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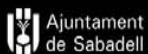
**S10. II Simposi sobre
Fertilitat Femenina i
Masculina: Genètica i
Ambient**

Sabadell, 7 de juliol

**MECHANISMS OF ANEUPLOIDY INDUCTION IN HUMAN
OOGENESIS AND EARLY EMBRYOGENESIS**

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Mechanisms of Aneuploidy Induction in Human Oogenesis and early Embryogenesis

Joy Delhanty



A decorative graphic at the top of the slide consists of two groups of three circles. The left group has a solid light purple circle on the left, a white circle with a light purple outline in the middle, and a solid light purple circle on the right. The right group has a solid light purple circle on the left, a white circle with a light purple outline in the middle, and a solid light purple circle on the right. The text 'Human Fertility' is written in a bold, dark red font, overlapping the first solid circle of the left group.

Human Fertility

- As a species, humans are not very fertile - fecundity rate 20-25% for young couples
- One major factor is aneuploidy arising during oogenesis, closely tied to maternal age
- Embryos from IVF - over 50% chromosomal mosaics by day 3. In vivo - ?



Human Fertility - Aneuploidy mechanisms

- To illustrate mechanisms that cause aneuploidy arising during oogenesis - I will present data on our oocyte study using CGH analysis
- To illustrate mechanisms causing chromosome abnormalities in embryos from IVF - I will present data after aneuploidy screening at our Centre



Aneuploidy rates in human oocytes

- Recent karyotyping and limited FISH analyses suggest 11% for maternal ages 32-35 yrs.
- Chromosomes preferentially involved: 13-22 and X
- Expected rate from miscarriage data: 20-25% (all maternal ages)



Aims of our study using CGH analysis

- To investigate the variety of anomalies arising during maternal meiosis I by analysing unfertilised or in vitro matured oocytes and their corresponding first polar bodies, when available
- Gain insight into aneuploidy mechanisms especially those affecting younger women
- Validate prediction of oocyte status via 1st PB analysis for clinical and research use

Aneuploidy rates in human oocytes

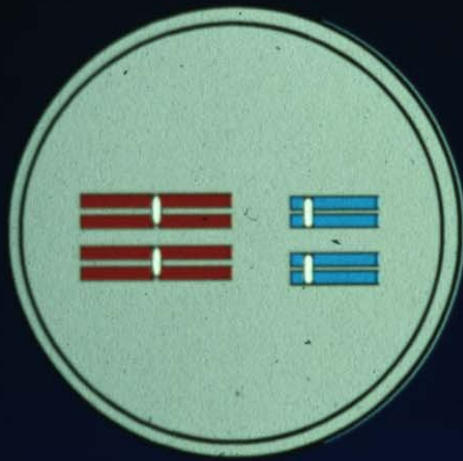


- All karyotyping and FISH analyses involve spreading chromosomes from a single cell - risk of loss of chromosomal material
- Advantages of DNA based method such as CGH are that the whole genome is analysed. Losses as well as gains are reliably detected.

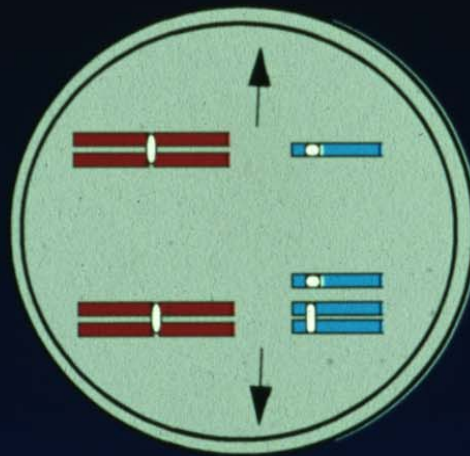
Non Disjunction in Human Oocytes at Meiosis I

