MALDI-TOF mass spectrometry analysis of lipids in single bovine oocytes revealed differences before and after in vitro maturation

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Abstract Body

Oocyte contains intracellular lipids which are involved in membrane composition, intracellular signaling and energy storage. We previously showed that Intact Cell Matrix-assisted laser desorption/ionization time of flight Mass Spectrometry (ICM-MS) analysis of lipid profiles of cumulus cells was able to discriminate immature and mature bovine oocytes (Sanchez-Lazo et al. Mol Endocrinol 2014, 28(9):1502-1521). The objectives of this work were to adapt ICM-MS technology to single bovine oocytes and to compare lipid contents in the oocytes before and after IVM.ICM-MS was performed on individual immature oocytes (n=12) and mature oocytes after 24h IVM (n=12), completely denuded from CC and cocrystallized with DHAP matrix. Lipid spectral profiles were acquired using an UltrafleXtreme MALDI-TOF/TOF instrument (Bruker) in positive reflector mode. M/z peaks were detected (160 to 1000 m/z range) and values of the normalized peak heights (NPH) were quantified (Progenesis MALDI™, Nonlinear Dynamics). Multivariate Principal Component Analysis (PCA) and Student test were applied to NPH values of immature and mature oocytes. Peaks were annotated using high resolution MS/MS and LipidMaps database.A total of 266 distinct peaks ranging from m/z 163.27 to 951.62 were detected. Mean coefficient of variation for all the peaks was 32%. 72 peaks were differential between immature and mature oocytes (38 up- and 34 down-regulated during IVM, $p<0.01$, fold change >2.0). Several up-regulated peaks (2-68 fold after IVM) ranging from m/z 700 to m/z 815 were identified as different phosphatidylcholines and sphingomyelins. Among the down-regulated there were fatty acids C14:0 (16-fold decrease during IVM) and C17:0 (2-fold decrease). PCA clearly discriminated immature and mature oocytes.In conclusion, lipid content significantly varied in the oocytes before or after IVM due to both changes of oocyte follicular environment to in vitro culture and to proper intracellular fatty metabolism (lipogenesis, lipolysis, oxidation) leading to structural modifications in the oocyte.