Oocyte polarity: a sign of oocyte quality?

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DISCLOSURE

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Presentation outline

1. Oocyte quality: impact, acquisition and approaches
2. Oocyte polarity: a feature of oocyte maturation
3. Oocyte polarity: a reflection of oocyte quality?
4. Future challenges

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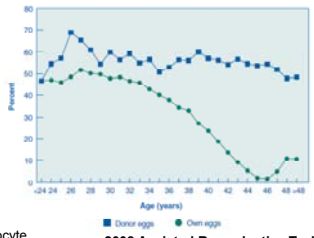
Oocyte Quality: a major determinant of **Embryo Quality** and **Reproductive Success**

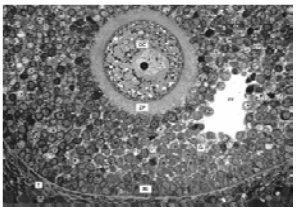
% Transfers that resulted in Live Births for ART Cycles using Fresh Embryos, from Own and Donor Eggs, by Woman's Age



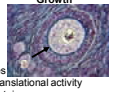
Oocyte Quality is an oocyte attribute of major relevance in the efficiency of the reproductive process, which strongly decreases with age !

2008 Assisted Reproductive Technology Report
Centers for Disease Control and Prevention, USA Department of Health
American Society for Reproductive Medicine
Society for Assisted Reproductive Technology
Dec 2010

During **Oogenesis** the oocyte acquires **critical funtionalities** for initial **Development**

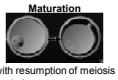


Growth



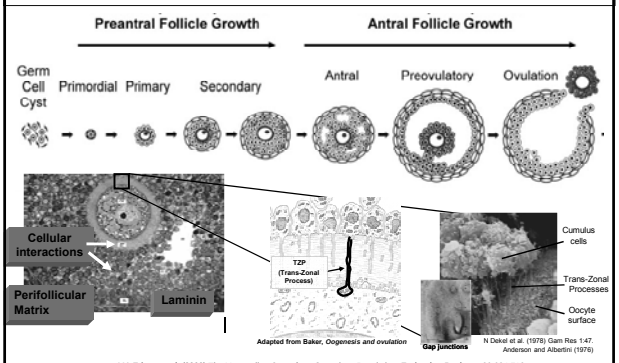
◆**Oocyte growth phase**:
- Oocyte diameter increases
- High transcriptional and translational activity
- Accumulation of RNA / proteins
- Building of new structures
- (zona pellucida, cortical granules)

Maturation



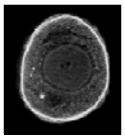
◆**Oocyte maturation phase**:
- Nuclear / cytoplasmic events with resumption of meiosis and arrest at M_{II} shortly before ovulation
- Organelle redistribution
- Cell polarity and asymmetric division

Successful oogenesis and folliculogenesis require **complex paracrine connectivity**



MA Edson et al. (2009) The Mammalian Ovary from Genesis to Revelation. Endocrine Reviews, 30:624-712.
SG Hillier (2009) Paracrine support of ovarian stimulation. Mol Hum Reprod 15: 843–850.

Oocyte Quality can be viewed as the aquisition of a series of competences during oogenesis



1. Meiotic
a. competence to reach the metaphase II arrest
b. competence to allow correct meiotic chromosome segregation

2. Activation
competence to fuse with sperm, finish meiosis,
block polyspermy, and form pronuclei

3. Developmental
competence to trigger and support embryonic development

Oocyte Quality is a very complex attribute !

- decreases strongly with age !

... and ...

- depends on the female clinical situation !
- varies within the same egg cohort !
- Can be affected by culture conditions !

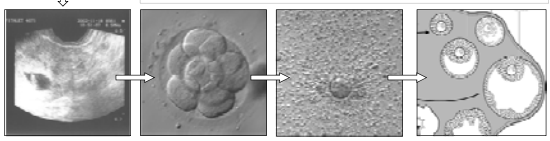
However, we infer Oocyte Quality a posteriori

Can we learn to predict Oocyte Quality ?

