Impact of Oocyte Quality on Embryo Viability

ESHRE Campus Symposium
"Everything you forgot about gamete physiology and its impact on embryo quality"
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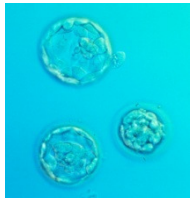


GMJ A.R.T. Solutions

ΓΕΝΕΣΙΣ
ΑΘΗΝΩΝ
ΙΑΤΡΙΚΗ ΜΕΡΙΜΝΑ ΓΥΝΑΙΚΟΛΟΓΙΑ
ΚΑΙ ΧΕΙΡΟΥΡΓΙΚΗ ΑΝΩΝΥΜΗ ΕΤΑΙΡΕΙΑ

miscl monash immunology and stem cell laboratories

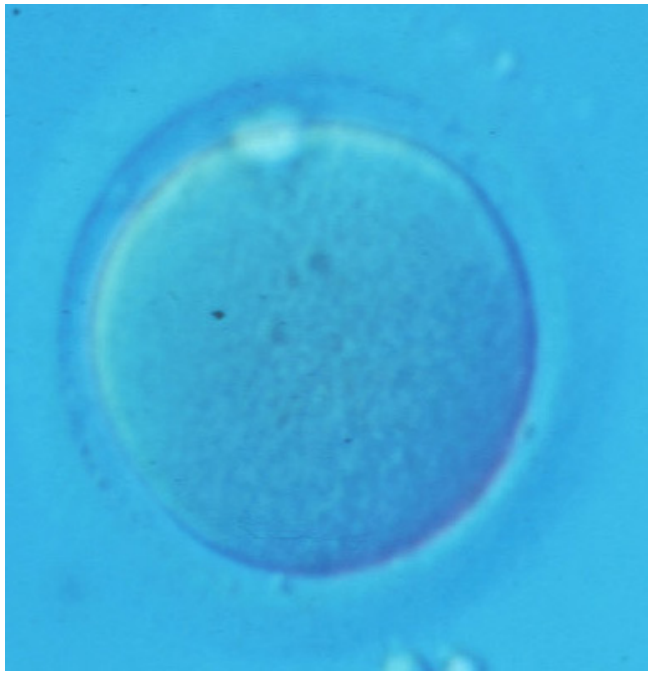
Learning Objectives

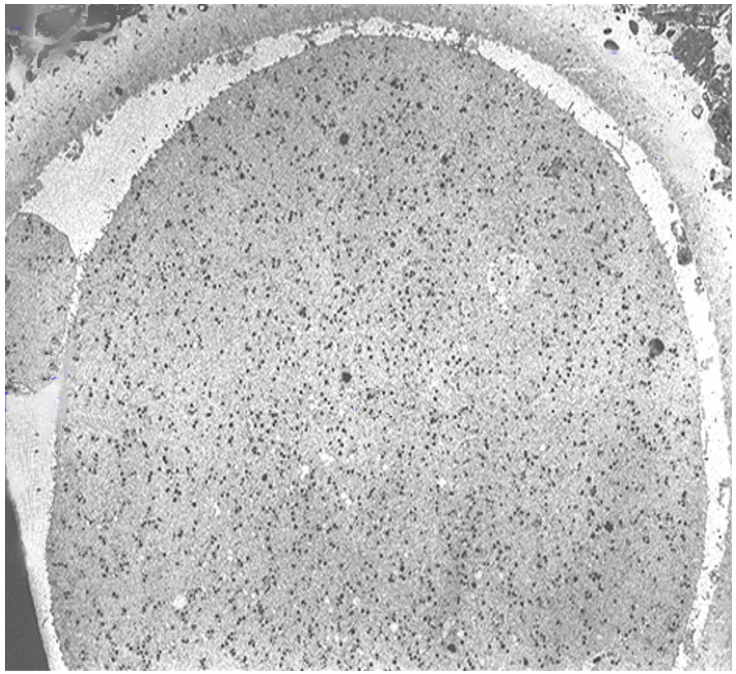
- An understanding of both the structural and molecular make-up of oocytes that contributes to ongoing health and viability of the embryo
- How deviations in any of these components can contribute to ongoing pathology
- Understanding that incorrect accumulation or temporal utilization of molecular message can result in pathologies such as cleavage failure or loss of viability
- The effect of maternal ageing on the structural and molecular components of the oocyte and how this contributes to developmental incompetence

Maternal Contribution to Embryo Development

- Structural
  - Chromosomes
  - Microtubules
  - Organelles
- Molecular
  - DNA
  - RNA
  - Protein

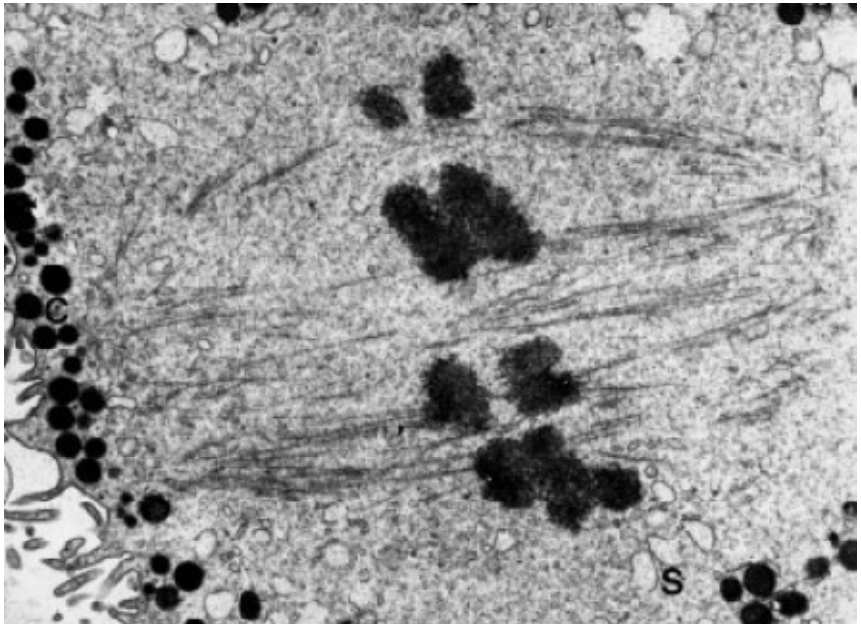
Mature Human Oocyte





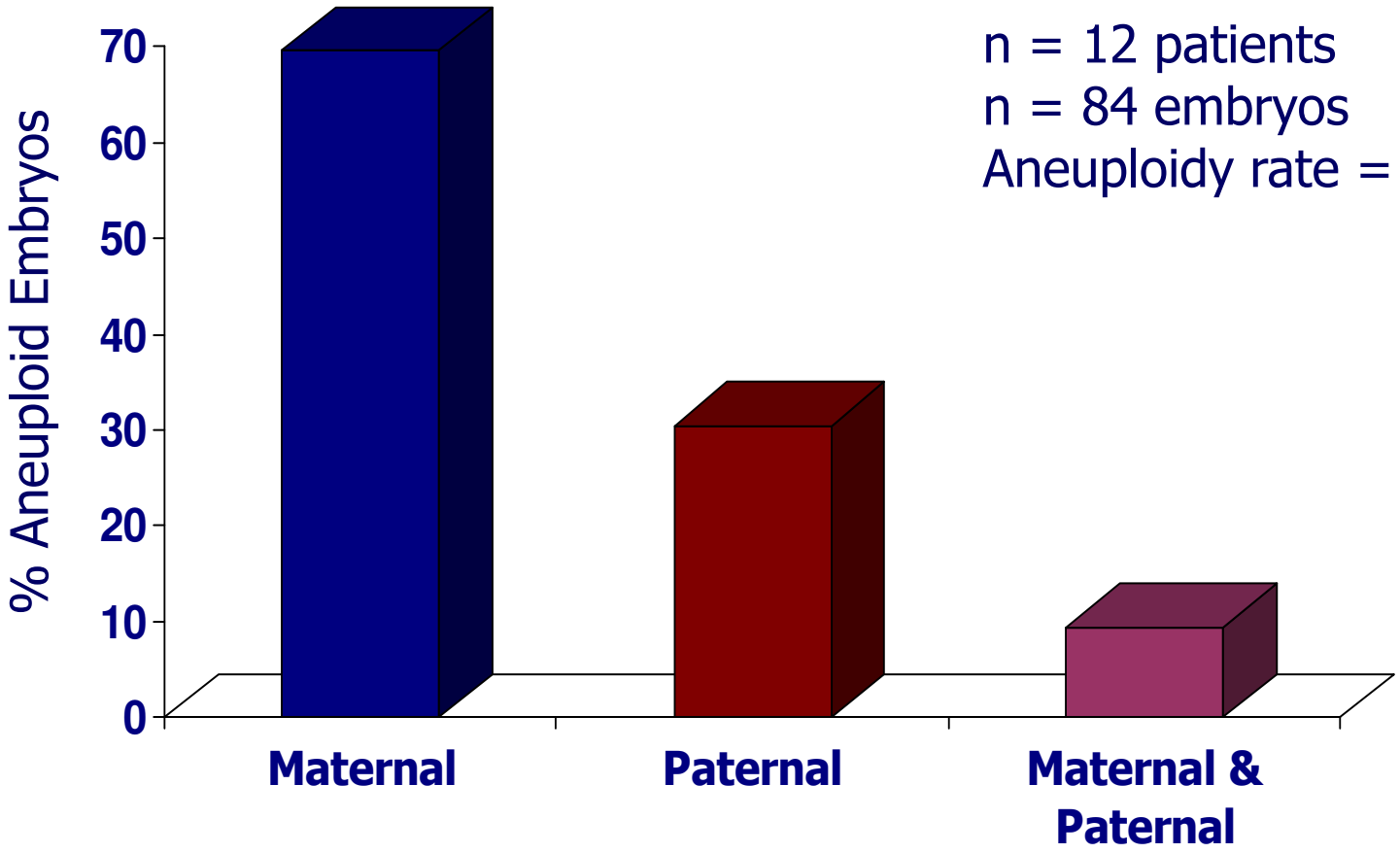
- Packaged with all the structural elements required for complete preimplantation development
- Exceptions: Paternal chromosomes and centrosome

Maternal Chromosomes



- Incidence of aneuploidy in first trimester abortions as high as 65% (Menasha et al., 2005)
- Aneuploidy in oocytes arises from both chromatid predivision and whole chromosome non-disjunction
- Karyotyping of oocytes from infertile women reveals aneuploidy rate around 11%
- CGH analysis of mature oocyte and its 1st PB reveals an aneuploidy rate >22%

Origin of Aneuploidy in Embryos



n = 12 patients
n = 84 embryos
Aneuploidy rate = 63%

Based on PCR detection of aneuploidy in patients with a chromosomal translocation

Centre for Human Reproduction, Genesis Athens Clinic & Genoma

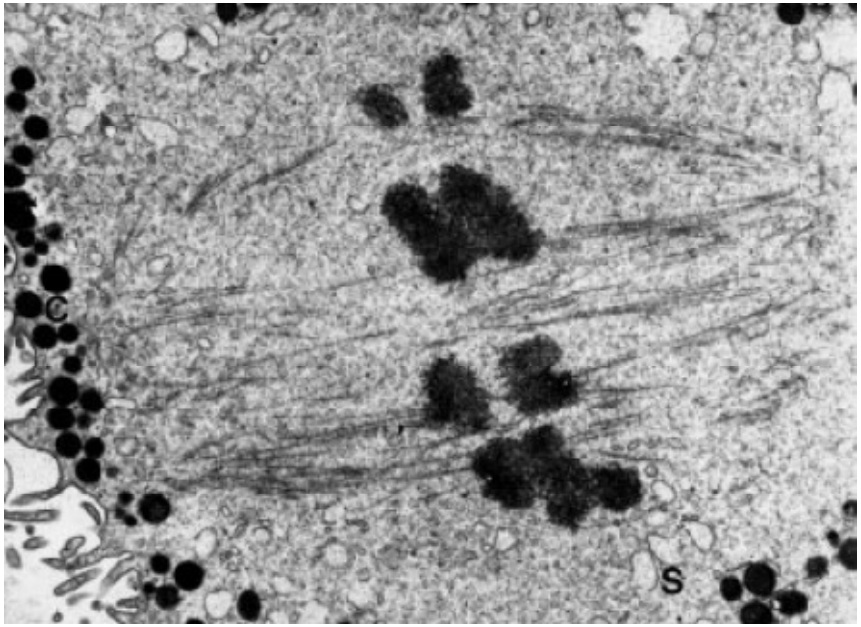
Origin of Aneuploidy in Human Oocytes

Chromosome	Total Abnormal*	Meiois I origin	Meiosis II origin	Meiosis I & Meiosis II origin
13	302 (8.5%)	167 (55.3%)	95 (31.5%)	40 (13.2%)
16	361 (10.1%)	127 (35.2%)	171 (47.4%)	63 (17.4%)
18	317 (8.9%)	212 (66.9%)	87 (27.4%)	18 (5.7%)
21	477 (13.4%)	248 (52.0%)	158 (33.1%)	71 (14.9%)
22	514 (14.4%)	178 (34.6%)	236 (45.9%)	100 (19.5%)

* Of 3598 oocytes with PB1 & PB2 results

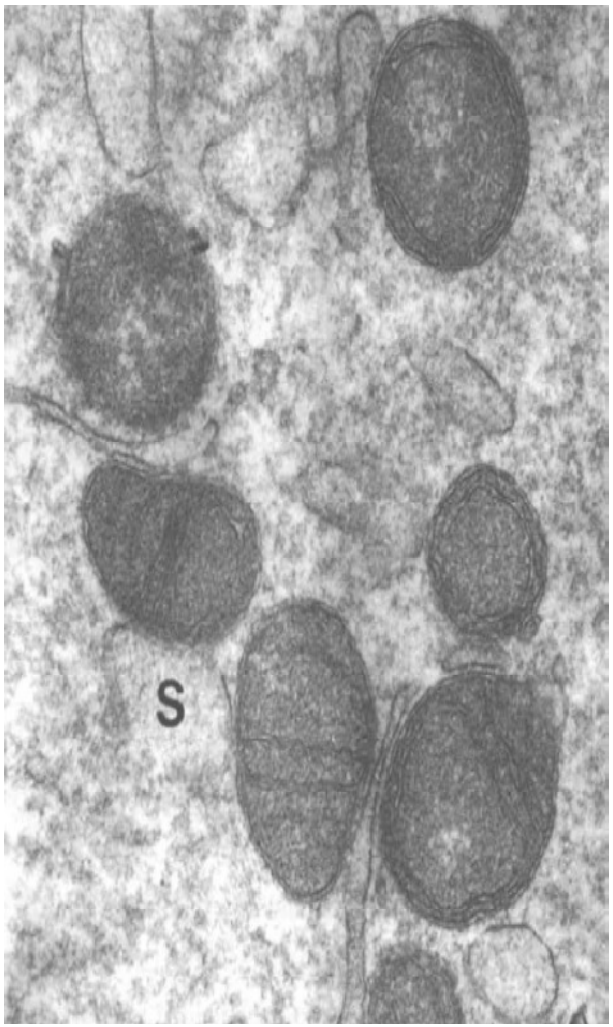
Kuliev et al 2005

Cortical Granules

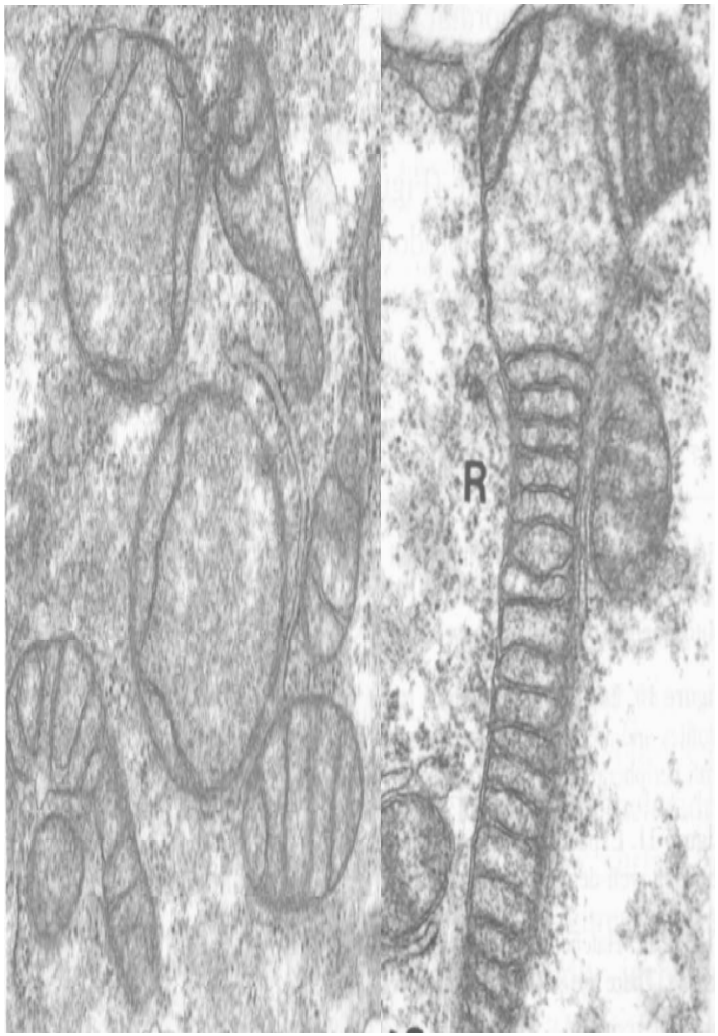


Upon fertilization cortical granules are exocytosed into the perivitelline space where the contents react with the zona pellucida to establish a block to polyspermy

