Why test for Oxidative Stress and DNA damage in sperm?

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ESHRE Campus, Thessaloniki, 2009
Semen analysis

• Essential for initial diagnosis

• Limited as prognostic tool for ART

• Only 20% of young Norwegian men achieve WHO values
  Jorgensen et al, 2006

• Only 46% of older men >45yrs (n=1174) meet all WHO values
  Hellstrom et al, 2006

• In infertility diagnosis-
  Many men with ‘below normal’ values can be fertile
  Haugen et al, 2006

• Men with ‘normal’ values can be infertile
  Bonde et al, Lancet 1998
Clinical significance of semen profiles

No single parameter was diagnostic of infertility (n=1461)
Extensive overlap between fertile and infertile ranges
Morphology most powerful
Guzick et al, 2001

Morphology most powerful but volume and motility of limited value
Probability of pregnancy ↑ as concentration ↑ up to 40 x 10⁶ /mL
then no further association (n=430)
extensive overlap between fertile and infertile ranges
Bonde, Skakkebaek et al, 1998

Concentration and motility were most powerful
Morphology poorest predictive power
- 50% of fertile men had abnormal morphology (n=719)
Nallella, Agarwal et al, 2006

243 fertile men had a mean of only 20% normal morphology by WHO 1992 criteria
Chia et al, 1998
Regional and world-wide variation of semen parameters

- Within USA, New York had highest concentrations \((134 \times 10^6/mL)\)
  - Iowa had lowest concentrations \((48 \times 10^6/mL)\)
  - Thailand \((52 \times 10^6/mL)\)
- In Japan, fertile men had lower semen quality, similar to Norway \((20\% < \text{WHO})\)
- In Europe, Finland and Denmark’s fertile men have markedly different semen profiles

**Fisch et al, 1996, Swan, 2006; Jorgensen et al, 2006; Iwamoto et al, 2006**
Variability of semen parameters between and within individuals

- Marked biological heterogeneity of semen between 243 fertile men
  *Chia et al, 1998*

- Even consecutive samples from same individuals (twice a week for 120 weeks)
  *WHO, 1990* 
  (673 samples from 7 men over 324 weeks)
  *Mallidis et al, 1991*

*Reference values have limited diagnostic value for infertility and are not predictive for ART*
Intracytoplasmic sperm injection
ISCI- 1992
Success for men with poor semen quality
Only requirement is sperm viability
Natural barriers (poor motility or defective sperm zona binding) removed
Usable with immature sperm
Pregnancy rates of 30-50%
The ‘ISCI Escalation’- almost twice as many cycles as IVF
-reduction in andrological research

ESHRE’s European IVF Monitoring Consortium, 2008
Male Infertility

Environment
Endocrine disruptors
- xenoestrogens
- Anti-androgens
- Toxic compounds

Occupation
- Plastics and resins, solvents,
- wood processing, metal industry,
- Automobile, truck and aircraft mechanics
- Sedentary or stressful job

Genetic Inheritance
- CABVD
- Robertsonian translocations
- Y-chromosome deletions
- Paternal Age

Lifestyle
- diet
- smoking
- alcohol
- recreational drugs
- STDs
- injury
- infection

Sperm DNA damage
High levels of sperm DNA damage have some correlation with

- **Oligozoospermia**
  

- **Poor motility and morphology**
  

- **OAT**
  

- **Cytoplasmic retention**
  

- **mtDNA damage**
  
  O’Connell et al, 2003
DNA reproducibility compared to conventional parameters

- DNA is more consistent than SA
  
  Schrader et al. 1988; Evenson et al. 1991; Zini et al. 2001; Loft et al. 2003

- Sperm DNA has lower CV (20% cf >40%)
  
  De Jonge et al, 2004

- DNA has ‘high monthly repeatability’ within donors CV 10% cf 44% for conc, 78% for motility and 69% for morphology
  
• Retrospective study (n=282 consecutive patients)
• Attending for IUI, IVF or ICSI with 2-5 DNA tests
• Mean CV of DFI was 29%
• 37% (95% CI: 27%, 49%) of patients with DFI>30% in 1st test had DFI<30% in 2nd test
• 27% (95% CI: 16%, 40%) of patients with 21-30% DFI in 1st test had DFI>30% in 2nd test
• Intra-individual variation in DFI is significant
• Repeated DNA tests are necessary
Does Sperm DNA influence Fertility outcomes?

