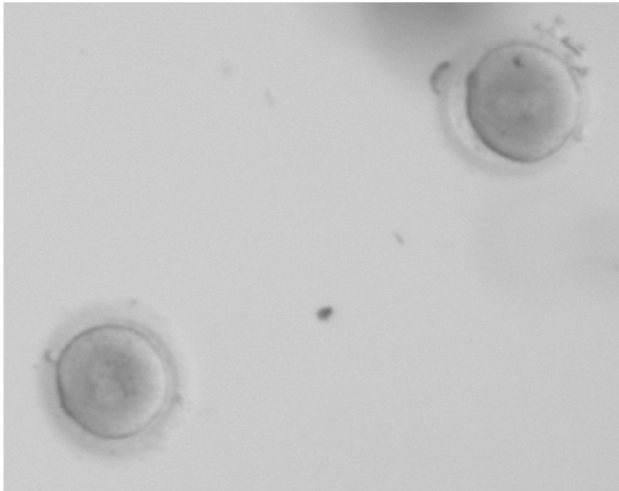


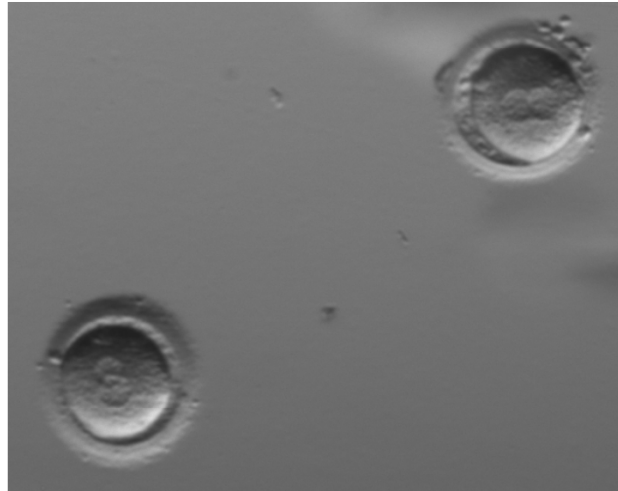
# **Morphological aspects at zygote stage**

