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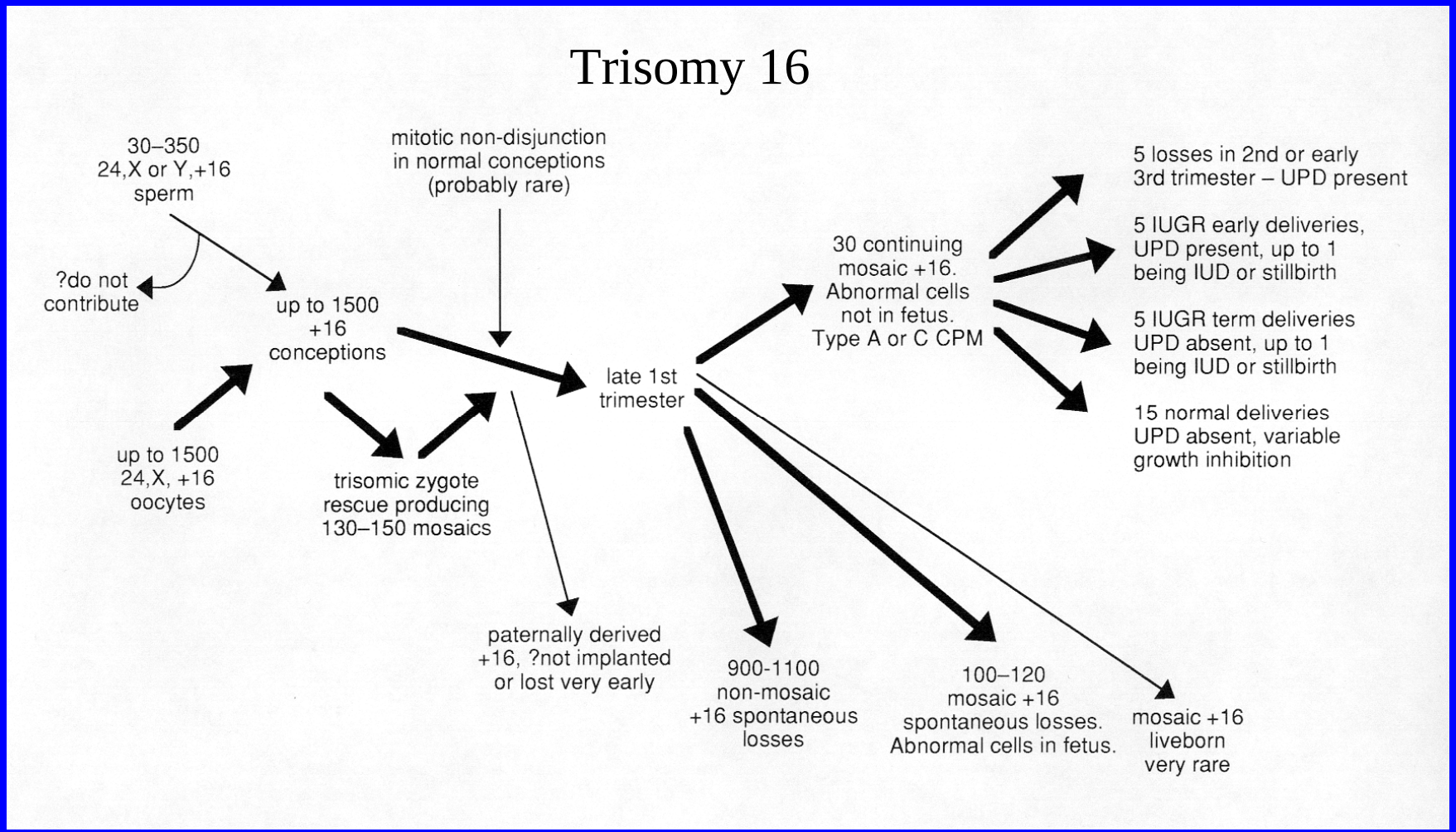
Hosted By:
U.S. Department of Health and Human Services
National Institutes of Health

