VII MAMMALIAN
FOLLICULOGENESIS AND
OOGENESIS ESHRE WORKSHOP

# Insights into oocyte competence from cumulus cells gene expression

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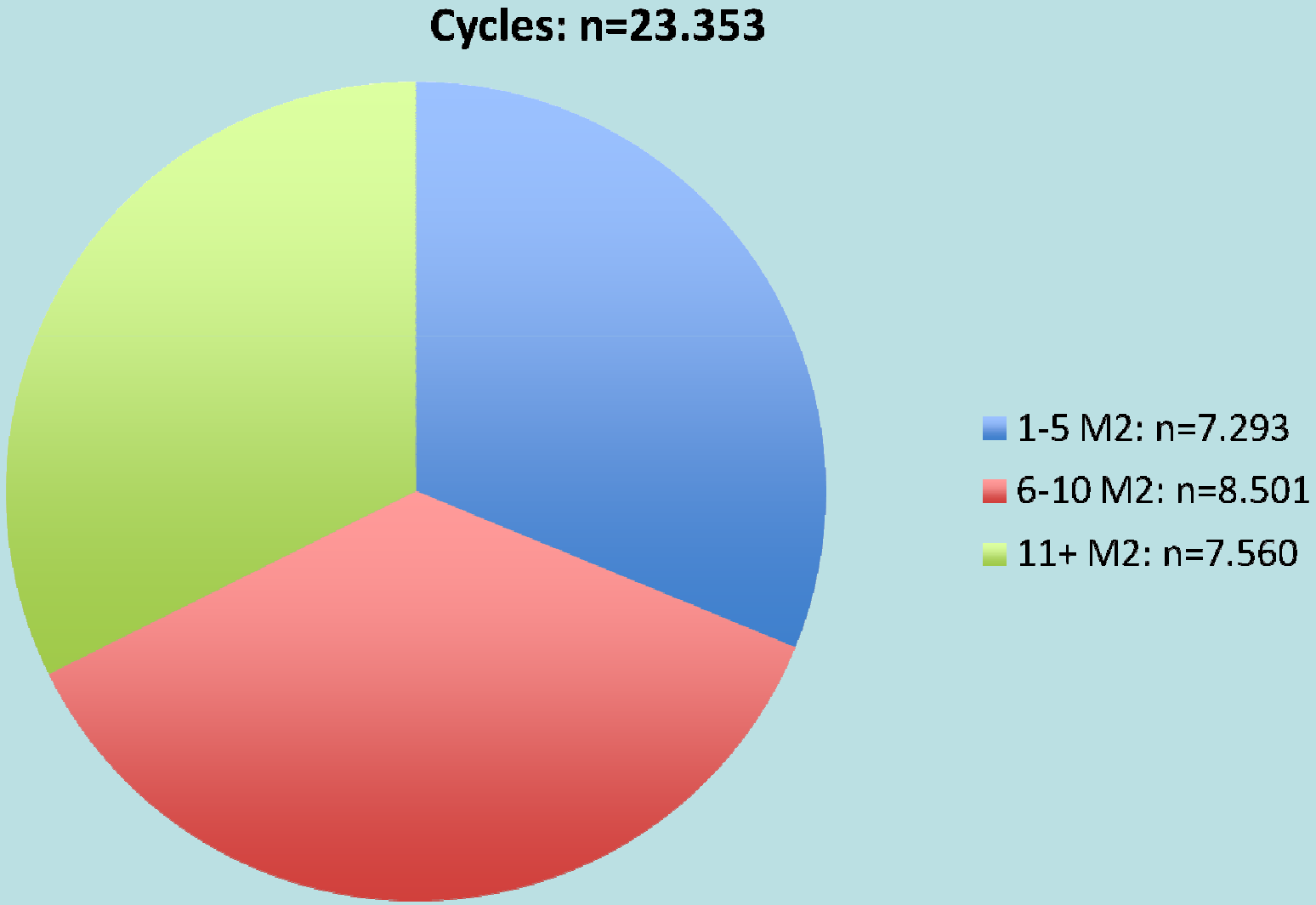
# CONTENTS

