

Evaluation of Genetic Diversity at GDF9 and BMP4 Gene by Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) in Indian Goats

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Abstract

Genetic diversity of three Indian goat breeds namely Barbari, Sirohi and Black Bengal goats (*Capra hircus*) was evaluated using Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) method to detect polymorphism in two candidate genes (GDF9 and BMP4) in a sample of 90 goats with 30 goats of each breed. Both of the studied loci were polymorphic, having three variants for GDF9 and two variants for BMP4, in the studied population. Polymorphic information content (PIC) criterion revealed an intermediate polymorphism for all analysed phenotypic groups for Barbari, Sirohi and Black Bengal goats. Effective allele number criterion also revealed equally diverse nature of these genes. This preliminary study provides insight into the genetic diversity of Barbari, Sirohi and Black Bengal goats at GDF9 and BMP4 gene locus, which can be utilized for further study polymorphism and association of these polymorphic variants with traits of economic traits in future.

Keywords: BMP4, GDF9, Goat, PCR, Polymorphism, SSCP



Introduction

Goat occupies a special niche in the Indian agricultural production system as it is a multi-purpose animal producing meat, milk, skin and hair; can utilize poor quality grass and crop residues and commonly regarded as the “poor man’s cow”. In addition to wholesome and nutritious milk-based products, dairy goats provide sustainable livelihoods, especially in limited resource areas and enable smallholders to accumulate assets. Being so beneficial, genetic improvement in goats is needed, therefore detection of genetic diversity and the use of this diversity in marker-assisted selection (MAS) is the basis of present work. The studies on genetic diversity of candidate genes allow the genetic characterization of breeds and documentation of correlations between genotypic variations and related phenotypic traits of economic importance. Growth and differentiation factor 9 (GDF9) and Bone Morphogenetic protein 4 (BMP4) genes are potential candidate genes in goats which affects various production and reproductive traits. Growth and differentiation factor 9 gene (GDF9), located on chromosome 5 in goat (Nicol *et al.*, 2009), is one of fecundity genes which have been shown to affect prolificacy in goat (Ran *et al.*, 2009). GDF9 gene was first identified as an oocyte-derived growth factor required for ovarian somatic cell function (Dong *et al.*, 1996), belongs to transforming growth factor- β (TGF- β) superfamily. This oocyte-secreted paracrine factor plays a critical role during folliculogenesis (Juengel *et al.*, 2004). BMP4 gene is known to have significant effect in growth and reproduction (Sharma *et al.*, 2013). India is having 34 well recognized and a number of an unexplored goat breeds of local areas. As it is difficult to propagate high quality domestic breeds of goats over a short period by the traditional breeding and genetics methods, the modern breeders recently focusing on using DNA markers for developing breeds through marker-assisted selection (MAS). The candidate gene approach, employed in identifying the polymorphisms in genes likely to cause phenotypic variation based on physiological and biochemical evidence, could accelerate the improvement of goat growth and reproductive traits. Thus, it is important to first explore the genetic diversity in the form of polymorphisms in candidate genes.

In the present paper, the genetic diversity of two candidate genes (GDF9 and BMP4) was detected by Single Strand Conformational Polymorphism (SSCP). SSCP is a highly cost effective, simple and rapid method to detect variations in DNA. The SSCP protocol is capable of producing different banding patterns on polyacrylamide gels in the presence of single nucleotide variations in the target (Sheffield *et al.*, 1993) allowing rapid identification of genetic diversity in organisms.

Polymorphism in BMP4 gene had been reported in Jining Grey goat, Boer, Angora and Inner Mongolia Cashmere goats, in Barbari, Beetal, Black Bengal, Malabari, Jakhrana, Osmanabadi, Sangamneri, Sirohi and Ganjam and in Barki lambs (Chu *et al.*, 2011; Sharma *et al.*, 2013; Ibrahim, 2018) respectively. Polymorphic study on GDF9 gene was conducted in seven breeds of Indian goats (Ahlawat *et al.*, 2016) and in Markhoz goats (Niazi *et al.*, 2018; Shokrollahi *et al.*, 2018)

