METABOLOMIC ANALYSIS OF BOVINE PRE-IMPLANTATION EMBRYOS

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Abstract

Metabolomics is the study of small molecules or metabolites present in biological samples. The metabolism of the bovine embryo is marked by a transition from the oocyte and early stage embryo, which are entirely dependent on TCA cycle activity for the generation of ATP, toward a significantly greater input of glycolysis during morula compaction and blastocyst formation. The aim of this study was to describe the changes in metabolic profiles of bovine embryos across pre-implantation development. Five pools of 100 embryos at the 2- to 4-cell, 8-cell, 16-cell, morula, and blastocyst stages (i.e. 500 embryos in total per developmental stage) were produced by IVM, IVF, and IVC. Each pool was snap frozen and stored at -80°C until analysis. Extraction of metabolites was performed using 6% perchloric acid. ^{1}H spectra were acquired on a 600-MHz Varian NMR spectrometer operating at 25°C (Varian Inc., Palo Alto, CA, USA). All spectra were processed, baseline corrected, and integrated into bins of 0.02 ppm width. The water region was excluded and data were normalized to the sum of the spectral integral. Data were analyzed using multivariate statistics. Principal component analysis (PCA), an unsupervised pattern recognition technique, was performed initially to assess variation and expose any trends or outlying data. Partial least squares-discriminant analysis (PLS-DA) was subsequently performed to define the maximum separation between the different developmental stages. Data were visualized by constructing principal component scores and loadings plots, where each point on the score plot represented an individual sample and each point on the loadings plot represented a single ^{1}H NMR spectral region. The quality of all models was judged by the goodness-of-fit parameter (R^2) and the predictive ability parameter (Q^2). PCA analysis of all the data showed distinct separation of the 2- to 4-cell, and 8-cell (i.e. pre-embryonic genome activation, EGA) from 16-cell, morula and blastocyst (i.e. post-EGA) extracts. Pair-wise comparisons between successive developmental stages were performed. PCA analysis showed separation of 2- to 4-cell and 8-cell extracts. A PLS-DA was built with an R^2 of 0.97 and a Q^2 of 0.55. The main discriminating metabolites were acetate, acetoacetate, and an unidentified peak at 1.25 ppm. Similarly, PCA analysis showed separation of 8-cell and 16-cell embryo extracts. A PLS-DA model was built with an R^2 of 0.99 and a Q^2 of 0.92. The discrimination was due to higher levels of acetate and an unidentified peak in 8-cell extracts and the appearance of a new peak in 16-cell extracts, tentatively assigned to oxalacetate. PCA analysis showed no separation of the 16-cell, morula, and blastocyst extracts. In conclusion, ^{1}H NMR spectroscopy allows the simultaneous measurement of small-molecular-weight molecules in complex biological samples. Given that the metabolome is downstream of gene function it may represent a superior measure of cellular activities compared to transcriptomic and proteomic approaches.

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