Precocious division of chromatids

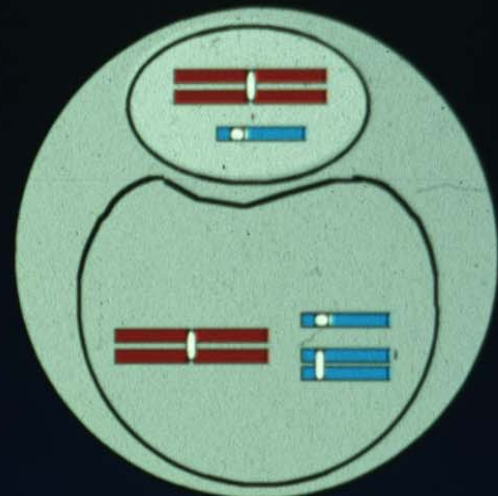
- Angel, 1991 -



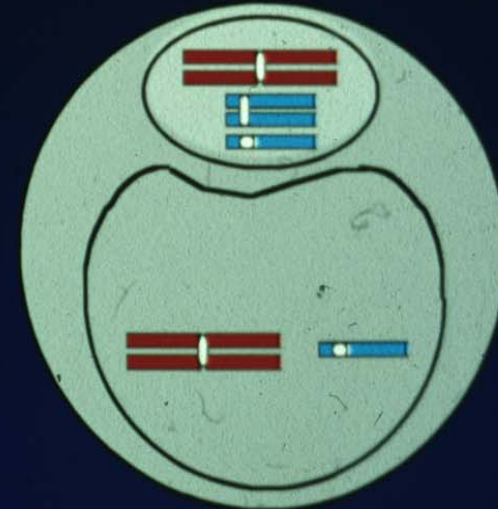
Diakinesis I



Anaphase I



OR



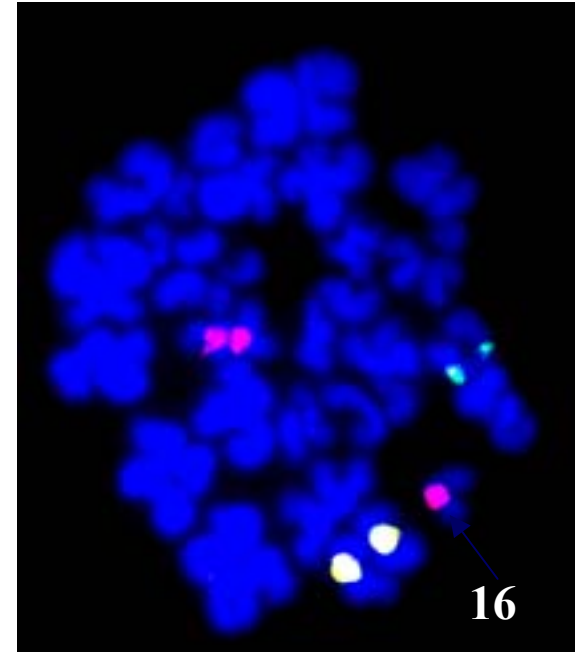
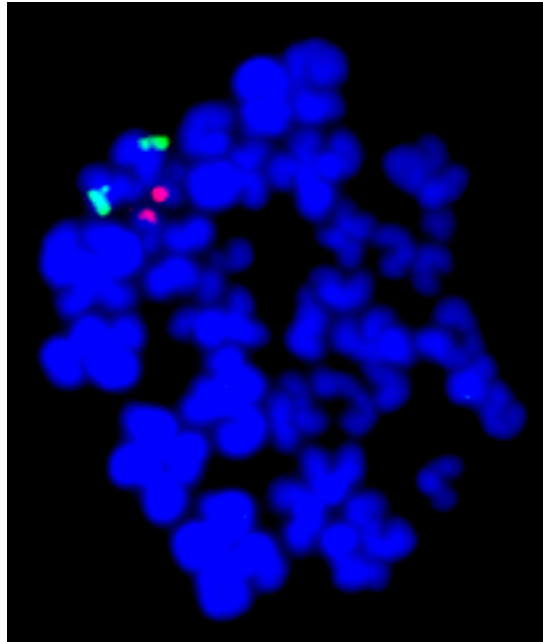
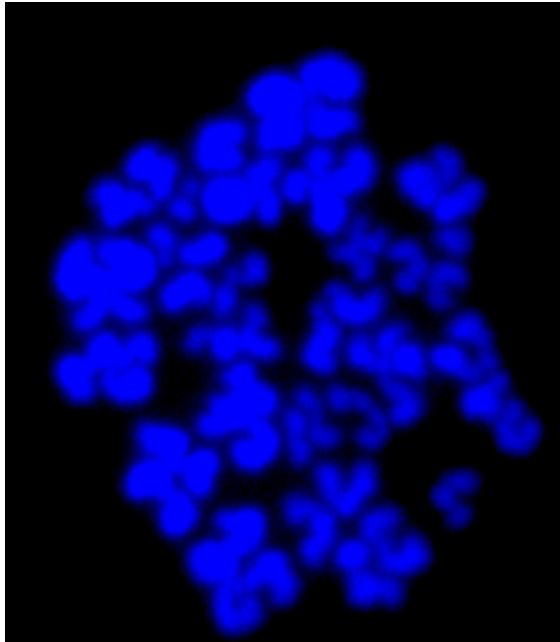
Metaphase II

