Current recommendations for genetic testing in the infertile male

Sjoerd Repping, PhD

S.Repping@amc.uva.nl

Universiteit van Amsterdam

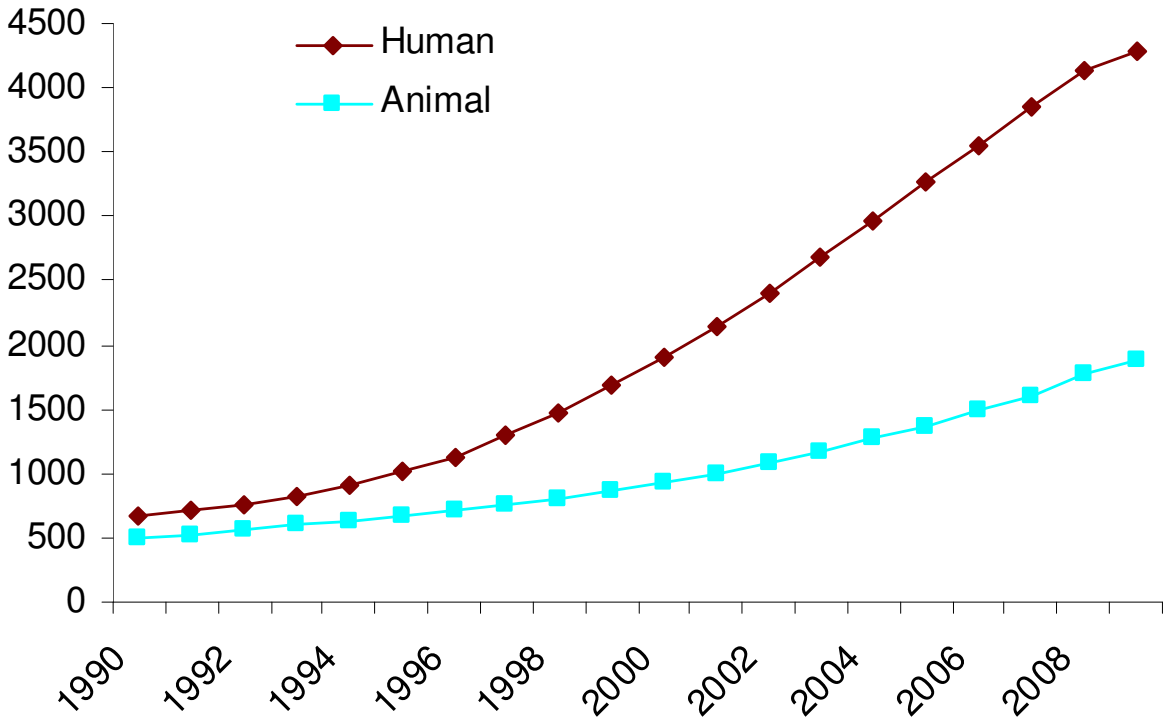
amc center for reproductive medicine

Layout of course

- Basic aspects of genetics and male infertility
  - Definitions
  - Study flaws
  - Biological difficulties
- Established genetic causes
  - Numerical / structural chromosome abnormalities
  - Y-chromosome aberrations
- Conclusions
  - Recommendations
  - Critical remarks

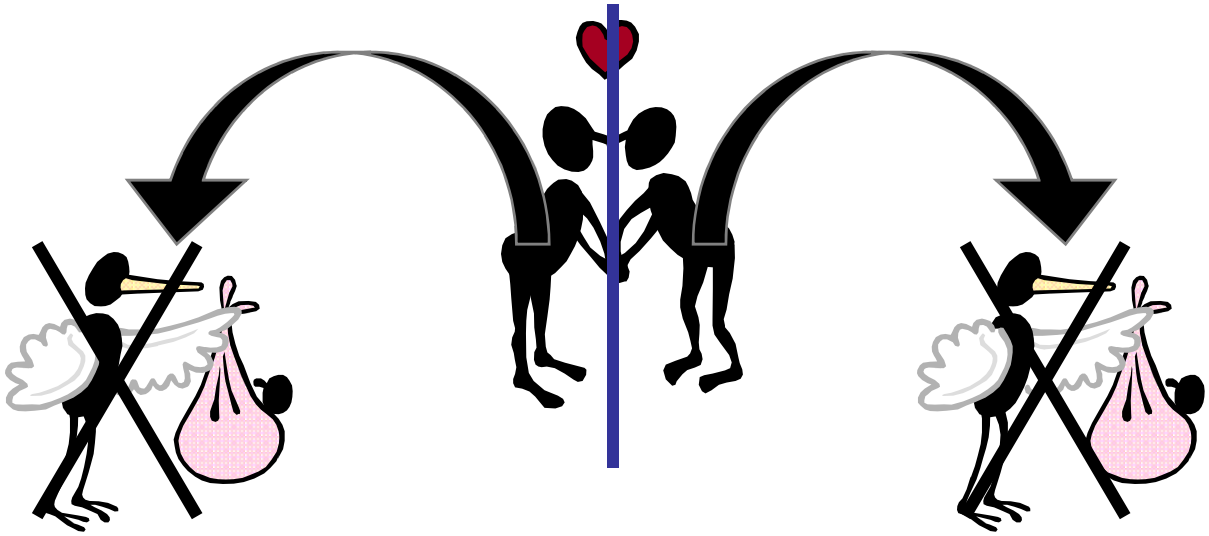
Search for genetic causes

- PubMed search “male infertility AND genetics”



Only a handful of genetic factors have unequivocaly been demonstrated to affect spermatogenesis in humans

