GENETIC POLYMORPHISM IN THE ALDO-KETO REDUCTASE FAMILY 1 MEMBER B1 (AKR1B1) GENE OF MURRAH BUFFALO BULLS (*Bubalus bubalis*)

V.B. Ahir¹, M.T. Panchal², A.K. Tripathi¹, P.G. Koringa¹ and C.G. Joshi¹

ABSTRACT

The present study was conducted to investigate the existence of polymorphism at the AKR1B1 locus by the PCR-RFLP method. The genotypes were confirmed by checking specific, clearly distinguishable DNA band patterns resulting from digestion with the restriction enzyme *Nde* I. Among the 41 animals studied, 18 samples produced two bands of 463 bp and 333 bp referred as AA genotype, whereas 23 animals produced three bands of 796 bp, 463 bp and 333 bp referred as AG genotype. None of the animals revealed GG genotype. The allelic frequencies of ‘A’ and ‘G’ alleles were found to be 0.725 and 0.275, respectively. Association analysis revealed that none of the genotypes were associated with any semen quality traits studied. The current study is the first report of DNA based genotyping of AKR1B1 gene of this indigenous buffalo breed of India.

Keywords: AKR1B1 gene, Murrah buffalo, PCR-RFLP, genotype

INTRODUCTION

The variation in DNA sequence, as it causes the variation in the performance of animals is the basic material for improving livestock through selection. Embryo implantation is an important step in the establishment of pregnancy and is marked by distinctive biological processes that occur during the preimplantation and early post implantation periods. Preimplantation development directs the formation of an implantation-, or attachment-competent embryo so that metabolic interactions with the uterus can occur, pregnancy can be initiated, and foetal development can be sustained (Watson, 1992; Watson and Barcroft, 2001). Preimplantation development encompasses the period from fertilization to implantation, which occurs on 17-34 days for cow (Ko, 2004). Bovine preimplantation embryo development is under constant control of genes activated from either the maternal or the embryonic genome. Large-scale association studies by genotyping many single nucleotide polymorphisms (SNPs), in individuals with well-characterized phenotypes are considered as promising methods for identifying the cause of many complex traits. Ideally, such studies should be free of biological hypotheses and be done at the whole genome level to maximize the likelihood of success (Tsuchihashi and Dracopoli, 2002).

AKR1B1 is an enzyme in carbohydrate metabolism that converts glucose to its sugar alcohol form, sorbitol, using NADPH as the reducing agent (Chung and LaMendola, 1989).

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This reaction, in particular the sorbitol produced, is important for the function of various organs in the body. Sorbitol is subsequently metabolized to fructose by sorbitol dehydrogenase. Fructose produced from sorbitol is used by sperm cells; also, fructose can be used as an energy source for glycolysis and glyconeogenesis. AKR1B1 participates in glucose metabolism and osmoregulation and is supposed to play a protective role against toxic aldehydes derived from lipid peroxidation and steroidogenesis that could affect cell growth or differentiation when accumulated (Lefrancois-Martinez et al., 2004). High-level glucose concentration which triggers apoptosis during preimplantation in murine embryos (Riley et al., 2004) has led to the over expression of the AKR1B1 gene (Mohan et al., 2002). The AKR1B1, gene known for its 20α-hydroxysteroid dehydrogenase activity, was found to be upregulated in both biopsies derived from blastocysts resulting in no pregnancy and resorption. It may determine the fate of the embryo through the involvement in apoptic pathway (El-Sayed et al., 2006).

**MATERIALS AND METHODS**

The experimental material for the present study comprised of 41 semen ejaculates collected from 41 Murrah buffalo bulls using AV method and evaluated for various semen quality traits, viz., ejaculate volume (ml), individual motility (%), sperm concentration (106/ml), live and dead sperm count (%) and post-thaw motility (%) at Amul Research and Development Association (ARDA), Ode centre, Anand. Genomic DNA was extracted using the Proteinase K method as described by Aravindakshan et al. (1998) and dissolved in 0.3X TE (pH 8.0) buffer. The primer sequences (AKR1B1 F: 5’- ACCAGGGCTTACCTGGAAGT -3’ and AKR1B1 R: 5’- GGTAATGGGCCCTTAGGATT -3’) for amplification of the AKR1B1 gene (Kia, 2007) were used. The PCR was carried out in a final volume of 25 μl reaction mixtures in 0.2 ml thin wall PCR tubes. Each PCR tube contained 12.5 μl of 2X master mix (MBI, Fermentas) containing 0.05 U/μl Taq DNA polymerase (recombinant), MgCl2 (4 mM) and dNTPS (0.4 mM of dATP, dCTP, dGTP, dTTP of each), 3 μl (30 ng/μl) genomic DNA, 1 μl of 10 picomole of each forward and reverse primer and 7.5 μl of DNase-RNase free water. The protocol for PCR amplification for AKR1B1 consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds. and extension at 72°C for 60 seconds. with final extension at 72°C for 5 minutes. The amplification was carried out in Minicycler (MJ Research) PCR machine.

