

# Effect of age on the transcriptome of the human MII oocyte

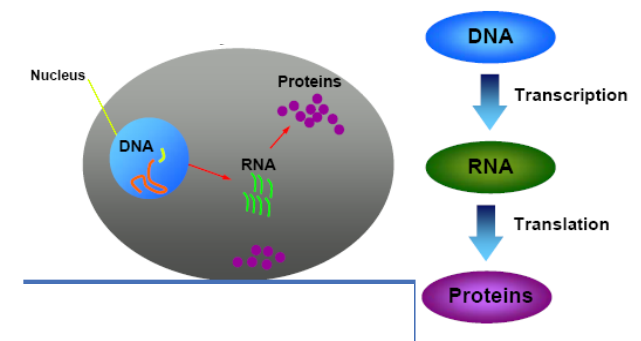
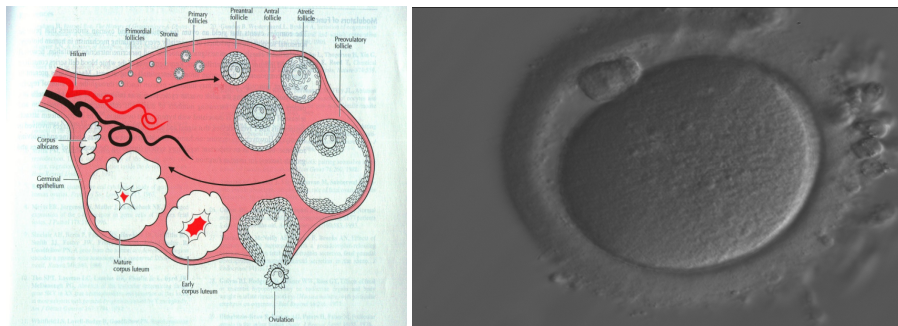
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# Contents

- Cytoplasmic maturation
- The transcriptome of the human MII oocyte
- Effect of age on the MII transcriptome

# Maturity of the oocyte

- **‘Nuclear maturation’** is used to describe the oocyte maturation stages of the chromosome compartment: GV, MI and MII
- **‘Cytoplasmic maturation’** covers the rest: the organelles, the **transcriptome** and the proteins stored in the cytoplasm during oogenesis.



# Developmental competence

- timely translation of **stored maternal transcripts** provide the ooplasm with new proteins
- post translational modification of stored or newly synthesized protein sets the exact timing for cellular events
- processes involved in degradation of proteins and mRNAs remove no longer needed molecules

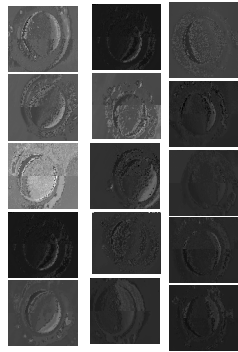
**The microarray technique has made it possible to approach the transcriptome part of the 'cytoplasmic maturation'.**



# The transcriptome of the human MII oocyte

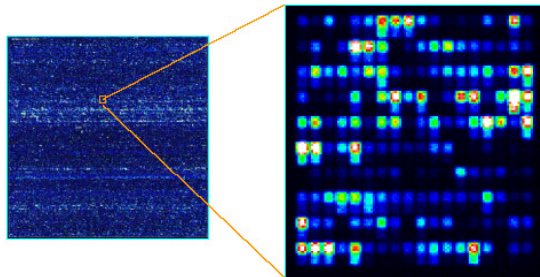
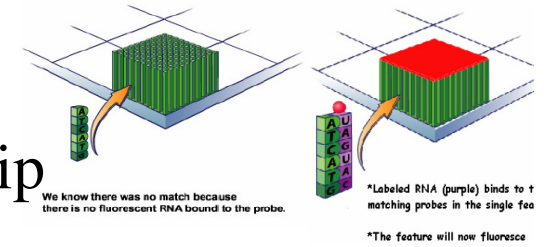
# Materials and Methods

- 15 Women undergoing IVF/ICSI treatment
- Short antagonist protocol, Puregon and Orgalutran (Organon, Denmark)
- One COC and follicular fluid with mural granulosa cells was donated, if  $> 6$  COCs available for the treatment
- Zona were removed with Tyrodes solution (MediCult, Denmark)
- The oocyte was flash frozen within one hour after ovum pick up



→ extraction and  
 amplification and  
 labelling of  
 mRNA (cRNA)  
 (PicoPure™ Isoolation Kit  
 (Arcturus, USA))

→ hybridization  
 to the gene chip



(Human Genome U133 2.0 Plus array from  
 Affymetrix (Affymetrix Inc., USA))



Probe  
Array  
(chips)



Fluidics  
Station

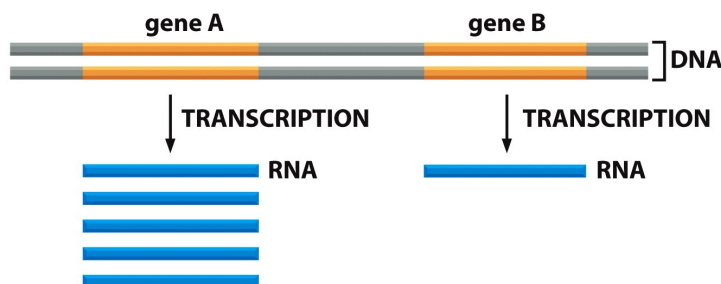


Scanner



Software  
Data Analysis

(R, dChip, Partek,  
 Ingenuity)



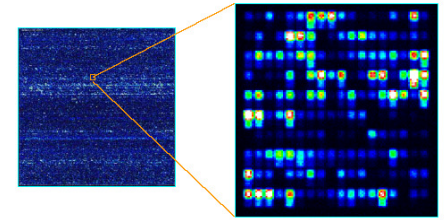
# Results

## Patient and treatment data

Number of donated follicles	15
Age (years)	33.1± 2.7 (27-39)
FSH CD2-3 (IU/l)	5.9±2.0 (2.0-13.0)
BMI	22.6± 2.7
Reason for treatment	Male factor: 10 Ovulationdefect: 3 Unexplained: 2
Cycle number	1.2± 0.4
FSH total (IU)	1785±383 (1200-4050)
FSH days	8.9±1.6
No of oocytes	11.6±4.6
“Follicle size” (ml)	2.5±0.9



# Results / Discussion



- **10,428** transcripts representing 7,470 genes were present in the human MII oocyte