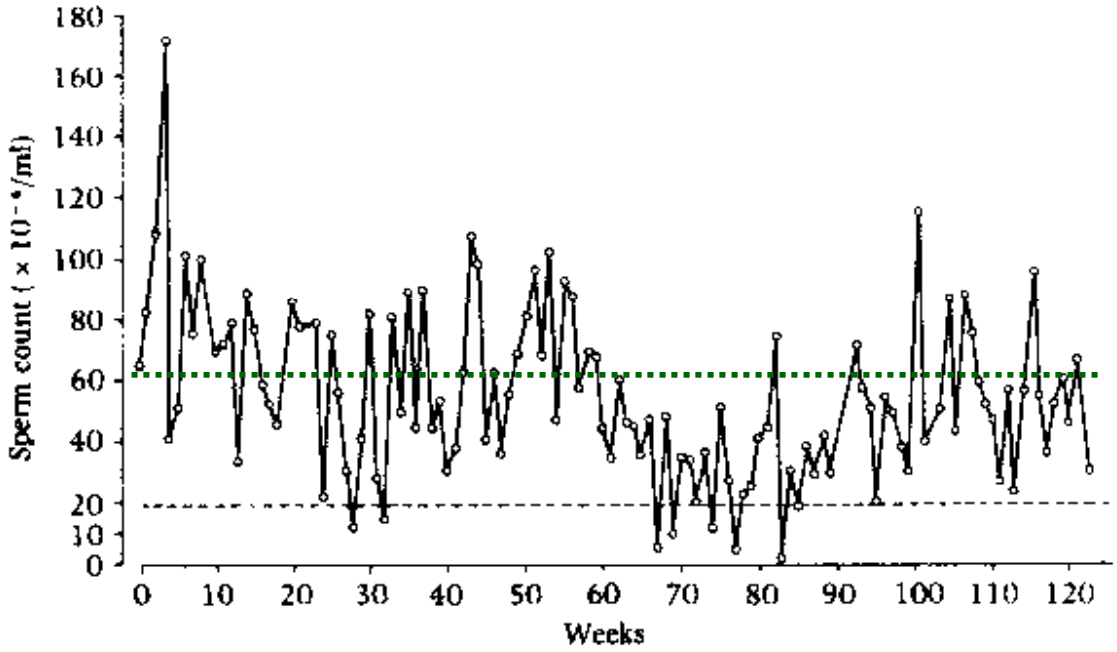
~~Male infertility~~



Spermatogenic failure

(= impaired sperm production)

Variability in sperm count



WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction (1999)

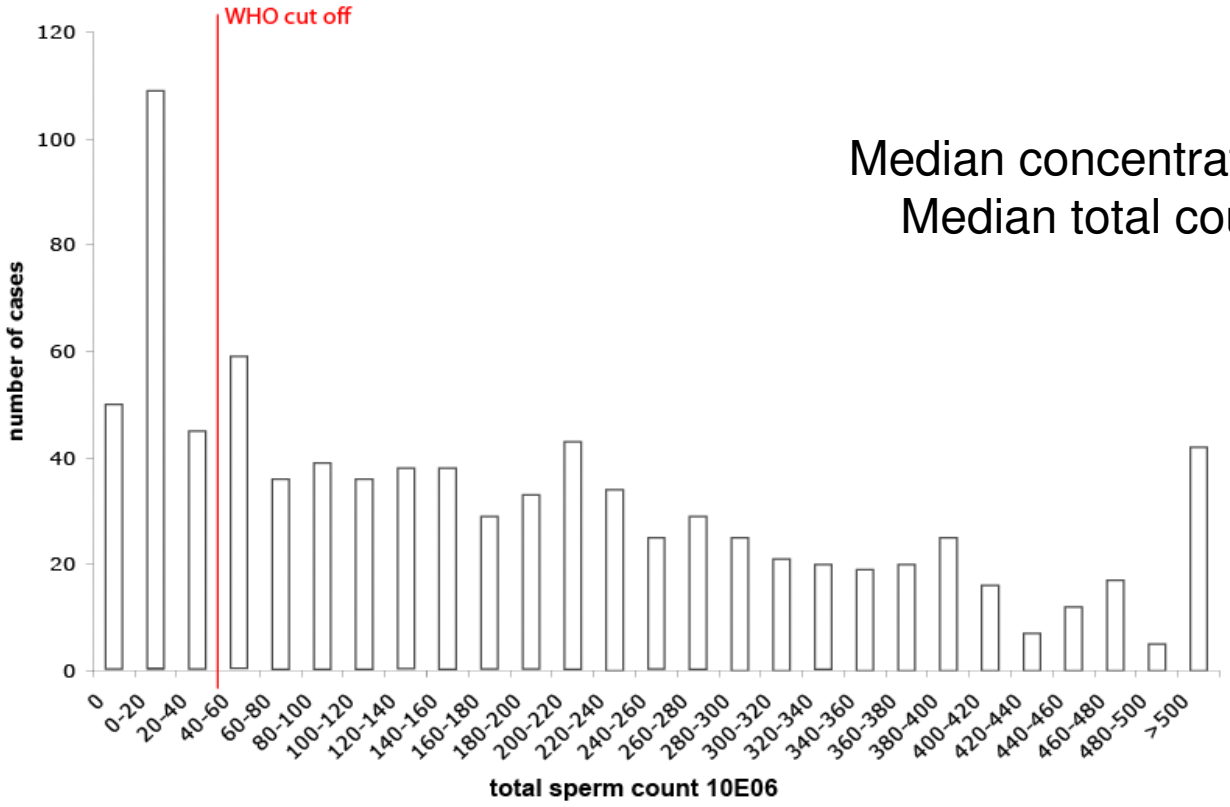
Patients and controls

- Patients
  - Men with reduced semen quality according to WHO criteria
  - Known causes of spermatogenic failure excluded
- Controls
  - Proven fertile fathers
    - Non-paternity excluded?
    - (severely) oligozoospermic men can father children
  - Vasectomy reversals
- Cohort based approach
  - Reproductive goals
  - Men with proven monogenetic spermatogenesis

- Preoperative sperm count unknown
- Methodologically more powerful
- Sperm count unknown
- A priori distinction between healthy and ill
- Comparison of genetically affected vs. unaffected

Sperm production variation

Consecutively included cohort male partners of subfertile couples (n=1,041)

