Genetic and Age-Related Contributions of the Male Gamete

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Disclosures and Conflicts of Interest

- **Disclosure:**
  - **On ABU/AUA Written Exam Committee (paid)**
  - **Board member of ASRM**
  - **Chair of AUA Reproduction Urology Care Foundation**
  - **Chair of American Society of Andrology Public Affairs and Health Policy Committee**

- **Conflicts of interest**
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The Optimal Evaluation of the Infertile Male: AUA Best Practice Statement

Revised, 2010

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Severe Male Factor

- Azoospermia
  - Absence of sperm in ejaculate
  - Identified in 10-15% of infertile males
- Severe oligospermia
  - <5 million sperm per milliliter
- Genetic disorders identified in ~15% of infertile men

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Lab Testing – Blood

- **Genetic Testing:**
  - Karyotype
  - Y chromosome micro-deletion (AZF)
  - CF testing

- **Indications:**
  - Karyotype/AZF: when azoo or conc. <5M/cc
  - CF – CBAVD/unexplained obstrn

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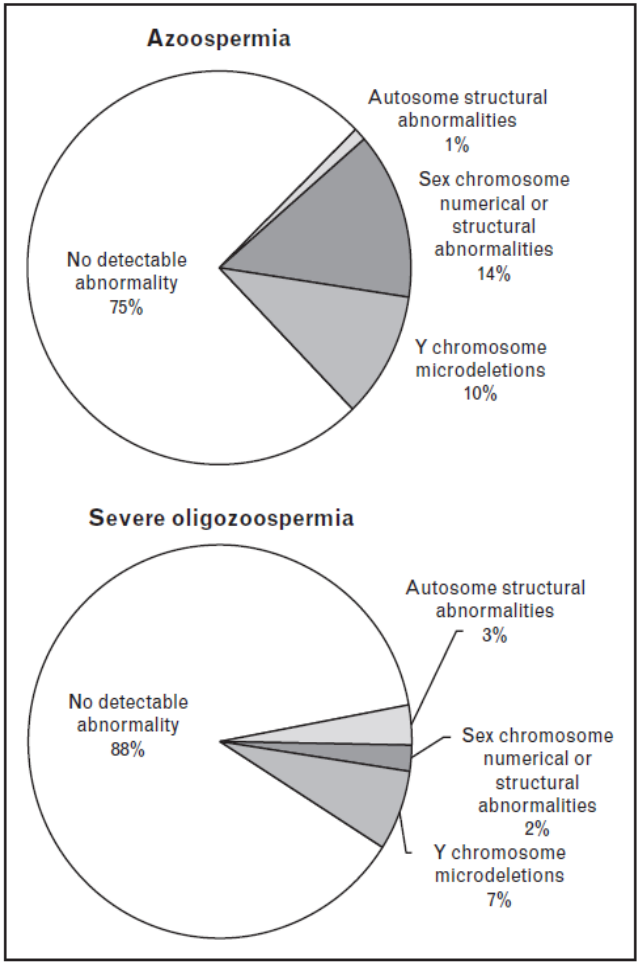
Nonobstructive Azoospermia/Severe Oligospermia

- **Karyotype and Y chromosome microdeletion assay**
  - **Up to 30% of men will have a genetic abnormality**



Stahl and Schlegel, *Curr Opin Obstet Gynecol*, 2012

Why Perform Genetic Evaluation?

- Etiology
- Prognosis/surgical sperm retrieval success
- Health risks
  - To patient
  - To future offspring

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Genetics and Male Infertility

- **Association of male infertility with known and unknown genetic abnormalities**

- **IVF-ICSI: Risk of transmission of these to the next generation or even later (epigenetic)**

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Implications of Genetic Causes of Male Infertility

- Genetic counseling
- PGD often indicated

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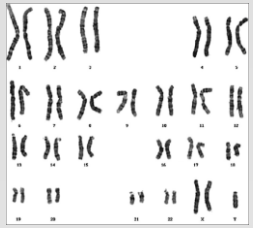
Karyotypic Abnormalities



- **14% of men with azoospermia and 4.6% of men with severe oligospermia have karyotypic abnormality**
- **47 XXY (Klinefelter syndrome) most common, but also see translocations, deletions**
- **These can impact outcome of ART via miscarriage, birth defects**

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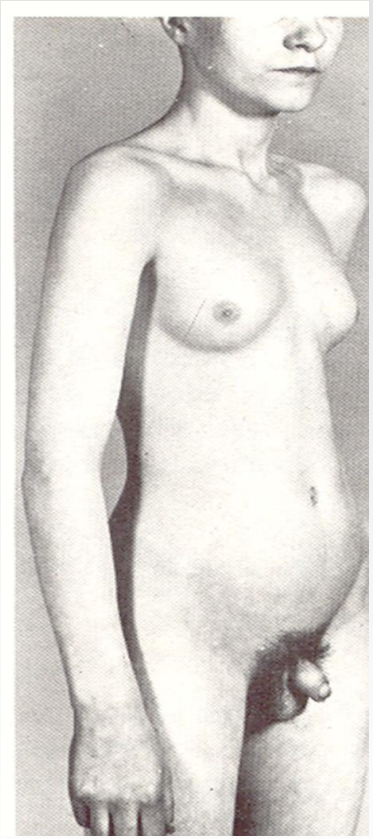
De Braekeleer M, Dao TN. Hum Reprod. 1991 Feb;6(2):245-50.

Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, Van Steirteghem A, Liebaers I. Hum Reprod. 1996 Dec;11 Suppl 4:1-24

Karyotypic Abnormalities

- **Klinefelter Syndrome (47XXY or 46XY/47XXY mosaic)**
  - **Small, firm testes**
  - **Usually azoospermic**
  - **Elevated FSH**
  - **Other medical issues: diabetes, osteoporosis, breast cancer, gynecomastia, extragonadal germ cell tumors**



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Klinefelter Syndrome

- First described in 1942
- Most common genetic cause of male infertility
- 1:600 men
- Prevalence 0.1-0.2% of the general population
- Up to 3.1% of male infertility population
- ~15% of NOA cases
- 80% due to XXY
- 20% due to higher grade aneuploidy eg 48 XXXY and mosaicism or X chromosome abnormality
- Mosaics may be underestimated ie XXY in testis but normal blood/leucocyte karyotype

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- **Klinefleter Syndrome may remain underdiagnosed**
  - **10% diagnosed prenatally**
  - **30% diagnosed in childhood or adult life**
  - **60% still remain underdiagnosed**

PATHOGENESIS

Non-dysjunction in meitotic division in germ cell development or mitotic cell division in the embryo

- **50% maternal or paternal origin – maternal error in meiosis 1 or 2, but only meiosis 1 for paternal origin**

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Sperm Retrieval in Klinefelter Syndrome Patients

- Systematic review of nonmosaic KS
- 338 patients
- Results: **44% overall successful sperm retrieval with TESE or mTESE**
  - TESE 42%
  - mTESE 55%

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Fullerton et al Hum Reprod 2010;25:588.

Role of micro-TESE in Klinefelter syndrome

114 attempts at sperm retrieval (in 88 men)

- **Sperm retrieved: 78/114 (68%) attempts**
  - **Fertilization & transfer: 66 cycles**
- **Clinical pregnancies: 33/78 (42%)**
  - **52% pregnancy rate/ET**
- **Forty-four children born (46,XX or 46,XY)**
- **Higher sperm retrieval rates than previously reported**

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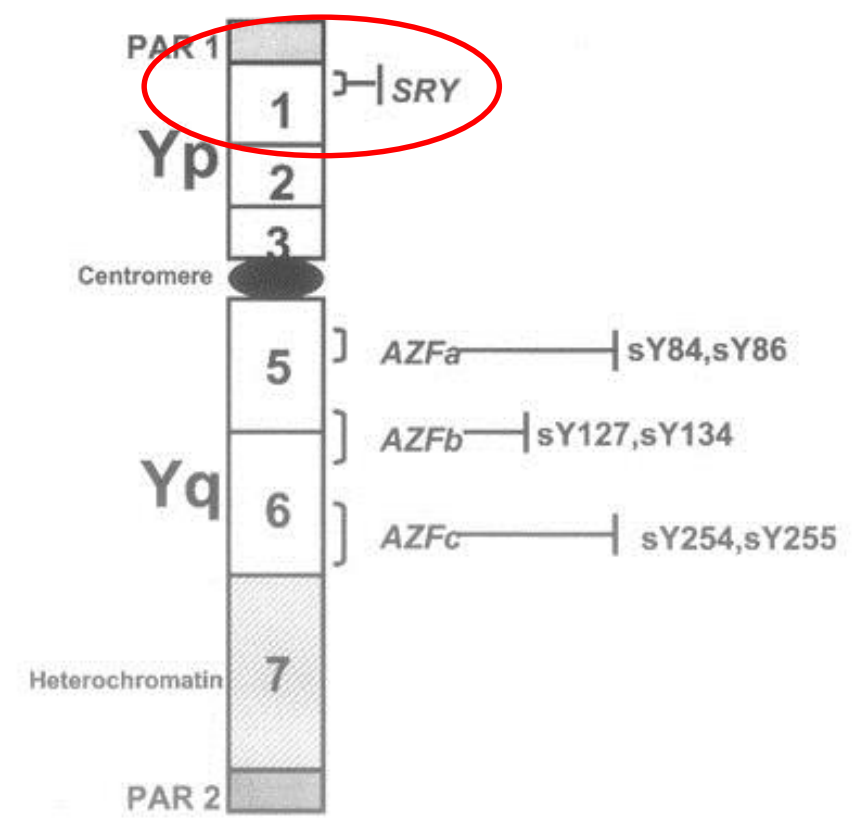
Karyotypic Abnormalities

