Current State of the Art and Evidence in Andrology Testing

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President SMRU
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

Conflicts of interest
- No financial involvement with Pharma or otherwise
Acknowledgements

- Erma Drobnis
- Mary Samplaski
- Jay Sandlow
- Craig Niederberger
Sperm parameters – WHO 2010 values...
... “It’s just a number... right?”
The Issue

- There is a complex relationship between semen analysis and pregnancy outcome.

- Fertility and infertility are NOT defined by the semen analysis reference values alone.

- BUT: Semen analysis parameters and reference values aim to provide evidence-based thresholds that aid the clinician in calculating the relative fertility of the patient through correlation with outcomes.

- BUT: There are functional factors that are beyond just numbers.
Where have we been to see where we are going?

- The World Health Organization (WHO) periodically releases manuals for laboratory examination of human semen:
  - The first one was published in 1980, with subsequent updates in 1987, 1992, 1999 and now 2010
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (cc)</td>
<td>-</td>
<td>≥2</td>
<td>≥2</td>
<td>≥2</td>
<td>1.5</td>
</tr>
<tr>
<td>Sperm conc (M)</td>
<td>20-200</td>
<td>≥20</td>
<td>≥20</td>
<td>≥20</td>
<td>15</td>
</tr>
<tr>
<td>Total Motility (%)</td>
<td>≥60</td>
<td>≥50</td>
<td>≥50</td>
<td>≥50</td>
<td>39</td>
</tr>
<tr>
<td>Morphology</td>
<td>80.5</td>
<td>≥50</td>
<td>≥30</td>
<td>&gt;14*</td>
<td>&gt;4*</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>&lt;4.7</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>
Before 2010:

- Up until 2010 the criteria were consensus based:
  - based on the clinical experience of investigators who have studied populations of healthy fertile men of unknown TTP
  - Previous WHO manuals acknowledge the limitations by stating that each laboratory should determine its own reference values
How the 2010 WHO Reference Values Differ from Previous Versions:

- For the first time, multi-country data from recent fathers with known time-to-pregnancy (TTP)

- Evidence-based

- Standardized methods for semen analysis used according to the WHO manual

- Laboratories that practiced internal and external quality control.
BUT!

- Not an accurate representation of the fertile man across the globe…this was acknowledged by Cooper et al.
  - Only one center from southern hemisphere
  - Nothing from China, India, Africa, Middle East or South America

- Not clear how data was pooled from 5 studies

- Female age and fertility status were not considered

- A single sample semen sample was used to represent each man in the reference studies.
  - WHO recommends two to three samples to establish a baseline
BUT! (continued)

- Not all studies used TTP as the end point

- Not all of the studies on morphology were conducted according to Kruger’s strict criteria

<table>
<thead>
<tr>
<th>Study</th>
<th>Year [reference]</th>
<th>Country</th>
<th>TTP &lt; 12 months clearly stated</th>
<th>Sperm morphology evaluation criterion</th>
<th>Overlapping authorship or collaboration among authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stewart et al.*</td>
<td>2009 [6]</td>
<td>Australia</td>
<td>Yes</td>
<td>Tygerberg</td>
<td>Yes</td>
</tr>
<tr>
<td>Slama et al.</td>
<td>2002 [7]</td>
<td>France, Denmark, UK, Finland</td>
<td>Yes</td>
<td>David, Tygerberg</td>
<td>Yes</td>
</tr>
<tr>
<td>Swan et al.*</td>
<td>2003 [8]</td>
<td>USA</td>
<td>No</td>
<td>Tygerberg</td>
<td>Yes</td>
</tr>
<tr>
<td>Jensen et al.</td>
<td>2001 [9]</td>
<td>France, Denmark, UK, Finland</td>
<td>Yes</td>
<td>David</td>
<td>Yes</td>
</tr>
<tr>
<td>Haugen et al.*</td>
<td>2006 [10]</td>
<td>Norway</td>
<td>Yes</td>
<td>Tygerberg</td>
<td>No</td>
</tr>
<tr>
<td>Jørgensen et al.</td>
<td>2001 [12]</td>
<td>France, Denmark, UK, Finland</td>
<td>No</td>
<td>David</td>
<td>Yes</td>
</tr>
<tr>
<td>Bonde et al.</td>
<td>1998 [13]</td>
<td>Denmark</td>
<td>Yes</td>
<td>David</td>
<td>Yes</td>
</tr>
</tbody>
</table>

TTP = Time to pregnancy  
UK = United Kingdom  
*Studies contributing to data on sperm morphology.
Percentiles

- Use of the cut-off of the lowest 5th percentile adequate?

That is the question!

This is the Holy Grail of Andrology.....every sperm is sacred but how many and which ones?

- A certain number of functioning sperm are needed for normal physiology/fertilization.....what is that?
So how far have we come?

- Only 5% of laboratories in the United Kingdom were compliant with the techniques set by the WHO guidelines for assessing sperm morphology\(^1\).

- Keel et al\(^2\):
  - 60% of laboratories indicated the criteria used for sperm morphology
  - 77% reported sperm count
  - 59% reported motility according to the WHO guidelines
  - 35% of laboratories were either not familiar with the WHO manual or did not have a copy of it in their

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Where are with WHO 1999 10 year later?

National semen analysis reference range reporting: adherence to the 1999 World Health Organization guidelines 10 years later

Heidi A. Penn, M.D.,a Andrew Windsperger, M.D.,a Zachary Smith, B.S.,a Sijo J. Parekattil, M.D.,b Wayne W. Kuang, M.D.,c Peter N. Kolettis, M.D.,d and Ajay K. Nangia, M.B.B.S.a

a Department of Urology, University of Kansas Medical Center, Kansas City, Kansas; b Department of Urology, University of Florida Gainesville, Gainesville, Florida; c Department of Urology, University of New Mexico, Albuquerque, New Mexico; and d Department of Urology, University of Alabama, Birmingham, Alabama

- 111 labs, 31 states
So how far have we come?