FISH Analysis of Unfertilised Oocytes and First Polar Bodies

DAPI

13, 21

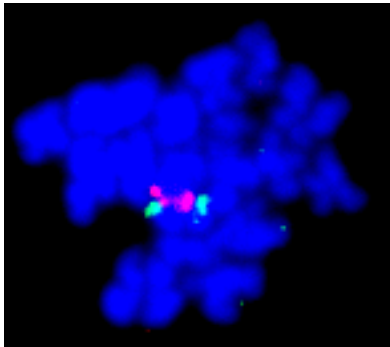
16, 18, X



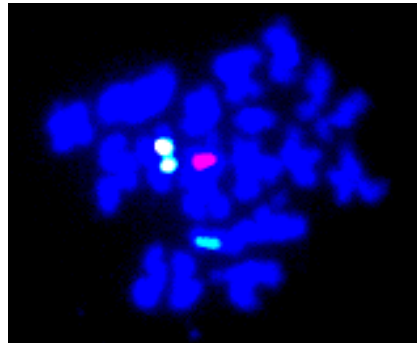
Oocyte with extra chromatid 16

FISH Analysis of Unfertilised Oocytes and First Polar Bodies

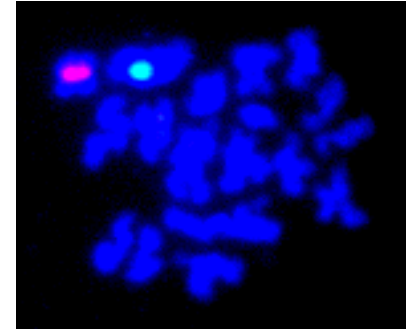
oocyte



13, 21

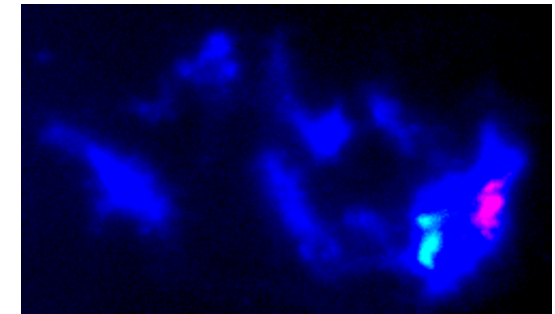
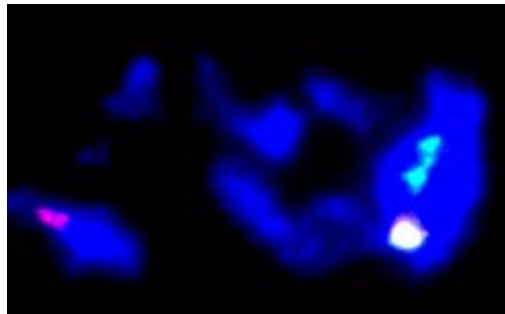
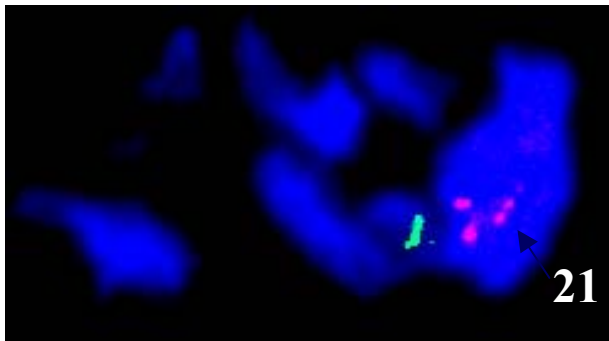


16, 18, X



1, 9

PBI



PBI with an extra chromosome 21 (oocyte appears normal)

