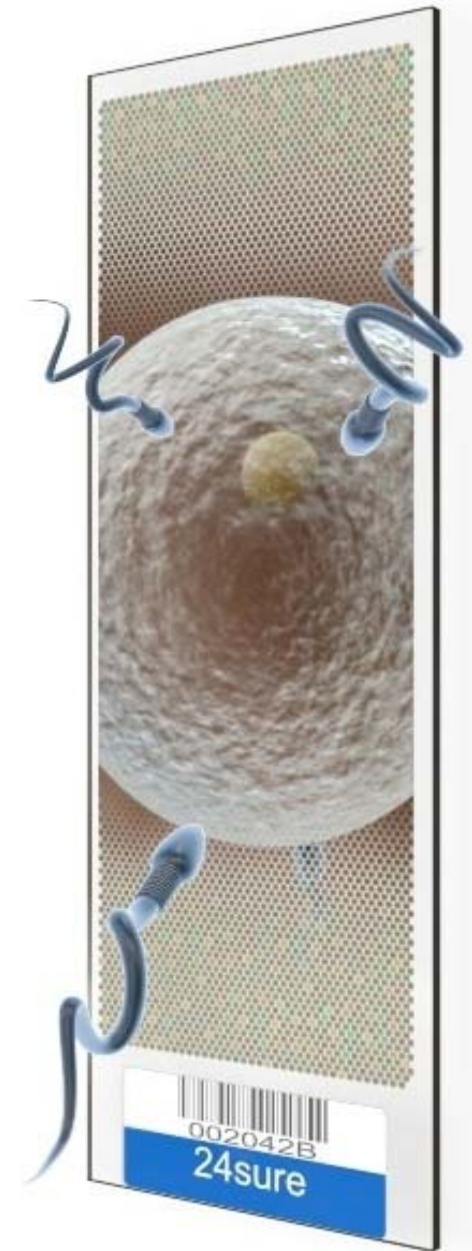


Pollegemebiopsi @ OUS - Rikshospitalet

Mette Haug Stensen

Gareth Greggains



Polar Body Biopsy

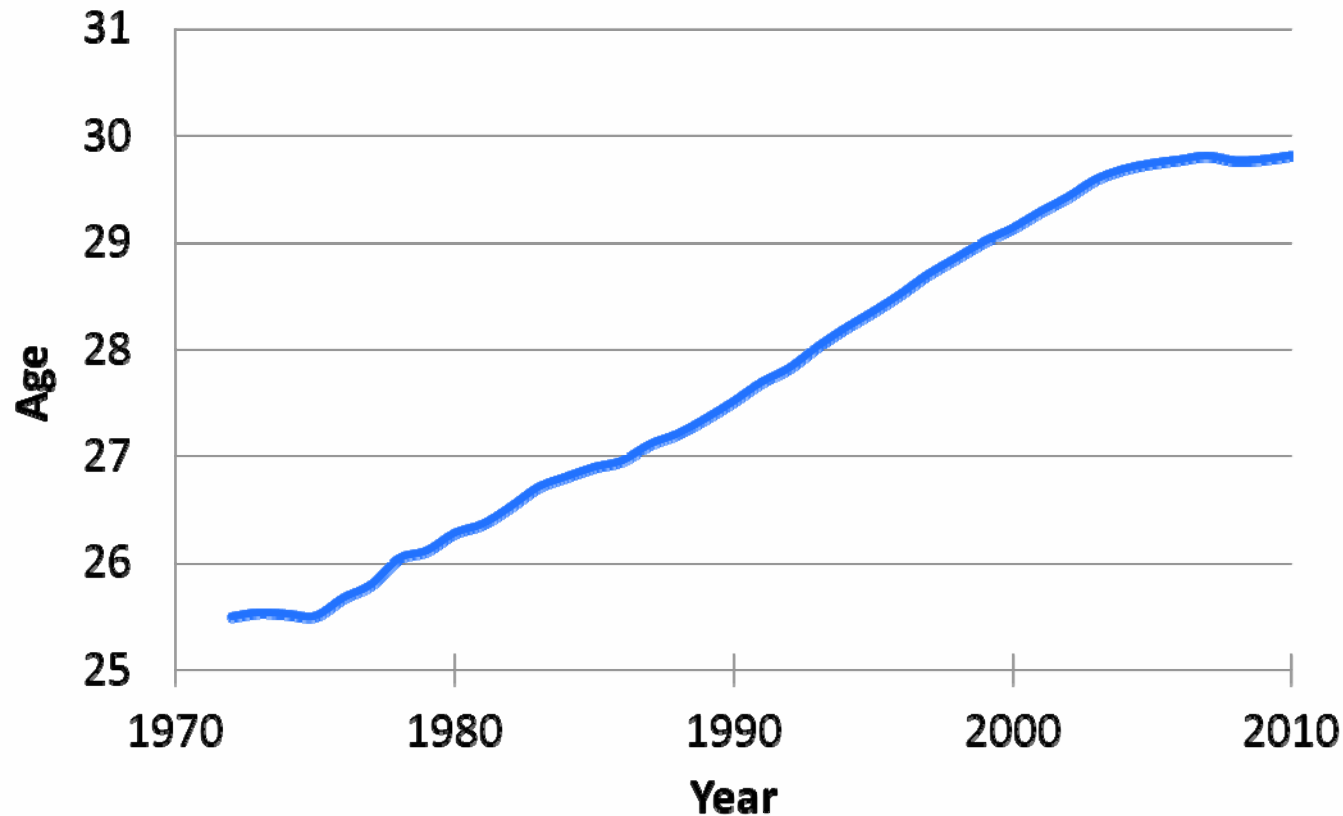
Part 1

- Age-related female infertility?
- What is a polar body?
- Why are we interested in polar bodies?

Part 2

- How we do polar body biopsy?
- What can a polar body tell us about the egg?

Age of Women at Childbirth

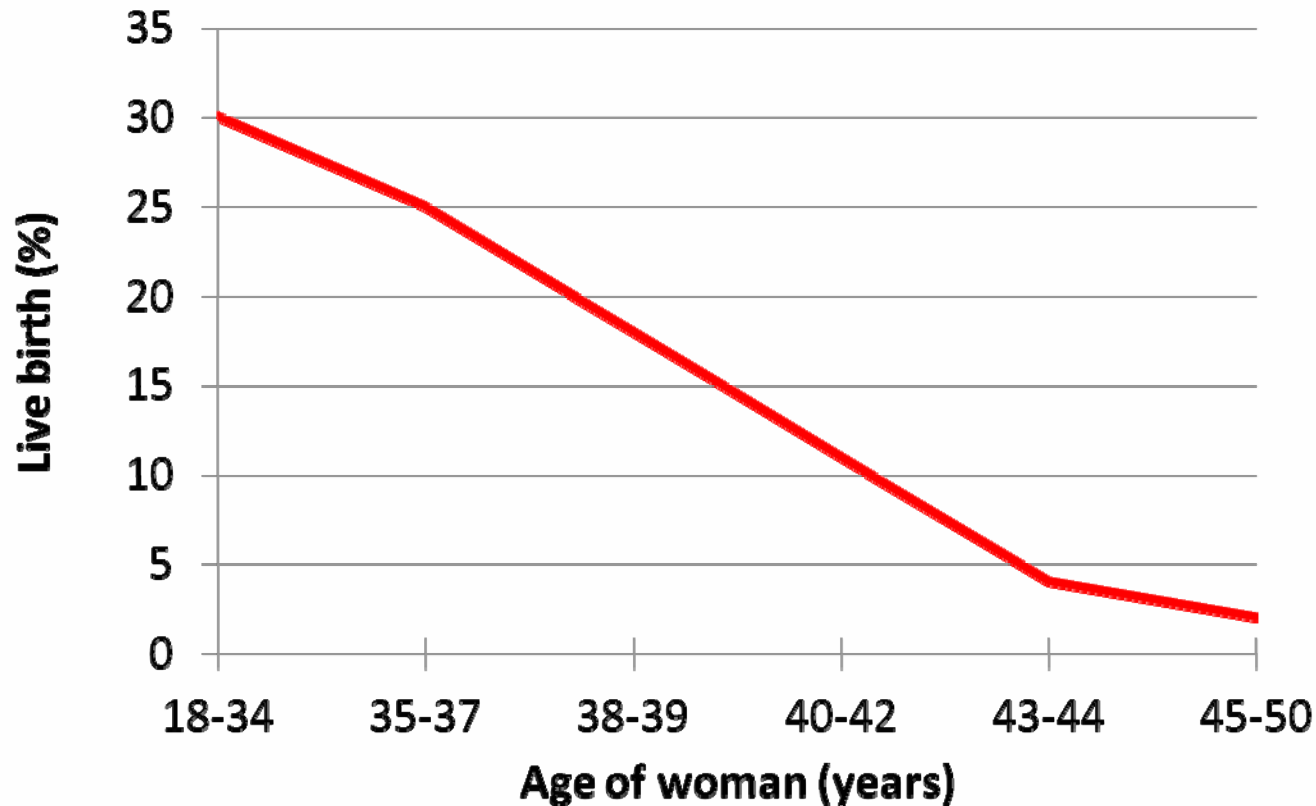


- Average age of woman at childbirth has increased
- 20% of women in Norway give birth over age 35