## Measure gene expression in cumulus

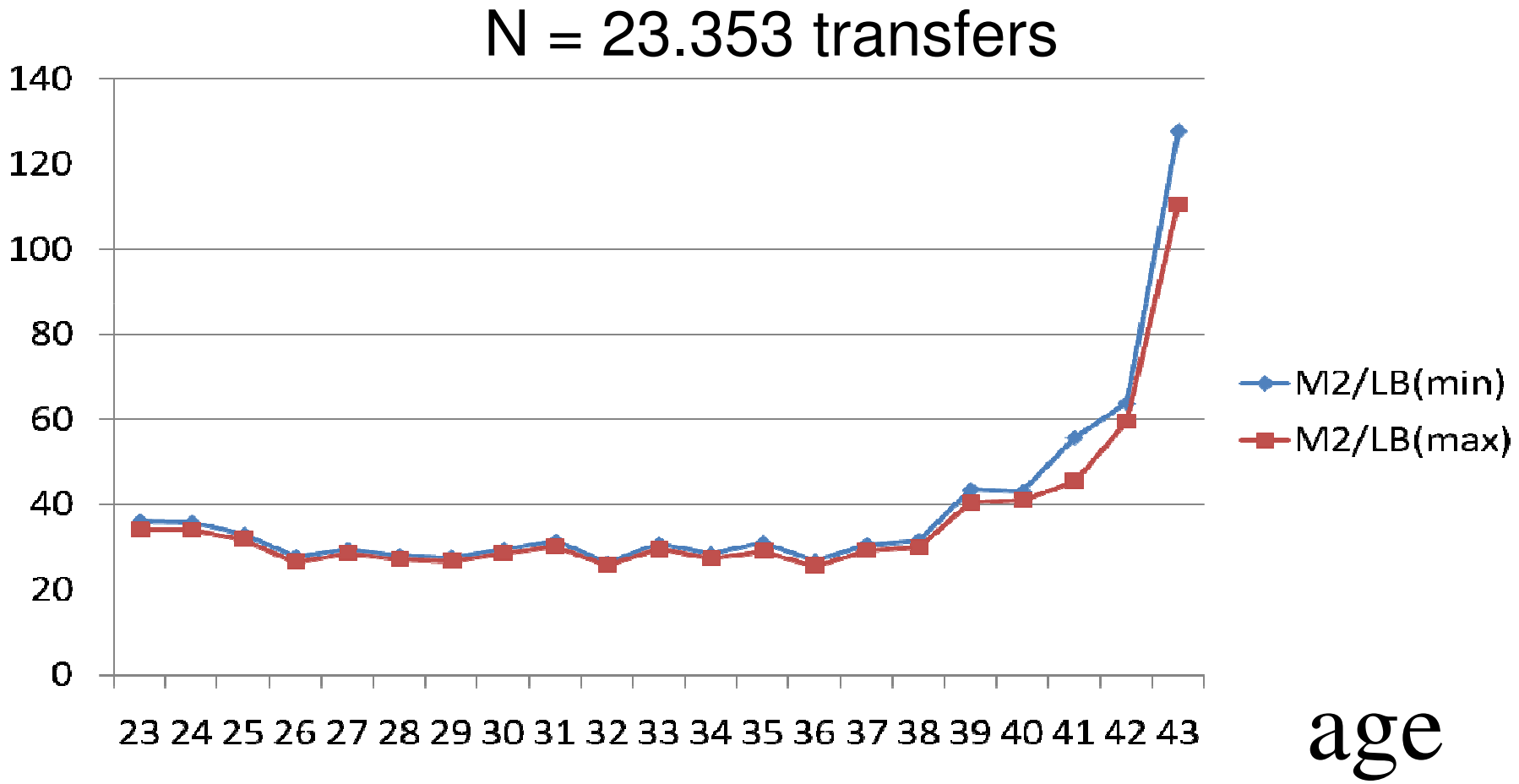
- to help in selecting oocytes
  - for maturation capacity
  - choose embryo to transfer

- help to make decisions in ART (culture / SET)
- help developing better culture conditions