Markus Montag, Prof., Ph.D.  
University of Bonn  
Germany

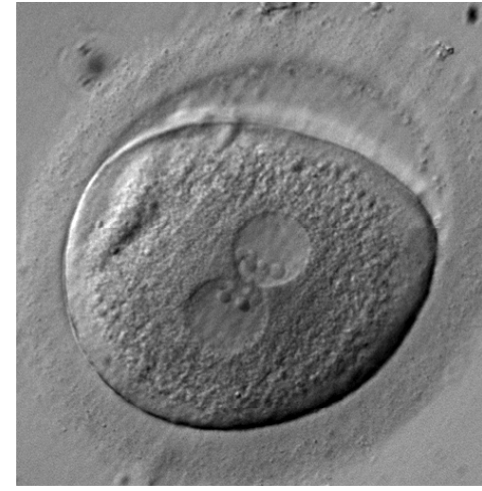
# Imaging zygotes



Stereo microscope  
- Standard contrast -

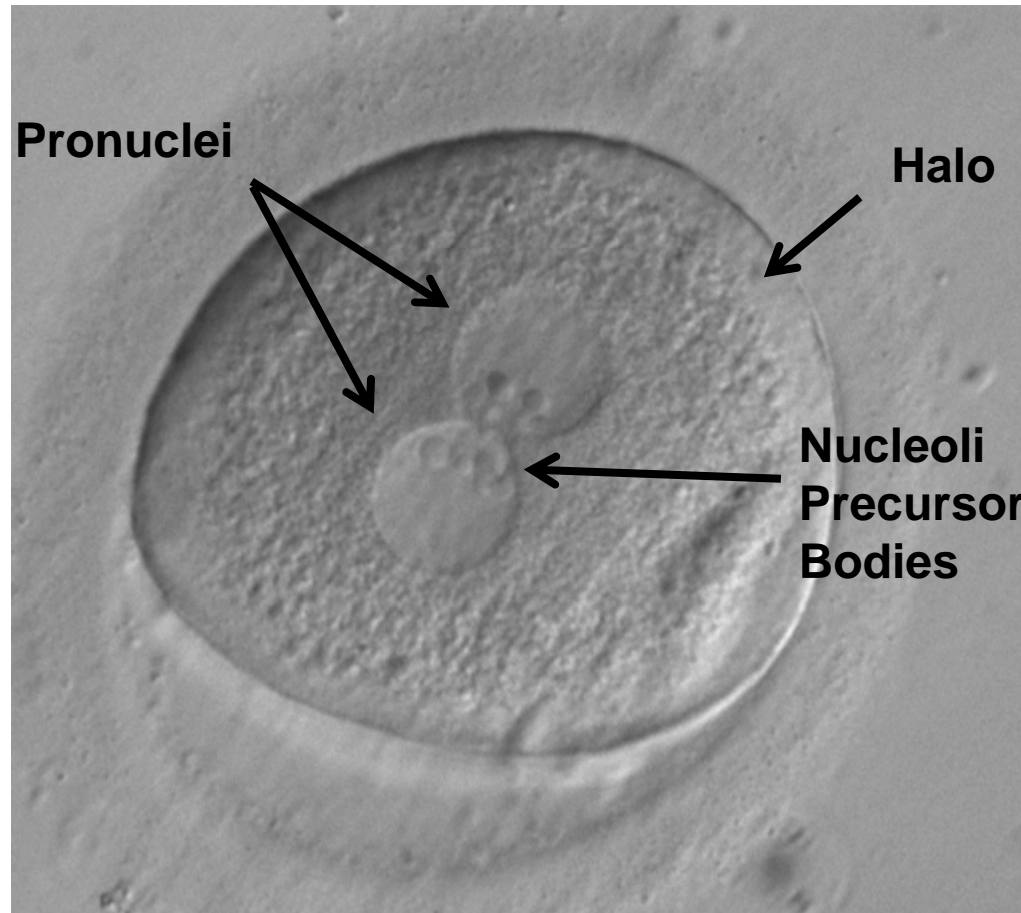


Stereo microscope  
- Relief contrast -



Inverted microscope  
- Hoffman contrast -

# Zygotes - what can we see?

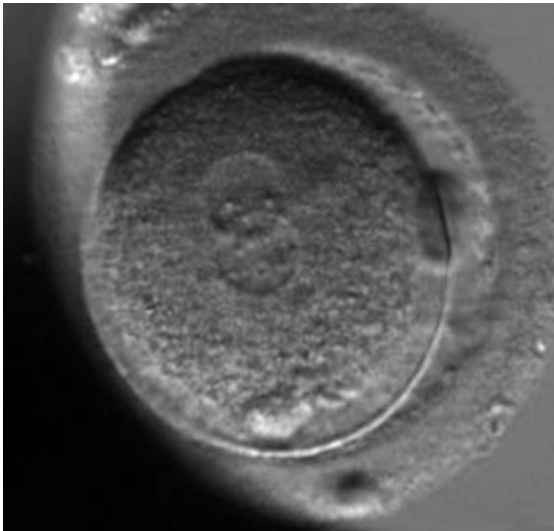


# Number of pronuclei

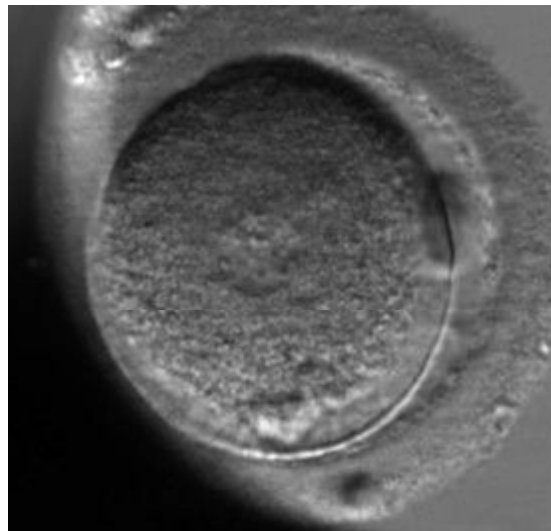


# Mechanism of 1 PN formation

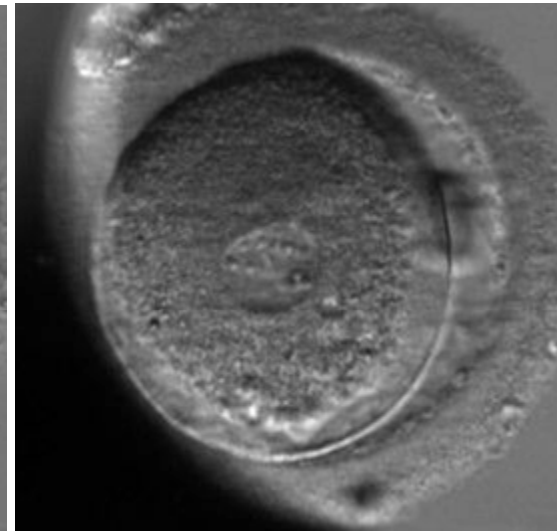
## - Fusion of 2PN -



11.4h

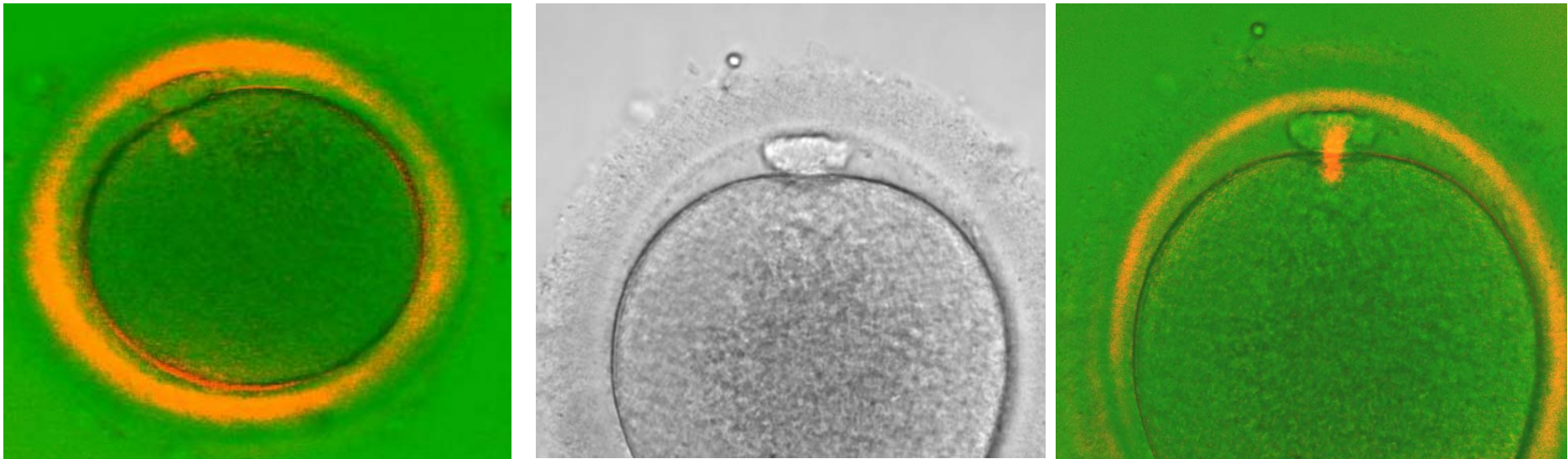


11.7h



16.1h post ICSI

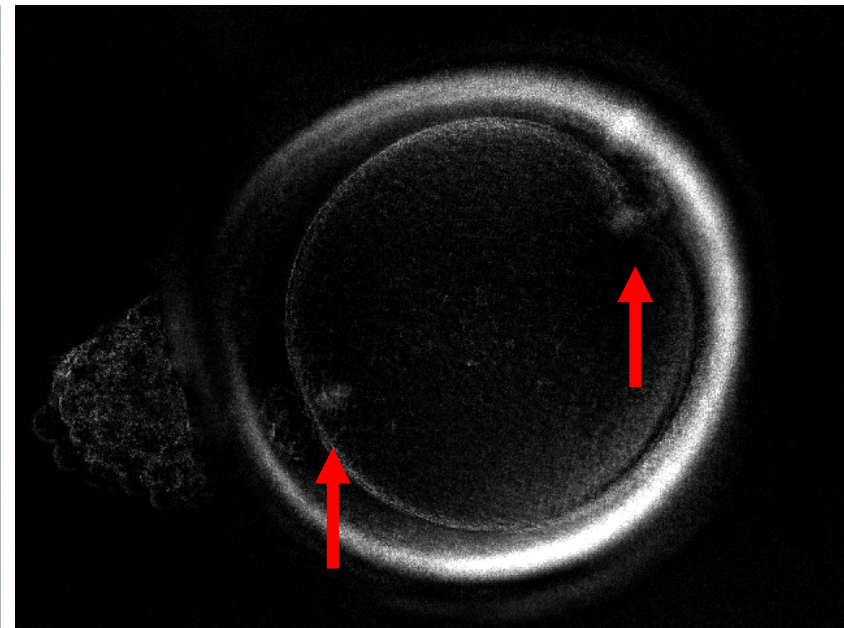
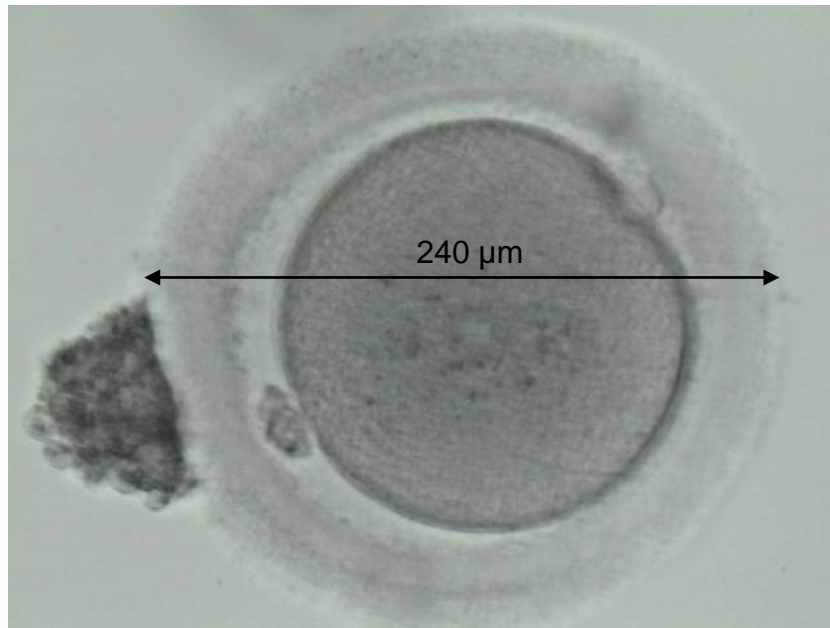
# Mechanism of 3PN formation in ICSI cycles I



