IJAS Iranian Journal of Applied Animal Science

Genetic Polymorphism of GDF9 Gene in Iranian Moghani Sheep Breed

Short Communicaion

F. Ala Noshahr[1*] and A. Rafat[1]

[1] Department of Animal Science, University of Tabriz, Tabriz, Iran

Received on: 7 Nov 2013
Revised on: 15 Jan 2014
Accepted on: 30 Jan 2014
Online Published on: Dec 2014

*Correspondence E-mail: fatemehala@rocketmail.com

Online version is available on: www.ijas.ir

ABSTRACT

The families of TGF-β proteins are the most important growth factors in the ovary for growth and differentiation of early ovarian follicles. Three related oocyte-derived members of the transforming growth factor-β superfamily namely growth differentiation factor 9 (GDF9), BMP15 and BMPR-IB have been shown to be essential for follicular growth and ovulation. Different mutations in the GDF9 gene cause increased ovulation rate and infertility in a dosage-sensitive manner in sheep. In this study, blood samples were collected from 150 Moghani sheep breed using venojects treated with the anti-clot substance (EDTA) and subsequently their DNA content were salted out and extracted. The quantity and quality of extracted DNA was examined using spectrophotometery and gel electrophoresis, respectively. With using a pair of specific primers, the DNA fragment was amplified from exon 1 of GDF9 (462 bp). Digested PCR products with *HhaI* enzyme showed a G to A substiuation in GDF9 locus. The wild type allele of this gene (G/+) with two restriction site resulted DNA fragments of 156, 52 and 254 bp while the mutant allele (G/-) with one restriction site resulted two DNA fragments with the size of 52 and 410 bp. Genotype frequencies for G (+/+), G (+/-) and G (-/-) were 0.66, 0.24 and 0.1, respectively. From studied luci, GDF9 was polymorphic in Iranian Moghani sheep breed.

KEY WORDS GDF9, Moghani sheep, PCR, polymorphism.

INTRODUCTION

Sheep is a highly diverse species with > 900 different breeds that substantially differs at economical production and genetic traits point of view (McNatty *et al.* 2004). Iran has 27 sheep breed populations and Moghani sheep is afattailledmeet breed with carpet quality wool and considered to be an important animal for meat, wool and milk production (Tavakkolian, 2000). Low efficiency is common in sheep due to the low heritability of the traits (5-10%) (Javanmard *etal.* 2011). In sheep, genetic variation in ovulation rate has been widely documented. Evidence shows substantial difference among breeds and in a number of cases exceptional variations within breeds/strains (Galloway *et al.* 2000). Now-a-days mutations with major effects on ovulation rate and litter size were identified in Transforming Growth Factor Beta (TGFβ) superfamily ligands and receptors. The molecules contain over 35 members and many of which play a pivotal role in regulating fertility (Juengel *et al.* 2004). Growth differentiation factor 9 (GDF9) is a growth factor and a member of TGF-β superfamilythat is secreted by oocytes in growing ovarian follicles (Laitinen *et al.* 1998). The GDF gene has been mapped to sheep chromosome 5 and it spans approximately 2.5 kb and contains two exons and one intron (Davis *et al.* 1991). Eight different point mutations (G1-G8) have been identified in the GDF9 gene. Three (G2, G3 and G5) out of the eight polymorphisms are nucleotide

changes that do not result in an altered amino acid. The five remaining nucleotide changes, give rise to amino acid changes. The G1 arginine to histidine change at amino acid residue 87 in exon 1 substitutes one basic charged polar group with another and occurs at a position before the furin cleavage site for the mature peptide, so is unlikely to affect the activity of the mature protein (Hanrahan *et al.* 2004). Ewes with a single copy of the mutated GDF9 (FecGH) gene are fertile and have an increased ovulation rate. In contrast, ewes homozygous for this mutation are infertile with primary ovarian failure (Hanrahan *et al.* 2004). The GDF9 mutations are thought to result in reduced levels of mature protein or altered binding to cellsurface receptors (McNatty *et al.* 1997). From examination of phenotypes of these mutations and subsequent physiological studies, it is clear that GDF9 are essential for ovarian follicular development and normal ovulation or corpus luteum formation in sheep. Moreover, it is evident that GDF9 (Hanrahan *et al.* 2004; Hsueh *et al.* 2000) an X-linked gene that increased ovulation rate by about 1.0 but caused sterility in homozygous carrier females was first described in Romney sheep and named the Inverdale gene (FecX) (Davis *et al.* 1991; Davis *et al.* 1992). The infertile ewes have small undeveloped 'streak' ovaries which never ovulate. Measurements from a small sample of ewes suggest that the effect of the GDF9 mutation on ovulation rate is significant and with one copy of FecGH increasing ovulation rate by about 1.4 in the Cambridge and Belclare breeds (Davis *et al.* 2006). The aim of this study was to investigate the presence of polymorphism in GDF9 in the Moghani sheep breed.