Comparative Genomic Hybridisation (CGH)



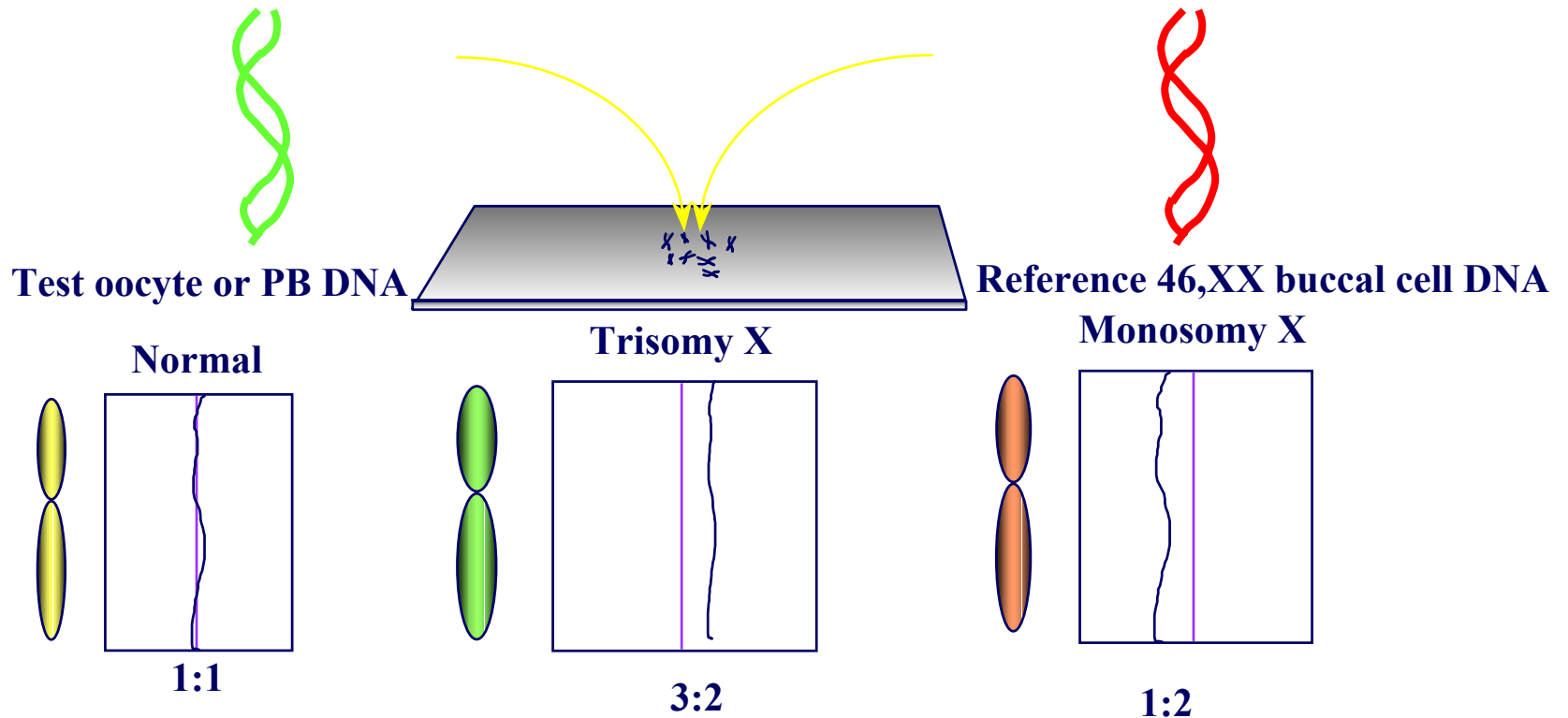
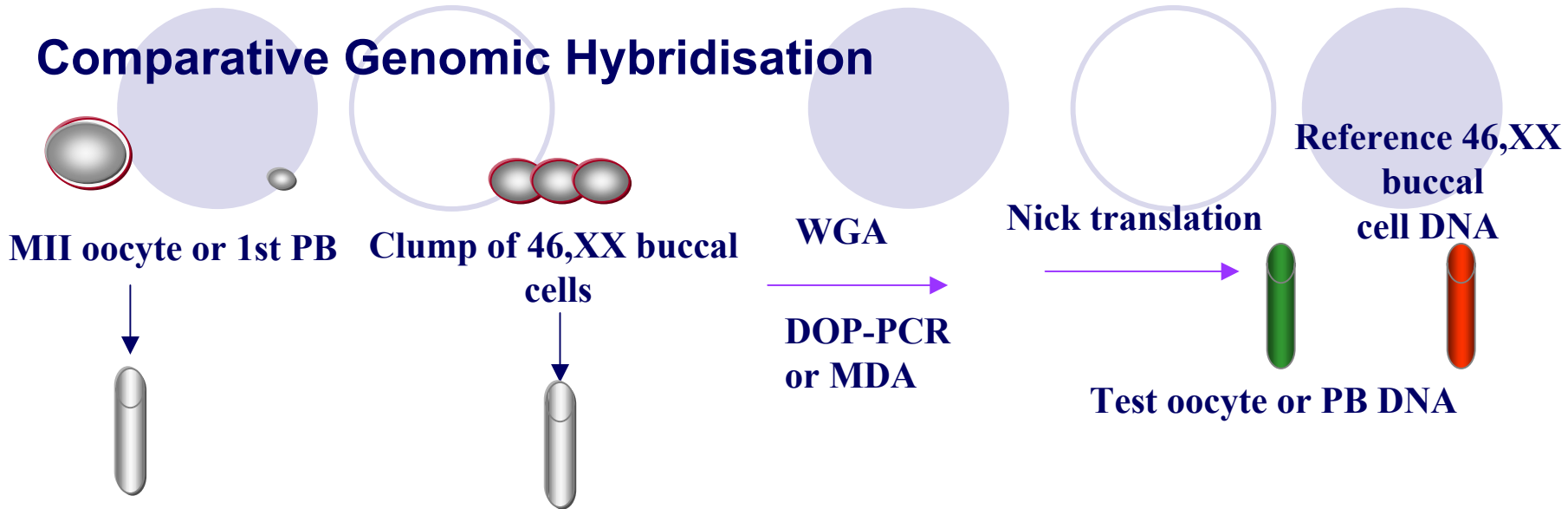
- Technique related to FISH
- Enables the screening of an entire genome in a single hybridisation step
 - Leads to identification of imbalance in chromosomal material
 - Sample DNA labelled in green
- Sample DNA is mixed with an equal amount of chromosomally normal DNA (46,XY or XX), labelled in red
- DNA mixture is hybridised to normal male (46 XY) metaphase spreads on a microscope slide