- **46XX male**
  - 1:20,000
  - SRY translocated to X chromosome or autosome
  - Phenotype = male
  - No spermatogenesis



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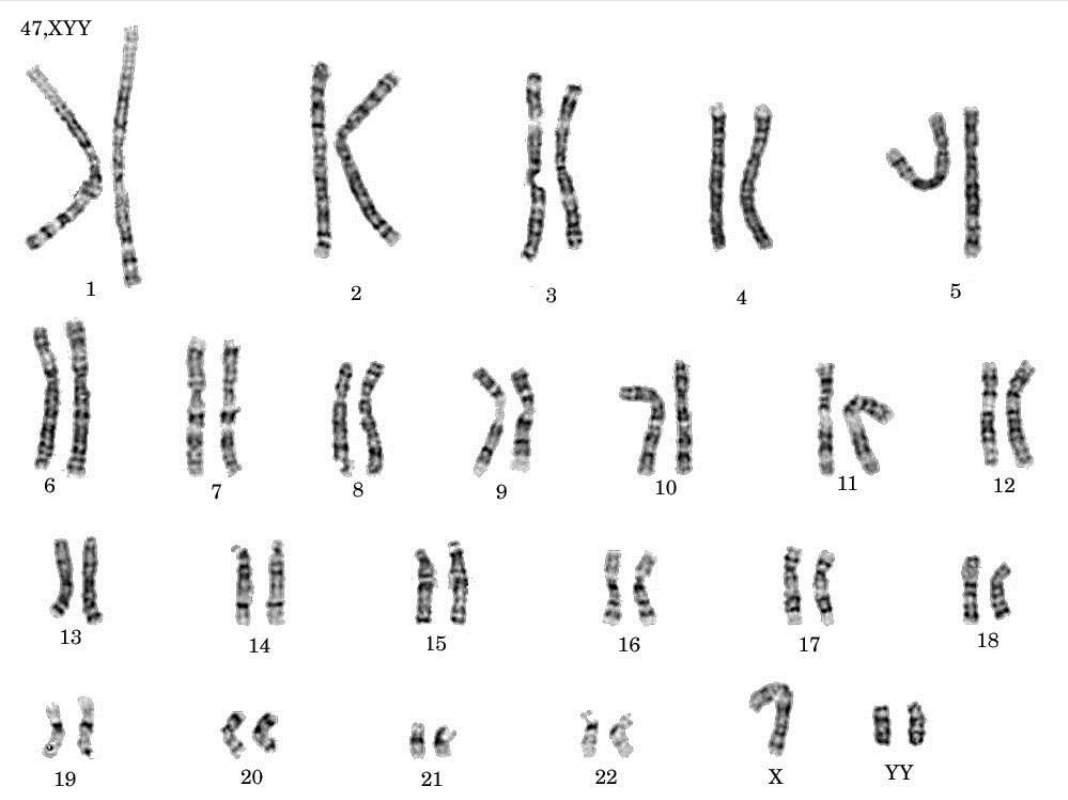
Karyotypic Abnormalities

- **47XYY male**
  - Tall
  - Normal or hypotrophic testes
  - Variable endocrine profile
  - Variable semen parameters



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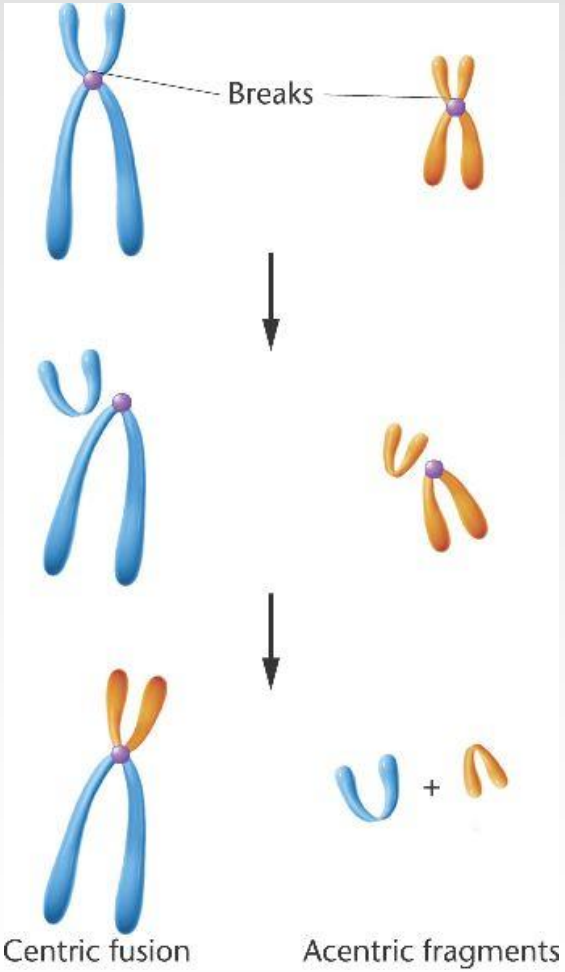
Kim et al, *Rev Urol*, 2013
Abdel-Razic et al, *Andrologia*, 2012

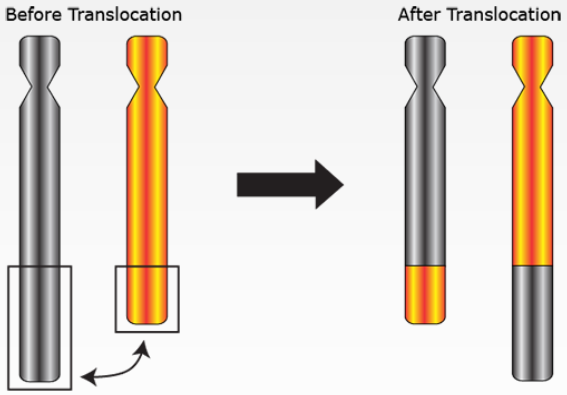
Karyotypic Abnormalities

- **Robertsonian translocations**
  - **Chromosomes 13, 14, 15, 21, 22**
  - **Unbalanced exchange of genetic material**
  - **1.5% oligospermic men**



Karyotypic Abnormalities

- **Reciprocal translocations**
  - **Balanced exchange of genetic material**
  - **0.7% of men with azoo- or oligospermia**



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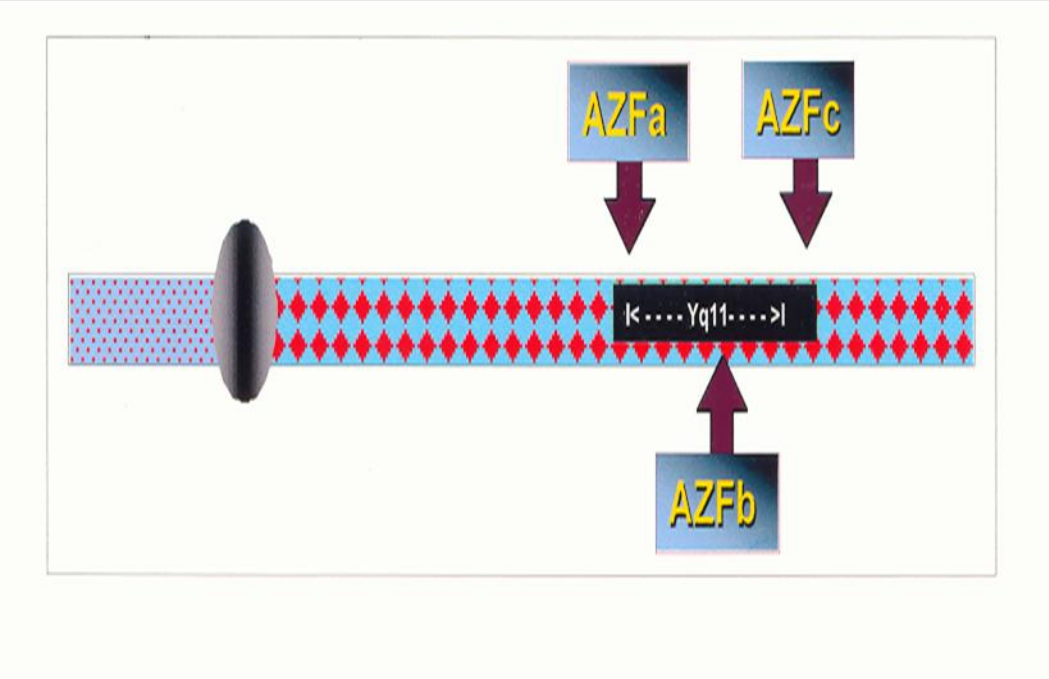
Y Chromosome Micro-deletion



