Biology of Oocyte Maturation

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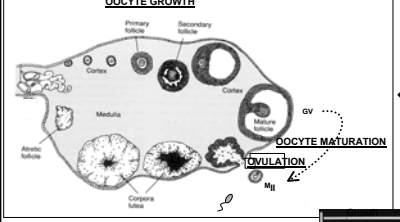
Oogenesis

OOCYTE GROWTH



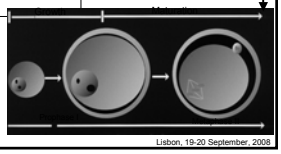
FERTILIZATION

Resumption of meiosis → PN formation
Embryo development

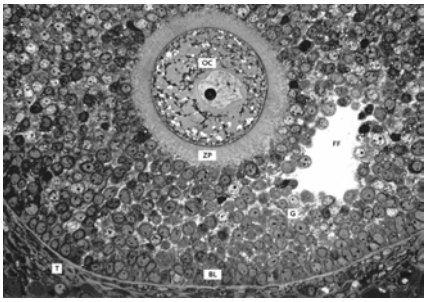
◆**Growth Phase**:
- Oocyte diameter increases
- Organelle redistribution
- High transcriptional and translational activity
- Accumulation of RNA / proteins
- Incompetent → Competent ooc.

◆**Oocyte Maturation**:
Complex series of nuclear and cytoplasmic events with resumption of the 1st meiotic division and **arrest at M_{II}** shortly before ovulation

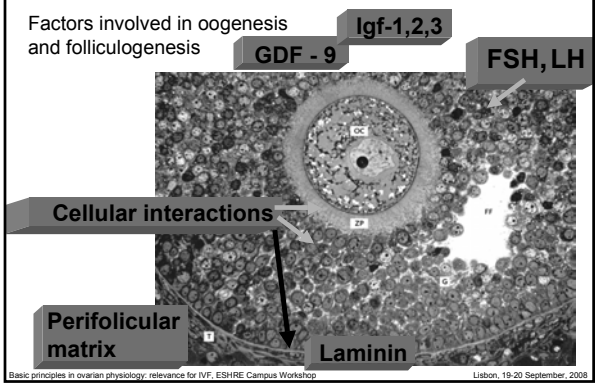
Ovulation



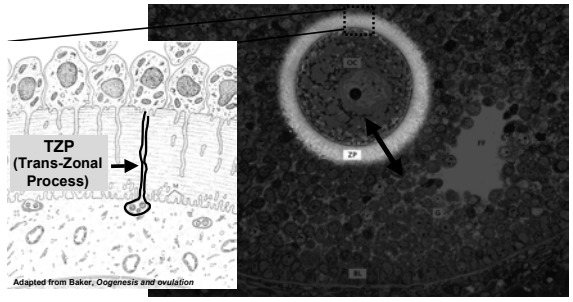
Oogenesis *in vivo* (including oocyte maturation) takes place inside a morfo-functional unit: **The Ovarian Follicle**



Factors involved in oogenesis and folliculogenesis

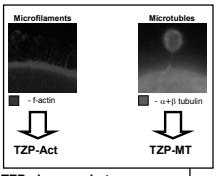


Somatic-germinal cellular interactions

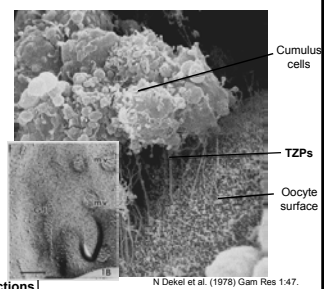


What are TZPs ?

Granulosa cell extensions that transverse the zona pellucida and terminate at the oocyte cell surface
(Hertig and Adams, 1967; Anderson and Albertini, 1976)



TZPs have an heterogeneous cytoskeletal content

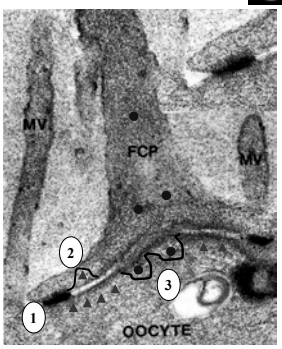


N Dekel et al. (1978) Gam Res 1:47.

