Gamete quality and ovarian reserve as markers for early pregnancy loss
Special Interest Group Early Pregnancy

1 July 2012
Istanbul, Turkey
Gamete quality and ovarian reserve as markers for early pregnancy loss

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Organised by
the Special Interest Group Early Pregnancy
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Course coordinators

Ole B. Christiansen (Denmark)

Course description

There is uncertainly whether low quality of spermatozoa and low sperm count as well as low oocyte number and quality reflected in markers for ovarian reserve is predictive for an increased risk of biochemical pregnancies and miscarriages in subsequent pregnancies conceived with and without the use of ART.
The course will review the current knowledge about whether such associations exist and whether some of the markers for sperm and oocyte quality can be helpful in clinical practice.

Target audience

Reproductive physicians and biologists
Scientific programme

Chair: Mariette Goddijn (The Netherlands)

09.00 – 09.10 Introduction – Ole B. Christiansen (Denmark)
09:10 – 09:40 Subfertility, mode of conception and their effect on miscarriage rate – Monique Brandes (The Netherlands)
09.40 – 09.50 Discussion
09.50 – 10.20 Transmission electron microscopy and FISH studies of sperm in couples with recurrent miscarriage – Gaia Terzuoli (Italy)
10.20 – 10.30 Discussion
10.30 – 11.00 Coffee break

Chair: Siobhan Quenby (United Kingdom)

11.00 – 11.30 Markers of sperm quality and miscarriage rate – Nicolas Garrido (Spain)
11.30 – 11.40 Discussion
11.40 – 12.10 Sperm DNA damage and its effect on miscarriage after IVF/ICSI – Armand Zini (Canada)
12.10 – 12.30 Panel discussion with Nicolas Garrido, Gaia Terzuoli, Armand Zini, and delegates on the role of sperm factors in post-conception reproductive failure and its clinical implications
12.30 – 13.30 Lunch break

Chairs: Roy Farquharson (United Kingdom)

13.30 – 14.00 Are ovarian reserve tests predictive of miscarriage in women undergoing ART? – Jayaprakasan Kannamannadiar (United Kingdom)
14.00 – 14.10 Discussion
14.10 – 14.40 Ovarian reserve and early pregnancy – Maaike Haadsma (The Netherlands)
14.40 – 14.50 Discussion
15.00 – 15.30 Coffee break

Chair: Ole B. Christiansen (Denmark)

15.30 – 16.00 Anti-Müllerian hormone levels and miscarriage rates after IUI – Kelton Tremellen (Australia)
16.00 – 16.30 Anti-Mullerian hormone levels in women with recurrent miscarriage and their value in predicting another miscarriage – Elisabeth Clare Larsen (Denmark)
16.30 – 17.00 Panel discussion with Banchhita Sahu, Maaike Haadsma, Kelton Tremellen, Elisabeth Clare Larsen and delegates on the role of ovarian reserve tests in miscarriage and their clinical importance
Subfertility, mode of conception and their effect on miscarriage rate

M. Brandes, MD PhD

Infertility

Normal fertility

Pregnancy chance in months after discontinuation of birth control
Diagnoses in the infertility clinic

Hull et al. 1985

- Male infertility: 35%
- Ovulation disorder: 11%
- Unexplained infertility: 7%
- Tubal infertility: 5%
- Cervical infertility: 5%
- Other: 19%

N=706

Brandes et al. 2010

- Male infertility: 26%
- Male & ovulation: 5%
- Ovulation disorder: 3%
- Unexplained infertility: 2%
- Tubal infertility: 2%
- Cervical infertility: 17%
- Endometriosis: 12%
- Other: 1%

N=2476

Overall outcome

Percentage ongoing pregnancies infertile patients

Brandes et al. 2010

Pregnancy chance per diagnosis

Brandes et al. 2010
Miscarriage rate

General population 10-15%
Infertile population 18-30%

Infertility and miscarriage

1572 women, 3269 pregnancies (1980-1990)

<table>
<thead>
<tr>
<th></th>
<th>Infertile couples</th>
<th>Fertile couples</th>
<th>Adj OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscarriage</td>
<td>23%</td>
<td>14%</td>
<td>1.71 (1.26-2.94)</td>
</tr>
</tbody>
</table>

Infertile women experience more frequently a miscarriage compared to normal fertile women

Gray and Wu 2000, Am J Publ Health

Risk factors for miscarriage
Maternal age and miscarriage

Nybo Anderson et al. 2000, BMJ


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No of Pregn</th>
<th>Spont abortion Rate %</th>
<th>Odd Ratio (95% CI)</th>
<th>Waiting Time &gt;1y %</th>
<th>Odd ratio of Infertility (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at conception, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>1001</td>
<td>10.4</td>
<td>1.0</td>
<td>5.8</td>
<td>1.0</td>
</tr>
<tr>
<td>29-39</td>
<td>1277</td>
<td>13.6</td>
<td>1.36 (1.04-1.78)</td>
<td>9.6</td>
<td>1.73 (1.34-2.24)</td>
</tr>
<tr>
<td>30-34</td>
<td>373</td>
<td>23.3</td>
<td>2.48 (1.85-3.32)</td>
<td>14.5</td>
<td>2.72 (1.88-3.93)</td>
</tr>
<tr>
<td>&lt;35</td>
<td>116</td>
<td>22.4</td>
<td>2.49 (1.50-4.13)</td>
<td>13.8</td>
<td>2.65 (1.38-4.68)</td>
</tr>
</tbody>
</table>

Gray and Wu 2000, Am J Publ Health
### Maternal and Paternal Age and Miscarriage

#### Paternal Age and Maternal Age

<table>
<thead>
<tr>
<th>Paternal age</th>
<th>Maternal age</th>
<th>20-29</th>
<th>30-34</th>
<th>35-44</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>1.00</td>
<td>1.72</td>
<td>9.18</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>1.06</td>
<td>1.62</td>
<td>3.87</td>
<td></td>
</tr>
<tr>
<td>35-39</td>
<td>1.31</td>
<td>1.06</td>
<td>3.38</td>
<td></td>
</tr>
<tr>
<td>40-64</td>
<td>1.80</td>
<td>2.90</td>
<td>6.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.52-6.24</td>
<td>1.26-6.67</td>
<td>3.50-12.95</td>
</tr>
</tbody>
</table>

Source: de la Rochebrochard and Thonneau 2002, Hum Reprod

#### Maternal and Paternal Age and Miscarriage

<table>
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<th>35-44</th>
</tr>
</thead>
<tbody>
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<td>1.00</td>
<td>1.72</td>
<td>9.18</td>
<td></td>
</tr>
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<td>1.06</td>
<td>1.62</td>
<td>3.87</td>
<td></td>
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<tr>
<td>35-39</td>
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<td>1.06</td>
<td>3.38</td>
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</tr>
<tr>
<td>40-64</td>
<td>1.80</td>
<td>2.90</td>
<td>6.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.52-6.24</td>
<td>1.26-6.67</td>
<td>3.50-12.95</td>
</tr>
</tbody>
</table>

Source: de la Rochebrochard and Thonneau 2002, Hum Reprod

#### Maternal Age

**Distribution of Maternal Age in Our Cohort**

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>200</td>
</tr>
<tr>
<td>25-30</td>
<td>800</td>
</tr>
<tr>
<td>30-35</td>
<td>1100</td>
</tr>
<tr>
<td>35-38</td>
<td>700</td>
</tr>
<tr>
<td>38-40</td>
<td>400</td>
</tr>
<tr>
<td>&gt;40</td>
<td>1200</td>
</tr>
</tbody>
</table>

Source: Brandes et al., unpublished data
Maternal age and miscarriage

![Maternal age and miscarriage graph]

Brandes et al. unpublished data

Maternal age and BMI and miscarriage

<table>
<thead>
<tr>
<th></th>
<th>Abn karyo</th>
<th>p value</th>
<th></th>
<th>Abn karyo</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AGE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>93</td>
<td>40.5</td>
<td>0.003</td>
<td>ART</td>
<td>99</td>
</tr>
<tr>
<td>≥35</td>
<td>111</td>
<td>67.6</td>
<td></td>
<td>IVF</td>
<td>111</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>153</td>
<td>63.4</td>
<td>0.040</td>
<td>ICSI</td>
<td>53</td>
</tr>
<tr>
<td>≥25</td>
<td>51</td>
<td>47.1</td>
<td></td>
<td>yes</td>
<td>58</td>
</tr>
<tr>
<td><strong>PCOS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>156</td>
<td>61.5</td>
<td>0.244</td>
<td>RPL</td>
<td>173</td>
</tr>
<tr>
<td>yes</td>
<td>48</td>
<td>52.1</td>
<td></td>
<td>yes</td>
<td>30</td>
</tr>
</tbody>
</table>

Landres et al. 2010, Hum Reprod

Maternal BMI

![Maternal BMI distribution graph]

Brandes et al. unpublished data
Maternal BMI and miscarriage

Brandes et al. unpublished data

Tobacco and Cocaine and miscarriage

400 women with miscarriage, aged 14-40 years

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous abortion</th>
<th>No spontaneous abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco use</td>
<td>34.6</td>
<td>21.8</td>
</tr>
<tr>
<td>Cocaine use</td>
<td>28.9</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Tobacco and cocaine use were associated with spontaneous miscarriage.

