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Short communication

Genome-wide association studies of cryostability of semen in roosters

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Abstract

Conservation of genetic resources by semen cryopreservation is essential for biodiversity conservation and storage of rare poultry breeds. Despite the widespread use of this method not all individuals presentia similar capacity for semen to be used after defrosting. The aim of the current study was to identify SNP markers and linked candidate genes potentially associated with rooster (*Gallus gallus*) sperm motility after cryopreservation. Genome-wide association studies were performed using 33 roosters from four breeds genotyped using Illumina Chicken 60K SNP BeadChip Calculations were performed using PLINK and EMMAX software. Significant SNP associations rs15557972 ($p<1.36E-07$) on chromosome 10 in the LOXL1 gene and rs15751385 ($p<6.10E-06$) on chromosome 6 in the intron of the ENSGALG00000052127 gene were identified. These findings associated with sperm motility SNPs will help to develop strategies for the selection of valuable individuals and the efficient conservation of the gene pool.

Key words: Genome-Wide Association Studies, cryostability, rooster sperm

Introduction

Sperm cryopreservation increases the efficiency of poultry breeding and is necessary for gene pool preservation (Thélie et al. 2018). Studies have shown that variants of SNP loci can be effectively used as genetic markers for various breeding traits (Meuwissen et al. 2017). Determining the genetic basis of mutations affecting the fertility of roosters has attracted the attention of researchers (Thélie et al. 2019). However, these works are aimed at studying the relationships of the genome with native sperm quality traits and defects in spermatogenesis. The search for genomic associations with sperm quality during cryopreservation has been carried out only on bulls and on a small number of indicators (membrane integrity using SYBR-14/PI staining and mitochondrial function using JC1/PI staining) (Kamiński et al. 2016, Kaminski et al. 2019).

In our study, we studied genetic determinants of sperm motility by genome-wide association studies (GWAS) in 33 roosters (Gallus gallus) of the Russian White, Silkie White, Rhode Island and Aurora breeds from the RRIFAGB Biological Resource Collection

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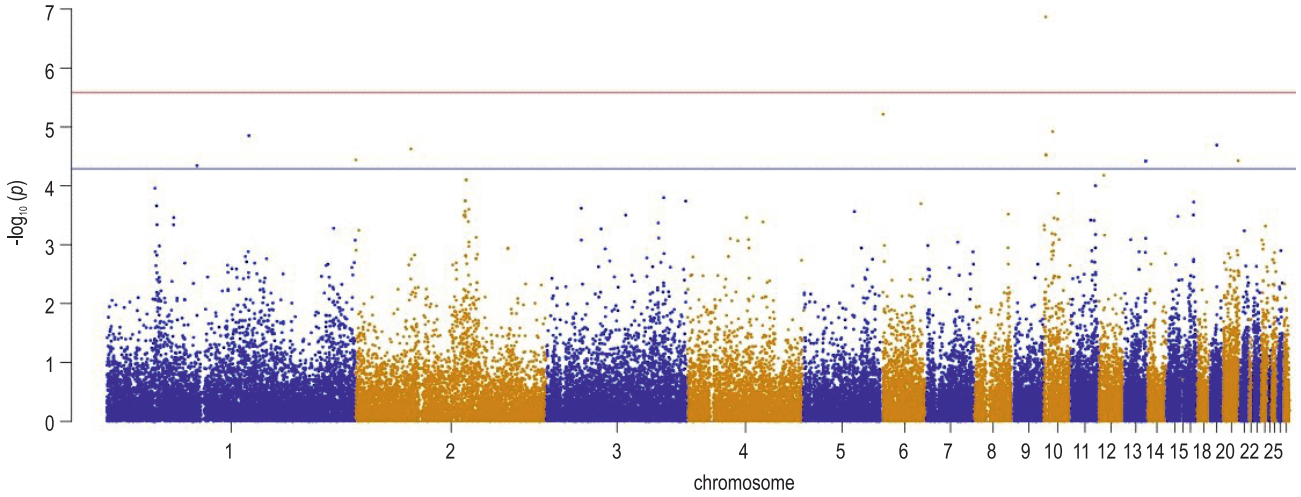

Fig. 1. Manhattan graph for the distribution of SNP significance level for chromosomes for the trait of sperm activity after cryopreservation genotyped with the Illumina Chicken 60K SNP iSelectBeadChip.

"Genetic Collection of Rare and Endangered Chicken Breeds" (RRIFAGB, Pushkin, St. Petersburg).

