

Cumulus gene expression and oocyte competence

D. Royere, P Feuerstein

Biologie de la Reproduction CHRU Tours  
UMR6175 INRA/CNRS/Haras nationaux/Université de Tours

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Two major events take place during follicular development :

1. Oocyte acquisition of meiotic competence as well as une developmental competence ? Oocyte quality
2. Granulosa cell proliferation and differentiation with specific differentiation of cumulus cells ? specific gene expression

→ Oocyte-cumulus dialog

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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)

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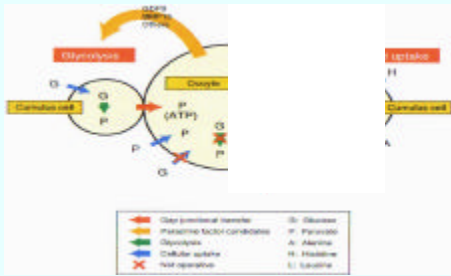
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## Metabolism

∞ Oocyte is very active to control the metabolism of granulosa cells for its own development, in mice



(Sugiura et Eppig, Reprod Fertil Dev, 2005)

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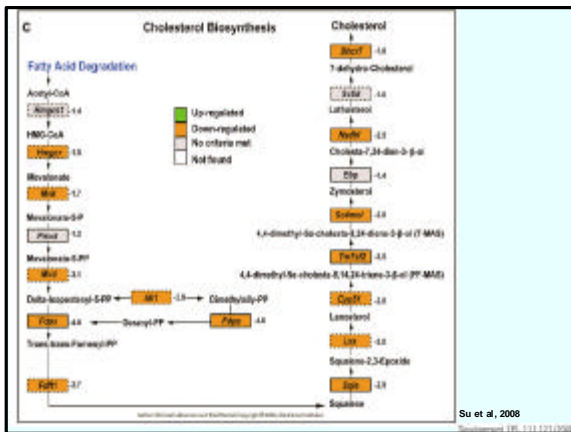
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Su et al, 2008

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## Early follicular growth

∞ Kit-KL pair

∞ GDF9/BMP15

∞ GPR3

## Perioviulatory period

∞ GDF9/BMP15

∞ PDE3A

∞ EGF-like factors

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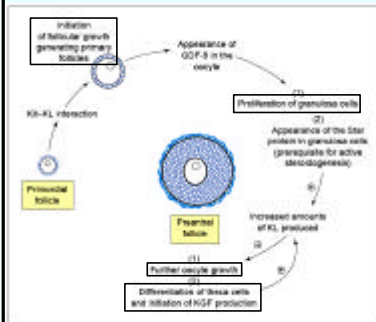
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### 1. Kit/Kit-ligand (KL) pair



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

(Driancourt et al., Rev Reprod 2000)  
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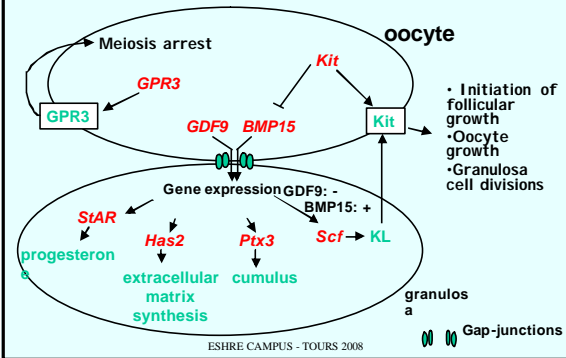
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### Interactions between oocyte and granulosa cells during early folliculogenesis



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### Early follicular growth

- Le couple Kit-KL
- GDF9/BMP15
- GPR3

### Periovulatory period

- GDF9/BMP15
- PDE3A
- EGF-like factors

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### 1. GDF9 et BMP15

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



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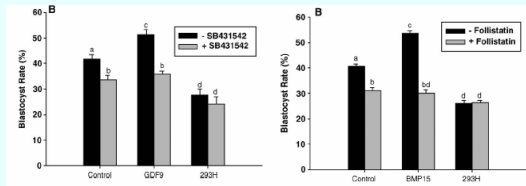
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Intact OCCs + GDF9 ou BMP15 + **specific inhibitor** : decreased blastocyst rate