Nuclear DNA anomalies lead to:-

• Failure of fertilization in IVF
  *Bianchi et al, 1993; Sun et al, 1997*

• Failure to implant in ICSI
  *Sakkas et al, 1996; Lopes et al, 1998*

• Increased time to conception

• Poor embryo development
  *Morris et al, 2002; Tomsu et al, 2002*

• Post-implantation loss and malformations
  *Robaire et al, 1985*

• Increased miscarriage rate
  *Evenson et al, 1999; Carrell et al, 2003*

• Childhood cancers
  *Knight and Marrett, 1997*
Sites and Causes of Sperm DNA Damage

Seminiferous tubules

Abortive apoptosis
*Sakkas et al, 1999*

Abnormal chromatin packaging
*Manicardi et al., 1995, Carrell and Liu, 2001; Zhang et al, 2006*

Epididymis

Incomplete repair of physiological nicks
*Sakkas et al., 1999*

Assault by senescent sperm and toxics
*Hess, 1998; Moore, 1999*

Aberrant SCF pathway
*Shaman et al, 2007, Yamauchi et al, 2007*

Post ejaculation

Clinical hazards imposed in ART labs

Oxidative Stress..........................
Oxidative Stress and Fertility

$\text{H}_2\text{O}$ $\text{ATP}$ $\text{O}_2$ $\text{e}^-$ $\text{H}_2\text{O}_2$ $\text{OH}^-$ $\text{e}^-$ $\text{H}^+$ $\text{H}_2\text{O}$  

$\text{O}_2^{-}$ $\text{e}^-$ $2\text{H}^+$  

Aitken et al, De Illius et al, 2006

Alvarez et al, 1987

De Lamirande et al, 1997

Supraphysiological ROS levels

Spermatozoa
- Lipid peroxidation
- DNA fragmentation
- Apoptosis

Oocytes
- Meiotic spindle damage
- † Oocyte quality

Embryos
- Mitochondrial alterations
- Embryo cell block
- ATP depletion
- † Cleavage
- Apoptosis

Fertilization
- † Oocyte penetration
- † Fertilization
- † Implantation
- † Early pregnancy loss

Figure 1. Effects of pathological levels of reactive oxygen species during IVF. 
ROS: Reactive oxygen species.

Du Plessis et al, Expert Reviews, 2009
Oxidative Stress is a major cause of DNA damage


Aitken and De Illius, 2009
Implications of sperm DNA

Because the aberrant repair event preceded S phase the mutation will be in every cell in the body.

Oxidative DNA damage in the fertilizing spermatozoon

Aberrant DNA repair in the zygote prior to the initiation of S-phase generates a CG→UA→TA transition in the FGFR3 gene.

Aitken and de Iulius, 2007
Risk of Diseases in Offspring from Damaged Sperm DNA

- Sperm DNA damage increases with ♂ Age
  *Singh et al, 2003; Wyrobek et al, 2006; Aitken and de Iulius, 2007*

- Oxidative damage increases with Age
- ↑ ♂ age is associated with ↑ incidence of disease
  - miscarriage *de Rochebrochard and Thonneau, 2002*

- dominant genetic mutations-Achondroplasia and Apert Syndrome
  *Crow, 2000; Wyrobek et al, 2006*

- neurological Disorders -Schizophrenia, Autism and Bipolar Disease
  *Sipos et al, 2004; Frans et al, 2008*

- Birth defects- neural tube defects and even Downs Syndrome
  *McIntosh et al, 1995*
Methodologies to Evaluate Sperm DNA Damage

Strand breaks
• Sperm Chromatin Structure Assay (SCSA)
• TUNEL
• Single-cell gel electrophoresis assay (Comet)
• Sperm Chromatin Dispersion Test (SCD)

Chromatin packaging defects
• Acid Aniline blue
• Chromomycin A3
Novel tests- for biomarkers of OS in DNA
8-Hydroxy-2’-deoxyguanosine (8-OH2dG) - the most abundant DNA adduct

• In sperm, no repair and little antioxidant protection
• DNA exposed to ROS $\rightarrow$ DNA adducts
• Adducts are highly mutagenic
• 8-OH2dG can lead to a GC to TA transversion
• valuable biomarker of sperm health
• High Performance Liquid Chromatography
DNA damage caused by OS

