Cryotolerance markers: Proteomics of boar semen from good and bad freezers

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The identification of high and low cryotolerance ejaculates is one of the biggest difficulties in the cryopreservation of boar semen, because an optimal sperm quality pre-cryopreservation does not necessarily guarantee a good quality after thawing. Cryopreservation of boar semen in pig farming remains restricted, because the sperm cell is highly sensitive to thermal and osmotic changes that occur during cryopreservation protocols, which directly interferes with the fertility of the sperm cells. The particularities that induce greater cryotolerance of some boar ejaculates remain poorly understood. The present study aims to identify which proteins act in the differentiation of boars with high and low cryotolerance and verify how they act for such differentiation. The study was based on evaluation of semen quality of 27 boars, evaluated before and after freezing. The criterion for dividing the groups was the quality of the semen after thawing, being divided into good and bad freezer boars. After classifying the groups, proteomic analyses of seminal plasma and spermatozoa were performed under LC-MS/MS. Mass-spectrometric data were processed with MaxQuant software; once identified and quantified proteins, intensity values of label-free quantification were analyzed with Perseus software and expression data of groups and treatments, 0 and 24 hours of holding time, were compared. Spermatozoon and seminal plasma were analyzed separately. Proteins with significant differences were submitted to Pathway enrichment analysis, to Protein-Protein Interaction and Social Network Analysis to identify biological pathways, comprehend molecular interactions and to identify biological entities who contribute to sperm cryotolerance at systemic level. The method of least squares discriminant partial analysis (PLS-DA) and principal component analysis (PCA) were used for multivariate analysis and an analysis of variance (ANOVA) with Bonferroni correction were applied for significance analysis and P-values ? 0.05 were considered significant. A total number of 2105 proteins were identified in boar semen, 1538 in spermatozoon and 695 in seminal plasma and 69 and 15 proteins were significative expressed in spermatozoon and seminal plasma, being catalytic activity, heterocyclic compound binding and ATP-dependent protein folding chaperone related with molecular functions and with biological processes involved generation of precursor metabolites and energy, metabolic process of small molecules and organic substances and sexual reproduction. Proteomic studies of ejaculates with high and low cryotolerance can elucidate the differences in the cryotolerance of the sperm cell and propose molecules to enhance cryopreservation protocols.

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