MATERIALS AND METHODS

In this study, blood samples (150 ewes) were initially taken from 150 Moghani sheep breed using venojects treated with the anti-clot substance (EDTA) and subsequently their DNA content were salted out and extracted. Genomic DNA was dissolved in TE buffer and kept at -20 ˚C.

In a total volume of 25 µL which template PCR reaction contained: 2.5 µL PCR buffer 10-X, 2.5 µL Mgcl2, 10 pm of each primers, 0.2 µL Taq DNA polymerase, 0.2 µL dNTPs and 0.8 µL template DNA. The amplification GDF9 for primers G9-1734: GAA GAC TGG TAT GGG GAA ATG (forward) and G9-2175: CCA ATC TGC TCC TAC ACA CCT (reverse) was carried out using 35 cycles at 94 ˚C for 5 min, 94 ˚C for 45 s, 58 ˚C for 40 s and 72 ˚C for 1 min, followed by 72 ˚C for 10 min. Digestion with restriction enzyme used for GDF9 is *HhaI*. Digestion reaction contain 5 µl of PCR product, 5 U appropriate enzyme, 2 µL buffer 10 × in 20 µL final volume incubated for 3 to 6 h at 37 ˚C (Hanrahan *et al.* 2004).

An x^2 test for goodness-of-fit was performed to verify if genotype frequencies agreed with Hardy-Weinberg equilibrium expectations. Analysis was performed using the GLM procedure of SAS (2002) program.

RESULTS AND DISCUSSION

A DNA fragment with the expected size of 462 bp was amplified from exon I of GDF9 gene. The G to A nucleotide change in GDF9 exon I disrupt a *HhaI* restriction enzyme cleavage site (GCGC to GCAC) at nucleotide 260 of the 462-bp PCR fragment produced by primers G9-1734 and G9-2175. Restriction digestion of the PCR product from wild-type animals with *HhaI* resulted in cleavage of the 462-bp product (at two internal *HhaI* sites) into fragments of 52, 156 and 254 bp ($FecG^+$). But, DNA fragments containing the A nucleotide yield only two fragments of 52 and 410 bp ($FecG^-$).

Animals heterozygous for the mutation have fragments of all four sizes (52, 156, 254 and 410 bp). All three possible genotypes were observed in Moghani sheep population (Figure 1). The allele and genotype frequencies of GDF9 gene was presented in Table 1. For the GDF9 gene, allele $FecG^+$ had the highest frequency (0.78), whereas allele $FecG^-$ had the lowest frequency (0.22) in Moghani sheep flock.