Strand breaks

Subjects
Type 1 diabetics (n=27)
Non diabetics attending for investigation of infertility (n=29)

oxidised bases

Increased concentrations of the oxidative DNA adduct 7,8-dihydro-8-oxo-2'-deoxoguanosine in the germ-line of men with type 1 diabetes
DNA damage caused by OS

8OHdG vs Comet

\[ R^2 = 0.54 \]
Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis

L.K. Thomson¹,², S.D. Fleming², R.J. Aitken³, G.N. De Iuliis³, J.-A. Zieschang¹, and A.M. Clark¹

<table>
<thead>
<tr>
<th>Sperm DNA fragmentation and 8OHdG</th>
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<tbody>
<tr>
<td>Native semen</td>
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<tr>
<td>Post DCG</td>
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</table>
Are sperm DNA tests useful as diagnostic or prognostic clinical tests?
For a test to be useful, it must have strong predictive capacity for pregnancy outcome and little overlap between fertile and infertile samples.
(Diagnostic) Odds Ratios

An Odds Ratio gives us the chance of a pregnancy occurring if the test result is above our specified threshold. Odds ratios need to be > 2.0 to be useful. If CIs include 1.0, relationship is usually NS.

Sensitivity - 1.00, if DNA damage above threshold prevents achievement of pregnancy in all cases.

Specificity - 1.00, if all samples with DNA damage below threshold achieve pregnancy so their sum should approach 2.0.

If Sensitivity plus Specificity > 1.0, ORs are generally significant.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Sens</th>
<th>Spec</th>
<th>Sens + Spec</th>
<th>Abnormal tests (%)</th>
<th>DOR (95% CI)</th>
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<tbody>
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<td>IVF</td>
<td>0.36</td>
<td>0.97</td>
<td>1.03</td>
<td>5</td>
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<td>Borini et al., 2006 (52)</td>
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<td>Cheek et al., 2005 (47)</td>
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Note: CI = confidence interval; DOR = diagnostic odds ratio; Sens = sensitivity; Spec = specificity.
## Sperm DNA Damage and IUI Outcomes

<table>
<thead>
<tr>
<th>Author</th>
<th>Assay</th>
<th>n</th>
<th>Design</th>
<th>Threshold (%)</th>
<th>&lt; Threshold Pregnancy (%)</th>
<th>&gt; Threshold Pregnancy (%)</th>
<th>Pregnancy</th>
<th>OR</th>
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<td>&lt;0.001</td>
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Very useful test for IUI
## Sperm DNA Damage and IVF Outcomes

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<th>design</th>
<th>Female sel</th>
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</table>
So is DNA damage a useful test for IVF?

• Combined odds ratio 1.67 for no pregnancy with high DNA damage (1.27-2.20) $p<0.01$

