# What has PGD-A taught us about ART and preimplantation development?

Dr. Leeanda Wilton
Scientific Director
Preimplantation Genetics
Melbourne IVF

leeanda.wilton@mivf.com.au

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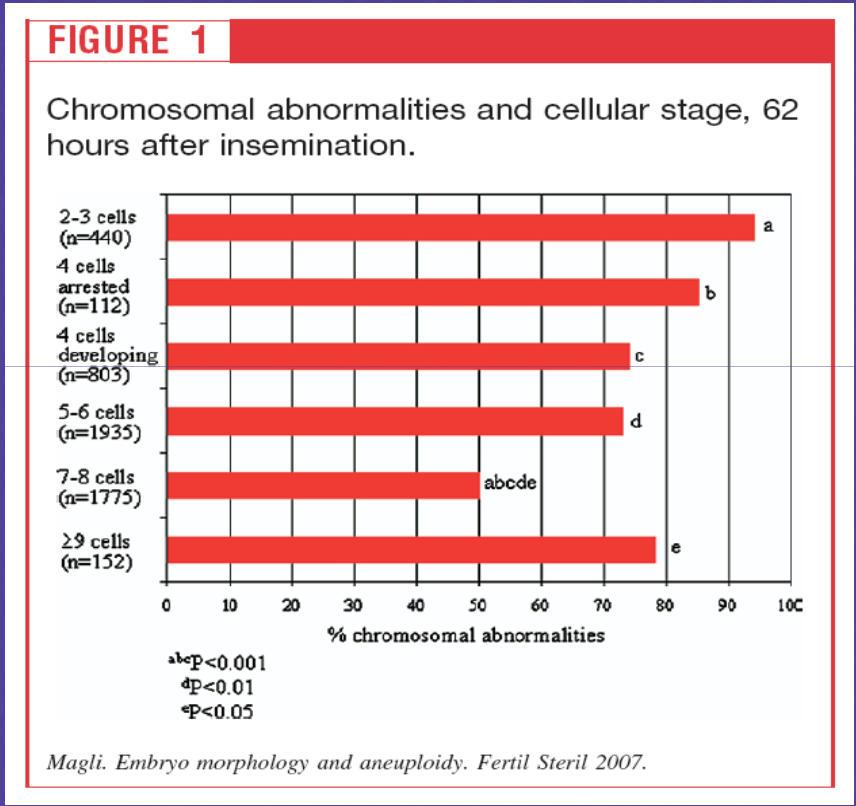
# Embryo morphology/development and chromosome error

- Some features of embryo development are associated with increased chromosome error
- Increased abnormality if development is:
  - Delayed or arrested
  - Accelerated
- Embryos that grow at expected rate have lowest frequency of error

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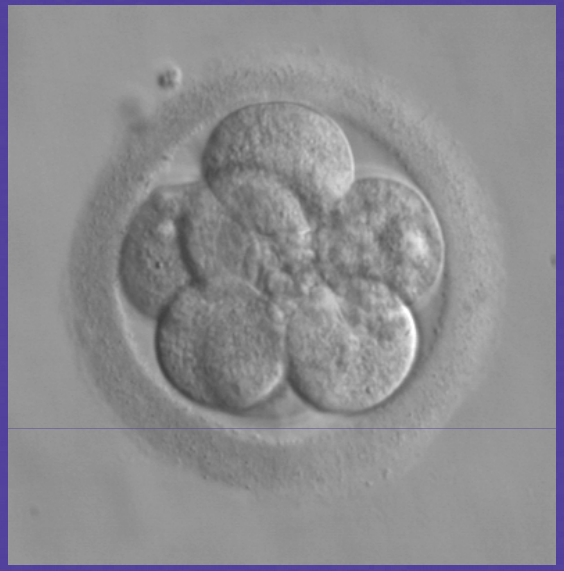
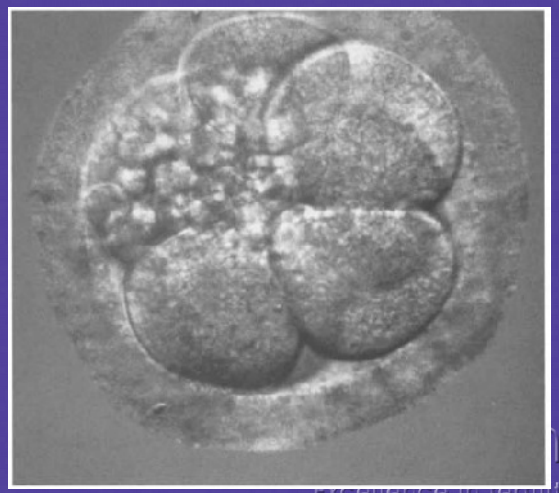
# Fragmented embryos

