Indian Journal of Animal Sciences **88** (2): 200–205, February 2018/Article

Genetic characterization of three fertility genes in Egyptian sheep and goat breeds

OTHMAN E OTHMAN[1], HEBA A ABD EL-KADER[2], ABEER H ABD EL-RAHIM[3], OMAIMA M ABD EL-MONEIM[4] and SALLY S ALAM[5]

National Research Centre, Dokki, Egypt

Received: 17 August 2017; Accepted: 2 September 2017

ABSTRACT

One of the effective approaches for genetic improvement of productivity traits in farm animals is marker-assisted selection (MAS) depending on the genetic markers that are associated with superior productivity traits. The improvement of fertility trait is one of the main targets in small ruminant breeding programs. This work aimed to identify RFLPs and SNPs variations among three fertility genes in Egyptian sheep and goat breeds. RFLP analysis of the amplified fragments at 462-bp from exon 1 of *GDF9* using *Hpa*II endonuclease showed the presence of two genotypes GG and AG. Depending on the presence of the restriction site of *Taq*I endonuclease (T^CGA) in the 348-bp amplified fragment from exon 5 of *GPR54* gene, the results showed the presence of two alleles, C and T with three genotypes, viz. CC, TT and CT. The PCR amplified fragments of 190-bp from *FecB* gene were digested with *Ava*II restriction enzyme and the results showed that all tested animals had the same homozygous non-carrier genotype (++). It was concluded that the identification of genetic structure and nucleotide sequences of *GDF9*, *GPR54* and *FecB* genes is considered the first step towards the genetic improvements of fertility trait in Egyptian small ruminants where these genes are associated with different fertility traits parameter like ovulation rate, ovarian follicular development, puberty and litter size in small ruminant breeds.

Key words: DNA sequencing, Egyptian, FecB, GDF9, GPR54, PCR-RFLP, Small ruminants

Marker assisted selection depending on the genetic markers associated with reproduction traits became the most effective tool for genetic improvement of economically important traits in different livestock (Kolosov *et al.* 2015). The ovulation rate (Hanrahan *et al.* 2004) and litter size (Cao *et al*. 2011) are two important indicators for the fertility and reproduction performances in farm animals especially sheep and goat (Tang *et al.* 2012, Dinçel *et al*. 2015). The detection of genes associated with fertility and reproduction traits and the identification of their genetic variation effects on these traits phenomena will help in the reproduction enhancement of sheep and goat breeds.

Growth differentiation factor 9 (*GDF9*) gene is expressed in the developing oocytes in the ovaries of ruminants (Bodensteiner *et al.* 1999, 2000) and plays an essential role in ovarian follicular development, ovulation rate and prolificacy in different mammalian species (Chung and Davis 2012, Tang *et al.* 2013). *GPR54* is one of the G protein-coupled receptors and the endogenous receptor of *KISS-1* peptide (Chu *et al.* 2012). *GPR54* gene is highly expressed in placenta, pancreas and in brain whereas it is expressed at low level in adrenal glands, testes and spleen (Funes *et al*. 2003, Cao *et al.* 2011). The kisspeptin/GPR54 pathway has an essential role in puberty process and is considered the key for GnRH secretion regulation. This pathway stimulates LH and FSH secretion to initiate the puberty (Kuohung and Kaiser 2006, Tena-Sempere 2006). Many reports focused on the role of the Booroola fecundity (*FecB*) gene in reproductive endocrinology, ovary development, ovulation rate and litter size (EL-Hanafy and El-Saadani 2009). This gene increases the ovulation rate and litter size in small ruminants and the identification of the *FecB* mutation is of great interest in the studies of mammalian fertility (Wilson *et al*. 2001). The present study aimed to identify the genetic structure and nucleotide sequences of three genes associated with fertility and reproduction traits in Egyptian small ruminant breeds.

