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EXPLORING POLYMORPHISM AND EFFECTS OF THE IGFIR GENE ON PRODUCTIVE LIFE IN BARKI EWES

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In sheep production systems, 20% of ewes are replaced annually and this contributes about 21% of the total inputs in sheep production (Ktbl, 2009). If ewes have long productive life, the flock needs fewer replacement ewes and this reduces the cost of maintaining flock size and increases flock efficiency. If ewes leave the flock prematurely through death or culling, fewer ewe lambs can be sold and higher number of lambs needs to be reared or purchased for replacement (Walker and Young, 2009). The length of the ewe's productive life in the breeding flock is impacted by age and reproductive performance (FAO, 2008).

To date, the extent to which age of ewe is due to genetic is not known, however, reproductive traits are low to moderately heritable and have low repeatability across parities (Matos *et al*., 1997; Safari and Fogarty, 2003). Traditional phenotypic selection, based on reproductive records, is less effective. Marker assisted selection is one method to improve lowly heritable traits. The identification of molecular marker significantly associated with long age and high reproductive performance of ewe would allow breeders to select lambs at early ages-prior to the entry of the flock, and that would have the best opportunity for increasing the productive life of ewes.

Age and reproductive performance of many species were found to be affected by some biological processes that are reg-

ulated by hormones and hormone receptors. The insulin like growth factor I receptor (IGFIR) is a tyrosine-protein kinase receptor, constitutively expressed in reproductive tissues and organs and binds the insulin like growth factor I (IGFI) and the insulin like growth factor II (IGFII) which have important roles in both tissues maintenance and development processes (Martinelli *et al*., 2008), primordial neuroendocrine system which integrates information from environmental stressors (Gerisch *et al*., 2001) and activation of MAPK and PI3K pathways. These biological processes were found affecting cell proliferation, protein synthesis, skeletal mass, immune function, metabolic rate, cancer suppression and reproductive status (Courtney *et al*., 2010; Pearson *et al*., 2001; Cargnello and Roux, 2011). The IGFIR signaling pathway participates in the regulation of secreting both gonadotropin-releasing hormone (GnRH) and luteinizing hormone **(**LH**)** - (Daftary and Gore, 2005). Infusion of IGFIR antagonist induced these two hormones, partially decreased progression of puberty and inhibited sexual behavior (Quesada and Etgen, 2002; Etgen and Acosta-Martinez, 2003). It is suggested that these phenomena affect both age and reproductive performance of organisms.

Many studies proved that the variation in IGFIR gene was associated with the longevity in nematodes (Braekman and Van-fleteren, 2007), *C. elegans* (Kaletsky and Murphy, 2010), fruit flies (Paaby and Schmidt, 2009), vertebrates (Tatar *et al*., 2003), mice (Holzenberger *et al*., 2003; Liang *et al*., 2003) and human (Suh *et al*., 2008; Pawlikowsk *et al*., 2009). Also, this variation was associated with reproductive performance of sows (Terman, 2011) and superovulation performance and pregnancy rates after embryo transfer in cattle (Yang *et al*., 2013). In New Zealand sheep, Byun *et al*. (2008 & 2012) have reported 3 allelic polymorphisms in ovine IGFIR gene and proved an association for these polymorphisms on lifespan and fecundity of ewes.

In view of the important role of IGFIR on age and reproductive performance of many species, the IGFIR gene is considered as a strong functional candidate gene affecting these traits in sheep. The objectives of the present study were to identify the allelic and genotypic polymorphisms within a variable fragment in intron2-exon3 of the ovine IGFIR gene and to test the effect of these polymorphisms on age and reproductive traits in Barki ewes.

MATERIALS AND METHODS

Source of data and flock management

A total number of 95 Barki ewes, (4-10 years; from 8 sires), raised at Maryout Research Station, Desert Research Center, were used to carry out this study. Ewes were housed in semi open sheds; and mainly fed concentrate feed mixture (16% crude protein) plus berssem (*Trifolium alexantrinum*) during the period from October to May and berssem hay and wheat straw during the rest of year.

Usually, the breeding season starts in September for a period of 35 days. Ewes and rams are allowed to breed for the first time at about 18-19 months of age. All rams and ewes in the flock are weighted and then selected for mating according to their visual appraisal of general health and conditions as well as their individual performance and parent offspring. The selected rams and ewes are divided into mating groups depending on their pedigree to avoid inbreeding. Ewes are joined in pens with single rams in groups of 20-25 ewes. After the mating period, ewes are separated from rams and kept as one group until lambing. The lambing season usually starts in March. At birth, each lamb is tagged and weighted. Lambs suckle until weaning at about 90 days.

