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Abstract Title
Partitioning Variability in Proteomic Identification in a Rat Model of Congenital Diaphragmatic Hernia

Background
Acknowledging and attributing sources of variability is particularly important when working with precious neonatal samples with limited replication capacity. To draw reliable inferences from proteomic snapshots taken from tandem mass spectrometry (MS/MS), accounting for variation arising from experimental design complexity, sample preparation and analytical platform factors is required.

Objective
In this study, we used a variance component model to partition variability among experimental sources, and examined influences on nitrofen-induced differential protein abundance due to batch, technical or biological effects in a rat model of congenital diaphragmatic hernia.

Methods
A total of 12 Sprague-Dawley rat dams were randomly assigned to either nitrofen (n=6) or control conditions (n=6), sacrificed at embryonic days 15, 18 and 21. Lungs were extracted from 2 randomly chosen pups (M/F). The whole E15 lung was used, whereas lung sides were separated into individual samples for E18 and E21. E21 samples were further partitioned into biological and technical replicates. Proteins were extracted from samples and processed with LC-MS/MS and the resulting peptide intensities were standardized. R packages were used to perform statistical and computational analyses.

Results
Variance percent analysis showed substantial variation due to gestational stage and residual factors, whereas biological sampling and technical procedures seemed to be well-controlled. Using a linear mixed model with condition as fixed effect and no sex-gestation interaction terms, 1,432 proteins were identified as significantly differentially expressed due to nitrofen treatment ($p < 0.05$).

Conclusion
Insights gained from variance partitioning can be used for quality control of proteomics experiments, which improves the trustworthiness of conclusions drawn from downstream analyses. Following statistical analysis of variability, computational approaches such as gene ontology classification of identified differentially abundant proteins can be conducted to compare proteomic snapshots in relevant biological effects.

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