

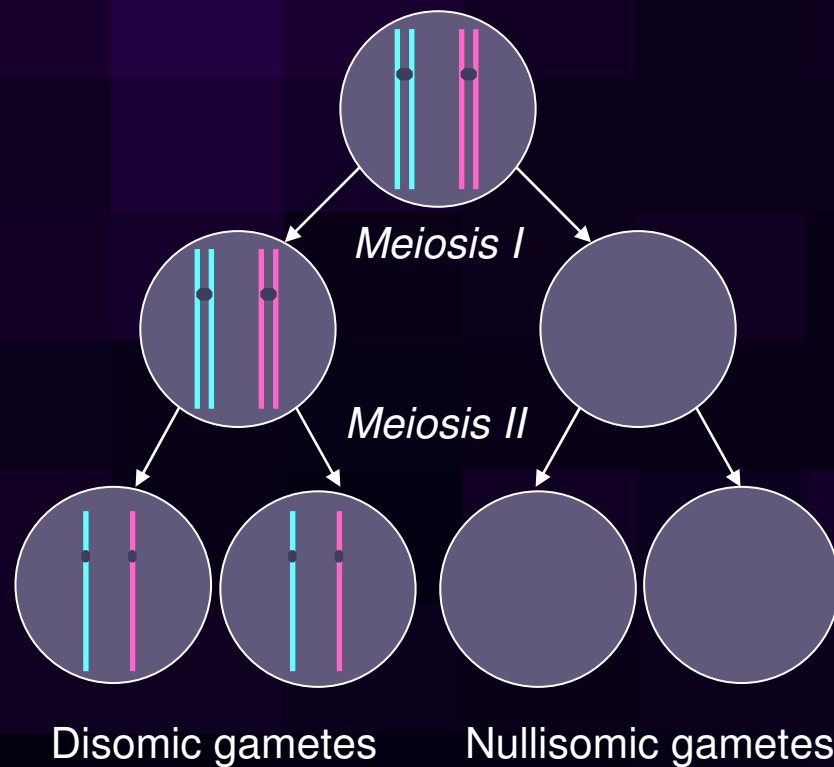
Investigation into foetal ovarian mosaicism

