Ovarian Stimulation and Aneuploidy

Any Correlations?

Darren K Griffin
University of Kent

Overview

- Hospital Pass
- Oocyte maturation – the process
  - What makes a good egg go bad?
- Evidence for differences associated with stimulation
  - Thank you Bart Fauser
- What can we do about it?

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Dear Prof. Griffin,

Thank you once again for accepting to be a speaker at the next PGDIS Congress that will take place in Bologna from the 8th to the 11th May 2016.

On behalf of the Scientific Committee, Dr. Gianaroli and Dr. Ferraretti would like to invite you to be a speaker at the Pre Congress Course "Clinical aspects of PGD/PGS", that will take place on the 8th May in the afternoon.

Due to your expertise in this field, the topic suggested for your lecture is "Ovarian stimulation and aneuploidy: any correlations?".

We really hope that you will be able to accept this invitation and we are looking forward to hearing from you soon in order to finalize the the program.

Best regards,
Serena Sgargi
Congress Executive Assistant

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Ovarian Stimulation and Aneuploidy

- Any correlations?
- Short answer:
- *Not a lot that I can see*

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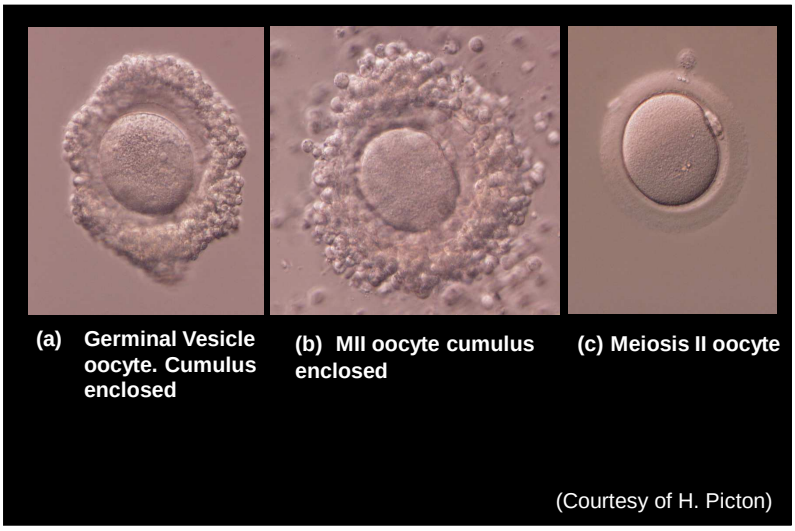
Oocyte maturation

3 aspects

- Nuclear maturation
  - Modification of chromatin
  - Dictyate phase (GV) to MII
- Cytoplasmic maturation
  - Changes in distribution/function/organisation of organelles
- Molecular maturation
  - Instructions accumulated during Germinal Vesicle stage
  - Controls nuclear and cytoplasmic progression
    - pre- and post-fertilisation
- All could affect chromosome segregation



(a) Germinal Vesicle oocyte. Cumulus enclosed

(b) MII oocyte cumulus enclosed

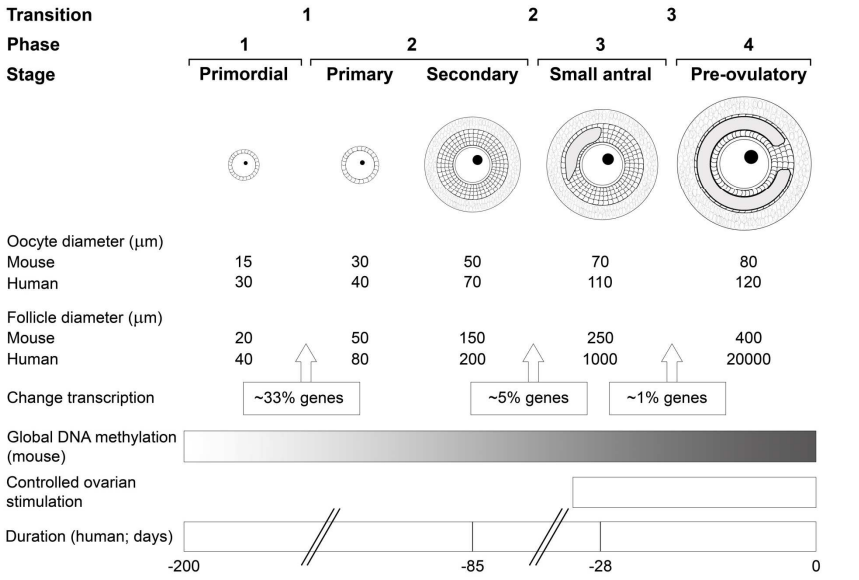
(c) Meiosis II oocyte

(Courtesy of H. Picton)

Oocyte Maturation and Meiosis

- GV (primordial) oocyte (prenatal)
  - Rapid division
- Primary oocyte (prenatal)
  - Initiates meiosis
  - Arrests before birth (diplotene)
  - Remains through childhood, adolescence and adulthood
  - Resumes at ovulation
- Secondary oocyte (ovulation)
  - Resumption of meiosis I
  - Extrusion of 1st polar body
  - Enters meiosis II
  - Arrests again at metaphase II
  - Will not complete meiosis unless fertilised
- Fertilised oocyte
  - Completes meiosis II
  - Extrusion of 2nd polar body

Ovarian folliculogenesis and oocyte growth



(Sinclair and Kwong, 2010)

Hunt, P.A. and T.J. Hassold. **Human female meiosis:**

What makes a good egg go bad?

