PRECONGRESS COURSE 14

Is the oocyte the main determinant of embryo quality? Strategies for the selection of the most competent oocyte

Middle East Fertility Society Exchange course

Geneva – Switzerland, 2 July 2017

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Is the oocyte the main determinant of embryo quality? Strategies for the selection of the most competent oocyte

Geneva, Switzerland
2 July 2017

Organised by
the Middle East Fertility Society (MEFS)

Contents

Course coordination

Johnny Awwad (Lebanon) and Mohammad Aboulghar (Egypt)

Course type

Advanced

Course description

The oocyte is the key player in the sperm-egg interaction and the major determinant of embryo developmental potential. In addition to transmitting the maternal chromosomal complement, it also contributes the mitochondrial genome to the developing embryo. Surprisingly little research have focused on studying the oocyte contribution to a successful implantation. Determining oocyte quality remains restricted to a morphological analysis, a well-proven inaccurate science.
Alternative innovative strategies, the outcome of extensive research, could prove useful in enhancing the ability of the treating team to select the most competent oocytes for fertilization and subsequent embryo transfer. In addition to advancing our ability to alter reproductive pathways, such technologies have also greatly expanded our understanding of the biology of reproduction.
Oocyte competence could hence be better identified on the basis of minimally invasive enhanced diagnostic modalities, such as distribution pattern and function of mitochondria, polar body genomic analysis, cumulus cell molecular signature and many others. Some of these developments have also led to focused interventions designed to improve oocyte reproductive performance, namely mitochondrial enhancement and androgen priming.
This pre-congress course discusses biologic pathways which influence oocyte competence and evaluates diagnostic and therapeutic interventions designed to promote oocytes with the highest reproductive potential.

Target audience

Fertility Specialists and Reproductive Endocrinologists

Educational needs and expected outcomes

At the completion of this pre-congress course, participants should be able to:
Describe the biologic pathways which determine oocyte competence
Evaluate the merits of minimally invasive diagnostic modalities in enhancing the selection of the most competent oocyte for fertilization
Develop an evidence-based assessment of the value of proposed interventions in improving the reproductive capability of women

Scientific programme

Chairman:	Mohamed A. Aboulghar, Egypt
09:00 - 09:30	Oocyte competence: The mitochondria hypothesis Dagan Wells, United Kingdom
09:30 - 09:45	Discussion
09:45 - 10:15	Does oocyte mitochondrial injection improve outcomes in women with multiple IVF failures? An assessment of biological rational and clinical data Kutluk H. Oktay, U.S.A.
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee break
Chairman:	Michel Abou Abdallah, Switzerland
11:00 - 11:25	Oocyte competence: The hypoxia hypothesis Jeremy G. Thompson, Australia
11:25 - 11:50	Oocyte competence: The androgen hypothesis Wiebke Arlt, United Kingdom
11:50 - 12:15	Androgen priming of antral follicles prior to assisted reproduction: An oocyte rejuvenating therapy? Johnny Awwad, Lebanon
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch break
Chairman:	Johnny Awwad, Lebanon
13:30 - 14:00	Oocyte competence: The aneuploidy hypothesis Elpida Fragouli, United Kingdom
14:00 - 14:15	Discussion
14:15 - 14:45	Does polar body analysis accurately predict the aneuploidy status of the developing embryo? Alan H. Handyside, United Kingdom
14:45 - 15:00	Discussion
15:00 - 15:30	Coffee break
Chairman:	Mohamed A. Aboulghar, Egypt
15:30 - 16:00	Oocyte competence: The follicle environment hypothesis Jeremy G. Thompson, Australia
16:00 - 16:15	Discussion
16:15 - 16:45	Human cumulus cells molecular signature: Does it predict oocyte competence and embryo implantation potential? Samir Hamamah, France
16:45 - 17:00	Discussion

Oocyte competence: The mitochondria hypothesis

Dagan Wells, United Kingdom

Contribution not submitted by the speaker

Does oocyte mitochondrial injection improve outcomes in women with multiple IVF failures? An assessment of biological, translational and clinical data

Kutluk Oktay, MD, PhD, FACOG
Professor of Obstetrics & Gynecology, Medicine, Cell Biology & Anatomy, and Pathology
Vice Chair, Department of Obstetrics & Gynecology
New York Medical College
Director, Division of Reproductive Medicine and Innovation Institute for Fertility Preservation and IVF

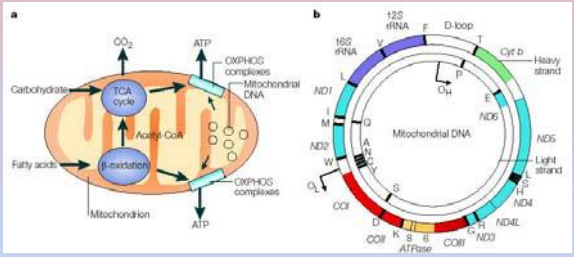
Disclosures

- Nothing to disclose (No conflicts pertinent to this presentation.

Objectives

- **To update the audience on mitochondria physiology**
- **Discuss whether mitochondrial dysfunction is relevant to reproduction**
- **Review proposed treatments for mitochondrial dysfunction**

Mitochondria: Battery for the Living



- Multicopy genome, circular ds-DNA molecule *Anderson et al., 1981*
- Codes for 13 essential subunits of the respiratory chain complexes *Wallace, 1992*
- Maternally-inherited
- 10x more prone to DNA damage

Striking Differences Between Mitochondrial and Nuclear Genome

Comparison between the human nuclear and mitochondrial genomes*

Characteristic	Nuclear genome	Mitochondrial genome
Size	~3.3 x 10^9 bp	16,569 bp
Number of DNA molecules per cell	23 in haploid cells; 46 in diploid cells	Several thousand copies per cell (polyploidy)
Number of genes encoded	~20,000–30,000	37 (13 polypeptides, 22 tRNAs and 2 rRNAs)
Gene density	~1 per 40,000 bp	1 per 450 bp
Introns	Frequently found in most genes	Absent
Percentage of coding DNA	~3%	~93%
Codon usage	The universal genetic code	AUA codes for methionine; TGA codes for tryptophan; AGA and AGG specify stop codons
Associated proteins	Nucleosome-associated histone proteins and non-histone proteins	No histones; but associated with several proteins(for example, TFAM) that form nucleoids
Mode of inheritance	Mendelian inheritance for autosomes and the X chromosome; paternal inheritance for the Y chromosome	Exclusively maternal
Replication	Strand-coupled mechanism that uses DNA polymerases α and δ	Strand-coupled and strand-displacement models; only uses DNA polymerase γ
Transcription	Most genes are transcribed individually	All genes on both strands are transcribed as large polycistrons
Recombination	Each pair of homologues recombines during the prophase of meiosis	There is evidence that recombination occurs at a cellular level but little evidence that it occurs at a population level

Taylor and Trumbull, Nature Review Genetics 2005

Peculiarities of Mitochondria

- Most genes needed for mitochondrial function are coded by the nucleus; traverse mitochondrial membrane to function
- Mitochondrial numbers can change depending on energy needs:
  - High energy need: grow and divide
  - Low energy need: destroyed and become inactive

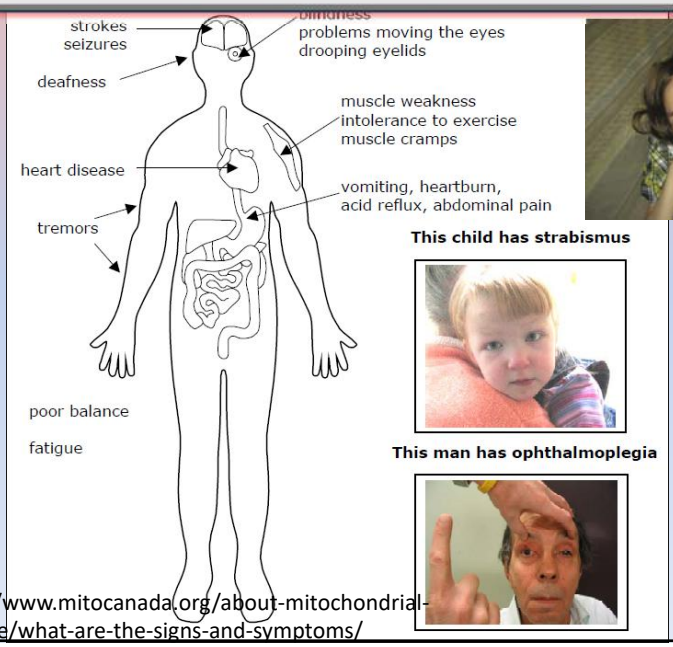
Wallace, 1992

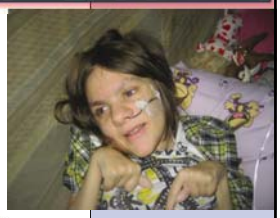
Mitochondrial Function not Limited to Just Being an "Energy Plant"

- Redox functions
- Oxygen sensing
- Fatty-acid oxidation (B-oxidation)
- Calcium hemostasis
- Cell Signaling
- Programmed Cell Death

Van Blerkom J Mitochondrion 2011

When mitochondria do not function…





This child has strabismus

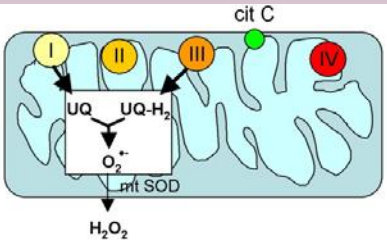
This man has ophthalmoplegia

http://www.mitocanada.org/about-mitochondrial-disease/what-are-the-signs-and-symptoms/

How do ROS Form in Mitochondria?

Stimuli inducing increased mitochondrial generation of ROS:

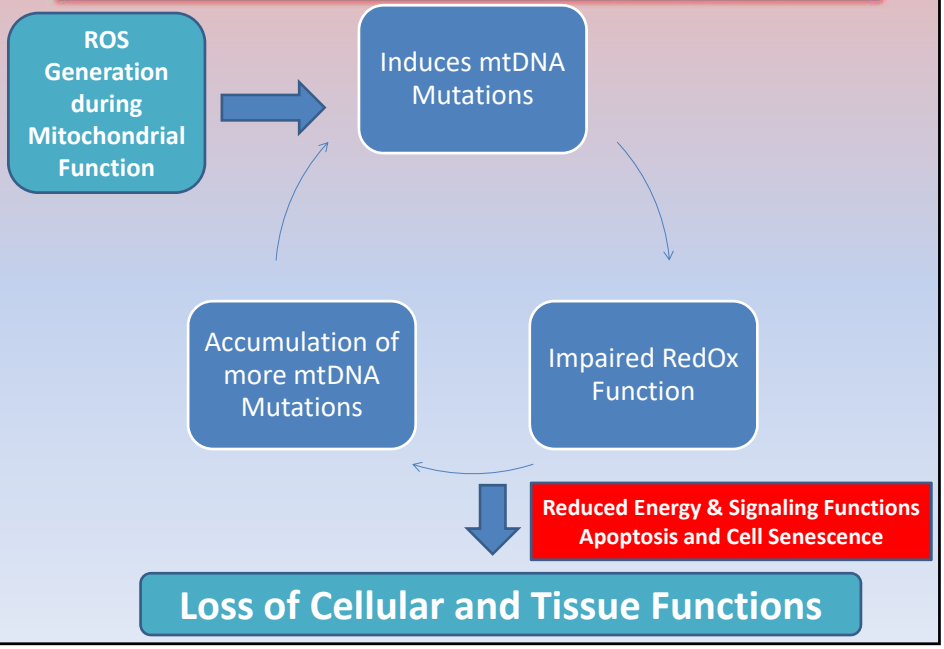
- serum deprivation
- integrin signalling
- apoptosis
- TNFα
- hypoxia
- ceramide
- p53
- oncogenic Ras



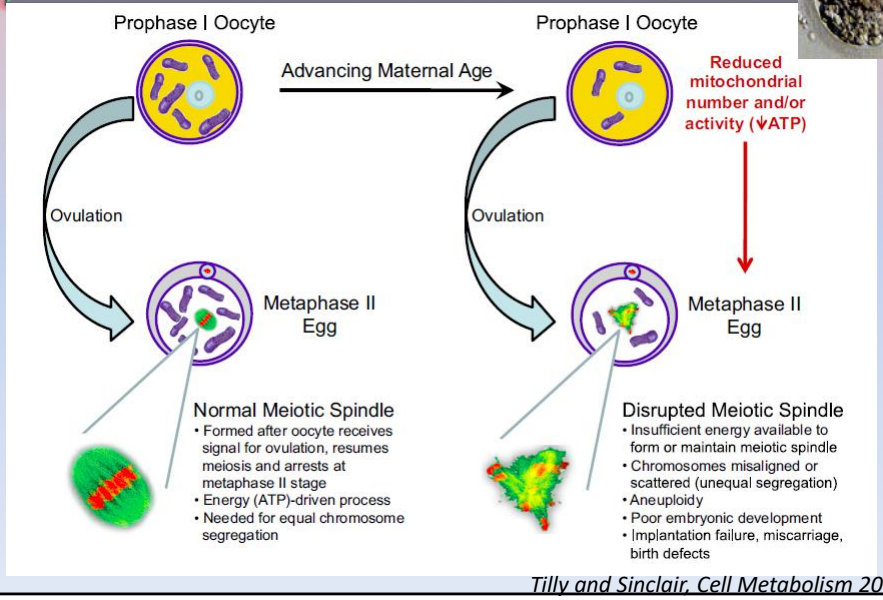
- Normally O_2 is reduced to H_20 through redox reactions
- ~ 0.1–2% of electrons passing through the chain oxygen is prematurely and incompletely reduced to give rise to superoxide radical (*0-2)
- The pronated form hydroxyperoxyl (HO*) inactivates enzymes or initiate lipid peroxidation

Novo and Parola Fibrogenesis & Tissue Repair 2008 1:5 doi:10.1186/1755-1536-1-5

Mitochondrial Theory of Aging



Mitochondrial Function and Oocyte Quality: Is there a Connection?



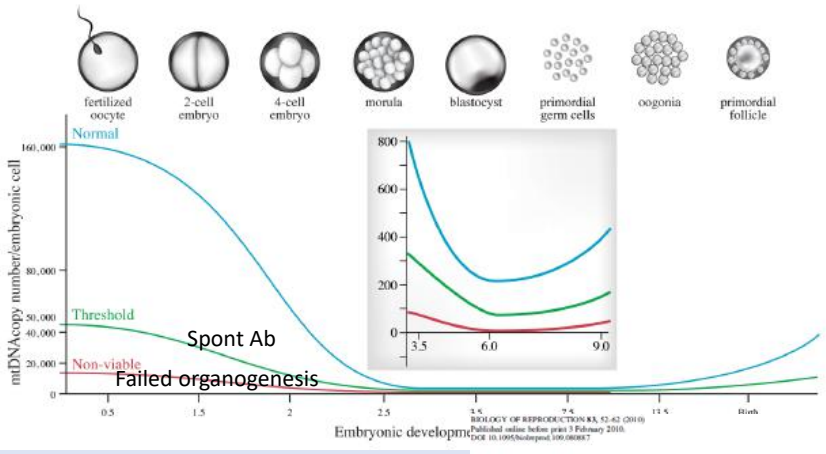
Tilly and Sinclair, Cell Metabolism 2013

Mitochondria in Oocytes

- Large endowment of mitochondria: 100K->600K
- Round/few cristae in oocytes vs. elongated/many cristae in morula/blastocyst
- Localization changes based on stage:
  - GV/M-I perinuclear (to support meiotic activities, spindle formation, chromosomal segregation?)
  - More peripheral in M-II
  - Perinuclear at 2-PN stage
  - Transient microzonation

Va Blerkom, RBM Online 2008

Mitochondrial Threshold for Viable Embryo Development in Mice



The Role of Mitochondrial DNA Copy Number in Mammalian Fertility[1]

Timothy Wai,[3,4] Asangla Ao,[3,4] Xiaoyun Zhang,[3,4] Daniel Cyr,[5] Daniel Dufort,[5,7] and Eric A. Shoubridge[2,3,4]

BIOLOGY OF REPRODUCTION 83, 52–62 (2010)
Published online before print 3 February 2010.
DOI 10.1095/biolreprod.109.080887

Mitochondria directly influence fertilisation outcome in the pig

Shahinaz H El Shourbagy, Emma C Spikings, Mariana Freitas and Justin C St John

The Mitochondrial and Reproductive Genetics Group, The Medical School, The University of Birmingham, Birmingham B15 2TT, UK

Reproduction (2006) **131** 233–245

Table 5 Fertilisation rates after IVF or ICSI on oocytes with (supplemented) and without (sham injection) mitochondrial supplementation.

Treatment	IVF fertilisation rate (%)	ICSI fertilisation rate (%)
BCB^+	37.5^a	40.4^c
BCB^-	17.6^b	19.8^d
BCB^- supplemented	31.0^a	34.0^c
BCB^- sham injected	17.0^b	10.0^d

^{a,b}Values in the same column with different superscripts differ ($P < 0.002$); ^{c,d}values in the same column with different superscripts differ ($P < 0.001$).

BCB: Brilliant Cresyl Blue Dye

Aging Cell (2002) 1, pp40–46

Mitochondrial dysfunction leads to telomere attrition and genomic instability

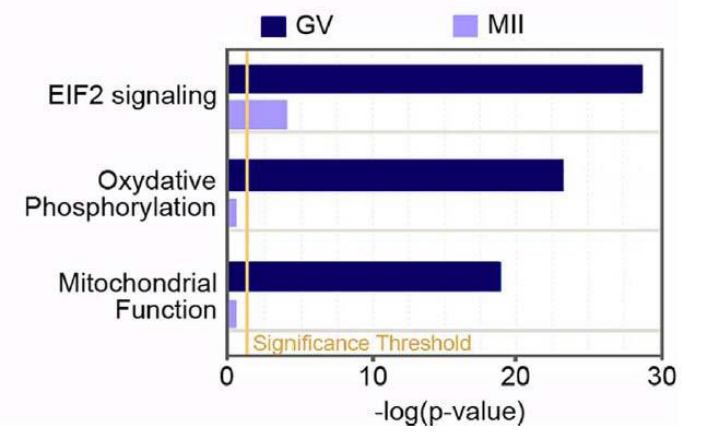
Lin Liu,*‡ James R. Trimarchi,*‡ Peter J. S. Smith† and David L. Keefe*‡

programmed cell death or apoptosis and mitochondria appear

n most somatic cell systems

Mitochondrial dysfunction and oxidative stress have been implicated in cellular senescence, apoptosis, aging and aging-associated pathologies. Telomere shortening and genomic instability have also been associated with replicative senescence, aging and cancer. Here we show that mitochondrial dysfunction leads to telomere attrition, telomere loss, and chromosome fusion and breakage, accompanied by apoptosis. An antioxidant prevented telomere loss and genomic instability in cells with dysfunctional mitochondria, suggesting that reactive oxygen species are mediators linking mitochondrial dysfunction and genomic instability. Further, nuclear transfer protected genomes from telomere dysfunction and promoted cell survival by reconstitution with functional mitochondria. This work links mitochondrial dysfunction and genomic instability and may provide new therapeutic strategies to combat certain mitochondrial and aging-associated pathologies.

Shift in Mitochondria Function in Bovine Oocyte?



The Adenosine Salvage Pathway as an Alternative to Mitochondrial Production of ATP in Maturing Mammalian Oocytes[1]

Sara Scantland, et al

BIOLOGY OF REPRODUCTION (2014) 91(3):75, 1–11

Oocyte Mitochondrial DNA Copy Number is Reduced in Ovarian Insufficiency

Low oocyte mitochondrial DNA content in ovarian insufficiency

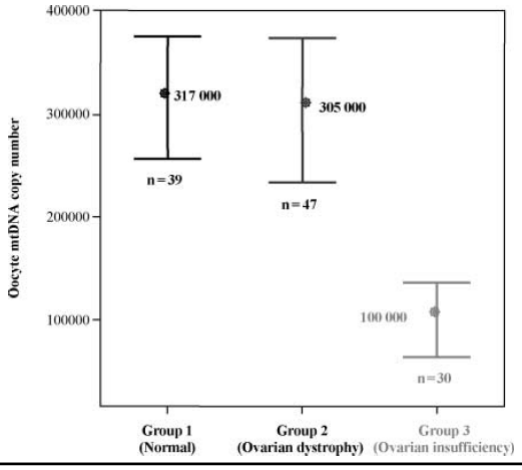
Human Reproduction Vol.20, No.3 pp. 593–597, 2005

P.May-Panloup[1,4], M.F.Chrétien[1], C

[1]Biologie de la Reproduction–Laboratoire FIV, [2]INS
Gynécologie Obstétrique (UF Médecine de la Repro
F-49033 Angers cedex 01, France

[4]To whom correspondence should be addressed. E-n

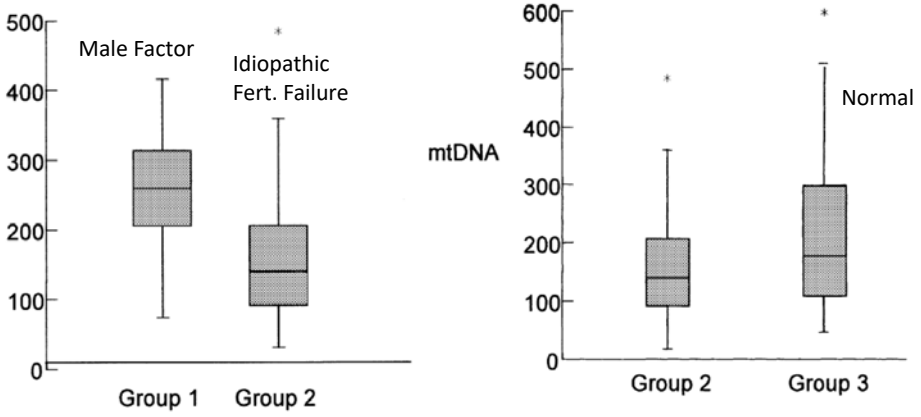
BACKGROUND: Mitochondrial biogenesis
embryo development. We have investigated
lack of oocyte maturity observed during IVF
ian insufficiency. **METHODS:** We used real
116 oocytes obtained from 47 women underg
from women with a normal ovarian profile
insufficiency. **RESULTS:** We found an aver
mtDNA copy number was not significantly d
nificantly lower in oocytes from women with
Our results suggest that low mtDNA conten
insufficiency.



Molecular Human Reproduction Vol.7, No.5 pp. 425–429, 2001

Mitochondrial DNA content affects the fertilizability of human oocytes

P.Reynier[1,4], P.May-Panloup[2], M-F.Chrétien[2], C.J.Morgan[1], M.Jean[3], F.Savagner[1], P.Barrière[3] and Y.Malthièry[1]



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< Previous Article Volume 364, No. 9437, p875–882, 4 September 2004 Next Article >

Mechanisms of Disease

Parkinsonism, premature menopause, and mitochondrial DNA polymerase γ mutations: clinical and molecular genetic study

Petri Luoma, MSc, Atle Melberg, MD, Juha O Rinne, MD, Jyrki A Kaukonen, MD, Nina N Nupponen, PhD, Richard M Chalmers, MD, Prof Anders Oldfors, MD, Ilkka Rautakorpi, MD, Prof Leena Peltonen, MD, Prof Kari Majamaa, MD, Hannu Somer, MD, Dr Anu Suomalainen, MD

DOI: http://dx.doi.org/10.1016/S0140-6736(04)16983-3

Article Info

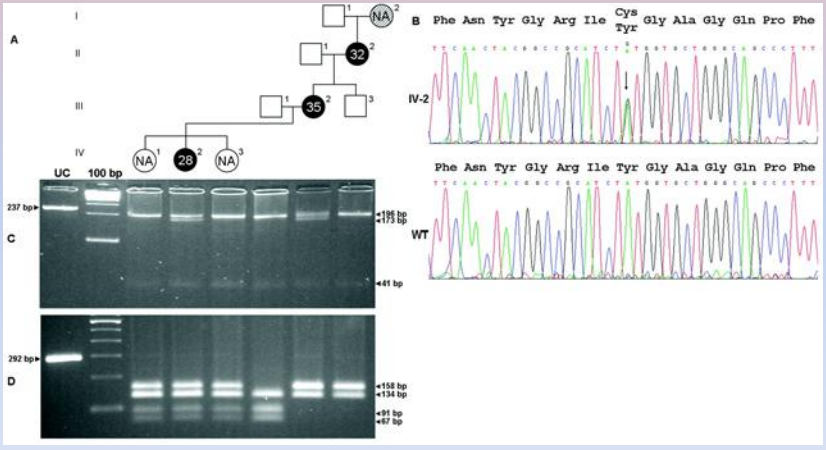
Summary Full Text Tables and Figures References Glossary

Summary

Background

Mutations in the gene encoding mitochondrial DNA polymerase γ (*POLG*), the enzyme that synthesises mitochondrial DNA (mtDNA), have been associated with a mitochondrial disease—autosomal dominant or recessive progressive external ophthalmoplegia—and multiple deletions of mtDNA. Mitochondrial dysfunction is also suspected to participate in the pathogenesis of Parkinson's disease. However, no primary gene defects affecting mitochondrial proteins causing mendelian transmission of parkinsonism have been characterised. We aimed to analyse the gene sequence of *POLG* in patients with progressive external ophthalmoplegia and their healthy relatives.

Premature Ovarian Failure in Women with Mitochondrial Mutations



Alistair T. Pagnamenta et al. Hum. Reprod. 2006;21:2467-2473

human reproduction

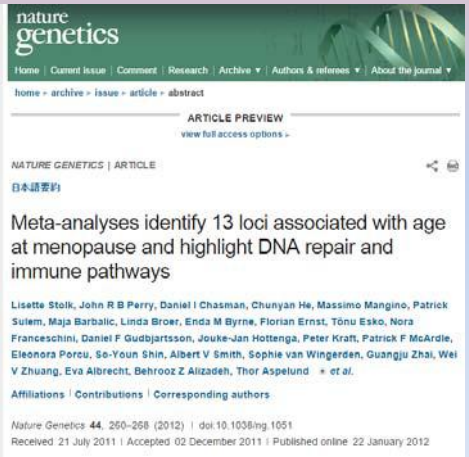
Clues from Women with Mitochondrial Diseases

Table 1. Fertility Rates in Women with Inherited Pathogenic Mitochondrial DNA Mutations, as Compared with Rates in the General Population.*

Age Range	Women with Mitochondrial DNA Mutations			General Population	P Value
	No. of Live Births	No. of Person-Years	Live Birth Rate (95% CI) *no./1000 person-yr*	Live Birth Rate	
15–19 yr	17	761	22.3 (13.0–35.8)	32.2	0.14
20–24 yr	69	713	96.8 (75.3–122.5)	100.4	0.82
25–29 yr	91	651	139.8 (112.5–171.6)	117.6	0.12
30–34 yr	41	588	69.7 (50.0–94.6)	86.9	0.17
35–39 yr	12	516	23.3 (12.0–40.6)	38.5	0.08
40–44 yr	2	446	4.5 (0.54–16.2)	8.0	0.62
15–44 yr	232	3674	63.1 (55.2–71.8)	67.2	0.36

* Values for women with inherited pathogenic mitochondrial DNA mutations are compared with the equivalent age- and era-weighted fertility rates (live births per 1000 women) and for the general population obtained from the U.K. Office for National Statistics.

Menopause Timing is Tied to Mitochondrial Dysfunction


nature genetics

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NATURE GENETICS | ARTICLE

日本語要約

Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways

Lisette Stolk, John R B Perry, Daniel I Chasman, Chunyan He, Massimo Mangino, Patrick Sulem, Maja Barbalic, Linda Broer, Enda M Byrne, Florian Ernst, Tõnu Esko, Nora Franceschini, Daniel F Gudbjartsson, Jouke-Jan Hottenga, Peter Kraft, Patrick F McArdle, Eleonora Porcu, So-Youn Shin, Albert V Smith, Sophie van Wingerden, Guangju Zhai, Wei V Zhuang, Eva Albrecht, Behrooz Z Alizadeh, Thor Aspelund + et al.

Affiliations | Contributions | Corresponding authors

Nature Genetics 44, 260–268 (2012) | doi:10.1038/ng.1051

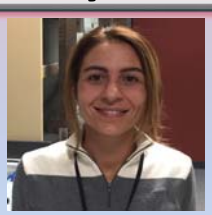
Received 21 July 2011 | Accepted 02 December 2011 | Published online 22 January 2012

- Meta-analysis of 22 genome-wide association studies (GWAS) in 38,968 women of European descent, with replication in up to 14,435 women.
- Gene-set enrichment pathway analyses using the full GWAS data set identified exoDNase, NF-κB signaling and mitochondrial dysfunction as biological processes related to timing of menopause

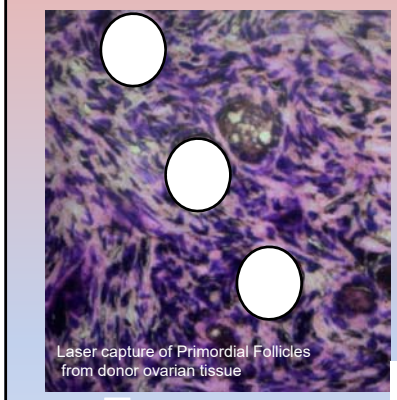
Summary

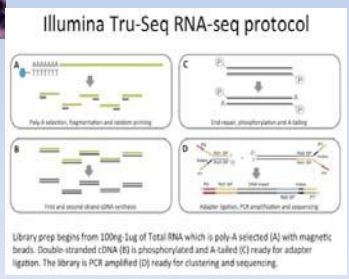
- Indirect evidence in human and some direct evidence in animals support essential role for intact mitochondrial function in oocyte health.
- Because mitochondria plays a multitude of functions in cell viability, the impact of mitochondrial dysfunction may not just be through reduced energy production.
- Direct evidence that mitochondrial function declines with age in human oocytes is missing.

qRT-PCR Analysis Indicates Age-Induced Increase in Mitochondrial DNA Damage in Mouse Oocytes



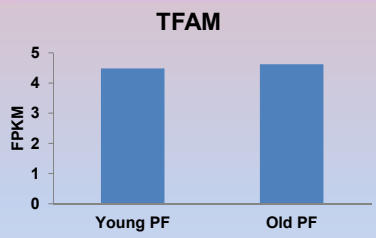
Bahar Kartal, PhD, Thesis Work, Oktay Lab

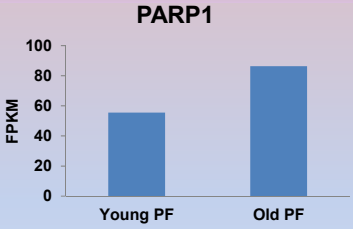


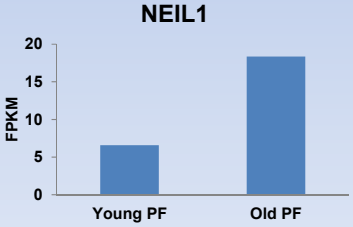


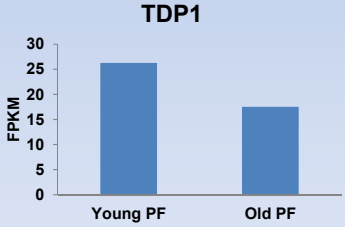
Library preparation for
Next generation sequencing

MtDNA Damage in Human Primordial Follicles: RNA Sequencing









Results are the average of 3 biological replicates

FPKM: Fragments per kilobase of transcript per million mapped reads

Proposed ART Treatments for Mitochondrial Disorders

- Donor cytoplasmic transfer — Abandoned
- Pronuclei transfer
- Spindle transfer

  Treatment for Mitochondrial Diseases
- Autologous mitochondria injections — Egg Quality Treatment?