Charlotte Morris

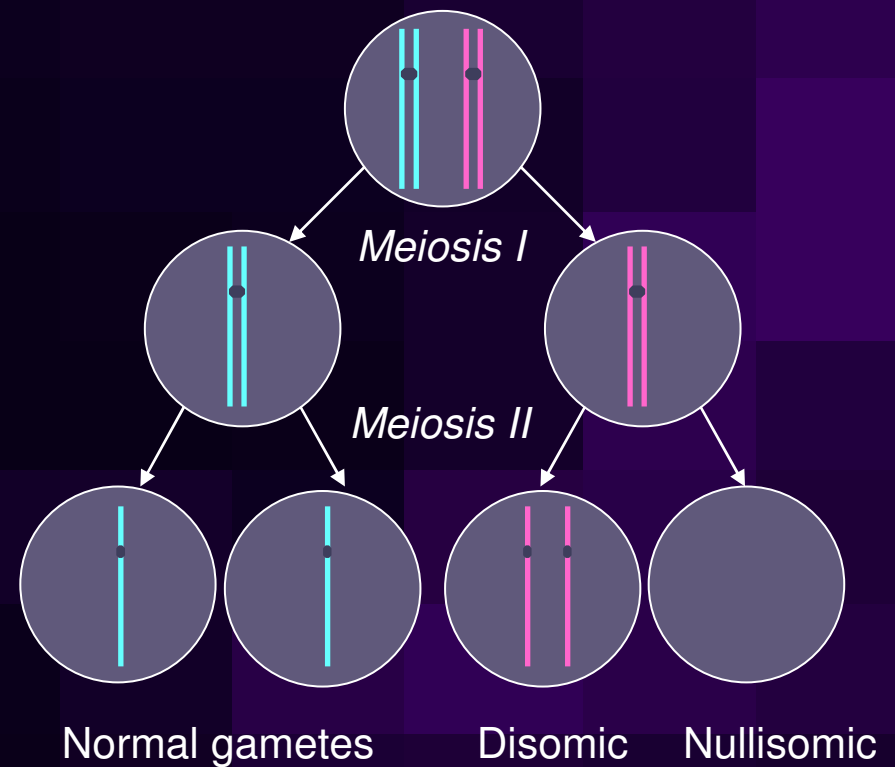
Origin of trisomy

- Meiotic non-disjunction

Meiosis I error

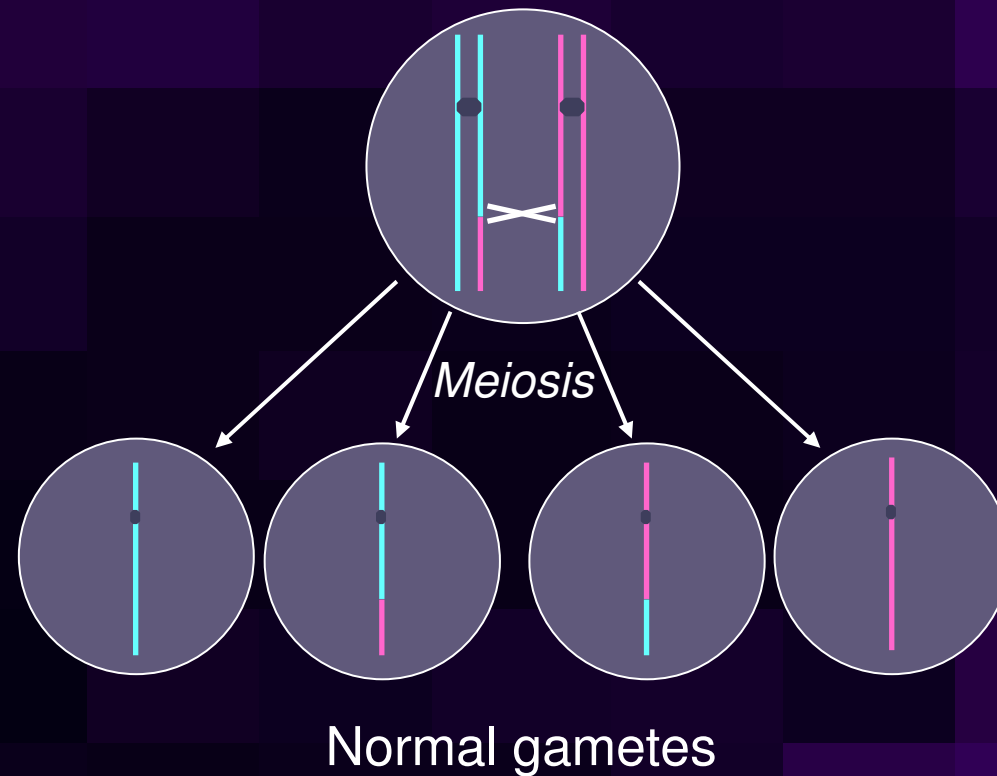


Meiosis II error



Origin of trisomy

- Recombination – Meiosis I

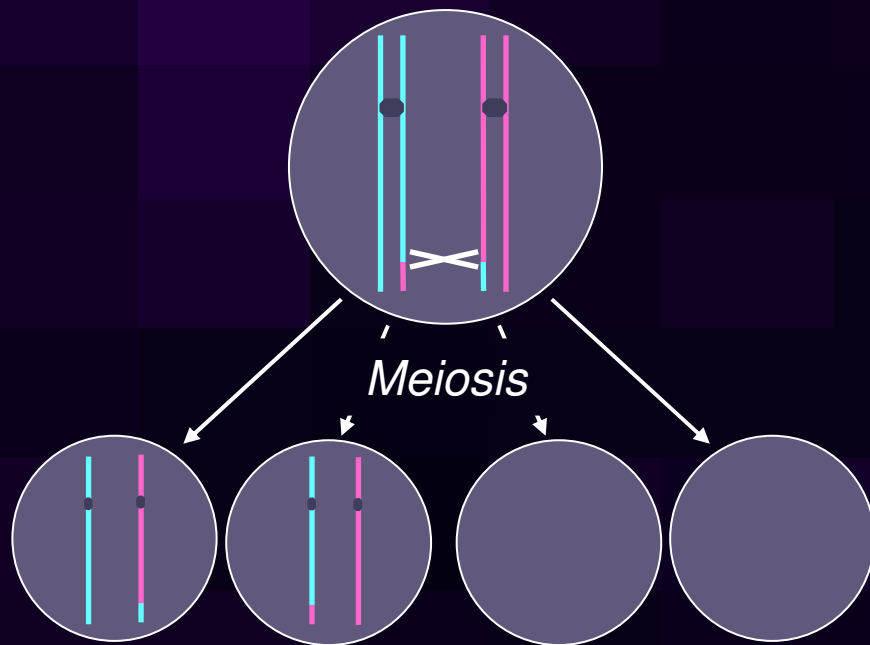


Origin of trisomy