Gap junctions

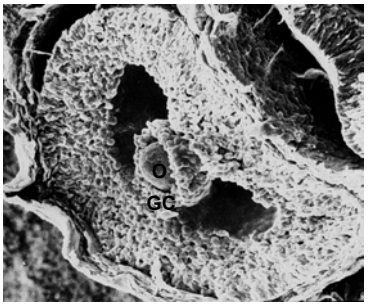
The oocyte-granulosa cell interface

1. Gap/adherens jxns
2. Oocyte exocytosis
3. GC exocytosis



Anderson and Albertini, 1976

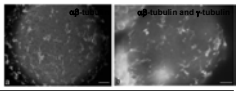
Antral ovarian follicle



In antral follicles, meiotic arrest is maintained by granulosa cell signaling

FSH modulate TZP phenotype in GCs

1. FSH-like priming reduces cumulus TZP-MT density

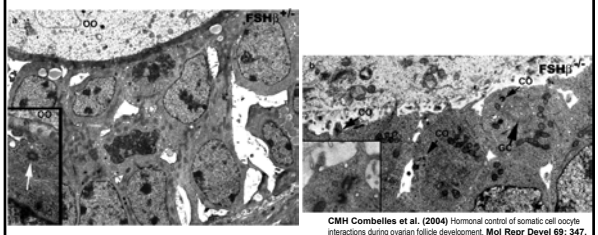


Genotype	Unprimed	Primed
FSHβ +/-	10.52 ± 0.75 (83)[a]	9.81 ± 0.61 (79)[a]
FSHβ -/-	24.3 ± 1.45 (74)[b]	8.94 ± 0.57 (83)[a]

CMH Combelles et al. (2004) Hormonal control of somatic cell oocyte interactions during ovarian follicle development. **Mol Repr Devel 69: 347.**

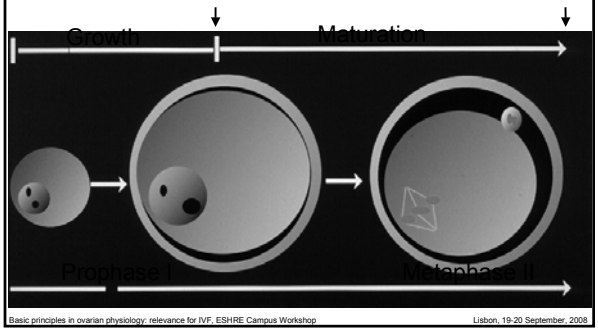
FSH modulate TZP phenotype in GCs

2. Absence of FSH enhances cumulus TZP-MT density

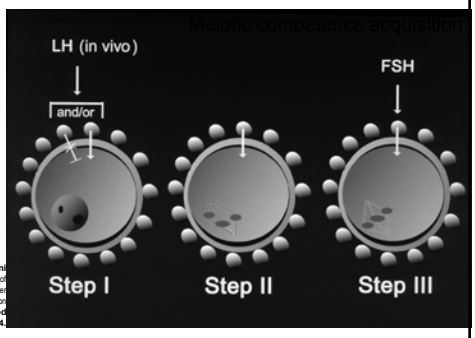


CMH Combelles et al. (2004) Hormonal control of somatic cell oocyte interactions during ovarian follicle development. **Mol Repr Devel 69: 347.**

Meiotic cell cycle progression



Lessons from the hamster model

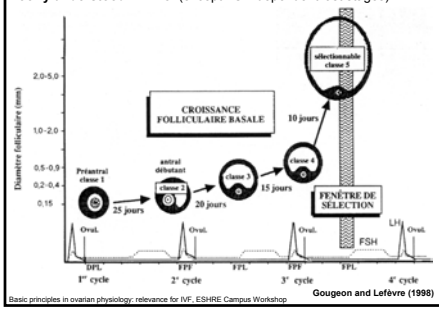


CE Plancha, DF Albertini (1994) Hormonal regulation of meiotic maturation in the hamster oocyte involves a cytoskeleton mediated process. **Biol Reprod 51:852-864.**