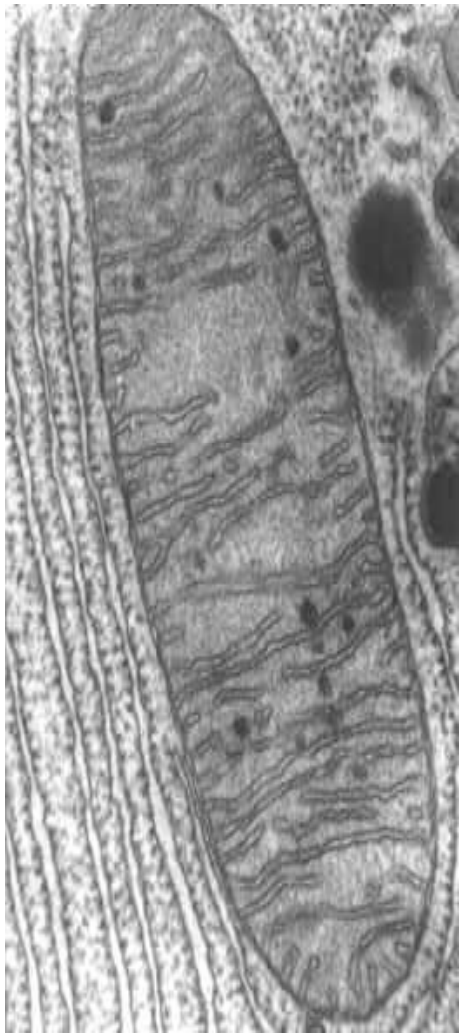
Mitochondria



Oocyte



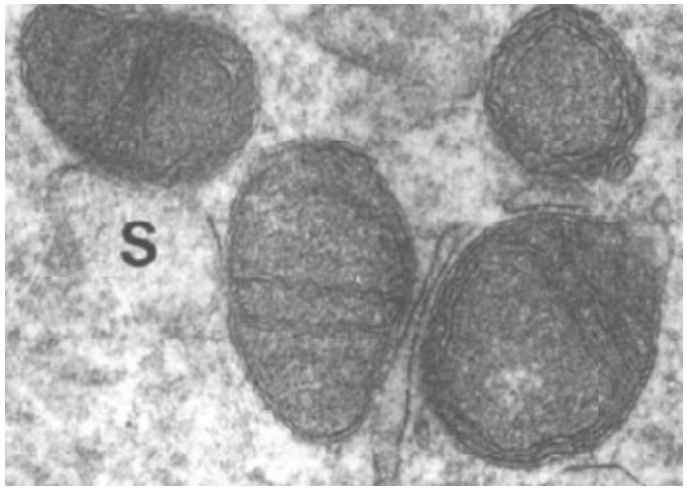
Blastocyst

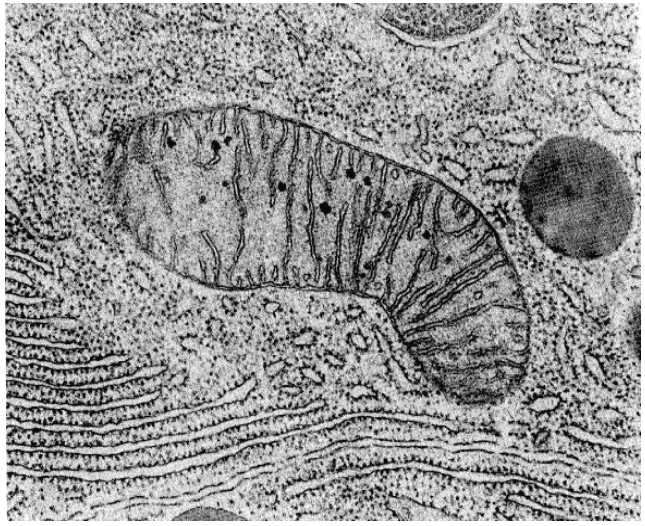


Somatic Cell

Somatic cell mitochondria:

- High metabolic activity
- Located close to energy demand in cells
- Over 200 varieties with tissue specific morphologies





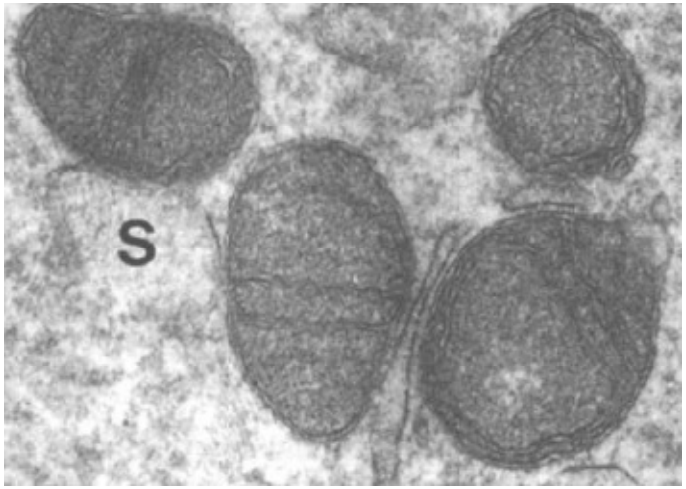
Oocyte mitochondria:

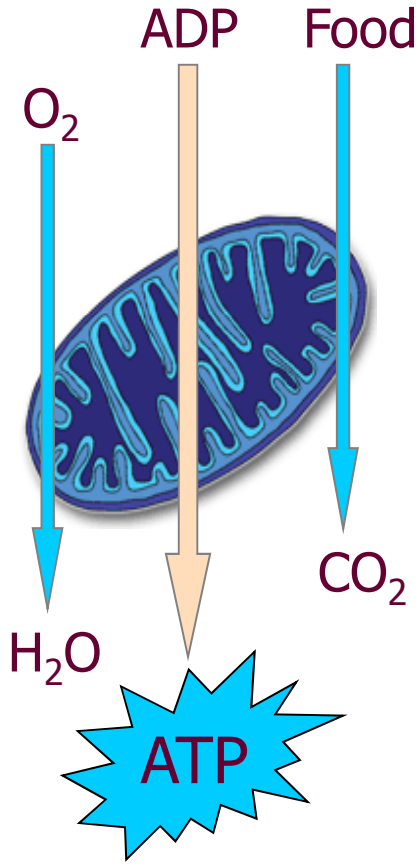
- Large numbers (>$1x10^5$)
- Not yet mature in structure
- Metabolically quiescent
- Related to oocyte viability

Oocyte Mitochondria – Biogenesis

- Maternally inherited
- mtDNA - Genetic selection and population sorting during oogenesis ('bottleneck')
- Multiply during oogenesis to 10^5 to 10^6 per mature oocyte
- Maternally derived mitochondria preferred – (selective proteolysis of midpiece mitochondria from sperm)
- Stage-specific redistributions during development
- Structural remodelling during preimplantation stages
- Replicate after blastulation

Mitochondria – Cell Batteries



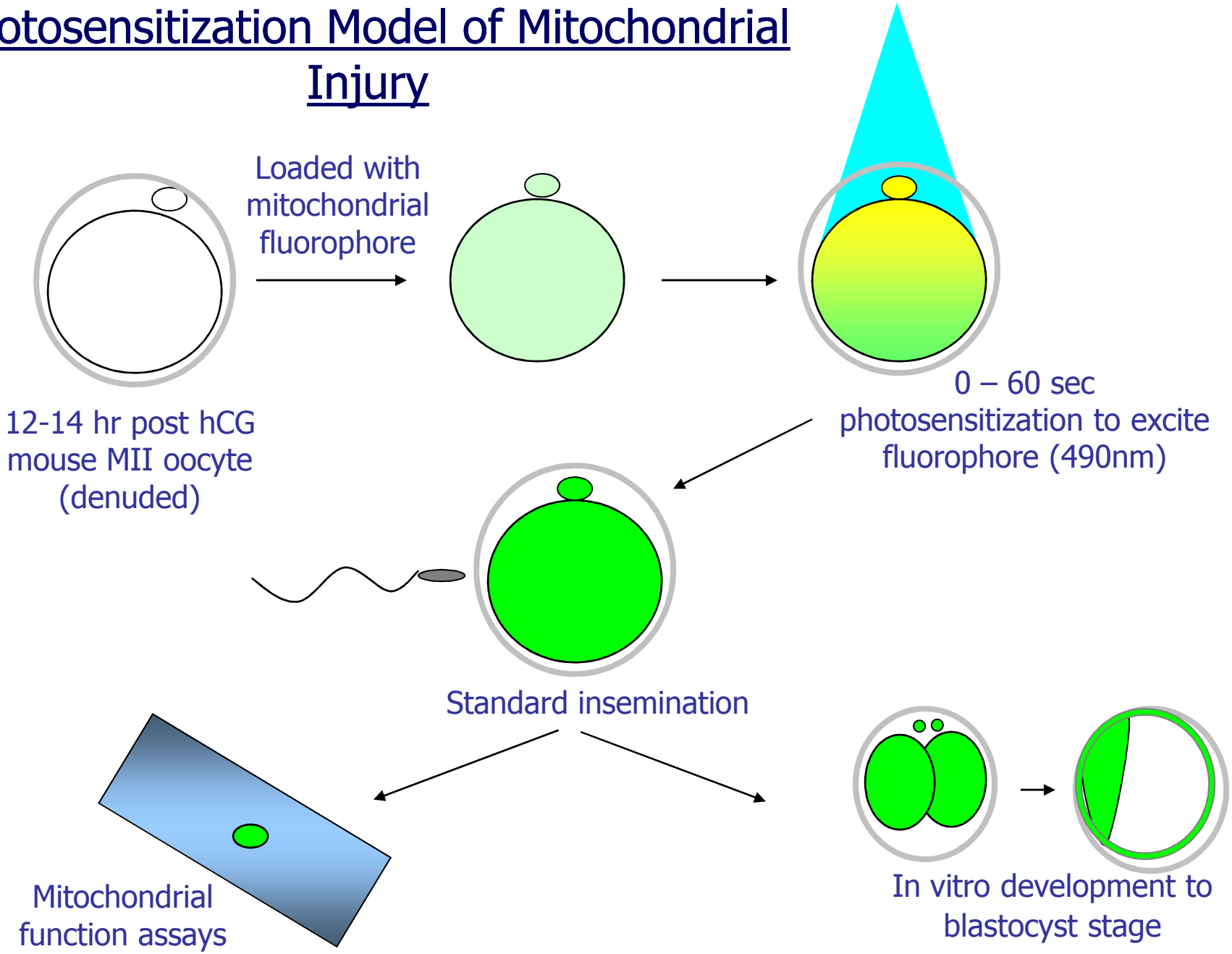


- Oxidative generation of ATP (respiration)
  - TCA cycle
  - Electron transport chain
- Calcium homeostasis/signalling
- Fatty acid metabolism
- Apoptosis

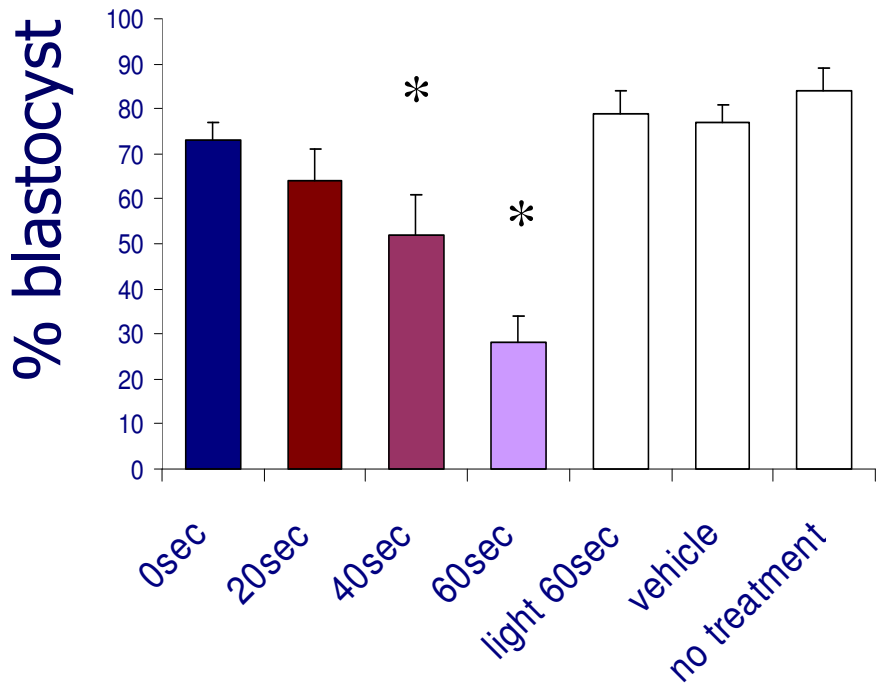
Mitochondrial Function Related to Developmental Competence

- Mitochondrial maturation corresponds to increased oxygen consumption (human, rodent)
- No *in vitro* development without oxygen or pyruvate in culture environment (mouse)
- Aerobic metabolism more closely correlated with blastocyst formation and postimplantation (hamster)
- Specific mitochondrial patterning predictive of developmental competence
- Irregular distributions in oocytes are maintained in early embryo (human)
- Altered pHi can disturb patterning (hamster)
- Oocyte ATP production correlated with morphology, embryo development and viability (human)

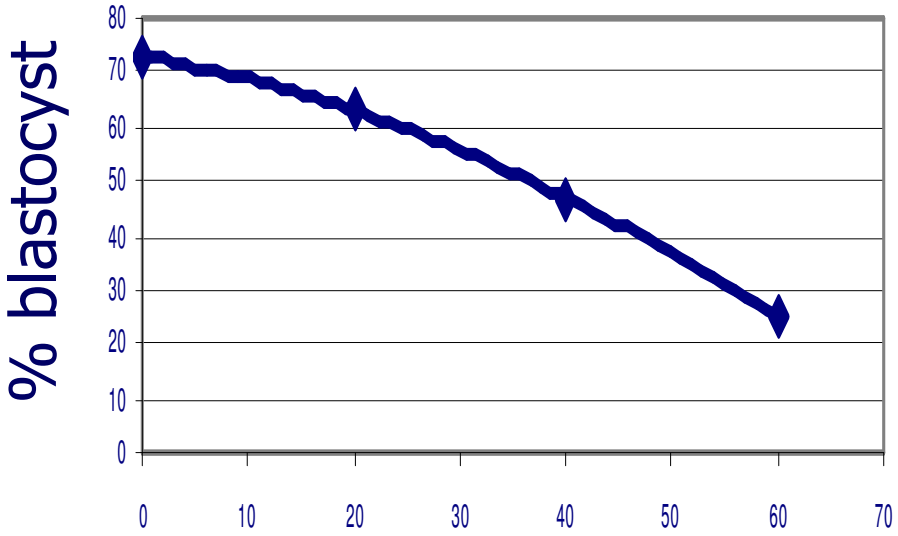
Photosensitization Model of Mitochondrial Injury



Preimplantation Developmental Arrest After Mitochondrial Perturbation



*P < 0.05

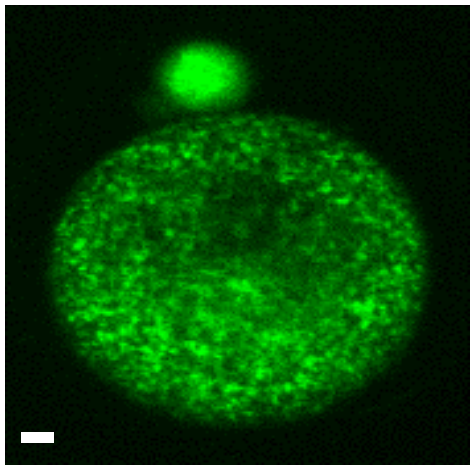


Photosensitization time (sec)