The amplified PCR products of the AKR1B1 gene were resolved on 2% agarose gel electrophoresis along with MassRular Law range DNA Ladder (Range, 80-1031 bp). Restriction digestion of the PCR products was carried out to confirm the identity of the PCR products. The PCR product of AKR1B1 gene was digested with 5.0 U of Nde I restriction enzyme by incubating in a water bath for 14-16 h at 37°C in a 200 μl capacity PCR tube. After restriction digestion, the PCR products were electrophoresed on 2% agarose gel (according to the expected size of fragments) along with 100 bp molecular weight marker and undigested PCR product as a positive control.
Figure 1. Polymorphic pattern of AKR1B1 gene 796 bp PCR fragment digested with Nde I. PC: Positive control, L: Molecular marker. Lane 1, 2, 3, 4, 5, 6, 7, 810, 12, 14, 15, 17 Homozygous AA type. Lane 9, 11, 13, 16, 18 Heterozygous AG type.

Table 1. Genotypes, genotype frequencies and allelic frequencies in Murrah buffalo bulls.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Sample No.</th>
<th>Genotype Frequencies</th>
<th>Allele Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>Murrah</td>
<td>41</td>
<td>0.44</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 2. Mean (±SEM) of different semen quality traits for the AKR1B1 loci.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sperm Concentration (10⁹/ml)</th>
<th>Volume (ml)</th>
<th>Motility (%)</th>
<th>Motility after Thawing (%)</th>
<th>Live and Dead Sperm Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA Mean ± SEM</td>
<td>1494.83±119.99a</td>
<td>3.81±0.48a</td>
<td>73.06±0.92a</td>
<td>53.89±1.036a</td>
<td>84.67±0.77a</td>
</tr>
<tr>
<td>AG Mean ± SEM</td>
<td>1523.78±89.38a</td>
<td>2.99±0.28a</td>
<td>73.91±1.75a</td>
<td>54.78±0.86a</td>
<td>86.04±0.57a</td>
</tr>
</tbody>
</table>

* (p< 0.05)
RESULTS AND DISCUSSION

The PCR amplification generated a 796 bp segment from the buffalo AKR1B1 gene homologous to the bovine AKR1B1 gene of similar length (Figure 1). The target sequence which includes part of intron 7 of the bovine AKR1B1 gene has one polymorphic Nde I site due to a A to G transition mutation (Kia, 2007). Allele A of bovine AKR1B1 having one internal site for Nde I was represented by two fragments of 463 and 333 bp, while allele G is comprised of an intact fragment of 796 bp with no internal site for Nde I restriction enzyme. Genotype AA results in a two fragments of 463 and 333 bp, AB in three fragments of 796, 463 and 333 bp and AG in one fragment of 796 bp on 2.0% agarose gel electrophoresis (Figure 1).

In the present study, the amplified product of 796 bp when digested with Nde I enzyme revealed two distinct genotypes, viz. AA and AG, in 18 and 23 Murrah buffaloes, respectively, while the GG genotype was absent in Murrah buffaloes. For unambiguous typing, some of the samples were digested with excess unit of enzymes (20 U) and for extended duration of digestion to confirm that there was no partial digestion. The allele and genotype frequencies for the AKR1B1 locus were calculated using POP GENE32 software (Yeh et al., 1999) and are given in Table 1.

Because of the paucity of the literature on the AKR1B1 gene in the buffalo, it is difficult to compare with other observations. However, Kia (2007) reported the allele frequencies were 0.82 and 0.18, respectively, for the ‘A’ and ‘G’ alleles in *bos taurus*. The genotype GG was not detected. These frequencies are not much different from our findings and indicate ‘A’ as predominant over the ‘G’ allele.

Association analysis of AKR1B1 gene with various semen quality traits

To study the association the AKR1B1 gene with semen quality traits, genotypes were compared with various semen quality parameters using SPSS software version 12 for calculating means and standard errors of mean (SEM) and the F-test was used at the 5% level of significance. The means for various semen quality parameters, viz., volume (ml), concentration (10⁶/ml), motility (%), motility after thawing (%) and live and dead count (%) were found to be 3.35±0.27, 1511.07±112.25, 73.54±1.05, 54.39±0.66 and 85.44±0.47, respectively. The results were found to be non-significant (P>0.05) (Table 2) and hence it was concluded that there was no association of genotypes GG and GH with any of the semen quality traits studied.

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REFERENCES