- 3 PN from oocytes in a transition from telophase to metaphase

# Mechanism of 3PN formation in ICSI cycles II

- 3 PN from diploid (giant) oocytes



# Micro-pronuclei

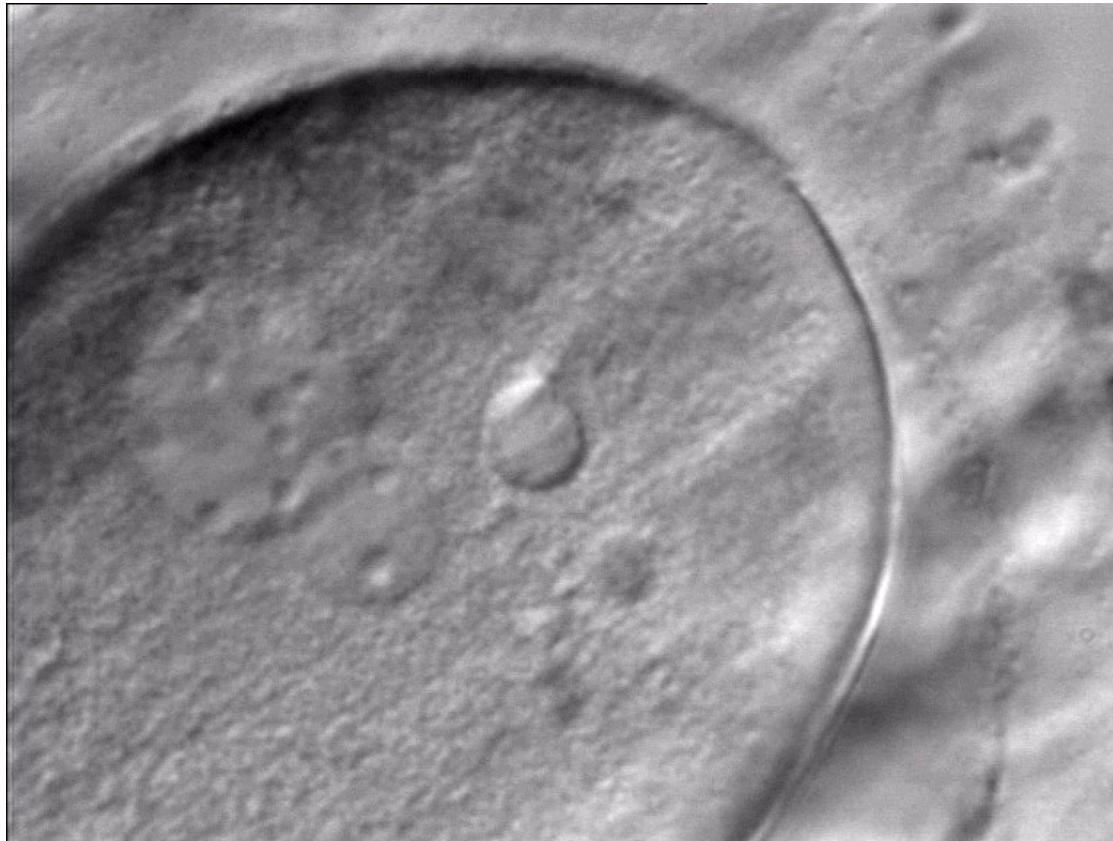
- May arise by chromatid/chromosome lagging during division
  - High risk for aneuploidy





# Shape and size of pronuclei

- Equal - good / unequal - bad



Zollner et al., 2002

# Position of pronuclei

- PN in the periphery or apart from each other are considered developmentally abnormal



