Animal Science Papers and Reports vol. 30 (2012) no. 4, 373-381
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Estrogen receptor α (ER-α) gene and bovine performance: is there any relation?

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(Received March 13, 2011; accepted July 2,2012)

The objective of the study was to investigate *ER-α* gene polymorphism (A/G transition) in 5′ region in consensus promoter for exon C, determine its allelic and genotypic frequencies and assess its association with cattle performance. Genomic DNA was extracted from 200 dairy Holstein cows in four farms in Isfahan province of Iran and the individuals were genotyped by PCR-RFLP technique. The DNA fragments were PCR-amplified and then digested with *Bgl*I endonuclease. Restriction fragments with 245 bp represented allele A and those with 168 bp and 77 bp indicated allele G. Allelic frequencies were 0.0742 and 0.9257, respectively for allele A and G, and the total distribution of AA, AG and GG genotypes was 0.010, 0.129 and 0.861, respectively. The population was in Hardy-Weinberg equilibrium. Using SAS software (Proc GLM), the effect of *ERα* gene polymorphism on cows' reproduction traits and calves' birth weight was investigated, but no significant relation was found. So it could be concluded that this SNP has no effect on traits under study and also is not in a linkage disequilibrium with other mutations affecting the reproduction traits or birth weight of cattle.

KEY WORDS: birth weight / cattle / estrogen receptor alpha / gene polymorphism / reproduction traits

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The response to phenotypic selection of some traits would be low because of their low heritability [Rothschild and Bidanel 1998]. Marker-assisted selection (MAS) can increase the selection response [Larzal *et al*. 1997] by detection of QTL [Wilkie *et al*. 1999] or mutations in genes with important biological functions [Andersson and Georges 2004]. One way to determine genetic factors affecting quantitative traits is to use as markers the candidate genes which take part in physiological processes leading to expression of such traits [Kmieć *et al*. 2002]. Estrogen receptor (*ER*)gene is one of such candidate genes [Rothschild *et al*. 1995].

Estrogen is important hormone affecting reproduction [Genuth 2000]. It also influences growth, differentiation and function of reproductive tissues like mammary gland [Hewitt and Korach 2003], uterus, ovary [Rosselli and Dubey 2006], testes [Cooke *et al*. [1991], Saunders 2005] and prostate [Ebling 2000]. It works as a mitogen to increase the proliferation of uterine [Hruska *et al*. 2000, Hewitt and Korach 2002], vaginal [Hruska *et al*. 2000] and mammary gland [Clarke 2000, Li *et al*. 2006] epithelial cells. Estrogens also increase sexual behavior, regulate secondary sexual traits [Genuth 2000, Hruska *et al*. 2000] and play a major role in female post-natal physiology and pathology [Fritsch and Murdoch 1998, Rybaczyk *et al*. 2005].

Estrogen receptors like other members of nuclear receptors' superfamily are transcription factors which bind a proper ligand (usually 17-β estradiol) and regulate gene expression [Fuller *et al*. 1991, Carr 1998, Jakimiuk *et al*. 2007]. After entry the cell nucleus, the receptor-steroid complex takes an active form and, after dimerization, binds to estrogen- responsive *cis* elements in promoter regions of estrogen target genes. This leads to increase or decrease in gene transcription, and finally changes protein production and begins a physiological response to this stimulation [Deroo and Korach 2006].

There are two isoforms known for estrogen receptor: ER-α and ER-β [Enmark *et al*. 1997, Hruska *et al*. 2000, Hewitt and Korach 2002] each encoded by separate genes located in different chromosomes [Enmark *et al*. 1997]. The major sites of ER expression are reproductive organs [Cooke *et al*. 1991, Wang *et al*. 2000, Neville *et al*. 2002] and also stomach [Matsuyama *et al*. 2002], kidney [Sharma and Thakur 2004], lung, liver, intestine [Yu *et al*. 2006] and pituitary [Schreihofer *et al*. 2000]. All genes coding for nuclear receptors, including ER-α, have a special characteristic which is the complicated structure of their 5′ region [Kos *et al*. 2001]. ER protein is coded by 8 exons but in the 5′ region there are additional exons which encode different transcripts with specific expressions in different tissues or developmental stages [Gronemeyer 1991].

In database (e.g. GenBank®) complete sequences of *ER-α* gene of human, mouse and rat are available. Also partial sequences of *ER* coding region and mRNA of pig and sheep are known. Part of 5' non-coding region and partial coding sequences of bovine *ER-α* gene are also known and available [Szreder and Zwierzchowski 2004ab]. Szreder and Zwierzchowski [2004a] sequenced a 2853 bp of bovine *ER-α* gene including non coding exons A, B, C and their putative promoters and also partial sequence of coding exon 1. They also found a single nucleotide polymorphism (SNP) which is an A/G

transition probably in the promoter region of exon C which was recognized by PCR-RFLP with *Bgl*I restriction nuclease [Szreder and Zwierzchowski 2004a].

The present study is implemented based on this SNP to determine *ER-α* allelic and genotypic frequencies and to find the relation between *ER-α* polymorphism and reproduction traits and birth weight in Iranian HF cattle.

