Cumulus cells as non-invasive predictor of oocyte/embryo quality

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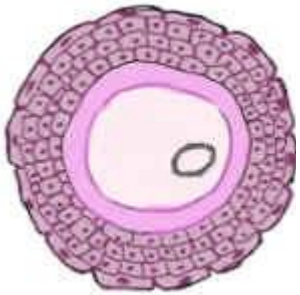
Linz, Austria

TAJEV 2014
April 30th – May 4th
Antalya

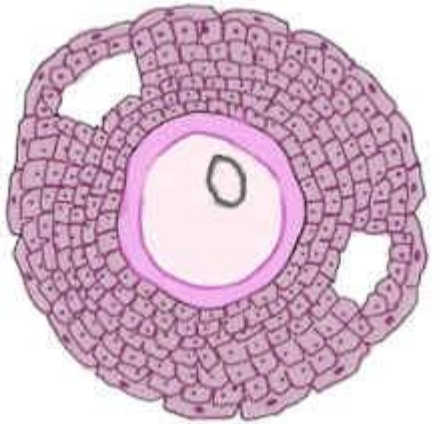
Correlation between oozyte and cumulus cells?

primordial follicle

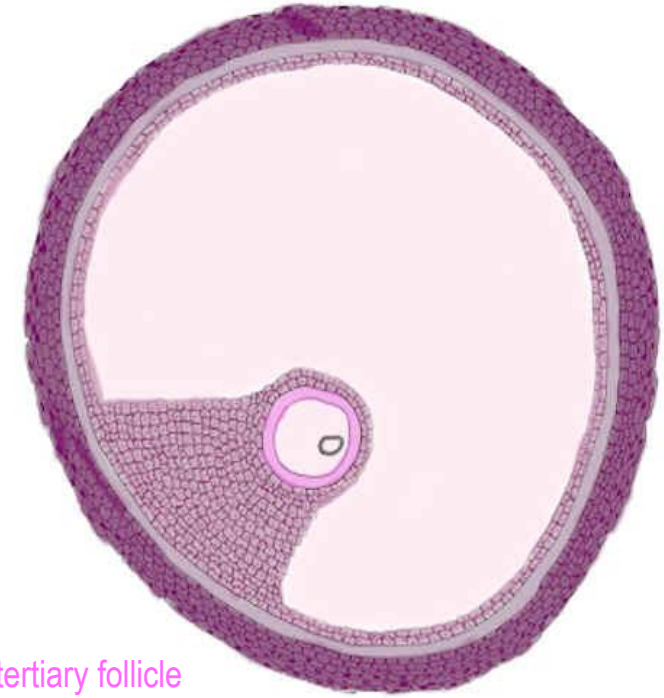
primary follicle



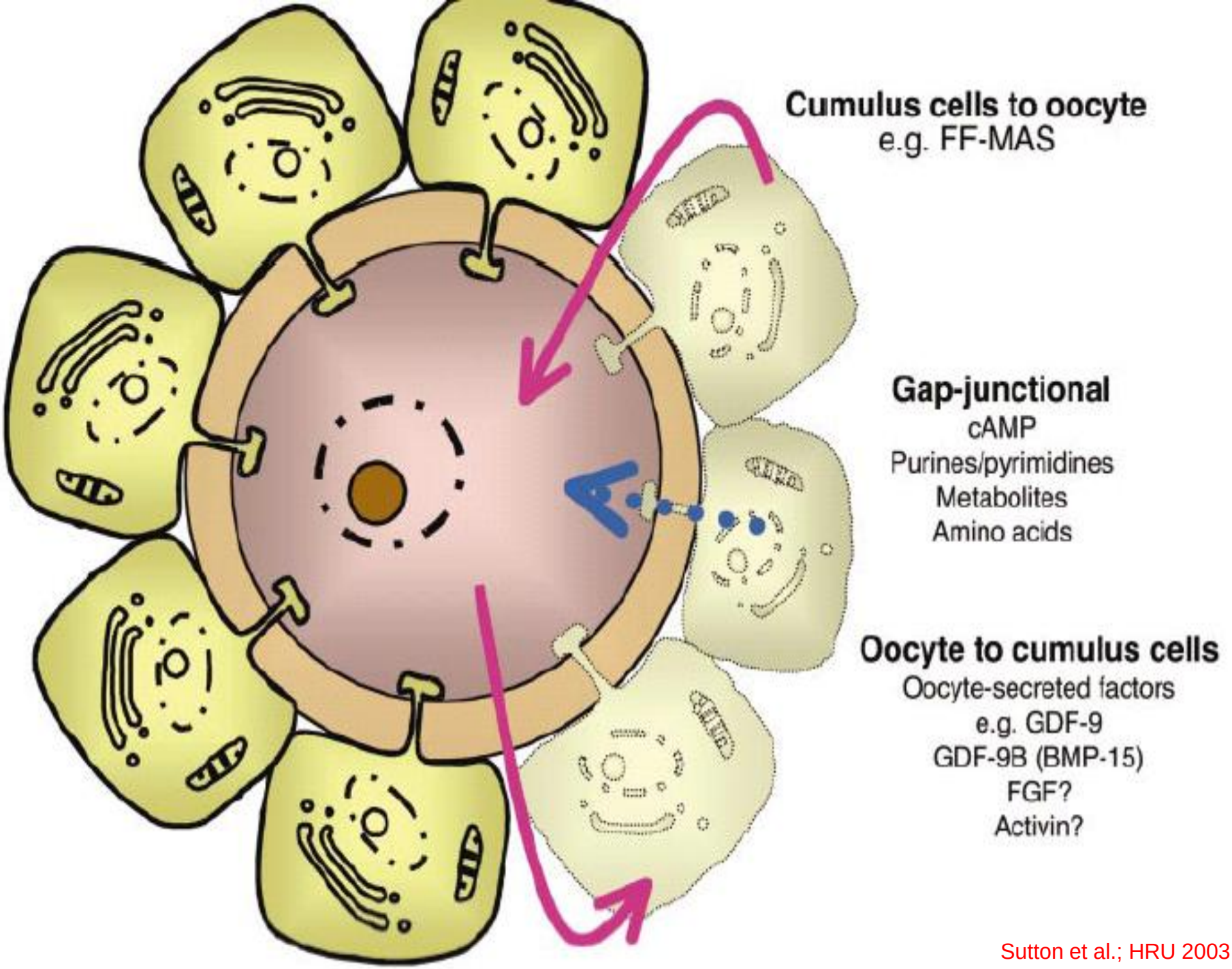
secondary follicle



secondary follicle



tertiary follicle



Sutton et al.; HRU 2003

Cumulus cells as marker of oocyte/embryo potential

- I. Morphology of COC
- II. Apoptosis in CC
- III. Telomere lenght of CC
- IV. Gene expression of CC

