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Identification of Unique Biomarkers of Human Oocyte Maturation by Non-Invasive Metabolomic Profiling.

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Introduction: According to the SART statistics, there were 122,683 IVF cycles performed in US in 2005 and three times more of that number can be estimated world-wide. Average number of embryos transferred per cycle ranged between 2.4 (<35 years of maternal age) and 3.3 (41-42 years). At the same time, pregnancy rates ranged from 43% to 18% and multiple gestations ranged between 37.3% and 15.7% in the same age categories, respectively. These outcomes, low pregnancy and high multiple gestation rates, highlight the difficulties of selecting embryos for transfer that have the highest potential for implantation and the inability to correctly estimate embryo viability. It is imperative to improve on our ability to more accurately measure/estimate reproductive potential of male/female gametes and embryos. Recently, non-invasive methods, using biospectroscopy-based metabolomics, have been employed to assess biomarkers of oxidative metabolism (OM) in embryo culture medium. Moreover, the distribution of these biomarkers correlated well with embryo viability, implantation and pregnancy. However, no data exist regarding the metabolomic status of human oocytes prior to fertilization. For this reason, in the present study, we collected culture medium from different maturational stage oocytes maintained individually in culture after ovarian stimulation. Corresponding metabolomic profiles were obtained for each group of maturational stage oocytes and evaluated for levels of oxidative metabolism.

Materials & Methods: Two IVF programs were collaborated in the project. Patient treatment and laboratory and study conditions were standardized. Patient inclusion criteria: <38 years of age with 10 or more retrieved oocytes assigned for ICSI. A total of 21 cycles and 184 oocytes were involved in the study. Oocytes were retrieved 36 hours after HCG administration (following a luteal down regulation or antagonist controlled ovarian stimulation). Following enzymatic and mechanical cleansing (Cumulase or Sigma Fraction IV), nuclear maturity was recorded and the oocytes were allocated into individual culture media drops of 60 μ l. Three hours later mature oocytes were ICSI'd and spent oocyte media was collected representing the different maturation stages. Fertilized eggs were cultured until day-3 or day-5 when selected embryos were transferred and/or frozen. Individual profiles were obtained from 5 μ l media samples using Near Infrared (NIR) spectroscopy. Specific biomarkers, corresponding to unique functional groups (NH₂, aromatic -CH, -OH, -SH) were identified yielding exclusive metabolomic profiles which were then quantified using a wavelength selective algorithm (Molecular Biometrics, LLC, Chester, NJ). Resulting metabolomics data were correlated with morphological assessed nuclear maturity of the corresponding oocytes.

Results: Metabolomic profiles were obtained for all oocytes and subjected to bioinformatic analysis. Unique metabolomic profiles of the specific OM biomarker populations were reliably identified in all culture samples by NIR spectroscopy. Profiles of Metaphase I and II oocytes were significantly different from each other and from profiles of Prophase I (GV) oocytes. Statistical analysis performed on the metabolomic profiles established significance at the 95% confidence interval ($P < 0.001$). Analysis of biomarkers by NIR required a minimum of three wavelength regions, and resulted in a specificity of 83% and a sensitivity of 85% for GV versus MI oocytes; and 93% specificity and 100% sensitivity between MI and MII oocytes.

Conclusions: A detectable and significant difference exists in the metabolomic profiles OM biomarkers found in culture media obtained from oocytes. Furthermore, the biomarker profiles corresponded to distinct maturational stages of oocytes. The reported metabolomic parameters were established using proprietary spectroscopic analysis and bioinformatic algorithms, with samples obtained from two different centers, and achieved high sensitivity and specificity. Strikingly, just three hours of individual culture of oocytes proved to be sufficient to produce clearly detectable metabolomic "fingerprints" that distinguish early stages of oocyte maturation. Our findings suggest that the oxidative pathways of oocytes are more active at the earliest stages of development than originally thought and that the metabolism of these cells increases significantly *in-vitro* in the first hours following retrieval. These data also demonstrate that metabolomic profiling may serve as a useful methodology for rapid, non-invasive gamete assessment that, together with a similarly descriptive embryo viability test, may favorably impact outcomes of assisted reproduction by enhancing gamete and embryo selection procedures.