Materials and Methods

Sample Collection

Blood sample from 90 female goats representing three breeds i.e., Barbari, Sirohi and Black Bengal collected aseptically from jugular vein in sterile EDTA coated vacutainer tubes (Vacutte, Greiner bio-one GmbH, Austria). Goats were selected randomly from goat breeding farm of Nanaji Deshmukh Veterinary Science University. Genomic DNA was extracted from blood samples manually by method described by John *et al.* (1999) with slight modification. Extracted DNA was quantified using nanodrop spectrophotometer (ND 1000) whereas quality check of DNA samples was done by running on 0.8% agarose gel electrophoresis. Polymerase Chain Reaction was used to amplify gene fragments of two candidate genes (GDF9 and BMP4) of the *Capra hircus* genome using published primers by Hadizadeh *et al.* (2014) and Ariyaratne *et al.* (2016) respectively. The details of the gene fragments, primer pairs used and respective annealing temperatures are given in the Table 1. PCR reactions were performed in a 25 μ l reaction mixture containing 100- 120 ng genomic DNA, 1.0 μ M of forward and reverse primer each, 12.5 μ l of PCR master mix (Promega) and nuclease free water for each gene. Amplified PCR products were visualized on 2% agarose gel stained with ethidium bromide (1 μ g/ ml) and size of the amplified PCR product was judged by using a 100 bp ladder.

Table 1: Details of gene fragments amplified, primer sequences and the respective annealing temperatures used in PCR reactions

Gene	Gene Region	Primer sequence	Fragment size (bp)	Annealing Temperature (°C)
GnRHR	Exon2	F- ATCCCACCCTGACGTTTAAGGC R- TCCTCCCAAAGGCATAGACAGG	1069	62
BMP4	Intron 2	F-CTGGGGAAATGTTTGGTA R-GCTAAGAGTTGGGTGATGAG	380	57

Single Strand Conformational Polymorphism (SSCP) Analysis

A sample (5 µl) of each PCR product was mixed with 15 µl of denaturation buffer (10 mM/L EDTA, pH 8, 80 % m/V N, N-dimethyl formamide, 1mg/ml Bromophenol blue, 1mg/ml Xylene Cyanol) in PCR tubes, denatured at 95 °C in water bath for 5 minutes, snap chilled on ice for 15 minutes and loaded on polyacrylamide gels (6%). The electrophoresis was carried out in a vertical unit (130 V, 5 W, 6 mA), in 1x TBE buffer at 4°C for 7 hours. Gel was then stained by silver staining to visualize the bands. The gel was visualised manually for band patterns and scored manually to obtain PCR-SSCP genotype frequency.

Statistical Analysis

Various population genetics parameters like genotypic frequency, allelic frequency, observed homozygosity, expected heterozygosity, Polymorphism information content (PIC) value and Chi square test for HWE at GDF9 and BMP4 in Barbari, Sirohi and Black Bengal goat populations were analysed using software POPGENE V1.32 (Yeh *et al.*, 1999) and online software to explore the genetic diversity in three goat population.

Results and Discussion

The gene fragments of GDF9 and BMP4 gene were amplified with the specific primers as expected. The PCR product of GDF9 and BMP4 gene were found to be approx. of 1069 bp (Fig. 1a) and 380bp (Fig. 2a), size respectively.



Figure 1a: Amplified PCR product of GDF9 gene in Barbari goats on 2% agarose. (M-100 bp ladder; Lanes - 1-10 GDF9 PCR products)

The GDF9 and BMP4 gene fragments were observed polymorphic, GDF9 gene showed three SSCP patterns (Fig. 1b) whereas BMP4 gene fragment (Fig. 2b) showed only two SSCP patterns which are designated as AA, AB and BB and AA and BB genotypes respectively. The distribution of genotypes, alleles, Chi Square values, observed Heterozygosity (H_o), Expected heterozygosity (H_e), Effective allele number (N_e) and Polymorphism information content (PIC) among two genes are presented in Table 2.

