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Genome-wide association study for sperm membrane integrity in frozen-thawed semen of Holstein-Friesian bulls

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ABSTRACT

The aim of the study was to screen the entire bull genome to identify SNP markers and propose candidate genes potentially involved in the variation of sperm membrane integrity in Holstein-Friesian bulls. Two hundred eighty eight bulls kept in one AI center were included in the study. Each bull was genotyped for 54.001 Single Nucleotide Polymorpisms (SNP) by the Illumina BovineSNP50 BeadChip. Commercial straws of frozen-thawed semen were used for the evaluation of sperm plasma membrane integrity (SYBR-14/PI staining) and sperm mitochondrial function (JC1/PI staining). An additive model for Linear Regression Analysis was applied to estimate the effect of SNP marker for sperm membrane integrity (by the use of GoldenHelix SVS7 software). Five significant markers (encompassing 2,2 MB region located on chromosome 6) for SYBR-14/PI were found. Among them one marker—rs41570391 passed Bonferroni correction test. Within approximately 3 Mb genomic region including significant markers three candidate genes: SGMS2 (Sphingomyelin Synthase 2), TET2 (Methylcytosine dioxygenase 2) and GSTCD genes (Gluthatione S-transferase C terminal domain) were proposed as potentially involved in sperm membrane integrity in frozen-thawed semen of Holstein-Friesian bulls.

1. Introduction

Genetic background of sperm quality is a subject of genome-wise studies but mostly for basic traits of fresh semen, like low sperm concentration and motility (Aston and Carnell, 2009; Aston et al., 2010; Hering et al., 2014a), low sperm motility (Hering et al., 2014b), variation in semen volume and total number of sperms (Hering et al., 2014c) or concentration of hormones involved in Braham bull fertility (Fortes et al., 2012, 2013). Semen with standard quality can however show reduced fertility (subfertility) (Gnoth et al., 2005). Among many factors contributing to bull fertility (Mathevon et al., 1998), status of sperm membranes should be considered as significant factor in overall sperm function (Pena, 2007; Amaral et al., 2013). Sperm plasma membrane reacts on physiological signals and protects sperm against extracellular damage, participates in sperm capacitation and sperm-egg interaction, both having crucial role in effective fertilization (Flesch and Gadella, 2000; Primakoff and Myles, 2002). Most of the research was focused on how cryopreservation technology affects sperm membrane integrity (Tapia et al., 2012). Genetic factors influencing variation in this trait were not studied, with the exception of the work pub-

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lished by Pausch et al. (2014) in which a nonsense mutation within TMEM95 encoding a nondescript transmembrane protein was described as the cause of the idiopathic male subfertility in Fleckvie bulls.

In this paper we screened a population of Holstein-Friesian bulls by 54 thousand SNPs evenly spread across the genome and perform GWAS to find markers associated with variation of sperm membrane integrity.

2. Materials and methods

2.1. Animals

The analyzed data set originates from the Holstein-Friesian dairy cattle population and consisted of 288 bulls from one AI station. The bulls included in the analysis were at a similar age (12–18 months) and were kept in uniform feeding and housing conditions. All bulls underwent routine evaluation of testis and none of them showed clinical symptoms affecting the volume of ejaculate or total number of sperm. Sperm motility was also assessed in AI center and all analyzed bulls achieved at least 70% of motile sperm. Finally, each bull was approved by AI company as producing semen with standard quality.

2.2. Phenotypic data

Sperm plasma membrane integrity was assessed using dual fluorescent staining, SYBR-14 and PI (Live/Dead Sperm Viability Kit; Molecular Probes), as described by Garner and Johnson (1995), with slight modifications. Briefly, aliquots of sperm samples (20×10^6 spermatozoa/cm^3) were incubated with SYBR-14 (1 mM SYBR-14 in DMSO) and PI solutions (2.4 μM PI in Tyrode's salt solution) for 10 min at 37 °C. Following incubation, stained sperm cells were placed on microscopic slides and examined at 600× magnification under a fluorescence microscope (Olympus CH 30 RF-200, Tokyo, Japan). Sperm cells displaying only bright green fluorescence were considered viable spermatozoa with an undamaged plasma membrane. A minimum of 100 cells per slide was examined in random fields of each aliquot.