1. Learn from the oocyte / follicle that produced a viable embryo

Ongoing implantation ⇩

Individual culture - follow-up : find markers



⇧

Live healthy birth

METABOLIC MARKERS :
Production / Consumption
(H. Leese)

SECRETOME
(M. Katz-Jaffe)

CHROMOSOMAL ANALYSIS
PGS / PGD

CUMULUS CELL FUNCTION
Gene Expression
Metabolic Function

OOCYTE (Invasive)
Fluorescence Microscopy

OOCYTE (Non-Invasive)
Polscope

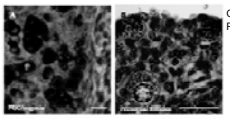
BIOMARKERS
Serum / Follicle Fluid

ULTRASOUND
Follic. Vol. Increase

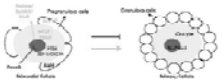
DOPPLER
Peri-follicular Vascular Function

2. Study and manipulate oogenesis in experimental models

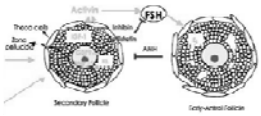
- early stages -



Oogonia cluster breakdown and
Primordial follicle formation



Primordial to primary follicle transition



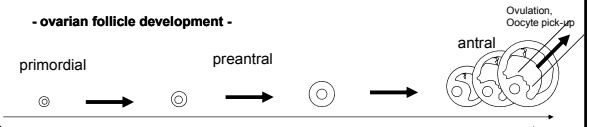
Preantral to antral follicle transition

Rodrigues et al. (2008) Oogenesis: prospects and challenges for the future. J Cell Physiol 216:355-365.

2. Study and manipulate oogenesis in experimental models

- late stages -

- ovarian follicle development -



Time

- weeks, months -

oocyte maturation

- hours -

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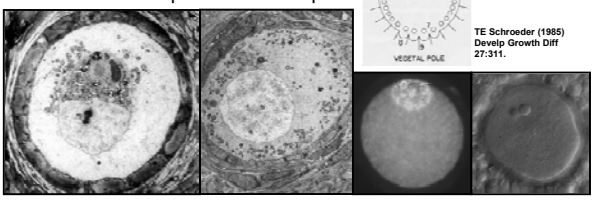
Immature oocytes exhibit polarity
Eccentric GV specifies animal pole

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



Human primary oocyte in primordial follicle

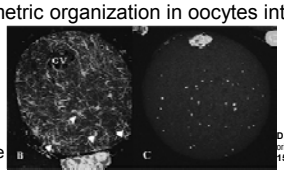
Rabbit primary oocyte in primordial follicle

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

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

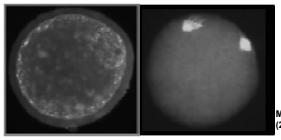
Asymmetric organization in oocytes intensifies with maturation

mouse

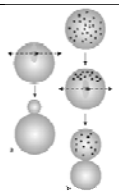


DF Albertini, SL Barrett (2004) The developmental origins of mammalian oocyte polarity. **Sem Cell Dev Biol 15:599.**

hamster



Mafalda Rato, CE Plancha (2005) Personal results



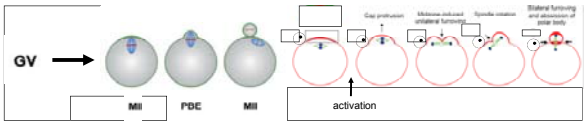
Mechanisms of asymmetric cell division

a) different volumes

b) different components

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

During oocyte maturation, asymmetry in the two divisions of meiosis ensures one big gamete and two small polar bodies

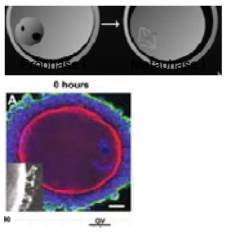


J Azoury et al. (2008) Spindle Positioning in Mouse Oocytes Relies on a Dynamic Meshwork of Actin Filaments. Current Biology 18:1514-1519.