I. MORPHOLOGY

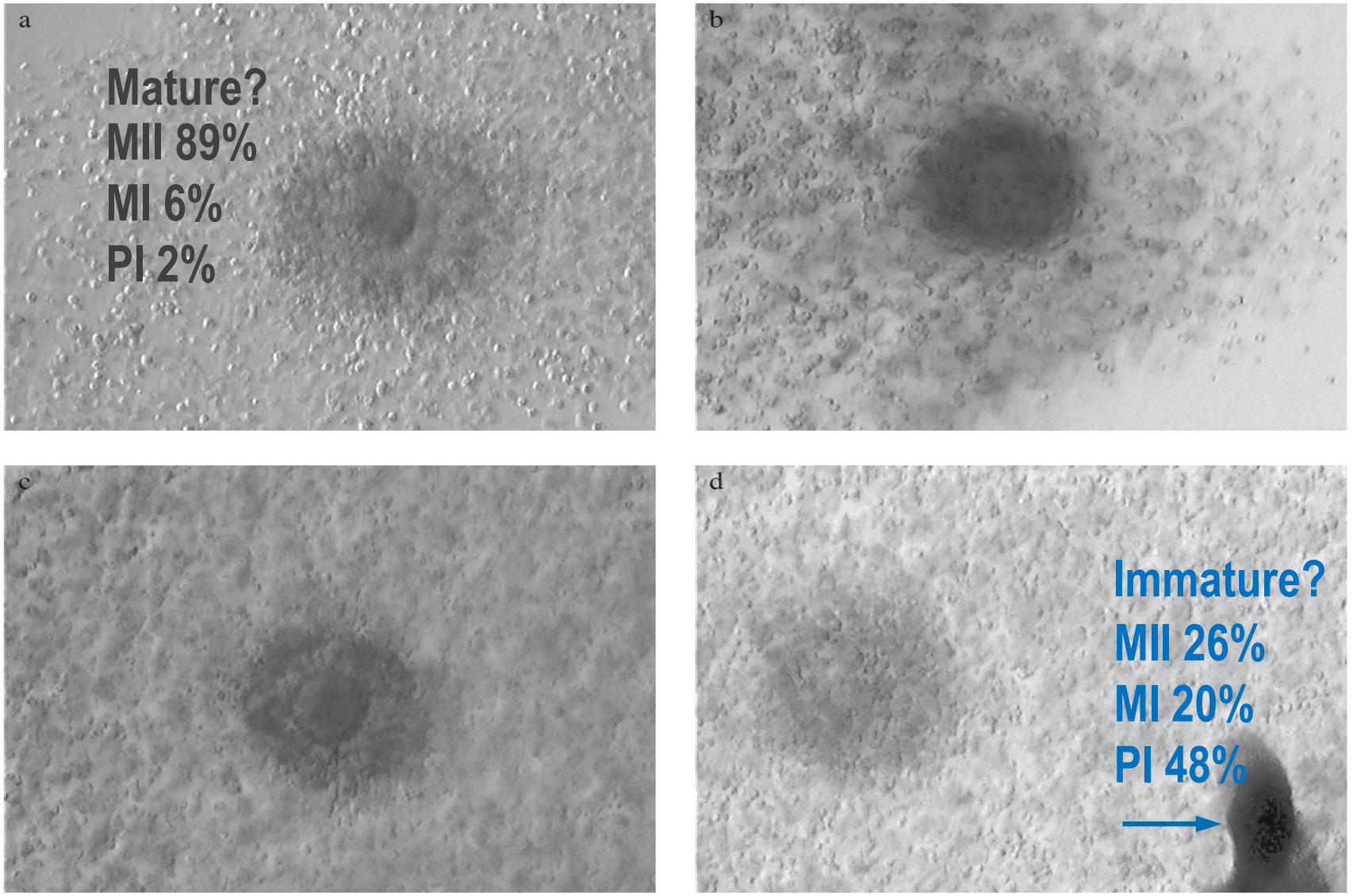


Figure 1. Grading of cumulus–oocyte complexes according to the expansion of corona radiata and cumulus matrix (×40). (**a**) Grade 1 (suspected mature): fluffy and radiant corona and cumulus with visible oocyte; (**b**) Grade 2: dense corona (oocyte hardly visible) but fluffy cumulus; (**c**) Grade 3: radiant corona (oocyte visible) and rather dense cumulus; (**d**) Grade 4 (suspected immature): dense corona and cumulus without visible oocyte with blood clot (arrow).

Ebner et al., RBM Online, 2009

II. APOPTOSIS

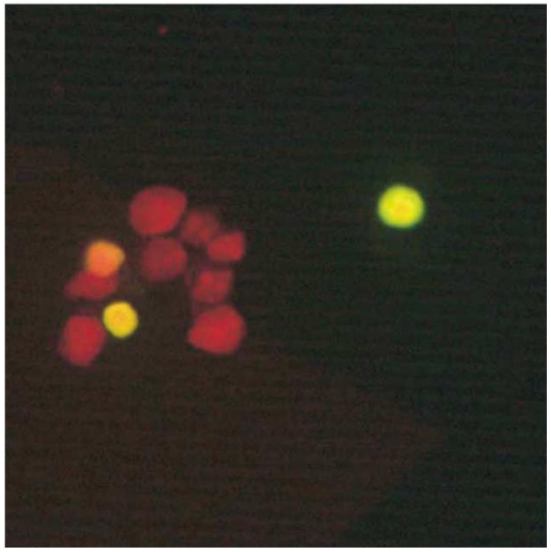
Predictive value of cumulus cell apoptosis with regard to blastocyst development of corresponding gametes

Claudia Maria Corn, B.Sc.,[a] *Cornelia Hauser-Kronberger, Ph.D.,*[b] *Marianne Moser, Ph.D.,*[c] *Gernot Tews, M.D.,*[c] *and Thomas Ebner, Ph.D.*[c]

[a] University of Salzburg, Institute of Zoology, Salzburg; [b] General Hospital and Paracelsus University, Institute of Pathology, Salzburg; and [c] IVF-Unit, Women's General Hospital, Linz, Austria

FIGURE 1

Apoptosis in cumulus cells analyzed using a TUNEL assay. Apoptotic cells appear *yellowish green* compared with *red* nonapoptotic cells.



Corn. Cumulus cell apoptosis and development. Fertil Steril 2005.

TABLE 2

Morphologic features in MII oocytes and their association with apoptotic processes in corresponding cumulus cells.