Ness et al. 1999, N Eng J Med

Cigarette, alcohol, caffeine and miscarriage

330 women with spontaneous abortion
1168 women with ongoing pregnancy

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥5 U alcohol/week</td>
<td>4.94 (2.87-8.16)</td>
</tr>
<tr>
<td>375 mg caffeine/day</td>
<td>2.21 (1.53-3.18)</td>
</tr>
<tr>
<td>10-19 cigarettes</td>
<td></td>
</tr>
<tr>
<td>≥20 cigarettes</td>
<td></td>
</tr>
</tbody>
</table>

Consumption of ≥5 units alcohol p/wk and ≥375 mg caffeine p/day during pregnancy may increase the risk for spontaneous abortion.

Rasch et al. 2003, Acta Obstet Gynecol Scan
Fertility diagnosis and miscarriage

PCOS and miscarriage
Single center, retrospective study
PCOS: 631 patients
Controls (tubal path): 1423 patients
Pregnancies after IVF
409 miscarriage (23% vs 17%, p<0.05)

Yan et al. 2011, Zhonghua Fu Chan ke Za Zhi

PCOS and miscarriage
Single study, 1018 patients
Overall incidence of miscarriage 21%
Univariate:
  PCOS vs non-PCOS: 25% vs 18% p<0.01
Multivariate logistic regression:
  PCOS vs non-PCOS: not significant!

Wang et al. 2001, Hum Reprod
Endometriosis and miscarriage

140 patients with endometriosis
182 IVF cycles

Relation of mode of conception to miscarriage

Infertility treatment and miscarriage

Single center, historical cohort, 418 patients

Spontaneous vs OI, vs IVF, vs non-OI depending treatment

No increased risk for miscarriage after either treatment

Pezeshki et al. 2000, Fertil Steril
Infertility treatment and miscarriage

Multicenter, 6759 patients

Spontaneous vs ART pregnancies (2503 patients)

Corrected by female age (RR 1.20, 95%CI 1.03-1.46)

Wang et al. 2004, Hum Reprod

---

Study design

2476 patients

1809 clinical pregnancies

1523 ongoing pregnancies

286 miscarriages

Brandes et al. 2011 RBM Online
**Results**

<table>
<thead>
<tr>
<th></th>
<th>Ongoing pregnancy</th>
<th>Early pregnancy loss</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age, years</td>
<td>30.1 ± 4.1</td>
<td>32.1 ± 4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male age, years</td>
<td>33.1 ± 5.1</td>
<td>34.1 ± 5.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Female BMI kg/m²</td>
<td>24.2 ± 4.8</td>
<td>23.8 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility, months</td>
<td>16.8 ± 12.3</td>
<td>17.9 ± 13.2</td>
<td>NS</td>
</tr>
<tr>
<td>Time to pregnancy, months</td>
<td>25.8 ± 16.3</td>
<td>27.3 ± 16.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Brandes et al. 2011 RBM Online

**Multivariate analysis**

- Female age (p<0.001)
- Male age (p=0.006)
- Obstetrical history (p=0.10)
- Male alcohol use (p=0.02)
- Type of menstrual cycle (p=0.14)
- Diagnosis (p=0.05)
- Secondary or tertiary hospital (p=0.03)
- Female smoking behaviour (p=0.12)

Brandes et al. 2011 RBM Online

**Infertility treatment and miscarriage**

<table>
<thead>
<tr>
<th>Mode of conception</th>
<th>n</th>
<th>Miscarriage n (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>864</td>
<td>125 (14.5)</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>OI</td>
<td>266</td>
<td>42 (15.8)</td>
<td>1.3</td>
<td>0.79-2.18</td>
</tr>
<tr>
<td>IUI</td>
<td>20</td>
<td>5 (25.0)</td>
<td>2.4</td>
<td>0.82-7.42</td>
</tr>
<tr>
<td>IUI/COH</td>
<td>203</td>
<td>37 (18.2)</td>
<td>1.3</td>
<td>0.85-2.10</td>
</tr>
<tr>
<td>IVF</td>
<td>190</td>
<td>31 (16.3)</td>
<td>1.1</td>
<td>0.67-1.75</td>
</tr>
<tr>
<td>ICSI</td>
<td>202</td>
<td>30 (14.9)</td>
<td>1.0</td>
<td>0.60-1.62</td>
</tr>
<tr>
<td>FET</td>
<td>61</td>
<td>16 (26.2)</td>
<td>2.2</td>
<td>1.14-4.19</td>
</tr>
</tbody>
</table>

Corrected for: female and male age, obstetric history, diagnosis, male alcohol use, female smoking

Brandes et al. 2011, RBM Online
Infertility treatment and miscarriage

Distribution of the embryo quality for pregnancies after the different treatments.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Embryo</th>
<th>Misc/pr %</th>
<th>Misc/pr %</th>
<th>Misc/pr %</th>
<th>Misc/pr %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IVF</td>
<td>3/31</td>
<td>9.7</td>
<td>19/104</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7/37</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/4</td>
<td>25.0</td>
</tr>
<tr>
<td>2</td>
<td>ICSI</td>
<td>4/21</td>
<td>19.0</td>
<td>22/128</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3/27</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0/1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>FET</td>
<td>1/10</td>
<td>10.0</td>
<td>10/32</td>
<td>31.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2/7</td>
<td>28.6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0/1</td>
<td>-</td>
</tr>
</tbody>
</table>

Conclusions

The incidence of miscarriage in an infertile population is 18-30%.

But, most studies not corrected for risk factors, like female age.

Age and lifestyle most important factors in an infertile population.

Diagnoses give contradictory results.

Conclusions

Modes of conception did not show an increased miscarriage rate, except for the replacement of frozen-thawed embryos.

The quality of the embryo influences the chance of pregnancy but it does not influence the pregnancy outcome.

This information can be useful in counseling infertile couples.
Thank you for your attention!
Transmission electron microscopy and FISH studies of sperm in couples with recurrent miscarriage

Gaia Terzuoli
Disclosure of commercial and financial relationships and conflict of interest: none

RECURRENT MISCARRIAGE (RM)
• Three or more consecutive miscarriages before 20 weeks post-menstruation with the same biological father
• Around 1% of fertile couples
• More pregnancies are lost spontaneously than pregnancies carried to term
• The most common complication of pregnancy
• The experience can be painful for the couple

CAUSES OF RM
• Around 50% are associates with parental chromosomal anomalies, maternal thromophilic disorders, structural uterine anomalies, maternal immune dysfunction, endocrine abnormalities
• Around 50% are idiopathic
Any possible treatment for known causes?

• Treatment of genetic problems: in vitro fertilization
• Treatment of immunological factors (Antiphospholipid antibody syndrome and Systemic lupus erythematosus): Aspirin, Low-molecular-weight heparin, Prednisone
• Treatment of hormonal causes:
  • Luteal phase defect: Progesterone supplements, Clomiphene citrate
  • Polycystic ovary disease: Metformin

Male contribution to RM?

SEMEN ANALYSIS

ANALYSIS OF SEMEN PARAMETERS (WHO guidelines)

• Liquefaction
• Volume
• pH
• Motility
• Concentration
• Viability
• Morphology

Is this information enough?
Some studies, available in the literature, using different kind of sperm analysis to investigate male contribution

- Sperm aneuploidy and recurrent pregnancy loss, Bernardini et al. 2004
- Possible role of male factors in recurrent pregnancy loss, Saxena et al. 2008
- TEM and FISH studies in sperm from men of couples with recurrent pregnancy loss, Collodel et al. 2009
- Evaluation of sperm’s chromatin quality with acridine orange test, chromomycin A3 and aniline blue staining in couples with unexplained recurrent abortion, Kazerooni et al. 2009

Some studies, available in the literature, using different kind of sperm analysis to investigate male contribution

- Assessment of sperm factors possibly involved in early recurrent pregnancy loss, Gil-Villa et al. 2010
- Y chromosome microdeletions are not associated with spontaneous recurrent pregnancy loss in a Sinhalese population in Sri Lanka, Wettasinghe et al. 2010
- Y chromosome microdeletions, sperm DNA fragmentation and sperm oxidative stress as causes of recurrent spontaneous abortion of unknown etiology, Bellver et al. 2010
- Value of sperm chromatin dispersion test in couples with unexplained recurrent abortion, Abasalan et al. 2012
Why TEM analysis on spermatozoa?