- Atypical recombination – Meiosis I errors

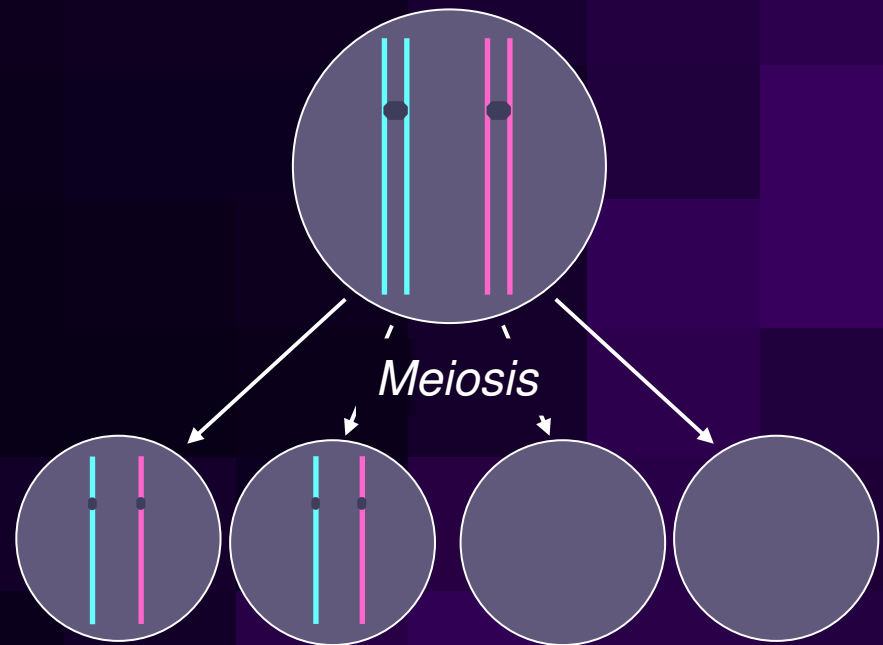
Movement of chiasmata

Achiasmate



Disomic gametes

Nullisomic gametes



Disomic gametes

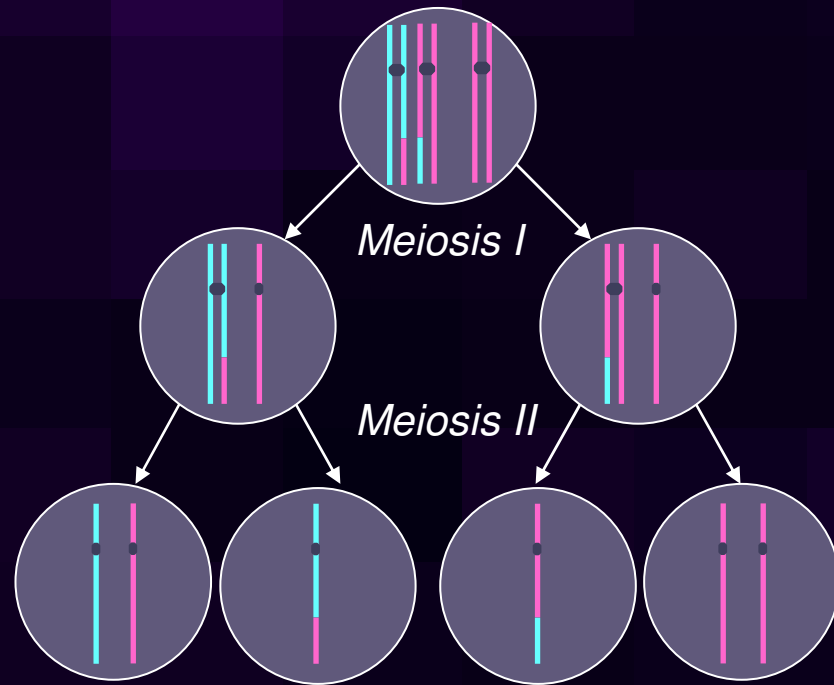
Nullisomic gametes

Origin of trisomy

- Ovarian mosaicism (Hultén et al., 2008)