CGH protocol/Single cell level

- Amplification of single cell DNA with the degenerate oligonucleotide primed (DOP) polymerase chain reaction (PCR)
- Labelling of amplified DNA by nick translation
- Hybridisation onto 46,XY metaphase slides
- Post-hybridisation washes after 72 hours

Comparative Genomic Hybridisation





Oocytes investigated

Total number of patients: 46

Age range: 18-42 years

Average age: 32.5 years

Mature MII unfertilised oocytes – IVF or ICSI used (28)

Mature MII uninjected oocytes (11)

Immature oocytes, matured in vitro (56)



Total Results

Results from 62 oocytes 81 first PBs and 2 second PBs

Representing 100 eggs (oocyte-PB complexes)

12 oocytes and 17 first PBs & 1 second PB showed abnormalities
(22 eggs)

Aneuploidy rate: 22%

Chromosomes affected in order of frequency: X, 21, 20, 13, 8,
2, 4, 1, 22, 5, 9, 12, 17, 19

Structural abnormality identified in one PB (4q duplication)



Total Results

Number of whole chromosome aneuploidies: 15

Fertilised egg at risk of trisomy in 4 and monosomy in 11

Number of chromatid aneuploidies: 10

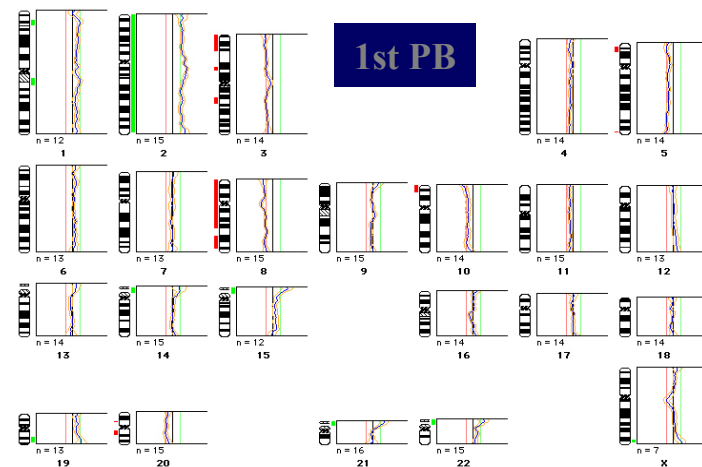
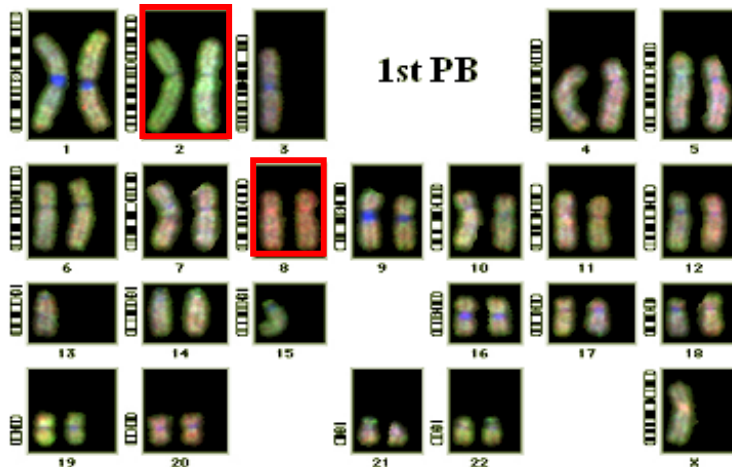
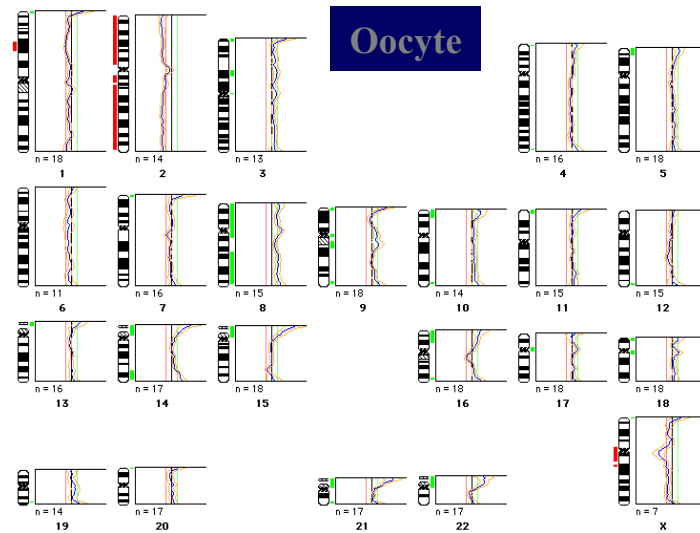
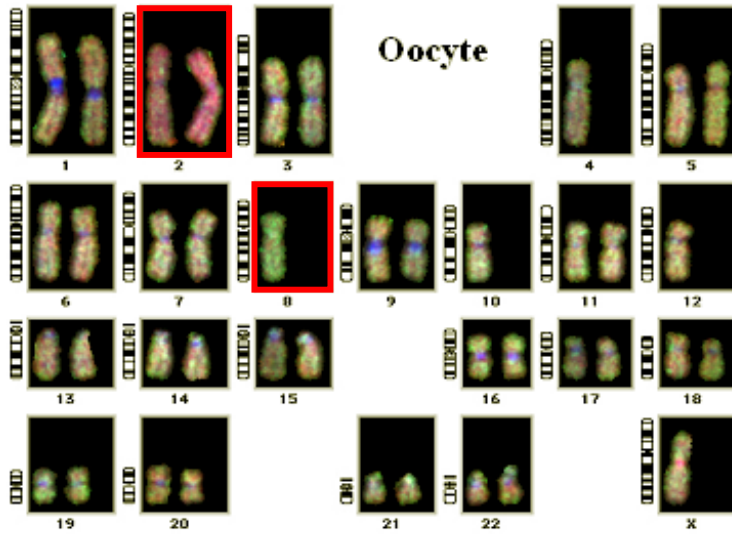
Fertilised egg at 50% risk of aneuploidy (trisomy 3.5; monosomy 1.5)

Overall: risk of trisomy 7.5; risk of monosomy 12.5

Number of reciprocal gains/losses in oocyte and 1st PB: 8

Patient A

- Age: 28.6, male factor, polycystic ovaries
- Three eggs investigated, reciprocal loss and gain of chromosomes 2 and 8 identified in one egg



Conclusions: Patients with abnormalities

- 15 patients have abnormalities
- Average age 31 yrs., range 18-40
- Effect of advanced age not seen.
? Due to unequal age distribution of pt. group
- Particular patients appear prone to frequent anomalies
- Suggests predisposition to aneuploidy

Predisposition to aneuploidy



- Gonadal mosaicism: young couples with 3 conceptions with identical trisomies. Confirmed by studies on oocytes
- Recurrent miscarriage couples: analysis of embryos shows increased aneuploidy risk, independent of age
- Women under 35 with previous aneuploid conception: embryos show increased rate of aneuploidy

Predisposition to aneuploidy- data from this study



- Presence of multiple abnormalities in one or more analysed cells

OR

- Same type of abnormality in all of their cells



Summary of mechanisms

- Whole chromosome non-disjunction
- Chromatid non-disjunction caused by predivision
- Germinal/gonadal mosaicism for a trisomic cell line
- Partial aneuploidy due to chromosome breakage

Summary - oocytes



- Largest oocyte & PB CGH study to date
- Findings confirm and extend previous cytogenetic investigations of human oocytes
- Reliability of CGH demonstrated. Protocol for PB CGH analysis to predict status of oocyte ready for clinical and research application
- Age-independent mechanisms acting in a group of younger infertile patients; possible link with infertility
- Detailed investigation of meiotic regulation needed

Chromosomes of Human Embryos



Normal - all cells uniformly diploid

Abnormal - all cells uniformly abnormal, e.g trisomy 21

Mosaic - two or more cell lines present -
often diploid / aneuploid mosaic

Chaotic - different chromosome content in every nucleus -
at least 3 chromosome pairs affected

(Harper and Delhanty, 1995; Delhanty et al., 1997)

Chromosomes of Human Embryos



Abnormal - all cells uniformly abnormal, e.g trisomy 21-
due to meiotic error.

