Use of Testicular Sperm for ICSI in Non-Azoospermic Men: How Far Should we Go?

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McGill University

Disclosure: Shareholder in YAD Tech – Neutraceuticals Co.
Sperm Retrieval: Overview

Historical Perspectives
Classical Indication(s) for TESA & TESE
Evolving Indication(s) for TESA & TESE
Techniques
CFAS Survey on New Indication(s)
Summary
IVF/ICSI: Historical Perspectives

- First report of successful ICSI in 1992
- Most significant advance in treatment of male-factor infertility

Palermo et al, Lancet 1992
IVF/ICSI: Early Experience

- Presence of any number of motile sperm - OK
  The Belgium group reported “sperm motility (progressive and non-progressive) does not affect ICSI results, except in cases where only immotile spermatozoa are available for injection”

- Presence of any morphologically normal sperm - OK

- Sperm DNA damage may be bad for ICSI?
  Virro et al, *Fertil Steril* 2004
  Zini et al, *Hum Reprod* 2005
IVF/ICSI and Sperm DNA Damage

Systematic Review and Meta-analysis (1998-2014)
24 Estimates (24 studies) on Sperm DNA damage & ICSI

Combined $\text{OR} = 1.31$ (95% CI, 1.08-1.59, $P=0.006$)

- PPV = 64% ($++++$ Sperm DNA damage – CPR = 36%)
- NPV = 40% (No Sperm DNA damage – CPR = 40%)
- Heterogeneous studies???

Zini et al, unpublished data

**OA – Obstructive azoospermia**
- PESA - Percutaneous epididymal sperm aspiration
- TESA - Testicular sperm aspiration

**NOA – Non-obstructive azoospermia**
- TESE (open biopsy) or micro-TESE

**Anejaculation**
- TESA
- TESE
TESE to Rescue ICSI Failure?

Greco et al, *Hum Reprod* 2004

- Evaluated couples with repeated ICSI failure and sperm DNA damage
  - 18 couples: 2 failed ICSI & sperm DNA damage (>15% of sperm by TUNEL assay)
  - Performed TESE/ICSI: clinical pregnancy rate of 44% (8/18)

Greco et al, speculated that some sperm DNA damage may be caused by a post-testicular event (infection, partial obstruction, epididymal dysfunction, heat)
Rationale for TESA & TESE

Suganuma, Yanagimachi & Meistrich *Hum Reprod* 2005

- Conducted experimental studies: created *mutant* mice with abnormal spermatogenesis (*mutant* mice with minimal levels of transition nuclear proteins).
- These mice have poor sperm nuclear compaction (due to the higher levels of residual histones and lower levels of disulphide bonds) and the sperm DNA is not fully protected during epididymal passage.

![Normal Sperm](image1.png) ![Abnormal Sperm](image2.png)
Rationale for TESA & TESE

Suganuma, Yanagimachi & Meistrich *Hum Reprod* 2005

The passage of abnormal sperm through the epididymis is associated with a loss of sperm DNA integrity and fertilizing capacity (lower implantation rates and live birth rates with ICSI).
IVF/ICSI: Importance of Sperm Quality

- Presence of any number of motile sperm - OK

The Belgium group reported that “sperm motility (progressive and non-progressive) does not affect ICSI results, except in cases where only immotile spermatozoa are available for injection”

IVF/ICSI: Importance of Sperm Quality

- Presence of any number of motile sperm - OK
  The Belgium group reported that “sperm motility (progressive and non-progressive) does not affect ICSI results, except in cases where only immotile spermatozoa are available for injection”
  

- ICSI outcomes are influenced by sperm quality?
- TESA(TESE)-ICSI is a good option in some cases?

  Strassburger et al, *JARG* 2000
  Greco et al, *Hum Reprod* 2004
  Weissman et al, *RBM online* 2008
  Ben-Ami et al, *Fertil Steril* 2013
  Alrabeeah et al, *Urology* 2014
IVF/ICSI: Importance of Sperm Quality

1. TESA-ICSI for couples with severe-complete asthenozoospermia

TESTICULAR SPERM ASPIRATION (TESA) FOR INFERTILE COUPLES WITH SEVERE OR COMPLETE ASTHENOOZOOSPERMIA
Division of Urology, McGill University, OVO Fertility Clinic
and Department of Obstetrics and Gynecology, University of Montreal