Trisomic pre-meiotic cell

Bivalent & univalent

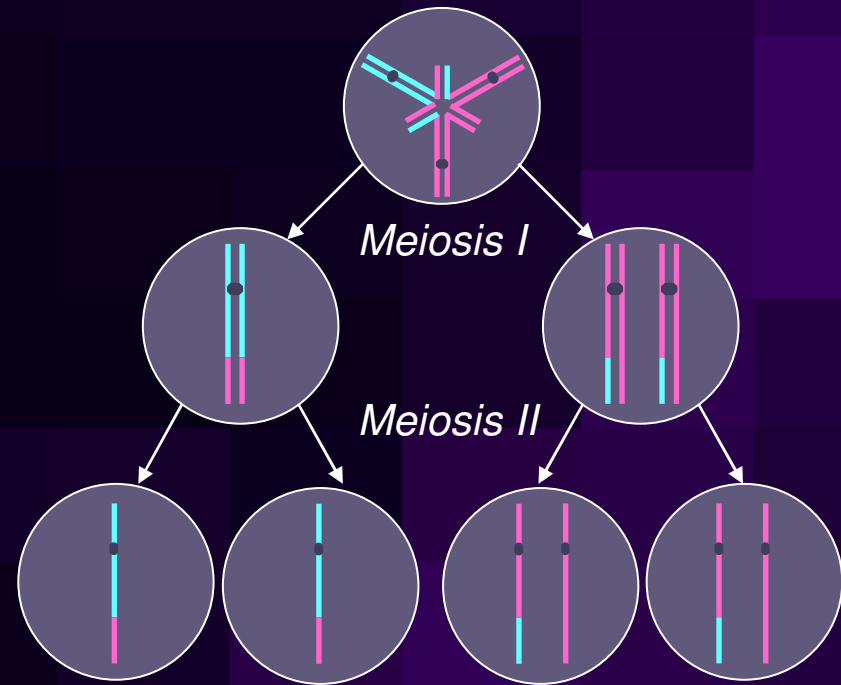


Disomic

Normal gametes

Disomic

Trivalent



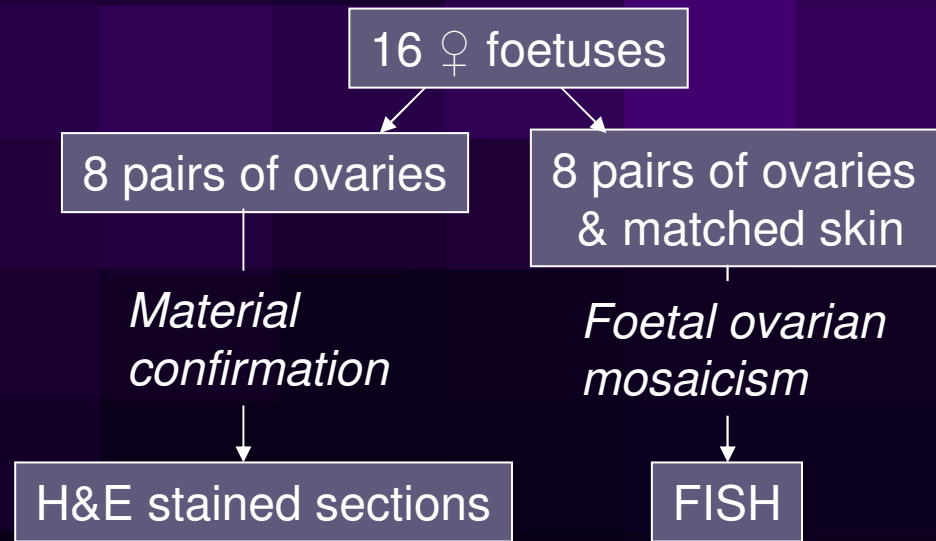
Normal gametes

Disomic gametes

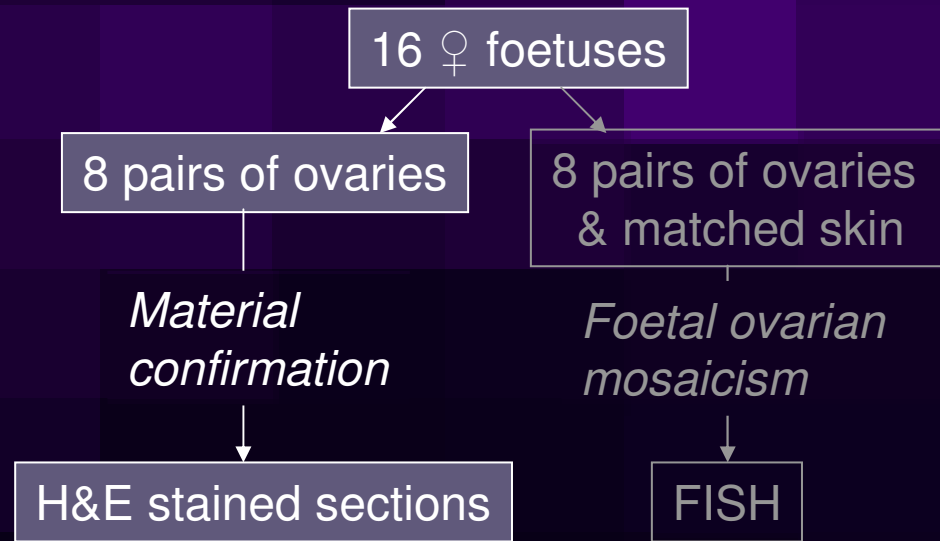
Aims

- Confirm the findings of foetal ovarian mosaicism