# Cycles at UZBRUSSEL from Age group 23-43 YEARS

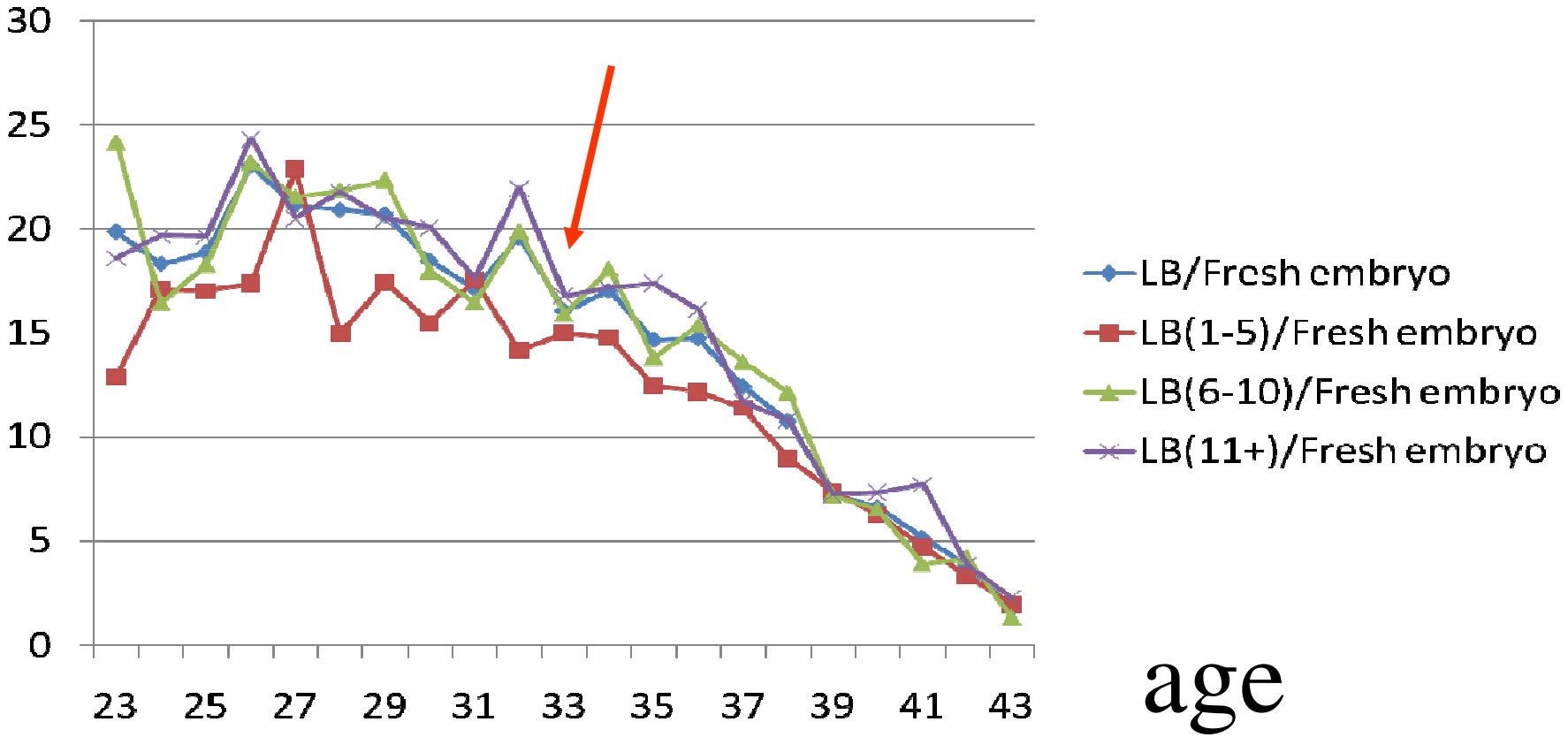


# Number mature oocytes needed per Live Birth (23-43)

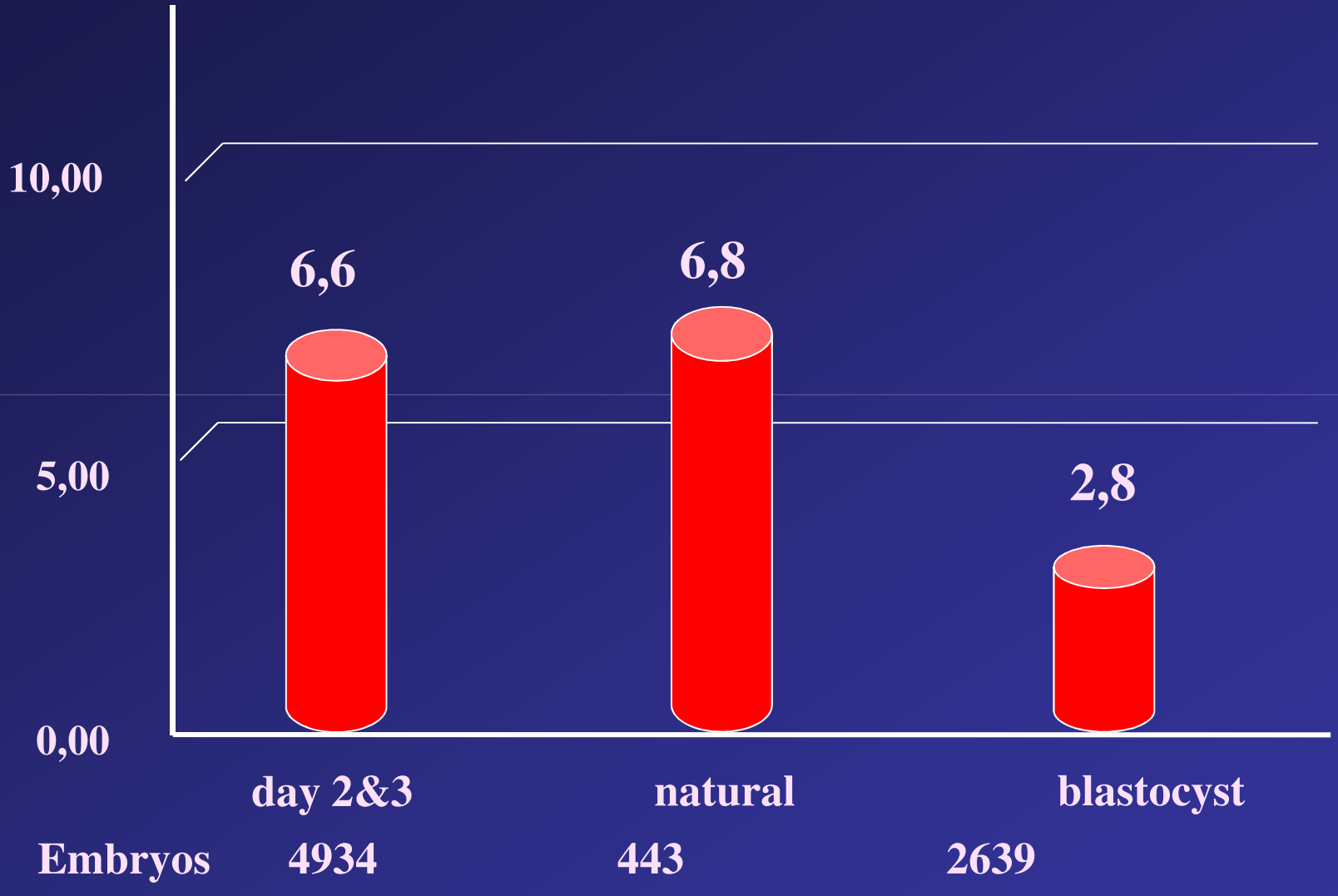


# Live Birth / Fresh embryo transferred

N = 23.353 transfers



# Number of embryos required for transfer per baby born




*V. Vlaisavljevic (2010)*

# Difficulties to make the right choice

## Recent data from actual culture systems

MEGASET
MENOPUR in GnRH Antagonist Cycles with Single Embryo Transfer

- Day 3 morphology : consider 3 categories :
  - TOP, GOOD , MINIMAL
- Blastocyst consider 3 gradings :
  - excellent and good (1)
  - other type (2)
  - No blastocyst was formed (3)

**RESULTS :**

TOP on day 3 : 46% became 1, 27% : 2, and 27% : 3
GOOD on day 3 : 24% became 1, 28 % : 2, and 47 % : 3
MINIMAL on day 3 : 6% became 1, 17% : 2, and 76% : 3

# Blasto culture , 1 blasto transferred

multicentric study on 619 patients

MEGASET
MENOPUR in GnRH Antagonist Cycles with Single Embryo Transfer

Blastocyst criteria :

best prognostic ones = expansion capacity en hatching

| | pregnancy chance % | tot prevalence % | prevalence/pt % |
|---|---|---|---|
| grade 1 | 5 | 20 | 8 |
| grade 2 | 10 | 12 | 8 |
| grade 3 | 18 | 26 | 17 |
| grade 4 | 42 | 32 | 41 |
| grade 5 | 47 | 9 | 26 |

