Gene expression profile of cumulus cells investment of bovine oocytes at different stage of differentiation based on the degree of their chromatin compaction

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Introduction

Oocytes acquire their competence during antral follicle development. During this period, chromatin configuration within the germinal vesicle (GV) changes dynamically and is indicative of oocyte's developmental potential. Since interactions between somatic and germ cells are critical for the acquisition of oocyte competence and serve modulate chromatin morphology and function, we hypothesized that features of cumulus cells (CC) investment change along with oocyte chromatin compaction, reflecting oocytes quality. Therefore this study aimed at assessing gene expression profile by microarray platform of CC isolated from oocytes with different chromatin configurations

Methods

Bovine cumulus-oocyte complexes were isolated from 0.5-6 mm antral follicles. Oocytes were separated from surrounding CC and chromatin configuration was assessed under fluorescent microscopy. Oocytes were classified as GV0, GV1, GV2 and GV3 according to the degree of chromatin compaction. RNA extracted from CC belonging to each group was, amplified and hybridized on a bovine embryo-specific 44K Agilent slide (EmbryoGene). The CC_GV1, CC_GV2 and CC_GV3 classes were each hybridized against the CC_GV0, representing a stage of early oocyte differentiation with poor development competence. Data were normalized with Loess and fold

changes of differentially expressed genes were determined with Limma procedure. Microarray data were validated using quantitative RT-PCR on selected targets. Microarray data were further analysed through: 1) between-group analysis (BGA), which classifies the samples according to their transcriptomic profiles; 3) cluster analysis according to the expression profile of each gene using the mFuzz Bioconductor package; 4) Ingenuity Pathway Analysis (IPA) to study regulation pattern genes and predicted function. Finally CC belonging to each GV group were cultured for 3hrs and apoptotic cells were assessed by CaspaTag Pan-Caspase in situ Assay.

Results

Overall, qRT-PCR results of a subset of 5 selected genes (Thrombospondine-1, Serpine-2, regulator of G-protein signaling-2, inhibin alpha and solute carrier family 39 member-8) were consistent with the microarray data.

Clustering analysis generated 16 clusters representing the main profiles of transcription modulation. Of the 5571 significantly differentially expressed probes, the majority (25.49%) best fitted with cluster #**6** (down regulation between CC_GV0 and CC_GV1 and stable low levels in successive groups). IPA identified the most relevant functions associated to each cluster. Genes included in cluster #1 were mostly related to biological processes such as ‘cell cycle’ and ‘cell death and survival’, while genes included in cluster #5 were mostly related to ‘gene expression’. Interestingly, ‘lipid metabolism’ was the most significant function identified in cluster # 6, #9 and #12.

IPA of gene lists obtained from each contrast individually (CC_GV0 vs CC_GV1; CC_GV0 vs CC_GV2; CC_GV0 vs CC_GV3), which considered the fold change difference for each gene, revealed that the main affected functions in each contrast was ‘cell death and survival’. Importantly, IPA revealed that apoptosis is predicted to be inhibited in CC_GV1 and CC_GV2, while it is activated in CC_GV3.

Caspase Assay results indicated that a low percentage of CC_GV0 are prone to undergo apoptosis, while it significantly increases in CC from oocytes with

condensed chromatin, reaching the highest level in CC_GV3 (ANOVA, $p<0.05$).

Conclusions

This study confirms that oocyte competence is related to the transcriptomic profile of somatic compartment and provides multiple non-invasive biomarkers that can predict oocyte quality. This has important implications in treating human infertility as well as developing breeding schemes in domestic mammals.