Association between GDF9, FecB and Prolactin gene polymorphisms and prolificacy of Awassi sheep

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ABSTARCT: Prolificacy data of 208 ewes and 27 rams were used to investigate the effect of growth differentiation factor, fecundity and prolactin genes on prolificacy. Genotyping process was achieved by PCR-RFLP technique. Genotypic frequencies of growth differentiation factor 9 gene were 0.889, 0.12, and 0.00 for MM genotype, MN genotype, and NN genotype, respectively. The genotypic frequencies of prolactin gene were 0.646 for AA, 0.221 to AB and 0.131 for BB genotypes. Fecundity gene was monomorphic. Prolificacy records were adjusted for the effect of parity, season and dam weight at lambing. Mixed model procedure was used for the association analysis by including the sires as random. Prolificacy only differed ($P<0.05$) between prolactin gene variants, BB genotype had 0.16 and 0.11 more prolificacy than AA and AB, respectively. The association results suggested the ability of using prolactin gene to assist the selection for improving Prolificacy.
Keywords: Sheep**;** Fecundity**;** Genetics

Introduction

In general, reproductive activity of Awassi ewes is considered low. Gootwine *et al*. (2008) investigated the prolificacy of improved Awassi sheep and found that lambs born/ lambing equals to 1.31. In Jordan, prolificacy was reported to be 1.16 lambs born/ lambing (Jawasreh *et al*., (2010) while in another study published later by Jawasreh *et al*. (2013) it was found to be 1.29 , 2.086 and 1.68 in Local, Afec and Improved Awassi sheep, respectively . Prolificacy is controlled by one or many genes such as : fecundity gene (FecB) (Baird & Campbell, 1998; McNatty & Henderson, 1987; Montgomery *et al*., 1992; Yan *et al*., 2005), growth differentiation factor (GDF9 gene) (Hanrahan *et al*., 2004; Moradband *et al*., 2011 ;Liandris *et al*., 2012), and prolactin gene (PRL) (Oul *et al*., 2007;Chu *et al*., 2008). The FecB gene was investigated in several studies (Davis *et al*., 2002; Yan *et al*., 2005; Guan *et al.,* 2006; Ghaffari *et al*., 2009; Asadpour *et al*., 2012) to be a major gene controlling prolificacy. In Jordan, a genetic selection program was started 10 years ago and as a result milk production was elevated from 0.5 to 1.2 kg (Jawasreh *et al.* 2010). Current study was conducted to investigate the possible polymorphisms of fat tailed Awassi sheep Prolactin, Fecundity and GDF9 genes and to investigate the relationship between genes polymorphisms and Awassi sheep prolificacy.

Materials and Methods

Experiment was conducted at Al-Khanasry Research Station, Jordan. Five ml of blood samples were collected from the jugular vein of 208 Awassi ewes and 27 Awassi rams. Blood samples were placed in ice box and then stored at 4°C until analysis. For optimization, of the genotyping process of FecB gene, two Afec Awassi ewes (known as heterozygous for FecB gene) blood samples were collected and their FecB genotypes were compared with all local Awassi FecB genotypes. DNA was extracted from the collected blood using E. Z. N. A Blood DNA kit (OMGA- Bio-Tek, Inc.).

Gene sequences, expected size and annealing temperatures are illustrated in Table 1. The PCR-RFLP method was carried out to detect the gene polymorphisms in the growth differentiation factor gene (GDF9), FecB gene, and prolactin gene (PRL). For the modified PCR-RFLP assay, PCR reactions were set up in a final volume of 20µl using 10µl nuclease free water, 2µl of genomic DNA (50ng), 2µl primer (forward), 2µl primer (reverse) and 4µl (5 U/µl) of HOT FIRE Pol® DNA Polymerase, then specific PCR protocols for each primer were applied. The annealing temperatures were 56°, 60 and 58 °C, respectively for PRL, FecB and GDF9. The PCR product of each of PRL, FecB and GDF9 genes were digested by *Hae III, AvaII and HhaI,* enzymes respectively.

Table 1 Primer sequences, annealing temperatures and PCR product size of the targeted genes conducted under this study

(Primers 5'→3')	Annealing	Product length
PRL-F: ACCTCTCCTCG-GAAATGTTCA PRL-R:GGGACACTGAAGGACCAGAA	56°C	1,209 bp
FecB-F: CCAGAGGACAA-TAGCAAAGCAAA FecB-R: CAA-GATGTTTTCATGCCTCATCAACACGGTC	60°C	190 bp
GDF9-F: GAAGACTGG-TATGGGGAAATG GDF-R:CCAATCTGCTCCTACACACCT	58°C	462 bp

Alleles and genotype frequencies were calculated manually according to Falconer & Meckay, (1996).

Data collection and genetic association analysis

A total number of 360 lambing records of208 Awassi ewes through different lambing seasons were retrieved from the station performance records including pedigree information's, date of mating, date of birth, season, parity, dam weight at lambing, and prolificacy. After detecting the genotypes of all genes for all individuals, the data was assigned with genes polymorphisms for analysis. PROC MIXED procedure of SAS, (2004) (version 9.1, 2000, SAS Inst. Inc., Cary, NC) was used to investigate the markers effect (FecB, PRL, and GDF9). The model included the effect of parity, dam weight at lambing (covariate) and the effect of season as fixed effects. The obtained genotypes were re-coded to numeric values (AA=1, AB=0 and BB= -1) for obtaining the marker effect, Sires were included in the model as Random effect. The least square means of the mixed model were obtained for comparison purposes.