• Positive predictive value 74% but wrongly predicts failure in 26%

*Collins et al, 2008; Zini et al, 2009*
# Sperm DNA Damage and ICSI Outcomes

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
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<th>assay</th>
<th>Threshold (%)</th>
<th>&lt; Preg (%)</th>
<th>&gt; Preg (%)</th>
<th>Fert</th>
<th>Preg</th>
<th>OR</th>
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<td>30</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>0</td>
<td>1.34</td>
<td>0.52,3.43</td>
</tr>
<tr>
<td>Zini ’05</td>
<td>60</td>
<td>Pro</td>
<td>SCSA</td>
<td>30</td>
<td>51</td>
<td>55</td>
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<td>0</td>
<td>0.87</td>
<td>0.23,3.22</td>
</tr>
<tr>
<td>Boe-Hansen ’06</td>
<td>47</td>
<td>Pro</td>
<td>SCSA</td>
<td>27</td>
<td>27.6</td>
<td>33.3</td>
<td>0</td>
<td>0</td>
<td>0.76</td>
<td>0.21,2.72</td>
</tr>
<tr>
<td>Borini ’06</td>
<td>50</td>
<td>-</td>
<td>TUNEL</td>
<td>10</td>
<td>45</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>7.36</td>
<td>1.67,32.4</td>
</tr>
<tr>
<td>Muriel ’06</td>
<td>85</td>
<td>Pro</td>
<td>SCD</td>
<td>-</td>
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<td>NA</td>
<td>0</td>
<td>0</td>
<td>0.79</td>
<td>0.28,2.25</td>
</tr>
<tr>
<td>Benchaib ’07</td>
<td>218</td>
<td>pro</td>
<td>TUNEL</td>
<td>15</td>
<td>37.4</td>
<td>27.8</td>
<td>0</td>
<td>0</td>
<td>1.55</td>
<td>0.70,3.41</td>
</tr>
<tr>
<td>Bungum ’07</td>
<td>223</td>
<td>Pro, consec</td>
<td>SCSA</td>
<td>30</td>
<td>37.3</td>
<td>47.9</td>
<td>0</td>
<td>0</td>
<td>0.65</td>
<td>0.37,1.14</td>
</tr>
<tr>
<td>Lin ’07</td>
<td>86</td>
<td>pro</td>
<td>SCSA</td>
<td>27</td>
<td>52.3</td>
<td>47.6</td>
<td>0</td>
<td>0</td>
<td>1.21</td>
<td>0.45,3.23</td>
</tr>
<tr>
<td>Bakos ’07</td>
<td>68</td>
<td>-</td>
<td>TUNEL</td>
<td>35</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>0</td>
<td>0.79</td>
<td>0.28,2.25</td>
</tr>
</tbody>
</table>
Combined Odds ratio = 1.20 (0.91, 1.59)  
p > 0.05

so there is no clinical application as sperm DNA damage does not affect pregnancy rates after ICSI

- ICSI appears to bypass poor sperm DNA too

Zini et al, 2009
# Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

<table>
<thead>
<tr>
<th>Author</th>
<th>ART</th>
<th>n</th>
<th>Threshold</th>
<th>&lt; Preg loss (%)</th>
<th>&gt; Preg loss (%)</th>
<th>Preg loss (%)</th>
<th>Risk</th>
<th>OR</th>
<th>CI</th>
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<tbody>
<tr>
<td>Virro '04</td>
<td>IVF and ICSI</td>
<td>30%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check '05</td>
<td>ICSI</td>
<td>104</td>
<td>--</td>
<td>--</td>
<td>47</td>
<td>↑</td>
<td>2.27</td>
<td>0.45,1.59</td>
<td></td>
</tr>
<tr>
<td>Zini '05</td>
<td>ICSI</td>
<td>60</td>
<td>30%</td>
<td>12</td>
<td>33</td>
<td>16</td>
<td>↑</td>
<td>3.67</td>
<td>0.46,29.42</td>
</tr>
<tr>
<td>Borini '06</td>
<td>IVF</td>
<td>82</td>
<td>10%</td>
<td>15.8</td>
<td>50</td>
<td>6</td>
<td>↑</td>
<td>32.0</td>
<td>0.62,1663</td>
</tr>
<tr>
<td>Borini '06</td>
<td>ICSI</td>
<td>50</td>
<td>10%</td>
<td>0</td>
<td>62.5</td>
<td>25</td>
<td>↑</td>
<td>108.0</td>
<td>1.73,6729</td>
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<tr>
<td>Benchaib '07</td>
<td>IVF</td>
<td>84</td>
<td>30%</td>
<td>2.6</td>
<td>25</td>
<td>13</td>
<td>↑</td>
<td>10.0</td>
<td>0.87,114.8</td>
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<tr>
<td>Benchaib '07</td>
<td>ICSI</td>
<td>218</td>
<td>30%</td>
<td>2.8</td>
<td>8.3</td>
<td>13</td>
<td>↑</td>
<td>3.51</td>
<td>0.89,23.28</td>
</tr>
<tr>
<td>Lin '07</td>
<td>ICSI</td>
<td>137</td>
<td>27%</td>
<td>11.8</td>
<td>40</td>
<td>12</td>
<td>↑</td>
<td>2.56</td>
<td>0.44,15.03</td>
</tr>
<tr>
<td>Lin '07</td>
<td>IVF</td>
<td>86</td>
<td>27%</td>
<td>8.5</td>
<td>16.7</td>
<td>12</td>
<td>↑</td>
<td>5.00</td>
<td>0.97,25.77</td>
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<tr>
<td>Frydman '07</td>
<td>ICSI</td>
<td>117</td>
<td>35%</td>
<td>10</td>
<td>36.8</td>
<td>19</td>
<td>↑</td>
<td>5.25</td>
<td>1.31,21.11</td>
</tr>
<tr>
<td>Bungum '07</td>
<td>IVF</td>
<td>388</td>
<td>30%</td>
<td>24.4</td>
<td>19</td>
<td>22</td>
<td>0</td>
<td>0.73</td>
<td>0.23,233</td>
</tr>
<tr>
<td>Bungum '07</td>
<td>ICSI</td>
<td>223</td>
<td>30%</td>
<td>15.6</td>
<td>23.8</td>
<td>22</td>
<td>↑</td>
<td>1.69</td>
<td>0.63,4.49</td>
</tr>
</tbody>
</table>
So is DNA damage a useful test for predicting pregnancy loss?