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ART</th>
<th>Non-ART</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. laboratories</td>
<td>65</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Laboratories reporting all parameters</td>
<td>21/65 (32)</td>
<td>5/46 (11)</td>
<td>.008</td>
</tr>
<tr>
<td>recommended</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratories reporting recommended</td>
<td>62/65 (95)</td>
<td>31/46 (67)</td>
<td>.0004</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratories reporting recommended</td>
<td>50/65 (77)</td>
<td>28/46 (61)</td>
<td>.069</td>
</tr>
<tr>
<td>motility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratories reporting recommended</td>
<td>26/65 (40)</td>
<td>5/46 (11)</td>
<td>.001</td>
</tr>
<tr>
<td>morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Values are number (percentage).*

Male Factor Infertility

- 36% of ART cycles in the US now report male factor as a contributory diagnosis\(^1\)

- This is an increase of 46% as compared to 1996\(^1\)

- As male factor continues to become a more prevalent diagnosis for infertility, it is increasingly important that men are properly diagnosed

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As updates to laboratory manuals occur the issue of defining male factor could potentially worsen:

- in terms of national and international agreement by the community to use WHO reference ranges
- criteria to define infertile versus sub-fertile men
- thresholds to define treatment options.

The controversy continues and ultimately large regional studies to define fertile ranges are needed and disseminated to labs.
Will we see a decrease in the number of referrals for male infertility?

The effect of the new 2010 World Health Organization criteria for semen analyses on male infertility

Katie S. Murray, D.O.,a Andrew James, M.D.,a James B. McGeady, M.D.,b Michael L. Reed, Ph.D.,c Wayne W. Kuang, M.D.,b,d and Ajay K. Nangia, M.B.B.S.a

a Department of Urology, University of Kansas Medical Center, Kansas City, Kansas; and b University of New Mexico, c Center of Reproductive Medicine of New Mexico, and d Southwest Fertility Center for Men, Albuquerque, New Mexico
A total of 184 men had at least two semen analyses

A total of 501 men had one semen analysis

Overall, 103 patients (15.1%) who had one or more parameter below the reference value on the original analysis were converted to having all parameters at or above the 2010 reference values.
Morphology

I take it you haven't had sex for a while.

NORMAL SPERM

YOUR SPERM
15.7% to 19.3% of men would be reclassified as having normal morphology of greater than 4% from having been abnormal in the past i.e. less than 14%.

- The change in this parameter in determining the use of ART, especially ICSI, is controversial.
- Many reproductive endocrinologists already determine the need for ICSI based on 4% normal morphology and not 14%.
Sperm Morphology—what is the clinical use?

- Strict morphology originally used to predict fertilization for IVF

- Has been extrapolated to be a predictive factor for pregnancy outcome, both naturally and with ART

- Many recent studies refute this, especially with isolated teratozoospermia

- Should not base treatment solely on strict morphology

The Concerns:

- Men may be classified as fertile by many providers especially in idiopathic cases.

- This will affect reporting data for research or even demographics and outcomes e.g. to CDC/SART.

- This may under represent the cause and subsequent work up and treatment of male infertility in a couple e.g. varicocele.
• Semen analysis alone is not an absolute marker of male infertility.

• Timeline of greater than 1 year and overall clinical picture still defines infertility and over-rides any semen analysis - abnormal or normal

• Providers should also appreciate that male factor may also still exist even with normal semen parameters, especially if functional sperm abnormalities are present
Possible Solutions

- Regional definition of normal fertility – would require a large study:
  - Stratified by female age range
  - Stratified by male age range
  - Race/ethnicity
  - ?BMI

- Defining the odds of pregnancy by the percentile range not just lowest 5th percentile

- Regional definitions based on accurate data with ART outcomes.....what do we know now?
Functional Testing:

- Antisperm Antibodies
- Sperm DNA fragmentation
- Elevated oxidative stress (e.g. leucocytes)
- Viability
- Acrosome studies
- Genetic testing
Antisperm Antibody Testing
Many Clinicians do not Test for ASA-Why?

- Lack of standardized & universally accepted assay
- Unclear that results will change therapy
- No mechanistic explanation of how ASA decrease conception
- No consensus on clinical consequences of ASA
I disagree with that stance...

- ASA can be a reason for unexplained infertility – male OR female...and is a definable cause
- ASA with delayed pregnancy post vas reversal with normal parameters
- If an IUI prep does not prep well with or without agglutination – a reason to suspect antibodies.
- Unsuccessful IUI or poor fertilization from IVF – may be ASA
- ...We are looking for answers to explain the problem – immune/ASA is part of that work up.
| TABLE I.  
Indications for antisperm antibody testing  |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility and at least one of the following</td>
</tr>
<tr>
<td>Identifiable risk factors (see text)</td>
</tr>
<tr>
<td>Abnormal semen analysis, especially</td>
</tr>
<tr>
<td>Clumping/agglutination of sperm</td>
</tr>
<tr>
<td>Low motility</td>
</tr>
<tr>
<td>Shaking-in-place motility</td>
</tr>
<tr>
<td>Poor sperm viability</td>
</tr>
<tr>
<td>Abnormal postcoital test, including</td>
</tr>
<tr>
<td>Low numbers of sperm in mucus</td>
</tr>
<tr>
<td>Poor motility</td>
</tr>
<tr>
<td>Shaking-in-place motility</td>
</tr>
<tr>
<td>Abnormal in vitro cervical mucus penetration test</td>
</tr>
<tr>
<td>Failed or low fertilization during in vitro fertilization</td>
</tr>
<tr>
<td>Abnormal sperm penetration assay</td>
</tr>
<tr>
<td>Unexplained infertility after male and female evaluation</td>
</tr>
</tbody>
</table>
Mechanism of ASA

