Genetic and epigenetic aspects of male infertility:
What should every andrologist know?

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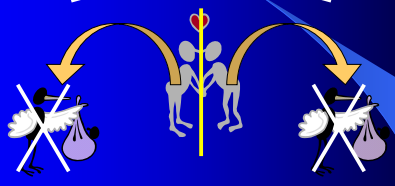
Layout of course

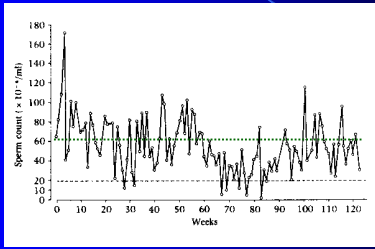
- Basic aspects of male infertility
  - Important for study design
- Genetics
  - Numerical / structural chromosome abnormalities
  - Single gene disorders
  - Y-chromosome aberrations
  - Others?
- Epigenetics
- Summary

~~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)

Patient definition

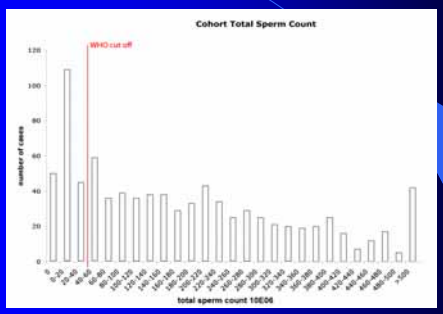
- Men with reduced semen quality
  - volume <2 ml,
  - and/or <20x10^6 /ml spermatozoa, Oligozoospermia
  - and/or <40x10^6 spermatozoa in total,
  - and/or <30% spermatozoa with normal morphology,
  - and/or <50% motile spermatozoa
- Known causes of spermatogenic failure excluded

WHO Manual (1992/1999)

Control definition

- Often used in literature
  - Proven fertile fathers
    - Non-paternity excluded?
    - (severely) oligozoospermic men can father children
  - Vasectomy reversals
    - Pre-operative sperm count unknown
  - Population controls
- Only correct control group
  - Men with proven normal spermatogenesis
- But great variation.....

Sperm production variation

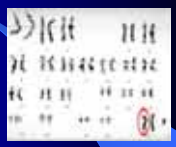


Spermatogenesis is a quantitative trait and many (genetic) factors control the rate of sperm production

Evidence of genetic causes

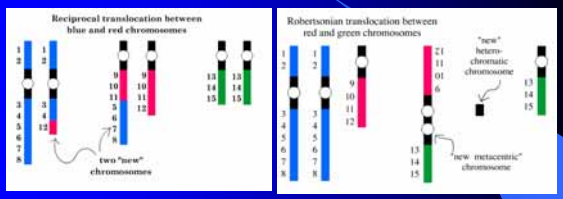
- Karyotype abnormalities
  - Klinefelter syndrome (47, XXY)
  - Translocations
- Single gene disorders
- Y-chromosome aberrations

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

Translocations



- Meiotic disturbance

Egozcue, et al. HumRepUpdate (2000)

Single gene disorders

- *CFTR* mutations
  - Obstructive azoospermia (CBAVD)
- Sporadic mutations
  - *FSH receptor*
  - *USP9Y*
  - *NALP14*
  - *SYCP3*
  - *SPATA16*

Tapanainen, et al., Nat. Genet. (1997), Sun, et al., Nat. Genet. (1999), Miyamoto, et al., Lancet (2003), Westerveld, et al., HumRep (2006), Dam et al. AJHG (2007)

Y chromosome and male fertility

- 1976 Microscopic deletions
  - AZoospermia Factor (AZF) on Yq
    Tiepolo and Zuffardi, Hum Genet (1976)
- 1995 AZF candidate-gene
  - Deleted in AZoospermia (*DAZ*) on Yq11-23
    Reijo, et al., Nat Genet (1995)
- 1996 Three non-overlapping deletion intervals
  - *AZFa, AZFb, AZFc*
    Vogt, et al., Hum Mol Gen (1996)

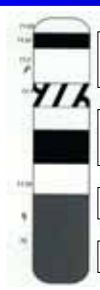
Complete sequence of the Y

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

Skaletsky, et al., Nature (2003)

Genes on the Y-chromosome

- male specific -



Inability to express affection over the telephone (ME TOO)

Refusal to ask for directions when lost (anti-STOP)

pseudo Self-Confidence (ΨSC)

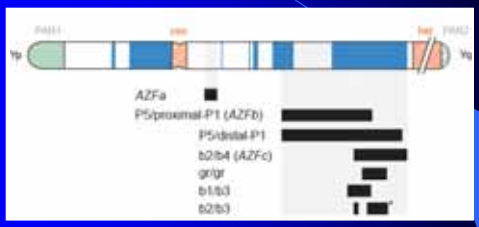
Air guitar (AIR)