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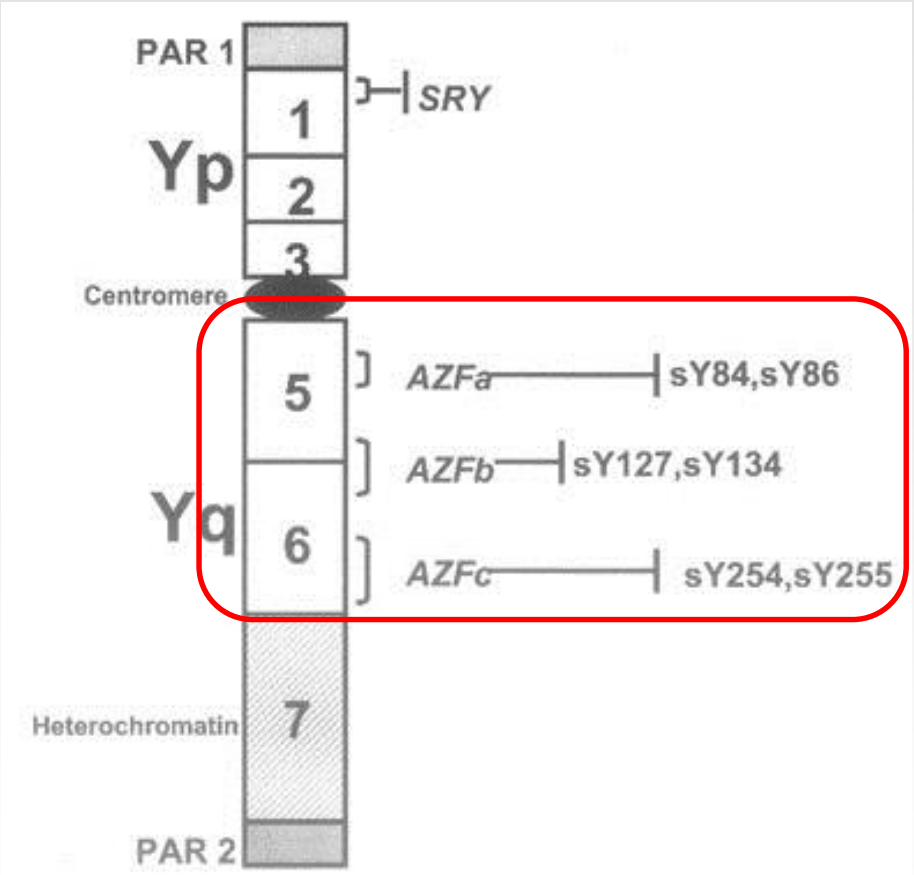
Y Chromosome Microdeletions

- Yq deletions
  - 10% NOA
  - 5-10% oligospermic men



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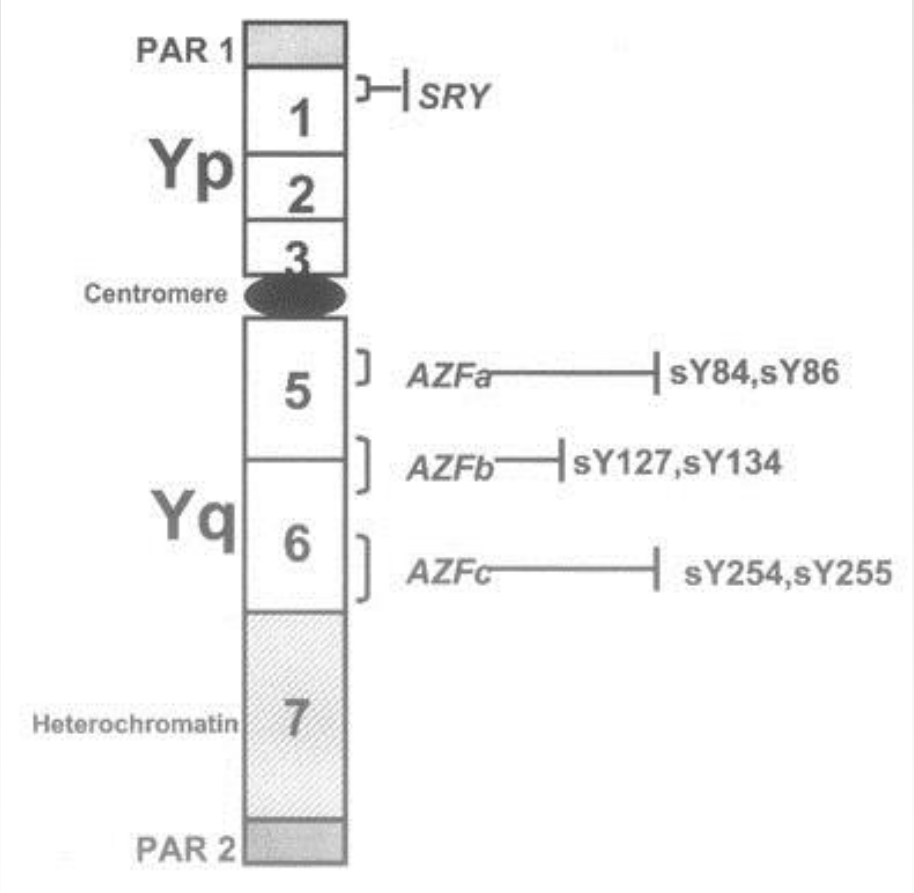
Reijo et al, *Nat Genet*, 1995
Reijo et al, *Lancet*, 1996

Y Chromosome Microdeletions

- Yq deletions
  - 10% NOA
  - 5-10% oligospermic men
- Implications for parent
  - Etiology
  - Prognosis



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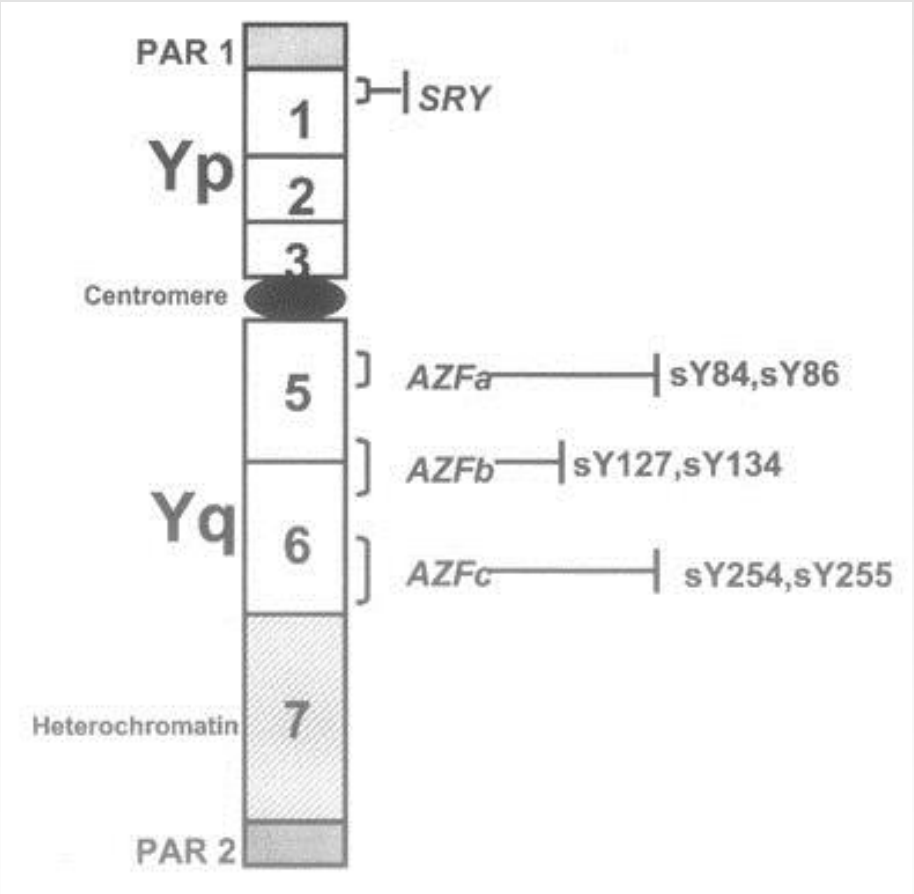
Y Chromosome Microdeletions

- AZFa → no sperm
- AZFb → no sperm
- AZFc → potential for sperm (ejaculated or via sperm retrieval)



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Hopps et al, *Hum Reprod*, 2003

Sperm Retrieval in Microdeletion in AZF

- mTESE/TESE not performed in AZFa and AZFb
- TESE and micro-TESE in 42 oligospermic and azoospermic men with AZFc deletions
  - 66% retrieval rate
- 21 patients with AZFc microdeletions from a single center
  - 43% retrieval rate with TESE
  - 72% retrieval rate with micro-TESE
  - 46% pregnancy rate

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Oats et al Hum Reprod 2002;17:2813.
Choi et al Korean J Urol 2013;54:111.

Sperm Retrieval Rates: Maturation Arrest

- Retrospective studies varying from 15-151 patients
  - Sperm retrieval – 23-51%
- Weedin et al:
  - Decreased retrieval rates among men with early MA as compared with those with late MA
  - Sperm retrieval 50% of 119 micro-TESE
  - Pregnancy rate 29%

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Kalsi et al BJU Int 2012;109:418
Weednin et al J Urol 2011; 186:621
Tsai et al Urology 2012:80:826

Sperm Retrieval Rates: Sertoli Cell Only

- Overall sperm retrieval rates 29-43%
- Weill Cornell experience
  - 670 micro- TESE
  - 44% sperm retrieval rate
  - 46% clinical pregnancy rate
  - Patients with normal testis volume (>15cc) and FSH between 10 and 15 IU/L had sperm retrieval rate of 5.9%

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Kalsi et al BJU Int 2012;109:418
Berookhim et al Urol Clin N Am 41 (2014) 97–113

The Next Generation

Molecular Human Reproduction vol.2 no.12 pp. 943–950, 1996

The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers

Ethical Question: Are we affecting the future of Men's Health?