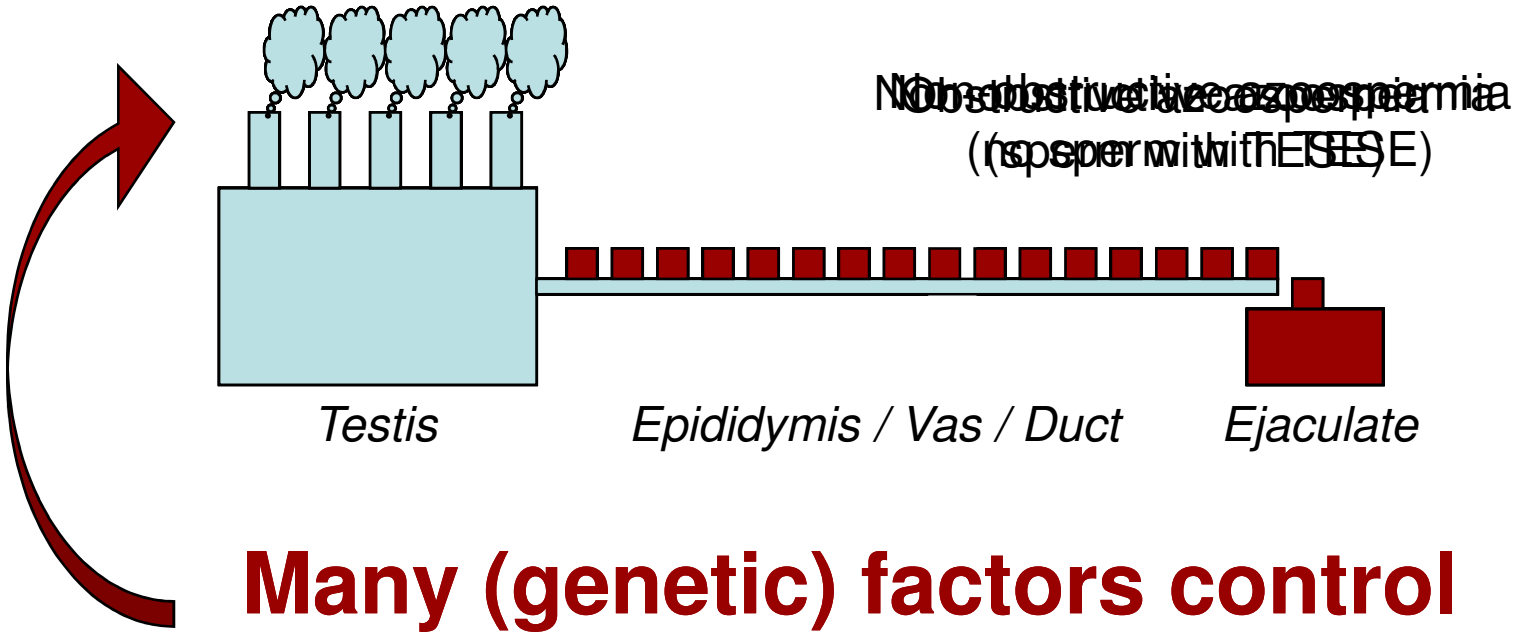


Median concentration: 52 x10^6/ml
Median total count: 149 x10^6

amc enter for reproductive medicine

Our view on spermatogenesis

Spermatogenesis is a quantitative trait



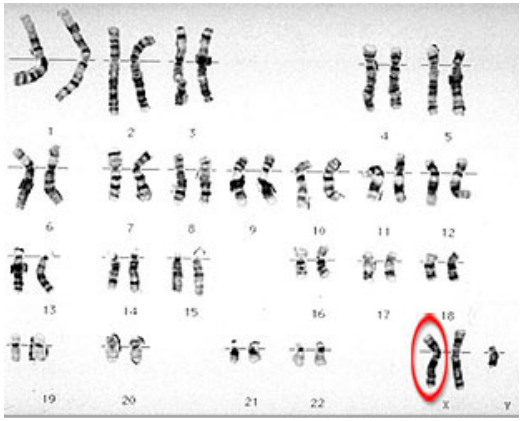
Many (genetic) factors control the rate of sperm production

Established genetic causes

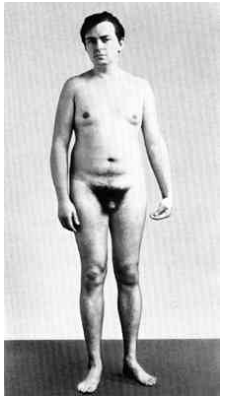
- Karyotype abnormalities
  - Klinefelter syndrome (47, XXY)
  - Translocations
- Y-chromosome aberrations
  - Deletions
  - Isodicentric Y-chromosomes

Klinefelter syndrome

- Extra X-chromosome
  - Meiotic inbalance
  - Overexpression X-Y genes

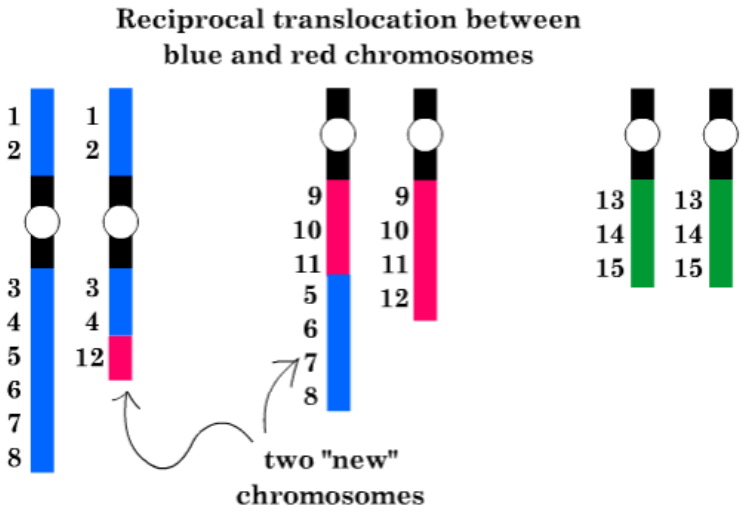


- Phenotype
  - Increased height
  - Disproportional arms/legs
  - Breast formation
  - Small testicles
    - (complete) absence of sperm production
    - Often mosaic genotype

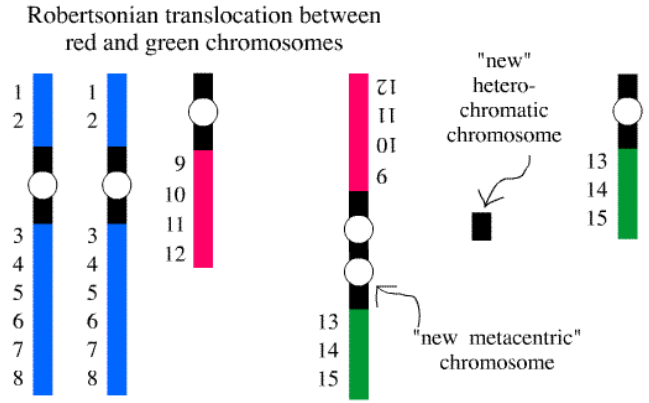


Translocations

- Reciprocal translocations



- Robertsonian translocations



Egozcue, et al. HumRepUpdate (2000)

Y chromosome and spermatogenesis

- 1976 Microscopic deletions
  - AZoospermia Factor (AZF) on Yq
- 1995 AZF candidate-gene
  - Deleted in AZoospermia (*DAZ*) on Yq11-23
- 1996 Three non-overlapping deletion intervals
  - *AZFa, AZFb, AZFc*

Tiepolo and Zuffardi, Hum Genet (1976) Reijo, et al., Nat Genet (1995)
Vogt, et al., Hum Mol Gen (1996)