$y = -0.0075x^2 - 0.35x + 73$

$R^2 = 1$

Photosensitization Induction of Mitochondrion-Specific Injury



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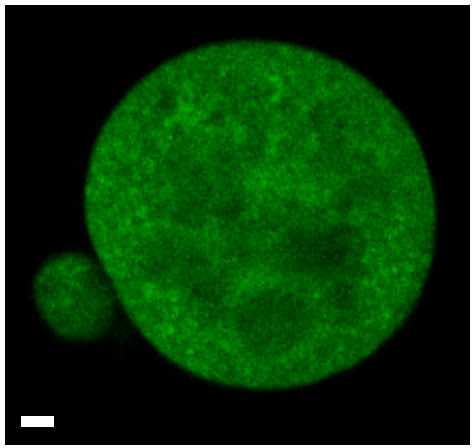
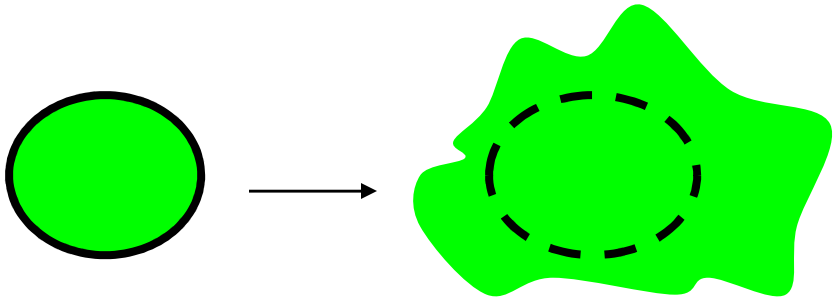
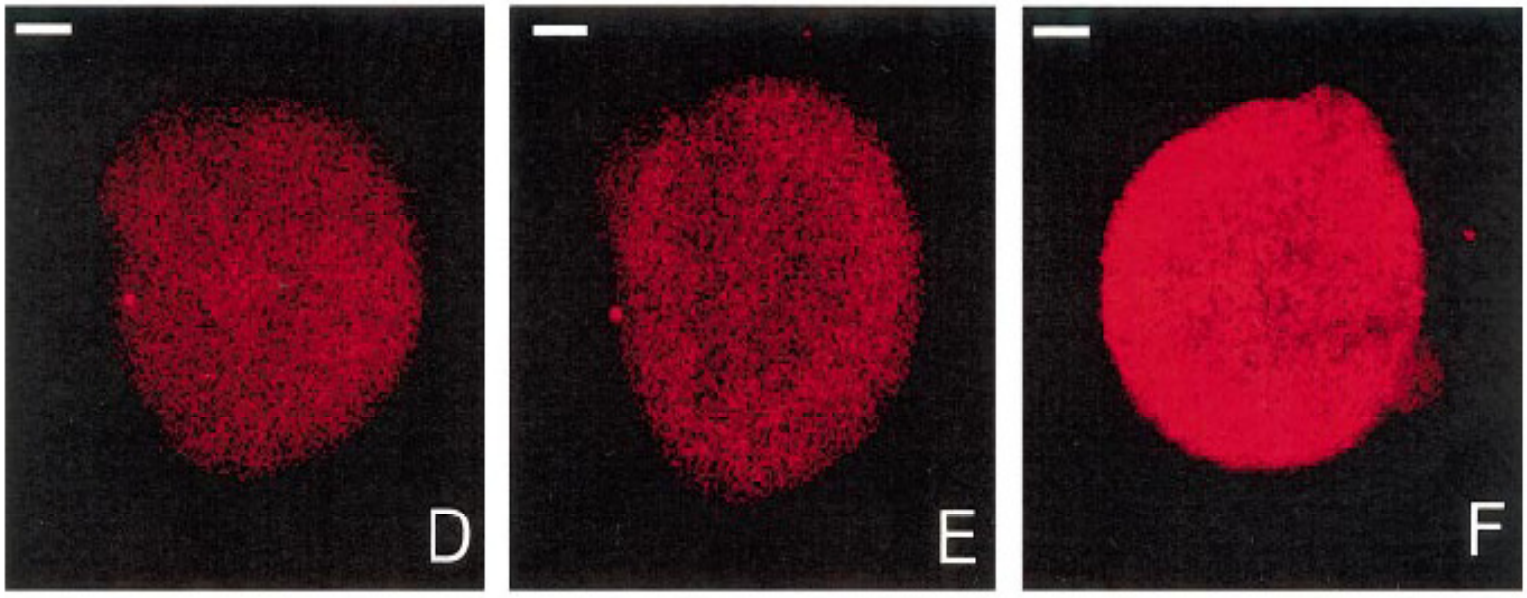


60



Punctate-to-diffuse cytoplasmic staining, indicating seepage of fluorophore from mitochondria via membrane permeabilization

Photosensitization Induction of Mitochondrion-Specific Injury



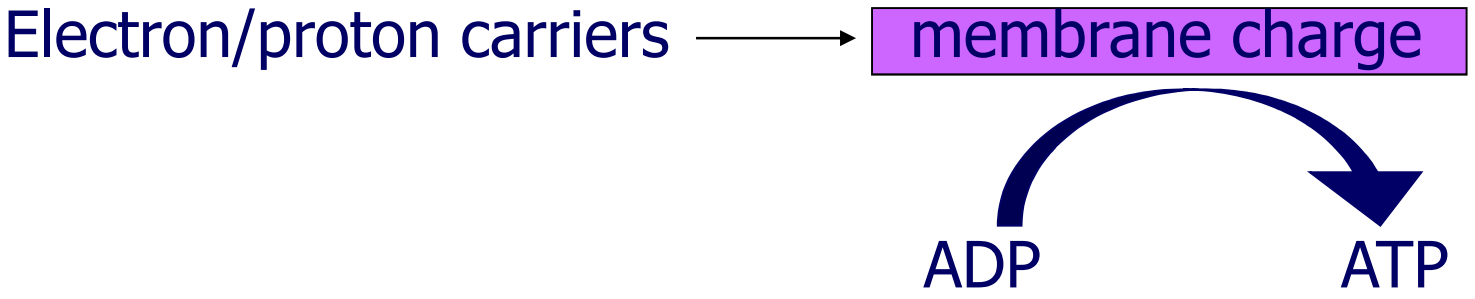
Untreated R123 only R123 + 60sec

Caspase-3 antibody staining

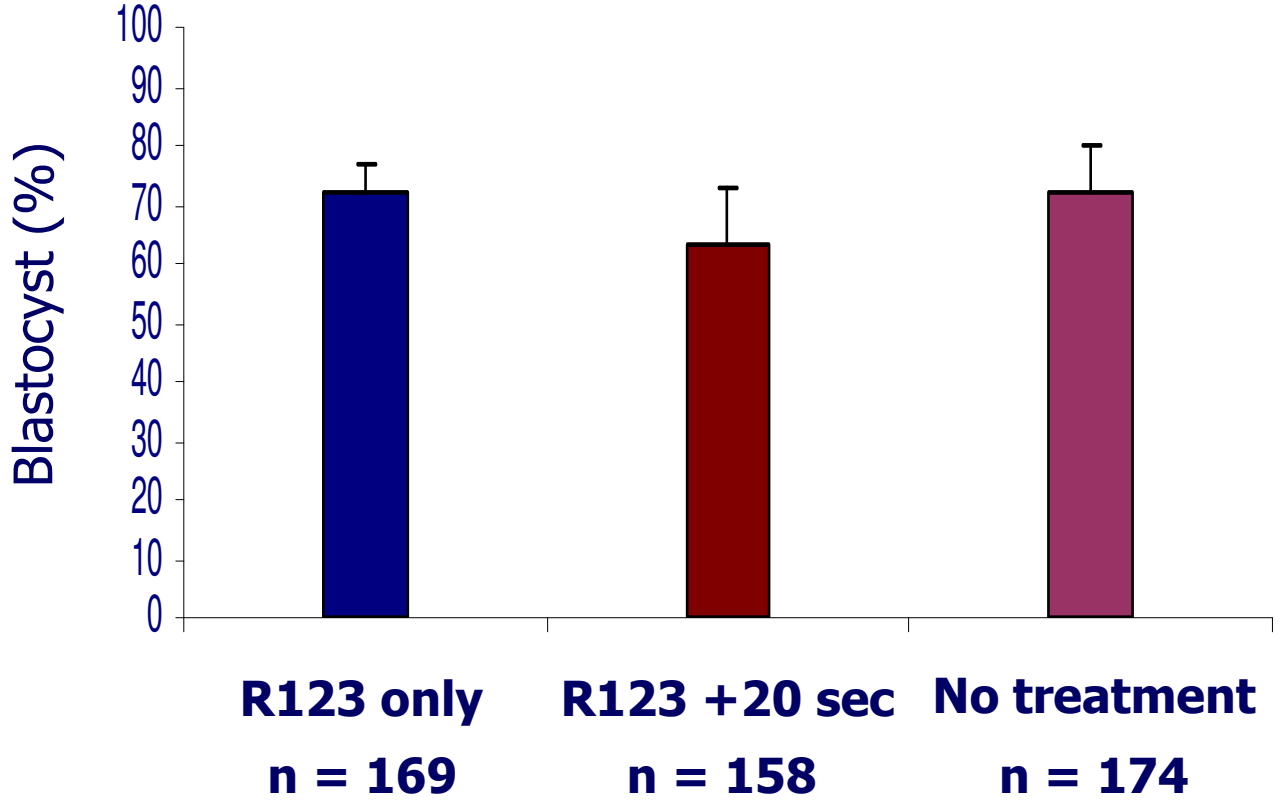
Photosensitization Induction of Mitochondrion-Specific Injury

	Mitochondrial Charge (AU)	**NADH/NADPH (AU)**	ATP (pmol/zygote)
R123	8.2 ± 0.3 (n = 26)	158 ± 12 (n = 38)	3.0 ± 0.8 (n = 30)
R123 + 60sec	4.3 ± 0.2* (n = 42)	125 ± 10* (n = 39)	1.0 ± 0.1* (n = 30)

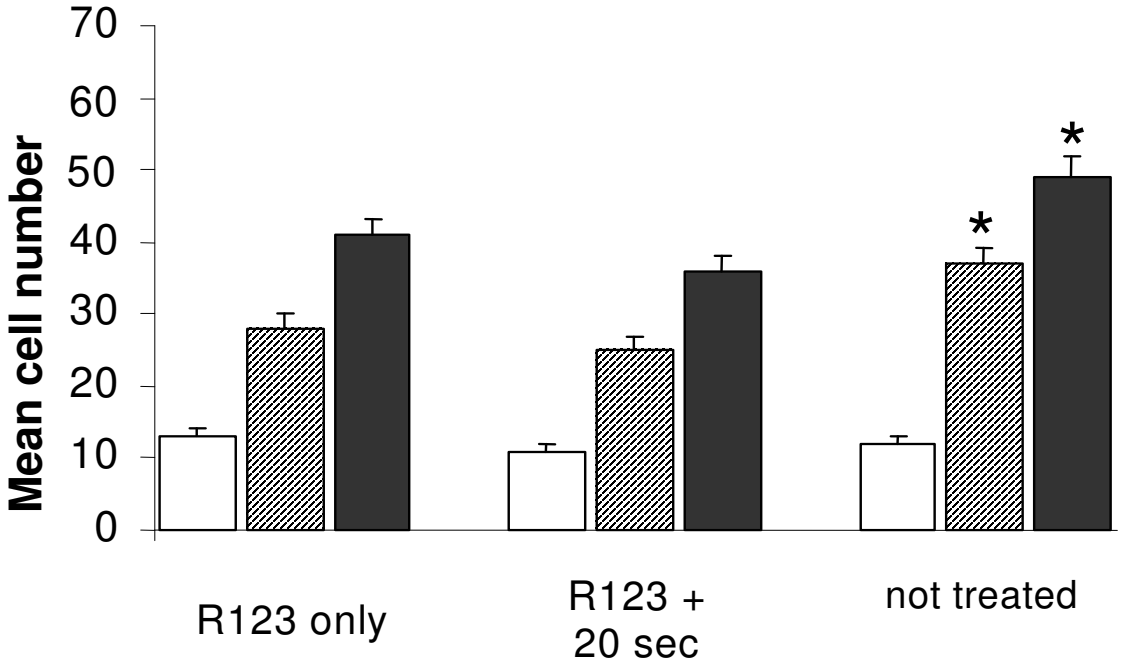
*P < 0.05

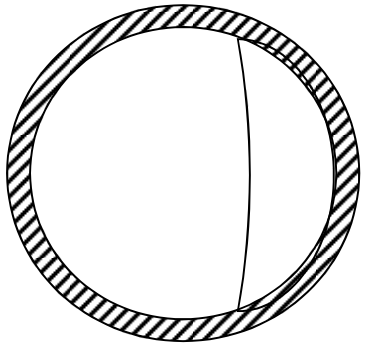


Blastocyst Development Following Sublethal Injury to Mitochondria



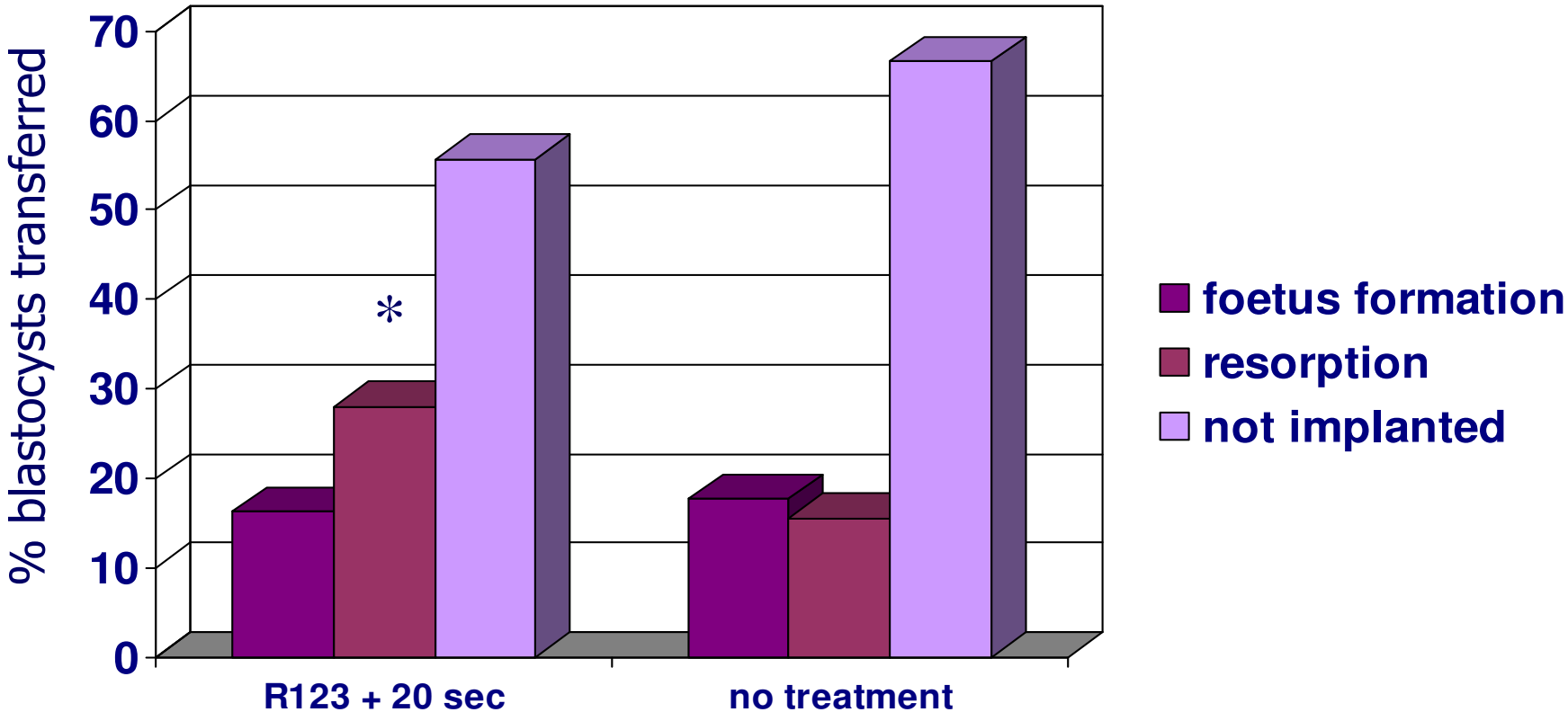
Blastocyst Cell Numbers Following Sublethal Injury to Mitochondria