for trisomy 21 (as found by Hultén et al. (2008))
- Elucidate the origin of trisomies 16 and 22

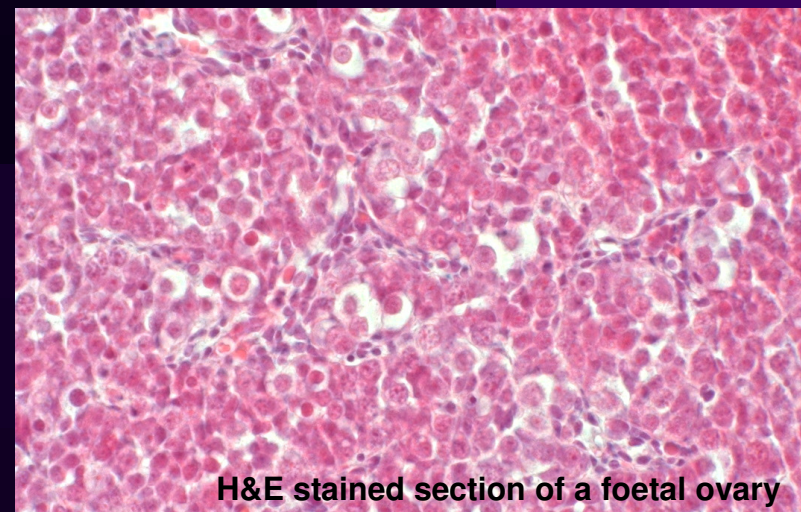
Foetal samples



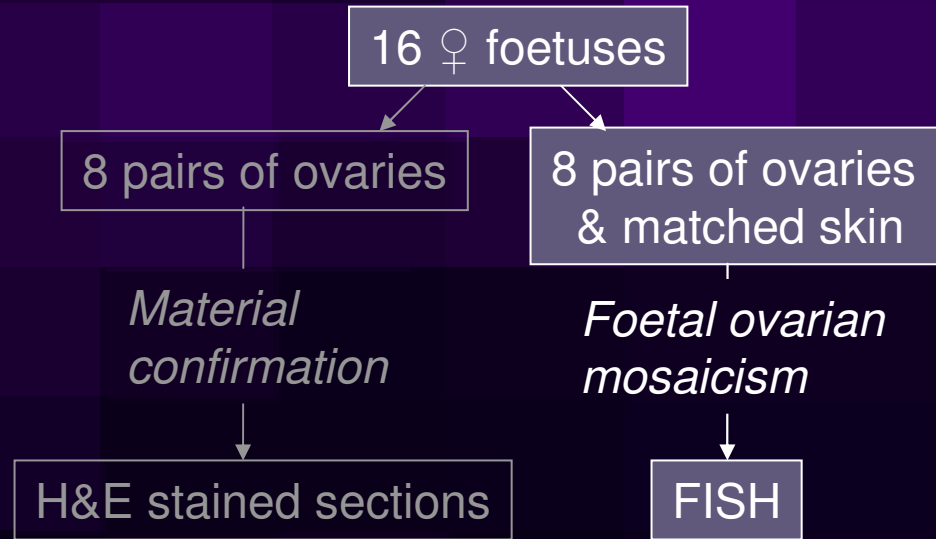
Material confirmation



- H&E stained sections
 - Material confirmed as ovarian by a Consultant Histopathologist