Complete sequence of the Y

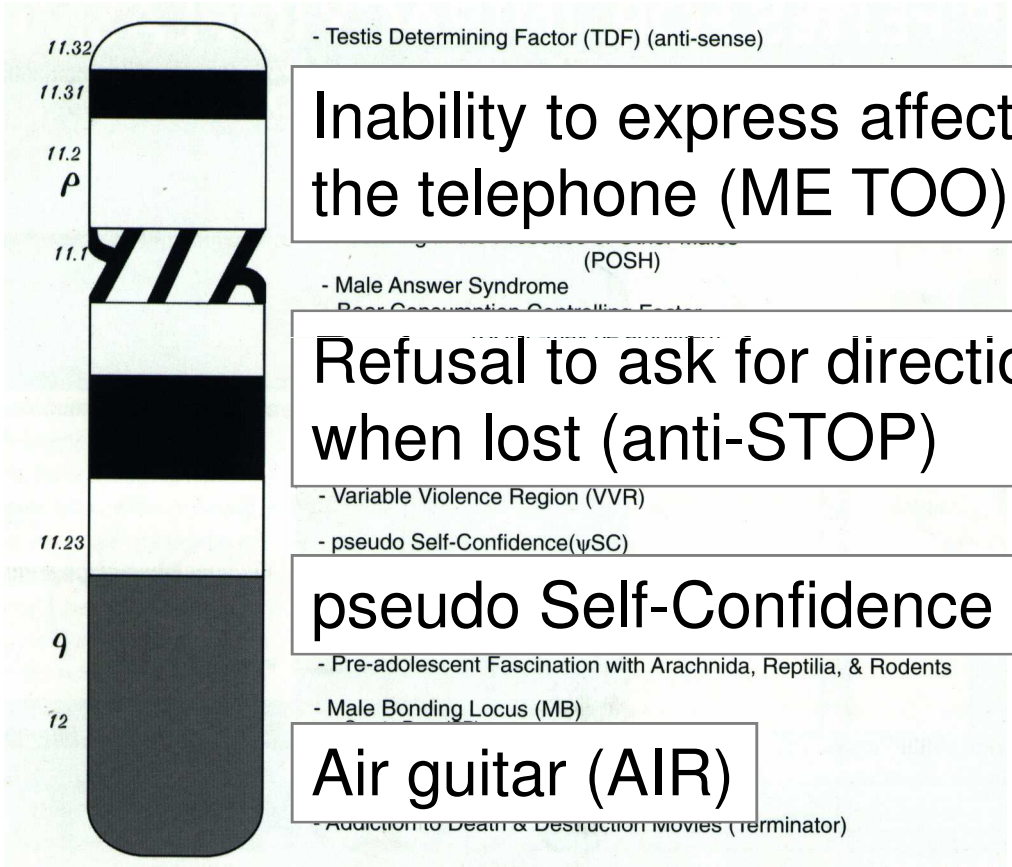
The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes

Helen Skaletsky*, Tomoko Kuroda-Kawaguchi*, Patrick J. Minx†, Holland S. Cordum†, LaDeana Hillier†, Laura G. Brown*, Sjoerd Repping‡, Tatyana Pyntikova*, Johar Ali†, Tamberlyn Bieri†, Asif Chinwalla†, Andrew Delehaunty†, Kim Delehaunty†, Hui Du†, Ginger Fewell†, Lucinda Fulton†, Robert Fulton†, Tina Graves†, Shun-Fang Hou†, Philip Latrielle†, Shawn Leonard†, Elaine Mardis†, Rachel Maupin†, John McPherson†, Tracie Miner†, William Nash†, Christine Nguyen†, Philip Ozersky†, Kymberlie Pepin†, Susan Rock†, Tracy Rohlfing†, Kelsi Scott†, Brian Schultz†, Cindy Strong†, Aye Tin-Wollam†, Shiaw-Pyng Yang†, Robert H. Waterston†, Richard K. Wilson†, Steve Rozen* & David C. Page*

** Howard Hughes Medical Institute, Whitehead Institute, and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA*
† Genome Sequencing Center, Washington University School of Medicine, 4444 Forest Park Boulevard, St Louis, Missouri 63108, USA
‡ Center for Reproductive Medicine, Department of Gynaecology and Obstetrics, Academic Medical Centre, Amsterdam 1105 AZ, the Netherlands

Skaletsky, et al., Nature (2003)

Genes on the Y-chromosome



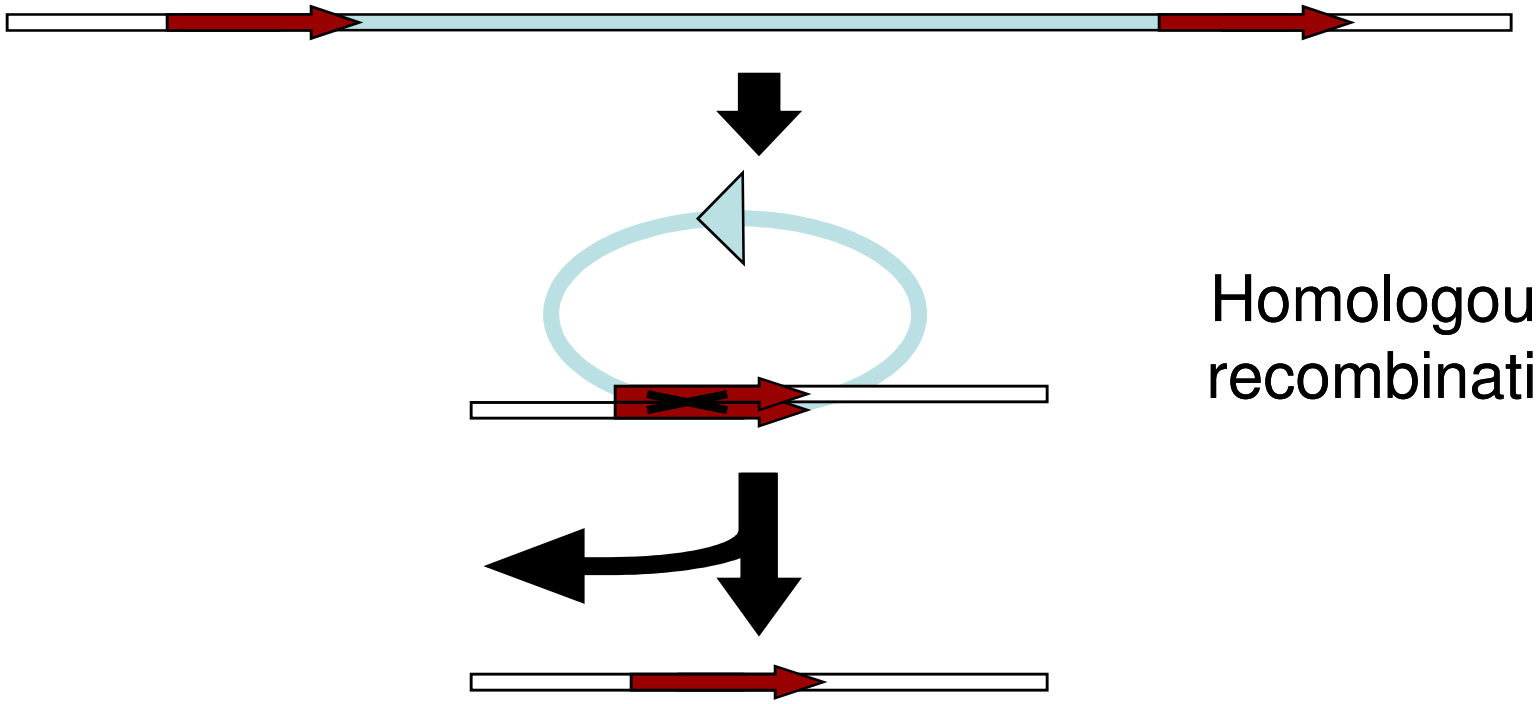
MSY genes

- 156 transcription units
  - 78 likely to encode proteins

- Gene classes:
  - X-Y homologs
    - 18 genes
    - Widely expressed
  - MSY specific
    - 60 genes
    - Testis-specific expression
    - Present in multiple copies