Wang et al. (2011) Mechanism of the chromosome-induced polar body extrusion in mouse eggs. Cell Division 6:17.

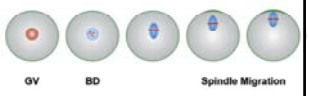
Two views on how the first meiotic spindle becomes cortically located

The first meiotic spindle starts from a cortical location



SL Barrett, DF Albertini (2010) Cumulus cell contact during oocyte maturation in mice regulates meiotic spindle positioning and enhances developmental competence. J Assist Reprod Genet 27:29–39.

The first meiotic spindle starts from a central position and moves towards a cortical location



S Brunet, B Maro (2007) Germinal vesicle position and meiotic maturation in mouse oocyte. Reproduction 133:1069-1072.

In any case at MI the first meiotic spindle is cortically located, with involvement of the actin microfilament system

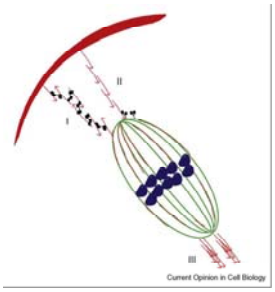


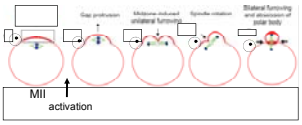
Diagram of possible mechanisms of actin-dependent spindle translocation.

(I) Contraction of anti-parallel actin filaments connecting the cortex to the spindle by bipolar myosin II mini thick filaments.
(II) Cargo-like transport of the spindle along actin filaments with barbed ends oriented toward the cortex, with myosin II localized at the spindle pole.
(III) Actin polymerization-driven motility from the rear.

Actin – red, Microtubules – green, Chromosomes – blue, Myosin II – black.

AS Fabritius, ML Ellefson, FJ McNally (2011) Nuclear and spindle positioning during oocyte meiosis. Curr Opin Cell Biol 23:78-84.

The second meiotic spindle seems cortically located from the beginning



Wang et al. (2011) Mechanism of the chromosome-induced polar body extrusion in mouse eggs. Cell Division 6:17.

Oocyte polarity ensures asymmetrical division of cytoplasmic content between the gamete and the polar bodies in oocyte maturation and activation

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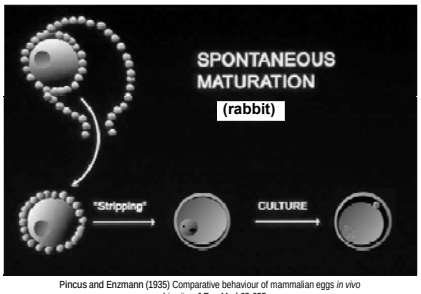
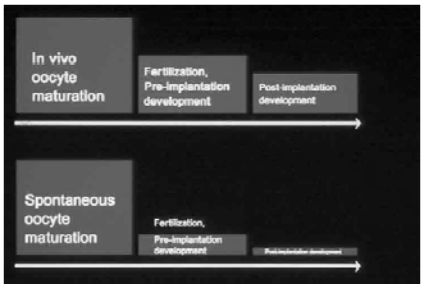
• Oocyte IVM was observed about 50 years before clinical IVF has started.

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.

