09.00 – 09.30
The search for excellence in IVF laboratories: towards "the best"
ESHRE Campus symposium
Bologna, Italy 23-24 January 2009

Zygote morphology as a reflection of embryo polarity

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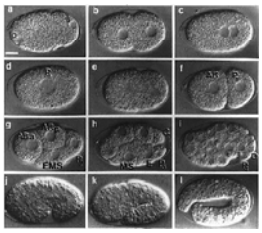
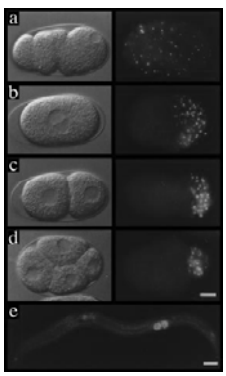
Learning Objectives

- **Define polarity in mammalian oocytes and embryos**
- **Define zygote morphology, what it means biologically**
- **Relate polarity and zygote morphology to continued development**
- **Discuss how Polarity and Zygote morphology can affect clinical outcome**

Oocyte and Embryo Polarity is well established in lower order animals: C. Elegans, Xenopus, Avians etc
- **Disputed in mammalian development….**



Alan Scott, 2000



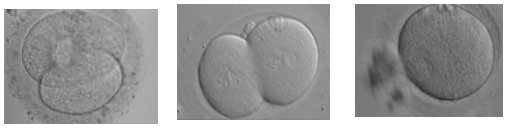
L. Seydoux, 1997

Published Literature

- ***Gardner 1996/ Edwards 1997*** **suggested that it does exist in oocytes which dictates all further polarity and axes and that an animal-vegetal pole exists, directed by the polar body position**
- ***Zernika-Goetz*** **stated "there are no essential components that are localized uniquely to the animal or the vegetal pole *1998***
- ***Zernika-Goetz, 2002*** **suggest that the axis is set up by Sperm Entry site = first axis**
- ***Hiiragi and Solter, 2004, 2005:*** **Alternately, it is the plane separating the 2 pronuclei as they move to the center of the oocyte which sets up the first axis (in the mouse)**
- ***Hansis and Edwards, 2003/2005:*** **The only report on polarity in human 4-cell embryos, not repeated, but its not related to the oocyte or zygote**

Empiric observations

- **Uneven cleavage at the first mitotic division is not compatible with delivery**
- **Muti-nucleation in blastomeres at the 2-4 cell stage is not compatible with delivery**
- **Off-Center Nuclei at the 1 cell stage is not compatible with delivery**
  - ***Scott et al 2007***



Investigating Human Oocyte Polarity

- **Look at human oocytes and embryos and elucidate impact of any observed polarity in cleavage on outcome using common/ known parameters of determining embryo morphology as predictors of outcome measures.**
- **The ideal method is Time-Lapse videos or frequent sequential still observations**
- **Relate this to known morphological features that impact outcome**

Time Lapse of Human Oocytes and Embryos

- **Immature GV and M1 oocytes, fresh**
- **1PN and 3 PN abnormal fertilized oocytes, fresh**
- **Thawed 2PN embryos donated to research**
- **Thawed 2-8 cell embryos, donated to research**

- **2 Systems were utilized:**
- **Octax, in continual streaming video within a closed chamber**
- **Unisense-EmbryoScope in a dedicated incubator system with time lapse.**

- **All Oocytes and Embryos were set up as individuals in both systems and monitored for 24-120 hours continually**

GV to M1 Maturation

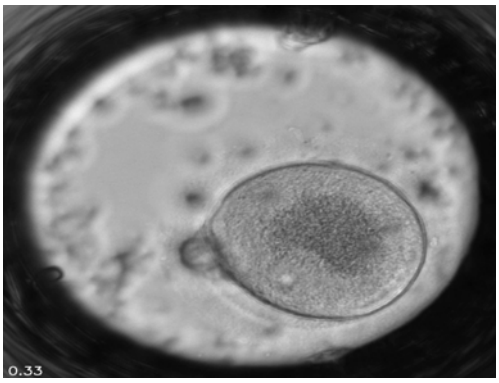
- **15/19 GV oocytes matured to MI with**
- **Disillusion of the GV membranes, considerable movement of the cytoplasm, including a pulsing as the oocyte appeared to slightly shrink and re-expand**
- **Polarity? It would be hard to tell unless there were fluorescent markers to indicate a top and bottom in the oocyte**

