Abstract

Analysis of DNA polymorphisms and expression of selected genes associated with different freezability of boar spermatozoa

This thesis was performed in three studies. In the first study, variant calling of transcriptome sequencing data (RNA-Seq), which allowed the comparison of sperm transcriptome profiles from boars differing in freezability, was performed to identify single nucleotide polymorphisms (SNPs). Variant calling identified more than 1000 SNPs, in which most of them were detected at the 3′-untranslated regions (3′-UTRs). Forty SNPs were used for SNP genotyping by the KASP assay. Analysis showed associations of SNPs with some of the selected parameters of sperm functions following freezing-thawing. Analysis confirmed significant associations of *APPL1*, *PLBD1*, *FBXO16*, *EML5*, *RAB3C*, *OXSR1*, *PRICKLE1* and *MAP3K20* gene polymorphisms with the quality characteristics of frozen-thawed (FT) spermatozoa. It can be suggested that polymorphisms in the candidate genes are potential markers for post-thaw quality of boar semen.

In the second study, the 5′-flanking region sequences of *STK35*, a stress-related gene, and *IFT27*, a motility-related gene, were PCR amplified and analyzed by Sanger sequencing method to identify polymorphisms in spermatozoa of boars considered as having good and poor semen freezability (GSF and PSF, respectively). Bioinformatics was used to study the interactions of the identified SNPs with transcription factors (TFs) on the gene promoter activity. One SNP, rs327863835 (C > T) was detected in the *STK35* promoter, while two SNPs (rs337563873; A > T; rs331520020, T > C) were detected in the *IFT27* promoter. Significant allele frequency differences of *STK35* and *IFT27* promoter polymorphisms were observed between the freezability groups. It was predicted that *STK35* rs327863835 and *IFT27* rs331520020 resulted in the generation of additional binding sites for TFs, such as NFATC2, ELK1 and GR-β, which might regulate the transcriptional activity of the genes in either

freezability group. Furthermore, protein expression of STK35 and IFT27 varied between the fresh and PT semen, however, IFT27 protein expression was more marked in FT spermatozoa of the GSF group. It could be suggested that the allelic variants in *STK35* and *IFT27* can serve as potential markers for predicting the freezability of boar semen.

In the third study, it was investigated whether the expression of stress-related genes (*TXNRD1*, *HSPA4L* or *ATP1B1*) was associated with sperm cryotolerance. Higher relative mRNA expression levels of *TXNRD1* and *HSPA4L* were more marked in FT semen of the PSF group compared with the fresh semen. Likewise, HSPA4L protein expression was markedly higher in FT semen of the PSF group, while the fresh semen exhibited significantly higher expression of TXNRD1 protein in the GSF group. The significantly higher level of *ATP1B1* mRNA expression in the fresh semen of good freezability ejaculates could be used as a promising marker for semen freezability. Furthermore, variations in TXNRD1 and HSPA4L protein expression, and the electrophoretic protein profiles of FT semen between the freezability groups provide more insights into the role of sperm proteins in cryo-damage.

These studies indicate that the application of genetic markers for sperm freezability would contribute to the improvement in the cryopreservation of boar semen through the selection of individuals with high freezability ejaculates.