Animal Reproduction

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Multiple mutations can be found in the exon 11 of prolactin receptor gene in crossbred bovine embryos

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Cattle with the SLICK haplotype have been characterized by a sleek and short hair coat, and one of the primary benefits of the SLICK haplotype is its role in improving thermoregulation in cattle, particularly in hot and humid climates. Causal variant responsible for the slick phenotype in cattle is primarily located in the 11th exon of the prolactin receptor gene, however it should be noted that not all variants found in this region result in the slick phenotype (Porto-Neto et al., Front. Genet., 9:57, 2018). Nevertheless, these single alleles remain crucial for matters of guide design in CRISPR experiments, particularly those aimed at knocking out or modifying the prolactin receptor gene. The identification of these single alleles contributes to a more comprehensive understanding of genetic variation in the region and can assist researchers in designing more precise and effective guide RNAs for their experiments. Consequently, even alleles that do not directly contribute to the slick phenotype possess significant value in advancing our knowledge of the underlying genetic mechanisms implicated in this essential trait. The aim of this study was to assess the genome sequences of in vitro fertilized (IVF) Bos taurus x Bos indicus crossbred cattle embryos, with a particular focus on the PRLR region. Blastocysts were individually collected and subjected to DNA extraction using a two-step incubation method with proteinase K (1,5ug/uL) lysis buffer. Subsequently, PCR amplification was conducted in duplicate and the PCR fragments were submitted to Sanger sequencing. Sequence analysis was performed using Unipro Ugene software (Okonechnikov K., et al. Bioinformatics, 28 (8):1166-7, 2012). A total of 15 samples were analyzed and it was observed that 33.3% (5/15) of the samples exhibited a single mutation (C>T) at position 39099463, resulting in a substitution from a serine to a stop codon that has not been reported before. In addition, a pair of missense mutations were identified in a closely located region, with position 39099322 showing a mutation (G>T) from an arginine to a leucine in 60% of the samples, and position 39099190 exhibiting a mutation (C>T) from a serine to a leucine in all samples. Lastly, a silent mutation was identified at position 39099368, potentially resulting in a substitution of cytosine by thymine in 60% of the samples, which would lead in both cases to the synthesis of a tyrosine. Based on the results obtained from the initial analysis, it can be inferred that this region has an increased potential for genetic variation. Therefore, it is recommended to inspect the target genomic region of crossbred animals and compare with Bos taurus one before designing guide RNAs aiming to introduce indels to promote the slick phenotype. In conclusion, this study's findings provide valuable insights into the genetic variation of the PRLR region in cattle, which may influence the gene editing efficiency.

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