Foetal ovarian mosaicism



- Tissues dissociated
 - FISH with break apart probes:
 - CBF β (16q22)
 - RUNX1 (21q22)
 - EWSR1 (22q12)
- >1000 nuclei scored on each slide
» independently checked ~ 500 nuclei

Results

- 26095 ovarian, 25051 skin cells examined
- 13 trisomic cells (3 ovarian, 10 skin)

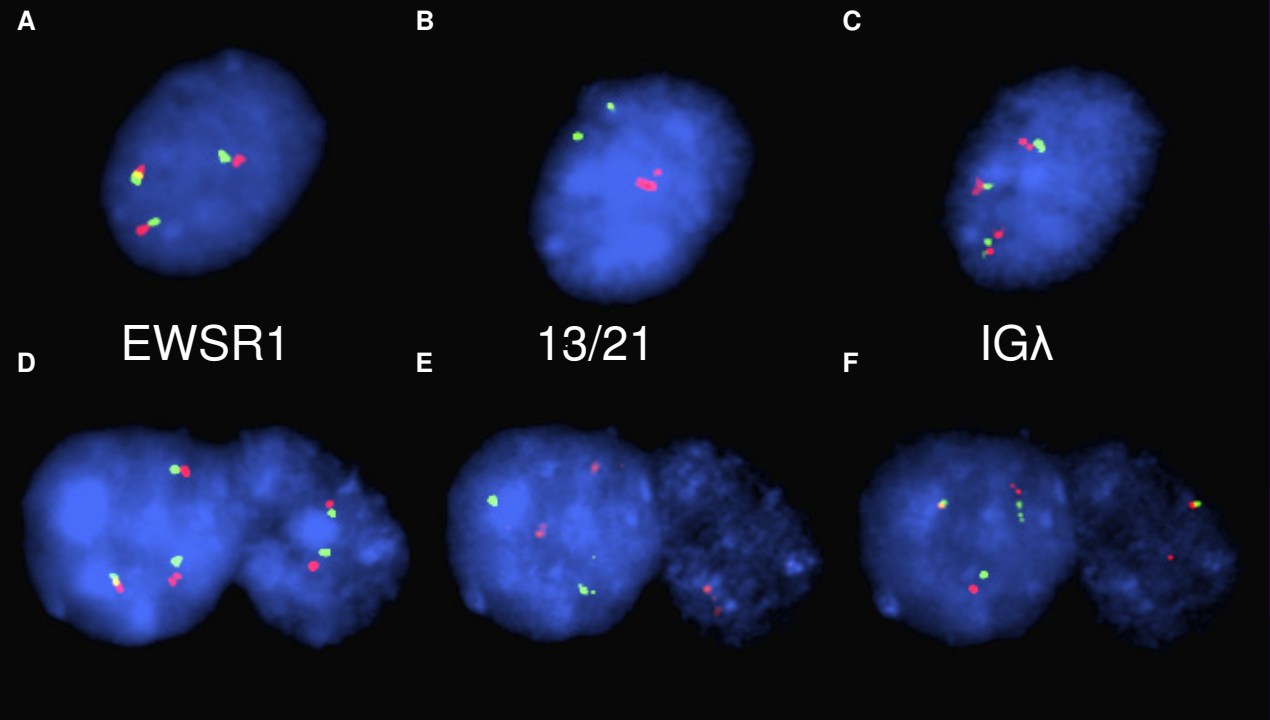
Sample	Proportion of trisomic cells					
	CBF β (16q)		RUNX1 (21q)		EWSR1 (22q)	
	Ovary	Skin	Ovary	Skin	Ovary	Skin
N1444	0/1806	0/1103	0/1096	0/1051	1/1263	1/1087
N1499	0/1001	0/1030	1/1019	1/1021	0/1030	0/1045
N1510	0/1072	0/1048	1/1047	0/1008	0/1062	0/1040
N1520	0/1021	1/1032	0/1027	0/1081	0/1045	0/1020
N1526	0/1008	0/1029	0/1020	3/1005	0/1010	0/1013
N1528	0/1074	0/1054	0/1012	0/1013	0/1084	0/1016
N1529	0/1026	0/1046	0/1022	0/1012	0/1015	0/1103
N1530	0/1077	1/1044	0/1122	1/1054	0/1136	2/1096
Total	0/9085	2/8386	2/8365	5/8245	1/8645	3/8420

