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79 Boar seminal plasma proteomic profiling for biomarker discovery

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As an important technology in the swine industry, artificial insemination is of great significance in the preservation of elite breeds and the improvement of the utilisation rate of elite breeds, which makes the quality of preserved pig semen particularly critical. This abstract mainly evaluates a proteomic method to profile boar seminal plasma of different breeds and evaluate potential candidate biomarkers. It has considerable practical value, new technical methods, reasonable test steps, and important theoretical reference value for the test results. In this study, various sample preparation methods were compared (acetone precipitation [urea and SDS resuspension] vs non-precipitated) to develop a standard workflow that can be used for large proteomics studies; the optimal method (non-precipitated) was then tested on a pilot study that was composed of seminal plasma from eight boars belonging to three different breeds (two Large White, three Kolbroek, and three Windsnyer). Briefly, proteins from seminal plasma were digested on an automated FlexDuo system using trypsin. The peptides were pefractionated into six fractions using the hydrophilic interaction liquid chromatography/strong anion exchange method. The fractionated peptides were then analysed on the Evosep One connected to a 6600 TripleTOF Sciex mass spectrometer. A data-independent acquisition method was used. Spectronaut software was used for protein identifications and differential protein analysis. A total of 1,314 peptides were identified across runs from 233 protein groups. The proteins identified covered a wide range of biological functions related to sperm function, such as AQN-1, AWN, and SOD. To mention a few candidate proteins of interest, superoxide dismutase, glutathione transferase, β hexosaminidase, and the prostaglandin isomerase were diferentially expressed among breeds and also in individual boars within the same breed. The in-solution method used in this study is semi-automated and can be used for high throughput biomarker and validation studies.

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