Trends Genet, 2008. 24(2): p. 86-93

3 vulnerable stages of oogenesis:

- Meiotic prophase
  - synapsis and recombination
  - fetal ovary
- Follicle formation,
  - 2nd trimester of fetal development and
  - associated with dramatic loss of oocytes
- Oocyte growth
  - adult ovary
  - culminates in resumption/completion of
    - meiosis I
    - ovulation of a metaphase II arrested egg

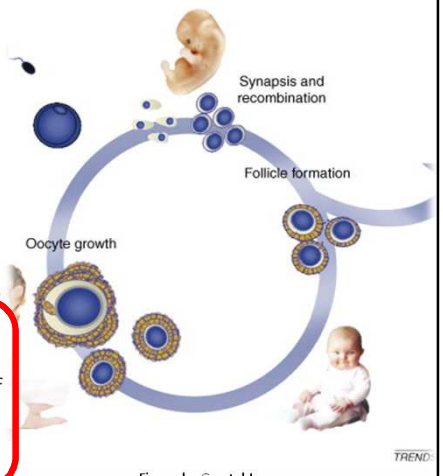
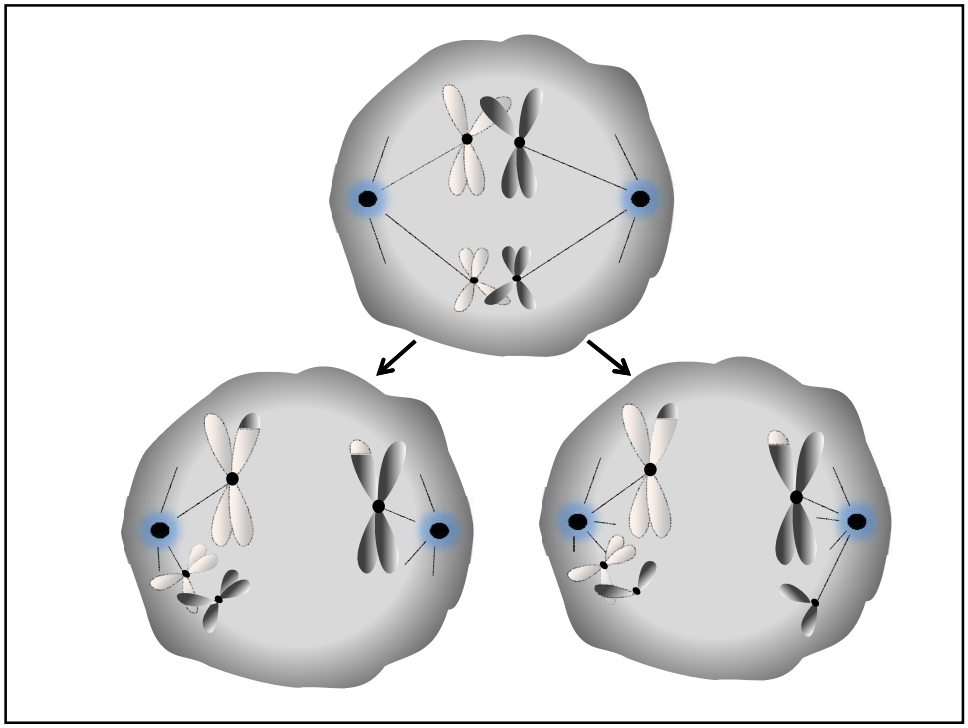


Figure by Crystal Lawson



Any evidence that endocrine-related chemicals can affect aneuploidy?

- **Hunt, P. A.**, et al. (2012). "Bisphenol A alters early oogenesis and follicle formation in the fetal ovary of the rhesus monkey." Proc Natl Acad Sci U S A **109**(43): 17525-17530.
- *"BPA induces subtle disturbances in the prophase events that set the stage for chromosome segregation at the first meiotic division"*
- Endocrine disruptors
- BPA is a "poster child"
- So it's certainly biologically feasible that drugs that mess with our endocrine system *could* lead to increased aneuploidy levels

