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The effect of polymorphism in gene of insulin-like growth factor-I on the serum periparturient concentration in Holstein dairy cows

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ABSTRACT

Objective: To investigate the relationship between polymorphism within the 5′-untranslated region (5′-UTR) of IGF-I gene and its periparturient concentration in Iranian Holstein dairy cows. **Methods:** Blood samples (5 mL, n = 37) were collected by caudal venipuncture from each animal into sample tubes containing the EDTA and DNA was extracted from blood. In order to measure IGF-I concentration the collection of blood samples (n = 111) was also done at 14 d before calving (prepartum), 25 and 45 d postpartum. **Results:** We found evidence for a significant effect of C to T mutation in position 512 of IGF-I gene on its serum concentration in dairy cows in Iran. Cows with CC genotype had significantly higher concentration (Mean±SD) of IGF-I at 14 d prepartum (91.8±18.1) μg/L compared to those with TT genotype (73.3±14.4) μg/L (P=0.04). A significant trend (quadratic) was found for IGF-I concentration, as higher in CC cows compared to ones with TT genotype, during the 14 d before calving to 45 d postpartum (P=0.01). **Conclusions:** We concluded that C/T transition in the promoter region of IGF-I gene can influence the serum concentration of IGF-I in periparturient dairy cows.

1. Introduction

Reproductive events such as follicular development and ovulation are controlled by several hormones and metabolites. The periparturient period is critical for fertility and milk production after calving. Postpartum ovarian activity was impaired following hormonal and metabolic changes especially after calving and eventually the cattle's reproductive performance will be affected[1]. One of the most important metabolic factors affecting the reproductive activity is insulin-like growth factor-I (IGF-I) concentration changes after calving[2,3].

Insulin-like growth factor-I is produced in organs of reproductive significance such as hypothalamus, ovaries, oviducts, and uterus[4]. It can change reproductive activity by affecting on neural pathway which control production of GnRH or effect on the secretion of pituitary gonadotropin; it has also direct effects on the ovary and influences its susceptibility to FSH and LH[5]. In addition, IGF-I can influence on the proliferation and differentiation of granulosa cell, postpartum follicular growth and consequently first ovulation of the dominant follicle, development of corpus luteum and preimplantation embryo development[6,7]. Correspondingly, a positive relationship has been reported between reproductive performance and the concentration of IGF-I after calving in dairy cows[8,9].

IGF-I gene is located on chromosome 5 in cattle. Although different polymorphisms have been reported in IGF-I gene so far[10,11], the point mutation (C/T transition) at nucleotide position 512 within the 5′-untranslated region (5′-UTR) has attracted the most attention[12]. This type of mutation was first identified by Ge *et al*[13]. Recently, Maj *et al* discovered a significant association between the IGF-I genotypes based on this region and the IGF-I blood level[14]. Heritability of IGF-I is moderate and significant[15]. Postpartum peripheral IGF-I levels are affected by breed/genotype[16]. Different genotypes can alter the endocrine and metabolic profiles of the transition dairy cow under grazing condition[17]. Several studies reported periparturient changes of IGF-I blood concentration in cattle[18,19]. It has been reported significant associations between single nucleotide polymorphisms (SNPs) in IGF-I and production traits in Holstein-Friesian cattle[20-22]. However, the effect of IGF-I genotypes on its

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blood concentration during periparturient period (before and after calving) in dairy cow has not yet been investigated. During postpartum period, concentrations of IGF-I has links with many aspects of reproduction, is altered. With these in mind, the present investigation was undertaken to study the relationship between polymorphism within 512 region of IGF-I gene and its periparturient concentration in Iranian Holstein dairy cows.

2. Materials and methods

2.1. Animals and sample collection

This study was carried out on registered multiparous Iranian Holstein cows at the farm of Farzis milk and meat producing complex in Shiraz, Fars province, south of Iran. Shiraz is located at latitude of 29→38′ and longitude 52→36′ E. Its altitude is 1296 m above sea level. The cows were kept under the same weather and management conditions in a similar manner. Cows were fed standard rations (total mixed ration) including mainly alfalfa, corn silage, beet pulp, cotton seed, soybean, corn and barley. Oestrus was detected four times daily by visual detection. Cow was artificially inseminated (AI), about 12 h after heat detection. Pregnancy diagnosis was carried out by ultrasound examination (real time B-mode linear array scanner with a 5 MHz transducer, SIUI, China) 25-35 d after AI. In this study, 37 high producing Holstein dairy cows with the history of acceptable physical conditions in previous lactation were chosen. The cows with 3 to 6 lactation number were selected. Selection criteria for studied cows included acceptable body condition score and general health during the various production stages in previous lactation. Cows had an average daily previous lactations milk yield of (30±2) kg.