Risk increases with maternal age or genetic predisposition

Mosaic and **Chaotic** - on average over 50% of embryos
affected by day 3. Some patients have higher risk e.g
those with repetitive IVF failure.

Embryos with low level mosaicism may survive but high
levels leads to arrested development.

Chromosomes of Human Embryos



Diploid mosaics - mechanisms

- Chromosome loss
 - 2 cell lines: e.g. XX 18 18/ XX 18
- Chromosome gain
 - 2 cell lines: e.g. XX 18 18/ XX 18 18 18
- Mitotic non-disjunction
 - 3 cell lines: e.g. XX 18 18/ XX 18 18 18/ XX 18

Chromosomes of Human Embryos

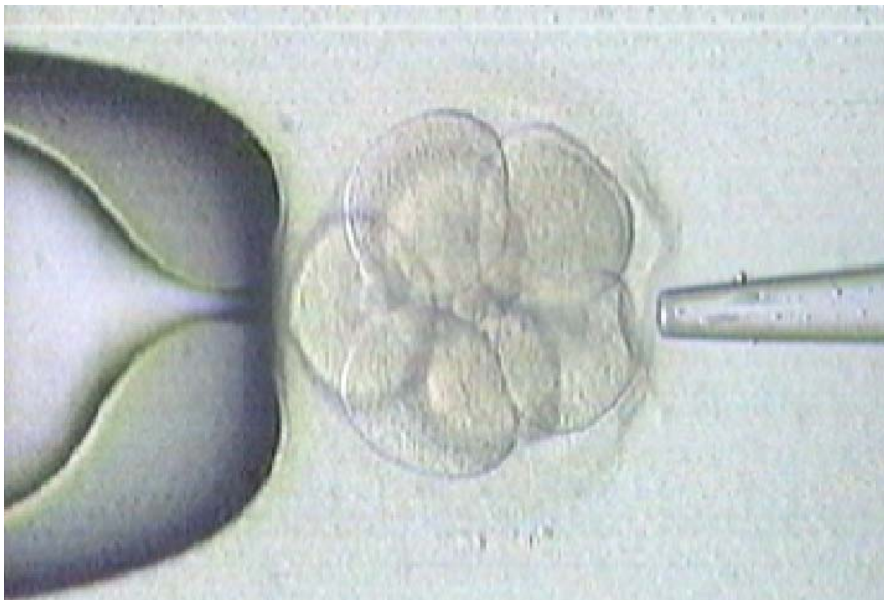
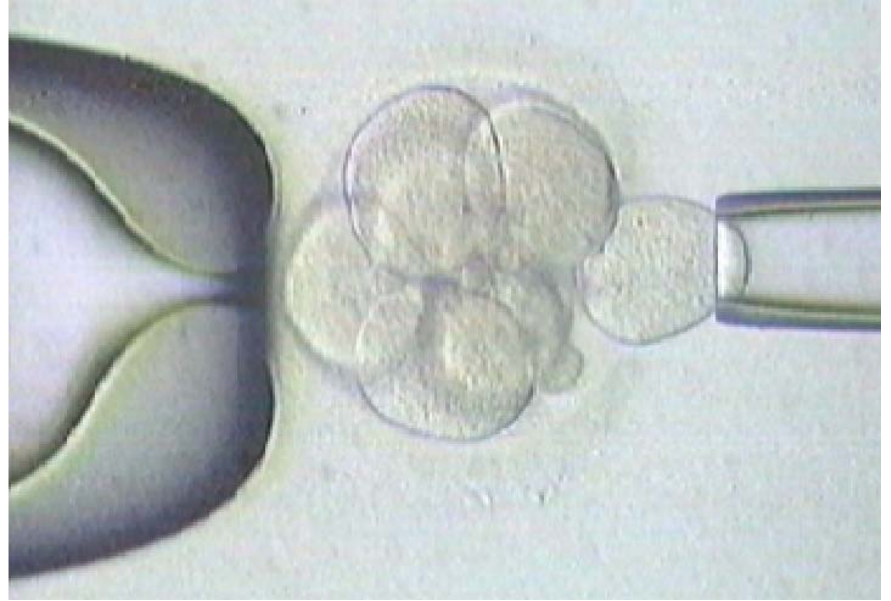
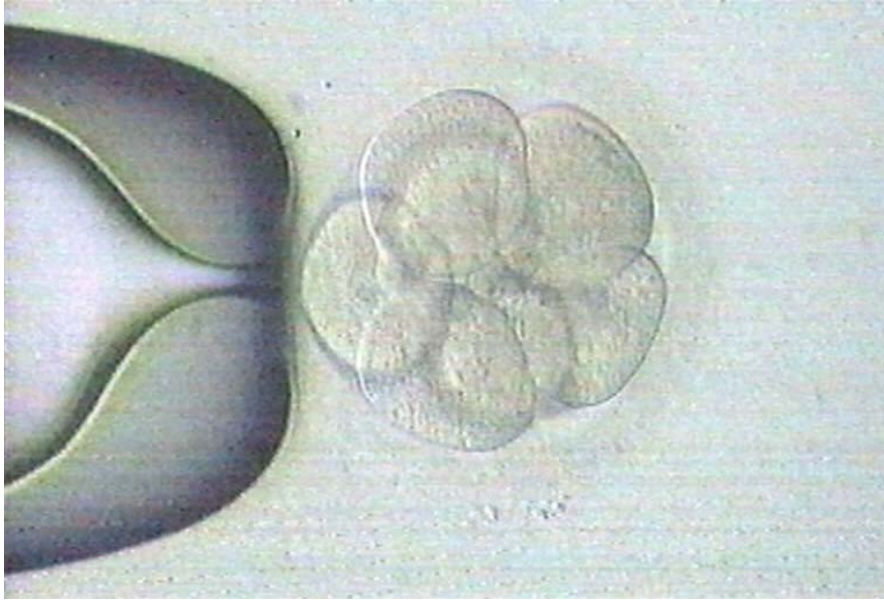
Chaotic mosaics - mechanisms -?