- 12% of infertile men have ASA – serum, seminal plasma, direct to sperm

- Antibodies are primary/“idiopathic” or secondary – due to a known cause: exposure of autoantigens

Hendry WF et al; BJU 1977: 44; 757
## Known Associations

<table>
<thead>
<tr>
<th>Obstruction:</th>
<th>Trauma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasectomy and reversal</td>
<td>Coital</td>
</tr>
<tr>
<td>Idiopathic epididymal obstruction</td>
<td>Torsion</td>
</tr>
<tr>
<td>Ejaculatory duct obstruction</td>
<td>Testis Biopsy</td>
</tr>
<tr>
<td>CBAVD</td>
<td>Oral, rectal exposure</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td><strong>Thermal</strong></td>
</tr>
<tr>
<td>Orchitis</td>
<td>Varicocele</td>
</tr>
<tr>
<td>STIs</td>
<td>Cryptorchism</td>
</tr>
<tr>
<td>Prostatitis</td>
<td>Hot tubs, baths</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
</tr>
<tr>
<td><strong>Genetic</strong></td>
<td></td>
</tr>
<tr>
<td>Thymic maldevelopment</td>
<td></td>
</tr>
<tr>
<td>HLA-B28 haplotype</td>
<td></td>
</tr>
</tbody>
</table>
The limitations of the test:

- What type of antibody matters?
- What test is most “accurate”?
- What degree of binding and to what matters?
- What epitopes matter?
- Why is the test so polyclonal in this day and age with no advancement in the science?

- Valid concerns but I still use the test while thinking about the above issues in unexplained cases or known associations
Comparing ASA Results

“The confusion over the role of ASA in infertility...reflects the inadequacies of the current diagnostic techniques.”


1. Different tests give different results for the same specimen
2. Test results sensitive to specific methodology, e.g., sperm preparation effect on surface ASA
3. What is positive? Different cut-off values constituting a positive test
4. Tests are polyclonal: test for ASA in general, but variable effect of each ASA on fertility
## Types of ASA Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>What is tested?</th>
<th>Used clinically?</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Immunobead Test (IBT)</td>
<td>Sperm</td>
<td>Yes</td>
<td>Uses washed sperm</td>
</tr>
<tr>
<td>Mixed Agglutination Reaction (MAR)</td>
<td>Sperm</td>
<td>Yes</td>
<td>Sperm in semen</td>
</tr>
<tr>
<td>Indirect IBT or MAR test</td>
<td>Fluids</td>
<td>Yes</td>
<td>Donor sperm treated with fluid</td>
</tr>
<tr>
<td>Tray Agglutination Test (TAT)</td>
<td>Fluids</td>
<td>No longer</td>
<td>Donor sperm treated Agglutination detected</td>
</tr>
<tr>
<td>Sperm Immobilization Test (SIT)</td>
<td>Fluids</td>
<td>No</td>
<td>Only detects ASA that fix complement</td>
</tr>
<tr>
<td>ELISA</td>
<td>Fluids</td>
<td>Yes</td>
<td>Sperm Ags on a plate, Nonspecific &amp; internal Ags recognized</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>Sperm</td>
<td>Not yet</td>
<td>Nonmotile and motile sperm used</td>
</tr>
</tbody>
</table>

If unfixed, washed, motile sperm are used, results equivalent, regardless of the probe (immunobead, fluorescence, enzyme)

**Sperm MAR Test** (mixed antiglobulin reaction)

- Immunobeads coated with IgG are added to whole semen
- In the cartoon, the sperm is coated with IgG ASA
- Linker anti-IgG antibodies are added & bind to IgG on bead & sperm
- Motile sperm with linked beads are counted

**The Sperm MAR Test**
Immunobead Test (IBT)

- Immunobeads with bound anti-IgG antibodies are added to washed sperm
- The beads bind directly to the IgG ASA on the sperm
- Motile sperm with bound beads are scored
- This requires more time for washing the patient & control sperm
The two tests agree reasonably well & each is appropriate for routine testing.

Note that the IBT tends to give lower values, likely because some Ag recognized by SpermMAR are adsorbed Ags that are removed by washing.

Adsorbed proteins can be from accessory glands; some are important in sperm transport & capacitation.

Scoring ASA Tests - Cut-off Value

- The consensus cut-off value for clinical significance is 50% of sperm having ASA
- There are few clinical data to support this value, but it is the value recommended by WHO, 1999; 2010


<table>
<thead>
<tr>
<th>% Sperm with immunobeads</th>
<th>IUI Pregnancy Rate in 12 cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 50%</td>
<td>15.3%</td>
</tr>
<tr>
<td>&lt; 50%</td>
<td>66.7%</td>
</tr>
</tbody>
</table>


- However, researchers and clinical laboratories use cut-off values from 10% to 50%
- Probably not the %sperm with ASA but what epitopes are ASA bound
## Variation in ASA Cutoff Values