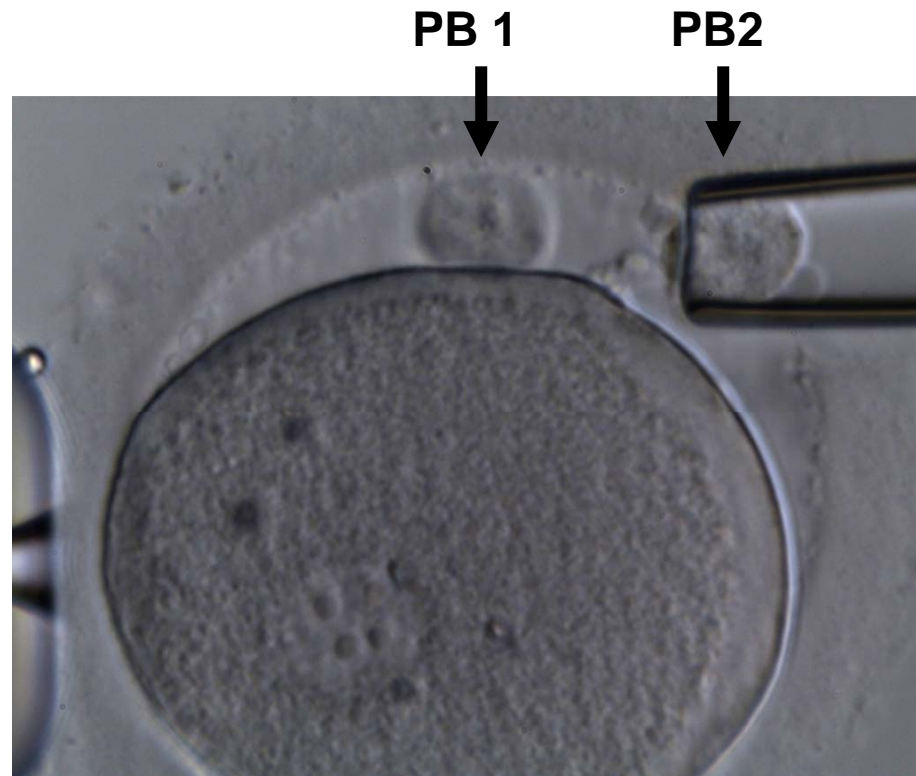
Garello et al., 1999



Scott & Smith, 1998

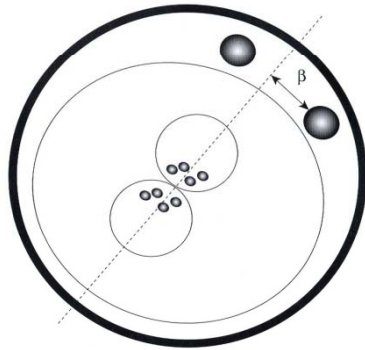
# Polar bodies: 1st and 2nd

Videos of polar body formation



# Position of pronuclei versus polar bodies

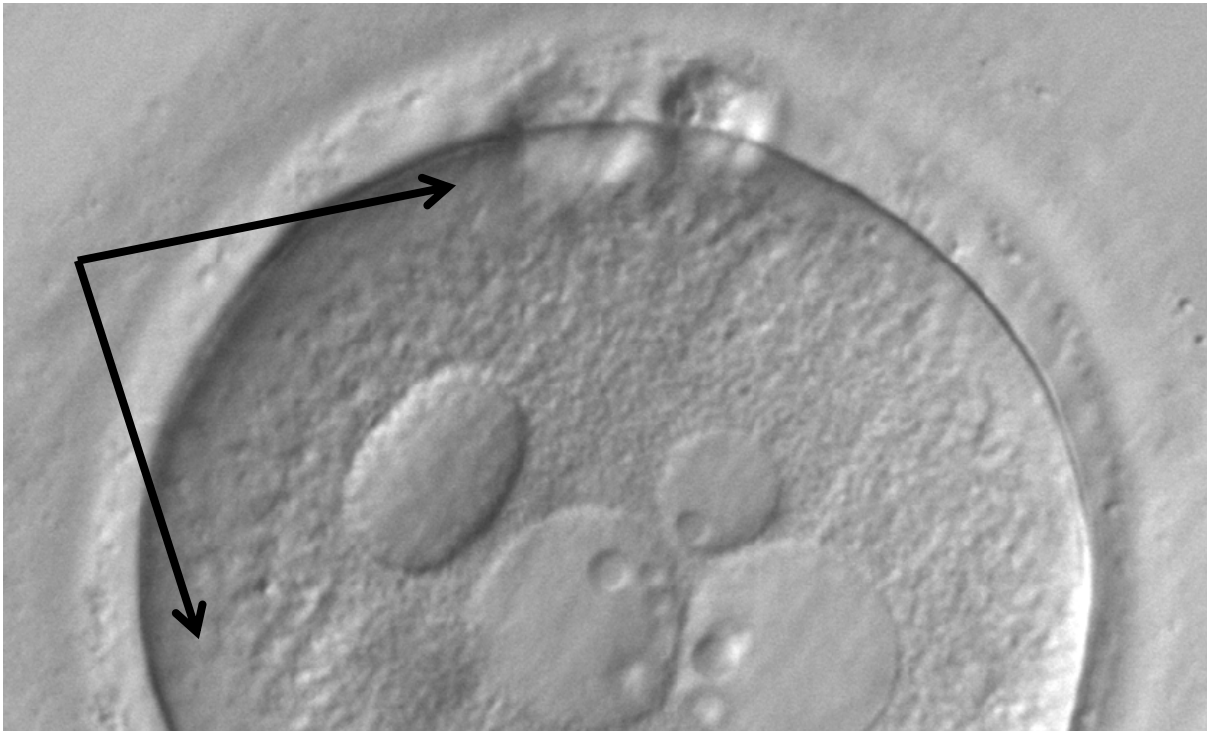
- Alignment (Garello et al., 1999)



- The possible benefit of this criterion is questioned due to a possible impact of denudation on polar body position (Taylor et al., 2008)
- Polar bodies move (Scott et al., 2008)

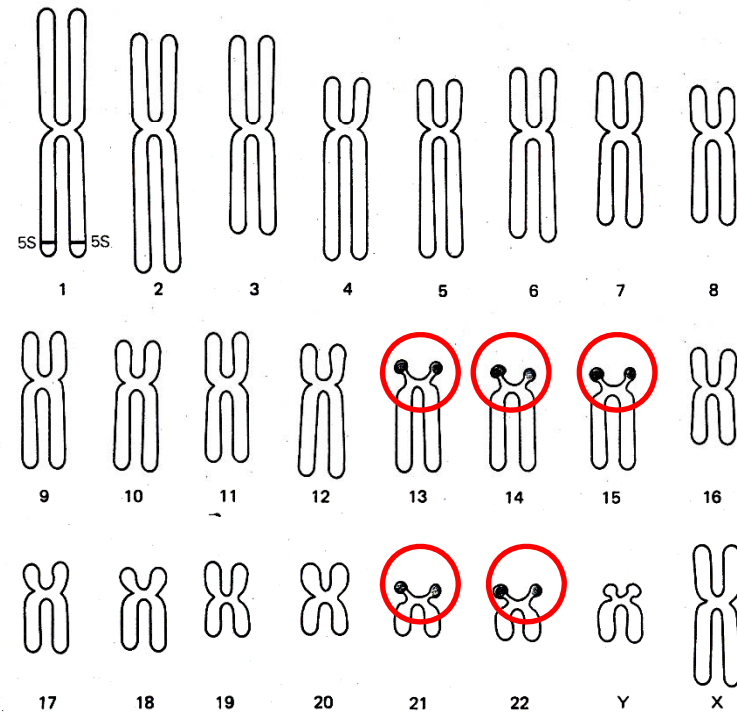
# The HALO

- The Halo is primarily a sign that the cytoskeleton of the developing zygote is functionally active