The reproductive traits calculated from the ewes' records were: the total number of lambs born per ewe (TNLB), the total weight of lambs born per ewe (TWLB), the total number of lambs weaned per ewe (TNLW), the total weight of lambs weaned per ewe (TWLW), the rearing ability of ewe (TNLW/TNLB), the lambing rate (lambing number/joining number) and the twining rate of ewe (TNLB/lambing number).

Polymerase chain reaction (PCR)

Blood samples of the phenotyped ewes were collected from the jugular vein using 5 ml heparinized tubes and the genomic DNA was extracted using DNA extraction kit (Promega).

Two specific primers were used to amplify a fragment of ovine IGFIR gene containing a part of intron 2 and exon 3. The sequence of these primers as described by Byun et al. (2008) were as follows: (F: 5`- CTCACACC CTGCCTGTC -3`) and (R: 5`- CACACTGACCTCTGGCTC- 3`). Polymerase chain reaction (PCR) amplification was performed in 20 µl reactions containing 50 ng of genomic DNA, 2 mM $MgCl_2$, 200 µM dNTP, 0.7 U of *Taq* DNA polymerase, 0.30 µM of each primer and 1 x reaction buffer. Reaction conditions were: one cycle of 94°C for 3 min; 35 cycles of 94°C for 30 sec, 62°C for 30 sec and 72°C for 30 sec; and a final extension for 10 min at 72°C.

Single strand conformational polymorphism (SSCP) analysis

Single strand conformational polymorphism (SSCP) analysis was performed using a Protean II xi cells electrophoresis apparatus (Bio-Rad, USA). Volumes of 2 µl of PCR products were mixed with 8 µl of denaturation dye (98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 10 mM EDTA. The mixtures were denatured at 105°C for 6 min, rapidly chilled on wet ice and then loaded on 16 × 18 cm; 12% acrylamide: bisacrylamide (37.5: 1; Bio-Rad, USA) gels with the addition of 3% glycerol. The electrophoresis was run in 0.5 x TBE buffer for 19 h at 300 V and 28°C. Gels were silver stained using the method of Sanguinette *et al*. (1994).

Statistical Analysis

Traits and IGFIR genotypes were statistically analyzed by least square analysis of variance using the General Linear Mixed Model (GLMM) procedure of SPSS software, version 19 (SPSS Science Inc., Chicago, IL, USA). Three different sets of modeling approaches were used to test the effect of IGFIR genotype on the studied traits.

The first set of GLMMs was used to assess the effect of IGFIR genotypes on age and reproductive traits, the second set of GLMMs was used to explore the effect of the presence/ absence of each IGFIR allele on age and reproductive traits and the third set of GLMMs was performed to test the effect of the number of IGFIR allele copies present on age and reproductive traits. IGFIR genotype was fitted as a fixed factor while sire was fitted as a random factor in each model. Age of ewe at first mating and weight of ewe at first mating were included in the model as covariates. Where significant, these were further explored using pairwise comparisons (Duncan test; $P \leq 0.05$).

The generalized statistical model that was used: $Y_{ijk} = \mu + G_i + S_j + \varepsilon_{ijk}$

Where Y_{ijk} = trait value, μ = general mean, G_i = *the* fixed effect of IGFIR genotype in the first set of GLMMs, the presence/ absence of each IGFIR allele in the second set of GLMMs or the number of IGFIR allele copies present in the third set of GLMMs, S_j is the random effect of sire and ε_{ijk} = the random error associated with each observation, assumed to be normally and independently distributed with zero mean and variance σ^2.

RESULTS AND DISCUSSION

The Allelic and genotypic variation in the intron2-exon3 region of ovine IGFIR gene that was examined using the SSCP analysis is shown in Fig. (1). Only three genotypes (coded as AA, AB and BB with frequencies of 0.05, 0.28 and 0.67, respectively) were observed. These genotypes were representing two alleles A and B with frequencies of 0.19 and 0.81, respectively. Our results are quite different from the results obtained by Byun *et al.* (2008) who detected 3 alleles (A, B and C with frequencies of 0.845, 0.053 and 0.093, respectively) and six SSCP genotypes (AA, AB, AC, BB, BC and CC with frequencies of 0.725, 0.094, 0.146, 0.002, 0.009 and 0.016, respectively). This comparison reflectes a high level of genetic differentiation between Barki and New Zealand breeds of sheep at intron2-exon3 region of the IGFIR gene.