- To improve the knowledge about their structure and physiology
- To verify pathologic conditions
- To verify the efficacy of treatments
- To investigate the presence and the causes of defects

What it is possible to see through TEM Analysis

Axoneme and periaxonemal structures

Mitochondria and fibrous sheath
TEM analysis is NOT a quantitative analysis

TEM data were elaborated using a mathematical formula, based on the Bayesian method (Baccetti et al. 1995), able to quantify electron microscopy results by calculating the number of spermatozoa probably free of structural defects in a semen sample (the fertility index) and the percentage of sperm pathologies as apoptosis, necrosis and immaturity (Collodiel and Moretti 2008).

Bartoov et al. 1999 performed the quantitative ultramorphological methodology (QUM)

Fertility Index (FI) >2x10^6

IMMATURETY <55%  NECROSIS <21%  APOPTOSIS <5%

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

FISH is a simple technique able to highlight aneuploidy in sperm cells

**PRO**:
- quick, sensitive, specific
- it allows analysis of a large number of spermatozoa in a short time

**CONS**:
- you cannot analyze all the chromosomes at the same time
- it does not allow you to visualize the structural alteration of chromosomes
Centromeric probes (18, X, Y) were utilized on interphasic nuclei

**FLUORESCENCE IN SITU HYBRIDIZATION (FISH)**

**WHY a TEM and FISH study?**

Presence of ultrastructural sperm defects and altered meiotic segregation
Their relevance in assisted reproduction techniques

- Ultrastructural studies of spermatozoa from infertile men with robertsonian translocation and 18, X, Y aneuploidy. Baccetti et al. 2005
- Necrosis in human spermatozoa. I. Ultrastructural features and FISH study in semen from patients with ure-genital infection. Collodel et al. 2005
- Necrosis in human spermatozoa. II. Ultrastructural features and FISH study in semen from patients with recovered infections. Moretti et al. 2005

- Fluorescence in situ hybridization and molecular studies in infertile men with displasia of the fibrous sheath. Baccetti et al. 2005
- TEM, FISH and molecular studies in infertile men with pericentric inversion of chromosome 9. Collodel et al. 2006
- Cryptorchidism and semen quality: a TEM and molecular study. Moretti et al. 2007
PATIENTS:

- 22 Italian couples with at least three prior pregnancy losses after natural conception at less than 20 weeks of gestation
- All pregnancies were fathered by the same partner
- None of these couples have ever had a live birth
- Semen samples obtained from 25 men of proven fertility (aged 22–40 years) were used as controls for semen parameters [WHO guidelines], for TEM indices and for FISH values (Collodel and Moretti 2008)

WOMEN:

- 22 women aged 29–38 years
- Normal 46, XX karyotype (evaluated using conventional cytogenetic analysis)
- Normal Hysterosalpingogram, Thyroid function analysis and reproductive endocrine evaluation, Factor V Leiden status, lupus anticoagulant anticardiolipin antibody levels

MEN:

- 22 men aged 28–46 years
- Nonazoospermic patients
- Normal 46, XY karyotype (evaluated using conventional cytogenetic analysis)
- Normal hormonal profile
- No history of radiotherapy, chemotherapy, chronic illness or medication
- Absence of sperm defects of possible genetic origin

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<th>Subject</th>
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<th>PAP %</th>
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## TEM analysis mathematically elaborated

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<th>SUBJECT</th>
<th>APOPTOSIS %</th>
<th>NECROSIS %</th>
<th>IMMATURETY %</th>
<th>FERTILITY INDEX</th>
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</table>

*Controls, Cabioli and Moretti 2006*  
*Patients versus Controls Apoptosis P<0.01*  
*Range: 25° - 75° percentiles*
Detection of membrane PS externalisation and membrane integrity using the Annexin V/Propidium Iodide Assay

Annexin V+/Propidium Iodide+ Apoptotic and Necrotic cell

Results from screening with Annexin V (Av)-FITC and Propidium Iodide (PI) assay performed in sperm nuclei

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<th>Av−/PI−</th>
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Mean ± SD 78.6 ± 2.5 ** 7.4 ± 1.9 * 11 ± 2.2 3.1 ± 1.5

*Controls, Collodel and Moretti 2008

Mean ± SD 84.8 ± 4.8 2.8 ± 2.2 8.5 ± 2.2 3.9 ± 1.6

FISH RESULTS

<table>
<thead>
<tr>
<th>Subject</th>
<th>% Diploidy</th>
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</table>

Mean ± SD 0.087 ± 0.039 0.075 ± 0.043 0.115 ± 0.048 0.103 ± 0.052 0.06 ± 0.032 0.137 ± 0.071

* Controls, Collodel and Moretti 2008

Diploidy 1818XY and disomy 18XY were significantly higher in the study group compared to the control group.
When in each patient disomies and diploidies are evaluated, approximately 50% of patients presented a higher percentage diploidy and disomy compared to controls.

FISH data, considered as total diploidy, total disomy, and total aneuploidy, were not significantly different from the control group.

CONCLUSIONS

- Routinary semen parameter analysis is not enough in couples with unexplained RM
- Methods of investigations are reported in the literature to deeply investigate seminal samples of couple with RM
- FISH and TEM analyses of sperm are suggested in RM work-up when no other cause has been detected
THANK YOU FOR YOUR ATTENTION
Markers of sperm quality and miscarriage rate

Dr. Nicolás Garrido Puchalt, PhD
nicolas.garrido@ivi.es
Andrology Laboratory and Semen Bank Director, Statistics Advisor
Instituto Universitario IVI Valencia
Plaza de la Policía Local, 3, 46015, Valencia (Spain)

Learning objectives:
To provide information about the relevance of early pregnancy loss in ART failures and describe the impact of male factor in assisted reproduction results
To define sperm quality markers involved in reproductive success
To describe the current knowledge about the link between early pregnancy loss and markers of sperm quality

Course description:
"There is uncertainty whether low quality of spermatozoa and low sperm count ....... is predictive for an increased risk of biochemical pregnancies and miscarriages in subsequent pregnancies conceived with and without the use of ART."

What is quality, when referred to a sperm cell?
A quality indicator should be a feature linked to optimal results under a functional viewpoint

Optimal results — Healthy newborn achievement
Markers of sperm quality and miscarriage rate

The upper limits of Assisted Reproduction Techniques: the male’s perspective

Highest cumulative livebirth rates using donor sperm

- H1 - donor sperm
- IVF - donor sperm

TAKE HOME MESSAGE:
EVEN USING THE BEST SPERM, THERE IS ROOM FOR IMPROVEMENT IN ART

Biochemical pregnancies and early miscarriages can account up to 40% of all implantational events in natural pregnancies

hCG produced by a conceptus is usually detected in maternal blood from implantation time onwards

Clinical evidence of pregnancy is obtained from week 6th onwards

Biochemical pregnancies and early miscarriages can account up to 40% of all implantational events in natural pregnancies

Cumulative probability of live-birth (%)

- Survival function
- ESHRE 2010

The limits to succeed:
- Implantation failure
- Pregnancy loss: pre-clinical
- Clinical

Where did the embryos go?

Survival function
About 26% in IVF conceived pregnancies

Those patients needing a high number of embryos to achieve newborn are able to get pregnant, but experience several early pregnancy losses. About 26% in IVF conceived pregnancies.

Cumulative probability of live-birth (%) IN OVUM DONATION

Diagnosis of male infertility:
Semen analysis, as stated by the World Health Organization is up to now the only worldwide accepted tool to diagnose male fertility, however its predictive value to forecast a male or a sperm sample possibilities to reach livebirth is relatively low.
Markers of sperm quality and miscarriage rate

Regarding the number as a quality marker...

IUI with donor sperm depending on the total motile sperm inseminated

Regarding the number and motility as a quality marker...

Retrospective analysis of AI with donor donation pregnancies (n=4423, 27% miscarriages) from 2006

Predictive power of sperm parameters to forecast livebirth

Predictive power of sperm parameters to forecast livebirth

Regarding the morphology as a quality marker...

Basic sperm analysis is unrelated to EPLs
Regarding the molecular profiles described as sperm quality markers...seems difficult to be a successful sperm!

Molecular markers

- Nucleic acid-linked
  - Aneuploidies, DNA fragmentation, gene expression (Microarrays), epigenetic
- Non-nucleic acid-linked
  - Apoptosis, Oxidative stress, Hialuronic acid, Ubiquitin, Platelet Activating Factor

The most challenging research in Andrology is on the causes making sperm cells unable to reach a term pregnancy with competent oocytes at any level, conception, implantation, or pregnancy maintenance:

- DIAGNOSTIC TOOLS TO ASSESS SPERM AND FORECAST LIVEBIRTH
- SELECTION TOOLS TO UTILIZE THE MOST COMPETENT SPERM CELLS

Sperm aneuploidies

FISH studies: up to 50% of the males with severe oligospermia or azoospermia and normal karyotype present FISH abnormalities (Rubio et al., 2001)

Linked to recurrent pregnancy loss

Effects mitigated by PGs
Sperm DNA fragmentation

The theory:

Sperm DNA is the vehicle to transmit males’ genetic information to the offspring, several internal and external factors have been demonstrated to affect sperm DNA integrity.