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



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

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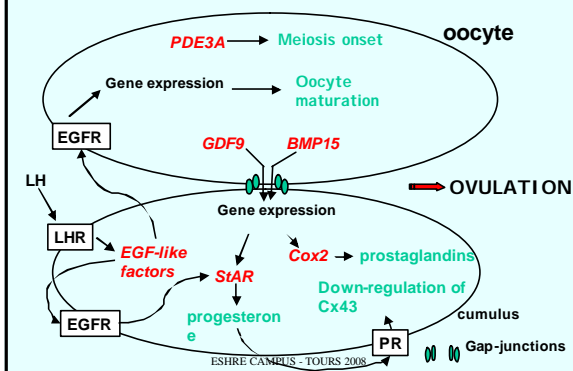
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### Oocyte - cumulus dialog during periovulatory period




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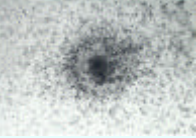
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Preliminary statement

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)

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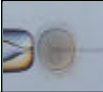
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Why working on human cumulus cells ?

- 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

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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

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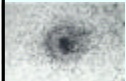
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## Material and Methods

- 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



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## 2 parts

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

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## 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 endogenous reference gene

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## Target genes

- Steroidogenic Acute Regulatory protein (**STAR**) :
  - Progesterone synthesis (Cholesterol transport)
- Cyclooxygenase 2 (**PTGS2** or **COX2**) :
  - Prostaglandin (PGE<sub>2</sub>) synthesis
  - Cumulus expansion
  - Ovulation

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- Amphiregulin (**AREG**) :
  - Cumulus expansion
  - Oocyte nuclear maturation (GVBD)
- Stearoyl-CoenzymeA Desaturase 1 and 5 (**SCD1** and **SCD5**) :
  - Monounsaturated fatty acids synthesis

Endogeneous reference gene :

- Ribosomal Protein L19 (**RPL19**)

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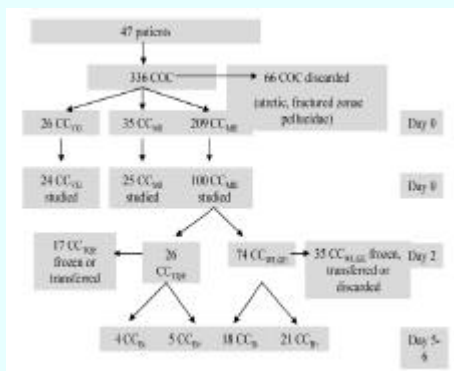
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Feuerstein et al,HR2007

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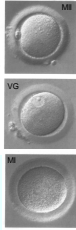
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## 1.1 Transcripts levels of target genes according to oocyte nuclear maturity

- Various situations :

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



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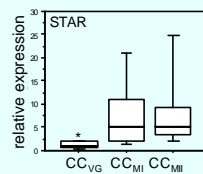
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? Transcripts levels of all target genes significantly higher in cumulus cells from mature oocytes than in cumulus cells from GV oocytes



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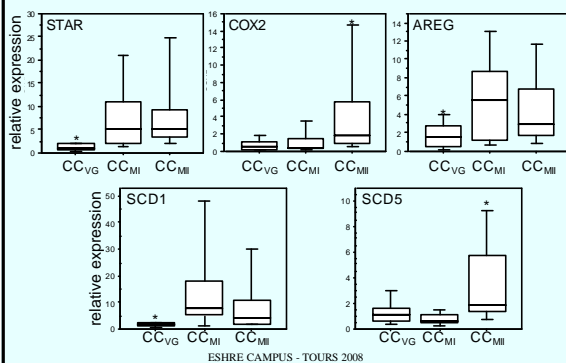
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? Variation between 2.4- and 7.6-fold (\*: p<0.05)