- Combined odds ratio 2.48 (1.52-4.04) p<0.0001
- Positive predictive value of loss of 37% (high DNA damage) or 10% (low DNA damage) with sensitivity of 0.4
- However, 67% of couples with high DNA damage had normal offspring

*Zini et al, 2009*
Ito summarise the relationship between sperm DNA damage and pregnancy

- in IUI: strong negative effect (OR=9.9)
- in IVF: mild negative effect (OR=1.7)
- in ICSI: no effect (OR=1.2)

Thus

↑Intervention from IUI to IVF to ICSI, the less impact sperm DNA damage has on early fertility checkpoints

BUT in IVF and ICSI pregnancy loss: DNA damage has a moderate positive effect (OR=2.5)

ie an effect on fetal development

*Systematic review and meta-analysis by Zini et al, 2008*
Are we expecting too much from one test?

Other factors with important roles-

- Sperm function
- Oocyte quality
- Embryo quality
- Uterine competence
- ORs are based on thresholds-
  - how accurate are they?
Single Cell Gel Electrophoresis

Comet assay

• more sensitive - detecting just 50 SSB/cell
• Inexpensive
• reproducible
• Requires low no of sperm (60,000/slide)
• Measure SSB + DSB and alkali labile sites
Another test for DNA adducts

- Formamidopyrimidined-DNA glycosylase; FPG
- Converts 8OHdG to single strand breaks
- They can then be measured by Comet assay

*FPG extract kindly donated by Gunnar Brunborg, Institute of Public Health, Oslo, Norway*
### Relationship between sperm DNA fragmentation and pregnancy rates in IVF

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sample</th>
<th>n</th>
<th>ROC</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comet</td>
<td>Native</td>
<td>146</td>
<td>0.649</td>
<td>0.57-0.79</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>DCG</td>
<td>149</td>
<td>0.634</td>
<td>0.54-0.75</td>
<td>0.025</td>
</tr>
<tr>
<td>Comet + FPG</td>
<td>Native</td>
<td>64</td>
<td>0.698</td>
<td>0.60-0.91</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>DCG</td>
<td>63</td>
<td>0.697</td>
<td>0.53-0.87</td>
<td>0.029</td>
</tr>
</tbody>
</table>

- Native semen – 39.6 v 52.3 %
- DGC sperm – 28.0 v 36.5%
- Potential breaks constitute additional 12 – 20 %
- Adducts present in both native and DGC sperm
- No pregnancies when DNA damage > 48/62 %
Relationship between sperm DNA fragmentation and pregnancy rates in ISCI

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sample</th>
<th>n</th>
<th>ROC</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comet</td>
<td>Native</td>
<td>90</td>
<td>0.637</td>
<td>0.46-0.72</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>DCG</td>
<td>89</td>
<td>0.553</td>
<td>0.43-0.69</td>
<td>0.271</td>
</tr>
<tr>
<td>Comet + FPG</td>
<td>Native</td>
<td>51</td>
<td>0.686</td>
<td>0.51-0.86</td>
<td>0.042</td>
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<tr>
<td></td>
<td>DCG</td>
<td>51</td>
<td>0.702</td>
<td>0.53-0.87</td>
<td>0.027</td>
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</table>

- No relationship between Comet and pregnancy
- Significant rel between Comet plus adducts and pregnancy
### Clinical significance of Comet using thresholds for native and DGC sperm in IVF & ICSI

