Non-Obstructive Azoospermia

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DISCLOSURE

- Lilly (consultant, legal)
- Thesan (consultant, toxicity)
- Viamet (consultant, toxicity)
- Merck (consultant, legal)
- Abbvie (consultant, legal)
- Regeneron (consultant, toxicity)
- Theralogix (nutraceuticals; medical advisory board)

LEARNING OBJECTIVES

- To describe appropriate evaluation of men with non-obstructive azoospermia
- To identify the potential of men with azoospermia to have cryptozoospermia and avoid surgery
- To appreciate and discuss the effect of sperm morphology on results of ICSI for men with non-obstructive azoospermia
**Treatable abnormalities in men**

- Mechanical problems (hypospadias, impotence)
- Hormonal abnormalities
- Infections
- Autoimmune (antibodies)
- Toxic factors (heat, gonadotoxins)
- Varicoceles
- Testicular failure (sperm retrieval)
- Obstruction (microsurgical reconstruction, TUR)

**Non-obstructive azoospermia**

- Sperm production so impaired that no sperm appear in the ejaculate.
- Small testicular volume
- Markedly elevated FSH (nl FSH <8 IU/L)

- Exceptions
  - Maturation arrest (nl testes, nl FSH)
  - Sertoli cell-only (some with nl testes, FSH)
Genetics
What are the causes of NOA?

- Karyotype
- Y chromosome microdeletions
- Other (gene) defects (currently “idiopathic”)
- Insults to the testis
  - Chemotherapy
  - Cryptorchidism/devascularization

Use of Y chromosome maps for microdeletion testing

AZFa
A=Awful
AZFb
B=Bad
AZFc
C=Cool

AZF deletions & sperm retrieval

- AZFa deletion: 0% sperm retrieval (all SCO)
- AZFb deletion (complete) or AZFb/c:
  - 0% sperm retrieval rate
    - Hopps et al., Human Reprod 18:1660, 2003
- AZFc deletions: % of men with AZFc deletions

- Sperm in ejaculate
- Sperm found by microTESE
- No sperm
Can you optimize sperm production for men with NOA?

- Low testosterone – can hormonal intervention improve sperm production?
- Treatment of conditions that can affect sperm production - role of varicocelectomy
- Men with azoospermia commonly have rare sperm detected in the ejaculate with more careful semen analysis (Extended sperm prep)

Ron-El et al., Hum Reprod 12:1222, 1997

Low serum testosterone

- Low serum testosterone:
  - Low production
  - Excess metabolism
- Aromatization
  - Testosterone → Estradiol
  - Measured by T/E₂ ratios
  - Lower T/E in aging, testicular failure/infertility

Klinefelter Management

- Men with Klinefelter syndrome (KS):
  - Baseline sperm retrieval: 68-73%
  - On testosterone replacement: Testic T level low
  - Sperm retrieval on/after T replacement: 25%
- Stop exogenous T or add hCG
  - If exogenous T used, evaluate 17-OH Progesterone
- If total testosterone <250 ng/dL
  - Rx aromatase inhibitor (testolactone, anastrozole)
  - If inadequate response, add hCG
Response to T Rx predicts sperm retrieval in KS patients

Response to T Rx predicts sperm retrieval in KS patients

Ramasamy et al., 2009

SERM Use in NOA

- Studies suggest up to 50% of men with NOA will have return of sperm to the ejaculate with use of clomiphene citrate
  - Hussein et al., J Androl 26:787, 2005
- No controls
- Treated men had MA/hypo; likely to have rare sperm in the ejaculate without treatment
- Randomized controlled trials suggest some increase in spermatogenesis, fertility with SERMs
  - Chua et al, Andrology 1:749, 2013
- No benefit of SERM/AIs for sperm retrieval
  - Reifsnyder et al., J Urol 188:532, 2012

Varicocelectomy in NOA

- Isolated case series report that up to 55% of men with NOA will have sperm detectable (on at least one analysis) after varicocelectomy repair
- No controls in these studies (up to 35% of NOA pts have rare sperm in ejaculate on ESP)
  - Ron-El et al., Hum Reprod 1997
- Less than 10% of men with NOA who have varicocelectomy will have enough sperm to avoid TESE; Need to defer IVF ≥ 6 months after repair
  - Schlegel & Kaufman, Fertil Steril 81:1588, 2004
**Cornell approach**

- Men with low serum testosterone & infertility
  - Rule out significant PRL, pituitary abnormalities
  - For T<300 ng/dL
- Treat with anastrozole if T/E <10
- Treat with clomiphene or tamoxifen if T/E>10
- Only need to treat 1-2 months for response
- Follow T/E on therapy
- SA repeated on day of planned sperm retrieval

**Non-obstructive azoospermia**

- Severely defective sperm production
- Identification of limited areas of sperm within the testicle

**Techniques for sperm retrieval**

- Fine needle aspiration
- Percutaneous biopsy
- Open biopsy
- Multiple open biopsies
Distribution of sperm

- Larger biopsies taken to retrieve sites with sperm production
- Excessive tissue removed
- Rare sperm in these tissues difficult to find
- Optimal approach to identify sites of sperm production?