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Assay</th>
<th>ASA cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Almeida et al, 1989</td>
<td>15</td>
<td>IBT</td>
<td>70%</td>
</tr>
<tr>
<td>Rahah et al, 1992</td>
<td>36</td>
<td>IBT &amp; MAR</td>
<td>20%</td>
</tr>
<tr>
<td>Lahteenmaki, 1993</td>
<td>156</td>
<td>IBT &amp; MAR</td>
<td>1%</td>
</tr>
<tr>
<td>Acosta et al, 1994</td>
<td>67</td>
<td>IBT</td>
<td>10%</td>
</tr>
<tr>
<td>Pagidas et al, 1994</td>
<td>435</td>
<td>IBT</td>
<td>10%</td>
</tr>
<tr>
<td>Sukcharoen &amp; Keith, 1995</td>
<td>167</td>
<td>IBT</td>
<td>20%</td>
</tr>
<tr>
<td>Vazquez-Levin et al., 1997</td>
<td>18</td>
<td>MAR</td>
<td>20%</td>
</tr>
<tr>
<td>Vijisic et al, 2005</td>
<td>52</td>
<td>IBT</td>
<td>20%</td>
</tr>
<tr>
<td>Clarke, 2006</td>
<td>89</td>
<td>IBT</td>
<td>80%</td>
</tr>
<tr>
<td>Van Weert et al, 2008</td>
<td>473</td>
<td>MAR</td>
<td>20%</td>
</tr>
<tr>
<td>Nagy et al, 1995</td>
<td>1822</td>
<td>MAR</td>
<td>80%</td>
</tr>
<tr>
<td>Lahteenmaki et al, 1995</td>
<td>49</td>
<td>MAR</td>
<td>10%</td>
</tr>
<tr>
<td>Clarke et al, 1997</td>
<td>179</td>
<td>IBT</td>
<td>80%</td>
</tr>
<tr>
<td>Mercan et al, 1998</td>
<td>207</td>
<td>IBT &amp; MAR</td>
<td>30%</td>
</tr>
<tr>
<td>Check et al, 2000</td>
<td>93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esteves et al, 2007</td>
<td>351</td>
<td>IBT</td>
<td>50%</td>
</tr>
</tbody>
</table>

Serum or Seminal ASA most relevant?

Women - serum?
Men – seminal plasma/direct?
Serum/Semem

- **In women:**
  - Uterine/serum may be more relevant?
  - Local – cervical mucus

- **In men:**
  - Less significance for serum – vasectomy/reversal data
Isotypes of ASA – Relevance in Decision Making?

- **Ig A:**
  - In seminal plasma and virtually never in serum – produced local and secretory in genital tract
  - Generated by local antigen inoculation
  - Most clinically relevant

Isotypes (continued)

- **Ig G**: – most common
  - Primarily an transudate/exudate from serum
    - Only 1% of serum IgG observed in seminal plasma
    - Most produced from systemic antigen inoculation
  - Produced locally in the male genital tract in situ

- **IgM**:
  - Large pentomer – prevents transudation into seminal fluid but have been found
  - Role in infertility most likely limited

References:
- Bronson RA. J Reprod Immunol 1999; 45: 159-183
Is there any correlation for ASA location?

- ASA on sperm is poorly correlated to ASA in blood serum
- ASAs in cervical secretions are poorly correlated to ASA in blood serum
- Location on the sperm the ASA are located has significance – head binding vs tail
- Variation in results for one patient over time - patients with ASA have “flares” and remissions

Value of Serum Antisperm Antibodies in Diagnosing Obstructive Azoospermia

Richard Lee, Marc Goldstein,* Brant W. Ullery, Joshua Ehrlich, Marc Soares, Renee A. Razzano, Michael P. Herman, Mark A. Callahan, Philip S. Li, Peter N. Schlegel and Steven S. Witkin

From the The Center for Male Reproductive Medicine and Microsurgery, Department of Urology and Cornell Institute for Reproductive Medicine (RL, BWU, JE, MS, MPH, PSL, PNS, MG), Department of Public Health (RAR, MAC), and Department of Obstetrics and Gynecology, Division of Immunology (SSW), Weill Cornell Medical College, and The Population Council, Center for Biomedical Research (PNS, MG), New York, New York
Dilemmas

- How long do you pursue natural conception; IUI; and/or conventional IVF in “unexplained” infertility...when it may actually be explained i.e. positive ASA?

- Is early use of ICSI the correct treatment for ASA infertility and thereby cost effective in these situations?
ASA – Functional Evidence

- Some men with ASA will have normal fertility with intercourse, IUI or IVF BUT overall, ASA inhibits every sperm function:
  - Penetration of cervical mucus
  - Sperm storage in the oviduct (where they normally reside for up to 3 days awaiting the oocyte)
  - Binding to the ZP
  - The acrosome reaction (an absolute requirement for fertilization)
  - Fusion with the oolemma
  - Post-fusion events are less clear (which is why ICSI is very successful).
Cumulative Spontaneous Pregnancy Rates

A population of patients with sperm surface ASA

*Normal populations are from 1) Canadian church registries from 17th and 18th centuries, 2) Population in NY in 1950’s, 3) Population in Germany in in the 1990’s

Figure from: Nieschlag E, Behre HM. Andrology 2nd Ed. 2001. Springer New York.
ASA and IUI Outcomes

- If cervical ASA – IUI may be possible*.