YES! Need for better research

S. DEJAGER, H. BRY-GAUILLARD, E. BRUCKERT, B. EYMARD, F. SALACHAS, E. LEGUERN, S. TARDIEU, R. CHADAREVIAN, P. GIRAL, AND G. TURPIN

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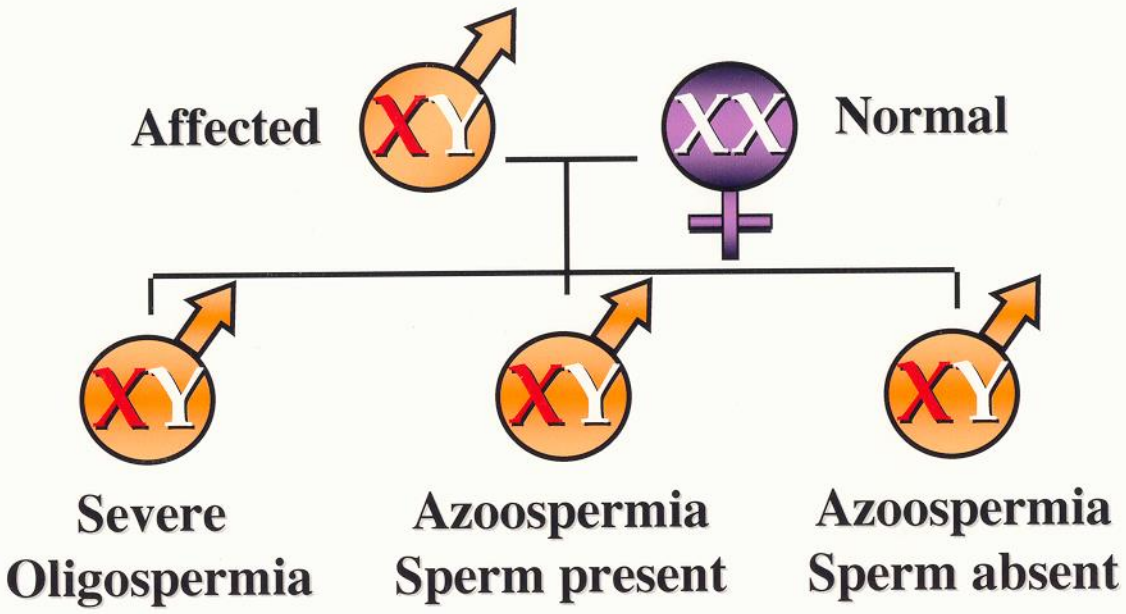
Transmission of DAZ Deletion with ICSI +/-TESE



Congenital absence of the vas

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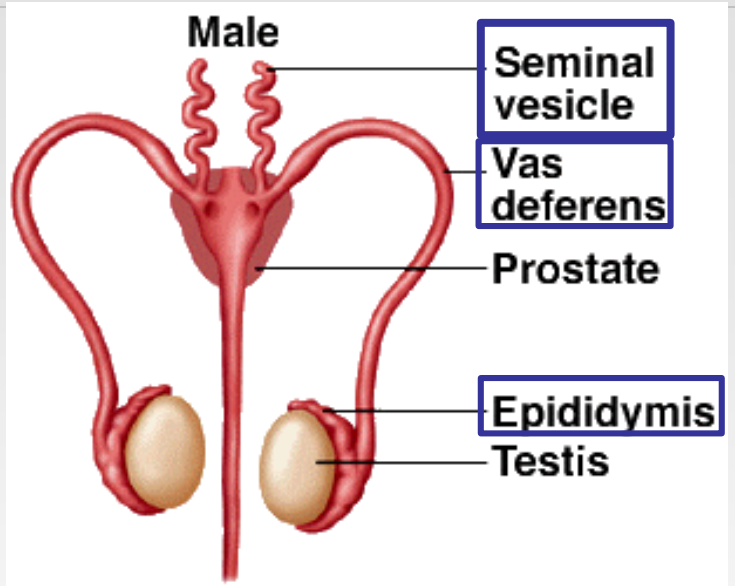
Vasal Absence

- **Abnormal development of mesonephric (Wolffian) duct structures in the setting of CFTR gene mutation**
- **98% of compound heterozygotes have mesonephric abnormalities**



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Jarow et al, *AUA Best Practice Statement*, rev 2011

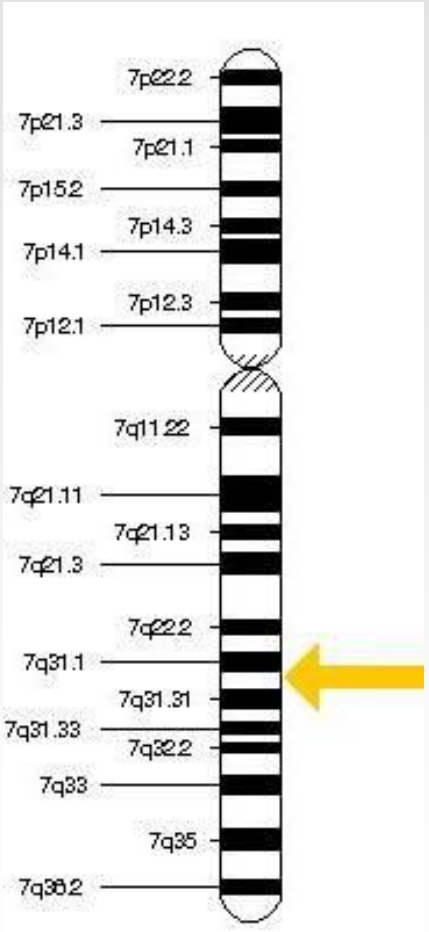
Ratjen and Doring, *Lancet*, 2003

Congenital Bilateral Absence of the Vas Deferens

- Low volume (< 1.0 mL) azoospermia
- Seminal vesicles often aplastic
- Acidic pH, fructose low or negative

CFTR testing

- **Mutation of CFTR gene (chromosome 7) coding for transmembrane chloride ion transport**
  - **Most common mutation = ΔF508**
  - **Almost all males with clinical CF will have CBAVD**
  - **~70-80% of men with CBAVD have no clinical evidence of CF**
  - **Over 1500 possible mutations**
  - **Degree of symptomatology depends upon which mutations are present**



Transmission of Cystic Fibrosis

Female Genotype	Male Genotype: ΔF508	Male Genotype: 5 T (Intron 8)
ΔF508	ΔF508 / ΔF508 Cystic Fibrosis	ΔF508 / 5 T CBAVD
+	ΔF508 / + Carrier	5 T/ + "Carrier"

CBAVD - Summary

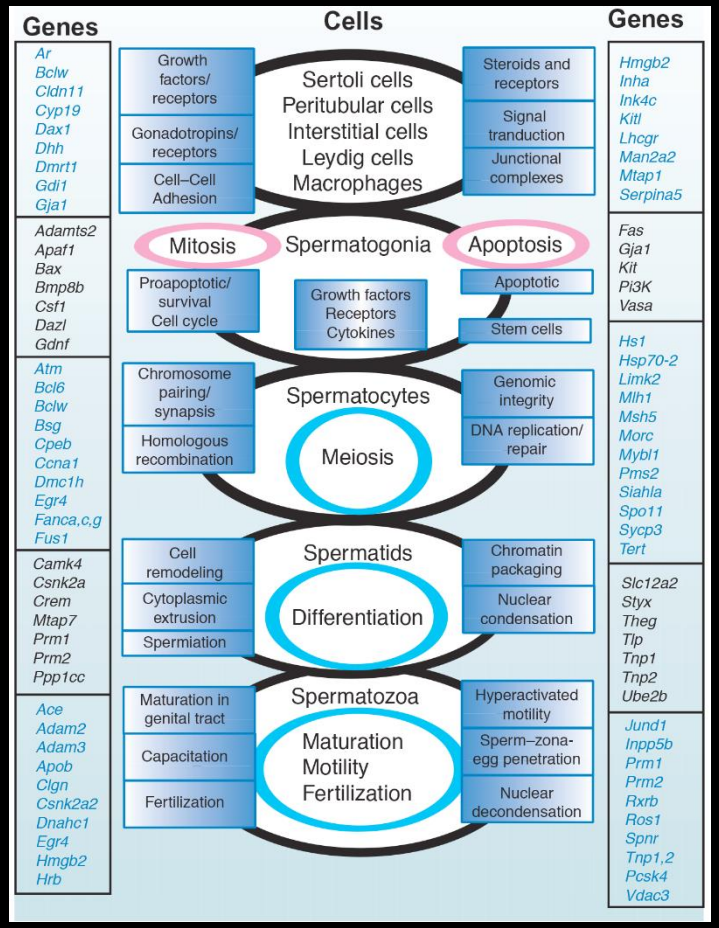
- Accounts for 6% of cases of obstructive azoospermia
- Most common cause = mutations of CFTR gene (chromosome 7) for transmembrane transport chloride ion transport
  - Test for common mutations and polyT mutations in intron 8
  - Almost all males with CF will have CBAVD
  - ~70-80% of men with CBAVD have no clinical evidence of CF
- Diagnosis
  - Physical examination
    - Prominent caput
    - Absent distal 2/3 of epididymis
    - Atrophy/hypoplasia of seminal vesicles
  - Imaging and surgical exploration not necessary to confirm diagnosis