CPR per cycle started (with 1.3 ETs) = 30%

2. TESA-ICSI for couples with oligozoospermia & prior ICSI failures

TESTICULAR SPERM ASPIRATION (TESA) FOR INFERTILE COUPLES WITH OLIGOASTHENOTERATOZOOSPERMIA (OAT) AND PRIOR ICSI FAILURE
Division of Urology, McGill University, OVO Fertility Clinic
and Department of Obstetrics and Gynecology, University of Montreal

CPR per embryo transfer (with 1.4 ETs) = 36%
1. ICSI outcomes in couples with oligozoospermia and high DFI: TESA-ICSI vs. Ejac-ICSI


**Prospective observational study**

91 couples opted for **Ejac-ICSI**
*Live birth rate* per ET (with 1.9 ETs) = 26%

81 couples opted for **TESA (or TESE)-ICSI**
*Live birth rate* per ET (with 2.0 ETs) = 47% (*P*=0.007)
Sperm Retrieval Techniques

**TESA**
- Evidence of mild-mod spermatogenesis defect
  - Clinical evaluation (History, Exam, Hormones, ?Genetics)
  - Normal testicular volume, normal FSH
  - Sperm concentration >1 million/ml

**TESE or microTESE**
- Evidence of severe spermatogenesis defect
  - Clinical evaluation (History, Exam, Hormones, Genetics)
  - Reduced testicular volume, high FSH
  - Sperm concentration <<1 million/ml
TESA – Testicular Sperm Aspiration

- Testis
  - TESA (testicular sperm aspiration)
Normal spermatogenesis may be distinguished from areas of Sertoli cell-only by microscopic examination

- dilated tubules usually indicate normal spermatogenesis (but may also contain maturation arrest pattern)
- thin, scarred tubules suggest Sertoli cell-only pattern

Schlegel et al, *Hum Reprod* 1999
## Sperm Retrieval Rates

<table>
<thead>
<tr>
<th>Category</th>
<th>Procedure</th>
<th>Success Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mild OAT</strong></td>
<td>TESA</td>
<td>90-99%</td>
</tr>
<tr>
<td><strong>Severe OAT-Cryptozoospermia</strong></td>
<td>TESA</td>
<td>50-70%</td>
</tr>
<tr>
<td></td>
<td>MicroTESE</td>
<td>85-95%</td>
</tr>
</tbody>
</table>

*Alrabeeah et al, Urology 2015*
*Alrabeeah et al, Andrology 2015*
# CFAS Survey on Use of TESA & TESE

1. What SIG do you belong to?  

<table>
<thead>
<tr>
<th>SIG</th>
<th>Total Respondents: 71</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART Lab</td>
<td>52.11% (37)</td>
</tr>
<tr>
<td>Andrology</td>
<td>39.44% (28)</td>
</tr>
<tr>
<td>IVF Medical Directors</td>
<td>16.90% (12)</td>
</tr>
<tr>
<td>Scientists in Reproductive Endocrinology</td>
<td>7.04% (5)</td>
</tr>
<tr>
<td>Fertility Preservation</td>
<td>5.63% (4)</td>
</tr>
<tr>
<td>Ethics and Law</td>
<td>4.23% (3)</td>
</tr>
<tr>
<td>Nurses</td>
<td>2.82% (2)</td>
</tr>
<tr>
<td>Administrative, Imaging</td>
<td>2.82% (2)</td>
</tr>
</tbody>
</table>
CFAS Survey on Use of TESA & TESE

2. How do you define your role?

- Clinician
- Andrologist
- Embryologist
3. Do you believe that the use of testicular sperm for ICSI may be a better option than ICSI with ejaculated sperm in some non-azoospermic couples?
4. Do you work in a clinic or laboratory that performs testicular sperm retrieval (e.g. TESA or TESE) or performs ICSI with testicular sperm?
5. If you answered yes to question 4, does your clinic or laboratory occasionally offer-perform TESA or TESE with ICSI for non-azoospermic men?
6. If you answered yes to question 4, does your clinic or laboratory occasionally offer-perform TESA or TESE with ICSI in couples with severe OAT or cryptozoospermia (rare sperm in the pellet)?
CFAS Survey on Use of TESA & TESE

7. If you answered yes to question 4, does your clinic or laboratory occasionally offer-perform TESA or TESE with ICSI in couples with **complete asthenozoospermia** (absent sperm motility)?
8. If you answered yes to question 4, does your clinic or laboratory occasionally offer-perform TESA or TESE with ICSI in couples with significant sperm DNA damage?
9. If you answered yes to question 4, is your clinic’s decision to proceed to TESA or TESE with ICSI (for non-azoospermic men) influenced by prior failed ICSI attempt(s) using ejaculated sperm?
CFAS Survey on Use of TESA & TESE

