

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

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1



2



3



# INTRODUCTION

✓ Most embryos produced in vitro fail to implant

	ESHRE	ASRM	RED LARA
Pregnancy per ovum pick up (%)	29.4	35.0	30.2
Pregnancy per embryo transfer (%)	30.9	43.2	33.5

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doi:10.1093/humrep/dew082

human  
reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

## International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010<sup>†</sup>

S. Dyer<sup>1,\*</sup>, G.M. Chambers<sup>2</sup>, J. de Mouzon<sup>3</sup>, K.G. Nygren<sup>4</sup>,  
F. Zegers-Hochschild<sup>5</sup>, R. Mansour<sup>6</sup>, O. Ishihara<sup>7</sup>, M. Banker<sup>8</sup>,  
and G.D. Adamson<sup>9</sup>

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2008, 2009 e 2010

> 4 x 10<sup>6</sup> cycles

> 1 x 10<sup>6</sup> live births

Take home baby < 20,0%

# INTRODUCTION



The American College of  
Obstetricians and Gynecologists  
WOMEN'S HEALTH CARE PHYSICIANS

## COMMITTEE OPINION

Number 671 • September 2016

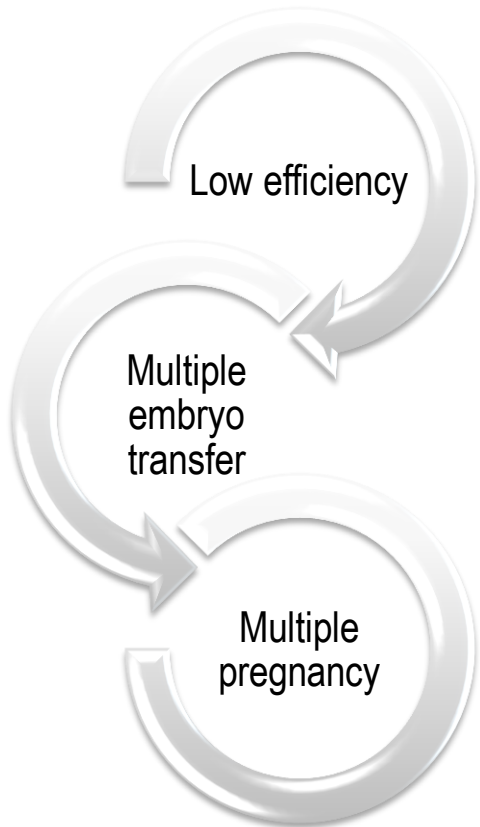
(Replaces Committee Opinion No. 324, November 2005)

### Committee on Obstetric Practice Committee on Genetics

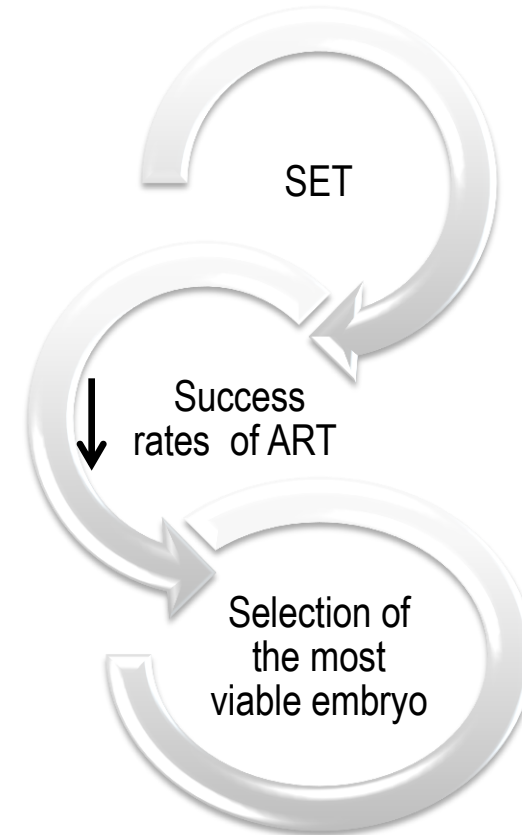
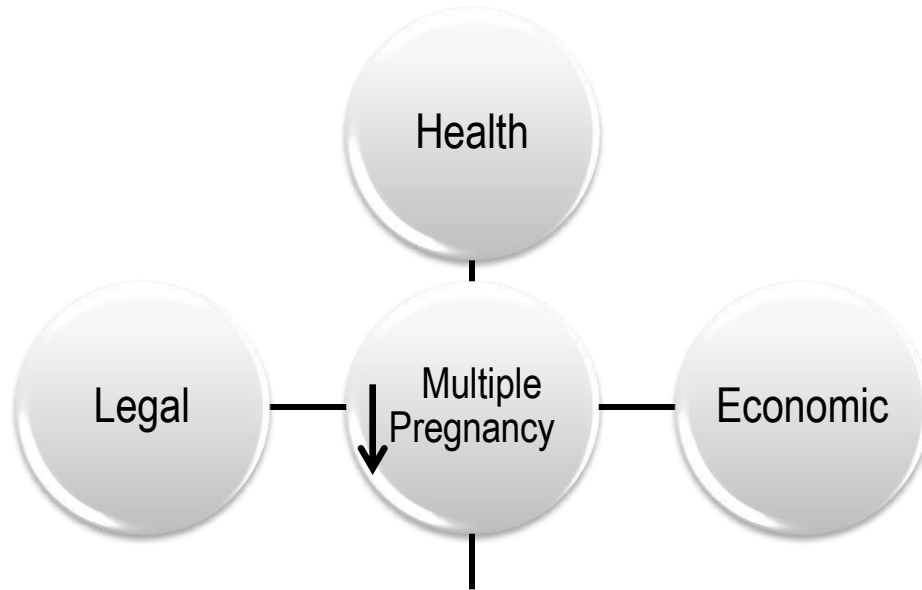
*The American Society for Reproductive Medicine and the Society for Maternal-Fetal Medicine endorse this document. This Committee Opinion was developed by the American College of Obstetricians and Gynecologists' Committee on Obstetric Practice and the Committee on Genetics in collaboration with committee members James Summers, MD and Jeffrey L. Ecker, MD, and the U.S. Food and Drug Administration's representative member Rhonda Hearn-Stokes, MD. The views do not necessarily represent those of the Food and Drug Administration or the U.S. government.*