- There are NO CONTROLLED PROSPECTIVE STUDIES OF IUI IN TREATING IMMUNE INFERTILITY

- IUI for head binding shown to be worse than tail binding**

*Bronson RA. J Reprod Immunol 1999; 45: 159-83
**Margalloth EJ et al. Fertil Steril 1988; 50: 441-446
Combined OR = 1.85 (0.88-3.88)  
(NS – but a trend to significance)
EFFECT OF TREATING ANTIBODY-COATED SPERM WITH CHYMOTRYPSIN ON PREGNANCY RATES FOLLOWING IUI AS COMPARRED TO OUTCOME OF IVF/ICSI

J. H. CHECK, W. HOURANI, M. L. CHECK, V. GRAZIANO, and E. LEVIN

*University of Medicine/Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics/Gynecology, Division of Reproductive Endocrinology/Infertility, Camden, New Jersey, USA*

**Table 1.** Comparison of two treatment modalities for males with 100% of their sperm demonstrating antisperm antibodies

<table>
<thead>
<tr>
<th></th>
<th>Chymotrypsin treated group (n = 17)</th>
<th>IVF with ICSI treated group (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>47</td>
<td>38</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Pregnancy rate/cycle</td>
<td>10.6%</td>
<td>28.9%</td>
</tr>
<tr>
<td>Pregnancy rate/patient</td>
<td>29.4%</td>
<td>44%</td>
</tr>
<tr>
<td>Average number of cycles/patient</td>
<td>2.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>0 (0%)</td>
<td>1 (9.0%)</td>
</tr>
</tbody>
</table>
ASA and IVF Fertilization Rate Outcomes

Fertilization Rate - Odds Ratio

- Mandelbaum et al (1987) IBT > 20%
- Matson et al (1988) IBT > 20%
- Chang et al (1993) IBT > 10%
- Lähteenmäki (1993) MAR > 0%
- Rajah et al (1993) MAR > 20%
- Acosta et al (1994) MAR > 10%
- Sukcharoen & Keith (1996) IBT > 20%
- Ford et al (1996) IBT > 20%
- Vazquez-Levin (1997) MAR > 20%
- Vujisic et al (2005) MAR > 20%
- Clarke (2006) IBT > 80%

Combined 6563 Oocytes

ASA-positive better ← ASA-negative better
Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis

Armand Zini¹,*, Nader Fahmy¹, Eric Belzile², Antonio Ciampi²,³, Naif Al-Hathal¹, and Ahmed Kotb¹
Craig Niederberger said it best…

“My concern with the study is that an odds ratio for pregnancy failure of 1.00 for ICSI is different than that of 1.22 for IVF, with IVF being worse; it is just that the number of included studies did not achieve statistical significance. It is a common problem with meta-analyses that by mixing together differing studies we may be throwing out the baby with the bathwater.”
ASA and IVF-ICSI Outcomes

- ICSI leads to similar fertilization and pregnancy rates in ASA positive and negative cases
  - Approx 78% for ASA+ vs 69% for ASA-

- Concern about embryo quality with ASA positive sperm
  - e.g. ICSI with sperm with >80% MAR binding. 38% preg loss in ASA+ vs 0% in ASA-

Nagy ZP et al. Human Reprod 1995; 10; 1775-1780
FISH and Male Infertility

- Fluorescent in situ hybridization (FISH) detects sperm aneuploidy, even in men with normal karyotype.

- May explain reproductive failure, including recurrent miscarriage and failed fertilization.

- Numerous clinical syndromes found to be related to abnormal FISH.

- Some authors advocate sperm FISH prior to sperm retrieval (NOA), as well as in couples with unexplained failed IVF cycles.

Sperm DNA testing

- DNA fragmentation: Tests available and how do they differ and what value/limitations do they have?
  - Predictive for failed fertilization/poor implantation/recurrent miscarriages?
Introduction

- Sperm with DNA damage take a longer time to conceive. (Spano 2000)
  - But ultimately these sperm are able to fertilize. (Lopes 1998, Gandini 2004, Aitken 1998)

- Both oocyte and embryo are equipped with mechanisms to repair some paternal DNA anomalies. (Wells 2005, Gasca 2007)
  - depends on the quality of the oocyte
  - impacted dramatically by increasing female age.

- Sperm DNA quality is increasingly being linked to paternal age. (Wyrobek 2006)
  - This may further exacerbate the decrease in pregnancy rate observed in women of advanced age. (Belloc 2008)

- Sperm have few repair mechanisms. (Aitken 2006)

Aitken RJ. Biol Reprod 1998; 59: 1037–46
How does sperm DNA damage occur?

(i) Apoptosis during spermatogenesis
(ii) DNA strand breaks during spermiogenesis
(iii) Post-Testicular DNA fragmentation via ROS
(iv) DNA fragmentation induced by endogenous caspases and endonucleases;
(v) DNA damage induced by radio and chemotherapy; and
(vi) DNA damage induced by environmental toxicants.

Oxidative stress

- Oxidative stress has long been implicated as the major etiological factor in sperm DNA damage.

- Reactive oxygen species (ROS): need some but not too much.

- Oxidative stress leads to base modifications, which may lead to discrete DNA strand breaks. (Croteau 1997)

Type of DNA damage: single vs double-stranded

- **Single-stranded DNA damage:**
  - Better prognosis, easier to repair
  - Caused by:
    - Unrepaired DNA nicks generated during chromatin remodeling
    - Oxygen radical-induced damage

- **Double-stranded DNA damage:**
  - Caused by:
    - Apoptosis
    - Hydrolysis by caspases and endonucleases
    - Oxygen radical-induced DNA damage through the activation of caspases and endonucleases.
      - Damage depends on levels of antioxidant enzymes present in the lumen of the epididymis.

(Britan 2006)
DNA Fragmentation Tests

- **Different assays** measure different aspects of sperm DNA and chromatin:
  - Degree of DNA fragmentation, protamination, DNA denaturation.

- **Assay conditions** can greatly influence the accessibility of the dye or enzyme to the sites of damaged DNA and, therefore, impact on the final results.
  - Reagents themselves can alter the reactions
  - The concentration of reducing agents can alter sperm nuclear decondensation
  - Sample preparation and handling (centrifugation, prolonged incubation) can impact the test results.

- Assays do not identify the DNA fragmentation in an individual cell.
Sperm Chromatin Dispersion

- Measure the rate at which denatured single stranded DNA form from native double stranded DNA.
- Sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with nonfragmented DNA.
- Assessed by fluorescence and brightfield microscopy.