Material and methods

DNA samples

Blood samples were withdrawn from 200 Holstein dairy cows from 4 different farms in Isfahan province and DNA was extracted.

Genotyping

PCR-RFLP method was used to genotype the cows. The 25 μL PCR reaction mixture used for amplification of partial *ER-α* (245 bp) contained 20-40 ng genomic DNA, 10 pmol of each primer (METABION), 160 μM dNTPs, 1.6 mM $MgCl_2$, 2.5 μL 10X PCR-buffer and 3 units of Taq polymerase (all from CINNAGEN). Used were the following sequences of forward (ERF) and reverse (ERR) primers: 5′-TTTGGT TAACGAGGTGGAG-3′ and 3′-TGTGACACAGGTGGTTTTTC-5′, respectively, as earlier reported by Szreder and Zwierzchowski 2004a]. The PCR reaction was run in TECHNE TC512 thermocycler with the programme adjusted as follows: 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. Ten μL of PCR-amplified DNA was digested by 5 units of *Bgl*I restriction enzyme in a reaction volume of 20 μL in 37°C for 6 h. Digested fragments were run on 12% polyacrylamide gel and different genotypes were determined.

Statistical

After genotyping and determining the genotype frequencies, Hardy-Weinberg proportions were tested using the chi-square test. To determine the association of reproduction traits and birth weight with *ER-α* gene polymorphism, the SAS software and the GLM procedure were used and the least squares means of genotypes were compared. The linear model used to extract different effects additional of genotype was as follows:

$$Y_{ijk} = \mu + ER_i + H_j + b_1(X_{ijk}-X) + b_2(Z_{ijk}-Z) + b_3(W_{ijk}-W) + e_{ijk}$$

where:

Y_{ijk} – reproduction traits and birth weight;

μ – population 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, 4);

X_{ijk} – days of milking;
b_1 – the linear regression coefficient of days of milking;
Z_{ijk} – number of dry days;
b_2 – the linear regression coefficient of dry days;
W_{ijk} – number of dry days;
b_3 – the linear regression coefficient of open days;
e_{ijk} – random residual.

Results and discussion

ER-α genotyping

The Polymerase Chain Reaction showed fragments with 245 bp in length. After digestion and electrophoresis, two variants were found. The 245-bp variant represented allele A and those with two fragments with 168 and 77 bp indicated allele G. So the digestion reaction resulted in two restriction fragments for GG, three fragments for AG and one uncut fragment for AA genotype (Fig 1). The present study showed that GG and AA genotypes had the highest (0.771-0.937) and the lowest (0-0.021) frequencies in all herds, respectively, and the frequency of AG genotype ranged from 0.042 to 0.214. Allelic frequency was calculated based on genotypic frequency and showed the

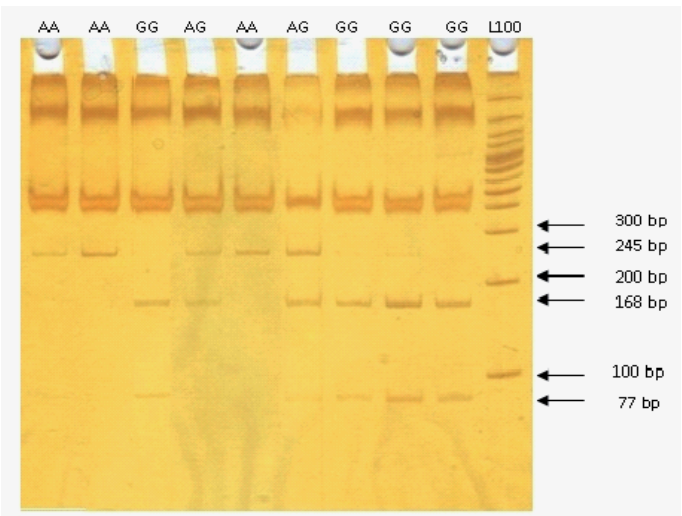

Fig 1. The PCR-RFLP analysis of the A/G polymorphism in the bovine estrogen receptor α (*ERα*) gene. Electrophoresis in the 12% polyacrylamide gel stained with silver: the genotypes are shown above each line and the length of the bands is denoted at the right side.

range of 0.031-0.121 for allele A and 0.878-0.968 for allele G. Comparing the overall genotypic frequency with that expected from Hardy-Weinberg equilibrium, showed that there was no significant difference between the two (Tab. 1), so the population was in equilibrium probably because of no selection was ever performed for this gene.

Table 1. Genotypic and allelic frequencies of *ER-α* gene in Holstein and chi-square test calculations

Herd	Genotype			Allele	
	AA	AG	GG	A	G
1	0	0.162	0.838	0.081	0.919
2	0.014	0.214	0.771	0.121	0.878
3	0	0.062	0.937	0.031	0.968
4	0.021	0.042	0.936	0.042	0.957
Total	0.010	0.129	0.861	0.0742	0.9257
Expected in Hardy-Weinberg equilibrium	0.005	0.138	0.857	chi-square = 0.0056	

Effect on reproduction and birth weight

Reproduction traits investigated in this study were gestation length, calving interval and interval between calving and first successful insemination. The results showed that ER-α genotypes have no significant effect on these traits (P>0.1).