*This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.*

### Perinatal Risks Associated With Assisted Reproductive Technology



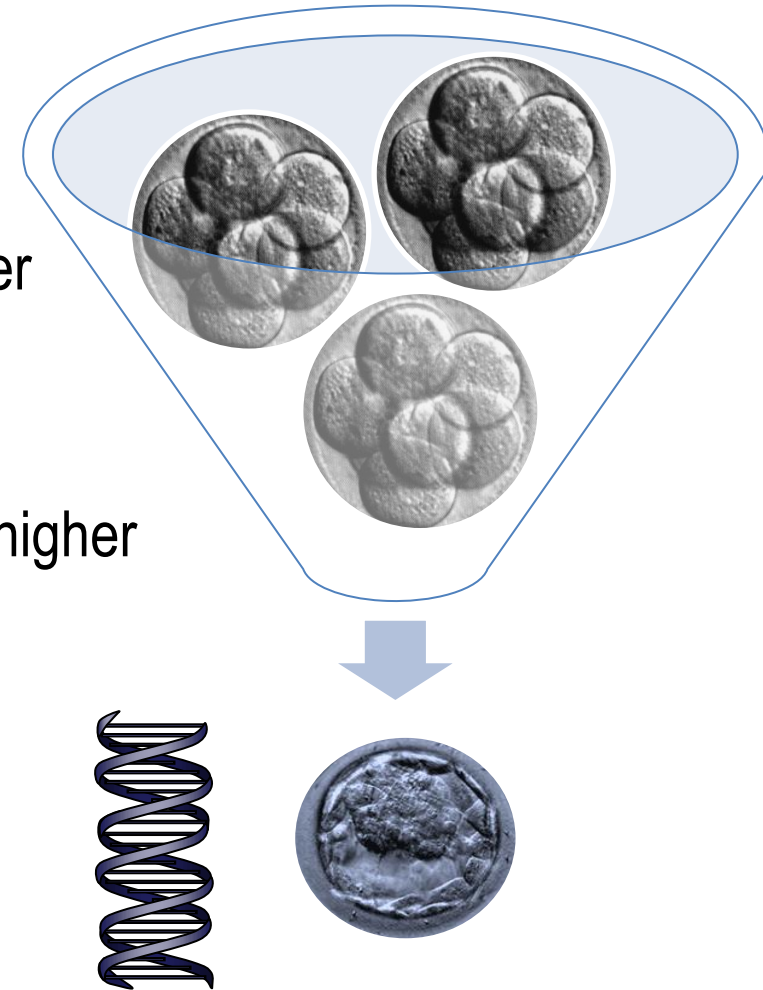
# INTRODUCTION



**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 ?



embryo genome expression

CRUCIAL

# INTRODUCTION

Non-invasive approaches for embryo development assessment

**GENOMIC**  
~25,000 GENES

**TRANSCRIPTOMIC**  
~100,000

**PROTEOMIC**  
~1,000,000

**METABOLOMIC**  
~2,500

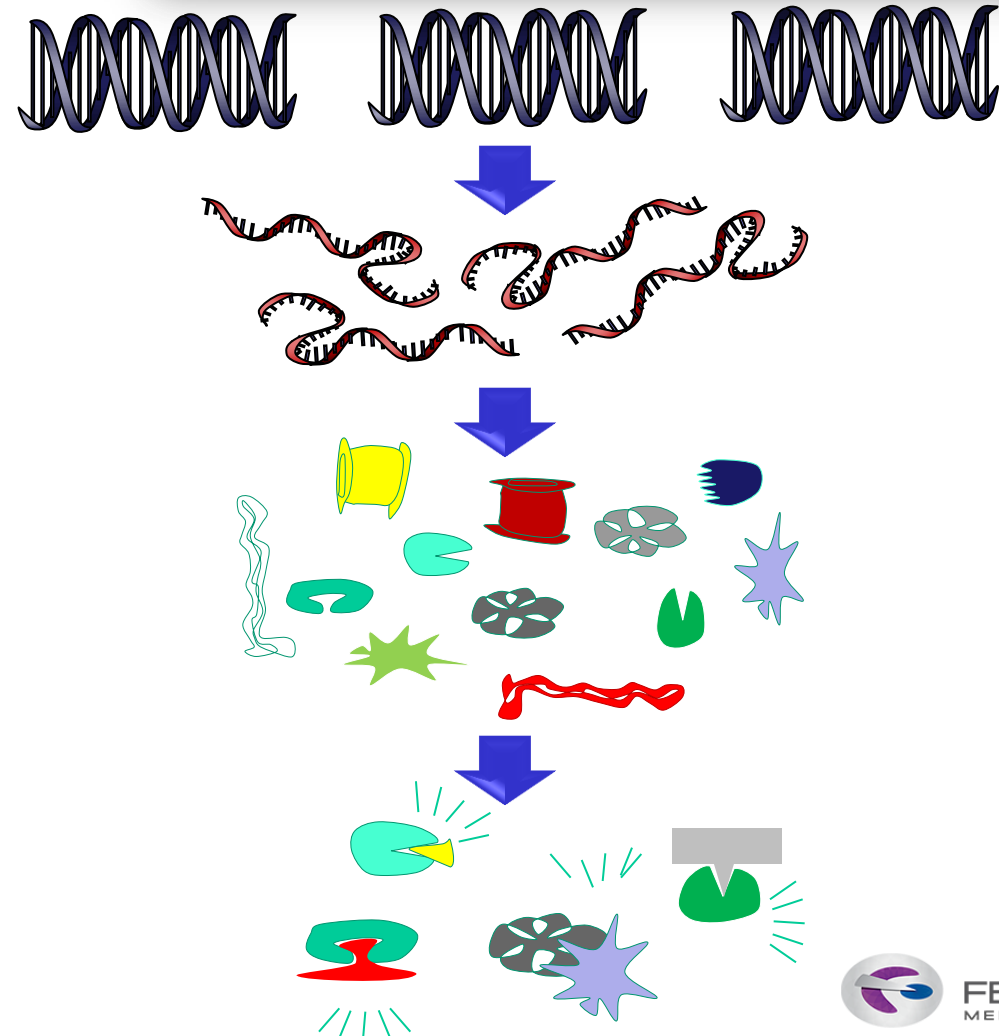
Molecular Human Reproduction, Vol.16, No.8 pp. 513–530, 2010  
Advanced Access publication on June 10, 2010 doi:10.1093/molehr/gaq041

**MHR**  
The Journal of Assisted Reproduction

NEW RESEARCH HORIZON Review

## OMICS in assisted reproduction: possibilities and pitfalls

Emre Seli<sup>1</sup>, Claude Robert<sup>2</sup>, and Marc-Andre Sirard<sup>2\*</sup>

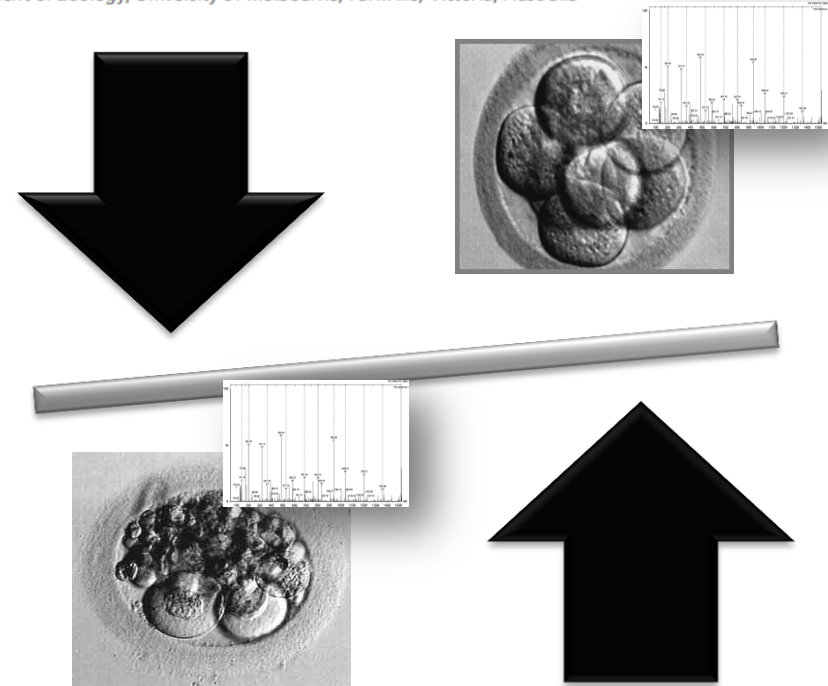
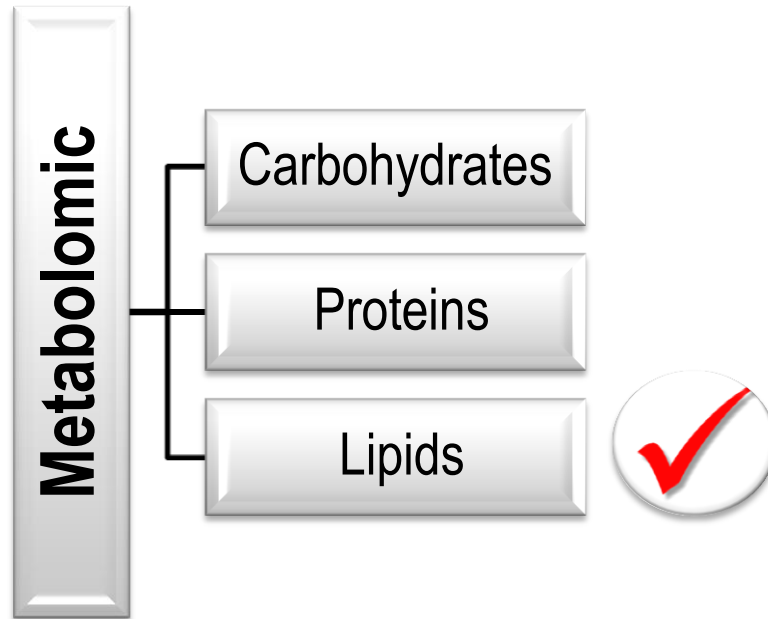


# INTRODUCTION

## Analysis of metabolism to select viable human embryos for transfer

David K. Gardner, D.Phil. and Petra L. Wale, B.Sc.

Department of Zoology, University of Melbourne, Parkville, Victoria, Australia



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.

