MALE INFERTILITY AND
SURGICAL SPERM RETRIEVAL / ICSI

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MALE INFERTILITY

- 80 millions infertile patients (WHO)
- 1/6 couples are infertile
- 1/10 in ART
- Up to 50% “male” factor
- 2-10% “infertile men”
- 10% infertile men with <1M/ml
- 10-15% infertile men with azoospermia (1% among all men)
- France: 30 000 ICSI / 1800 surgical sperm retrieval

De Kretzter DM, Lancet 1997

Anamnesis

- Relevant medical history
- Lifestyle and environment

Physical examination

- BMI
- Testis
- Complete clinical examination

Ultrasonography

- Color flow Doppler ultrasonography
- Transrectal ultrasonography
- Scrotal ultrasonography
- Renal tract evaluation

Hormones

- Serum total testosterone
- FSH, LH, PRL
- Serum inhibin B, AMH

EVALUATION

Sperm

- ≥ 2 ejaculates – 3 months
- No sperm after centrifugation
- Microbiological evaluation
- Seminal biochemical markers

Genetics

- Karyotype
- Yq microdeletion
- CFTR mutations
- FISH on gametes

Surgical exploration
Diagnosis
MESA/TESE
**EVALUATION**

**Anamnesis**
- Prior fertility
- Childhood illnesses and disorders such as viral orchitis; cryptorchidism
- Genital trauma or pelvic or inguinal surgery
- Infections such as epididymitis or urethritis
- Exposure to gonadotoxins such as radiation or chemotherapy, recent fevers or heat exposure or current or recent medications
- Family history of birth defects, mental retardation, reproductive failure, or cystic fibrosis

**Physical examination**
- Presence of inguinal or scrotal scars
- Testis size (normal volume > 19 mL) and consistency
- Secondary sex characteristics including body habitus, hair distribution, and gynecomastia
- Presence and consistency of the vasa deferentia
- Consistency of the epididymes
- Presence of varicoceles
- Masses palpable on digital rectal examination

**Measurement of selected hormones**

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>FSH</th>
<th>LH</th>
<th>Testosterone</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal spermatogenesis</td>
<td>High</td>
<td>High</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal spermatogenesis</td>
<td>High</td>
<td>High</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal spermatogenesis</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal spermatogenesis</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
<td>Normal</td>
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<td>Normal</td>
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</tr>
<tr>
<td>Normal spermatogenesis</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal spermatogenesis</td>
<td>Low</td>
<td>Low</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Azoospermia or cryptozoospermia?

- Extended sperm preparation with careful examination of the pellet droplets ESP
- Surgical sperm retrieval SSR
- Two semen analyses performed at least 3 months apart

### TABLE 1
Summary of findings of extended sperm preparation (ESP) in azoospermic men.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Present study</th>
<th>Rue et al. 1997</th>
<th>Teun et al. 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>57</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>Spermatozoa men, %</td>
<td>18 (31%)</td>
<td>11 (19%)</td>
<td>10 (25%)</td>
</tr>
<tr>
<td>Spermatozoa women, %</td>
<td>16.6</td>
<td>16.6</td>
<td>15 (21%)</td>
</tr>
</tbody>
</table>

Note: n/a = not available.

Pre-testicular causes (rare): secondary testicular failure
- Endocrine abnormalities: hypogonadotropic hypogonadism

Testicular causes: primary testicular failure
- External factors
- Infection
- Malformation
- Genetics
- Idiopathic male infertility...

Post-testicular causes (40%): Correctable
- Ejaculatory dysfunction
- Ductal obstructions

**CAUSES....**

Pre-testicular causes (rare): secondary testicular failure

Testicular causes: primary testicular failure

- External factors
- Infection
- Malformation
- Genetics
- Idiopathic male infertility...

Post-testicular causes (40%): Correctable
- Ejaculatory dysfunction
- Ductal obstructions

**External factors**

Microscopic level:
- Concentration
- Motility
- Morphology

Molecular level:
- Aneuploidy
- Oxidative stress
- DNA fragmentation

**Drugs**

- Endocrine disruptors
- Smoking
- Alcohol
- Recreational drugs
- Genital heat stress
- Psychological stress
- Cellular telephone use
- Weight and nutrition
- ....
Among couples in ICSI for male infertility: 17% with genetic male factor

100 azoospermic patients:
- 29% genetic anomaly (karyotype, CFTR, Y microdeletion)
- 22% external factor or illness
- 27% cryptorchidism
- 22% idiopathic

Human spermatogenesis: > 4000 genes!