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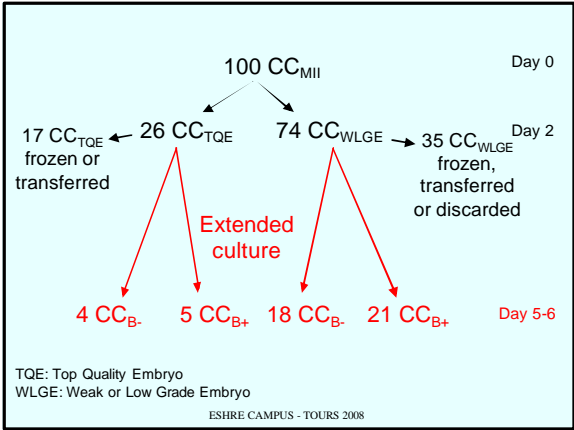
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**Table II.** STAR, COX2, CX43, AREG, SCD1, SCD5 mRNA relative expression, using real-time RT-PCR with RPL19 as an endogenous reference, in individual human cumulus cells as a function of embryo status at Day 2 following fertilization.

Target gene	CC <sub>WLGE</sub> (n = 71-74)	CC <sub>TQE</sub> (n = 26)
STAR	9.2 ± 1.3	8.8 ± 1.7
COX2	4.6 ± 0.9	6.9 ± 1.9
CX43	2.8 ± 0.2	3.2 ± 0.5
AREG	4.8 ± 0.6	7.1 ± 2.2
SCD1	9.5 ± 1.5	10.5 ± 2.9
SCD5	3.6 ± 0.4	4.0 ± 0.9

Values are presented as means ± SEM.  
CC<sub>WLGE</sub>: Cumulus cells from oocytes yielding a weak or low grade embryo at Day 2. CC<sub>TQE</sub>: Cumulus cells from oocytes yielding a top-quality embryo at Day 2.

Feuerstein et al, HR2007

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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<sub>B-</sub> (n=22)
  - Cumulus cells enclosing oocytes which achieved blastocyst development = CC<sub>B+</sub> (n=26)



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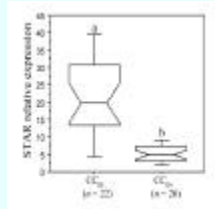
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? Transcripts levels of all target genes significantly lower in cumulus cells from oocytes which achieved blastocyst development



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Blastocyst

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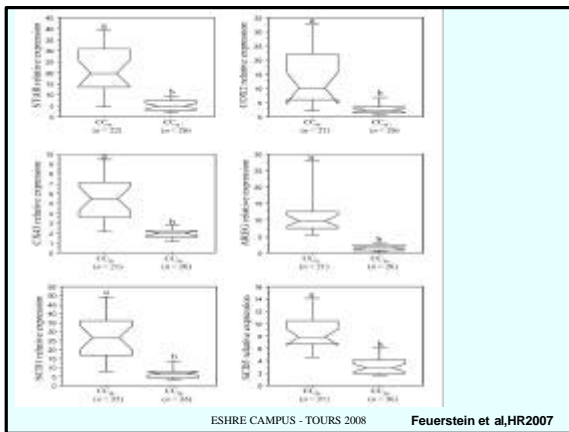
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Feuerstein et al,HR2007

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### Conclusion part1

- 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)

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### 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

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### 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

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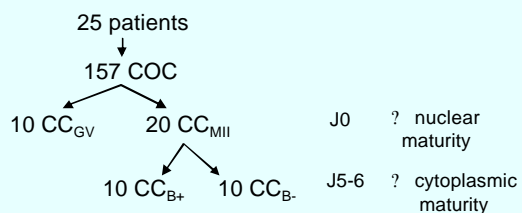
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? Various situations:



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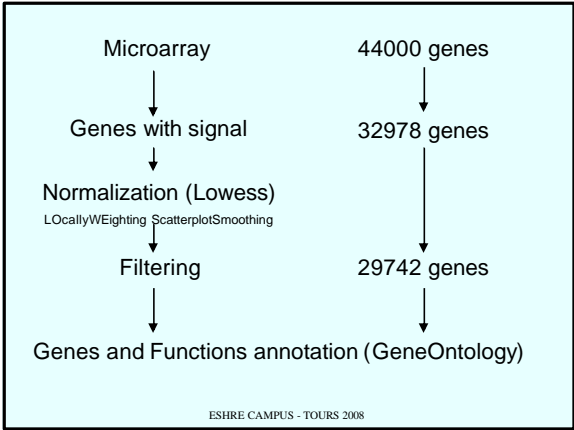
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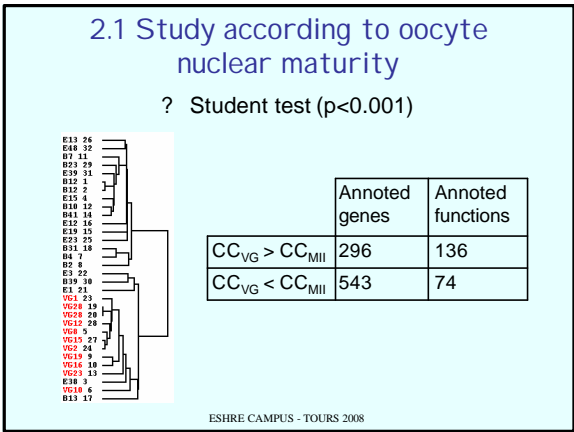
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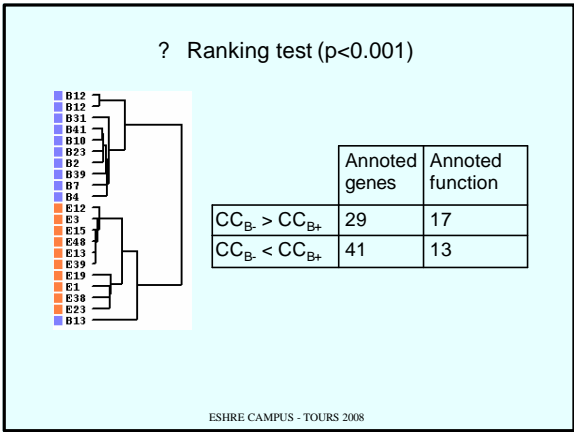
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1. Down regulated functions in CC<sub>B+</sub>

Function	p
antigen_processing	0
antigen_presentation_exogenous_antigen	0,0003
antigen_processing_exogenous_antigen_via_MHC_class_II	0,0003
MHC_class_II_receptor_activity	0,0003
response_to_stimulus	0,0007
visual_perception	0,0018
sensory_perception_of_light_stimulus	0,0018
organismal_physiological_process	0,0018
antigen_presentation	0,0024
development	0,0129
skeletal_development	0,016
structural_molecule_activity	0,0175
immune_response	0,0182
sensory_perception	0,0185
defense_response	0,0233
response_to_biotic_stimulus	0,0283
organ_development	0,0462

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2. Up-regulated functions in CC<sub>B+</sub>

Function	p
basement_membrane	0,0035
nucleic_acid_binding	0,0212
regulation_of_transcription_DNA-dependent	0,0226
transcription_DNA-dependent	0,0266
GTPase_activity	0,0292
regulation_of_transcription	0,0305
regulation_of_nucleobase_nucleoside_nucleotide_and_nucleic_acid_metabolism	0,0335
transcription	0,0379
regulation_of_cellular_physiological_process	0,0413
regulation_of_cellular_metabolism	0,0458
nucleobase_nucleoside_nucleotide_and_nucleic_acid_metabolism	0,0459
regulation_of_physiological_process	0,0494
nucleoside-triphosphatase_activity	0,0499

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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

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 **Acknowledgments** 

**• Laboratoire de Biologie de la Reproduction, CHRU Bretonneau, Tours**  
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P Feuerstein

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R Houlgatte  
R Teusan

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