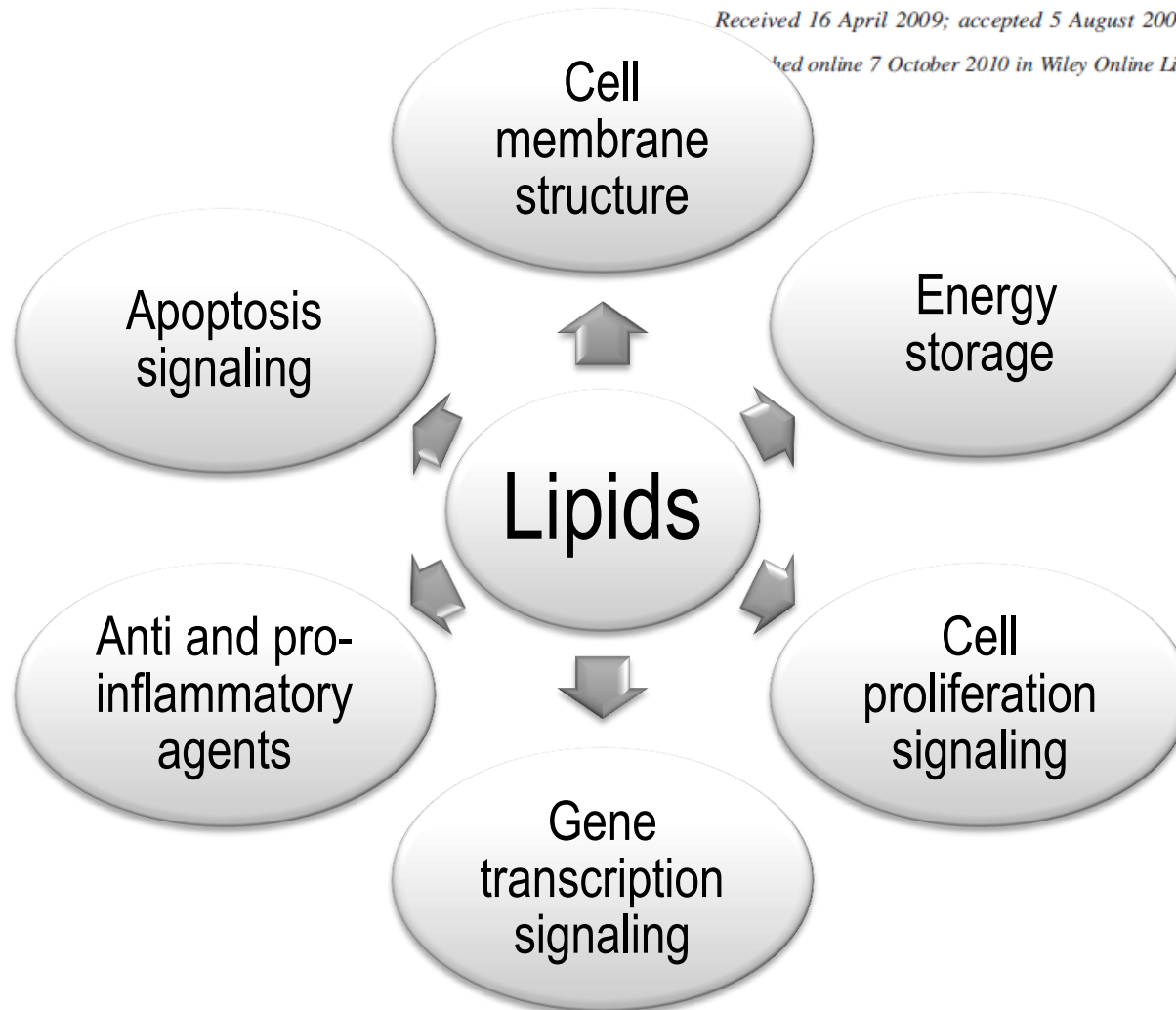
# INTRODUCTION

**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*

*Received 16 April 2009; accepted 5 August 2009*

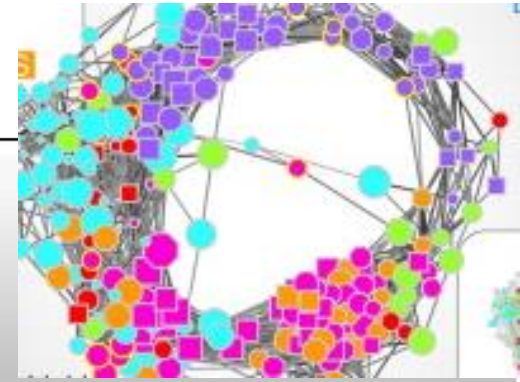
*Published online 7 October 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mas.20294*





# 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

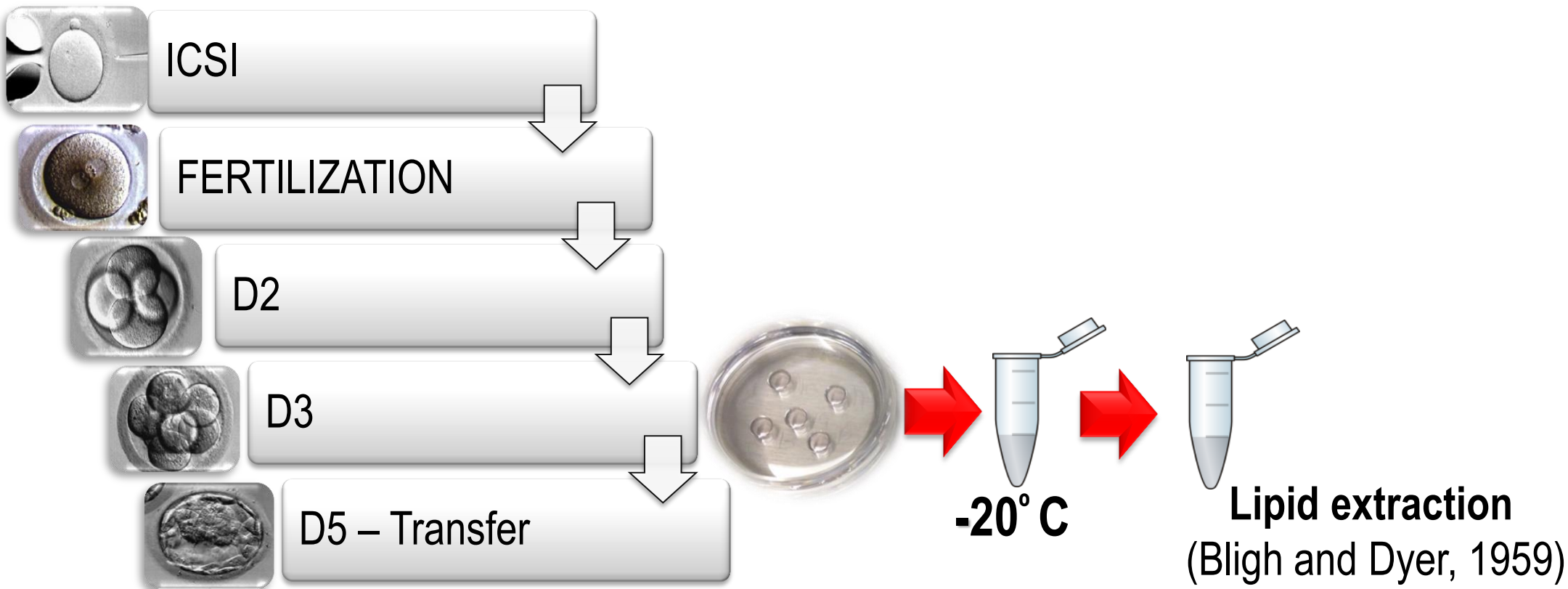
Samples of D3  
embryo culture  
media

```
graph TD; A[Samples of D3 embryo culture media] --- B[Live births];
```

Live births

# MATERIALS AND METHODS

- SAMPLES COLLECTION

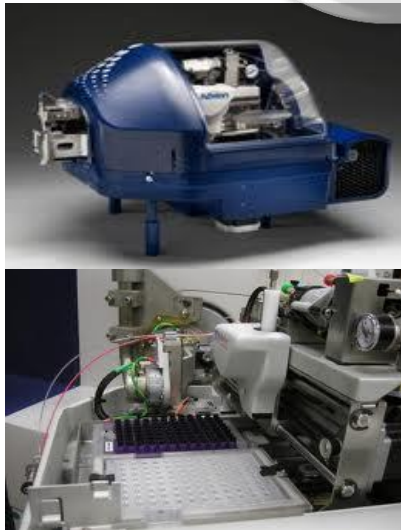
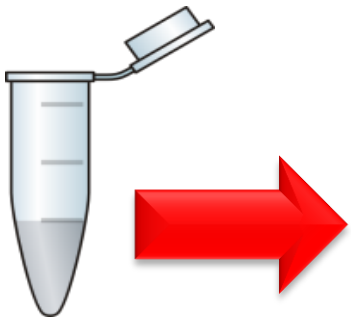