Type	n	Apoptosis
No anomaly	96	11.9 ± 10.6
Cytoplasmic anomalies		
Aggregation of ER	2	4.5 ± 0.7
Discoloration	7	12.5 ± 14.1
Central granulation	19	14.2 ± 10.5
Incorporations	20	13.7 ± 9.0
Refractile body	25	10.9 ± 9.5
Bull's eye	7	11.8 ± 12.3
Vacuole	11	7.6 ± 7.0
Anomalies of outer layer		
Granules in PVS	9	12.7 ± 8.1
Giant oocyte	2	4.0 ± 2.8
Ovoid shape	11	15.6 ± 17.2

Note: All values are mean ± SD; $P>.05$, oocytes with anomalies compared with unaffected gametes. ER = endoplasmic reticulum; PVS = perivitelline space.

Corn. Cumulus cell apoptosis and development. Fertil Steril 2005.

TABLE 3

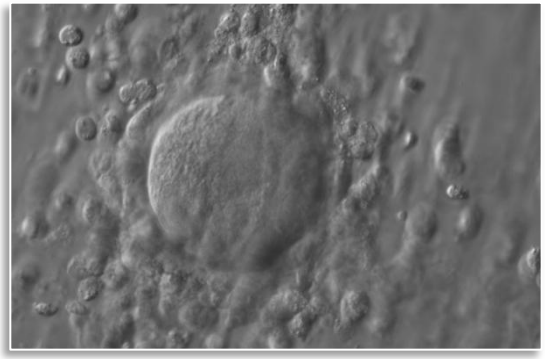
Relation between embryo morphology at cleavage stages and programmed cell death in corresponding cumulus cells.

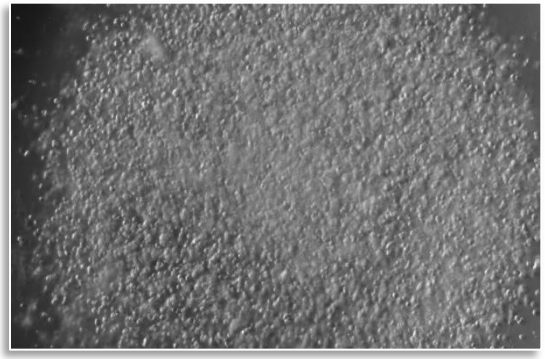
Morphologic feature	n	Apoptosis
Day 2		
<4 blastomeres	28	14.2 ± 12.4
≥4 blastomeres	96	11.2 ± 9.5
No fragmentation	58	11.6 ± 11.1
Moderate fragmentation	45	12.2 ± 10.6
Severe fragmentation	21	12.2 ± 10.3
Multinucleation/unequal cells	8	15.0 ± 12.6
Day 3		
<6 blastomeres	19	13.3 ± 12.0
≥6 blastomeres	105	11.3 ± 10.5
No fragmentation	57	11.3 ± 11.2
Moderate fragmentation	49	12.3 ± 10.4
Severe fragmentation	18	13.0 ± 10.2
Day 4		
Compacting	48	10.0 ± 8.9
Not compacting	41	10.7 ± 11.0
Day 5		
Blastocysts	44	7.5 ± 6.2[a]
Arrested	45	14.2 ± 11.2[a]
Good quality blastocysts	23	5.7 ± 5.0
Bad quality blastocysts	21	9.3 ± 6.8

[a] $P<.001$.

Corn. Cumulus cell apoptosis and development. Fertil Steril 2005.

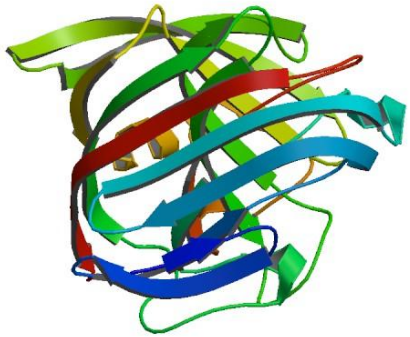
THEORY





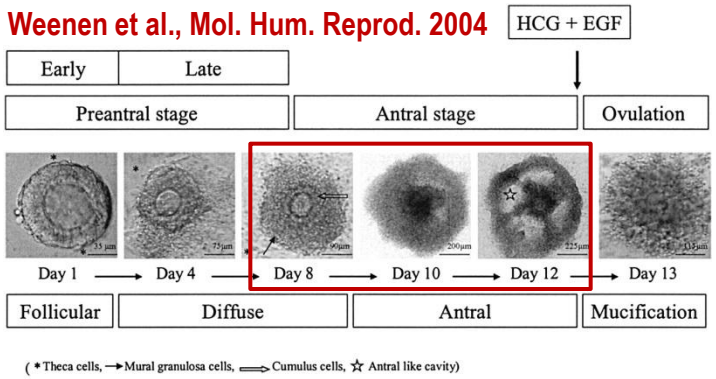
Ebner et al., Hum. Reprod. 2006

?