	Sample	Number of transcripts	whole genome arrays
<b>Human</b>	Individual MII oocytes	8,125	(Woods et al. 2007)
	Pooled (3-10) MII oocytes	4,801 - 12,031	(Wells and Patrizio, 2008), (Jones et al., 2008), (Assou et al., 2006) (Jaroudi et al., 2009). (Kocabas et al., 2006).
<b>Murine</b>	Pooled (25-500) MII oocytes	10,977- 13,892	(Hamatani et al., 2004) (Pan et al., 2008)

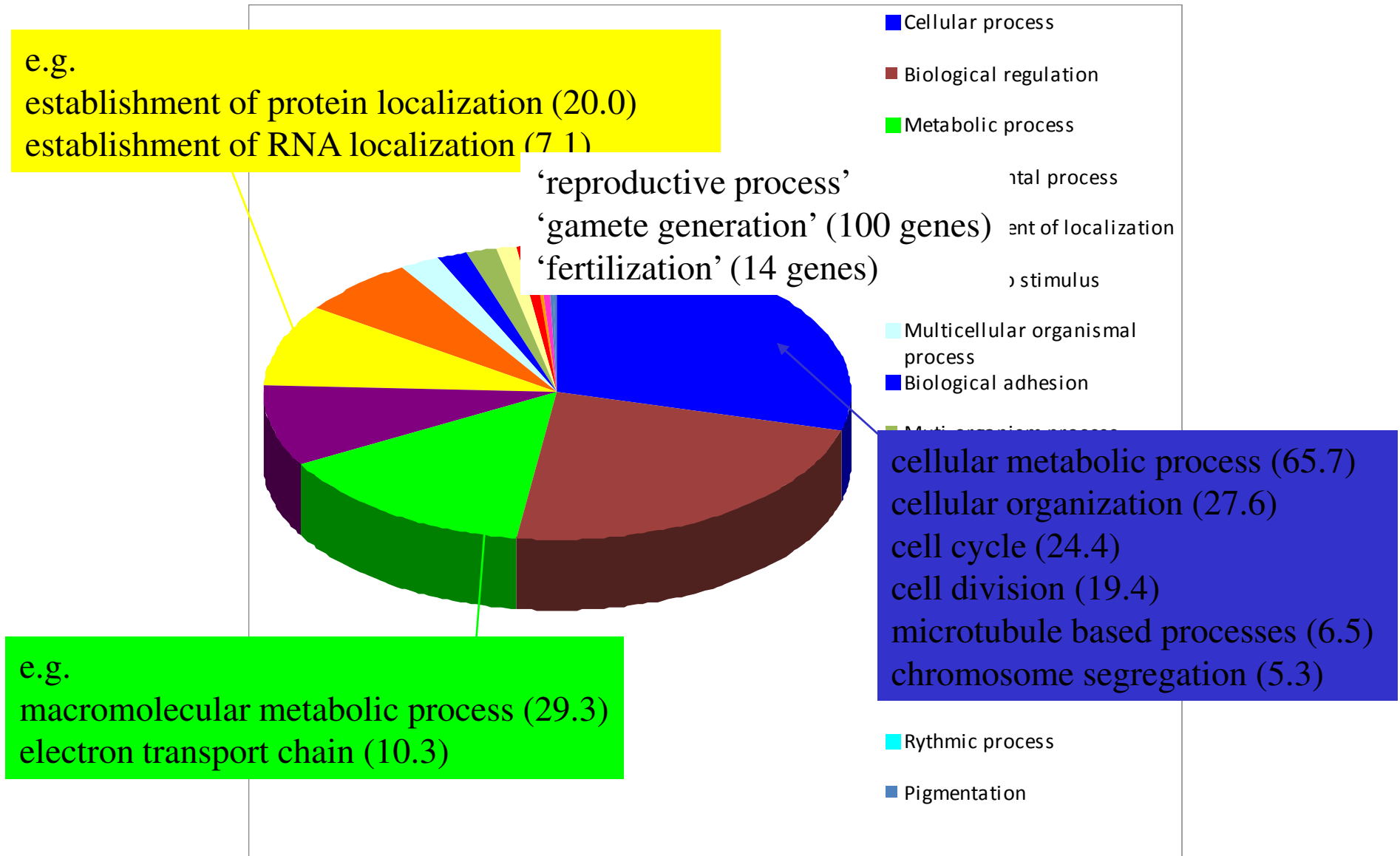
# Results

## Enrichment analysis

- In this list of expressed genes in the MII oocytes, 5,213 of the expressed genes have a biological and a molecular process term assigned
- 2,257 genes have no functional term assigned, yet.

# Results

## Biological function



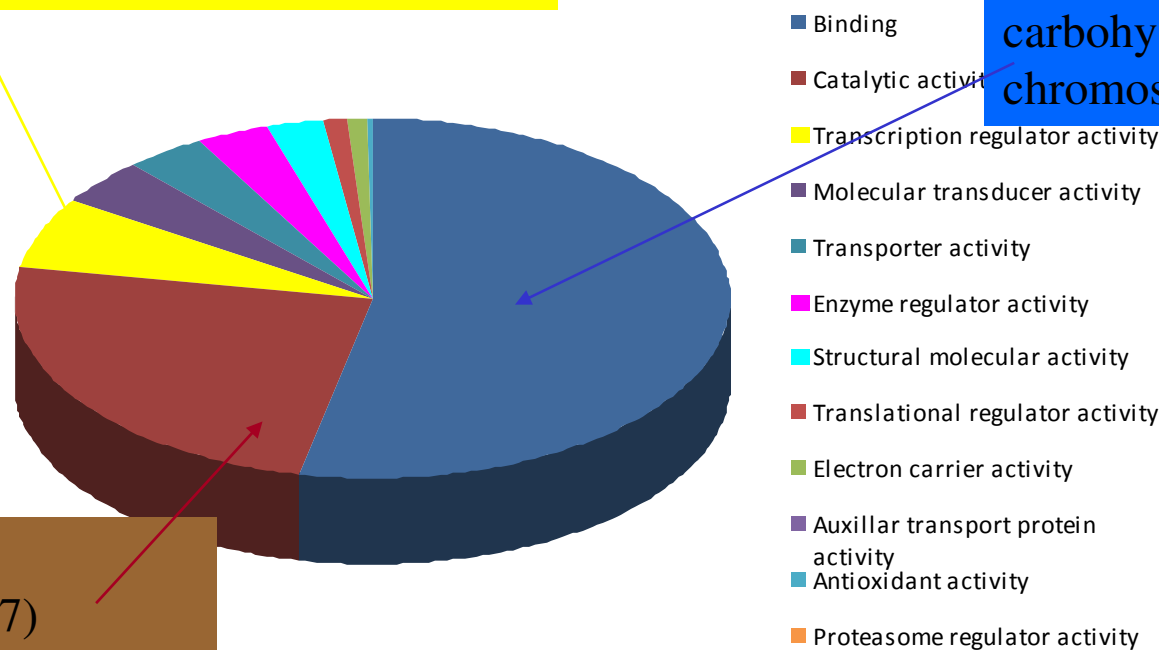
Figures in ( ): enrichment score > 1 is significant;  $p < 0.001$

