Two major events take place during follicular development:

1. Oocyte acquisition of meiotic competence as well as une developmental competence.

2. Granulosa cell proliferation and differentiation with specific differentiation of cumulus cells.

Specific gene expression

Oocyte-cumulus dialog

Two types of gap-junctions are installed:

1. Junctions between oocyte and closest somatic cells: they involve the connexins 37 (Cx37).

2. Junctions in-between cumulus cells: they involve connexins 43 (Cx43).

These gap-junctions allow exchanges between the two compartments involved in oocyte-cumulus complex (OCC).
Metabolism

* Oocyte is very active to control the metabolism of granulosa cells for its own development, in mice (Sugiura et Eppig, Reprod Fertil Dev, 2005)

Early follicular growth

* Kit-KL pair
* GDF9/BMP15
* GPR3

Periovulatory period

* GDF9/BMP15
* PDE3A
* EGF-like factors
1. Kit/Kit-ligand (KL) pair

- **Kit**: Transmembranal receptor, expressed by oocyte once meiosis is blocked
- **KL**: Kit ligand, expressed on granulosa cells

(Driancourt et al., Rev Reprod 2000)

**Interactions between oocyte and granulosa cells during early folliculogenesis**

- Meiosis arrest
- **GDF9**/**BMP15**
- **Ptx3**/**Has2**
- Progesterone
- **BMP15**
- Exocellular matrix synthesis
- **PDE3A**
- **EGF-like factors**

**Early follicular growth**
- Le couple Kit-KL
- GDF9/BMP15
- GPR3

**Periovulatory period**
- GDF9/BMP15
- PDE3A
- EGF-like factors
1. GDF9 et BMP15

Role of GDF9 and BMP15 in the acquisition of developmental competence of the bovine oocytes:

- Blastocyst rate

Intact OCCs + GDF9 ou BMP15 + specific inhibitor:
- Decreased blastocyst rate

Inhibitors were able to neutralize the effect of endogenous as well as exogenous factors.

GDF9 and BMP15 are involved in the acquisition of oocyte developmental competence, through cumulus cells.

Oocyte - cumulus dialog during periovulatory period

- Meiosis onset
- Oocyte maturation
- Gene expression
- EGF-like factors
- Progesterone
- Prostaglandins
- Gap-junctions

PDE3A
EGFR
LHR
LHR
EGF-like factors
PR
Cox2
Cox43
STAR
CYP21A2
StAR
P450
Ovulation
Preliminary statement

Various factors inside OCC are involved in the regulation of follicular growth and differentiation
2 consequences for human purpose (as well as domestic mammals):
1. IVM of OCCs (adding various factors to culture medium)
2. Indirect assessment of oocyte quality (studying the expression level of cumulus specific genes as a function of oocyte follow up once fertilized)

Why working on human cumulus cells?

- Major role of the cumulus on oocyte maturation
- Cumulus cells easily accessible during IntraCytoplasmic Sperm Injection (ICSI) procedure
- Human oocytes uneasy to use for research purpose

Aim of this study

- To evaluate the expression of specific genes in human cumulus cells according to oocyte maturity
- Objectives:
  - To relate these expression patterns with developmental competence of the oocyte
  - To select embryos with high implantation potential
**Material and Methods**

- Human cumulus cells individually retrieved shortly before ICSI
- Cumulus stored at -80°C with lysis buffer of the extraction kit of total RNA (Stratagene or Qiagen)
- Individual follow up of oocyte and embryo quality assessment

**2 parts**

- Study of expression of target genes by real time Polymerase Chain Reaction (PCR)
- Study of cumulus cells transcriptome according to oocyte maturity

**1. Study of specific genes expression by real time PCR**

- Total RNA extraction [Absolutely RNA Nanoprep® kit (Stratagene)]
- Reverse Transcription of all RNA [iScript first-strand cDNA Synthesis kit (Bio-Rad Laboratories)]
- Quantitative PCR amplification [iQ™ SYBR® Green Supermix kit (Bio-Rad Laboratories)]
- Results normalized to an endogeneous reference gene
Target genes

- **Steroidogenic Acute Regulatory protein (STAR):**
  - Progesterone synthesis (Cholesterol transport)

- **Cyclooxygenase 2 (PTGS2 or COX2):**
  - Prostaglandin (PGE\textsubscript{2}) synthesis
  - Cumulus expansion
  - Ovulation

- **Amphiregulin (AREG):**
  - Cumulus expansion
  - Oocyte nuclear maturation (GVBD)

- **Stearoyl-CoenzymeA Desaturase 1 and 5 (SCD1 and SCD5):**
  - Monounsaturated fatty acids synthesis

Endogenous reference gene:
- **Ribosomal Protein L19 (RPL19)**

Feuerstein et al, HR2007
1.1 Transcripts levels of target genes according to oocyte nuclear maturity

- Various situations:
  - Cumulus cells from mature oocytes at Metaphase II stage = CC<sub>MII</sub> (n=100)
  - Cumulus cells from immature oocytes at Germinal Vesicle stage = CC<sub>GV</sub> (n=24)
  - Cumulus cells from immature oocytes without GV or 1<sup>st</sup> polar body, arbitrarily called Metaphase I = CC<sub>MI</sub> (n=25)

Transcripts levels of all target genes significantly higher in cumulus cells from mature oocytes than in cumulus cells from GV oocytes

Variation between 2.4- and 7.6-fold (*: p<0.05)
1.2 Transcripts levels of target genes according to developmental ability of the fertilized oocytes at day 5-6

- Two situations:
  - Cumulus cells enclosing oocytes which stopped their development at embryo stage = CC\(_B\) (n=22)
  - Cumulus cells enclosing oocytes which achieved blastocyst development = CC\(_B\) (n=26)
Transcripts levels of all target genes significantly lower in cumulus cells from oocytes which achieved blastocyst development.