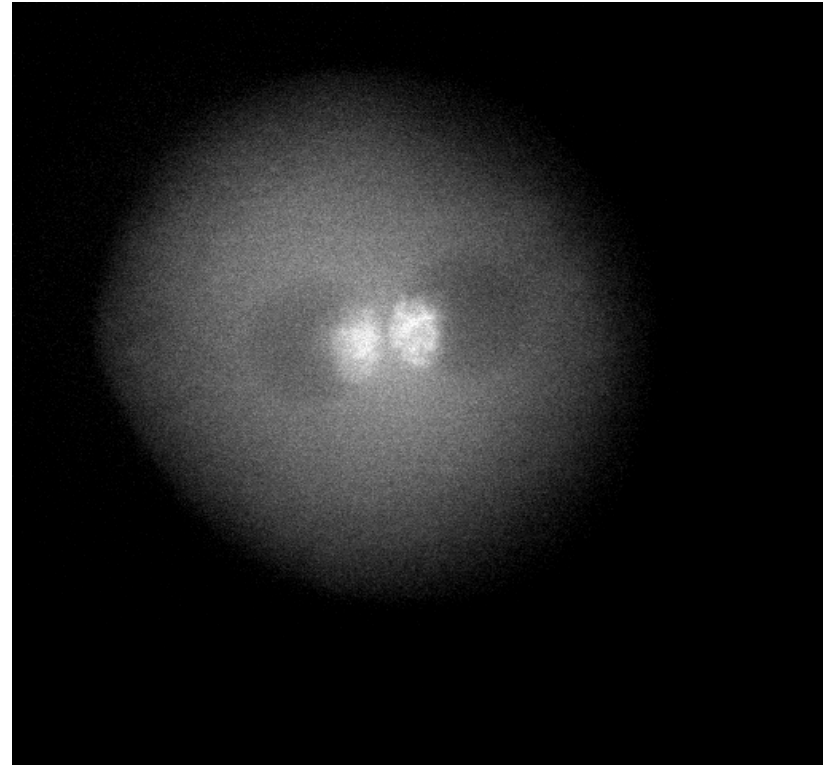


- The Halo is not correlated to babies born (Scott, 2006)

# The nature of the Nucleolar Precursor Bodies (NPBs)



# Distribution of DNA in relation to the NPBs



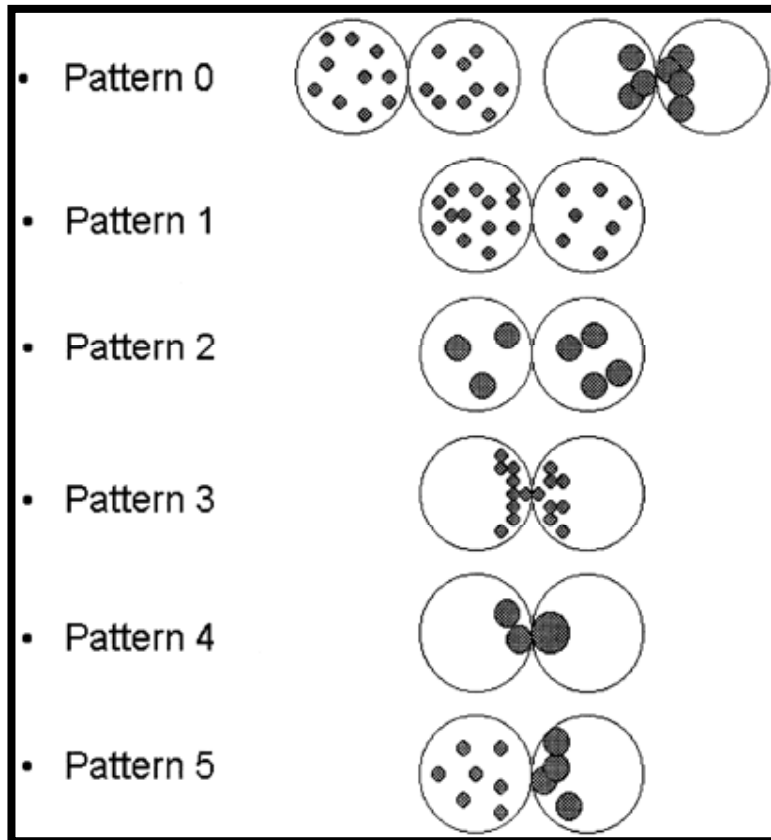
# Morphological aspects and zygote scoring

## Zygote scoring systems

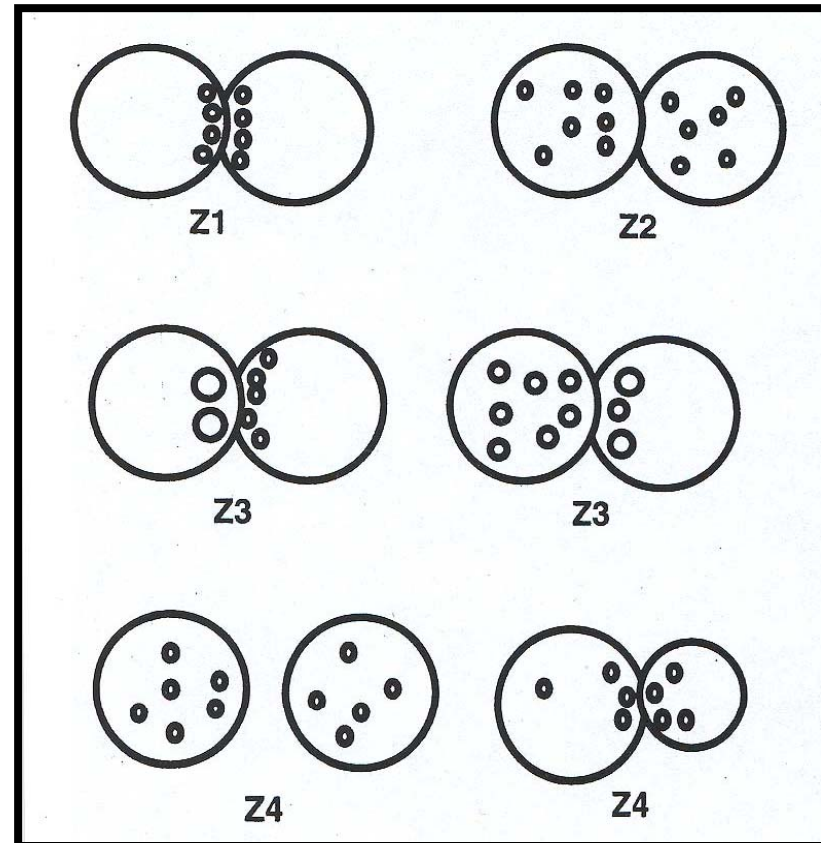
- Scott & Smith, 1998      PN, NPB, Halo
- Tesarik & Greco, 1999      PN, NPB
- Garello et al., 1999      PN, PB
- Balaban et al., 2001      PN, NPB
- Montag et al., 2001      PN, NPB
- Scott et al., 2001      PN, NPB
- Zollner et al., 2002      Multi-factorial, Score
- Ebner et al., 2003      PN, Halo
- Senn et al., 2006      Multi-factorial, Score



