Ovarian stimulation and inflammation, not always friends!

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Conflict of interest

• Controlled Ovarian Stimulation Timing Test (COST2)GFI project 2014-2016
Our main hypothesis

“making an competent oocyte requires the optimal follicular environment which is very dynamic and can act on positively and negatively on the oocyte”
The pathway to competence

Growth

Follicle growth
Follicle differentiation

Acquisition of oocyte competence

Transcription arrest

Oocyte maturation
Fertilization
Embryo development
Oocyte acquires competence in a multi-step fashion

Done in animals…. (and for IVM in humans)

- Full oocyte size: 0% competence
- Early antral
  - FSH Stimulated growth
  - Competence potential
    - Low growth
    - LH effect dominant
- Competent
  - LH pulses
  - LH surge
The apple analogy

FSH

LH

10mm

low

high
COS plus FSH arrest under low LH

Bovine model (nivet et al. 2012)
Blastocyst rate

% blastocyst dispersion/coasting duration

Bovine model (nivet et al. 2012)
Raised and decreased quality
Do we see this in humans?

- We need individual follicular aspiration to correlate the follicular status with the outcome
- We need to see if a suboptimal follicular environment can comprise oocyte quality
Follicle-oocyte analysis

- Individual follicle aspiration, oocyte IVF and culture.
Genomic analysis to find markers of success and failure?

(Hamel et al 2008, 2010 a,b)

Markers of success and..
Follicular size and development

Markers of small and large follicles

**Small follicles**
- Antral growth
- Luteinization
- Steroidogenesis

**Medium follicles**
- Follicle growth
- Pro-apoptosis

**Large follicles**
- Steroidogenesis
- Cellular proliferation
- Extracellular matrix maintenance
- Apoptosis

**Over-expressed in M+/-**
- TGFB2, ANAPC11
- PDE8B
- THBD, A2M
- ANXA3
- HLA MHC
- TLR2, CHODL
- SERPINA3
- TIMP1
- VANGL1

**Under-expressed in M+/-**
- ACE2, CCNB2, FSCN1
- LGALS12
- SPTAN1
- DUOX2
- EMP3
- VTN, ACE2
- FILIP1, YBX1, TGFB2
- PRDM5
- NLRP12, BCL2L13

Conclusion 1

• We can now assess (biomarkers) why an individual follicle is successful or not (not very useful clinically)

• Can we use this info to document a pool of all the follicles of a failed cycle?
COST-2 study (GFI)

- Control ovarian stimulation timing test
- Concept: When a cycle fails, collect and use the normally discarded granulosa/follicle cells for biomarkers of follicular failure
- Making a diagnostic with such cells
- Changing the stimulation protocol at 2\textsuperscript{e} trial
- Learning the influences of treatment on the pattern.
Trial design:

The biomarkers must be validated by a retrospective and a prospective trial.

• For the retrospective study, 200 samples were used from 4 different clinics to identify the genes associated with failure to derive the best markers for the panel of 24.

• For the prospective study, 320 samples will be tested from 4 clinics using the 12 best markers selected from phase 1.
Phase 1

200 samples obtained from 4 fertility clinics

Insufficient RNA quality
Discarded samples
n=44

Good RNA quality
Usable genetic material
n=156

Grouped based on outcome
Usable for analysis
n=132

Pregnancy
n=69

Positive (A)
 n=69

Negative (B)
 ET day 5
 n=35

Negative (C)
 ET day 3
 n=28

No pregnancy
n=63

No embryo transfer
n=12

Still have frozen embryos
n=12

Unclassified
No outcome result
n=24

Microarray
n=16

RT-qPCR
n=16
Demographic features and cycle parameters in both groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Positive group</th>
<th>Negative group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Age</td>
<td>16</td>
<td>34.44</td>
</tr>
<tr>
<td>Prior cycles</td>
<td>16</td>
<td>0.75</td>
</tr>
<tr>
<td>FSH day 2 to 4 (IU/ml)(^a)</td>
<td>12</td>
<td>6.94</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>16</td>
<td>9.38</td>
</tr>
<tr>
<td>Total IU FSH used (IU)</td>
<td>16</td>
<td>2582.84</td>
</tr>
<tr>
<td>E2 on the day of trigger (pmol/l)(^a)</td>
<td>12</td>
<td>6982.08</td>
</tr>
<tr>
<td>Endometrial thickness (mm)(^a)</td>
<td>12</td>
<td>11.17</td>
</tr>
<tr>
<td>No. of follicles on day of trigger</td>
<td>16</td>
<td>14.25</td>
</tr>
<tr>
<td>No. of COC retrieved</td>
<td>16</td>
<td>9.38</td>
</tr>
<tr>
<td>Oocyte maturity (%)</td>
<td>14</td>
<td>82%</td>
</tr>
</tbody>
</table>

Prior cycles = number or prior IVF cycles for the patient, Endometrial thickness = thickness of endometrium within a few days of trigger, Oocyte maturity = no. of MII oocytes/no. of COC retrieved.