10. If you answered yes to question 4, does your clinic or laboratory have a specific protocol or guideline on the use of TESA or TESE with ICSI for non-azoospermic men?
CFAS Survey on Use of TESA & TESE

Summary

Canadian Fertility Clinics
~90% perform TESA-ICSI

Regarding TESA-ICSI for non-azoospermic men
~20-30% are uncertain about this indication

1. 65% perform TESA-ICSI in this context
2. 70% perform TESA-ICSI for severe OAT-cryptozoospermia
3. 55% perform TESA-ICSI for complete asthenozoospermia
4. 35% perform TESA-ICSI for sperm DNA damage
5. 50% perform TESA-ICSI after prior failed ICSI (ejac sperm)
6. 30% perform TESA-ICSI based on guidelines???

To date, there are no guidelines on this topic
Important Considerations

Guidelines/Indications
- Threshold motility, concentration, DNA damage, prior ICSI

Reliability of Sperm DNA Test
- Validated Assay, Accurate Threshold

Complications of TESA, TESE, MicroTESE
- Bleeding, infection, testicular pain
- Hypogonadism, genetic?

Improve Sperm Quality and Avoid TESA-TESE

How to Improve Sperm Quality (Concentration, Motility, DNA Integrity)

➔ Minimize gonadotoxins and hyperthermia
  – E.g. smoking, saunas, hot-tubs, occupational hazards

➔ Antibiotics for semen or genital tract infection
  Ochsendorf, *Hum Reprod Update* 1999

➔ Vitamin (AOXs) supplements (*in vivo, in vitro*)
  – Vitamins E, C, selenium, folate, zinc...

➔ Varicocelectomy
  – Sperm DNA damage may decrease after varicocele repair
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# Effect of AOX on Pregnancy Rates

*(Showell et al, *Cochrane Rev* 2011)*

<table>
<thead>
<tr>
<th>Study</th>
<th>Rx</th>
<th>Ctl</th>
<th>Duratn</th>
<th>Vitamin(s)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suleiman, ‘96</td>
<td>11/52</td>
<td>0/35</td>
<td>6 Mos</td>
<td>VitE</td>
<td>6.6 (1.8, 23.9)</td>
</tr>
<tr>
<td>Lenzi, ‘03</td>
<td>6/43</td>
<td>0/43</td>
<td>6 Mos</td>
<td>L-carnitine</td>
<td>8.4 (1.6, 43.6)</td>
</tr>
<tr>
<td>Balercia, ’05</td>
<td>2/15</td>
<td>1/5</td>
<td>6 Mos</td>
<td>L-carnitine</td>
<td>0.6 (0.04, 9.6)</td>
</tr>
<tr>
<td>Pievandi, ‘10</td>
<td>3/15</td>
<td>0/15</td>
<td>6 Mos</td>
<td>L-carnitine</td>
<td>8.6 (0.8, 89.5)</td>
</tr>
<tr>
<td>Lenzi, ‘04</td>
<td>4/30</td>
<td>0/26</td>
<td>6 Mos</td>
<td>L-carnit, L-acetyl</td>
<td>7.2 (1.0, 54.3)</td>
</tr>
<tr>
<td>Cavallini,’04</td>
<td>9/39</td>
<td>1/47</td>
<td>6 Mos</td>
<td>L-carnit, L-acetyl</td>
<td>7.5 (2.0, 28.0)</td>
</tr>
<tr>
<td>Balercia, ’05</td>
<td>5/15</td>
<td>1/5</td>
<td>6 Mos</td>
<td>L-carnit, L-acetyl</td>
<td>1.8 (0.2, 15.7)</td>
</tr>
<tr>
<td>Sigman, ‘06</td>
<td>1/12</td>
<td>1/9</td>
<td>6 Mos</td>
<td>L-carnit, L-acetyl</td>
<td>0.7 (0.04, 13.0)</td>
</tr>
<tr>
<td>Balercia, ’09</td>
<td>6/30</td>
<td>3/30</td>
<td>6 Mos</td>
<td>CoQ10</td>
<td>2.2 (0.5, 8.8)</td>
</tr>
<tr>
<td>Omu, ‘98</td>
<td>11/49</td>
<td>2/48</td>
<td>3 Mos</td>
<td>Zn</td>
<td>4.8 (1.5, 15.2)</td>
</tr>
<tr>
<td>Galatioto, ’08</td>
<td>1/20</td>
<td>0/22</td>
<td>3 Mos</td>
<td>Combined AOX</td>
<td>8.2 (0.2, 413.4)</td>
</tr>
<tr>
<td>Tremellen, ’07</td>
<td>20/38</td>
<td>4/20</td>
<td>3 Mos</td>
<td>Combined AOX</td>
<td>3.8 (1.3, 11.2)</td>
</tr>
</tbody>
</table>

