NEW DEVELOPMENTS ON BIOMARKERS OF ENDOMETRIAL RECEPTIVITY

Prof. Carlos Simón MD; PhD
Professor Obs/Gyn, University of Valencia. Spain
Scientific Director, Fundación IVI. Spain
Adjunct Clinical Professor Obs/Gyn, Stanford University. USA
Merck Serono GFI supports the advancement of science and INNOVATIVE TECHNOLOGIES in fertility with the potential to improve TAKE HOME BABY RATES for the benefit of patients.

Our goal is to demonstrate our COMMITMENT and LEADERSHIP IN FERTILITY.

INDUSTRIAL SUPPORTED LUNCH SYMPOSIUM
Supporting the advancement of science and INNOVATIVE TECHNOLOGIES in fertility with the potential to improve TAKE HOME BABY RATES for the benefit of patients.
GFI & Endometrial Receptivity Diagnostics

1. Carlos Simon, Spain - **New non-invasive diagnostic method of human endometrial receptivity: lipidomics** The development of new diagnostic approaches to using biologic fluids has opened up a new field of investigation in non-invasive endometrial diagnosis techniques. Using mass spectrometry and nuclear magnetic resonance precise detection of lipids is now possible. Lipidomics analysis of endometrial fluid opens a new door to possible non-invasive diagnosis and this project is designed to determine the lipidemic profile expressed during the WOI. With the specific detection of PGs in the endometrial fluid it will be possible to develop a new non-invasive technique to help us to predict the state of endometrial receptivity.

2. Nick Macklon, UK - **Endometrial gene expression profile associated with recurrent implantation failure** Using a recently identified profile of 70 genes which are highly dysregulated in women who fail to achieve implantation despite the transfer of multiple high quality embryos after IVF the study aims to understand the genomic footprint of the highly refractory human endometrium and this information to be developed into a diagnostic test of endometrial receptivity, or to provide reliable pointers for new clinical interventions, the profile needs to be tested in different populations.

3. Roberto Matorras, Spain - **Endometrial fluid proteomics to assess uterine receptivity** The hypothesis of the present study is that the different situation of endometrial development under hormonal stimulation might yield a different protein secretion pattern, and some of these proteins could be related with the implantation outcome. Consequently, a favourable and unfavourable protein pattern could be determined with important clinical applications. Concerning the comparison with oocyte donors, in some IVF patients an adverse proteomic pattern could be detected, that perhaps could be related with the cause of the infertility.

4. Ellen Greenblatt, Canada - **Validation of endometrial receptivity biomarkers predictive of success** The objectives of the study are to 1) correlate the expression of candidate biomarkers of endometrial receptivity to pregnancy outcomes and determine patterns of gene expression predictive of implantation and pregnancy in natural cycles; 2) compare the endometrial gene expression profile in natural cycles to controlled ovarian hyperstimulation (COH) cycles; and 3) determine gene expression patterns predictive of pregnancy in COH-IVF cycles.

5. Lois A. Salamonsen, Australia - **Uterine receptivity: the final hurdle in IVF** The aim of this project is to discover and validate biomarkers of uterine receptivity. This project will apply innovative highly targeted proteomic analyses to uterine fluid. It will compare protein glycoforms present in pooled fluid taken at the relevant time of the menstrual cycle, from fertile and infertile women. Those that are altered between the two groups will be validated on cohorts of individual women. Subsequent development of novel glycoform assays will provide for quantitative analysis.