Source: Norwegian birth register



Female Age and Fertility

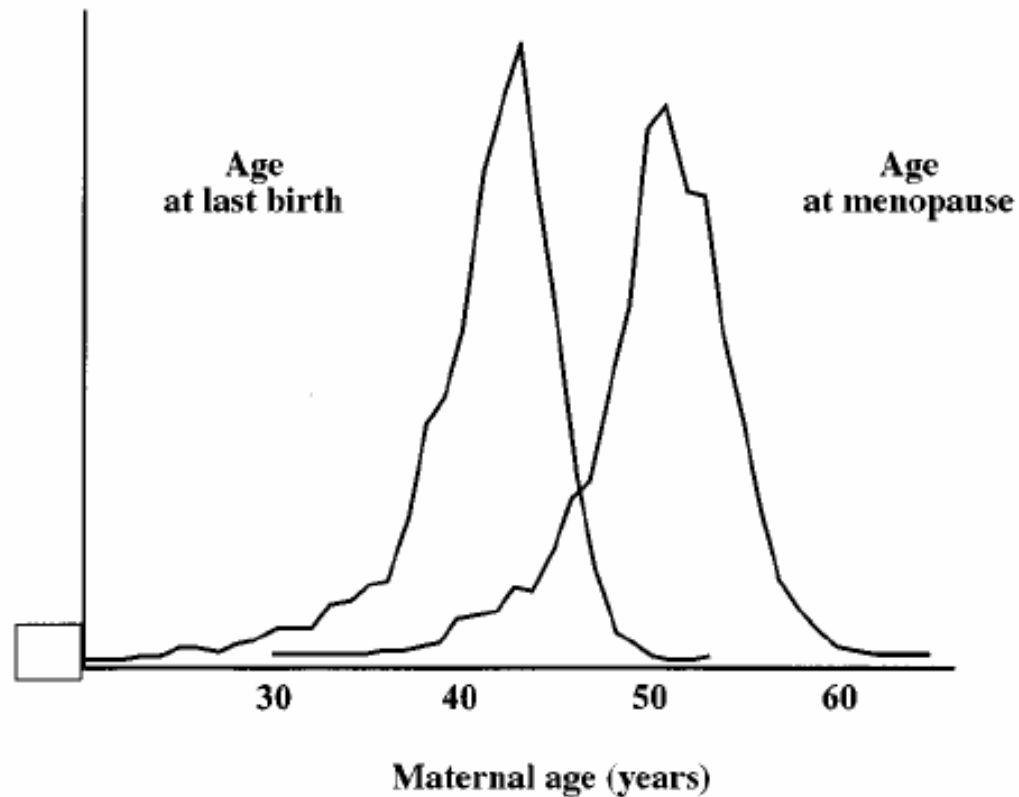


- IVF success rate declines with female age
- Live birth rate falls from age 35 to menopause

Source: HFEA UK (2004-2008)

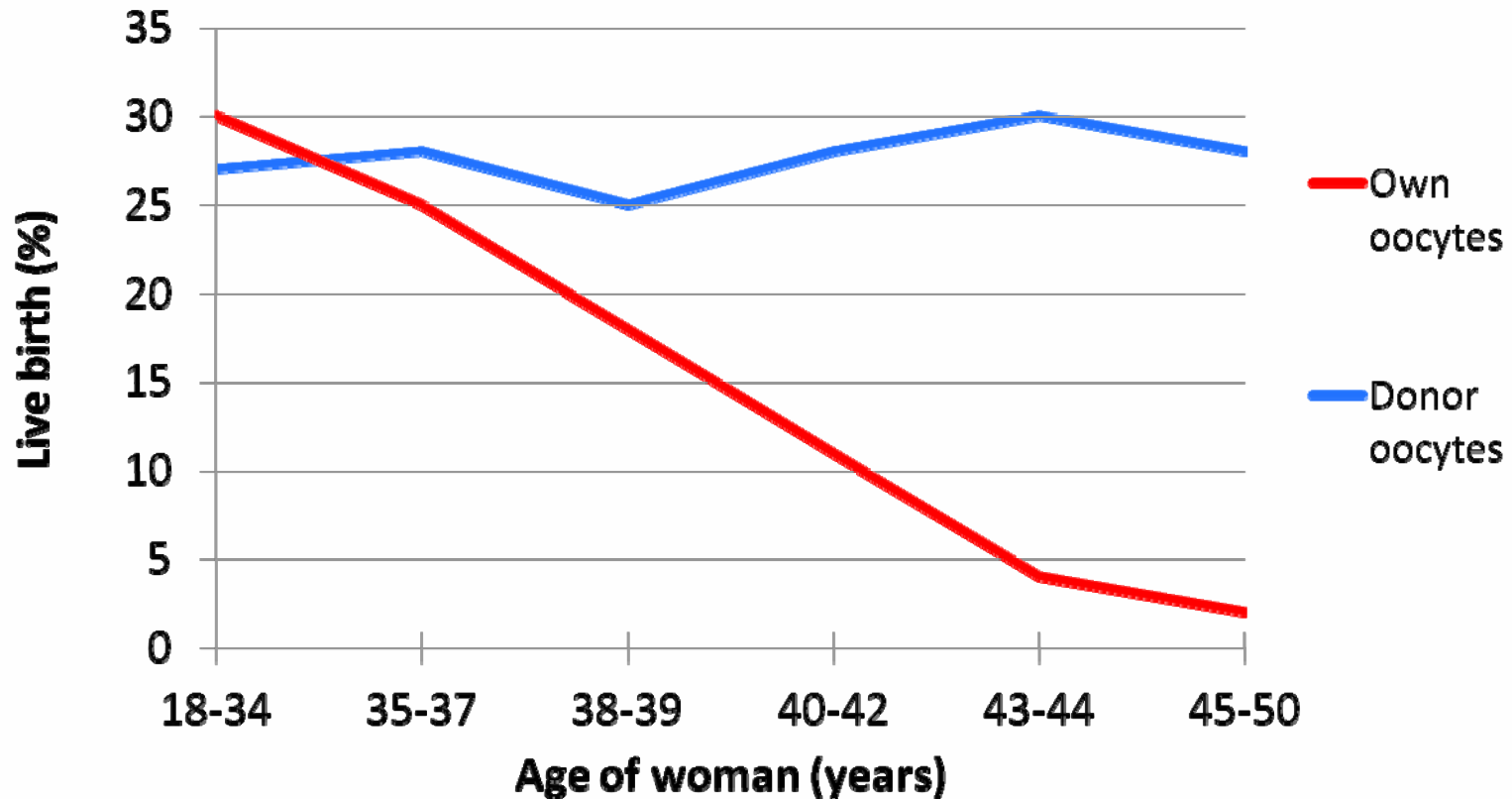


Fertility and Menopause



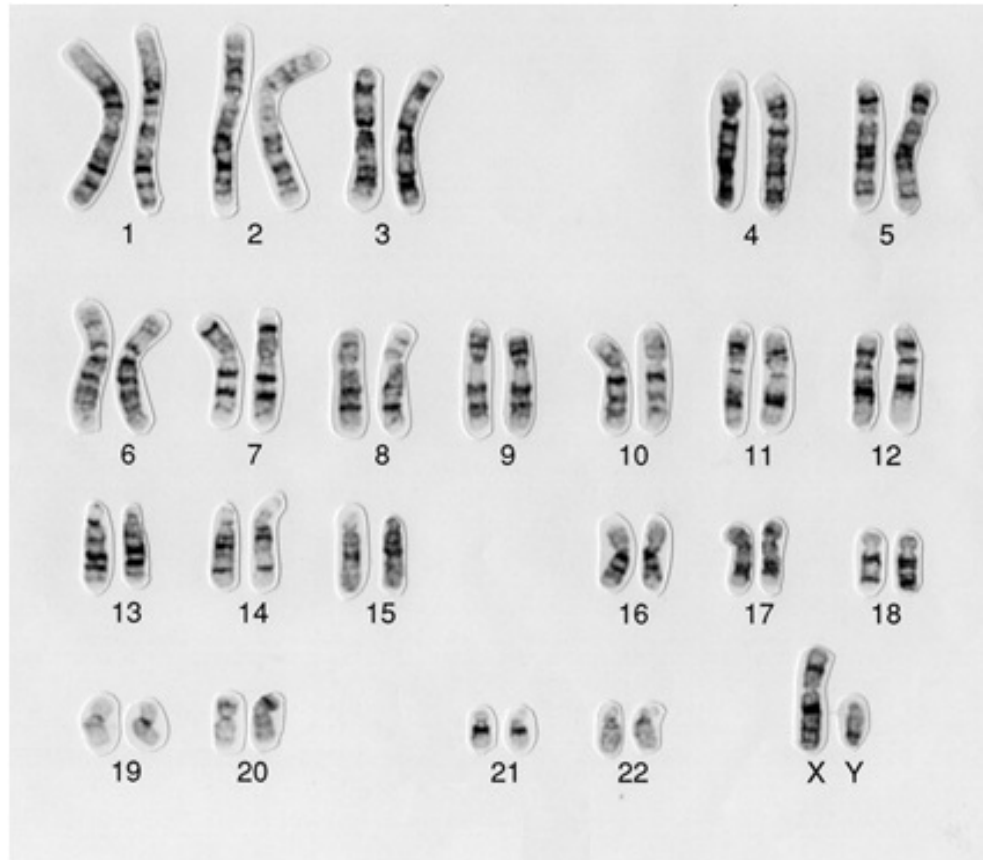
- Functional loss of fertility occurs ~10 years before menopause.