MI to MII

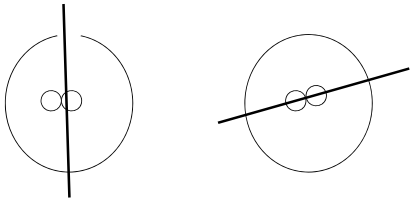
28/35 MI matured to MII accompanied by

- **the extrusion of the polar body (PB)**
- **which was accompanied by deep shrinking followed by expansion, but never to its original size, with formation of the perivitaline space.**
- **Where the oocyte did not shrink, the PB was small or appeared to be sequestered into the oocyte (Abnormal??)**

GVBD to MI to MII



Axis Designations

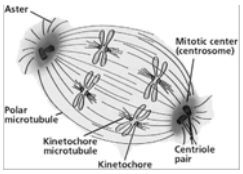


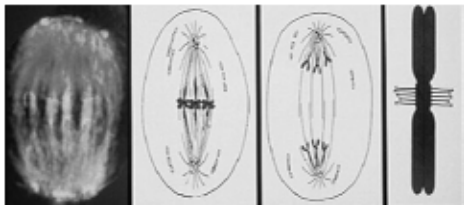
Vertical Axis
PB through Center of Oocyte

Pronuclear Axis
Through Centers of
Pronuclei

Origin of Centrioles

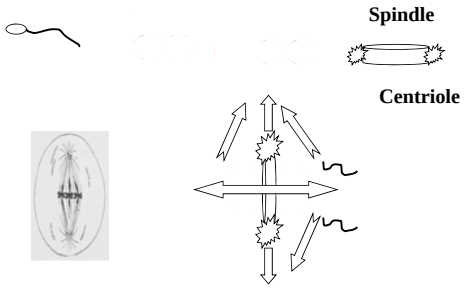
- **Mice: Centrioles are formed in the oocyte**
- **In humans they come from the sperm**
- **Can this be a source of difference in patterning and polarity between mouse and human oocytes?**
  - » ***Review Chatzimeletiou, K et al. 2008***

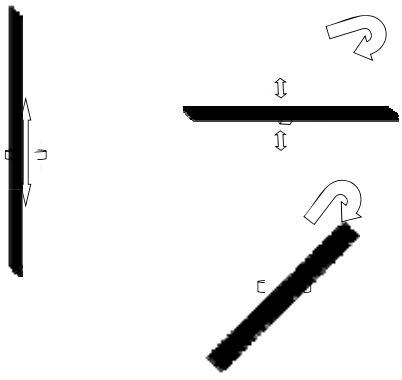




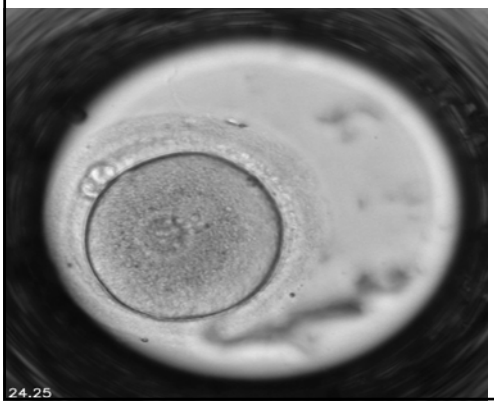
Courtesy of David Keefe

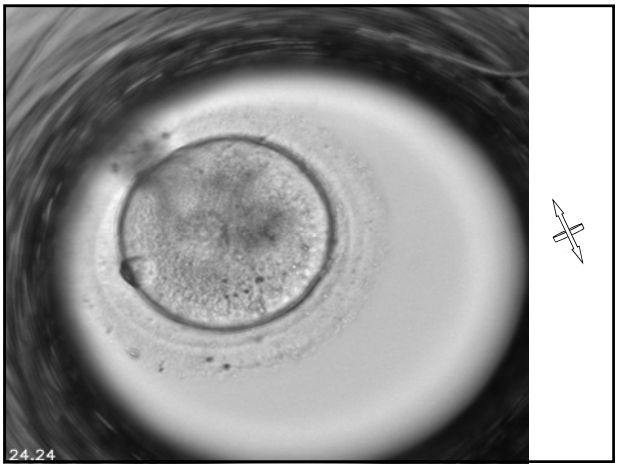
Spindle, Pronuclear Formation, First Mitotic Division



