCR production of FecXH mutation in BMP15 gene was 240 bp (Figure 2). After digestion with restriction endonuclease (SpeI), the mentioned restriction site was not detected (Figure 3).

Table1: Primers sequences for BMP15 gene and PCR product length (Hongcai et al., 2010).

Gene	Allele	Sequences : 5'3'	Length
BMP15	FecX ^L	5'-GAAAGTAACCAGTGTTCCCTCCACCCTTTTCT-3'	150 bp
		5'- CATGATTGGGAGAATTGAGACC -3'	
	FecX ^H	5'-TATTTCAATGACACTCAGAG-3'	240 bp
		5'-GAGCAATGATCCAGTGATCCCA-3'	

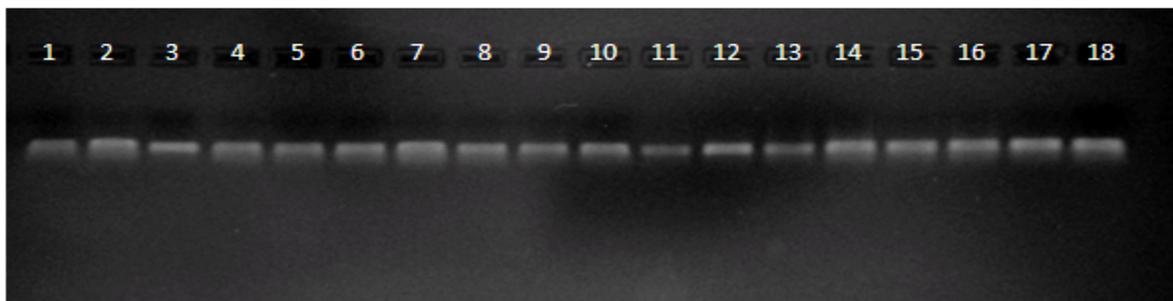


Fig. 1: Electroporesis on agarose gel for extracted DNA. 1 to 18: barbarine ewes

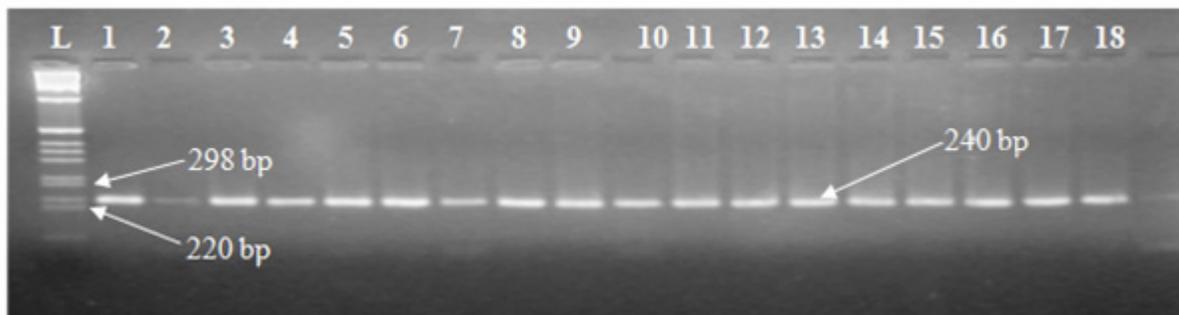


Fig. 2: PCR product of FecXH. L: ladear (Invitrogen 1 kb), 1 to 18 Barbarine ewes.