To date, there are a lot of studies concerning DNA analysis of human semen suggesting that the determination of DNA fragmentation can be a diagnostic parameter of semen quality, directly implicated in male fertility (Agarwal et al 2003).

Highly controversial results
Different techniques to be employed
Different cut-off values and odds ratio of achieving a term pregnancy
Outcomes not always related to miscarriage or livebirth

There is a mild correlation between the sperm DNA integrity and embryo quality, that could potentially affect success.

The prognostic value of this test is very low, and it seems unnecessary to complement each semen analysis.

Further information is needed.
Markers of sperm quality and miscarriage rate

All in one about recurrent pregnancy loss:

- **Y chromosome microdeletions**
- DNA fragmentation
- 8Oh guanosine (oxidative stress attack symptom on DNA: DNA oxidation)

No Y-chromosome microdeletions found

Oxidative stress in sperm: the basis

- **Oxidative stress** vs ROS physiological levels
- ROS lacking

**Antioxidants:**
- Superoxide dismutase
- Catalase
- GSH peroxidase
- GSH reductase
- Taurine and hypotaurine
- Alpha-tocopherol
- Lactate and pyruvate

ROS: Antioxidants: Superoxide anion

Hydroxyl radicals

Hydrogen peroxide

GSH: Glutathione (reduced form)

Superoxide dismutase

Catalase

GSH peroxidase

GSH reductase

Taurine

Alpha-tocopherol

Lactate and pyruvate

June 16th, 2009
Markers of sperm quality and miscarriage rate

Oxidative stress damage to cells:
- Plasma membrane
- Sperm DNA
- Cytoskeleton
- Motility

Proteomics in sperm:
- Very initial phase
- Very expensive equipment
- No clinical consideration
Markers of sperm quality and miscarriage rate

**Intracytoplasmic Morphologically Selected Sperm injection: the basis**

The morphological normality of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm

Arti Schakiltse, Fino Ergi, Sobrini Yarin, Nathan Ratt, Alex Roer, and Fishman

- A new method of unstained, real-time, high magnification motile sperm organellar morphological examination (MSOME)

- The average length and width of this configuration were estimated to be 4.75 ± 0.28 and 3.28 ± 0.20 mm, respectively

- The nuclear chromatin content was considered normal if it contained no more than one vacuole, which occupies 4% of the nuclear area (Bartoov et al., 1981, 2002, 2003)

**IMSI: the methods**

- Improved enhancement of the microscope resolution allowing 6300x.

- The strict descriptive criteria for normally shaped nuclei were based on those defined by scanning electron microscopy (Bartoov et al., 1981, 2002, 2003).

- The average length and width of this configuration were estimated to be 4.75 ± 0.28 and 3.28 ± 0.20 mm, respectively

- The nuclear chromatin content was considered normal if it contained no more than one vacuole, which occupies 4% of the nuclear area (Bartoov et al., 1981)

**IMSI: the clinical aspects**

- Pregnancy rates were higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic sperm injection

- Table showing the results of the clinical aspects

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Experimental group</th>
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<tbody>
<tr>
<td>Retrieved oocytes</td>
<td>13.3 ± 5.2</td>
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<td>Injected oocytes</td>
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<td>10.2 ± 5.5</td>
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<tr>
<td>Fertilization rates (%)</td>
<td>63.7 ± 18.9</td>
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<tr>
<td>Optimal embryos (%)</td>
<td>31.1 ± 16.4</td>
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<td>Transferred embryos (%)</td>
<td>3.6 ± 1.1</td>
<td>3.5 ± 1.2</td>
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<td>Implantation rates (%)</td>
<td>-9.5 ± 15.3</td>
<td>26.4 ± 27.9</td>
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<td>Pregnanies</td>
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<td>miscarriages</td>
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</table>
Markers of sperm quality and miscarriage rate

**IMSI: the clinical aspects**

- Good scientific evidences, clinically related to miscarriage
- Selected patient population
- Costs of the equipment
- Embryologists training
- Time-consuming (new microinjecting schedules?)
Hyaluronan binding assay (PICSI): the basis

- Diminished sperm fertility is associated with the retention of the surplus cytoplasm that is extruded from elongating spermatids in the course of normal sperm development.

- Only mature sperm without cytoplasmic retention were able to bind to the zona pellucida of oocytes. This finding led to hypothesize that the sperm plasma membrane undergoes a maturation-related remodeling.

- This remodeling step facilitates the formation of the sperm binding sites for the zona pellucida of oocytes. Indeed, immature sperm that fail to undergo membrane remodeling are unable to bind to immobilized HA, as is the case with immature sperm that fail to bind to the zona pellucida.

PICSI: the methods

HA-bound plates are able to immobilize mature sperm

These can be selected for ICSI

PICSI: the clinical aspects

Intracytoplasmic sperm injection: a novel selective method to improve the pregnancy rate in chromosomally normal couples.
**Markers of sperm quality and miscarriage rate**

**PICSI: the clinical aspects**

"Physiologic ICSI": Hyaluronic acid (HA) favors selection of embryos without DNA fragmentation

<table>
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<tr>
<th>Study</th>
<th>NO-PICSI</th>
<th>PICSI</th>
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<tr>
<td>No. Treatments</td>
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<td>115</td>
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<tr>
<td>Clinic selection age ≤ 32 of cycles retrieve</td>
<td>31.1 (1.8)</td>
<td>31.3 (2.4)</td>
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<tr>
<td>Fertilized embryos (%)</td>
<td>293/375 (78.2)</td>
<td>300/330 (90.6)</td>
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<tr>
<td>Clinical pregnancy rate (%)</td>
<td>33/129 (25.5)</td>
<td>40/126 (31.7)</td>
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<td>Mean pregnancy development rating ≤ 32</td>
<td>88.9 ± 2.1*</td>
<td>88.3 ± 3.0*</td>
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<tr>
<td>No. of embryos transferred</td>
<td>1.65</td>
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<tr>
<td>Clinical pregnancy rate per transfer (%)</td>
<td>23/109 (21.1)</td>
<td>21/126 (16.6)</td>
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<td>Implantation (%)</td>
<td>45/125 (35.2)</td>
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<td>No. of live births</td>
<td>9/32</td>
<td>9/30</td>
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Source: [ICSI = intracytoplasmic sperm injection, other abbreviations are Table 1.](#)

**The -omics in sperm: the basis**

Paternal contribution is not only one half of the embryo’s genome: exclusive mRNAs, proteins, and structures are delivered into the oocytes.

**IMPORTANT UNTIL THE EMBRYOS GENOMIC ACTIVATION**

Sperm has no transcriptional activity, but possesses a pool of active mRNAs synthesized during spermatogenesis.

Moreover, the fertilized oocyte has mRNAs not found in the unfertilized oocytes. Failure of partenogenetic development.

**Markers of sperm quality and miscarriage rate**

**Molecular factors involved those involved in:**

- Spermatogenesis in the testis
- Male factors in the epididymis
- Female factors in the endometrium
- Assisted Reproduction

**Markers**

**Access Reproduction**
Markers of sperm quality and miscarriage rate

Sperm Fertility Array (SFA) project:

Hypothesis:
Sperm cells with or without reproductive success present different transcriptomes

The molecular requirements of sperm cells to achieve pregnancies are different in vivo, or in vitro, and even different among different ART techniques.

THERE ARE MANY MOLECULAR FEATURES POTENTIALLY ABLE TO BE EMPLOYED IN SPERM TO BETTER DIAGNOSE AND IMPROVE RESULTS

FERTILITY AND INFERTILITY

XXVIII Congreso de la Sociedad Española de Fertilidad, Valencia, 19, 20, 21 Mayo 2010

SIG Early Pregnancy Pre-congress Course 1 July 2012

The genomics of sperm: how can we read and interpret the results?

- Lists of genes
  - Those Differentially Expressed
  - Those present/absent
  - Sequences implicated in reproductive success or newly associated

- Functional analysis:
  - MICROARRAY RESULTS CAN PROVIDE YOU WITH RELEVANT INFORMATION ABOUT THE KEY MOLECULAR FACTORS AND PROCESSES INVOLVED IN FERTILITY

TAKE HOME MESSAGE:

Page 45 of 140
**Markers of sperm quality and miscarriage rate**

**Microarray analysis of Intrauterine Insemination samples:**

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<thead>
<tr>
<th>Name</th>
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**Markers of sperm quality and miscarriage rate**

**Intrauterine insemination**

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**Markers of sperm quality and miscarriage rate**

**Intrauterine insemination**

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Microarray analysis of IVF samples:

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Top10 genes differentially expressed

IN VITRO FERTILIZATION

Exclusively found on sperm from pregnant couples | Exclusively found on sperm from NON-pregnant couples

Fertility markers?

