

EFFECT OF POLYMORPHISM G(129)R IN GROWTH DIFFERENTIATION FACTOR 9 GENE (GDF9) ON AWASSI EWES THAT BREED OUT OF SEASON

Ali Aziz Abid¹, Laith Sofian Younis^{2*} and Saad Tawfiq Rasheed³

¹Department of Theriogenology, University of Tikrit, Wasit, Iraq.

²Department of Theriogenology, University of Falluja, Baghdad, Iraq.

³Department of Veterinary Assisted Reproductive Technology, Tikrit University, Tikrit, Iraq.

e-mail : laith.vet89@tu.edu.iq, ali.aziz81@gmail.com, drsaadrasheed@tu.edu.iq

(Received 23 April 2019, Revised 22 June 2019, Accepted 29 June 2019)

ABSTRACT : The present study was performed to test the linkage between GDF9 mutation (G(129)R) and seasonality in Awassi sheep. Thirty mature non-pregnant ewes were used in this study in the period between September/ 2018 to January/2019. Fifteen ewes were lambd at September and November/2018 and this was considered as the Seasonal group, and the Non-Seasonal group consisted of 15 ewes which were lambd in mid-December and January/2019. A double primers were utilized to magnificate the 1st exon of GDF9 gene by a polymerase chain reaction (PCR) after the DNA extraction was done from blood specimens. The single nucleotide polymorphism (SNP) were detected through sequencing (by matching with the GDF9 gene sheep sequencing in NCBI data bases. The results revealed one single nucleotide polymorphism G(129)R in chromosome 5 of GDF9 gene (exon I) when compared with Sequence ID: FJ429111.1, without resulting in an amino acid. Two genotypes (GG and GA) were showed, and high significant differences ($P < 0.001$) were find between the genotypic frequencies of G(129)R locus. The results showed that the mutant heterozygote genotype (GA) recorded a higher significant rise ($P < 0.001$) in non-seasonal ewes (60%) as compared with the wild homozygote (GG) genotype (40%). In contrast, the genotype frequency (GG) recorded a higher significantly increment ($P < 0.001$) in seasonal ewes (63%) in comparison with (GA) genotype (37%). As a conclusion, exon I GDF9 gene polymorphism has an effect on breeding out of season in Awassi breed and the mutant genotype find mostly in out of season Awsassi ewes.

Key words : GDF9, genotypes, polymorphism, seasonality.

INTRODUCTION

The Bone morpho-genetic,protein (BMP) and GDF9 are belong to the transforming, growth factors (β family) (Elvin *et al*, 2000). BMP, 15 and GDF9 modulate other, hormones and factors, to control follicular, growth and apoptotic, signaling (McNatty *et al*, 2005; Demars *et al*, 2013). In addition, the GDF9 activate, follicular development, granulosa, cells proliferation, and follicular, luteinization and they keep up typical, follicular condition in ovine ovary (Juengel *et al*, 2002). The GDF9 expression, in oocytes, happens earlier (from initial phase until ovulation) throughout, folliculogenesis (Hanrahan *et al*, 2004); therefore, GDF9 acts as an intraovarian regulator for an early antral follicle transition from preantral follicle (Kobayashi *et al*, 2009).

As well as the function of these factors (BMP15 and GDF9) in cumulus cell expansibility, maturation of oocyte and final ovulation (Yoshino *et al*, 2006), GDF9 controls the early folliculogenesis by effect on many cellular mechanisms like prostaglandin, F2 alpha and

estrogen (E2) receptor expansion, it also enhances progesterone and E2 synthesis by preovulatory follicle particularly in granulosa cells (Silva *et al*, 2005).

The GDF9 protect follicular structure and conserve growth of follicles. According to Hayashi *et al* (1999); using of GDF9 with follicle stimulating hormone or alone had a positive effect on mice preantral follicles growth. As well, the daily growth of goat preantral follicular diameter is related with GDF9 treatment (Almeida *et al*, 2011).

The *Ovine* GDF9 gene is an autosomal, which codes the mRNA GDF9 that emerge from the oocyte, its fundamental for maintenance the folliculogenesis and process of ovulation (Juengel *et al*, 2004; Sadighi *et al*, 2002). The chromosome five which contain the GDF9 gene (Sadighi *et al*, 2002). The size of whole GDF9 gene about 2500 base pairs (bp) and has two exons, and a single intron that interposes between the two exons; exon I size is 397 bp (encodes 134 amino acids), in addition, the exon II lasts about 968 bp (encodes 135-456 amino

*Corresponding author : e-mail: laith.vet89@gmail.com, ORCID ID : <https://orcid.org/0000-0001-7686-6167>