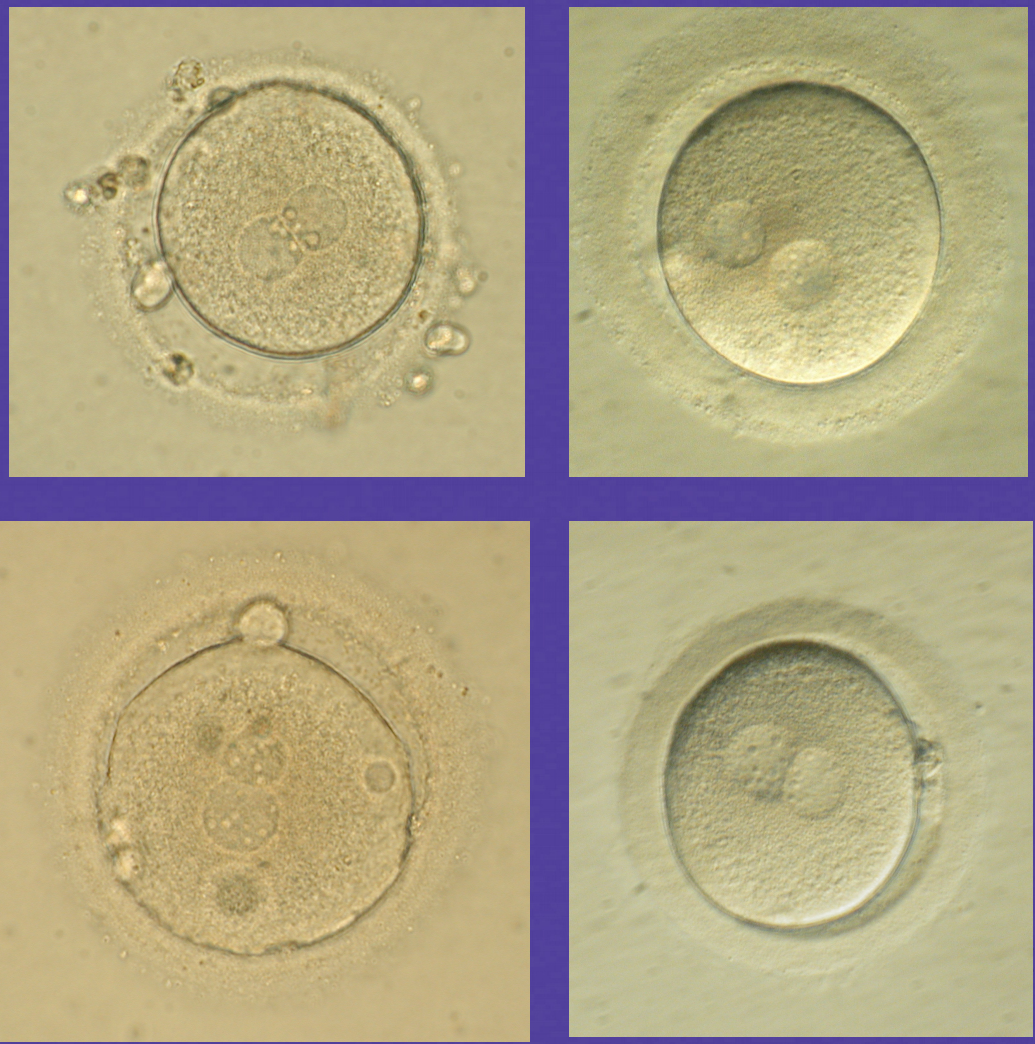
- Fragmentation is common in early embryos
- Strong correlation with implantation and pregnancy
- Munné 2006: high (>35%) fragmentation
  - Polyploidy/haploidy
  - Mosaicism
- Type of fragmentation matters in good embryos (Magli et al., 2007)
  - Scattered fragments associated with increased chromosomal errors





