

Differential growth of human embryos *in vitro*: role of reactive oxygen species

Bedaiwy M.A.¹, Miller K.¹, Falcone T.¹, Nelson D.¹, AbdelAleem A.², Mohamed M.², Al-Hussaini T.², and Agarwal A.¹

¹Center for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Department of Obstetrics-Gynecology and Urological Institute, The Cleveland Clinic Foundation, Cleveland, Ohio, USA and ²Department of Gynecology-Obstetrics, Assiut School of Medicine, Egypt.

Aim: Pregnancy rates in assisted reproductive technologies are sub-optimal despite substantial technical improvements in the last decade. Better understanding of *in vitro* embryonic growth is very important as little is known about the biological, biochemical, and metabolic functions of pre-implantation embryos. Recently, the presence of various oxidative/antioxidant systems in a number of reproductive tissues has sparked an interest in studying the relationship of oxidative stress parameters with different aspects of reproduction. The objective of this study was to examine the relationship of early human embryonic development with day 1 culture media reactive oxygen species (D-1 ROS) levels and parameters.

Material and Methods: Patients undergoing *in vitro* fertilization (IVF; n = 182; 93 with intracytoplasmic sperm injection (ICSI) and 89 without ICSI in 108 cycles) were included. Fertilization and early culture were performed in HTF with 5% serum substitute supplement. D-1 ROS levels in the central well (sample) and the outer well (control) of each embryo culture dish were measured after overnight incubation by enhanced chemiluminescence assay using luminol as the probe. Fertilization rate embryo quality parameters (day 3 and day 5) were recorded for each cycle. Patients were comparable regarding age, parity, and demographic features.

Results: After controlling for all demographic and clinical variables, D-1 ROS levels were not related to fertilization rate in both patient groups. D-1 ROS levels were significantly related to increased embryonic fragmentation at day 3 (p = 0.03), and increased % of arrested embryos at day 5 in ICSI cycles (p = 0.005) but not in the IVF cycles (p = 0.4 and 0.7, respectively).

Conclusions: D-1 ROS level in day 1 culture media is an important biochemical marker for early embryonic growth. ROS levels in day 1 culture media are related with an increased embryo fragmentation and embryonic arrest in ICSI cycles. Whether this relationship is a cause or effect needs further assessment. Differential growth of ICSI embryos incubated under the same conditions may be due to differences in ROS levels surrounding them. Mechanisms to intercept ROS production may prevent this phenomenon.