MATERIALS AND METHODS

Animals and DNA extraction: The blood samples were collected from 140 animals belonging to three sheep breeds, viz. Barki (32 animals), Ossimi (28 animals) and Rahmani (22 animals) in addition to three goat breeds namely Baladi (16 animals), Barki (20 animals) and Zaraibi (22 animals). Genomic DNA was extracted from the whole blood according to the method described by Miller *et al.* (1988) with minor modifications. Briefly, blood samples were mixed with cold 2× sucrose-triton and centrifuged at 5,000 rpm for 15 min at 4°C. The nuclear pellet was suspended in lysis buffer, sodium dodecyl sulfate and proteinase K and

Present address: [1](othmanmah@yahoo.com), [2](mayamo2013 @yahoo.com), [3](abeerabdel_rahim@yahoo.com), [4](emy.monem @yahoo.com), [5](sallyalam@yahoo.com).

incubated overnight in a shaking water bath at 37°C. Nucleic acids were extracted with saturated NaCl solution. The DNA was picked up and washed in 70% ethanol. The DNA was dissolved in 1× TE buffer. DNA concentration was determined using Nano Drop1000 Thermo Scientific spectrophotometer, and then diluted to the working concentration of 50 ng/µl, which is suitable for polymerase chain reaction.

Polymerase chain reaction (PCR): The DNA fragments from the tested genes were amplified using polymerase chain reaction technique developed by Mullis *et al*. (1986). A PCR cocktail consisted of 1.0 µM upper and lower primers (Table 1), 0.2 mM dNTPs and 1.25 U of *Taq* polymerase. The cocktail was aliquot into PCR tubes with 100 ng of sheep or goat DNA. The reaction was cycled with the following conditions: initial denaturation for 5 min at 94°C followed by 30 cycles of denaturation at 94°C (1 min), annealing at optimum temperature for each tested gene (1 min) and extension at 72°C (2 min) and the final extension for 10 min at 72°C. The amplification was verified by electrophoresis on 2% agarose gel in 1× TBE buffer using GeneRuler^{TM} 100-bp ladder as a molecular weight marker for confirmation of the length of the PCR products. The gel was stained with ethidium bromide and visualized on UV trans-illuminator.

Restriction fragment length polymorphism (RFLP): Ten microlitres of PCR products were digested with 1 µl of FastDigest restriction enzyme specific for each tested gene (Table 1) at 37°C for 5 min. The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gel (GIBCO, BRL, England) in 1× TBE buffer (0.09 M Tris-boric acid and 0.002 M EDTA). Gels were visualized under UV light and documented in FX Molecular Imager apparatus (BIO-RAD).

Sequence analysis: The PCR products representing detected genotypes of each tested gene were purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using NCBI/BLAST/blastn suite. Results of endouclease restriction were carried out using FastPCR.

RESULTS AND DISCUSSION

In recent decades, marker assisted selection became one of the most essential tools in practical genetics where the selection of animals depends on the genetic markers associated with economically important production and reproduction traits in livestock (Yang *et al*. 2010 and Klimenko *et al.* 2014). The identification of fertility genes and their polymorphism effects on reproduction traits in different farm animals including small ruminant is considered the first step towards the improvement of these traits (Iwanowska *et al.* 2011, Chu *et al.* 2012). The aim of the present work was the identification of genetic structure and nucleotide sequences of three genes - *GDF9*, *GPR54* and *FecB* - associated with fertility traits in six Egyptian small ruminants.

Growth differentiation factor 9 (GDF9) gene: The digestion process of the amplified fragments from *GDF9* gene at 462-bp using *Hpa*II resulted into two different genotypes GG and AG according to the presence of restriction sites (CC^GG) in these fragments. AG genotype with four fragments at 410-, 254-, 156- and 52-bp and GG genotype with three fragments at 254-, 156-bp and 52-bp (Fig. 1).

Table 2 summarize the frequencies of two detected genotypes in tested small ruminant breeds. The total frequencies for GG and AG genotypes were 85.4% and 14.6% in 82 tested sheep animals whereas in 58 goat animals, the frequencies for GG and AG genotypes were 83.6% and 19%, respectively. The total frequencies for GG and AG genotypes as well as G and A alleles in tested small ruminants were 83.6%, 16.4%, 91.8% and 8.2%, respectively.

The sequence analysis of GG (Fig. 2) and AG (Fig. 3) genotypes showed the appearance of a SNP (G/A) at position 209 in AG genotype which leads to the presence of four digested fragments at 410-, 254-, 156- and 52-bp in these genotype.