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

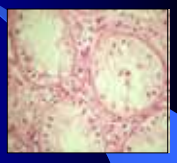
Skaletsky, et al., Nature (2003)

Y-chromosome deletions



Noordam & Repping, COGD (2006)

AZFa deletions



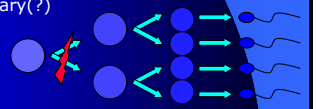
- very infrequent
  - only few cases reported
- remove 2 single copy genes
- 800 kb in size
- phenotype: Sertoli Cell Only
  - no germ cells → no ICSI
  - no testis biopsy necessary(?)

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)

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

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

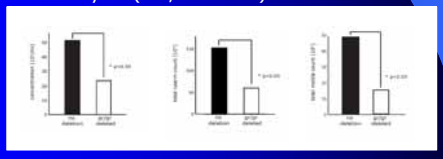
gr/gr deletions

- 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
  - High frequency in Japan
- Variable phenotype
  - not always *de novo* (natural transmission)
  - risk factor for spermatogenic failure

Repping, et al., Nat Genet (2003)

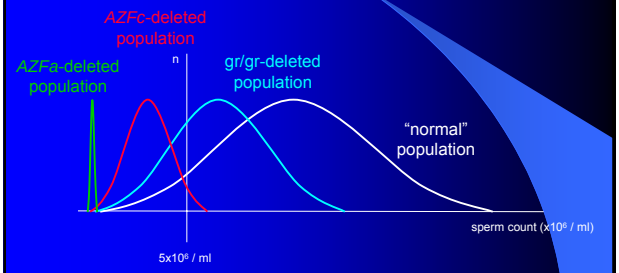
Phenotypic effect gr/gr deletions

- Conflicting results in literature
  - Many studies of poor design
- Meta analysis shows an increase of gr/gr deletions in patients (OR 2.77 / 95% CI 1.61 – 4.77)
- Cohort analysis (>1,000 men)



Visser, et al. Poster PS8, Florence 2007

Phenotypic effect

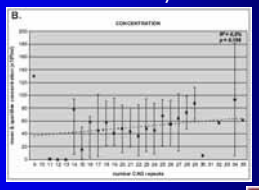


The search for others...

- A lot of research on genetics
  - PUBMED: [male infertility AND genetics] 7-11-2007
    - 3522 Hits
- Numerous mouse candidate genes
  - *Pog, at/at, c-kit, Pin1, Scf, Gja1, Eif2s3y, Cit-k, Dazl, Gdnf, Plzf, bclxl, bax, p27, stra8, Msh5, Atm, scid, dmc1, spo11, Mlh1, Mlh3, Cks2, Bsg, Siah 1a, Crem, sys, hop, Trf2, miwi, Hrb, Tlf, Gopc, azh, Hr6b, Csnk2a2*
- Numerous association studies in humans
  - Pro185Ala, POLG, androgen receptor, (MTHFR) C677T, DAZL, DQB1*0604, USP26 …
- But very few established causes

Associations revisited
CAG repeat in the androgen receptor

- Increase in number of CAG repeats associated with spermatogenic failure
  - Many conflicting results
- Genotype driven cohort analysis:



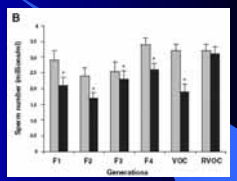
Tut, et al., JCEM (1997), Westerveld, et al., Fertil.Steril. (2007)

Epigenetics

- Limited research on epigenetics
  - PUBMED: [male infertility AND epigenetics]
    - 51 Hits (of which 20 are reviews)
- Some reports show an increase in frequency of imprinting disorders in ICSI offspring
    - But also in IVF (effect of culture?)
- Methylation seems to be complete before meiosis is finished
  - Spermatozoa used for ICSI / TESE
- Endocrine disruptors can affect imprinting
  - epigenetic effects can be transgenerational

Mol, et al., Lancet (2003), Gosden, et al., Lancet (2003)
Hartmann, et al., MolHumRep (2006)

Transgenerational effect



Anway, et al., Science (2005)

What should you remember?

- Study spermatogenic failure rather than male infertility
  - Male infertility ≠ spermatogenic failure and vice versa
- Spermatogenesis is a quantitative trait
  - Cohort design preferential
- Limited genetic causes established
  - Chromosome abnormalities
  - Single gene effect
  - Y-chromosome deletions
    - Variable phenotype (from sterility to risk factor)
- Role of epigenetics seems limited
  - Requires further research

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