Autologous Mitochondria from Oogonial Precursor Cells (OPCs)

- Described in cortical tissue of the ovary
- Unipotent, germline cells
- Because of slow self-renewing nature, proposed to be less predisposed to age-related mitochondrial damage

Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women

Yvonne A R White, Dori C Woods, Yasushi Takai, Osamu Ishihara, Hiroyuki Seki & Jonathan L Tilly

articles

Germline stem cells and follicular renewal in the postnatal mammalian ovary

Nature Med. 2004

Origin of germ cells and formation of new primary follicles in adult human ovaries

Antonin Bukovsky*[1,2], Michael R Caudle[1,2], Marta Svetlikova[1] and Nirmala B Upadhyaya[2]

Differentiation potential of germ line stem cells derived from the postnatal mouse ovary ☆

Jason Pacchiarotti[a], Chad Maki[a], Thomas Ramos[a], Joel Marh[a], Kyle Howerton[a], Jadelind Wong[a], Jane Pham[a], Sandra Anorve[a], Yung-Chiong Chow[b], Fariborz Izadyar[a]

Neo-oogenesis Has Been Refuted

Human Reproduction, Vol.26, No.8 pp. 2129–2139, 2011

human reproduction ORIGINAL ARTICLE *Reproductive biology*

No evidence for the presence of oogonia in the human ovary after their final clearance during the first two years of life

A.G. Byskov[1,*], P.E. Høyer[2], C. Yding Andersen[1], S.G. Kristensen[1], A. Jespersen[3], and K. Møllgård[3]

PNAS

Experimental evidence showing that no mitotically active female germline progenitors exist in postnatal mouse ovaries

Hua Zhang[a,1], Wenjing Zheng[a,1], Yan Shen[a], Deepak Adhikari[a], Hiroo Ueno[b,2], and Kui Liu[a,2]

[a]Department of Chemistry and Molecular Biology, University of Gothenburg, SE-405 30, Gothenburg, Sweden; and [b]Department of Stem Cell Pathology, Kansai Medical University, Moriguchi City, 570-8506, Osaka, Japan

Edited* by John J. Eppig, The Jackson Laboratory, Bar Harbor, ME, and approved June 5, 2012 (received for review April 19, 2012)

Fidelity of DDX4 Ab Questioned

Characterization of extracellular DDX4- or Ddx4-positive ovarian cells

Silvia F Hernandez[1,2,6], Nima A Vahidi[4,6], Solji Park[4], R Patrick Weitzel[5], John Tisdale[5], Bo R Rueda[1–3,7] & Erin F Wolff[4,5,7]

To the Editor:

A few groups[1–5] have now reported that ovarian-derived stem cells (OSCs; also known as oogonial stem cells or oocyte precursor cells) have been isolated from adult mouse[5] and rat[4] ovaries; these cells are able to undergo meiosis after transplantation back into recipient ovaries and give rise to offspring. These cells have also been isolated from human adult ovaries, where they can give rise to oocytes after xenotransplantation[2]. The marker used to isolate viable cells with germ cell characteristics, DDX4 in humans or Ddx4 in mice and rats (hereafter referred to collectively as DDX4/Ddx4), is controversial because it was historically considered to be exclusively an intracellular protein distributed in the cytoplasm of germ cells. However, DDX4/Ddx4 was reported to have a C-terminal domain that is expressed extracellularly, whereas the N terminus is expressed intracellularly[2,3]. DDX4/Ddx4 expression was reported in freshly isolated OSCs and after propagation for 18 months (mouse) and 4 months (human) in defined cultures by immunostaining, reverse-transcription PCR (RT-PCR), and fluorescence-activated cell sorting (FACS)[2]. Here we further characterize the expression of DDX4/Ddx4 in mouse, rhesus macaque and human ovarian cells using a polyclonal antibody specific to DDX4 (ab13840: Abcam, generously provided by Jonathan Tilly and purchased from Abcam).

By immunohistochemical analysis of paraffin-embedded tissue, we observed staining for DDX4/Ddx4 in the expected locations in

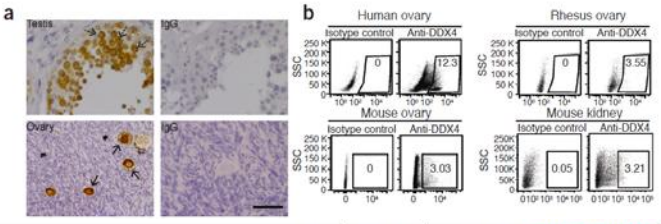


Figure 1 Cells isolated by FACS using a DDX4-specific antibody do not express DDX4/Ddx4. (**a**) Representative immunostaining in human testis ($n = 1$) and ovary ($n = 11$). Right, the IgG control. Scale bar, 50 μm. Dashed arrows show spermatogonia and spermatocytes; solid arrows show oocytes. (**b**) Representative ($n = 5$ pooled per each mouse sample, $n = 17$ for rhesus macaque, $n = 5$ for human) flow cytometry plots of cells from human, rhesus macaque and mouse

Debate on DDX4

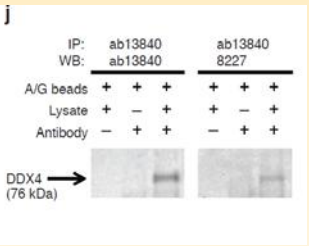
Woods and Tilly reply:

Zhang *et al.*[1] state that they were unable to repeat findings presented in our 2012 publication in *Nature Medicine* regarding the characterization of oogonial stem cells (OSCs) in mouse and human ovaries[2], using methods further detailed a year later[3]. Separately, Hernandez *et al.*[4] question the specificity of antibodies that target the C terminus of DDX4 (DEAD box polypeptide 4) to viably sort OSCs from adult mouse, monkey and human ovaries, as we reported[2,3]. Although these two correspondences focus on our work from 2012, DDX4-specific antibody–based sorting of OSCs was first published in 2009 by another laboratory[5]. A year before this publication, Richards *et al.*[6] reported isolation of viable germ cells from cultures of human embryonic stem cells using fluorescence-activated cell sorting (FACS) coupled with DDX4-specific antibodies. Our 2012 study therefore represents independent methodological verification of these two earlier reports.

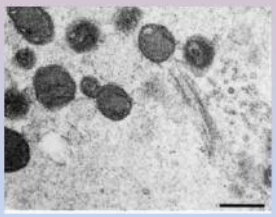
prepared by DDX4 antibody-based sorting. Because, however, th ovarian cells sorted and used by Zhang *et al.*[1] differ from the OSC others and we have isolated and described, it is not surprising tha their downstream endpoint analyses would not reproduce what ha been already reported using purified OSCs as starting material.

With that said, apparent differences between our findings an those of Zhang *et al.*[1] and Hernandez *et al.*[4] regarding the ability DDX4-specific antibodies to isolate OSCs highlight a fundamen tal issue raised by both sets of authors. Namely, is DDX4 entirel cytoplasmic in all germ cells at all stages of differentiation or d OSCs differ from other germ cells in their membrane localizatio of DDX4, thus making the protein available to be targeted in puri fication schemes involving magnetic-assisted cell sorting or FACS If the latter case is true, why have some groups been able to repea the DDX4-specific antibody–based approach to isolate OSCs whil others have failed?

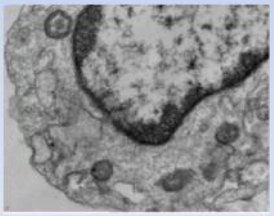
Species	Sample	Mass spectrometry: DDX4 homology
Mouse	Total ovarian cell lysate	Not detected
Mouse	Excised gel band from total ovarian cell lysate	Not detected
Mouse	IP, excised gel band from total ovarian cell lysate	Detected
Mouse	IP, excised gel band from total cultured OSC lysate	Detected



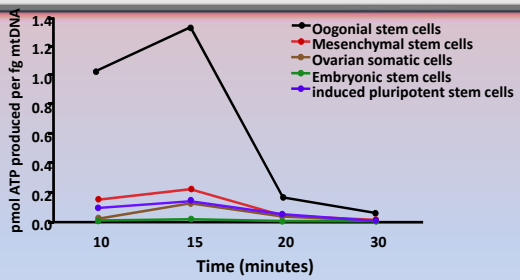
Indirect Evidence Supporting OPC May Have Healthy Functional Mitochondria

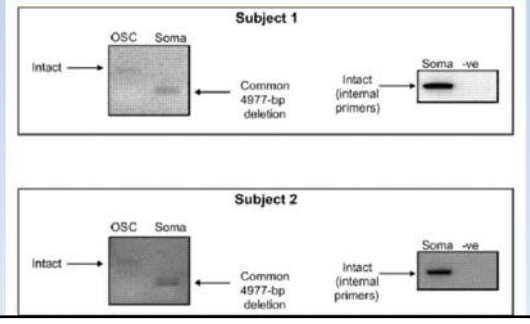


Human Oocyte

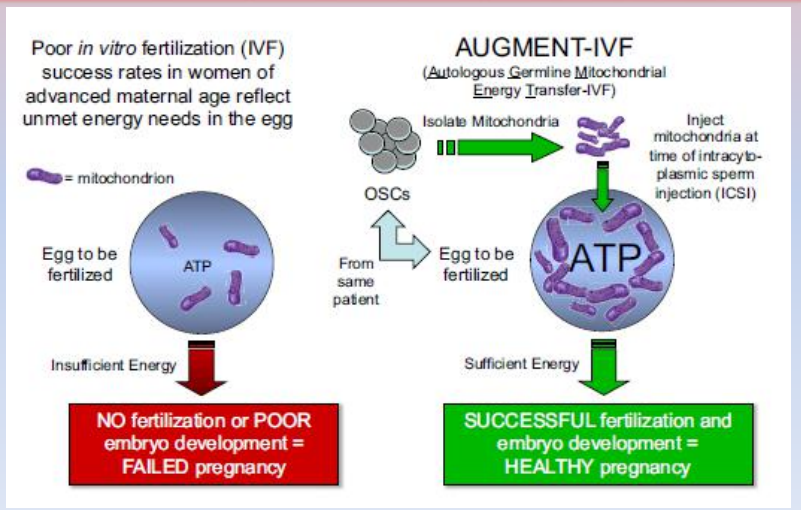


OPC

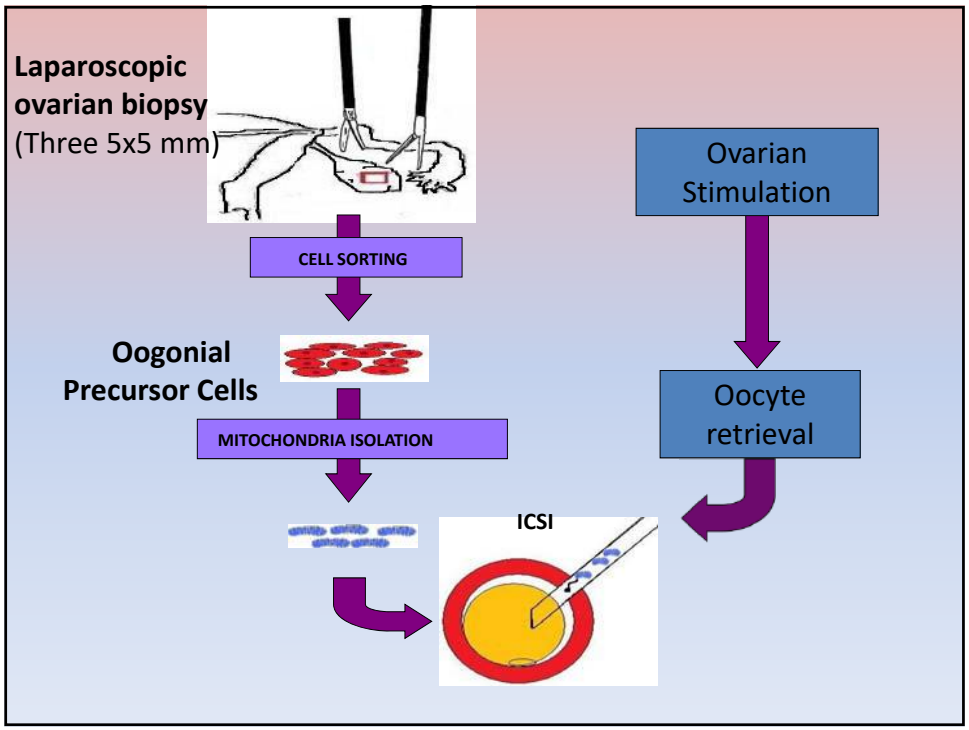




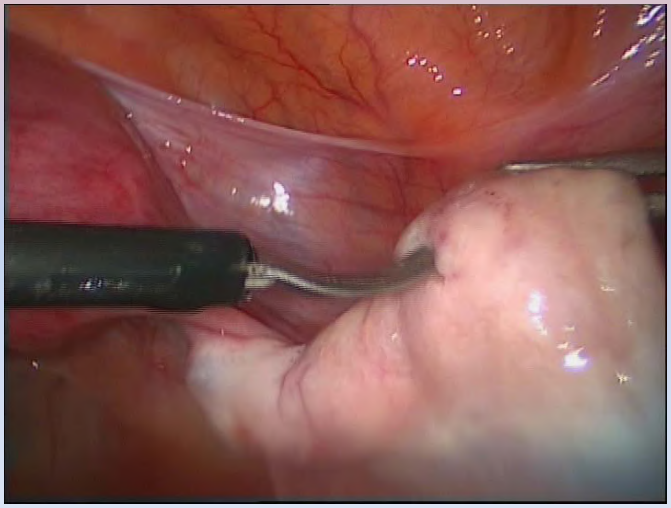
Autologous Germline Mitochondrial Energy Transfer



Tilly and Sinclair, Cell Metabolism 2013



Ovarian Tissue Harvesting for AMI/Augment



Original Article

Reproductive Sciences
1-6

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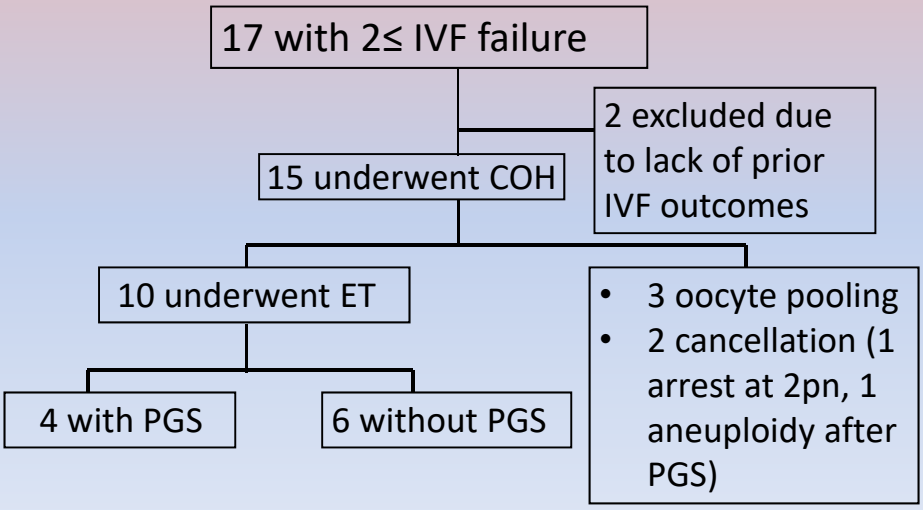
Oogonial Precursor Cell-Derived Autologous Mitochondria Injection to Improve Outcomes in Women With Multiple IVF Failures Due to Low Oocyte Quality: A Clinical Translation

Kutluk Oktay, MD, FACOG[1,2,3], Volkan Baltaci, MD[3], Murat Sonmezer, MD[4], Volkan Turan, MD[1,2], Evrim Unsal, PhD[3], Aysun Baltaci, MD[3], Suleyman Aktuna, PhD[3], and Fred Moy, PhD[1,2]

Abstract

Background: Mitochondrial dysfunction has been suggested as a major cause of age-induced decline in oocyte quality. In the past, donor oocyte cytoplasmic transfer showed some success but was abandoned due to the concerns with heteroplasmy. Recent studies indicated presence of oogonial precursor cells (OPCs) in the human ovary, which could be an autologous source of "healthy mitochondria." We sought to investigate the clinical efficacy of OPC-derived autologous mitochondrial injection (AMI) to improve oocyte quality in women with multiple in vitro fertilization (IVF) failures. **Methods:** The OPCs were isolated from laparoscopically obtained ovarian cortical pieces by cell sorting using a monoclonal anti-DDX antibody. They were then disrupted and mitochondria were isolated. Reconstituted mitochondria were injected into each oocyte during intracytoplasmic sperm injection. Paired comparisons were made between the first failed cycles and the post-AMI cycles. **Results:** Of the 15 women undergoing ovarian stimulation, 2 were canceled and 3 decided to pool oocytes for later AMI. In remaining 10 (mean age 34.7 ± 4.1), AMI significantly improved fertilization rates (49.7 ± 31.3 vs 78.3 ± 18.9; $P = .03$) with a trend for better embryo grades (2.3 ± 0.3 vs 3.1 ± 0.7; $P = .08$). Four of 10 women conceived after single frozen embryo transfer and 3 after confirmation of diploidy via aCGH (clinical pregnancy/embryo transfer = 4/10). **Conclusion**: These data show encouraging results for AMI in comparison to previous failed IVF cycles and a historical control group.

AMI Study-Flow chart

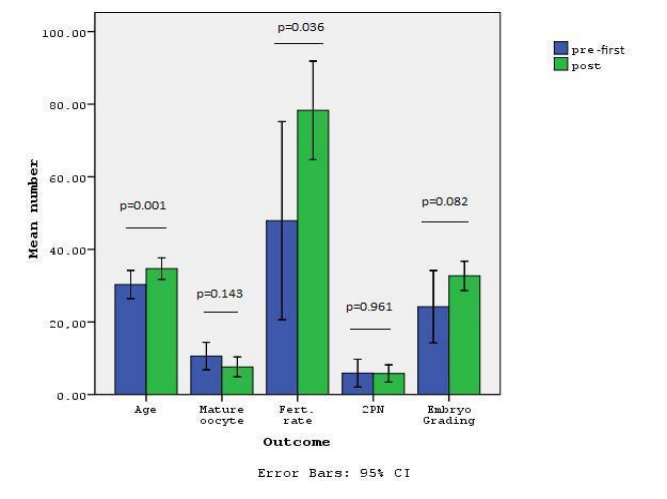


Oktay et al Reprod Sciences, 2015;22(12):1612-7

Characteristics of Patients

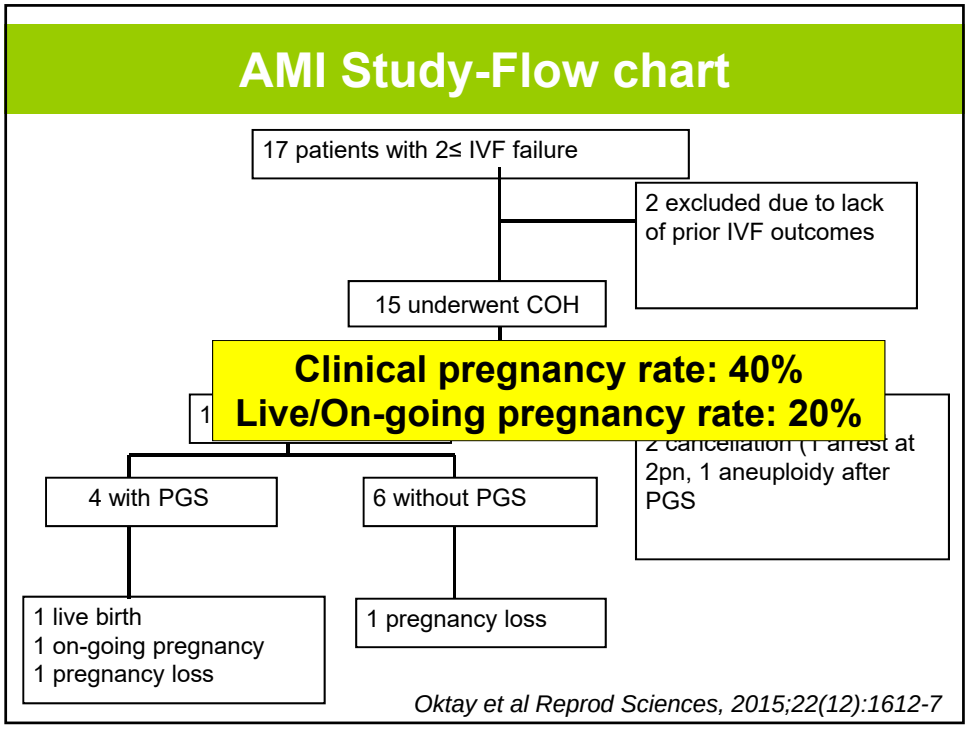
Age (years)	D2-3 FSH (IU/ml)	D2-3 E2 (pg/ml)	AMH (ng/ml)	N of IVF failures
27	6.1	57	2.5	6
31	7.2	39	3.5	3
32	5.9	36	NA	2
34	15.2	100	1.4	7
35	7.4	47	1.8	5
35	6.9	NA	NA	4
36	NA	NA	NA	2
36	5.5	134	1.1	3
40	9	NA	NA	7
41	5.6	39	0.7	3

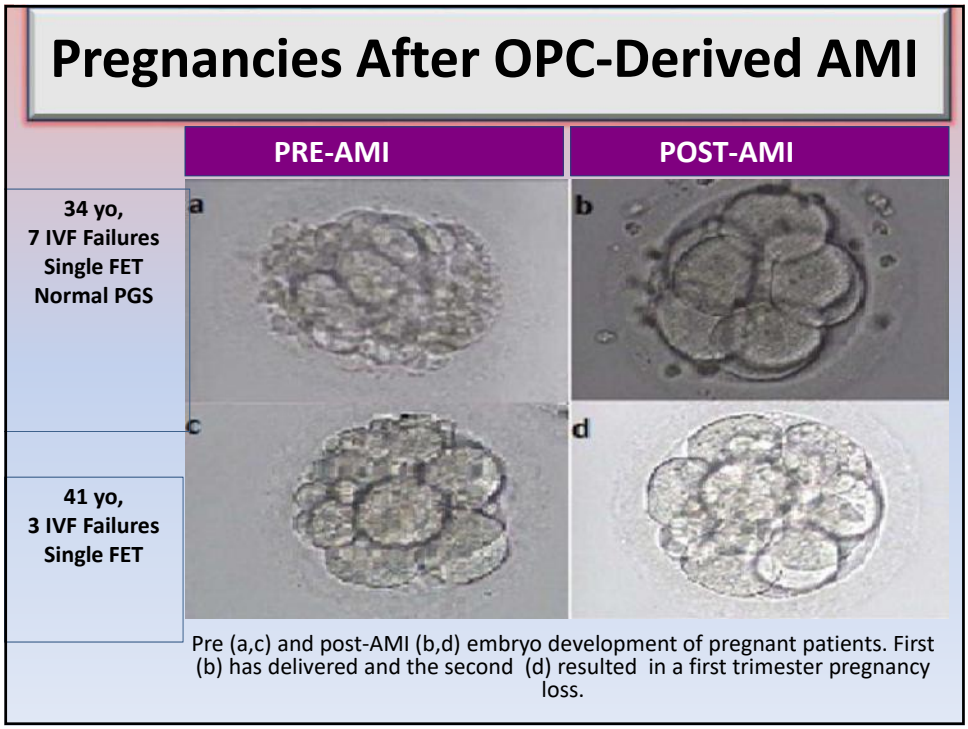
AMI Impact on Oocyte Quality



Pregnancy outcomes

Age (years)	Fresh/ Frozen	PGS (N of embryos)	N of embryos transferred	Pregnancy outcome
27	Fresh	NA	2	-
31	Frozen- thawed	2 normal out of 7	2	-
32	Frozen-thawed	4 normal out of 8	1	Pregnancy loss
34	Frozen-thawed	1 normal out of 3	1	Live birth
35	Fresh	NA	2	-
35	Fresh	NA	2	-
36	Frozen-thawed	1 normal out of 7	1	Ongoing Pregnancy
36	Frozen- thawed	NA	2	-
40	Fresh	NA	2	-
41	Frozen- thawed	NA	1	Pregnancy Loss


AMI Study-Flow chart
17 patients with 2≤ IVF failure
2 excluded due to lack of prior IVF outcomes
15 underwent COH
Clinical pregnancy rate: 40%
Live/On-going pregnancy rate: 20%
2 cancellation (1 arrest at 2pn, 1 aneuploidy after PGS
4 with PGS
6 without PGS
1 live birth
1 on-going pregnancy
1 pregnancy loss
1 pregnancy loss
Oktay et al Reprod Sciences, 2015;22(12):1612-7


Pregnancies After OPC-Derived AMI
PRE-AMI
POST-AMI
34 yo,
7 IVF Failures
Single FET
Normal PGS
41 yo,
3 IVF Failures
Single FET
a
b
c
d
Pre (a,c) and post-AMI (b,d) embryo development of pregnant patients. First (b) has delivered and the second (d) resulted in a first trimester pregnancy loss.

“Augment” Baby: Elanur

Comparison Of Augment with Age Matched Historical Control Group

	Augment (n=10)	Control (n=20)	P value
Mean Age	34.7±4.1	35.1 ±3.6	0.91
# Previous IVF Failures	4.3± 2.0	3.4± 0.5	0.53
# Embryos Transferred	1.6± 0.5	1.6± 0.5	1.00
Clin. Pregnancy Rate (%)	4/10 (40)	3/20 (15)	0.18
Live/Ong. Birth Rate (%)	2/10 (20)	1/20 (5)	0.25

Data From other Augment Centers

THE AUGMENT EXPERIENCE		
	TCART	FAKIH IVF
Total AUGMENT cycles initiated	34	60
Average cycles per patient	1	1
Total embryo transfers	26	34
Clinical pregnancy rate: • per cycle initiated • per embryo transfer	 12/34 (35%) 12/26 (46%)	 13/60 (22%) 13/34 (38%)
Ongoing clinical pregnancy and live birth rate: • per cycle initiated • per embryo transfer	 9/34 (26%)* 9/26 (35%)*	 11/60 (18%)** 11/34 (32%)**

*includes 1 live birth
**includes 2 live births (two sets of twins)
Note: All on-going clinical pregnancies reported here were continuously on-going pregnancies at the time of publication submission

Fakih MH, Shmoury MEl, Szeptycki J, dela Cruz DB, Lux C, et al. (2015) The AUGMENTSM Treatment: Physician Reported Outcomes of the Initial Global Patient Experience. JFIV Reprod Med Genet 3: 154. doi:10.4172/2375-4508.1000154

Data From other Augment Centers

	Patient History	Clinical Pregnancy Rate per Initiated AUGMENT Cycle	Clinical Pregnancy Rate per AUGMENT Embryo Transfer	Ongoing Clinical Pregnancy Rate/ Live Birth Rate per Initiated AUGMENT Cycle	Ongoing Clinical Pregnancy/ Live Birth Rate per AUGMENT Embryo Transfer
Canada	• Average age: 36.0 • 1-5 prior IVF cycles	35%*	46%*	26%	35%
United Arab Emirates	• Average age: 37.3 • 1-16 prior IVF cycles	22%	38%	18%	32%

- No Control group
- Patients with single IVF failure treated

Fakih MH, Shmoury MEl, Szeptycki J, dela Cruz DB, Lux C, et al. (2015) The AUGMENTSM Treatment: Physician Reported Outcomes of the Initial Global Patient Experience. JFIV Reprod Med Genet 3: 154. doi:10.4172/2375-4508.1000154

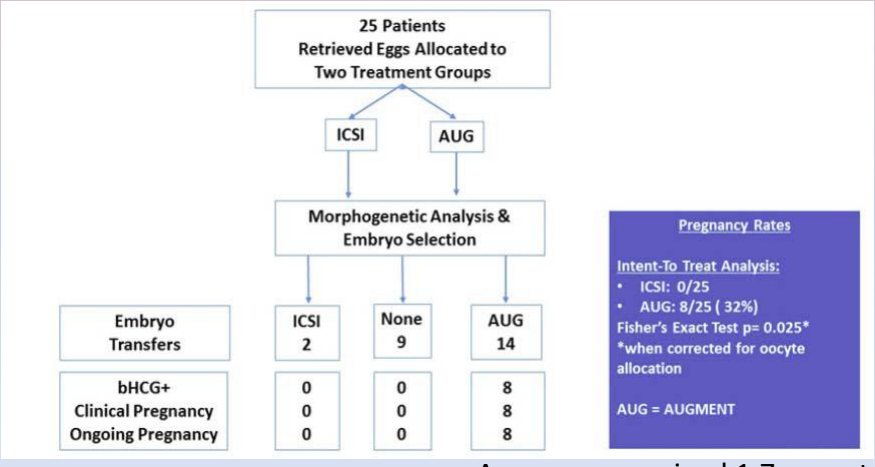
Augment: Lack of Livebirths > Age 40

Age (years)	# of Patients	# bHCG +	# Clinical Pregnancies	# Ongoing Clinical Pregnancies
20-30	5	3*	3*	2*
31-35	10	7*	6*	6*
36-40	14	5	3	1
41-45	5	-	-	-
46-48	-	-	-	-
Totals	34	15	12	9

*Includes one pregnancy each from a subsequent frozen embryo transfer

	FAKIH IVF			
Age (years)	# of Patients	# bHCG +	# Clinical Pregnancies	# Ongoing Clinical Pregnancies
20-30	5	3	2	2
31-35	12	3	3	3
36-40	28	7	7	5
41-45	10	2	1	1
46-48	4	-	-	-
Totals	59	15	13	11

Data From other Augment Centers



Fakih MH,, et al. (2015) JFIV Reprod Med Genet 3: 154. doi:10.4172/2375-4508.1000154

- Aug group received 1.7x oocytes
- Non-blinded or randomized
- No IRB; "clinical experience"

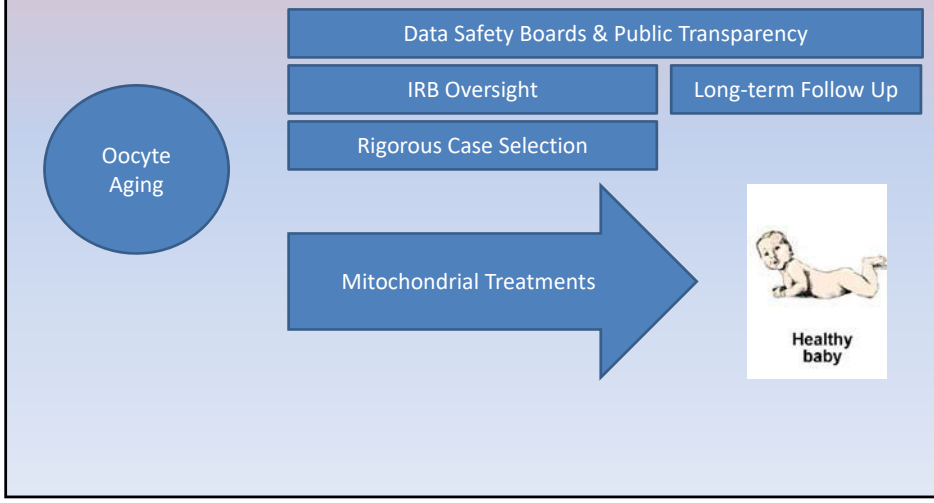
Sumary & Conclusions

- Initial non-randomized studies suggest some improvement in fertilization, embryo quality and possibly pregnancy rates < age 40 with OPC-derived mitochondria injection.
- Specifity of these improvements cannot be proven from the current data
- Further prospective-randomized data are needed before this treatment can be considered effective and safe.

Future Work

- Comparative data on mitochondrial health: OPC-derived vs. aged oocyte
- Better quantification and correlation with outcome of mitochondria numbers placed in the oocyte
- Randomized-blinded study:
  - Straight randomization of oocytes vs. patients
  - Cross-over design?
- A registry of children born from “Augment”

Mitochondrial Treatments: There is a Responsible Way



- **Laboratory of Molecular Reproduction & Fertility Preservation**
  - Shiny Titus, PhD
  - Fred Moy, PhD (Biostat)
  - Enes Taylan, MD
  - Yodo Sugishita, MD, PhD
  - Robert Stobezki, PhD
  - Tai Kawahara, MD

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Extra-Mural Collaborators:

✓ Sumanta Goswami, PhD, *(Yeshiva Univ/AECOM)*

✓ Maura Dickler, MD & Mark Robson,MD, *(Memorial Sloan Kettering Cancer Center)*

- **Innovation Institute for Fertility Preservation**
  - Kutluk Oktay, MD, PhD
  - Enes Taylan, MD
  - Giuliano Bedoschi, MD
  - Allison Rosen, PhD
  - Anitra Miraglia

❖ **Past Fellows:**

Fernanda Pacheco, MD
Biran Musul, B.Sc.
Youn Chung, MD
Volkan Emirdar, MD
Jhansi Reddy, MD
Kyungah Jeong, MD
Volkan Turan, MD
Enis Ozkaya, MD
Ozgur Oktem, MD

Kenny Rodriguez, MD, PhD
Elke Heytens, PhD
Aylin Cil, MD
Murat Sonmezer, MD
Sanghoon Lee, MD
IlginTurkcuoglu, MD
Margalida Sastri, MD
Sinan Ozkavukcu, MD, PhD
Heesuk Chae, MD

www.fertilitypreservation.org

Contact: info@fertilitypreservation.org

- Molecular Reproduction & Fertility Preservation Laboratory at NYMC:
  - Shiny Titus, PhD
  - Fred Moy, PhD (Biostat)
  - Robert Stobezki, PhD
  - Biran Musul, B.Sc.
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Evrim Unsal, PhD, & Volkan Baltaci, MD, PhD, Yeni Yuzyil Univ, Istanbul, TR

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  - Aylin Cil, MD
  - Murat Sonmezer, MD
  - Sanghoon Lee, MD
  - Ozgur Oktem, MD
  - Kenny Rodriguez, MD, PhD
  - Elke Heytens, PhD
  - IlginTurkcuoglu, MD
  - Margalida Sastri, MD
  - Sinan Ozkavukcu, MD, PhD

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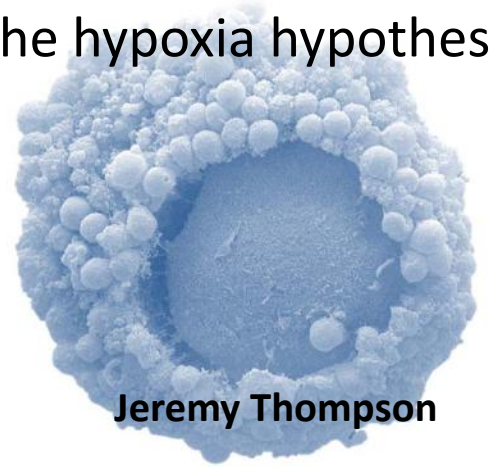
1. Bentov Y, Yavorska T, Esfandiari N, Jurisicova A, Casper RF. The contribution of mitochondrial function to Reproductive aging. J Assist Reprod Genet. 2011;28(9):773-783. 2. Keefe DL, Niven-Fairchild T, Powell S, Buradagunta S. Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. Fertil Steril. 1995;64(3):577-583. 3. Liu L, Trimarchi JR, Smith PJ, Keefe DL. Mitochondrial dysfunction leads to telomere attrition and genomic instability. Aging Cell. 2002;1(1):40-46. 4. Eichenlaub-Ritter U, Wieczorek M, Luke S, Seidel T. Age related changes in mitochondrial function and new approaches to study redox regulation in mammalian oocytes in response to age or maturation conditions. Mitochondrion. 2011;11(5):783-796. 5. Tilly JL, Sinclair DA. Germline energetics, aging, and female infertility. Cell Metab. 2013;17(6):838-850. 6. Oktay K. Ovarian tissue cryopreservation and transplantation: preliminary findings and implications for cancer patients. Hum Reprod Update. 2001;7(6):526-534. 7. Fujioka T, Yasuchika K, Nakamura Y, Nakatsuji N, Suemori H. A simple and efficient cryopreservation method for primate embryonic stem cells. Int J Dev Biol. 2004;48(10):1149-1154. 8. World Health Organization. Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva, Switzerland: WHO; 2010. 9. Henkel R. Sperm preparation: state-of-the-art–physiological aspects and application of advanced sperm preparation methods. Asian J Androl. 2012;14(2):260-269. 10. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature. 2004;428(6979):145-150. 11. Pacchiarotti J1, Maki C, Ramos T, et al. Differentiation potential of germ line stem cells derived from the postnatal mouse ovary. Differentiation. 2010;79(3):159-170. 12. Bukovsky A, Caudle MR, Svetlikova M, Upadhyaya NB. Origin of germ cells and formation of new primary follicles in adult human ovaries. Reprod Biol Endocrinol. 2004;2:20. 13. Tilly J, Johnson J; Compositions comprising female germline stem cells and methods of use thereof. US patent number US7955846 B2, Jun 7 2011. 14. Van Blerkom J. Mitochondrial function in the human oocyte and embryo and their role in developmental competence. Mitochondrion. 2011;11(5):797-813.

