

## Short Communication

# Analysis of FecG gene in lohi sheep breed using PCR-RFLP technique

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### Abstract

The growth and follicles differentiation in ovary is controlled by the FecG gene of TGF- $\beta$  superfamily proteins. FecG (GDF9), FecB (BMP1B) and FecX (BMP15) are three oocyte-derived genes of TGF- $\beta$  superfamily, which have essential role in ovulation and follicular growth. This study was intended to identify the FecG mutation in 30 ewes of Lohi sheep. A fragment length of 139 bp of FecG gene at exon 2 was amplified with a forced PCR-RFLP primer pairs and products were digested with *Ddel* enzyme. The results revealed that there is no mutation at this locus, because *Ddel* enzyme could not recognize restriction site. In this study, we found wild type (++) alleles. The polymorphism analysis of Lohi sheep indicates that the reported mutation for litter size is not present in this breed. Therefore, it is important to identify other SNPs for FecG gene in Lohi sheep.

**Keywords:** Allele; FecG; Lohi sheep; Mutation; Polymorphism

### Introduction

Growth differentiation factor 9 (FecG) is a fecundity gene and belongs to transforming growth factor (TGF- $\beta$ ) family. FecG gene is mapped on Chromosome-5 in sheep (*Ovis aries*) and spans 2.5 kb and contains two exons separated by single intron (1126 bp), encodes 453 amino acid residues of a prepropeptide [1]. FecG mutation increase fertility and ultimately effects on ovulation rate in sheep. FecG gene expresses from follicular development until ovulation in

sheep [2]. However, fertility trait in small ruminants has important contribution in breeding program. Major fecundity genes including FecG have significant role in reproductive performance of sheep around the world [3]. FecG is an active member of TGF- $\beta$  superfamily and identified as fertility regulators in different species. FecG mutation in mice is important for follicular development and involve in normal cumulus cells development [2]. However, Davis et al. [4] described that

FecGH mutation may involve in follicular luteinization at ovulation. In sheep FecG protein and mRNA present in germ cells during formation of follicles and in primordial follicles of oocytes at all follicular growth stages. Modern animal breeding practices based on the DNA technologies are routinely used in livestock industry with success in developed countries [5]. Pakistan is blessed with 28 diverse sheep breeds across the country. Lohi is an important dual purpose (mutton and wool) sheep breed of Punjab [6]. There are some reported studies that investigate fecundity genes in goat breeds in Pakistan, but lacking any information about sheep breeds [7]. The use of molecular markers of FecG gene at early age of lambs will enhance the breeding efficiency in sheep industry. Thus, the present study was conducted to scan FecG gene polymorphism in Lohi sheep using PCR-RFLP technique.

### Materials and methods

#### Animal's sampling and DNA extraction

Thirty Lohi sheep breed ewe's samples were collected from livestock production research institute, Bahadurnager, Okara, Punjab. 10 ml venous jugular blood samples were collected in 50 ml falcon

tubes contains EDTA (0.5 M). DNA extraction was carried out by method described at Mustafa et al. [8]. The quality and quantity of the genomic DNA was assessed by agarose gel-electrophoresis and Nano Drop (ND1000-Spectrophotometer) apparatus.

A 139 bp region of FecG gene was amplified using forward (*Ddel*F: 5' ATGGATGATGTTCTGCACCATGGTGTGAACCTG-3') and reverse (*Ddel*R: 5'CTTTAGTCAGCTGAAGTGGGACAA C-3') primers (Table 1) [1]. The PCR reaction was carried out in a 25 µl reaction mixture consisting 3 µl genomic DNA (50 ng/ µl), 2.5 µl of 10x buffer (50 mM KCl, 10 mM Tris HCL (pH 8.0), 0.1 % Triton X-100), 3.0 mM MgCl<sub>2</sub>, 200 µM each dNTPs, 2 µM of each primer and 0.2 µl Taq polymerase (5 U/ µl). The amplification reaction conditions were carried out using the following steps described in a study of Debnath and Singh [9]. 15 µl of PCR-RFLP product reaction mixture was used for digestion along with 2µl of 10x buffer at 37 °C for overnight with 1 µl of *Ddel* restriction enzyme (10 µg/ µl). Digested - products were resolved in a 3.5% agarose gel stained with ethidium bromide and visualized under UV light.