Blood samples (n = 37; 5 mL) were collected by caudal venipuncture from each animal into sample tubes containing the anticoagulant potassium ethylenediaminetetraacetic acid (EDTA K3E 15%, 0.12 mL; BD Vacutainer, BD Vacutainer systems, Plymouth, UK) and stored at -20 ℃ for subsequent DNA extraction. In order to measure periparturient serum IGF-I concentration, the collection of blood samples (n = 111) was also done at 14 d before calving (prepartum), 25 and 45 d postpartum of studied cows in the Spring (from April to June). Within 4 h after collection, serum was separated by centrifugation (1 700 × g for 15 min) and stored at -22 ℃ until assayed. Serum IGF-I was measured using ELISA kits (UK immunodiagnostic systems Ltd, IDS). The inter- and intra-assay coefficients of variation were 6.5% and 7.2%, respectively, and the sensitivity was 3.1 ng/mL.

2.2. Genomic DNA extraction

Total genomic DNA was extracted from blood using the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Briefly, 50 μL of blood added to 170 μL of PBS then digested using 200 μL of a lysis solution (AL buffer, Qiagen) containing guanidine hydrochloride and 20 μL of proteinase K (50 μg/mL) at 56 ℃ for 10 min. after adding 200 μL Ethanol, the mixture was vortexed for 15 s and then added to a DNA-binding column and spun down for 1 min. The column was then washed several times using AW1 and AW2 buffers (Qiagen). Finally, the genomic DNAs were eluted in a volume of 100 μL supplied elution buffer. The extracted DNA was diluted to a working concentration of 10-20 ng/μL, and 2.4 μL of it was used as a template in PCR.

2.3. IGF-I polymorphism

The amplification of the 5′-flanking region of the IGF-I gene was carried out with primer sequences designed by Ge *et al*[13]. In this method, the IGF-I/SnaBI polymorphism was identified using the Amplification Created Restriction Site (ACRS) methods. The 249-bp fragment of the IGF-I gene was amplified using primers IGF677F: 5′-ATTACAAAGCTGCCTGCCCC-3′, and IGF897R: 5′-AC CTTACCCGTATGAAAGGAATATACGT-3′ (The underlined introduce a restriction site near the point mutation).

The following PCR conditions were applied to each assay; 50 mM KCl, 10 mM Tris-HCl (pH = 9.0), 1.5 mM $MgCl_2$, 200 μM dNTPs, 10 pmol of each primer, and 1 U Taq DNA polymerase (Fermentas, USA) per 20 μL reaction using 2.4 μL of DNA extracted as template. PCR were carried out using a Bio-Rad thermocycler (Bio-Rad Laboratories Inc., Hercules, USA) with the following conditions: with the initial denaturation at 94 ℃ for 5 min, followed by 35 cycles, denaturation at 94 ℃ for 45 s, annealing at 62 ℃ for 1 min and extension at 72 ℃ for 30 s. A final extension at 72 ℃ for 7 min at the end of the amplification cycles was included. Sterile water was used as the negative controls. The PCR products were analyzed by agarose gel 1.5%. The positive reaction products were used for enzymatic digestion by SnaBI (TAC. GTA) restriction endonuclease.

Digestion of the products of RFLP-PCR with the SnaBI nuclease (Fermentas Inc., USA) was carried out in a mixture consisting of 10 μL of the PCR product, 1.5 μL 10× Buffer Tango, 2 μL (30 U) of the enzyme, and 1.5 μL distilled water to a final volume of 15 μL. The reaction was at 37 ℃ for 5 h followed by 20 min of inactivation at 65 ℃. The digested PCR products were analyzed by agarose gel 3% in 1 × Tris-Acetic Acid- EDTA (TAE) buffer. The gels were stained with ethidium bromide (0.5 μg/mL) and visualized under UV light on a transilluminator.

2.4. Statistical analysis

The results were analyzed from the aspect of relationship between the polymorphism within the 5′-UTR of IGF-I gene and its concentration. The serum concentration of IGF-I was compared between cows with different IGF-I genotypes by Kruskal Wallis test. Three categories of cow genotypes include TT, TC and CC. With the observed mean, the effects of C/T transition on the periparturient concentration of IGF-I was statistically analyzed with ANOVA for repeated measures using the GLM procedure of SPSS statistical software. The statistical package SPSS for Windows was used (SPSS for Windows, version 15, SPSS Inc, Chicago, Illinois). Probability values of $P<0.05$ were considered significant.

3. Results

Point mutation and marker genotyping showed that a

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