# MATERIALS AND METHODS

- MASS SPECTROMETRY AND LIPID PROFILE

Positive  
ionization  
mode

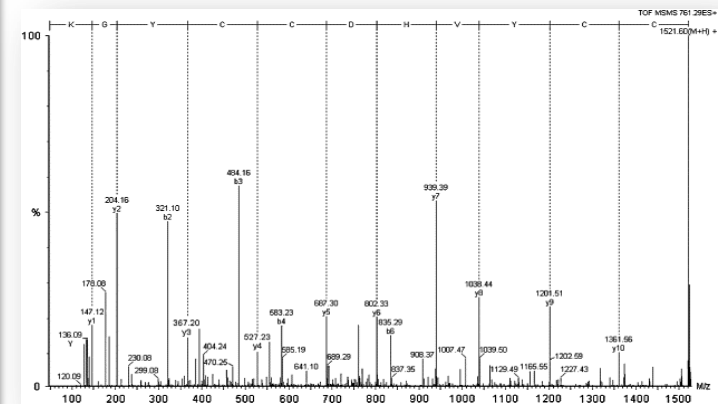
Negative  
ionization  
mode



**Nanomate**  
automated injector



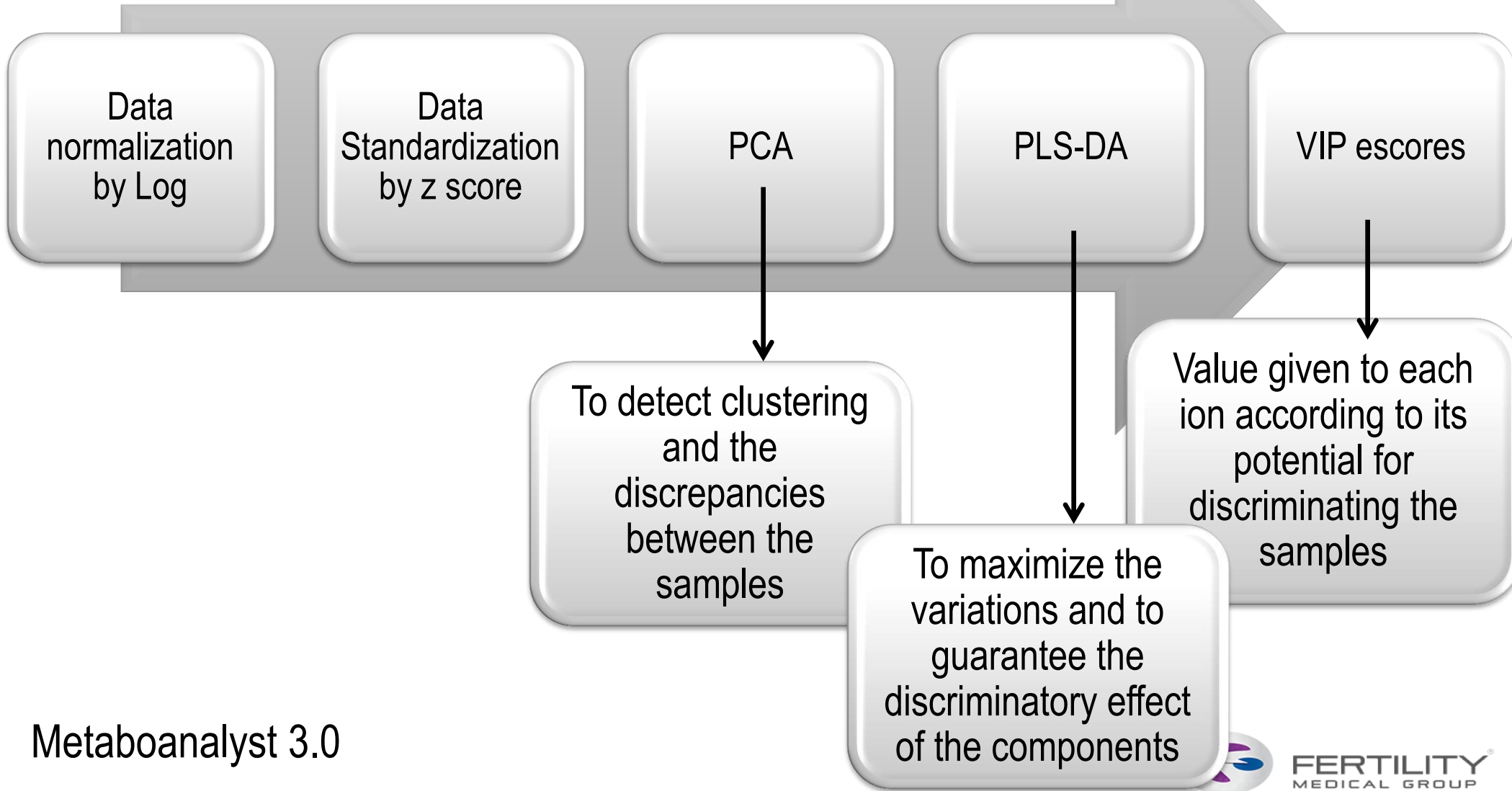
**Q-TOF**



**Data acquisition**

# MATERIALS AND METHODS

- DATA ANALYSIS

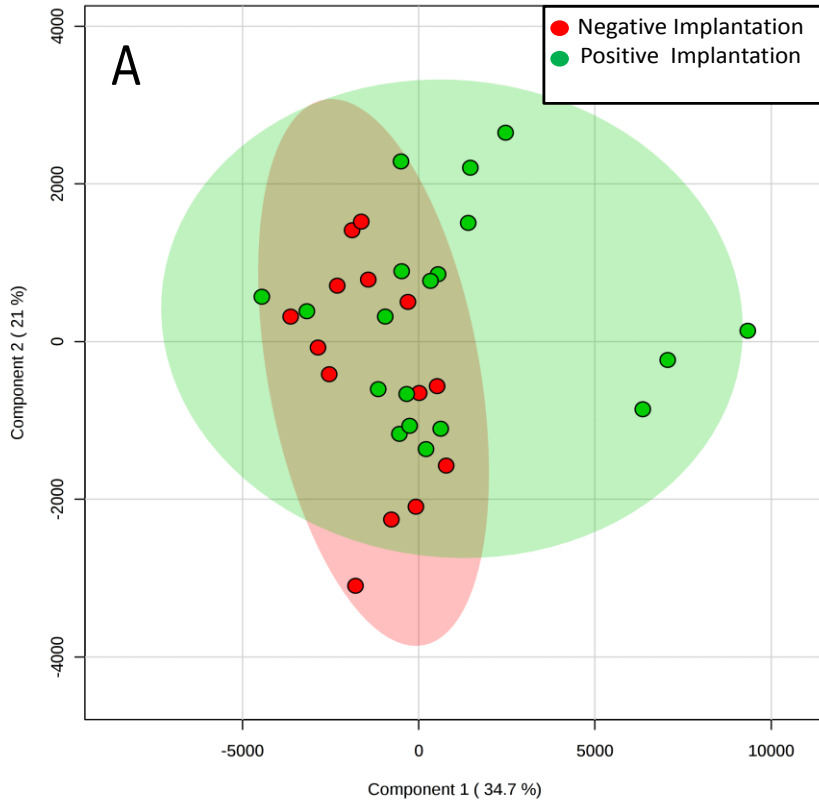


# RESULTS

PCA

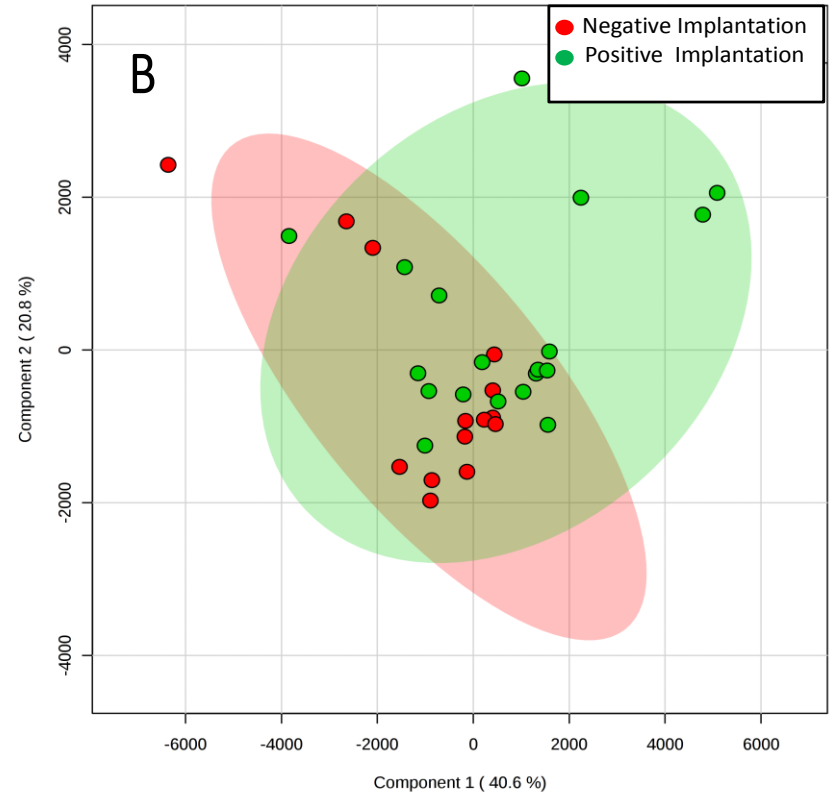
Clustering of the samples from the negative implantation group on positive and negative ionization modes