CBAVD - Summary

- Counseling
  - Both partners should undergo genetic counseling and some degree of testing to ascertain future health effects to offspring
  - Failure to identify mutation does not rule it out
- Fertility
  - Normal spermatogenesis, but low semen volume
  - Sperm retrieval via percutaneous or open surgical approach of testis or epididymis
- Renal anomalies
  - Association with unilateral vasal agenesis and ipsilateral renal agenesis (25%)
  - Weaker in bilateral vasal agenesis (10%)

Spermatogenesis Genes

Interactions and Targets



Matzuk MM and Lamb DJ. Nat Cell Biol. 2002 Oct;4 Suppl:s41–9

Evolving

- **Sperm FISH/cell sorting**
- **PGS/PGD for male/paternal origin**

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Advanced Paternal Age and Fertility

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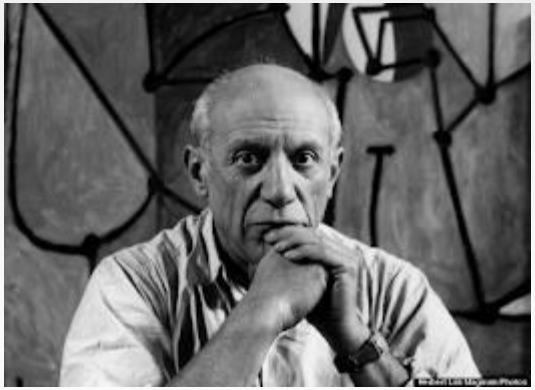
When are the reproductive years?

- Need to ask the patient…he may not be done!
  - e.g. oldest celebrity fathers: Charlie Chaplin (73); Robert DeNiro (68); Pablo Picasso (68)



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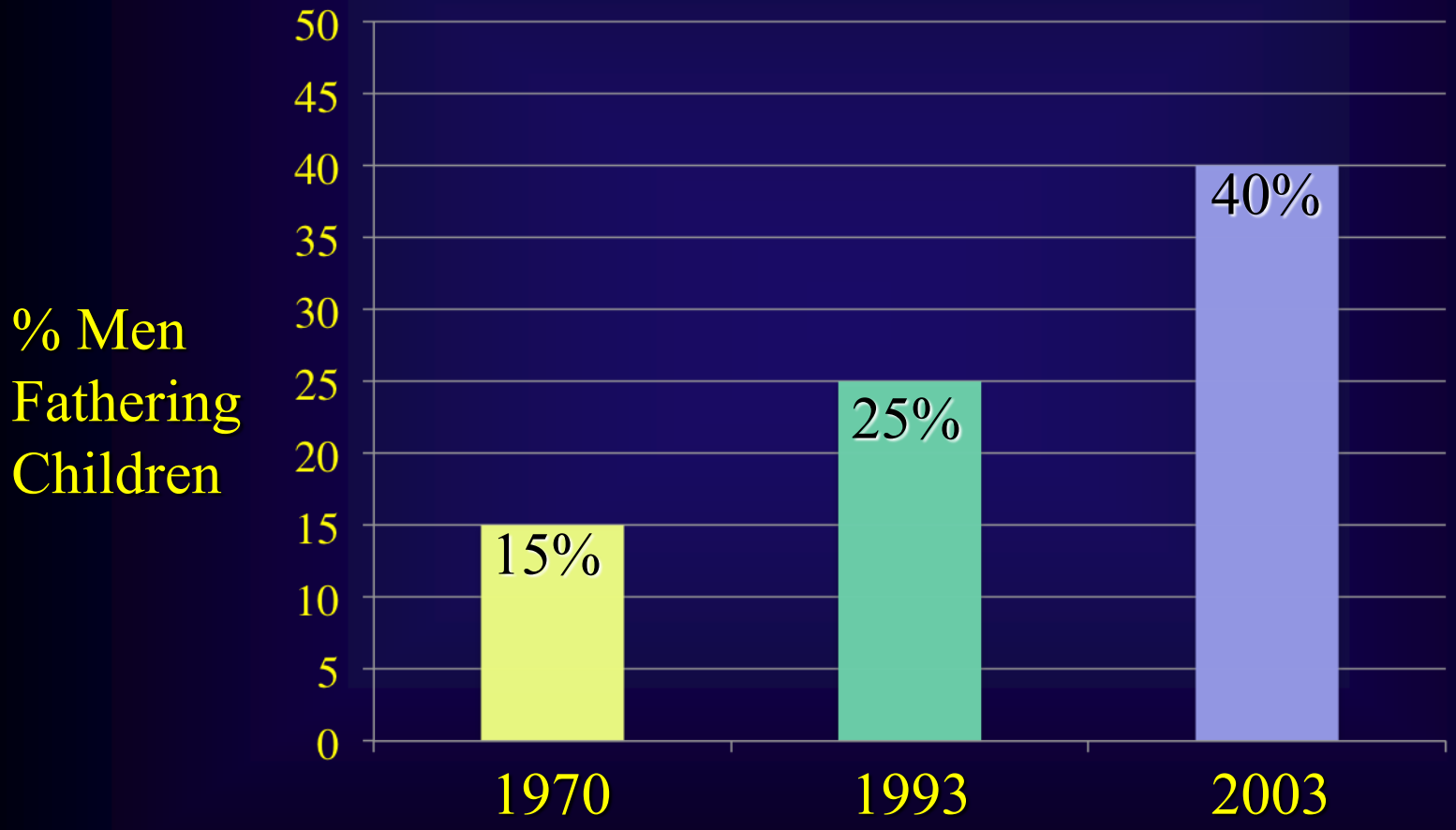
What's Happening to Fathers?

Proportion of fathers over 35 years old in U.K.



Bray I et al. J Epidemiol Comm Health 2006; 60: 851–3

National Health Statistics Reports
Number 51 ■ April 12, 2012

Fertility of Men and Women Aged 15–44 Years in the United States: National Survey of Family Growth, 2006–2010

- **By age 30, 50% of men had fathered their first child.**
- **By age 40, 76%**
- **The average number of children born to married or formerly married men was two .**

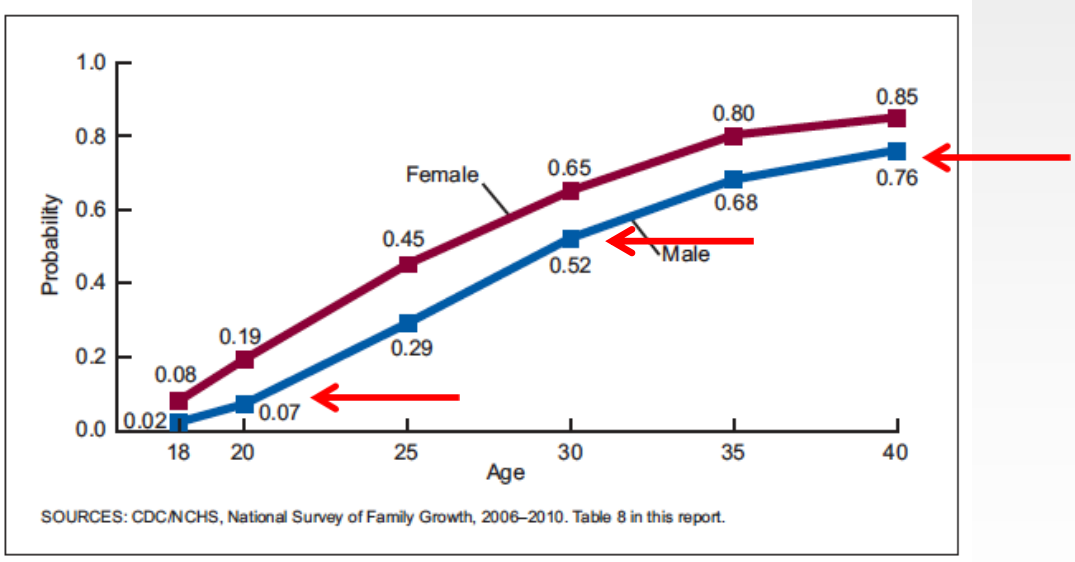


Figure 4. Probability of a first birth, by selected ages for males and females aged 15–44 years: United States, 2006–2010

Martinez G, Daniels K, Chandra A. Natl Health Stat Report. 2012 Apr 12;(51):1-28.