Towards a longtitudinal picture of the origins and fates of specific chromosome abnormalities throughout human development

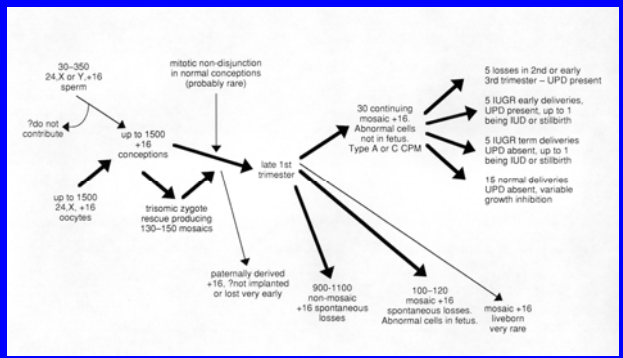
John Wolstenholme

10 years ago….


Trisomy 16
30–350
24,X or Y,+16
sperm
?do not
contribute
mitotic non-disjunction
in normal conceptions
(probably rare)
up to 1500
+16
conceptions
up to 1500
24,X, +16
oocytes
trisomic zygote
rescue producing
130–150 mosaics
late 1st
trimester
paternally derived
+16, ?not implanted
or lost very early
30 continuing
mosaic +16.
Abnormal cells
not in fetus.
Type A or C CPM
5 losses in 2nd or early
3rd trimester – UPD present
5 IUGR early deliveries,
UPD present, up to 1
being IUD or stillbirth
5 IUGR term deliveries
UPD absent, up to 1
being IUD or stillbirth
15 normal deliveries
UPD absent, variable
growth inhibition
900-1100
non-mosaic
+16 spontaneous
losses
100–120
mosaic +16
spontaneous losses.
Abnormal cells in fetus.
mosaic +16
liveborn
very rare

Why?

- Not new, but timely - new data about CPM and UPD, gametogenesis and early development
- Everything should add up
- You should (eventually) be able to incorporate all observations into a single model
- Trisomy 16 had some good data available
- It is interesting clinically
- Potential for 23 follow-up papers



Is audit useful?

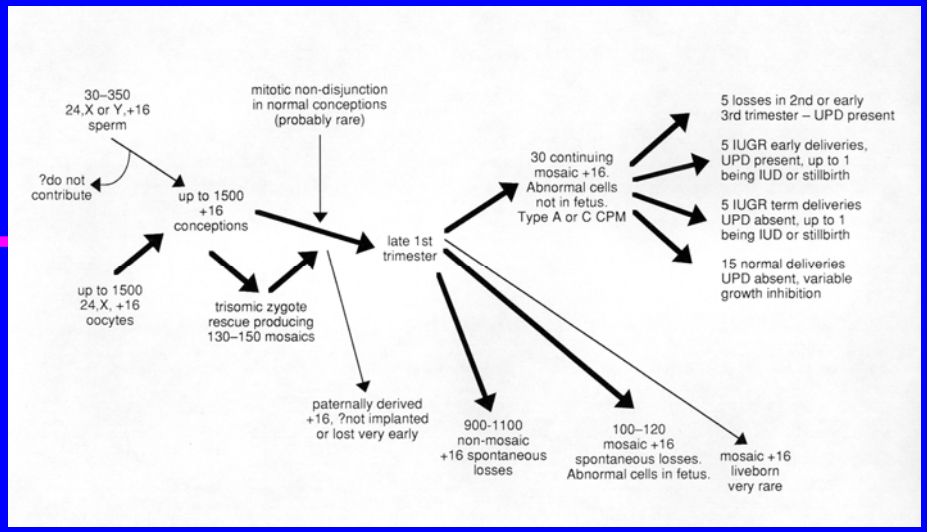
- Quantifies changing patterns
- Quantifies chromosome abnormality specific characteristics
- Highlights inconsistencies, impossibilities and areas requiring re-interpretation
- Puts mosaicism in prenatal diagnosis in context
- Tells you where there are gaps in knowledge

What does audit demonstrate?