IN VITRO FERTILIZATION
Microarray analysis of ICSI samples:

Markers of sperm quality and miscarriage rate

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Overexpressed on sperm from pregnant couples

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Overexpressed on sperm from NON-pregnant couples

Exclusively found on sperm from pregnant couples

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Exclusively found on sperm from NON-pregnant couples

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Top10 genes differentially expressed

ICSI

XXVIII Congreso de la Sociedad Española de Fertilidad, Valencia, 19, 20, 21 Mayo 2010
SIG Early Pregnancy Pre ‐ congress Course 1 July 2012

Fertility markers? Infertility markers?
Markers of sperm quality and miscarriage rate

Is there any possibility to avoid early pregnancy losses?

Once defined the “ideal” molecular features in sperm, we could select those appropriate for ART

Why are sperm cells interesting?

a) excess: it is not a limiting factor (it is the oocyte!)

b) genetic uniqueness in each sperm provides a wide variety of sperm phenotypes (natural selection?)

Each sperm is different

TAKE HOME MESSAGE:

WITHIN AN EJACULATE, WE MAY FIND GOOD AND BAD SPERM
What more we can do to understand the process and improve results?

- **SBT**
- **Microfluidics**
- **Birrefringence**
- **Electrophoresis**
- **PICSI**
- **IMSI**

**SPERM SELECTION IS A CRUCIAL STEP: DEPENDING ON THE SPERM SELECTED, EMBRYO QUALITY AND RESULTS MAY BE VERY DIFFERENT**

**TAKE HOME MESSAGE:**
- “self-selection” (IUI and IVF)
- SWIM UP
- DENSITY GRADIENT CENETRIFICATION
- subjective sperm selection (ICSI)

**CLASSIC SPERM SELECTION METHODS IN ART**

**SPERM SELECTION METHODS UNDER RESEARCH:**

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**TAKE HOME MESSAGE:**
- CURRENTLY AVAILABLE SPERM SELECTION METHODS ARE FOCUSED IN A SINGLE SPERM CHARACTERISTIC BYPASSING OTHER FEATURES
Markers of sperm quality and miscarriage rate

Sperm selection methods by molecular features:

- **Self/Subjective Sperm Selection** → **SMART SELECTION**
- **Magnetic Activated Cell Sorting: MACS**
  - MICROBEADS: colloidal superparamagnetic particles (50 nanometers)
  - Coupled to highly specific monoclonal antibodies or molecules recognizing targets of interest
  - Specialized magnetic affinity-type columns to isolate sperm
  - 2 fractions: bound and unbound, depending on molecular features
    - High purity

MACS, what’s next?

**Evaluation of new molecular candidates**
- Based on the previously available literature
- Obtained information from the SFA project

Gene lists of mRNA involved in reproductive success

Molecular Candidate's required characteristics:
- Having been related to successful livebirth achievement
  - To be present in sperm cells
  - To be located at the plasma membrane
  - Ab availability
Markers of sperm quality and miscarriage rate

MACS, what’s next?

Ubiquitin:
Defective mammalian spermatozoa become ubiquitinated during epididymal passage, a mechanism that may mark the abnormal spermatozoa for proteolytic destruction.

Those ubiquitinated sperm present in an ejaculate, or in a TESE sample (have not passed through the epididymes), could be used involuntarily in ART, impairing the results.

Then, removing those ubiquitinated sperm could help.

Phospholipase A2

Has been demonstrated to be >40x downregulated in samples achieving pregnancy in IVF.
Then, the depletion of PLA2G2A presenting sperm cells may increase reproductive results (negative selection)

Stabilin-2
Multifunction receptor with seven domains FAS1, four repeats EGF-like, and a domain able to identifying and digesting old cells in apoptosis in macrophages.
Also a phosphatidilserine recognizing receptor (Linked to apoptosis process)
Information from microarrays experiments: it has been linked to infertility

Complex sperm selection:
Successive positive selection rounds of appropriate sperm cells

Massive depletion of inadequate sperm cells (Ab cocktail)
Markers of sperm quality and miscarriage rate

**MACS, what’s next?**

Complex sperm selection:
Study/characterization of molecular sperm defects in a patient

**TAKE HOME MESSAGE:**
MACS OPEN A UNIVERSE OF POSSIBILITIES IN SPERM SELECTION, BEING A PROMISING TOOL TO BE IMPLEMENTED IN ART

Individualized sperm selection strategy?

**SUMMARIZING:**
Sperm cells present very interesting and unique properties to enable the development of sperm selection strategies as a part of the ART.

The development of objective sperm selection methods to be employed, and the design of complex strategies of sperm selection could improve ART results.

MACS versatility enables sperm selection depending of very different molecular features, and can also be employed in addition to other techniques.

In the future, the design of customized sperm selection methods, even individualized per patient, could solve the male infertility problems caused by altered molecular factors in the sperm samples.

**Conclusions:**
Early pregnancy losses represent a significant % of all ART failures.

There are clinical evidences about the sperm relevance on ART results, and several molecular sperm quality markers seem relevant.

There are very few data directly linking sperm quality with pregnancy loss (biochemical or clinical), although the definition of the ideal features for a sperm may help to detect characteristics leading to pregnancy wastage.

The deep knowledge and identification of those molecular factors in sperm factors involved in ART may open the possibility of designing adequate sperm selection...
Sperm DNA Damage and Pregnancy Loss after IVF and ICSI

Armand Zini, MD
Associate Professor, McGill University

Disclosure: Shareholder in YAD Tech – Neutraceuticals Co.

Learning Objectives

- Recognize the etiologies of sperm DNA and chromatin damage
- Evaluate the relationship between sperm DNA damage and pregnancy outcomes
- Recognize the controversies regarding the studies on sperm DNA and reproduction
- Apply the results of sperm DNA damage tests into clinical practice

Overview

- Etiology of sperm DNA damage
- Rationale for examining sperm DNA
- Tests of sperm DNA damage
- Relationship between sperm DNA damage and reproductive outcomes
  - Pregnancy and Pregnancy loss (in IVF & ICSI)
- Clinical utility of sperm DNA tests
Sperm DNA Damage: Etiology

Multi-factorial

- Temperature
- Toxins
- Oxidants

- Testis dysfunction
- Idiopathic
- Genetic
- Developmental

2 Step Theory

1st step
Poorly protaminated spermatozoa
(Defective spermatogenesis)

2nd step
Oxidative injury
(extrinsic or intrinsic)

Gatewood et al., J Biol Chem 1990
Carrell & Liu, J Androl 2001
Aoki et al, J Androl 2005
Aitken et al, Mol Hum Reprod 2010
Suganuma et al, Hum Reprod 2005
Sperm DNA Integrity

Potential applications of sperm DNA tests?

1. To more accurately diagnose male infertility
   Current markers of male fertility potential (i.e. conventional semen parameters) are inadequate
   – Exhibit a high degree of variability
   – Modest predictors of male fertility potential
     Guzik et al, NEJM 2001
     Menkveld et al, Hum Reprod 2001
     Cooper et al, Hum Reprod Update 2009

   Sperm DNA test results have a lower degree of variability
     Oleszczuk et al, Hum Reprod 2011
     Smit et al, Int J Androl 2011
     Evenson et al, Reprod Toxicol 2011

2. To help predict pregnancy outcomes after ARTs (fertilization, pregnancy)
   – Conventional semen parameters are not predictive (only need viable & morphologically normal sperm)
     Nagy et al, Hum Reprod 1995
     Creus et al, Hum Reprod 2000
     De Vos et al, Fertil Steril 2003
     Bartov et al, Fertil Steril 2003

In animal studies, sperm DNA is a predictor of ART outcomes

Sperm DNA Damage (Animal studies): Influence on IVF outcomes

- Sperm DNA damage was induced by gamma radiation
- Spermatozoa (mouse model) were then used in IVF cycles
  Ahmadi & Ng, J Exp Zool 1999

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<tr>
<td>Blastocyst (%)</td>
<td>50</td>
<td>20</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Live Fetus</td>
<td>34</td>
<td>21</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fatehi et al, J Androl 2006 – Bovine Model

Sperm DNA Damage (Animal studies):
Influence on IVF outcomes

- Sperm DNA damage was induced by gamma radiation
- Spermatozoa (mouse model) were then used in IVF cycles
  Ahmadi & Ng, J Exp Zool 1999

<table>
<thead>
<tr>
<th>Gamma radiation dosage (GY)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization(%)</td>
<td>53</td>
<td>64</td>
<td>60</td>
<td>59</td>
<td>61</td>
</tr>
<tr>
<td>Blastocyst (%)</td>
<td>50</td>
<td>20</td>
<td>8</td>
<td>3</td>
<td>2</td>
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<tr>
<td>Live Fetus</td>
<td>34</td>
<td>21</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Perez-Crespo et al, J Androl 2008 – Mouse Model – frozen-thawed sperm

Experimental sperm DNA damage ≠ Clinical sperm DNA damage

Sperm DNA Integrity

Potential applications of sperm DNA tests?
3. To evaluate the relationship between sperm DNA damage and post-natal health (of the IVF - ICSI child) because:
   - Natural barriers to fertilization are removed at ICSI
   - Infertile men exhibit high levels of sperm DNA damage
   - Pregnancy is possible despite high levels of DNA damage
   - Experimental (animal) studies suggest that sperm DNA damage might adversely impact the health of the child
Sperm DNA Integrity