- >30% - poor fertility prognosis
TUNEL

- Terminal deoxynucleotidyl transferase dUTP nick end labeling

- DNA fragmentation detected by labeling the terminal end of nucleic acids.

- Nicks in the DNA are identified by TdT, an enzyme that will catalyze the addition of a fluorescent nucleotide marker.

- Threshold for subfertility variable: 4-20%
Detects DNA damage at the level of the individual sperm

Allows for quantitative measurement of DNA damage.

Cells are lysed with detergent and salt to form nucleoids containing loops of DNA.

Electrophoresis → look at the pattern of DNA migration through the gel, observed by fluorescence microscopy.

The intensity of the comet tail relative to the head reflects the number of DNA breaks.

More sensitive than other tests.
### TABLE 5

Number of couples and pregnancy rates for different values of DFI and HDS.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DFI (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–≤9</td>
<td>23</td>
<td>8.7</td>
</tr>
<tr>
<td>9.01–≤18</td>
<td>32</td>
<td>40.6</td>
</tr>
<tr>
<td>18.01–≤27</td>
<td>21</td>
<td>33.3</td>
</tr>
<tr>
<td>27.01–≤36</td>
<td>11</td>
<td>54.5</td>
</tr>
<tr>
<td>&gt;36</td>
<td>8</td>
<td>37.6</td>
</tr>
<tr>
<td><strong>HDS (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5</td>
<td>15</td>
<td>40.0</td>
</tr>
<tr>
<td>5.01–≤10</td>
<td>50</td>
<td>32.0</td>
</tr>
<tr>
<td>10.01–≤15</td>
<td>15</td>
<td>40.0</td>
</tr>
<tr>
<td>15.01–≤20</td>
<td>7</td>
<td>28.6</td>
</tr>
<tr>
<td>&gt;20</td>
<td>8</td>
<td>12.5</td>
</tr>
</tbody>
</table>
The predictive value of DNA fragmentation testing is likely the sum of many factors:

- Percent of sperm with DNA damage
- Extent of DNA damage per spermatozoon
- Whether there is combined nucleotide damage and DNA fragmentation
- Ability of the oocyte to repair DNA damage in the fertilizing sperm
- Type of sperm DNA fragmentation test used
- Sperm processing in ART
- Oocyte number
- Oocyte quality
Numerous studies have shown that higher DNA fragmentation rates are associated with impaired fertility:

- longer times to conceive (Spano et al., 2000)
- impaired embryo cleavage (Morris et al., 2002)
- higher miscarriage rates (Evenson et al., 1999)
- increased risk of pregnancy loss after both IVF and ICSI (Zini 2008)

The impact of sperm DNA damage on ART outcomes decreases with invasiveness:

- **SP > IUI > IVF > ICSI** (least useful in ICSI) (Collins 2008, Zini 2009)
- Hypothesis: ICSI is able to bypass genetic (and functional) defects. (Ozmen 2007, Bungum et al., 2008)
**Spontaneous Pregnancy & IUI**

- **Spontaneous Pregnancy:**
  - **Prolonged time to pregnancy** (Evenson 1999, Giwercman 2010, Loft 2003, Spano 2000)
    - **Failure to achieve a natural pregnancy** (OR = 7.01, p < 0.001; Table 1)

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Assay</th>
<th>%hDD</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evenson et al. 1999</td>
<td>144</td>
<td>SCSA</td>
<td>7</td>
<td>0.19</td>
<td>0.96</td>
<td>0.60</td>
<td>0.81</td>
<td>6.54</td>
<td>(1.72, 24.92)</td>
</tr>
<tr>
<td>Spano et al. 2000</td>
<td>215</td>
<td>SCSA</td>
<td>13</td>
<td>0.23</td>
<td>0.96</td>
<td>0.86</td>
<td>0.55</td>
<td>7.59</td>
<td>(2.54, 22.67)</td>
</tr>
<tr>
<td>Giwercman et al. 2010</td>
<td>257</td>
<td>SCSA</td>
<td>12</td>
<td>0.21</td>
<td>0.96</td>
<td>0.83</td>
<td>0.58</td>
<td>6.82</td>
<td>(2.52, 18.47)</td>
</tr>
</tbody>
</table>

Abbreviations: %hDD = proportion of samples with high sperm DNA damage; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; OR = odds ratio; SCSA = sperm chromatin structure assay.

- **IUI:**
  - **Lower IUI pregnancy rates** (Bungum 2007, Duran 2002, Muriel 2006)
  - **OR = 9.9 (p < 0.001)**

---

• Zini et al. meta-analysis: Sperm DNA damage is associated with lower IVF pregnancy rates.
  - Combined OR 1.70 (p < 0.05)
ICSI & DFI

- Zini et al. meta-analysis: Sperm DNA damage is not related to ICSI pregnancy rates
  - Combined OR 1.15 (p = 0.65)

- The careful selection of the sperm and embryo during ICSI may negate the adverse effect of sperm DNA damage on reproductive outcomes.

