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Does Uridine Monophosphate Synthase Polymorphism Cause Recurrent Pregnancy Loss

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Abstract

In cattle an inherited Deficiency of Uridine Monophosphate Synthase (DUMPS) due to a single nucleotide polymorphism (SNP), causes stillbirth or death shortly after birth. To determine if this SNP is associated with recurrent pregnancy loss in humans, we analyzed DNA samples from 32 couples with recurrent pregnancy loss (RPL) and 18 fertile control couples using PCR-RFLP and identified their genotype. The results revealed no difference in the genotype between the two groups suggesting that, the DUMPS SNP may not be associated with RPL.

Keywords: Uridine Monophosphate Synthase; DUMPS; Human; Recurrent pregnancy loss

Introduction

Deficiency of Uridine Monophosphate Synthase (DUMPS) enzyme activity has been identified in humans in chromosome 3q13, as an autosomal recessive disease, hereditary orotic aciduria. It is a rare, autosomal recessive disorder of pyrimidine metabolism characterized by elevated orotic acid secretion in the urine, megaloblastic anemia, and failure to thrive [1-3]. The deduced amino acid sequence of Uridine Monophosphate Synthase cDNA clone from bovine shows 84% identity in addition to a high degree of conservation in regions of putative catalytic sites with that of its human counterpart [4]. In cattle, Embryos homozygous for DUMPS do not survive to birth and usually die early in gestation and hence so far, no homozygous recessive animal was detected. The embryos appear to be aborted or reabsorbed approximately 40 days after conception, leading to repeat breeding problems [5-7].

This characteristic deficiency is inherited as a single, two-allele, autosomal locus in which normal males and females are homozygous for the normal allele; deficient males and females are heterozygotes or homozygous genotype for the deficient allele and is lethal in calves with DUMPS. A single nucleotide polymorphism (SNP), transition from C to T in codon 405 that changes CGA (arginine) to TGA (stop), which subsequently produces a functionally impaired enzyme [5,7-11] causes DUMPS.

Awareness of DUMPS and the possible effect on couples with recurrent pregnancy loss (RPL) in humans is currently unknown. The objective of the present study was to ascertain the occurrence of SNP at codon 405 in couples with previous history of RPL in comparison to couples with no history of reproductive failure (fertile controls).

Material and Methods

Buccal scrapings were obtained with sterile swabs from the buccal mucosa, of 32 couples with recurrent pregnancy loss and 18 fertile control couples. The DNA extraction was then performed with QI quick DNA isolation kit (Qiagen, Germany) following manufacturer's protocol. We followed ethical principles in the Declaration of Helsinki in the current study.

Polymerase Chain Reaction (PCR)

A set of primers flanking the SNP was designed to give product of 536 bp.

Forward Primer: 5'- ATTGCGGGAAGGGAAGGGAAAGGGT -3'

Reverse Primer: 5'- TTGGAAGAGCCACGACCAGTGACCAG - 3'

Amplification was performed using 1 µM of each primer, 0.4 mM dNTPs, 2 mM MgCl2 and 0.5U Taq DNA polymerase (Qiagen, Germany) in a 25µl s reaction volume containing 50 ng of genomic DNA template. The amplification conditions were 94°C for 5 minutes, 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 45 seconds for 30 cycles followed by final extension for 5 minutes at 72°C in an ABI 2720 (Applied Bio systems, USA) thermal cycler. The PCR products were resolved on 1.5 % agarose gel and pictures were taken using UVP, Biodoc-it system.

Restriction Fragment Length Polymorphism (RFLP)

An RFLP was performed on the 536 bp PCR product to confirm the C- to T- polymorphism. Two restriction enzymes viz., AvaI which can cut both C or T alleles and a second restriction enzyme XhoI

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which cuts only T- allele, the mutant type was used. The digested products were resolved on a 5% non-denaturing PAGE and stained with ethidium bromide.

Results and Discussion

DUMPS prevalence in cattle appears to be limited to a few countries and the frequency of the mutant allele has been estimated at 1-2% in US Holstein cattle, 0.96% in Argentinian Holstein bulls and 0.11% in Argentinian Holstein cows in studies performed during the1990s [12]. So far, no homozygous recessive animal has been detected. The homozygous recessive embryos appear to be aborted or reabsorbed approximately 40 days after conception, leading to repeat breeding problems [5,6].

Our PCR-RFLP approach was slightly different and used a set of new PCR primers and one additional restriction enzyme XhoI. The digested products were analyzed by non-denaturing Polyacrylamide Gel Electrophoresis (PAGE) as opposed to the use of higher percentage agarose gel, reported in cattle. In the previous reports, the researchers amplified a 108 bp product to detect point mutation in a gene coding for uridine monophosphate synthase and digested using the restriction enzyme AvaI to obtain 3 bands of size 53, 36 and 19 bp for normal animals and 4 bands of 89, 53, 36 and 19 bp, in carrier of the disease [5,7,10,12]. Our current approach is simpler and included a confirmatory step by digesting the PCR products with XhoI and thus improving the specificity of the assay.

The PCR amplification yielded a 536 base pairs (bp) product spanning the point mutation and was subjected to restriction enzyme digestion using AvaI and XhoI in two separate reactions in parallel, for each PCR product. The restriction enzyme AvaI recognizes and digests both wild type- C- and mutant allele- T yielding 440 bp and 96 bp products if present, whereas XhoI specifically recognizes mutant allele T. In the current study, only AvaI restriction enzyme digestion yielded two products, 440 and 96 bp respectively for all the samples. None of the PCR products were digested by XhoI, indicating that, the RPL samples and fertile controls tested were all homozygous -C for the MPS gene, the normal genotypes. This indicates that, factors other than DUMPS were involved in the recurrent pregnancy loss, for the study population with RPL.

Declaration of Interest

Conflicts of interest: None.

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