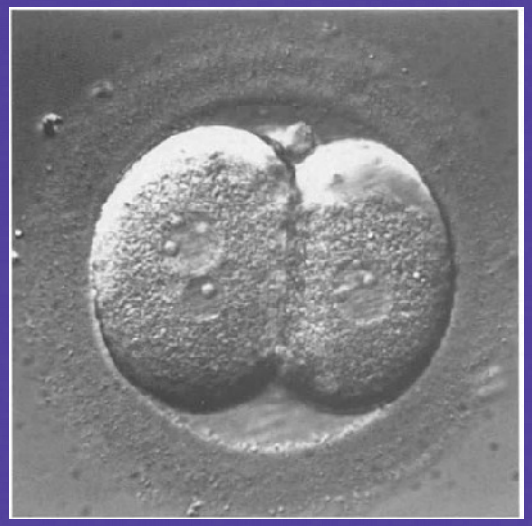
# Pronuclear morphology and chromosome error

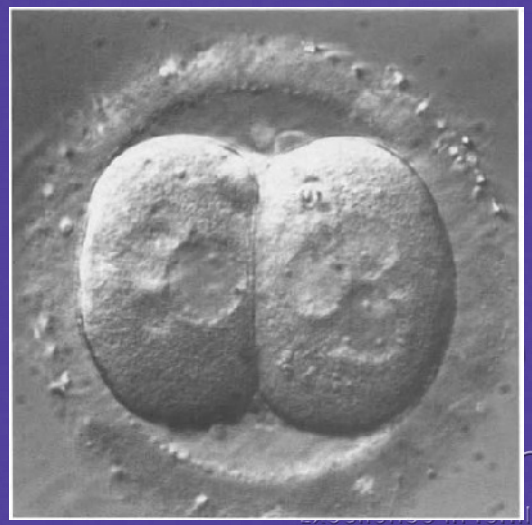
- Pronuclear morphology associated with better outcomes after IVF
- Number of studies have demonstrated correlation with of pronuclear morphology with chromosomal error
- Synchrony of pronuclei and NPBs is important



# Multinucleated embryos

- 15-20% of embryos exhibit some degree of multinucleation up to day 3
- Seem to have increased frequency of error
  - Extensive mosaicism
  - Polyploidy
  - Haploidy
- Indicative of poor embryo development
  - Karyokinesis without cytokinesis



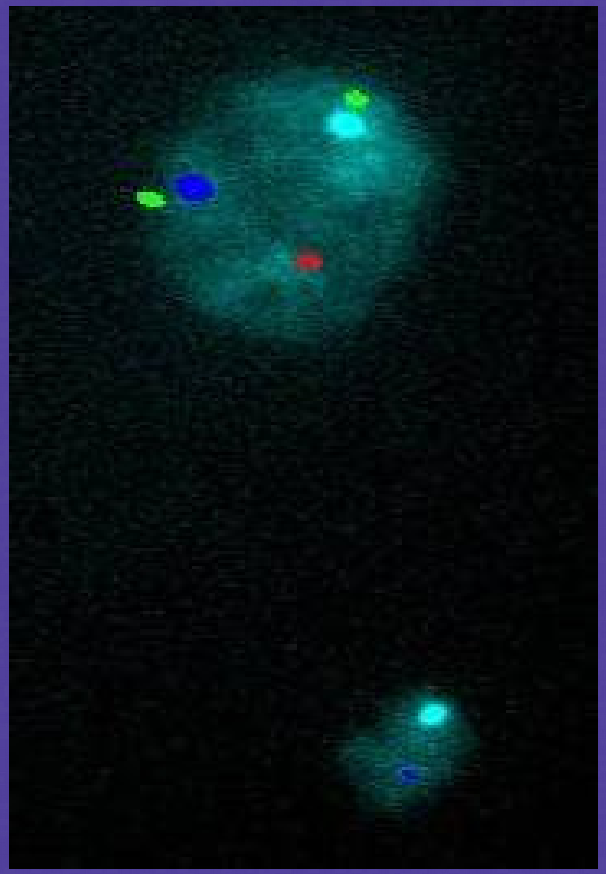


# Frequency and types of chromosome error

- Most studies report 20-80% of human embryos have ≥ 1 chromosomal error(s)

