Noninvasive metabolomic profiling of embryo culture media using proton nuclear magnetic resonance correlates with reproductive potential of embryos in women undergoing in vitro fertilization

Emre Seli, M.D.,[a] *Lucy Botros, M.Sc.,*[b,c] *Denny Sakkas, Ph.D.,*[a,d] *and David H. Burns, Ph.D.*[b]

[a] Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut; and [b] Department of Chemistry, McGill University, Montreal, Quebec, Canada; [c] Molecular Biometrics LLC, Quebec, Canada; and [d] Molecular Biometrics LLC, New Haven, Connecticut

Objective: To identify biomarkers associated with reproductive outcome using proton nuclear magnetic resonance (^{1}H NMR) metabolomic profiling of embryo culture media.
Design: Retrospective study.
Setting: An academic assisted reproductive technology (ART) program; a university research center.
Patient(s): Women undergoing ART treatment.
Intervention(s): Spent media samples from embryos that resulted in pregnancy and delivery (n = 17) and samples (n = 17) from embryos that failed to implant were individually collected on day 3, and evaluated using ^{1}H NMR spectroscopy. The spectra obtained were quantified by integrating six biomarker signals in the aliphatic region after baseline subtraction. Using a multivariate analysis, a model that calculates a viability index for each spectrum was developed. Sensitivity and specificity of predicting pregnancy (described as implantation and delivery) were calculated.
Main Outcome Measure(s): The ^{1}H NMR metabolomic profile of embryo culture media and embryo viability.
Result(s): Glutamate concentrations determined by ^{1}H NMR were significantly higher in spent culture media of embryos that resulted in pregnancy and delivery compared to those that failed to implant. Similarly, viability indices calculated by ^{1}H NMR using the weighted coefficients of glutamate and alanine/lactate ratio quantities were higher for embryos that implanted and resulted in a delivery. Proton NMR spectroscopy predicted viability of individual embryos with a sensitivity of 88.2% and a specificity of 88.2%.
Conclusion(s): Metabolomic profile of spent embryo culture media using ^{1}H NMR correlates with the reproductive potential of embryos. (Fertil Steril® 2008;90:2183–9. ©2008 by American Society for Reproductive Medicine.)

Key Words: Metabolomics, proton NMR, spectroscopy, embryo viability, reproductive potential, culture media, IVF

Infertility, defined as the inability to conceive after 1 year of unprotected intercourse, is estimated to affect 15% of the reproductive age population (1). Among the treatment modalities offered to infertile couples, those using assisted reproductive technologies (ART) are associated with the highest success rates. Consequently, ART use has been increasing steadily within the past decade. In the United States, more than 125,000 ART cycles started in 2004 (2), accounting for 1% of births (2) and 18% of multiple births (3).

Despite the widening use of ART, approximately 2 of 3 ART cycles fail to result in pregnancy, and 8 of 10 transferred embryos fail to implant (2, 4, 5). Failed ART cycles seem to be at least in part due to our inability to determine the embryos with the highest reproductive potential as ART cycles using thawed embryos following failed fresh cycles result in 7%–11 % implantation rate, and 13%-17% ongoing pregnancy rate (PR) per transfer (6, 7).

To prevent failures, centers have historically chosen to perform simultaneous transfer of multiple embryos, accepting the related risk of multiple pregnancies. In the United States, a mean number of 2.45 embryos were transferred in ART cycles using fresh nondonor oocytes in 2006, leading to a 34.3% live birth rate per transfer, of which 32% were multiple infant live births (2). Similarly, a mean number of 2.3 embryos were transferred in ART cycles using fresh donor oocytes, achieving a 52.3% live birth rate per transfer, 40.8% of which were multiple infant live births (2). In total, more than 30% of ART pregnancies are twins or higher-order multiple gestations, and 51% of all ART neonates are the products of multiple gestations (3), a frequency 15- to 20-fold greater than with spontaneous conceptions (8).

The high multiple PRs associated with ART has significant public health consequences. The increased rate of preterm

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Reprint requests: Emre Seli, M.D., Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, 300 George Street, Suite 770J, New Haven, CT 06511 (FAX: 203-785-7134; E-mail: emre.seli@yale.edu).

delivery in neonates from multiple infant pregnancies compromises their survival chances and increases their risk of lifelong disability. Although multiple births constitute only 3% of all births in the United States, they account for 13% of preterm births (<37 weeks), and 25% of very low birthweight infants (<2,000 g) (9). The incidence of cerebral palsy is increased 8-fold in twins and 47-fold in triplets (10), whereas infant deaths (birth to 1 year) are increased 6-fold in twins, and 17-fold in triplets and higher-order gestations (11). Multiple pregnancies also constitute a health risk for the mother including a 2- to 4-fold increase in pregnancy-induced hypertension and postpartum hemorrhage (12).