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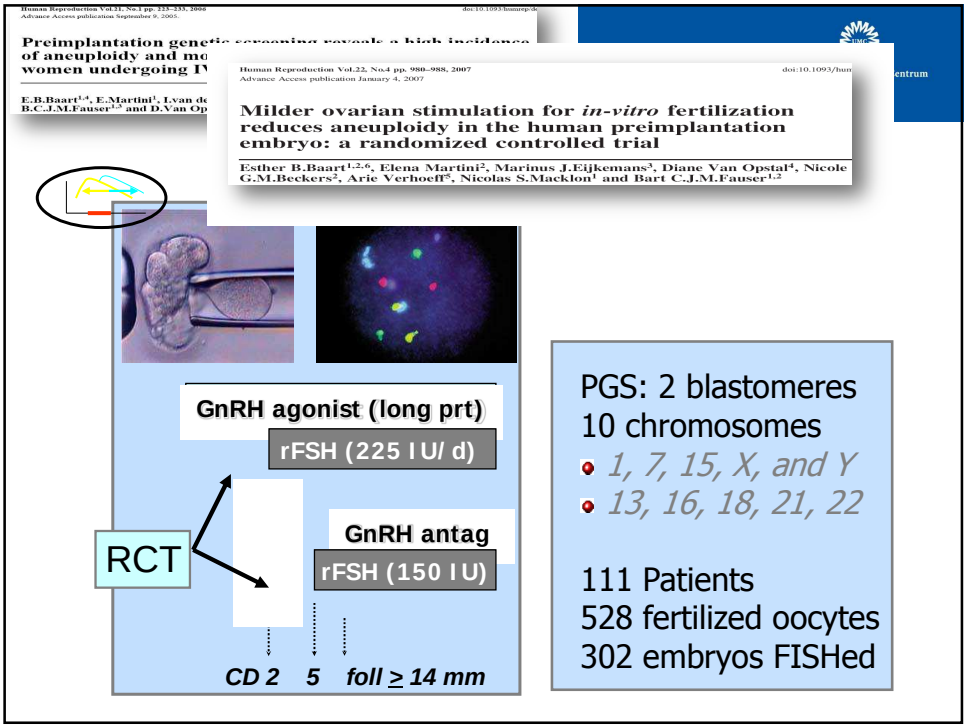
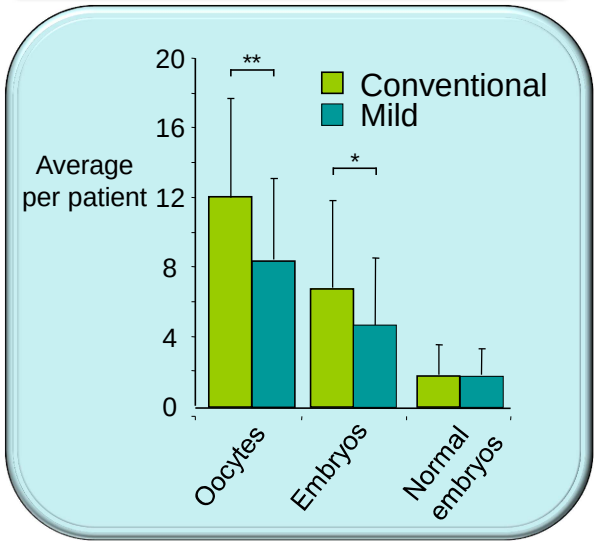
Thank-You Bart Fauser

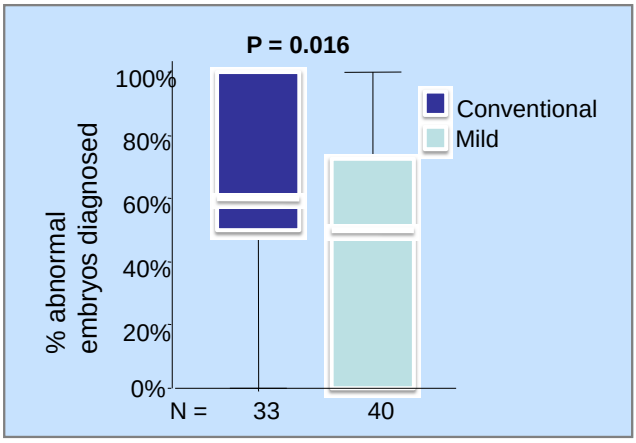


Milder ovarian stimulation for *in-vitro* fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial HR 2007

Esther B.Baart[1,2,6], Elena Martini[2], Marinus J.Eijkemans[3], Diane Van Opstal[4], Nicole G.M.Beckers[2], Arie Verhoeff[5], Nicolas S.Macklon[1] and Bart C.J.M.Fauser[1,2]