Source of fertility decline



- Oocyte is the source of age-related decline in fertility

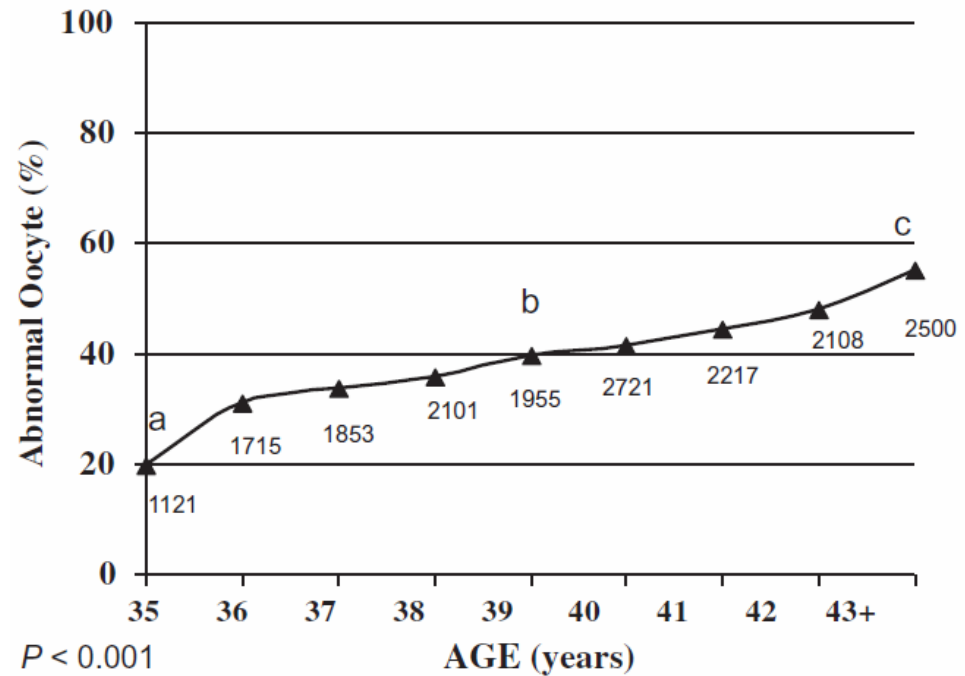
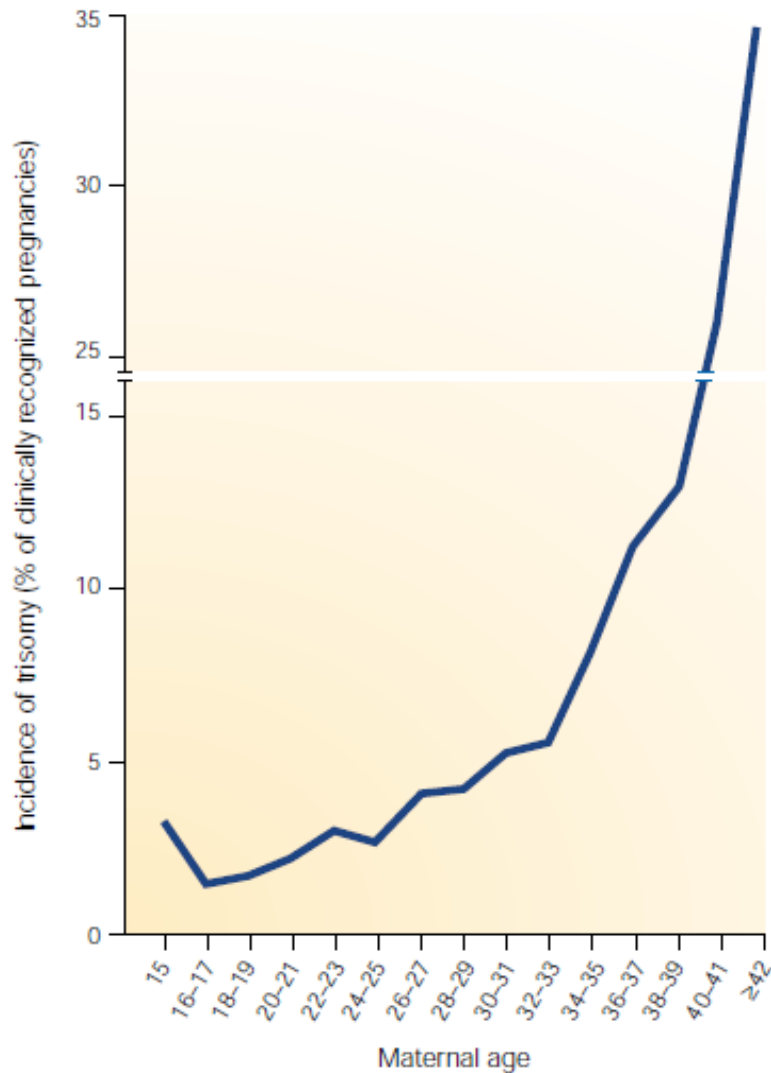
The Role of Egg and Sperm



- Humans have 23 pairs of chromosomes
- One inherited from mother (egg) and father (sperm)



Chromosome Abnormality And Age

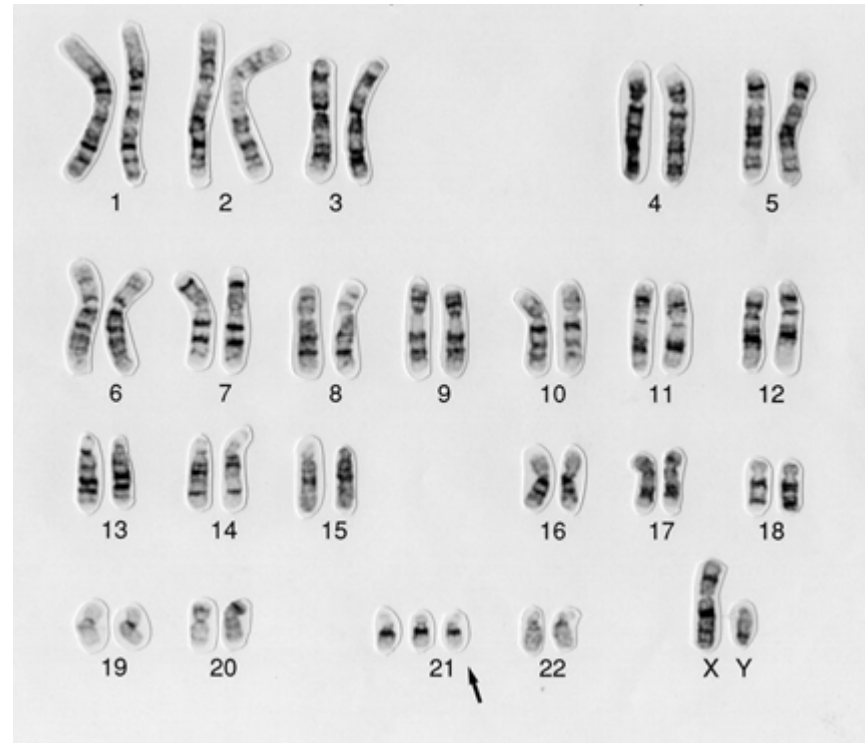


- Increase in chromosome abnormalities come from oocytes

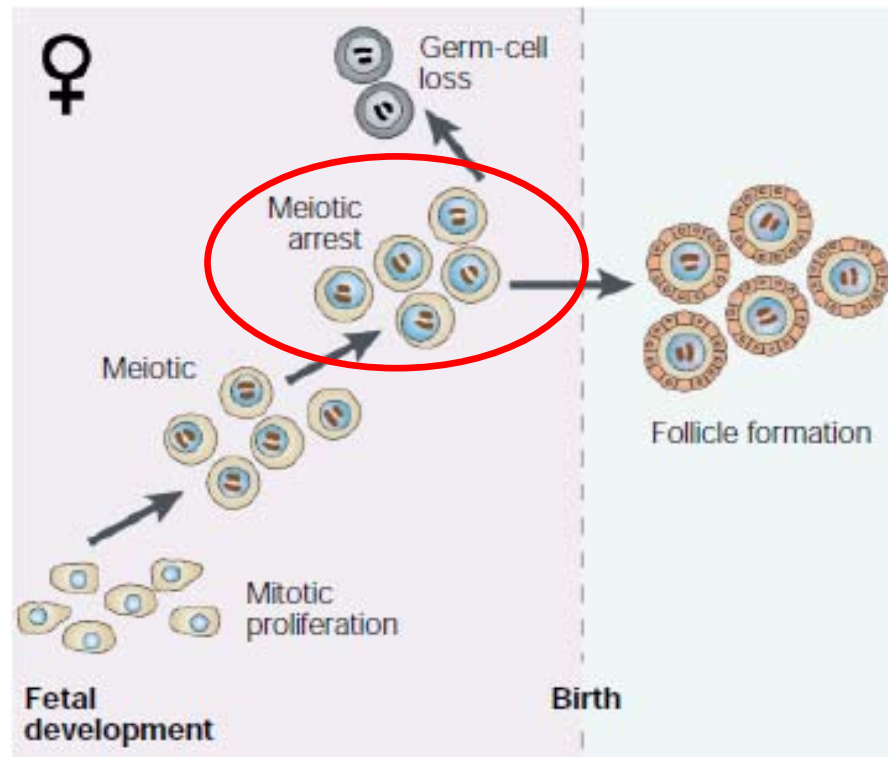
1. Hassold and Hunt (2001), Nat Rev Genet 2, 280-91
2. Kuliev et al. (2010).RBM Online 22, 8-2.