Kolosov *et al.* (2015) determined *GDF9* polymorphism in two Russian sheep breeds-Salskaya and Romanov- using

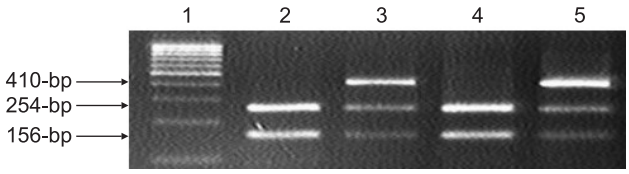


Fig. 1. Electrophoretic pattern after digestion of *GDF9* PCR products with *Hpa*II endonuclease. Lane 1, 100-bp ladder marker; lanes 2 and 4, GG genotype with 3 digested fragments at 254- 156- and 52-bp; lanes 3 and 5. AG genotype with 4 digested fragments at 410-, 254-, 156- and 52-bp; small fragment at 52-bp is not shown in the figure.

Table 1. Primer sequences used for PCR

Gene	Primer sequences 5'---------- -3'	Annealing temperature	PCR product size	Restriction enzyme	Reference
GDF9	GAAGACTGGTATGGGGAAATG CCAATCTGCTCCTACACACCT	63°C	462-bp	*HpaII*	Kolosov *et al.* (2015)
GPR54	ACCTGGCATCCGCGCAGTT CTCAGAGGGGCCCGTCTTGAT	58°C	348-bp	*TaqI*	Cao *et al.* (2011)
FecB	CCA GAG GAC AAT AGC AAA GCA AA CAAGATGTTTTCATGCCTCATCAACAGGTC	60°C	190-bp	*Ava*II	Wilson *et al.* (2001)

Table 2. The genotype and allele frequencies of *GDF9* gene in Egyptian sheep and goat breeds

Species	Breed	No. of animals	Genotype frequencies				Allele frequencies	
			GG		AG		G	A
			No	Frequency	No	Frequency	Frequency	Frequency
Sheep	Barki	32	28	87.5%	4	12.5%	93.75%	6.25%
	Ossimi	28	23	82.1%	5	17.9%	91.1%	8.9%
	Rahmani	22	19	86.4%	3	13.6%	93.2%	6.8%
	Sub-total	82	70	85.4%	12	14.6%	92.7%	7.3%
Goat	Baladi	16	13	81.3%	3	18.7%	90.6%	9.4%
	Barki	20	17	85.0%	3	15.0%	92.5%	7.5%
	Zaraibi	22	17	77.3%	5	22.7%	88.6%	11.4%
	Sub-total	58	47	81.0%	11	19.0%	90.5%	9.5%
Total		140	117	83.6%	23	16.4%	91.8%	8.2%

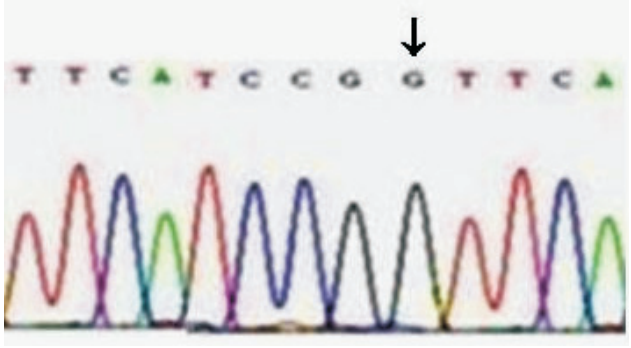


Fig. 2. Genotype GG of *GDF9* with the nucleotide G at position 209.

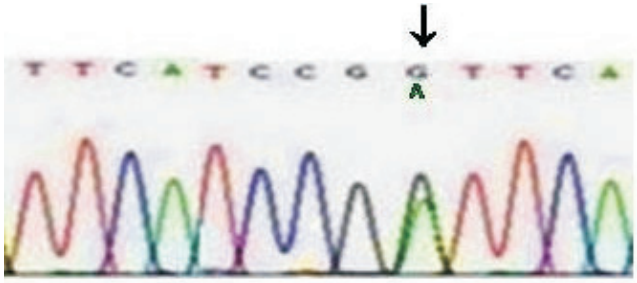


Fig. 3. Genotype AG of *GDF9* with the nucleotide A/G at position 209.

PCR-RFLP technique. They reported the appearance of GG and AG genotypes in exon 1 which was tested in the present study and AA and AG genotypes in exon 4. At exon 1, the frequencies of GG and AG genotypes were 90% and 10%, in Salskaya breed and 60.9% and 39.1% in Romanov breed, respectively. This result declared that our sheep breeds were genetically closer to Salskaya breed other than Romanov breed, where the frequencies of GG and AG genotypes in our sheep breeds were 85.4% and 14.6%, respectively.