Ovarian follicle development in humans

Primordial → ~120-270 days → Preantral → ~85 days → ovulation

Poorly understood *in vivo* (except FSH-dependent last stages)



Gougeon and Lefèvre (1998)

***In vivo* oocyte maturation:**

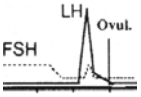
(natural cycle)

-Trigger: LH surge

-Duration: 36h

-Meiotic progression: from P_I to M_{II}

-Ovulation of M_{II} ooc.



Ovarian follicle development in humans

Primordial → ~120-270 days → Preantral → ~85 days → ovulation

ART

Assisted Reproduction Technologies

***In vivo* oocyte maturation:**

(ART cycle)

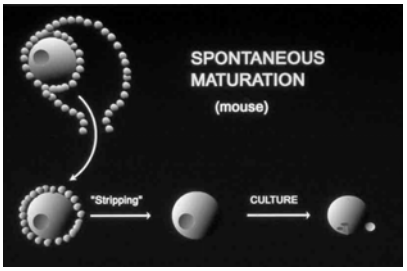
-Trigger: hCG or LH

-Duration: <36h

-Antral follicle aspiration of M_{II} ooc.

- Oocyte IVM was observed about 50 years before clinical IVF has started.
- These oocytes were initially considered to be similar to *In Vivo* matured ones.

Oocytes spontaneously resume meiosis after removal from the follicular environment



Pincus and Enzmann (1935) Comparative behaviour of mammalian eggs *in vivo* and *in vitro*. **J Exp Med 62:655**.

Mouse

However,

Efficiency of meiotic cell cycle progression is lower in IVM oocytes

Maturation Rates	
IVM	40%
IVO	99%

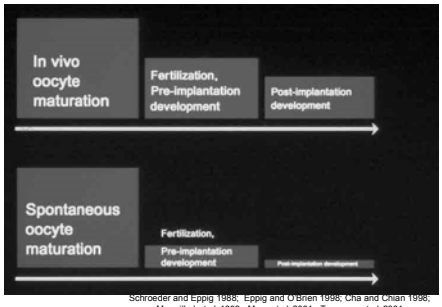
A Sanfins *et al.* (2003) Biol Reprod 69:2059-2067.

This means,

Signs of meiotic competence loss in IVM oocytes

Partial loss in oocyte quality in IVM

However, accumulating evidence shows that IVM oocytes exhibit less fertilization and developmental competence then *In Vivo* matured ones



Schroeder and Eppig 1988; Eppig and O'Brien 1998; Cha and Chian 1998; Mermillod *et al.* 1999; Moor *et al.* 2001; Trounson *et al.* 2001

Our group has been interested in finding both experimental and morphological evidence to explain functional differences between IVM and IVO oocytes, and to improve culture conditions

Morphological signs of oocyte polarity loss may explain the observed decrease in oocyte quality in IVM oocytes

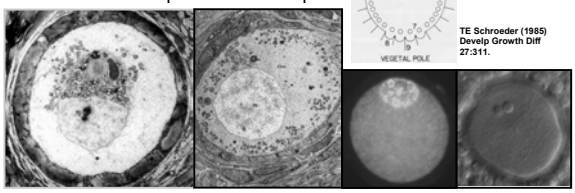
Oocyte polarity may be essencial for oocyte function

Oocyte polarity

Evidence in Mammals

Immature oocytes exhibit polarity
Eccentric GV specifies animal pole

TE Schroeder (1985) Develp Growth Diff 27:311.