Changes in Testicles and Semen with Age

- Morphometric studies:
  - Leydig cells: fall 80 million/testis/decade
  - Age-related decline in Sertoli cells
  - Decreased germ cell proliferation

Hellstrom et al. J. Androl 2006
Wyrobek AJ. PNAS, 2006; 103:9601

Reduced Semen Quality with Age

- Lower ejaculate volume. Changes in prostate protein and water content
- Increased risk of infection
- Decreased fructose from seminal vesicles
- Sperm motility falls (gradually)
- Concentration changes harder to show

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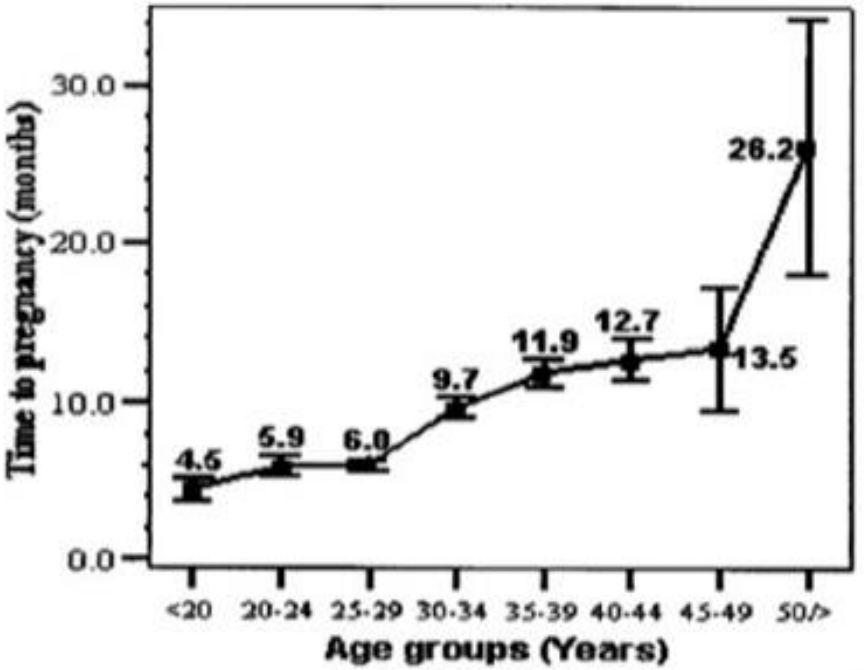
Kuhnert. Hum Reprod Upd. 2004, 10:327-339
Wyrobek AJ. PNAS. 2006, 103:9601

Paternal Age Effects: Achieving Pregnancy

- Studies in non-clinical populations (Irish, Mormon, The Avon Longitudinal Study of Parents and Children [ALSPAC])
- Demonstrate increased time to pregnancy
- Odds ratio (OR) for fertility falls:
  2%/year of age



Kidd S. Fert Steril. 2001, 75:237-48

Ford W. Hum Reprod. 2000, 15:1703-8

Paternal Age Effects: Achieving Pregnancy

Confounders

- Female age
- Erectile and sexual dysfunction
- Coital frequency
- Comorbid conditions

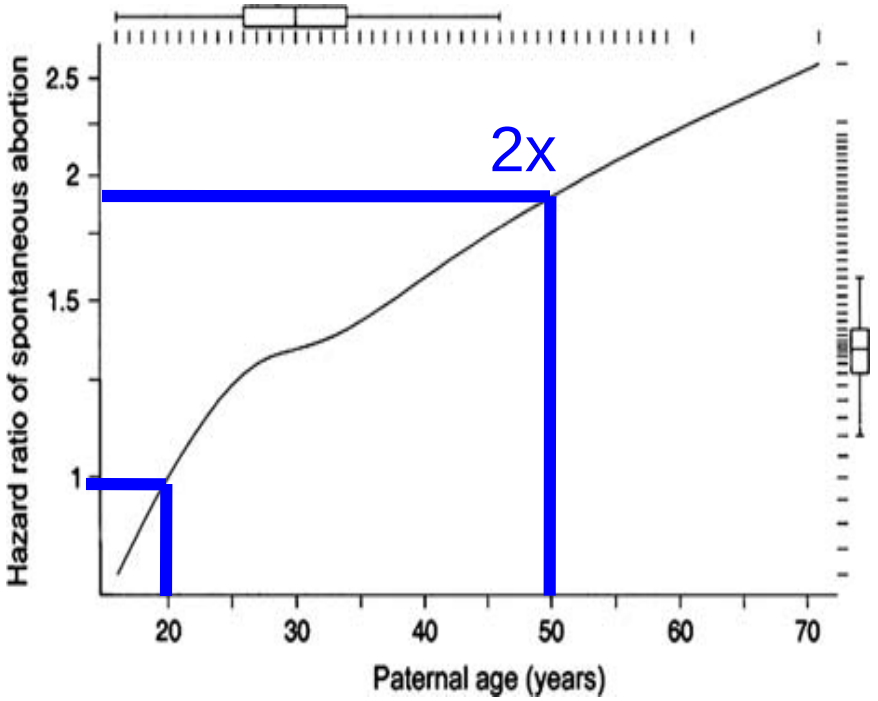
Kidd S. Fertil Steril. 2001, 75:237-48 Ford W. Hum Reprod. 2000, 15:1703-8

Paternal Age Effects: Pregnancy Outcomes

- Miscarriages
- Preterm birth
- Fetal death

Paternal Age Effects: Pregnancy Outcomes

- Miscarriages



- Danish Birth Cohort (n=23,000)
- Adjusted for lifestyle, maternal confounders
- **Fathers >50 yrs old associated with 2x increased risk**

Kuhnert. Hum Reprod Update. 2004, 10:327-339.

Lambert. World J Urol. 2006, 24:611-617.

Paternal Age Effects: Pregnancy Outcome

- Preterm births (<32 weeks)

Country	Years	Maternal Ages (yrs)	Findings
Italy	1990-98	20-29	**OR 1.7** (>45yrs)
Denmark	1986-96	20-29	**OR 2.1** (>50yrs)
USA	1995-2000	20-35	**No effect**

Kuhnert. Hum Reprod Update. 2004, 10:327-339.

Lambert. World J Urol. 2006, 24:611-617.

Paternal Age Effects: Pregnancy Outcome

- Fetal death

Danish study 1997-1999

23,831 births; n=124 with fathers >50 yrs

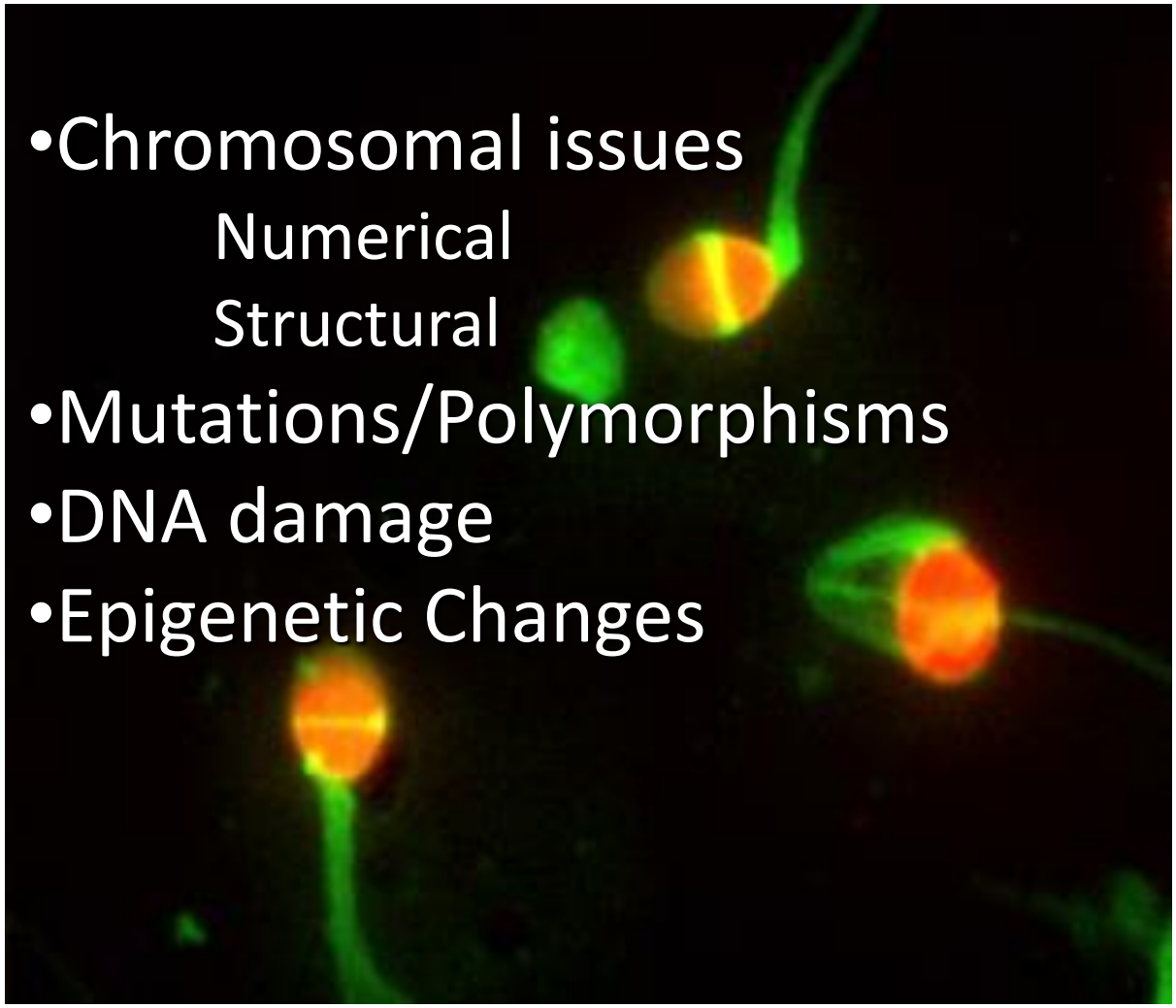
Adjusted for maternal age, lifestyle and reproductive history

Fathers ≥ 50 yrs old associated with **HR 1.88** for fetal death (CI 0.93, 3.82)