AZOOSPERMIA

13%: chromosomal anomalies
   (21 X male newborn)

93%: sex chromosomal abnormalities

47,XXY Klinefelter syndrome 67%
   and mosaic 33%
   Structural defect X and Y
   Men 46,XX 7%

Abnormal karyotypes of the azoospermic male
   Gonadal failure and genitale

Meschede et al, 1996
Fedder, 2004
OLIGOZOOSPERMIA

4.6%: chromosomal anomalies
(7 X male newborn)

- 67% autosomal translocation and inversion
  - Robertsonian T 35%
  - Eciprocal autosomal T 16%
- 33% sex anomalies
  - KS 12%
  - 47, XYY 8%

Mau-Holzmann, 2005. Cytogenet Genome Research

Robertsonian translocation
45,XX,der(13;14)(q10;q10)
**Yq MICRODELETIONS**

- Non obstructive male infertility (de novo)
- Azoospermia AND < 1 M SPZ/ml
- FSH high or normal
- 10-15% azoospermia, 5-10% oligozoospermia

**Yq MICRODELETIONS**

- Correlation genotype – sperm concentration

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of men</th>
<th>O</th>
<th>D</th>
<th>D+ and T+</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZFa</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AZFa-1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AZFa+</td>
<td>48</td>
<td>22 (45.8%)</td>
<td>16 (33.3%)</td>
<td>2 (4.2%)</td>
</tr>
<tr>
<td>AZFa+A</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AZFa+A+</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>69</td>
<td>50 (72.4%)</td>
<td>20 (29.1%)</td>
<td>3 (4.3%)</td>
</tr>
</tbody>
</table>

CA: cryptorchidism

- Correlation genotype – testicular histology

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of patients</th>
<th>Testicular histology/testericular sperm retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZFa</td>
<td>3</td>
<td>0/0</td>
</tr>
<tr>
<td>AZFa-1</td>
<td>1</td>
<td>0/0</td>
</tr>
<tr>
<td>AZFa+</td>
<td>48</td>
<td>0/0</td>
</tr>
<tr>
<td>AZFa+A</td>
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<tr>
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<td>1</td>
<td>0/0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>69</td>
<td>0/0</td>
</tr>
</tbody>
</table>

**References**

- Krausz and Degl’Innocenti 2006
- Patrat et al., 2008
ICSI can be performed but risk of transmission of the Y deleted chromosome to the offspring

- More nullisomic gametes for sex chromosomes
  - Turner syndromes
- Among 12 46,XY/45,X men : 3 AZFc deletions

Y CHROMOSOME INSTABILITY

PGD Sex selection?

Patsalis et al. Lancet 2002; Siffroi et al., 2000

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Future fertility

Possible decline in spermatogenesis over time in AZFc deleted men:

- Patient : sperm cryopreservation for future fertility!
- ICSI - conceived sons : follow-up and sperm cryopreservation in early adulthood?

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BILATERAL CONGENITAL ABSENCE OF VASA DEFERENTIA

- 1-2% male infertility
- 99% if cystic fibrosis
- 25% OA
  - CF (AR) 1:2500 birth incidence
  - BCAVD in isolation

CFTR* mutations

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* Cystic fibrosis transmembrane conductance regulator (CFTR) gene
* Positive detection in 50 - 80% of men with BCAV (4% general pop.)
Atrophy during the fetal life of the Wolffian duct derivatives (seminal vesicles, ejaculatory ducts, vasa, epididymal body/tail)

- OA with normal testis volume, thin/absent scrotal vasa
- Ejaculate: low volume, low fructose, acidic ejaculation
- Renal tract anomalies in 10% of BCAV patients

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**SPERM RETRIEVAL**

**INDICATIONS**

<table>
<thead>
<tr>
<th>AZOOSPERMIA</th>
<th>CRYPTOZOOSPERMIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non obstructive NOA C/A</td>
<td>NECROZOOSPERMIA</td>
</tr>
<tr>
<td>Obstructive OA C/A</td>
<td>IMMOTILE SPERMATOZOA / KARTAGENER</td>
</tr>
</tbody>
</table>

DNA FRAGMENTATION

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**SPERM-RETRIEVAL**

**TECHNIQUES**

- Percutaneous epididymal sperm retrieval (PESA)
- Percutaneous biopsy
- Testicular sperm extraction (TESE)
SPERM-RETRIEVAL TECHNIQUES