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Sperm DNA Damage and Fertility

Infertile men have higher levels of sperm DNA -
Chromatin damage than fertile men

- Chromatin Structure: Evenson et al, Hum Reprod 1999
  - Spano et al, Fertil Steril 2000
  - Zir et al, Fertil Steril 2001

- DNA Fragmentation: Hughes et al, Hum Reprod 1996
  - Irvine et al, J Androl 2000


- Protamine Deficiency: Gatewood et al, J Biol Chem 1990
  - Carrell & Liu, J Androl 2001
  - Zhang et al, J Androl 2006

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   - Experimental (animal) studies suggest that sperm DNA damage might adversely impact the health of the child

Sperm DNA Integrity: Influence on Health of the Offspring

- Mouse ICSI studies (fresh [N] and frozen-thawed spz [DFS])
- CD1 and B6D2F1 mouse strains
  
  Fernandez-Gonzalez et al, Biol Reprod 2008

ICSI with DFS (compared to N sperm)

- Reduced embryo development
- Reduced number of live pups
- Development of atypical tumors in 33% of females (CD1)
- Reduced longevity (85% vs. 100% surviving at 25 weeks)
- Altered behavioral responses (“anxiety-like reactions”)

Sperm DNA Integrity/Damage

What do sperm DNA damage tests measure?

- Damage to the double stranded DNA
  - Fragmentation, oxidation or denaturation
  - Presence of DNA adducts
- Defects in the sperm chromatin
  - Improper or incomplete compaction of the chromatin (DNA and nuclear proteins [protamines and histones])
### Estimation of Sperm DNA Damage

#### Direct Tests
- Sperm DNA fragmentation
  - TUNEL assay - labeling of fragmented DNA
  - COMET assay - Single cell gel electrophoresis
- Sperm DNA oxidation
  - 8-hydroxy deoxyguanosine (8-OHdG)

#### Indirect Tests
- Sperm chromatin integrity/maturity
  - SCSA (susceptibility to DNA damage & chromatin compaction)
  - Aniline / toluidine blue (detects histones)
  - Chromomycin A3 (detects under-protamination)
  - SCD – sperm chromatin dispersion (chromatin compaction)

---

#### Advantages of sperm DNA damage tests
- Provide information on the quality of spermatogenesis
  - Complementary to the conventional sperm parameters
- Exhibit a low degree of biological variability
  - Lower variability than conventional sperm parameters (SCSA)
- Testing cryopreserved semen does not alter test results
  - Cannot be done with conventional sperm parameters

---

#### Limitations of Current Tests of DNA damage
- No test (direct or indirect) can measure:
  - The full extent or degree of damage (quantitatively)
  - Clinically relevant damage (e.g. gene-specific damage)
- Results are dependent on chromatin compaction
  - Assay conditions can influence accessibility of the dye or enzyme to the target sites
- No test can allow for use of sperm (e.g. for ICSI) after DNA testing

---

**References:**
- Sakkas et al., Hum Reprod 1996
- Hughes et al., Mol Hum Reprod 1996
- Erenpreiss et al., J Androl 2001
- Zini, SBRM 2011
- Barrett et al., Hum Reprod 2010
Sperm DNA Damage & Pregnancy Loss: What is the Evidence?

**Are measures of sperm DNA damage associated with pregnancy loss after IVF-ICSI?**

- Experimental-Animal Studies (Indirect)
  - Indirect Clinical Evidence:
    - Relationship between paternal age & sperm DNA damage
    - Relationship between paternal age & pregnancy loss (P-Loss)
    - Prospective Case-control studies on sperm DNA damage & P-Loss (natural pregnancy)
- Direct Clinical Evidence:
  - Systematic review of studies relating sperm DNA damage to pregnancy loss after IVF or ICSI

---

### Sperm (DNA) Damage (Animal studies): Influence on Pregnancy Loss

**Administration of a germ cell toxin (drug) to the male leads to an increased risk of Post-implantation (PI) loss**

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Species</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balasinor '02</td>
<td>Tamoxifen</td>
<td>rat</td>
<td>Increased PI loss</td>
</tr>
<tr>
<td>Salian '09</td>
<td>Bisphenol A</td>
<td>rat</td>
<td>Increased PI loss</td>
</tr>
<tr>
<td>Eustache '09</td>
<td>Endocrine Dis</td>
<td>rat</td>
<td>Increased PI loss</td>
</tr>
<tr>
<td>Anjum '11</td>
<td>Lead Acetate</td>
<td>rat</td>
<td>Increased PI loss</td>
</tr>
</tbody>
</table>

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### Sperm (DNA) Damage (Animal studies): Influence on Pregnancy Loss

**Administration of a germ cell toxin (chemo) to the male leads to Post-implantation (PI) loss and sperm DNA damage**

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Species/Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doerkson '96</td>
<td>5-azacytosine</td>
<td>rat/DNA methyl</td>
<td>Increased PI loss &amp; hypomethyl</td>
</tr>
<tr>
<td>Vaishvva '07</td>
<td>CHOP</td>
<td>rat/SICSA+Tunel</td>
<td>Increased DD &amp; PI loss</td>
</tr>
<tr>
<td>Debies '10</td>
<td>CHOP</td>
<td>rat/COMET</td>
<td>Increased DD &amp; PI loss</td>
</tr>
</tbody>
</table>
### Sperm DNA Damage & Pregnancy Loss

**Indirect Evidence**

**Relationship between paternal age & sperm DNA damage**

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Assay</th>
<th>Population</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisini '03</td>
<td>257</td>
<td>COMET</td>
<td>Infertile men</td>
<td>Increased sperm DD with age</td>
</tr>
<tr>
<td>Singh '03</td>
<td>66</td>
<td>COMET</td>
<td>Infertile men</td>
<td>Increased sperm DD with age</td>
</tr>
<tr>
<td>Vagnini '07</td>
<td>508</td>
<td>TUNEL</td>
<td>Infertile men</td>
<td>Increased sperm DD with age</td>
</tr>
<tr>
<td>Singh '03</td>
<td>1169</td>
<td>TUNEL</td>
<td>Infertile men</td>
<td>Increased sperm DD with age</td>
</tr>
<tr>
<td>Nikk '09</td>
<td>320</td>
<td>Flow-Pi</td>
<td>Infertile men</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Belloc '09</td>
<td>140</td>
<td>TUNEL</td>
<td>Infertile men</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Hammiche '11</td>
<td>227</td>
<td>SCSA</td>
<td>Infertile men</td>
<td>Increased sperm DD with age</td>
</tr>
<tr>
<td>Nijs '11</td>
<td>278</td>
<td>SCSA</td>
<td>Infertile men</td>
<td>No significant relationship</td>
</tr>
<tr>
<td>Varshini '11</td>
<td>504</td>
<td>TUNEL</td>
<td>Infertile men</td>
<td>Increased sperm DD with age</td>
</tr>
<tr>
<td>Wyrobek '06</td>
<td>97</td>
<td>SCSA</td>
<td>Healthy men</td>
<td>Increased sperm DD with age</td>
</tr>
</tbody>
</table>

Most studies report higher sperm DNA damage with increasing paternal age.

**But, these are largely from highly selected populations (infertile men)**

### Sperm DNA Damage & Pregnancy Loss

**Indirect Evidence**

**Relationship between paternal age & pregnancy loss (natural--IUI)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selvin &amp; Garfinkel '76</td>
<td>Retrospective</td>
<td>1.5 million certified</td>
<td>Increased late P-Loss with age</td>
</tr>
<tr>
<td>Rochdil &amp; Richard '12</td>
<td>Retrospective</td>
<td>3.174 pregnancies</td>
<td>Increased P-Loss with age</td>
</tr>
<tr>
<td>Srame '05</td>
<td>Prospective</td>
<td>5,126 (early preg)</td>
<td>Increased P-Loss with age</td>
</tr>
<tr>
<td>Kleinhans '06</td>
<td>Case/Control</td>
<td>1,006 / 12,539</td>
<td>Increased P-Loss with age</td>
</tr>
<tr>
<td>Macnaboe '06</td>
<td>Case/Control</td>
<td>833 / 8,116</td>
<td>Increased P-Loss with age</td>
</tr>
</tbody>
</table>

**IUI Pregnancy**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bellver '08</td>
<td>Retrospective</td>
<td>2,204 cycles</td>
<td>Increased P-Loss with age</td>
</tr>
<tr>
<td>Bellic '09</td>
<td>Retrospective</td>
<td>17,502 cycles</td>
<td>Increased P-Loss with age</td>
</tr>
</tbody>
</table>

**Natural-IUI studies report higher rates of P-Loss with increasing paternal age**
Sperm DNA Damage & Pregnancy Loss

Indirect Evidence

Relationship between paternal age & pregnancy loss (IVF-ICSI)

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Population</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dain '11</td>
<td>Meta-analysis</td>
<td>7 studies (IVF)</td>
<td>No significant relationship</td>
</tr>
</tbody>
</table>

How do we reconcile the opposite findings of the Natural & IUI studies on the relationship between P-Loss and paternal age and those of the IVF/ICSI studies on the same relationship?