# Current way of blasto scoring

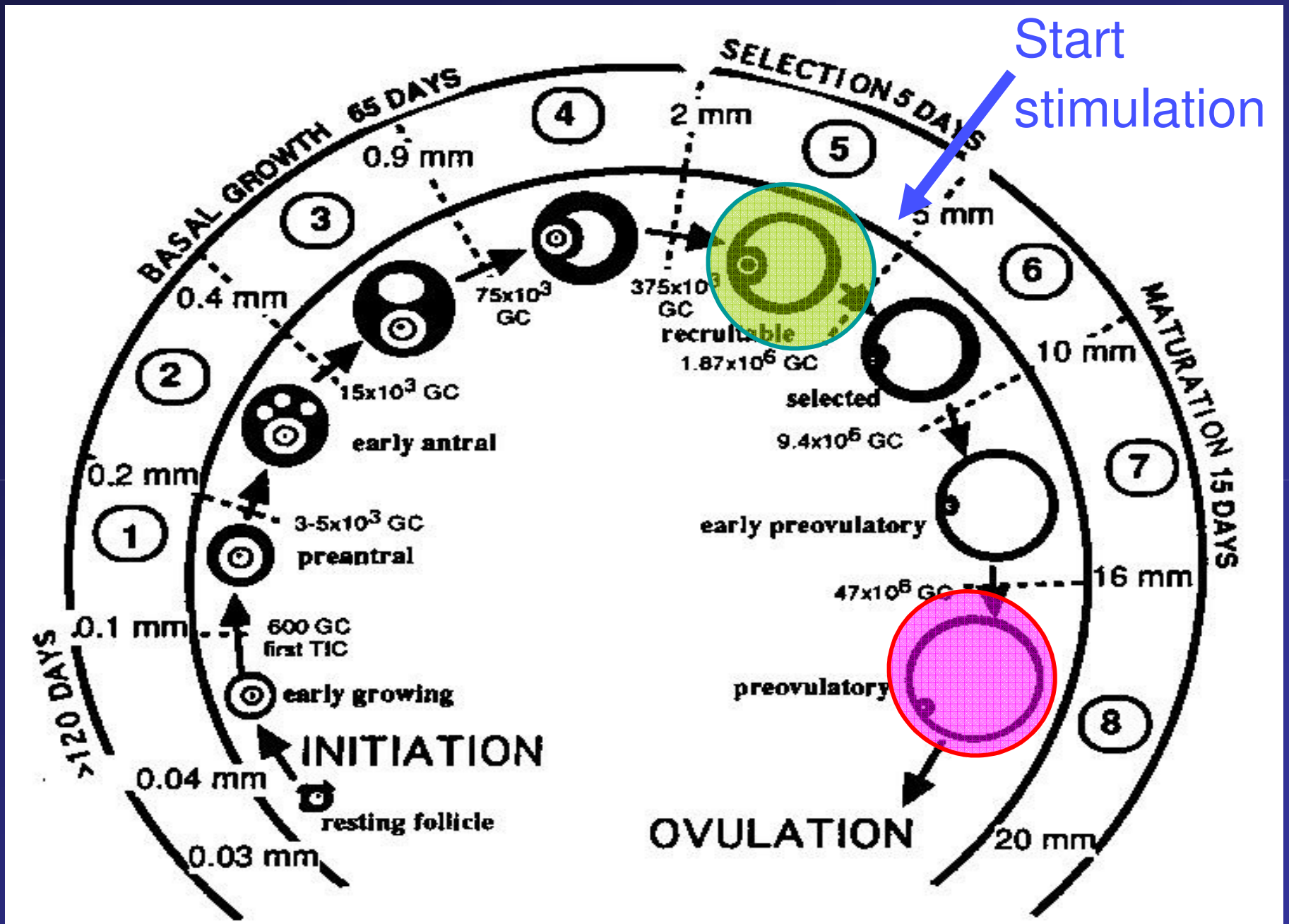
# PPV - NPV - Accuracy

MEGASET
MENOPUR in GnRH Antagonist Cycles with Single Embryo Transfer

multicentric study on 619 patients

Best PPV : 47 %  chance when looking good for a Preg
Best NPV : 90 %  chance of no implantation if poor morphology

Best Accuracy : 50 % ( = % correct classifications)