Skaletsky, et al., Nature (2003)

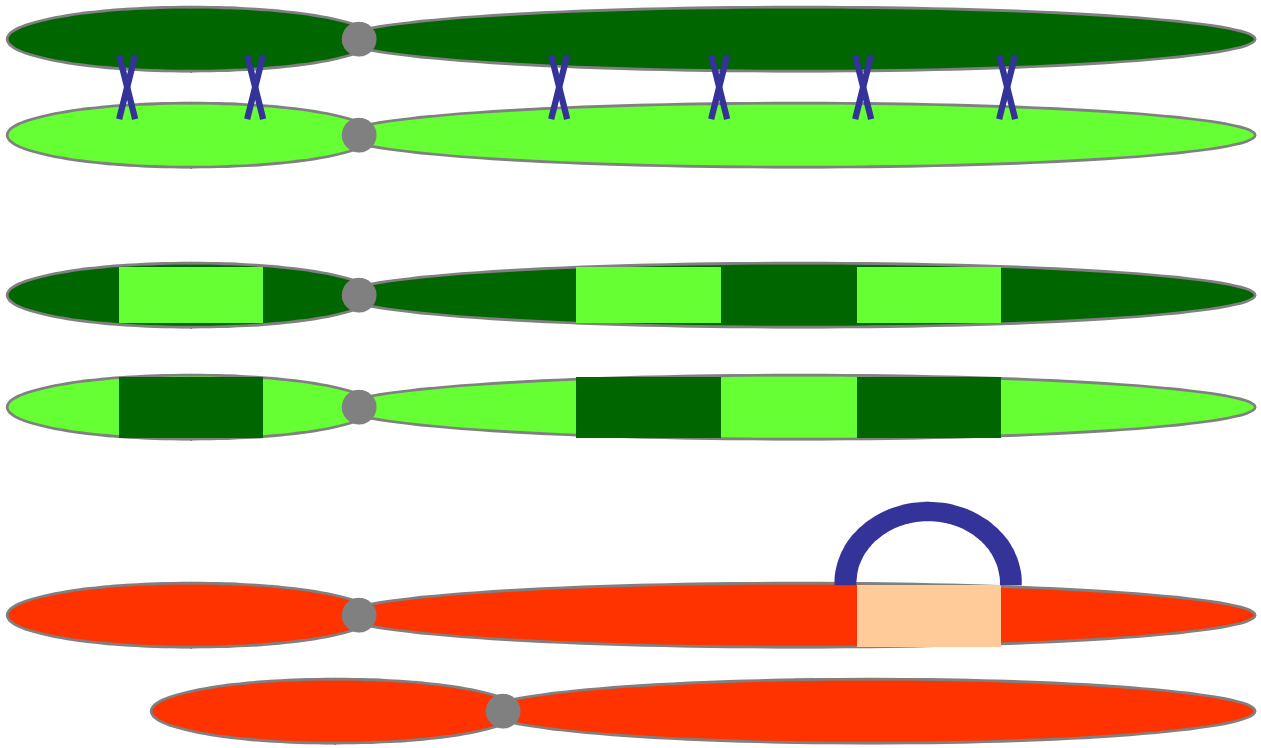
Y-chromosome deletions



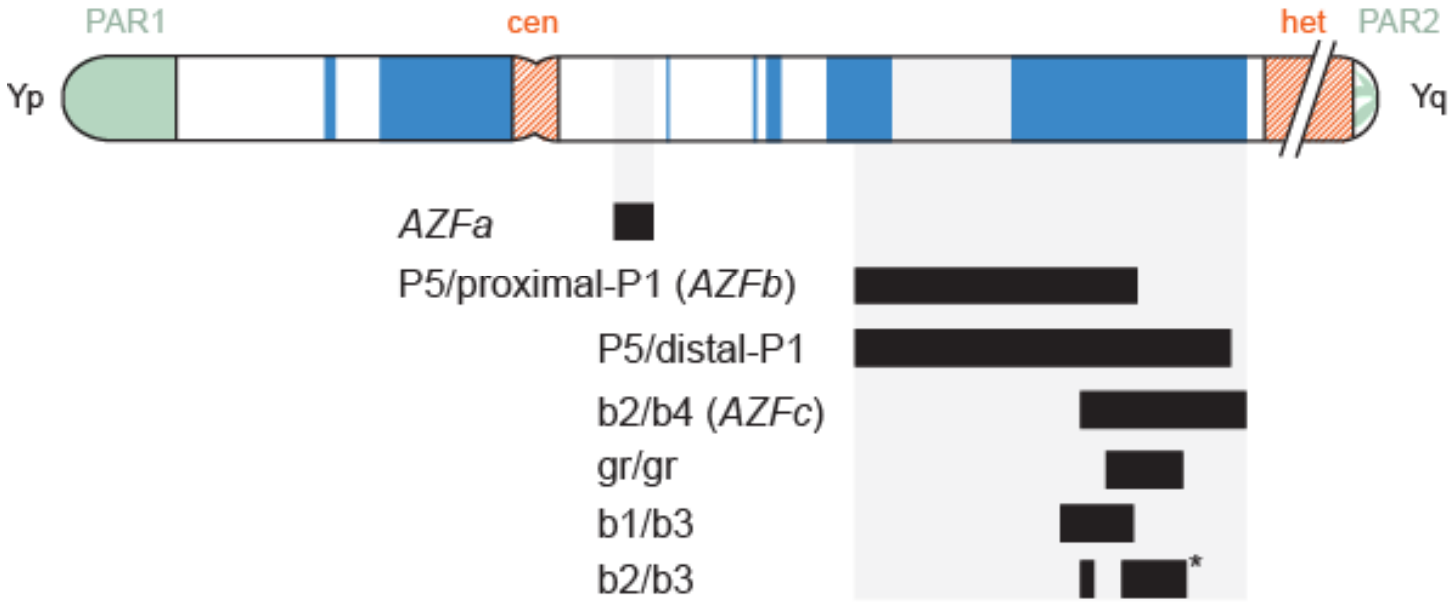
Homologous recombination

Sun, et al., Hum Mol Genet (2000), Kuroda-Kawaguchi, et al., Nat Genet (2001)
Repping, et al., AJHG (2002), Nat.Genet (2003), Genomics (2004)

Homologous recombination



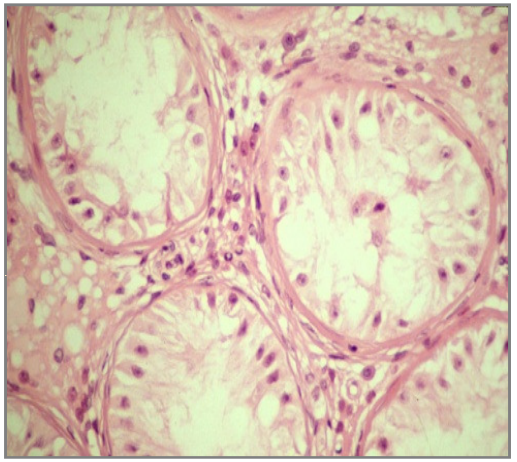
Y-chromosome deletions



Noordam & Repping, COGD (2006)

AZFa deletions

- very infrequent (<1%)
- remove 2 single copy genes
- 800 kb in size
- phenotype: Sertoli Cell Only
  - no germ cells → no ICSI
  - no testis biopsy necessary (?)