# Molecular function

e.g.

translation initiation factor activity (9.3)  
translation elongation factor activity (2.8)

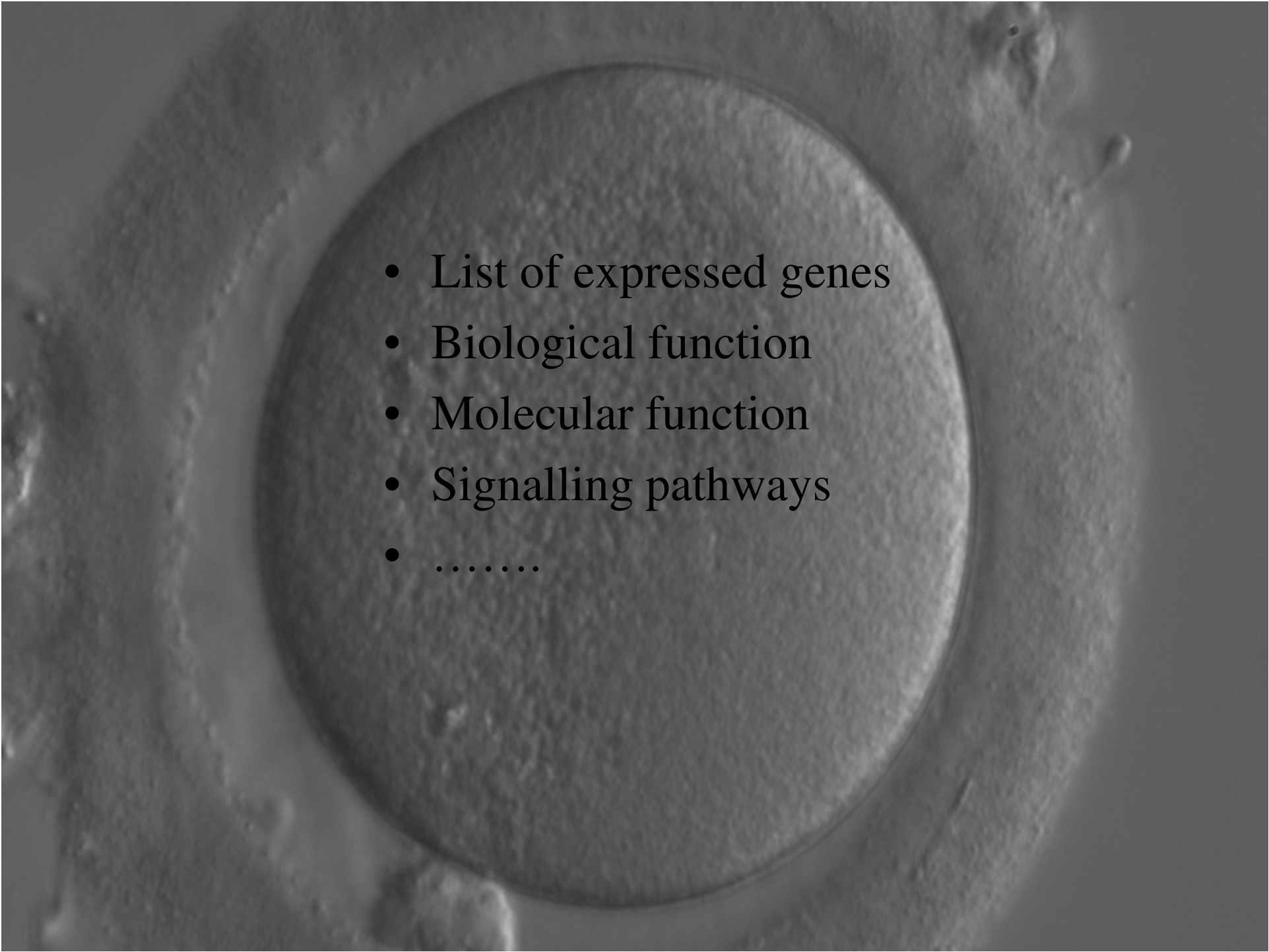
protein binding (45.5)  
antigen binding (44.8)  
nucleotide binding (21.8)  
nucleic acid binding (20.7)  
carbohydrate binding (10.4)  
chromosome binding (6.8)



e.g.

ligase activity (15.7)  
isomerase activity (7.4)  
transferase activity (6.5)  
RNA splicing activity (5.5)