Selection process?

Natural & IUI pregnancies are from men with relatively homogeneous (and "normal") sperm parameters whereas with IVF/ICSI the population of men is so heterogeneous that an age effect may be diluted.

Sperm DNA Damage & Pregnancy Loss

Indirect Evidence

Sperm DNA damage & P-Loss (Natural pregnancies)

Prospective Case-control studies

(Cases: Couples with recurrent P-Loss, Ctls: Fertile Couples)

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases/Ctls</th>
<th>Assay(s)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhattacharya '08</td>
<td>74/60</td>
<td>AO</td>
<td>Higher DNA damage in Cases</td>
</tr>
<tr>
<td>Saxena's '08</td>
<td>35/20</td>
<td>NCD</td>
<td>Poorer NCD in Cases</td>
</tr>
<tr>
<td>Kazerooni '09</td>
<td>30/30</td>
<td>CMA3, AB, AO</td>
<td>Higher DNA damage in Cases</td>
</tr>
<tr>
<td>Bellver '10</td>
<td>30/30</td>
<td>SCD</td>
<td>Higher DNA damage in Cases</td>
</tr>
<tr>
<td>Talebi '11</td>
<td>40/40</td>
<td>5 tests of DD</td>
<td>Higher DNA damage in Cases</td>
</tr>
<tr>
<td>Absalan '12</td>
<td>30/30</td>
<td>SCD</td>
<td>Higher DNA damage in Cases</td>
</tr>
</tbody>
</table>

All studies report higher levels of sperm DNA-chromatin damage in cases

Sperm DNA Damage & Pregnancy Loss

Direct Evidence

Systematic Review & Meta-analysis:

Examined all studies on sperm DNA and ...

- Pregnancy loss (after IVF and ICSI)
- But also sperm DNA and ...
- IVF pregnancy
- ICSI pregnancy
Systematic Review & Meta-analysis

Diagnostic test
- Sperm chromatin / DNA damage
  (by SCSA, TUNEL, SCD, COMET)

Reproductive outcomes (after IVF and ICSI)
- Pregnancy rate (clinical pregnancy)
- Pregnancy loss

---

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease + (no preg)</td>
</tr>
<tr>
<td>Test + (&gt;cutoff)</td>
<td>a</td>
</tr>
<tr>
<td>DNA damage</td>
<td>c</td>
</tr>
<tr>
<td>Test - (&lt;cutoff)</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = a/(a+c)
Specificity = d/(b+d)
Odds Ratio = ad / bc
PPV (pos. predictive value) = a/(a+b)
NPV (neg. predictive value) = d/(c+d)

---

<table>
<thead>
<tr>
<th></th>
<th>Disease + (no preg)</th>
<th>Disease - (preg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test + (&gt;cutoff)</td>
<td>a (true + test)</td>
<td>b (false + test)</td>
</tr>
<tr>
<td>DNA damage</td>
<td>c (false - test)</td>
<td>d (true - test)</td>
</tr>
<tr>
<td>Test - (&lt;cutoff)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = a/(a+c)
Specificity = d/(b+d)
Odds Ratio = ad / bc
PPV (pos. predictive value) = a/(a+b)
NPV (neg. predictive value) = d/(c+d)
<table>
<thead>
<tr>
<th>DNA damage</th>
<th>Test + (&gt;cutoff)</th>
<th>Test - (&lt;cutoff)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>(true + test)</td>
<td>c</td>
</tr>
<tr>
<td>b</td>
<td>(false + test)</td>
<td>d</td>
</tr>
</tbody>
</table>

Sensitivity = \( \frac{a}{a+c} \) (true + test rate)
Specificity = \( \frac{d}{b+d} \) (true - test rate)
Odds Ratio = \( \frac{ad}{bc} \) (measure of assoc. b/n test and disease)
PPV (pos. predictive value) = \( \frac{a}{a+b} \) (disease prob if + test)
NPV (neg. predictive value) = \( \frac{d}{c+d} \) (no disease prob if - test)

Sperm DNA Damage and IVF Outcomes
### Sperm DNA Damage and IVF Outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Design</th>
<th>Fem - Selection</th>
<th>Fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filatov '99</td>
<td>176</td>
<td>not specified</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Host '00</td>
<td>175</td>
<td>prospective, consecutive</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Tomlinson, '01</td>
<td>140</td>
<td>not specified</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Henkel, '03</td>
<td>208</td>
<td>prospective</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Huang, '05</td>
<td>217</td>
<td>retrospective</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Boe-Hansen, '06</td>
<td>139</td>
<td>prospective</td>
<td>fsh&lt;10</td>
<td>NA</td>
</tr>
<tr>
<td>Boe, '06</td>
<td>82</td>
<td>not specified</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Benchaib, '07</td>
<td>84</td>
<td>prospective</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Bungum, '07</td>
<td>388</td>
<td>prospective, consecutive</td>
<td>&lt;40 yo, fsh&lt;12</td>
<td>0</td>
</tr>
<tr>
<td>Lin, '07</td>
<td>137</td>
<td>prospective</td>
<td>&lt;40, fsh&lt;10</td>
<td>0</td>
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<tr>
<td>Frydman, '07</td>
<td>117</td>
<td>prospective</td>
<td>&lt;38, fsh&lt;15</td>
<td>0</td>
</tr>
<tr>
<td>Gu, '09</td>
<td>136</td>
<td>prospective</td>
<td>Tub obstrn</td>
<td>0</td>
</tr>
<tr>
<td>Tarozzi, '08</td>
<td>82</td>
<td>not specified</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Speyer, '10</td>
<td>192</td>
<td>prospective</td>
<td>&lt;45 yo</td>
<td>0</td>
</tr>
<tr>
<td>Simon, '10</td>
<td>224</td>
<td>prospective</td>
<td>none</td>
<td>0</td>
</tr>
<tr>
<td>Nijs, '10</td>
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<td>prospective</td>
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<td>0</td>
</tr>
<tr>
<td>Jiang, '11</td>
<td>116</td>
<td>not specified, consecutive</td>
<td>&lt;38, fsh&lt;12</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>studies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Sperm DNA Damage and IVF Outcomes

<table>
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### Sperm DNA Damage and IVF Fertilization

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### Sperm DNA Damage and IVF Outcomes

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### Sperm DNA Damage and IVF Pregnancy

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14 studies
### Sperm DNA Damage and IVF Pregnancy

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**Fixed Effects Model:** (Test for Homogeneity: p > 0.1)

Combined Odds ratio = 1.89 (1.48, 2.41), p < 0.01

### Clinical Application?

- **Positive predictive value (PPV median):** 79% no PR (21% PR)
- **Negative predictive value (NPV median):** 34% PR

**Clinical significance of an 13% difference in PR?**
### Sperm DNA Damage and ICSI Outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Design</th>
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<th>Fertilization</th>
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Systematic Review – March 2012
## Sperm DNA Damage and ICSI Outcomes

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### Sperm DNA Damage and ICSI Outcomes

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### Sperm DNA Damage and ICSI Pregnancy

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## Sperm DNA Damage and ICSI Pregnancy

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### Odds Ratio Summary

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**Total** 40% 0.40 0.71

**Fixed Effects Model:** (Test for Homogeneity: P > 0.1)

Combined Odds ratio = 1.30 (1.02, 1.65), P < 0.05
Sperm DNA Damage and ICSI Pregnancy

Fixed Effects Model:
Combined Odds ratio = 1.30 (1.02, 1.65), P <0.05

Clinical Application?
Positive predictive value (PPV median): 70% no PR (30% PR)
Negative predictive value (NPV median): 37% PR
Clinical significance of a 7% difference in PR?

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

<table>
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<th>Preg</th>
<th>P-Loss</th>
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Total 16 studies
2079 776
Systematic Review – March 2012

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

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Total 14 studies
2079 776
Systematic Review – March 2012
### Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

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Test for Homogeneity: Q test non-significant
Fixed Effects Model:
Combined Odds ratio = 2.58 (1.67, 3.97), p = 0.0001
Sperm DNA Damage and Pregnancy Loss (All) after IVF and/or ICSI

Combined Odds ratio = 2.58 (1.67, 3.97), p < 0.0001

Clinical Application?

Positive predictive value (PPV median): 35% PL
Negative predictive value (NPV median): 90% no PL (11% PL)
Sperm DNA Damage: Practical Application

What is clinical utility of sperm DNA tests?
In infertile couples with pregnancy loss post-IVF

Test Characteristics:
- Median prevalence of a + test is 25-30%
- Median sensitivity 40% → many other causes for PLoss
- Median specificity 85 % → + test points to male factor in PL

If +test → Increased risk of PL with IVF or ICSI

Evaluate the male & correct any male factor

Summary
The relationship between sperm DNA damage & pregnancy loss after IVF & ICSI is supported by...