Microsurgical identification of sperm-producing tubules by appearance

- Dissection deep within testicular tissue to examine all areas of testis

Microdissection TESE
Sperm retrieval techniques (NOA)

- Meta-analysis of all published studies
- 1,890 patients treated
- Studies involved comparison (within patients)
  - TESA/FNA mapping vs. conventional TESE
  - Conventional TESE vs. microTESE

- cTESE 2x better than TESA
- microTESE 1.5x better than cTESE


Sperm retrieval in non-obstructive azoospermia

- Use of a microscope can identify sites of sperm production without removal of large areas of the testis

- Microdissection TESE (testicular sperm extraction)
Mincing of tissue specimens

Processing enhances sperm yield 300-fold

Ostad et al., Urology, 1995
24 gauge angiocatheter

TESE-ICSI
Non-obstructive azoospermia

<table>
<thead>
<tr>
<th>Retrieval attempts</th>
<th>&gt;2,600</th>
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<tbody>
<tr>
<td>Sperm retrieval</td>
<td>54%* (830/1,540)</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>45% (4,603/9,280)</td>
</tr>
<tr>
<td>Clin Preg/retrieval</td>
<td>45% (369/816)</td>
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* - only first time attempts evaluated for sperm retrieval rate


Average male FSH level: 27.5 IU/L
Average female age: 31.7 years

Updated 1/2018

Samples examined in OR

- Sperm seen – stop dissection when adequate sperm observed for ICSI
- No sperm seen: In-lab processing
  - Supernatant separated from precipitate
  - Supernatant spun at 1800g
  - Precipitate digested with 0.1% collagenase x 1 hr
  - Examined in microdroplets
- If no sperm seen in OR, sperm found <3%
Chance of finding sperm: NOA
Dependent on most advanced region

- Patient parameters:
  - FSH
  - Testicular volume

Reflects overall testicular function, not the focus of most active sperm production in a dysfunctional testis

Effect of FSH on sperm retrieval
Ramasamy et al., F&S, 92:590, 2009

Sperm retrieval: Testicular volume

55%
Extent of dissection – chance of finding sperm

- Initial opening – 60% of sperm
- Deep dissection – 30% of sperm
- Contralateral – 8% of cases with sperm found

Sertoli cell-only (testes ≥ 15 cc)

- Berookhim et al., ASRM, 2013

TESE: Repeat attempts

- Repeat TESE only done if sperm found
- Repeat TESE attempt after 6 months:
  80% successful retrieval
- Attempts <6 mos: 25% success
- Wait at least 6 months between TESE procedures
**Cornell experience: 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
  - Low risk of genetic anomalies in offspring


**Cryptorchidism: Azoo after Orchidopexy**

- Repair >6 months before TESE (FSH=29)
- 74% (35/47) men with sperm retrieved
- MicroTESE 77%, std TESE 63%

Schlegel & Raman, J Urol 170, 1287, 2003

**NOA after chemotherapy**

- 73 men azoospermic after chemoRx/radiation
  - Hodgkins, non-Hodgkins lymphoma
  - Wilms tumor; Ewing’s sarcoma
  - Germ cell tumor;
  - Leukemias, nephrotic syndrome
- Sperm retrieval 43% (36/84)
- Clinical pregnancy 50% (18/36)
- Lower retrieval rate with lymphoma (37%) than for germ cell tumors (85%)

Husko. JCO, 2011
Success of microTESE after prior unsuccessful biopsies

Ramasamy & Schlegel, J Urol 177:1447, 2007

Non-obstructive azoospermia

- Sperm from men with NOA are different
  - 0% normal morphology
  - Fertilization rate very low (e=45%)
- Sperm characteristics affect ICSI success
  - No motility at the time of injection
  - Short/blunt tails (elongating spermatids)
  Mean fertilization rate of 45% can be limiting
  - In an individual cycle, 20% fert: Only 2 of 10 oocytes may fertilize
  - Repeat sperm retrieval, ability to use frozen sperm not guaranteed

Implications for IVF success

- Factors that affect fertilization rate can dramatically decrease the chance of IVF success
  - Lower fertilization using frozen sperm
  - Lower fertilization using frozen oocytes
- Success of IVF cycle: dependent on #oocytes
- With limited embryos, day 3 transfer often preferred
Maturation arrest: A critical frontier

- Diffuse maturation arrest
  - Commonly a low prognosis for sperm retrieval
- Large volumes of germ cells, making isolation of rare sperm identification within tissue difficult
- Different sperm processing needed
- Cell sorting techniques being improved
- Substantial promise for treatment success of many couples previously unable to be helped

SYTO-17 selected marker for sperm identification and sorting combined with SD-1

Change in FSC and SSC to log. for initial gating of sperm, then compared sperm (1N) and WBCs (2N)

Do you need fresh sperm?

- Sperm retrieval with IVF can be logistically challenging
- Use of frozen sperm (cryoTESE) common
- Some literature suggest cryoTESE equivalent
  - Often exclude patients with rare sperm on TESE
  - Exclude men with sperm that don’t survive F/T
  - Lower pregnancy rates with cryoTESE than fresh
- 70% of men with sperm on TESE: non-viable sperm or inadequate sperm for 2nd ICSI

Schiegel et al., Urology 64:1069, 2004
Summary: NOA treatment

- Men with NOA have severely impaired sperm production; “testicular failure”
- Attempt to optimize production before retrieval
- Most men have a heterogeneous pattern of sperm production; detailed surgical search
- Treatment possible for a majority of men; microTESE safe and effective
- Preoperative evaluation of patients informs chance of successful sperm retrieval
- Future developments evolving