AMH

Weenen et al., Mol. Hum. Reprod. 2004



Preovulatory follicle??

Reproductive BioMedicine Online (2011) **22**, 389–398

Anti-Müllerian hormone remains highly expressed in human cumulus cells during the final stages of folliculogenesis

ML Grøndahl [a,b,*], M Eilsø Nielsen [c], MB Dal Canto [d], R Fadini [d], IA Rasmussen [c], LG Westergaard [c], SG Kristensen [a], C Yding Andersen [a]

[a] *Laboratory of Reproductive Biology, The Juliane Marie Centre for Women, Children and Reproduction, Copenhagen University Hospital, Copenhagen University, Denmark;* [b] *The Fertility Clinic,*

- Preovulatory follicles produce less AMH than smaller follicles
- Expression of AMH-receptor 2 ist also reduced
- In follicles that contain a MII-oocyte CC produce more AMH than mural GC
- But less AMH as compared to CC from follicles with an immature egg

Reproductive BioMedicine Online (2012) **24**, 540–546

Anti-Müllerian hormone is highly expressed and secreted from cumulus granulosa cells of stimulated preovulatory immature and atretic oocytes

Alon Kedem-Dickman [a,*], Ettie Maman [a], Yuval Yung [a], Gil M Yerushalmi [a], Rina Hemi [b], Mirit Hanochi [b], Jehoshua Dor [a], Ariel Hourvitz [a]

[a] *IVF Unit and Fertility Research Laboratory, Department of Obstetrics and Gynecology, Sheba Medical Center, Tel Hashomer, Affiliated with the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel;* [b] *Institute of Endocrinology, Sheba Medical Center, Tel Hashomer, Affiliated with the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

FACTORS influencing AMH

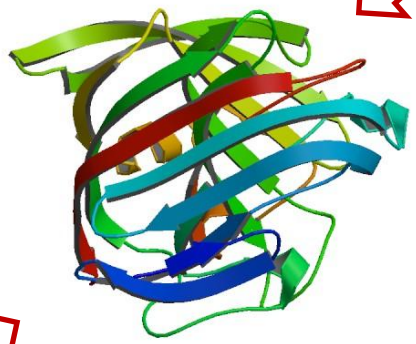
Maternal Age

oxidative stress
oxidative phosphorylation
MITOCHONDRIA

Tropomyosin-related kinase
APOPTOSIS

ovarian reserve

Steroid metabolism
IGF
Energy production
Anti-apoptotic BCL2
OOCYTE COMPETENCE



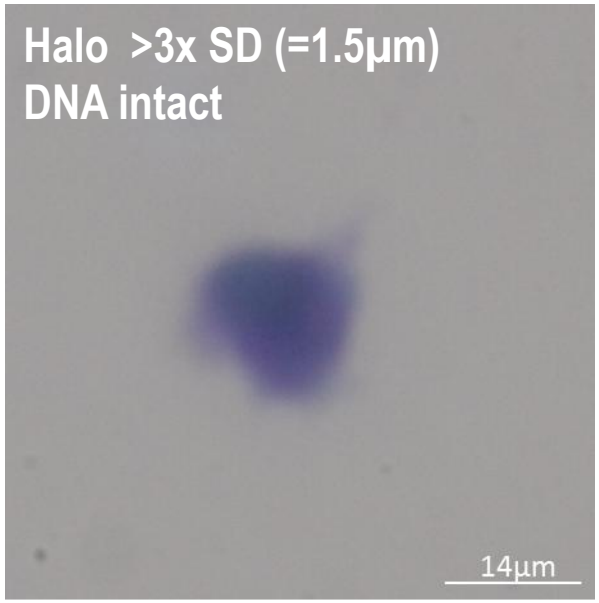
AMH

basal FSH

Indication

MAT & MET

- Strand break rate
- Sperm Chromatin Dispersion (SCD)Test (=Halo-Test)
  - Quantification of DNA-damage not possible
  - Are strand breaks related to apoptosis (single strand breaks)?
  - Approved for sperms and not for somatic cells
  - DNA in sperms is packed differently as compared to cumulus cells
  - Is there a difference in the size of the halo?
- Reference values of CC halo have to be established