Chromosome Abnormality

- Three copies of a chromosome (2 egg, 1 sperm)
 - Chr 21: Down syndrome
 - Chr 18: Edwards
 - Chr 13: Patau
 - Sex chromosomes
XXX, XXY, XYY
 - Chr 1: no development
 - Others: spontaneous abortion (varying times)
- One copy of a chromosome (0 egg, 1 sperm)
 - usually results in embryo loss



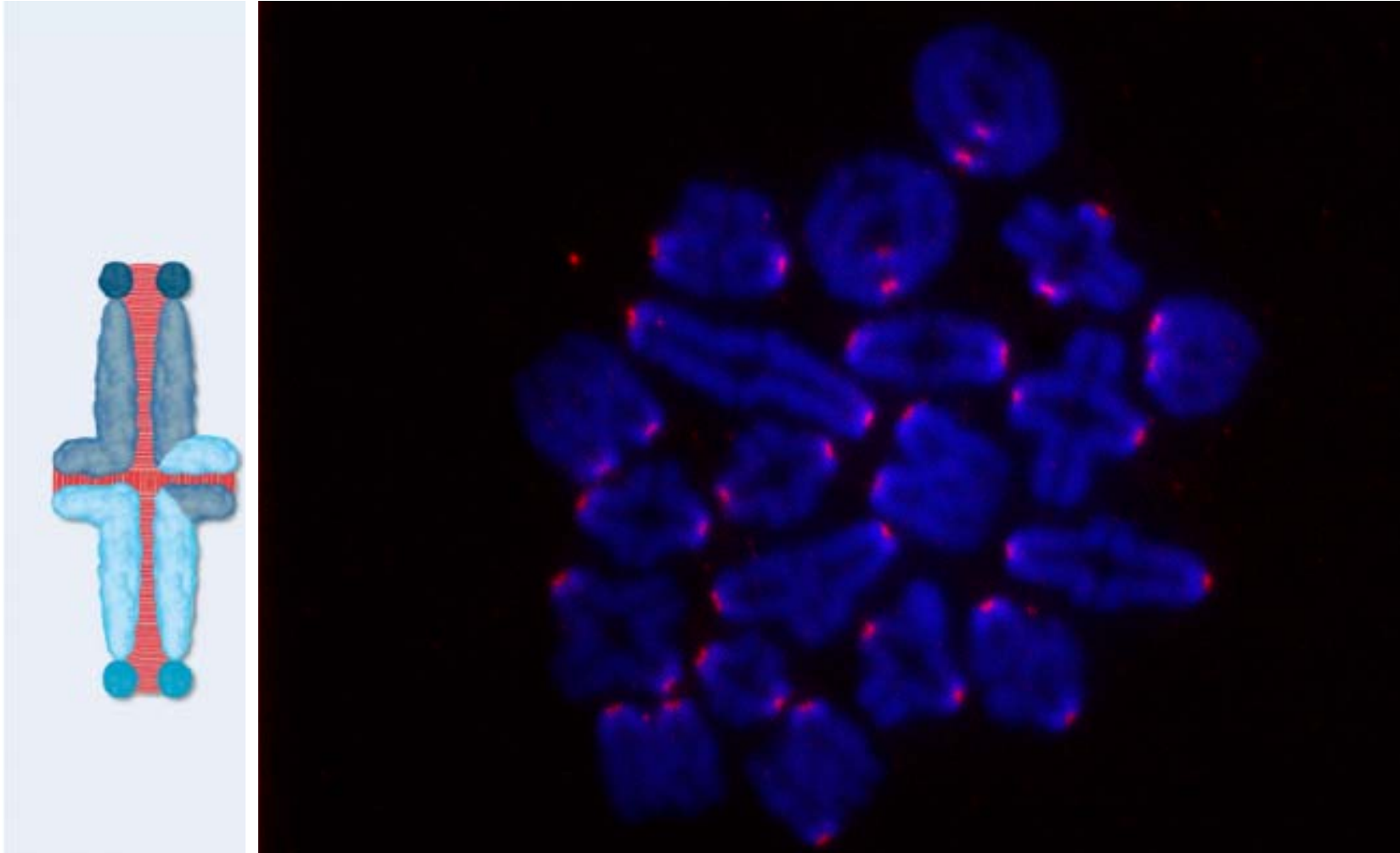
The Making of an Egg



- Journey starts early during foetal development
- Cells enter meiosis: special form of cell division that creates eggs and sperm – become oocytes – and wait
- Follicles form around oocytes

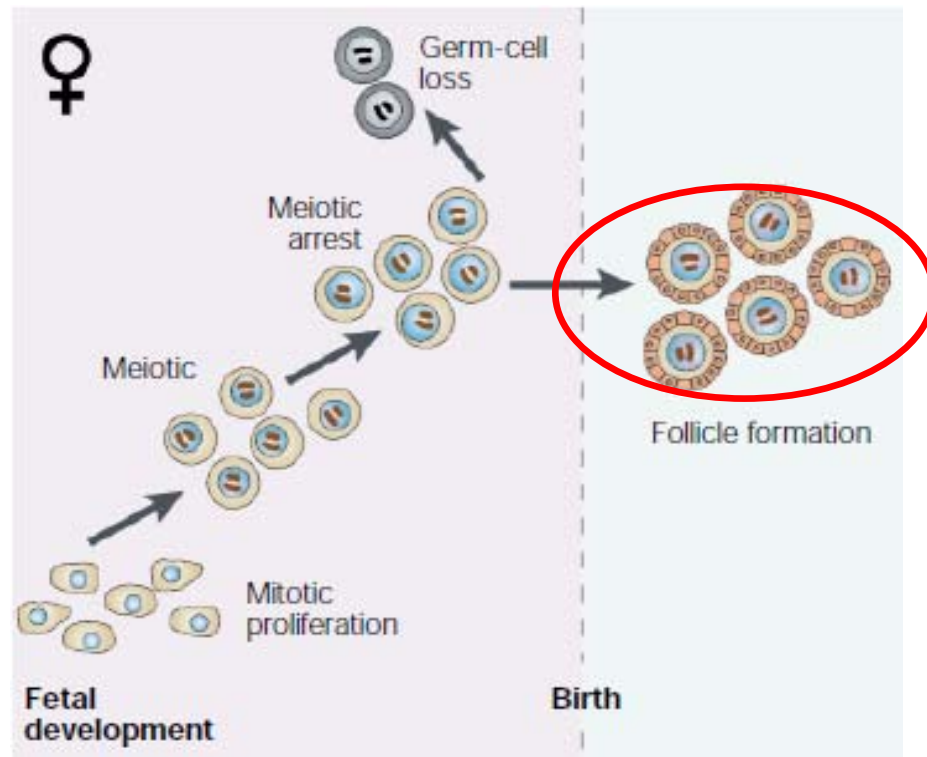
Hassold and Hunt (2001), Nat Rev Genet 2, 280-91

Chromosomes in Meiosis



Hunt and Hassold (2010). *Curr Biol* 20, 699-702
Greggains/Herbert, unpublished

The Making of an Egg

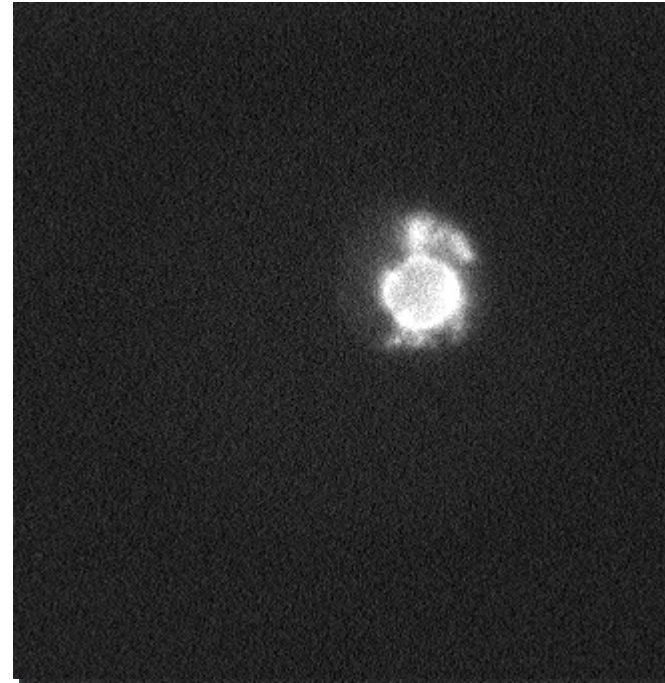


- Wait for a hormonal signal to prepare for fertilisation
- Can wait for up to 50 years before ovulation occurs
- At ovulation the meiotic cell begins dividing