Oocyte competence: The hypoxia hypothesis



Jeremy Thompson

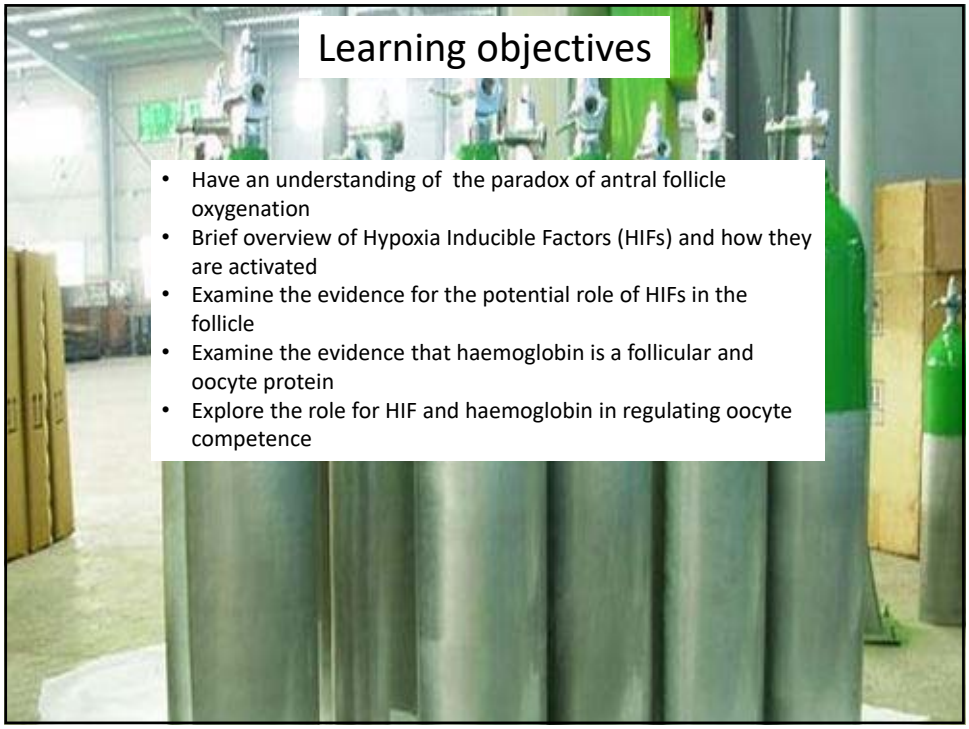
Robinson Research Institute
Healthy children for life.

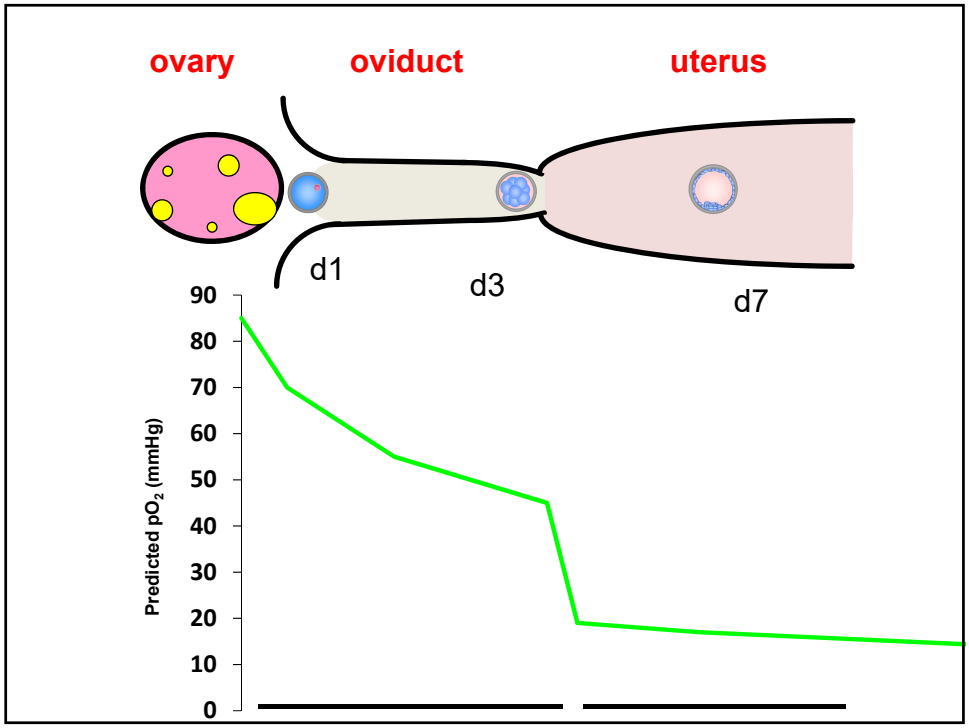
The University of Adelaide

Centre for Nanoscale BioPhotonics
ARC Centre of Excellence

Conflicts of Interest Statement

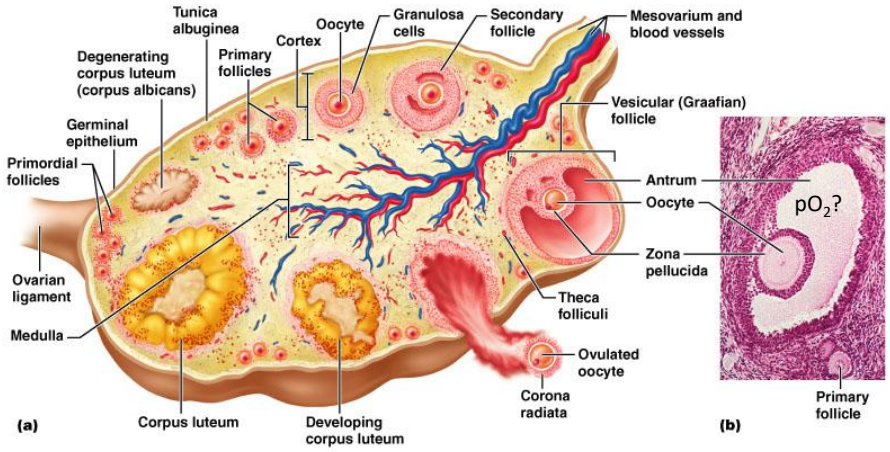
The University of Adelaide receives consultation funds from Cook Medical LLC for use by Jeremy Thompson for research expenditure.


Learning objectives
• Have an understanding of the paradox of antral follicle oxygenation
• Brief overview of Hypoxia Inducible Factors (HIFs) and how they are activated
• Examine the evidence for the potential role of HIFs in the follicle
• Examine the evidence that haemoglobin is a follicular and oocyte protein
• Explore the role for HIF and haemoglobin in regulating oocyte competence


ovary
oviduct
uterus
d1
d3
d7
90
80
70
60
50
40
30
20
10
0
Predicted pO2 (mmHg)

Is the antral follicle hypoxic?

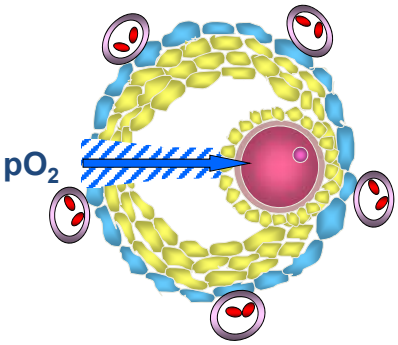
8-10%



Oocytes need O_2

- Totally dependent on oxidative phosphorylation – little glycolytic capacity
  - Biggers et al (1967) PNAS
  - Thomson (1967) J Exp Zool
- Low follicular O_2 associated with poor development
  - Van Blerkom et al (1997) Hum Reprod

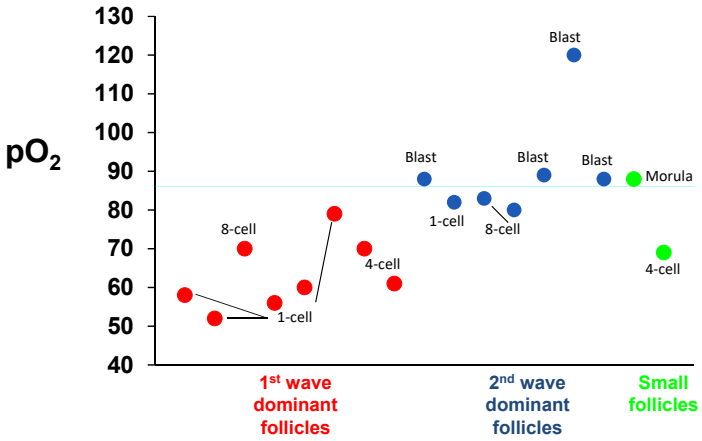
Follicular pO_2



↓pO_2 <2.5% O_2 associated with:
↓developmental competence
↑ meiotic spindle defects

Van Blerkom et al Hum Reprod (1997)

Intrafollicular pO_2 determines developmental competency



Berg et al (unpublished)

REPRODUCTION
RESEARCH

Mathematical modelling of oxygen transport-limited follicle growth

G P Redding[1], J E Bronlund[1] and A L Hart[2]

[1]Institute of Technology and Engineering, Massey University, Private Bag 11222, Palmerston North 4410, New Zealand [2]Food and Health, AgResearch Grasslands, Private Bag 11008, Palmerston North 4410, New Zealand

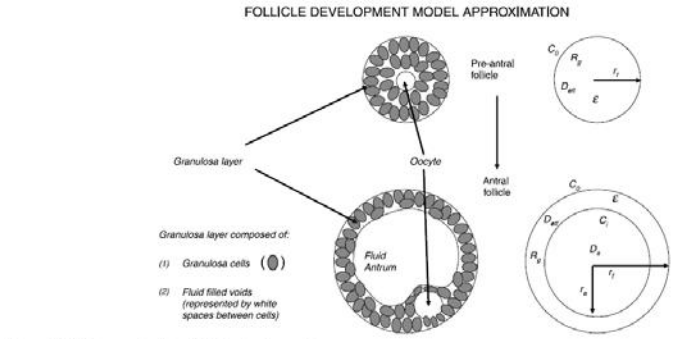


Figure 1 Model approximation of follicle development.

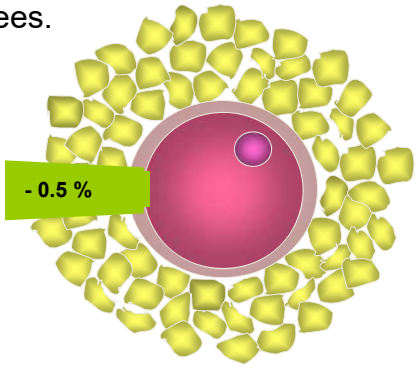
Redding et al (2007) Reproduction

Mathematical modelling

Cumulus cells consume relatively small amounts of oxygen.

The follicular fluid oxygen concentration is close to what the oocyte sees.

2 – 20% O_2 **- 0.5 %**



Clark et al (2006) Reproduction

Metabolism of the Cumulus-oocyte Complex

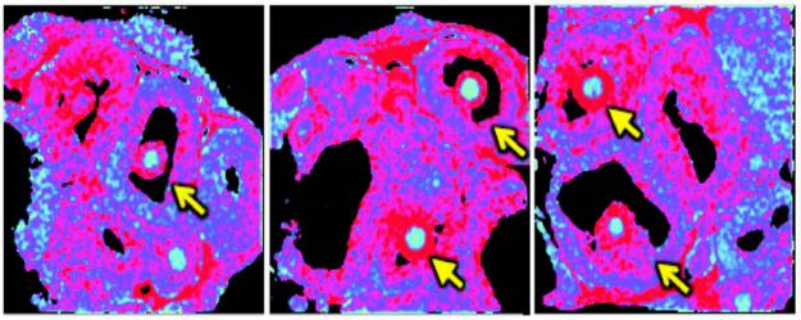
BIOLOGY OF REPRODUCTION (2016) 95(6):129, 1–12
Published online before print 28 September 2016.
DOI 10.1095/biolreprod.116.142141

Spatial Characterization of Bioenergetics and Metabolism of Primordial to Preovulatory Follicles in Whole Ex Vivo Murine Ovary[1]

Rachel Cinco,[3] Michelle A. Digman,[4,5] Enrico Gratton,[4,5] and Ulrike Luderer[2,3,6,7]

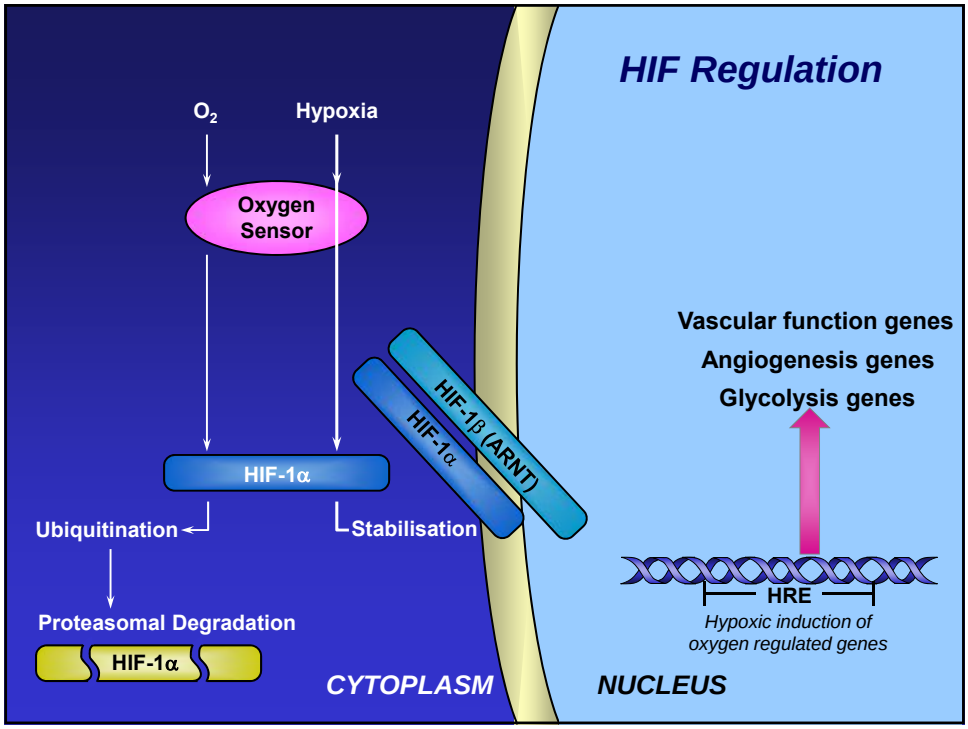
More Bound NADH — More Free NADH

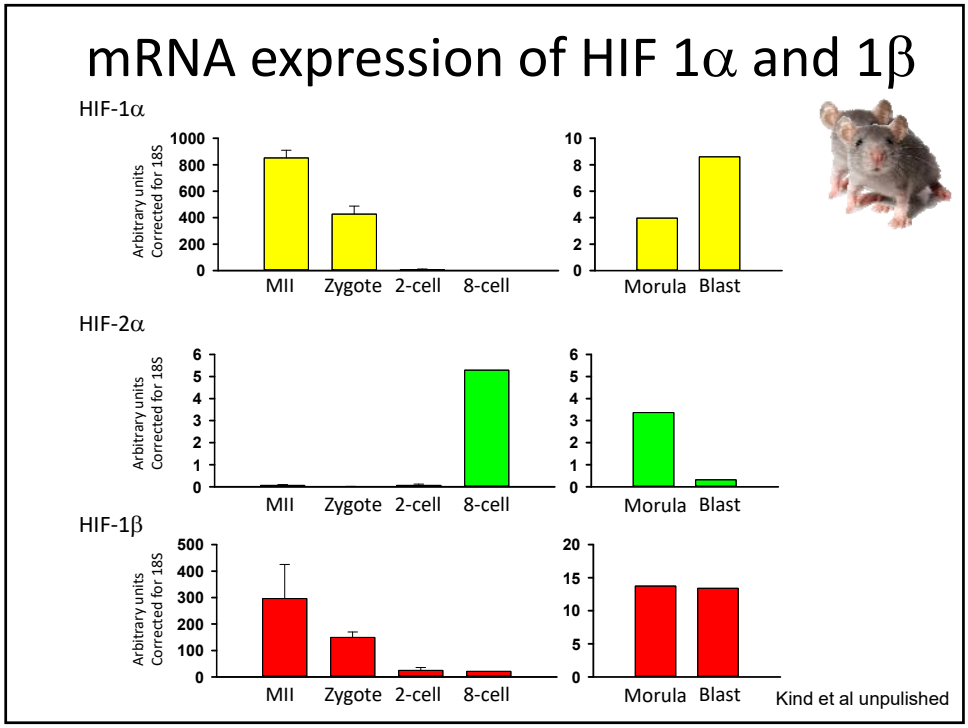
Bound NADH = Oxidative metabolism
Free NADH = Glycolytic metabolism

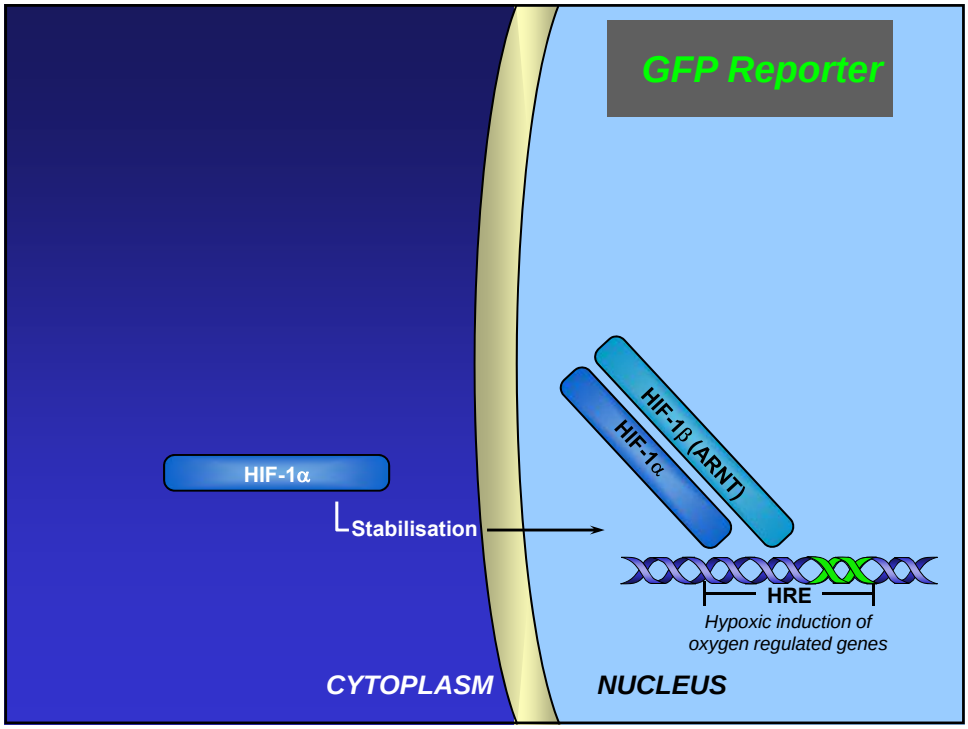


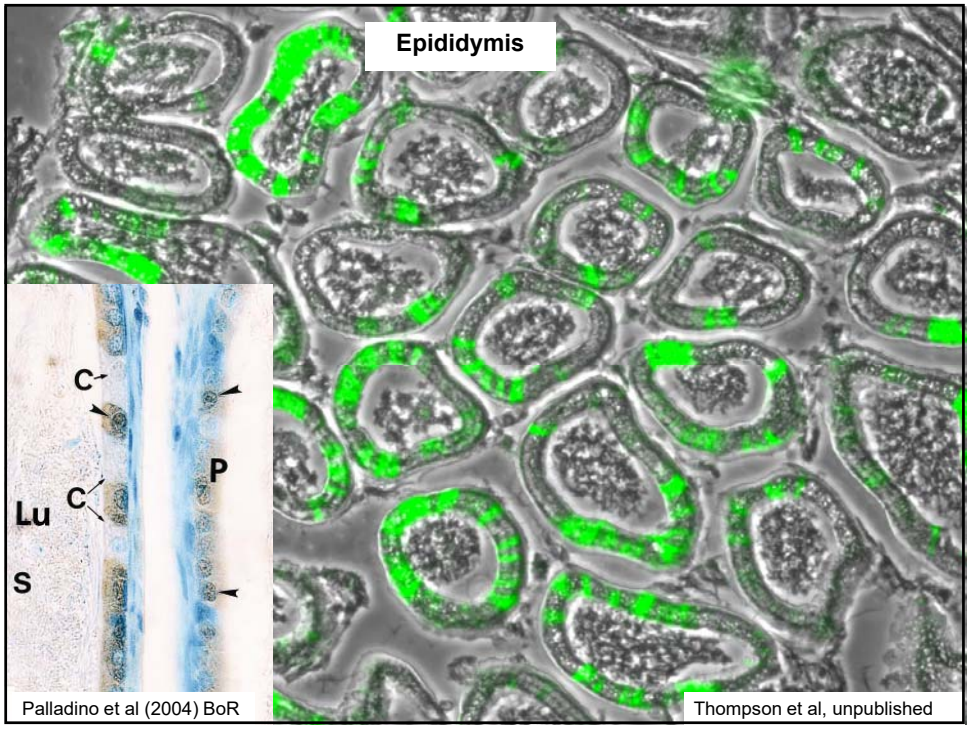
Oxygen-regulated gene expression

- Hypoxia inducible factors (HIFs)
- bHLH-PAS transcription factors
  - Heterodimeric proteins
  - HIF-1, -2, -3α
  - HIF-1β (ARNT)
  - Binding to HRE's (5'-RCGTG)
- Activates transcription of genes involved in response to low oxygen

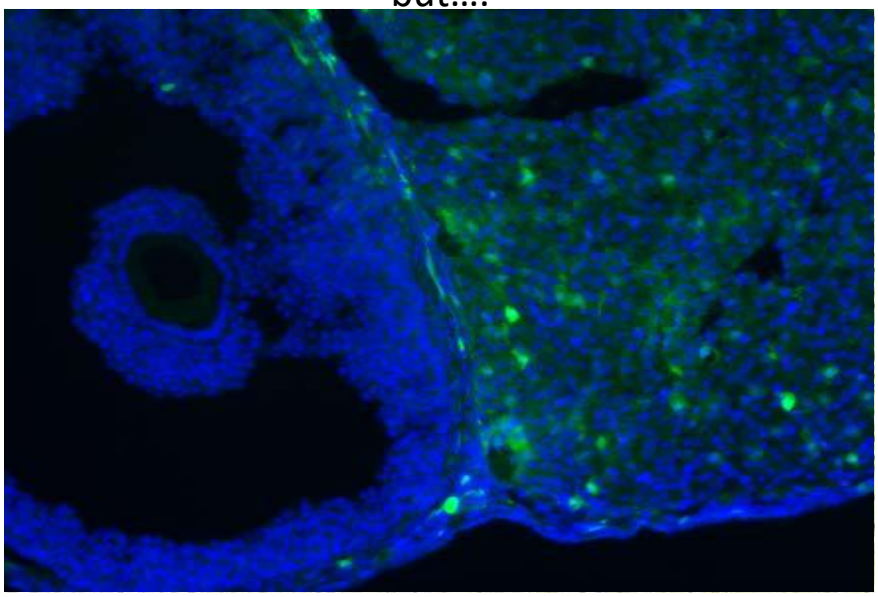

HIF Regulation
O2
Hypoxia
Oxygen Sensor
HIF-1α
HIF-1β (ARNT)
HIF-1α
Ubiquitination
Stabilisation
Proteasomal Degradation
HIF-1α
CYTOPLASM
Vascular function genes
Angiogenesis genes
Glycolysis genes
HRE
Hypoxic induction of oxygen regulated genes
NUCLEUS


mRNA expression of HIF 1α and 1β
HIF-1α
HIF-2α
HIF-1β
Arbitrary units Corrected for 18S
MII
Zygote
2-cell
8-cell
Morula
Blast
Kind et al unpulished


GFP Reporter
HIF-1α
Stabilisation
HIF-1β (ARNT)
HIF-1α
HRE
Hypoxic induction of
oxygen regulated genes
CYTOPLASM
NUCLEUS


Epididymis
C
C
Lu
S
P
Palladino et al (2004) BoR
Thompson et al, unpublished

HIF1α protein is found in both follicle and CL, but….

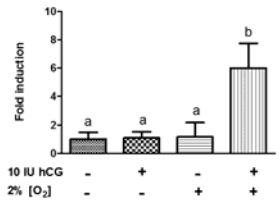


eCG/hCG treated SCID mouse ovary: mAB HIF1α

Tam et al (2010) Mol Cell Endo

Is HIF regulated by gonadotrophins?

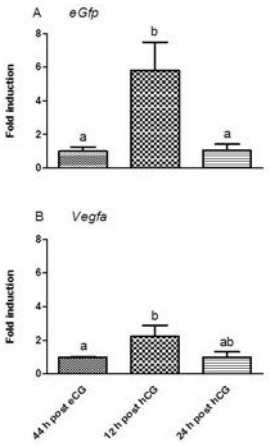
- FSH
  - No evidence that FSH influences HIF1
- LH/hCG
  - Combination of hCG and low O_2 stabilises HIF1 protein

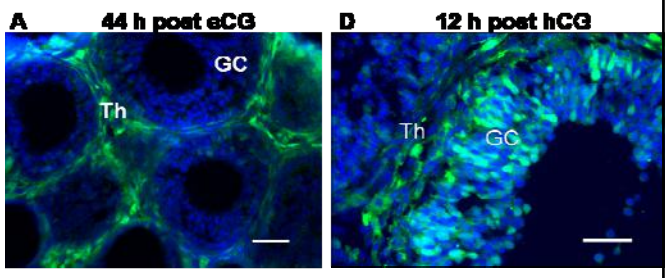


  - Temporally regulated by LH *in vivo*

Tam et al (2010) Mol Cell Endo

GFP increases following LH surge



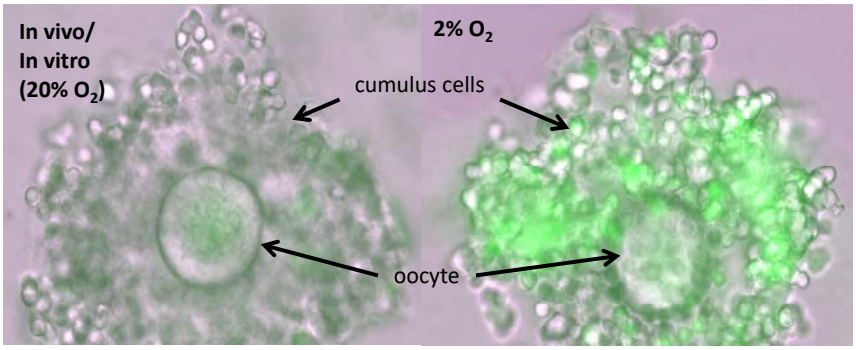


Tam et al (2010) Mol Cell Endo

The paradox of HIF activity in the ovary

- Large antral ovarian follicles are supposed to be hypoxic, or at least close to hypoxia?
  - No measurable HIF-induced response in both pre-antral and antral
- The developing corpus luteum is HIF active
  - Does this mean the developing CL is hypoxic?
  - CL formation is dependent on VEGF
- Is there a further regulatory mechanism that could help explain this paradox?

Low O_2 IVM turns on HIF



- Low O_2 IVM increases mRNA of many classic HIF1/2 regulated genes in cumulus cells, but not *in vivo*

Banwell et al unpublished
Kind et al (2014) RFD

CSIRO PUBLISHING
Reproduction, Fertility and Development
http://dx.doi.org/10.1071/RD13305

Microarray analysis of mRNA from cumulus cells following *in vivo* or *in vitro* maturation of mouse cumulus–oocyte complexes

Karen L. Kind[A,B], *Kelly M. Banwell*[A], *Kathryn M. Gebhardt*[A], *Anne Macpherson*[A], *Ashley Gauld*[A], *Darryl L. Russell*[A] *and Jeremy G. Thompson*[A,C]

Table 2. List of selected genes differentially regulated, as detected by microarray analysis, in cumulus cells derived from *in vitro*- compared with *in vivo*-matured cumulus–oocyte complexes

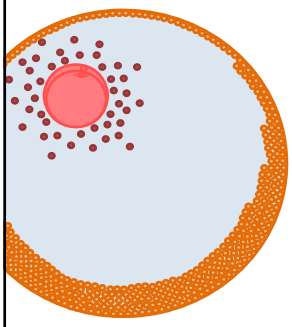
Genes with a positive fold change (fold change = 2^{M}) were found to be higher in cumulus cells derived from *in vitro*-matured (IVM) oocytes, whereas genes with a negative fold change were higher in cumulus cells derived from *in vivo*-matured (IVV) oocytes. BMP, bone morphogenetic protein; TGF-β, transforming growth factor-β

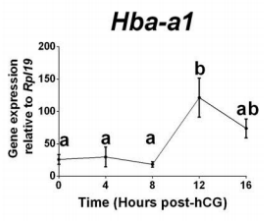
Gene name	Accession no.	M-value	*P*-value	Gene function (biological process)
Phosphodiesterase 7B (*Pde7b*)	NM_013875	7.5	0.00016	cAMP phosphodiesterase activity
Mitogen-activated protein kinase 10 (*Mapk10*)	NM_009158	6.6	0.00015	ATP binding, kinase activity
Insulin-like growth factor binding protein 5 (*Igfbp5*)	NM_010518	6.5	0.0003	Growth factor binding
Bone morphogenetic protein 4 (*Bmp4*)	BC013459	5.7	0.0012	BMP receptor binding, growth factor activity
Growth arrest specific 6 (*Gas6*)		5.0	0.0015	Calcium ion binding, metal ion binding
Anti-Müllerian hormone type 2 receptor (*Amhr2*)	NM_144547	4.8	0.00042	Hormone binding, TGF-β activity
A disintegrin-like and metallopeptidase (*Adamts1*)	NM_009621	−4.1	0.00048	Heparin binding
LH/choriogonadotrophin receptor (*Lhcgr*)	NM_013582	−4.9	0.00064	ATPase binding, LH receptor activity
Pentraxin-related gene (*Ptx3*)	NM_008987	−5.2	0.00059	Inflammatory response
Hyaluronan synthase 2 (*Has2*)	NM_008216	−5.3	0.0008	Hyaluronan synthase activity
Interleukin-6 (*Il-6*)	NM_031168	−7.8	0.000067	Cytokine activity, growth factor activity
Betacellulin (*Btc*)	NM_007568	−7.2	0.0026	Growth factor activity
Amphiregulin (*Areg*)	NM_009704	−7.7	0.000068	Growth factor activity, cytokine activity
Epiregulin (*Ereg*)	NM_007950	−10.2	0.000021	Growth factor activity
Haemoglobin β, 2 (*Hbb,b2*)	NM_0033234	−10.9	0.000016	Gas transport
Haemoglobin α, 1 (*Hba,a1*)	NM_008218	−11.9	6.59E-07	Gas transport

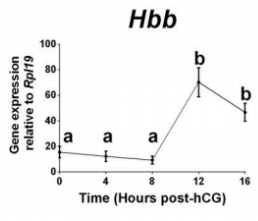
Kind et al (2013) RFD

Haemoglobin mRNA regulated in the mouse follicle

GRANULOSA CELLS



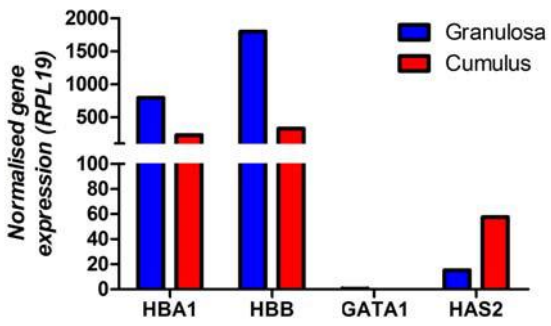


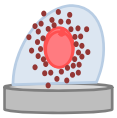


Brown et al (2015) BoR

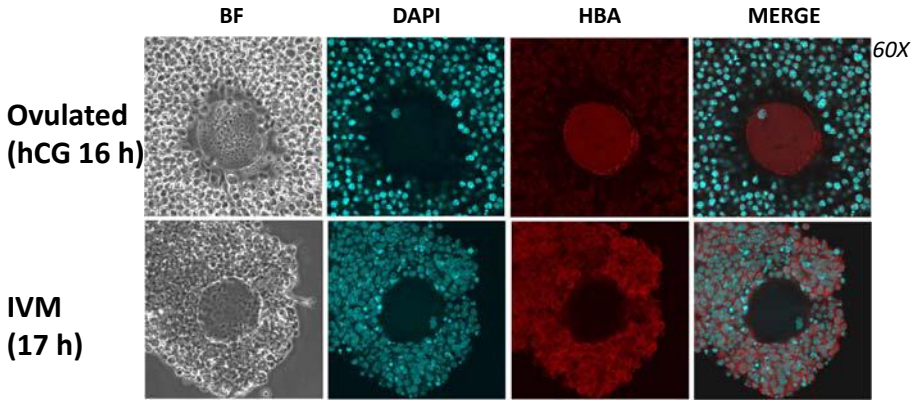
Human granulosa and cumulus cells

- Pooled human granulosa and cumulus cells collected from women undergoing ART.