SURGICAL SPERM RETRIEVAL

- Transport in culture medium
- Measurement and washing

SURGICAL SPERM RETRIEVAL

- Dilaceration
- Extraction
- Centrifugation

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**Table 1:** Advantages and disadvantages of sperm retrieval techniques.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESA</td>
<td>High clinical pregnancy rate</td>
<td>Requires microsurgical expertise, increased cost, increased risk of infection, increased risk of hematomas, increased risk of infection, increased risk of infection.</td>
</tr>
<tr>
<td>TESA</td>
<td>No microsurgical expertise required</td>
<td>Large number of sperm retrieved, low number of sperm retrieved, increased risk of infection, increased risk of infection, increased risk of infection.</td>
</tr>
<tr>
<td>PESA</td>
<td>No microsurgical expertise required</td>
<td>Local anesthetics, increased risk of infection, increased risk of infection, increased risk of infection, increased risk of infection.</td>
</tr>
<tr>
<td>Prolapse, TESA, TESA-TESE</td>
<td>Risk of hemorrhage, increased risk of infection, increased risk of infection, increased risk of infection, increased risk of infection.</td>
<td></td>
</tr>
</tbody>
</table>

Note: MESA = microsurgical epididymal sperm aspiration, PESA = percutaneous epididymal sperm aspiration, TESA = testicular sperm aspiration, TESE = testicular sperm aspiration, TESA-TESE = testicular sperm aspiration followed by testicular sperm extraction.
**OBSTRUCTIVE AZOOSPERMIA**

### EPIDIDYMAL/TESTICULAR?

View image of slide.

- Better results with epididymal sperm!

- **Buffat et al., 2007; FIVNAT**

- Better results with testicular sperm!

- **Dozortsev et al., 2006**

<table>
<thead>
<tr>
<th>N = 171 OA (368 ICSI) Testicular Epididymal</th>
<th>Fertilization</th>
<th>Clinical pregnancy</th>
<th>Spontaneous abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization %</td>
<td>51.9</td>
<td>58.9</td>
<td>35</td>
</tr>
<tr>
<td>Clinical pregnancy %</td>
<td>24.3</td>
<td>22.1</td>
<td>12</td>
</tr>
<tr>
<td>Spontaneous abortion %*</td>
<td>12.5</td>
<td>7</td>
<td>12.5</td>
</tr>
</tbody>
</table>

### NON OBSTRUCTIVE AZOOSPERMIA

View image of slide.

- Testis volume
- Serum FSH, inhibin B, AMH
- Seminal inhibin B, AMH

**CONSENSUS:**

- No ideal marker!
- Combined markers

**TABLE 1**

<table>
<thead>
<tr>
<th>Testis volume</th>
<th>Seminal inhibin B concentration</th>
<th>Seminal AMH concentration</th>
<th>Serum FSH</th>
<th>Serum inhibin B</th>
<th>Combined markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4.7 ± 5.7</td>
<td>9.64 ± 30.94</td>
<td>11.4 ± 7.3</td>
<td>62.0 ± 35.8</td>
<td>35 (10-70)</td>
</tr>
<tr>
<td>Negative</td>
<td>33.3 ± 24.6</td>
<td>3.3 ± 6.7</td>
<td>28.5 ± 14.0</td>
<td>22.2 ± 13.0</td>
<td>12 (5-35)</td>
</tr>
</tbody>
</table>

**Note:** Mann-Whitney-U test for comparison between groups. Fertilization rate, implantation rate, mean, SEM.

**CRYO-TESE-ICSI: CRUDE CUMULATIVE RATE**

<table>
<thead>
<tr>
<th>N sperm/straw</th>
<th>Sertoli Cell Only</th>
<th>Maturation arrest</th>
<th>Hypospermatogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° sperm/straw</td>
<td>8 (10)</td>
<td>15 (3-50)</td>
<td>25 (10-60)</td>
</tr>
<tr>
<td>Fertilization rate %</td>
<td>61%</td>
<td>40%</td>
<td>49%</td>
</tr>
<tr>
<td>Implantation rate %</td>
<td>17.1</td>
<td>25.3</td>
<td>24.0</td>
</tr>
</tbody>
</table>

