NOVEL MEASURES OF SPERM DNA DAMAGE INCREASE ITS USEFULNESS TO DIAGNOSE MALE INFERTILITY AND PREDICT LIVE BIRTHS FOLLOWING BOTH IVF AND ICSI

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

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Semen analysis has limited diagnostic value for male infertility and prognostic value for ART

The New England Journal of Medicine

SPERM MORPHOLOGY, MOTILITY, AND CONCENTRATION IN FERTILE AND INFERTILE MEN

David S. Guzick, M.D., Ph.D., James W. Overstreet, M.D., Ph.D., Pam Factor-Litvak, Ph.D., Charlene K. Brazil, B.S., Steven T. Naka, M.D., Christos Coutifaris, M.D., Ph.D., Sandra Ann Carson, M.D., Pauline Figueroa, Ph.D., Michael P. Steinkamp, M.D., Joseph A. Hill, M.D., Dong Xu, M.P.H., and Donna L. Vogel, M.D., Ph.D., for the National Cooperative Reproductive Medicine Network

Counting sperm does not add up any more: time for a new equation?


Focus on Determinants of Male Fertility

Is sperm evaluation useful in predicting human fertility?

Sheena E M Lewis
SPERM DNA TESTING

- Diagnostic of male infertility (Zini et al, Urol 2002: Simon et al, FS 2011)
- Increased time to conception (Evenson et al, HR 1999)
- Poor embryo development (Simon et al, HR 2014)
- Poor IVF and ICSI outcomes (Simon et al, BPRCOG, 2017)
- Increased miscarriage rate (Robinson et al, HR 2012)
DETECTION OF SPERM DNA DAMAGE

Comet assay
(Hughes et al., 1996)

Sperm Chromatin Structure assay
(Evenson et al., 1999; SPZ LAB, Christensen)

TUNEL assay
(Henkel et al., 2004)

Halo assay
(Sperm Chromatin Dispersion test)
(Fernandez et al., 2003)
BENEFITS OF SPERM COMET

• Second generation test
• Detects damage in 70 v 15% of sperm
• Quantifies damage per individual sperm
• Strong diagnostic tool
• Strong predictive value for ART
• Ongoing Research Program
• Utilisation with severe OATS
• Utilisation with testicular samples
BACKGROUND

• Lister has been using the SpermComet test since 2011

• Initial clinical thresholds seen as a limitation of the test:
  – 0-25% No significant DNA damage
  – 25-50% Consider IVF
  – >50% ICSI recommended

• Majority of clinic results fall in the 25-50% range

• Tighter thresholds would be more clinically useful in guiding treatment selection.
OBJECTIVES

• Determine and compare novel Comet Plot parameters to identify which has the highest ability to predict a live birth following IVF and also ICSI.

• Analyze the relationship between treatment pathway, live birth and sperm DNA damage for clinic patients who have had the SpermComet test.

• Define clinic specific thresholds to better guide the selection of treatment pathway.
IDENTIFICATION OF NOVEL COMET MARKERS

ACS = Average Comet Score (mean of all comets scored)
LCS = Low Comet Score
HCS = High Comet Score

COMET PLOT FOR TYPICAL FERTILE MAN

COMET PLOT FOR TYPICAL INFERTILE MAN

Source: SpermComet fertile donor database (N= 76); SpermComet Unexplained database (N= 166) .
METHODS

• 3 COMET markers
  – % Average COMET score
  – % Low COMET score (LCS)
  – % High COMET score (HCS)

• Receiver Operator Curve Analysis (ROC) for potential to diagnose male infertility
  – 76 fertile donors / 166 men from couples with idiopathic subfertility

• Receiver Operator Curve Analysis (ROC) for effect on ART outcome
  – 381 male partners of subfertile couples undergoing ART
METHODS

TOTAL SINGLE CYCLES
381

ICSI CYCLES
280

ICSI ET
229

IVF CYCLES
101

IVF ET
79

- Split IVF/ICSI, abandoned and converted to IUI excluded.
- Single cycle selected for each couple based on treatment cycle closest to SpermComet test.
- Only cycles resulting in embryo transfer included.
- 'Ongoing' outcome classification assumed to be live birth as majority >40 weeks.
RESULTS
## Diagnosis of Male Infertility

