Cryopreservation of boar semen plays an important role in the introduction and preservation of superior genetic resources from males with extremely high breeding values. However, its efficiency has often been compromised by the substantially reduced fertilizing capacity of frozen-thawed spermatozoa. Optimal sperm cryosurvival depends not only on the cryopreservation technologies, but also on individual variations to sustain cryo-induced injury. Irrespective of breed or genetic line, important boar-to-boar differences in semen freezability have been well established, and boars can be objectively classified as good or poor freezers, according to their post-thaw semen quality. It should be emphasized that boar variability in semen freezability has a genetic basis. Therefore, the main objective of this project will be to genetically improve post-thaw boar sperm quality by the identification of markers linked to genes controlling semen freezability. This task will be performed by the high-throughput RNA sequencing (RNA-Seq) technology with the Illumina NGS platforms. The proposed RNA-seq will i) provide *de novo* assembly of sperm genome of the Polish Large White (PLW) breed, ii) enhance significantly the current genome annotation of *Sus scrofa* and improve our understanding of the reproductive traits in the boar that are associated with semen freezability and iii) identify annotated and un-annotated genes linked to markers associated with good and poor freezability boar ejaculates.

A total of 296 ejaculates were collected from 40 Polish Large White boars. Using a variety of phenotypic sperm parameters, such as CASA-analyzed motility characteristics (TMOT, PMOT), viability (SYBR-14/PI and CFDA assays, HOS test), mitochondrial status (JC-1/PI assay, ATP content) and DNA integrity (neutral Comet assay), provided a comprehensive assessment of post-thaw semen quality, and will increase the chances of identifying potential markers associated with freezability. Thorough analysis of the post-thaw semen quality showed that 21 boars were classified as good freezers and 19 boars were poor freezers. According to the research objectives, the project will be divided into four main work programs: i) isolation of total RNA from boar spermatozoa and its quality control (QC)**,** ii) RNA-seq experiment of *Sus scrofa* sperm transcriptome using NextSeq Illumina, iii) bioinformatics analysis of sperm transcriptome of Polish Large White boars, and iv) validation of differentially expressed (DE) genes and SNPs markers potentially associated with freezability of boar semen. A modified version of the TRIzol protocol will be used to isolate high quality total RNA from spermatozoa of each boar. However, isolated sperm RNA from 6 boars, that is, 3 boars each with good and poor semen freezability, respectively, will be subjected to RNA-seq, using NextSeq 500 Illumina, followed by advanced bioinformatics analysis. Validation of differentially expressed (DE) genes by qRT-PCR analysis and SNPs markers will be performed on a larger animal population (n=40 boars) to identify markers showing an association with semen freezability. It is expected that about 20 candidate genes (both up-regulated, down-regulated and with no regulation according to RNA-seq data) will be subjected to detailed analyses. In addition, the levels of expression of the sperm proteins of the respective mRNA transcripts (approximately 20 transcripts) will be determined by Western blotting analysis to unravel their biological functions in sperm cryotolerance.

Traditionally, pig genomics and reproduction scientific community in Poland and worldwide have been targeting their research goals through the effective implementation of breeding programs for reproduction traits, using marker-assisted selection (MAS), gene-assisted selection (GAS) or breeding for genomics selection (BGS) in the pig breeding populations. Therefore, the overall objective of the proposed project will be to utilize the novel RNA-Seq technology on *Sus scrofa* sperm transcriptome to effectively implement the above-mentioned programs in the Polish pig breeding practices. It is expected that such an approach will help to improve the technology of boar semen cryopreservation through the selection and identification of markers linked to genes controlling freezability. Furthermore, it is envisaged that both dbESTs and dbSNPs of porcine sperm genome will be disseminated to the NCBI database, contributing to the genetic improvement in the economic traits associated with reproductive technologies in the boar, such AI and semen cryopreservation. The realization of this project will also ensure genetic improvement in boar semen cryopreservation for AI stations in Poland and may serve as an impulse to create regionalized cryobanks. The existence of such cryobanks will allow for the international exchange of cryopreserved boar semen and for expanding the gene pool. In addition, the effect of this project will be a cycle of original works that will be published in scientific journals at national and international level, as well as in papers that will be presented at international conferences and seminars. An element of the comprehensive application of the project results will be their use as the basis for further projects on semen cryopreservation in other domestic animal species.