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Editorial

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A Novel Approach to Assess Semen Freezability

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Cryopreservation of semen plays an important role in the preservation of genetic resources and its efficiency has been affected by compromised post-thaw sperm quality.[1] Considerable evidence suggests that genetic differences have contributed to the wide variations in post-thaw semen quality, and have encouraged the selection of animals whose semen can withstand the cryopreservation procedure.[2] In the case of boar, variations in semen freezability have been shown to be related to molecular genetic markers.[2] However, a thorough analysis of the specific genetic markers facilitating freezability will be the most efficient approach to improve the technology of semen cryopreservation. Specifically in the boar, such novel approach is based on transcriptome analysis of ribonucleic acid-sequencing or RNA-sequencing (RNA-seq) data from spermatozoa from individuals with poor and good semen freezability. Moreover, transcriptome analysis of spermatozoa has identify potential messenger RNA (mRNA) profiles that could serve as marker for bull semen freezability has been reported.[3] So far, RNA-seq data have been widely used in the analysis of different cellular tissues, but its application in transcriptome study on semen freezability is limited. Therefore, it is hypothesized that transcriptome study on poor and good freezability ejaculates, in conjunction with bioinformatics studies, will provide a more comprehensive approach for the identification of novel sperm-specific gene transcripts facilitating sperm cryo-tolerance.

In animal breeding, even though there has been some progress in characterizing the major genes involved in sperm function,[3] there has been no genetic selection of animals for semen cryopreservation. As regard boar semen, selection is important in an efficient cryopreservation protocol, and is mainly performed by the assessment of post-thaw sperm quality, such as computer-assisted semen analysis (CASA)-analyzed motion parameters, mitochondrial membrane status, functional and structural integrity of membranes, nuclear deoxyribonucleic acid (DNA) integrity, and the application of protein markers.[4] Even though some sperm protein markers have been shown to be associated with high cryo-tolerance, their function is dependent on the presence of mRNA.[1] It has been suggested that the analysis of RNA from spermatozoa might provide valuable information about their biological function.[3] In this respect high-throughput RNA-seq (Illumina RNA-seq), using the high-throughput next generation sequencing (HT-NGS) technology that provides greater resolution for transcriptome profiling compared with other microarray technologies,[5] is performed to identify candidate genes associated with poor and good semen freezability. By utilizing sperm transcriptome profiling of RNA-seq data from different freezability groups, in combination with advanced bioinformatics screening, including transcriptome assembling**,** differentially expressed (DE) gene analysis**,** detection of markers for single nucleotide polymorphisms (SNPs)**,** gene annotation and gene ontology analysis**,** multiple candidate genes associated with the sperm biological functions will be selected for further investigations, using a large animal population. Moreover, a validation procedure is required to reaffirm whether the candidate genes and putative SNP markers could contribute to high semen freezability. The DE genes are validated by quantitative real-time transcription polymerase chain reaction (qRT-PCR) analysis, whereas the KASP™ assay (LGC Genomics, Middlesex, UK) is used to analyze the SNP markers. Since the combination of transcriptome and proteomic studies is necessary to provide more insights in the mechanisms responsible for differences in sperm cryo-tolerance among individuals,[1] Western blotting analysis is performed to determine the expression levels of the sperm proteins of the respective mRNA transcripts.

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RNA-seq of spermatozoa will lay the foundation for more detailed investigation on a number of sperm-specific gene transcripts associated with poor and good semen freezability. Moreover, sperm transcriptome analysis, coupled with bioinformatics studies, will enable better understanding of the roles of the genetic markers in semen freezability, thus providing a new opportunity to efficiently improve the selection of animals for cryopreservation. It is envisaged that the genetic markers will have a great potential to improve the cryopreservation technology of semen from different animal species. Supported by a project from the National Science Centre, Poland (2015/19/B/NZ9/01333).

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