Human primary oocyte in primordial follicle

Rabbit primary oocyte in primordial follicle

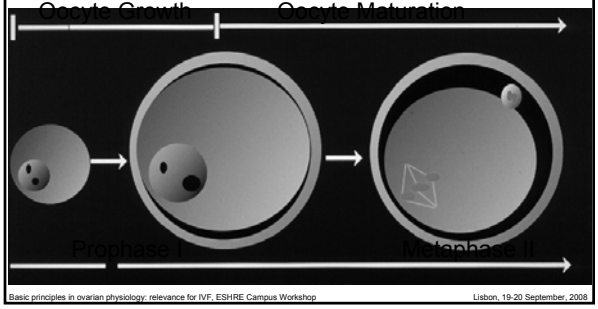
Hamster and **Mouse** full grown primary oocytes from antral follicles

"All animal eggs have a polar structure" **(Raven, 1961)**

Oocyte polarity

Evidence in Mammals

Asymmetric organization in oocytes intensifies during maturation

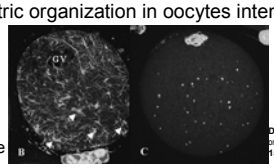


Oocyte polarity

Evidence in Mammals

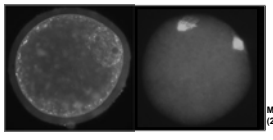
Asymmetric organization in oocytes intensifies during maturation

mouse



DF Albertini, SL Barrett (2004) The developmental origins of mammalian oocyte polarity. **Sem Cell Dev Biol 15:599.**

hamster

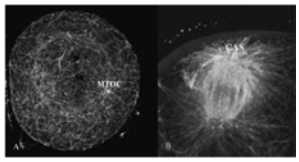


Mafalda Rato, CE Plancha (2005) Personal results

Oocyte polarity

Evidence in Mammals

Cortical anchoring of GV and meiotic spindle



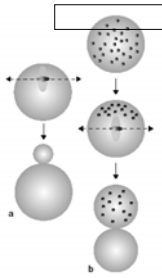
DF Albertini, SL Barrett (2004) The developmental origins of mammalian oocyte polarity. **Sem Cell Dev Biol 15:599.**

A Sanfins, DF Albertini, CE Plancha (2005) unpublished results.

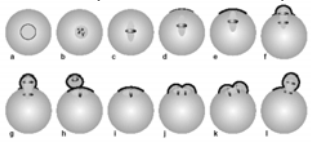
Oocyte polarity

Eccentric meiotic spindle determines polar body emission

Mechanisms of asymmetric cell division
. a) different volumes
. b) different components



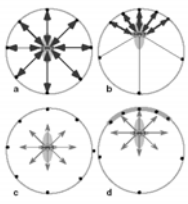
Polar body formation in mouse oocytes



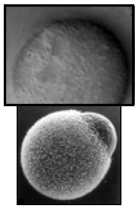
B Maro, M-H Verlhac (2002) Polar body formation: new rules for asymmetric divisions. **Nature Cell Biol 4:E281.**

Oocyte polarity

Eccentric positioning of the meiotic spindle requires formin-2



Putative role of formin-2 in polar body formation



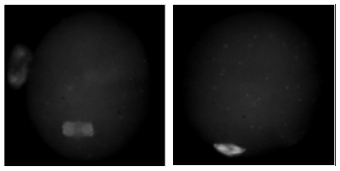
B Maro, M-H Verlhac (2002) Polar body formation: new rules for asymmetric divisions. **Nature Cell Biol 4:E281.**

Oocyte polarity

Signs of oocyte polarity loss in IVM oocytes

a. GV and spindle anchoring at the cortex is partially lost during IVM

IVM IVO

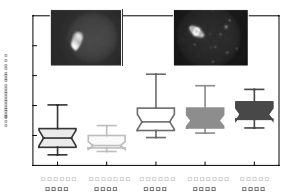


A Sanfins, DF Albertini, CE Plancha (2004) unpublished results.