Haemoglobin lost during IVM



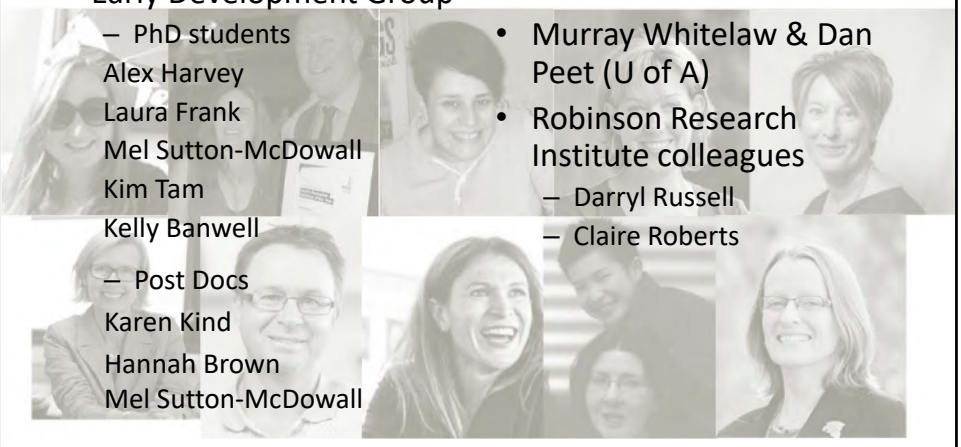
Protein aberrantly located in cumulus cells and lost from oocyte

Conclusions

- Follicular O_2 is:
  - Correlated to oocyte health
  - Difficult to measure
  - Oocyte most likely in a "hypoxic" range – but not hypoxic
- Hypoxia Inducible Factors in antral follicles
  - Do not appear to regulate pre-LH surge follicle growth
  - Regulates post-LH surge differentiation
- Haemoglobin present in follicular cells
  - Gas transport? – but which gas? Or other function?
- HIF and Haemoglobin
  - How does Hb interact with HIF, if at all?
  - Not present at all during IVM - what does this mean for oocyte *in vitro* maturation?
  - Present during embryo development? Yes!

Many people need to be thanked!

- Early Development Group
  - PhD students
    - Alex Harvey
    - Laura Frank
    - Mel Sutton-McDowall
    - Kim Tam
    - Kelly Banwell
  - Post Docs
    - Karen Kind
    - Hannah Brown
    - Mel Sutton-McDowall
- Murray Whitelaw & Dan Peet (U of A)
- Robinson Research Institute colleagues
  - Darryl Russell
  - Claire Roberts



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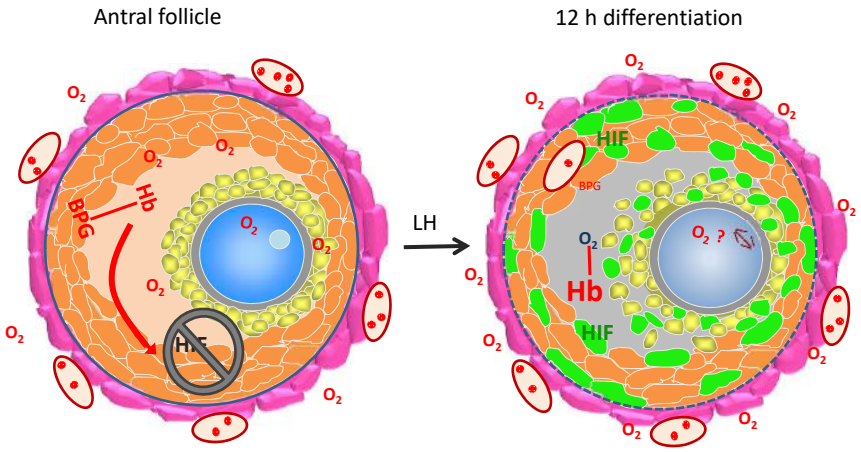
Centre for Nanoscale BioPhotonics
ARC CENTRE OF EXCELLENCE

IPAS
Institute for Photonics and Advanced Sensing

Current hypothesis



...RMING OF ANTRAL FOLLICLES TO ASSISTED REPRODUCTION: AN OOCYTE REJUVENATING THERAPY?

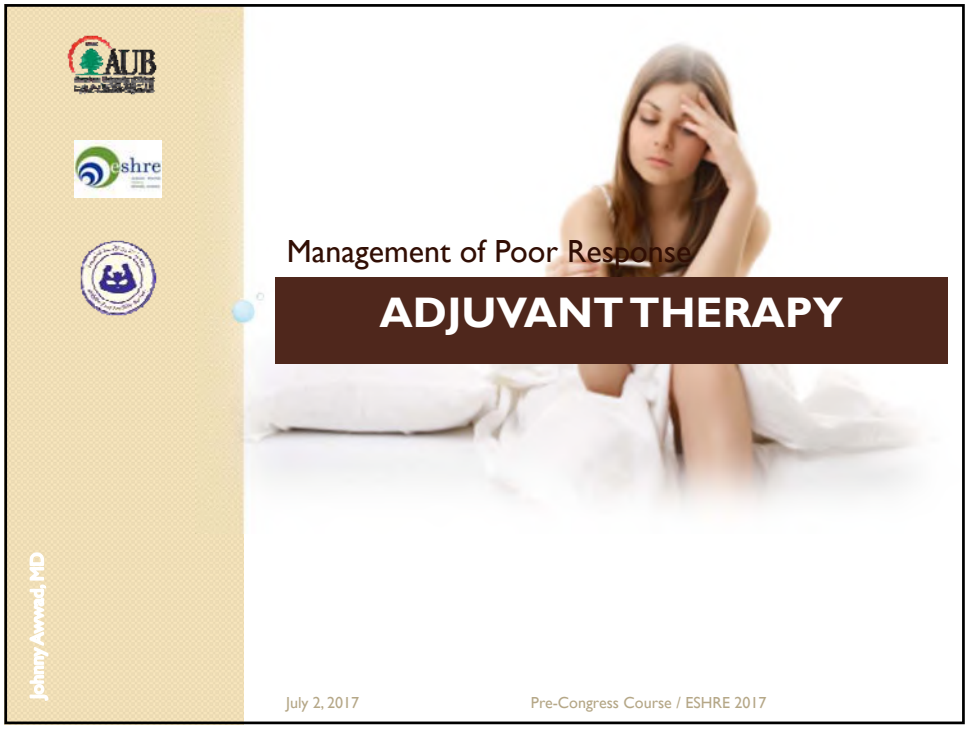
Johnny Awwad, MD
Professor of Obstetrics and Gynecology
Head, Division of Reproductive Endocrinology and Infertility
American University of Beirut Medical Center

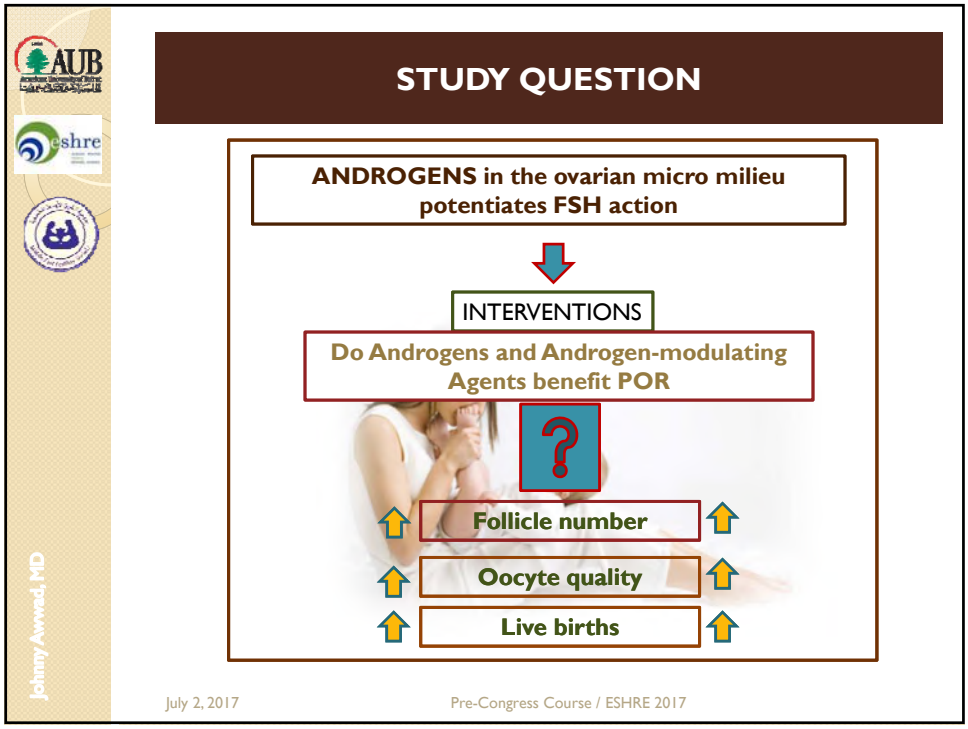
Johnny Awwad, MD

Non Disclosure Statement

NO CONFLICT OF INTEREST

Johnny Awwad, MD


Management of Poor Response
ADJUVANT THERAPY
Johnny Awwad, MD
July 2, 2017
Pre-Congress Course / ESHRE 2017


STUDY QUESTION
ANDROGENS in the ovarian micro milieu potentiates FSH action
INTERVENTIONS
Do Androgens and Androgen-modulating Agents benefit POR
Follicle number
Oocyte quality
Live births
Johnny Awwad, MD
July 2, 2017
Pre-Congress Course / ESHRE 2017

A 38 year old woman G0P0 was referred to you for primary infertility of 8 years duration.
AMH 0.9 ng/dl. CD3 FSH 13.0 IU/l. E2 45pg/ml. AFC 5.

She reports a previous ART cycle failure in which she received 300 IU daily dose of rec-FSH, developed 2 pre-ovulatory follicles and produced 2 oocytes.

She is planning her third ART cycle, and has heard about androgen therapy. To maximize benefit, you propose testosterone transdermal patches should be started:

A. On the first day of ovarian stimulation and until hCG.
B. Two weeks prior to ovarian stimulation and until hCG.
C. Six weeks prior to ovarian stimulation and until hCG.
D. Twelve weeks prior to ovarian stimulation and until hCG.

Reproductive BioMedicine Online (2012) 25, 450–459
www.sciencedirect.com
www.rbmonline.com
ELSEVIER

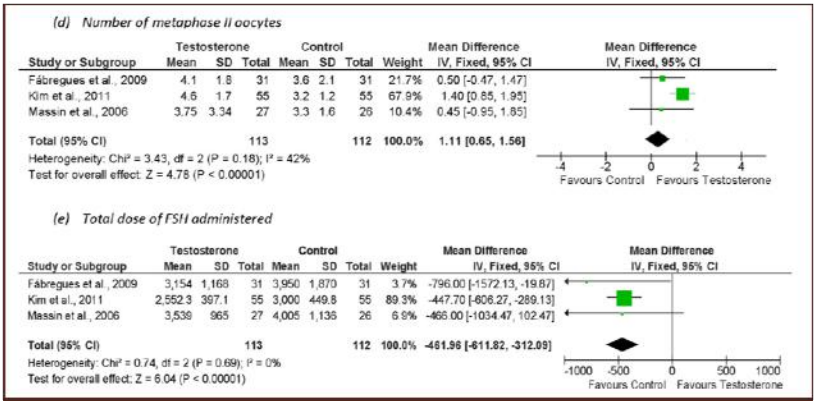
REVIEW

Effects of transdermal testosterone in poor responders undergoing IVF: systematic review and meta-analysis

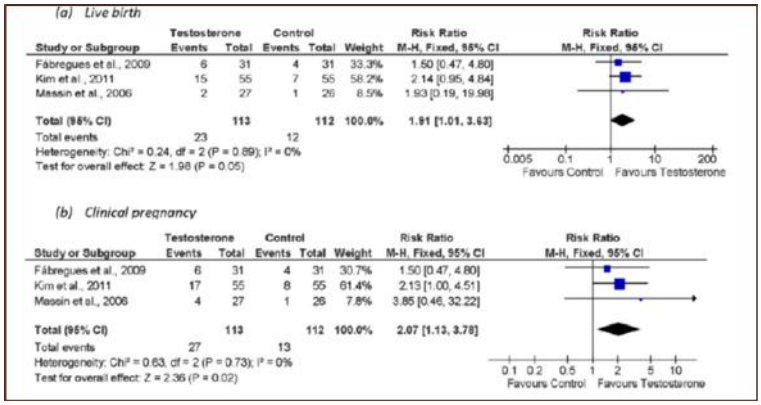
Mireia González-Comadran [a,b], Montserrat Durán [b], Ivan Solà [c,d], Francisco Fábregues [e], Ramón Carreras [a,f], Miguel A Checa [a,f,g,*]

Johnny Awwad, MD
July 2, 2017 Pre-Congress Course / ESHRE 2017

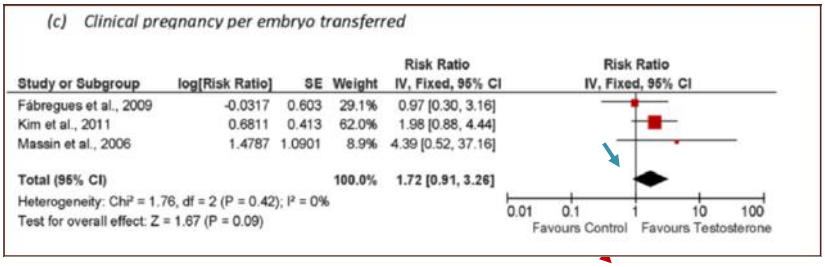
Testosterone-treated women achieved significantly higher live birth rate, clinical pregnancy rate and required significantly lower doses of FSH.



Testosterone-treated women achieved significantly higher live birth rate, clinical pregnancy rate and required significantly lower doses of FSH.



When the clinical pregnancy rate was adjusted per embryo transferred, differences among the two groups were not statistically significant.



Cochrane Library

Cochrane Database of Systematic Reviews

Cochrane Database of Systematic Reviews 2015, Issue 11. Art. No.: CD009749.
DOI: 10.1002/14651858.CD009749.pub2.

Androgens (dehydroepiandrosterone or testosterone) for women undergoing assisted reproduction (Review)

Nagels HE, Rishworth JR, Siristatidis CS, Kroon B

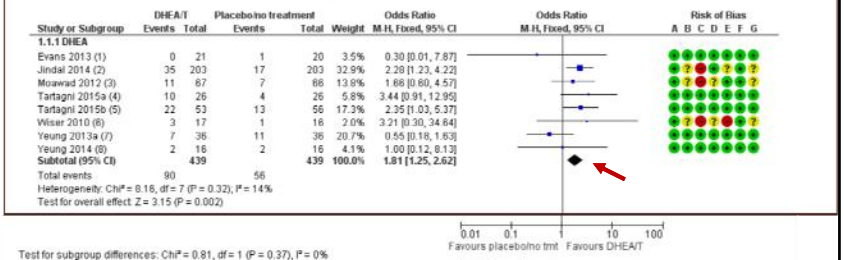
DHEA or testosterone versus placebo/no treatment for women undergoing assisted reproduction

Population: Women undergoing assisted reproduction
Settings: Outpatient clinic
Intervention: DHEA or testosterone versus placebo/no treatment

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo/no treatment	DHEA or testosterone				
Live birth/ongoing pregnancy rate - DHEA	116 per 1000	192 per 1000 (141 to 256)	OR 1.81 (1.25 to 2.62)	878 (8 studies)	⊕⊕⊕○ moderate[1]	
Live birth/ongoing pregnancy rate - Testosterone	82 per 1000	188 per 1000 (104 to 317)	OR 2.6 (1.3 to 5.2)	345 (4 studies)	⊕⊕⊕○ moderate[1]	

When compared with placebo or no treatment, pre-treatment with DHEA was associated with higher live birth rates.

Figure 4. Forest plot of comparison: 1 DHEA or testosterone versus placebo/no treatment, outcome: 1.1 Live birth/ongoing pregnancy rate.



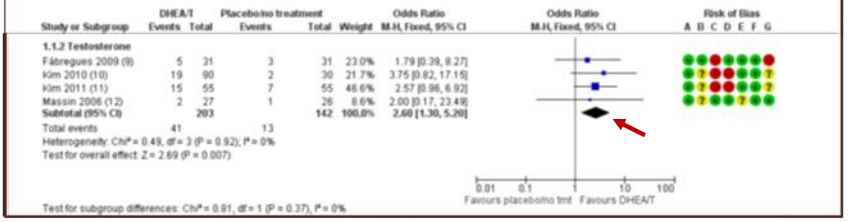
Test for subgroup differences: Chi² = 0.81, df = 1 (P = 0.37), I² = 0%

Footnotes
(1) Comparison was placebo
(2) Comparison was no treatment. This study transferred more embryos in the intervention arm
(3) Comparison was no treatment; reported as ongoing pregnancy rates
(4) Comparison was placebo; participants were infertile but not poor responders
(5) Comparison was placebo
(6) Comparison was no treatment
(7) Comparison was placebo; reported as ongoing pregnancy rates; participants were normal...
(8) Comparison was placebo

Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

When compared with placebo or no treatment, pre-treatment with testosterone was associated with higher rates of live birth.

Figure 4. Forest plot of comparison: 1 DHEA or testosterone versus placebo/no treatment, outcome: 1.1 Live birth/ongoing pregnancy rate.



Test for subgroup differences: Chi² = 0.81, df = 1 (P = 0.37), I² = 0%

(9) Comparison was no treatment. This study used a different stimulation protocol in the...
(10) Comparison was no treatment. 3 treatment groups: 2 weeks, 3 weeks or 4 weeks of T
(11) Comparison was no treatment
(12) Comparison was placebo

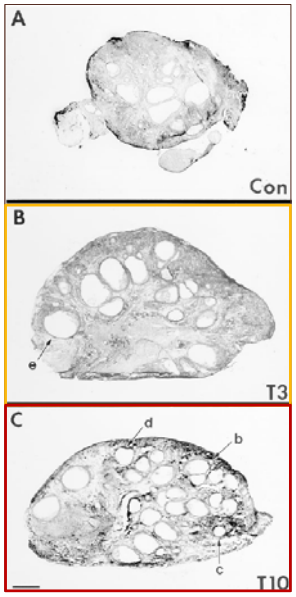
4 Live birth rate by length of T administration	4		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Up to 7 days	1	62	Odds Ratio (M-H, Fixed, 95% CI)	1.79 [0.39, 8.27]
4.2 14 to 20 days	2	113	Odds Ratio (M-H, Fixed, 95% CI)	2.10 [0.50, 8.88]
4.3 21 to 28 days	2	200	Odds Ratio (M-H, Fixed, 95% CI)	3.16 [1.38, 7.23]

Androgens Stimulate Early Stages of Follicular Growth in the Primate Ovary

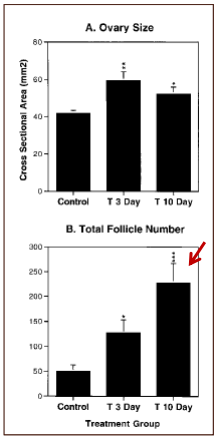
Keith A. Vendola, Jian Zhou, Oluyemisi O. Adesanya, Stacie J. Weil, and Carolyn A. Bondy
Developmental Endocrinology Branch, National Institute of Child Health and Development, National Institutes of Health, Bethesda, Maryland 20892

The Journal of Clinical Investigation
Volume 101, Number 12, June 1998, 2622–2629
http://www.jci.org

Experimental animals. Female rhesus monkeys (*Macacca mulatta*), 6–13 yr of age, from the NIH Poolesville colony were used in accordance with a protocol approved by the NICHD animal care and use committee. Animals had pellets (Innovative Research of America, Toledo, OH) inserted subcutaneously between their shoulder blades under ketamine anesthesia. In the first set of experiments, groups (n = 4–6) received pellets containing vehicle, high-dose testosterone (4 mg/kg per day for 3 d), or testosterone (400 μg/kg per day for 10 d). In a subsequent set of experiments, animals received pellets with placebo, low-dose testosterone (20 μg/kg per day for 5 d), or dihydrotestosterone (DHT; 145 μg/kg per day for 5 d). At the end of the dosing periods, ovariectomies were performed on the monkeys under ketamine anesthesia via a midventral laparotomy.



Testosterone treatment increased
- Ovarian size
- Follicular number



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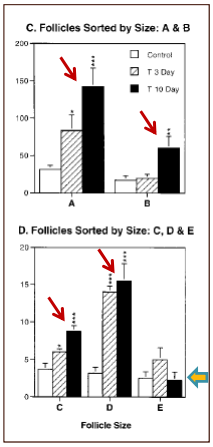
Androgen treatment stimulated early stages of primate ovarian follicular growth, independently of cycle stage or gonadotropin effect.

E	Large antral	> 1000	Mature graffian follicles with well developed granulosa, thecal, and antral elements.



Testosterone treatment

- Significantly increased the numbers of small follicles (primary to small antral)
- Did not increase the abundance of large antral follicles (pre-ovulatory)

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The Journal of Clinical Endocrinology & Metabolism

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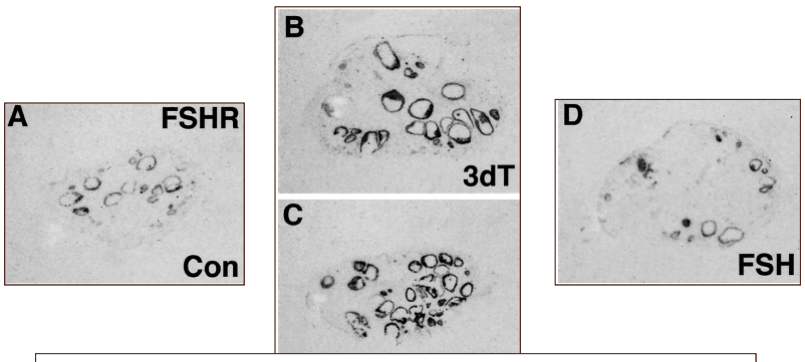
Androgen and Follicle-Stimulating Hormone Interactions in Primate Ovarian Follicle Development

STACIE WEIL, KEITH VENDOLA, JIAN ZHOU, AND CAROLYN A. BONDY

Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

Female Rhesus monkeys, 6–13 yr of age (from the NIH Poolesville, MD, colony) were studied under a protocol approved by the NICHD Animal Care and Use Committee. Monkeys were treated with sc pellets (Innovative Research of America, Toledo, OH) containing vehicle (n = 8) or sustained release T (4 mg/kg for 3 days, n = 4; or 0.4 mg/kg for 10 days, n = 4), as previously described (3). Another group (n = 4) received sc injections of recombinant FSH (Metrodin, Serono, Norwell, MA, 35 IU) for 2 days. Ovariectomies were performed under ketamine anesthesia via a ventral laparotomy.

Androgens promoted follicular growth indirectly by amplifying FSH effect.



FSHR mRNA expression was
- Significantly increased following testosterone treatment
- Only modestly increased following FSH treatment

Androstenedione induces abnormalities in morphology and function of developing oocytes, which impairs oocyte meiotic competence

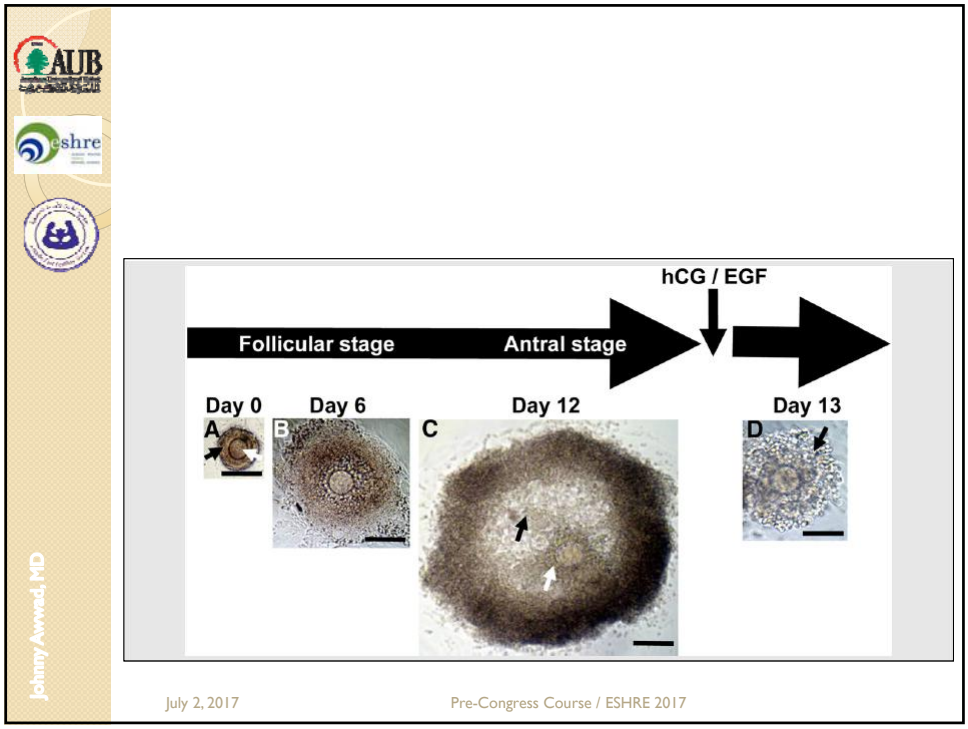
Wataru Tarumi, M.Sc.,[a,b] Sanae Tsukamoto,[a] Yuki Okutsu, M.D.,[a] Noriyuki Takahashi, Ph.D.,[a] Toshitaka Horiuchi, Ph.D.,[c] Masanori T. Itoh, Ph.D.,[d] and Bunpei Ishizuka, M.D.[a]

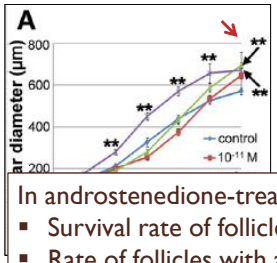
[a] Department of Obstetrics and Gynecology and [b] Department of Anatomy, St. Marianna University School of Medicine, Kawasaki; [c] Graduate School of Comprehensive Scientific Research, Prefectural University of Hiroshima, Hiroshima, and [d] Department of Biology, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Ichikawa, Japan

Fertility and Sterility® Vol. 97, No. 2, February 2012 0015-0282/$36.00

doi:10.1016/j.fertnstert.2011.11.040

Setting: St. Marianna University School of Medicine.
Animal(s): Prepubertal (14-day-old) BDF1 female mice.
Intervention(s): Early secondary follicles were isolated from the ovaries and were cultured individually in vitro with or without androstenedione (10^{-11} to 10^{-5} M) for 12 days. Thereafter, the follicles were treated with hCG and epidermal growth factor (EGF).



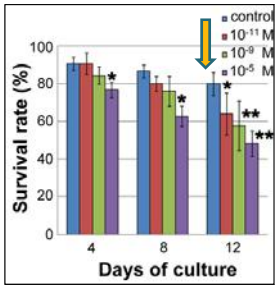


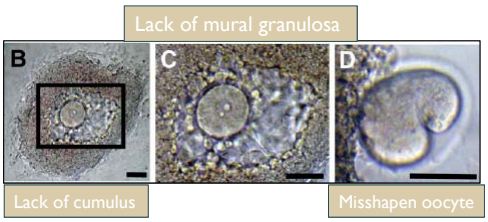
Equivalent to ovarian follicular fluid concentrations:

- PCOS - 10^{-5} M
- Healthy women - 10^{-9} M

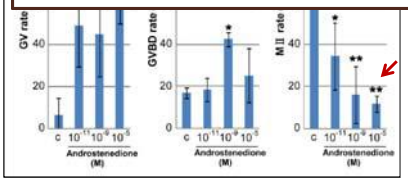
In androstenedione-treated follicles,

- Survival rate of follicles decreased in a dose-dependent manner
- Rate of follicles with abnormal morphology higher

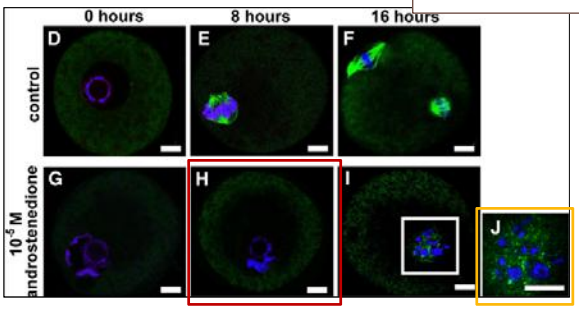




Excess androgen induced abnormalities in the morphology and function of developing oocytes, which impairs oocyte meiotic competence.



lower
- Failure of spindle assembly higher
- Misaligned chromosomes more frequent



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Position Paper

Testosterone for Poor Ovarian Responders: Lessons From Ovarian Physiology

Reproductive Sciences
1-3

DOI: 10.1177/1933719116660849
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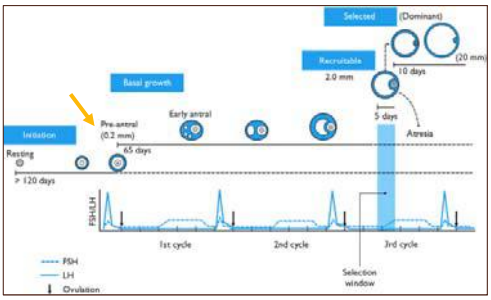
Nikolaos P. Polyzos, MD, PhD[1,2], Susan R. Davis, MD, PhD[3], Panagiotis Drakopoulos, MD, MSc[1], Peter Humaidan, MD, DMSc[2], Christian De Geyter, MD[4], Antonio Gosálvez Vega, MD[5], Francisca Martinez, MD, PhD[6], Evangelos Evangelou, PhD[7,8], Arne van de Vijver, MD[1], Johan Smitz, MD, PhD[1], Herman Tournaye, MD, PhD[1], Pedro Barri, MD, PhD[6], and The T-TRANSPORT Investigators Group

Johnny Awwad, MD



Does clinical research follow principles of ovarian physiology

The transition from preantral to antral follicular stage ≈ 70 days

of Transdermal Testosterone Preceding Gonadotropin Treatment in Poor Ovarian Responders.

Study	Sample Size	Duration, days	Dose (per day)
Bosdou et al[7]	50	21	10 mg
Kim et al[4]	110	21	12.5 mg
Fábregues et al[5]	62	5	0.02 mg/kg
Massin et al[6]	49	15	10 mg

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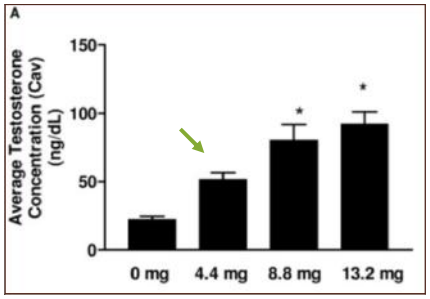


Testosterone in excess to 5.5 mg/d may be detrimental to follicle development

Johnny Awwad, MD

A randomized, controlled, pilot trial on the effect of dehydroepiandrosterone on ovarian response markers, ovarian response, and in vitro fertilization outcomes in poor responders

Tracy Wing Yee Yeung, M.B., B.S., Joyce Chai, M.B., Ch.B., Raymond Hang Wun Li, M.B., B.S., Vivian Chi Yan Lee, M.B., B.S., Pak Chung Ho, M.D., and Ernest Hung Yu Ng, M.D.