The association between IGFIR genotypes and productive life traits are given in Table (1). The IGFIR genotype showed high significant effect ($P < 0.01$) on age of ewe and significant effect ($P < 0.05$) on both TNLB and TNLW. The results of least square means showed that ewes with the genotype AA have longer age, higher TNLB and higher TNLW than ewes with the genotypes AB and BB. No associations were found between the genotype and other traits.

The least square means of productive life traits for the absence and presence of the identified alleles are summarized in Table (2). The presence/absence of A or B allele had high significant effect ($P < 0.01$) on age of ewe and significant effect ($P < 0.05$) on lambing number, TNLB and TNLW. The presence of allele A and the absence of allele B was associated with longer age (0.89 year; $P < 0.01$), higher lambing number (0.70; $P < 0.05$), higher TNLB (0.86; $P < 0.05$) and higher TNLW (0.65; $P < 0.05$) of ewes. These results are in contrast to the results obtained by Byun *et al*. (2012) who reported that ewes carrying allele B tended to have longer age and higher fecundity than ewes carrying allele A.

Data included in Table (3) shows the effect of number of allele copy present on the productive life traits of Barki ewes. Also, the number of allele copy present proved significant effect on age, TNLB and TNLW. Age was considerably longer ($P < 0.01$) in ewes that had two copies of allele A in comparison with the other genotypes, as well as ewes with two copies of allele A had higher ($P < 0.05$) TNLB and TNLW.

According to our results, the variation in ovine IGFIR gene mainly affected age of ewes. This result is in agreement with the results of previous studies that concerned the effect of variation in human IGFIR gene on longevity. Suh *et al*. (2008) detected an over-representation of two nonsynonymous mutation in the offsprings of a cohort of Ashkenazi Jewish centerian. Two single nucleotide polymorphisms (SNPs) were detected by Barbieri *et al*. (2012) and found to be associated with all-cause mortality risk (e.g., metabolic rate, energy expenditure, respiratory quotient, oxiditive stress). Albani *et al*. (2011) identified another two SNPs affected the longevity of a sample of Italian people.

The extension of age and increasing lambing number of ewes that correlated with the variation in IGFIR gene might due to the crucial roles of IGFIR in mediating the biological functions of the IGFI and the IGFII that have major effects on oxidative stress resistance (Holzenberger *et al*., 2003; Thakur *et al*., 2013), cancer suppression (Reinmuth *et al*., 2002; Wang *et al*., 2005; Ji *et al*., 2007; Singh *et al*., 2014), carbohydrate and lipid metabolism (Cornu *et al*., 2010; Janku *et al*., 2013; Burkhardt *et al*., 2014), growth (Akis *et al*., 2010; Szewczuk *et al*., 2013) and survival (Epaud *et al*., 2012). It is assumed that, these biological actions prevent or postpone age-related diseases and extend age of organisms.

Another effect for the IGFIR genotype was observed on TNLB and TNLW. The TNLB was found to be highly correlated with ovulation rate, pregnancy rate and embryo survival (Schoenian and Burfening, 1990; Stellflug *et al*., 2001). In mammals, the level of IGFIR proved to have significant effect on the differentiation of cumulus granulosa cells

(Baumgarten, 2014), follicular development (Mŭnoz-Gutierrez, 2004), ovulation rate (Yang *et al.*, 2013), pre-implementation and development of embryo (Wang *et al*., 2009 & 2012) and litter size (Terman, 2011). These reproductive phenomena might explain the effect of variation in IGFIR gene on TNLB in Barki ewes.

CONCLUSION

The results of this study suggest that if the breeding program is to be done for improving the lambing number, TNLB and TNLW in Barki ewes based upon the IGFIR polymorphism, the AA genotype is recommended to increase its frequency through the marker assisted selection. However, further studies on associations between the IGFIR genotype and productive life in sheep breeds are necessary to assure our findings.