The PCR amplified fragments from exon 1 of *GDF9* (462-bp) in Iranian Sangsari sheep breed were digested using *Hha*I and the results showed a G to A substitution in *GDF9* locus with allele frequencies for G and A as 80.16% and 19.84%, respectively. The results showed that this Iranian sheep breed possess a rare genotype AA which does not appear in Egyptian or Russian sheep breeds suggesting that the domestication of sheep breeds may have occurred in Iran and surrounding area of Fertile Crescent. Some reports declared that the sheep animals with GG genotype of *GDF9* possess high fertility than animals with AG genotypes while the animals with AA genotypes have low fertility (Kasiriyan *et al.* 2011). This finding showed that our animals had high fertility rate where most of them possess GG (85.4%) and AG (14.6%) genotypes of *GDF9* with the absence of AA genotype.

G protein-coupled receptor 54 (GPR54) gene: A fragment of 348-bp from *GPR54* exon 5 was amplified using PCR and the restriction analysis of these fragments using endonuclease *Taq*I declared the presence of three genotypes,

Table 3. The genotype and allele frequencies of *GPR54* gene in Egyptian sheep and goat breeds

Species	Breed	No. of animals	Genotype frequencies						Allele frequencies	
			CC		CT		TT		C	T
			No	Frequency	No	Frequency	No	Frequency	Frequency	Frequency
Sheep	Barki	32	10	31.25%	20	62.5%	2	6.25%	62.5%	37.5%
	Ossimi	28	9	32.1%	18	64.3%	1	3.6%	64.3%	35.7%
	Rahmani	22	6	27.3%	15	68.2%	1	4.5%	61.4%	38.6%
	Sub-total	82	25	30.5%	53	64.6%	4	4.9%	62.8	37.2%
Goat	Baladi	16	6	37.5%	10	62.5%	0	00.0%	68.75%	31.25%
	Barki	20	7	35.0%	12	60.0%	1	5.0%	65.0%	35.0%
	Zaraibi	22	9	41.0%	12	54.5%	1	4.5%	68.2%	31.8%
	Sub-total	58	22	37.9%	34	58.6%	2	3.5%	67.2%	32.8%
Total		140	47	33.6%	87	62.1%	6	4.3%	64.6%	35.4%

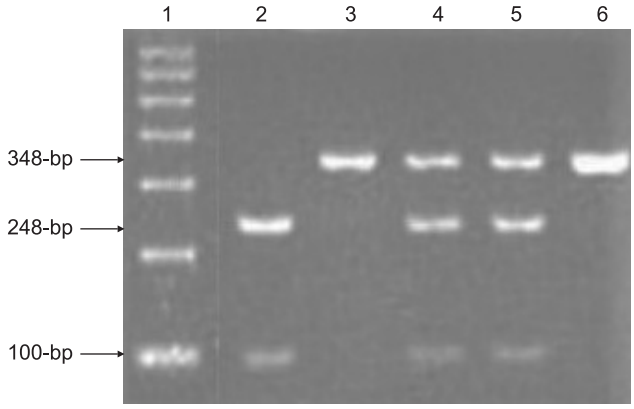
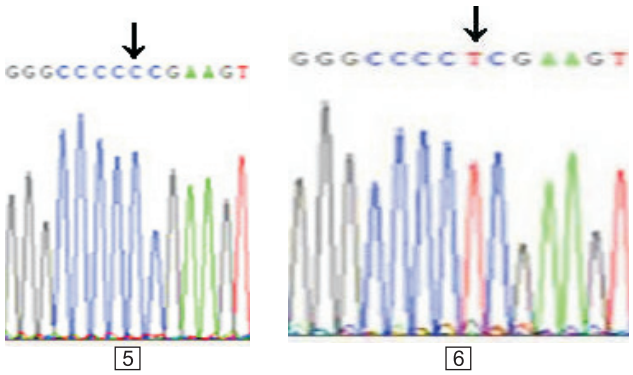


Fig. 4. Electrophoretic pattern after digestion of *GPR54* PCR products with *Taq*I endonuclease. Lane 1, 100-bp ladder marker; lane 2, TT genotype with 2 digested fragments at 248- and 100-bp; lanes 3 and 6, CC genotype with undigested fragment at 348-bp; lanes 4 and 5, CT genotype with 3 digested fragments at 348-, 248- and 100-bp.