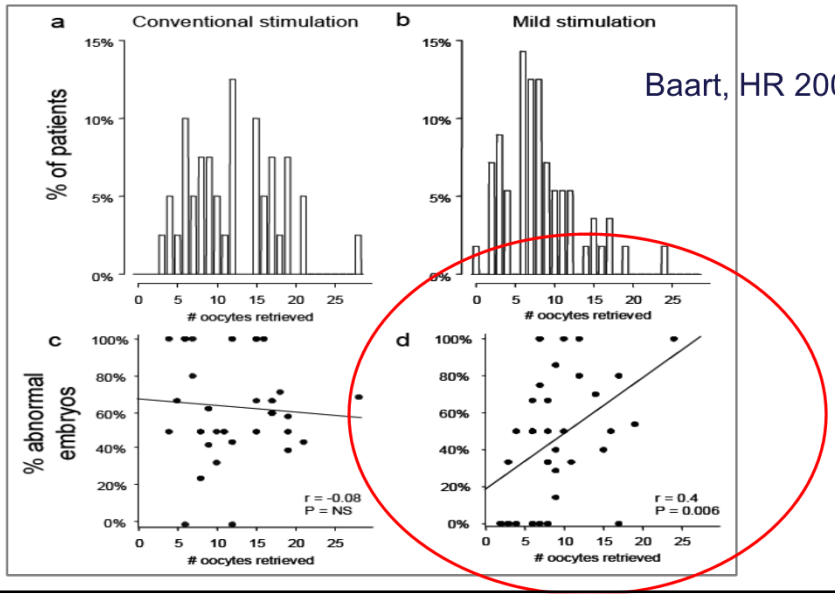


Embryo aneuploidy for mild vs conventional stimulation



Correlation between oocyte number and embryo aneuploidy

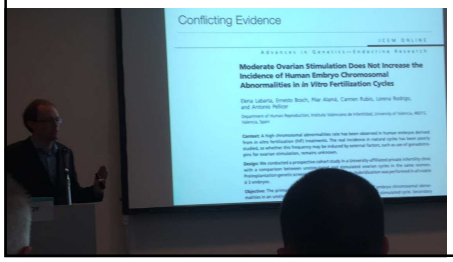
Universitair Medisch Centrum Utrecht

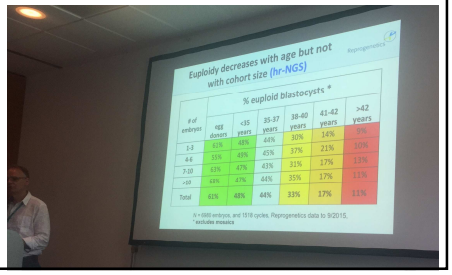


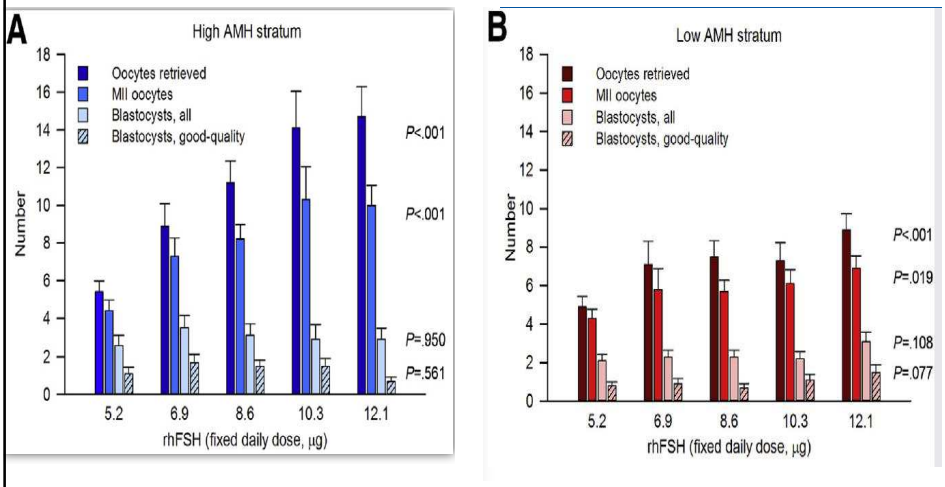
Baart, HR 2007

But

- FISH based study
  - …… and we all know how popular they are!
- Some conflicting evidence
- Difficult to measure magnitude of effect
- Contemporary tools can give a better idea of mechanisms







Arce et al 2014, Fert Ster

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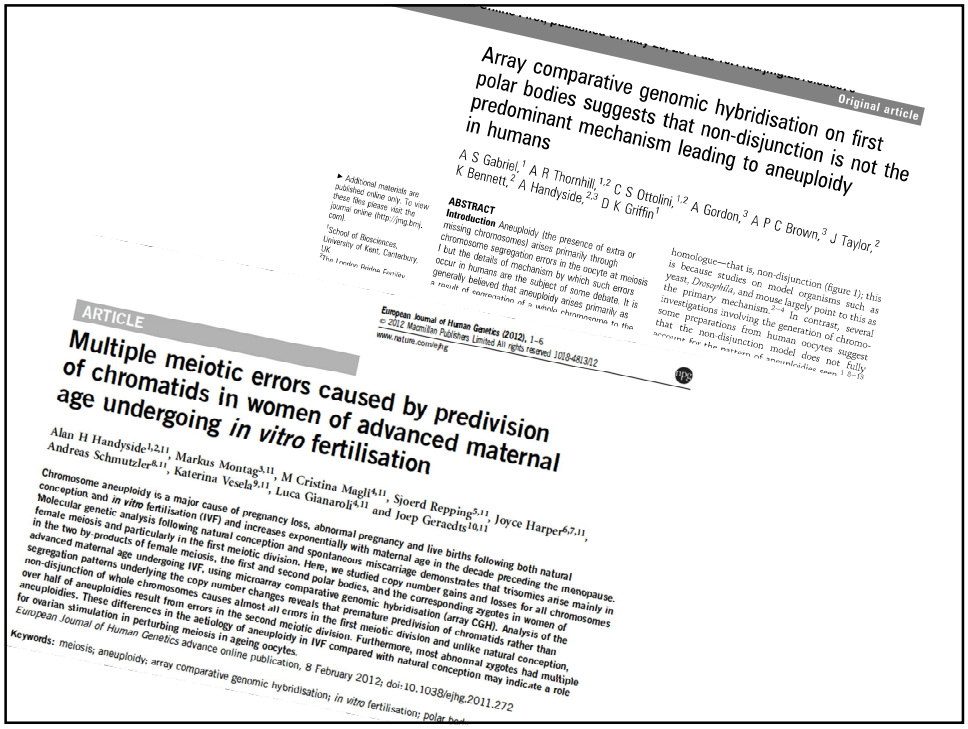

Original article

Array comparative genomic hybridisation on first polar bodies suggests that non-disjunction is not the predominant mechanism leading to aneuploidy in humans

A S Gabriel,[1] A R Thornhill,[1,2] C S Ottolini,[1,2] A Gordon,[3] A P C Brown,[3] J Taylor,[2] K Bennett,[2] A Handyside,[2,3] D K Griffin[1]

ABSTRACT

Introduction Aneuploidy (the presence of extra or missing chromosomes) arises primarily through chromosome segregation errors in the oocyte at meiosis I but the details of mechanism by which such errors occur in humans are the subject of some debate. It is generally believed that aneuploidy arises primarily as

homologue—that is, non-disjunction (figure 1); this is because studies on model organisms such as yeast, *Drosophila*, and mouse largely point to this as the primary mechanism.[2–4] In contrast, several investigations involving the generation of chromosome preparations from human oocytes suggest that the non-disjunction model does not fully