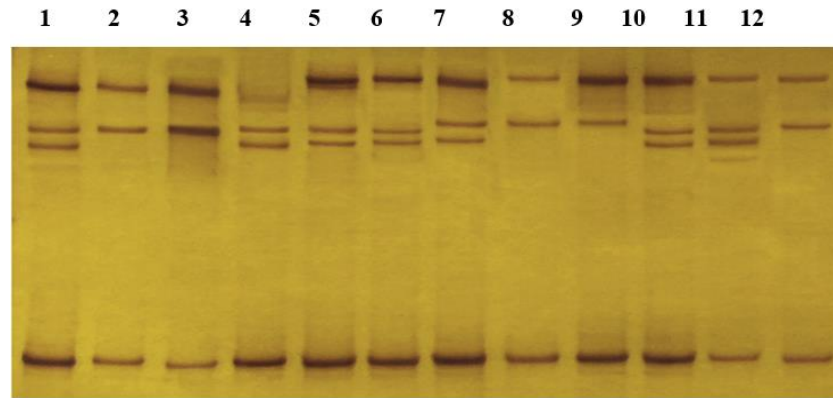


Figure 1b: GDF9 gene SSCP Patterns in Barbari goats. (Lane - 1, 5-6, 7, 10-11 AA Genotype; Lane- 2-3, 8-9, 12 BB Genotype; Lane - 4 BB Genotype)

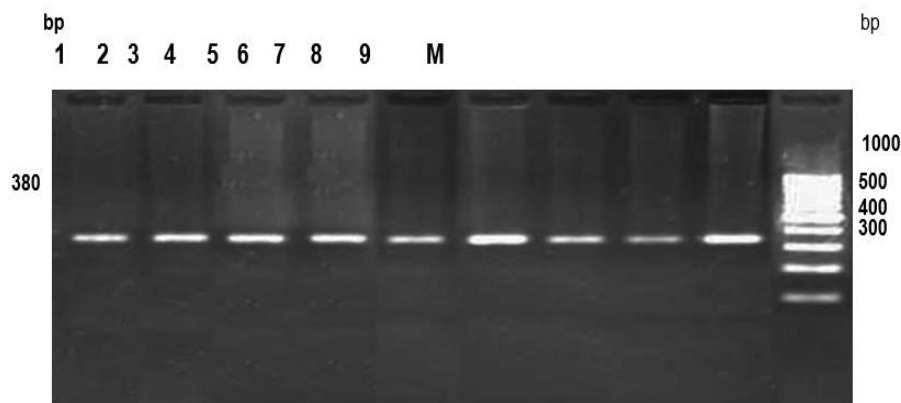


Figure 2a: Amplified PCR product of BMP4 gene in Sirohi goats on 2% agarose. (M -100 bp ladder; Lanes - 1-10 BMP4 gene PCR products)

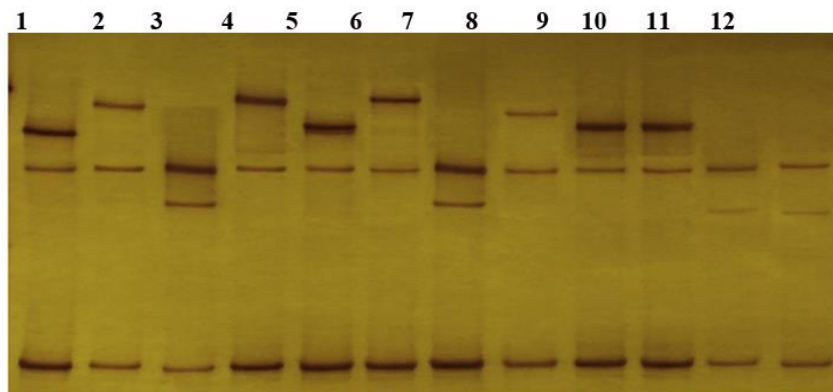


Figure 2(b): BMP4 gene SSCP Patterns. (Lane - 1-2, 4-6, 8-10 AA Genotype; Lane - 3, 7, 11-12 BB Genotype)

