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Comparison of lipid profiles and gene expression in granulosa and cumulus cells in bovine

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Cumulus cells (CC) derive from granulosa cells (GC) during follicular growth and antrum formation and are coupled with an oocyte. Fatty acid (FA) synthesis and oxidation in GC impact cell proliferation and steroidogenesis (Elis et al, 2015 *Theriogenology*. 2015, 83(5):840-53) whereas in CC these processes are crucial for oocyte maturation (Sanchez-Lazo et al, 2014, *Mol Endocrinol*. 2014 28(9):1502-21). Both GC and CC contribute to oogenesis and reflect oocyte quality. The objective of our study was to compare intracellular lipids and lipid related transcripts between these compartments. Lipid profiles obtained using MALDI-TOF mass spectrometry were compared between GC from individual follicles (n=12) and pools of CC (n=12) aspirated from 4-5 mm follicles of slaughtered cows. Freshly isolated cells were analysed using UltrafleXtreme MALDI-TOF/TOF instrument (Bruker) in positive (+) and negative (-) reflector mode, with 2,5-dihydroxyacetophenone matrix. Peaks were detected in 100-1000 m/z range and values of the normalized peak heights (NPH) were quantified using Progenesis MALDI™ (Nonlinear Dynamics). Student's t-test was applied to NPH values for hunting lipid content variations between GC and CC. Peaks were annotated using MS/MS fragmentation confronted to lipid databases. Lipid fingerprints from CC and GC gathered 462 peaks in (+) and 486 peaks in (-) modes, with coefficients of variation = 27% for CC and 18% for GC. 143 species were significantly upregulated in CC (P<0.01, fold change >2.0). Among them, 2 lysophosphatidylcholines (LPC 20:4 and 20:3) 12 phosphatidylcholines (PC), and 12 sphingomyelins (SM) were identified. Among 44 molecular forms which were more abundant in GC, we identified LPC (14:0), 4 PC, ceramide (22:1), SM (15:1), phosphatidylethanolamines (28:0 and 38:7) and phosphatidylserine (29:0). 4 peaks were annotated as triglycerides. Gene expression in pools of CC (n=4) and GC (n=4) was analyzed using a customized 60K bovine microarray (Agilent technology, 61326 probes). Differential analysis revealed 2009 differentially expressed genes (DEG) which were up-regulated in CC and 694 in GC (P<0.05, Benjamini-Hochberg correction). DEG showed significant enrichment in the pathways related to carbon metabolism, glycolysis /gluconeogenesis, ATP-binding cassette transporters, amino acid and O-glycan biosynthesis, thyroid hormone, PI3K-Akt signaling, p53 and PPAR signaling pathway (corrected P<0.05). Among the DEG related to lipid metabolism and regulated by PPARs, genes *ACOX2*, *LPL*, *SCD*, *PPARG*, *FABP3*, *FADS2*, *ACADL*, *SLC27A2* were up-regulated in CC and *CPT1A*, *CPT1B*, *SCD5*, *PLTP* were more expressed in GC. Ten sphingolipid metabolism genes were over-expressed in CC. In conclusion, numerous genes related to lipid metabolism were differently expressed in CC and GC. This corroborates differences in GC and CC lipids and may reflect different involvement of GC and CC in glyceroneogenesis, lipogenesis, oxidation and steroid production.

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