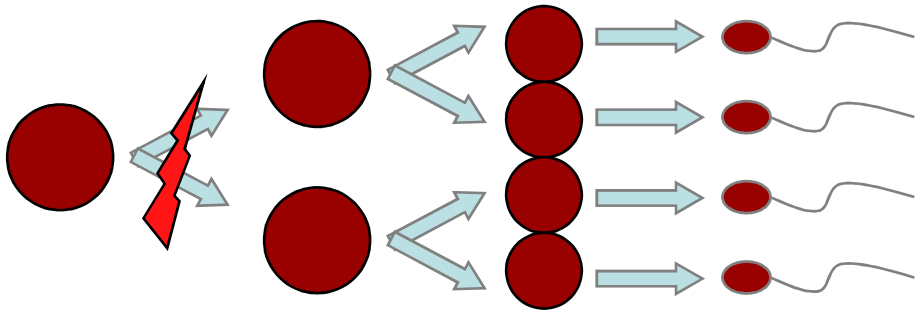


- Partial deletions and point mutations also described
  - Less severe phenotype

Sun, et al., Nat Genet (1999), Sun, et al., Hum Mol Gen (2000)
Krausz, et al., HumMolGen (2006), Luddi, et al., NEJM (2009)

P5/P1 (*AZFb*) deletions

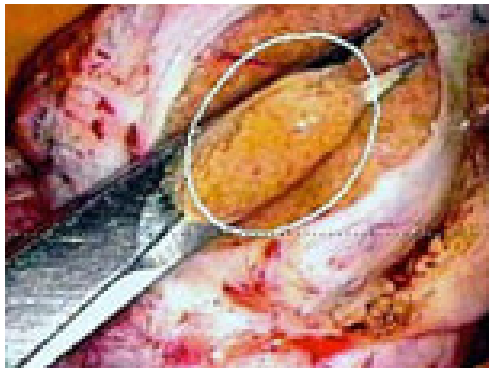
- infrequent (1-2%)
- remove 33-44 transcripts
- 6.2-7.7 Mb in size
  - largest characterized deletion in the human genome
- phenotype: early meiotic arrest
  - no mature spermatozoa → no ICSI
  - no testis biopsy necessary (?)



Vogt, et al., Hum Mol Genet (1996), Repping, et al., AJHG (2002)
Oates, et al., Hum Rep (2002)

AZFc deletions

- frequent (5-10%)
  - most common molecular cause of spermatogenic failure
- remove 21 genes
- 3.5 Mb in size
- Variable phenotype
  - SCO … oligozoospermia
- ICSI possible
  - ejaculated spermatozoa
  - testicular derived spermatozoa (TESE)
    - ~80% retrieval success
- Transmission to offspring (all males)



Kuroda-Kawaguchi, et al., Nat Genet (2001)
Oates, et al., Hum Rep (2002)

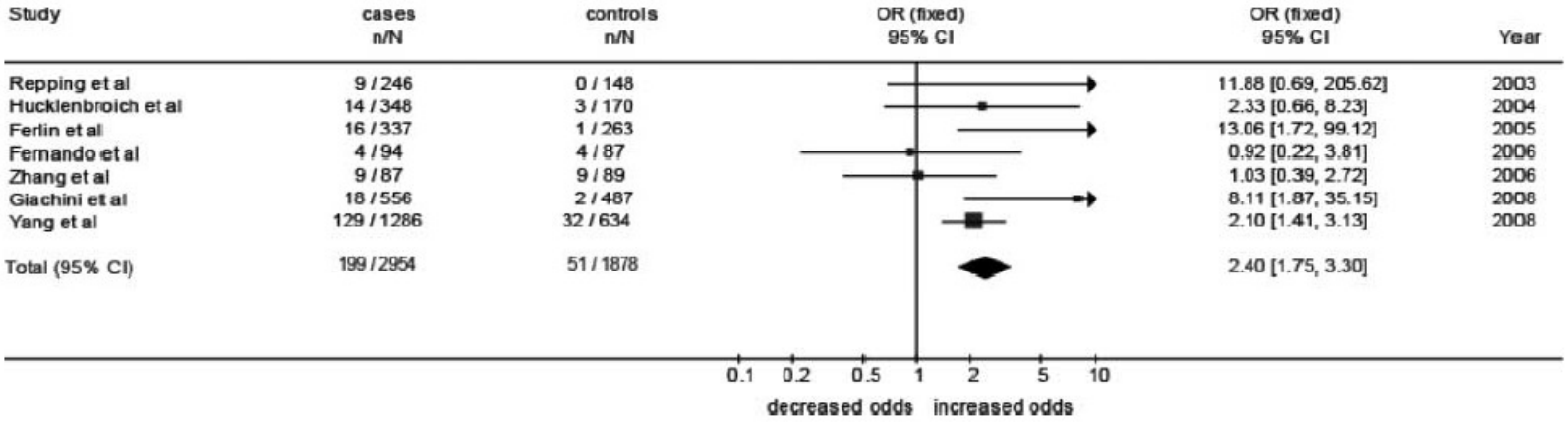
gr/gr deletions

- frequent (~4%)
- remove 9 genes
  - no complete gene family
  - reduce copy number of 8 families
- 1.6 Mb in size
- Persist in population as polymorphism
  - Variable frequency among populations
- Variable phenotype
  - not always *de novo* (natural transmission)
  - risk factor for spermatogenic failure

Repping, et al., Nat Genet (2003)

gr/gr deletions

- Meta-analysis
  - 26 studies published
    - 16 studies methodologically flawed