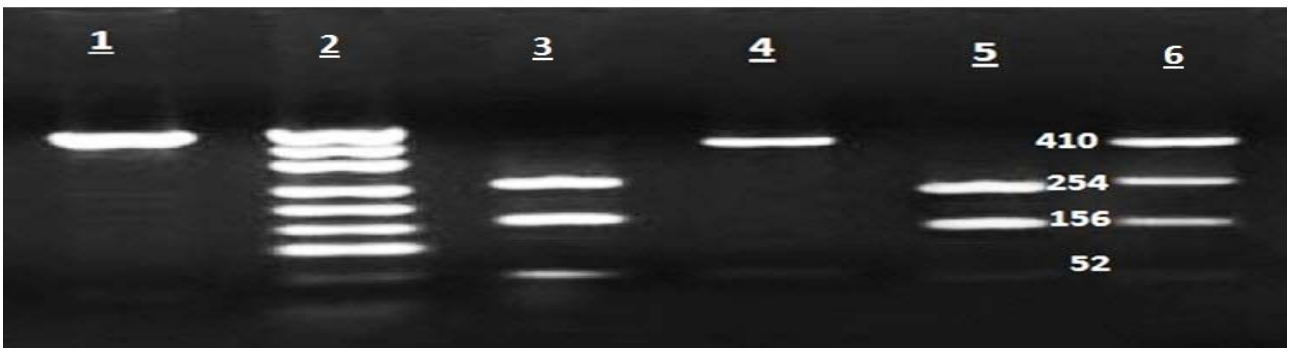

Figure 1 Agarose gel electrophoresis (3%) of digested product of GDF9 gene exon I. Lane 1: PCR product (462 bp); Lane 2: molecular weight marker; Lanes 3 and 5: wild type genotypes ($FecG^+/FecG^+$); Lanes 4: mutant genotype ($FecG^-/FecG^-$) and Lane 6: heterozygous genotype ($FecG^+/FecG^-$)

Table 1 Estimated allelic and genotypic frequencies of GDF9 gene in Moghani sheep population used in this study

Gene	Totalof animals	Allele frequency (No. of animals)		Genotype frequency (No. of animals)		
		$FecG^+$	$FecG^-$	$FecG^+$ / $FecG^+$	$FecG^+$ / $FecG^-$	$FecG^-$ / $FecG^-$
GDF9	150	0.78 (117)	0.22 (33)	0.66 (99)	0.24 (36)	0.10 (15)

The Genotype frequencies of $FecG^+$ / $FecG^+$, $FecG^+$ / $FecG^-$ and $FecG^-$ / $FecG^-$ were 0.66, 0.24 and 0.10, respectively (Table 1). X^2 test (11.59) confirmed the Hardy-Weinberg's equilibrium in this population.

PCR-RFLP is a rapid, simple and exact technique for single nucleotide polymorphism (SNP) genotyping. This approach has been used previously to genotype prolific sheep and goat by several research groups (Guan *et al.* 2006).

In our experiment, a forced PCR-RFLP approach was used to detect the genotype based on the method described by Hanrahan *et al*. (2004) and Davis *et al.* (2006).

The present study showed similar results reported by Hanrahan *et al.* (2004), Juengel *et al*. (2004), Chu *et al*. (2004), Chu *et al*. (2007) and Liao *et al*. (2004). Our result showed that homozygous genotype had reduced fertility rate, and similar result as reported by Galloway *et al.* (2000).

The presence of one copy of mutant GDF9 gene increase fecundity rate in Moghani sheep and the same result reported by Davis *et al.* (2006), Hanrahan *et al.* (2004), Juengel *et al*. (2004) and Liao *et al*. (2004). Ewes heterozygous for GDF9 mutations have increased ovulation rates, where as homozygous ewes are sterile due to a failure of normal ovarian follicular development (Davis *et al.* 1991; Galloway *et al*. 2000; Hanrahan *et al*. 2004).

Generally, many different loci effect reproduction and ovulation rate between different breeds of sheep, more than genetic background is under control of age, season and nutrition.

According to these and the high prolificacy in these-breeds, it is concluded that high prolificacy may be under control of other factors such as age; season and nutritionor maybe there is another major gene in Moghani sheep.

CONCLUSION

Identifying genes of major effect offers the opportunity to improve production efficiency, product quality and product diversity in livestock industry, through utilizing them in breeding programs. Preliminary polymorphism analysis performed on $FecG^-$ mutation in GDF9 locus suggested a major gene inheritance of prolificacy in this selected population. In conclusion, we cannot completely exclude the possibility that the observed effects could be caused by linkage disequilibrium with other functional SNP in a nearby regions of GDF9 gene or by linkage disequilibrium with other nearby loci.

ACKNOWLEDGEMENT

We appreciate and acknowledge the sincere cooperation Officials of Agricultural Research Center and Iranian Laboratory staff.

REFERENCES

Chu M.X., Wang S.C.Y.E. and Fang L. (2004). Association between PCR-SSCP of growth differentiation factor 9 genes and high prolificacy in Small Tail Han sheep. *Anim. Biotechnol*. **15,** 111-120.