<table>
<thead>
<tr>
<th></th>
<th>Native</th>
<th></th>
<th></th>
<th>DGC</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>IVF</td>
<td>ICSI</td>
<td>IVF</td>
<td>ICSI</td>
<td>IVF</td>
<td>ICSI</td>
</tr>
<tr>
<td></td>
<td>&lt;62%</td>
<td>&gt;62%</td>
<td>OR (CI)</td>
<td>&lt;62%</td>
<td>&gt;62%</td>
<td>OR (CI)</td>
</tr>
<tr>
<td>Cycles started</td>
<td>114</td>
<td>35</td>
<td>--</td>
<td>43</td>
<td>47</td>
<td>--</td>
</tr>
<tr>
<td>Clinical pregnancies</td>
<td>25 (80.7%)</td>
<td>4 (36.4%)</td>
<td>3.54 (1.07-12.89)</td>
<td>16 (88.9%)</td>
<td>12 (54.6%)</td>
<td>1.73 (0.64-4.70)</td>
</tr>
<tr>
<td>Deliveries to date</td>
<td>17 (68.0%)</td>
<td>2 (50.0%)</td>
<td>5.46 (0.86-44.04)</td>
<td>8 (50.0%)</td>
<td>8 (66.7%)</td>
<td>1.40 (0.33-6.07)</td>
</tr>
<tr>
<td>Early pregnancy loss</td>
<td>3 (12.0%)</td>
<td>1 (25.0%)</td>
<td>2.44 (0.00-50.80)</td>
<td>5 (31.3%)</td>
<td>2 (16.7%)</td>
<td>2.27 (6.28-22.03)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>IVF</th>
<th>ICSI</th>
<th>IVF</th>
<th>ICSI</th>
<th>IVF</th>
<th>ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;48%</td>
<td>&gt;48%</td>
<td>OR (CI)</td>
<td>&lt;48%</td>
<td>&gt;48%</td>
<td>OR (CI)</td>
</tr>
<tr>
<td>Cycles started</td>
<td>114</td>
<td>35</td>
<td>--</td>
<td>51</td>
<td>39</td>
<td>--</td>
</tr>
<tr>
<td>Clinical pregnancies</td>
<td>26 (74.3%)</td>
<td>3 (37.5%)</td>
<td>4.97 (1.06-32.03)</td>
<td>19 (86.4%)</td>
<td>9 (50.0%)</td>
<td>1.98 (0.71-5.62)</td>
</tr>
<tr>
<td>Deliveries to date</td>
<td>18 (69.2%)</td>
<td>1 (33.3%)</td>
<td>7.41 (0.80-177.8)</td>
<td>10 (45.5%)</td>
<td>6 (66.7%)</td>
<td>1.67 (0.40-7.40)</td>
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<tr>
<td>Early pregnancy loss</td>
<td>3 (11.5%)</td>
<td>1 (33.3%)</td>
<td>6.00 (0.0-328.36)</td>
<td>5 (26.3%)</td>
<td>2 (22.2%)</td>
<td>1.25 (0.14-12.40)</td>
</tr>
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</table>
Strategies to Reduce Oxidative Stress

Antioxidant treatment

• ZnSO$_4$/ folic acid and semen quality
  *Wong et al, 2002*

• Zn and Selenium and DNA quality
  *Menezo et al, 2007*

• Vit C and E and ICSI outcome
  *Rolf et al, 1999; Greco et al, 2005*

• Menovit and IVF/ICSI outcome
  *Tremellen et al, 2007*
Sperm DNA: organisation, protection and vulnerability – from basic science to clinical application

ESHRE Campus symposium
Stockholm, Sweden
21-22 May 2009

Organised by the ESHRE Special Interest Group “Andrology” in collaboration with the Karolinska Institutet (Centre for Andrology and Sexual Medicine, Department of Medicine, Huddinge, Stockholm, Sweden) with support from the Swedish Research Council (Vetenskapsrådet).

Consensus document:
edited by Chris Barratt
Recommendations from Consensus Document

1. Fundamental research is urgently required
2. Standardization of clinical assays
3. Animal Models
4. High quality clinical data is urgently required
5. Long term follow up of ART children
Acknowledgements

Prof. Gunnar Brunborg,
Institute of Public Health, Norway
Luke Simon
Ishola Agbaje
Ciara Hughes
Lauren Dalzell

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Carmel McVicar
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