Single nucleotide polymorphism analysis of the bone morphogenetic protein receptor IB and growth and differentiation factor 9 genes in Rayini goats (*Capra hircus*)

Y. Mehdizadeh Gazooei[1*], A. Niazi[2] and M. J. Zamiri[3]

[1]Former Post-graduate Student, Department of Animal Science, College of Agriculture, Shiraz University, Shiraz, Iran.[2]Institute of Biotechnology, College of Agriculture, Shiraz University, Shiraz, Iran.
[3]Department of Animal Science, College of Agriculture, Shiraz University, Shiraz, Iran.

* Corresponding author, E-mail address: yassermehdizadeh@gmail.com

Abstract   The *FecB*, a mutation in the bone morphogenetic protein receptor IB (*BMPR-IB*) gene, which increases the fecundity of Booroola Merino sheep, and *FecG^H*, a mutation in the growth and differentiation factor 9 (*GDF9*), which affects the fecundity of Cambridge and Belclare sheep in a dose sensitive manner, were analyzed as candidate genes associated with the prolificacy in Rayini goats. These polymorphisms were analyzed by PCR-RFLP method in 110 individuals randomly selected out from a flock of Rayini goats with a mean litter size of 1.29. These mutations were not detected in this flock. All individuals were wild type homozygous for *FecB*and*FecG^H*. These results suggested that fecundity of this breed is not linked to the same loci in *BMPR-IB* or *GDF9* as in some sheep breeds, and other polymorphism(s) may be involved in Rayini goat prolificacy. Therefore, searching for other polymorphisms in these genes or any polymorphisms in known fecundity genes is suggested to develop marker assistance selection techniques and to study the genetic mechanism of prolificacy in goat.

Keywords: fecundity gene, polymorphism, Rayini goat

Introduction

Several genes controlling ovulation rate /litter size are reported in rabbit (Peiró et al., 2008), pig (Isleret al., 2002), human (Montgomery et al.,2004), sheep (Davis 2004) and goat (Ran et al., 2009). Point or deletion mutations in main genes affect fecundity of the carriers. For example, Bone Morphogenetic Protein Receptor IB (*BMPR-IB*), Bone Morphogenetic Protein 15 (*BMP15*), and Growth and Differentiation Factor 9 (*GDF9*) have been shown to control fecundity in several sheep breeds (Davis, 2004). In sheep, *BMPR-IB, BMP15* and *GDF9* are mapped to chromosome 6 (Montgomery et al., 1994), X (Galloway et al., 2000) and 5 (Sadighi et al., 2002), respectively. One copy of *FecX^I*, *FecX^H* (Galloway et al., 2002), *FecX^L* (Bodin et al., 2007), *FecX^B*, *FecX^G* (Hanrahan et al., 2004) or *FecX^R* (Monteagudo et al., 2009) mutation in *BMP15* and also one copy of *FecG^H*mutation in *GDF9* increases the litter size in the carries, but two copies cause infertility due to streak ovaries. The effect of *FecB* mutation in *BMPR-IB* is additive so that one copy of the

FecB locus increases the ovulation rate by about 1.5 and two copies by about 3.0. These extra ovulations increase litter size by about 1.0 and 1.5, respectively (Davis, 2004).

Measurements from a small sample of Cambridge and Belclare ewes suggested that the effect of *GDF9* mutation on ovulation rate was greater than that of *BMP15* mutations, and lower than the effect of *BMPR-IB* mutations, where each copy of *FecG^H* increased the ovulation rate by about 1.4 in the Cambridge and Belclare sheep (Davis, 2004). The *FecB* mutation causes A to C transition at position 830, which replaces glutamine with argenine at position 249 of the mature protein (Wilson et al., 2001). In *GDF9* gene, *FecG^H* mutation changes C to T at position 1184, with serine being replaced with phenylalanine at position 77 of the mature protein; it is suggested that this amino acid variation is located in the receptor binding domain (Hanrahan et al., 2004). Folding change of this domain due to substitution of an uncharged polar amino acid with

a nonpolar amino acid disturbs the biological action of the protein and removes its negative effect on ovulation. Therefore, this mutation increases ovulation rate /litter size.