The sperm mitochondrial function was assessed using dual staining with fluorescent probes, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide, JC-1 (Molecular Probes, Eugene, USA) with propidium iodide (PI, Sigma Chemical Co., St. Louis, MO, USA), according to a previously described method (Thomas et al., 1998), with some modifications (Dziekońska et al., 2009). Aliquots of sperm samples (20×10^6 spermatozoa/cm^3) were incubated with JC-1 solution (1 mg JC-1/cm^3 dimethylsulfoxide, DMSO) for 15 min at 37 °C. Following incubation, sperm samples were stained with PI (10 μl of PI solution in 0.5 mg/cm^3 phosphate buffered solution) for 10 min at 37 °C, washed (600× g, 5 min at room temperature) and the sperm pellets were re-suspended in a HEPES buffered solution (10 mM HEPES, 0.85% NaCl, 0.1% bovine serum albumin, pH 7.4). Stained sperm samples were placed on microscopic slides, covered with coverslips (22 × 22 mm) and examined under a fluorescence microscope (Olympus CH 30 RF-200). Viable spermatozoa with functional mitochondria emitted orange-red fluorescence. Two slides were evaluated per sample and 200 spermatozoa were counted per slide.

For evaluation of both traits a mix o 3 randomly chosen semen straws were used.

2.3. Genotypic data

Genomic DNA was isolated from the half volume of one commercial semen straw using the Wizard Plus Megapreps DNA Purification System (Promega). DNA was then used to genotype each bull by Illumina BovineSNP50 BeadChip, which consists of 54,001 SNP markers (Version 1) or 54,609 SNP markers (Version 2). In the final analysis, only common SNPs included in both panels were used. For the combined set of SNPs present on both panels, quality data analysis using GenomeStudio (version 2011.1, Illumina, San Diego, CA) was performed.

2.4. Data analysis/statistical tests

The associations between SNP markers and traits analyzed were tested using GoldenHelix SVS7 software (Bozeman, MT, USA). Initial data cleanup was done to remove poorly-performing SNPs or/and samples giving an insufficient number of informative genotypes. Markers from 29 autosomes and X chromosome were included in the analysis. All samples with individual genotypes had call rates over 98%. Monomorphic SNPs (n = 6514) and SNPs which showed a significant ($P < 0.001$) deviation from the Hardy-Weinberg equilibrium (HWE) (n = 1860) were removed prior to association analysis. Additionally, the SNP selection criteria were applied: a minor allele frequency (MAF) of at least 0.01 and a minimum call rate of 98%. Linkage disequilibrium pruning was not used. After all editing, 38 089 SNPs were selected for statistical analysis.

An additive model for Linear Regression Analysis was applied to estimate the effect of SNP marker for both sperm membrane integrity traits (by the use of GoldenHelix SVS7 software). Each trait was analyzed separately and two genotype association tests were performed. Bonferroni correction at $p < 0.05$ was applied.

Our model did not require corrections for additional fixed and random effects since bulls were at similar age, kept in the same feeding and welfare conditions and the semen was collected and evaluated by the standardized procedures.

Kruskal-Wallis test was used to assess the significance of differences of plasma membrane integrity between bulls having different genotype for the most GWAS-significant marker.

2.5. Searching for candidate genes

Candidate genes were searched in immediate vicinity to GWAS-significant SNP markers. The genomic positions of SNPs included in the Illumina BovineSNP50 BeadChip (Version 2) were taken from the Illumina publication (www.illumina.com). The genomic positions of candidate genes were assigned based on the UMD 3.1 bovine genome