Meiosis I: Ovulation



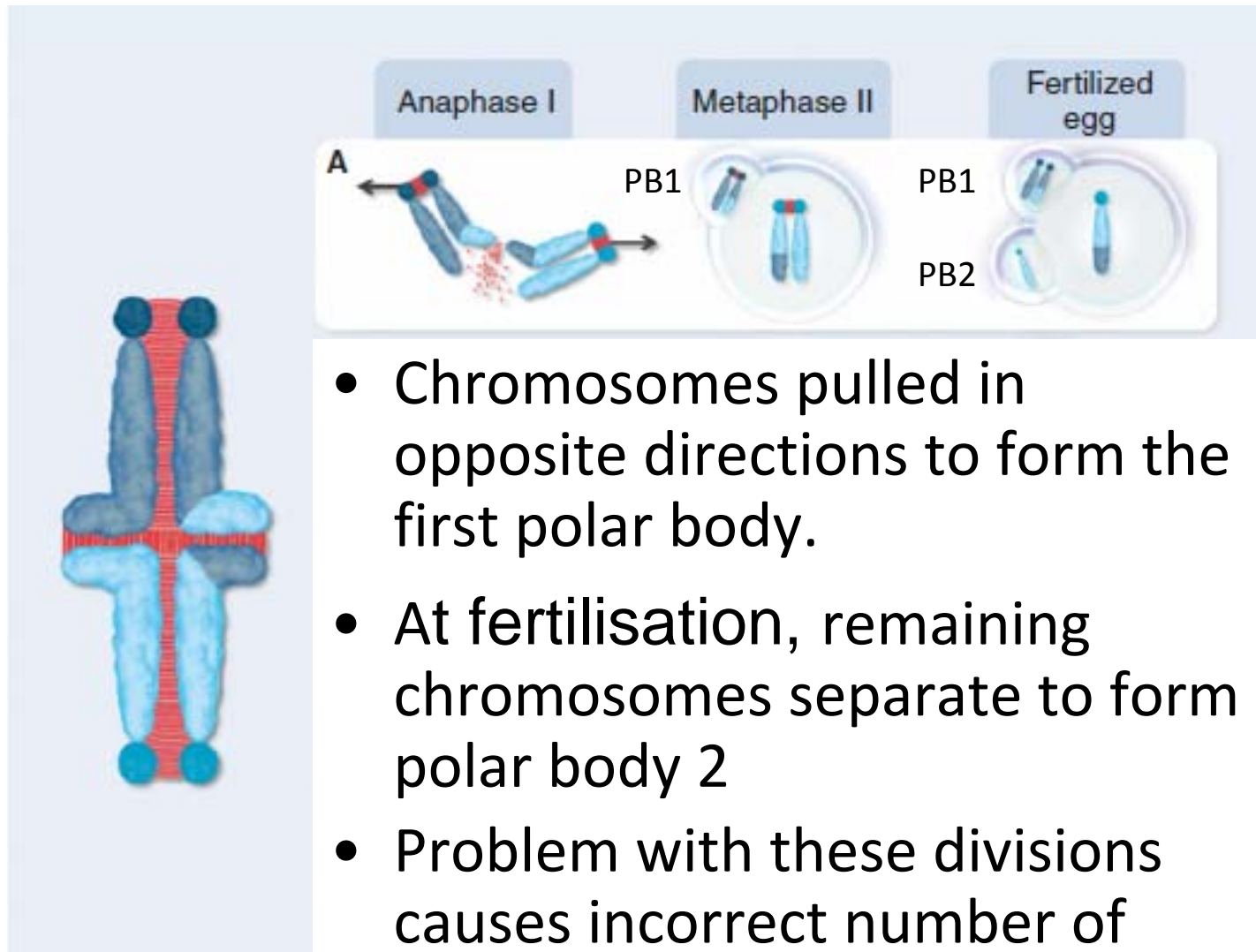
Mouse egg



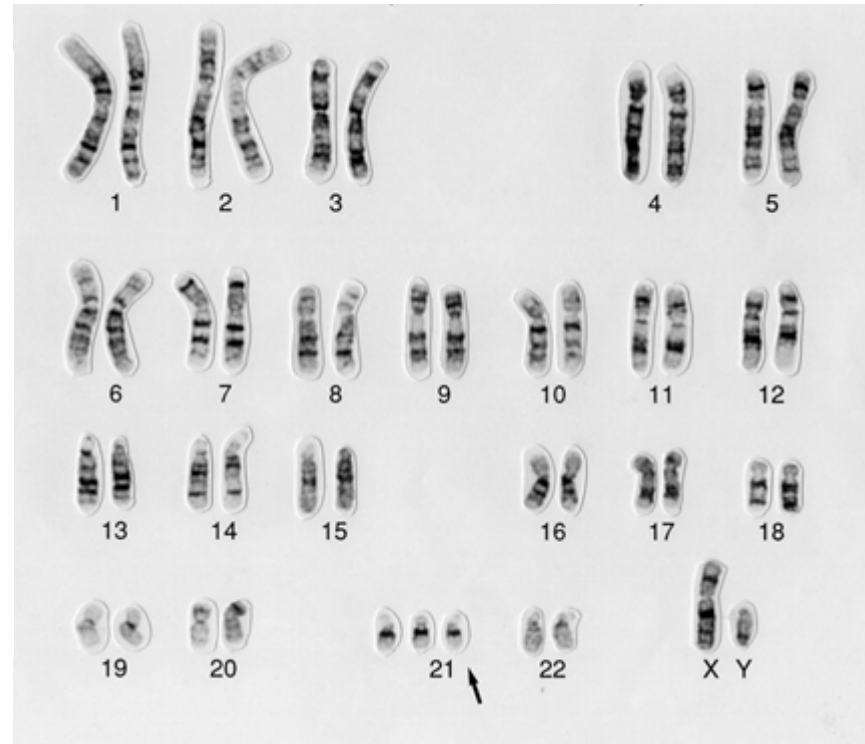
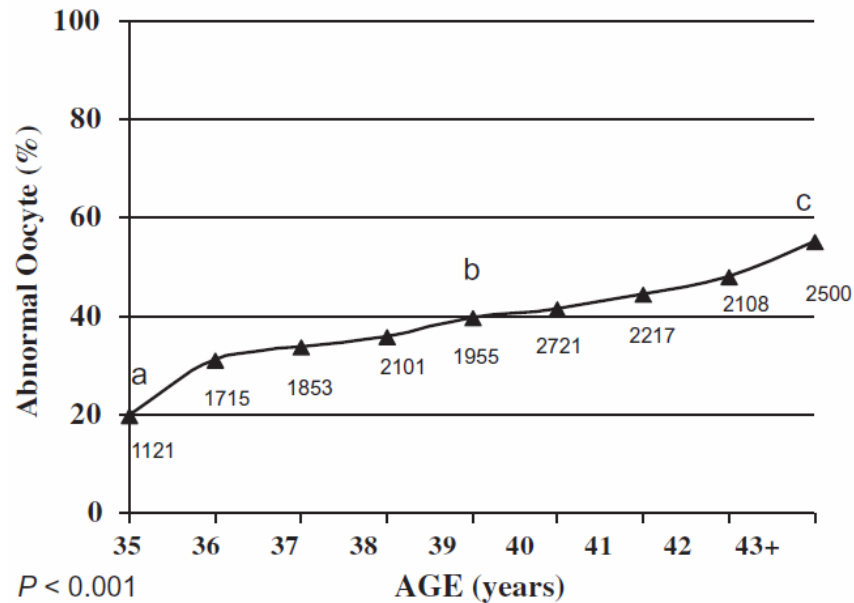
Chromosomes

- 1st polar body contains half of the chromosomes

Chromosomes and Polar Bodies



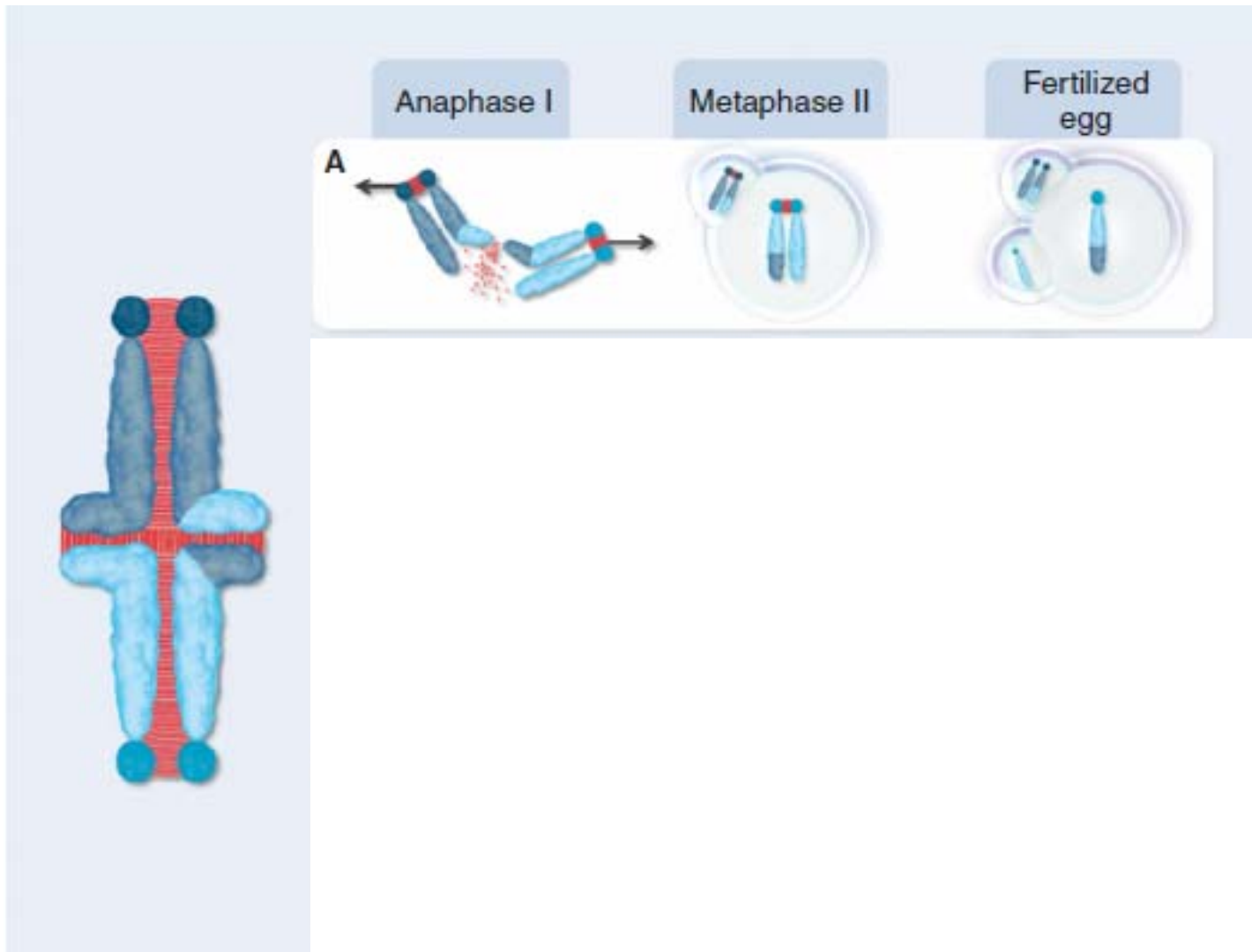
Chromosome Abnormality



- Problems with divisions increase with woman's age
- Incorrect divisions results in chromosome abnormalities
- Embryo loss, spontaneous abortions, developmental defects

1. Kuliev et al. (2010).RBM Online 22, 8-2.
2. Wellcome Collection (<http://www.wellcomecollection.org>)

Errors and Polar Bodies



- Polar bodies show what is left in the oocyte

Summary

- Age of women at childbirth has increased
- Fertility declines with age
- First and second divisions of meiosis are more likely to fail in eggs of older women
- Results in eggs with incorrect numbers of chromosomes
- Causes embryo loss, spontaneous abortion, and developmental defects
- But...We can check eggs for chromosome defects by looking at the polar bodies!