IVM oocytes exhibit less fertilization and developmental competence then *In Vivo* matured ones (IVO)



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

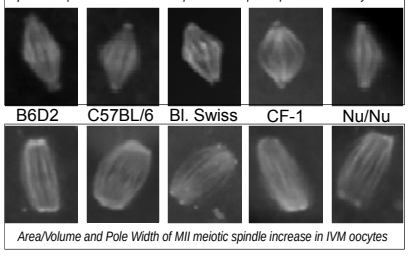
Strategies to find morphological evidence to explain functional differences between IVM and IVO oocytes

IVO
M_{II} Spindles

≠

IVM
M_{II} Spindles

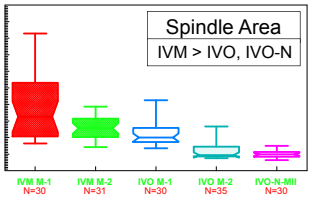
γ-tubulin / pericentrin restricted to pointed MII spindle poles in IVO oocytes



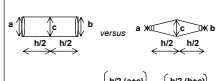
Area/Volume and Pole Width of MII meiotic spindle increase in IVM oocytes

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.

Area (Volume) of MII meiotic spindle is higher in non-gonadotropin supplemented IVM



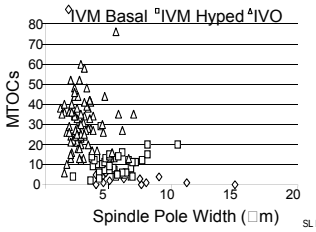
Quantification of Spindle Size and Shape

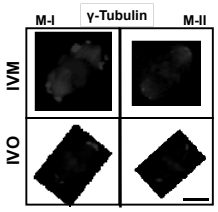


Area of spindle = $\left(\frac{h/2\,(a+c)}{2}\right) + \left(\frac{h/2\,(b+c)}{2}\right)$

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.

Pole width of MII meiotic spindle is higher in non-gonadotropin supplemented IVM (Basal), intermediate in gonadotropin supplemented IVM (Hyped) and lower in IVO oocytes





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.

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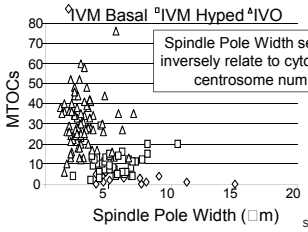
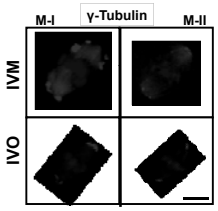
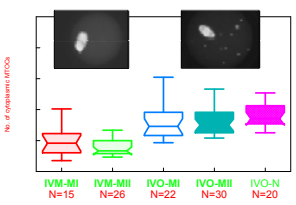


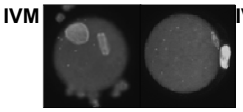


SL Barrett, DF Albertini (2007) Allocation of gamma-tubulin between oocyte cortex and meiotic spindle influences asymmetric cytokinesis in the mouse oocyte. Biol Reprod 76:949-57.

Number of cytoplasmic MTOCs decreases in non-gonadotropin supplemented IVM

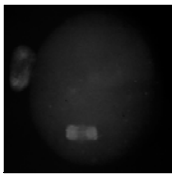
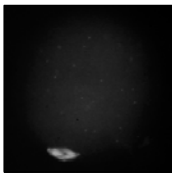


Centrosome Numbers
IVM < IVO



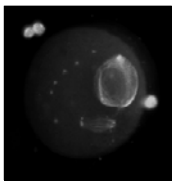
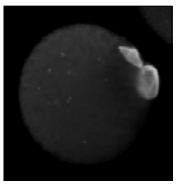
A Sanfins et al. (2003) Distinctions in meiotic spindle structure and assembly during *in vitro* and *in vivo* maturation of mouse oocytes. Biol Reprod 69: 2059.

Spindle anchoring at the cortex is partially lost during non-gonadotropin supplemented IVM

IVM   **IVO**

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

Retention of MTOCs and oocyte volume is partially lost during non-gonadotropin supplemented IVM

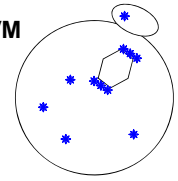
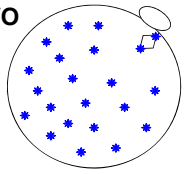
IVM   **IVO**

Polar Bodies formed *in vitro* contain MTOCs

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.