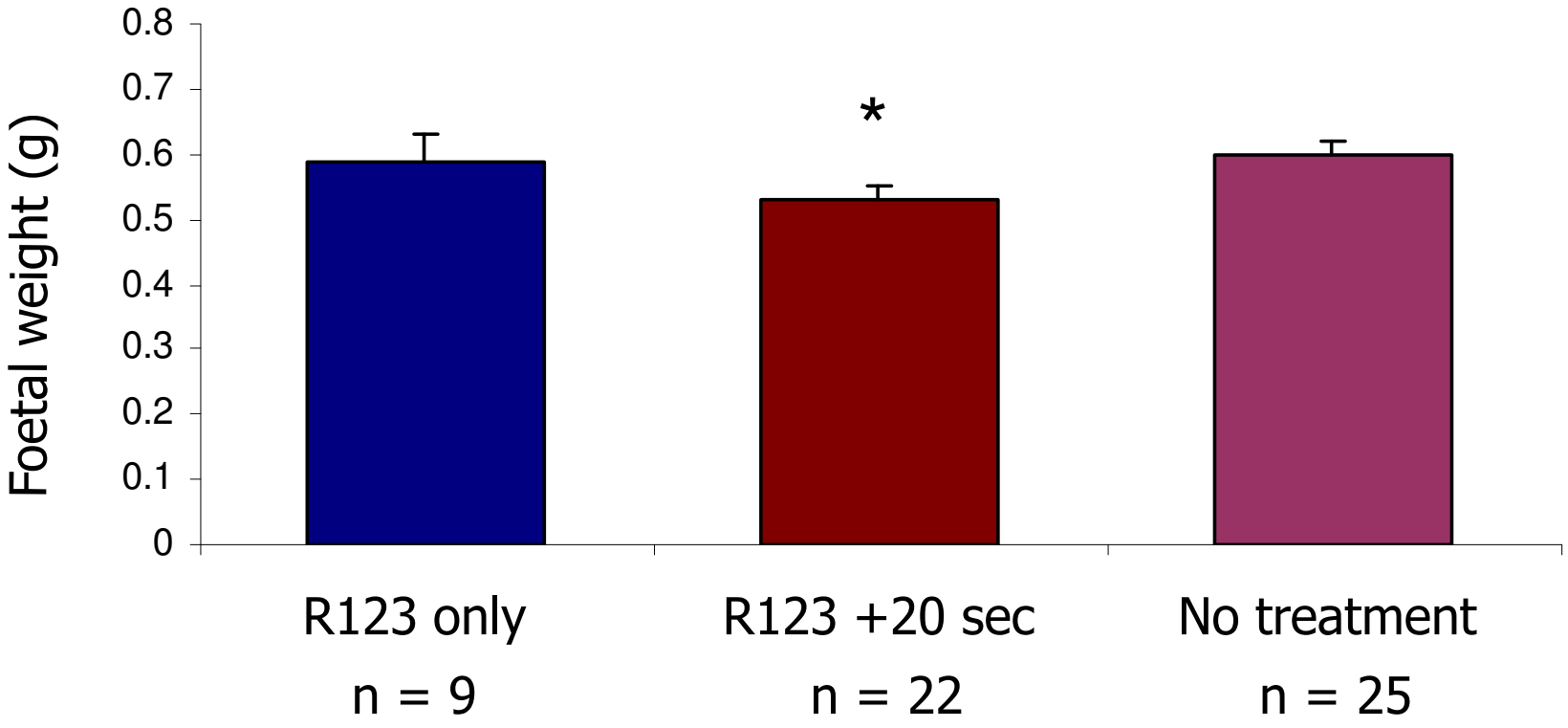
*P < 0.05

Post-implantation Development Following Sublethal Injury to Mitochondria



*P < 0.05

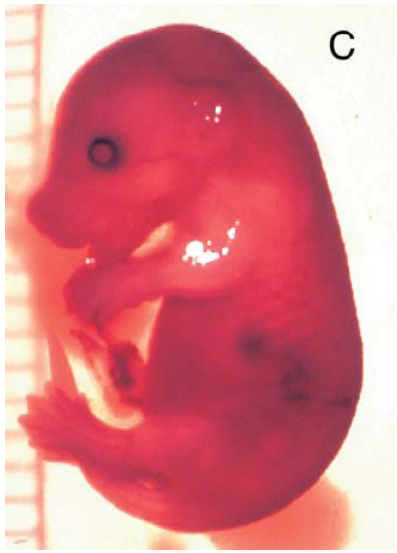
Foetal Weights Following Sublethal Injury to Mitochondria

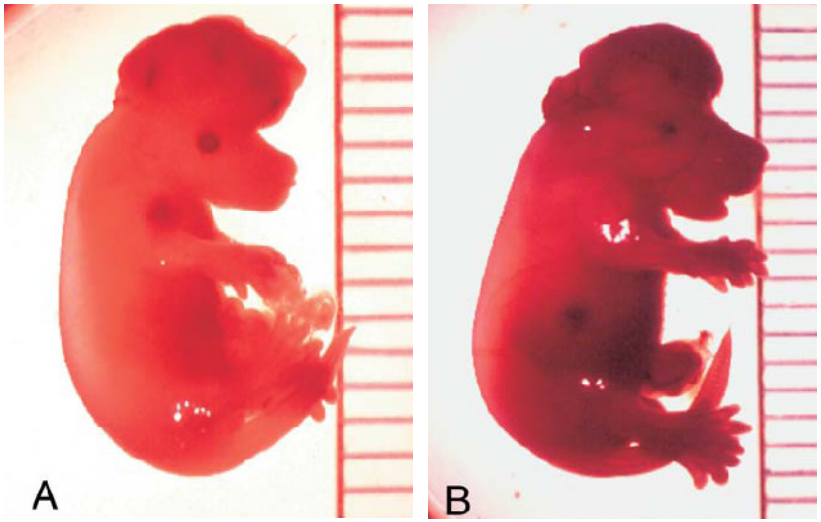


*P < 0.05

Foetal Abnormalities Resulting from Sublethal Injury to Mitochondria

- Evidence of foetal exencephaly after low-dose photosensitization
- Associated with reduced fetal weight

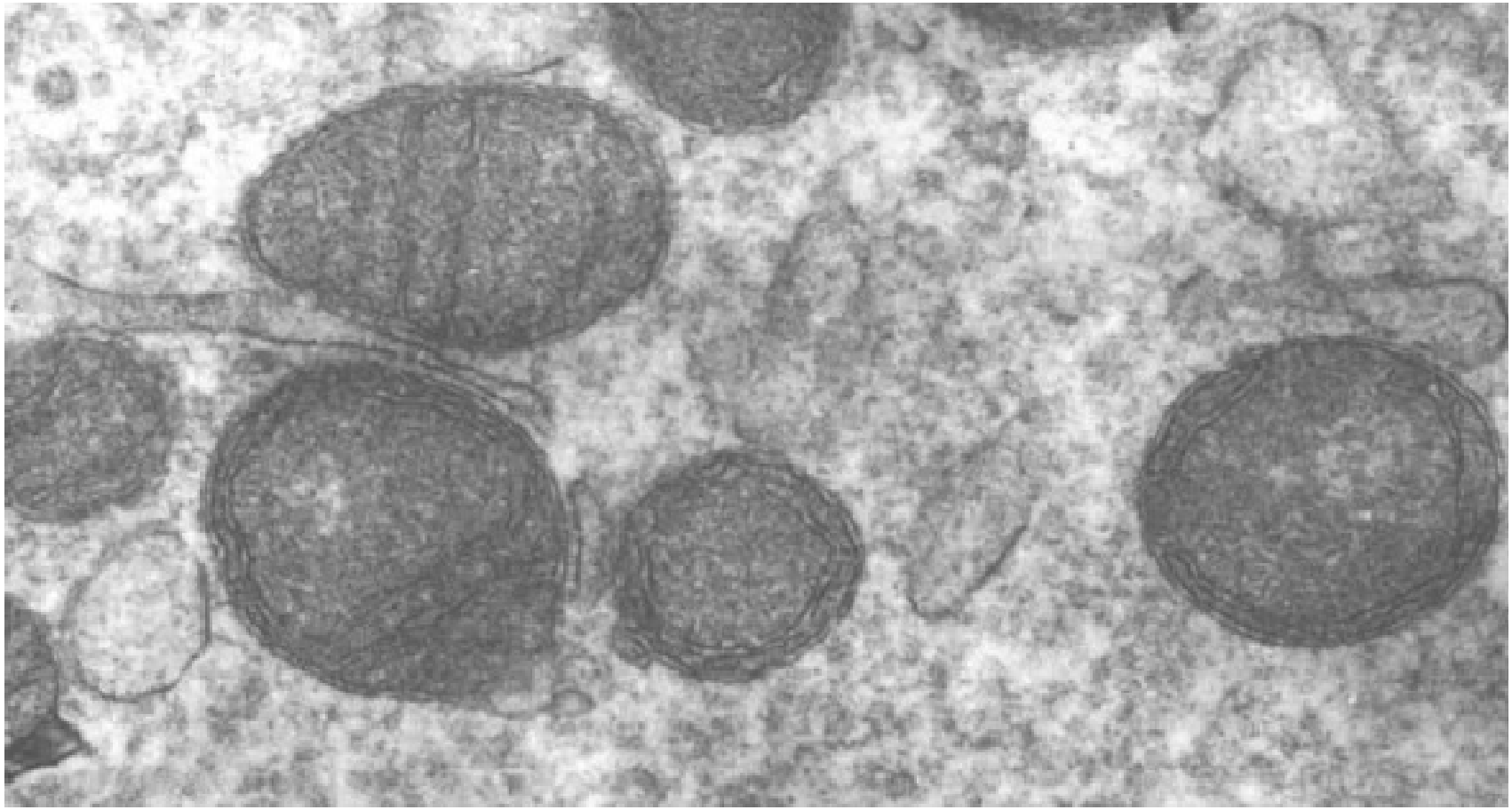




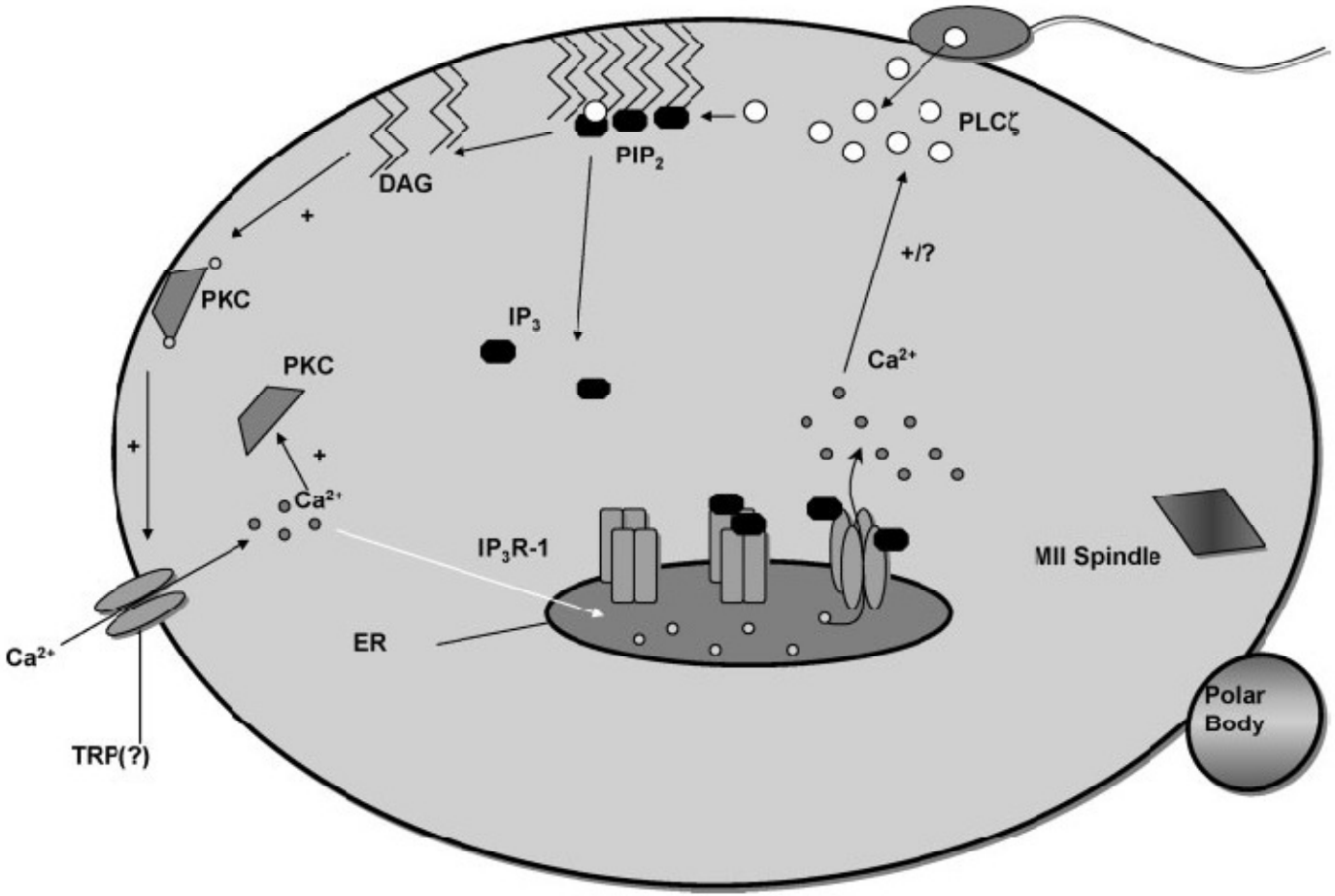
Summary – Mitochondrial Injury Experiments

- Severe injury to oocyte mitochondria can be manifest as immediate developmental arrest and activation of apoptosis
- More subtle injury to oocyte mitochondria can be permissive to complete pre-implantation development but may manifest as decreased/abnormal foetal development post-implantation

Oocyte
Mitochondria – ER complexes



Fertilization Induction of Phosphoinositide Signalling System



Malcuit et al., 2006

Fertilization Induced $Ca^{2+}_{[i]}$ Oscillations in the Oocyte

- Cortical granule exocytosis
  - Block to polyspermy
- Oocyte activation & cell cycle resumption
  - Completion of meiosis
  - Initiation of the mitotic cell cycles
- Recruitment of maternal mRNA's
  - Polyadenylation in preparation for protein translation
  - Degradation of transcripts
    - Deadenylation and microRNA's
- Protein Degradation & Phosphorylation
- Cytoskeletal Rearrangements

Fertilization Induced $Ca^{2+}_{[i]}$ Oscillations in the Oocyte

- Amplitude, frequency and duration of oscillations are important for induction of downstream events
- Demonstrated to effect the differential cell numbers in the blastocyst
  - Single large Ca^{2+} rise results in ↑TE & ↓ICM
  - Oscillations for 2h from activation results in ↑ICM
  - Oscillations extended until pronuclear formation and/or during first mitosis results in ↑↑ICM

Bos-Mikich et al., 1997

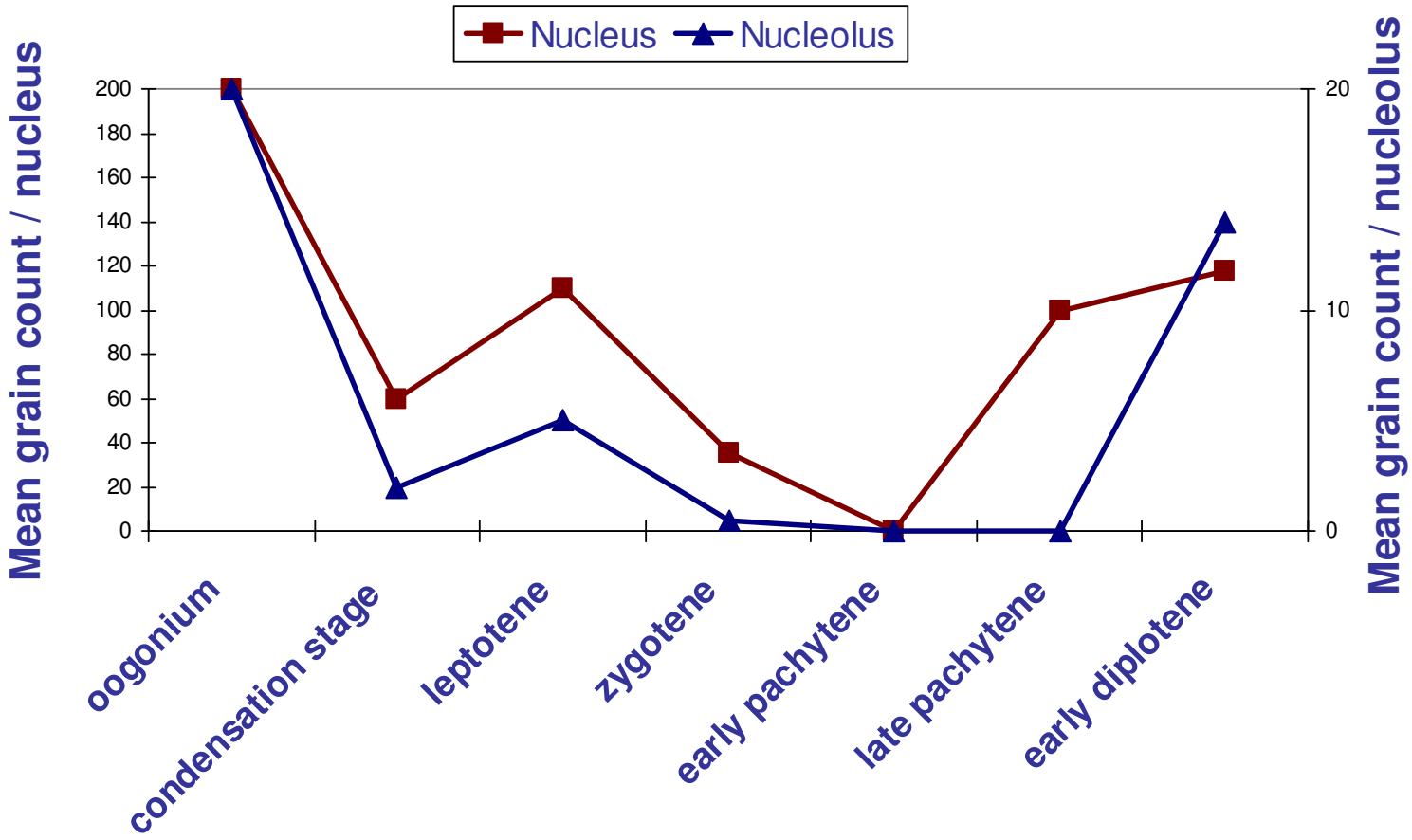
- Demonstrated to have post-implantation effects on foetal viability and normality