HR=hazard ratio
CI=confidence interval

Andersen et al., Am J Epi. 2004, 160: 1214

Paternal Age Effects: Sperm Genetics



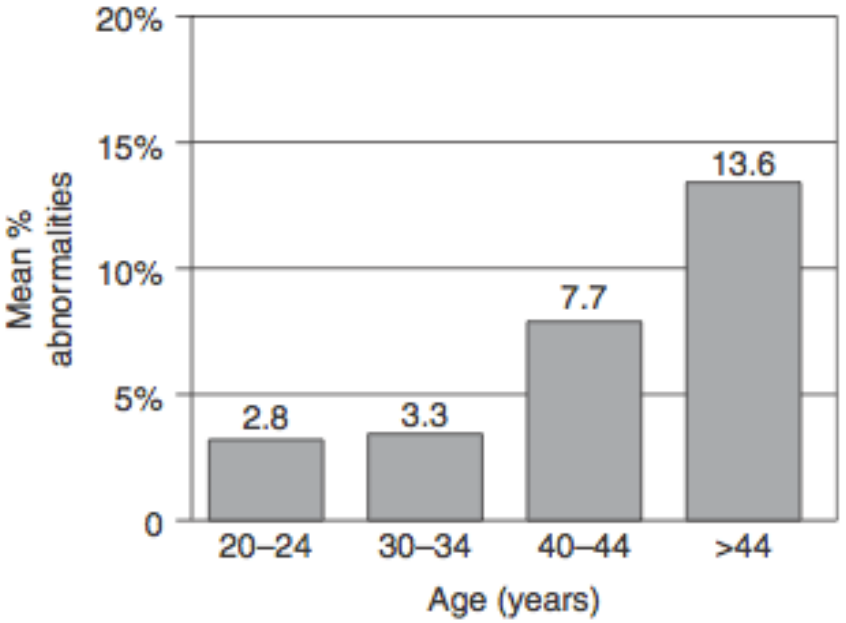
Paternal Age Effects: Sperm Genetics

- Chromosomal Aneuploidies
  - Aneuploidy occurs in 30-50% of all pregnancies
  - Most are lethal
  - Arise from non-dysjunction during meiosis (I and II)
  - Definite increase in aneuploidy in infertile vs. fertile sperm
  - **Autosomal aneuploidy: No consensus on** whether it increases with paternal age
  - **Sex chromosomal aneuploidy and disomy**: Clear evidence that they increase with paternal age (2-3x)
    - **XY diploidy** (meiosis I) and **XX/YY diploidy** (meiosis II)

Sloter et al., Fertil Steril. 2004, 81:925
Templado C. Cytogenet Genome Res. 2003, 111:199-205

Paternal Age Effects: Sperm Genetics

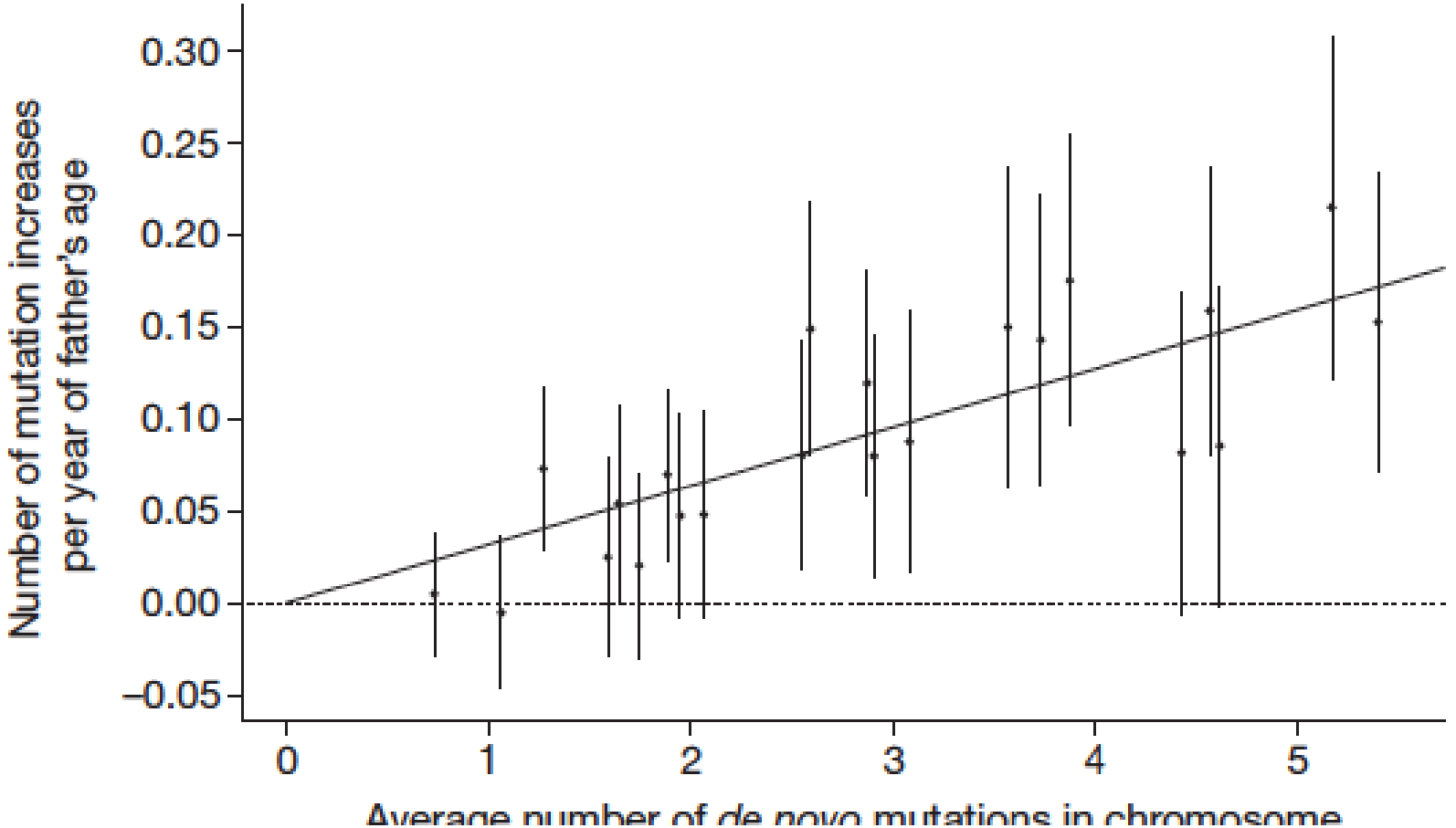
- Chromosomal issues: Structural
  - Comprise **0.25%** of births
  - **Chromosomal breaks & fragments** increase with age
  - Pronounced relationship**: r=0.63**
  - Especially chromosome 1 and acentric fragments
  - **Not evident** in offspring



Sloter et al., Fertil Steril. 2007, 87: 1077
Martin and Rademaker. Am J Hum Genet. 1987, 41: 484

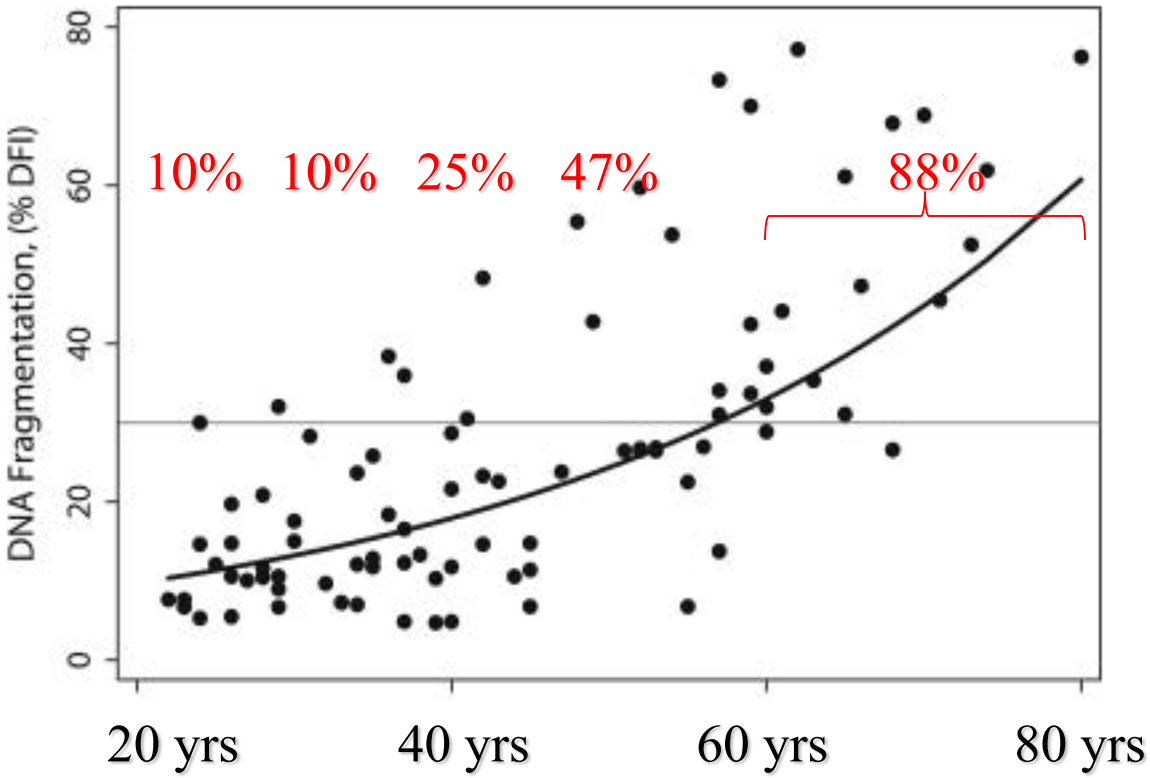
Effect of Paternal Age on de novo Mutations by Chromosome

Kong et al., 2012



Paternal Age Effects: Sperm Genetics

- Sperm DNA fragmentation:



- N=88 healthy non-smokers
- r=0.72; p<0.001
- Predicted change of 3.1%/year of age
- Associated with defective mismatch repair?