\(^a\) Some data are missing for these parameters because they were not routinely measured on all patients of the clinic.

No significant difference was found between the two groups following Mann-Whitney t-test.
Differently expressed genes (DEG)

Gene networks analysis:
- Upstream regulator
- Functionnal analysis of gene function
The biological functions affected most significantly by the measured changes in gene expression patterns, based on ingenuity pathway analysis:

<table>
<thead>
<tr>
<th>Biological function</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer-related</td>
<td>1.82E-13 – 5.15E-03</td>
</tr>
<tr>
<td>Organismal injury and abnormalities</td>
<td>1.82E-13 – 5.15E-03</td>
</tr>
<tr>
<td>Haematological system development and function</td>
<td>5.51E-08 – 5.15E-03</td>
</tr>
<tr>
<td><strong>Immune cell trafficking</strong></td>
<td>5.51E-08 – 5.15E-03</td>
</tr>
<tr>
<td>Cellular movement</td>
<td>5.51E-08 – 5.15E-03</td>
</tr>
<tr>
<td><strong>Inflammatory response</strong></td>
<td>5.51E-08 - 5.15E-03</td>
</tr>
<tr>
<td>Tissue development</td>
<td>6.42E-08 – 4.53E-03</td>
</tr>
<tr>
<td>Skeletal and muscular system development and function</td>
<td>1.76E-07 – 5.15E-03</td>
</tr>
<tr>
<td>Cell-to-cell signalling and interaction</td>
<td>4.43E-07 – 4.81E-03</td>
</tr>
<tr>
<td><strong>Cellular growth and proliferation</strong></td>
<td>3.02E-06 – 5.15E-03</td>
</tr>
<tr>
<td>Cell death and survival</td>
<td>5.14E-06 – 5.15E-03</td>
</tr>
<tr>
<td>Cellular development</td>
<td>8.24E-06 – 4.74E-03</td>
</tr>
<tr>
<td>Organ development</td>
<td>8.24E-06 – 4.53E-03</td>
</tr>
<tr>
<td>Hematopoiesis</td>
<td>1.35E-05 – 4.62E-03</td>
</tr>
<tr>
<td>Cardiovascular system development and function</td>
<td>1.67E-05 – 5.15E-03</td>
</tr>
</tbody>
</table>

*The P value is the probability that the association between the change in gene expression and the suggested biological function is due to chance. It is presented as a range since each function includes several sub-functions.*
Inflammation is key

- anti-inflammation  Pro-inflammation
But do all factor of failure applies to all patients

- Probably not
- How to segregate different types of response (bio informatics)
- Clustering analysis
- Finding indicative genes in each cluster to characterize the failure
Differently expressed genes (DEG)

Total = 165

- Upregulated: 63
- Downregulated: 102

Selection of 24 biomarkers of failure associated with
Small
Medium
Large
Follicles

RT-PCR on 132 patients
Cluster analysis of the fail cycles

1 (heterogenous/big)
2 (growing)
3 (inflammation)
Patient charts analysis

• Age
• How much FSH was given and how long?
• Follicles number and size
• Number of mature eggs
• Estradiol level
• Endometrium thickness
AGE

1 (heterogenous/big)
2 (growing)
3 (inflammation)
Days of FSH

1 (heterogenous/big)
2 (growing)
3 (inflammation)
Total FSH UI

1 (heterogenous/big)
2 (growing)
3 (inflammation)
Total Follicles

1 (heterogenous/big)
2 (growing)
3 (inflammation)
Estradiol

1 (heterogenous/big)
2 (growing)
3 (inflammation)
Endometrium

1 (heterogenous/big)
2 (growing)
3 (inflammation)
Conclusion-2

• Follicle size may be misleading
• IVF cycles may fail due to
  1. Premature harvest of follicles
  2. Excess heterogeneity
  3. Increased inflammatory reaction
• These categories may not be obvious looking at patients files and represent new and useful information to clinician
Acknowledgements

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Students

Isabelle Gilbert, Mélanie Hamel, Anne-Laure Nivet, Chloé Fortin
Inclusion/exclusion criteria:

Should be excluded from the study: Patients over 40 years of age, PCOs patients and severe male factors.

These first 2 conditions are known to influence the response to gonadotropins or in the case of the severe male factors, it might create false negative samples.