Two types:

- Chaotic throughout; error in first cleavage division. Possible centrosome duplication error leads to multipolar spindle and aberrant segregation in mitosis
- Partially diploid; chaotic divisions affect some cells.
- Cause?

Both types common in RIVFF group

Embryo Biopsy



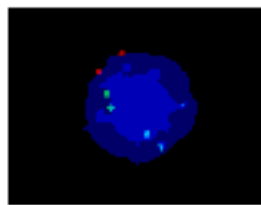
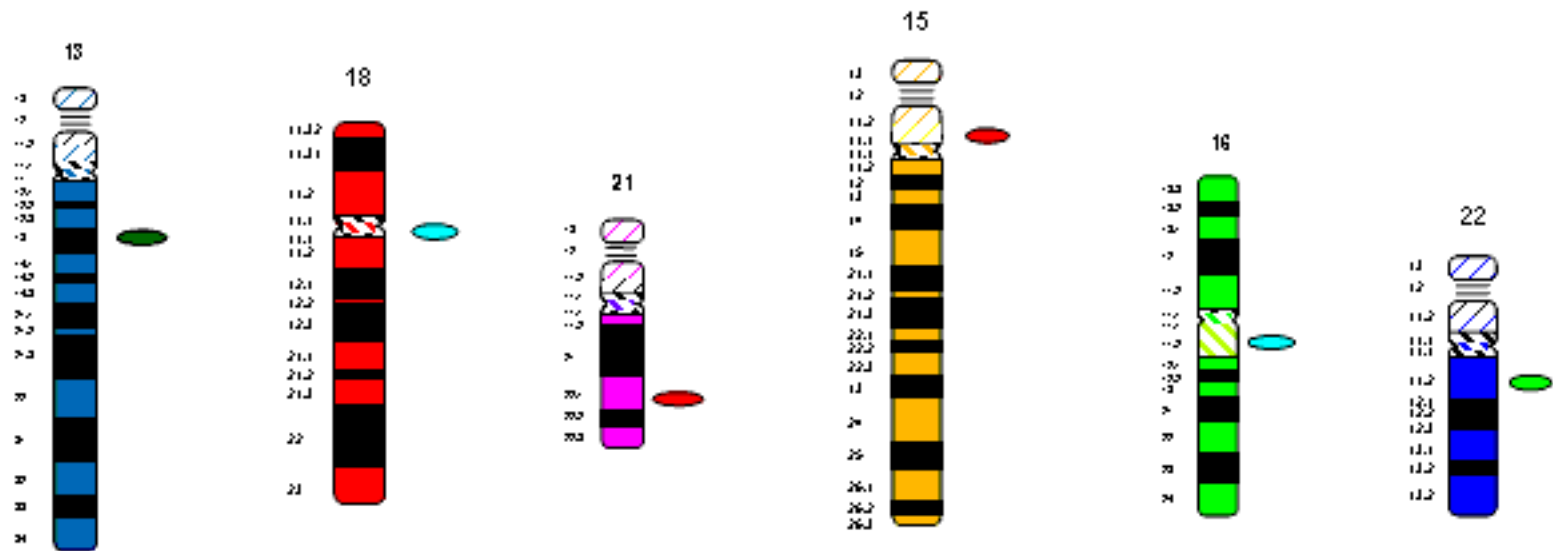


UCL Centre for PGD

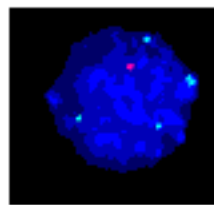
PGS - referral reasons

- 3 or more failed IVF cycles
- 3 or more unexplained miscarriages with or w/o IVF
- Advanced maternal age alone (at UCL 40 yrs +)

