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# Bovine ER-α polymorphism in relation with production traits

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**Abstract.** This study was implemented to investigate ER-α gene polymorphism in 5′ region, in consensus promoter for exon C, A/G transition, and determine its effect on production traits. DNA was extracted from 200 dairy Holstein in four farms in Isfahan province and the individuals were genotyped by PCR-RFLP/ BglI technique. Fragments with 245 bp indicated allele A and those with 168 bp and 77 bp represented allele G. Using SAS software (Proc GLM), the effect of ERα gene polymorphism on production traits was investigated. No relation of this SNP was found with milk production and milk protein, but AG genotype significantly increased milk corrected fat yield and fat percentage ($p<0.05$). The reason might be the linkage of this polymorphism with other mutations affecting fat yield and fat percentage or maybe ERα gene transcription factors' binding sites is changed and so the gene expression is altered and consequently estrogen may affect other essential hormones in metabolism or also expression of their receptors, in a different way.

**Key Words:** ***E***R-α polymorphism, mammary gland, production traits, milk fat, milk protein.

## 1. Introduction

Selection response by phenotypic selection would be low because of low heritability of some traits (Rothschild and Bidanel, 1998). MAS can increase the selection response (Larzal et al., 1997) by finding a QTL (Wilkie et al., 1999) or mutations in genes with physiological functions (Andersson and Georges, 2004). One way to determine the genetic base of quantitative traits is to use candidate gene polymorphism as a marker which its product take part in physiological processes (Kmieć et al., 2002). *Estrogen receptor* (*ER*) gene is one of these candidate genes (Rothschild et al., 1995).

Estrogen is one of the most important hormones affecting growth, differentiation and function of reproductive tissues like mammary glands (Hewitt and Korach, 2003), uterus, ovary (Rosselli and Dubey, 2006), testis and prostate (Szreder et al., 2007) and as a mitogen increases the uterine epithelial (Hruska et al., 2000; Hewitt and Korach, 2002), vagina (Hruska et al., 2000) and mammary gland cell proliferation (Clarke, 2000; Li et al., 2006).

Estrogen receptors like other members of nuclear receptors' superfamily, are transcription factors which bind to estrogen and regulate gene transcription (Szreder and Zwierzchowski, 2004a; Szreder and Zwierzchowski, 2004b; Jakimiuk et al., 2007). There are two isoforms known for estrogen receptor; ER-α and ER-β (Hruska et al., 2000; Hewitt and Korach, 2002; Szreder and Zwierzchowski, 2004b; Szreder et al., 2007) each coded by a separate gene (Szreder and Zwierzchowski, 2004a; Szreder and Zwierzchowski, 2004b). The major sites of ER expression are reproductive organs (Neville et al., 2002; Szreder and Zwierzchowski, 2004a; Szreder et al., 2007) and also liver, intestine, lung, stomach, kidney, and pituitary gland (Szreder and Zwierzchowski, 2004a). All genes coding nuclear receptors including ER-α have a special characteristic which is the complicated structure of their 5′ region (Kos et al., 2001). In all known species ER protein is coded by 8 exons. In the 5′ region, there are additional exons which do not code for

protein but code different transcripts with specific expressions in different tissues or developmental stages (Szreder and Zwierzchowski, 2004a).

Szreder and Zwierzchowsky, sequenced a 2853 bp of bovine *ER-α* gene including partial sequence of coding exon1and also non coding exons A, B, C and their putative promoters. They also found a single nucleotide polymorphism (SNP) which is an A/G transition probably in the putative promoter of exon C, in the 5′ non coding region and it was recognized by PCR-RFLP and *BglI* restriction enzyme (Szreder and Zwierzchowski, 2004a).

The present research is based on this SNP to determine *ER-α* allelic and genotypic frequencies and find the relation between *ER-α* polymorphism and production traits.

## 2. Materials and Methods

**2.1. DNA Extraction**. Blood samples were collected from 200 Holstein dairy cattle from 4 different farms in Isfahan province and DNA was extracted.

