MALDI-TOF mass spectrometry analysis of lipids in single bovine oocytes during IVM

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Keywords: oocyte, lipids, MALDI mass spectrometry

Bovine oocyte is rich in intracellular lipids which are involved in membrane composition, intracellular signaling and energy storage. We have recently showed that level of neutral lipids containing in lipid droplets was diminished during oocyte *in vitro* maturation, IVM (Auclair et al. *Am J Physiol Endo Metab*, 2013,304(6): E599-613). We also reported that 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 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.

IVM was performed on bovine oocyte-cumulus complexes from 4-6 mm ovarian follicles in culture medium containing 10% of fetal bovine serum (MP Biomedicals, Illkirch, France), growth factors and gonadotropins. ICM-MS was performed on individual immature (n=12) and mature (n=12) oocytes, completely denuded from CC. Lipid spectral profiles (3000 shots per spectra) were acquired from each oocyte, cocrystallized with 2,5-dihydroxyacetophenone (DHAP) matrix, using an UltrafleXtreme MALDI-TOF/TOF instrument (Bruker) in positive reflector mode. M/z peaks were detected in the range of 160 to 1000 m/z and values of the normalized peak heights (NPH) were quantified using Progenesis MALDI™ (Nonlinear Dynamics). Coefficient of variation (CV %) was calculated for each m/z peak from 3 technical replicates using 20 immature oocytes. Multivariate Principal Component Analysis (PCA) and Student test were applied to NPH values for hunting lipid content variations between immature and mature oocytes. Lipids were extracted from follicles; several peaks were fragmented by high resolution MSMS top-down analysis using LTQ Velos Orbitrap operating in positive mode and annotated using LipidMaps.

A total of 266 distinct peaks ranging from m/z 163.27 to 951.62 were detected. Mean CV% of 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). Among them, several up-regulated peaks (2-68 fold increase during IVM) ranging from m/z 700 to m/z 815 were identified as phosphatidylcholines (32:0, 32:1, 33:1, 34:2, 36:2, etc) and sphingomyelins (36:1, 42:2). Among the down-regulated peaks, fatty acids C14:0 (16-fold decrease during IVM) and C17:0 (2-fold decrease) were annotated. Groups of immature and mature oocytes could be clearly discriminated by PCA.

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…) leading to structural modifications in the oocyte.

Financial support: Bovomega3 project funded by Val de Loire Region, France; thesis of P. Bertevello is funded by University do Estado do Rio Grande do Norte (Brazil).