European Journal of Human Genetics (2012), 1–6
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www.nature.com/ejhg

ARTICLE

Multiple meiotic errors caused by predivision of chromatids in women of advanced maternal age undergoing *in vitro* fertilisation

Alan H Handyside[1,2,11], Markus Montag[3,11], M Cristina Magli[4,11], Sjoerd Repping[5,11], Joyce Harper[6,7,11], Andreas Schmutzler[8,11], Katerina Vesela[9,11], Luca Gianaroli[4,11] and Joep Geraedts[10,11]

Chromosome aneuploidy is a major cause of pregnancy loss, abnormal pregnancy and live births following both natural conception and *in vitro* fertilisation (IVF) and increases exponentially with maternal age in the decade preceding the menopause. Molecular genetic analysis following natural conception and spontaneous miscarriage demonstrates that trisomies arise mainly in female meiosis and particularly in the first meiotic division. Here, we studied copy number gains and losses for all chromosomes in the two by-products of female meiosis, the first and second polar bodies, and the corresponding zygotes in women of advanced maternal age undergoing IVF, using microarray comparative genomic hybridisation (array CGH). Analysis of the segregation patterns underlying the copy number changes reveals that premature predivision of chromatids rather than non-disjunction of whole chromosomes causes almost all errors in the first meiotic division. Furthermore, most abnormal zygotes had multiple over half of aneuploidies result from errors in the second meiotic division. Unlike natural conception, aneuploidies. These differences in the aetiology of aneuploidy in IVF compared with natural conception may indicate a role for ovarian stimulation in perturbing meiosis in ageing oocytes.

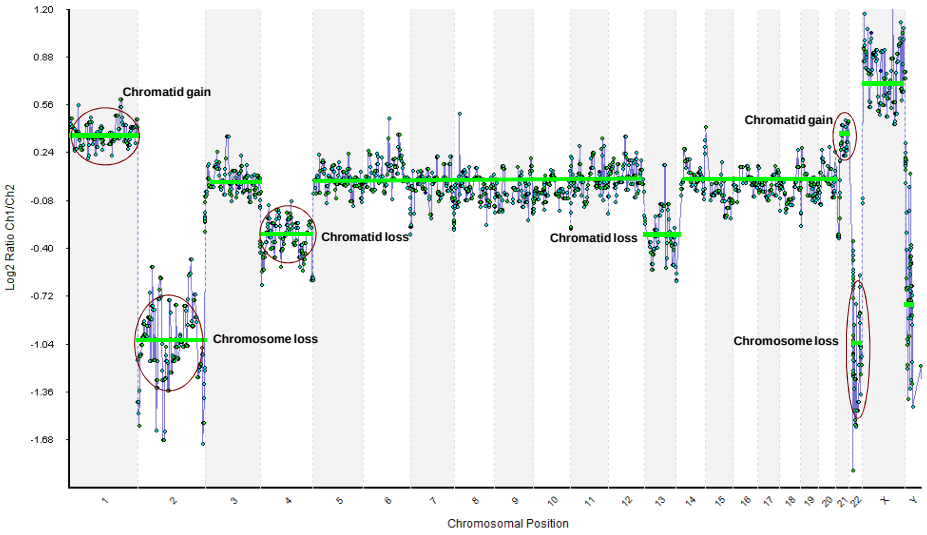
European Journal of Human Genetics advance online publication, 8 February 2012; doi:10.1038/ejhg.2011.272

Keywords: meiosis; aneuploidy; array comparative genomic hybridisation; *in vitro* fertilisation; polar bod

Screening polar bodies by aCGH

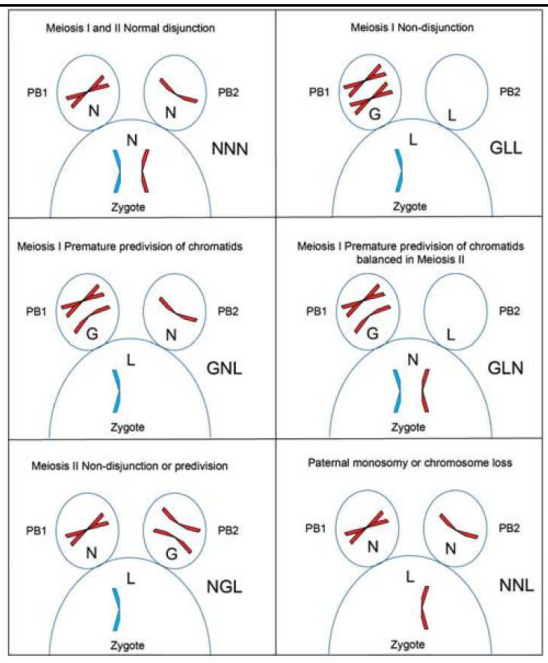
- Looking at PB1 only
  - Gabriel et al. 2011
  - Meiosis I only
- Looking at PB1, PB2 and blastomere
  - Handyside et al. 2012
  - Meiosis I and II
- Unencumbered by cytogenetic preparation/FISH errors