# Widely used scores

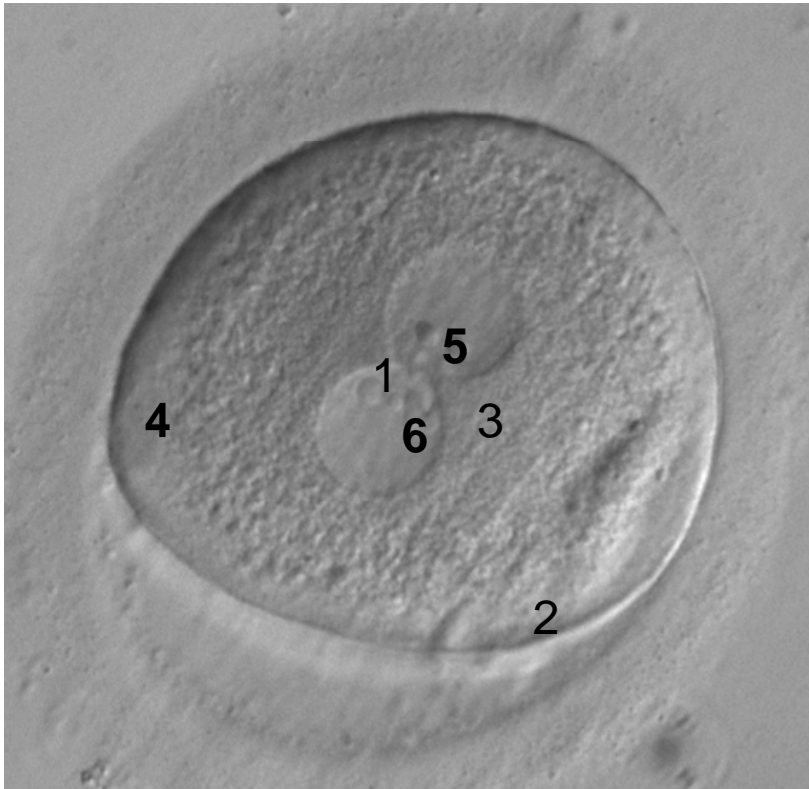


Tesarik & Greco, 1999



Scott et al., 2002

# Automatic scores



Senn et al., 2006

**1-3 points per criterion:**

1. Distance of PN
2. Orientation of PN versus PB
3. Position of PN

4. Cytoplasmic Halo
5. Number of Nucleoli
6. Polarisation of Nucleoli

Relevant for pregnancy rate

# Criticism of PN score

## Limited value of zygote (PN) scoring systems

- Payne et al., 2005
- James et al., 2006
- Nicoli et al., 2007
- Brezinova et al., 2009
- Nicoli et al., 2010

# The time-point

- Zygote assessment is usually performed by a static observation
- Difference between IVF and ICSI cycles in timing (Montag et al., 2001)
- In ICSI cycles: strict timing required post-ICSI for studies on inter-cycle comparison

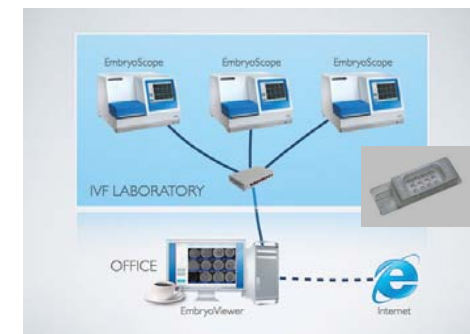
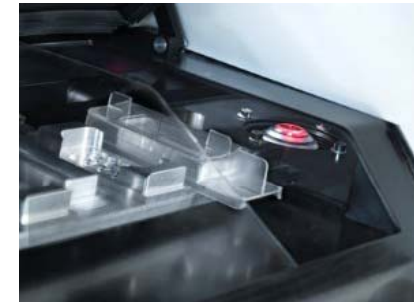
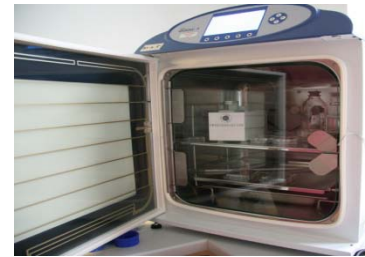
# Imaging zygote development

Payne et al., 1997

- The male pronucleus is shortly formed prior to the female pronucleus
- The female pronucleus is closer to the 2nd polar body and contains less NOR-precursor bodies compared to the male pronucleus

# Imaging of embryo development

- Time-lapse studies
  - 1<sup>st</sup> cleavage cycle
  - Embryo development
  - Blastocyst formation
- Adequate cleavage timing
  - Constant cleavage intervals
- Being fast is not always good
  - Too fast development: low IR