Morphological signs of oocyte polarity and asymmetric division partially lost in mouse non-gonadotropin supplemented IVM

Working Model

IVM  **IVO** 

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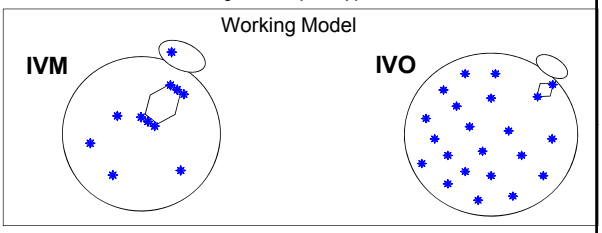
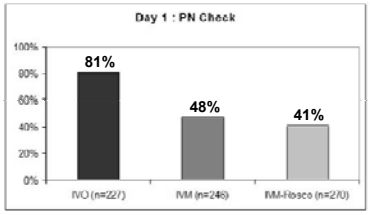
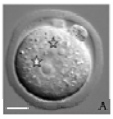


Are these morphological differences correlated with the described decrease in quality in IVM oocytes ?

Fertilization rate: presence of 2-PN 6h post-IVF

Mouse



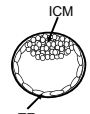


CE Plancha et al. (2005) Developmental competence of differently matured mouse oocytes. Poster at XXI ESHRE Annual Meeting, Copenhagen, Denmark

Evidence of fertilization competence loss !

Blastomere numbers at the blastocyst stage

Mouse



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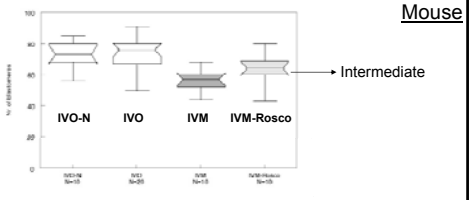
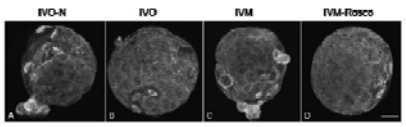




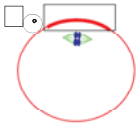
Evidence of developmental competence loss!

CE Plancha et al. (2005) Developmental competence of differently matured mouse oocytes. Poster at XXI ESHRE Annual Meeting, Copenhagen, Denmark

Oocyte polarity

1. Is an important feature of oocyte maturation

2. May reflect oocyte quality, since partial loss of morphological signs of polarity correlate with a decrease in fertilization and developmental competences



Presentation outline

1. Oocyte quality: impact, acquisition and approaches

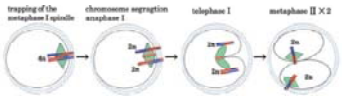
2. Oocyte polarity: a feature of oocyte maturation

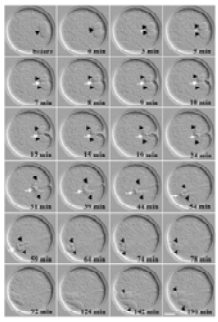
3. Oocyte polarity: a reflection of oocyte quality?

4. Future challenges

To assess if manipulating polarity in mouse oocyte maturation will reflect upon the fertilization and developmental competence levels

Symmetric first meiotic division can be forced by compressing cortical ooplasm near the MI meiotic spindle

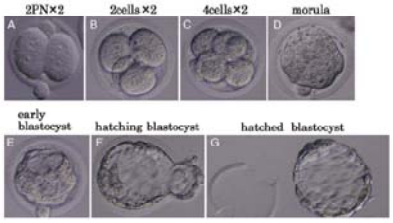




Two mini MII oocytes originate instead of one MII oocyte and one PB

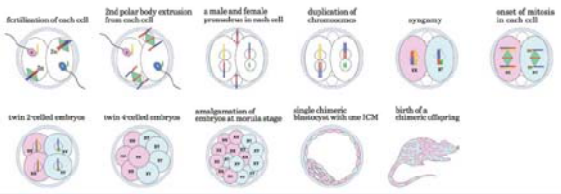
J Otsuki *et al.* (2012) Symmetrical division of mouse oocytes during meiotic maturation can lead to the development of twin embryos that amalgamate to form a chimeric hermaphrodite. Hum Repr 27(2):380-387.