Table 4. Selected Diagnostic Properties of 14 Studies on Sperm DNA Damage and Pregnancy After ICSI.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Assay</th>
<th>%hDD</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hammadeh et al. 1996</td>
<td>60</td>
<td>ABlue</td>
<td>44</td>
<td>0.50</td>
<td>0.71</td>
<td>0.82</td>
<td>0.35</td>
<td>2.40</td>
<td>(0.72, 7.96)</td>
</tr>
<tr>
<td>Host et al. 2000</td>
<td>61</td>
<td>TUNEL</td>
<td>59</td>
<td>0.57</td>
<td>0.38</td>
<td>0.58</td>
<td>0.36</td>
<td>0.79</td>
<td>(0.28, 2.25)</td>
</tr>
<tr>
<td>Henkel et al. 2003</td>
<td>54</td>
<td>TUNEL</td>
<td>48</td>
<td>0.68</td>
<td>0.63</td>
<td>0.79</td>
<td>0.50</td>
<td>3.67</td>
<td>(1.12, 12.0)</td>
</tr>
<tr>
<td>Gandini et al. 2004</td>
<td>22</td>
<td>SCSA</td>
<td>41</td>
<td>0.31</td>
<td>0.44</td>
<td>0.44</td>
<td>0.31</td>
<td>0.36</td>
<td>(0.06, 2.08)</td>
</tr>
<tr>
<td>Huang et al. 2005</td>
<td>86</td>
<td>TUNEL</td>
<td>57</td>
<td>0.64</td>
<td>0.50</td>
<td>0.55</td>
<td>0.60</td>
<td>1.80</td>
<td>(0.76, 4.27)</td>
</tr>
<tr>
<td>Zini et al. 2005</td>
<td>60</td>
<td>SCSA</td>
<td>18</td>
<td>0.17</td>
<td>0.81</td>
<td>0.46</td>
<td>0.51</td>
<td>0.87</td>
<td>(0.23, 3.22)</td>
</tr>
<tr>
<td>Check et al. 2005</td>
<td>104</td>
<td>SCSA</td>
<td>28</td>
<td>0.29</td>
<td>0.76</td>
<td>0.72</td>
<td>0.34</td>
<td>1.34</td>
<td>(0.52, 3.43)</td>
</tr>
<tr>
<td>Boe-Hansen et al. 2006</td>
<td>47</td>
<td>SCSA</td>
<td>38</td>
<td>0.36</td>
<td>0.57</td>
<td>0.67</td>
<td>0.28</td>
<td>0.76</td>
<td>(0.21, 2.72)</td>
</tr>
<tr>
<td>Borini et al. 2006</td>
<td>50</td>
<td>TUNEL</td>
<td>60</td>
<td>0.71</td>
<td>0.75</td>
<td>0.90</td>
<td>0.45</td>
<td>7.36</td>
<td>(1.67, 32.4)</td>
</tr>
<tr>
<td>Benchaib et al. 2007</td>
<td>218</td>
<td>TUNEL</td>
<td>17</td>
<td>0.19</td>
<td>0.87</td>
<td>0.72</td>
<td>0.37</td>
<td>1.55</td>
<td>(0.70, 3.41)</td>
</tr>
<tr>
<td>Bungum et al. 2007</td>
<td>223</td>
<td>SCSA</td>
<td>33</td>
<td>0.29</td>
<td>0.61</td>
<td>0.52</td>
<td>0.37</td>
<td>0.65</td>
<td>(0.37, 1.14)</td>
</tr>
<tr>
<td>Lin et al. 2008</td>
<td>86</td>
<td>SCSA</td>
<td>24</td>
<td>0.26</td>
<td>0.77</td>
<td>0.52</td>
<td>0.52</td>
<td>1.21</td>
<td>(0.45, 3.23)</td>
</tr>
<tr>
<td>Micinski et al. 2009</td>
<td>50</td>
<td>SCSA</td>
<td>35</td>
<td>0.40</td>
<td>0.85</td>
<td>0.91</td>
<td>0.28</td>
<td>3.73</td>
<td>(0.74, 18.77)</td>
</tr>
<tr>
<td>Tarozzi et al. 2009</td>
<td>50</td>
<td>CMA3</td>
<td>56</td>
<td>0.49</td>
<td>0.27</td>
<td>0.61</td>
<td>0.18</td>
<td>0.34</td>
<td>(0.09, 1.29)</td>
</tr>
</tbody>
</table>

Abbreviations: %hDD = proportion of samples with high sperm DNA damage; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; OR = odds ratio; ABlue = aniline blue; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; SCSA = sperm chromatin structure assay; CMA3 = chromomycin A3.
SCSA and Fertilization Rate

100 IVF or ICSI cycles

Pregnancy loss after IVF/ICSI

- Zini et al. meta-analysis: Sperm DNA damage is related to pregnancy loss after IVF and ICSI
  - Combined OR 2.48 (p < 0.0001)

- No difference in the OR according to the type of ART (IVF or ICSI).
- Possible cause: impaired embryo/blastocyst development associated with sperm DNA.
## Miscarriage Rates