- Meiotic error
  - Arises in gametes
  - Consistent in every cell in embryo
  - Non-disjunction, anaphase lag etc
  - ***10-20% of errors are meiotic***

- Post-zygotic error
  - Mosaicism
  - Different chromosome complement in every cell
  - Complex error
  - Non-disjunction
  - Nuclear fragmentation
  - ***30-60% of errors are post-zygotic***



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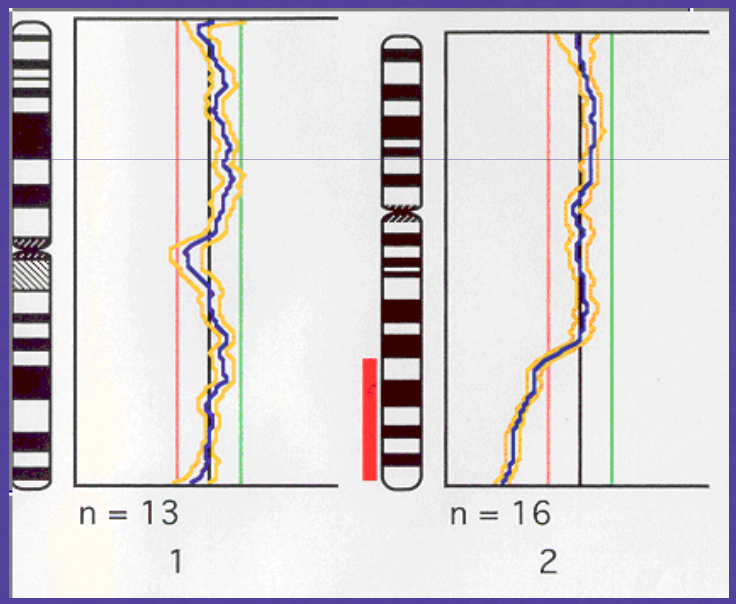
# Types of chromosome error

Chromosome breakage

- Identified by CGH
- Accounts for ~15% of all errors seen
- May be a “hotspot” on long arm of chr 2



# Aetiology of infertility and chromosome error

- Advanced maternal age:
  - extensive FISH evidence that aneuploidy in embryos increases with advancing age (Munné et al., 1995; Marquez et al., 2000; Bielanska et al., 2002)
    - Particularly chromosomes 16, 18 and 21
  - CGH evidence shows no change in complex abnormality but increase in aneuploidy of 1-2 chromosomes in women ≥ 38 years

| | <38 years | ≥ 38 years |
|---|---|---|
| Embryos with aneuploidy of 1-2 chromosomes | 20% | 38% |
| Complex abnormality (≥ 3 chromosomes) | 29% | 30% |

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# Complex abnormality and RIF

- RIF = recurrent implantation failure
  - Criteria = ≥ 10 (mean = 16) embryos transferred without pregnancy
- Complex aneuploidy is not associated with AMA
- Suggestion from our earlier work that complex abnormality may be increased in RIF patients

# Voullaire et al., 2007

| | RIF | No RIF (RMC, AMA) | |
|---|---|---|---|
| Embryos with aneuploidy of 1-2 chromosomes | 24.6% | 32% | NS |
| Embryos with complex abnormality (≥ 3 chromosomes) | 31.6% | 11.8%* | P=0.035 |

CGH data from ~100 abnormal embryos

# RIF and complex abnormality

- Complex abnormality
  - Independent of maternal age
  - more likely to be associated with RIF
- Confirmed by Mantzouratou et al., 2007
  - FISH for 6 chromosomes
  - Reanalysis of 350+ embryos
- Suggestive of different cellular or molecular pathology for RIF compared to AMA/RMC
  - More generalized phenomenon
  - Lack of cell cycle checkpoints?