Figures in ( ): enrichment score > 1 is significant;  $p < 0.001$

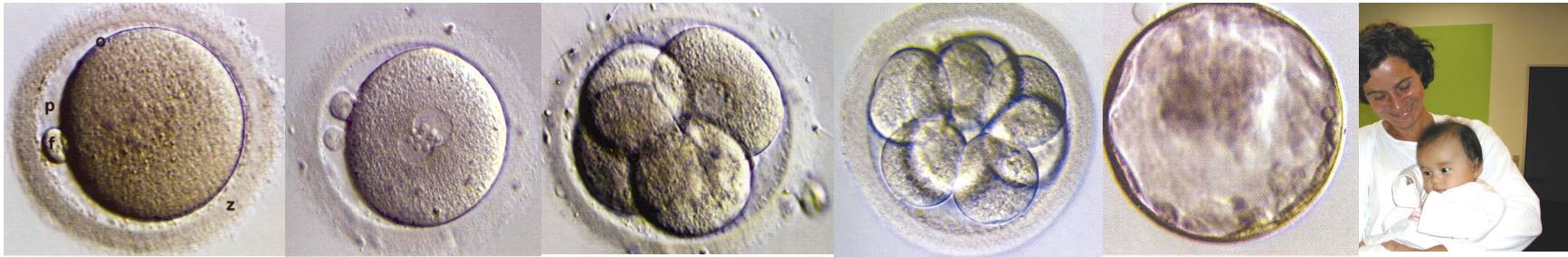
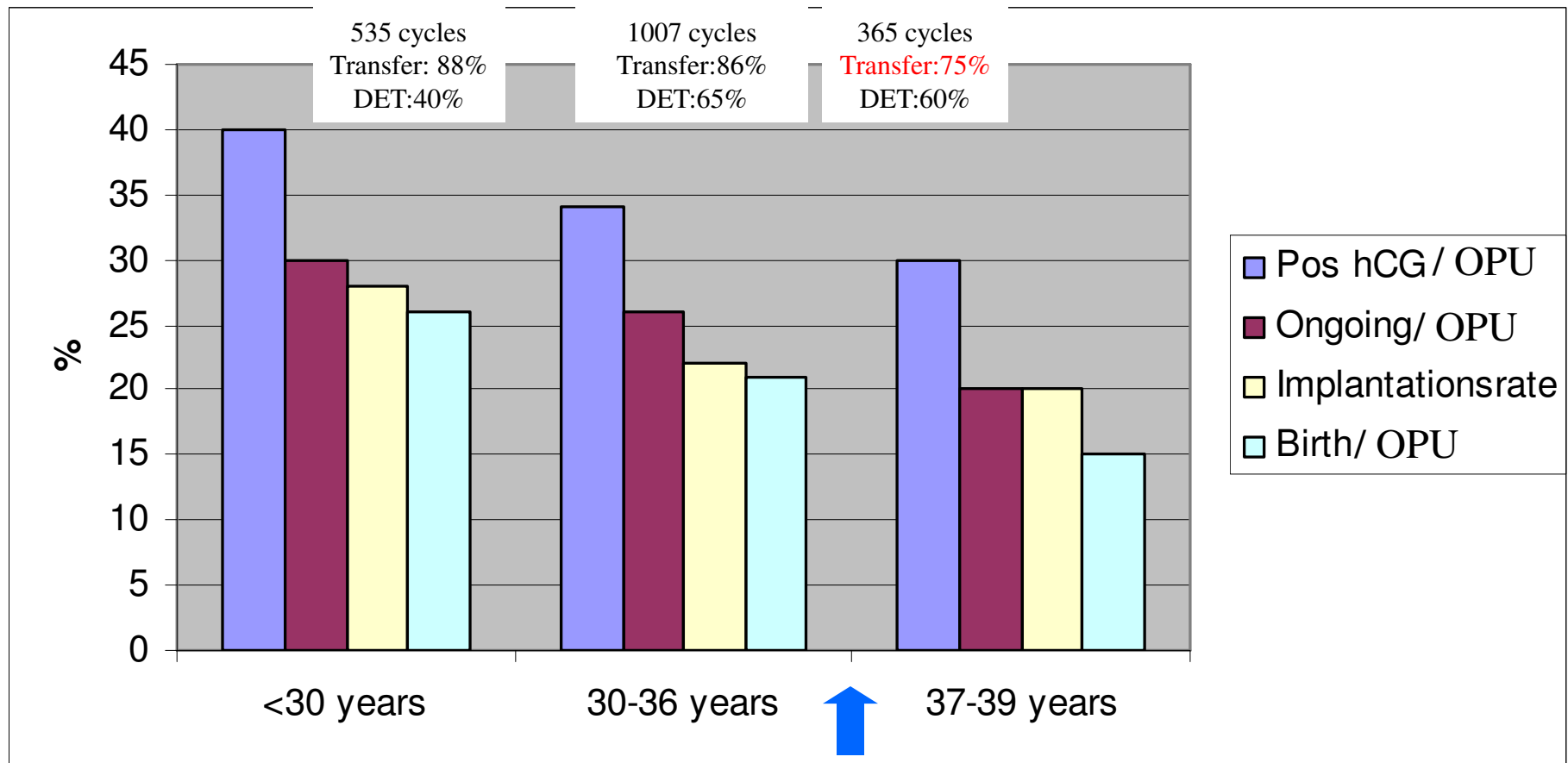
- 
- A grayscale electron micrograph of a cell nucleus. The nucleus is a large, roughly circular structure with a dense, granular interior (nucleolus) and a surrounding nuclear envelope. The background shows the cytoplasm with various organelles and membrane structures.
- List of expressed genes
  - Biological function
  - Molecular function
  - Signalling pathways
  - .....

# Competent or incompetent oocyte?

- What characterize the developmental machinery in a competent oocyte?
- Does the **transcriptome** part of the ‘cytoplasmic maturation’ differ?
- Age is a significant quality parameter!

# Hvidovre Hospital, 2003-2007

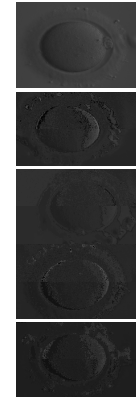
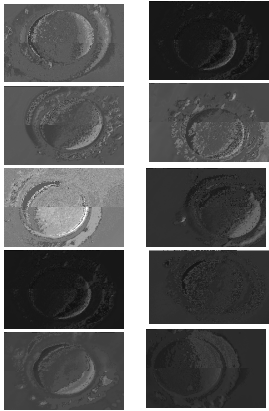
## 1st cycle