Figs 5–6. 5. The nucleotide C at position 100 in allele C of *GPR54* gene. 6. The nucleotide T at position 100 in allele T of *GPR54* gene.

CC, CT and TT (Fig. 4). The appearance of these genotypes resulted from the presence of T^CGA restriction site at position 100^101.

In sheep breeds, the total frequencies of CC, CT and TT genotypes were 30.5%, 64.6% and 4.9%, respectively whereas in goat breeds, the frequencies of CC, CT and TT genotypes were 37.9%, 58.6% and 3.5%, respectively. The total frequencies for CC, CT and TT genotypes in all sheep and goat animals were 33.6%, 62.1% and 4.3%, respectively. The total frequencies for C and T alleles in all tested animals were 64.6% and 35.4%, respectively (Table 3).

The sequence analysis of the two different alleles, C (Fig. 5) and T (Fig. 6), showed the presence of a SNP (C/T) at position 100 in allele T yielding two digested fragments at 248- and 100-bp in this allele.

Six pairs of primers were used to detect goat *GPR54* polymorphisms in Chinese goat breeds and their association with some fertility parameters (Cao *et al*. 2011). The only polymorphism was detected in exon 5 where there were three SNPs, G4014A, G4136A and C4152T which may have associations with sexual precocity in goat breeds. Also the association between C4152T polymorphism and fertility trait was reported in Jining Grey goat where goats with genotypes TT and CT had more kids than those with genotype CC.

Tang *et al*. (2012) reported the presence of different mutations in *GPR54* gene in four Chinese sheep breeds, viz. Small Tail Han, Chinese Merino, Hu and Corriedale. In first sheep breed, there are three genotypes namely AA with frequency of 0.25, AG (0.50) and GG (0.25) with negative effect on the litter size. On the other hand, the frequencies of CC (0.175), CD (0.125) and DD genotypes (0.700) were reported in this breed with positive effect where sheep ewes with genotype CC had more lambs than those with genotype DD or CD. These results declared that allele C of *GPR54* gene may be considered as a candidate marker for improving litter size in sheep.

FecB gene: A 190-bp fragment from *FecB* gene of sheep and goat was amplified using PCR. The digestion process of these fragments by *Ava*II restriction enzyme revealed the absence of the restriction site (G^GACC) at position 160^161 in tested animals yielding the presence of uncut fragments at 190-bp. This result showed that all tested Egyptian sheep and goats have the same homozygous non-carrier genotype (++) (Fig. 7).

The sequence analysis of the purified PCR products (Fig. 8) representing the detected monomorphic ++ non-carrier genotype showed the presence of a nucleotide at position 160 (Fig. 9), which is responsible for the absence of restriction site (G^GACC) at position 160^161 and consequently the presence of undigested fragments at 190-bp in all tested animals.

Litter size and lamb growth are two important reproduction parameters with economic importance in small breeding programs. Souza *et al*. (2003) reported the presence of genetic polymorphism in *BMPR-IB* gene and

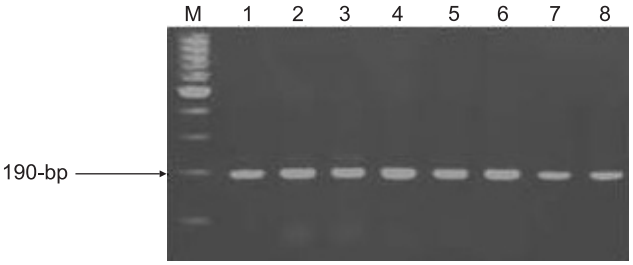


Fig. 7. The electrophoretic pattern obtained after digestion of PCR amplified fragment of *FecB* gene from sheep and goat DNA with *Ava*II restriction enzyme. Lane M, 100-bp ladder marker; lanes 1–8, ++ non-carrier homozygous genotype with uncut fragment at 190-bp.

CCAGAGGACAATAGCAAAGCAAATTCAGATGGTGAAACAGA
TGGAAAAGGTCGCTATGGGGAAGTTTGGATGGGAAAGTGG
GTGGCGAAAAGGTAGCTGTGAAAGTGTTCTTCACTACAGAG
AGGCCAGCTGGTTCCGAGAGACAGAAATATATCAGACCGTG
TGATGAGGCATGAAAACATCTTG

Fig. 8. The nucleotide sequence of ++ non-carrier genotype (190-bp). The nucleotide A at position 160.