**2.2. Genotyping.** PCR-RFLP method was used to genotype the cows. The 25 µL PCR reaction mixture that was used for amplification of partial *ER-α* (245 bp), contained of: 20-40 ng genomic DNA,160 µM dNTPs, 1.6 mM $MgCl_2$, 2.5 µL 10X PCR-buffer, 3 units of Taq polymerase enzyme (all purchased from Cinnagen) and 10 pmol of each primer (Metabion). The sequences of forward (ERF) and reverse (ERR) primers were respectively: 5′- TTTGGTTAACGAGGTGGAG -3′ and 3′-TGTGACACAGGTGGTTTTTC -5′, as previously reported (Szreder and Zwierzchowski, 2004a). The PCR reaction was run with the program adjusted in Techne-TC512 thermocycler as following: 2 min initial denaturation in 95°C, 30 cycles of 1 min denaturation in 94 °C, 1 min annealing in 53 °C, and 1 min extension in 72 °C. At the final step, 5 min was considered for final extension in 72 °C. 10 µL of PCR amplified fragments was digested by 5 units of *BglI* restriction enzyme in a reaction volume of 20 µL in 37 °C for 6 hours. Digested fragments were run on a 12% polyachrylamide gel and different genotypes were determined.

**2.3. Statistical Analysis.** After genotyping and determining the allelic and genotypic frequencies, the SAS software (GLM procedure) was used for association studies, and the least square means of genotypes were compared. The linear model used to extract different effects in addition of genotype is as followes: $Y_{ijk} = \mu + ER_i + H_j + b_1(X_{ijk}-X) + b_2(Z_{ijk}-Z) + b_3(W_{ijk}-W) + e_{ijk}$, where $Y_{ijk}$ = production traits, µ = overall mean, $ER_i$ = fixed effect of the *i*th genotype (GG, AG and AA), $H_j$ = fixed effect of the *j*th herd (1, 2, 3 and 4), ), $X_{ijk}$ is days of milking, $b_1$ is the linear regression coefficient of days of milking, $Z_{ijk}$ is dry days , $b_2$ is the linear regression coefficient of dry days, $W_{ijk}$ is open days, $b_3$ is the linear regression coefficient of open days, and $e_{ijk}$ is the random residual.

## 3. Results

After digestion and electrophoresis, two variants of *ER-α* were found. The fragment with 245 bp indicates allele A and two fragments with 168 and 77 bp represent allele G. So the digestion reaction resulted in two restriction fragments for GG, 3 fragments for AG and one uncut fragment for AA genotype (Figure 1). Total allelic frequencies were 0.0742 and 0.9257, for allele A and G respectively and the total distribution of AA, AG and GG genotypes were 0.010, 0.129 and 0.861, respectively.

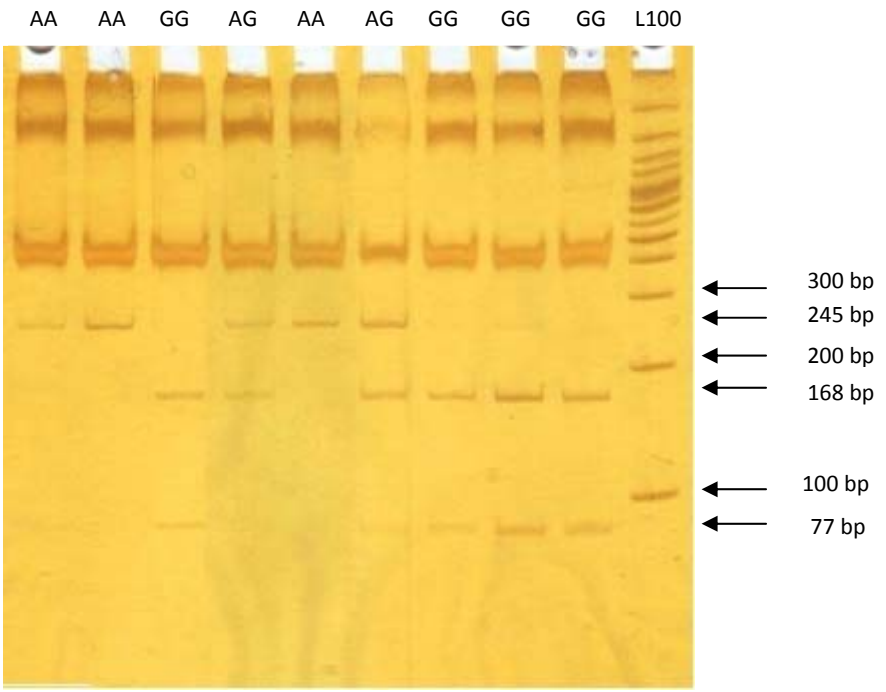


Fig 1. 12% polyachrylamide gel stained by silver staining: the related genotypes are shown above each line and the length of the bands is denoted at the right side.