# The effect of age on the transcriptome of the human MII oocyte

<36 years x 37-39 years





# Results

## Patient and treatment data

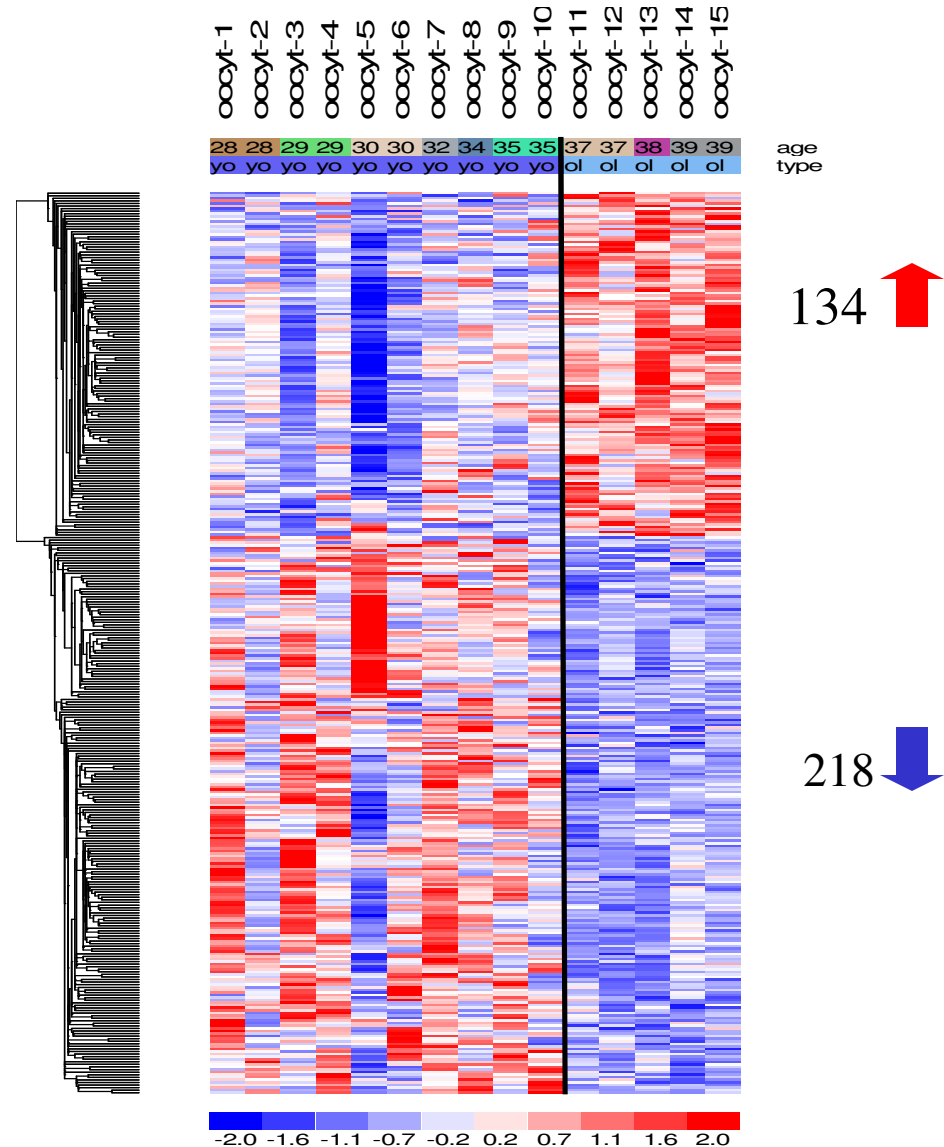
	<i>Younger</i>	<i>Older</i>
<b>Number of donated oocytes</b>	10	5
<b>Age (years)</b>	31.1±2.7 (27-35)	37.8±1.3 (37-39)
<b>FSH<sub>CD2-3</sub> (IU/l)</b>	4.5±1.7	8.9±3.3 *
<b>Length of infertility (years)</b>	2.1±1.5	1.6±0.9
<b>BMI</b>	23.4± 2.5	21.0 ±1.4
<b>Reason for treatment</b>	Male factor: 8 Ovulation defect: 2	Male factor: 2 Un-explained: 2 Ovulation defect: 1
<b>Cycle number</b>	1.2± 0.4	1.2± 0.4
<b>FSH total (IU)</b>	1,505±383	2,345±1,003*
<b>FSH days</b>	8.9±1.6	8.8±0.8
<b>No of oocytes</b>	11.9±3.8	11.2± 4.6
<b>“Follicle size” (ml)</b>	2.5±0.8	2.5±1.0



Data are mean ± standard deviation. \* P< 0.05

# Results / Discussion

- 351 transcripts, 342 unique genes, (4,5%) showed significant expression change with age, fold change >1.5,  $p < 0.05$
- 103 genes > 2 fold
- 239 genes >1.5 fold



# Results / Discussion

	Age groups (number of biological replicates)	Number of Differentially expressed genes	Array platform	
Human	<36 years (10) >36 years (5)	351 transcripts / <b>4.5%</b>  103 genes > 2 fold, 239 genes >1.5 fold	<b>48,000</b> transcripts Affymetrix, Human Genome U133 2.0 Plus	Present study
Human Oocytes failed to fertilize after IVF/ICSI	<32 years (2) 32-40 years (1) > 40 years (2)	608 transcripts / <b>28%</b>  > 1.5 fold change	<b>8,500</b> transcripts Affymetrix 'Human Genome Focus Array'	Steuerwald et al., 2007
Murine MII oocytes harvested 18 hrs after hCG	5-6w (3) 42-45w (3)	530 transcripts / <b>5%</b>  99 transcripts > 2.0 431 transcripts > 1.5	<b>22,000</b> transcripts Agilent technologies NIA 22K array	Hamatani et al., 2004
Murine MII oocytes harvested 16 hrs after hCG	6 weeks (4) MII 66 weeks (4)  GV	2129 transcripts/ <b>13%</b> 2001 transcripts >1.4 128 transcripts>2.0  335 transcripts / 2% 298 transcripts >1.4 47 transcripts > 2.0 fold	<b>39,000</b> transcripts Affymetrix 'murine genome array' MOE430 v2	Pan et al., 2008