Ozil, 1990

Maternal Contribution to Embryo Development

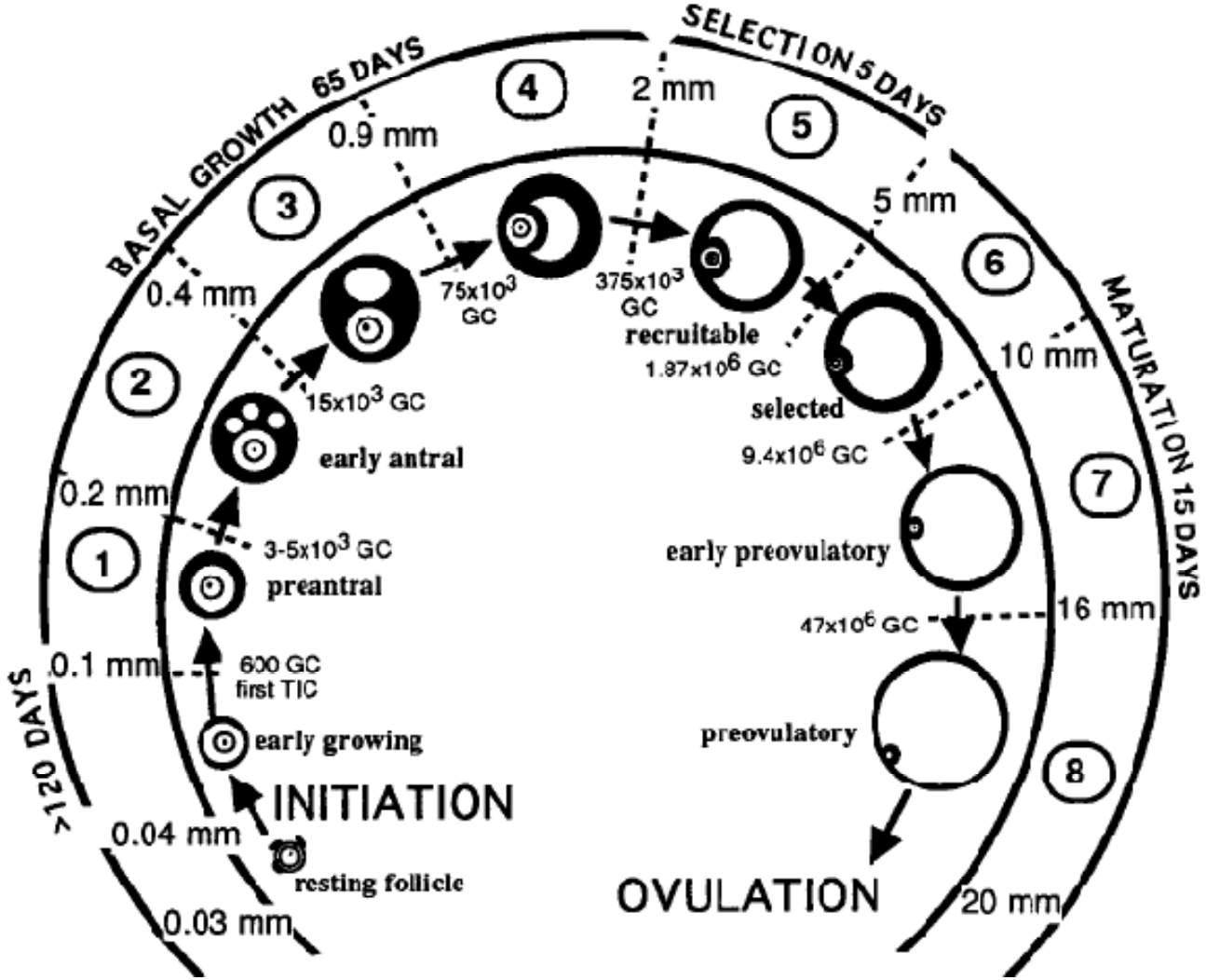
- Structural
  - Chromosomes
  - Microtubules
  - Organelles
- Molecular
  - DNA
  - RNA
  - Protein

Transcription During Prophase I of Meiosis Pre-natal Development



Hartung & Stahl, 1978

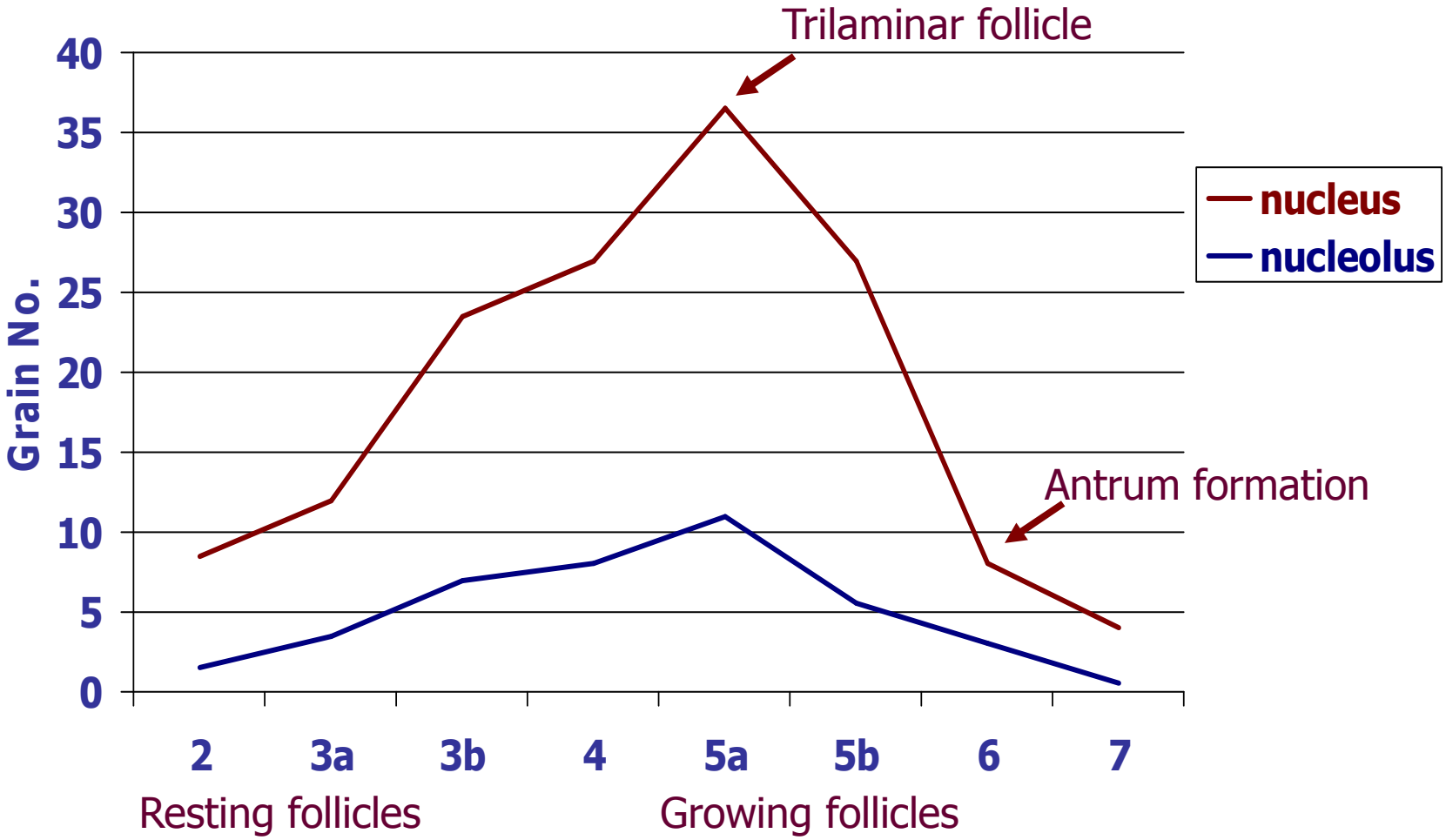
Folliculogenesis



Primordial Follicle to Ovulation > 6.5 months

Gougeon, 1996

Transcription During the Growth Phase



Moore et al., 1974

Transcription During Meiotic Maturation

- Once the maximal oocyte diameter is reached there is a sharp decline in transcription but RNA synthesis continues to within 2hours of GVBD
- Transcription virtually ceases once the germinal vesicle breaks down and meiosis is reinitiated
- 20% of total RNA is degraded during meiotic maturation
- Total degradation or deadenylation of one half of the accumulated Poly(A) RNA during meiotic maturation

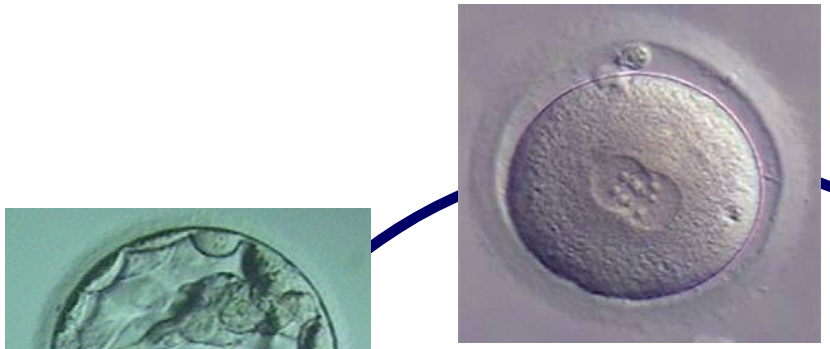
Transcripts Acquired During the Human Oocyte Growth Phase

- Completion of meiosis
- Entry into and completion of first 2-3 mitotic cell cycles
- Modification of chromatin structure and epigenetic properties
- Creation of an embryonic genome
- Initiation of transcription of the correct array of genes to begin the developmental program
- Basic homeostatic and metabolic processes

Oocyte Maternal mRNA's

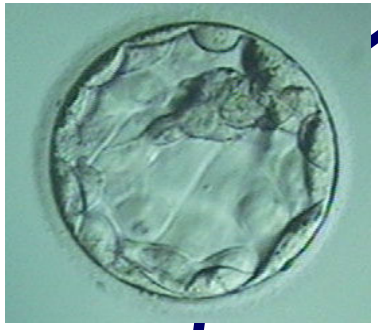
- Stored in inactive, masked form and recruited for translation in a stage-specific manner during oocyte maturation and early embryogenesis
- Relative abundance differs between species and may account for difference in timing of zygotic genome activation between species
- Failure to accumulate and regulate the maternal message acquired during oogenesis may result in incorrect temporal utilization of message and is likely to cause delays or failure in progression through preimplantation development

Embryonic Genome Transcription



Mouse

Wang et al., 2004
Hamatani et al., 2004



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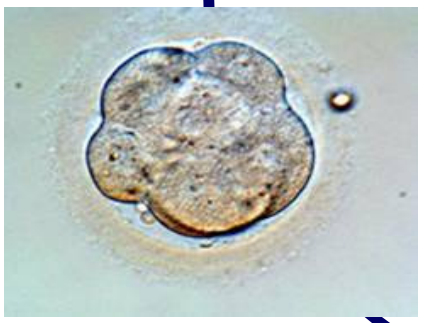
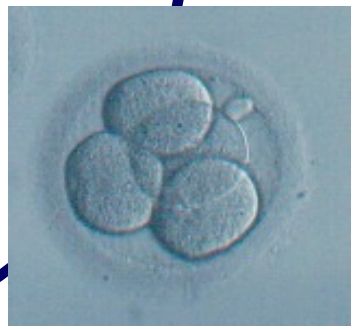
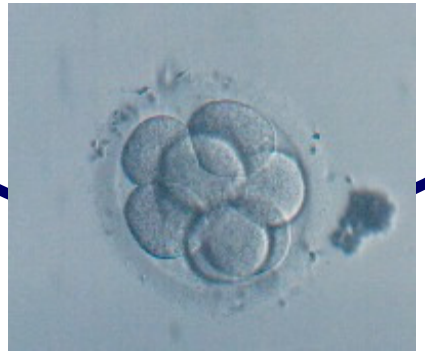






Human

Braude et al., 1988

Maternal mRNA Expression & Regulation Rhesus Monkey Oocytes & Embryos

Zheng et al., 2005

- Oocytes from 3 sources were used
  - In vivo matured oocytes following FSH + hCG stimulation = **high** developmental competence
  - In vitro matured oocytes from large follicles primed with FSH = **moderate** developmental competence
  - In vitro matured oocytes from small follicles in the absence of stimulation = **low** developmental competence

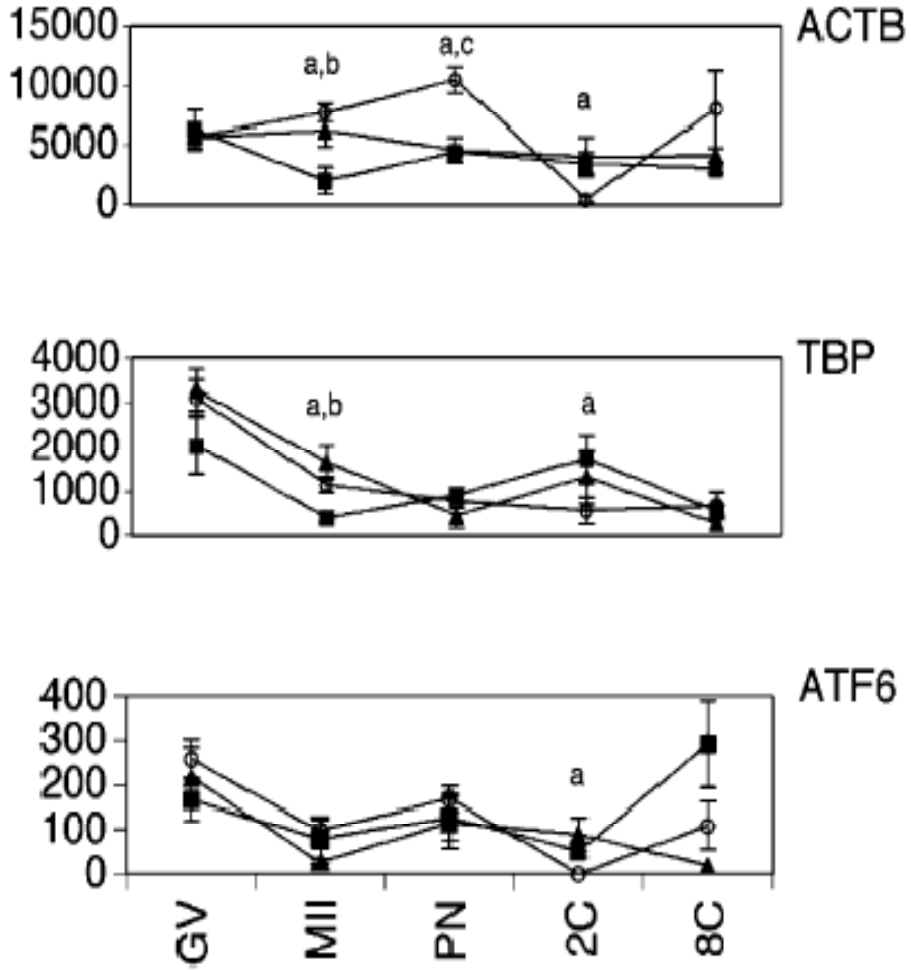
Maternal mRNA Expression & Regulation Rhesus Monkey Oocytes & Embryos

Zheng et al., 2005

- Non-stimulated oocytes showed aberrant accumulation of a number of maternal mRNAs with precocious loss by 2-cell stage
- FSH primed oocytes also showed aberrant gene expression relative to FSH + hCG stimulated oocyte but much less severe