Rayini is an Iranian native goat reared for meat and cashmere production. In the experimental flock, the adult mean body weight of males and females is 40 and 35 kg, respectively. The mean cashmere diameter and yield is about 17-18 µm and 540 g, respectively. The mean twining rate is about 1.29 when estrous synchronization and superovulation programs are not practiced. Occasionally, triplets and quadruplets are also reported. The aim of this study was to screen the *FecB* and *FecG^H* mutations and to study their association with prolificacy in Rayini goats.

Materials and Methods

Experimental flock, sampling, and DNA extraction

One hundred randomly-selected adult does, giving birth to single, twin or triplet lambs, and 10 adult bucks were used in this study. The goats belonged to Rayini Goat Breeding Station in Baft city, 180 km south-west of Kerman, Iran. Baft city is about 2270 m above sea level with an altitude of $56°$ 36' and latitude of $29°$ 17'. Approximately 10 ml blood was collected from the jugular vein in EDTA-containing tubes. Blood samples were transferred to the laboratory on ice. Genomic DNA was extracted from leucocytes, using DNP™ kit (high yield DNA purification kit) purchased from CinnaGen Inc., Tehran, Iran. The DNA samples were dissolved in Elution buffer (pH = 8.0)

and stored at -20 °C for further analysis.

Primer synthesis and PCR-RFLP reactions

Based on the methods described by Wilson et al., (2001) and Hanrahan et al., (2004), forced PCR-RFLP was used to detect the *FecB* and *FecG^H* mutations. In this method, a point mutation is deliberately introduced into one of the primers. The primers for *FecB* assay were synthesized by Metabion Co., Ltd., Germany, and those for *FecG^H* were produced by CinnaGen Inc., Tehran, Iran. The restriction endonucleases (RE) and other reagents were purchased from Promega Co., Ltd., USA. Total volume of 26 µl of each PCR reaction contained 2 µl of genomic DNA, 2 mM of $MgCl_2$ (0.8 µl of a solution containing 2.5 mM $MgCl_2$ per µl), 1 µl (10 µM) of each of forward and reverse primers, 0.5 µl (5 µM) of dNTPs, 1 unit (0.2 µl) of Taq DNA polymerase, 3 µl of 10 × PCR buffer and 17.5 µl deionized water.

In *FecB* assay, the fragments of 140 bp were amplified throughout initial denaturing at 94°C for 5 min; 35 cycles at 94°C for 40 sec, an annealing step for 40 sec, and extension at 72 °C for 40 sec, followed by a final extension of 72 °C for 10 min. In *FecG^H* assay, the fragments of 139 bp were amplified throughout 30 cycles of 94 °C for 30 sec; an annealing step for 45, and extension at 72 °C for 30 sec. The initial denaturing and final extension conditions were the same as for *FecB* amplifying assay. The primer sequence, RE, and annealing temperature of both PCR reaction programs are summarized in Table 1.

Table 1. Primers sequence, restriction endonucleases (RE), and annealing temperature of the assays

Mutation	No.	Primer sequence (5'→3')	Annealing temperature (°C)	RE
FecB	F1	GTCGCTATGGGGAAGTTTGGATG	63	*Ava*II
	R1	CAAGATGTTTTCATGCCTCATCAACACGGTC		
FecG^H	F2	CTTTAGTCAGCTGAA GTGGGACAAC	62	*Dde*I
	R2	ATGGATGATGTTCTGCACCATGGTGTGAACCTGA		

Resulting products (5 ml) were separated by electrophoresis on a 2% agarose gel and visualized with ethidium bromide to confirm amplification.

Forced restriction fragment length polymorphism PCR for FecB

The PCR was carried out using a modification of the forced RFLP method described by Wilson et al. (2001). The primer R1 has been designed to introduce a point mutation, resulting in PCR products with *FecB* mutation containing an *Ava*II restriction site (G/GACC), whereas products from non-carriers lack this site. The

140 bp product was digested by *Ava* II. If products contain the *FecB* mutation, they will be cut to yield fragments of 110 bp and 30 bp, whereas non-carrier products will remain uncut at 140 bp.