6. Hamamah - **Prospective study of the ‘Win Test’ (Window Implantation Test).**

7. Simon - **Analysis and functionality of miRNAs on endometrial fluid.**
Refining the Fingerprint

- Cytokines
- Glycoproteins
- Nanoparticles
- Prostaglandins
- Lipids
- Signaling Factors
- Growth Factors
- Chemokines

- Salamonsen 2012
- Greenblatt 2011
- Hamamah 2013
- Simon 2010
- Simon 2013
- Matorras 2011
Studies in early phase development
Proteomic assessment of the endometrial liquid as a prognostic factor of the embryo implantation in In Vitro Fertilization (IVF)

Roberto Matorras Weinig
University of the Basque Country, Vizcaya, Spain

- Study aims to perform proteome analysis on endometrial fluid (taken by embryo transfer catheter immediately before embryo transfer)
- Attempt to identify endometrial protein secretion pattern related to implantation outcomes

Technology used:
- Proteomics analysis with two dimensional electrophoresis
- Mass spectrometry
- ELISA
- xMAP
Validation of endometrial receptivity biomarkers predictive of success

Ellen Greenblatt
University of Toronto, Department of Obstetrics and Gynaecology, Canada

- Aims to correlate the expression of candidate biomarkers of endometrial receptivity to pregnancy outcomes and determine patterns of gene expression predictive of implantation and pregnancy in natural cycles
- Will allow us to compare the endometrial gene expression profile in natural cycles to controlled ovarian hyperstimulation (COH) cycles; and determine gene expression patterns predictive of pregnancy in COH-IVF cycles

Technology used:
- Microarray
- qPCR
- Gene networking software
Uterine receptivity: the final hurdle in IVF
Lois A. Salamonsen
Prince Henry’s Institute of Medical Research, Melbourne, Australia

- Study aims to apply innovative highly targeted proteomic analyses to uterine fluid, to discover and validate biomarkers of uterine receptivity
- It will compare protein glycoforms present in pooled fluid from fertile and infertile women
- Differences between the two groups will be validated on cohorts of women; the most promising markers will also be tested in samples from women undergoing IVF and egg donation

Technology used:
- Mass spectrometry for proteomic analysis
Endometrial gene expression profile associated with recurrent implantation failure

Nick Macklon
Director of the Complete Fertility Centre Southampton, Chair in Obstetrics and Gynaecology in Southampton, UK

- Study aims to evaluate a profile of 70 genes previously identified as highly dysregulated in women who fail to achieve implantation despite the transfer of multiple high quality IVF embryos
- The team hope to understand the genomic footprint of the highly refractory human endometrium, to ultimately develop a diagnostic test of endometrial receptivity, or to provide reliable pointers for new clinical interventions

- Technology used:
  - PCR

Images from online public sources including Wikipedia
Prospective study of the ‘Win Test’ (Window Implantation Test)
Samir Hamamah
Medical School and University-Hospital Montpellier, France

- Study aims to validate the relevance of the ‘Win Test’ in a prospective study using two populations: fertile or with multiple implantation failures

- Technology used:
  - Win Test

RNA-seq mapping of short reads over exon-exon junctions, depending on where each end maps to, it could be defined a Trans or a Cis event.
Analysis and functionality of miRNAs on endometrial fluid

Carlos Simón
Valencia University & Fundación IVI, Spain

- Study aims to determine the expression profiles of secreted miRNAs in endometrial fluid samples obtained at different phases of the menstrual cycle.
- To identify, by *in silico* analysis, the putative gene targets and functional pathways affected by the miRNAs in the receptivity phase.

Technology used:
- *In silico* analysis

Interaction of microRNA with protein translation process

Images from online public sources including Wikipedia
Study in pre-clinical phase
Lipidomics as an emerging tool to predict endometrial receptivity
Carlos Simón, Fundacion Instituto Valenciano de Infertilidad and Instituto Universitario IVI/INCLIVA, Valencia University; and Iviomics, Paterna, Valencia, Spain

- Lipid analysis of endometrial secretions is a new, non-disruptive method for the diagnosis of endometrial receptivity
- Study aimed to develop a non-invasive diagnostic technique to diagnose endometrial receptivity, based on the detection of two specific lipids found in the endometrial fluid of the receptive endometrium
- Techniques used:
  - NMR, nuclear magnetic resonance
  - MALDI, matrix assisted laser desorption/ionization
  - ESI, electrospray ionization
  - MS-MS, tandem mass spectrometry

NMR, nuclear magnetic resonance; MALDI, matrix assisted laser desorption/ionization; ESI, electrospray ionization; MS-MS, tandem mass spectrometry
Aspiration of endometrial secretion does not affect pregnancy rates