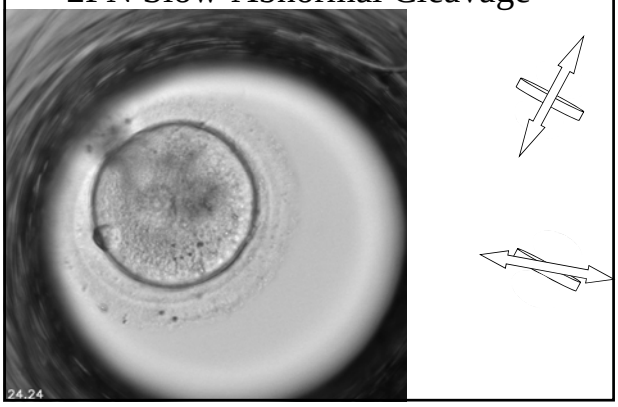


PN Fast with Movement

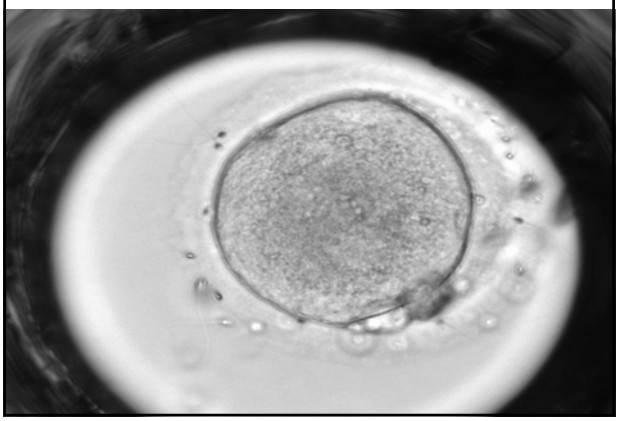




2PN Slow-Abnormal Cleavage

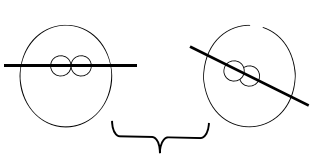


3PN Exploding

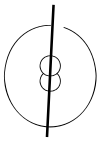


Results: Cleavage and Development

20/26 2PN Cleaved through the Pronuclear Axis (77%)



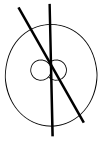
N= 14 (54%)
14 8-cell
6 blastocyst



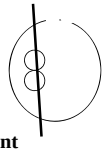
N= 12 (46%)
10 8-cell
3 blastocyst

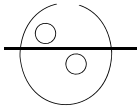
Abnormal Development

6/26 2PN Cleaved "Off" the Pronuclear Axis = 23%



3 Spontaneous 3 cell, arrested
2 cleaved to uneven 2-cell, arrested
1 4-cell, arrested



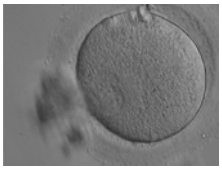


Other abnormal cleavage
Results in arrested development

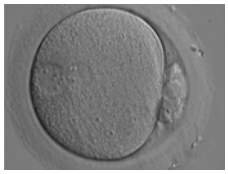
2 PN Oocytes

- **Cleavage was generally on the Pronuclear Plane**
- **The first cleavage division is very dynamic and can only be observed with *rapid* time-lapse (short interval) or continual video**
- **Blastomeres undergo pulsing, blebbing and rearrangement but settle into a 2-cell embryo by +/-2 hours**
- **The polar body rapidly rotates into the cleavage plane**
- **This pattern is repeated at the second/third division and then no more**
- **At the 2 cell stage, multinucleated blastomeres can explode into multiple cells**

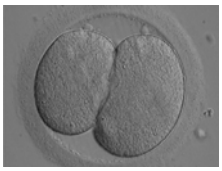
PN Position, PB Morphology, Day 2 Score

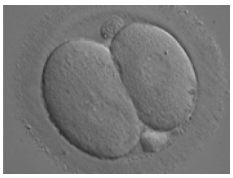


Off center, Uneven cleavage



Off center, abnormal PB, MN





Polarity: Relevance to ART?