- chromosomal variation in incidences of meiotic errors
- lots of correction of trisomy going on, all chromosomes?, all 1 in 3 UPD?, all the same incidence?
- not very well linked to ensuring a normal fetus
- chromosomal variation in incidences of somatic errors
- ?inverse relationship between meiotic and somatic errors
- predicts UPD rates
- potential hazards of PGD

Gaps in knowledge

- Spermatogenesis ●●
- Oogenesis ●●
- Fertilisation ●
- cleavage stage ●●
- blastocyst -
- spontaneous losses ●●●●
- CVS and amniocentesis ●●●●●
- late losses ●●●●
- term ●●●●



Blastocyst series

- Complement other studies of oocytes and studies at cleavage stage
- looking at a more viable population
- most trisomy correction will be in place
- many of the somatic errors will be in place
- initial selection against some abnormalities will have occurred
- make useful comparisons with earlier and later stages

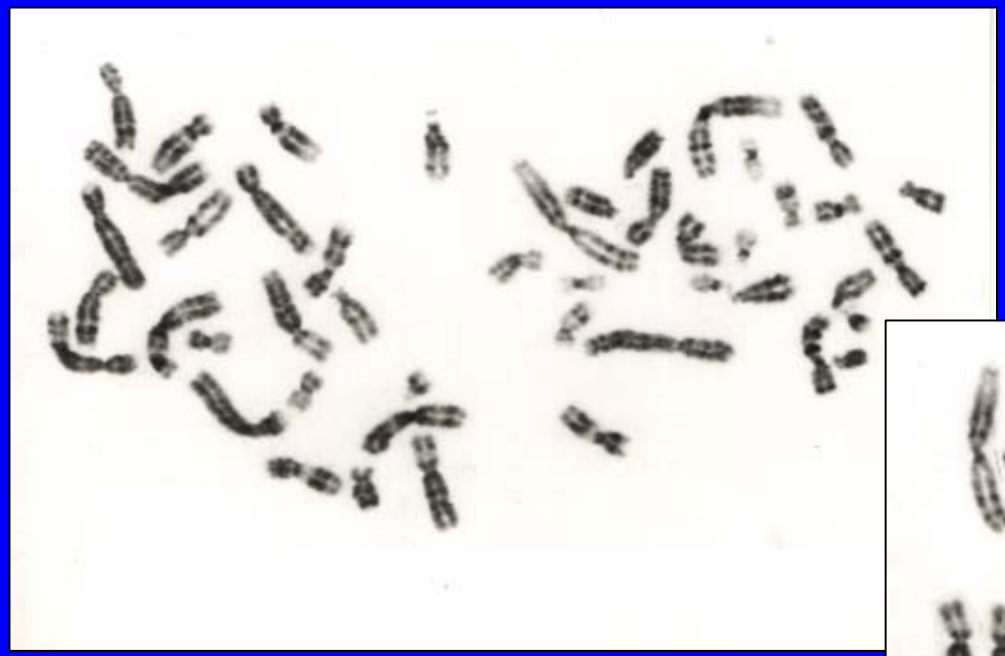
Method: Cytogenetics + FISH

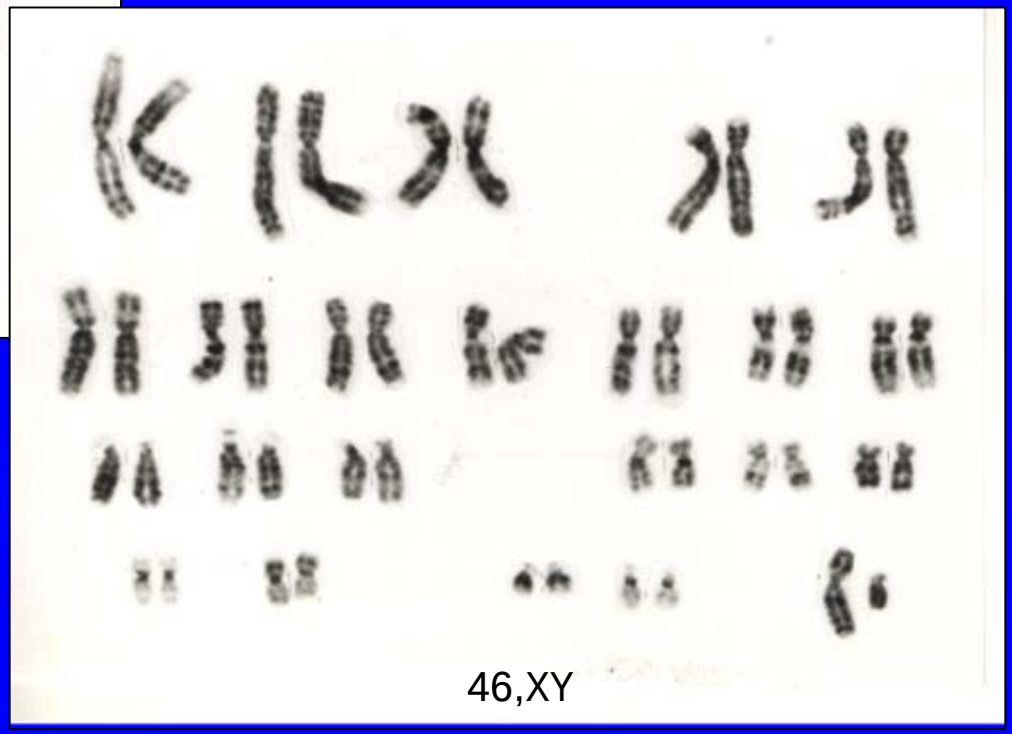
- Synchronise cell division
  0.5mg/ml thymidine
- Arrest cell cycle in metaphase
  0.1μg/ml Colcemid
- Fix intact blastocysts
- Disaggregate cells
  70% acetic acid
- G-band metaphases
- Sequential FISH

Results - first 438 published

See Clouston et al. (2002) *Prenatal Diagnosis*, **22**,1143-1152

for published data





46,XY

Comparison with cleavage stage embryos

- Jamieson et al, 1994.