Start stimulation
SELECTION 5 DAYS
BASAL GROWTH 65 DAYS
MATURATION 15 DAYS
>120 DAYS
0.9 mm
2 mm
0.4 mm
5 mm
10 mm
0.2 mm
16 mm
0.1 mm
20 mm
0.04 mm
0.03 mm
75x10³ GC
375x10³ GC
recruitable
1.87x10⁶ GC
selected
9.4x10⁵ GC
15x10³ GC
early antral
3-5x10³ GC
preantral
600 GC
first TIC
early growing
early preovulatory
47x10⁶ GC
preovulatory
INITIATION
resting follicle
OVULATION
1
2
3
4
5
6
7
8

## GENE EXPRESSION in human FOLLICLE or OOCYTE useful to predict further development ?

- We TREAT : from Gougeon stage 5 up to stage 8
- Determine oocyte dev stage of maturation :
  - competence to GVBD : **useful for IVM**
- oocyte dev potential: cytoplasmic competence
  - capacity to fertilise
  - capacity to become embryo
  - capacity to become an healthy child

**useful for IVF / ICSI**

# Actual NEEDS in IVF (ICSI) and IVM

IVF :

- Which oocyte has developmental potential ?
- choose ‘the best’ among the embryos

IVM :

- make selection in the aspirated COC
- is the oocyte nuclear competent ?