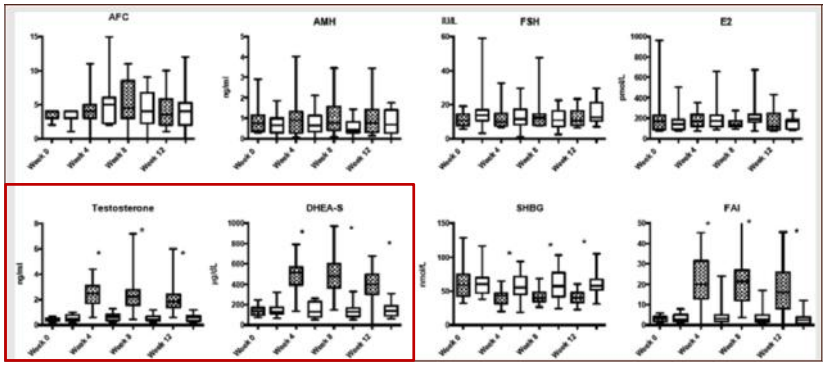
Department of Obstetrics and Gynecology, University of Hong Kong, Hong Kong, People's Republic of China

Fertility and Sterility® Vol. 102, No. 1, July 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.03.044

Design: Randomized, double-blind, placebo-controlled pilot study.
Setting: Tertiary reproductive medicine unit.
Patient(s): Thirty-two women with anticipated poor ovarian response.
Intervention(s): Randomization into DHEA group (n = 16) receiving GNC (25 mg three times a day) or placebo (n = 16) starting from at least 12 weeks before the scheduled IVF treatment according to a computer-generated randomization list.
Main Outcome Measure(s): Measurement of monthly ovarian response markers, including antral follicle count (AFC), serum anti-müllerian hormone (AMH), and follicle-stimulating hormone (FSH) levels; comparison of ovarian response to a standard dose of gonadotropin stimulation at week 8 and IVF outcomes; and AFC after 12 weeks (primary outcome).

Serum testosterone and DHEA-S levels were statistically significantly higher after DHEA supplementation



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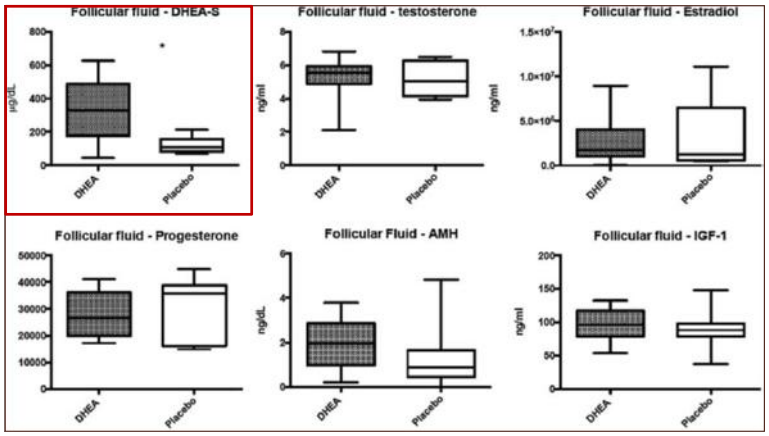
Median follicular DHEA-S level, but not testosterone, was statistically significantly higher in the DHEA group.



No significant difference in the oocytes obtained, clinical pregnancy, ongoing pregnancy, live birth or miscarriage was observed.

IVF cycle characteristics of the DHEA and placebo groups.

Cycle characteristic	DHEA group (n = 16)	Placebo group (n = 16)	*P* value
Insemination			
IVF	10	11	.648
ICSI	4	2	
Gonadotropin			
Duration (d)	10 (9–12)	12 (9–15)	.114
Dose (IU)	2,475 (2,194–3,206)	3,150 (2,475–4,388)	.069
E_2 on day of hCG	3,947 (2,781–4,408)	5,101 (1,479–6,222)	.347
Follicle size			
14–15 mm	0.5 (0–1)	0 (0–0.5)	.169
16–17 mm	0 (0–2)	0 (0–1)	.550
≥18	1 (1–2)	2 (1–2)	.430
Endometrial thickness (mm)	11.2 (9.4–13.8)	10.7 (9.3–12.5)	.705
Number of			
Oocytes obtained	3 (1.25–6.75)	2.5 (1–3)	.186
Fertilized embryos	3 (0.5–4)	1 (0–2)	.155
Cleaved embryos	3 (0.5–3.75)	1 (0–2)	.169
Transferred embryos	2 (0.25–2)	1 (0–2)	.430
Frozen embryos	0 (0–1.75)	0 (0)	.202
TQE	1 (0–2)	0 (0–0.75)	.141

Note: Data are expressed as median (25th to 75th centile) or number (percentage) as appropriate. $P < .05$ was considered statistically significant. TQE = top-quality embryos.

Yeung. Effect of DHEA in poor responders. Fertil Steril 2014.

Human Reproduction, Vol.31, No.5 pp. 977–985, 2016
Advanced Access publication on March 7, 2016 doi:10.1093/humrep/dew028

human reproduction ORIGINAL ARTICLE *Endocrinology*

Transdermal testosterone pretreatment in poor responders undergoing ICSI: a randomized clinical trial

J.K. Bosdou[1], C.A. Venetis[2], K. Dafopoulos[3], L. Zepiridis[1], K. Chatzimeletiou[1], G. Anifandis[3], A. Mitsoli[1], A. Makedos[1], I.E. Messinis[3], B.C. Tarlatzis[1], and E.M. Kolibianakis[1,*]

[1]Unit for Human Reproduction, 1st Department of Obstetrics and Gynecology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece [2]School of Women's and Children's Health, UNSW Medicine, University of New South Wales, Sydney, Australia [3]Unit for Human Reproduction, Department of Obstetrics and Gynecology, Medical School, University of Thessaly, Larissa, Greece

STUDY DESIGN, SIZE, DURATION: The present RCT was designed to detect a difference of 1.5 COCs (sample size required = 48 patients). From 02/2014 until 04/2015, 50 poor responders fulfilling the Bologna criteria have been randomized (using a randomization list) to either testosterone pretreatment for 21 days ($n = 26$) or no pretreatment ($n = 24$).

All patients underwent a long follicular protocol with GnRH agonist triptorelin (Arvekap, Ipsen Ltd, France) 3.75 mg depot, starting on the first day of the menstrual cycle, followed by daily injections of triptorelin (Arvekap, Ipsen Ltd, France) 0.1 mg, if necessary. In the testosterone group, a daily dose of 10 mg of testosterone gel (Tostran 2% Gel, ProStrakan) was applied transdermally onto the inner thigh daily, for 21 days as suggested by Kim *et al.* (2011), starting from the GnRH agonist initiation. Testosterone was supplied in a canister with a dosing pumping mechanism, which delivered one half gram of gel containing 10 mg of testosterone each time the piston was depressed.

Table IV Clinical outcome between the testosterone pretreatment group and the no pretreatment group.

	Testosterone pretreatment ($n = 26$)	No pretreatment ($n = 24$)	Difference % 95% CI P-value
	% (n)		
Proportion of patients with at least one top quality embryos	20.0 (4)	23.8 (5)	−3.8 −28.2 to +21.5 0.72
Patients with embryo transfer	83.3 (20)	91.3 (21)	−8.0 −28.2 to +12.7 0.47
Cancelation rate	7.7 (2)	4.2 (1)	+3.5 −13.5 to +20.3
Clinical pregnancy per embryo transfer	10.0 (2)	9.5 (2)	−0.5 −20.2 to +21.7 1.00
Live birth rate (ITT analysis)	7.7 (2)	8.3 (2)	−0.6 −19.0 to +16.9 1.00
Live birth per embryo transfer	10.0 (2)	9.5 (2)	−0.5 −20.2 to +21.7 1.00

The non-significant increase in the number of COCs, following transdermal testosterone pretreatment was not associated with the probability of embryo transfer.

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Table II Embryological outcome in the testosterone pretreatment and the no pretreatment groups.

	Testosterone pretreatment ($n = 26$)	No pretreatment ($n = 24$)	95% CI of the difference between medians	P-value
Primary outcome measure				
Cumulus oocyte complexes (COCs), Intention to treat (ITT) analysis	3.5 (4.0, 2.0–5.0)	3.0 (3.0, 2.7–4.3)	−1.0 to +1.0	0.76
COCs per protocol analysis	4.0 (4.0, 2.0–5.3)	3.0 (3.0, 3.0–4.7)	−1.0 to +2.0	0.66
Secondary outcomes				
Metaphase II oocytes (MII) ITT analysis	3.0 (2.0, 2.0–3.5)	3.0 (2.0, 1.7–3.0)	−1.0 to +1.0	0.63
Maturation rate (MII/COCs) % per patient with COCs retrieved	100.0 (25.0, 78.3–100.0)	80.4 (50.0, 65.5–100.0)	0.0 to +25.0	0.77
2-pronuclei oocytes (2pn) ITT analysis	2.0 (3.0, 1.0–3.0)	2.0 (2.0, 1.0–2.3)	−1.0 to +1.0	0.50
Fertilization rate (2pn/COCs) % per patient with COCs retrieved	66.7 (32.5, 50.0–78.5)	66.7 (42.9, 48.8–75.0)	−16.7 to +25.0	0.73
Fertilization rate (2pn/MII) % per patient with COCs retrieved and treated by ICSI	85.4 (38.3, 66.7–100.0)	81.7 (33.3, 66.7–100.0)	−15.9 to +10.0	0.99
Number of embryos transferred	2.0 (1.0, 2.0–3.0)	2.0 (2.0, 1.0–2.0)	0.0 to +1.0	0.27

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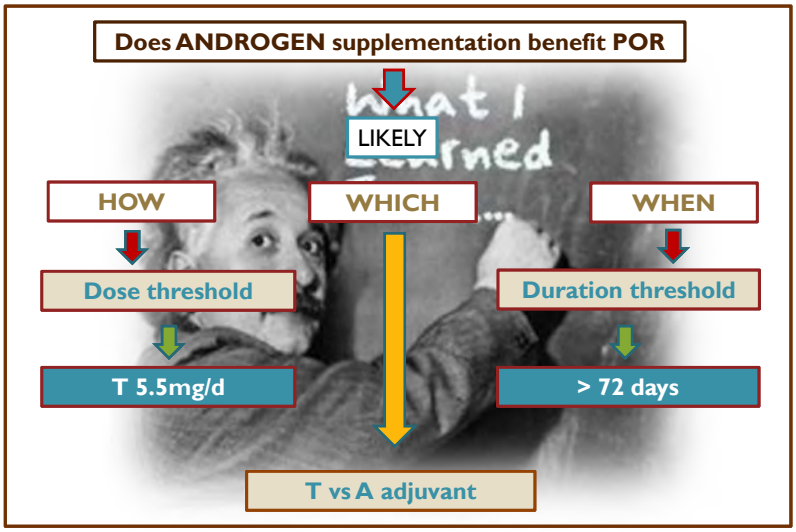
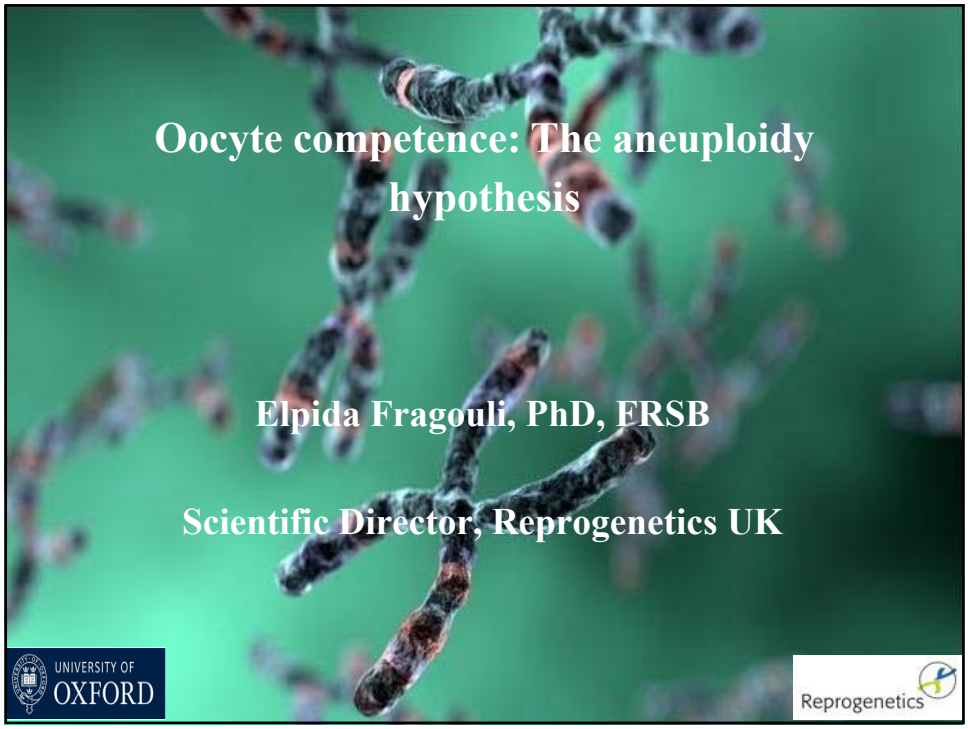
TAKE HOME MESSAGES





Disclosure

Employee (scientific director) of Reprogenetics UK, a PGD service provider

Learning objectives

- **Origin of aneuploidy & relevance to reproductive failure**
- **Oogenesis & meiosis**
- **Mechanisms leading to aneuploidy of female meiotic origin:**
  1. Whole chromosome non-disjunction
  2. Unbalanced chromatid pre-division
- **Methods employed for oocyte/ PB analysis:**
  Advantages & disadvantages
- **Oocyte analysis data:**
  1. Karyotyping & FISH
  2. Comprehensive molecular cytogenetic methods (CGH & aCGH)
- **Why is female meiosis so error prone?**
  Recombination

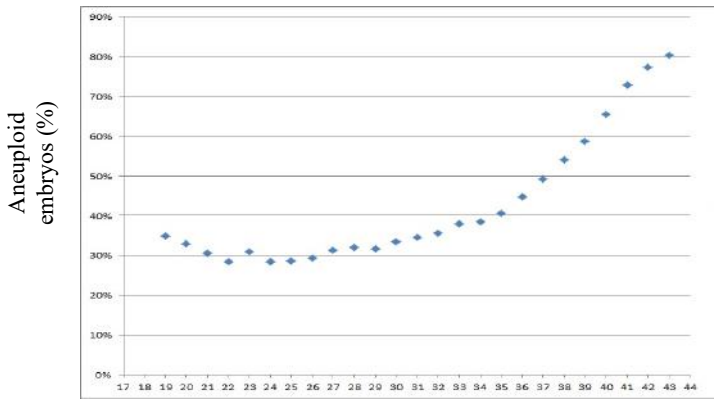
The impact of aneuploidy in reproductive success & failure

- **Numerical chromosome abnormalities: common & clinically significant**
- **5% of clinically recognised pregnancies carry a trisomy or monosomy**
- **Aneuploid pregnancies mostly miscarry**
- **A few trisomies & sex chromosome abnormalities lead to live births**
- **Relevance of aneuploidy to IVF**
  1. **Embryonic arrest**
  2. **Implantation failure**
  3. **Spontaneous abortion**

Hassold and Hunt, 2001: Hassold et al., 2007

Origin of aneuploidy

- **Aneuploidies arise during oogenesis (mostly) & post-fertilization**
- **Meiotic aneuploidy principally influenced by female age**



Reprogenetics UK data from > 2000 oocytes

Meiosis

- **Specialised cell division taking place in reproductive tissue**
- **Reduces diploid chromosome number by half- haploid gametes created**

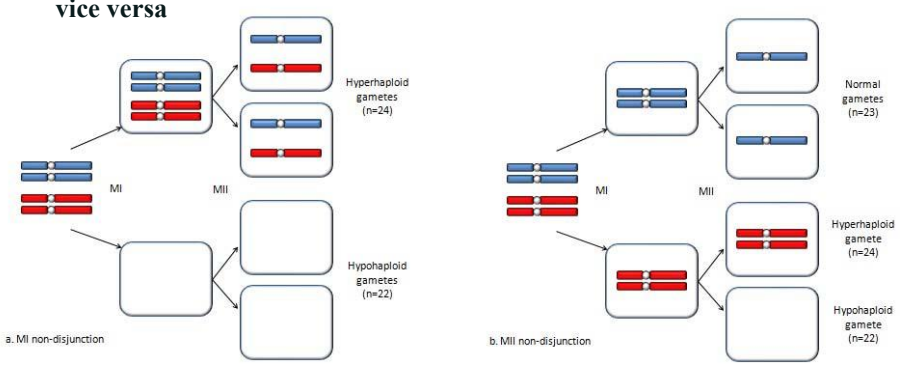
Oogenesis

- Females born with complete oocyte set
- Oogenesis starts during fetal development
- Mitotic divisions form primordial follicles containing diploid primary oocytes
- MI begins on 12th week of fetal development
- Diplotene prophase I arrest:
  - homologous chromosome recombination
  - germinal vesicle (GV) formation
- Puberty & menstrual cycle:
  - one oocyte released each month
  - uterus prepares for implantation

Jones, 2008

Whole chromosome non-disjunction

- Takes place during MI or MII
- Homologous chromosomes move towards same meiotic spindle pole
- Oocyte with extra chromosome & 1st or 2nd PB with missing chromosome or vice versa

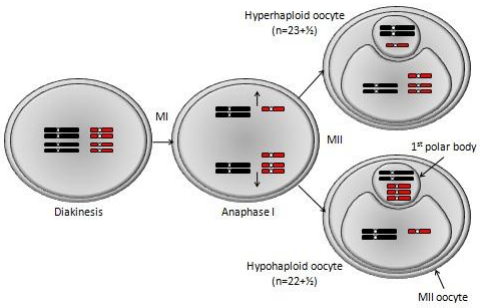


- Due to reduced or no recombination & position of chiasmata
- Female age affects recombination patterns

Delhanty, 2005: Hassold et al., 2007

Unbalanced chromatid predivision

- Takes place during MI
- Sister chromatids divide prematurely & segregate randomly
- Lead to aneuploidy in 50% of cases

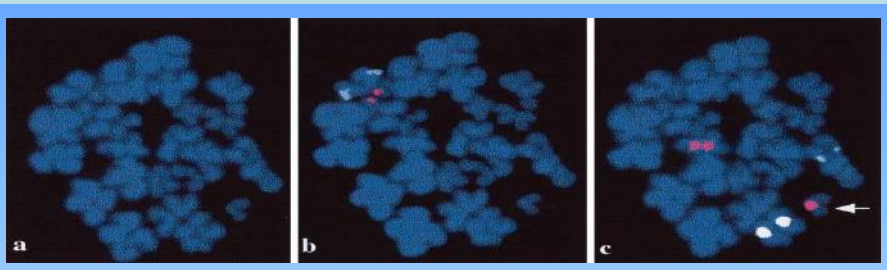


- Due to reduced or no recombination & female age

Angell et al., 1993

Cytogenetic analysis of oocytes & polar bodies

Oocyte & polar body fixation on a slide



- Required for karyotyping or FISH analysis
- Involves hypotonic treatment followed by fixation on slide
- Enables visualisation of chromosomes & chromatids
- Risks artefactual chromosome loss
- Accurate hyperhaploidy scoring only

Picture from Mahmood et al., 2000; Fragouli et al., 2011

Oocyte analysis via classical cytogenetic methods

- Pellestor et al. (2003) analysed 1397 oocytes via R banding
- 792 women of average age 34 years
- Modified fixation minimised artefactual chromosome loss
- 10.8% oocytes abnormal
- Aneupoidy increased with advancing female age
- Unbalanced chromatid pre-division affected by advancing female age
- IVF indication did not influence aneuploidy rates

Pellestor et al., 2003

Oocyte analysis via classical cytogenetic methods

- **Karyotyping results conclusions:**

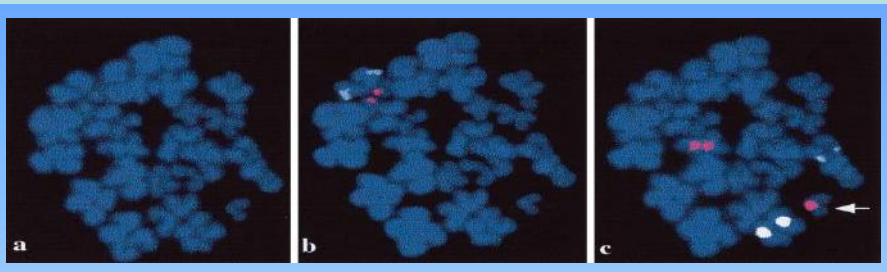
1. Determined how advancing female age affects whole chromosome non-disjunction & unbalanced chromatid pre-division

2. Smaller chromosome groups D-G malsegregated more frequently

- **Poor oocyte metaphase morphology meant that specific chromosomes could not be identified**

Pellestor, 1991; Zenzes and Casper, 1992; Angell, 1997; Pellestor et al., 2002

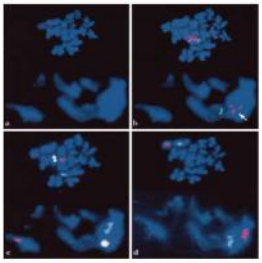
Oocyte analysis via fluorescent in situ hybridisation



- **FISH provided results for ALL types of oocyte metaphase spreads**
- **FISH enabled assessment of polar bodies**
- **FISH probes targeted smaller chromosomes**
- **Limited number of chromosomes assessed**

Picture from Mahmood et al., 2000

Oocyte analysis via fluorescent in situ hybridisation



- **Mahmood et al. (2000) & Cupisti et al. (2003) analysed 236 oocyte-PB complexes via FISH**
- **124 women of average age 32.5 years**
- **Chromosomes 1, 9, 12, 13, 16, 18, 21, X assessed**
- **Chromosome gains assessed only**
- **Hyperploidy rate: 4%**
- **Chromosomes 13, 16, 18 and 21 malsegregated more frequently**
- **Chromosome non-disjunction, chromatid predivision, germinal/gonadal mosaicism identified**

Mahmood et al., 2000; Cupisti et al., 2003

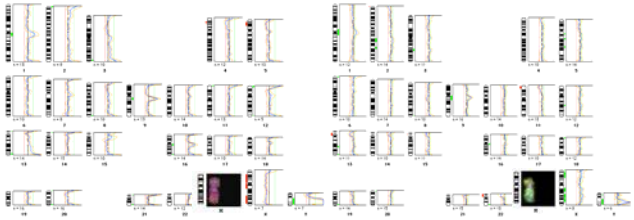
Oocyte analysis via FISH/ clinical results

- **Verlinsky et al. assessed first & second PBs for PGS purposes**
- **Chromosomes 13, 16, 18, 21, 22 targeted**
- **Data from 7,103 first and second PB pairs:**

1. Chromatid errors more frequent than whole chromosome errors in older women (27.1% vs. 2.4%)
2. MI and MII abnormality rates similar in older women (42% vs. 38%)
3. Malsegregation affecting chromosome 16 taking place mostly in MII
4. Malsegregation affecting chromosome 18 taking place mostly in MI
5. MII correction of MI chromatid error for 33% of oocytes

Kuliev et al., 2003; Kuliev and Verlinsky, 2004

Oocyte analysis via comprehensive molecular cytogenetic methods



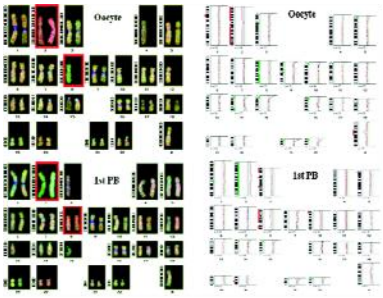
- **Karyotyping and FISH technical issues**

1. Artefactual loss of chromosomes due to slide spreading- inability to determine global oocyte aneuploidy rate
2. Inability to examine entire chromosome complement

- **Combination of whole genome amplification & comparative genomic hybridisation overcame issues**

Wells et al., 1999; Wells and Delhanty, 2000

Oocyte & 1st PB analysis via CGH

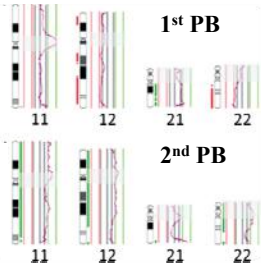


- **Fragouli et al. (2006) analysed 107 oocyte-PB complexes via CGH**
- **46 women of average age 32.5 years**
- **Reciprocal chromosome & chromatid errors identified in oocyte-PB pairs**
- **Aneuploidy rate: 22%**
- **Malsegregation affected all chromosome groups**
- **Smaller chromosomes (13-22 &X) more frequently abnormal**
- **Larger chromosomes (1-12) affected by whole chromosome non-disjunction only**
- **Smaller chromosomes (13-22 & X) affected by whole chromosome non-disjunction & unbalanced chromatid pre-division**
- **Structural abnormalities identified**

Fragouli et al., 2006

Oocyte analysis via CGH/ clinical results

- Fragouli et al. (2011) analysed 308 first & second PB pairs via CGH for PGS purposes
- 70 women of average age 41 years
- Total aneuploidy rate: 70%
- MI aneuploidy rate: 40% vs. MII aneuploidy rate: 50%
- Unbalanced chromatid pre-division more frequent than whole chromosome non-disjunction during MI (62% vs. 38%)
- Chromosome losses more frequent than chromosome gains (MI 68% losses vs. 32% gains; MII 60% losses vs. 40% gains)
- Malsegregation affected all chromosome groups
- Smaller chromosomes (13-22 &X) more frequently abnormal
- Advancing female age affected MII more



Fragouli et al., 2011

Is analysis of both PBs predictive of embryo's chromosome complement?

- Christopikou et al. (2013) analysed 34 first & second PB pairs & corresponding cleavage stage embryos via aCGH
- Aim: determine predictive ability of PB1 & PB2 analysis for corresponding embryo's chromosome status
- 30/34 cleavage stage embryos confirmed as aneuploid- 100% concordant with PB1 & PB2 results
- 12% PB copy number changes were not detected in corresponding embryos
- False positive copy number changes were more common in PB1

Christopikou et al., 2013

Why is female meiosis so error prone?

Why is female meiosis so error prone?

- **Female meiosis stops & starts during foetal & adult life**
- **Strict regulation of oocyte nuclear & cytoplasmic maturation essential**
- **Advancing female age & aberrant genetic recombination affect accurate oocyte chromosome segregation**
- **Recombination patterns predisposing to aneuploidy:**
  1. Chiasmata formation absence
  2. Chiasmata formation too close or too far from chromosome centromere

Fisher et al., 1995; Hassold et al., 1995; Nicolaidis and Petersen, 1998 ; Sherman et al., 2005

Recombination rates in oocytes

- Ottolini et al., (2015) mapped 2,032 female & 1342 male crossovers to infer 529 chromosome pair segregations
- 23 sets of PB1, PB2 & corresponding oocytes/embryos & 29 embryos analysed by karyomapping
- > 4 million SNPs genotyped after sample WGA
- 39 instances of whole chromosome aneuploidy & 3 segmental errors identified
- Unbalanced chromatid pre-division as main MI aneuploidy causing mechanism
- New “reverse” segregation detected
- Normal oocytes/embryos with ~6x more recombination events than aneuploid
- Higher global recombination rates protect again chromosome malsegregation

Ottolini et al., 2015

Crossover maturation in oocytes

- Wang et al. (2017) examined male and female meiosis via computer modelling approach
- Oocyte & sperm crossover patterns simulation analysis
- Female recombination affected by crossover maturation inefficiency
- Phenomenon not observed for male recombination
- Phenomenon creates vulnerable chromosome configurations
- Phenomenon contributes significantly to oocyte aneuploidy
- Is aneuploidy an evolutionarily favoured trait?

Wang et al., 2017

Conclusions

- **Chromosome abnormalities of female meiotic origin contribute significantly to reproductive failure**
- **Large numbers of oocytes/PBs examined with various cytogenetic methods**
- **Main mechanisms of female meiotic aneuploidy:**
  1. Whole chromosome non-disjunction
  2. Unbalanced chromatid pre-division
  3. Germinal/gonadal mosaicism (?)
- **All chromosomes affected by aneuploidy, but smaller groups (D-G) more frequently abnormal**
- **Advancing female age affects both meiotic divisions, but MII especially**
- **Crossover frequency & maturity influence meiotic chromosome segregation**
- **Is aneuploidy an evolutionarily favoured trait?**
- **Does the use of PB1 & PB2 provide accurate representation of embryo?**

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Christopikou D, Tsorva E, Economou K, Shelley P, Davies S, Mastrominas M, Handyside AH. Polar body analysis by array comparative genomic hybridization accurately predicts aneuploidies of maternal meiotic origin in cleavage stage embryos of women of advanced maternal age. Hum Reprod 28:1426-1434 (2013).

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Reading list

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Does polar body analysis accurately predict the aneuploidy status of the developing embryo?