Van der Gaast et al. RBmOnline 2002

Glycodelin levels correlate with the menstrual cycle phase of endometrial aspirations

Van der Gaast MH, et al. BJOG 2009

The profile of cytokines can be determined in endometrial secretions

Boomsma CM et al. RBmOnline 2009

The lipidomics is the large-scale study of lipid species present in a cell or biological fluid and their interacting pathways

Wenk MR. Nature reviews Drug Discovery 2005
The lipidomics can be defined as large-scale study of lipid species present in a cell or biological system and their interacting pathways and metabolic networks.
ANIMAL MODELS

Multiple female reproductive failures in cyclooxygenase 2-deficient mice.
Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK.
Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City 66160, USA.

Molecular Human Reproduction Vol.10, No.4 pp. 215–221, 2004
M. Maccarrone¹,², M. DeFelici², F. G. Klinger², N. Battista³, F. Fezza¹, E. Dainesi¹, G. Siracusa² and A. Finazzi-Agrò³

Maternal disturbance in activated sphingolipid metabolism causes pregnancy loss in mice.
Mizugishi K, Li C, Olivera A, Bielawski J, Bielawska A, Deng CX, Proia RL.
INTRODUCTION
Expression of prostaglandins

Levels of PGs in implantation and interimplantation sites on day 5 of pregnancy.

Mouse

Hamster

Human

Wang et al. JBC, 2004

Downie et al. J. Physiol, 1974
1) To demonstrate the specificity and consistency of the lipidomic profile in endometrial fluid in natural, COS and HRT cycles, and in refractory endometrium induced by an IUD.

2) To understand the localization, regulation and function of these prostaglandins (PGs) and their receptors in embryo adhesion at the maternal-embryo interface.

3) To assess the sensitivity and specificity of the diagnostic accuracy of PGE$_2$ & PGF$_2$a levels in endometrial fluid (EF) obtained 24 hours before embryo transfer to predict pregnancy outcomes.
A transfer catheter is gently introduced into the uterine cavity and suction is applied with a 10 mL syringe. To obtain between 10-80 μl of EF.

Lipids from EF extracts were identified by liquid chromatography combined with tandem mass-spectrometry (LC/MS/MS).
1) To demonstrate the specificity and consistency of the lipidomic profile in endometrial fluid in natural, COS and HRT cycles, and in refractory endometrium induced by an IUD.

2) To understand the localization, regulation and function of these prostaglandins (PGs) and their receptors in embryo adhesion at the maternal-embryo interface.

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10 lipids were identified and only 2 were differentially secreted

**Normal menstrual Cycle**
- Group I: days 0-8
- Group II: days 9-14
- Group III: days 15-18
- Group IV: days 19-23
- Group V: days 24-30

**EF**
- EF (n=51)
  - *** (p<0.001)
The same ovum donors underwent consecutive treatments (n=30)

Day of endometrial fluid collection
THE LIPIDOMIC PROFILE DURING THE WOI IS CONSISTENT IN HRT CYCLES

Concentration (nmol/g)

PGE₂  PGF₂α

HRT (n=30)  *(p<0.05)
THE LIPIDOMIC PROFILE DURING THE WOI IS CONSISTENT IN COS CYCLES

The lipidomic profile during the WOI is consistent in COS cycles, with notable differences in concentration levels across different time points after hCG administration. The diagram illustrates the concentration (nmol/g) of PGE₂ and PGF₂α at various stages: hCG+0, hCG+3, hCG+5, hCG+7, hCG+9, and hCG+11, with each sample group consisting of 5 individuals.

- hCG+0: Low concentration levels for both PGE₂ and PGF₂α.
- hCG+3: Minimal change from hCG+0.
- hCG+5: Slight increase in concentration levels.
- hCG+7: Significant increase in concentration levels, particularly for PGF₂α, marked with an asterisk (*) indicating statistical significance (p<0.05).
- hCG+9: Maintenance of concentration levels similar to hCG+7.
- hCG+11: Further increase in concentration levels, with PGF₂α showing a pronounced rise.

