NON-INVASIVE PREDICTION OF BLASTOCYST IMPLANTATION AND LIVE BIRTH, BY MASS SPECTROMETRY LIPID FINGERPRINTING

Amanda Setti Raize¹; Daniela Paes de Almeida Ferreira Braga¹,²; Daniela A. Montanni²; Elaine Cristina Cabral³; Marcos N. Eberlin³; Edson G. Lo Turco²; Edson Borges Jr¹
INTRODUCTION

✓ Most embryos produced in vitro fail to implant

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<th>ESHRE</th>
<th>ASRM</th>
<th>RED LARA</th>
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<tbody>
<tr>
<td>Pregnancy per ovum pick up (%)</td>
<td>29.4</td>
<td>35.0</td>
<td>30.2</td>
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<tr>
<td>Pregnancy per embryo transfer (%)</td>
<td>30.9</td>
<td>43.2</td>
<td>33.5</td>
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2008, 2009 e 2010

> 4 x 10^6 cycles

> 1 x 10^6 live births

Take home baby < 20.0%
INTRODUCTION

Low efficiency

Multiple embryo transfer

Multiple pregnancy

Perinatal Risks Associated With Assisted Reproductive Technology

ART

Multiple pregnancies: 30 fold increase
Selection of the most viable embryo: Main Challenge in ART
INTRODUCTION

• Prolonging the embryo culture period enables a better selection of embryos for transfer

• Inability to predict which blastocyst presents the higher implantation potential

• Development of reliable and non-invasive methods of embryo evaluation

CRUCIAL

embryo genome expression
INTRODUCTION

Non-invasive approaches for embryo development assessment

GENOMIC
~25,000 GENES

TRANSCRIPTOMIC
~100,000

PROTEOMIC
~1,000,000

METABOLOOMIC
~2,500
INTRODUCTION

Metabolites detected in the embryo culture medium carry important information about the embryo.

Changes in embryo metabolism: viable embryos present a different metabolic profile from non-viable embryos.
INTRODUCTION

Lipids

- Cell membrane structure
- Apoptosis signaling
- Energy storage
- Anti and pro-inflammatory agents
- Gene transcription signaling
- Cell proliferation signaling

LIPIDOMICS ERA: ACCOMPLISHMENTS AND CHALLENGES

Maroun Bou Khalil, Weimin Hou, Hu Zhou, Fred Elisma, Leigh Anne Swayne, Alexandre P. Blanchard, Zemin Yao, Steffany A.L. Bennett, and Daniel Figeys

Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON, Canada K1H 8M5

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FERTILITY MEDICAL GROUP
INTRODUCTION

LIPIDOMICS

- Large-scale study of lipid species and their related networks and metabolic pathways
OBJECTIVE

Identify lipid markers of blastocyst implantation and live birth by day three culture medium mass spectrometry fingerprinting
MATERIALS AND METHODS

• STUDY DESIGN

Samples of D3 embryo culture media

Live births
MATERIALS AND METHODS

• SAMPLES COLLECTION

ICSI

FERTILIZATION

D2

D3

D5 – Transfer

-20°C

Lipid extraction (Bligh and Dyer, 1959)
MATERIALS AND METHODS

• MASS SPECTROMETRY AND LIPID PROFILE

Positive ionization mode

Negative ionization mode

Nanomate automated injector

Q-TOF

Data acquisition
MATERIALS AND METHODS

• DATA ANALYSIS

- Data normalization by Log
- Data Standardization by z score
- PCA
- PLS-DA
- VIP scores

To detect clustering and the discrepancies between the samples
To maximize the variations and to guarantee the discriminatory effect of the components
Value given to each ion according to its potential for discriminating the samples

Metaboanalyst 3.0
RESULTS

PCA

Clustering of the samples from the negative implantation group on positive and negative ionization modes

+ ionization mode

- ionization mode
Discrimination of the samples from the negative implantation group on positive and negative ionization modes

+ ionization mode

- ionization mode
The main ions responsible for the discrimination of the negative implantation group

- **Positive mode**
  - AUC: 73.5%

- **Negative mode**
  - AUC: 72.0%
RESULTS

- Ions identification

- Glycerophosphoglycerol
- Unsaturated fatty acids
- Dicarboxylic acids,
- Phosphoethanolamine
- Glycerophosphocholine
RESULTS

LIPID INTERACTIONS

Biosynthesis of GPI anchors

Synthesis of inositol phosphate

Synthesis of glycerophospholipids

Lipids Involved in cellular restructuring

Embryos with poor implantation potential

Machinery to exhaustion
The Embryologist’s Dilemma....
Lipid profile in embryo culture medium

Implantation failure biomarkers
Noninvasive metabolomic profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study