Alan Handyside
The Bridge Centre, London, University of Kent, Canterbury and Illumina, Cambridge, UK

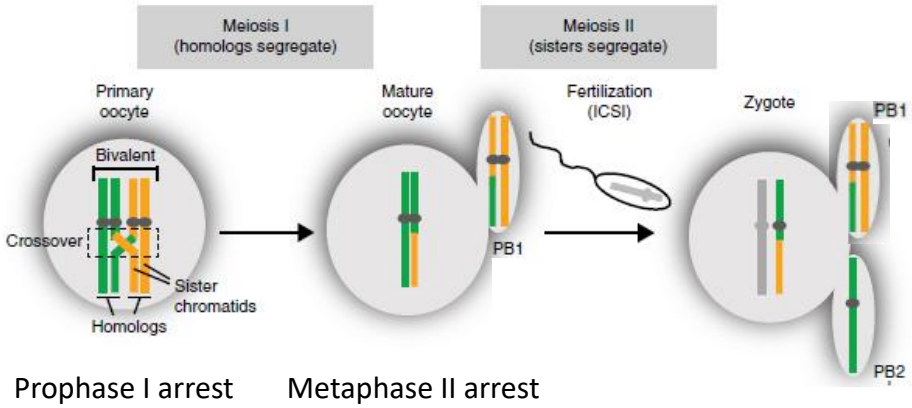
Disclosure

I am a part time employee of, and have share options in, Illumina, San Diego, CA, USA, based in Cambridge, UK, which manufactures equipment and reagents for DNA sequencing, diagnostics and preimplantation genetic testing

Learning objectives

- Normal and abnormal patterns of chromosome segregation in female meiosis
- Basis of polar body analysis, advantages and disadvantages
- Principles of copy number analysis by array comparative genomic hybridisation (array CGH) or next generation sequencing (NGS)
- Principles of genome-wide single nucleotide polymorphism (SNP) and meiomapping for polar body analysis
- Accuracy of polar body analysis for maternal aneuploidies arising in female meiosis

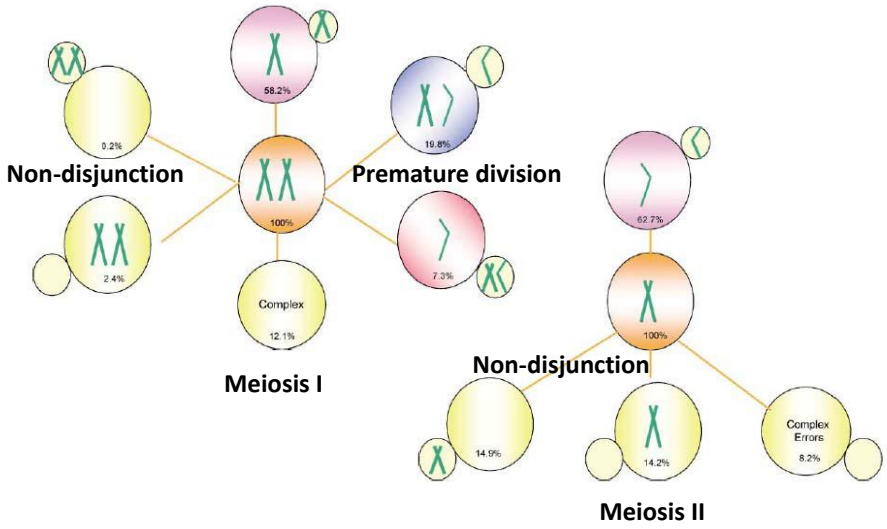
Chromosome segregation in female meiosis



Prophase I arrest
Fetal ovary

Metaphase II arrest
Ovulation post puberty

Multicolour FISH analysis of polar bodies



Kuliev and Verlinsky (2004) Hum Reprod Update 10, 401

Polar body testing for detection of female meiotic errors

Advantages

- Relatively non-invasive
- Early diagnostic results
- Direct detection of female meiotic errors
- Female meiotic errors 10x > paternal meiotic errors
- Highly likely to affect whole embryo
- No confusion with mitotic (mosaic) errors

Disadvantages

- Need to test first (PB1) and second (PB2) polar bodies
- Copy number analysis by array CGH less reliable

Single cell genomics

Single cell (or 3-10 cells)
~ pg DNA

Whole genome amplification (WGA)

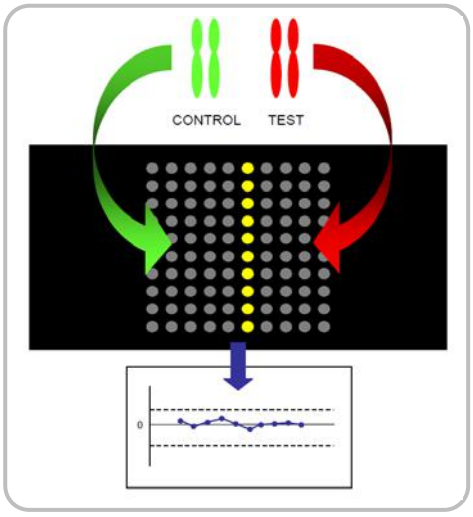
>ug DNA

PGD
SNP genotyping arrays and karyomapping

PGS
BAC arrays for Array CGH
Low read depth NGS

NGS based solutions for combined PGD and PGS

24 chromosome copy number analysis by array comparative genomic hybridisation (array CGH)

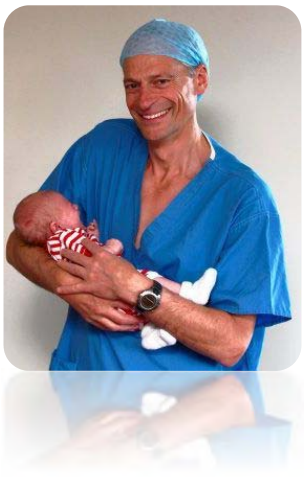


THE TIMES

September 2, 2009

New IVF test–Array CGH

Produces baby Oliver,
offering hope to infertile

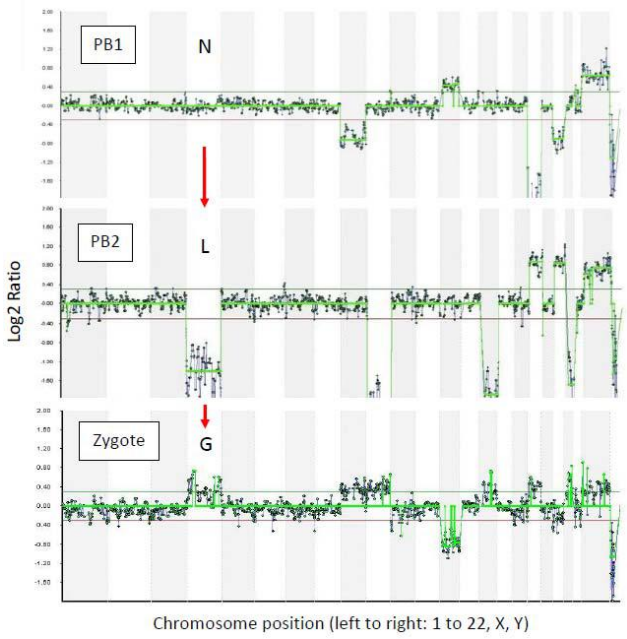


- 13 previous failed IVF cycles
- 7/9 first polar bodies aneuploid

PGS for 24 chromosomes by array CGH of the first (PB1) and second polar bodies (PB2) in advanced maternal age

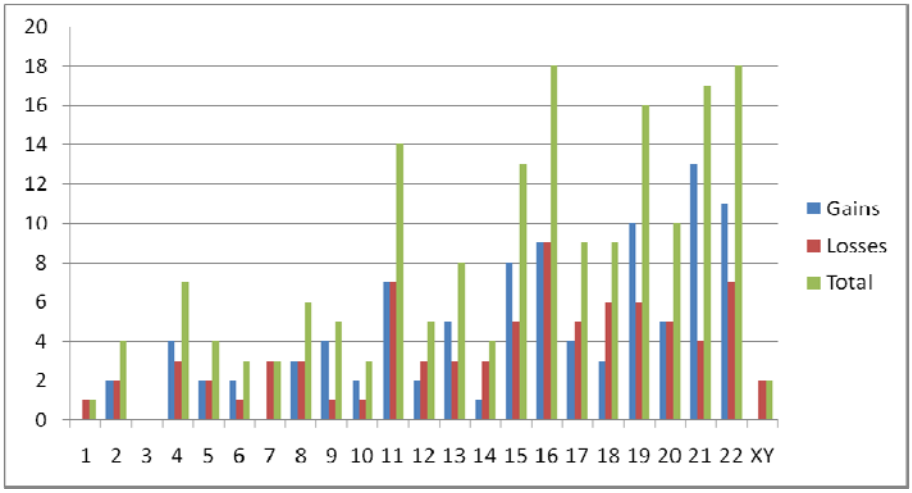
- 41 patients, 42 ICSI cycles
- Mean maternal age 40 years
- 226 oocytes/zygotes biopsied
- Array CGH of both polar bodies and the corresponding zygote analysed blind to confirm the diagnosis
- 55 (28%) euploid, 140 (72%) aneuploid
- All aneuploid in 19/42 cycles (42%)
- 8 clinical pregnancies, 1 livebirth, 7 ongoing
- 19% per cycle, 33% per ET

ESHRE PGS Task Force: Handyside et al (2012) EJHG 20, 742

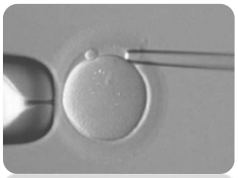


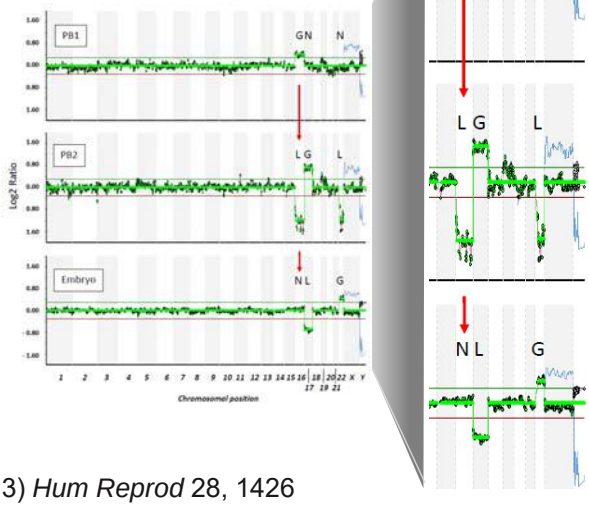
Segregation patterns of copy number gains and losses in the first and second polar bodies and corresponding zygotes (PB1/PB2/Zygote)

Zygote	Origin	Pattern	Cause	No with different patterns per chromosome																							
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	XY	Total	%*
		NNN		98	91	99	92	93	96	95	85	90	96	80	94	83	96	81	76	86	88	79	85	77	71	92	2023	
GAIN	MI	LGG	NDJ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2	1
		LNG	PD	0	0	0	3	1	1	0	1	3	0	3	1	1	1	4	3	1	2	6	2	4	5	0	42	23
		LLG		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	MI/MII	LGN		0	1	0	1	0	0	0	4	1	1	1	2	4	1	3	2	0	0	1	1	2	2	1	28	
	MII	NLG		0	2	0	1	1	1	0	2	1	2	4	1	4	0	4	6	3	1	4	3	9	6	0	55	31
	Other	NNG		0	0	1	1	1	0	0	0	1	1	2	1	1	1	0	1	0	0	2	1	3	0	1	18	
LOSS	MI	GLL	NDJ	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	1
		GNL	PD	0	1	0	3	0	0	2	0	1	0	2	2	1	0	3	3	2	1	2	1	2	1	2	29	16
		LGL		0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
	MI/MII	GLN		1	0	0	1	0	0	1	1	2	1	0	0	1	1	3	3	1	2	1	0	0	1	0	20	
	MII	NGL		0	1	0	0	2	1	1	1	0	1	5	1	2	3	1	5	3	4	4	4	2	6	0	47	26
	Other	NNL		0	1	1	2	0	0	2	1	0	0	1	2	0	2	2	0	4	0	1	3	0	3	5	30	
Total maternal aneusomies (excluding MI/MII compensation)				1	4	0	7	4	3	3	6	5	3	14	5	8	4	13	18	9	9	16	10	17	18	2	179	



Polar Body Biopsy With Follow Up at Cleavage Stages on Day 3





Christopikou et al. (2013) *Hum Reprod* 28, 1426

- 30/30 (100% concordant) embryos predicted to be aneuploid by array CGH of both polar bodies confirmed in day 3 embryos
- 69/73 (93%) of aneuploidies associated with copy number changes in polar bodies
- 68/69 (98.5%) of aneuploidies correctly predicted
- 19/20 reciprocal copy number changes in the first and second polar bodies resulted in normal copy number in the embryo
- 17 (12%) false positive copy number changes in polar bodies not associated with aneuploidy in the embryo
- Only 12/17 of these predicted aneuploidy

Increasing Live Birth Rate by Preimplantation Genetic Screening of Pooled Polar Bodies Using Array Comparative Genomic Hybridization

Michael Feichtinger[1], Tina Stopp[1], Christian Göbl[2], Elisabeth Feichtinger[1], Enrico Vaccari[1], Ulrike Mädel[1], Franco Laccone[3], Monika Stroh-Weigert[1], Markus Hengstschläger[1,3], Wilfried Feichtinger[1], Jürgen Neesen[3]*

1 Wunschbaby Institut Feichtinger, Vienna, Austria, **2** Department of Obstetrics and Gynecology, Medical University of Vienna, Vienna, Austria, **3** Institute of Medical Genetics, Medical University of Vienna, Vienna, Austria

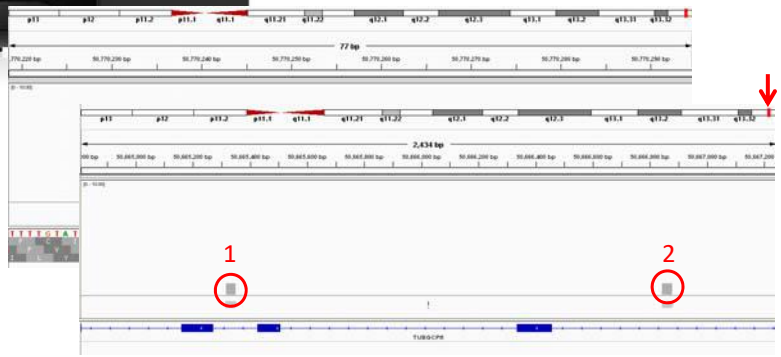
Fechtinger et al (2015) PLoS One 10 (5)

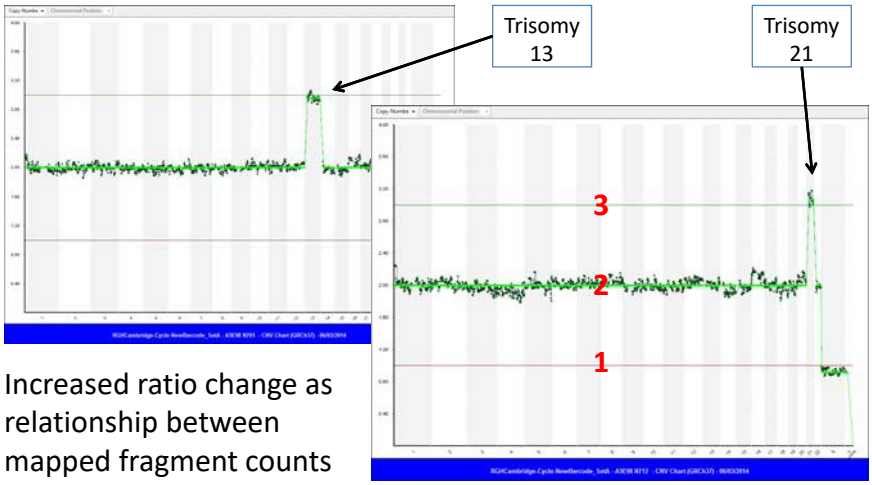
Retrospective study of 351 patients

	Control	PB aCGH	*p*
n	240	111	
Maternal age	38.4	39.5	<0.001
Live birth per embryo	14.9	26.4	0.015
Live birth per patient	22.7	35.7	0.031

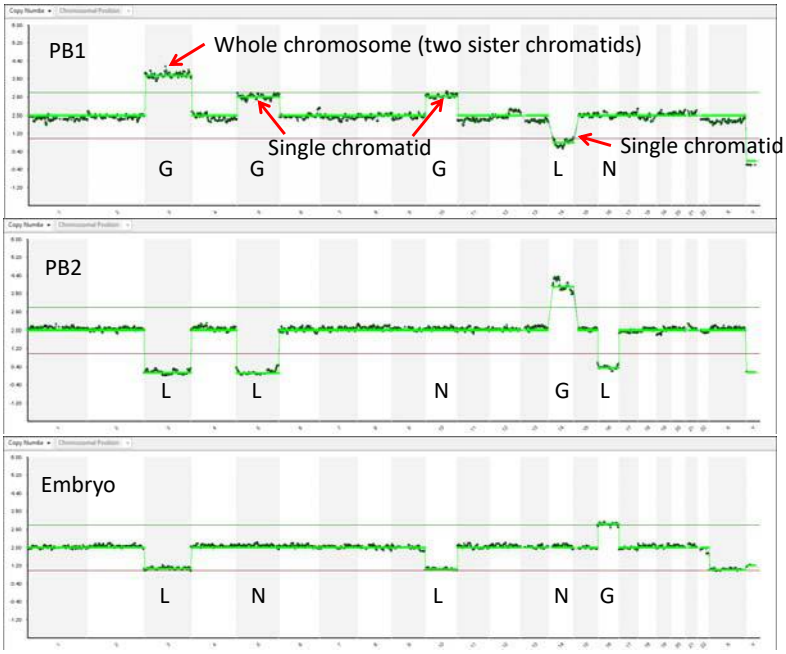
Fechtinger et al (2015) PLoS One 10 (5)

24 chromosome copy number analysis by low cost, low read depth (0.1x) next generation sequencing (NGS) and mapped fragment counting





Increased ratio change as relationship between mapped fragment counts and copy number is linear



nature genetics

Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates

Chris Hrish Alan Alan

PROTOCOL

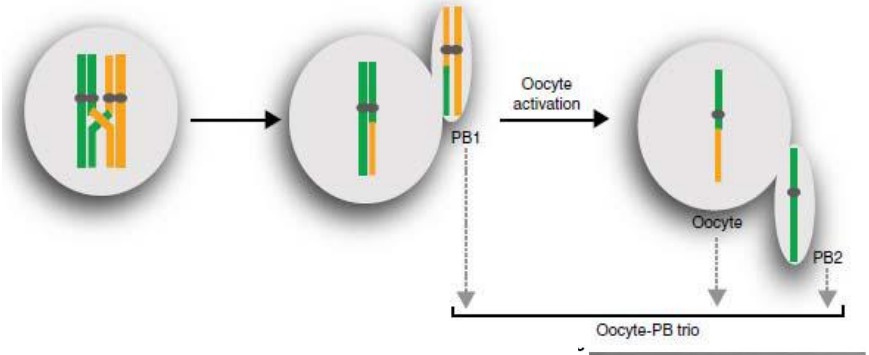
Generation of meiomaps of genome-wide recombination and chromosome segregation in human oocytes

Christian S Ottolini[1,2,8], Antonio Capalbo[3,4,8], Louise Newnham[5,8], Danilo Cimadomo[3,4], Senthilkumar A Natesan[7], Eva R Hoffmann[5,6], Filippo M Ubaldi[3,4], Laura Rienzi[3,4] & Alan H Handyside[1,2,7]

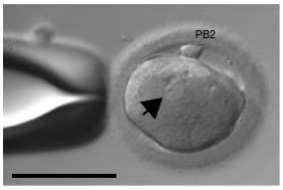
[1]The Bridge Centre, London, UK. [2]School of Biosciences, University of Kent, Canterbury, UK. [3]GENERA, Centers for Reproductive Medicine, Rome, Italy. [4]GENETYX, Molecular Genetics Laboratory, Marostica, Italy. [5]Genome Damage and Stability Centre, School of Life Sciences, University of Sussex, Brighton, UK. [6]DNRF Center for Chromosome Stability, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark. [7]Illumina, Cambridge, UK. [8]These authors contributed equally to this work. Correspondence should be addressed to A.H.H. (ahandyside@illumina.com).

Ottolini et al (2015) Nature Genetics 47, 727
Ottolini et al (2016) Nature Protocols 11, 1229

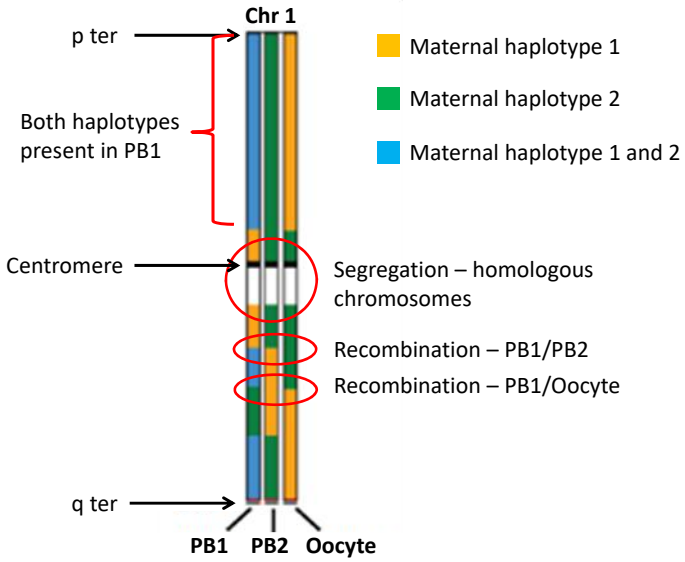
Analysis of all three products of meiosis: both polar bodies and oocyte (oocyte-PB trios)



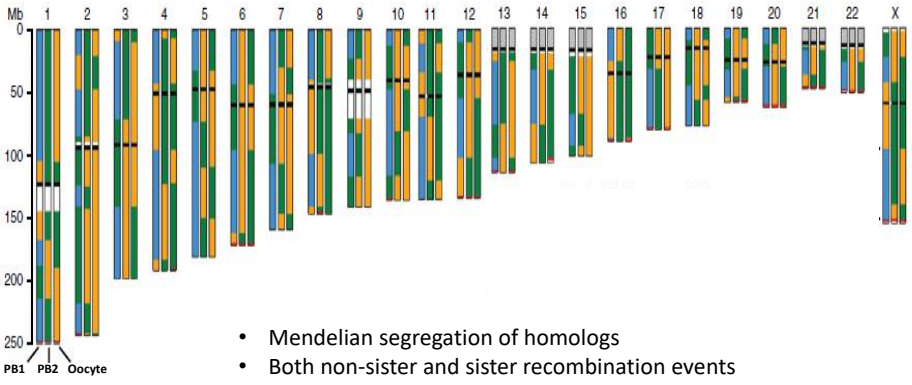
- Sequential polar body and oocyte biopsy
- Whole genome amplification (WGA)
- SNP genotyping
- Phasing of SNPs using a haploid reference



Typical MeioMap Visualisation

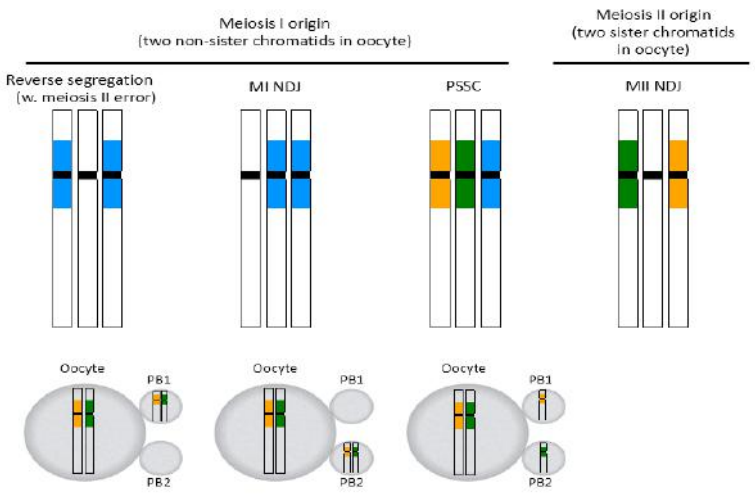


Genome-wide Meiomap

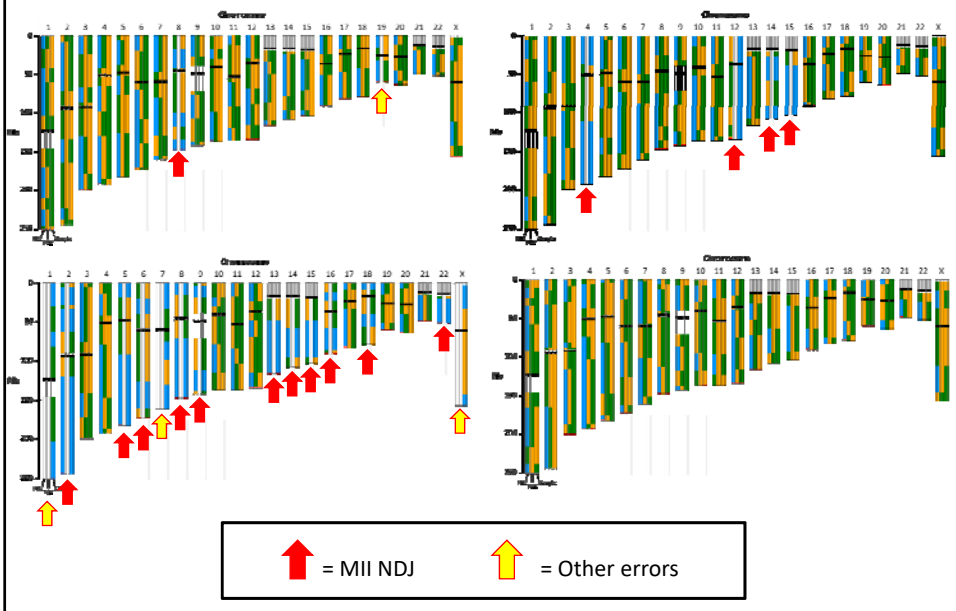


- Mendelian segregation of homologs
- Both non-sister and sister recombination events identified
- Harlequin pattern – unique oocyte/embryo and chromosome specific fingerprint

Examples of typical non-canonical segregation patterns in the pericentromeric region



- Non-disjunction (NDJ) relatively rare
- Premature separation of sister chromatids (PSSC) common
- Reverse segregation also common (**not detectable by copy number analysis**)



Summary

- Most aneuploidies in the human embryo arise as chromosome segregation errors in female meiosis
- Accurate diagnosis of maternal meiotic errors in the fertilised oocyte requires analysis of both the first and second polar bodies
- Copy number analysis by array CGH can result in false positives
- NGS based copy number analysis allows accurate discrimination of chromosome and chromatid gains and losses
- Meiomapping of all three products of meiosis allows accurate analysis of the mechanism of chromosome segregation errors

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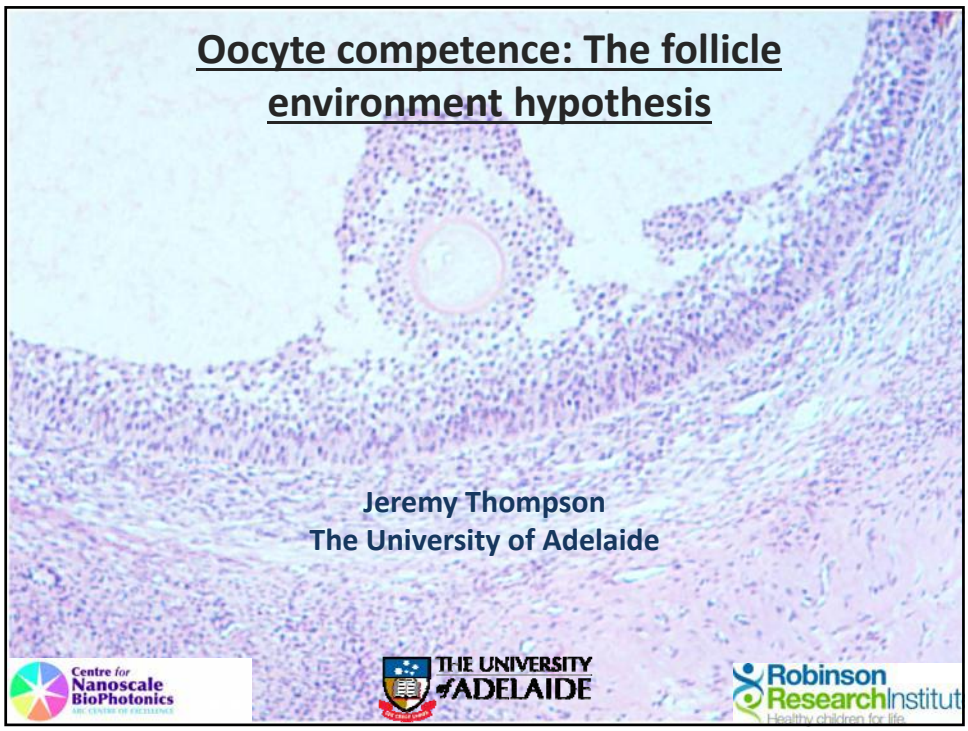
Genesis Genetics
Dr Tony Gordon



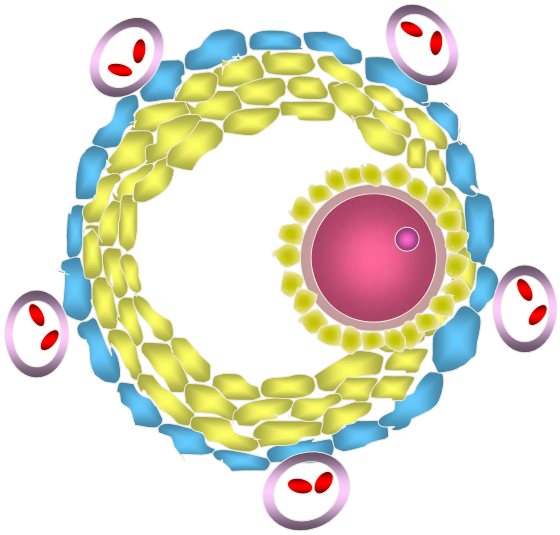
Conflicts of Interest Statement

The University of Adelaide receives consultation funds from Cook Medical LLC for use by Jeremy Thompson for research expenditure.

Learning objectives

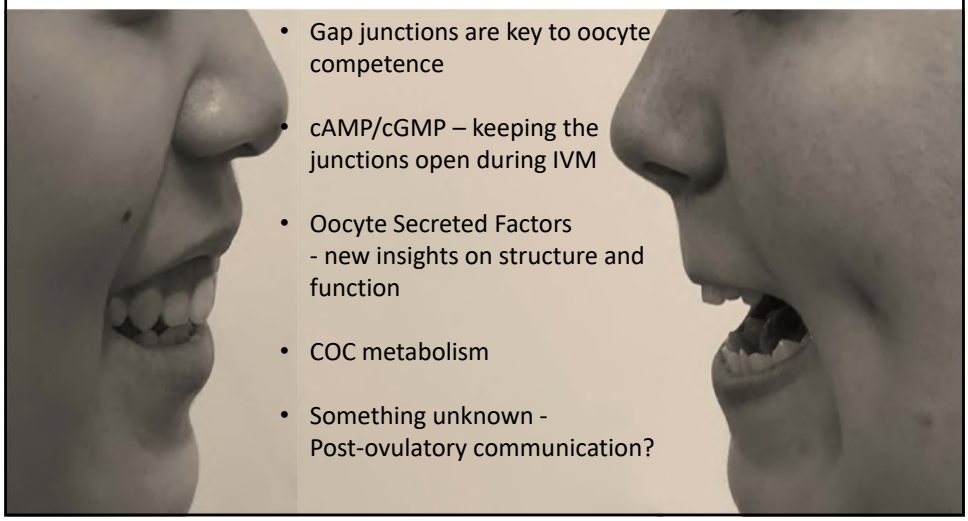
- Role of gap junctions in follicle communication with oocyte and its importance to oocyte competence
- Role of cAMP/cGMP in determining oocyte competence
- Role of oocyte secreted factors in determining oocyte competence
- Role of metabolism
- Question: is there such a thing as post-ovulatory follicular cell signalling?

Communication in the follicle is everywhere



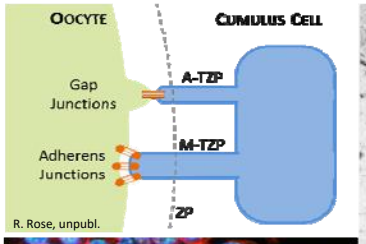
Communication across
- Stroma
- Basal lamina
- Mural granulosa cells
- Antral fluid
- Cumulus
- Oocyte

Cumulus – oocyte communication
It's a conversation…..and both sides benefit!

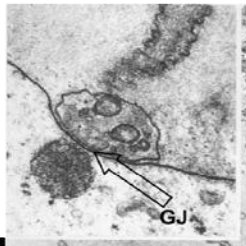


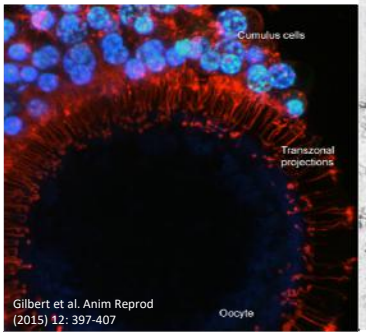
- Gap junctions are key to oocyte competence
- cAMP/cGMP – keeping the junctions open during IVM
- Oocyte Secreted Factors
  - new insights on structure and function
- COC metabolism
- Something unknown - Post-ovulatory communication?

Cumulus cell – oocyte communication
Gap junctions are key



R. Rose, unpubl.

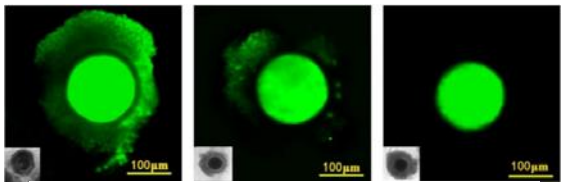


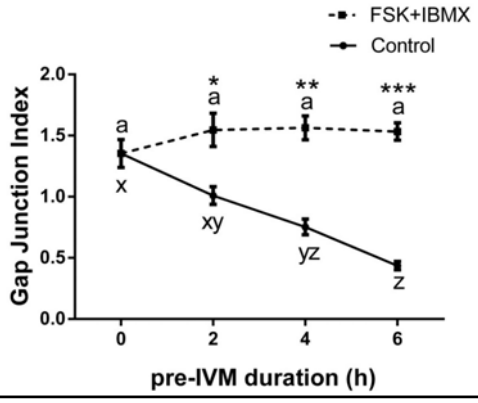


Gilbert et al. Anim Reprod (2015) 12: 397-407

Gilchrist et al. (2008) Hum Reprod Upd. 14: 159-77.

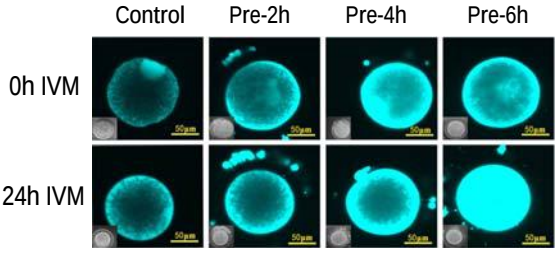
Gap junction communication can be measured

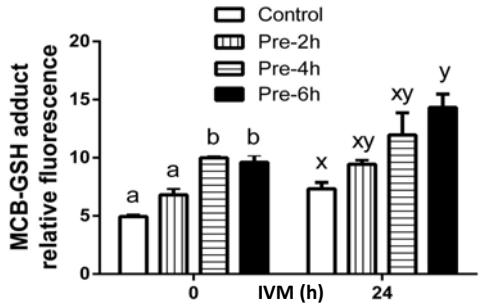




Li et al. (2016) Hum Reprod. 31:810-821

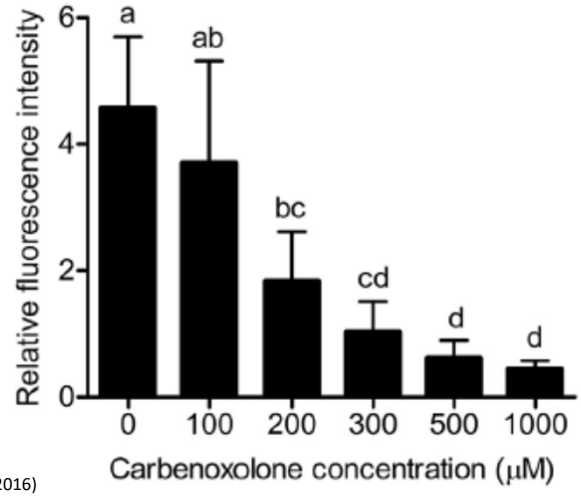
Gap junctions allow molecules to accumulate from cumulus to oocyte





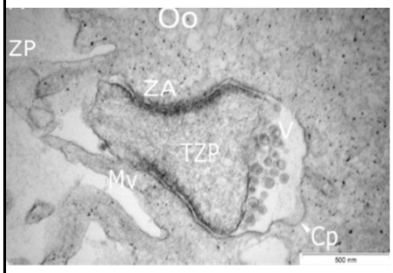
Li et al. (2016) Hum Reprod. 31:810-821

Gap junction communication can be blocked



Campen et al. (2016)
Mol Cell Endo 420:46-56

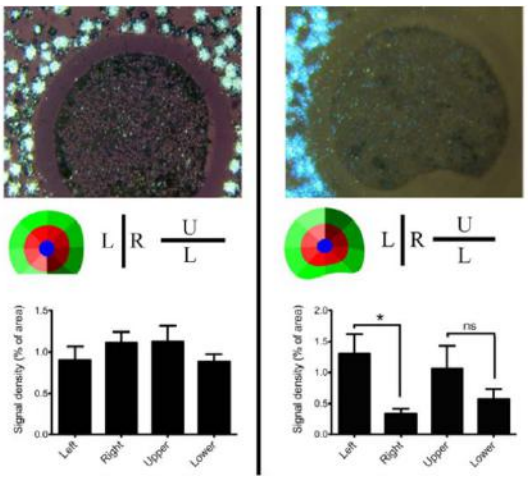
More than small molecules from the cumulus to oocyte?