At GDF9 gene, AA genotype was predominant in Sirohi (60%) and Barbari (43%) while BB in Black Bengal (37%) (Table 2). Similar to findings of present work polymorphism in the GDF9 locus was also reported in Beetal goats (Hadizadeh *et al.*, 2014) and in Markhoz goats (Shakrollahi and Moarammazi 2018, Niazi *et al.*, 2018, Ghoreishi *et al.*, 2019) while this locus was observed monomorphic in Black Bengal goats (Polley *et al.*, 2009) and in Assam hill goats (Dutta *et al.*, 2013). The present study substantiates the polymorphic nature of GDF9 locus in goat. The polymorphic nature of BMP4 by PCR-SSCP as reported in this study is in agreement with the results reported by Fang *et al.* (2010) in Xuhuai White goat, Boer goat and Haimen goats, Sharma *et al.* (2013) in nine different Indian breeds goat, Chu *et al.* (2011) in Jinning Grey goats and Ariyaratne *et al.* (2016) in Sri Lankan non-descript, crossbreds and Jamunapari goats. However, Sarma *et al.* (2019) reported GDF9 gene locus to be monomorphic in Assam hill goats.

Table 2: Genotypic, allelic frequencies and population genetic indices for GDF9 and BMP4 gene fragments

Gene	Breed	Number of Samples	Genotypic Frequency			Allelic Frequency		χ^2	Ho	He	Ne	PIC
			AA	AB	BB	A	B					
GDF9	Barbari	30	0.43	0.37	0.2	0.62	0.38	1.76 ^{NS}	0.4	0.33	1.89	0.36
	Sirohi	30	0.6	-	0.4	0.6	0.4	31.13 ^{**}	0.37	0.48	1.47	0.26
	Black Bengal	30	0.3	0.33	0.37	0.47	0.53	3.62 ^{NS}	0.33	0.51	1.99	0.37
BMP4	Barbari	30	0.47	-	0.53	0.47	0.53	31.04 ^{**}	0	0.45	1.8	0.37
	Sirohi	30	0.67	-	0.33	0.67	0.33	31.31 ^{**}	0	0.51	1.99	0.34
	Black Bengal	30	0.5	-	0.5	0.5	0.5	31.03 ^{**}	0	0.51	2	0.38
	Black Bengal	30	0.5	-	0.5	0.5	0.5	31.03 ^{**}	0	0.51	2	0.38

^{**} Significant ($p \leq 0.01$), NS- Non-Significant ; χ^2 value, Ho- observed Heterozygosity, He- Expected heterozygosity, Ne- Effective allele number and PIC- Polymorphism information content.

The significant Chi-square value in Sirohi indicates that the populations were not in Hardy Weinberg equilibrium where as non-significant Chi-square value in Black Bengal and Barbari shows that the population was in Hardy Weinberg equilibrium at GDF9 gene locus. The observed population disequilibrium is in accordance to the findings in Chinese goat breeds and Markhoz goats (Zhu *et al.*, 2013; Shakrollahi and Moarammazi, 2018). For BMP4 gene, AA genotype was predominant in Sirohi (67%), BB in Barbari (53%), while both genotypes were found equal in frequency in Black Bengal. The significant Chi-square value indicated HW disequilibrium in Sirohi, Barbari and Black Bengal goats at BMP4 gene locus. Similar findings were also reported by Ariyaratne *et al.* (2016) in Sri Lankan non-descript, crossbreds and Jamunapari goats. However, Hardy-Weinberg equilibrium was reported at this locus in Xuhuai White goat, Boer goat, Haimen goats and Jinning Xuhuai White goat, Boer goat and Haimen goats and Jinning Grey goats (Fang *et al.*, 2010; Chu *et al.*, 2011). Hardy Weinberg disequilibrium indicates that the locus is under selection pressure or may be effect of migration, mutation or genetic drift in small population had deviated the genotypic frequency from equilibrium. The observed heterozygosity values are lower than expected heterozygosity values in all the three breeds at both GDF9 and BMP4 gene locus examined except for GDF9 IN Barbari goats (Table 2). Ariyaratne *et al.*, 2016 in Jamnapari and non-descript goats of Sri Lanka reported higher observed heterozygosity than expected heterozygosity. The gene diversity (GD) of a locus, also known as its expected heterozygosity (*He*), is a fundamental measure of genetic diversity in a population, and describes the expected proportion of heterozygous genotypes under Hardy- Weinberg equilibrium (Nei, 1973).