  Karyotyped 195/816 cleavage stage embryos:

  – similar level of triploidy

  – 19% of diploid embryos were aneuploid

- FISH studies difficult to interpret, but compatible with this level or even higher levels
- In general levels of aneuploidy are significantly higher in embryos at the earlier cleavage stage

Comparison with FISH blastocyst series

- 202 blastocysts
- 23 equivalent abnormalities based on 3-9 probes
- 23/202 = 11.4%

- equates to 25-40% or more for all chromosomes????
- varies between studies

- higher than our study, but we are looking at older, less-selected and mitotically active blastocysts

Comparison with first trimester data

- 1 in 6 clinically recognised pregnancies are lost in the 1st trimester, including the majority of unbalanced chromosome abnormalities
- Pregnancy loss series* can be used to estimate the frequency of lethal abnormalities in all recognised pregnancies

- Allows estimation of minimum frequency of lethal abnormalities anticipated in a series of blastocysts

*3300 spontaneous abortions: Warburton et al (1991)

Comparison with first trimester data

- Not all chromosome abnormalities are lethal in the first trimester
- Less lethal, ongoing pregnancies are represented in data from early CVS* procedures
- Incorporate this data to give more accurate estimations of the frequencies of less lethal abnormalities

*18851 cases: Ledbetter et al (1992); ACC working party (1994)