SUMMARY

The insulin like growth factor I receptor (IGFIR) gene is known to be involved in the control of the insulin like growth factor I (IGFI) and the insulin like growth factor II (IGFII) through mediating their strong actions that affecting many biological processes (e.g. energy expenditure, metabolism, oxidative stress resistance, cancer suppression, follicle development, ovulation rate,…etc.). These processes proved significant effects on age and reproductive traits of many lived organisms. The allelic and genotypic polymorphisms of the IGFIR gene were identified in 95 Barki ewes using the polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) tool. Also, the effect of variation in IGFIR gene on age and reproductive traits of ewes was measured using three sets of general linear mixed effect models. The obtained results revealed two allelic, A (0.19) and B (0.81) and three genotypes, AA (0.05), AB (0.28) and BB (0.67) polymorphisms. Least square mean analysis revealed a significant statistical effect for the IGFIR genotype on age ($P < 0.01$), total number of lambs born per ewe (TNLB; $P < 0.05$) and total number of lambs weaned per ewe (TNLW; $P < 0.05$). The presence of allele A and absence of allele B was significantly associated with longer age (0.89 year; $P < 0.01$), higher lambing number (0.70; $P < 0.05$), increased TNLB (0.86; $P < 0.05$) and increased TNLW (0.65; $P < 0.05$). Also, the number of allele A was positively associated with age, TNLB and TNLW. Finding the association of variation in IGFIR gene with age and reproductive traits in Barki sheep may be useful for the prolonged productive life.

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Table (1): Least Square means and standard errors of productive life traits according to the IGFIR genotypes effects in Barki ewes.

Trait	LSM ± SE			P-value
	AA (5)	AB (27)	BB (63)	
Age	7.40 ± 0.67^{aa}	6.59 ± 0.23^{ab}	5.83 ± 0.17^{bb}	**
Lambing Number	4.80 ± 0.58	4.48 ± 0.27	3.83 ± 0.17	NS
Lambing Rate	0.74 ± 0.03	0.81 ± 0.03	0.80 ± 0.02	NS
Twining Rate	1.11 ± 0.04	1.04 ± 0.02	1.03 ± 0.01	NS
Rearing Ability	0.80 ± 0.12	0.85 ± 0.02	0.87 ± 0.02	NS
TNLB	5.40 ± 0.74^{aa}	4.70 ± 0.31^{ab}	3.95 ± 0.18^{bb}	*
TWLB	18.00 ± 2.97	16.57 ± 1.12	14.09 ± 0.71	NS
TNLW	4.60 ± 1.03^{aa}	3.93 ± 0.25^{ab}	3.38 ± 0.16^{bb}	*
TWLW	93.30 ± 20.54	81.40 ± 4.63	72.15 ± 3.29	NS

TNLB: total number of lambs born per ewe; TWLB: total weight of lambs born per ewe; TNLW: total number of lambs weaned per ewe; TWLW: total weight of lambs weaned per ewe; NS: no significance; *: refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$).

Table (2): Association of the absence/ presence of IGFIR alleles with various productive life traits of Barki ewes.

Trait	Allele being assessed	LSM ± SE				P-value
		Allele absent	No.	Allele present	No.	
Age	A	5.83 ± 0.17	63	6.72 ± .226	32	**
	B	7.40 ± 0.67	5	6.06 ± .145	90	**
Lambing Number	A	3.83 ± 0.17	63	4.53 ± .246	32	*
	B	4.80 ± 0.58	5	4.02 ± .148	90	*
Lambing Rate	A	0.80 ± 0.02	63	0.80 ± .030	32	NS
	B	0.74 ± 0.03	5	0.81 ± .020	90	NS
Twining Rate	A	1.03 ± 0.01	63	1.05 ± .023	32	NS
	B	1.11 ± 0.04	5	1.03 ± .011	90	NS
Rearing Ability	A	0.87 ± 0.01	63	0.84 ± .025	32	NS
	B	0.80 ± 0.12	5	0.86 ± .014	90	NS
TNLB	A	3.95 ± 0.18	63	4.81 ± .289	32	*
	B	5.40 ± 0.74	5	4.18 ± 0.16	90	*
TWLB	A	14.09 ± 0.71	63	16.79 ± 1.04	32	NS
	B	18.00 ± 2.97	5	14.83 ± 0.61	90	NS
TNLW	A	3.38 ± 0.16	63	4.03 ± 0.20	32	*
	B	4.60 ± 1.03	5	3.54 ± 0.13	90	*
TWLW	A	72.15 ± 3.29	63	83.26 ± 4.93	32	NS
	B	93.30 ±20.54	5	74.92 ± 2.71	90	NS

TNLB: total number of lambs born per ewe; TWLB: total weight of lambs born per ewe; TNLW: total number of lambs weaned per ewe; TWLW: total weight of lambs weaned per ewe; NS: no significance; *: refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$).