According to the classification of PIC, PIC value < 0.25 is considered as low polymorphism, PIC value between $0.25 - 0.5$ as intermediate polymorphism and PIC value > 0.5 as high polymorphism (Ma *et al* 2010). However, as described by the PIC criterion all analysed breeds in this study possessed intermediate polymorphism for all the three-goat population at GDF9 and BMP4 gene locus. Similarly, Ariyaratne *et al* 2016 observed intermediate diversity as per the PIC value in Srilankan goats at BMP4 gene locus. Higher effective allele number (Ne) indicates higher polymorphism (Lan *et al* 2007) and for all the genes taken in three breeds of goat it is approx. 2 showing all the three-goat population at GDF9 and BMP4 gene loci were equally diverse (Table 2). Nanekarani *et al.*, 2016 at GDF9 gene observed lower effective allele number in Lori sheep breeds whereas and Ariyaratne *et al.*, 2016 at BMP4 gene observed higher effective allele number in crossbred goats whereas lower effective allele number in non-descript and Jamunapari goats.

Here SSCP is used for genotyping of goat population to study genetic diversity among goats, SSCP is a reliable, reproducible and cost-effective method as compared to DNA sequencing for detecting structural variations in genome due to point mutations (Sheffield *et al.*, 1993).

Conclusion

GDF9 and BMP4 gene regions analysed are polymorphic in the sample of goats used in this study. PIC criterion revealed an intermediate polymorphism for all analysed phenotypic groups at all the gene loci taken in the study.

The effective allele number value is also showing diverse nature of these gene loci. This study provides evidence for genetic polymorphism of GDF9 and BMP4 gene in three Indian goats breeds, which can be utilized in future to develop single nucleotide polymorphism markers to be used in association studies and marker assisted selection. This will be very useful for improvement of production potential of Indian goat breeds which in turn will be a step towards the upliftment of rural economy.

Conflict of Interests

There is no conflict of interest.

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References

- Ahluwat, S., Sharma, R., Roy, M., Mandakmale, S., Prakash, V., and Tantia, M.S. (2016). Genotyping of novel SNPs in BMP1B, BMP15, and GDF9 genes for association with prolificacy in seven Indian goat breeds. *Animal Biotechnology*, 27(3):199-207.
- Ariyaratne, H.B.P.C., Lokugalappatti, L.G.S., Ariyaratne, H.B.S. and Munasinghe D.M.S. (2016). Detection of genetic diversity in four candidate genes by Polymerase chain reaction-single strand conformation Polymorphism (PCR-SSCP) analysis of goats in Sri Lanka. *International Journal of Applied Science and Biotechnology*, 4(1), 133-138.
- Chu, M.X., Lu, L., Feng, T., Di, R., Cao, G.L., Wang, P.Q., Fang, L., Ma, Y.H. and Li, K. (2011). Polymorphism of bone morphogenetic protein 4 gene and its relationship with litter size of Jining Grey goats. *Molecular Biology Reports*, 38(7), 4315-4320
- Dong, J., Albertini, D.F., Nishimori, K., Kumar, T.R., Lu, N. and Matzuk, M.M. (1996). Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature*, 383: 531-535.
- Dutta, R., Das B., Laskar, S., Kalita, D.J., Borah, P., Zaman, G. and Saikia, D.P. (2013). Polymorphism, sequencing and phylogenetic characterization of growth differentiation factor 9 (*GDF9*) gene in Assam Hill goat. *African Journal of Biotechnology*, 12(50): 6894-6900.
- Fang, X., Xu, H., Zhang, C., Chen, H., Hu, X., Gao, X., Gu, C. and Yue, W. (2010). Polymorphism in BMP4 gene and its association with growth traits in goat. *Molecular Biology Reports*, 36: 1339-1344.
- Ghoreishi, H., Fathi-Yosefabad, S., Shayegh, J. and Barzegari, A. (2019). Identification of mutations in BMP15 and GDF9 genes associated with prolificacy of Markhoz goats. *Archives of Animal Breeding*, 62: 565–570.
- Hadizadeh, M., Mohammadbadi, M. R., Niazi, A., Esmailzadeh, A. and Gazooei, Y. M. (2014). Search for polymorphism in growth and differentiation factor 9 (GDF9) gene in prolific Beetal and Tali goats (*Capra hircus*). *Journal of Biodiversity and Environmental Sciences*, 4(4): 186-191.
- Ibrahim, A. H. M. (2019): Association of growth performance and body conformational traits with BMP4 gene variation in Barkilamb. *Growth Factors*, 87(2):86-97.
- John, S.W., Weitzner, G., Rozen, R. and Scriver, C.R. (1991). A rapid procedure for extracting genomic DNA from leukocytes. *Nucleic Acids Research*, 19(2): 408.
- Juengel, J.L., Bodensteiner, K.J. and Heath, D.A. (2004). Physiology of GDF9 and BMP15 signalling molecules. *Animal Reproduction Science*, 83: 447–460.
- Lan, X.Y., Pan, C.Y., Chen, H. and Zhang, C.L. (2007). An *AluI* PCR-RFLP detecting a silent allele at the goat POU1F1 locus and its association with production traits. *Small Ruminant Research*, 73: 8-12.
- Ma, R.N., Deng, C.J., Zhang, X.M., Yue, X.P., Lan, X., Chen, H. and Lei, C.Z. (2010) A novel SNP of α -Lactalbumin gene in Chinese dairy goats. *Molecular Biology*, 44 (4):608-612.
- Nanekarani, S., Goodarzi, M., Khederzadeh, S., Torabi, S., and Landy, N. (2016). Detection of polymorphism in booroola gene and growth differentiation factor 9 in Lori sheep breed. *Tropical Journal of Pharmaceutical Research*, 15(8): 1605-1611
- Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of National Academia of Science. U.S.A.*, 70: 3321–3323.
- Niazi, A., Zamiri, M.J., Javanmard, A., Mehdizadeh, Y. and Arefnejad, B. (2018). Novel single nucleotide polymorphisms (SNPs) in two oogenesis specific genes and their association with litter size in markhoz goat