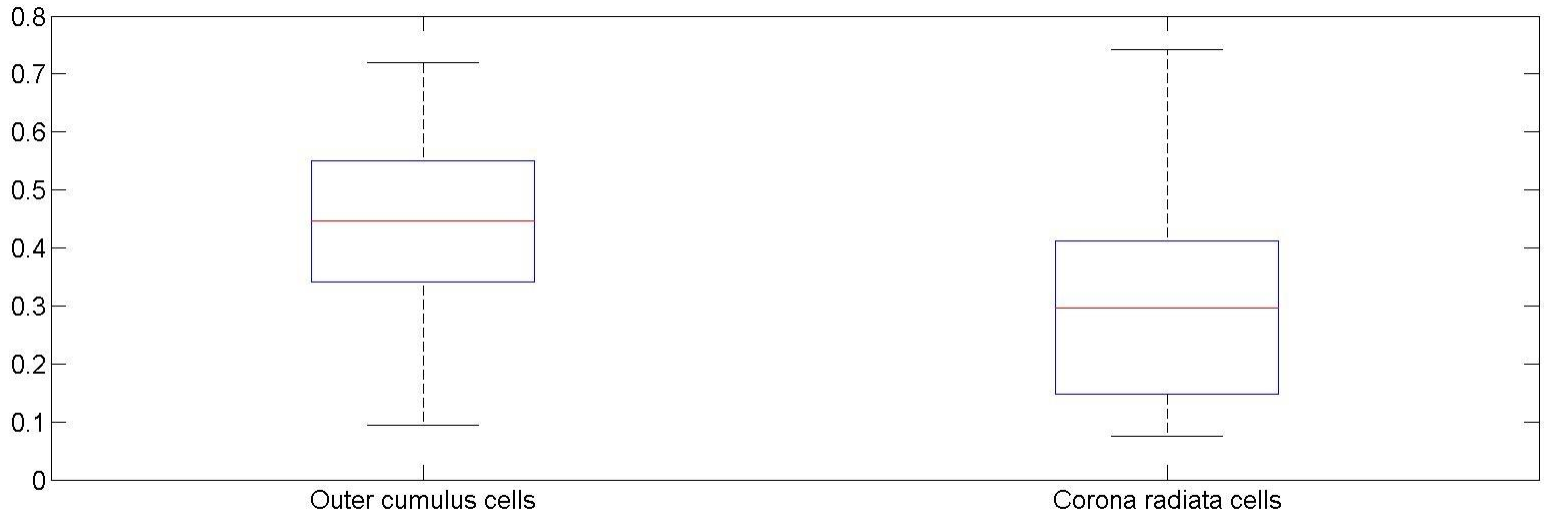
MAT & MET
Cumulus cells

- Short enzymatical denudation (15s) ⇒
- Harvesting of outer cumulus cells
- **Separate** pooling of corona radiata-cells
- 2x centrifugation ⇒
- Resuspension
- Cryopreservation ⇒
- Later analysis of strand breaks

NO effect on DNA

RESULTS

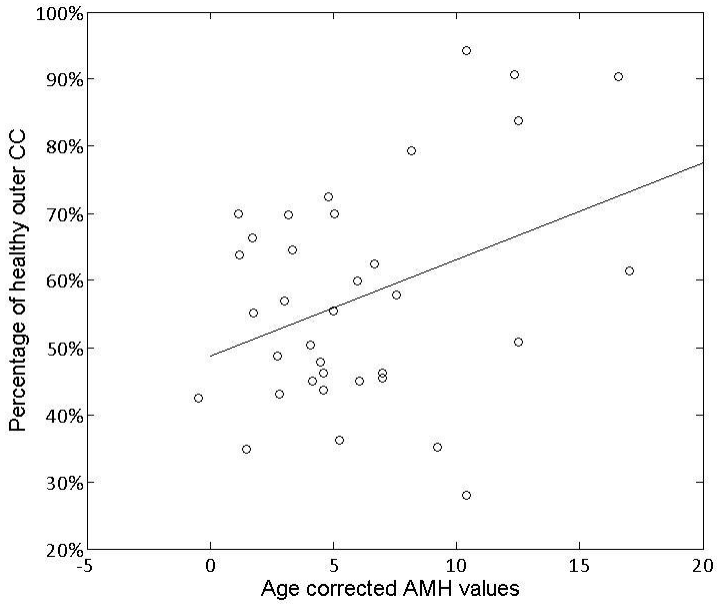
- >39,000 cells analyzed
- Significantly more outer CC were collected per patient than corona radiata cells ($p<0.01$)
- A total of 60% of all cells was found to be healthy (without strand breaks)
- Corona radiata cells next to oocyte should significantly less strand breaks ($p<0.01$)



- Percentage of healthy corona radiata cells correlates with blastocyst formation ($p<0.05$)

RESULTS II

- AMH correlated with E2, # COC, # MII und # 2Pn
- AMH was not related to IR and PR
- Strand break rate of outer CC and AMH showed no correlation