Maternal mRNA Expression & Regulation Rhesus Monkey Oocytes & Embryos

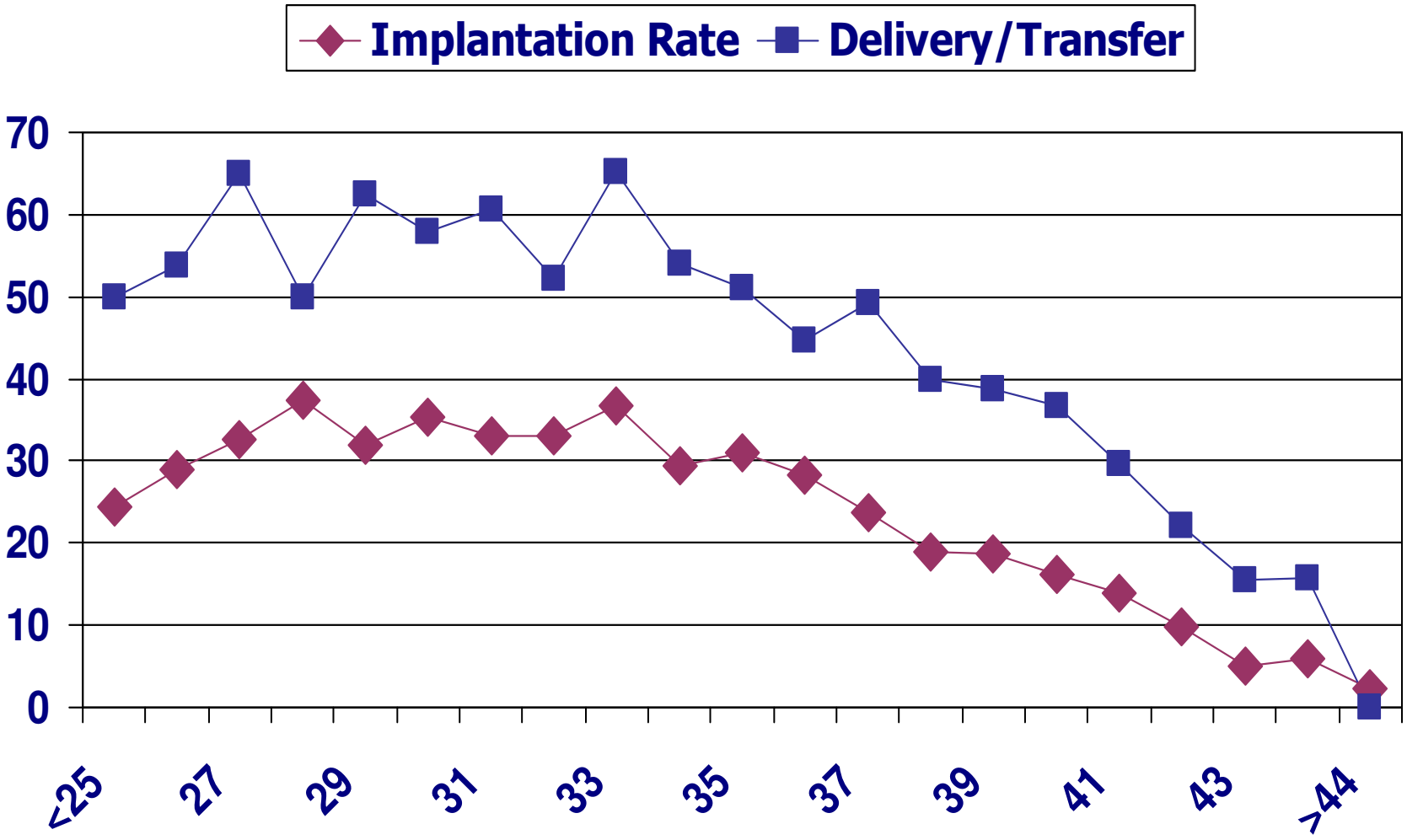
Zheng et al., 2005



■ FSH + hCG
▲ FSH only
○ No stimulation

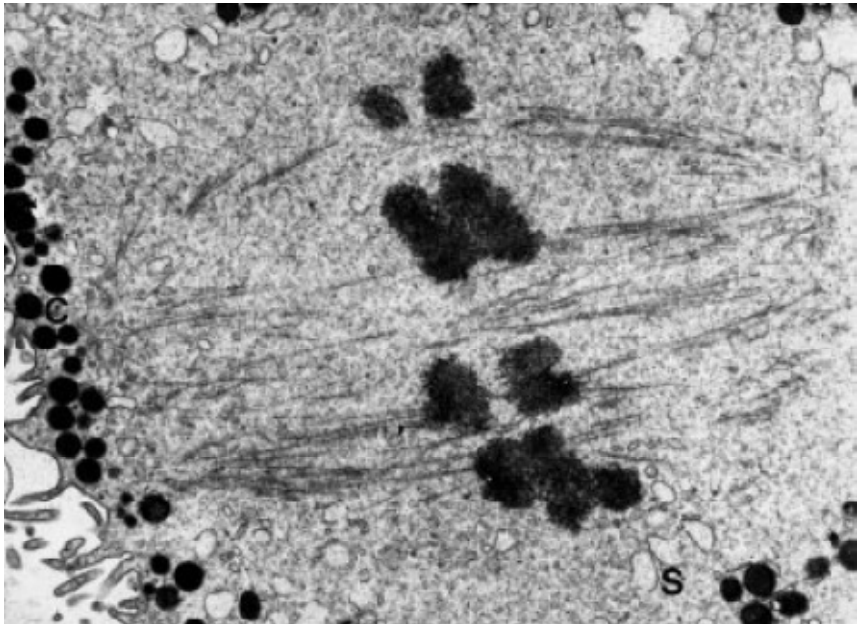
Maternal Ageing

IVF Outcome and Maternal Age



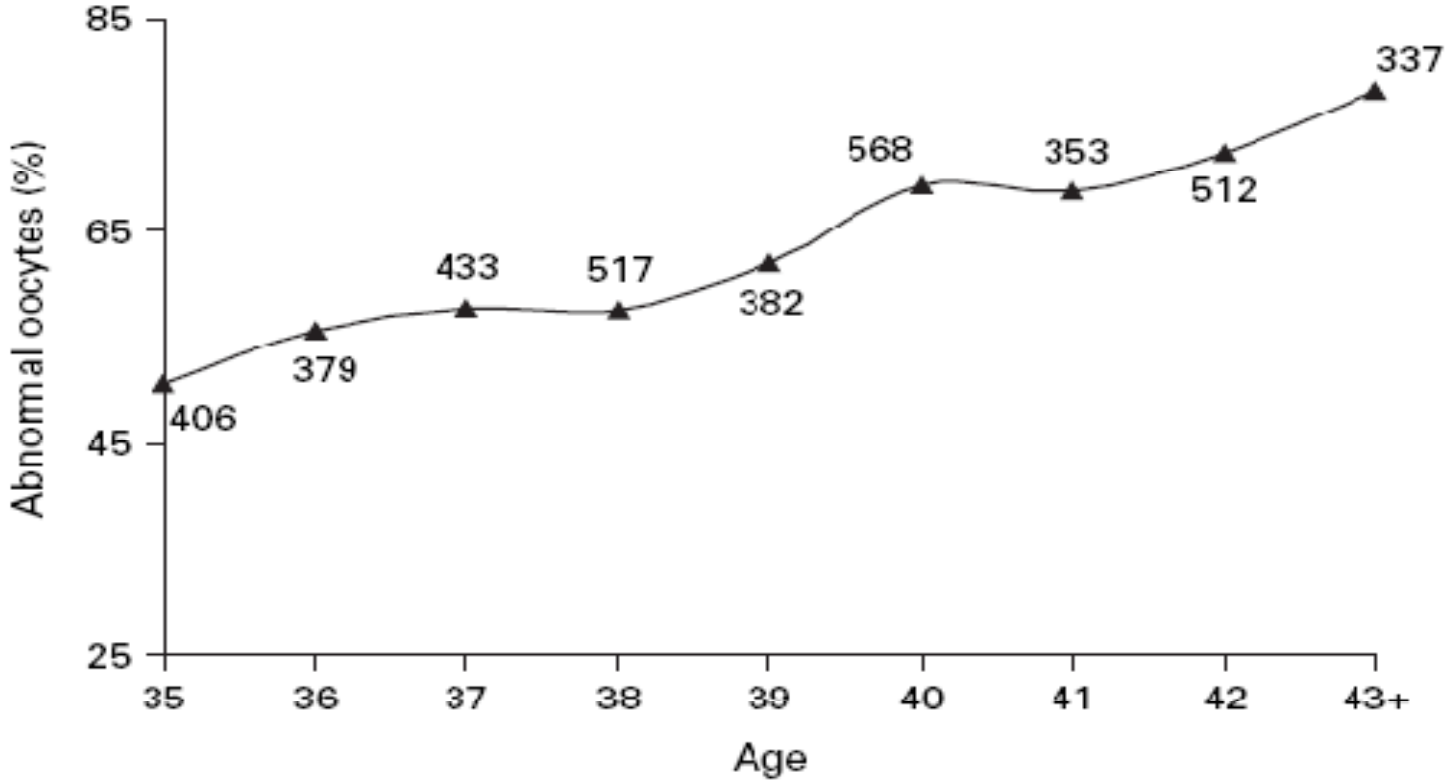
Spandorfer et al., 2000

Maternal Chromosomes



- Incidence of aneuploidy in first trimester abortions as high as 65% (Menasha et al., 2005)
- Aneuploidy in oocytes arises from both chromatid predivision and whole chromosome non-disjunction
- >50% aneuploidy rates in oocytes of women >40 years of age