Forced restriction fragment length polymorphism PCR for FecG^H

The *FecG^H* variant was screened using the method improved by Hanrahan et al. (2004). A point mutation was introduced in primer R2 to generate a restriction site for *Dde* I (C/TTAG) in the PCR products. Through digestion, the wild type fragments will be cut into 108
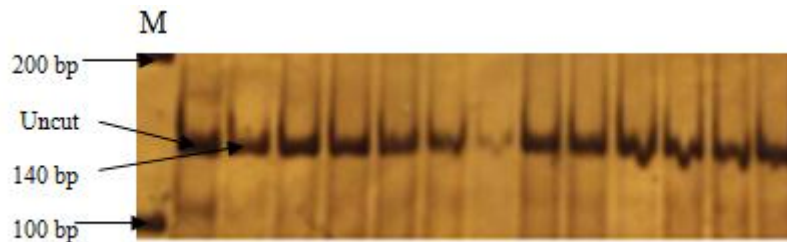
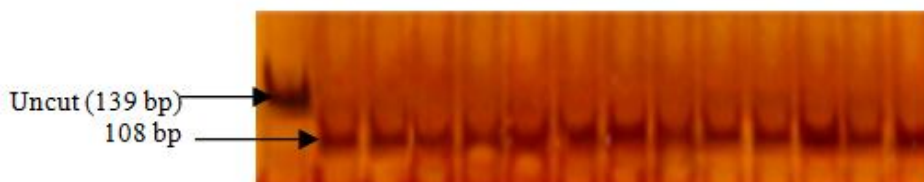bp and 31 bp fragments, whereas the mutant type ones with *FecG^H* variant will remain uncut at 139 bp.

Digestion of 8 ml of PCR products using 5 units RE in 20 ml final volume took place for 3 h at 37 °C. The 6-8 µl digestion products were loaded on 8% poly-acrylamide gel electrophoresis (PAGE) and visualized with silver staining. The gels were screened for the presence or absence of the mutations. Negative controls (uncut PCR product) were included with each assay.

Results

Forced PCR-RFLP electrophoretograms of *FecB* and *FecG^H* loci are shown in Figures 1 and 2, respectively. Mutations in *BMPR-1B* and *GDF9* genes were tested in the random samples from a flock of Rayini goats. After digesting with *Ava*II, samples from the animals with BB, B+ or ++ genotypes will reveal bands of 110 bp, 140 bp and 110 bp, and 140 bp, respectively. Digesting with *Dde*I results in 139 bp, 108 and 139 bp, and 108 bp bands for GG, G+, and ++ animals, respectively. Only the 140 bp product from *Ava*II digestion, and 108 bp product from *Dde*I digestion were observed in these goats. These results showed the presence of nucleotide A at position 746 on the *BMP15R-IB* sequence corresponding to glutamine at position 249 of the mature peptide, and the presence of nucleotide C at position 1184on the *GDF9* sequence corresponding to serine at position 77 of the mature peptide. Therefore, all 110 individuals screened in this experiment were wild-type homozygotes for *FecB* and *FecG^H* loci; none of the animals carried *FecB* or *FecG^H* mutations.

Discussion

Reproductive traits are amongst the most important economical parameters in sheep and goats. Heritability of the litter size is low and its improvement by traditional selection methods is a relatively slow process. Marker assisted selection, in conjunction with traditional selection methods, could accelerate the rate of genetic progress in litter size. Candidate gene markers for litter size have been identified in sheep including *BMPR-1B*, *BMP15* and *GDF9* (Davis, 2004), and in pig including estrogen receptor (Rothschild et al., 1996), prolactin receptor (Vincent et al., 1998), and follicle stimulating hormone- beta subunit (Li et al., 2001).

In the present study, *FecB* and *FecG^H* mutations, investigated as potential candidate genes, were not detected in a sample of male and female Rayini goats. Since result from a study by Wu et al., (2006) showed 99% homology of the nucleotide sequence of *GDF9* exon 2 between goat and sheep, we recruited primers previously used for screening of these genes in sheep by Wilson et al. (2001) and Hanrahan et al. (2004). Moreover, we used PAGE, instead of agarose gel electrophoresis (AGE), to obtain clearer electrophoretic results.

The results showed that Rayini goats with different kidding rates are wildtype for *FecB* and or *FecG^H* mutations. Therefore, these mutations most likely are not responsible for the prolificacy in Rayini goat, but other mutations in the same genes may be involved. Wu et al.

Figure 1. A PAGE electrophoretogram for *FecB*locus product digested by *Ava*II showing genotype. The 140 bp fragment comes from wild type products, while the mutation type with *FecB* cleaved to 110 and 30 bp fragments.