  if yes : induce maturation stimuli

  if no : pre-culture before inducing meiosis

# PATIENT BENEFITS ?

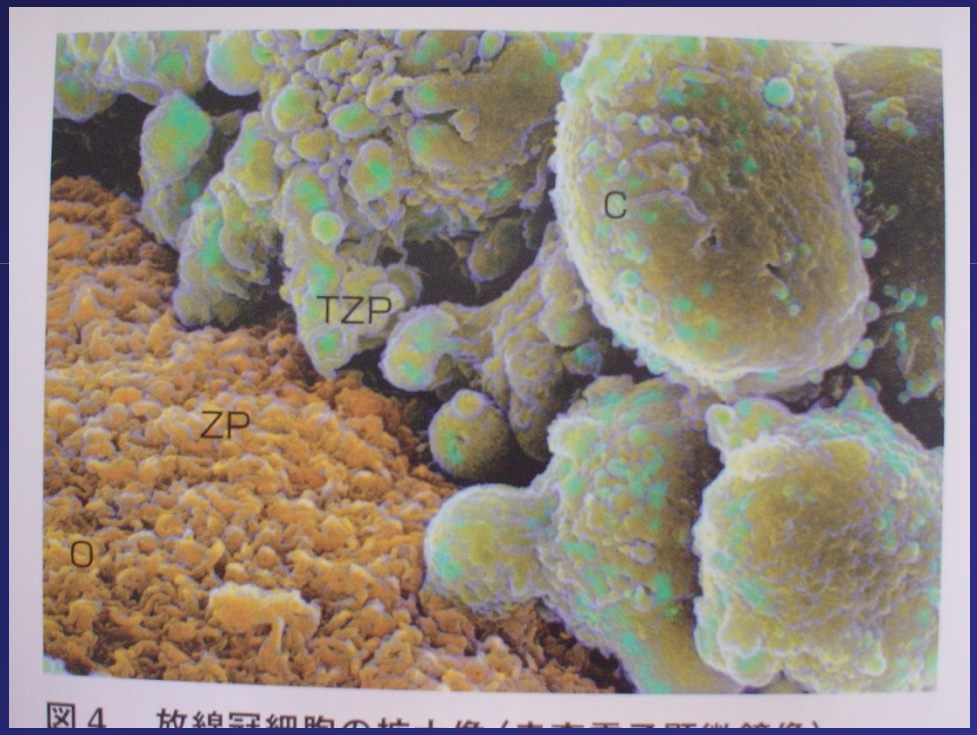
- zero risk for multiple pregnancy

and also

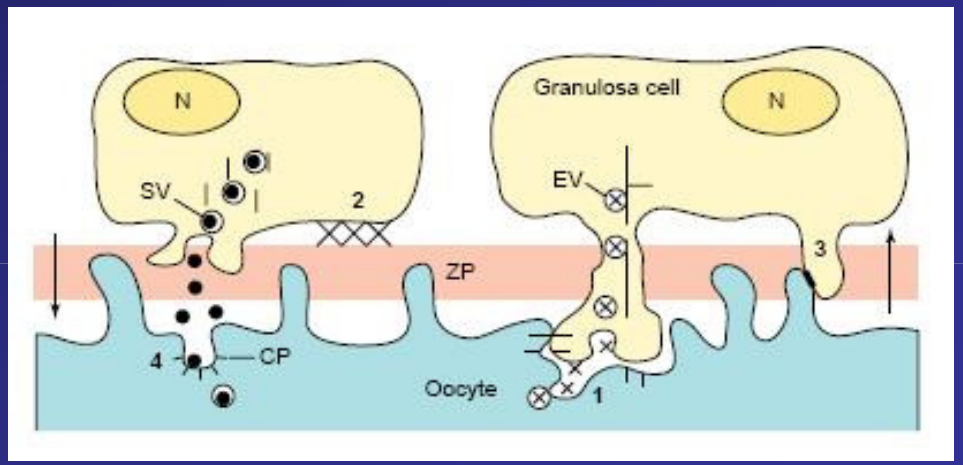
- limit time-to-pregnancy

help embryologist in decisions to plan
‘Oocyte & Embryo’ management

# TZP : communication




Courtesy Prof SUZUKI



# STRATEGIES :

- use discrete n° of genes : QPCR
- multiplex PCR
- use microarray

elements determining applicability in clinical setting:

- speed / ease / accuracy
- cost

# Find candidate genes.... as molecular markers HOW ?

detect mis-expression on a background of
normal gene-inherent variation in transcript levels

However over- or under expression are not dramatic (factor 2-4)

METHODOLOGICAL NEEDS
1- very accurate quantitative measurements
2- need to measure several genes

DNA Microarrays: to screen
QRT-PCR : to dose

# How to build candidate list of ‘quality’ genes

- collect cumulus prospectively
- analyse in relation to a posteriori outcomes :

  e.g. ongoing pregnancy or not / arrest of development

- Do array on sufficient n° of biol replicates
- Bioinformatics : find “significant” changes

  ‘hit’ gene list to be validated by QPCR on new samples

Today’s literature : estimation !

“At best 30 % of the differentially expressed genes found in microarray are confirmed by QPCR”

# Method: RT-PCR

Extraction

total-RNA

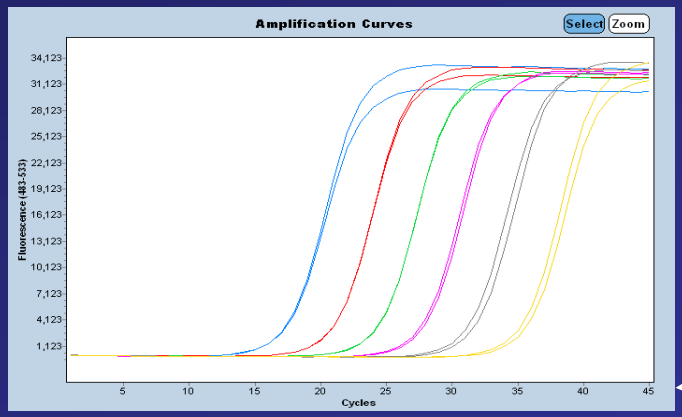
Reverse Transcription

cDNA

PCR using specific primers

2 # cycles copy's of 1 specific fragment corresponding with a specific mRNA (200-600 bp)

## Process optimization



# QPCR: cells used in Human ART Labs

- Floating granulosa cells : are ‘cumulus- alike’
- Cumulus-corona cells : first 3 layers
- Post hCG 36-40hr ( = sole occasion to obtain cells in IVF )
- Pre-hCG ( only in IVM laboratories )

- in literature CRITERIA for “GOOD outcome” were :
  - ‘Fast’ dividing embryos
  - morphological criteria of Day 3 embryos
  - Pregnancy outcome
    - very few data available, is long term research

# Work from Human ART Labs

- Post hCG material
  - Mc Kenzie (2004); Feuerstein (2007);
  - Van Montfoort (2008)
  - Hamel (2008); Anderson (2009); Assou (2009)

- GENES generally DIFFER BETWEEN THE REPORTS

- The FEW EXCEPTIONS ARE :

PTGS2, GREM1, HAS2, PTX3,
VCAN, GPX3, TNFAIP6, HSD3B1

# Predictive gene ‘families’

- mucification
- calcium metabolism
- glutathion ( ROS scavenging )
- TGF beta
- EGF related
- glucose transport
- luteinisation process

# Work from Human ART Labs 2004 - 2012

- Genes CONFIRMED by OTHERS

  Genes tested in an INDEPENDENT dataset:

FEW

- PROSPECTIVELY CHECKED

  Genes confirmed as useful in prosp clinical trial… :

NONE ?!