- (Iranian Angora). *Iranian Journal of Applied Animal Science*,12:91-99.
17. Nicol, L., Bishop, S.C., Pong-Wong, R., Bendixen, C., Holm, L., Rhind, S.M. and McNeilly, A.S. (2009). Homozygosity for a single base-pair mutation in the oocytespecific GDF9 gene results in sterility in Thoka sheep. *Reproduction*,138:921-933
 18. Polley, S., De, S., Batabyal, S., Kaushik, R., Yadav, P., Arora, J. S. and Datta, T. K. (2009). Polymorphism of fecundity genes (BMP1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. *Small Ruminant Research*, 85(2): 122–129.
 19. Ran, X.Q., Lin J.B., Du, Z.Y., Qing, C. and 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.
 20. Sarma, L., Nahardeka, N., Zaman, G., Aziz, A., Das,A., Akhtar, F., Upadhyay, S. and Borkolita, L. (2019). Analysis of BMP4 gene HaeIII polymorphism in Assam Hill goat. *Journal of Entomology and Zoology Studies*, 7(2): 34-37.
 21. Shokrollahi, B. and Morammazi, S.(2018).Polymorphism of GDF9 and BMP1B genes and their association with litter size in Markhoz goats. *Reproduction in Domestic Animals*,55(4): 971-978.
 22. Sharma, R., Ahlawat, S., Maitra, A., Roy, M., Mandakmale, S. and Tantia, M.S. (2013) Polymorphism of BMP4 gene in Indian goat breeds differing in prolificacy. *Gene*, 532(1):83-89.
 23. Sheffield, V., Beck, J., Kwitek, A., Sandstrom, D. and Stone, E. (1993). The sensitivity of single strand conformation polymorphism analysis for the detection of single base substitutions. *Genomics*,16: 325-332.
 24. Yeh, F.C., Yeng, R., Boyle Timothy, T.J., Ye, Z. H. and Mao Judy, X. (1999). POPGENE 32 version 1.32, the user-friendly software for population genetic analysis. Molecular biology and biotechnology center. University of Alberta, Canada. < <http://www.ualberta.ca/~fyeh/fyeh>>.
 25. Zhu, G. Q., Wang, Q. I., Kang, Y. G., Lv, Y. Z., and Cao, B. Y. (2013). Polymorphisms in GDF9 gene and its relationship with litter size in five Breeds of Black Goats. *Iranian Journal of Applied Animal Science*, 3(3): 625–628.
