CIRCULATING MIRNAS AS A NON-INVASIVE DIAGNOSTIC TOOL TO PREDICT IMPLANTATION FAILURE

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DIFFERENCES IN METABOLIC SIGNATURES BETWEEN THE INNER CELL MASS AND THE TROPHECTODERM IN DISCARDED HUMAN BLASTOCYSTS. Marta Venturas, M.Sc.,[1] Jaimin S. Shah, M.D.,[2] Tim H. Sanchez, Ph.D.,[3] Will Conway, Bs in Physics and Biology,[1] Denny Sakkas, PhD,[4] Dan Needleman, Ph.D.[1] [1]Harvard University, Cambridge, MA; [2]Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA; [3]None; [4]Boston IVF, Waltham, MA.

OBJECTIVE: To determine whether non-invasive metabolic imaging can detect differences in metabolic profiles between the inner cell mass (ICM) and the trophectoderm (TE) of discarded human blastocysts.

DESIGN: Prospective observational study.

MATERIALS AND METHODS: We used 192 morphologically normal human vitrified blastocysts from 126 patients (mean age 35.5 ±4.75 years) that were discarded and donated for research under an approved institutional review board protocol. These embryos were warmed, cultured for 2h and imaged, to analyze their metabolic signatures. Metabolic function was assessed using Fluorescence Lifetime Imaging Microscopy (FLIM). FLIM enables non-invasive imaging of the autofluorescent endogenous molecules, NADH and FAD+, essential coenzymes for cellular respiration and glycolysis. FLIM yields quantitative information on metabolite concentrations from fluorescence intensity and on enzyme engagement from fluorescence lifetimes. A single measurement provides 9 metabolic parameters (4 for NADH, 4 for FAD and Redox Ratio) which was used to separately analyze the metabolic signatures of the ICM and TE from each embryo. Metabolic parameters from the ICM were compared to those of the TE using paired t-test analyses while a sub analysis of embryo day (5 or 6) was performed using multilevel models.

RESULTS: Our data showed statistically significant variations in all metabolic parameters between the ICM and the TE from discarded human blastocysts. Both NADH and FAD+ intensities, that correlate with the concentration of these coenzymes, were significantly different between the ICM and the TE ($p<0.0001$). Furthermore, the lifetimes of these molecules and the fraction engaged with enzyme were significantly different between the ICM and the TE ($p<0.0001$). These findings are in line with previous evidence in mouse blastocysts showing that the ICM of mouse embryos have a relatively quiescent metabolism compared with that of the TE. Additionally, metabolic signatures from the ICM and the TE correlated with blastocyst day. When comparing day 5 and day 6 embryos, distinct metabolic signatures were visualized in the ICM (6/9 FLIM parameters, $p<0.002$) and also in the TE (6/9 FLIM parameters, $p<0.001$).

CONCLUSIONS: Non-invasive metabolic imaging can detect significant metabolic variations between the ICM and TE of discarded human blastocysts, suggesting different metabolic demands specific to the ICM and TE. Further studies on human embryo samples are planned which will investigate possible correlations between metabolic signatures of both the ICM and the TE with embryo morphology and pregnancy outcomes.

SUPPORT: Supported by the Blavatnik Biomedical Accelerator Grant at Harvard University. Becker and Hickl GmbH, and Boston Electronics sponsored research with the loaning of equipment for FLIM.

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CIRCULATING MIRNAS AS A NON-INVASIVE DIAGNOSTIC TOOL TO PREDICT IMPLANTATION FAILURE. Chloé Baron, PhD student,[1] Delphine Haouzi, PhD,[1] Frida Entezami, MD,[1] Sophie Brouillet, PharmD, PhD,[1] Samir Hamamah, MD, PhD[2] [1]Inserm U1203, CHU Montpellier, St-Eloi Hospital, Montpellier, France; [2]Arnaud de Villeneuve Hospital, CHU Montpellier, Montpellier, France.

OBJECTIVE: Do circulating miRNAs profile during the implantation window under the same hormone replacement therapy (HRT) treatment for frozen embryo replacement cycle,predict successful pregnancy?

DESIGN: Serum (n=14) were collected during the implantation window from repeated implantation failure (RIF) patients (mean ± SD, age: 38.6 ± 5.0 years) under HRT treatment for subsequent frozen embryo transfer. RNAs were extracted to perform the miRNA expression profile. Then, miRNA expression was analyzed according to the following pregnancy outcome: successful implantation (n=7) or implantation failure (n=7). Successful implantation was defined as both positive b-hCG and clinical pregnancy and implantation failure as negative b-hCG.