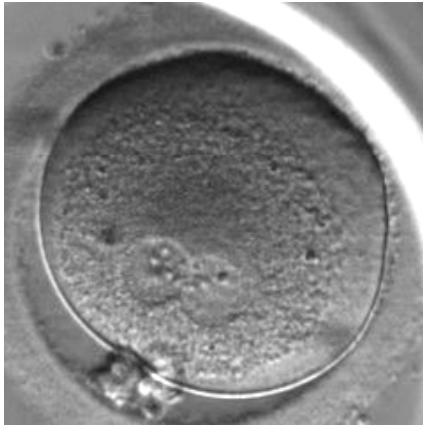


# Imaging zygote development

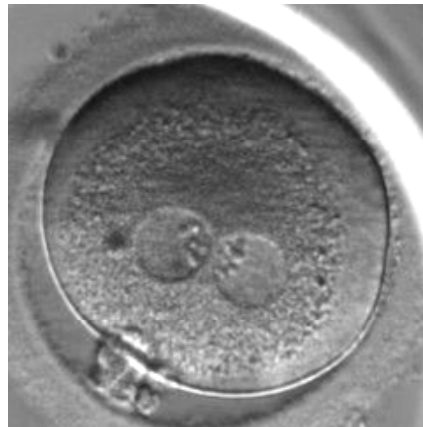
Videos showing zygote formation

# Imaging NPB variation

- Symetric from the beginning-



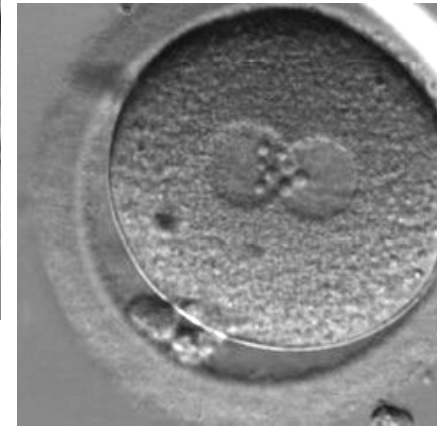
Post-ICSI: 11.7h



15.4h



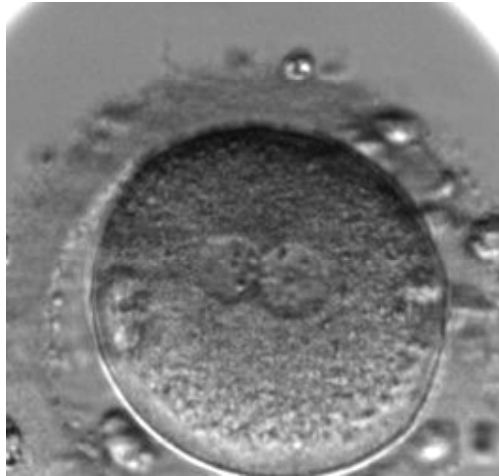
18.3h



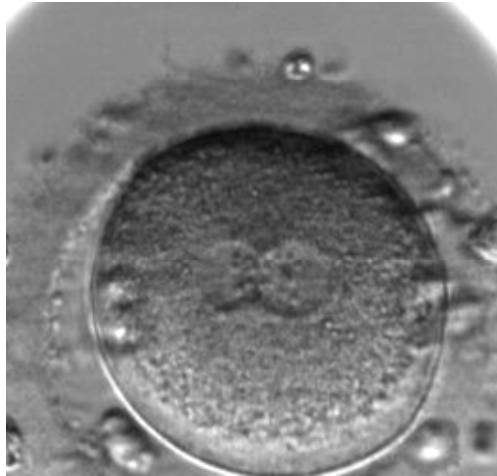
23.8h



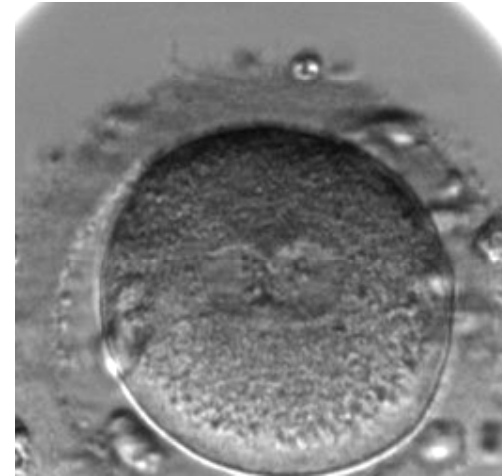
# Imaging NPB variation



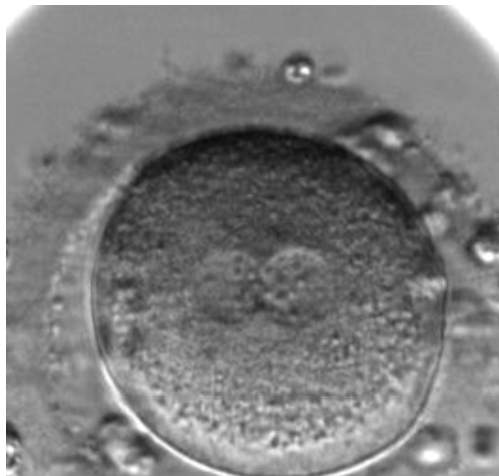
Post-ICSI: 12.7h



13.0h



13.7h



14.3h

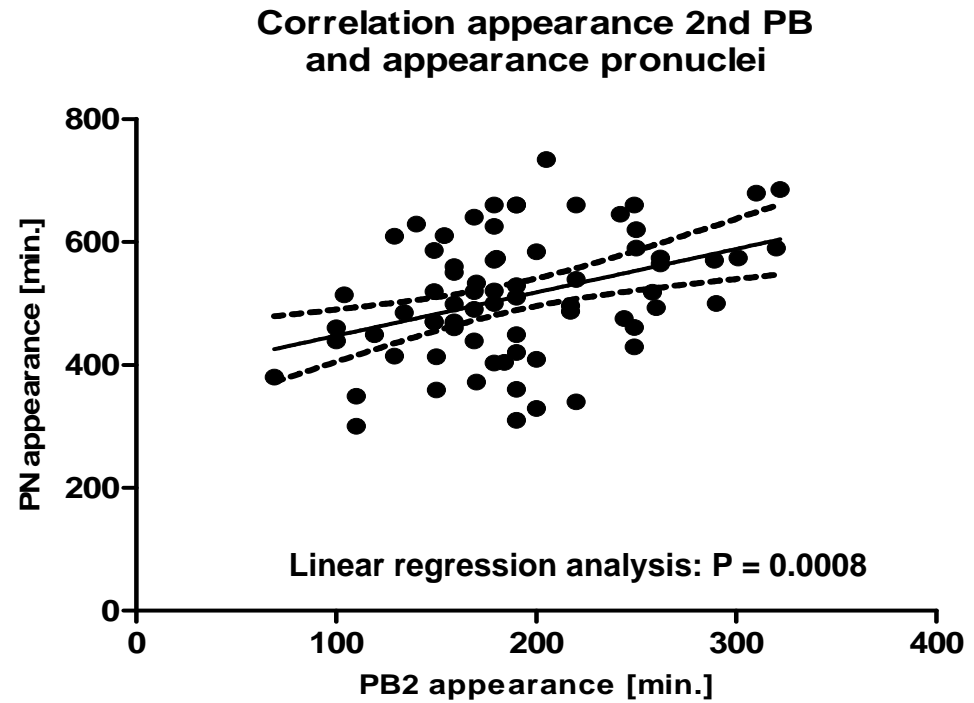
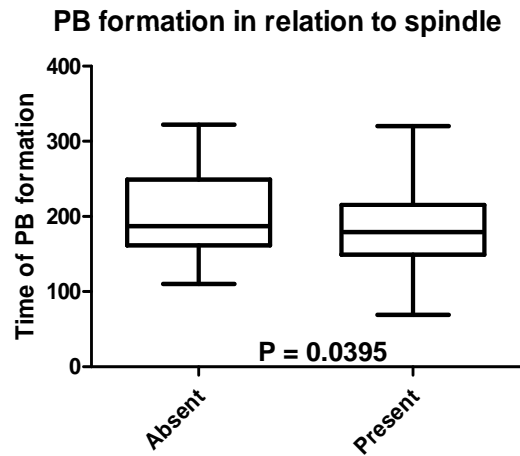