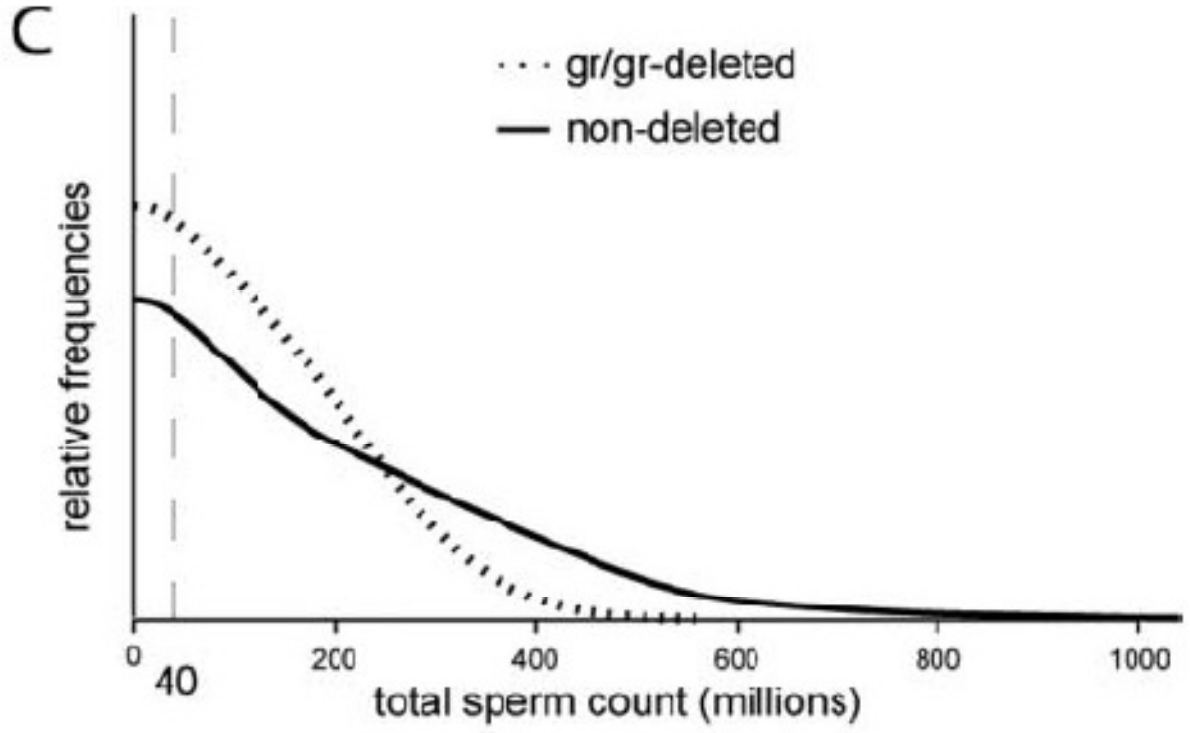


- Combined odds ratio 2.4 (1.75 – 3.30)
  - gr/gr deletion 2.4 times more common among azoo- oligozoospermic men than among normozoospermic men

Visser, et al., HumRep (2009)

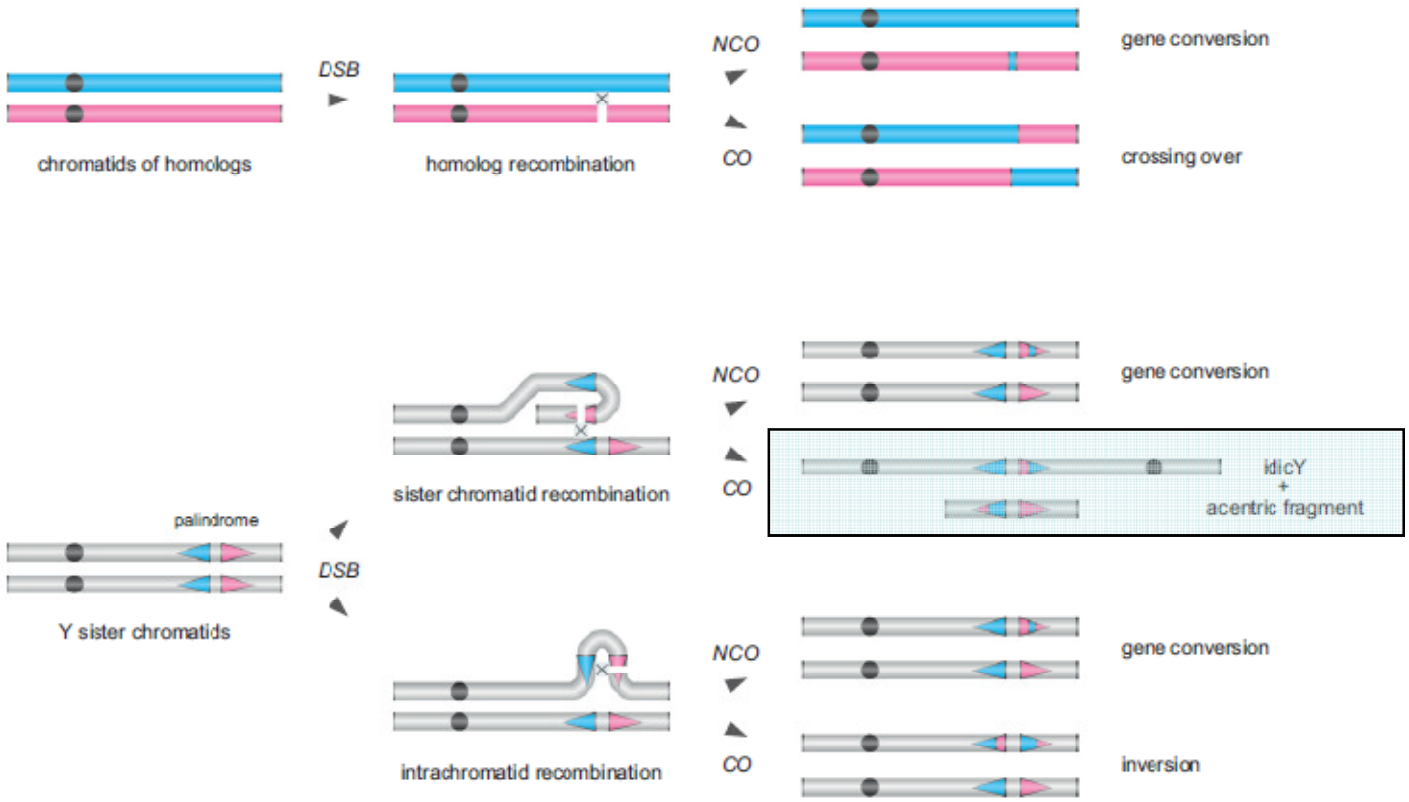
gr/gr deletions

- Cohort study
  - 1,041 male partners of subfertile couples
  - Compare semen quality gr/gr deleted vs non-deleted



Visser, et al., HumRep (2009)

