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METABOLOMIC ASSESSMENT OF OXIDATIVE STRESS IN FOLLICULAR FLUID AS A POSSIBLE PREDICTOR OF OOCYTE VIABILITY.

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INTRODUCTION: Since the introduction of assisted reproduction techniques (ART) in 1970s there has been a continuous search for biomarkers with the potential to predict a couple's chance of obtaining a pregnancy, for improving the relatively low baby-takehome rates, and to control the incidence of multiple pregnancies. With the exception of female age, no other factor of significant prognostic value for the outcome of ART has been identified. Oxidative stress (OS) and resultant reactive oxygen species (ROS) have been implicated in the etiology of female as well as male infertility. Previous studies using biochemical techniques have shown a negative correlation between levels of ROS in follicular fluid (FF) and the outcome of ART. Recent developments in the field of metabolomics now allow for the assessment of biomarkers of OS at the molecular level. The aim of this study was therefore to determine if metabolomic profiles of OS in FF could be used as independent predictors of ART outcome.

MATERIALS AND METHODS: Individual aspirates of follicular fluid (FF) (N=83) were collected during IVF and stored frozen at -80°C after the removal of oocytes. All subsequent parameters of the IVF procedure were documented. Each 10=1 sample of FF was analyzed by three different forms of spectroscopy: Nuclear Magnetic Resonance (NMR), Raman and Near Infrared (NIR) targeting OS biomarkers of NH, CH, OH and ROH at specific wavelengths. The individual spectra obtained from each sample were separately analyzed using a wavelength selective genetic algorithm (GA). Each metabolomic profile was then quantified using proprietary chemometrics and bioinformatics (Molecular Biometrics, LLC, Chester, NJ), coupled with logistic regression analysis, and correlated to multiple IVF outcome parameters. Total analysis time was ~1 min per sample.

RESULTS: Unique metabolomic profiles, reflective of OS, were reliably produced from discarded FF samples by all three forms of spectroscopy. The novel metabolomic data were correlated to pregnancy outcome but not to other IVF parameters. The ratio of CH to ROH content in the FF, that is reflective of oxidative stress, was also different between the pregnant and non-pregnant groups. Four spectral regions associated with the OS biomarkers were identified as predictive of pregnancy outcome. Compiled results from the leave-one-out cross-validation of the logistic regression from all three spectroscopic measurements resulted in a specificity and sensitivity of >80%.

CONCLUSIONS: This is the first study to establish the role of metabolomic profiles of OS in follicular fluid in assisted reproduction treatment. We found a strong correlation between the metabolomic profiles of OS biomarkers from FF specimens and pregnancy outcomes. There appear to be a unique metabolomic signatures in the FF that may be predictive of oocyte viability (and by inference embryo quality) and pregnancy. Further studies are needed to confirm these observations and to establish a possible role for metabolomic technology in oocyte assessment and selection.