### Table: DNA Damage and Miscarriage Rates

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>High DNA damage</th>
<th>Low DNA damage</th>
<th>Weight</th>
<th>Risk Ratio</th>
<th>Heterogeneity: Tau² = 0.03, Chi² = 5.05, df = 2 (P = 0.02); I² = 35%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCSA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Busn-Hansen, 2005</td>
<td>7</td>
<td>17</td>
<td>63</td>
<td>1.4%</td>
<td>0.23 (0.02, 3.45)</td>
</tr>
<tr>
<td>Bang, 2007</td>
<td>14</td>
<td>56</td>
<td>56</td>
<td>14.5%</td>
<td>1.05 (0.62, 1.77)</td>
</tr>
<tr>
<td>Check, 2005</td>
<td>5</td>
<td>11</td>
<td>56</td>
<td>11.3%</td>
<td>1.48 (0.73, 2.97)</td>
</tr>
<tr>
<td>Ewenstein, 1999</td>
<td>7</td>
<td>11</td>
<td>55</td>
<td>9.0%</td>
<td>1.68 (0.71, 3.99)</td>
</tr>
<tr>
<td>Gardini, 2004</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>15%</td>
<td>Not estimable</td>
</tr>
<tr>
<td>Lin, 2008</td>
<td>6</td>
<td>9</td>
<td>53</td>
<td>8.3%</td>
<td>2.32 (1.12, 7.06)</td>
</tr>
<tr>
<td>Vima, 2004</td>
<td>8</td>
<td>16</td>
<td>100</td>
<td>10.7%</td>
<td>1.72 (0.85, 3.54)</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>171</td>
<td></td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>40</td>
<td></td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heterogeneity: Tau² = 0.03, Chi² = 5.05, df = 2 (P = 0.02); I² = 35%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TUNEL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benich, 2007</td>
<td>5</td>
<td>14</td>
<td>7</td>
<td>7.5%</td>
<td>4.08 (1.51, 11.07)</td>
</tr>
<tr>
<td>Bono, 2008</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5.3%</td>
<td>5.00 (1.39, 17.99)</td>
</tr>
<tr>
<td>Estebert, 2011</td>
<td>5</td>
<td>11</td>
<td>8</td>
<td>8.3%</td>
<td>4.32 (1.72, 10.84)</td>
</tr>
<tr>
<td>Friedman, 2008</td>
<td>7</td>
<td>20</td>
<td>4</td>
<td>6.5%</td>
<td>3.59 (1.19, 10.84)</td>
</tr>
<tr>
<td>Greco, 2005</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.4%</td>
<td>13.59 (0.81, 224.24)</td>
</tr>
<tr>
<td>Ozmen, 2007</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>6.0%</td>
<td>2.36 (0.73, 7.66)</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>52</td>
<td></td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>22</td>
<td></td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heterogeneity: Tau² = 0.00, Chi² = 1.69, df = 5 (P = 0.69); I² = 0%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COMET</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. Simon et al., Unpublished results</td>
<td>9</td>
<td>74</td>
<td>2</td>
<td>4.4%</td>
<td>1.03 (0.25, 4.36)</td>
</tr>
<tr>
<td>Mora, 2002</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>1.4%</td>
<td>4.90 (0.30, 80.99)</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>83</td>
<td></td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>12</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heterogeneity: Tau² = 0.00, Chi² = 0.88, df = 1 (P = 0.32); I² = 0%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Acridine Orange</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zivi, 2005</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>3.9%</td>
<td>2.78 (0.58, 13.11)</td>
</tr>
<tr>
<td><strong>Subtotal (95% CI)</strong></td>
<td>6</td>
<td></td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>2</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Heterogeneity: Not applicable</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Test for overall effect: Z = 1.29 (P = 0.20)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>312</td>
<td>940</td>
<td>100.0%</td>
<td>2.16 (1.54, 3.03)</td>
<td></td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>76</td>
<td></td>
<td>149</td>
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<td><strong>Heterogeneity: Tau² = 0.13, Chi² = 21.15, df = 14 (P = 0.10); I² = 34%</strong></td>
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<td><strong>Test for overall effect: Z = 4.48 (P &lt; 0.00001)</strong></td>
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DNA damage is significantly lower in the seminiferous tubules compared with cauda epididymis or ejaculated sperm. (Suganuma 2005, Steele 1999, Greco 2005)

The use of testicular sperm in couples with repeated pregnancy failure in ART and high sperm DNA fragmentation in semen → a significant increase in PRs in these couples. (Greco 2005, Alvarez 2008)

- Eliminates the burden of sperm DNA repair by the oocyte

However, testicular sperm may not always solve the problem...

- DNA damage may also occur in the seminiferous tubules by apoptosis or be due to defects in chromatin remodeling during spermiogenesis.

Alvarez J. Argentina de Andrologia 2008;5.
The rate of aneuploidy: testicular sperm > ejaculated sperm.
- Concerns about ICSI sperm with a higher rates of chromosomal abnormalities.
- Possibly due to selective elimination of aneuploid sperm during passage through the epididymis. (Egozcue 2005)
- However, this data is from studies using sperm from severe oligospermic or azoospermic men.
- These men may have higher aneuploidy at baseline as compared with normospermic men.

Using testicular sperm in couples with high levels of sperm DNA fragmentation, PRs were higher and miscarriage rates lower than when using ejaculated sperm. (Borini 2006)

Egozcue J. Cytogenet Genome Res 2005;111:337–42.
- **Greco et al.**:
  - Men with TUNEL measured DFI >15%
  - Failed IVF with ejaculated sperm
  - IVF with testicular sperm
  - Clinical PR of 44.4% (vs- 0% with ejaculated spermatozoa)
So, Do All Roads lead to ICSI with High DFI?

• Problem with high DFI
  - Consistency with each SA
  - What threshold?
  - Pregnancies do happen with high DFI - natural /IUI/IVF

• BUT....we can’t really fix high DFI in most cases
  - Treat ROS/WBC/know toxins...maybe
  - Testicular sperm over ejaculated
  - MVI - no benefit really
  - Aren’t a lot of labs moving to all ICSI to prevent any chance of failed fertilization...controversial
Applications?...the real question

- Infertile couples who present to with:
  - a history of longstanding infertility
  - repeated IVF failure
  - recurrent miscarriages
Spontaneous Pregnancy:

• The prevalence of a positive test in first pregnancy planners is low (<10%) and 17% of couples with a positive test will achieve a pregnancy, indiscriminate sperm DNA testing in this context is not advised.

Screening in Mild Male-Factor (IUI Candidates):

• More studies are needed before routine DNA fragmentation testing is recommended prior to IUI.

Screening in Severe Male-Factor (IVF Candidates):

• Couples with sperm DNA damage may choose to proceed to ICSI, where pregnancy rates are independent of the test result.
• The clinical value of an 11% difference in pregnancy rates (34% vs. 23%) is modest and it may be hard to justify routine testing.
• However, clinicians may want to test select couples (e.g., with failed IVF) so as to better counsel these couples in future ART cycles.
Thank you!