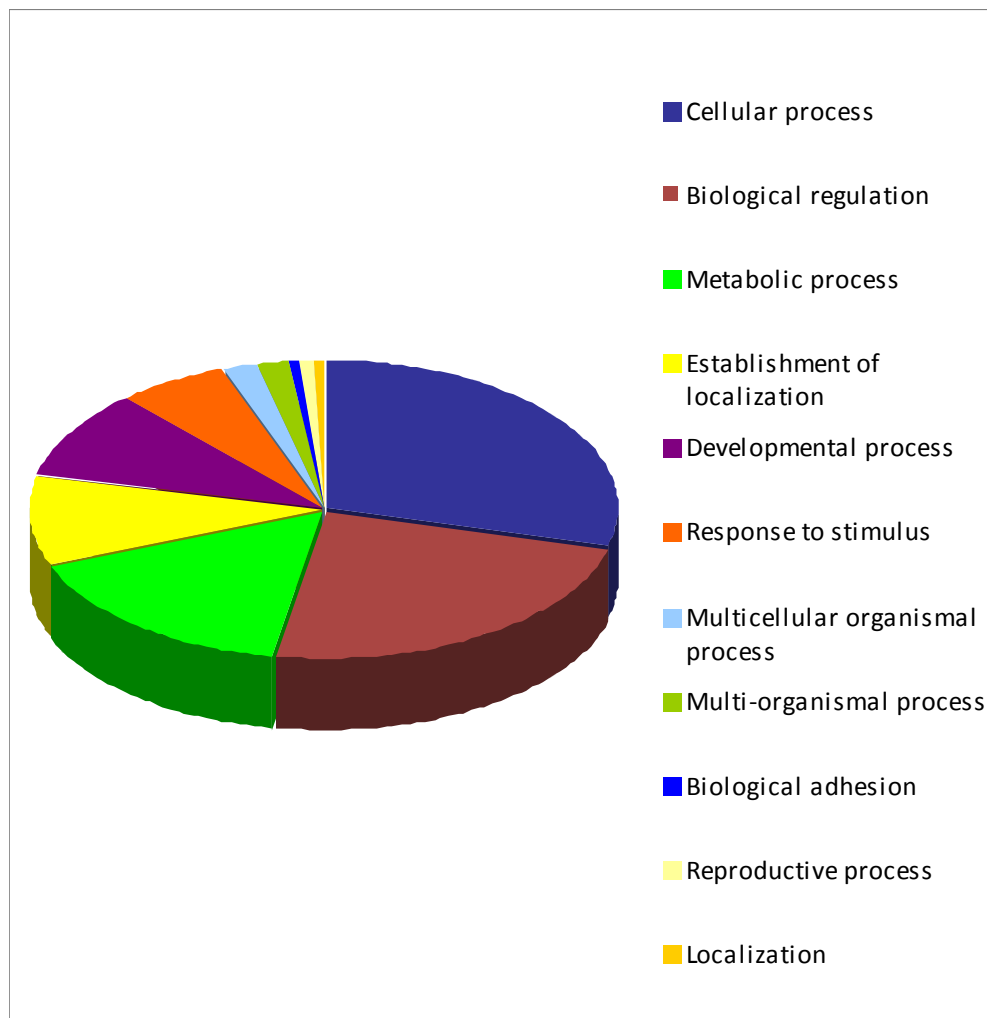
# Results

qRT-PCR confirmation of microarray data

Gene Symbol	Ratio by microarray	Ratio by real time PCR
	5 older oocytes (37-39 years) 10 younger oocytes (27-35 years)	2 older oocytes (39 years) 4 younger oocytes (26-35 years)
<b>SMAD2</b> <i>SMAD family member 2</i>	0.42	0.38
<b>MRPL43</b> <i>mitochondrial ribosomal protein L43</i>	2.50	1.40
<b>ANAPC4</b> <i>anaphase promoting complex subunit 4</i>	0.65	0.08

# Results

## Enrichment analysis of the differentially expressed genes



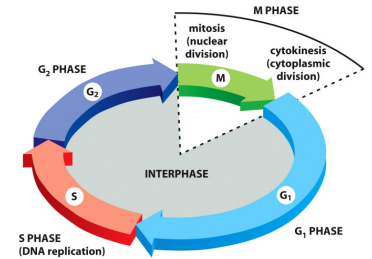
	<i>Annotational term</i>		<i>p-value</i>
<b>Cell compartment</b>	Intracellular	188	0.000074
	Nucleus	75	0.004391
<b>Cell cycle</b>	Cell cycle	27	0.002681
	Mitotic cell cycle	13	0.00148
	Regulation of mitosis	5	0.005004
<b>Spindle / microtubule</b>	Cytoskeleton organization and biogenesis	19	0.00078
	Spindle organization and biogenesis	4	0.000733
<b>Cellular organization</b>	Cellular component organization and biogenesis	61	0.004947
	Regulation of cellular component organization	6	0.002685
	Golgi vesicle transport	9	0.001402
<b>DNA</b>	DNA metabolic process	28	0.000248
	DNA replication	10	0.003983
	Nucleobase, nucleoside, nucleotide metabolic process	87	0.000507
	Hydrolase activity, acting on ester bonds	21	0.004135
<b>DNA repair</b>	DNA repair	10	0.016809
	Response to DNA damage stimulus	13	0.010501
<b>RNA</b>	Regulation of transcription from RNA polymerase II promoter	15	0.008099
	Negative reg of transcript from RNA polymerase II promoter	7	0.008154
<b>Metabolism</b>	Metabolic process	171	0.000035
	Biopolymer metabolic process	122	0.000000
	Catalytic activity	107	0.009242
<b>Post translational protein modification</b>	Protein modification process	46	0.000565
	Post-translational protein modification	43	0.000088
	Protein catabolic process	12	0.000749
<b>Ubiquitination</b>	Protein ubiquitination	6	0.00222
	Ubiquitin ligase complex	5	0.00569
<b>Energy</b>	ATP binding	36	0.007735
	Purine ribonucleotide binding	43	0.007701
<b>Apoptosis</b>	Programmed Cell death	20	0.035156
<b>Signalling and</b>	Protein kinase activity	28	0.000007

# Developmental competence

- timely translation of **stored maternal transcripts** provide the ooplasm with new proteins
- post translational modification of stored or newly synthesized protein sets the exact timing for cellular events
- **processes involved in degradation of proteins and mRNAs remove no longer needed molecules**



# Results ubiquitination



- The most prominent function of ubiquitination is labelling of proteins for proteasomal degradation by the ubiquitin ligases. The proteasomal degradation pathway is essential for many cellular processes including the cell cycle regulation and progression, where cyclins are ubiquitinated, i.e. cyclin A is degraded by the ubiquitin ligase, anaphase promoting complex
- Ubiquilin 1 (1.6 fold), an anaphase promoting complex subunit, ANAPC4 (1.6 fold) and two ubiquitin-conjugating enzyme genes UBE2D (1.9 fold) and UBE4B (1.8 fold) were down regulated while three genes for ubiquitin specific peptidases USP2, USP34 and USP42 were up-regulated (2.9, 1.6 and 1.6 fold, respectively).
- These findings showing mainly reduction in the ubiquitination by age is in accordance with previous findings on murine (Hamatani *et al.*, 2004) and human oocytes (Steuerwald, *et al.*, 2007).