Conclusion part 1

- Increased expression of all target genes in cumulus cells after resumption of meiosis of the oocyte
- Decreased expression in cumulus enclosing fertilized MII oocytes with a high developmental potential (expression and use of these transcripts)
Conclusion part 1

Expression of all investigated genes (STAR, COX2, AREG, SCD1 and SCD5) in cumulus cells in a precise chronological pattern to sustain or reflect further embryo development.

Part 2. Study of cumulus cells transcriptome

- Total RNA extraction [RNeasy® micro kit (Qiagen)]
- Reverse transcription, Amplification and labelling of all RNA [Low RNA Input Linear Amplification Kit, PLUS, Two-Color (Agilent)]
- Hybridization of cDNA onto microarray [4x44K microarrays (Agilent)]
- Scanning and Feature extraction

Various situations:

25 patients
157 COC
10 CC_{GV} 20 CC_{MII} J0 ? nuclear maturity
10 CC_{B+} 10 CC_{B-} J5-6 ? cytoplasmic maturity
Microarray  44000 genes
Genes with signal  32978 genes
Normalization (Lowess)
LOcally WEighting ScatterplotSmoothing
Filtering  29742 genes
Genes and Functions annotation (GeneOntology)

2.1 Study according to oocyte nuclear maturity

? Student test (p<0.001)

<table>
<thead>
<tr>
<th>Annotated genes</th>
<th>Annotated functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC_MII &gt; CC_VG</td>
<td>296</td>
</tr>
<tr>
<td>CC_MII &lt; CC_VG</td>
<td>543</td>
</tr>
</tbody>
</table>

? Ranking test (p<0.001)

<table>
<thead>
<tr>
<th>Annotated genes</th>
<th>Annotated function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC_MII &gt; CC_Rn</td>
<td>29</td>
</tr>
<tr>
<td>CC_MII &lt; CC_Rn</td>
<td>41</td>
</tr>
</tbody>
</table>
1. Down regulated functions in CC_{B+}

<table>
<thead>
<tr>
<th>Function</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen processing</td>
<td>0.0026</td>
</tr>
<tr>
<td>Antigen presentation</td>
<td>0.0068</td>
</tr>
<tr>
<td>B+ response to stimulus</td>
<td>0.0002</td>
</tr>
<tr>
<td>Response to stimulus</td>
<td>0.0007</td>
</tr>
<tr>
<td>Nucleic acid binding</td>
<td>0.0028</td>
</tr>
<tr>
<td>T helper cell activation</td>
<td>0.0018</td>
</tr>
<tr>
<td>Immune response</td>
<td>0.0014</td>
</tr>
<tr>
<td>MHC class II antigen presentation</td>
<td>0.0022</td>
</tr>
<tr>
<td>Development</td>
<td>0.0022</td>
</tr>
<tr>
<td>Antigen presentation</td>
<td>0.0018</td>
</tr>
<tr>
<td>Immune response</td>
<td>0.0018</td>
</tr>
<tr>
<td>Sensory perception of light stimulus</td>
<td>0.0008</td>
</tr>
<tr>
<td>Visual perception</td>
<td>0.0003</td>
</tr>
<tr>
<td>Antigen presentation</td>
<td>0.0002</td>
</tr>
<tr>
<td>Antigen presentation</td>
<td>0.0001</td>
</tr>
<tr>
<td>Antigen processing</td>
<td>0.0001</td>
</tr>
<tr>
<td>Antigen processing</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

2. Up-regulated functions in CC_{B+}

<table>
<thead>
<tr>
<th>Function</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basement membrane</td>
<td>0.0029</td>
</tr>
<tr>
<td>Nucleic acid binding</td>
<td>0.0032</td>
</tr>
<tr>
<td>Regulation of transcription, DNA-dependent</td>
<td>0.0026</td>
</tr>
<tr>
<td>Transcription, DNA-dependent</td>
<td>0.0036</td>
</tr>
<tr>
<td>GTPase activity</td>
<td>0.0028</td>
</tr>
<tr>
<td>Regulation of transcription</td>
<td>0.0029</td>
</tr>
<tr>
<td>Regulation of nucleic acid metabolism and nucleic acid metabolism</td>
<td>0.0033</td>
</tr>
<tr>
<td>Transcription</td>
<td>0.0026</td>
</tr>
<tr>
<td>Regulation of cell biological process</td>
<td>0.0024</td>
</tr>
<tr>
<td>Regulation of transcription</td>
<td>0.0019</td>
</tr>
<tr>
<td>Regulation of nucleic acid metabolism and nucleic acid metabolism</td>
<td>0.0025</td>
</tr>
<tr>
<td>Regulation of transcription</td>
<td>0.0019</td>
</tr>
<tr>
<td>Registrome and ribosome biosynthesis</td>
<td>0.0024</td>
</tr>
<tr>
<td>Registrome and ribosome biosynthesis</td>
<td>0.0024</td>
</tr>
</tbody>
</table>

Perspectives

- Validation by quantitative PCR of the expression pattern of identified genes (ongoing)
- Clinical application: use of cumulus biomarkers to better define and discriminate competent oocyte...to influence transfer strategy
- Analysis of mechanisms involved for these target genes to modulate oocyte competence
Acknowledgments

• Laboratoire de Biologie de la Reproduction, CHRU Bretonneau, Tours
  V Cadoret
  F Guerif

• UMR 6175 INRA/CNRS/Haras nationaux/Université de Tours, INRA, Tours
  R Dalbies-Tran
  P Feuerstein

• Plateforme Génopole-Ouest, Nantes
  R Houlgatte
  R Teusan