Figure 2. A PAGE electrophoretogram for *FecG^H* locus product digested by *Dde*I showing genotype. The 108 bp fragments come from wild type products, while the mutation type with *FecG^H* remains uncleaved at 139 bp.

(2006) found a new mutation in *GDF9* exon 2 in Jining Grey goats in which homozygote carriers had 0.57 and 0.62 kids more than the heterozygote- and non-carriers, respectively. Chu et al. (2004) found another new mutation in *GDF9* gene in Small Tail Han sheep that increased the litter size in this breed. A mutation in *GDF9* gene was also reported in Thoka sheep (Nicol et al., 2009). Mutation(s) in other genes may also affect prolificacy. For example, Small Tail Han sheep, the most prolific sheep in China, showed $FecX^G$ mutation in *BMP15* gene but not in $FecG^H$ mutation of *GDF9* (Chu et al., 2011). In addition, Davis et al. (2006) did not find *FecB* mutation in the 19 out of 21 prolific sheep sampled in 13 countries. Recently, He et al. (2010) concluded that inhibin α (INHα) gene can be regarded as a candidate gene for high prolificacy in goats. Moreover, there are possible differences in prolificacy inheritance patterns between sheep, goat, and other species (Yan et al., 2001; Wu et al., 2006). Studies have been performed to find mutations related to fecundity genes in Iranian sheep and goats. Results of a study searching for *FecB* and $FecG^H$ mutation in IranainShal sheep (Ghaffari et al., 2009a,b) are in line with our findings. The $FecX^G$ and $FecX^L$ mutations (on *BMP15*) were not found in this breed either (Zare et al., 2007). In another Iranian sheep, Lori-Bakhtiari, $FecX^L$ (Nejati-Javaremi et al., 2007), $FecX^I$ (on *BMP15*) and *FecB* (Amiriet al., 2007) were not detected. The $FecG^H$ mutation was not found in Arkha-Merino (Farajzadeh et al., 2007), Arab and Kordi sheep (Ghaderi et al., 2010). Deldar-Tajangookeh et al. (2009) detected neither $FecX^B$ nor $FecX^G$ in a population of Iranian native goats. Sangsari sheep had $FecG^H$ mutation, with no evidence for the presence of $FecX^G$ mutation (Kasiriyan et al., 2011). Moradband et al. (2011) found a new mutation in *GDF9* exon 2 that might influence prolificacy in Baluchi sheep; but did not detect $FecX^G$ and *FecB* in these sheep.

Conclusions

The absence of $FecG^H$ mutation in a prolific breed does not preclude major gene effects on prolificacy in other breeds; therefore, more extensive screening is required as tests for newly discovered mutations are developed. However, considering the important role of the genes affecting fecundity in ruminants (Vincent et al., 1998; Bodensteiner et al., 1999; Bodensteiner et al., 2000), PCR-RFLP and single strand conformation polymorphism (SSCP) analyses are suggested for detecting the polymorphisms in fecundity genes in goats.

References

Amiri, S., Rahimi, G., Vatankhah, M., 2007. No incidence of allelic mutation in Booroola (*FecB*) and Inverdale (*FecX^I*) genes in Lori-Bakhtiari sheep breed. Proceedings of the 5th National Biotechnology Congress of Iran, Tehran, Iran. pp. 495.

Bodensteiner, K.J., Clay, C.M., Moeller, C.L., Sawyer, H.R., 1999. Molecular cloning of the ovine growth/differentiation actor-9 gene and expression of growth/differentiation factor-9 in ovine and bovine ovaries. *BiologyofReproduction* 60, 381-386.

Bodensteiner, K.J., McNatty, KP, Clay, C.M., Moeller, C.L., Sawyer, H.R., 2000. Expression of growth and differentiation factor-9 in the ovaries of fetal sheep homozygous or heterozygous for the Inverdale prolificacy gene (*FecX^I*). *BiologyofReproduction* 62, 1479-1485.

Bodin, L., Di Pasquale, E., Fabre, S., Bontoux, M., Monget, P., Persani, L., Mulsant, P., 2007. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. *Endocrinology* 148, 393-400.