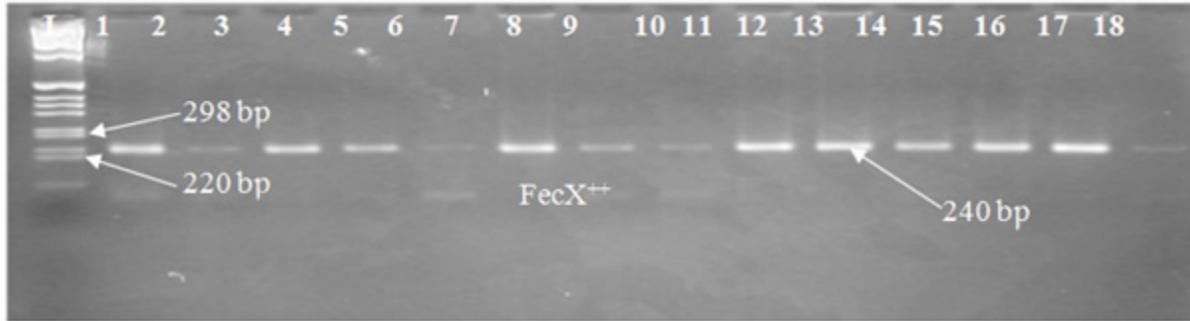


Fig. 3: RFLP digested PCR product using PstI. L: ladder (Invitrogen 1 Kb).

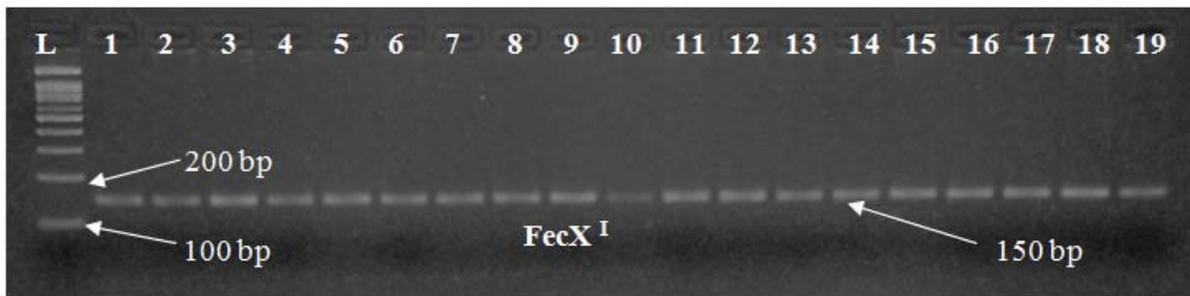


Fig. 4: PCR product of FecXI. L: ladder (Invitrogen 1 kb), 1 to 18 Barbarine ewes.

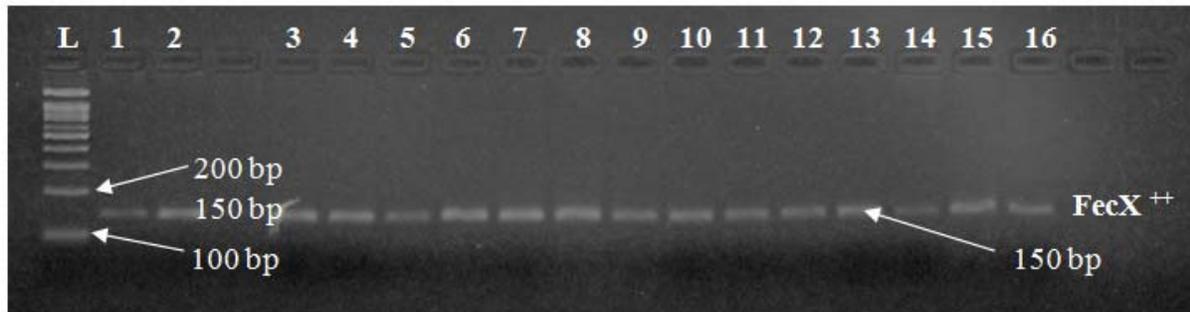


Fig. 5: RFLP digested PCR product using PstI. L: ladder (Invitrogen 1 Kb).

The resulted PCR production of FecXI in BMP15 gene was 150 bp (Figure 4). Digestion of PCR product indicated the absence of restriction site for all analysed ewes (Figure 5).

All 334 Barbarine ewes initially sampled were homozygous not carrier. All genotyped sheep had the wild allele (++) for FecX^H and FecX^I.