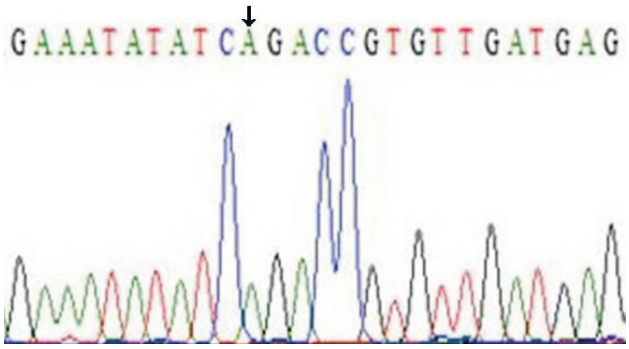


Fig. 9. The nucleotide A in the monomorphic ++ non-carrier genotype.

its association with the *FecB* gene and the high prolificacy in Booroola Merino sheep using PCR-RFLP technique (Souza *et al.* 2001 and Davis *et al.* 2002).

The genetic polymorphism in *FecB* gene and its association with some economically important growth parameters was identified by Guan *et al.* (2007). They reported that Hu sheep are homozygous carriers (BB) whereas in Merino prolific meat breed, the three genotypes, BB, B+ and ++ appeared with different frequencies. In Merino prolific sheep breed, the animals with genotypes BB and B+ had higher mean litter sizes of ewes, the heart girth and chest width than those with genotype ++.

The association between *FecB* gene and some reproduction and fertility parameters like reproductive endocrinology, ovary development, litter size, organ development and body mass was reported (Smith *et al.* 1993, Smith *et al.* 1996 and Cognie *et al.* 1998). The effects of *FecB* gene on these parameters are different where it has positive effects on litter size and ovulation rate and negative effects on fetal growth and development and body mass (Wang *et al*. 2003 and Liu *et al.* 2003).

In conclusion, the identification of genetic structure and nucleotide sequences of *GDF9*, *GPR54* and *FecB* genes is considered the first step towards the genetic improvements of fertility trait in Egyptian small ruminants where these genes are associated with different fertility traits parameter like ovulation rate, ovarian follicular development, puberty and litter size in small ruminant breeds.

REFERENCES

Bodensteiner K J, Clay C M, Moeller C L and Sawyer H R. 1999. Molecular cloning of the ovine growth/differentiation factor-9 gene and expression of growth/differentiation factor-9 in ovine and bovine ovaries. *Biology of Reproduction* **60**: 381–86.

Bodensteiner K J, McNatty K P, Clay C M, Moeller C L and 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 (*FecXI*). *Biology of Reproduction* **62**: 1479– 85.

Cao G L, Chu M X, Fang L, Feng T, Di R and Li N. 2011. Analysis on DNA sequence of GPR54 gene and its association with litter size in goats. *Molecular Biology Reproduction* **38**: 3839–48.

Chu M, Xiao C, Feng T, Fu Y, Cao G, Fang L, Di R, Tang Q, Huang D, Ma Y, Li K and Li N 2012. Polymorphisms of KiSS-1 and GPR54 genes and their relationships with litter size in sheep. *Molecular Biology Reports* **39**: 3291–97.

Chung H and Davis M. 2012. PCR-RFLP of the ovine calastatin gene and its association with growth. *Asian Journal of Animal and Veterinary Advance* **7**: 641–52.

Cognie Y, Benoit F, Poulin N, Khatir H and Driancourt M A. 1998. Effect of follicle size and of the FecB Booroola gene on oocyte function in sheep. *Journal of Reproduction and Fertility* **2**: 379–86.

Davis G H, Galloway S M, Ross L K, Gregan S M, Ward G, Nimbkar B V and Ghalsasi P M. 2002. DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. *Biology of Reproduction* **66**: 1869–74.

Dinçel D, Ardiçli S, Soyudal B, Er M, Alpay F, [a]amli H and Balci F. 2015. Analysis of FecB, BMP15 and CAST gene mutations in Sakiz sheep. *Kafkas Universitesi Veteriner Fakultesi Dergisi* **21**: 483–88.

EL-Hanafy A A and El-Saadani M A. 2009. Fingerprinting of FesB gene in five Egyptial sheep breeds. *Biotechnology in Animal Husbandry* **25**: 205–12.