MATERIALS AND METHODS: Serum were obtained during a mock HRT treatment cycle from 14 RIF patients. Then, miRNA expression profile between groups, successful *vs*. implantation failure after frozen embryo replacement was evaluated by Next Generation Sequencing using the HTG EdgeSeq miRNA Whole Transcriptome Assay (Illumina, Firalis). miRNA sequencing data were normalized using the method recommended by HTG molecular diagnostic. Then, statistical analysis and receiver operating characteristic (ROC) analysis were applied to miRNA sequencing data.

RESULTS: We identified 44 miRNAs differentially expressed between groups with a fold change > 2 and a p-value < 0.05. All miRNAs were overexpressed in serum from patients with successful implantation. Supervised hierarchical clustering of these 44miRNAs showed a good segregation of serum samples from patients with and without a successful implantation. Related to these 44 miRNAs, we note four of them which are members of the let-7 family [miR-1 (x2.4, $p = 0.017$, AUC = 0.88), miR-2 (x2.2, $p = 0.004$, AUC = 0.94), miR-3 (x2.1, $p = 0.001$, AUC = 0.90), miR-4 (x2.1, $p = 0.001$, AUC = 0.98)].The microRNA target filter function from Ingenuity software predicted that 1375 mRNAs were targeted by the let-7 family that are involved in cell invasion, proliferation, growth and survival via integrin subunit beta 3 (*ITGB3*), vimentin (*VIM*), B-cell CLL/lymphoma 2 like 1 (*BCL2L1*) that play a central role in endometrial receptivity acquisition and implantation process. These results might have potential clinical applications to develop a non-invasive diagnostic tool to predict successful implantation, to avoid endometrium biopsy and consequently, to increase IVF/ICSI success.

CONCLUSIONS: We identified a miRNA signature in serum during the implantation window that can predict successful implantation. This information is crucial and can lead to the development a prognostic tool of the attempt, opening new perspectives in the patient care management. Further investigations with a larger number of patients are in progress to validate theses results.

SUPPORT: This study was partially supported by a grant from Gedeon Richter company.

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DISRUPTION OF NRF2- MEDIATED STRESS RESPONSE AND DNA REPAIR PATHWAYS ARE ASSOCIATED WITH LIMITED DEVELOPMENTAL POTENTIAL OF TRISOMY EMBRYOS. Jason C. Parks, BS, Mary E. Haywood, PhD, Blair R. McCallie, BS, Sue McCormick, BS, William B. Schoolcraft, MD, Mandy G. Katz-Jaffe, PhD. Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Embryonic chromosomal trisomy is recognized as a leading cause of reproductive failure and pregnancy loss in human reproduction. Interestingly, specific trisomy's have differing implantation potential and only a handful of trisomies (chromosome 13, 18, 21, X and Y) will even develop past the first trimester. The aim of this study was to investigate the association between the embryonic transcriptome and a range of embryonic trisomy with differing implantation potentials.

DESIGN: Research study.

MATERIALS AND METHODS: Surplus, frozen blastocysts (n = 55) were donated to research with IRB and patient consent. Blastocysts underwent micromanipulation to isolate trophectoderm (TE) cells for transcriptome analysis. In addition to euploid blastocysts (n=17), the following trisomies were identified based on differing implantation potential: trisomy 7 (n=11) and trisomy 11 (n=5) that are most likely to result in implantation failure, as well as trisomy 15 (n=11) and trisomy 22 (n=11) which are able to implant but will always result in miscarriage. Individual TE total RNA (n = 25) was isolated for sequencing on the NovaSEQ 6000 (Illumina). Reads were aligned to hg38 using GSNAP and only reads mapping to non-trisomy chromosomes were included in downstream analysis (genes on chromosomes 7, 11, 15, and 22 were excluded in all samples). Differential gene expression was analyzed with edgeR and limma (FDR<5%), and interpreted using Ingenuity Pathway Analysis (Qiagen). Validation was performed on additional individual TE samples (n = 30; 6 per group) with gene expression confirmed using Real-Time PCR (ViiA 7 Real-Time PCR System; $P<0.05$).

RESULTS: An interplay of several biological processes were evident in all trisomy embryo groups compared to euploid, regardless of the specific chromosome involved in the meiotic error, with a total of 389 differentially expressed genes ($P<0.05$). There was no enrichment for chromosome type or cytoband. Pathway analysis identified a globally inhibited NRF2-mediated stress response including validation of the two central players in the pathway, NRF2 and KEAP1 ($P<0.001$) and 8 significantly decreased NRF2 targets