The association study also showed that *ER-α* genotypes have no significant effect on calves' birth weight (P>0.1) and all three genotypes were nearly the same. The least squares means of reproduction traits and birth weight in relation to *ER-α* genotypes are given in Table 2.

Table 2. Least squares means (LSM) and their standard errors of the reproduction traits (days) and birth weight (kg) obtained for the ER-α genotypes

ER-α genotype	Gestation length	Calving interval	Calving to first insemination	Birth weight
	LSM±SE	LSM±SE	LSM±SE	LSM±SE
AA	276.18 ± 3.09	384.98±19.96	60.69±13.85	43.57±2.65
AG	277.08± 0.90	388.62±5.80	55.13±6.13	41.06±0.90
GG	278.47 ± 0.34	394.35±2.23	59.69±1.49	41.08±0.33

No significant differences between genotypes.

Earlier we showed that the effect of *ER-α* genotypes on corrected milk fat and fat content was significant at P<0.05. Individuals with AG genotype yielded more milk fat than those with GG genotype. However, the AA genotype showed no significant differences in relation to other genotypes [Zahmatkesh *et al*. 2011].

Rothschild *et al.* [1994] found a relation between *ER-α* gene polymorphism and number of teats in the pig. They also showed in pigs a significant relation between *ER-α* gene polymorphism and litter size. This would indicate that *ER-α* might be a major gene or at least the closest linked marker for litter size [Rothschild *et al*. 1996]. Zhen-Fang *et al*. [2006] noted that *ER-α PvuI*I polymorphism in pigs is significantly related to the litter size, number of live-born piglets and their birth weight.

ER-α/*Bgl*I single nucleotide polymorphism was found in Aberdeen-Angus, Charolaise, Limousine, Simmental, Hereford, Friesian and Polish Red cattle and two genotypes, AG and GG were observed [Szreder and Zwierzchowski 2004a]. However, no relation of this SNP with reproduction traits was studied.

No significant relation between *ER-α* genotypes and reproduction traits was seen in the present study. However *ER-α* gene is expressed in many reproductive tissues and deficiency in estrogen receptor may cause disorders in male and female reproductive system [Hewitt and Korach 2002]. So it may be concluded that this SNP (A/G transition) is probably not a causative mutation and is not in linkage disequilibrium with other mutations affecting reproduction traits. Maybe it makes no change in *ERα* gene transcription factors' binding sites, although this should be confirmed by future studies.

Little information is available about *ERα* in relation to birth weight, and the data published are limited to pigs. Rothschild *et al*. [1996] found no significant relation between *ERα*/*Pvu*II polymorphism and average daily gain in pigs, but Short *et al.* [1997] showed a significant relation of this polymorphism with a decrease in backfat and average daily gain in pigs carrying allele B. Zhen-Fang *et al.* [2006] noted that the birth weight of piglets of sows carrying AA and AB genotypes were significantly higher than those of BB genotype in the first farrowing. No information is available about the relation of *ERα/Bgl*I polymorphism and birth weight in cattle. We have studied this relation and found no effect of this polymorphism on birth weight. Although ERα protein is expressed in pituitary [Schreihofer *et al*. 2000] and liver [Yu *et al*. 2006], and may influence GH and IGF secretion, this SNP probably does not change the mode of estrogen effect on these hormones.

Estrogen, along with progesterone, cortisol, prolactin, growth hormone and other growth factors, leads to cell proliferation and mammary duct extension in prematured females [Wilkie *et al*. 1999, Neville *et al*. 2002, Li *et al*. 2006] and the development of alveoli during gestation [Soyal *et al*. 2002]. *ER-α* gene is found to be expressed in cow′s mammary epithelial cells [Capuco *et al*. 2002]. The effect of *ER-α* gene polymorphism was found to be significant on corrected milk fat and fat percentage of milk [Zahmatkesh *et al*. 2011], suggesting this SNP to be an effective factor that probably changes the mode of estrogen effects on the mammary epithelial tissue or other hormones involved in milk production.

Concluding, the results presented here show that the *ER-α* gene A/G polymorphism had no significant effect on the studied reproduction traits in HF cows as well as on birth weight of calves. Because of higher milk fat production in AG individuals [Zahmatkesh *et al*. 2011], this genotype may have the possibility to go under selection

and AG individuals can be used to produce more fat of milk without changing the cows feed ration. AA genotype very rarely appeared in the population, so to identify its effects on the traits studied, more animals of a greater population are needed. It is suggested to check this SNP in association with other reproduction traits such as calving rate or number of inseminations per gestation. The SNP in question can also be considered in relation to estrogen content of serum to find other aspects of variation due to *ERα/Bgl*I polymorphism.

Acknowledgement. *The authors acknowledge the grant from Isfahan University of Technology and financial supporting of Ms. Azadeh Zahmatkesh's MSc thesis*.

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