To limit the rate of multiple pregnancies and associated complications, a number of countries have restricted the number of embryos transferred in ART cycles (5). However, the majority of countries have not yet adopted strict regulations with respect to ART practice; most probably due to high cost of treatment and patients' desire to increase their chances to achieve pregnancy in a given cycle. Consequently, decreasing multiple gestations while maintaining or increasing overall PRs is an important goal of contemporary infertility treatment, and an improvement over the currently used embryo assessment methods would be beneficial for this purpose.

The currently used embryo grading systems, largely based on embryo cleavage rate and morphology (13–18) were developed soon after the report of the first successful pregnancy after IVF (19), and the development of controlled ovarian hyperstimulation (COH) (20). Although these grading systems led to significant improvements in implantation rates and PRs (21), their accuracy remained insufficient to compel most patients and clinicians to reduce the number of embryos transferred to a point where twins are uncommon and high-order multiple gestations are rare or eliminated.

The limitations of embryo assessment based on morphology and cleavage rate have led many investigators to pursue adjunctive technologies to determine an individual embryo's reproductive potential. Several metabolic parameters of developing embryos and of the spent embryo culture media have been studied using a variety of noninvasive techniques (22). In one such study, Conaghan et al. (23) found an inverse relationship between pyruvate uptake and human embryo viability, whereas Gardner and colleagues (24) reported that glucose uptake was greatest in human blastocysts of highest grade. Brison et al. (25) determined the levels of 18 amino acids in embryo culture media, using high performance liquid chromatography (HPLC), and found that elevated asparagine and decreased glycine and leucine levels in embryo culture media correlate with pregnancy.

We have recently reported that noninvasive metabolomic profiling of embryo culture media using Raman and near-infrared (NIR) spectroscopy correlates with pregnancy outcome in women undergoing IVF (26). Our initial findings were confirmed in a prospective blinded study (27). In the current study, we applied proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to analyze the metabolomic profile of embryo culture media and to identify components of the media that correlate with reproductive potential.

MATERIALS AND METHODS

Patient Selection, Treatment, and Sample Collection

All patients participating in the study were treated at the Yale Fertility Center, New Haven, CT. Institutional Review Board approval was obtained before the initiation of the study. All patients undergoing IVF were considered for participation in the study. Patients with abnormal endometrial development (<6 mm in peak thickness or failure to develop a trilaminar pattern before hCG administration) were excluded.

Controlled ovarian hyperstimulation was performed using a variety of protocols as previously published (26–28). Patients were monitored per established protocol and were judged to have sufficient follicular maturation when they had 2 or more follicles 18 mm or greater in maximal diameter. Oocytes were collected by transvaginal ultrasound-guided needle aspiration of the follicles under deep conscious sedation. Retrieved oocytes were rinsed, graded, and placed in *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered human tubal fluid (HTF) (Irvine Scientific, Santa Ana, CA) at 37°C under 6% CO_2 in air. Conventional insemination or intracytoplasmic sperm injection (ICSI) was used as indicated.

On the day after oocyte retrieval and insemination (day 1), each oocyte was examined for evidence of fertilization. Those that were found to have two pronuclei were placed into individual droplets for culture to the cleavage stage. For culture from days 1–3, 30 μL of Scandinavian G1 media (VitroLife, Englewood, CO) supplemented with 5% human serum albumin (HSA; Irvine Scientific) was used. Embryos were cultured individually.

An embryo scoring system based on cleavage rate and morphology was used for the evaluation of embryo quality as previously described (15, 26, 27). The patients who did not have sufficient high quality embryos to justify extended culture (5 or more 4-cell grade 1 or 2 embryos on day 2) had their embryos selected for transfer on day 3. After removal of the embryos in preparation for transfer, the spent media were placed individually into labeled cryovials, snap frozen in liquid nitrogen, and then stored at −80°C.

Upon completion of the treatment cycle, the patients were characterized relative to their implantation rates. The transferred embryos from patients who had no implantation were labeled as having a zero percent implantation rate. The transferred embryos from patients who had all transferred embryos implant and subsequently delivered were labeled as having a 100% sustained implantation rate.