<table>
<thead>
<tr>
<th>Result parameter</th>
<th>Threshold value</th>
<th>ROC curve (95% CI)</th>
<th>$P$ value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Odds ratio</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS</td>
<td>$\geq 26%$</td>
<td>0.925 (0.893-0.956)</td>
<td>$&lt;0.0001$</td>
<td>0.735</td>
<td>1.000</td>
<td>1.000</td>
<td>0.632</td>
<td>N/A</td>
<td>2.7</td>
</tr>
<tr>
<td>LCS</td>
<td>$\leq 74%$</td>
<td>0.936 (0.908-0.964)</td>
<td>$&lt;0.0001$</td>
<td>0.783</td>
<td>0.934</td>
<td>0.963</td>
<td>0.664</td>
<td>51.2</td>
<td>2.7</td>
</tr>
<tr>
<td>HCS</td>
<td>$\geq 4%$</td>
<td>0.909 (0.872-0.942)</td>
<td>$&lt;0.0001$</td>
<td>0.843</td>
<td>0.803</td>
<td>0.903</td>
<td>0.701</td>
<td>21.9</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*Source: SpermComet fertile donor database (N= 76); SpermComet Unexplained database (N= 166).*
CONCLUSIONS

All COMET markers highly predictive of male subfertility confirming role for testing in unexplained infertility
**IMPACT ON IVF OUTCOME**

<table>
<thead>
<tr>
<th>AVERAGE COMET SCORE (ACS)</th>
<th>LOW COMET SCORE (LCS)</th>
<th>HIGH COMET SCORE (HCS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All IVF patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>&lt;=29%</td>
<td>&gt;=64%</td>
<td>&lt;=6%</td>
</tr>
<tr>
<td>31%</td>
<td>33%</td>
<td>38%</td>
</tr>
<tr>
<td>&gt;29%</td>
<td>&lt;64%</td>
<td>&gt;6%</td>
</tr>
<tr>
<td>13%</td>
<td>13%</td>
<td>13%</td>
</tr>
</tbody>
</table>

Source: Lister IVF dataset (N= 79). P value calculated using Chi square.
CONCLUSIONS

All COMET markers highly predictive of male subfertility confirming role for testing in unexplained infertility

IVF Livebirth rate declined sharply once sperm DNA damage exceeded all ROC threshold levels identified with HCS > 6% the most predictive (38% vs 13%)
IMPACT ON ICSI OUTCOME

Average Comet Score (ACS)

- All ICSI patients: N = 229
- <=27%: 50
- >27%: 179

Low Comet Score (LCS)

- All ICSI patients: N = 229
- >=68%: 41
- <68%: 188

High Comet Score (HCS)

- All ICSI patients: N = 229
- <=10%: 81
- >10%: 148

P value = 0.02

Source: Lister ICSI dataset (N= 229). P value calculated using Chi square.
Trends in IVF compared to ICSI

![Graph showing trends in IVF and ICSI live birth rates](image-url)
CONCLUSIONS

All COMET markers highly predictive of male subfertility confirming role for testing in unexplained infertility

IVF Livebirth rate declined sharply once sperm DNA damage exceeded all ROC threshold levels identified with HCS > 6% the most predictive (38% vs 13%)

ICSI Livebirth rate moderately declined once sperm DNA damage exceeded all ROC threshold levels identified with HCS > 10% the most predictive (43% vs 28%)
IMPACT ON TREATMENT CLINIC CYCLES

NORMOZOOSPERMIC PATIENTS SPLIT BY SPERM DNA DAMAGE THRESHOLDS
N = 184

SCENARIO 1: CURRENT LISTER PATHWAY

IVF ICSI

<=25% >25<=50% >50%
46 132 6

ONLY 3% directed to ICSI

SCENARIO 2: PREDICTED PATHWAY BY LISTER HCS

IVF ICSI

<6% >6%
56 128

70% directed to ICSI

Source: Lister Outcome Database.
IMPACT ON POTENTIAL LIVEBIRTH OUTCOME

SCENARIO 1: CURRENT USE

184 COUPLES

46 <=25% 132 >25<=50% 6 >50%

IVF

SCENARIO 2: LISTER HCS

56 <6% 31 7-10% 97 >10%

LIVE BIRTH RATE

28% <=25% 23% >25<=50% 31% >50%

IVF

38% <6% 43% 7-10% 28% >10%

ICS

13 <=25% 30 >25<=50% 2 >50%

ACTUAL LIVE BIRTHS

21 <6% 13 7-10% 27 >10%

TOTAL LIVE BIRTHS = 45

TOTAL LIVE BIRTHS = 61
SUMMARY

• We have identified clinic-specific thresholds for ACS and novel SpermComet parameters

• Role for testing in Unexplained Subfertility

• Role for prediction of ART outcome / improved treatment pathway
  – HCS parameter is most predictive of IVF and ICSI Livebirth
  – Recommend IVF over ICSI even with normal semen parameters with COMET scores above identified thresholds
  – ICSI livebirth also impacted by COMET scores suggesting role for urological intervention to reduce damage before treatment
  – Role for testicular retrieval with very high levels / recurrent cycle failures
THANKS

• Multidisciplinary team at the Lister caring for this group of patients

• Professor Lewis and her SpermComet team for testing and data analysis

• Peter Larsen (Cryos) for control group samples

• Urological input in ongoing management of these patients
  – Mr Suks Minhas
  – Mr Jonathan Ramsay