Chu, M.X., Li, B.X., Wang, J.Y., Ye, S.C., Fang, L., 2004. Association between PCR-SSCP of growth differentiation factor 9 gene and high prolificacy in Small Tail Han sheep. *AnimalBiotechnology* 15, 111-120.

Chu, M.X., Yang, J., Feng, T., Cao, G.L., Fang, L., Di, R., Huang, D.W., Tang, Q.Q., Ma, Y.H., Li, K., Li, N., 2011. GDF9 as a candidate gene for prolificacy of Small Tail Han sheep. *MolecularBiologyof Reproduction* 38, 5199-5204.

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

countries. *Animal Reproduction Science* 92, 87-96.

Davis, G.H., 2004. Fecundity genes in sheep. *Animal Reproduction Science* 82–83: 247–253.

Deldar-Tajangookeh, H., Zare, Shahneh, A., Zamiri, M.J., Daliri, M., Kohram, H., Nejati-Javaremi, A., 2009. Study of *BMP-15* gene polymorphism in Iranian goats. *African Journal of Biotechnology*8, 2929-2932.

Farajzadeh, M., Dehnad, A., Rahimi, G., Amiri, A., 2007. Genetic polymorphisms in oocyte-derived growth factor (*GDF-9*) gene in Arkha Merino sheep using RFLP-PCR. Proceedings of the 5th National Biotechnology Congress of Iran, Tehran, Iran. pp. 503.

Galloway, S.M., Gregan, S.M., Wilson, T., McNatty, K.P., Juengel, J.L., Ritvos, O., Davis, G.H., 2002.*BMP15* mutations and ovarian function. *Molecular and Cellular Endocrinology*191, 15–18.

Galloway, S.M., McNatty, K.P., Cambridge, L.M., Laitinen, M.P., Jennifer, J.L., Jokiranta, S., McLaren, R.J., Luiro, K., Dodds, K.G., Montgomery, G.W., Beattie, A.E., Davis, G.H., Ritvos, O., 2000. Mutations in an oocyte-derived growth factor gene (*BMP15*) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genetics*25, 2792-2783.

Ghaderi, A., BeigiNasiri M.T., Mirzadeh K.H., Fayazi J., Sadr A.S., 2010.Identification of the GDF9 mutation in two sheep breeds by using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) technique. *AfricanJournalofBiotechnology* 9, 8020-8022.

Ghaffari, M., Nejati-Javaremi, A., Rahimi, G., 2009a.Detection of polymorphism in oocyte derived growth factor (GDF-9) gene associated with twinning in Shal sheep breed. *South African Journal of Animal Science* 39, 355-360.

Ghaffari M., Nejati-Javaremi A., Rahimi G., 2009b. Detection of polymorphism in Booroola gene (*FecB*) associated with twinning in Shal sheep breed using PCR-RFLP method. *International Journal of Agriculture and Biology* 11, 97-99.

Hanrahan, P.J., 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 (*Ovisaries*). *Biology of Reproduction* 70, 900-909.

He, Y., Ma, X., Liu, X., Zhang, C., Li, J., 2010. Candidate genes polymorphism and its association to pro-lifi-cacy in Chinese goats. *Journal of Agricultural Science* 2, 88-92.

Isler, B.J., Irvin, K.M., Neal, S.M., Moeller, S.J., Davis, M.E., 2002. Examination of the relationship between the estrogen receptor gene and reproductive traits in swine. *Journal of Animal Science* 80, 2334-2339.

Kasiriyan, M.M., Hafezian, S.H., Hassani, N., 2011. Genetic polymorphism BMP15 and GDF9 genes in Sangsari sheep of Iran. *International Journal of Genetics and Molecular Biology* 3, 31-34.

Li, N., Zhao, Y.F., Xiao, L., Zhang, F.J., Chen, Y.Z., Dai, R.J., Zhang, J.S., Shen, S.Q., Jiang, Z., Gibson, J.P., Archibald, A.L., Haley, C.S., 2001.The porcine gonadotropin-releasing hormone receptor gene (*GNRHR*): genomic organization, polymorphisms, and association with the number of corpora lutea. *Genome*44, 7-12.

Monteagudo, L.V., Ponz, R., Tejedor, M.T., Lavina, A., Sierra, I., 2009. A 17bp deletion in the Bone Morphogenetic Protein 15 is associated to increased prolificacy in the Rasa Aragonesa sheep breed. *Animal Reproduction Science* 110, 139-146.

