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

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The Optimal Evaluation of the Infertile Male: AUA Best Practice Statement

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American Urological Association
Education and Research, Inc.
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
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
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
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)
Implications of Genetic Causes of Male Infertility

- Genetic counseling
- PGD often indicated
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


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

PATHOGENESIS
Non-dysjunction in meiotic 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
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%

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
Karyotypic Abnormalities

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

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

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
Y Chromosome Micro-deletion
Y Chromosome Microdeletions

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

Y Chromosome Microdeletions

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

- Implications for parent
  - Etiology
  - Prognosis
Y Chromosome Microdeletions

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

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

Oats et al Hum Reprod 2002;17:2813.
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%
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%

Kalsi et al BJU Int 2012;109:418
The Next Generation


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

Transmission of DAZ Deletion with ICSI +/- TESE

Affected (XY) - Normal (XX)

- Severe Oligospermia (XY)
- Azoospermia Sperm present (XY)
- Azoospermia Sperm absent (XY)

Potentially fertile with technology  Sterile
Congenital absence of the vas
Vasal Absence

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

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

Male Genotype

ΔF508

ΔF508 / ΔF508
Cystic Fibrosis
ΔF508 / +
Carrier
ΔF508 / 5 T
CBAVD
5 T/ +
“ Carrier”

Female Genotype

ΔF508

+
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

Evolving

- Sperm FISH/cell sorting
- PGS/PGD for male/paternal origin
Advanced Paternal Age and Fertility
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)
What’s Happening to Fathers?

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>15%</td>
</tr>
<tr>
<td>1993</td>
<td>25%</td>
</tr>
<tr>
<td>2003</td>
<td>40%</td>
</tr>
</tbody>
</table>

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

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
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

Ford W. Hum Reprod. 2000, 15:1703-8
Paternal Age Effects: Achieving Pregnancy

Confounders

Female age
Erectile and sexual dysfunction
Coital frequency
Comorbid conditions

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

# Paternal Age Effects: Pregnancy Outcome

- Preterm births (<32 weeks)

<table>
<thead>
<tr>
<th>Country</th>
<th>Years</th>
<th>Maternal Ages (yrs)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>1990-98</td>
<td>20-29</td>
<td><strong>OR 1.7</strong> (&gt;45yrs)</td>
</tr>
<tr>
<td>Denmark</td>
<td>1986-96</td>
<td>20-29</td>
<td><strong>OR 2.1</strong> (&gt;50yrs)</td>
</tr>
<tr>
<td>USA</td>
<td>1995-2000</td>
<td>20-35</td>
<td>No effect</td>
</tr>
</tbody>
</table>

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

- Chromosomal issues
  - Numerical
  - Structural
- Mutations/Polymorphisms
- DNA damage
- Epigenetic Changes
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
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
    Agrees with *sperm* sex chromosomal aneuploidy and disomy findings
    Trisomy 21: 9% of 352 cases paternal

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”
    - 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
<table>
<thead>
<tr>
<th>Single Gene Mutations: Sentinel Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achondroplasias  $(FGFR3)$</td>
</tr>
<tr>
<td>Apert syndrome  $(FGFR2)$</td>
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<tr>
<td>Crouzon syndrome  $(FGFR2)$</td>
</tr>
<tr>
<td>Hemophilia A</td>
</tr>
<tr>
<td>Marfan syndrome  $(FGFR3)$</td>
</tr>
<tr>
<td>Neurofibromatosis</td>
</tr>
<tr>
<td>Oculodentodigital syndrome</td>
</tr>
<tr>
<td>Pfeiffer syndrome  $(FGFR2)$</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
</tr>
<tr>
<td>Progeria</td>
</tr>
<tr>
<td>Treacher-Collins syndrome</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
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<tr>
<td>Aniridia</td>
</tr>
<tr>
<td>Bilateral retinoblastoma</td>
</tr>
<tr>
<td>Fibrodysplasia ossificans</td>
</tr>
<tr>
<td>Lesch-Nyhan syndrome</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia II (MEN II)</td>
</tr>
<tr>
<td>Osteogenesis Imperfecta $(FGFR3)$</td>
</tr>
<tr>
<td>Polyposis coli</td>
</tr>
<tr>
<td>Thanatophoric dysplasia $(FGFR3)$</td>
</tr>
<tr>
<td>Waardenburg syndrome</td>
</tr>
</tbody>
</table>
Paternal Age Effects: Offspring

• Birth defects

<table>
<thead>
<tr>
<th>Paternal Age (Yrs)</th>
<th>Added Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-35</td>
<td>4%</td>
</tr>
<tr>
<td>40-44</td>
<td>8%</td>
</tr>
<tr>
<td>45-49</td>
<td>8%</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>15%</td>
</tr>
</tbody>
</table>

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%

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autism</td>
<td>5.7</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>3 - 4.6</td>
</tr>
<tr>
<td>Autism spectrum disorder</td>
<td>1.4</td>
</tr>
<tr>
<td>Neurocognitive impairment</td>
<td>1.1</td>
</tr>
<tr>
<td>Dyslexia</td>
<td>?</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>?</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td>?</td>
</tr>
</tbody>
</table>
Paternal Age Effects: Offspring

• Diseases - Schizophrenia

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

Inventory of Donor Collections

- Collection 1
- Collection 2

Donor Age (yrs) vs. Collection