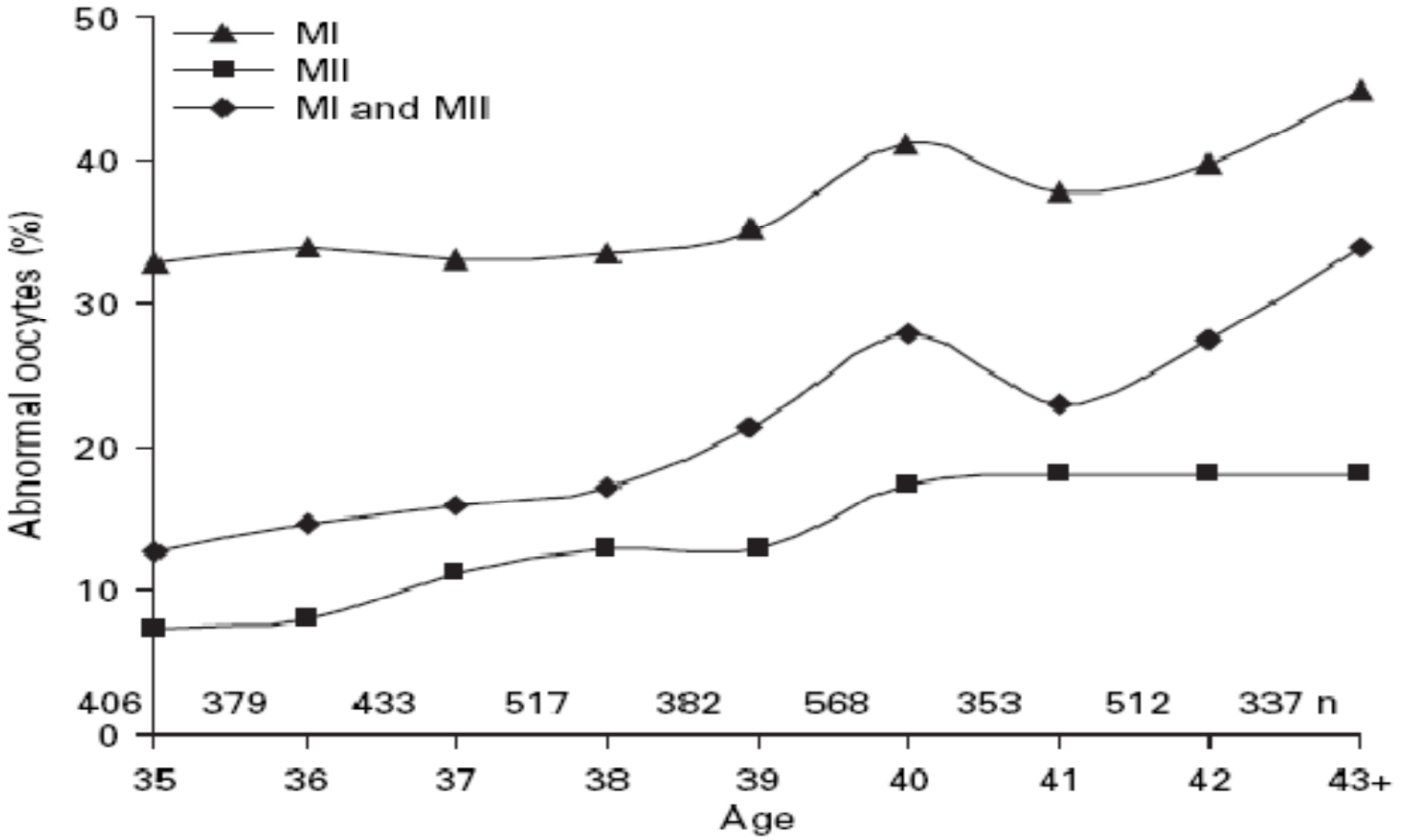
Aneuploidy and Maternal Age



Based on FISH results for chromosomes 13, 16, 18, 21 and 22

Kuliev et al 2005

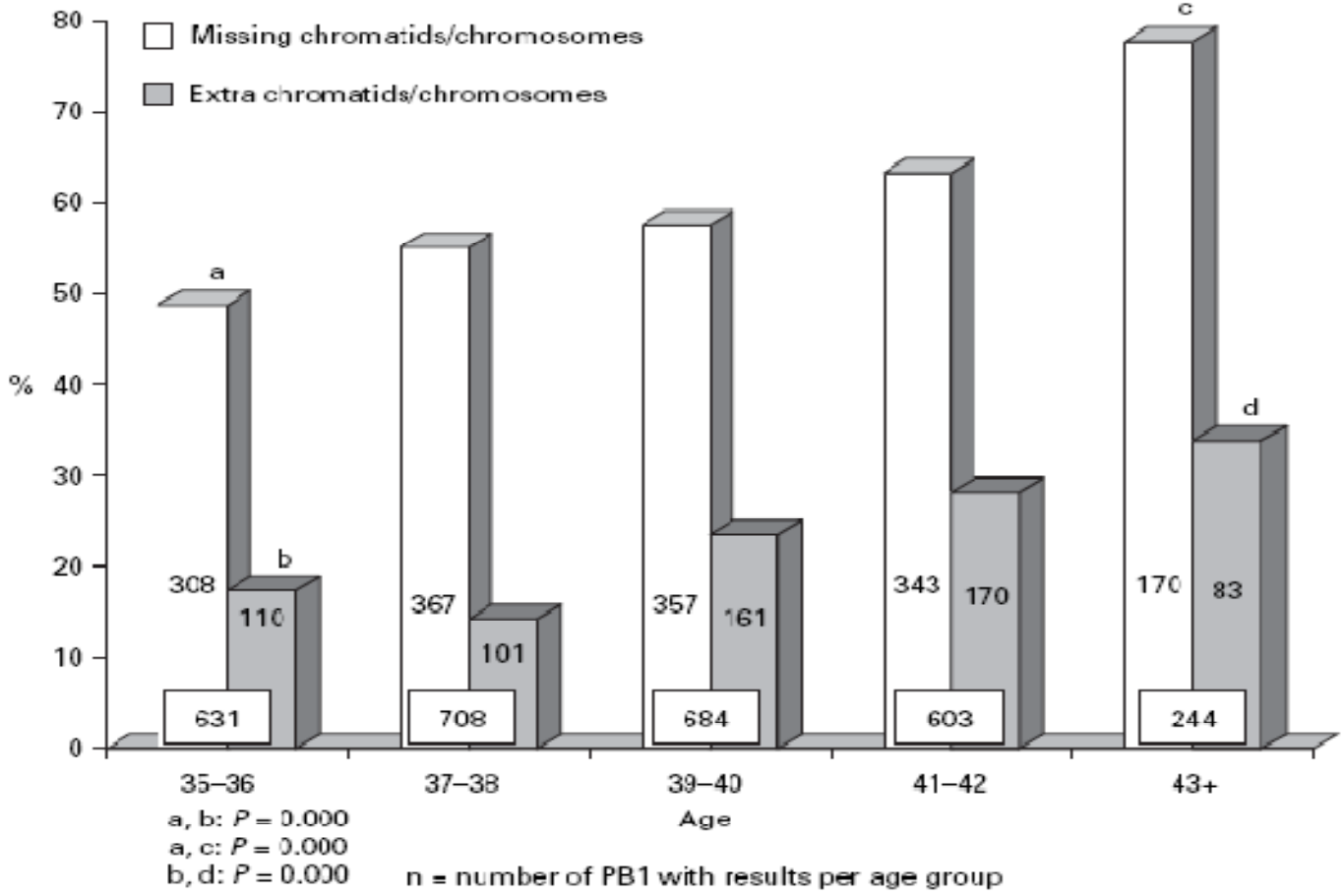
Meiotic Error and Maternal Age



Based on FISH results for chromosomes 13, 16, 18, 21 and 22

Kuliev et al 2005

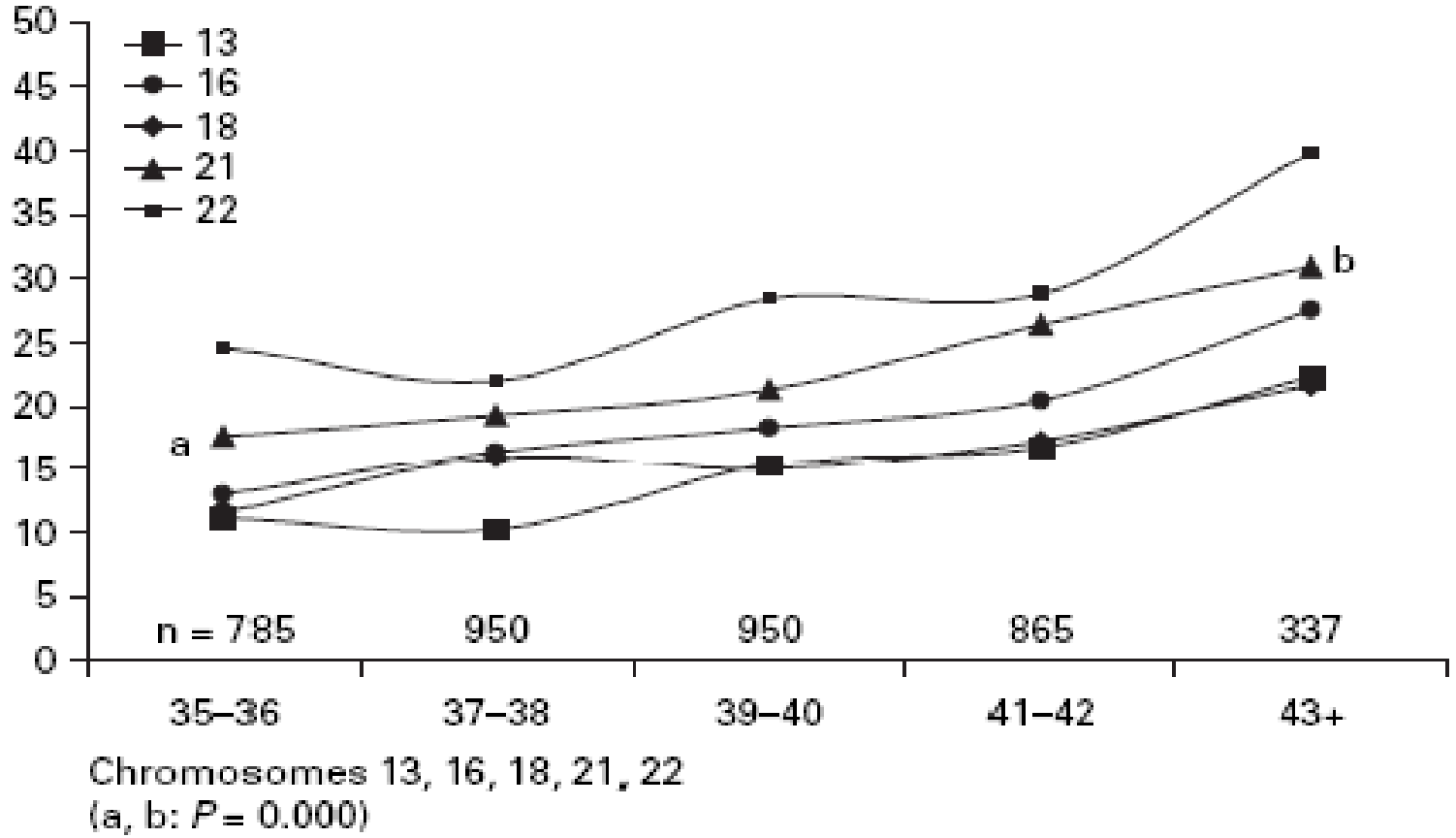
Missing and Extra Chromatids/ Chromosomes in PB1 and Maternal Age



Based on FISH results for chromosomes 13, 16, 18, 21 and 22

Kuliev et al 2005

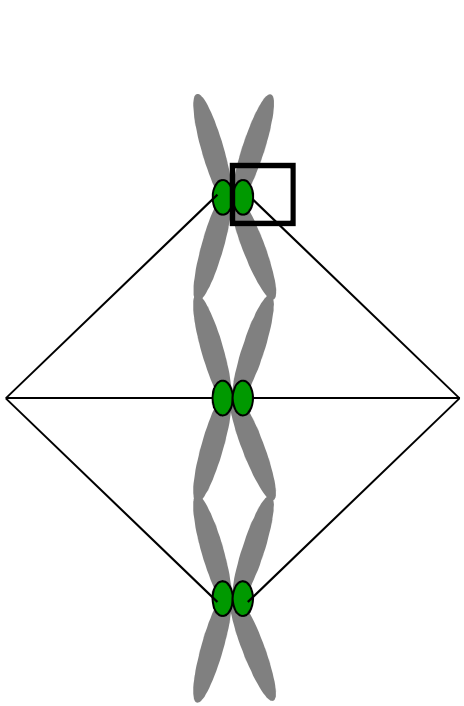
Frequency of Chromosome Specific Error and Maternal Age



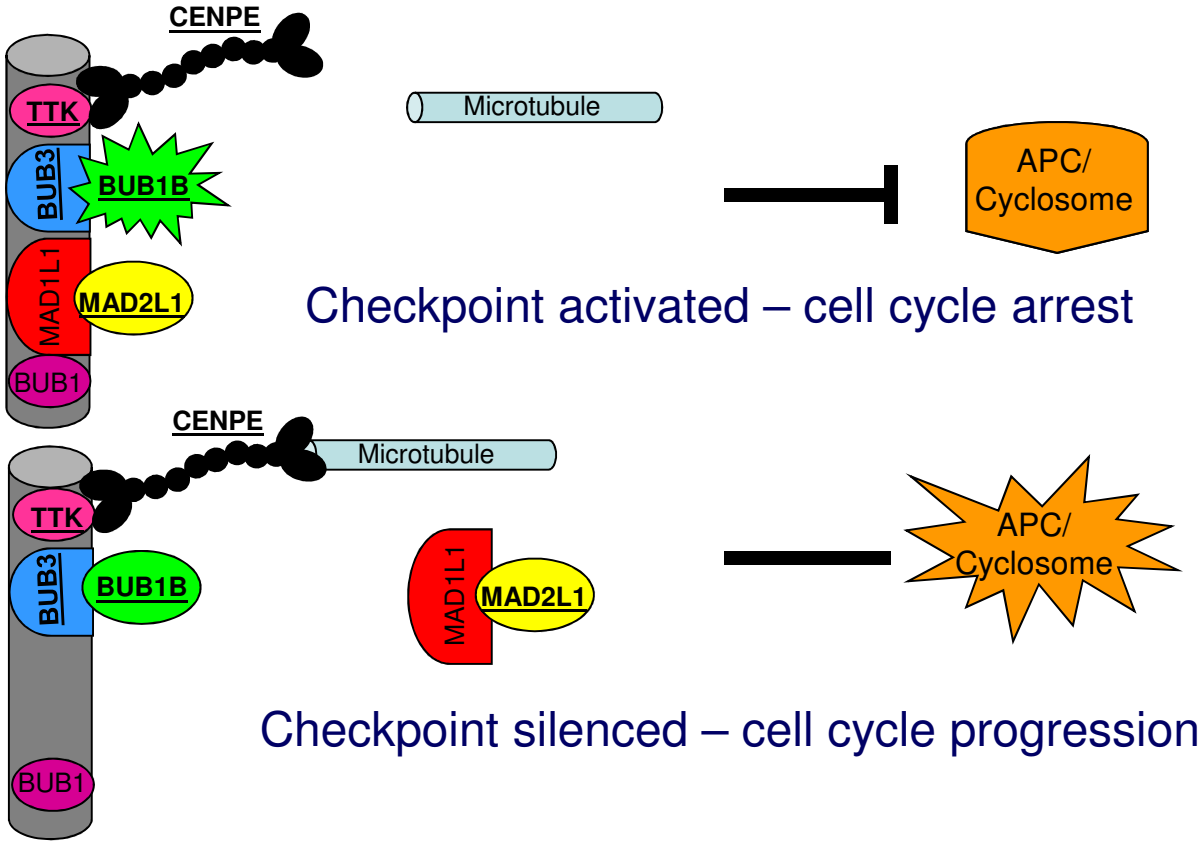
Based on FISH results for chromosomes 13, 16, 18, 21 and 22

Kuliev et al 2005

Spindle Checkpoint



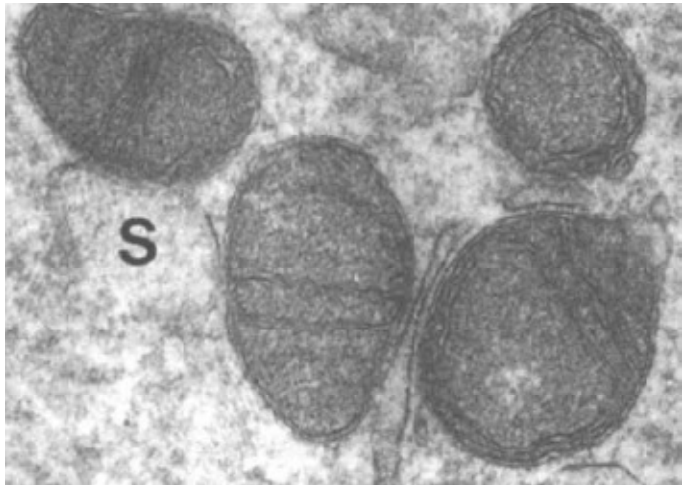
Metaphase

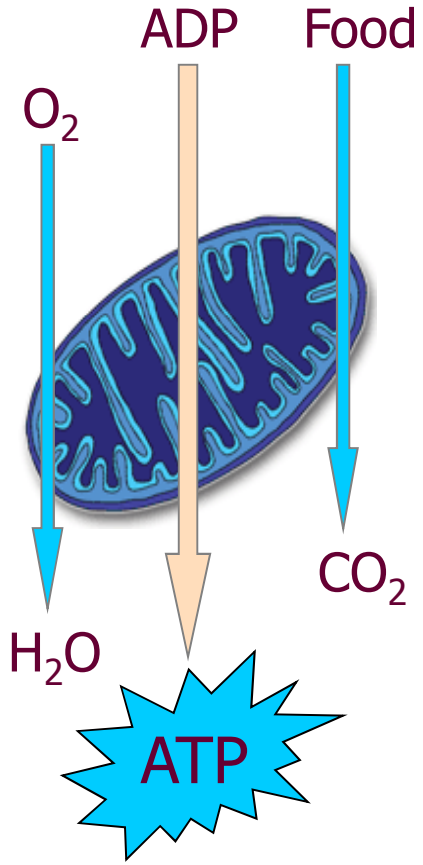


BUB1 and MAD2L1 levels decreases in oocytes with age

Steuerwald et al., 2001

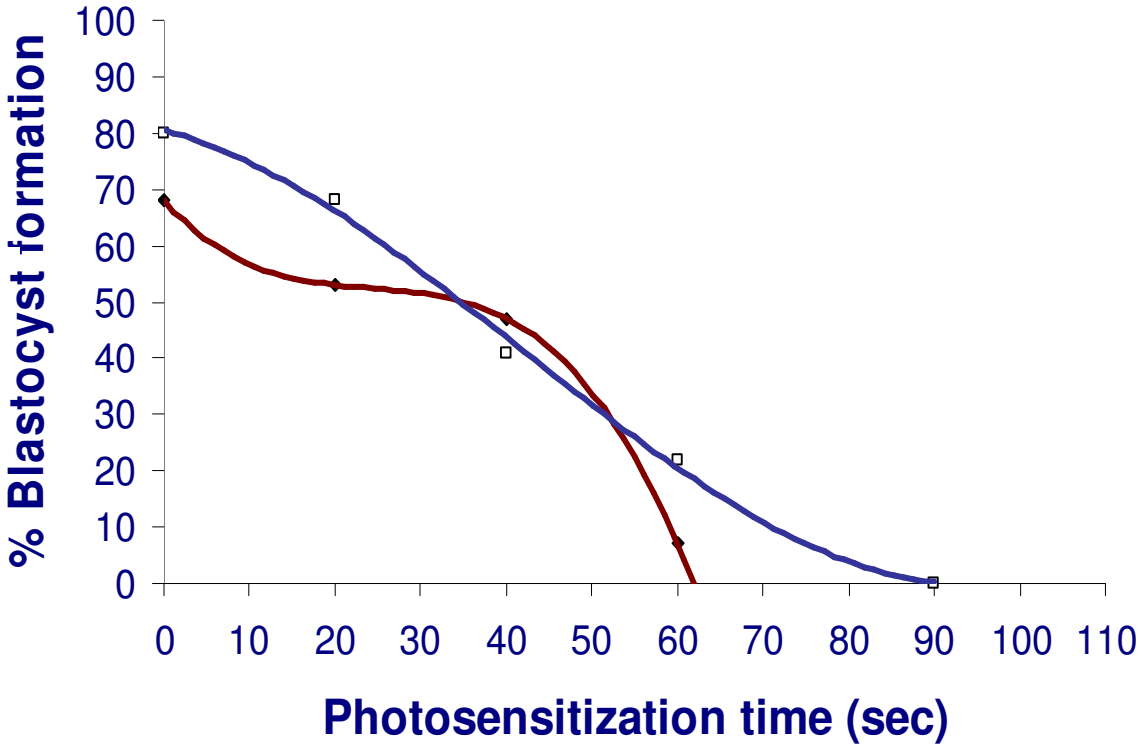
Mitochondria – Maternal Age

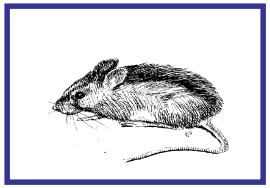


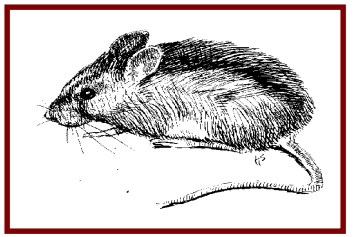


- ↑ hypoxic follicles
- ↑ mtDNA damage
- ↓ efficiency of oxidative phosphorylation
- ↓ $\Delta\Psi_{mt}$
- ↓ATP content

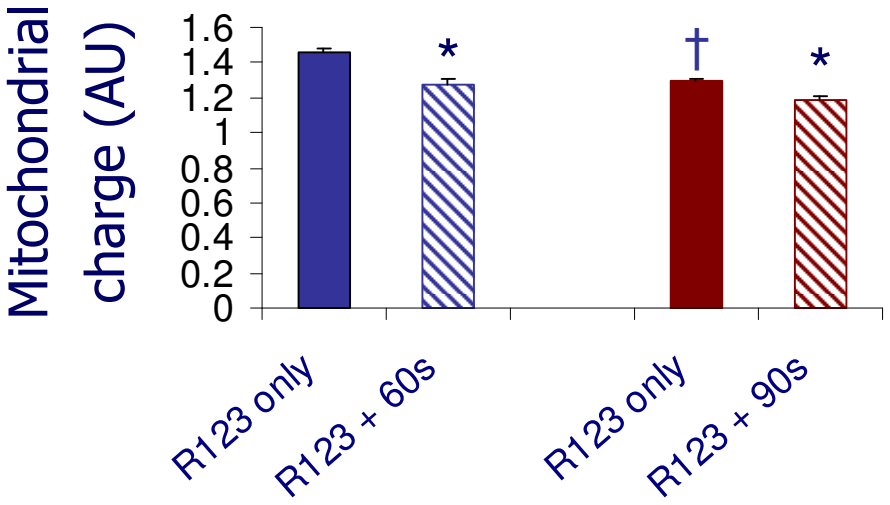
Maternal Ageing and Tolerance to Mitochondrial Injury