Scores Plot



+ ionization mode

Scores Plot

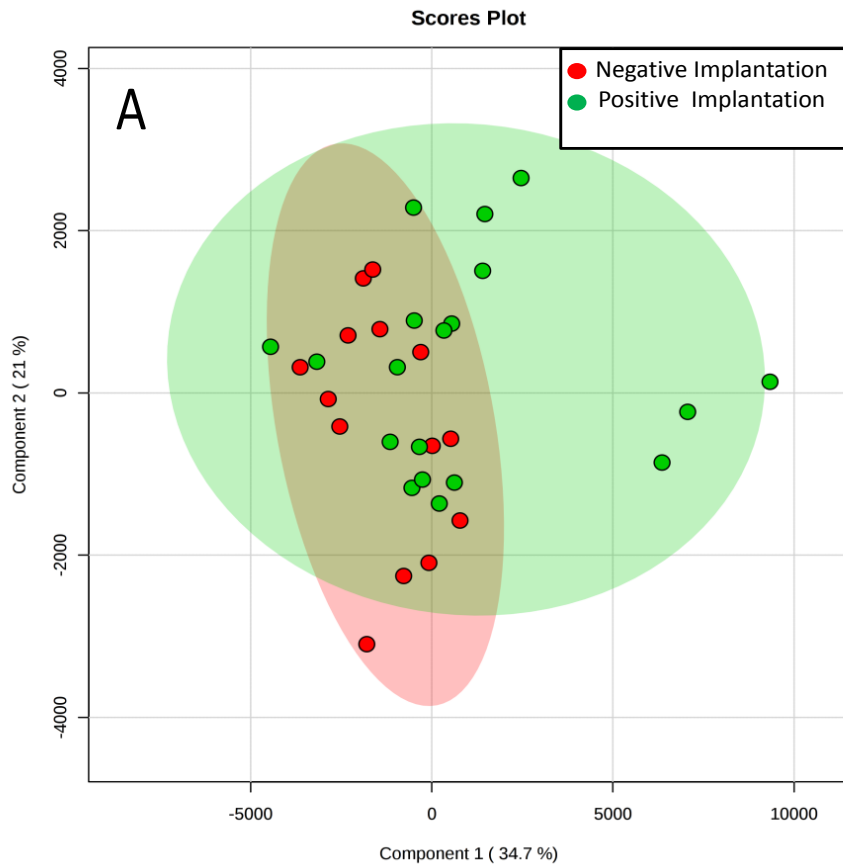


- ionization mode

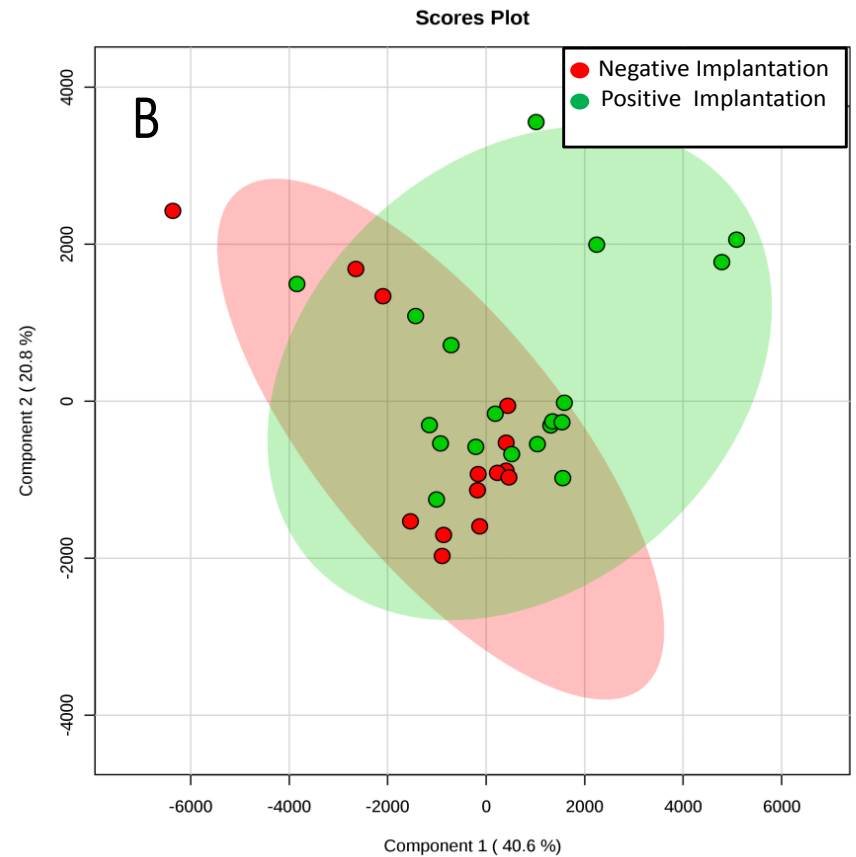
# RESULTS

PLS-DA

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



+ ionization mode



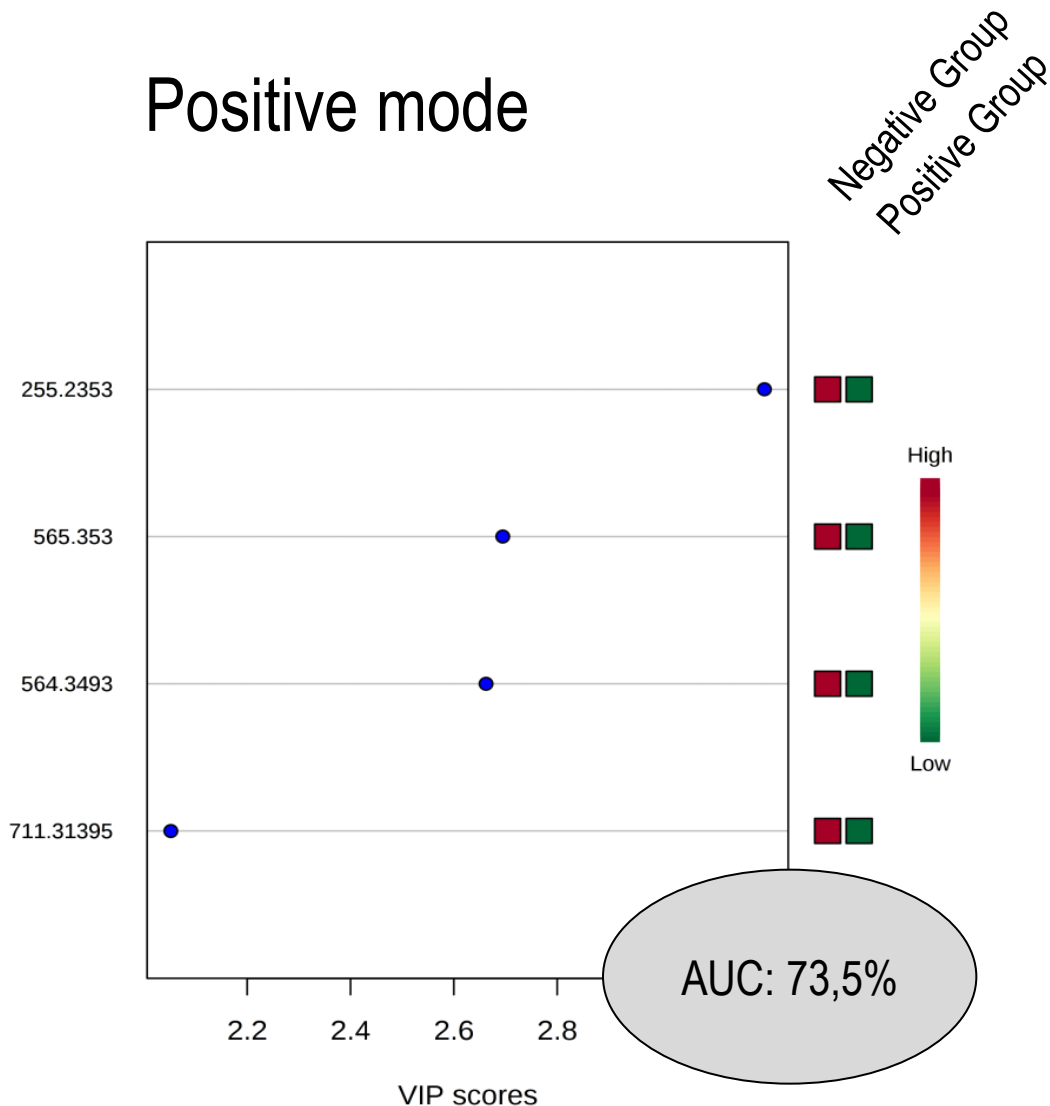