18.3h

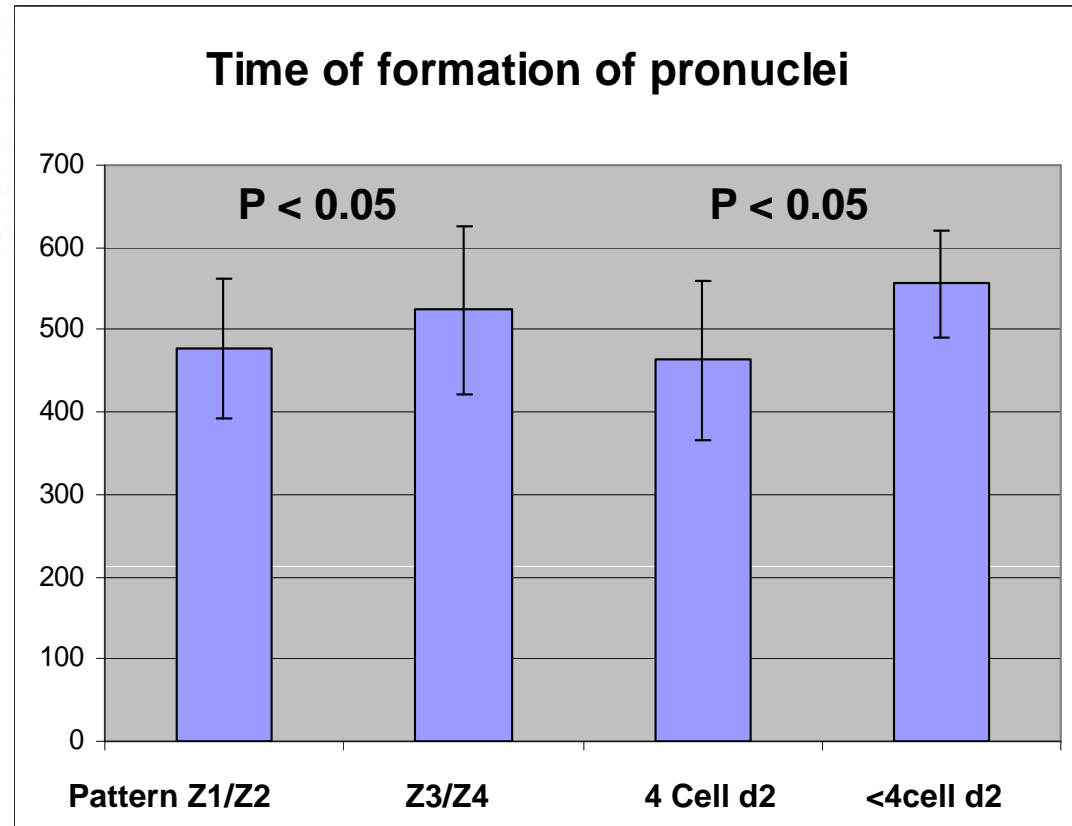
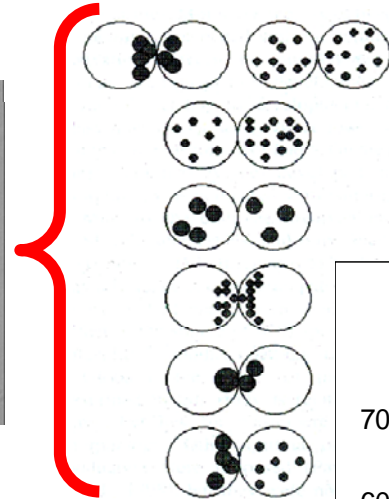


21.0h

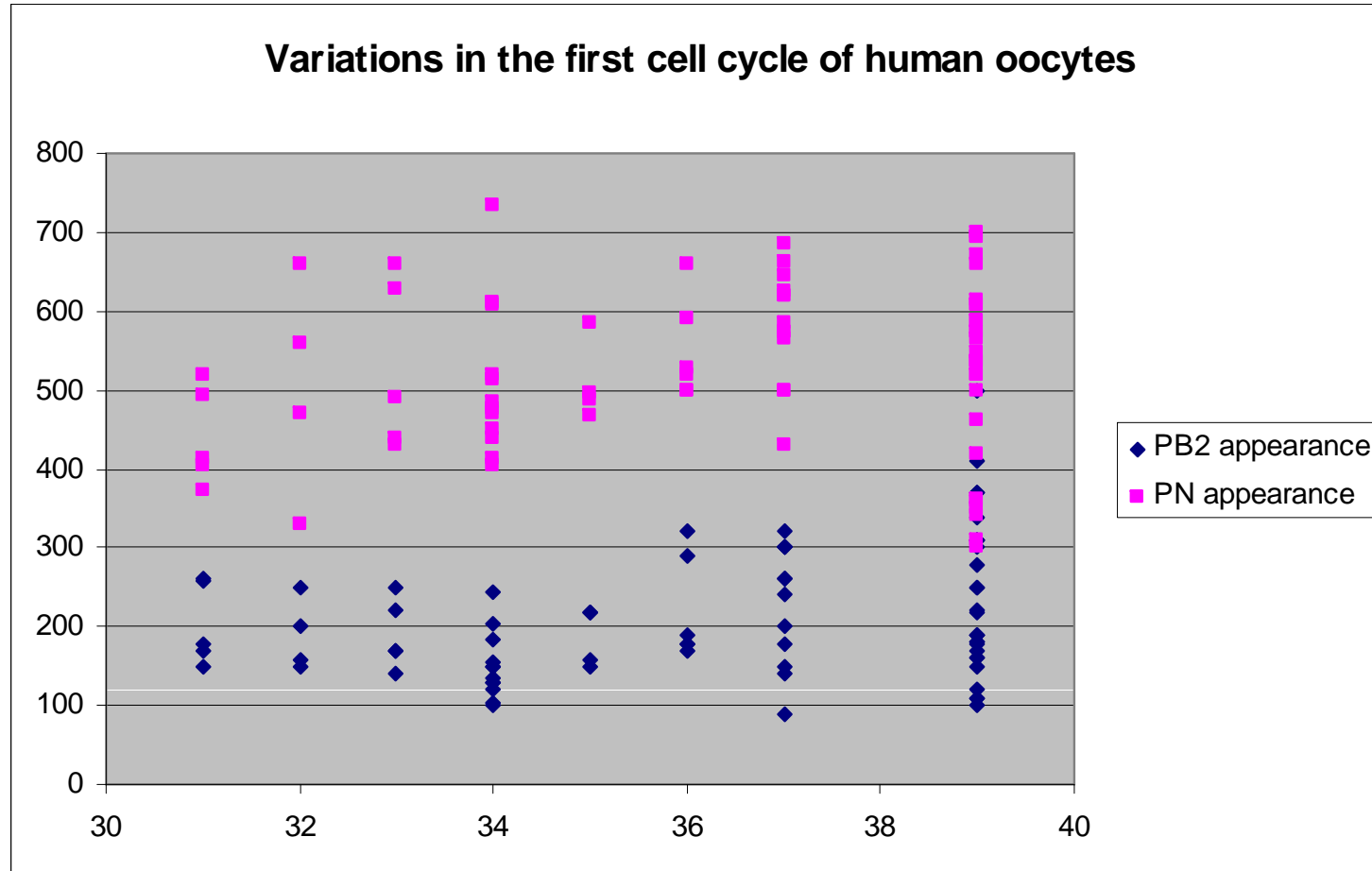
# Timing of PN formation



# PN formation and PN score



# Variations in PN formation



# Conclusions

- Certain zygote parameters are an indicator for the progression through the cell cycle
  - Pronuclear morphology,
  - Orientation of the polar bodies
  - Changes in the cytoplasm (e.g. halo)
- Scoring systems for these parameters are in place, but their absolute benefit is somehow questioned in the era of blastocyst culture
- Imaging zygote development may prove as a new parameter, however, it's relevance is under investigation