- But corrected for age a linear relationship was observed

DISCUSSION

- Obviously there are two entities of CC, inner and outer ones
- Eppig (2001) postulated a gradient of oocyte-derived proteins
- Hussein et al. (2005) were the first to confirm this theory in a bovine model (BMP 6, 7 und 15)
- In human, van der Ven et al. (2009) emphasized that Connexin 43 and BMP 15 follow an inside-outside gradient
- BMP 15 in corona radiata cells presumably protects the oocyte from apoptosis
- This is further strengthened by the finding that the protein PTX3 (downstream metabolite of BMP 15) is associated with clinical pregnancy rate (van Tol et al., 2010)

III. TELOMERE LENGHT

Human Reproduction, Vol.28, No.4 pp. 929–936, 2013
Advanced Access publication on February 1, 2013 doi:10.1093/humrep/det004

human reproduction

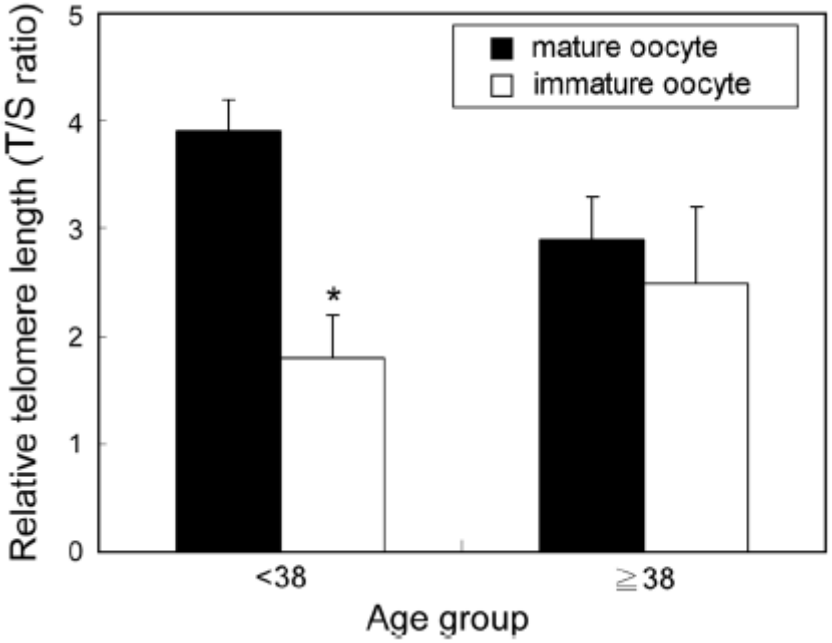
ORIGINAL ARTICLE *Embryology*

Evaluation of telomere length in cumulus cells as a potential biomarker of oocyte and embryo quality†

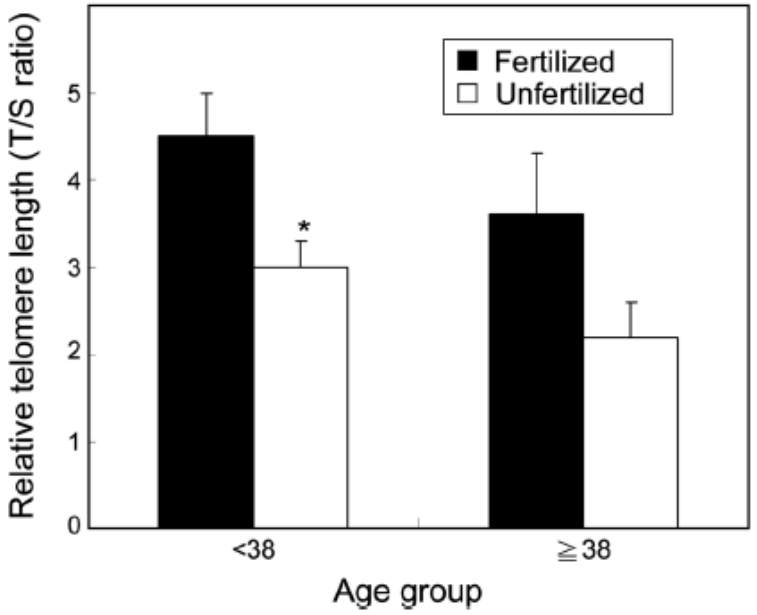
En-Hui Cheng[1], Shee-Uan Chen[2], Tsung-Hsien Lee[2,3,4], Yi-Ping Pai[1], Lii-Shung Huang[1,5], Chun-Chia Huang[1,6], and Maw-Sheng Lee[1,3,4,*]