Wyrobek et al., PNAS. 2006, 103:9601

Paternal Age Effects: Offspring

- Congenital illness/birth defects
- Diseases

Paternal Age Effects: Offspring

- Congenital illness/birth defects
  - Chromosomal

    General: No increase with paternal age

    Exception: Sex chromosomes **(47,XXY)**

    55% of sex chromosomal aneuploidies are paternal in origin

    Risk with paternal age less clear. **RR 1.3-2.7**[1]

    Agrees with **sperm** sex chromosomal aneuploidy and disomy findings

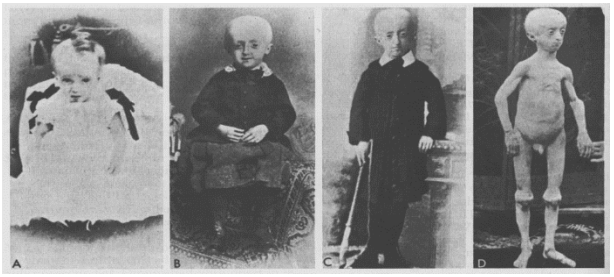
    Trisomy 21: 9% of 352 cases paternal[2]

[1]Toriello and Meck. Genet Med. 2008, 10-457

[2]Zaragoza et al., Hum Genet. 1994, 94:411

Paternal Age Effects: Offspring

- Congenital illness/birth defects
  - Single Gene Mutations: **"Sentinel phenotypes"**
    - **40** mutations; **40** diseases. Selfish gene issue.
    - Debilitating illnesses requiring lifelong care
    - Rare, ranging from **1:10K to 1:1million**
    - Fathers of affected children average **6-7 years older** than fathers of unaffected children
    - Diseases occur **10x more frequently** with fathers >50 yrs old vs. 20-30 yrs old
    - Overall prevalence is still **<1%**
    - Screening not recommended



Single Gene Mutations: Sentinel Phenotypes

Achondroplasias *(FGFR3)*
Apert syndrome *(FGFR2)*
Crouzon syndrome *(FGFR2)*
Hemophilia A
Marfan syndrome *(FGFR3)*
Neurofibromatosis
Oculodentodigital syndrome
Pfeiffer syndrome *(FGFR2)*
Polycystic kidney disease
Progeria
Treacher-Collins syndrome
Tuberous sclerosis

Aniridia
Bilateral retinoblastoma
Fibrodysplasia ossificans
Lesch-Nyhan syndrome
Multiple endocrine neoplasia II (MEN II)
Osteogenesis Imperfecta *(FGFR3)*
Polyposis coli
Thanatophoric dysplasia *(FGFR3)*
Waardenburg syndrome

Paternal Age Effects: Offspring

- Birth defects

Paternal Age (Yrs)	Added Risk
30-35	4%
40-44	8%
45-49	8%
> 50	15%

Comparison: **220%** increase with maternal age >45 yrs

- Pop. based, retrospective, cohort study
- 5.2 million U.S. subjects
- 1999-2000 birth registry
- Examined 22 serious birth defect categories
- Overall rate **1.5%**

- Pop. based, retrospective, cohort study
- U.S. Births from 1997-2004
- Overall rate **increases from 2% to 2.5%**

Green et al., Ann Epid. 2010, 20: 241

Yang et al., Hum Reprod. 2007, 22: 696

Paternal Age Effects: Offspring

- Birth defects: The usual suspects
  - Ventricular septal defects
  - Atrial septal defects
  - Pulmonary stenosis
  - Situs inversus
  - Neural tube defects (spina bifida)
  - Cleft palate
  - Diaphragmatic hernia
  - Tracheoesophageal fistula

Green et al., Ann Epid. 2010, 20: 241

Paternal Age Effects: Offspring

- **Congenital illness/birth defects**
- Diseases

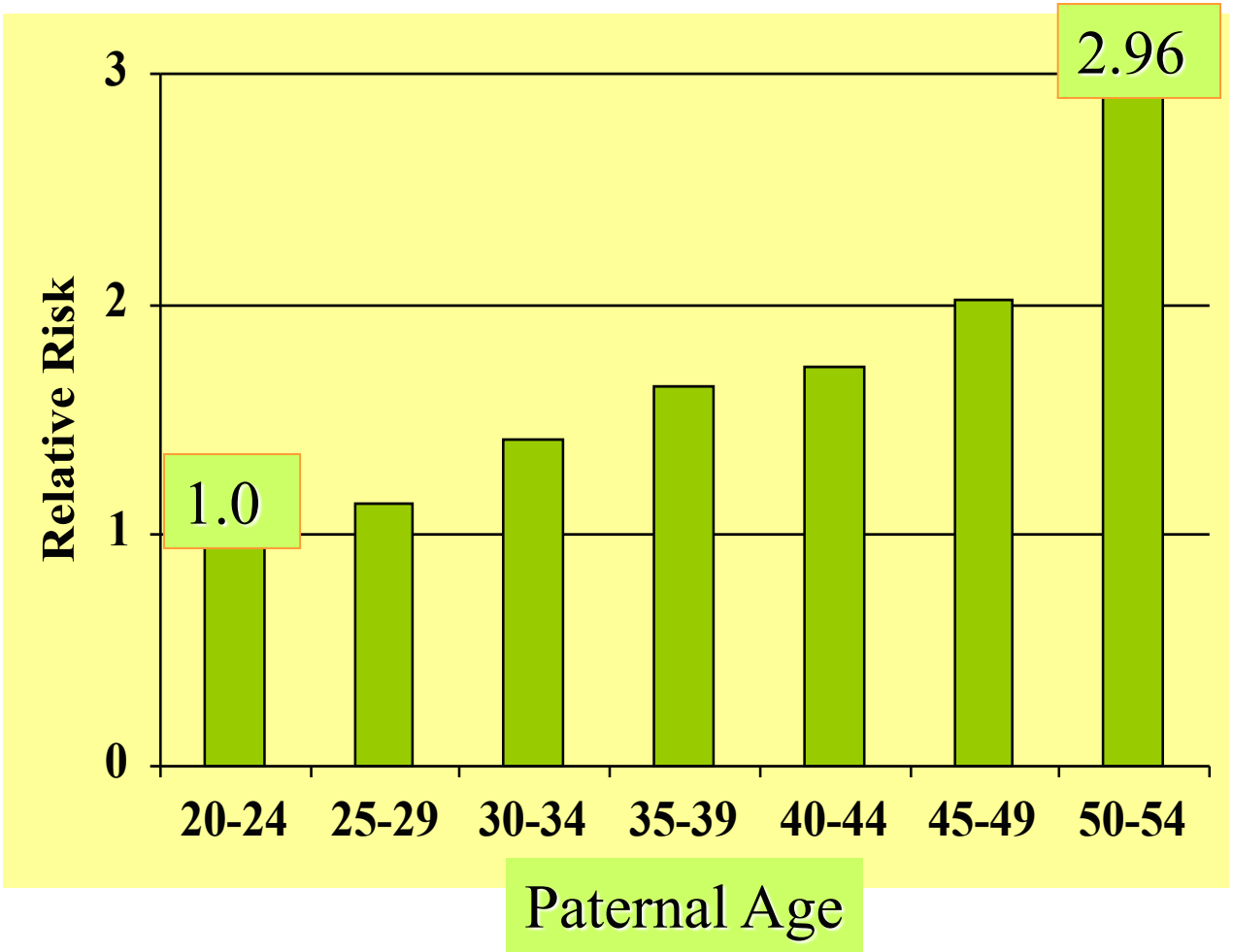
Paternal Age Effects: Offspring

- **Diseases:** Developmental, psychiatric conditions

Condition	Relative Risk
Autism	5.7
Schizophrenia	3 - 4.6
Autism spectrum disorder	1.4
Neurocognitive impairment	1.1
Dyslexia	?
Bipolar disorder	?
Alzheimer disease	?

Paternal Age Effects: Offspring

- Diseases-Schizophrenia



- Israeli registry
- n=87,907 births
- Reproduced in **5** other countries
- DeCode study suggests 20-30% is paternal age related

Malaspina et al. Arch Gen Psych. 2001, 58: 361

Paternal Age Effects: Summary

- The **sperm genome** is altered during aging.
  - Aneuploidy
  - DNA Damage (Breaks)
  - Mutations/Polymorphisms
  - Epigenetic Changes
- Paternal age effects on **offspring** include increased:

Single gene mutations	8-10x
Sex chromosome anomalies	1.3-2.7x
Miscarriages	2x
Preterm birth	1.7-2.1x
Fetal death	1.9x
Birth defects	1.25x
Adult diseases	1.1- 5.7x

- Prevalence rates remain low
- **No changes** to current genetic screening protocols

Evaluation of Methylation in Donor Over Time