## Is frequent embryonic aneuploidy a human phenomenon?

- Non-primate species have low frequency of chromosome error in newborns and fetuses
  - Mouse oocytes 1-2% aneuploid
- Limited data in non-human primates
  - Abnormal newborns
    - Trisomies of human homologous chromosomes 13, 18, 21 and X in chimps, baboons, rhesus, gorillas and others

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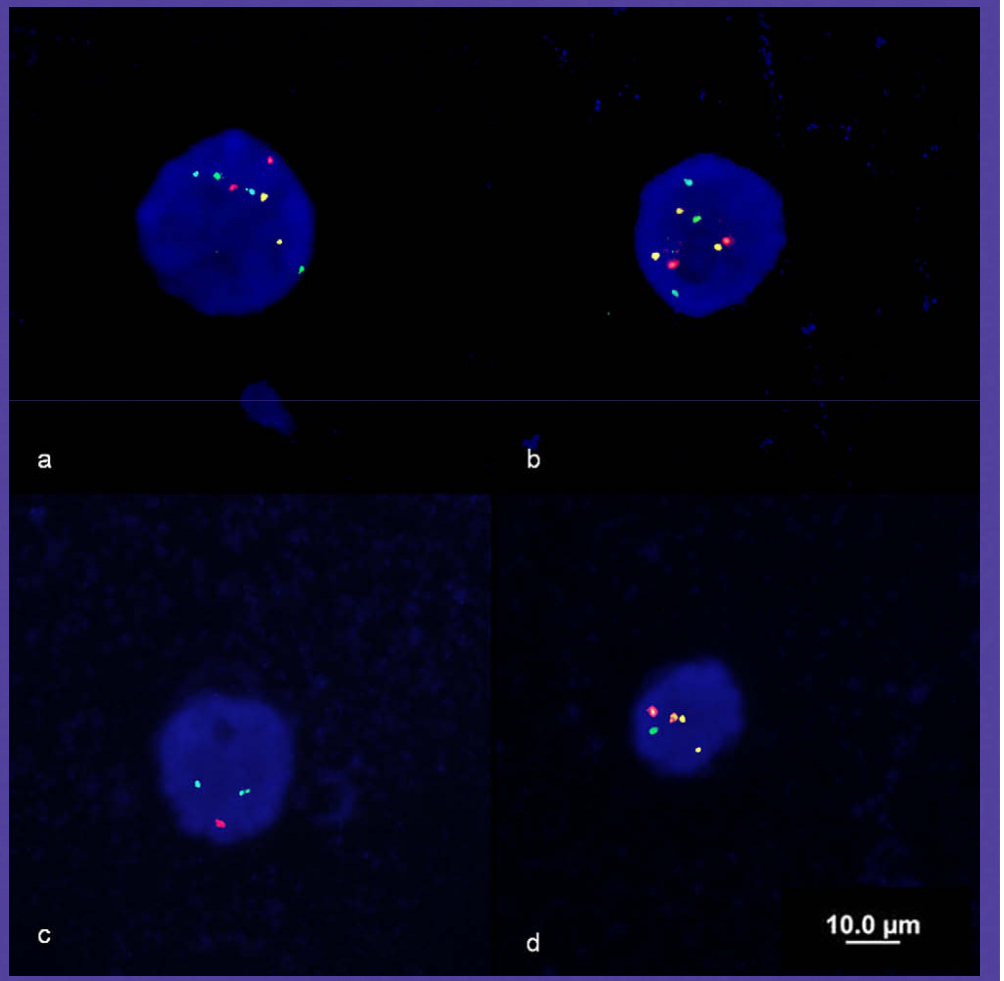
# Dupont et al., 2008

- Rhesus macaque preimplantation embryos
  - 50 good morphology, normally developing
  - From young, superovulated females
- FISH for chromosomes
  - X, Y, 17, 18, 20
  - Homologous to human X, Y, 13, 18, 16

# Dupont et al., 2008

- Results
  - 54% normal
  - 22% mosaic
  - 6% chaotic
  - 4% aneuploid
  - 6% haploid
  - 8% triploid
- Mirrors patterns seen in human embryos
- Superovulation thought to adversely affect rhesus oocyte quality
- Primate model for chromosome anomalies in early development



# Does superovulation influence aneuploidy?

- Superovulation might increase the frequency of aneuploidy in oocytes/embryos (Munné et al., 1997)
- Baart et al., 2007
  - RCT of conventional vs mild stimulation found
    - Fewer oocytes/embryos
    - Lower frequency of chromosome errors
- Verpoest et al., 2008
  - Unstimulated IVF in young women
    - 36% aneuploidy (single blastomere)

# Conclusion

- The past 20 years of PGD has informed us about
  - Early embryo development
  - Aetiology of infertility
- The next few years will be an exponential increase in knowledge with expanding ability to analyse all chromosomes
- Valuable because it benefits a much broader group of patients

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