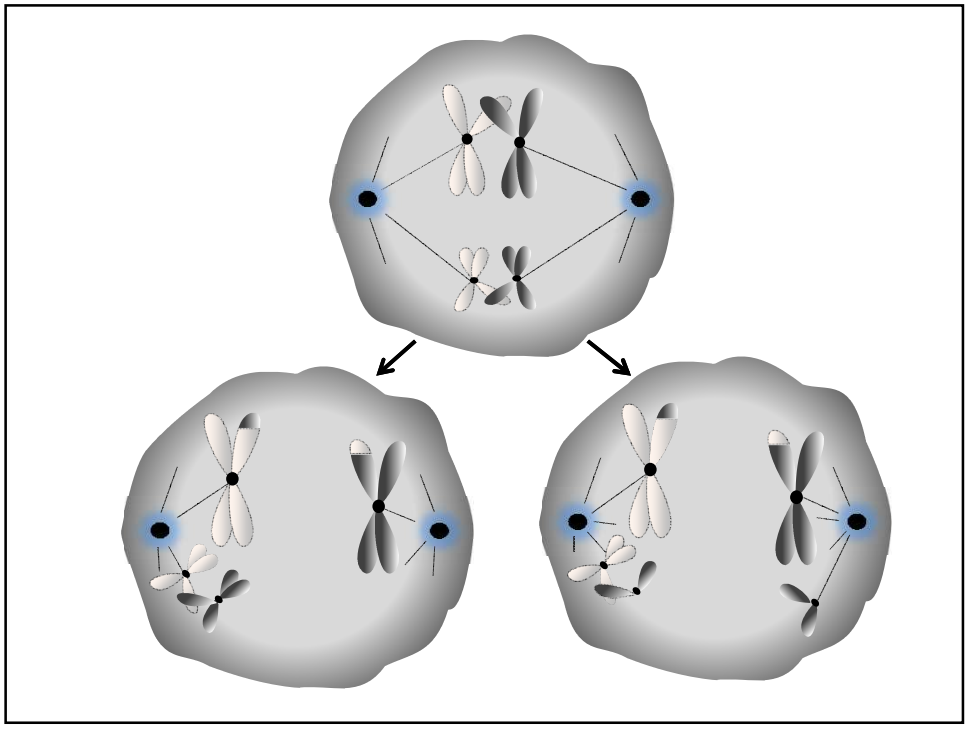
Gabriel et al 2011

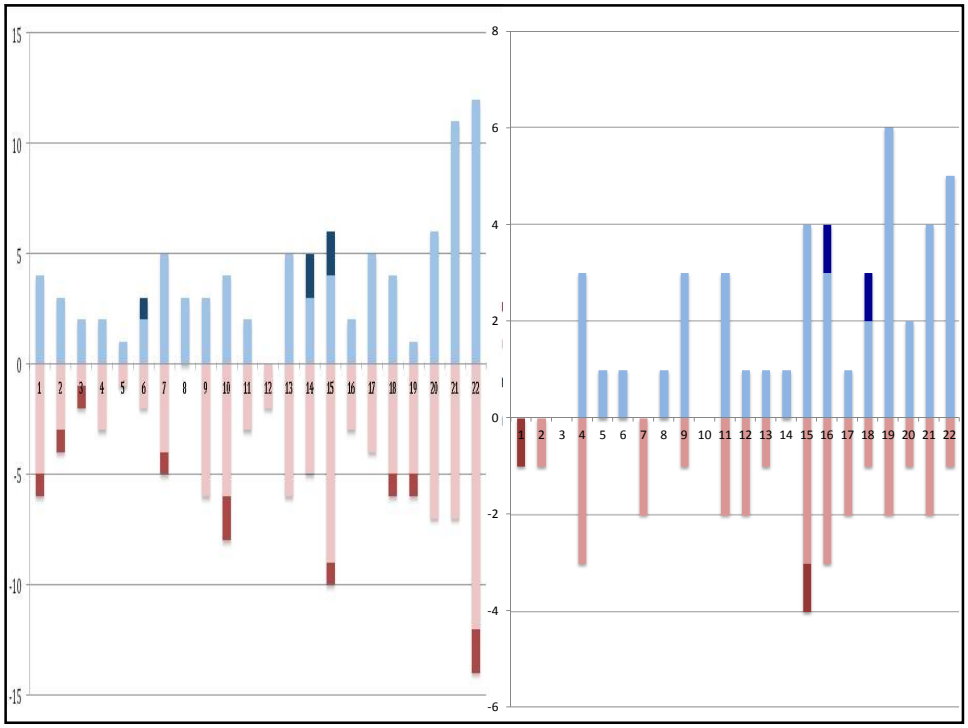


Handyside et al 2012

Segregation patterns in PB1 and PB2 and the corresponding zygote

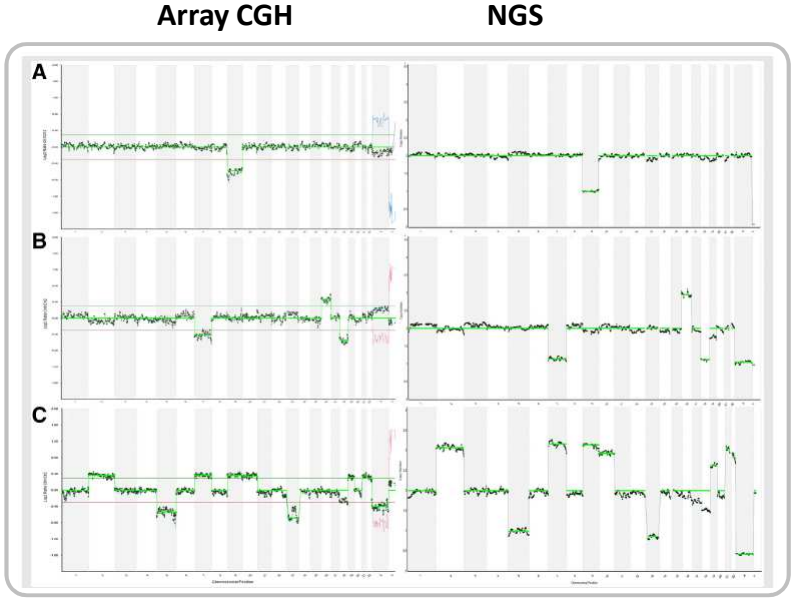





15
10
5
0
-5
-10
-15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
8
6
4
2
0
-2
-4
-6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Findings