The association study showed that ER-α genotypes have a significant effect on corrected milk fat ($p<0.05$) and milk fat percentage ($p<0.05$), as AG genotype increased fat yield and fat percentage in comparison with GG genotype. However AA genotype showed no significant difference with two other genotypes. No relation of this SNP with milk production, corrected milk protein and protein percentage was found (not shown). The least square means of milk fat in association with ER-α genotypes are given in Table 1.

Table 1: Least square means and standard errors of the milk fat yield obtained for the ER-α genotypes

| ER-α | Corrected fat (kg) | Fat percentage |
|---|---|---|
| Genotypes | LSMean ± SE | LSMean ± SE |
| AA | 228.68±25.00[a b] | 2.48±0.22[a b] |
| AG | 260.33±7.26[a] | 2.76±0.06[a] |
| GG | 240.01±2.82[b] | 2.58±0.02[b] |

a,b: significantly different least square means ( $p<0.05$).

## 4. Discussion

*ER-α*/*BglI* single nucleotide polymorphism was found in Aberdeen-Angus, Charolaise, Limousine, Simmental, Hereford, Friesian & polish Red breeds and two genotypes, AG and GG, were observed (Szreder and Zwierzchowski, 2004a). However no relation of this SNP with production traits was studied.

Estrogen in premature famales along with progesterone, prolactin, cortisol , growth hormone and growth factors causes the cell proliferation and the extension of mammary ducts (Wilkie et al., 1999; Neville et al., 2002; Li et al., 2006). In gestation helps the development of the alveoli (Soyal et al., 2002) and also has an effect on prolactin and somatomedin secretion (Genuth, 2000). *ER-α* gene is expressed in cow′s mammary epithelial cells (Capuco et al., 2002). So this idea has rised that *ER-α* gene polymorphism may have an influence on milk production and milk contents. Different gene polymorphisms have been studied to have effects on milk. Grochowska *et al* found that *AluI* heterozygote genotype of GH gene significantly had higher milk production than other genotypes (Grochowska et al., 2001). Aggrey *et al*. studied different polymorphisms in *GH receptor* gene in cattle and found that *AluI* homozygote genotype affects the increase in milk fat and *StuI* homozygote genotype has a significant effect on increasing the milk protein and milk fat breeding values (Aggrey et al., 1999). Zhou *et al*. in study of *AspI* polymorphism in *GH* gene showed that individuals with AA genotype had higher milk protein and lower milk fat percentage (Zhou and Jiang, 2006). There is no information about the effect of *ER-α* gene polymorphism on milk production and its contents. In the present research these associations were studied and resulted in AG genotype with higher corrected milk fat production and fat percentage, but no effect on milk production and milk protein was detected. As previously noted, the polymorphism is in the 5′ non coding region of *ER-α* gene, so it would not enter the protein structure of the receptor. So in order to explain its effect on milk fat, we may relate the position of this mutation in promoter region of exon C, to transcription factors' binding sites and so affecting the gene expression. This mutation may also influence other mutations in vicinity. However estrogen affects the secretion of metabolic hormones like GH, IGF and leptin, so as a transcription factor may affect the expression of their receptors. This polymorphism might have an influence on quantity or quality of estrogen effects on these hormones and their following effects on milk fat production. Probably interaction of transcription factors in this part of estrogen receptor alpha, causes estrogen to have influence on special parts of genome which changes the milk fat, without changing the milk production and milk protein.

As the results show, AA genotype was very rare in the population, so to significantly find its effect on the production traits, a greater population is needed. Although the milk production and milk protein were not altered, the milk fat production was higher in AG individuals, so this genotype may have the possibility to go under selection and produce more fat in milk without changing the cows' ration.

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