[1]Division of Infertility Clinic, Lee Womens' Hospital, Taichung City 406, No. 263, Bei Tun Road, Taichung, Taiwan [2]Department of Obstetrics and Gynecology, College of Medicine and the Hospital, National Taiwan University, Taipei, Taiwan [3]Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan [4]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan [5]School of Nursing, Chung Shan Medical University, Taichung, Taiwan [6]Department of Biotechnology, Central Taiwan University of Science and Technology, Taichung, Taiwan

Telomere lenght of CC in mature/immature oocytes



Telomere lenght of CC in 2Pn and 0Pn oocytes



IV. GENEXPRESSION

- ✓ Oocyte quality
  - ✓ COX2 (Cyclooxygenase 2)
- ✓ Positive correlation with embryo quality
  - ✓ GREM1 (gremlin1)
  - ✓ HAS2 (hyaloronic acid synthase 2)
  - ✓ STAR (steroidogenic acute regulatory protein)
  - ✓ SCD1
  - ✓ AREG (amphiregulin)
  - ✓ PTX3
- ✓ Negative correlation with embryo quality
  - ✓ GPX3 (gluthation peroxidase 3)
  - ✓ CXCR4 (chemokine receptor 4)
  - ✓ CCND2 (cyclin D2)
  - ✓ CTNND1 (catenin delta 1)
- ✓ Blastocyst formation
  - ✓ SDC4 (syndecan 4)
- ✓ Pregnancy
  - ✓ VCAN (versican)

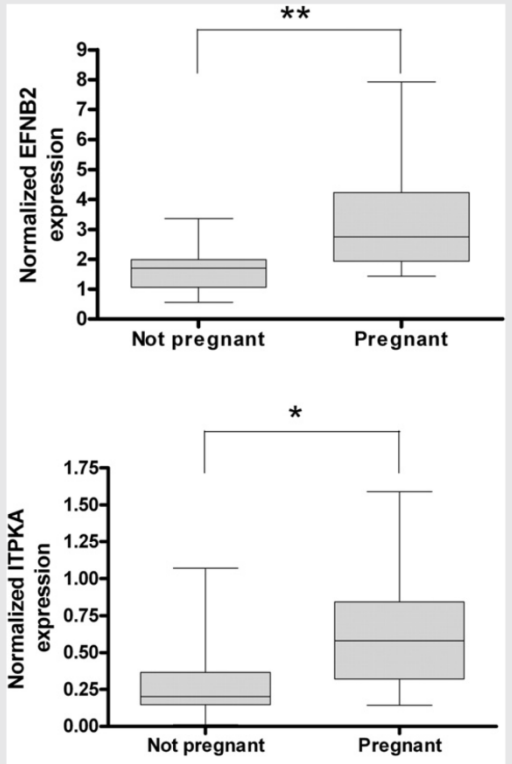
McKenzie et al., 2004
Zhang et al., 2005
Assou et al., 2006
Cilio et al., 2007
Hamel et al., 2008
Feuerstein et al., 2007
Assou et al., 2008
van Montfoort et al., 2008
Adriaenssens et al., 2010
Assou et al., 2010
Wathlet et al., 2011

New candidate genes to predict pregnancy outcome in single embryo transfer cycles when using cumulus cell gene expression

Sandra Wathlet, M.Sc.,[a] Tom Adriaenssens, M.Sc.,[a] Ingrid Segers, M.Sc.,[a] Greta Verheyen, Ph.D.,[b] Ronny Janssens, B.Sc.,[b] Wim Coucke, Ph.D.,[c] Paul Devroey, M.D., Ph.D.,[b] and Johan Smitz, M.D., Ph.D.[a]

[a] Follicle Biology Laboratory and [b] Center for Reproductive Medicine, Universitair Ziekenhuis Brussel; and [c] Department of Clinical Biology, Scientific Institute of Public Health, Brussels, Belgium

FIGURE 2



Not pregnant (n = 17) versus pregnant (n = 16) *t* tests on log-transformed expression values: *$P<.0045$; **$P<.001$. Boxes and whiskers represent the total range and quartiles with a line at the median.

Wathlet. Cumulus gene expression and pregnancy. Fertil Steril 2012.

gespag
OÖ. GESUNDHEITS- UND SPITALS-AG

Kinderwunsch Zentrum Linz
an der Landes- Frauen- und Kinderklinik

Thank you very much for your kind attention

Prof. Dr. OPPELT
Assoc.Prof. Dr. Omar SHEBL
Dr. Richard B. MAYER
Dr. Marianne MOSER
Fr. Manuela PUCHNER
Fr. Renate WIESINGER