Macauley et al.
(2014) Biol Reprod.
91: 90

Cumulus-enclosed

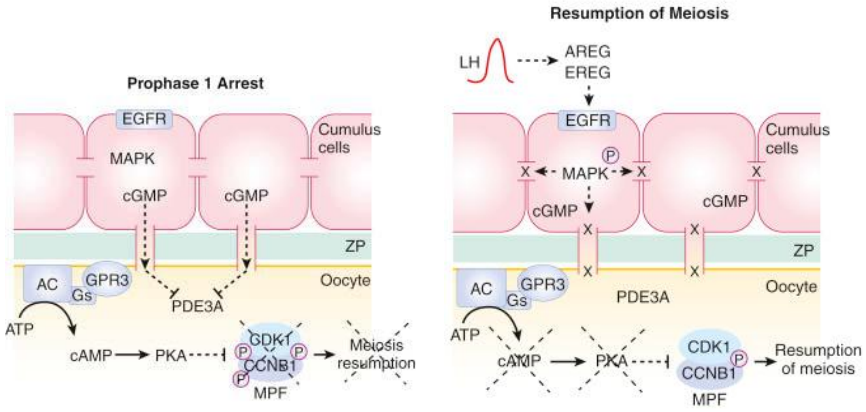
Hemi-denuded



RNA's

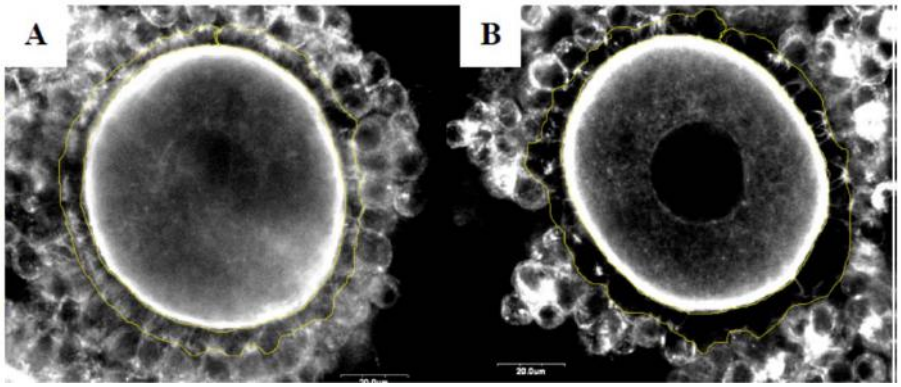
Macauley et al.
(2016) Biol Reprod.
94: 16

Keeping the gap junctions open during IVM



Straus & Williams. *In: Yen & Jaffe's Reproductive Endocrinology (Seventh Edition),* 2014.

Keeping the gap junctions open during IVM



Romero et al. 2016 Biol Reprod 95:64

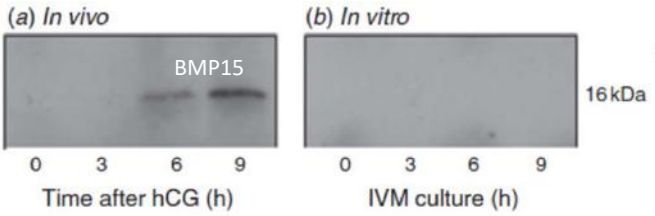
Oocyte Secreted Factors
- their structure is important

Maturation system

In vivo  **75%**

In vitro  **35%**

In vitro + OSFs



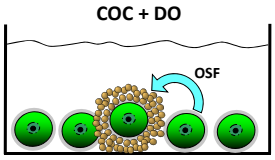
%

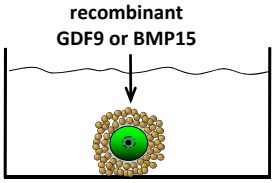
Mester et al (2015) RFD 27: 801-11

Oocyte-secreted factors enhance oocyte developmental competence

Tamer S. Hussein, Jeremy G. Thompson, Robert B. Gilchrist*

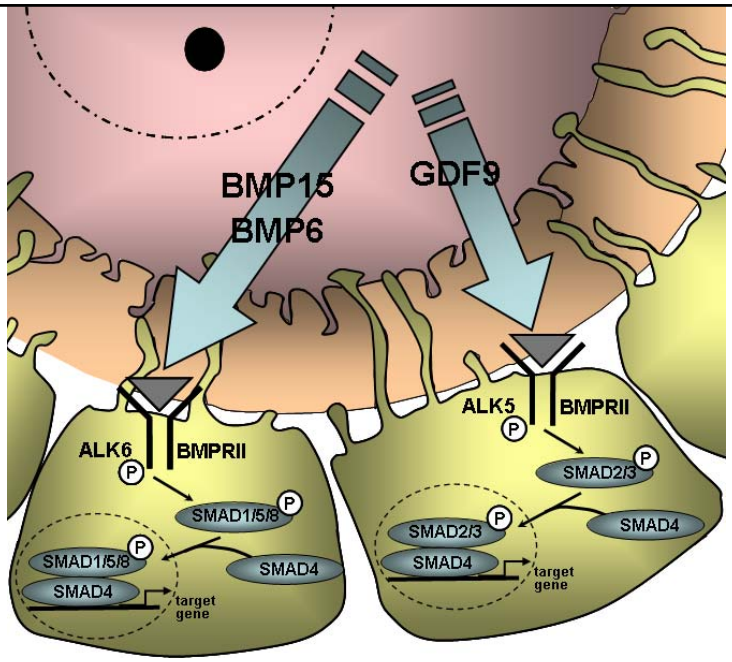
Research Centre for Reproductive Health, Discipline of Obstetrics and Gynaecology, The University of Adelaide, The Queen Elizabeth Hospital, South Australia 5011, Australia





- Bovine:
  - *Hussein TS (2006) Dev. Biol.*
  - *Hussein TS (2011) RFD*
  - *Dey SR (2012)*
  - *Sugimura S (2014) MHR*
- Murine:
  - *Sudiman J (2014) JARG*
- Caprine:
  - *Romaguera R (2010)*
- Porcine:
  - *Gomez MNL (2012)*

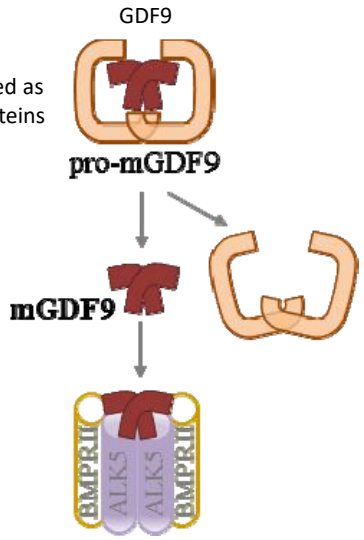
- Bovine:
  - *Hussein TS (2006) Dev. Biol.*
  - *Hussein TS (2011) RFD*
  - *Sutton-McDowall ML (2012) BOR*
  - *Sugimura S (2014) MHR*
  - *Sudiman J (2014) PLoS1*
  - *Sutton-McDowall ML (2015) BOR*
- Murine:
  - *Yeo CX (2008) Hum. Reprod.*
  - *Sudiman J (2014) JARG*
- Porcine:
  - *Li JJ (2014) Mol. Endocr.*
  - *Sugimura S (2015) Dev. Biol.*

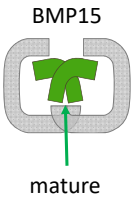


Gilchrist RB et al (2008)
Hum. Reprod. Update 14:159-177

GDF9 & BMP15 secreted as pro-mature protein

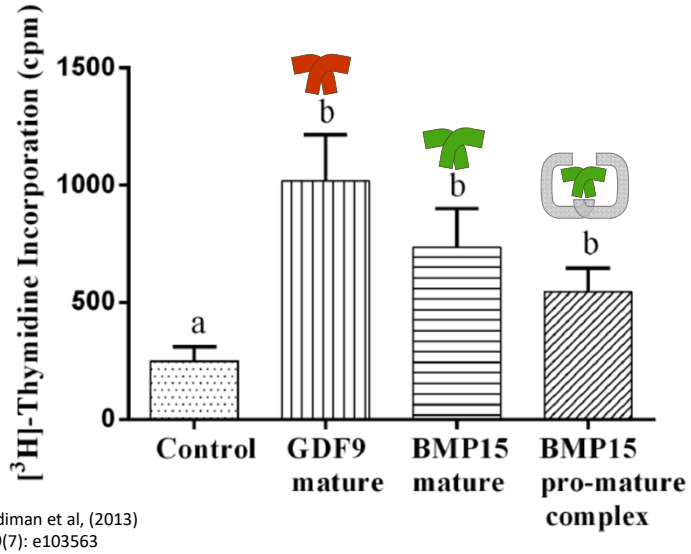
All TGFβ family proteins secreted as pro-mature proteins





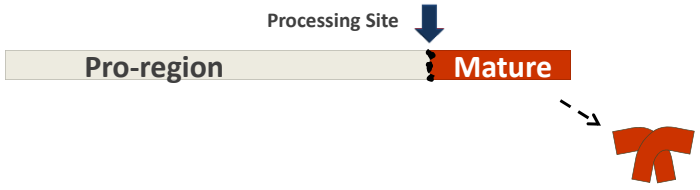
Simpson et al. (2012)
Endocrinology 153:1301–10

Mature proteins increase granulosa cell proliferation



Mouse: Sudiman et al, (2013) PLoS ONE 9(7): e103563

Mature homodimers of GDF9 and/or BMP15 do not improve oocyte quality



BMP15 or GDF9 mature region dimer (R&D Systems)

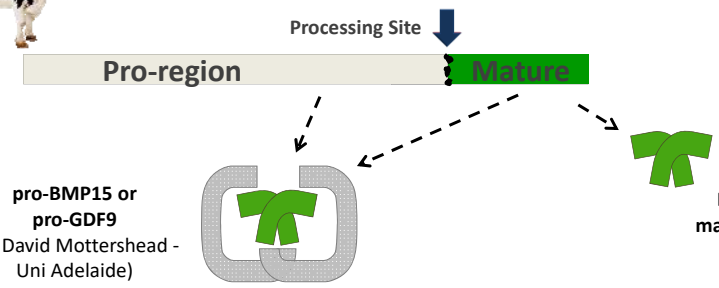
J Assist Reprod Genet

Table 3 Effect of graded doses of recombinant GDF9 during IVM on subsequent embryo development

Treatment	GDF9 (ng/ml)	Number of oocytes	Cleavage[a]	Blastocyst on day 6[b]	Hatching blastocyst on day 6[c]	ICM[d]	TE[d]	TCN[d]
Control	0	152	82.9±3.3	81.7±4.4	62.6±8.6	16.0±0.8	53.0±2.4	69.0±2.8
GDF9	50	118	74.6±5.2	72.6±8.9	61.8±8.9	16.8±1.1	54.2±3.6	71.0±4.4
GDF9	100	118	74.8±9.6	82.6±7.8	59.1±7.6	16.7±0.9	49.2±2.6	65.9±3.3
GDF9	200	147	86.1±4.7	82.5±8.3	64.3±7.1	17.9±0.7	56.8±3.5	74.8±3.9

Mouse: Sudiman et al, (2014) J. Assist. Reprod. Gen. 31:295-306

But pro-proteins improve oocyte quality



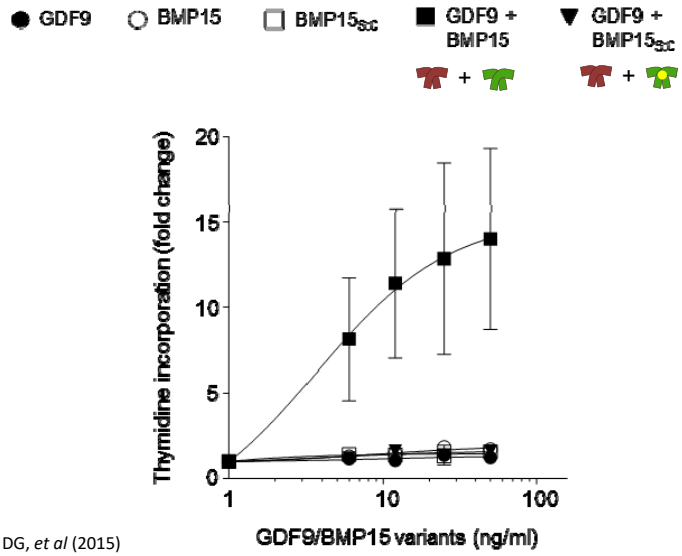
pro-BMP15 or pro-GDF9
(by David Mottershead - Uni Adelaide)

BMP15 or GDF9 mature region dimer
(R&D Systems)

	Form	Cleavage (%)	Blastocyst (%)
Control	-	94	43[a,b]
GDF9	mature	89	36[a]
BMP15	mature	92	50[b,c]
BMP15	pro-mature	89	58[c]

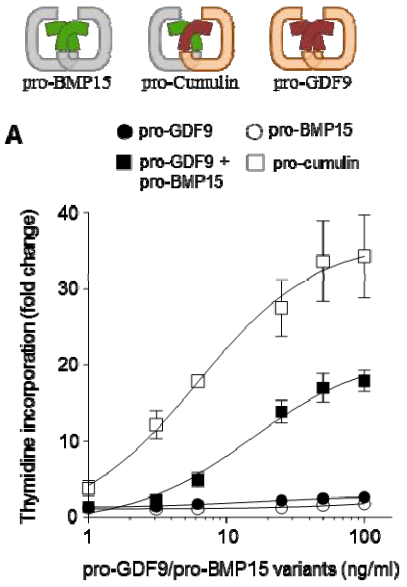
Bovine: Sudiman et al. (2014)
PLoS One 9(7):e103563

Low dose Pro-GDF9 and Pro-BMP15 synergism



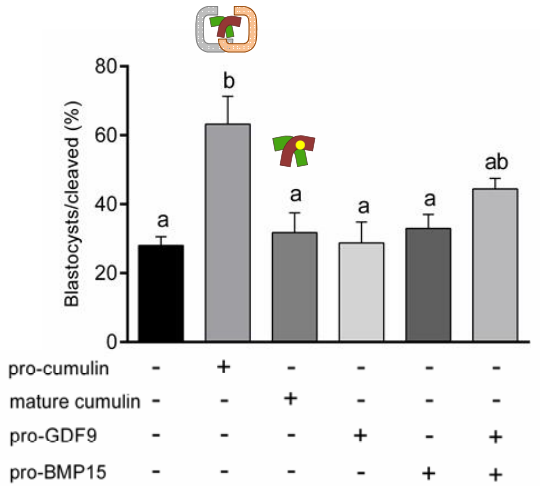
Mottershead DG, *et al* (2015)
J Biol Chem 290:24007–20

Pro-cumulin – A new potent OSF heterodimer



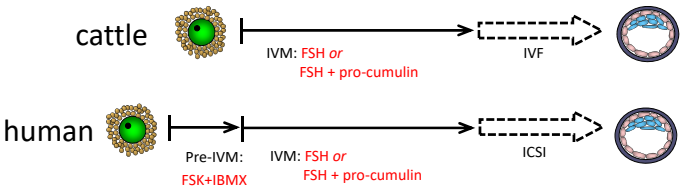
Mottershead DG, *et al* (2015) J Biol Chem 290:24007–20

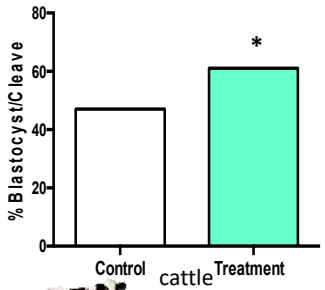
Pro-cumulin is a potent stimulator of gilt oocyte developmental competence



Mottershead et al (2015) J Biol Chem 290:24007–20

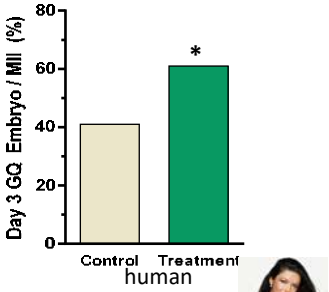
Pro-cumulin effective in other species: Embryo development in cow and human





Thompson et al
unpublished

*P<0.05



Gilchrist et al
unpublished

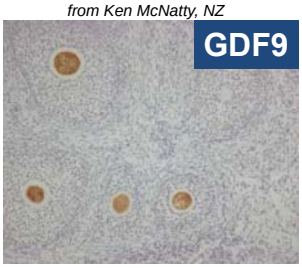
Is cumulin a natural GDF9 & BMP15 heterodimer?

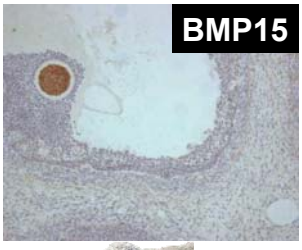
- **Genetic evidence:**
  - McNatty KP (2004); Yan C (2001); Hanrahan JP (2004)
- **Physical interactions of proteins:**
  - Liao WX (2003); McIntosh CJ (2008)
- **Functional studies:**
  - McNatty KP (2005a, 2005b); Mottershead DG (2012); Wigglesworth K (2013); Peng J (2014); Reader K (2016)

HOWEVER

A natural GDF9 and BMP15 form of a stable heterodimer, such as "Cumulin", has not been isolated from in vivo as yet

from Ken McNatty, NZ





The metabolic environment via FLIM

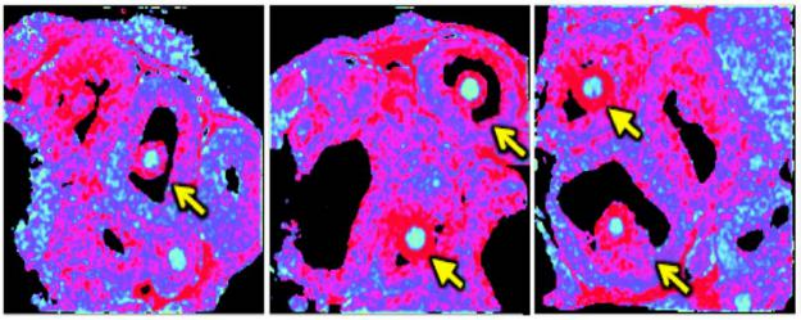
BIOLOGY OF REPRODUCTION (2016) 95(6):129, 1–12
Published online before print 28 September 2016.
DOI 10.1095/biolreprod.116.142141

Spatial Characterization of Bioenergetics and Metabolism of Primordial to Preovulatory Follicles in Whole Ex Vivo Murine Ovary[1]

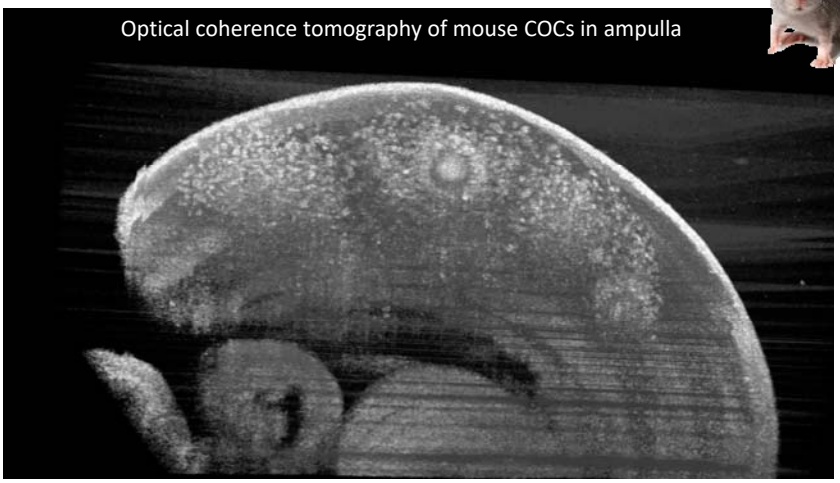
Rachel Cinco,[3] Michelle A. Digman,[4,5] Enrico Gratton,[4,5] and Ulrike Luderer[2,3,6,7]

More Bound NADH — More Free NADH

Bound NADH = Oxidative metabolism
Free NADH = Glycolytic metabolism



Post-ovulatory cumulus-oocyte communication?

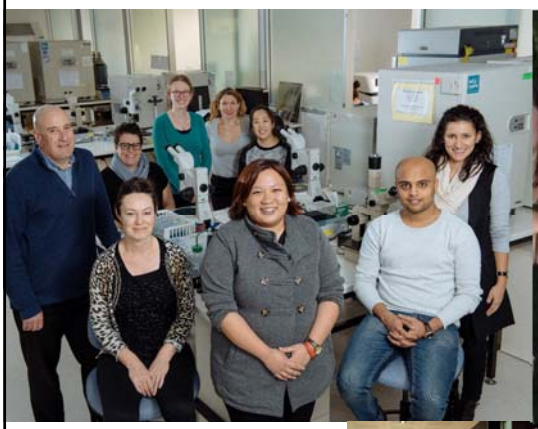


Kindly provided by Irina Larina, Baylor College of Medicine, Houston, USA

Conclusions

- Cumulus-oocyte communication is key to optimal oocyte competence
- Factors that enable prolonged cumulus-oocyte communication are:
  - Managing cGMP/cAMP levels during maturation – Pre-maturation is an additional step to achieve this
  - Oocyte secreted factors enhance communication - for IVM "Cumulin" is a new, powerful OSF
- Metabolic health of the COC is important
  - Also regulated by OSFs
- What communication occurs between cumulus-oocyte post ovulation?

Many thanks to:



Human cumulus cells molecular signature: Does it predict oocyte competence and embryo implantation potential?

Pr. Samir Hamamah

Chair: Reproductive Biology/PGD Department
Head: ART/PGD Division
Director: INSERM U 1203

ART/PGD Department
Arnaud de Villeneuve hospital
University-hospital of Montpellier
INSERM U 1203 'Early embryo developement and pluripontency'
Montpellier-34295, France

Conflict of Interest

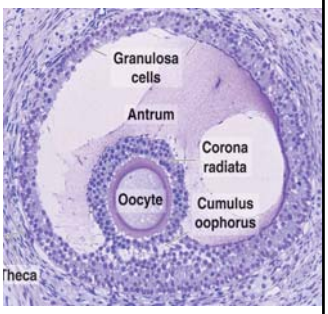
I declare that I have no commercial or financial interests in relation to the subject of this presentation or its content

Learning objectives

At the conclusion of this presentation, participants should be able to understand:

- The interest of cumulus cells (CCs)
- The knowledgment on human CCs
- The micro-RNA expression in CCs
- The impact of female aging on gene expression in human CCs
- The CCs as biomarkers of oocyte competence and pregnancy outcome

Concept to emerge today



Oocyte-cumulus complex

. Oocyte is not passive in ovarian follicule
. Fundamental regulator of somatic cell differentiation and function
. Oocyte-CC plays a central role in the regulation of folliculogenesis

Ovarian follicular microenvironment and maternal signals, mediated through GCs and CCs, are responsible for the gradual acquisition of oocyte competence

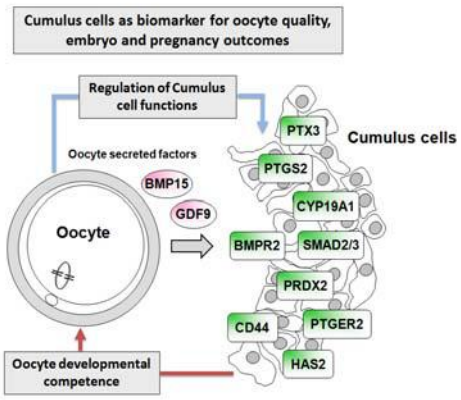
Why cumulus cells?

Table 1
Mouse models with defects in cumulus expansion. For more detailed information, refer to reference [4].

Gene (symbol)	Fertility status	Ref
Prostaglandin-endoperoxide synthase 2 (*Ptgs2*; Cox2)	Mostly infertile	[22,23]
Prostaglandin E receptor 2, subtype EP2 (*Ptger2*)	Subfertile	[24–26]
Pentraxin 3 (*Ptx3*)	Subfertile	[27,28]
Tumor necrosis factor α induced protein 6 (*Tnfaip6*)	Infertile	[29]
Sulfotransferase family 1E, member 1 (*Sult1e1*)	Subfertile	[30,31]
Alpha 1 microglobulin/bikunin (*Ambp*)	Subfertile	[32,33]
Amphiregulin (*Areg*)	Subfertile	[34]
Bone morphogenetic protein 15 (*Bmp15*)	Subfertile	[35]
Bone morphogenetic protein receptor, type IB (*Bmpr1b*)	Subfertile	[36]
Epiregulin ($Ereg^{wa2/wa2}$; hypomorph)	Subfertile	[34]
Mitogen-activated protein kinases 3 and 1 ($Mapk3^{-/-}$ *Mapk1* cKO)	Infertile	[37]
Nuclear receptor subfamily 5, group 2, member 1 (*Nr5a1*; Sf1, steroidogenic factor 1) (cKO)	Infertile	[38]
Nuclear receptor subfamily 5, group 2, member 2 (*Nr5a2*; Lrh1, liver receptor homolog 1) (cKO)	Infertile	[39]

cKO: conditional knockout.

Why cumulus cells?

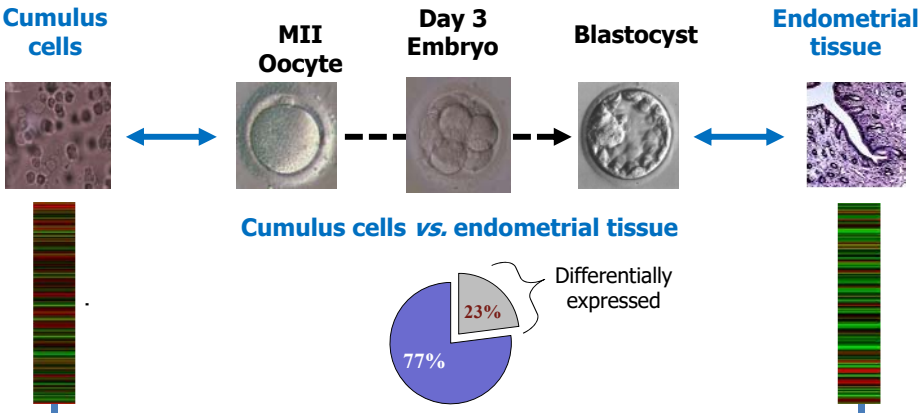


- Coordinates follicle development with oocyte maturation
- Provides energy substrate for oocyte meiotic resumption
- Regulates oocyte transcription
- Promotes nuclear and molecular maturation of the oocyte

Assou et al. 2010, MHR

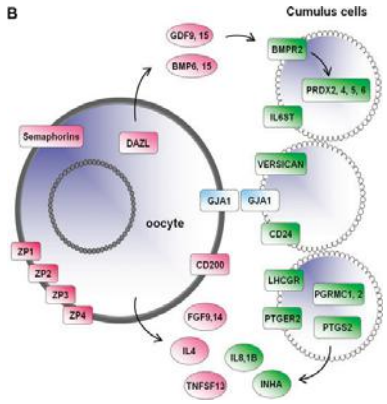
Oocyte and embryo within their niche

Somatic microenvironment



Both common and specific molecular signature between the two niches

WHAT ARE THE CUMULUS CELLS ?