Discussion

As proteins regulate oocyte secretion, the change of important sites in BMP15 can significantly affect ovulation in ewe. C/T mutation at E+67 bp of BMP15 in FecX^H carried ewe led to the change of Glu at amino acid 23 into termination codon (Galloway et al., 2000). T/A mutation at E+92 bp of BMP15 in FecX^I carried ewe led to the change of Val at amino acid 31 into Asp (Galloway et al., 2000).

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). 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 constitutes 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.

Conclusion

None of polymorphism of FecXI or FecXH loci was identified. It is supposed to seek for other genes (Booroola and GDF-9) or loci (FecXG, FecXB, FecXL) in Tunisian Barbarine sheep.

References

- Atti, N., BECQUER and F. Khaldi, G. 2004. Performance of the fat-tailed Barbarine sheep in its environment: adaptative capacity to alternation of underfeeding and re-feeding periods. *Review Animal Research*, 53: 165-176.
- Bedhiaf-Romdhani, S. Djemali, M. 2006. Estimation of sheep carcass trait by ultrasound technology. *Livestock science*, 101: 294-299.
- Davis, G.H., Bruce, G.D. and Dodds, K.G. 2001b. Ovulation rate and litter size of prolific Inverdale (FecXI) and Hanna (FecXH) sheep. *Proceeding Association Advance Animal Breeding and Genet*, 14: 175-178.
- Davis, G.H., Dodds, K.G., Wheeler, R. and Jay, N.P. 2001a. Evidence that an imprinted gene on the X chromosome increases ovulation rate in sheep. *Biology of Reproduction*, 64: 216-221.
- Davis, G.H. 2005. Major genes affecting ovulation rate in sheep. *Genetic Selection Evolution*, 37 (Suppl. 1): S11-S23.
- Djemali, M. et Alhadrami, G. 1997. Consideration beyond breeding goals in breeding sheep and goats in relation to their environment. *Options méditerranéennes, series cahiers*, 33:171-174.
- Djemali, M., Aloulou, R. and Ben Sassi, M. 1994. Adjustment factors and genetic and phenotypic parameters for growth traits of Barbarine lambs in Tunisia. *Small Ruminant Research*, 13: 41- 47.
- Fabre, S.A.P., Mulsant, P., Bodin, L., DiPasquale, E., Persani, L., Monget, P. and Monniaux, D.. 2006. Regulation of ovulation rate in mammals: contribution of sheep genetic models. *Reproduction, Biology and Endocrinology*, 4:20.
- Galloway, S.M., McNatty, K.P., Cambridge, L.M., Laitinen, M.P., Juengel, J.L., Jokiranta, T.S., McLaren, R.J., Luro, K., Dodds, K.G., Montgomery, G.W., Beattie, A.E., Davis, G.H. and Ritvos, O. 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature and Genetics*, 25: 279-283.
- Hanrahan, J.P., Gregan, S.M., Mulsant, P., Mullen, M., Davis, G.H., Powell, R. and 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*). *Biology of Reproduction*, 70: 900-909.
- Hongcai, S., Bai, J., Niu, Z., Muniresha, Fen, L and Jia, B. 2010. Study on candidate gene for fecundity traits in Xingjiang Cele black sheep. *African Journal of Biotechnology*, 9(49): 8498-8505
- Khaldi, G. 1989. Barbarine sheep. In: small ruminant in the Near East, vol III, North Africa FAO, Animal Production Health. 74: 96-135.
- Souza, C.J., MacDougall, C., MacDougall, C., Campbell, B.K., McNeilly, A.S. and Baird, D.T., 2001. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *Journal of Endocrinology*, 169: R1-R6.
- Wilson, T., Wu, X.Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., McEwan, J.C., O'Connell, A.R., McNatty, K.P. and Montgomery, G.W., 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biology of Reproduction*, 64: 1225-1235.