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Genome-wide association study for semen volume and total number of sperm in Holstein-Friesian bulls

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ABSTRACT

In artificial insemination industry bulls producing high volume of semen with relatively high concentration of sperm are very desirable since they ensure stable production of commercial straws especially in case of top bulls. The aim of the study was to screen the entire bull genome to identify markers and candidate genes underlying semen volume (SV) and total number of sperm (TNS) in ejaculate produced by Holstein-Friesian bulls. Data on semen production were retrieved from records of AI center and included a population of 877 Holstein-Friesian bulls. Each bull was genotyped using the Illumina BovineSNP50 BeadChip. Genome-wide association analysis was performed with the use of GoldenHelix SVS7 software. An additive model for Linear Regression Analysis was used to estimate the effect of SNP marker for SV and TNS. After Bonferroni correction, 3 markers located on chromosome 22 reached the highest significance (rs41625599, rs41584616, rs42012507) for both traits. In the vicinity of these significant markers 3 genes are located (*DCP1A*, *SFMBT1*, *TMEM110*). Moreover, marker rs110109069 located on chromosome 25 was significantly associated with TNS and marker rs42438348 located on chromosome 10 has been found to be associated with SV. Some additional candidate genes were suggested to be potentially involved in analyzed traits (*GALC*, *PRKCD*, *PHF7*, *TLR9*, *SPATA7*). Identifying SNPs associated with the lower total number of sperm may be very useful for early recognition of a young sire as less suitable for effective semen production in artificial insemination centers.

1. Introduction

Efficient and regular production of high quality semen is very desirable trait in bull used in artificial insemination (AI) industry. Unpredictable fluctuations in semen production can cause significant economic losses and disturb the distribution and marketing of semen. AI industry reports (personal communication) that there is a wide variation in semen volume and sperm concentration which make a risk of unexpected decrease in the number of straws produced, especially in the period of its high demand coming from the market. The simple way to assess semen production is to evaluate the testis weight (Coulter and Foote, 1979). Sperm output in young dairy bulls is highly correlated with the measurements of scrotal circumference (Hahn et al., 1969) and also is influenced by the shape of the testis (Bailey et al., 1996). Currently however, when pre-selection of bull calves is the routine practice, direct or indirect measurements of testis has limited value and therefore age-independent prediction of semen production are more desirable.

Although semen volume and total number of sperm depend on many environmental factors (Mathevon et al., 1998), they also have substantial genetic component (Druet et al., 2009; Fortes et al., 2012, 2013). So far, most of the research focused on genetics factors underling semen

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deficiencies in humans (Aston and Carnell, 2009; Aston et al., 2010; Sang et al., 2011; Hu et al., 2011; Wu et al., 2013; Xu et al., 2013). Genes underlying the volume of semen and total number of sperm in ejaculate of fertile bulls were not studied. In our earlier work (Hering et al., 2014) we found SNP markers associated with extremely poor sperm motility and suggest some candidate genes involved in this undesirable trait. The aim of this work is to identify markers and candidate genes associated with the variation in semen volume and total number of sperm – traits of high importance for AI stations.

2. Materials and methods

2.1. Phenotypic data

877 Holstein-Friesian bulls kept in one AI station were included in the analysis. The bulls have similar age (12–18 months) and were kept in uniform feeding and housing conditions. Semen was collected at weekly intervals, at least 19 times from each bull. Artificial vaginas maintained at 41 °C were used to collect ejaculates. Immediately after the collection, semen was evaluated on the basis of color, mass activity and individual motility. Semen volume (SV) was measured in glass buret scaled in accuracy of 0.1 ml. Sperm concentration was assessed in fresh spermatozoa by the standard spectroscopic method. Total number of sperm (TNS) was calculated by multiplying sperm concentration and semen volume. The average values for semen traits in the studied population of bulls are presented in Table 1.

All bulls underwent routine evaluation of testis and none of them showed clinical symptoms affecting the volume of ejaculate or total number of sperm. The general condition of bull's testis was evaluated visually and by palpation. All bulls have testis size and shape within physiological range, relatively to their age.

2.2. 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 using the 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, preliminary quality data analysis using GenomeStudio (version 2011.1, Illumina, San Diego, CA) was performed.

Table 1
Characteristics of basic traits of semen in 877 Holstein-Friesian bulls.

Traits	Unit	Mean	*SD*
Semen volume	ml	3.75	1.21
Concentration of sperms	mln/ml	1275.94	348.45
Sperm motility	%	56.94	15.24
Total number of sperm	mln	4974.93	2147.04

2.3. Data analysis/statistical tests

The association between SNP markers and traits analyzed was tested using GoldenHelix SVS7 analysis software (Bozeman, MT, USA). Initial data cleanup was performed to remove poorly performing SNPs or/and samples giving an insufficient number of informative genotypes. Markers from 29 autosomes were included in the analysis. All samples with individual genotypes had call rates over 98%. Monomorphic SNPs ($n = 5571$) and SNPs which showed a significant ($P < 0.001$) deviation from the Hardy–Weinberg equilibrium (HWE) ($n = 2209$) were removed prior to association analysis. Additionally, the SNP selection criteria were applied: a minor allele frequency (MAF) of at least 0.05 and a minimum call rate of 98%. Linkage disequilibrium pruning was not used. After all editing, 35,093 SNPs were selected for the final analysis.

An additive model of the Linear Regression Analysis was used to estimate the effect of SNP markers for SV and TNS. Each trait was analyzed separately and two genotype association tests were performed. Bonferroni correction at $P < 0.05$ was applied. Based of at least 19 samples from each bull, average values for SV and TNS were calculated and then used in statistical model.

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. Also, review of sperm collection dates in our dataset revealed that there was no significant seasonal trend in the variation of both traits (data no shown).

2.4. Searching for candidate genes

Candidate genes were searched in the vicinity of significant 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 assembly (Zimin et al., 2009) through the current Ensembl database (www.ensembl.org).

3. Results

3.1. SNP genotyping

The average SNP call rate across all autosomes was 99.89% ($SD = 0.2\%$) and the average call rate across SNPs (with non-autosomal markers) included in the analysis was 99.79% ($SD = 0.2\%$). The average heterozygosity rate across all autosomes and across SNPs (with non-autosomal markers) was 0.37 ($SD = 0.001$). For these sets of SNPs, the average MAF was 0.47 ($SD = 0.008$).

3.2. Association analysis

Manhattan plot (Fig. 1) presents an overview of all significant SNP effects for SV and TNS and shows the $-\log_{10}$(*P*-value) for the associations of 35,093 SNP. It is demonstrated that 46 SNPs and 37 SNPs reached