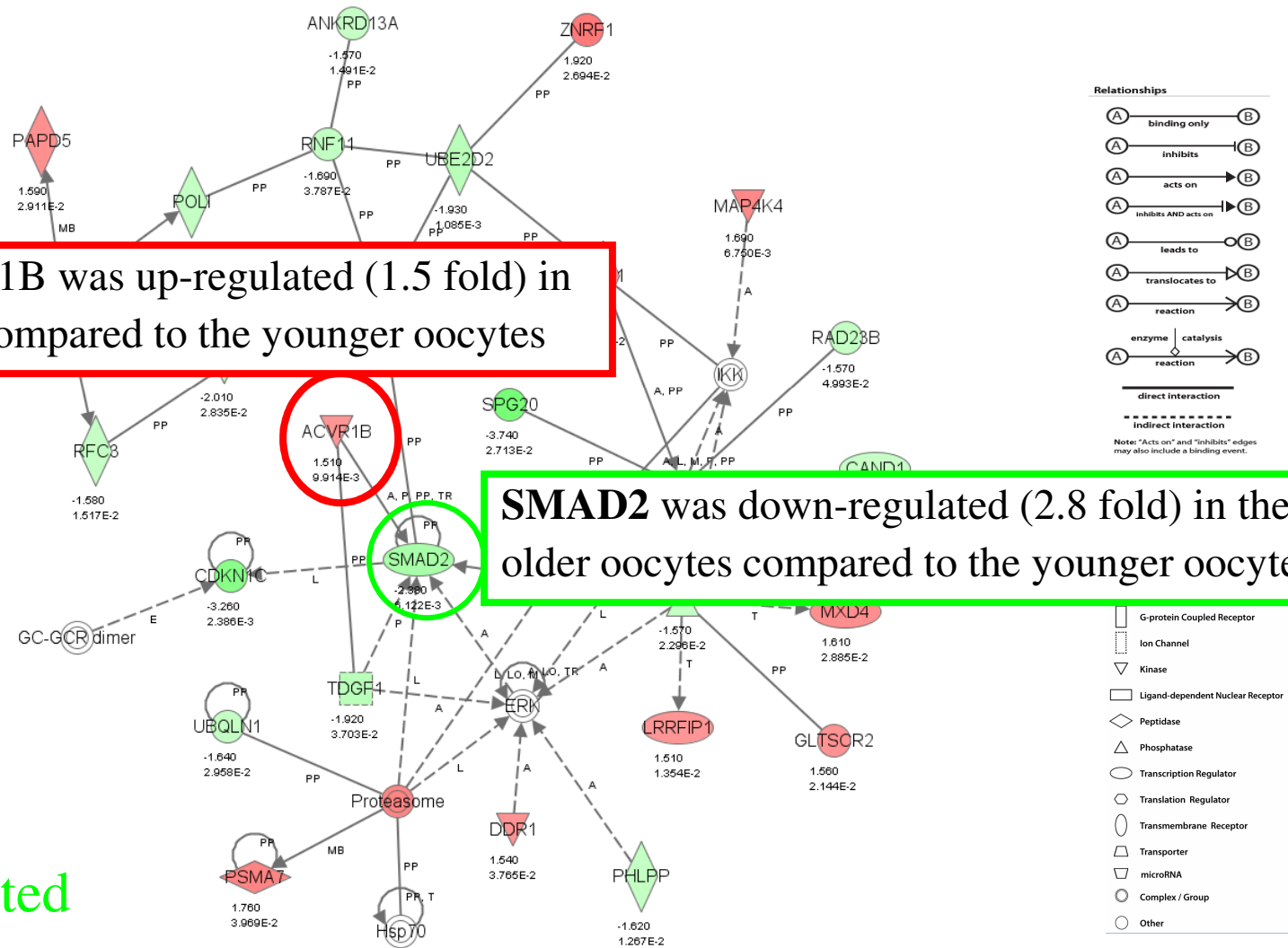
# Results cell cycle

Number one signalling network affected by age  
'organism development and cell cycle'

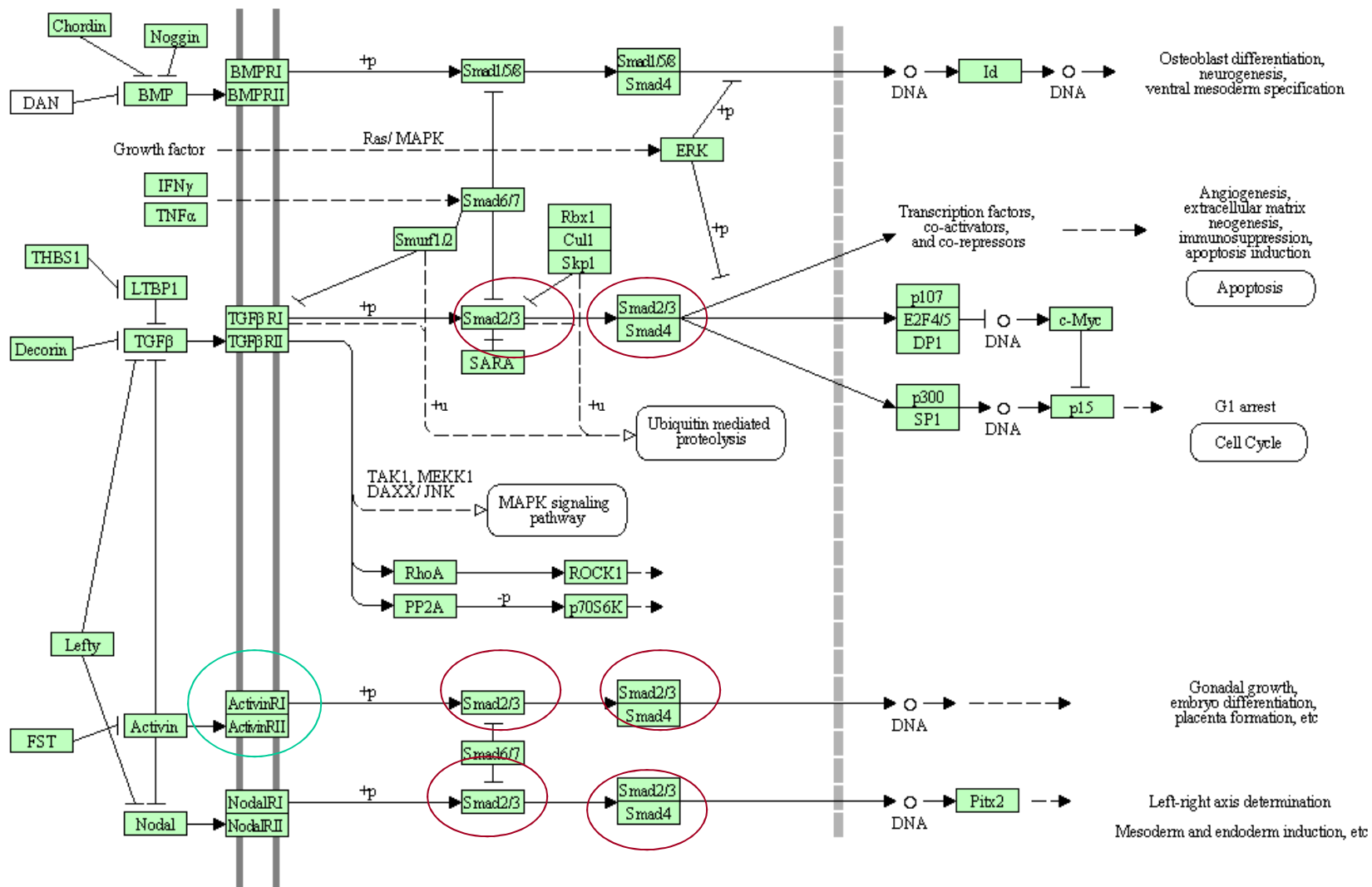
Activin A receptor 1B was up-regulated (1.5 fold) in the older oocytes compared to the younger oocytes

SMAD2 was down-regulated (2.8 fold) in the older oocytes compared to the younger oocytes