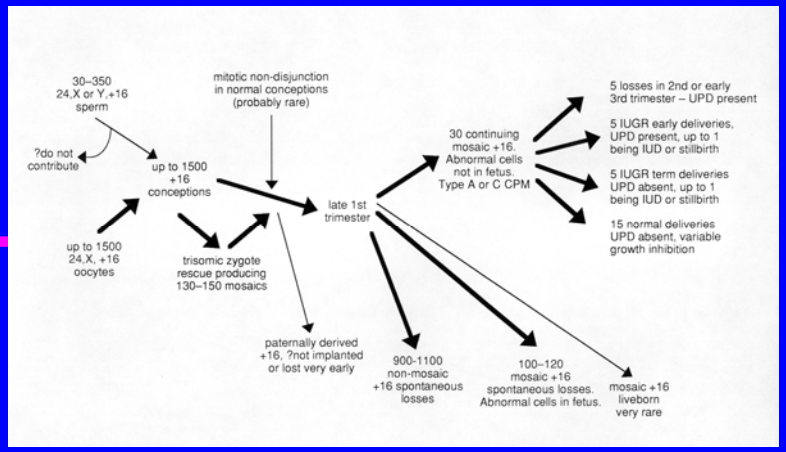
Karyotyped blastocysts

- Results fit with those from earlier and later stages of gestation and suggest:
  - a relatively constant level of triploidy and trisomy 16 throughout early development
  - significant selection against haploid, monosomic and some trisomic embryos prior to blastocyst stage.
  - less selection pressure between blastocyst stage and clinical pregnancy

Karyotyped blastocysts

- The general range and incidence of most main groups of chromosome abnormality observed in the first trimester appear to be in place by the blastocyst stage

Gaps in knowledge



- Spermatogenesis ●●●
- Oogenesis ●●●
- Fertilisation ●
- cleavage stage ●●●
- blastocyst ●●●
- spontaneous losses ●●●●●
- CVS and amniocentesis ●●●●●●●
- late losses ●●●●●
- term ●●●●●

Gaps in knowledge: pre-implantation stages

- Numbers are still small
- little abnormality-specific data
- 70-80% will fail to implant
- all data from surplus, sub-optimal IVF embryos/blastocysts
- implantation related to embryo quality
- significance of chaotic embryos

Gaps in knowledge: pre-implantation stages

- essentially we have no idea which abnormal embryos/blastocysts would have gone on to produce a recognised pregnancy
- little information as to how abnormal cells are distributed in blastocyst and how this is controlled
- no DNA studies
- rapidly changing denominator

Gaps in knowledge: pregnancy losses

- Cell lineage restricted data
- significant underestimation of mosaicism
- limited DNA analysis of origins of abnormality

Gaps in knowledge: prenatal diagnosis and later

- Picture continues to improve
- Case reports and series of case reports with corrected trisomies
- for +13, +18, +21, 45,X, XXX, XXY losses or at term, not clear how many non-mosaics are actually mosaics
- little data on origins of known mosaic cases

Gaps in knowledge

- For most abnormalities, origins are much more heterogeneous than trisomy 16
- far more complicated to disentangle all the separate components of what is going on
- also have have much less information to work with

Trisomy 2

- Can see +2 in spermatocytes
- ? Level in oocytes and at cleavage stage
- mat/pat, MI/MII known to occur but quite unclear to what levels in early stages
- trisomy 2 positively identified in our blastocysts
- also seen in mosaic form (? somatic error)
- about 5-10% of trisomy 2 is being corrected?????
- are all origins of trisomy being equally corrected?

Trisomy 2

- Most correct trisomies end up as pregnancy losses
- 50% of trisomy 2 is due to somatic errors, mostly CPM but also contributes to spontaneous losses
- why does most somatic trisomy 2 seem to be absent from the trophoblast?
- 1 in 10K continuing pregnancies are corrected trisomy 2 with 1 in 3 UPD
- outcomes of corrected trisomies ±UPD difficult to predict
- fetal mosaic trisomy is rare - possibly underestimated

Gaps in knowledge

- For most abnormalities, origins are much more heterogeneous than trisomy 16
- far more complicated to disentangle all the separate components of what is going on
- also have have much less information to work with

- we can get a feel for what is going on, but it is difficult to put hard figures on it

Conclusions

- For trisomy 16 and triploidy we can get a good picture of what is going on
- for trisomies 2, 3, 7, 8, 9, 13, 15, 18, 21, 22, X and Y, we can still only see part of the picture
- for the others, we really don’t have much of a picture at all

- It might be some while before I write the other 23 papers

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