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# Dr. John Wolstenholme

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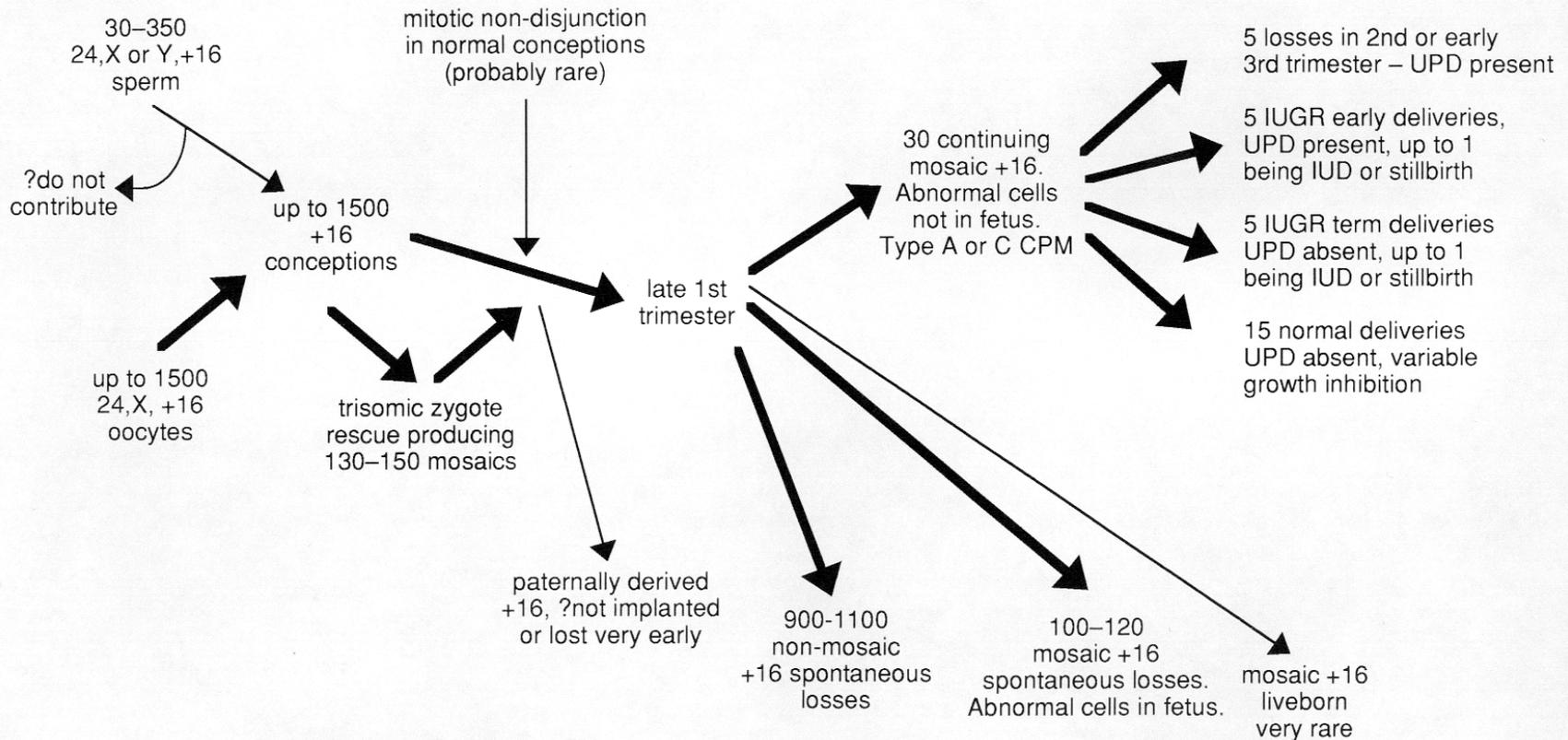
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Towards a longitudinal picture of the  
origins and fates of specific chromosome  
abnormalities throughout human  
development

John Wolstenholme

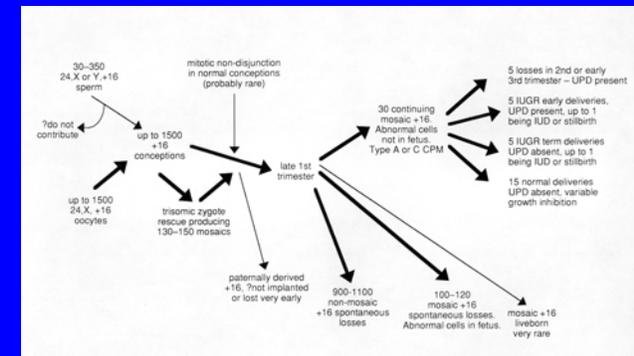
# 10 years ago....

## Trisomy 16



# 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?

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- ◆ 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?

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- ◆ 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 ● ●