# Practically : RNA in human cumulus potential study biases

- Effect of background characteristics : age / BMI, …
- Effect of stimulation protocol : FSH ± LH-activity

- Effect of culture medium
- Effect of cell sampling (Hyaluronidase)

- Laboratory conditions : T° , sterility , speed

# VARIABILITY

- Between-**patient** variability :
  - study pooled cumulus : from MII , M1, GV

- Between-**follicle** variability :
  - study cumulus from one-by-one cultured oocytes : MII

# between-patient variation

- There is important patient to patient variation by:
  - Age & BMI
  - Ovarian responsiveness
  - The stimulation protocol

- CONSEQUENCES for pregnancy prediction genes :
  - need to analyze a large number of patients
  - need to stratify patient population
    - Age (BMI)
    - Stimulation protocol
    - Serum FSH
    - …

Or : introduce correction factors evidenced by stepwise multivariate regression analysis

(Adriaensens et al , 2010)

# Predictive Power?

-Outcome is **Multifactorial**

-Gene expression is 'biased' by other factors

- Make linear regression model : $Y = a + bX + cZ + dT$
  - Y : gene expression
  - X, Z, T patient and treatment related variables
  - B, c, d indexes
  - a: intercept

Significance of models set at $P<0,01$

EXAMPLE :

for a specific stimulation protocol

Log ( **Grem** )= 1,608 -0,0009 ( Age ) -0,11 ( Prog )-0.0002 ( >7cell on Day 3 )

(Adriaensens et al , 2010)

# Predict developmental competence among sibling MII oocytes by CC transcript quantification ?

## Within-patient variation

Relation oocyte competence to expression profiles?

- "competence" outcome parameters are:
  - » Embryo development : D3 and/or D5
  - » Ongoing Clinical Pregnancy
  - » Live Birth

- Calculate Efficiency of prediction model :
  - PPV
  - NPV
  - Accuracy : % correct answers

Strongest predictive genes obtained after 3 studies

# Does oocyte competence relate to CC expression ?

## Analyse Human Cumulus Cells from Individual oocytes

*8 Genes analyzed:

| | |
|---|---|
| SDC4 | Extra cellular Matrix/Signaling |
| VCAN | Extra cellular Matrix |
| ALCAM | Signaling |
| PTGS2 | LH/EGF signaling |
| GREM1 | BMP (oocyte) signaling |
| TRPM7 | cation channel ($Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$,…) |
| CALM1,2 | $Ca^{2+}$ Signaling |
| ITPKA | $Ca^{2+}$ Signaling |

**7 additional Genes analyzed:

| | |
|---|---|
| CA1 | Ca2+ Signaling |
| CA2 | Regulates steroidogenesis and Ca2+ conc. |
| CA3 | Regulates steroidogenesis and Ca2+ conc. |
| CA4 | Regulates intracellular Ca2+ |
| NEU | Neuronal factor (transmembr. Ligand) |
| STER1 | Steroidogenesis |
| STER2 | Steroidogenesis |

(Wathlet et al, HR 2011)

(Wathlet et al, 2012, in revision)

# PRELIMINAR Conclusions

*CC gene expression is influenced by:*

- Age or BMI
- stimulation dependent biological parameters
- gonadotrophin preparation
- **oocyte "quality"**

oocyte competence prediction is possible using multiparametric gene model

- Ongoing Pregnancy (PPV and NPV >80%)

*Some promising genes for Pregnancy Prediction got identified*

*PROSPECTIVE STUDY needed for evaluating discrim. power*

# Future Work = *Prospective study*

*Do Single Embryo Transfer :*

*Group 1 : embryo selection day 3 : morph*

*Group 2 : gene panel ( 3-5 ) in cumulus*

*Evaluate prediction model with "top 5 genes":*

*expected increase : a 30 % gain in pregn rate*

*If we would want an increase from 40 (baseline) to 70% OPR*
*Needed : 40 to 50 patients in each arm of the study*