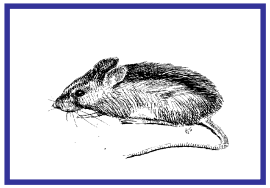


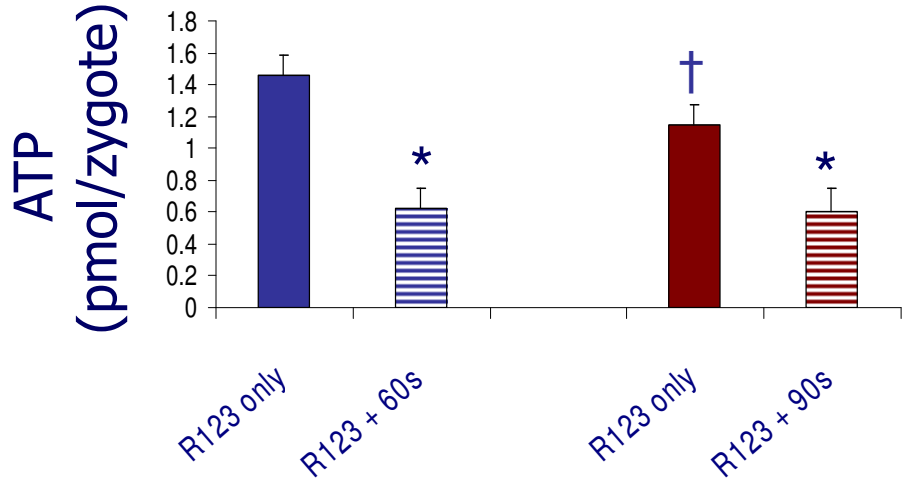




Maternal Ageing and Mitochondrial Function





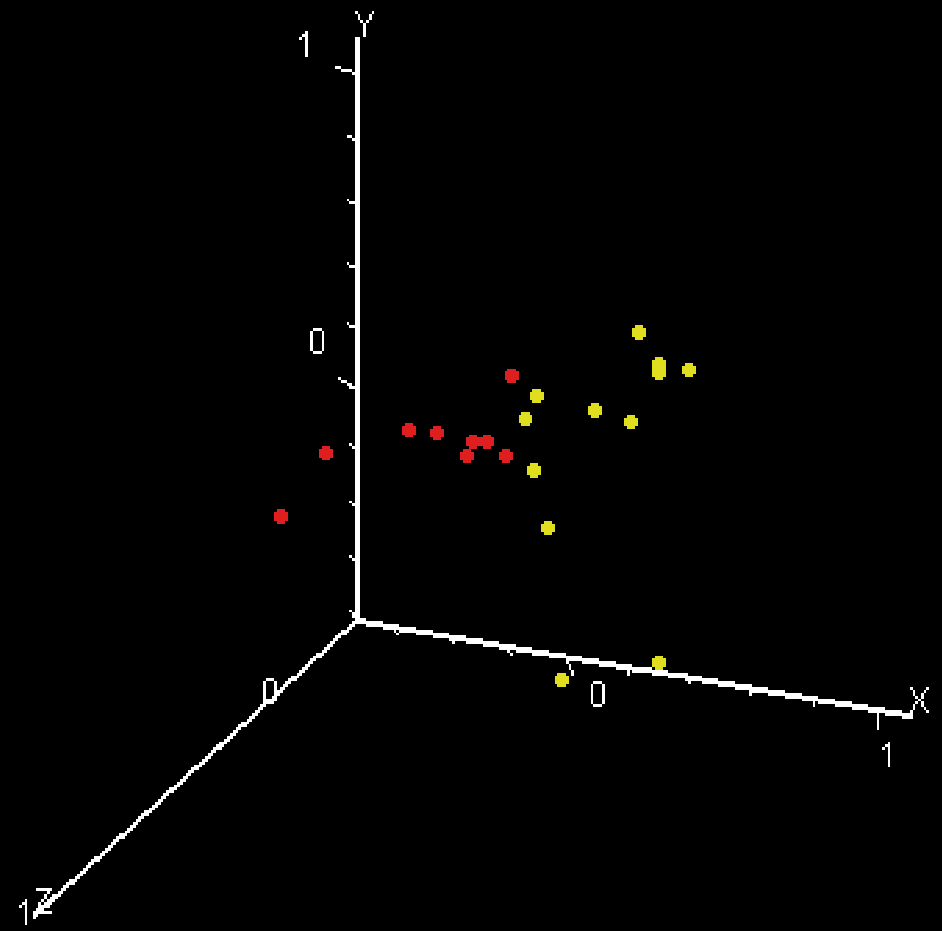


Gene Expression & Maternal Age

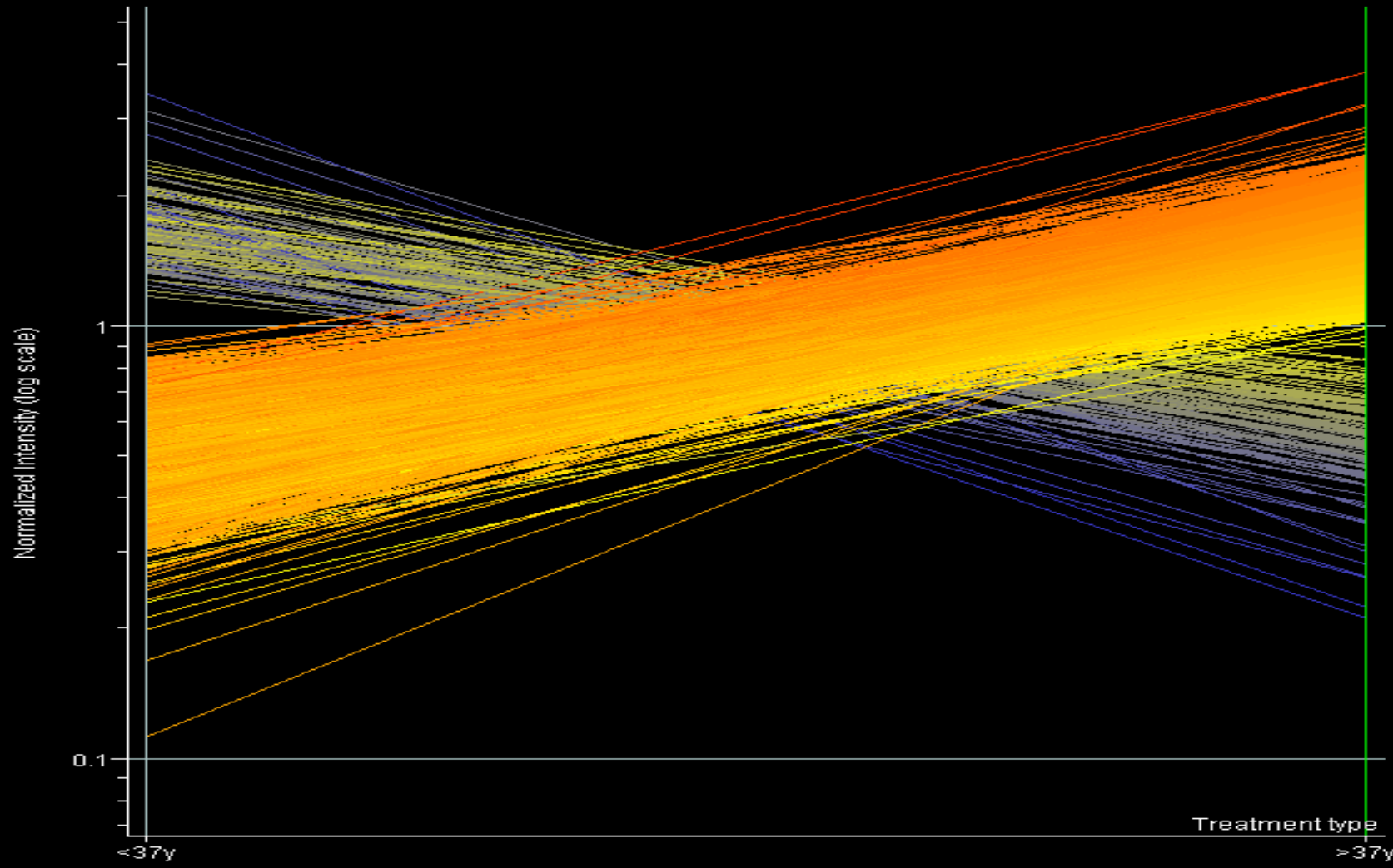
Human Oocyte Gene Expression Profiles & Maternal Age

- All mature MII oocytes from gonadotrophin stimulated cycles
- 9 replicates (45 oocytes) from women aged between 28-37
  - 3 replicates 28-34 years
  - 6 replicates 35-37 years
- 12 replicates (60 oocytes) from women aged 38-43
  - 6 replicates 38-40 years
  - 6 replicates >40 years

Principal Components Analysis



≤37 years vs >37 years

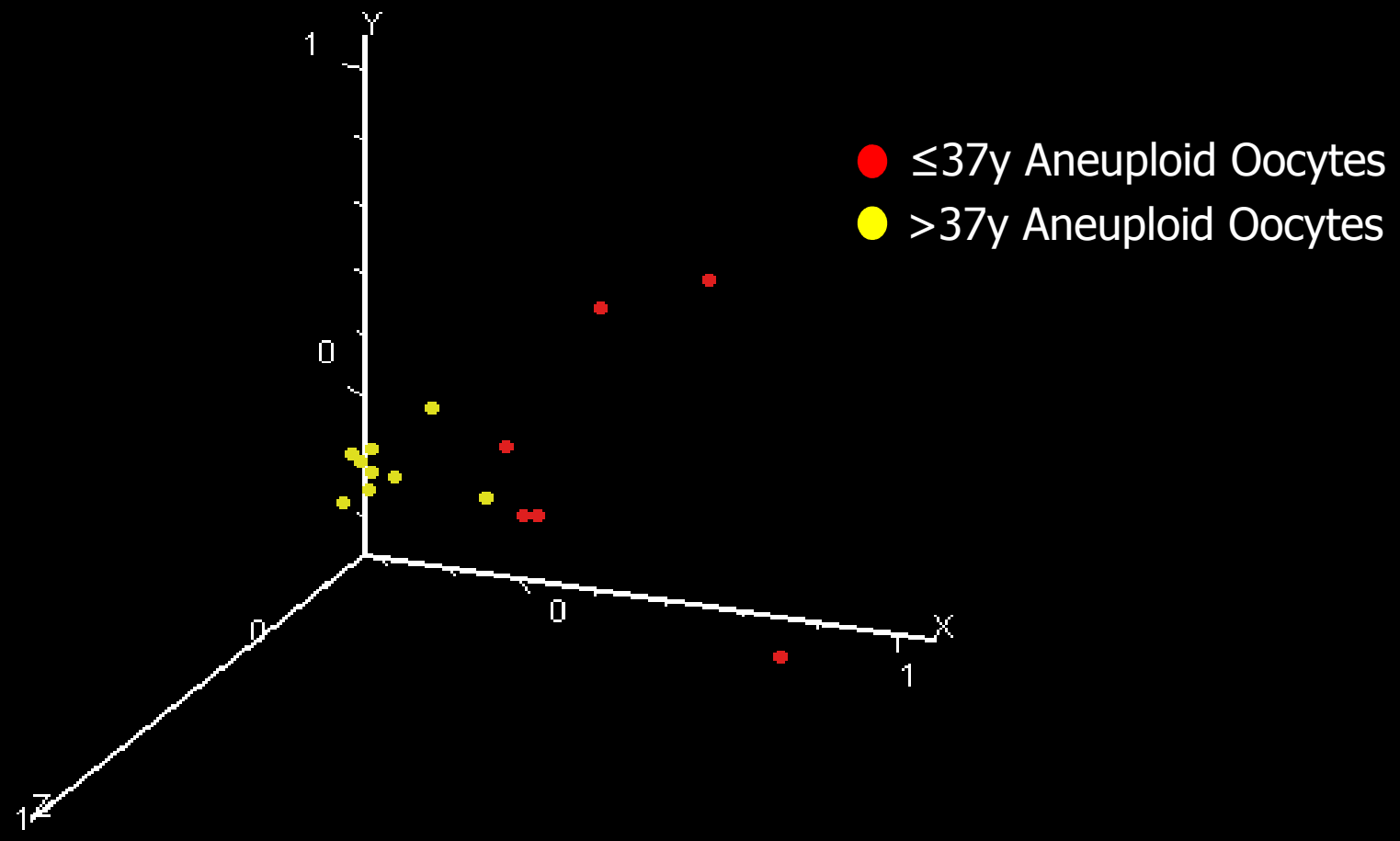


124 probes >2fold lower in >37 years; 7,136 probes >2fold higher in >37 years

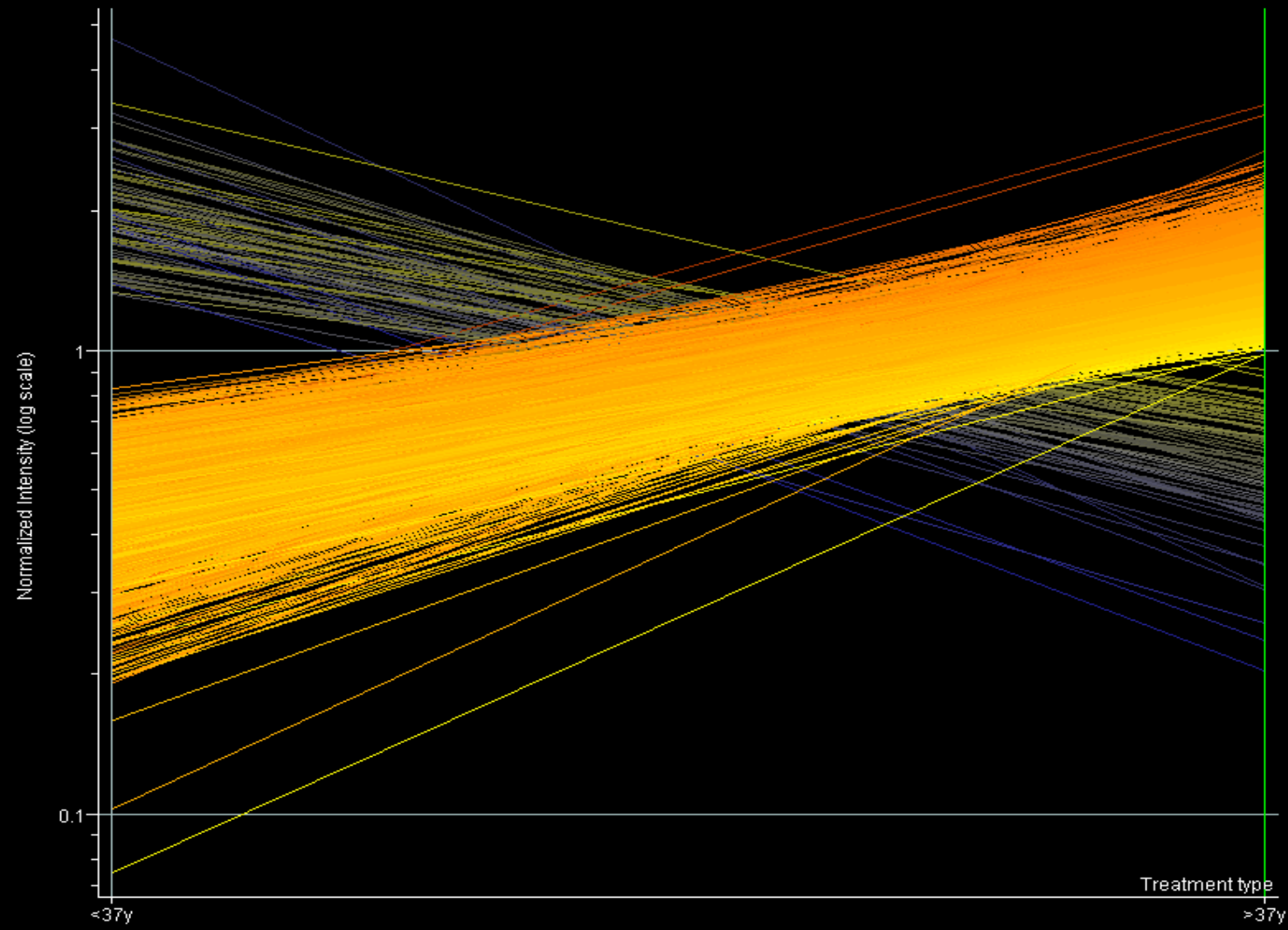
Gene Expression in Aneuploid Oocytes & Maternal Age

- All oocytes diagnosed as aneuploid by FISH following PB biopsy and staining for chromosomes X, 13, 15, 16, 18, 21, 22
- 5 oocytes per microarray sample
- Group 1 ≤37 years
  - 28-34y (n=1)
  - 35-37y (n=5)
- Group 2 >37 years
  - 38-40y (n=5)
  - > 40y (n=5)

Principal Components Analysis



≤37 years vs >37 years Aneuploid Oocytes



81 genes >2fold lower in >37 years; 4,849 genes >2fold higher in >37 years

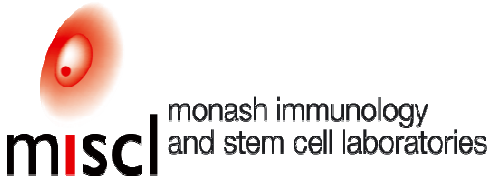
Summary – Maternal Ageing

- Oocytes from **older women** that are physiologically less developmentally competent are associated with **higher** expression of a significant number of genes compared to the oocytes of **young women**
- Over-representation of genes involved in **mitochondrial function and energy production** and genes involved in translation and RNA processing
- Aneuploidy is usually implicated as the major factor responsible for the reduced developmental competence of oocytes however there are other contributors as large gene expression differences are detected even when all oocytes are aneuploid from young women compared to older women

Conclusions

- Human oocyte comes pre-packaged to provide all the structural elements, with the exception of paternal chromosomes and the centrosome, required for development through to blastocyst
- Human oocyte comes pre-packaged to provide all the molecular elements required for development until the 4- to 8- cell stage when the embryonic genome is activated
- Pathology to any of these elements can be caused in vivo or in vitro and have significant consequences to downstream development

Acknowledgments



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Bibliography

- Bos-Mikich et al (1997) Dev Biol 182: 172-179
- Braude et al. (1988) Nature 332: 459-461
- Gougeon (1996) Endocr Rev 17: 121-125
- Hamatani et al. (2004) Dev Cell 6: 117-131
- Hartung & Stahl (1978) Cytogenet Cell Genet 20: 51-58
- Kuliev et al. (2005) Cytogenet Genome Res 111: 193-198
- Malcuit et al. (2006) J Cell Physiol 206: 565-573
- Menasha et al. (2005) Genet Med 7: 251-263
- Moore et al. (1974) J Cell Biol 60: 416-422
- Ozil (1990) Development 109: 117-127
- Spandorfer et al. (2000) J Assist Reprod Genet 17: 303-306
- Steuerwald et al (2001) Mol Hum Reprod 7: 49-55
- Thouas et al. (2004) Biol Reprod 71: 1936-1942
- Thouas et al. (2005) Biol Reprod 73: 366-373
- Thouas et al. (2006) Biol Reprod 74: 969-977
- Wang et al. (2004) Dev Cell 6: 133-144
- Zheng et al. (2005) Biol Reprod 72: 890-897