The COS cycles (n=30) demonstrate consistent lipidomic profiles with notable statistical significance at hCG+7.
THE LIPIDOMIC PROFILE DURING THE WOI IS ABROGATED IN REFRACTORY ENDOMETRIUM

Concentration (nmol/g)

PGE$_2$  PGF$_2$\textsubscript{a}

HRT (n=25)  HRT and IUD (n=25)  *(p<0.05)
1) To demonstrate the specificity and consistency of the lipidomic profile in endometrial fluid in natural, COS and HRT cycles, and in refractory endometrium induced by an IUD.

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SPECIFIC PG SYNTHASES ARE UP-REGULATED IN THE RECEPTIVE ENDOMETRIUM

PGE₂ and PGF₂α are regulated by AKR1C3, AKR1C1 and CBR1 PG synthases in R vs non-R endometrium.
**INHIBITION OF PG SYNTHASES BLOCKS THE SECRETION OF PGE2 AND PGF2α IN hEECs**

**PG synthases expression in hEEC**

**Concentration (ng/ml)**

**Concentration (pg/ml)**

**AKR1C1**  **AKR1C3**  **mPGES1**  **mPGES2**  **cPGES**  **CBR1**  **GAPDH**

**INHIBITION OF PG SYNTHASES BLOCKS THE SECRETION OF PGE2 AND PGF2α IN hEECs**
PGE2 AND PGF2α RECEPTORS ARE LOCATED IN THE BLASTOCYST
INHIBITION OF PGE2 AND PGF2α DECREASES EMBRYO ADHESION RATES WHILE ADDITION RESTORES THEM IN VITRO

Vilella et al., J Clin Endocrin Metab 2013
EP2 AND FP ARE THE EMBRYONIC RECEPTORS IMPLICATED IN EMBRYO ADHESION

Vilella et al., J Clin Endocrin Metab 2013
1) To demonstrate the specificity and consistency of the lipidomic profile in endometrial fluid in natural, COS and HRT cycles, and in refractory endometrium induced by an IUD.

2) To understand the localization, regulation and function of these prostaglandins (PGs) and their receptors in embryo adhesion at the maternal-embryo interface.

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**PGs LEVELS IN EF OBTAINED 24H BEFORE DAY 3 ET (S&S)**

**Concentration (nmol/g)**

- **Pregnant (n=5)**: PGE₂, PGF₂α
- **Not Pregnant (n=15)**: PGE₂, PGF₂α

<table>
<thead>
<tr>
<th></th>
<th>PGE₂</th>
<th>PGF₂α</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROC curve</strong></td>
<td>0.88</td>
<td>0.973</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>80.00%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>86.70%</td>
<td>93.30%</td>
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*(p<0.05)  ***(p<0.01)
ROC curve

Pregnant (n=8) vs Not Pregnant (n=9)

<table>
<thead>
<tr>
<th>Concentration (nmol/g)</th>
<th>Pregnant</th>
<th>Not Pregnant</th>
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<tbody>
<tr>
<td>0</td>
<td>PGE2</td>
<td>PGF2a</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
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<td>2</td>
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<tr>
<td>2.5</td>
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PGs LEVELS IN EF OBTAINED 24H BEFORE DAY 5 ET (S&S)

<table>
<thead>
<tr>
<th></th>
<th>PGE2</th>
<th>PGF2α</th>
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</thead>
<tbody>
<tr>
<td>ROC curve</td>
<td>0.694</td>
<td>0.653</td>
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<tr>
<td>Sensitivity</td>
<td>75.00%</td>
<td>37.50%</td>
</tr>
<tr>
<td>Specificity</td>
<td>77.80%</td>
<td>100%</td>
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Lipidomics as an emerging tool to predict endometrial receptivity

Carlos Simón

Results and conclusion

Results

- Prostaglandins E2 and F2a are especially abundant during the window of implantation

Conclusion

- Specific detection of prostaglandins in endometrial fluid will help predict the state of endometrial receptivity
Thanks for your attention