Chu M.X., Liu Z.H., Giao C.L., He Y.Q., Fang L., Ye S.C., Chen G.H. and Wang G.Y. (2007). Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (*Ovis aries*). *J. Anim. Sci*. **85,** 324-330.

Davis G.H., McEwan J.C., Fennessy P.F., Dodds K.G. and McNatty K.P. (1992). Infertility due to bilateral ovarian hypoplasia in sheep homozygous (FecXI, FecXI) for the Inverdale prolificacy gene located on the X chromosome. *Biol. Reprod.* **46,** 636-640.

Davis G.H., McEwan J.C., Fennessy P.F., Dodds K.G. and Farquhar P.A. (1991). Evidence for the presence of a gene influencing ovulation rate on the X chromosome of sheep. *Biol. Reprod.* **44,** 620-624.

Davis G.H., Balakrishnan L., Ross I.K., Wilson T., Galloway M., Lumsden B.M., Hanrahan J.P., Mullen M.X., Mao Z., Wang G.L., Zhao Z.S., Robinson J.J., Mavrogenis A.P., Papachristoforou C., Peter C., Baumung R., Cardyn P., Boujenane I., Cockett N.E., Eythorsdottir E., Arranz J.J. and Notter D.R. (2006). Investigation of the Booroola (FecB) and Inverdale (FecXI) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. *Anim. Reprod.* **92,** 87-96.

Galloway S.M., McNatty K.P., Cambridge L.M., Laitinen M.P. E., Juengel J.L., Jokiranta T.S., McLaren R.J., Luiro K., Dodds K.G., Montgomery G.W., Beattie A.E., Davis G.H. and Ritvos O. (2000). Mutations in an oocytederived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature*. **25,** 279-283.

Guan F., Liu S.R., Shi G.Q., Ai J.T., Mao D.G. and Yang L.G. (2006). Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. *Acta Genetic. Sci.* **33,** 117-124.

Hanrahan J.P., Gergan 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*). *Biol. Reprod.* **70,** 900-909.

Hsueh A.J., McGee E.A., Hayashi M. and Hsu S.Y. (2000). Hormonal regulation of early follicle development in the rate ovary. *Mol. Cell. Endocrinol*. **163(1),** 95-100.

Javanmard A., Azadzadeh N. and Esmailizadeh A. (2011). Mutations in bone morphogenetic protein 15 and growhdiffirentiation factor 9 genes are associated with increased litter size in fat-tailed sheep breeds. *Vet. Res. Commun*. **35,** 157-167.

Juengel J.L., Hudson N.L., Whiting L. and McNatty K.P. (2004). Effects of immunization against bone morphogenetic protein 15 and growth differentiation factor 9 on ovulation rate, fertilization, and pregnancy in ewes. *Biol. Reprod.* **70,** 557-561.

Laitinen M., Vuojolainen K., Jaatinen R., Ketola I., Aaltonen J., Lehtonen E., Heikinheimo M. and Ritvos O. (1998). A novel growth differentiation factor 9 (GDF9) related factors is co-expressed with GDF9 in mouse oocytes during folliculogenesis. *Mech. Dev*. **78,** 135-140.

Liao W.X., Moore R.K. and Shimasaki S. (2004). Functional and molecular characterization of naturally occurring mutations in the oocytesecreted factors bone morphogenetic protein-15 and growth and differentiation factor-9. *J. Boil. Chem.* **17,** 17391-17396.

McNatty K.P., Jungel J.L., Reader K.L., Lun S., Myllymaa S., Lawrence S.B., Western A., Meerasahib M.F., Mottershead D.G., Groome N.P., Ritvos O. and Laitinen M.P. (1997). Bone morphogenetic protein 15 and growth differentiation factor 9 co-operate to regulate granulose cell function in ruminants. *Reprod.* **129,** 481-487.

McNatty K.P., Moore L.G., Hudson N.L., Quirke L.D. and Lawrence S.B. (2004). The oocyte and its role in regulating ovulation rate: anew paradigm in reproductive biology. *Reprod.* **128,** 379-386.

SAS Institute. (2002). SAS®/STAT Software, Release 6.00. SAS Institute, Inc., Cary, NC.

Tavakkolian J. (2000). An Introduction to Genetic Resources of Native Farm Animals in Iran. Animal Science Research Institute, Kraj, Iran.