Variable IGF1R mRNA Expression in Individual GV and In Vitro Matured M2 Human Oocytes

Winston, N., Fierro, M., Zamah, A., Scoccia B., and Stocco, C.
University of Illinois at Chicago IVF Program / Department of Physiology,
Chicago, Illinois

Objective: Characterization of human insulin-like growth factor 1 receptor (IGF1R) mRNA expression in individual human oocytes to better understand the role of biologically significant mRNAs in establishing oocyte quality following ovarian hyperstimulation for in vitro fertilization (IVF) treatment.

Design: Germinal vesicle oocytes (GV) were identified after removal of all cumulus cells prior to ICSI and collected from consenting patients with IRB-approval.

Materials and Methods: Single GVs were placed directly into lysis buffer (5μl). Oocytes in vitro matured (IVM) to the M2 stage were placed into individual tubes containing lysis buffer after 24 to 48 hours (h) in culture. Total RNA isolated from cumulus granulosa cells (GC) using the TRIzol reagent (Invitrogen) as stated in the manufacturer's protocol served as a positive control. Reverse Transcription (RT) was performed using anchored oligo-dT primers (IDT, Coralville, IA) and Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen) on the entire 5μl oocyte lysate at 37°C for 1.5h or 1mg total GC RNA 42°C for 1h. Quantitative polymerase chain reaction (qPCR) was performed combining standards or 1μl cDNA with PCR buffer containing SYBR Green I (Sigma Chemical Co, St. Louis, Missouri), *Taq* polymerase (Genscript, Piscataway, NJ), and primers for IGF1R. Melting curves were routinely determined to ascertain generation of only the expected product.

Results: qPCR performed on GC cDNA demonstrated detection of the expected product under the reaction conditions in use. Gel electrophoresis of the PCR product confirmed the size of the DNA fragment generated. qPCR analysis detected a signal for IGF1R mRNA in 9 of the 15 GVs analyzed from 9 patients. Moreover, for 3 patients with multiple GVs, differences in IGF1R rnRNA expression were seen between individual GV oocytes, as well as between oocytes from different patients. Similarly, 8 of 12 IVM M2 oocytes from 11 patients gave a signal for the presence of IGF1R mRNA.

Conclusions: These results report the use of a direct-lysis method for the extraction and analysis of mRNA from single human oocytes. These data suggest that GV oocytes failing to respond to the hCG trigger during ovarian stimulation for IVF vary in their biological integrity. Furthermore, we observed that GV oocytes advanced to the M2 stage in vitro in the absence of any demonstrable IGF1R mRNA expression. Molecular assessment of single human oocytes can be used to better understand the low per-oocyte success rate in human IVF.

Disclosures: Nothing to disclose.