Erfaringer i Europa og Amerika

- Preimplantasjons genetisk screening (PGS) introdusert i 1993 (Munne *et al.*, 1993)
- 10 randomiserte studier: PGS ved fluorescent *in situ* hybridisering (FISH) øker ikke fødselsraten
- ASRM, BFS og ESHRE: PGS øker ikke fødselsraten til pasienter med gjentatte mislykkede IVF – forsøk, gjentatte spontanaborter eller økt kvinnelig alder
- ESHRE PGS Task Force 2008: Array – teknologi for analyse av 1. og 2.pollegeme

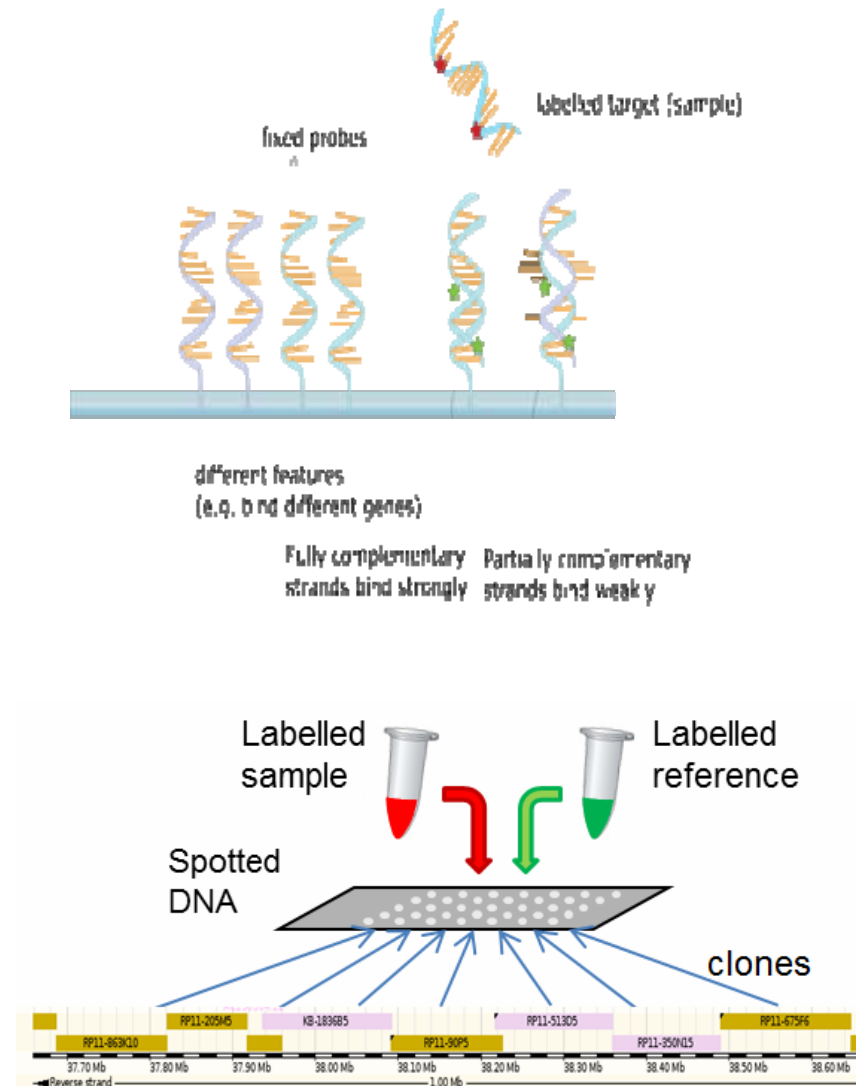


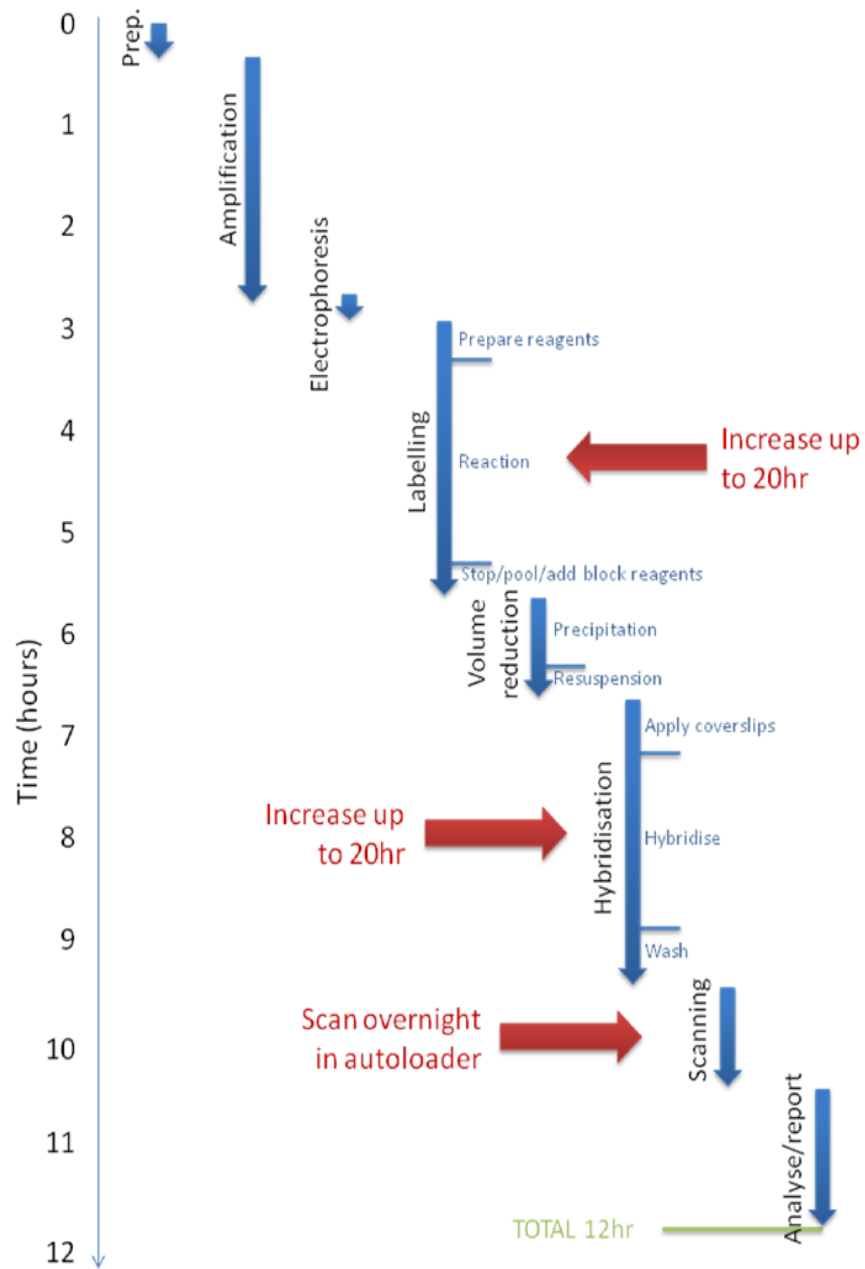
Pollegemebiopsi og array - teknologi

- Metode for å kunne kartlegge aneuploidier i oocytten – alle 23 kromosomer
- Må kunne analyseres på bakgrunn av èn enkelt celle (1.og evt. 2 pollegeme)
- Kort tidsaspekt (12 – 13 t)
- Riktig resultat i minst 90% av forsøkene