Richard Scott, M.D., a Enure Seli, M.D., b Kathy Miller, B.S., a Denny Sakkas, Ph.D., b Katherine Scott, B.S., a and David H. Burns, Ph.D. a

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

Enure Seli, M.D., a Lucy Botros, M.Sc., b,c Denny Sakkas, Ph.D., a,d and David H. Burns, Ph.D. b

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Noninvasive metabolomic profiling as an adjunct to morphology for noninvasive embryo assessment in women undergoing single embryo transfer

Enure Seli, M.D., a, b Carlijn G. Vergouw, M.Sc., b Hiroshi Morita, B.Agr., c Lucy Botros, M.Sc., a Pieter Roos, Ph.D., c Cornelius B. Lambalk, M.D., Ph.D., b Naoki Yamashita, M.D., a Osamu Kato, M.D., a and Denny Sakkas, Ph.D. a

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Metabolomic profiling by near-infrared spectroscopy as a tool to assess embryo viability: a novel, non-invasive method for embryo selection

C.G. Vergouw, 1,2,4 L.I. Botros, 2, P. Roos, 2, J.W. Lens, 1, R. Schats, 1, P.G.A. Hompes, 1, D.H. Burns, 3 and C.B. Lambalk 1
DISCUSSION

Prediction of embryo implantation potential by mass spectrometry fingerprinting of the culture medium

Sylvia Sanches Cortezzi\textsuperscript{1}, Elaine Cristina Cabral\textsuperscript{2}, Marcello Garcia Trevisan\textsuperscript{3,4}, Christina Ramires Ferreira\textsuperscript{2}, Amanda Souza Setti\textsuperscript{1,5}, Daniela Paes de Almeida Ferreira Braga\textsuperscript{1,5}, Rita de C\textsuperscript{\textsc{\textae}}sia S\textsuperscript{\textsc{\textae}}vio Figueira\textsuperscript{5}, Assumpto Iaconelli Jr\textsuperscript{1,5}, Marcos Nogueira Eberlin\textsuperscript{2} and Edson Borges Jr\textsuperscript{1,5}

\textsuperscript{1}Sapientiae Institute – Educational and Research Center in Assisted Reproduction, Rua Vieira Maciel, 62, 04503-040 São Paulo, SP, Brazil, \textsuperscript{2}Th\textsuperscript{OM}Son Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas - UNICAMP, 13083-970 Campinas, SP, Brazil, \textsuperscript{3}Institute of Chemistry, Federal University of Alfenas, 37130-000 Alfenas, MG, Brazil, \textsuperscript{4}National Institute of Science and Technology of Bioanalytical - INCTBio, 13084-971 Campinas, SP, Brazil and \textsuperscript{5}Fertility – Assisted Fertilization Center, Avenida Brigadeiro Luiz Antônio, 4545, 01401-002 São Paulo, SP, Brazil


- Metabolomic profile of embryo culture media by MS as a predictive toll of embryo viability
DISCUSSION

Non-Invasive Prediction of Blastocyst Formation by Day Three Embryo Culture Medium Mass Spectrometry Lipid Fingerprinting

Daniela Paes de Almeida Ferreira Braga¹,²,³, Amanda Souza Setti²,³, Elaine Cristina Cabral⁴, Marcos Eberlin⁵, Edson Guimarães Loturco¹, Edson Borges Jr²,³

¹Disciplina de Urologia, Departamento de Cirurgia – UNIFESP – Brasil
²Instituto Sapientiae – Centro de Estudos e Pesquisa em Reprodução Assistida – Brasil
³Fertility – Medical Group – Sao Paulo – Brasil
⁴Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas – CPQBA – UNICAMP – Brasil
⁵Laboratório ThoMson de Espectrometria de Massas – Instituto de Química – UNICAMP – Brasil

- Lipid profile was accessed using mass spectrometry to predict which embryo is able to reach the blastocyst stage
DISCUSSION

• To access the lipid profile and determine which blastocysts is able to implant

• Blastocysts capable of leading to a successful pregnancy and live births

• Predictive power of this model was not totally conclusive
• Culture media from embryos that reached the blastocysts stage and were selected for transfer, were analyzed.

• Embryos selected according with the morphology on days one, two, three and five

• The strategy used here was able to identify differentially represented lipids in the culture medium of blastocysts that implanted vs those that did not implant.
DISCUSSION

Expensive
Robust
Complicated
Difficult Access

New platforms Available in surgical rooms Real-time analysis Adapted in assisted reproduction centers
CONCLUSION

DAY THREE CULTURE MEDIA LIPIDOMIC BY MASS SPECTROMETRY

• Fast, non invasive and viable predictive toll for blastocyst implantation, successful pregnancy, and live birth;

• Incorporated in the laboratory routine, adjunct to morphology evaluation to identify embryos that should not be transferred;

• Increasing implantation and also reducing the rate of multiple pregnancies