Where ?
Surrounding oocyte cells

When ?
During oocyte maturation and first embryo cleavage

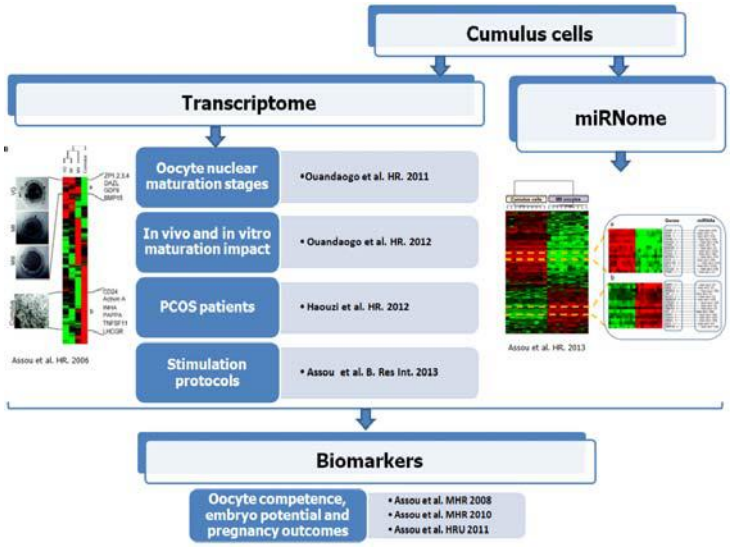
What role ?
Bi-directional communication

The human-cumulus oocyte complex gene expression profile

CC mirror of oocyte quality and competence

Assou et al. 2006 HR

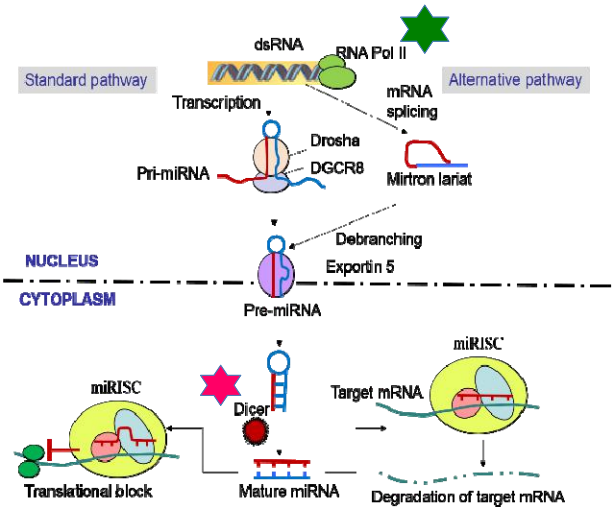
Knowledgment on human CCs



MicroRNAs: regulators of cellular functions

Background

MicroRNAs: biosynthesis and function



Joshi et al., 2011, modified

MicroRNAs are non coding sequences which are approximately 19-25 nucleotides in length

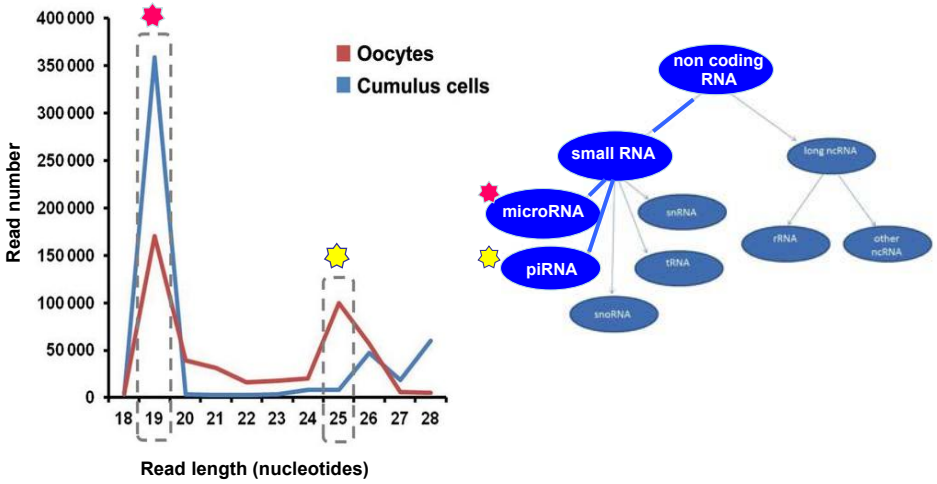
Hum. Reprod. Advance Access published July 30, 2013
Human Reproduction, Vol.0, No.0 pp. 1–12, 2013
doi:10.1093/humrep/det321

human reproduction ORIGINAL ARTICLE *Reproductive biology*

MicroRNAs: new candidates for the regulation of the human cumulus–oocyte complex

S. Assou[1,2], T. Al-edani[1,2], D. Haouzi[2], N. Philippe[2], C.-H. Lecellier[3], D. Piquemal[4], T. Commes[2,4], O. Aït-Ahmed[2], H. Dechaud[1,2,5], and S. Hamamah[1,2,5,*]

MicroRNA expression in cumulus cells and oocyte Important facts



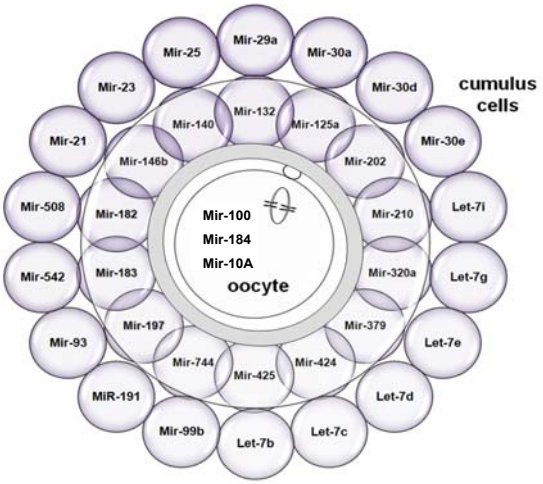
MicroRNA expression in cumulus cells and oocyte Important facts

3 miRNAs in MII oocytes

MIR184 (1988 reads)
MIR10A (555 reads)
} most abundant

32 miRNAs in cumulus cells

LET7b (51 reads)
MIR21 (28 reads)
} most abundant



Potential gene targets of the miRNAs identified by sequencing

Identified microRNAs: 32 in CCs and 3 in the oocyte

GeneGo MetaCore software

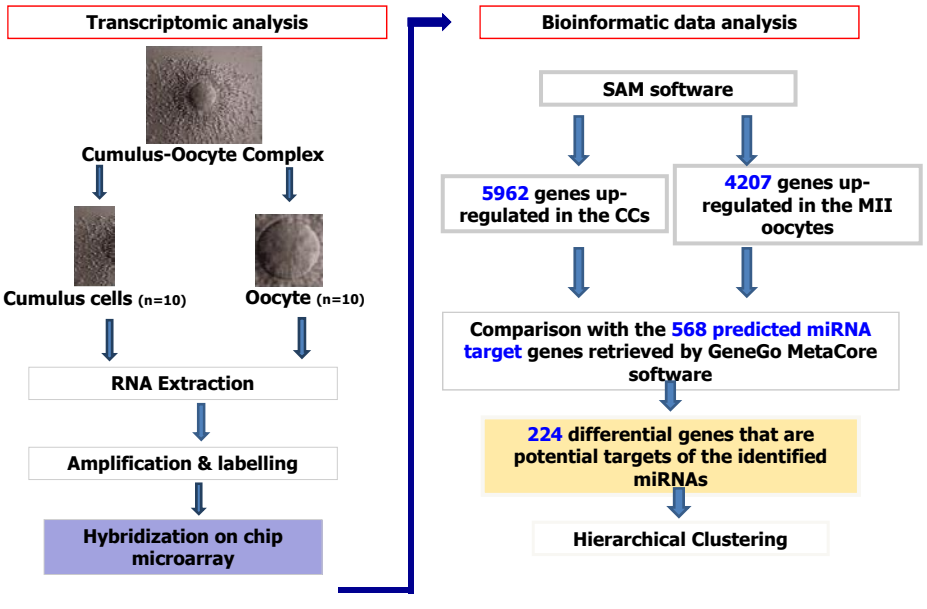
568 predicted miRNA target genes are retrieved

538 for the cumulus cells and 30 for the oocyte

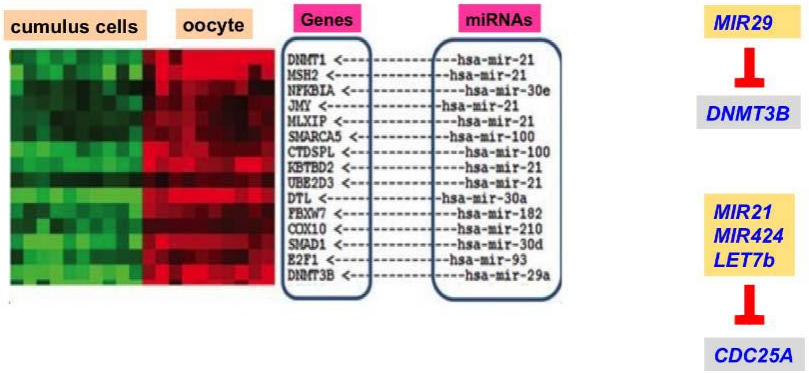
?

Among these genes, how many are differentially expressed between cumulus cells and the oocyte ?

Study design

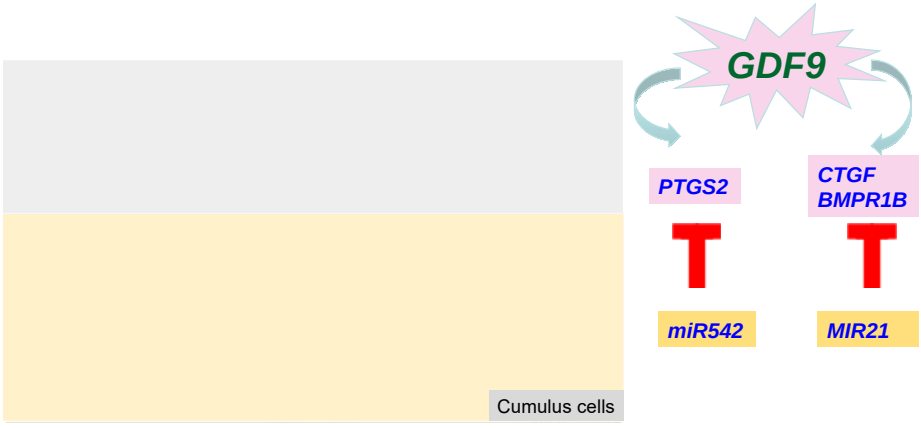


Important genes for chromosome or chromatin functions are up-regulated in the MII oocyte and are predicted targets of CC-miRNAs



This suggests a dialogue between the cumulus cells and the oocyte through microRNA action

Genes involved in a crosstalk between cumulus cells and oocyte are predicted targets of CC-miRNAs



GDF9 **is known as a regulator of** ***PTGS2, CTGF, BMPR1B*** **and to be essential in oocyte-cumulus cells crosstalk**

Conclusions

- We have reported the first sequencing data of small non coding RNAs in the human cumulus cells and oocyte.
- Our results illustrate the cellular specificity of the microRNAs. Some miRNAs are highly abundant in the oocyte and not even detected in the cumulus cells.
- CC-miRNA may be regulators of mRNAs over-expressed in the oocyte, illustrating the dialogue between cumulus cells and oocyte trough miRNA action.

The impact of female aging on gene expression in human cumulus cells

- Molecular signature according to female age
- Pathways significantly affected by female aging
- Predicted miRNAs that target genes impacted by female age

Female aging alters expression of human cumulus cells genes that are essential for oocyte quality.

Al-Edani, et al, 2014

Impact of female aging on oocyte quality

Decrease of proteins stored in the oocyte

mtDNA damage, reduction of ATP production
Reduction of oocyte metabolic function

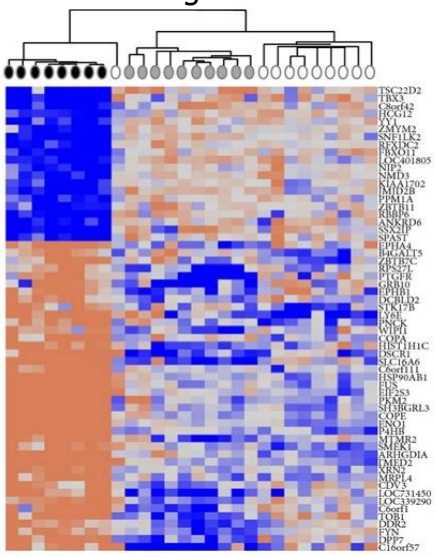
Increase of oxidative stress and apoptosis
Increase of oocyte aneuploidy

these modifications affect oocyte competence and quality

Bentov et al., 2011; Fragouli et al., 2010

The question is: which molecular changes account for these physiological changes ?
Impact of aging on gene expression in the cumulus cells

Molecular signature of cumulus cells according to female age



Identification of genes differentially expressed in the 3 age groups of cumulus cells

20 genes of each class with highest expression

Hierarchical Clustering

CC_{older} **CC >37 years**

CC_{median} **CC 31-34 years**

$CC_{younger}$ **CC <30 years**

Blue: Up-regulated genes
Pink: Down-regulated genes

Molecular signatures reveal that CC >37 years are distantly located from the other groups

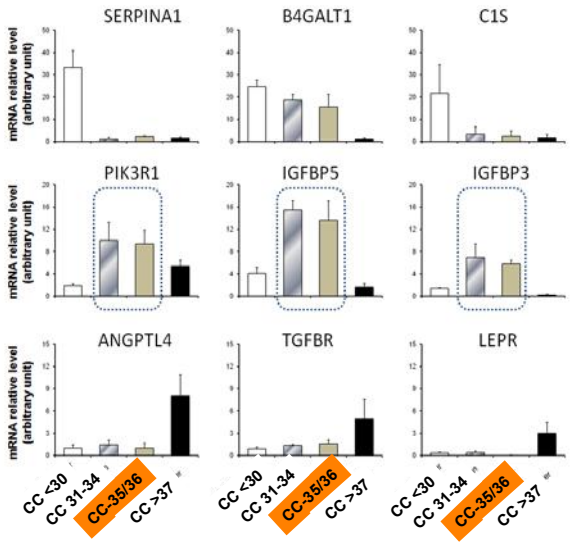
Al-Edani, et al, 2014

A molecular change occurs at age of 37 for essential biological processes

Inflammatory genes

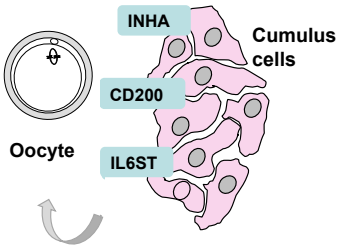
Insulin signaling pathway

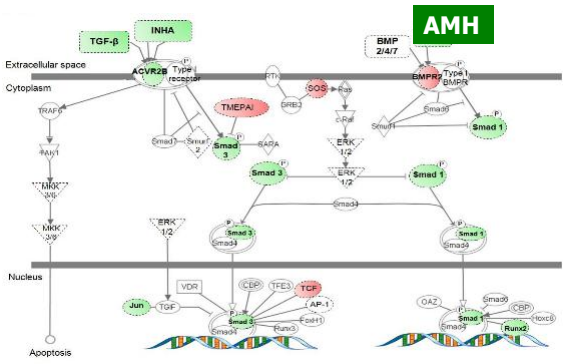
Angiogenesis



The 35/36 group behaves as the 31-34 group illustrating a molecular change at 37

Genes that play an essential role in the cumulus-oocyte dialogue and oocyte quality are down-regulated in older cumulus cells





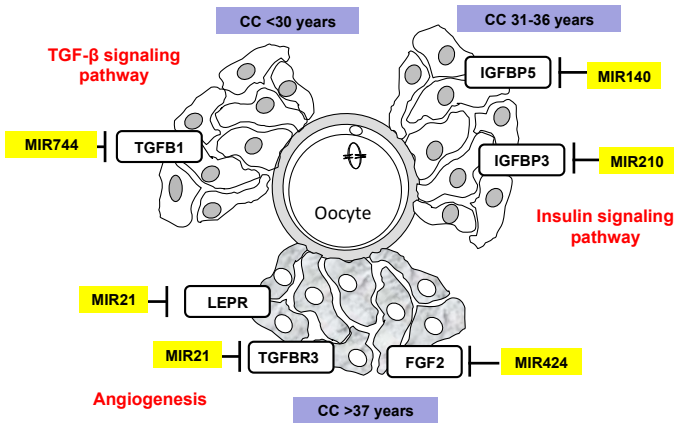
Many genes of the TGF-b pathway are down-regulated (green), a few of them are up-regulated (red)

Conclusions

Transcriptomic data show that:

- aging widely impacts gene expression
- the decrease in fertility that occurs at 37 is supported by a dramatic molecular change after the age of 36
- the physiological impact of aging is underlied by an alteration of expression of genes and pathways that are critical for oocyte quality and competence (insulin,TGF-beta etc)
- Genes that are essential to buffer the effect of hypoxia (angiogenic genes), which is linked with aging, are up-regulated in older CCs

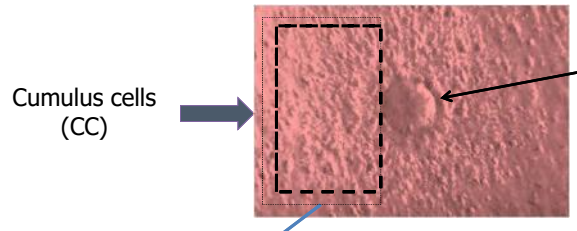
Genes impacted by aging in relation to CC-miRNAs



CC-miRNAs are validated regulators of the genes involved in pathways altered with aging

These miRNAs are potential biomarker candidates of follicle aging

Cumulus cells predictive value



As biomarker of oocyte potential and pregnancy outcome

Diagnostic tool in cIVF/ICSI

Expression of several genes in cumulus cells

COX2: cyclooxygenase 2

indicative of oocyte and embryo quality

GREM1 : gremlin 1,
HAS2 : hyaluronic acid synthase 2 ,
STAR : steroidogenic acute regulatory protein,
SCD1, 5: stearoyl-co-enzyme A desaturase 1 and 5,
AREG: amphiregulin,
PTX3: pentraxin 3

positively correlated with embryo quality

GPX3: glutathione peroxidase 3,
CXCR4: chemokine receptor 4,
CCND2: cyclin D2,
CTNND1: catenin delta 1

inversely correlated with embryo quality

McKenzie et al. Hum Reprod 2004
Zhang et al. Fertil Steril 2005
Feuerstein et al., 2007
van Montfoort et al., 2008

Gene expression profiling of human oocyte

Table I Microarray studies of oocytes and embryos.

Techniques	Samples	Number of identified genes	Targets	Study
Human oocytes and embryos				
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	1361 transcripts expressed in oocytes	Study oocyte transcriptomes	Bermudez et al. (2004)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	1514 overexpressed in oocytes compared with cumulus cells	Understanding of the mechanisms regulating oocyte maturation	Assou et al. (2006)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	533 transcripts enriched in metaphase II oocytes relative to somatic cells	Comprehension of genes expressed in in vivo matured oocytes	Kocabas et al. (2006)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	10 183 genes were expressed in germinal vesicle	Study of global gene expression in human oocytes at the later stages of folliculogenesis (germinal vesicle stage)	Zhang et al. (2007)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	Of the 8123 transcripts expressed in the oocytes, 374 genes showed significant differences in mRNA abundance in PCOS oocytes	Understanding of PCOS	Wood et al. (2007)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	—	Identify new potential regulators and marker genes which are involved in oocyte maturation	Gasca et al. (2007)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	283 genes found in the case report sample	Identify molecular abnormalities in metaphase II (MII) oocytes	Gasca et al. (2008)
Whole Genome Bioarrays printed with 54 840 discovery probes representing 18 055 human genes and an additional 29 378 human expressed sequence tags (EST)	Oocytes	2000 genes were identified as expressed at more than 2-fold higher levels in oocytes matured in vitro than those matured in vivo	Analysis of gene expression profile of oocytes following in vivo or in vitro maturation	Jones et al. (2008b)
Applied Biosystems Human Genome Survey Microarray (32 878 60-mer oligonucleotide)	Oocytes	Germinal vesicle, in vivo-MII and IVM-MII oocytes expressed 12 219, 9735 and 8510 genes, respectively	Characterized the patterns of gene expression in germinal vesicle stage and meiosis II oocytes matured in vitro or in vivo	Wells and Patrizio (2008)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes	342 genes showed a significantly different expression level between the two age groups [women aged 36 years (younger) and women aged 37–39 years (older)]	Investigate the effect of age on gene expression profile in mature oocytes	Grondahl et al. (2010)
Two cDNA microarrays, each containing about 20 000 targets (representing in total ~29 778 independent genes according to Unigene Build 133)	Oocytes and embryos	1896 significant changes in expression following fertilization through Day 3 of development	Global analysis of the preimplantation embryo transcriptome	Dobson et al. (2004)
cDNA microarrays containing 9600 cDNA spots	Oocytes and embryos	184, 29 and 65 genes were overexpressed in oocytes, 4- and 8-cell embryos, respectively	Identify differential expression profiles of genes in single oocytes, 4- and 8-cell preimplantation embryos	Li et al. (2006)
Genome Survey Microarrays V2.0 (Applied Biosystems)	Oocytes and embryos	107 DNA repair genes were detected in oocytes	Identify the DNA repair pathways that may be active pre- and post-embryonic genome activation by investigating mRNA in human in vitro matured oocytes and blastocysts	Jaroudi et al. (2009)
HG-U133 Plus 2.0 array (Affymetrix)	Oocytes and embryos	5477 transcripts differentially expressed into transition from mature oocyte (MII) to 2-day embryo and 2989 transcripts differentially expressed into transition from 2- to 3-day embryo	Study of global gene expression in human preimplantation development	Zhang et al. (2009)

Continued

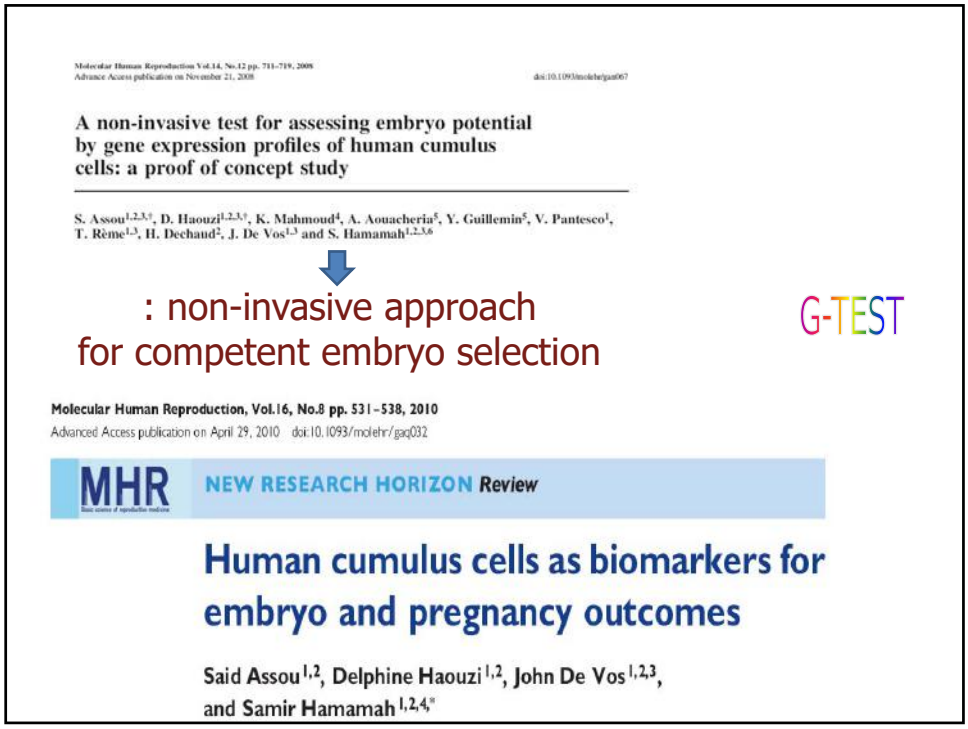
During IVM
According to female age
Under *in vivo or vitro* conditions
In PCOS patients

Assou et al. 2010, HRU

Studies analysing the genomics of CC or granulosa cells to identify reliable biomarkers for oocyte quality and competence, and for embryo development predictors

Biomarkers	Name	Function	Samples (individual or pooled)	Approaches	Outcome	Reference
STAR, AREG, Cx43, PTGS2, SCD1 and SCD5	Steroidogenic acute regulatory protein, amphiregulin, connexin 43, prostaglandin-endoperoxide synthase 2, stearoyl-CoA desaturase 1 and 5	STAR: regulated the cholesterol transport into the inner mitochondrial AREG: act as mediators of LH Cx43: permit the transfer of metabolites for growth and development and maintenance of meiotic arrest of the oocyte PTGS2: involved in inflammation and mitogenesis SCD: involved in biosynthesis of monounsaturated fatty acids from saturated acids	CCs from individual eggs	RT–PCR	Negatively associated with oocyte competence	Feuerstein *et al.* (2007)
HAS2, PTGS2, GREM1	Hyaluronan synthase 2, prostaglandin-endoperoxide synthase 2, gremlin 1		CCs from individual eggs	Quantitative RT–PCR	Positively associated with oocyte competence and embryo development	McKenzie *et al.* (2004)
BDNF, GREM1	Brain-derived neurotrophic factor, gremlin 1	BDNF: neurotrophic factor playing a role in regulation of stress response	CCs from individual eggs	Quantitative RT–PCR	Negative and positive predictors of embryo quality, respectively	Anderson *et al.* (2009)
PGK1, RGS2 and RGS3, CDC42	Phosphoglycerate kinase 1, regulator of G-protein signaling 2 and 3, cell division cycle 42	PGK1: involved in glycolysis RGS: hydrolyzed GTP to GDP	Mural GCs and CCs (individual)	Quantitative RT–PCR	Associated with pregnancy	Hamel *et al.* (2010)
VCAN, RPS6KA2, ALCAM, GREM1	Versican, ribosomal protein S6 kinase polypeptide 2, activated leukocyte cell adhesion molecule, gremlin 1	VCAN: plays a central role in tissue morphogenesis and maintenance RPSK6KA2: involved in the EGF signaling cascade ALCAM: involved in immune response	Pooled CCs from eggs	Quantitative RT–PCR	Correlated with oocyte maturity, low fragmentation and embryo development	Adriaenssens *et al.* (2010)

Assou et al. 2010, HRU



Molecular Human Reproduction Vol.14, No.12 pp. 711–719, 2008
Advance Access publication on November 21, 2008 doi:10.1093/molehr/gan067

A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study

S. Assou[1,2,3,†], D. Haouzi[1,2,3,†], K. Mahmoud[4], A. Aouacheria[5], Y. Guillemin[5], V. Pantesco[1], T. Rème[1,3], H. Dechaud[2], J. De Vos[1,3] and S. Hamamah[1,2,3,6]

: non-invasive approach
for competent embryo selection

G-TEST

Molecular Human Reproduction, Vol.16, No.8 pp. 531–538, 2010
Advanced Access publication on April 29, 2010 doi:10.1093/molehr/gaq032

MHR NEW RESEARCH HORIZON Review

Human cumulus cells as biomarkers for embryo and pregnancy outcomes

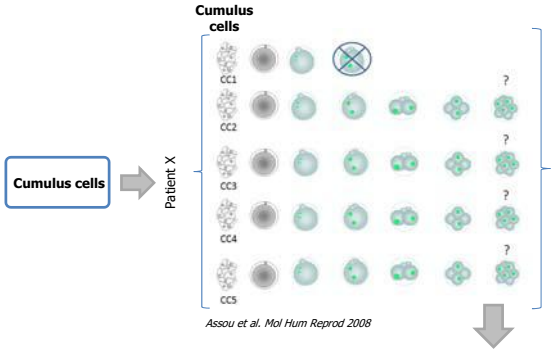
Said Assou[1,2], Delphine Haouzi[1,2], John De Vos[1,2,3], and Samir Hamamah[1,2,4,*]

Genomic TEST

NON INVASIVE TEST FOR EMBRYO SELECTION

G-TEST :

A NON INVASIVE TEST TO SELECT EMBRYO(S) WITH THE BEST POTENTIAL



What is the best embryo ?

RT-qPCR → 45 biomarkers

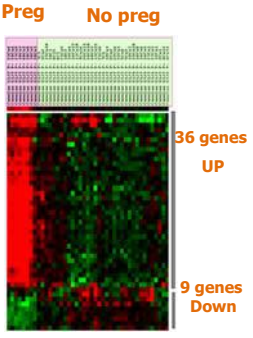
Algorithm prediction

Patent EP2010/054714 : « Methods for selecting oocytes and competent embryos with high potential for pregnancy »

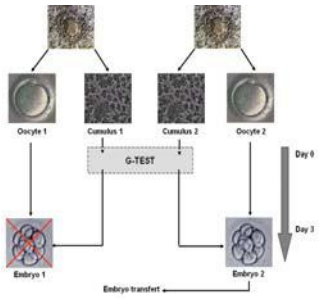
Embryo scoring (1 to 100) rank embryo accordind its competence for transfer

Cumulus cells biomarkers reflect oocyte and embryo developmental competence

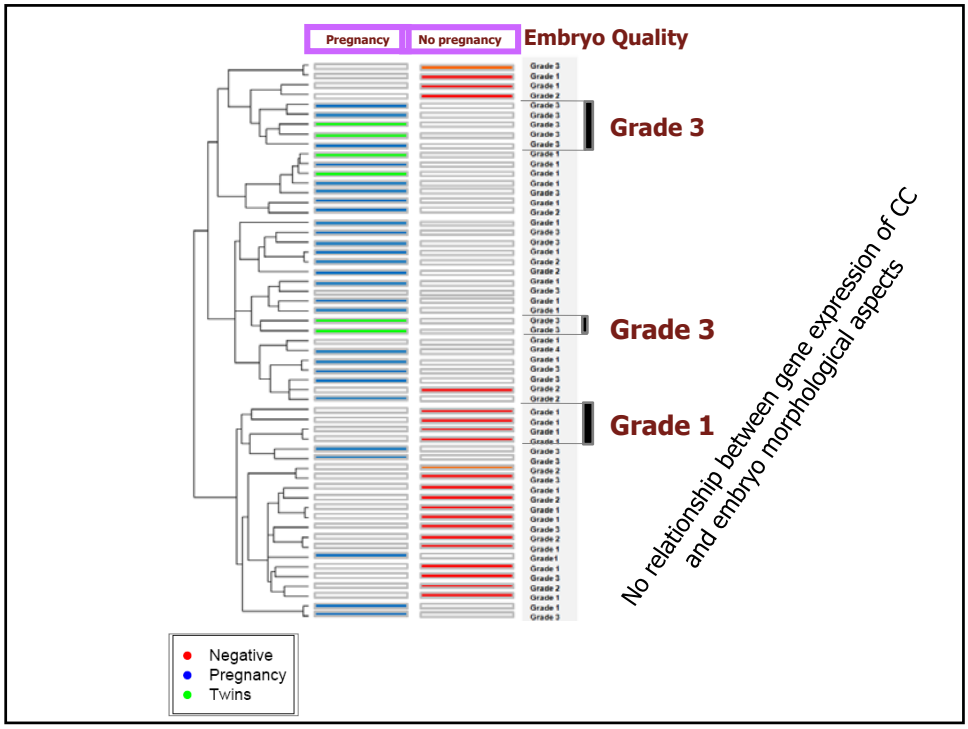
45 biomarkers



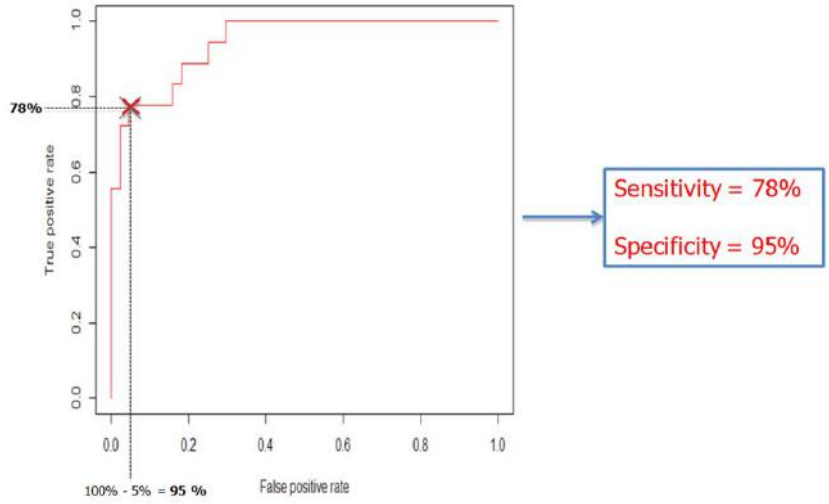
Clinical applications



⇨ Identify potential biomarkers of oocyte competence and pregnancy outcome that are expressed in CC

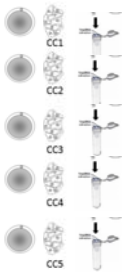


PREDICTIVE MODEL



REAL TIME PCR TECHNOLOGY

Patient X

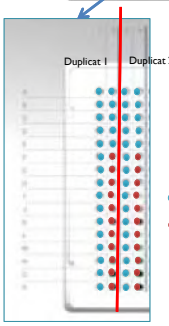


RNA extraction

RT-qPCR

ALGORITHM

EMBRYO SCORING



45 genes
Internal control

Cumulus cells	Score	Error
COC n° 5	90	0,18 %
COC n ° 3	80	0,22 %
COC n° 1	60	0,19 %
COC n° 4	56	0,30 %

STATE OF THE ART

OOCYTE MATURATION → 13 STUDIES

EMBRYO DEVELOPMENT → 15 STUDIES

PREGNANCY → 15 STUDIES

LIVE BIRTH → 4 STUDIES

META-ANALYSIS

Comparaison of 29 studies

No common gene in microarray analysis

11 genes in common in RT-qPCR analysis

	Oocyte maturation	Embryo competence	Pregnancy	Live birth
VCAN	+	+	+	+
PTGS2	+	+	+	
PTX3	+	+	+	
ALCAM	+	+		
GREM1	+	+		
HAS2	+	+		
RGS2		+	+	
BMP15		+	+	
GDF9		+	+	
STC2		+		+
SERPIN2	+		+	

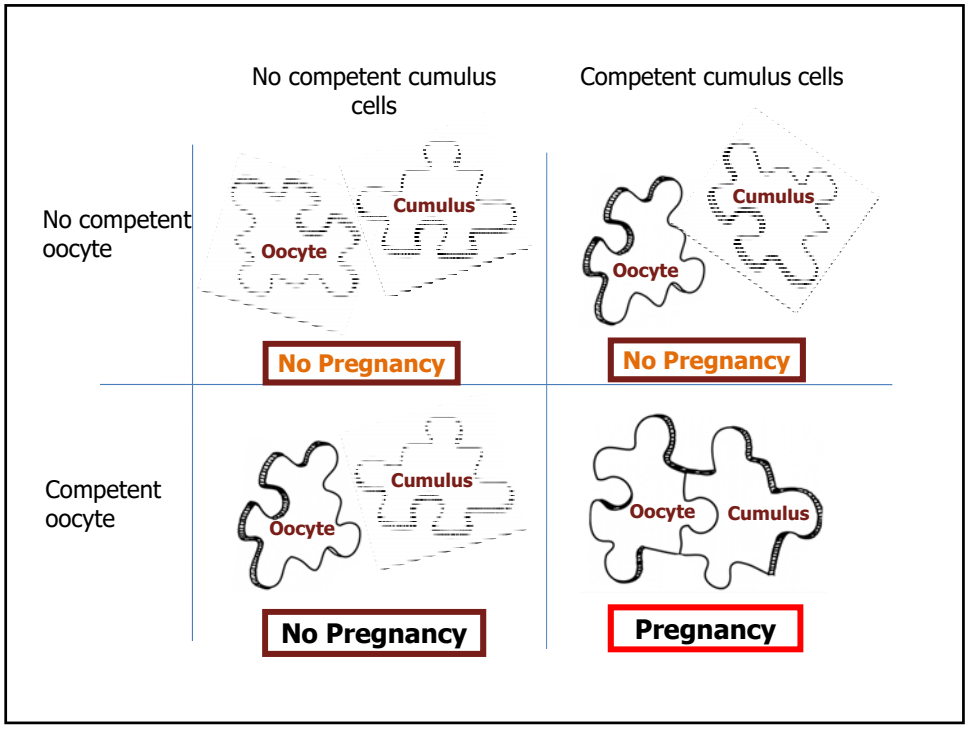
Reasons for this discrepancy between studies

Technical aspects

Number of samples
Type of DNA microarrays
Fold change thresholds
Statistical methodologies
Variability inter-platform
Variability inter-laboratory
Microarrays *vs.* selected candidates
…

Heterogeneous populations

Patient characteristics (age, BMI, diagnosis…)
Multiple stimulation types



Conclusions

Omics provides us with the opportunity to analyse human oocytes and CCs expression profiles on a genome scale and permitted significant progress in the understanding of the molecular events involved in the processes governing oocyte maturation.

Many of the genes described here are biomarkers to monitor health, viability and competence of oocytes.

Analysis of CC surrounding the oocyte can be a non-invasive approach for oocyte, embryon selection and pregnancy outcome

G-test: is a novel concept, providing a new potential strategy for competent oocyte and embryo selection

Conclusions

From basic discoveries into clinical applications

To improve efficiency of IVF (higher pregnancy rates, lower cost per child born), by the establishment of SET and improved cIVF/ICSI results

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