**Overall** 16% 3%
## Antioxidants and Sperm DNA Damage

### Controlled Trials of Oral Vitamin Supplements

#### Infertile men with high sperm DNA fragmentation levels

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Treatment</th>
<th>Duration</th>
<th>Outcome 1</th>
<th>Outcome 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greco '05</td>
<td>Infertility</td>
<td>vits C 1g, E 1g</td>
<td>32</td>
<td>Rx (2 months): 32 DD (22% → 9%)</td>
<td>Placebo group: no effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TUNEL&gt;15%</td>
<td></td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Tremellen'07</td>
<td>Male Infert</td>
<td>Menevit (lycopene, vits C, E, Zinc, Se, folate, garlic)</td>
<td>36</td>
<td>Rx (3 months): 39% ICSI pregnancy rate, But no in embryo quality, no post-Rx DD</td>
<td>Placebo group: 16% ICSI pregnancy rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TUNEL&gt;25%</td>
<td></td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

#### Unselected infertile men

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Treatment</th>
<th>Duration</th>
<th>Outcome 1</th>
<th>Outcome 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piomboni '08</td>
<td>Asthenosp.</td>
<td>vits C, E, β-glucan papaya, lactoferrin</td>
<td>36</td>
<td>Rx (90 days): motility &amp; morph but not DD</td>
<td>Control group: no change</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AO stain</td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Kodama '97</td>
<td>Male infert</td>
<td>Vit C, E (200mg) glutathione (400mg)</td>
<td>14</td>
<td>Rx (2 months): in 8-OHdG levels</td>
<td>Control group: no change</td>
</tr>
<tr>
<td></td>
<td>8-OHdG</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
How to Improve Sperm Quality (Concentration, Motility, DNA Integrity)

➔ Minimize gonadotoxins and hyperthermia
  – E.g. smoking, saunas, hot-tubs, occupational hazards

➔ Antibiotics for semen or genital tract infection
  Ochsendorf, *Hum Reprod Update* 1999

➔ Vitamin (AOXs) supplements (*in vivo, in vitro*)
  – Vitamins E, C, selenium, folate, zinc...

➔ Varicocelectomy
  – Sperm DNA damage may decrease after varicocele repair
  
  Zini & Dohle *Fertil Steril* 2011
Varicocelectomy and Sperm DNA Damage

### Retrospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Participants</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zini, '05</td>
<td>SCSA</td>
<td>37 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Werthman, '08</td>
<td>SCSA</td>
<td>11 High sperm DD</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Sakamoto, '08</td>
<td>TUNEL</td>
<td>11 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
</tbody>
</table>

### Prospective Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Participants</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasr-Esfahani, '07</td>
<td>CMA3</td>
<td>162 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Chen, '08</td>
<td>8-OHdG</td>
<td>30 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Dada, '10</td>
<td>COMET</td>
<td>11 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Smit, '10</td>
<td>SCSA</td>
<td>49 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Azedi, '10</td>
<td>CMA3</td>
<td>52 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Sadek, '11</td>
<td>A-Blue</td>
<td>72 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Lacerdo, '11</td>
<td>COMET</td>
<td>21 Adolescents</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>Zini, '11</td>
<td>SCSA</td>
<td>25 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
<tr>
<td>LaVignera, '11</td>
<td>TUNEL</td>
<td>30 Infertile men</td>
<td>↓ sperm DD post-varicocelectomy</td>
</tr>
</tbody>
</table>
TESA-TESE in Non-Azoospermic Men

HOW FAR SHOULD WE GO?

Need RCTs and Basic Research

Need to Establish Clear Indications-Guidelines
- Thresholds (sperm concentration, motility, DNA damage)
- Prior ICSI failures?

Need to Understand the Risks

Until then, TESA-TESE remains Empiric
TESA-TESE in Non-Azoospermic Men

- Sperm Quality Can Influence ICSI Outcomes
- Evolving Indication(s) for TESA & TESE?
- Evolving Indication(s) for Sperm DNA Testing?
- Retrieval Technique Depends on Diagnosis
- Retrieval Can Cause Serious Complications
- CFAS Survey: Canadians Use TESA & TESE
- No Clear Guidelines on use of TESA & TESE
- Future Studies Needed