Results

13 trisomic cells

- Sequential FISH: 1) AneuVysion 13/21 probe set
2) Chromosome specific break apart probe
- 11/13 failed
- Successful sequential FISH of two trisomy 22 cells

Ovarian (A-C)



Skin (D-F)

Discussion

0.025% of cells were trisomic in both the ovarian and skin samples

– only **0.011%** in ovarian tissue

Results discordant with previous study by Hultén et al. (2008)

→ **0.54%** of cells had trisomy 21

Differences between this study and Hultén et al. (2008):

- 1) Level of cell scrutiny and evaluation
- 2) FISH probes
- 3) Gestation of foetal samples

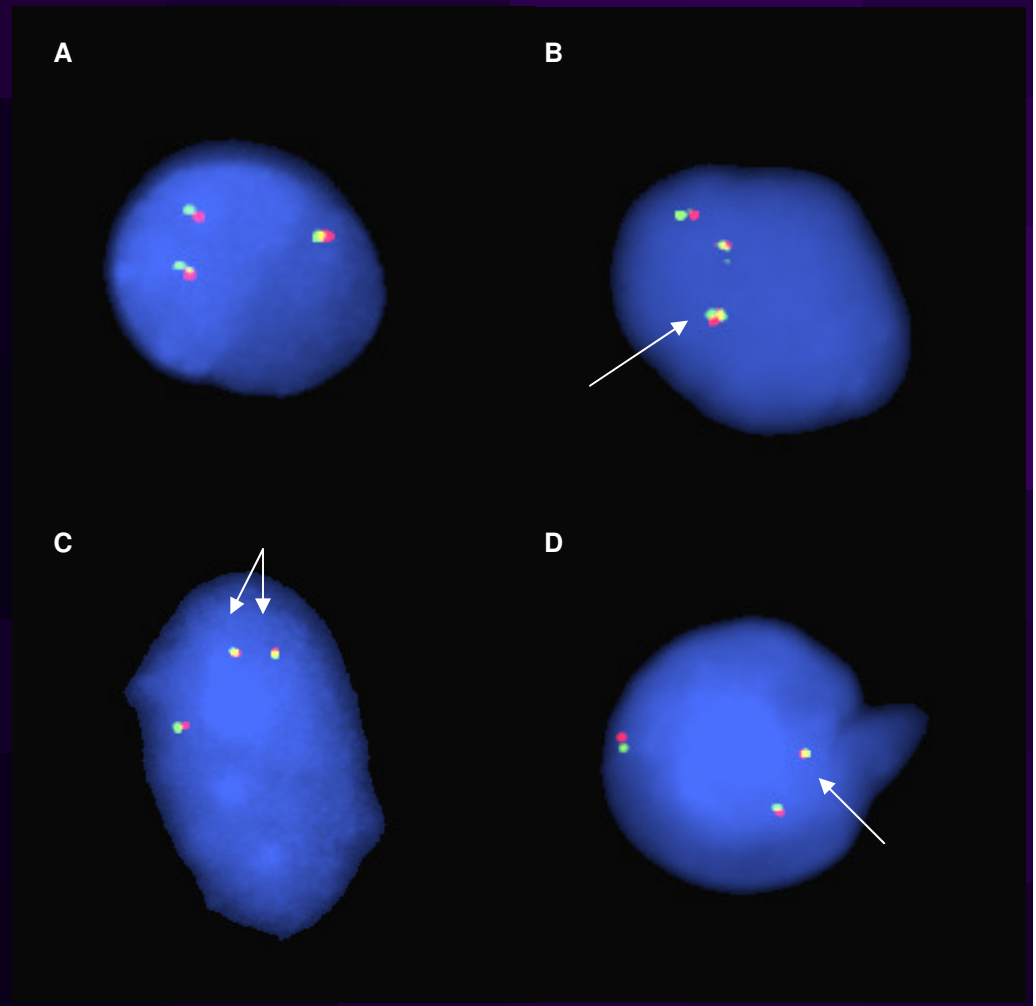
1) Cell scrutiny

Potential trisomic cells were evaluated by two experienced registered clinical cytogeneticists