Array CGH

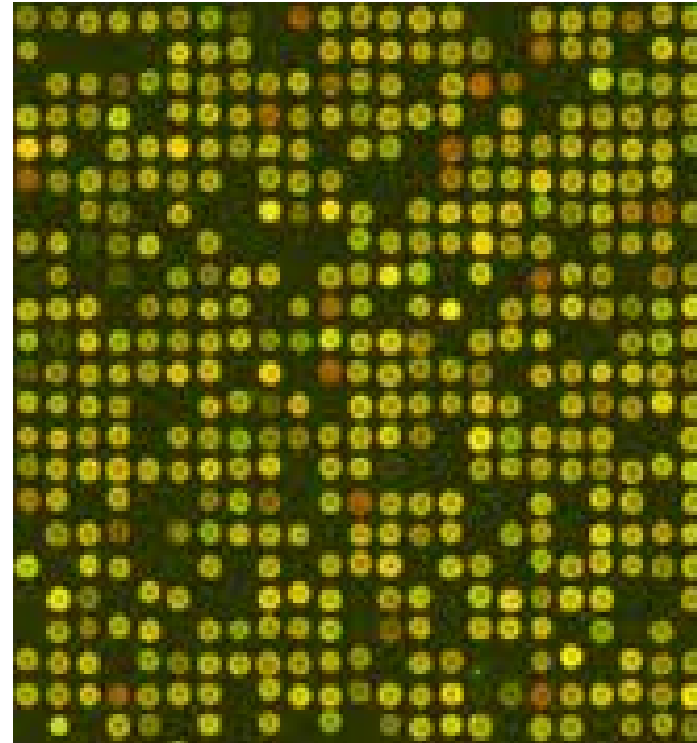
- Array comparative genomic hybridization (CGH)
- DNA ekstraktert fra en prøve amplifiseres og farges med en rød fluoreserende probe og en referanse DNA merkes med grønn farge.
- Begge hybridiseres med kloner fra en normal kontroll på overflaten av en glassplate (slide)
- Komplementære tråder bindes sterkt





Scanning og analyse

- Etter scanning: Rødt signal = ekstra genetisk materiale i prøven, grønt signal = tap av genetisk materiale i prøven, gult signal = samme som referansen, dvs normal
- Software