(this will be more evident in the second lecture)

- **MII Oocytes with abnormal PB and no PVS are likely to be dysfunctional even after fertilization**
- **Fertilized oocytes with PNs not centrally located are abnormal, can not adhere to polarity planes of cleavage**
- **Day 2 scoring is essential (in the absence of time-lapse) to eliminate cleavage errors**
- **The timing of Day 2 scoring is important and needs to be empirically defined per lab due to the dynamic nature of the first mitotic division**

Fertilized Oocyte Scoring-Zygote Morphology

Looks at a part of nucleoli in the early embryo

Nucleoli

- **Found in all actively dividing cells**
- **Sites of rRNA synthesis**
- **Develop on the DNA where the genes for ribosomes are located, rDNA**
- **These points on the DNA = NORs**
  - **Nucleolar Organizing Regions**

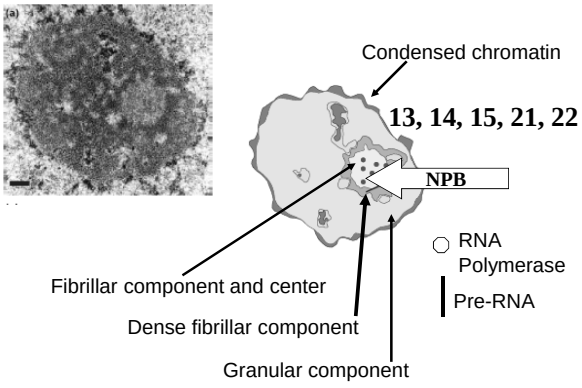
Nucleoli

- **During mitotic cell cycles the nucleoli fuse as the chromatin condenses**
- **Between 5-7 in human cells**
- **Always equality between mitotic daughter cells- size, number, fusion patterns**
- **Nucleoli are sites of protein synthesis, some mitogenic factors and growth regulatory proteins**
- **In oocytes the nucleoli are disaggregated at the GV stage and reform after fertilization during the first 2-3 mitotic divisions**

Human NORs

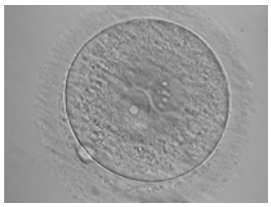
- **5 pairs of NOR-bearing chromosomes**
  - **13, 14, 15, 21, 22 (acrocentric)**
- **Generally 5-7 NORs in human cells**
- **Activation of the NORs induces pol 1 transcription**
- **NOR's are clustered and this is dependent on heterochromatin adjacent to rDNA genes**
- **Heterochromatin is *not* inactive and may be involved in developmental control (Dimitri, 2004)**
- **Transcription of rDNA results in 3 functional parts:**

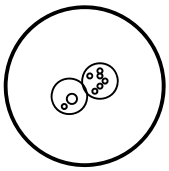
- **Dense fibrillar component (DFC)**
  - **Required for transcription of DNA**
  - **Pre-rRNA + proteins**
- ❖ **Fibrillar component (FC)**
  - **<u>Structural center</u> for transcription, storage of inactive transcription factors**
  - **Surrounded by DFC**
- **Granular component (GC)**
  - **Pre-ribosomal particles- cytoplasmic**



Sperm and NPB's

- **Sertoli cells are very mitotically active**
- **This means they have de-constructed nucleoli during mitosis**
- **There are +/- 6 NPB (FC regions) per daughter cell - easily visible**
- **This is what should be brought to the oocyte in the sperm**

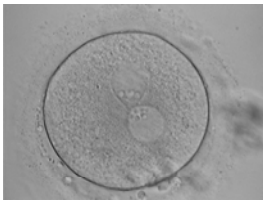




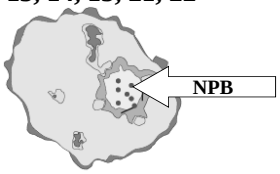
3 patients, 3-4 cycles each, no pregnancy
1-2 PGD cycles each
Donor IUI, 2 ongoing pregnancy