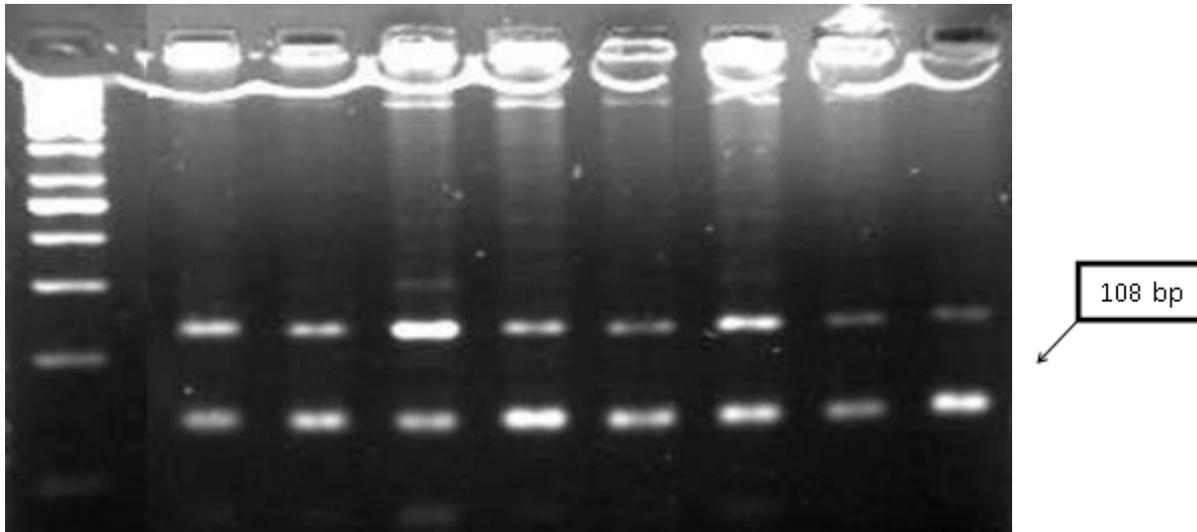
**Table 1. Primer pairs properties for FecG gene**

Primer	Sequence `5-3`	T <sub>m</sub> (°C)	length	GC %	Size	Ref
<i>Ddel</i> F	ATGGATGATGTTCTGCACCATGGTGTGAACCTG	62	33	52.6	139	[1]
<i>Ddel</i> R	CTTTAGTCAGCTGAAGTGGGACAAC	62	25	52.6		

### Results

A total of 30 ewe's samples from Lohi sheep breed were genotyped with the forced PCR-RFLP. The PCR-RFLP electrophoretic analysis of FecG gene is shown in (Figure

1). The PCR-RFLP results show that there is no polymorphic site exists in the exon 2 of FecG- gene in the selected samples of Lohi ewes and had wild type (++) genotype (Table 2).



**Figure 1. PCR-RFLP of FecG gene using *Ddel* restriction enzyme with 100 bp DNA ladder**

**Table 2. Gene and genotypic frequencies of FecG in Lohi sheep**

Gene frequency		Genotype frequency		
+	G	++	G+	GG
1	0	1	0	0

### Discussion

The PCR-RFLP is simple and rapid technique for SNPs genotyping and widely used for genotype screening in some prolific sheep [10]. In this study, we used PCR-RFLP method to identify the FecG mutation in Lohi sheep as defined by Hanrahan et al. [1]. However, we have used agarose gel in place of polyacrylamide gel. It is evident that RFLP results has a good stability and repeatability, but several factors such as enzyme quality and quantity manufactured by different companies, digestion time, gel concentration and PCR product quality might affects the results [8]. We used several concentrations of ingredients to maintain the optimal reaction conditions to produce repeatable and veracity of reaction. The results of used PCR products and gel electrophoresis revealed that these strategies are useful for the identification of FecG mutation in a sheep breed (Figure 1). The FecG gene could be considered as a candidate gene for litter size in sheep [3, 10]. However, Lohi sheep is considered as

prolific breed among other sheep breeds in Pakistan with 35 % twinning rate in many flocks [6]. The physiological characteristics of FecG mutation inactivation in sheep have not well studied [9]. Hanrahan et al. [1] reported that single FecGH mutation copy is responsible for increased ovulation rate in ewes, while homozygous copy of this mutation caused infertility. The heterozygous effect of FecG mutation on ovulation rate is additive in ewe and enhanced fertility [2]. The major gene of fecundity trait in Inverdale sheep was X-linked and homozygous carriers were sterile due to ovarian hypoplasia, an ovarian follicles failure to progress beyond the follicle development at primary stage [1].

### Conclusion

The present study results in Lohi sheep showed that same band pattern in all ewe's samples, inferring no mutation site in exon 2 of FecG locus. However, twinning rate in Lohi sheep breed is not controlled by genetic factor associated to FecG mutation, which already testified in some sheep

breeds. It may be concluded that twinning rate in Lohi sheep is controlled by other SNPs in FecG gene. Therefore, additional detail FecB genes characterization is recommended to confirm this hypothesis in Lohi sheep breed.

#### Authors' contributions

Conceived and designed the experiments: H Mustafa, K Javed & M Zeeshan, Performed the experiments: H Mustafa, A Ali & ZH Kuthu, Analyzed the data: H Mustafa, WA Khan & NU Ain, Contributed materials/analysis/ tools: H Mustafa & K Javed, Wrote the paper: H Mustafa, ZH Kuthu & A Ajmal.

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