Abstract # 2157

Cumulus-Oocyte Interaction Is Required to Maintain Active Suppression Of Glycine Transport In The Preovulatory Mouse Oocyte. Allison K. Tscherner, Ottawa Hospital Research Institute, Canada

Oocytes and early embryos are highly sensitive to changes in cell volume. It is now understood that cell volume dysregulation was a major cause of developmental arrest that occurred in traditional embryo culture. Early (1- to 2- cell) mouse embryos use a novel mechanism to control cell volume, in which glycine is accumulated intracellularly via the GLYT1 transporter (SLC6A9 protein). While SLC6A9 is expressed and localizes to the membrane of fully-grown oocytes, transport of glycine is absent until this transporter becomes activated by an unknown mechanism. In vivo, GLYT1 activation normally occurs in parallel with release of an oocyte from meiotic arrest that precedes ovulation. It also activates in vitro shortly after oocytes are removed from antral follicles, implying active suppression within follicles. The primary aim of this research is to identify the specific factor(s) responsible for the release of suppression of GLYT1 in oocytes, which are currently not known. To evaluate this, we have established a GLYT1 activity assay based on [3H]glycine uptake and adapted it for single oocyte measurements. Oocytes were cultured within COCs for 4 hours after removal from follicles. We have found for the first time that it is possible to maintain quiescence of GLYT1 in GV oocytes within isolated COCs, in a model where COCs are cultured individually and meiotic arrest is maintained by natriuretic peptide precursor C (NPPC). This suppressive effect is reversed when NPPC is removed. NPPC acts by inducing production of cGMP, which in turn mediates suppression of the oocyte's cAMP-specific phosphodiesterase, PDE3. GLYT1 suppression is similarly maintained when oocyte meiosis is arrested with milrinone, a direct inhibitor of PDE3. However, GLYT1 suppression is maintained only in intact COCs cultured in milrinone, whereas oocytes stripped of cumulus cells maintain meiotic arrest but GLYT1 is activated. Together, these findings indicate that maintaining GLYT1 suppression requires both meiotic arrest and the presence of cumulus cells, though either factor itself is insufficient to maintain active suppression. Finally, since gap junctions between the oocyte and cumulus cells play a major role in the physical association as well as chemical communication between these cells, we impaired gap junctional coupling with specific inhibitors and observed a partial activation of GLYT1 in COCs in the presence of milrinone. Overall, we have shown that the factor maintaining GLYT1 suppression before the resumption of meiosis requires the presence of cumulus cells. GLYT1 quiescence is only maintained under conditions of oocyte meiotic arrest and appears to involve gap junctional communication between cumulus cells and the oocyte. This study highlights the conditions required for glycine transport in vitro and provides insight into the signaling mechanisms likely involved in GLYT1 suppression in ovarian follicles in vivo.