- Gabriel et al. 2011
  - Precocious separation about 11x more likely than classical non-disjunction
  - Smaller chromosomes more prone to error
  - Clear maternal age effect
- Handyside et al. 2012
  - Precocious separation about 20x more likely than classical non-disjunction
  - Smaller chromosomes more prone to error
  - Clear maternal age effect

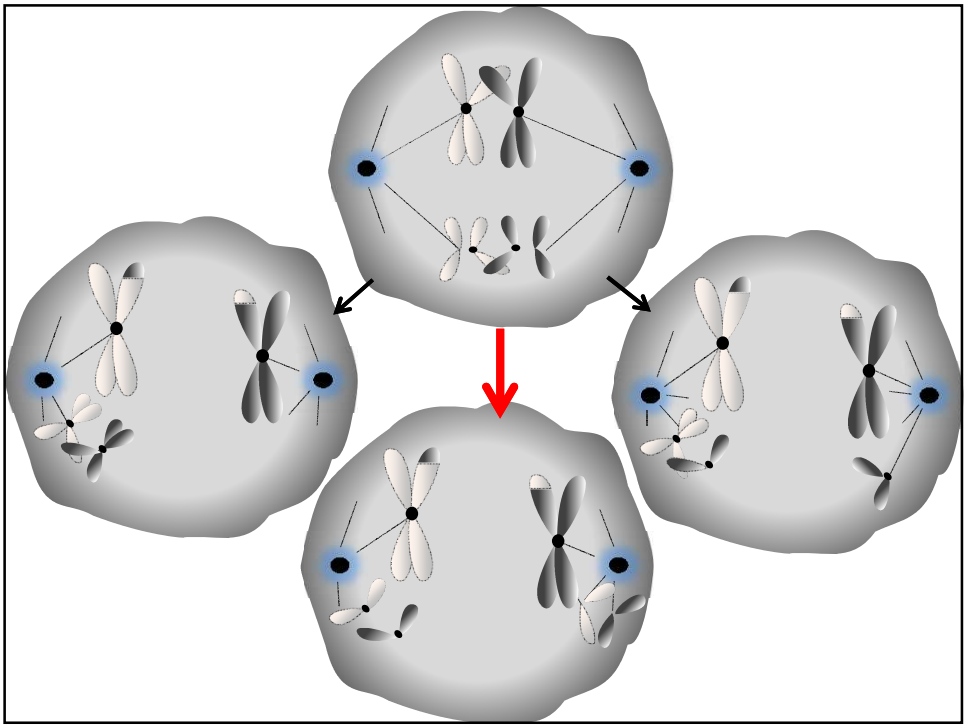


Analysis of polar bodies and oocytes by Karyomapping

- Ottolini et al 2015, Nature Genetics
- 27 chromosome segregation errors
  - Verified by array CGH
  - 11 PSSC (7 of which led to an aneuploidy oocyte)
  - 4 meiosis II errors
  - 0 classical meiosis I errors
  - 11 were a unique pattern of segregation error reminiscent of "inverted meiosis"
    - REVERSE SEGREGATION
  - Patterns of recombination

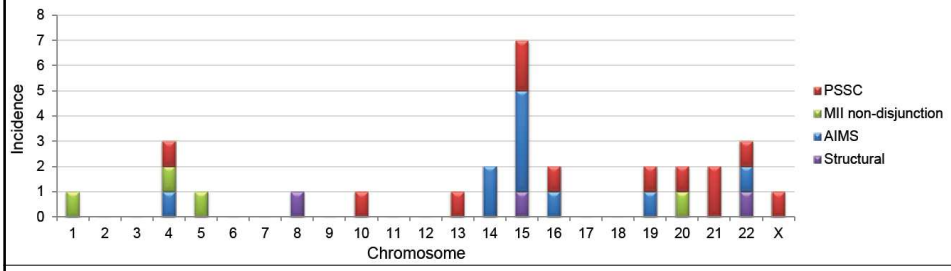

nature genetics

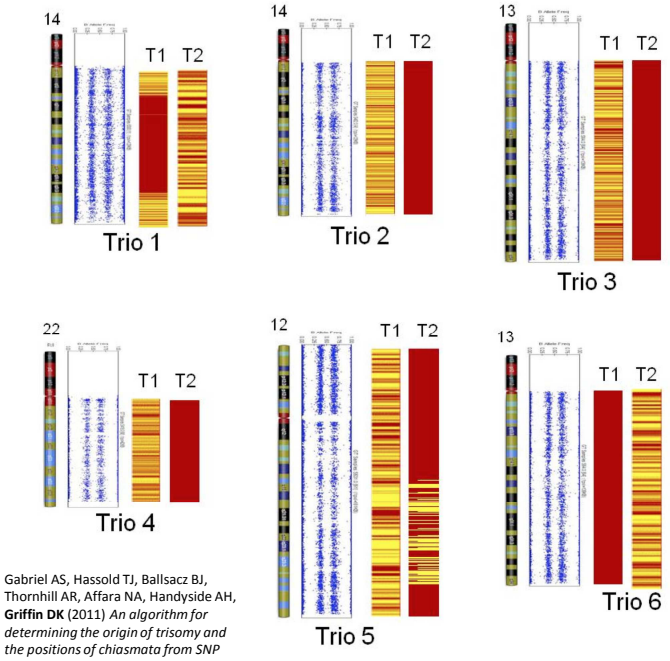
Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates



Reverse Segregation

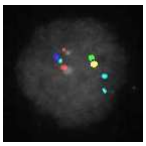
- Both homologues undergo precocious separation of sister chromatids in meioisis I (cf meiosis II)
- Led to **balanced** gametes in 9/11 cases



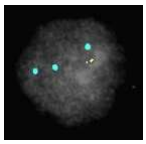


Gabriel AS, Hassold TJ, Ballsacz BJ, Thornhill AR, Affara NA, Handyside AH, **Griffin DK** (2011) *An algorithm for determining the origin of trisomy and the positions of chiasmata from SNP genotype data* **Chromosome Research** 19:155-63

"Karyomapping case" for PGS

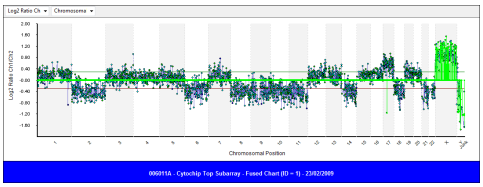


13, 16, 18, 21, 22
Monosomy 18, 21, 22

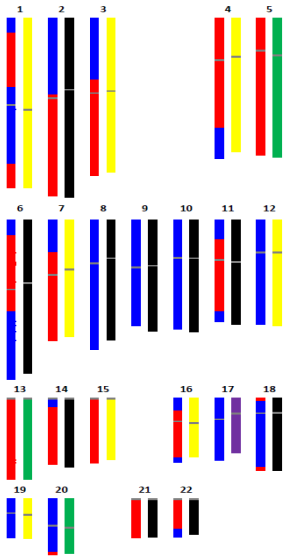


X, Y, 21
Trisomy X
Monosomy 21

Array CGH

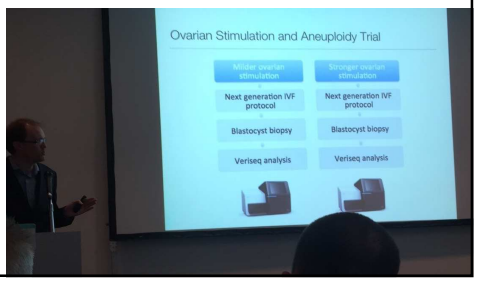


Karyomap



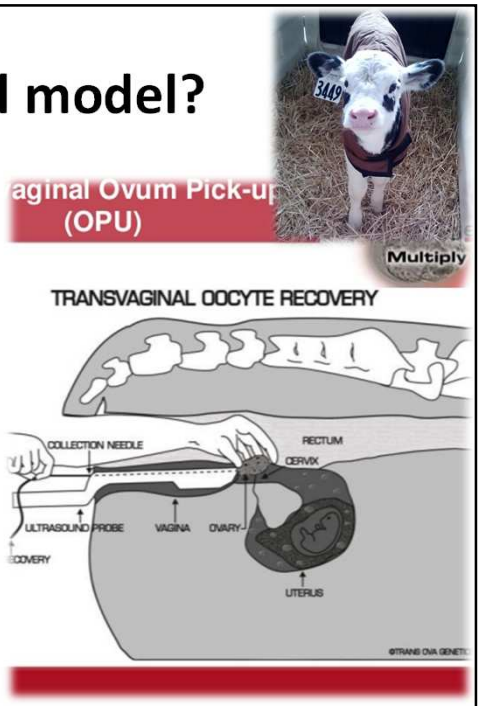
What can we do about it?

- Analysis of first polar bodies
  - a la Gabriel et al 2011
  - CGH, NGS and/or karyomapping
- Could analyze second polar body also
  - a la Handyside et al 2012
  - Induce to resume meiosis with calcium ionophore
    - Capalbo et al 2015
- Embryo analysis
  - Determine meiotic vs post-zygotic errors
    - Array CGH of NGS
    - Karyomapping
- Mild vs traditional stimulation
  - Retrospective
  - Prospective
  - Prospective randomized
- BUT WE DO NEED TO DO IT



Is there an animal model?

- Yes!
  - Cattle
- For food production reasons cattle IVF is commonplace
- Ovum pick up (OPU) similar to egg collection in humans
- Some companies now pioneering "coasting" protocols
  - Similar in principle to mild stimulation protocols
    - *"A period of no gonadotrophin administration after initial stimulation and before inducing ovulation"*
  - Opportunity to compare same animals (or close relatives) with same protocols



Conclusions

- It's certainly biologically that hormonal treatments can affect aneuploidy
  - Endocrine disruption
- Some evidence for an effect of ovarian stimulation on aneuploidy
  - Thank you Bart
  - But conflicting evidence
- But we need to do more studies
  - It couldn't be more crucial
  - We have the tools to do it in humans
    - Stimulated vs. mild stimulation
    - NGS and karyomapping
  - Animal models exist
    - Cattle
    - Coasting protocols vs normal stimulation
    - Similar detection methods

CISoR

CENTRE FOR INTERDISCIPLINARY STUDIES OF REPRODUCTION

University of Kent