Materials and Methods

DNA was isolated from poultry blood samples using the phenol/chloroform method. Blood samples were taken from the wing vein of chickens according to the standard venipuncture procedure. The chickens remained alive after taking blood and were not slaughtered after the study. The concentration of DNA ranged from 50 to 500 ng/µl. DNA samples were analyzed using the Illumina Chicken 60K SNP iSelect BeadChip. The object of the study was the semen of roosters (Gallus gallus) of Russian white, Silkie White, Rhode Island and Aurora breeds from the RRIFAGB bioresource collection "Genetic Collection of Rare and Endangered Chicken Breeds" (RRIFAGB, Pushkin, St. Petersburg). As a result of the selection of the best cocks for sperm quality, 33 individuals and 77 ejaculates before and after freezing were assessed by sperm motility. Sperm motility was evaluated visually. Of the DNA samples with genotyping quality at SNP loci, more than 95% were selected for analysis, which was carried out using the GenomeStudio program (Illumina, USA). SNP selection was carried out using PLINK with the following parameters for analysis: autosomes, MAF>0.01, Hardy-Weinberg error limits – HWE (p<0.0001). Genome-wide association studies were performed using EMMAX software (Kang et al. 2010) and kinship matrix (IBS). The following model was used to calculate effect of SNP on sperm motility: Y = Xb +u +Br+ e, where Y is a vector of phenotypes, b is an SNP effect, X is a design matrix of SNP genotypes, u is a vector of additive genetic effects assumed to be normally distributed with the mean equal to 0 and (co) variance σ2a G, with σ2 a as the additive genetic variance and G as the genomic relationship matrix, Br is breed effect, and e is a vector of random residual effects.

From the GWAS results using the qqman package in the R software, a Manhattan-plot graph was obtained. Genes within the candidate genomic region were determined using the chicken assembly Gallus_gallus-5.0 (https://www.ncbi.nlm.nih.gov/grc/chicken). SNP information in the corresponding genes was determined using the genomic browsers NCBI and Ensembl.

Results and Discussion

Total motility of fresh sperm ranged from 40% to 95% (87.9±0.83% , mean±SE). Post-thawed motility was from 0% to 75% (45.2±2.2 %). We conducted GWAS with a difference in the motility of fresh and post-thawed semen. We found some SNPs at the genome-wide level. SNP associations of rs15557972 (p<1.36E-07) were detected on chromosome 10 in the intron of the LOXL1 gene (Lysyl oxidase like 1) and rs15751385 (p<6.10E-06) on chromosome 6 in the intron of the ENSGALG00000052127 gene (Fig. 1) When G is replaced by T (rs15751385), we observe a significant (with a difference of 22%, p<0.01) decrease in sperm motility after cryopreservation. When replacing A with C (rs15557972), the decrease in motility also significantly (p<0.01) changed from 36% to 57% (Table 1). The best complex genotype for these substitutions was GGAA (p<0.01). Roosters with this genotype had the best result for maintaining sperm motility after freezing.

The suggested candidate gene, LOXL1, was pre-

Table 1. Indices of native sperm and assessment of the effect of cryopreservation on semen of roosters of various genotypes.

Rooster genotype	n	Concentration billion/ml	Volume of ejaculate, ml	Difference in motility between fresh and frozen sperm, %
rs15751385 – GG	24	2.17±0.24	0.59±0.05	39.17±3.83[a]
rs15751385 – GT	8	2.75±0.45	0.54±0.07	61.25±7.12[b]
rs15557972 – AA	19	2.1±0.3	0.64±0.06	35.79±4.05[c]
rs15557972 – AC	12	2.5±0.3	0.51±0.05	57.08±5.82[d]
GGAA (rs15751385, rs15557972)	19	2.1±0.3	0.64±0.06	35.79±4.05[e]
GGAC (rs15751385, rs15557972)	13	2.6±0.33	0.49±0.05	57.69±5.39[f]
TGCA (rs15751385, rs15557972)	8	2.75±0.45	0.54±0.07	61.25±7.12 [g]

a-b, c-d, e-f, e-g – $p<0.01$

viously identified as a constituent of eggshell membranes. (Du et al. 2015).

This study identified SNPs associated with the conservation of rooster sperm motility after cryopreservation, which may help in developing strategies for the selection of valuable individuals and the efficient conservation of the gene pool.

Our methods for detecting the candidate genes underlying the cryoresistence sperm were efficient, although the relatively few samples was one potential limitation of this study. Future sequencing of the candidate genomic region is necessary to confirm and extend our findings.

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