Each of the two mini MII oocytes can be fertilized and cleave, until one quimeric morula compacts and one blastocyst is formed



J Otsuki *et al.* (2012) Symmetrical division of mouse oocytes during meiotic maturation can lead to the development of twin embryos that amalgamate to form a chimeric hermaphrodite. Hum Repr 27(2):380-387.

This means that even a very small oocyte, in the limit of being a very large first polar body, is able to be fertilized and initiate embryo development.



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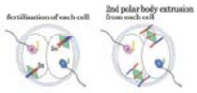


J Otsuki *et al.* (2012) Symmetrical division of mouse oocytes during meiotic maturation can lead to the development of twin embryos that amalgamate to form a chimeric hermaphrodite. Hum Repr 27(2):380-387.

- Asymmetry of the first meiotic division does not seem essential for embryo development.
- Asymmetry of the second meiotic division, however, was maintained.

Remaining questions :
- was there a decrease in fertilization and developmental rates?
- had zygotes been separated to develop independently, what would these rates be?
- what would happen in case we prevent asymmetry of second meiotic division?

To assess if manipulating polarity in mouse oocyte maturation will reflect upon the fertilization and developmental competence levels

To probe other models besides the mouse

In humans detectable signs of oocyte polarity loss do not seem to correlate with decrease in fertilization and developmental competence

L De Santis *et al.* (2005) Polar body morphology and spindle imaging as predictors of oocyte quality. RBM online 11:36-42.

Existing non-invasive approaches may not be enough to accurately discriminate different categories of oocytes

G Coticchio *et al.* (2010) Comparative analysis of the metaphase II spindle of human oocytes through polarized light and high performance confocal microscopy. Fertil Steril 93:2056–2064.

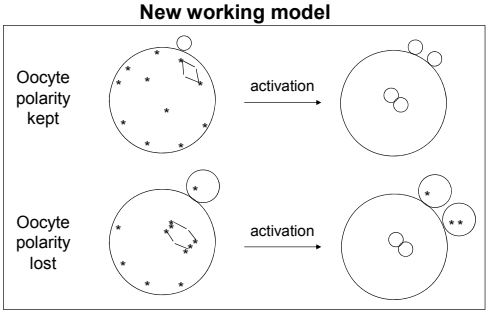
Pre- and post-ovulatory ageing associate with a loss of oocyte polarity

S Brunet MH Verlhac (2011) Positioning to get out of meiosis: the asymmetry of division. Hum Repr Update 17:68–75.

Open questions :

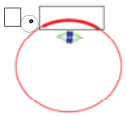
- what are the consequences of preventing asymmetry in the second meiotic division?
- should we focus our attention on the second polar body, besides the spindle?
- will more powerful non-invasive morphological approaches allow us to better associate oocyte polarity and quality?
- can we identify specific human situations or treatments where assessing oocyte polarity will reveal more important than in others?

New working model



Take-home messages

1. Oocyte quality is a relevant property acquired during oogenesis that has remained elusive to predictive studies
2. Oocyte polarity is an important feature of oocyte maturation
3. Oocyte polarity may reflect oocyte quality, since partial loss of morphological signs of polarity correlate with a decrease in fertilization and developmental competences
4. Future studies are needed to assess the value of oocyte polarity as a sign of oocyte quality



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Oocyte polarity: a sign of oocyte quality?

Probably yes, but it is still not clear how …

Thank You !