Funes S, Hedrick J A, Vassileva G, Markowitz L, Abbondanzo S, Golovko A, Yang S, Monsma F J and Gustafson E L. 2003. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. *Biochemical and Biophysical Research Communications* **312**: 1357–63.

Guan F, Liu S, Shi G and Yang L. 2007. Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. *Animal Reproduction Science* **99**: 44–52.

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**: 900–09.

Iwanowska A, GrzeÅ B, Miko Å, Ajczak B, IwaÅska E and Juszczuk-Kubiak E. 2011. Impact of polymorphism of the regulatory subunit of the µ-calpain (CAPN1S) on the proteolysis process and meat tenderness of young cattle. *Molecular Biology Reports* **38**: 1295–1300.

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

Klimenko A, Usatov A, Getmantseva L, Kolosov Y and Tretyakova O. 2014. Effect of melanocortin-4 receptor gene on growth and meat traits in pigs raised in Russia. *American Journal of Agricultural and Biological. Science*s **9**: 232–37.

Kolosov Y A, Getmantseva L V, Shirockova N V, Klimenko A, Bakoev S, Usatov A V, Kolosov A Y, Bakoev N F and Leonova M A. 2015. Polymorphism of the *GDF9* gene in Russian sheep breeds. *Journal of Cytology and Histology* **6**: 1–4.

Kuohung W and Kaiser U B. 2006. GPR54 and KiSS-1: role in the regulation of puberty and reproduction. *Reviews in Endocrine and Metabolic Disorders* **7**: 257–63.

Liu S F, Jiang Y L and Du L X. 2003. Study of BMPR-IB and BMP15 as candidate gene for fecundity in fittle tailed Han sheep. *Acta Genetica Sinica* **8**: 755–60.

Miller S A, Dykes D D and Polesky H F. 1988. A simple salting

out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16**: 1215.

Mullis K, Facoma F, Scharf S, Snikl R, Horn G and Erlish H. 1986. Specific amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harbor Symposium Quantitative Biology* **51**: 260.

Smith P O W S, Hudson N L, Shaw L, Heath D A, Condell L, Phillips D J and McNatty K P. 1993. Effects of the Booroola gene (*FecB*) on body weight, ovarian development and hormone concentrations during fetal life. *Journal of Reproduction and Fertility* **1**: 41–54.

Smith P, Hudson N L, Corrigan K A, Shaw L, Smith T, Phillips D J and McNatty K P J. 1996. Effects of the Booroola gene *FecB* (B) on bodymass, testis development and hormone concentrations during fetal life. *Journal of Reproduction Fertility* **2**: 253–61.

Souza C J, MacDougal C, Campbell B K, McNeilly A S and Baird D T. 2001. The Booroola (*FecB*) phenotype is ssociated with a mutation in the bone morphogenetic receptor type 1B (BMPR-1B) gene. *Journal of Endocrinology* **2**: R1–R6.

Souza C J, Campbell B K, McNeilly A S and Baird D T. 2003. Bone morphogenetic proteins and folliculogenesis: lessons from the Booroola mutation. *Reproduction* (Suppl. **61**): 361–70.

Tang Q Q, Chu M X, Cao G L, Fang L, Di R, Feng T, Huang D W and Li N. 2012. Association between polymorphism of GPR54 gene and litter size in Small Tail Han sheep. *Livestock Science* **143**: 97–103.

Tang K Q, Yang W C, Li S J and Yang L G. 2013. Polymorphisms of the bovine growth differentiation factor 9 gene associated with superovulation performance in Chinese Holstein cows. *Genetics and Molecular Research* **12**: 390–99.

Tena-Sempere M. 2006. GPR54 and kisspeptin in reproduction. *Human Reproduction Update* **12**: 631–39.

Wang G L, Mao X Z, Davis G H, Zhao Z S, Zhang L J and Zeng Y Q. 2003. DNA tests in Hu sheep and Han sheep (small tail) showed the existence of Booroola (FecB) mutation. *Journal of Nanjing Agriculture University* **1**: 104–06.

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**: 1225–35.

Yang W C, Li S J, Tang K Q, Hua G H and Zhang C Y. 2010. Polymorphisms in the 5' upstream region of the FSH receptor gene, and their association with superovulation traits in Chinese Holstein cows. *Animal Reproduction Science* **119**: 172–77.