Table (3): Association of the number of IGFIR allele copies present on productive life traits of Barki ewes.

Trait	Allele being assessed	LSM ± SE						P-value
		Allele absent	No.	Allele 1 copy	No.	Allele 2 copies	No.	
Age	A	5.83 ± 0.17^{bb}	63	6.59 ± 0.23^{ab}	27	7.40 ± 0.67^{aa}	5	**
	B	7.40 ± 0.67^{aa}	5	6.59 ± 0.23^{ab}	27	5.83 ± 0.17^{bb}	63	**
Lambing Number	A	3.83 ± 0.17	63	4.48 ± 0.27	27	4.80 ± 0.58	5	NS
	B	4.80 ± 0.58	5	4.48 ± 0.27	27	3.83 ± 0.17	63	NS
Lambing Rate	A	0.80 ± 0.02	63	0.81 ± 0.03	27	0.74 ± 0.03	5	NS
	B	0.74 ± 0.03	5	0.81 ± 0.03	27	0.80 ± 0.02	63	NS
Twining Rate	A	1.03 ± 0.01	63	1.04 ± 0.02	27	1.11 ± 0.04	5	NS
	B	1.11 ± 0.04	5	1.04 ± 0.02	27	1.03 ± 0.01	63	NS
Rearing Ability	A	0.87 ± 0.01	63	0.85 ± 0.02	27	0.80 ± 0.12	5	NS
	B	0.80 ± 0.12	5	0.85 ± 0.02	27	0.87 ± 0.01	63	NS
TNLB	A	3.95 ± 0.18^{bb}	63	4.70 ± 0.31^{ab}	27	5.40 ± 0.74^{aa}	5	*
	B	5.40 ± 0.74^{aa}	5	4.70 ± 0.31^{ab}	27	3.95 ± 0.18^{bb}	63	*
TWLB	A	14.09 ± 0.71	63	16.57 ± 1.12	27	18.00 ± 2.97	5	NS
	B	18.00 ± 2.97	5	16.57 ± 1.12	27	14.09 ± 0.71	63	NS
TNLW	A	3.38 ± 0.16^{bb}	63	3.93 ± 0.25^{ab}	27	4.60 ± 1.03^{aa}	5	*
	B	4.60 ± 1.03^{aa}	5	3.93 ± 0.25^{ab}	27	3.38 ± 0.16^{bb}	63	*
TWLW	A	72.15 ± 3.29	63	81.40 ± 4.63	27	93.30 ± 20.54	5	NS
	B	93.30 ± 20.54	5	81.40 ± 4.63	27	72.15 ± 3.29	63	NS

TNLB: total number of lambs born per ewe; TWLB: total weight of lambs born per ewe; TNLW: total number of lambs weaned per ewe; TWLW: total weight of lambs weaned per ewe; NS: no significance; *: refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$).

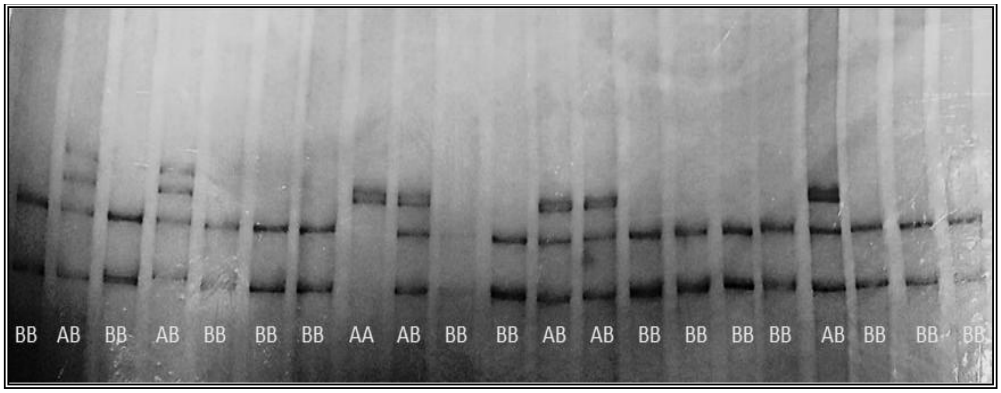

Fig. (1): Single strand conformational polymorphism analysis for (intron 2- exon 3) of IGFIR gene in Barki ewes.