**Dozortsev et al., 2006; De Croo et al., 2000**
### Y MICRODELETION

Table 5: Outcomes of ICSI cycles in couples with Y microdeletion

<table>
<thead>
<tr>
<th>Study</th>
<th>Couples (n)</th>
<th>ICSI cycle (n)</th>
<th>Pregnancy (%)</th>
<th>Live birth (%)</th>
<th>PFS per cycle (%)</th>
<th>Live birth per cycle (%)</th>
<th>Children born (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutkowska et al. (1995)</td>
<td>3</td>
<td>6</td>
<td>3 (100%)</td>
<td>1 (16.6%)</td>
<td>1 (16.6%)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Vassiliades et al. (2001)</td>
<td>8</td>
<td>19</td>
<td>1 (5.3%)</td>
<td>1 (5.3%)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collias et al. (2003)</td>
<td>26</td>
<td>49</td>
<td>12 (24.5%)</td>
<td>10 (20.4%)</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chai et al. (2004)</td>
<td>17</td>
<td>37</td>
<td>8 (21.6%)</td>
<td>7 (18.9%)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith et al. (2005)</td>
<td>16</td>
<td>40</td>
<td>7 (17.5%)</td>
<td>3 (7.5%)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Our study</td>
<td>23</td>
<td>42</td>
<td>13 (30.9%)</td>
<td>8 (19%)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TOTAL:** 93 192 48 36 48

*All ages for partner, ICSI delivery rates, including clinical pregnancies obtained after single embryo transfers.*

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### KLINEFELTER SYNDROME

### CRYO-ONCO-TESE-ICSI

**CASE REPORT**

**A SINGLE CANCEROUS TESTIS AND AZOOSPERMIA**
CRYPTOZOOSPERMIA

- Ejaculated/Testicular (NOA)
  - Better results in TESE
  - Bendikson et al., 2008

- Poor embryo quality and repeated implantation failures
  - Better results in TESE
  - Weissman et al., 2008

DNA FRAGMENTATION

- Ejaculated vs Testicular sperm (NOA)
  - Better results in TESE
  - Greco et al., 2005

IMMOTILE SPERMATOZOA

- HOS Test
  - Similar results in TESE
  - Westlander et al., 2003; Kaushal et al., 2007
CHILDREN

Patients ? Malformations ?

- 252 TESE (227) AND MESA (25)
  No influence of sperm !
  Ludwig et al, 2003

- 412 TESE (318) and MESA (94)
  More girls 45.4% vs 53.1% IVF (P < 0.005)
  Ludwig et Katalinic, 2002 ; Fedder et al, 2007

- 737 TESE (195) and MESA (542)
  More malformations and chromosomal abnormalities TESE
  6.48% vs MESA vs 2.38% vs ICSI 3.17% (p<0.0001)
  Bajirova et FIVNAT et al, 2001

Major malformations (%)

<table>
<thead>
<tr>
<th>Study</th>
<th>TESE</th>
<th>MESA</th>
<th>EJACULATED ICSI</th>
<th>EJACULATED IVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonduelle et al</td>
<td>6/16</td>
<td>4/16</td>
<td>8/16</td>
<td>13/255</td>
</tr>
<tr>
<td>Kallen et al</td>
<td>3/147</td>
<td>3/133</td>
<td>4/133</td>
<td>139/1428 (p&lt;.05)</td>
</tr>
<tr>
<td>Ludwig and Katalinic</td>
<td>3/122</td>
<td>1/26</td>
<td>2/26</td>
<td>284/19156 (p&lt;.05)</td>
</tr>
<tr>
<td>Palermo et al</td>
<td>1/67</td>
<td>4/53</td>
<td>4/53</td>
<td>33/1774 (p&lt;.05)</td>
</tr>
<tr>
<td>Wernersdott et al</td>
<td>3/31</td>
<td>3/16</td>
<td>3/16</td>
<td>33/554 (p&lt;.05)</td>
</tr>
<tr>
<td>Our study</td>
<td>3/176</td>
<td>0/26</td>
<td>105/1238 (p&lt;.05)</td>
<td>144/437 (p&lt;.05)</td>
</tr>
</tbody>
</table>

Woldringh et al, Aout 2009

Malformations
CHROMOSOMAL ABNORMALITIES IN MISCARRIAGES

- No difference ART (63.2%) versus natural conception (71.5%)
- No difference ICSI (61.5%) versus IVF (54.5%)
- ICSI TESE (80% abnormal) : 50% tri / tetraploidy

- immature diploid sperm ?
- incorrect oocyte activation ?
- incorrect oocyte maturation ?
- post-zygotic abnormality ?

CHROMOSOMAL ABNORMALITIES IN MISCARRIAGES

- Bettio et al, 2008

EPIGENETIC RISK?

- Epigenetic alterations of IgF2 – H19 in spermatozoa from infertile men
  - Genomic imprinting in disruptive spermatogenesis

- DNA methylation errors at imprinted loci after ART originate in the parental sperm

- Direct inheritance from the father’s sperm : a source of imprinting error in ART

- Marques et al, 2004 et 2008; Kobayashi et al., 2007; 2009; Chalas et al., 2010
THANKS !