- ionization mode



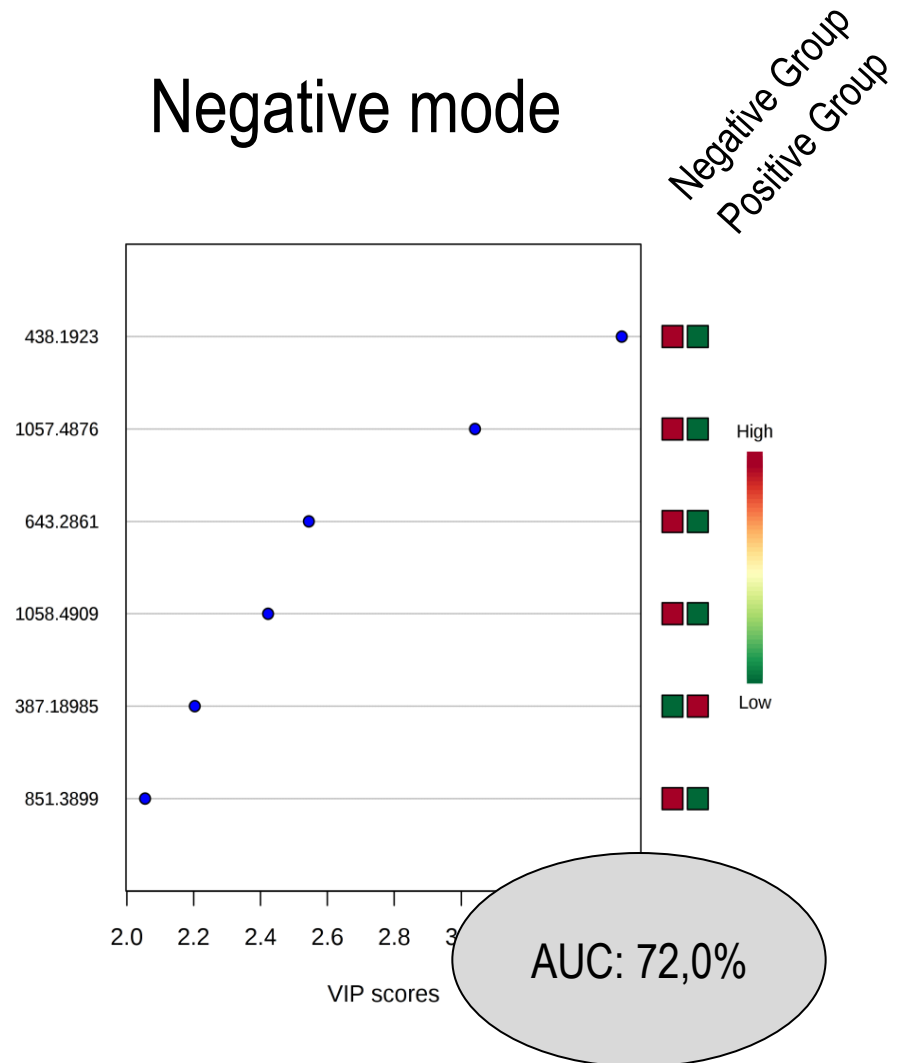
# RESULTS

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

## Positive mode

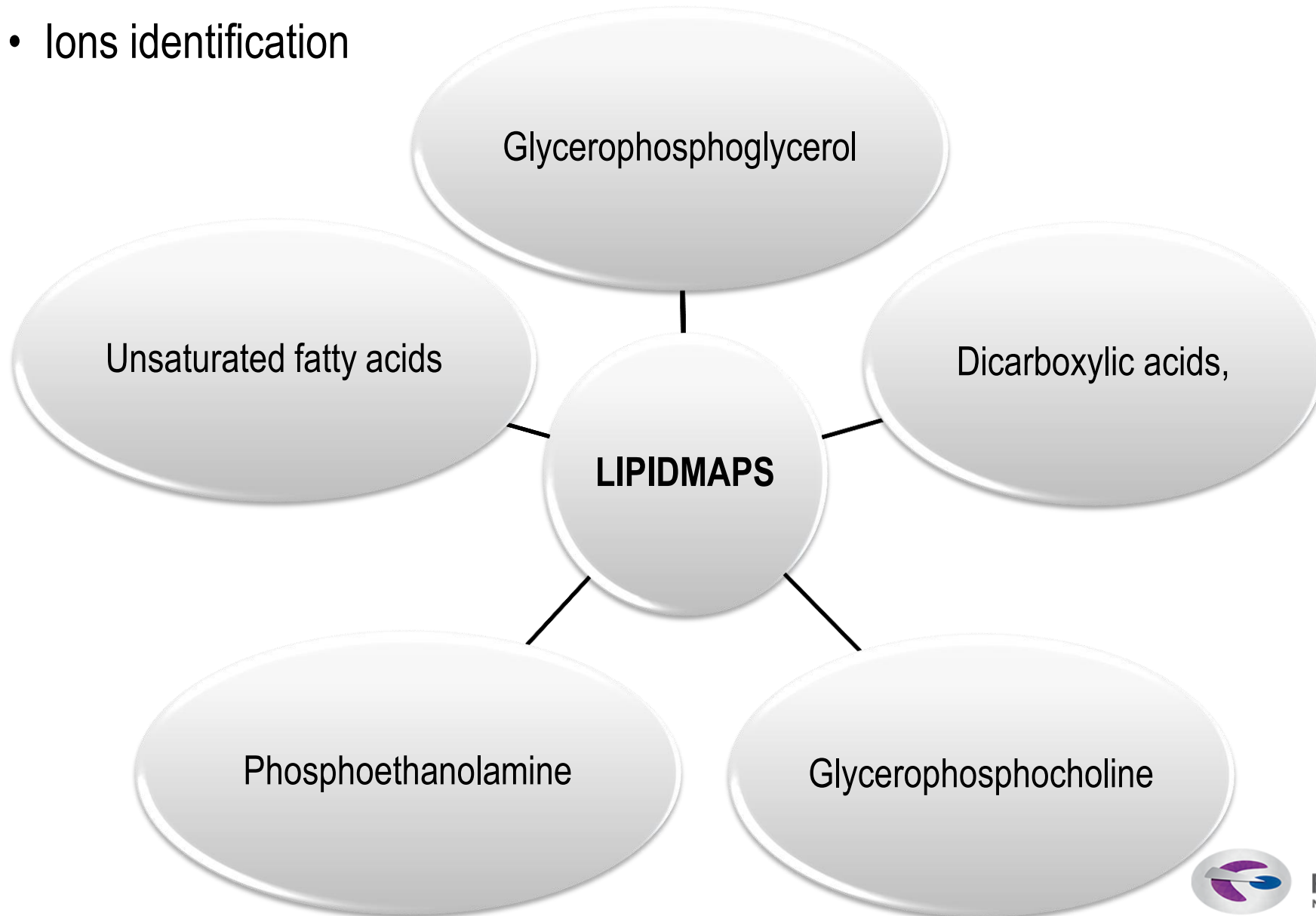


## Negative mode

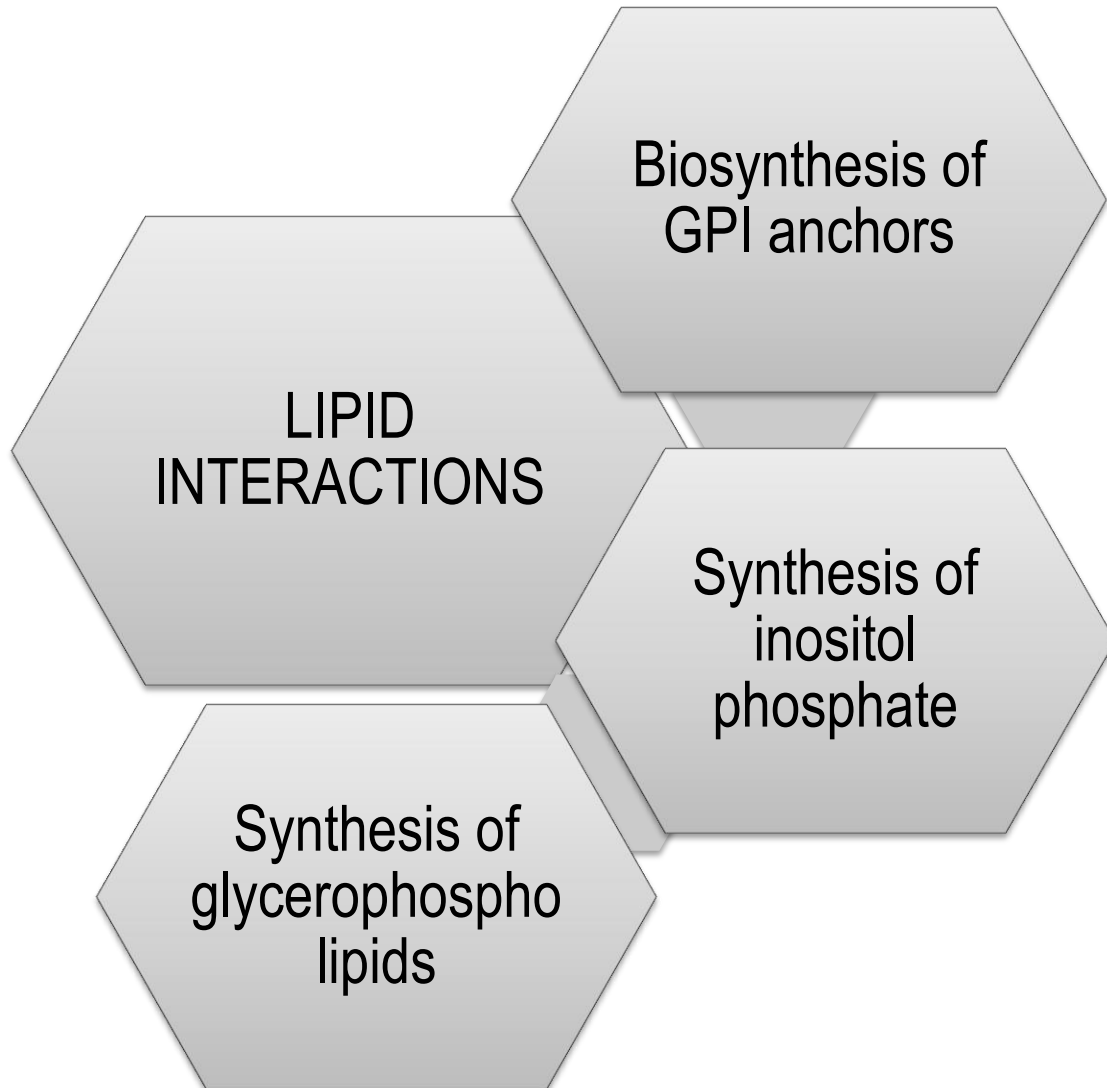