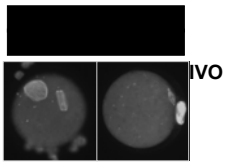
Oocyte polarity

Signs of oocyte polarity loss in IVM oocytes

b. Number of cytoplasmic MTOCs partially decreases during IVM



IVM IVO



A Sanfins et al. (2003) Distinctions in meiotic spindle structure and assembly during *in vitro* and *in vivo* maturation of mouse oocytes. **Biol Reprod 69: 2059.**

Oocyte polarity

Number of cytoplasmic centrosomes

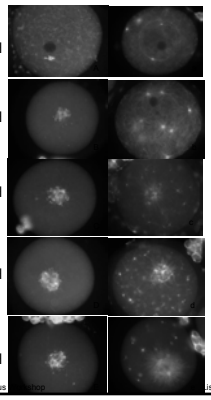
1H

2H

3H

4H

5H



Tubulin
Centrosomes
DNA

IVM

IVO

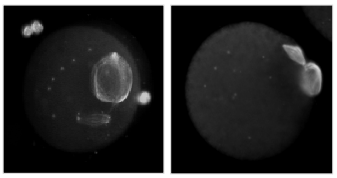
IVO: ++ MTOCs
IVM: - - MTOCs

A Sanfins et al. (2004) Meiotic spindle morphogenesis in *in vivo* and *in vitro* matured mouse oocytes: insights into the relationship between nuclear and cytoplasmic quality. **Hum Reprod 19: 2889-2998.**

Oocyte polarity

Signs of oocyte polarity loss in IVM oocytes

c. Retention of MTOCs and oocyte volume is partially lost during IVM

IVM  **IVO**

Polar Bodies formed *in vitro* contain MTOCs

Ibanez et al. (2005) Genetic strain variations in the metaphase-II phenotype of mouse oocytes matured *in vivo* or *in vitro*. **Reproduction 130:845-55**.

Oocyte polarity

SL Barrett, DF Albertini (2007) Allocation of gamma-tubulin between oocyte cortex and meiotic spindle influences asymmetric cytokinesis in the mouse oocyte. **Biol Reprod** 76:949-57.

Polar Bodies formed *in vitro* contain MTOCs

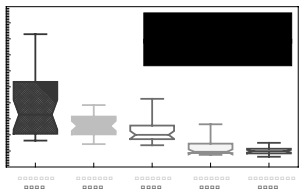
Polar Body Status	% of Total	% Int Mts	% W/ Spindles	% W/ MTOCs
IVO				
Undetectable/Degenerate	82.6	0	0	0
Present/ Not Dividing	13.5	13.5	0	0
Dividing/ Post Division	3.8	0	1.9	1.9
IVM Basal				
Undetectable/ Degenerate	0	0	0	0
Present/ Not Dividing	53	52.9	0	29.4
Dividing/ Post Division	47	11.8	35.3	17.6
IVM Hyped				
Undetectable/ Degenerate	16.6	0	0	2.3
Present/ Not Dividing	57.1	47.7	0	33.3
Dividing/ Post Division	28.6	2.3	23.8	23.8

IVM conditions do not promote stable MTOCs that bind to the oocyte cortex

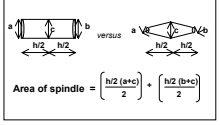
Oocyte polarity

Why is oocyte polarity important in ARTs?

d. Area of MII meiotic spindle increases during IVM



Quantification of Spindle Size and Shape



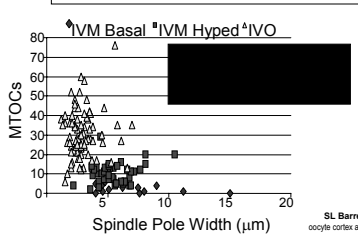
$$\text{Area of spindle} = \left(\frac{h/2\,(a+c)}{2}\right) + \left(\frac{h/2\,(b+c)}{2}\right)$$

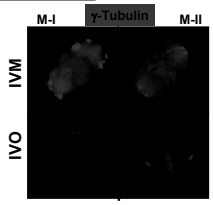
A Sanfins et al. (2003) Distinctions in meiotic spindle structure and assembly during *in vitro* and *in vivo* maturation of mouse oocytes. **Biol Reprod 69: 2059.**