Montgomery, G.W., Lord, E.A., Penty, G.M., Dodds, K.G., Board, T.E., Cambridge, L.M., Suden, S.L.F., Stone, R.T. Crawford, A.M., 1994. The Booroola fecundity (*FecB*) gene maps to sheep chromosome 6. *Genomics* 22, 148-153.

Montgomery, G.W., Zhao, Z.Z., Marsh, A.J., Mayne, R., Treloar, S.A., James, M., Martin, N.G., Boomsma, D.I., Duffy, D.L., 2004.A deletion mutation in *GDF9* in sisters with spontaneous DZ twins. *Twin Research* 7, 548-555.

Moradband, F., Rahimi, G., Gholizadeh, M., 2011. Association of polymorphisms in fecundity genes of GDF9, BMP15 and BMP15-1B with litter size in Iranian Baluchi sheep. *Asian-Australasian Journal of Animal Sciences* 24, 1179-1183.

Nejati-Javaremi, A., Rahimi, G., Amiri, A., 2007.Detection of polymorphism in *FecX^L* gene associated with twinning in Lori- Bakhtiari sheep breed using PCR-SSCP. Proceedings of the 5th National Biotechnology Congress of Iran, Tehran, Iran. pp. 520.

Nicol, L., Bishop, S.C., Pong-Wong, R., Bendixen, C., Holm, L., Rhind, S.M., McNeilly, A.S., 2009. Homozygosity for a single base-pair mutation in the oo-

cyte-specific *GDF9* gene results in sterility in Thoka sheep.*Reproduction* 138, 921–933.

Peiró, R., Merchán, M., Santacreu, M.A., Argente, M.J., García, M.L., Folch, J.M., Blasco, A., 2008. Identification of single-nucleotide polymorphism in the progesterone receptor gene and its association with reproductive traits in rabbits. *Genetics* 180, 1699-1705.

Ran, X.Q., Lin, J.B., Du, Z.Y., Qing, C., Wang, J.f., 2009. Diversity of Bmp15 and Gdf9 genes in White Goat of Guizhou province and evolution of the encoded proteins. *Zoological Research* 30, 593-602.

Rothschild, M., Jacobson, C., Vaske, D., Tuggle, C., Wang, L., Short, T., Eckhardt, G., Sasaki, S., Vincent, A., McLaren, D., Southwood, O., van der Steen, H., Mileham, A., Plastow, G., 1996. The estrogen receptor locus is associated with major gene influencing litter size in pigs. *Proceedingsof the National Academy of Sciences* (USA) 93, 201-205.

Sadighi, M., Bodensteiner, K.J., Beattie, A.E., Galloway, S.M., 2002. Genetic mapping of ovine growth differentiation factor 9 (*GDF9*) to sheep chromosome 5. *Animal Genetics* 33, 244-245.

Vincent, A.L., Evans, G., Short, T.H., Southwood, O., Plastow, G.S., Tuggle, C.K., Rothschild, M.F., 1998. The prolactin receptor gene is associated with increased litter size in pigs. Proceedingsof the 6th

World Congress on Genetics Applied to Livestock Production 27, University of New England, Armidale, Australia. pp. 15-18.

Wilson, T., Wu, X.Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., McEvan, J.C., O'Connel, A.R., McNatty, K.P., 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. *BiologyofReproduction* 64, 1225-1235.

Wu, Z.H., Chu, X., Li, X.W., Fang, L., Ye, S.C., Liu, Z.H., Chen, G.H., 2006. PCR-SSCP analysis on exon 2 of growth differentiation factor 9 gene in goats. *ScientiaAgriculturaSinica* 39, 802-808.

Yan, C., Wang, P., DeMayo, J., DeMayo, F.J., Elvin, J.A., Carino, C., Prasad, S.V., Skinner, S.S., Dunbar, B.S., Dube, J.L., Celeste, A.J., Matzuk, M.M., 2001. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Molecular Endocrinology* 15, 854-866.

Zare, Y., Nejati-Javaremi, A., Rahimi, G., 2007. Detection of polymorphisms in two point of gene associated with twinning (*BMP15*) in Shal sheep. Proceedings of the 5th National Biotechnology Congress of Iran, Tehran, Iran. pp. 483.

Communicating editor: Ali K Esmailizadeh