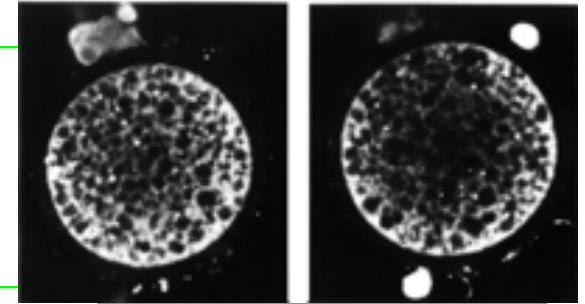
Down-regulated  
Up-regulated



# TGF-BETA SIGNALING PATHWAY



# SMAD2



**Table II.** mRNA expression pattern in human oocytes and preimplantation embryos. The figures show the number of oocytes and preimplantation embryos that were positive, and the figure in parentheses shows the number of patients that donated the material

Gene	Oocyte (3)	4-cell (3)	8-cell (4)	Morula (6)	Blastocyst (10)	
TGFβR-I	3/5	15/15	0/4	0/5	0/6	7/10
TGFβR-III		15/15	0/4	0/5	0/6	0/10
Smad2 <sup>a</sup>	5/5	15/15	4/4	5/5	6/6	8/10

TGF $\beta$ R-I = transforming growth factor (TGF) $\beta$  receptor type I;

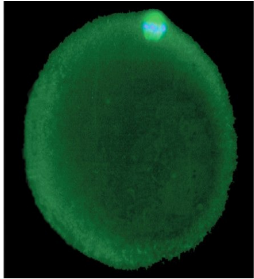
TGF $\beta$ R-II = TGF $\beta$  receptor type II.

<sup>a</sup>RT-PCR product obtained using primer for Smad 2/3 was by sequencing shown to be Smad2.

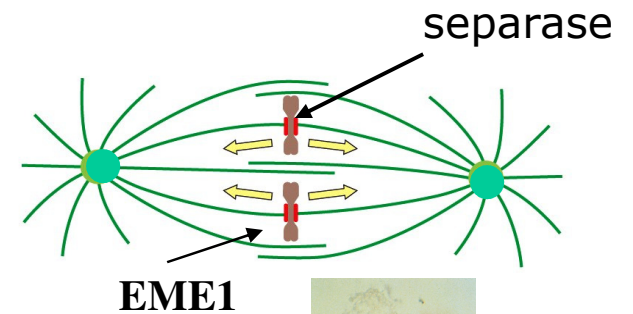
# Results/Discussion

## SMAD2

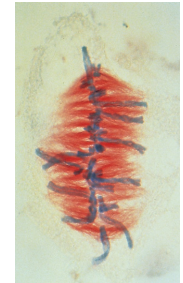
- SMAD2 was significantly down regulated (1.5 fold) in MII oocytes from aged compared to young mice. Pan et al 2008
- Presence and dosage of SMAD2 have been shown to have an essential role in early embryonic development in studies with mutant mice and in vitro culture of mice oocytes with inhibition of SMAD2 signalling. Weinstein, Yang *et al.*, 1998, Nomura and Li, 1998.



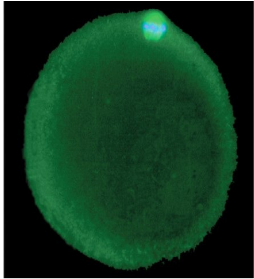
# Results meiosis, mitosis



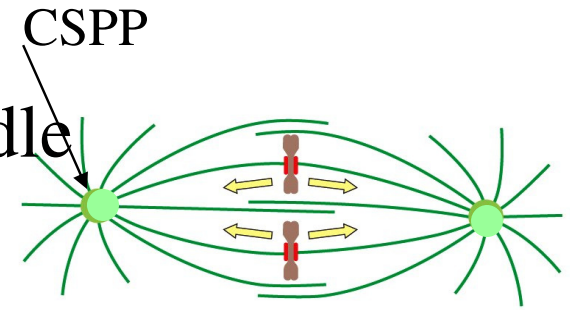
- **Separase** was up-regulated (1.6 fold) in the older oocytes compared to the younger oocytes  
Over-expression of separase induces premature separation of chromatids, lagging chromosomes, and anaphase bridges (Zhang, Kuznetsov *et al.*, 2008)



- **EME1** (essential meiotic endonuclease 1) gene expression was down-regulated (1.6 fold) in older oocytes compared to younger  
Small decreases in gene dosage of EME1 promote re-replication and polyploid cells in addition to chromosome aberrations as DNA gaps and breaks (Hiyama, Katsura *et al.*, 2006).

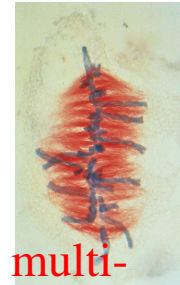


# Results meiosis, mitosis/spindle



- **CSPP** (centrosome/spindle pole associated protein) was up-regulated 1.7 fold) in the aged oocytes

Over-expression of CSPP has been shown to impair mitosis and promote multi-polar spindles (Patzke, Stokke *et al.*, 2006)



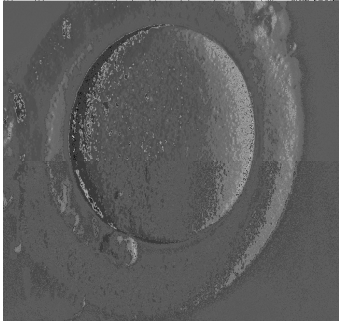
- **Ran** (member RAS oncogene family/GTP-binding nuclear protein Ran) was down regulated by 1.8 fold, ( $p = 0.0041$ )

-The Ran GTPase mediates chromatin signaling to control cortical polarity during polar body extrusion in mouse oocytes. Deng et al. 2008

-Involved in the acentriolar spindle assembly during meiosis i mouse oocytes.

-Ran was 1.4 fold up regulated in MII oocytes from aged mice as compared to younger. Pan et al 2008.





# Conclusion

- Significant difference in the transcriptome of the MII oocyte between younger and older oocyte
- **342** of 7470 expressed genes were differentially expressed between the two age groups
- Analysis of the these 342 genes indicates alterations in
  - ubiquination, generally reduced
  - cell cycle, top signalling network affected
  - mitosis/spindle, suggests molecular basis for the age-associated increase in aneuploidy in human oocytes and pre-embryos
- *Gene expression profiles in cumulus and granulosa cells are also affected by age*

# When and how are the changes induced in the oocyte?

- ‘limited oocyte pool’ hypothesis, where the limited number of antral follicles available in older women could lead to suboptimal oocyte for ovulation
- ‘over time’ hypothesis, while resting in the prophase, changes happen to the oocytes or its milieu inducing damage to the meiotic/developmental machinery





# Thanks

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