# RESULTS

- Ions identification



# RESULTS



Lipids Involved in cellular restructuring



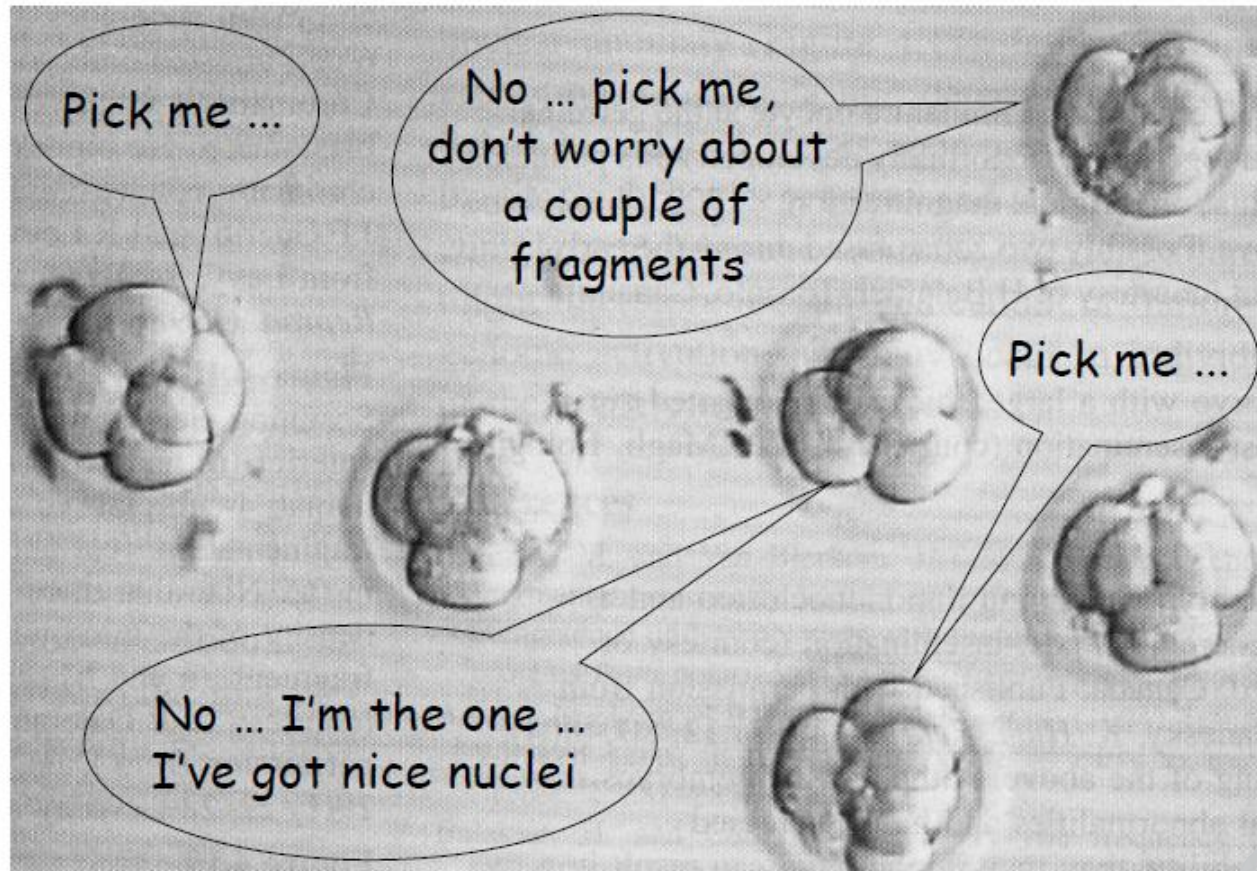
Embryos with poor implantation potential



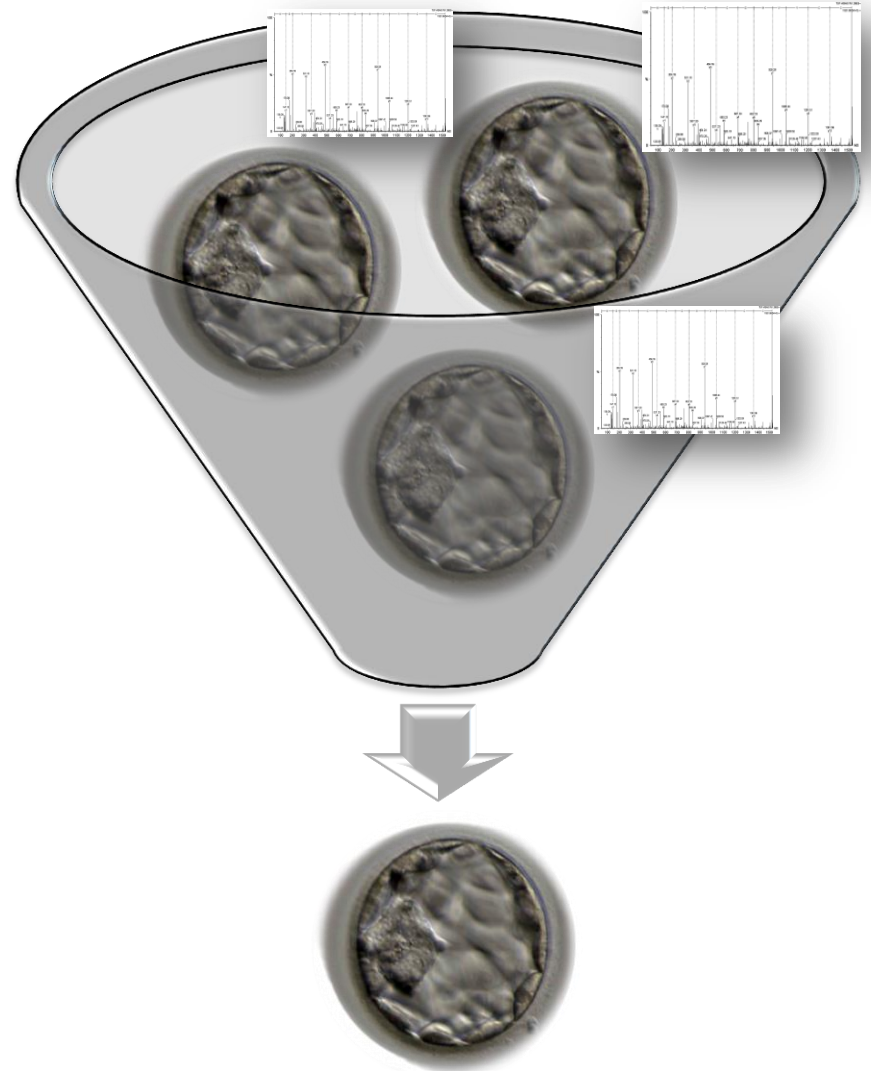
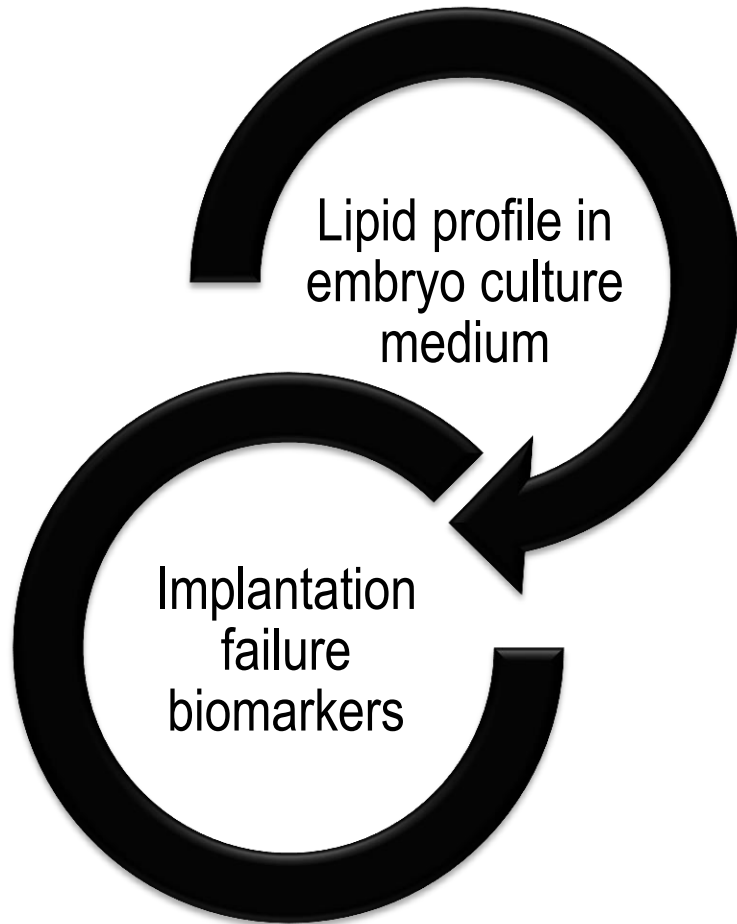
Machinery to exhaustion