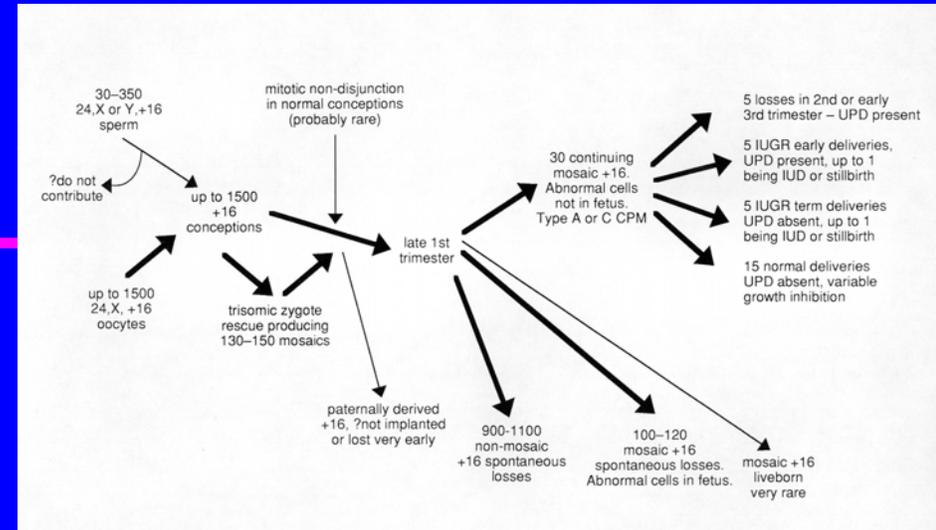
◆ blastocyst -

◆ spontaneous losses ● ● ● ●

◆ CVS and amniocentesis ● ● ● ● ●

◆ late losses ● ● ● ●

◆ term ● ● ● ●



# Blastocyst series

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- ◆ 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

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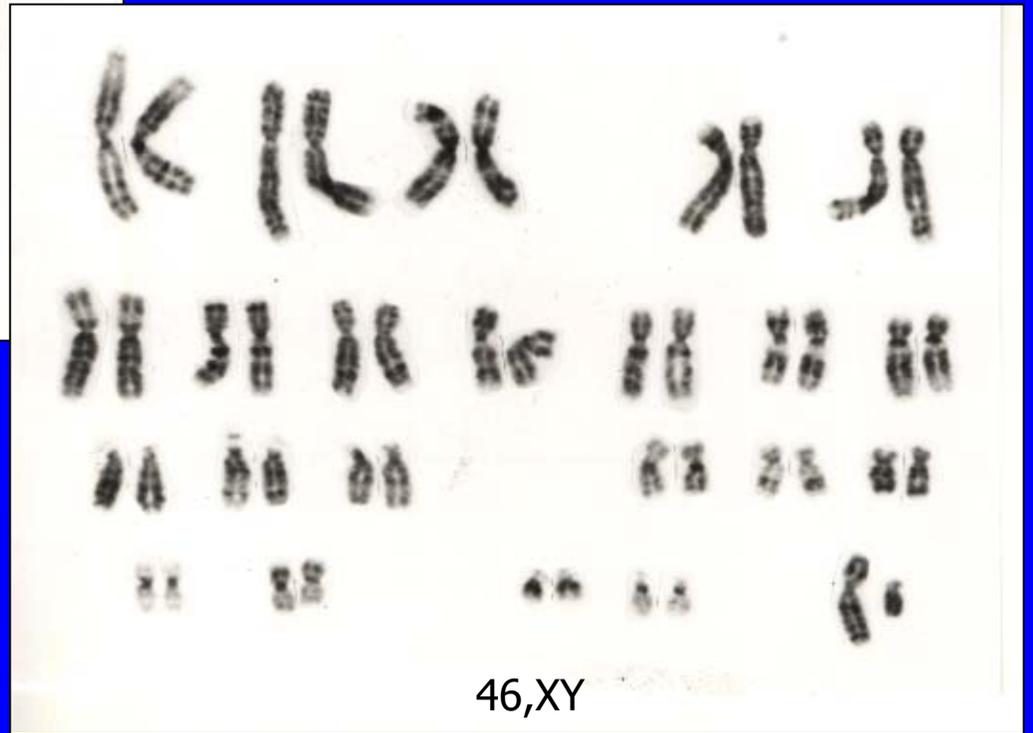
- ◆ Synchronise cell division  
0.5mg/ml thymidine
- ◆ Arrest cell cycle in metaphase  
0.1µg/ml Colcemid
- ◆ Fix intact blastocysts
- ◆ Disaggregate cells  
70% acetic acid
- ◆ G-band metaphases
- ◆ Sequential FISH

# Results - first 438 published

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

for published data



# Comparison with cleavage stage embryos

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- ◆ 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

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- ◆ 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

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- ◆ 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

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- ◆ 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

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- ◆ 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

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- ◆ 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



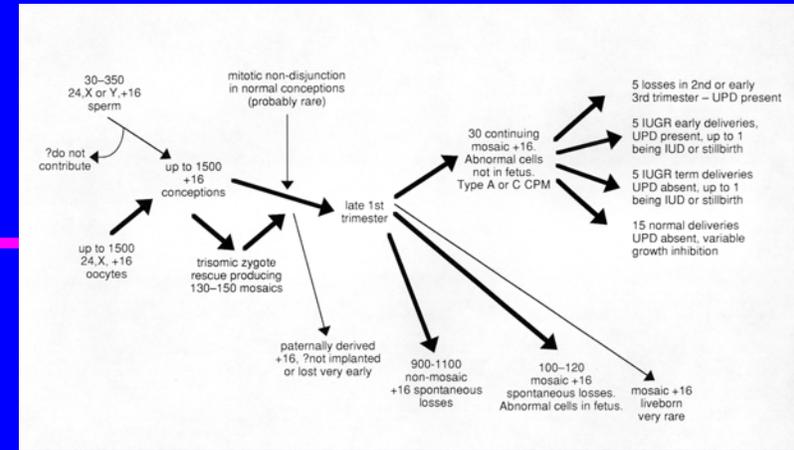
◆ CVS and amniocentesis



◆ late losses



◆ term



# Gaps in knowledge: pre-implantation stages

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- ◆ 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

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- ◆ 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

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

# Gaps in knowledge: prenatal diagnosis and later

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- ◆ 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

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- ◆ 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

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- ◆ 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

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- ◆ 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  $\pm$ UPD difficult to predict
- ◆ fetal mosaic trisomy is rare - possibly underestimated

# Gaps in knowledge

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- ◆ 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

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

# Acknowledgements

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