Oocyte polarity

Why is oocyte polarity important in ARTs?

e. Pole width of MII meiotic spindle increases during IVM





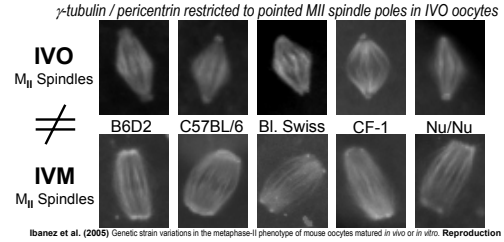
SL Barrett, DF Albertini (2007) Allocation of gamma-tubulin between oocyte cortex and meiotic spindle influences asymmetric cytokinesis in the mouse oocyte. **Biol Reprod** 76:949-57.

Oocyte polarity

Signs of oocyte polarity loss in IVM oocytes

d. Area
e. Pole width } of MII meiotic spindle increase during IVM

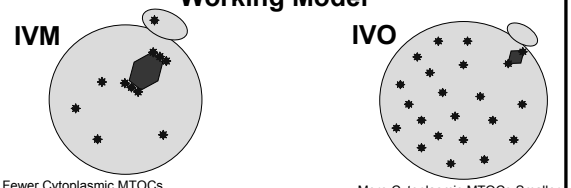
γ-tubulin / pericentrin restricted to pointed MII spindle poles in IVO oocytes



Ibanez et al. (2005) Genetic strain variations in the metaphase-II phenotype of mouse oocytes matured *in vivo* or *in vitro*. **Reproduction 130:845-55.**

Oocyte polarity and asymmetric division partially lost during IVM

Working Model



Fewer Cytoplasmic MTOCs
Larger Barrel Spindle
Larger PB
=
Low Glutathione, Low ATP
Embryonic failure

More Cytoplasmic MTOCs Smaller
Pointed Spindle Smaller PB
=
High Glutathione, High ATP
Embryonic Development

CE Plancha et al (2005) Cell polarity during folliculogenesis and oogenesis. **RBM online 10:478-484.**

Oocyte polarity

Why is oocyte polarity important in ARTs?

Oocyte polarity and efficiency of asymmetric meiotic division are partially lost during *in vitro* maturation

a. GV and spindle anchoring at the cortex
b. Number of cytoplasmic MTOCs
c. Retention of MTOCs and cell volume by oocyte
d. Area of meiotic spindle
e. Meiotic spindle pole width
f. functional differences? (meiotic, fertilization and developmental competence)

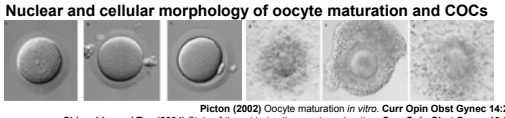
In vitro maturation of human oocytes (human IVM)

First successful IVM birth:
- from immature oocytes collected at cesarean section for oocyte donation.

Cha et al. (**1991**) Pregnancy after *in vitro* fertilization of human follicular oocytes collected from nonstimulated cycles, their culture *in vitro* and their transfer in a donor oocyte program. **Fertil Steril** 55:109-113.

IVM treatment have gained promise in ART since it diminishes the need of administrating fertility drugs to induce superovulation.

Nuclear and cellular morphology of oocyte maturation and COCs



Picton (2002) Oocyte maturation *in vitro*. **Curr Opin Obst Gynec 14:295-302**
Chian, Lim and Tan (2004) State of the art in *in-vitro* oocyte maturation. **Curr Opin Obst Gynec 16:211-219**

In vitro maturation of human oocytes (human IVM)

Although there are situations were IVM treatment is already recommended

Although there are situations were IVM treatment may turn out to be useful

IVM results need to be improved

Future directions

Clear need of basic Cell Biology studies on oocyte maturation in animal models and in humans, in order to integrate data into a coherent and scientific knowledge