# DISCUSSION

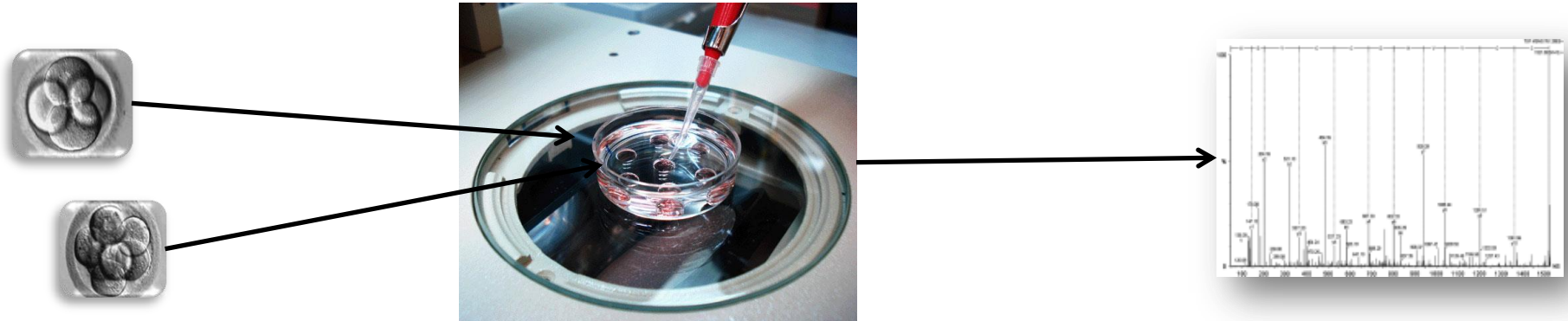
## The Embryologist's Dilemma....



# DISCUSSION



# DISCUSSION



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

Richard Scott, M.D.,<sup>a</sup> Emre Seli, M.D.,<sup>b</sup> Kathy Miller, B.S.,<sup>a</sup> Denny Sakkas, Ph.D.,<sup>b</sup> Katherine Scott, B.S.,<sup>a</sup> and David H. Burns, Ph.D.<sup>c</sup>

Fertility and Sterility® Vol. 90, No. 1, July 2008

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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

Emre Seli, M.D.,<sup>a</sup> Lucy Botros, M.Sc.,<sup>b,c</sup> Denny Sakkas, Ph.D.,<sup>a,d</sup> and David H. Burns, Ph.D.<sup>b</sup>

Fertility and Sterility®

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

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

Fertility and Sterility®

Human Reproduction Vol.23, No.7 pp. 1499–1504, 2008

Advance Access publication on April 18, 2008

doi:10.1093/humrep/den111

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

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

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

Sylvia Sanches Cortezzi<sup>1</sup>, Elaine Cristina Cabral<sup>2</sup>, Marcello Garcia Trevisan<sup>3,4</sup>,  
Christina Ramires Ferreira<sup>2</sup>, Amanda Souza Setti<sup>1,5</sup>, Daniela Paes de Almeida Ferreira Braga<sup>1,5</sup>,  
Rita de Cássia Sávio Figueira<sup>5</sup>, Assumpto Iaconelli Jr<sup>1,5</sup>, Marcos Nogueira Eberlin<sup>2</sup>  
and Edson Borges Jr<sup>1,5</sup>

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

Reproduction (2013) 145 453–462

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

# DISCUSSION

JBRA Assisted Reproduction 2015;19(3):119-124  
doi: 10.5935/1518-0557.20150027

Original Article

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

Daniela Paes de Almeida Ferreira Braga<sup>1,2,3</sup>, Amanda Souza Setti<sup>2,3</sup>, Elaine Cristina Cabral<sup>4</sup>, Marcos Eberlin<sup>5</sup>, Edson Guimarães Loturco<sup>1</sup>, Edson Borges Jr<sup>2,3</sup>

<sup>1</sup>Disciplina de Urologia, Departamento de Cirurgia – UNIFESP - Brasil

<sup>2</sup>Instituto Sapientiae – Centro de Estudos e Pesquisa em Reprodução Assistida - Brasil

<sup>3</sup>Fertility – Medical Group – Sao Paulo - Brasil

<sup>4</sup>Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas – CPQBA – UNICAMP - Brasil

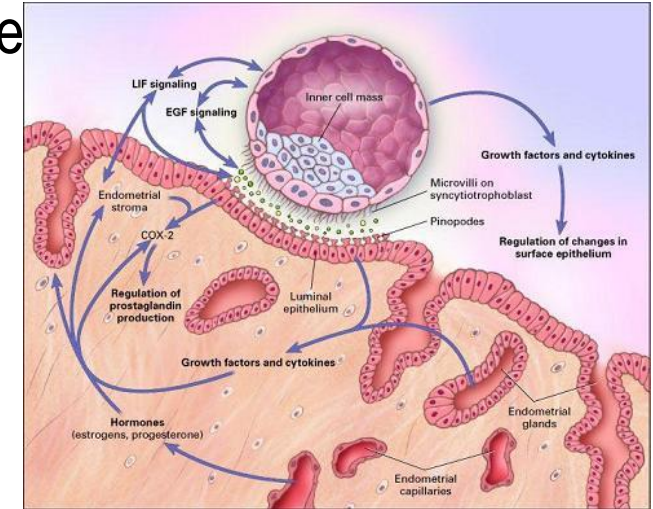
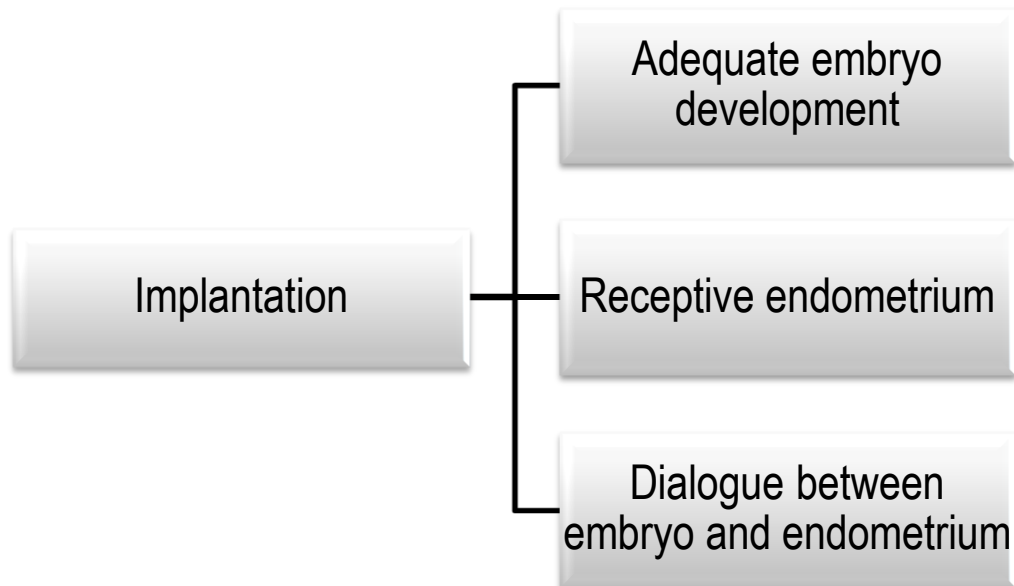
<sup>5</sup>Laboratório ThomSon de Espectrometria de Massas – Instituto de Química – UNICAMP - Brasil

- 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



Source: The cell

# DISCUSSION

- 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

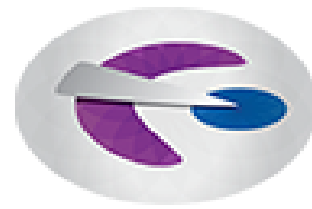


Expensive  
Robust  
Complicated  
Difficult Access

# 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



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