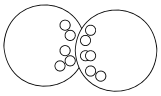
Male factor identified by PN morphology

NPB IN FERTILIZED OOCYTES



Looking at chromatin
13, 14, 15, 21, 22

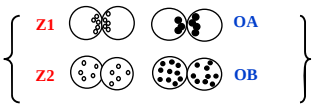


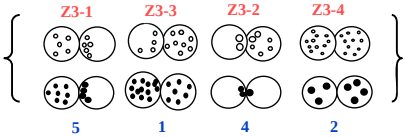


L = 5 R = 6

Nucleolar Precursor Body (NPB) Pattern

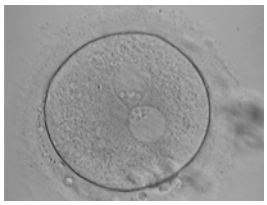
Normal = equality between nuclei

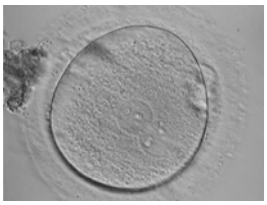


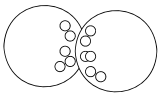


Abnormal = any inequality

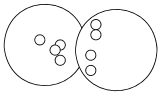
NPB RATIO







L = 5 R = 6



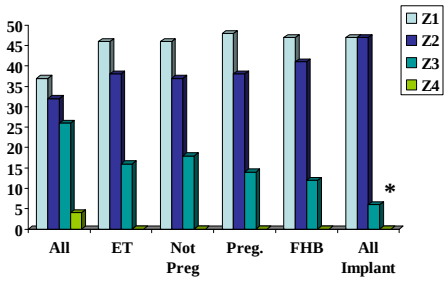
L = 4 R = 4

NPB Ratio vs. Outcome

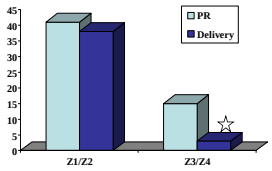
	ALL	ET	Not Preg	Preg	FHB	100% IR	>12 weeks
L/R 	4/9	5/8	4/9	5/7	6/7	6/7	6/7
Range	1-15	2-10	2-10	4-9	5-9	5-7	6-7 ☆

P<0.05

Pronuclear Score

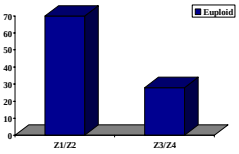


FCNE Application and Results

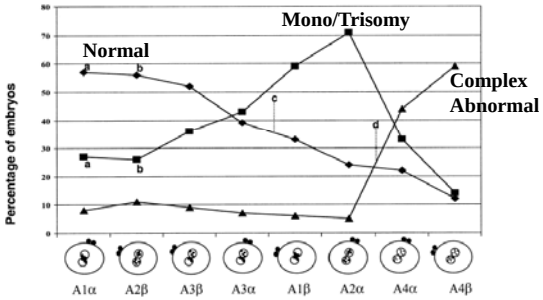


As a proportion of embryos transferred, Z3/Z4 result in fewer babies

More Z1/Z2 embryos are euploid *cf.* Z3/Z4 in the PGD/AS programme

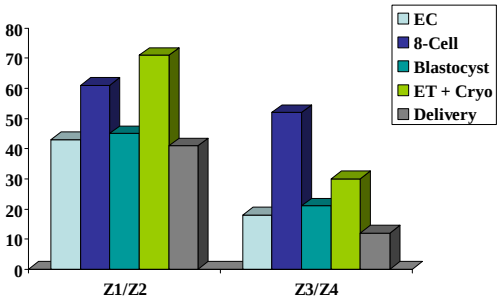


Chromosomal Status vs. PN Morphology



Gianaroli, 2003

Summarized Data



Conclusions

- **PN morphology may reflect the state of chromatin condensation onto the spindle**
- **May reflect the cell cycle and synchrony of the male and female nuclei**
- **Has a direct impact on early scoring parameters and blastocyst developmet**
- **Is correlated with euploidy/aneuploidy**
- **Has an impact on delivery rates**