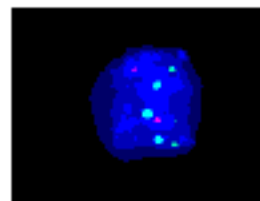
FISH enumeration probes



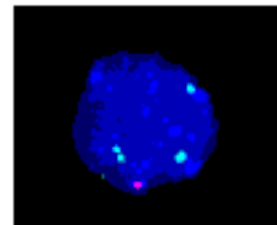
Normal blastomere



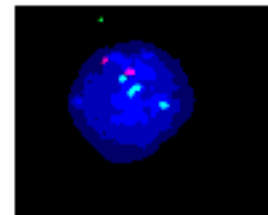
Monosomy 21



Trisomy 16

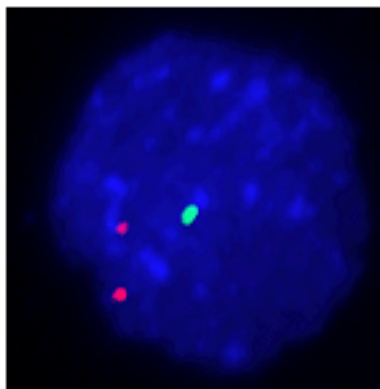


Monosomy 15

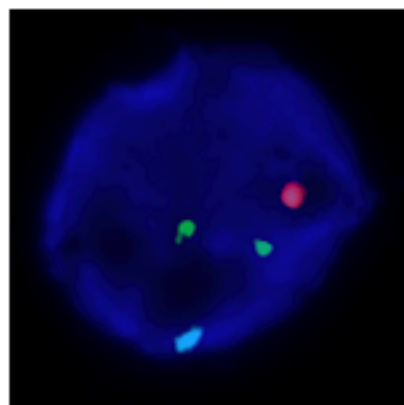


Normal blastomere

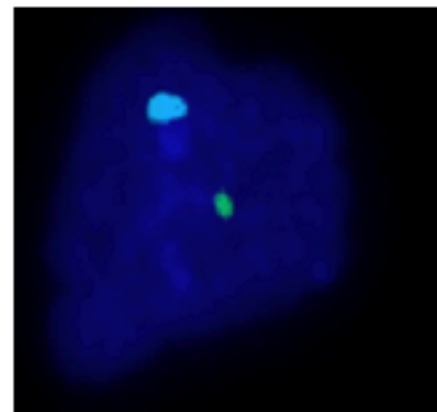
Abnormalities seen in embryos



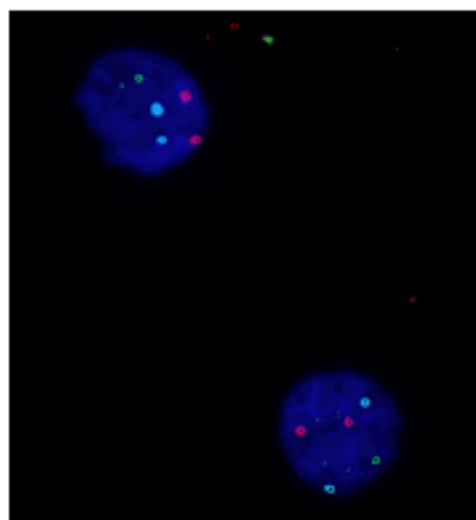
Monosomy 13 &
nullisomy 18



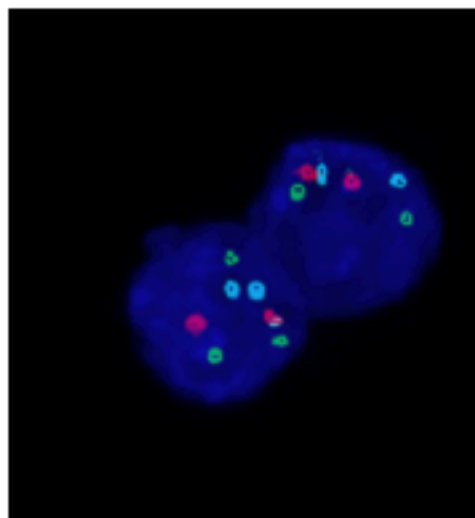
Monosomy 18 & 21



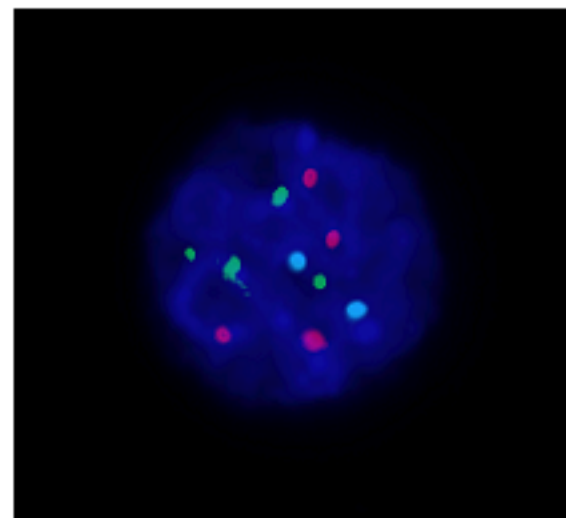
Monosomy 13 & 18, nullisomy 21



Birucleate- monosomy 22



Birucleate-diploid & trisomy 22



Tetrasomy 15 & 22

UCL Centre for PGD

PGS Follow up Data

- 475 embryos diagnosed; 84 'normal'
- Embryos abnormal on biopsy confirmed abnormal ; no false positives

- Follow up results on 352 abnormal embryos
- 65 with meiotic errors (18.5%)
- Remainder due to post-zygotic errors only



UCL Centre for PGD

PGS Follow up Data

- 70 meiotic errors (65 embryos)
- Chromosomes involved in order: 21, 22, 13, 18, 16, 15

Outcome of 60 cycles of Preimplantation Genetic Screening

Group	AMA	RIVFF	RM
Average mat age	42.5	36	36.5
Pregnancy rate/cycle to EC	20%	26.3%	33%
Normal on biopsy	14.3%	18.3%	17.5%
Mosaic on follow up	92.1%	99.5%	88.2%
Uniformly abnormal on follow up	7.8%	0.5%	11.8%
Embryos with meiotic errors/total abnormal	32.8%	9.9%	30%



UCL Centre for PGD

Conclusions

- In high risk patients 90% of embryos have post-zygotic errors
- Repetitive miscarriage patients have increased risk of meiotic errors, equal to those in women aged over 40 years



UCL Centre for PGD

Acknowledgements:

CGH study - E Fragouli, D Wells, MJW Faed

PGS - A Mantzouratou, A Mania, S Tashkandi,
L Xanthopoulou, J Harper, K Fordham and all the PGD team,
UCL

P Serhal, A Doshi, S Nuttall, S Gotts, and all the Staff of the
Assisted Conception Unit, UCLH