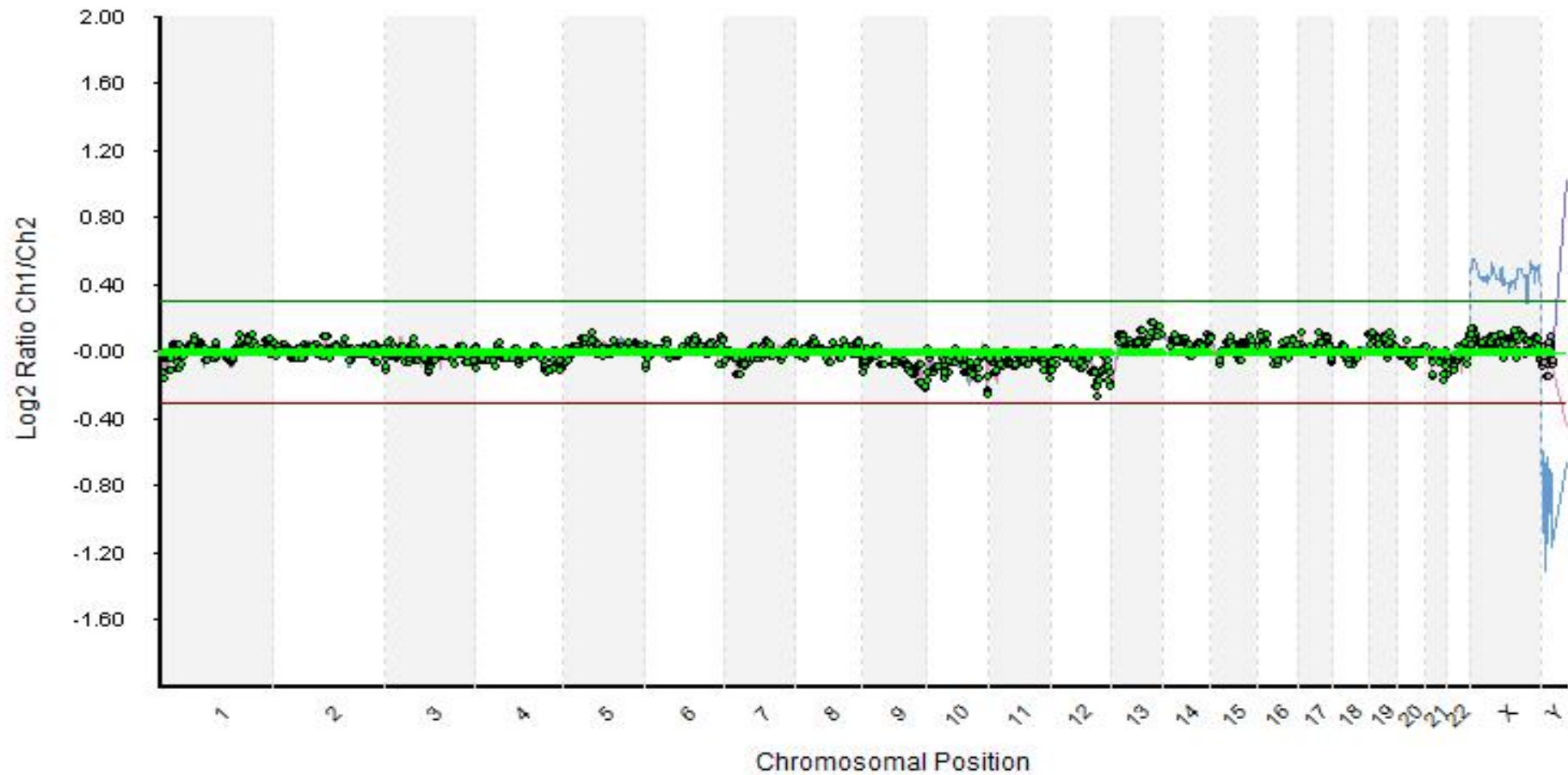
Pollegemebiopsi @ Rikshospitalet



Pollegemebiopsi - video



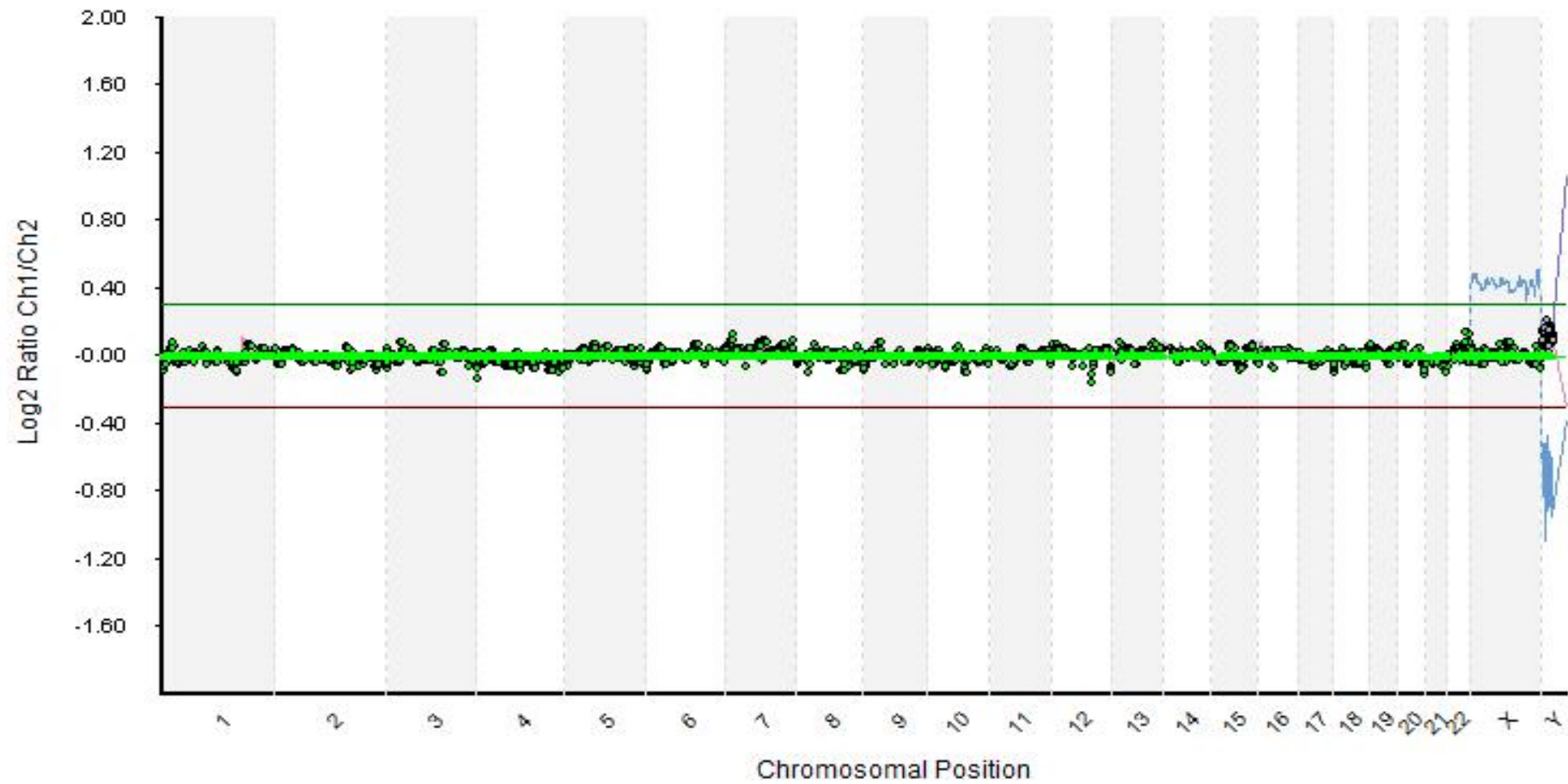
Eksempel 1: 1.pollegemet



Normalt – euploid
1.pollegeme



Eksempel 1: oocytten

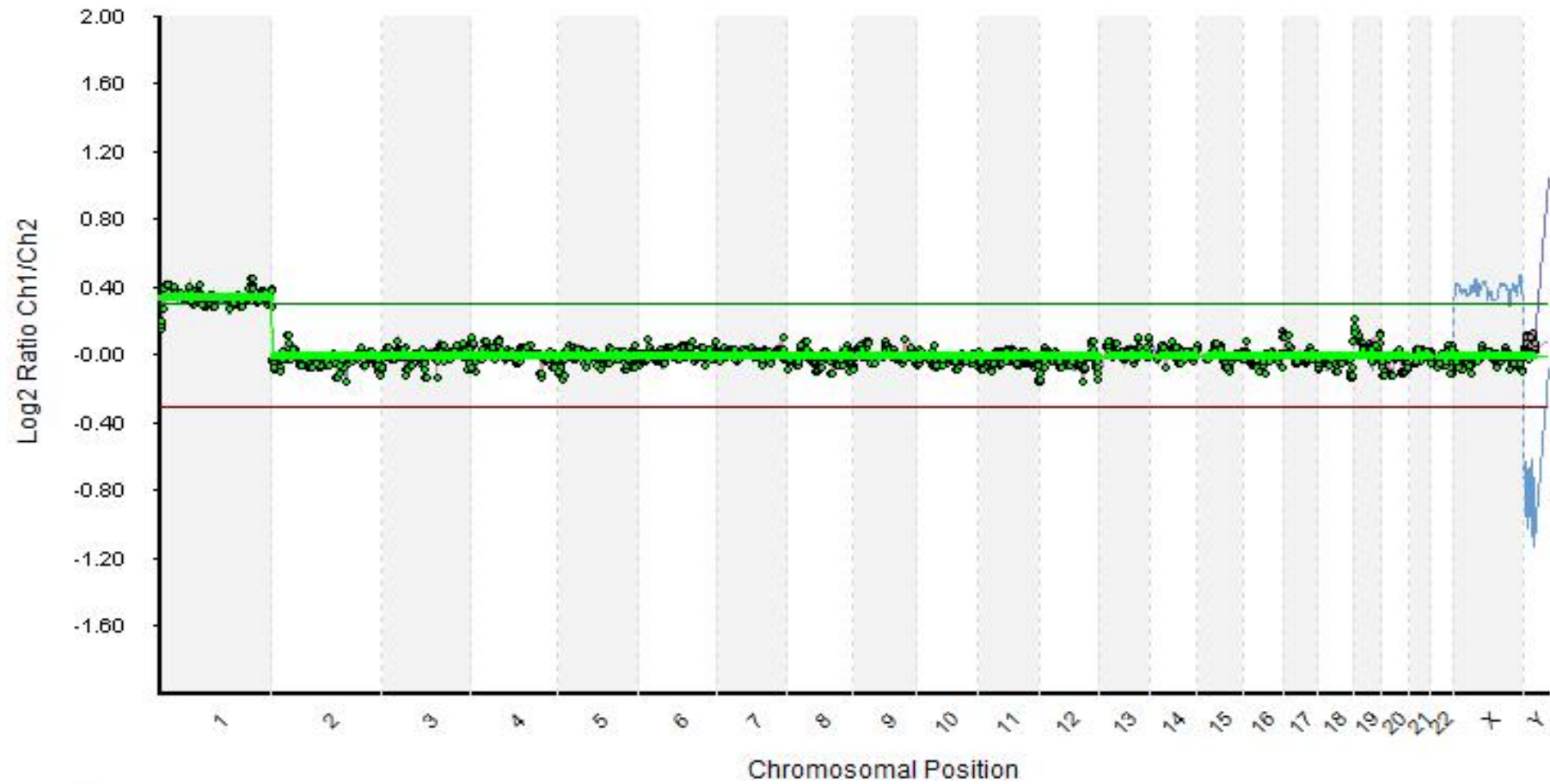


Normal - euploid oocyt

Konklusjon: Normal



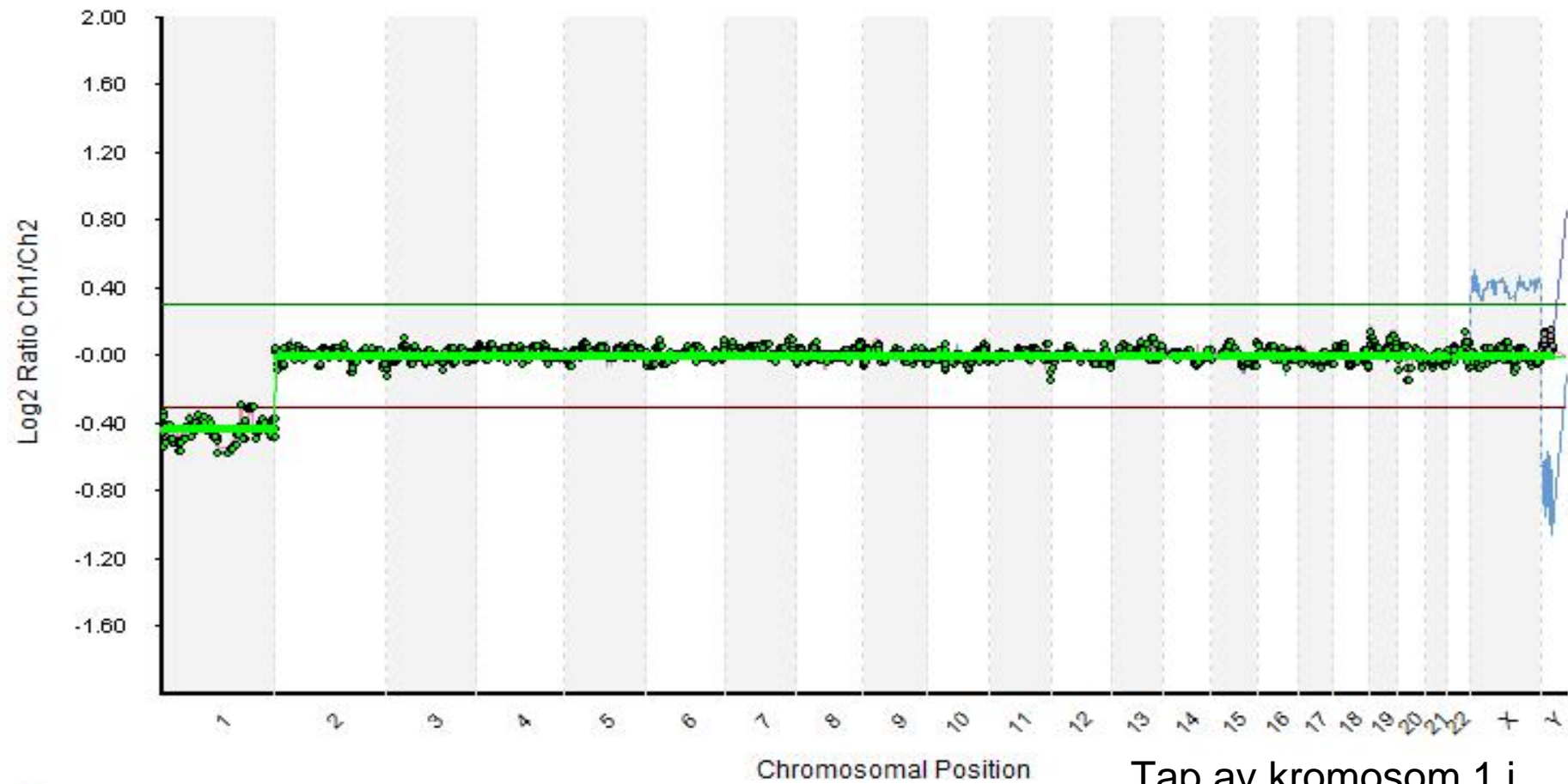
Eksempel 2: 1.pollegeme



Ekstra kromosom 1 i PB



Eksempel 2: oocytten



Tap av kromosom 1 i oocytten

Konklusjon: Missegresjon av Kromosom 1 i meiose I

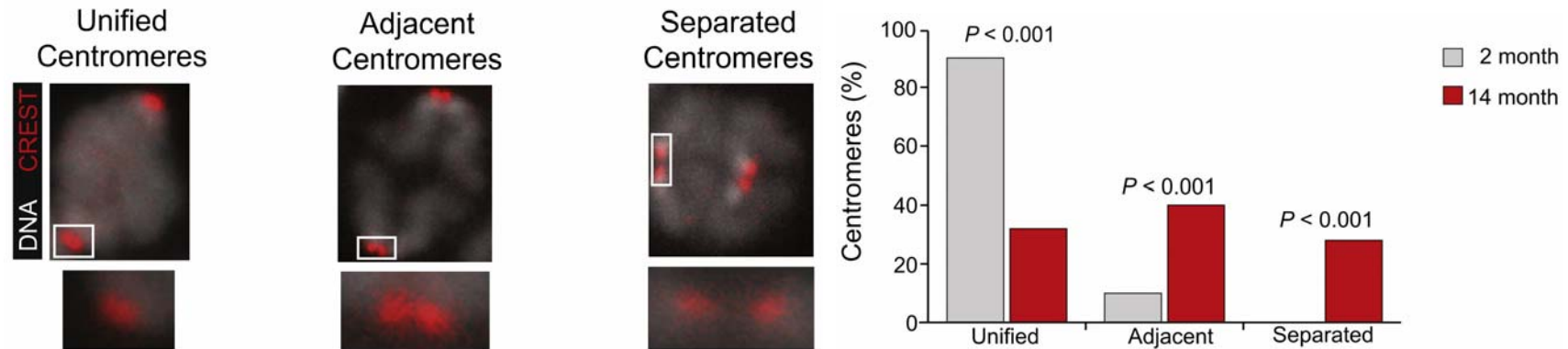


Ovarian senescence group

OUS, Rikshospitalet



Cause of Aneuploidy



- Chromosome structure changes with age
- Protein localisation is affected in older animals
- How can we detect aneuploidy in oocytes and embryos?