» 13 trisomic cells

– some had additional characteristics that could explain the trisomic signal pattern:

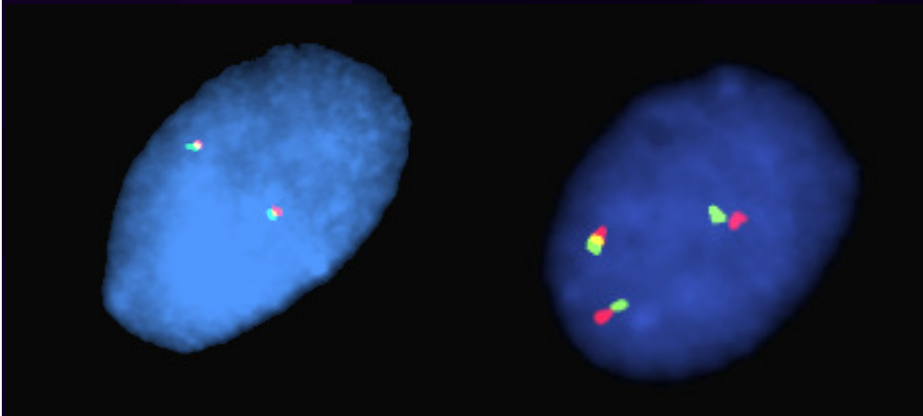
- 1) cryptic co-localisation of multiple cells (A)
- 2) grouping of two signals (B)
- 3) splitting of a single signal (C)
- 4) artificial appearance (D)



2) FISH probes

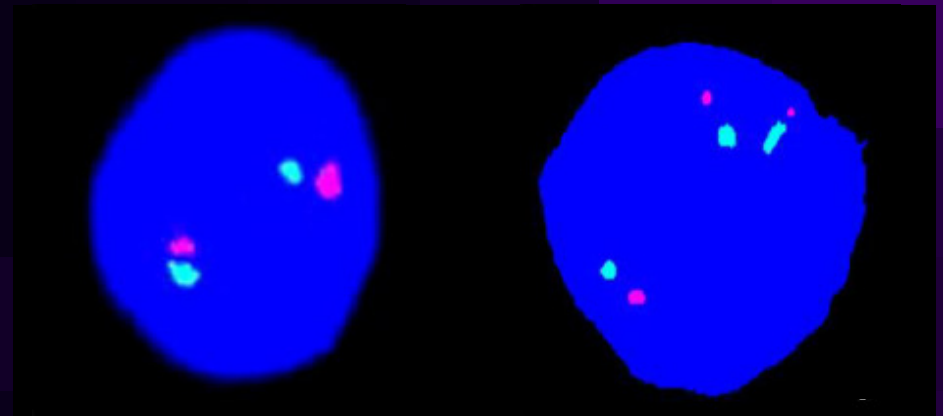
- Break apart vs. dual colour probes
- Break apart probes:
 - Easier to score
 - Larger proportion of cells give a clear signal pattern

Break apart probe



This study

Dual colour probe



From Hultén et al. (2008)

3) Foetal gestation

- 8-12 weeks vs. 14-22 weeks
 - Foetal ovaries consist of a large number of pre-meiotic germ cells between 8-12 weeks gestation

	Foetal gestation				
	6 weeks	9 weeks	4 months	5 months	Birth
Percentage of pre-meiotic germ cells in foetal ovaries	~10%	~15-20%	~29%	~26%	~14%

Percentages taken and calculated from Baker (1963) and Bendtsen et al. (2006)

- Hultén et al. (2008) potentially examined twice as many pre-meiotic germ cells per 1000 scored
 - might expect our incidence of trisomy 21 to be ~0.2-0.3%, half that measured by Hultén et al. (2008)
 - » 10 fold less than expected (0.024%)

Conclusion

- No evidence for foetal ovarian mosaicism for trisomies 16, 21 and 22

Acknowledgements

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