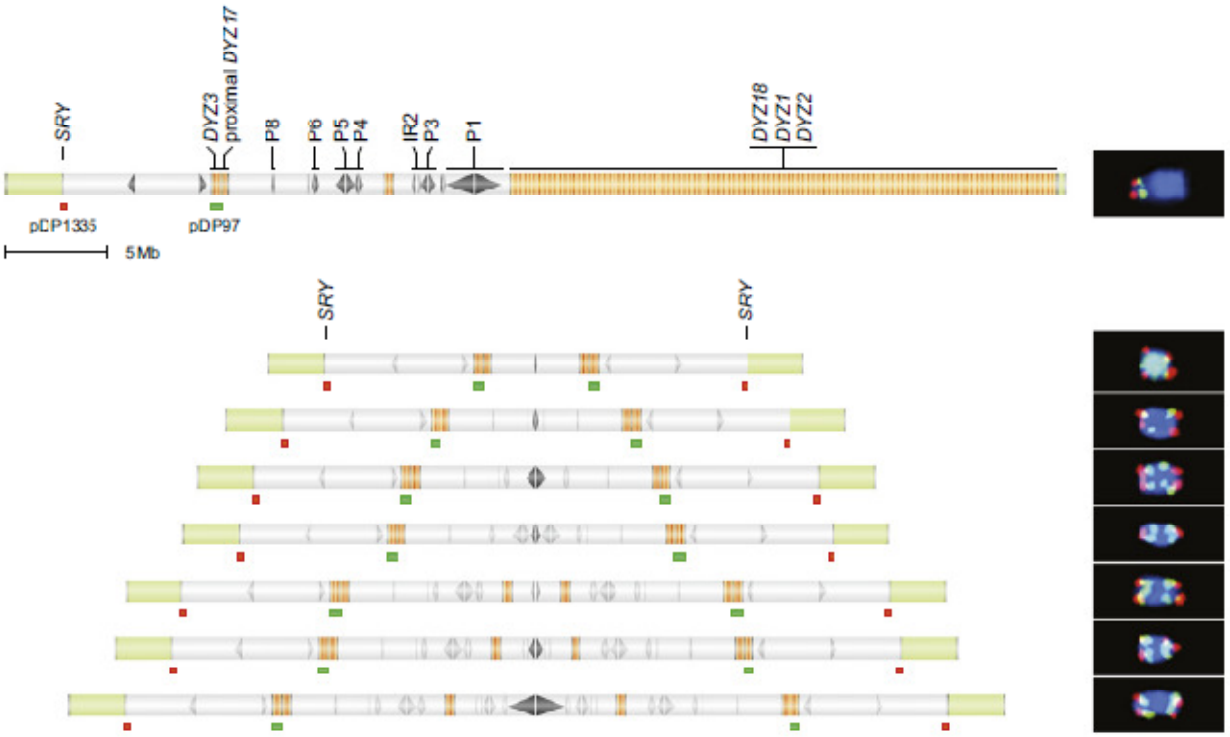
Isodicentric Y-chromosomes



Lange, et al., Cell (2009)

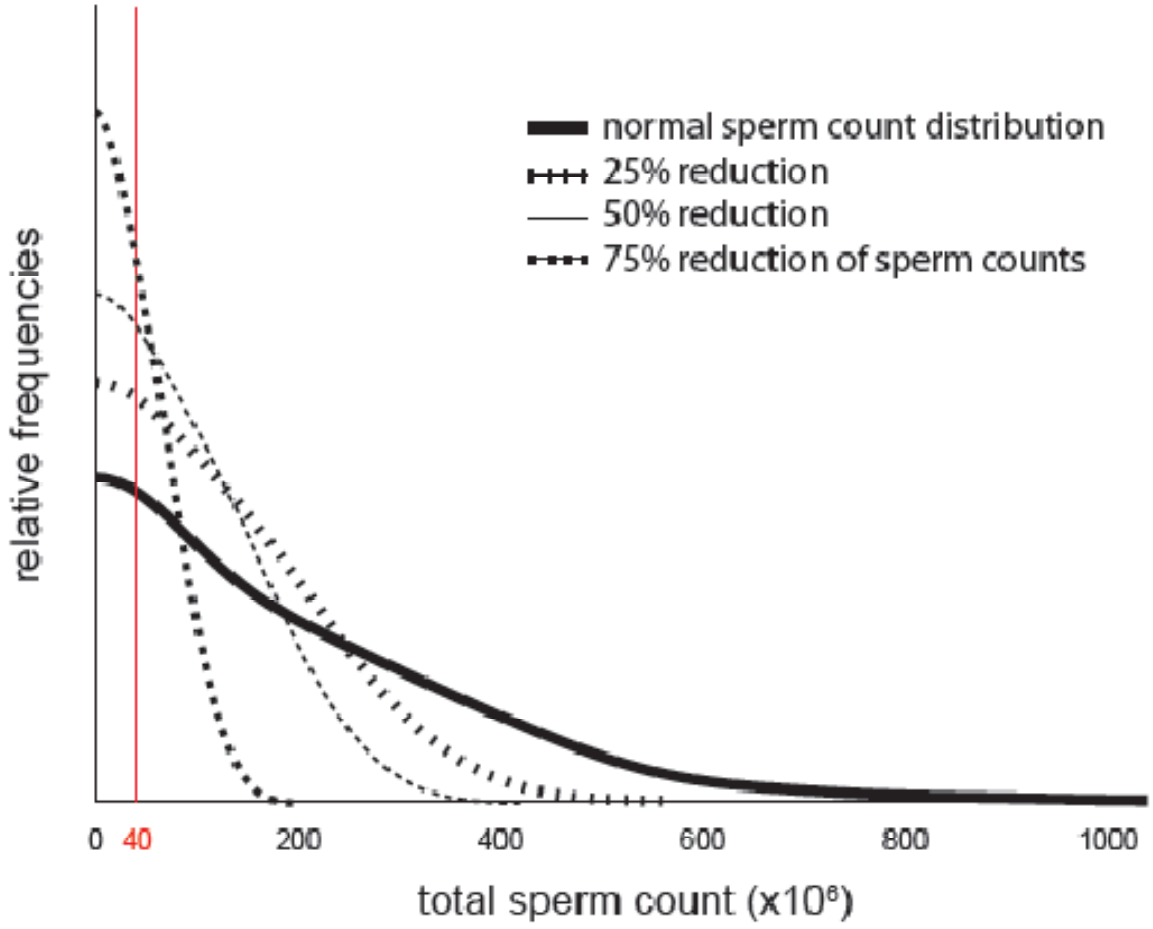
Isodicentric Y-chromosomes

- Present in 3% of azoospermic males
  - Some forms cause loss of Y and hence Turner Syndrome



Lange, et al., Cell (2009)

Phenotypic effect



Visser & Repping, Reproduction (2009)

Recommendations

- Screen azoo- and severely oligozoospermic men for
  - Karyotype abnormalities
  - Y-chromosome aberrations

- Future search
  - Homogeneous phenotype (SCO, astheno, globo)
  - Whole genome approach
    - SNPs / CNVs / whole genome sequencing
    - Large sample collections

Critical remarks

- What is our goal?
- Diagnosis of spermatogenic failure
  - Does it alter treatment strategy?
    - ….. no ……
  - Should we then screen at all?
- Treatment for spermatogenic failure
  - Thusfar no direct treatment options
  - Genetic screening may not be the way forward …….

Rucker, et al., J Urol (1998), Nap, et al., Hum Rep (1999)

Acknowledgements

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