Results and Discussion

The FecB gene was observed to be monomorphic; the wild type of this gene was detected in the Local Awassi sheep. Growth differentiation factor 9 gene (462 bp) was amplified by PCR at 58°C (as annealing temperature). The PCR-RFLP technique was applied using *HhaI* digestion enzyme to produce three bands of 52, 156, and 254 bp in case of homozygous animals for the M allele (MM), four bands of sizes of 52, 156, 254, and 410 bp in case of heterozygous genotype (MN) , while the homozygous case for the N allele (NN) 52 and 410 bp that reported by Moradband *et al*. (2011) in Baluchi breed; Liandris *et al*. (2012) in Chios breed; Hanrahan *et al*. (2004) in Belclare and Cambridge breeds, did not appeared in our Awassi sheep population but similar to Liandris *et al.* (2012) findings in Karagouniki.

Allelic frequencies of the studied population were 0.951 for M allele (mutant allele) and 0.049 for N allele and the genotypic frequencies were 0.889 for MM genotype and 0.123 for MN genotype (Table 2).. Prolactin gene (1200 bp) was amplified by PCR at 56°C, the A variants appeared with 540, 370, 147, and 152 bp amplified fragments while B variant displayed 517, 370, 147, and 152 bp and all three genotypes were detected in our population. These results were consistent with results obtained by Orford *et al*. (2010). The allelic frequencies were 0.757 for A allele and 0.243 for B allele, and the genotypic frequencies were 0.646 for AA, 0.221 for AB and 0.131 for BB (Table 1). Ramos *et al.* (2009) found that a value of 0.639 was recorded to the A allele and 0.361 value to the B allele in Serra da Estrela breed, 0.57 to A allele and 0.43 for B allele in white Merino breed and 0.722 to A allele and 0.278 for B allele in Black Merino.

Table 2 Allelic and genotypic frequencies of GDF9, PRL FecB and IGFBP genes

Genotypic frequencies			Allelic frequencies		No	Gene
NN (BB)	MN (AB)	MM (AA)	N (B)	M (A)		
0.0 (0)	0.123 (26)	0.889 (209)	0.049	0.951	235	GDF9
0.131 (31)	0.221 (52)	0.646 (152)	0.243	0.757	235	PRL
0.00	0.00	1.00	0.00	1.00	235	FecB

Genes Association

Non-significant differences were found between the different genotypes detected in GDF9 gene and Prolificacy (Table 3). Similarly, Liandris *et al.* (2012) reported non- significant association between the mutations in the GDF9 gene and prolificacy in Karagouniki breed. However, GDF9 gene was responsible for prolificacy in Baluchi breed (Moradband *et al.* 2011) and in Belclare and Cambridge breeds (Hanrahan *et al*. 2004).

Table 3 Least squares means ± standard error for prolificacy of different GDF9 and prolactin gene genotypes adjusted for different parities, seasons, and different dam weight at lambing (as covariate).

Locus/Genotype	No. of Records	Prolificacy* ± SEM
GDF9 locus		
MM	330	$1.20^{a} \pm 0.03$
MN	30	$1.28^{a} \pm 0.07$
Prolactin locus		
BB	47	$1.33^{a} \pm 0.06$
AB	70	$1.22^{ab} \pm 0.06$
AA	243	$1.17^{b} \pm 0.04$

*Within the same column, means with different superscripts (a and b) differ significantly at ($P < 0.05$).

Prolificacy seemed to be affected by Prolactin gene variants. The results of this study show that only BB genotype significantly ($P< 0.05$) differs from AA genotype and it gives more prolificacy of 0.16 lambs than AA genotype. The BB genotype was not significantly differs in Prolificacy from AB genotype, but it tends to be more than AB genotype by 0.11 lambs (Table 3). Chu *et al.* (2008) found that the AB genotype had 0.83 lambs more than those of AA genotype. Oul *et al*. (2007) found that the ewes with AA genotype had 0.39 or 0.98 more lambs than ewes with AB or BB genotypes, respectively, and ewes of AB genotype had 0.59 more lambs than those of BB genotype. Xiu-hua *et al.* (2007) reported that PRL gene had no significant effect on prolificacy of Jining Grey goats. Those results showed that the prolactin gene had high contribution in improving prolificacy of Awassi

sheep breed and it might have physiological effects on ovulation rate and the number and size of ovulatory follicles in the ovary (McNatty & Henderson, 1987; Montgomery *et al*., 1992; Baird &Campbell, 1998). Further study regards the effects of PRL gene in prolific Awassi sheep is needed to confirm this finding and this gene can be considered as a powerful tool for marker-assisted selection.

Conclusion

Prolactin locus found to has a significant effect on prolificacy. The BB genotype of Prolactin gene had the highest prolificacy in Awassi sheep.

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