Indirect Evidence:
- Experimental studies
- Paternal age & sperm DNA ↔ paternal age & pregnancy loss studies
- Case-control studies on sperm DNA damage & PLoss (natural)

Direct Evidence:
- Systematic analysis of studies relating sperm DNA damage to pregnancy loss after IVF or ICSI (OR=2.58)
Summary

The relationship between sperm DNA damage & pregnancy loss after IVF&ICSI is supported by...

But...

Indirect Evidence:

- Experimental studies
  - Experimental sperm DNA damage ≠ Clinical sperm DNA damage
- Paternal age & sperm DNA → paternal age & pregnancy loss studies
  - Large studies but relationship is speculative
- Case-control studies on sperm DNA damage & P-Loss (natural)
  - Small studies, that may not necessarily reflect ART P-Loss

Direct Evidence:

- Systematic analysis of studies relating sperm DNA damage to pregnancy loss after IVF or ICSI (OR=2.58)
  - Heterogeneous design, populations, sperm DNA tests

Conclusions

Sperm DNA damage is related to pregnancy loss after IVF&ICSI

Future directions

Large, well-designed prospective studies on IVF and ICSI pregnancy and pregnancy loss
  - Multivariate analysis, well-defined parameters
Are Ovarian Reserve Tests predictive of miscarriage in women undergoing ART?

Jayaprakasan K
MRCOG, PhD
Associate Professor & Subspecialist in Reproductive Medicine
Queen's Medical Centre, Nottingham, UK

Declaration
I have no conflicts of interest or any commercial relationship

Learning objectives (Plan)
Reproductive ageing and ovarian reserve
Ovarian Reserve Tests (ORT)
Ability of ORTs to predict miscarriage: evidence
Conclusions
Reproductive ageing

Natural fertility rates by age

Menken et al., 1986; Baird et al., 2005

Live birth rates by age per IVF cycle (2006)

(HFEA Database of 44,571 IVF cycles)

Live birth rates by age per IVF cycle (1992-2006)

(HFEA Database [http://www.hfea.gov.uk/2588.html])
Reproductive ageing

Live birth rates relate to egg quality

Van Voorhis, 2007 NEJM (IVF data)

Reproductive ageing

Rates of Miscarriage

CDC report 2008

Reproductive ageing

Rates of Aneuploidy

Reproductive ageing
Progressive loss of reproductive function

Reproductive ageing
OVARIAN AGEING
Decline in the quantity and quality of primordial follicles remaining within the ovaries
(Decline in “Ovarian Reserve”)

Ovarian reserve - Importance
✓ 'Fertility potential' of women
✓ Prediction of ovarian response/ IVF success
✓ Make an individualized treatment plan
✓ Miscarriage/ Aneuploidy/ Pre-eclampsia
✓ Reproductive life span
Primordial follicles
Gonadotrophin independent
Pre-antral follicles
Gonadotrophin responsive
Small antral follicles (0.25 to 4 mm)
Gonadotrophin dependent
Large antral follicles (4 to 10 mm)
Pre-ovulatory follicles (>10 mm)

Essentially quiescent; very little atresia

Ovarian reserve
Pre-antral follicles
Gonadotrophin independent
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Pre-ovulatory follicles (>10 mm)

Ovarian biopsy
Direct measure
Gonadotrophin independent
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Pre-ovulatory follicles (>10 mm)

Ovulation
in the presence of an HCG surge
also atresia after about 72 h.
Primordial follicles
Pre-antral follicles
Gonadotrophin independent
Small antral follicles (0.25 to 4 mm)
Pool of primordial follicles
Essentially quiescent; very little atresia
Direct measure (Ovarian biopsy)
Indirect measures (Clinical/Endocrine/Ultrasound measurements)
Pre-ovulatory follicles (>10 mm)
Ovulation
in the presence of an increase in estradiol
Gonadotrophin responsive follicles (0.25 to 2 mm)
Ovarian reserve:
Clinical
Age
Gonadotrophin independent follicles
Gonadotrophin responsive follicles (0.25 to 2 mm)
Gonadotrophin dependent follicles (>2-4 mm)
Ovulation
Anti-Mullerian Hormone
Ovarian reserve
Pre-antral follicles
Gonadotrophin independent
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Ovulation
in the presence of an increase in estradiol
Primordial follicles
Pre-antral follicles
Gonadotrophin independent

Pool of primordial follicles
Essentially quiescent; very little atresia

Ovarian reserve

Inhibin B
Gonadotrophin responsive

Estradiol
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent

FSH
Pre-ovulatory follicles (>10 mm)

DYNAMIC TESTS OF OVARIAN RESERVE

Clomiphene citrate challenge test
GnRH agonist stimulation test
Exogenous FSH stimulation test

ULTRASOUND MARKERS OF OVARIAN RESERVE

Antral follicle count

Ovulation
in the absence of an oocyte

FSH

Ovarian reserve

- Pre-antral follicles
  - Gonadotrophin independent
- Small antral follicles (0.25 to 4 mm)
  - Gonadotrophin responsive
- Large antral follicles (4 to 10 mm)
  - Gonadotrophin dependent
- Pre-ovulatory follicles (>10 mm)

Atresia

Prediction of poor ovarian response

AFC vs. AMH
Prediction: excessive response

AFC vs. AMH

Jayaprakasan et al., Fertility & Sterility 2008
Broer et al., Hum Repro update 2011

Prediction of conception

AFC & AMH are equally good at predicting ovarian response, but both less predictive of quality

Jayaprakasan et al., Fertility & Sterility 2009
Broer et al., Fertility & Sterility 2009

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<tr>
<td>Weghofer HR 2005</td>
<td>Age 25-40; FSH ≤ 10 IU/L</td>
<td>long protocol</td>
<td>Age specific FSH (lowest &amp; highest quartiles)</td>
<td>Not predictive, no difference in miscarriage rate (miscarriage not defined)</td>
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<td>Chuang FnS 2003</td>
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<td>Levi FnS 2001</td>
<td>All fertility patients</td>
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<td>FSH (&gt;14.2 &amp; &lt;14.2)</td>
<td>High 1st TM pregnancy loss (71.4% vs. 20%) in high FSH</td>
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<td>Abdalla HR 2004</td>
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<td>FSH 4 gps: &lt;15, 15-20, &gt;20</td>
<td>No difference in miscarriage (&lt;24 wks) rates across 4 gps</td>
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<td>vanMontfrans 2009</td>
<td>Age ≥30, natural conception</td>
<td>F/U of 12 mo. UPT on day 1 of cycle</td>
<td>FSH</td>
<td>No difference in FSH levels between nonpreg, EPL, miscarriage or age</td>
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<td>Age &gt;35, any age with unexplained infertility</td>
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<td>FSH CCCT</td>
<td>Similar reduced ovarian reserve (16%) in both RPL and non-RPL group</td>
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<td>Dubuis AGO 2004 N=40 RPL (58 unexplained vs. 22 cause found)</td>
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<td>High FSH/LH ratio and high E2 levels in unexplained RPL (1st TM)</td>
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<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasseri 1999</td>
<td>Karyotypes of miscarriage</td>
<td>Greater proportion of abnormal karyotypes had elevated FSH in women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Massie 2008</td>
<td>Karyotypes of TM miscarriages</td>
<td>55%IVF, 24%natural</td>
<td>FSH</td>
<td>High FSH not predictive of aneuploidy (70 euploid, 107 aneuploid)</td>
</tr>
<tr>
<td>van Montfrans 2001</td>
<td>Age &lt;41</td>
<td>FSH (11.5 IU/L cut-off)</td>
<td>High mean FSH levels in DS mothers (6.9 IU/L vs. 6.3) Higher proportion (14% vs. 5%) with high FSH levels</td>
<td></td>
</tr>
<tr>
<td>Van der Broom 2011</td>
<td>Same population as in van Montfrans 2001</td>
<td>1998 study group, FU in 2009</td>
<td>AMH (0.5 mcg/L cut-off)</td>
<td>Menopause Similar AMH levels (2.3 vs. 2.6 mcg/L) High proportion of DS (12% vs. 4%) with low AMH Similar menopausal status (15% vs. 13%) and age of menopause (47 vs. 45 yr)</td>
</tr>
</tbody>
</table>

**Miscarriage and poor response IVF cycles**

Haandsma et al., RBM online 2010 (N=1825; ongoing pregnancy=1468 & miscarriage = 357 pregnancy loss between 4 and 16 wks)

**Table 4**

<table>
<thead>
<tr>
<th>n (N)</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 years</td>
<td>0.9 (1.0)</td>
<td>0.8-1.1</td>
<td>0.49</td>
</tr>
<tr>
<td>&gt;2 years</td>
<td>1.0 (1.1)</td>
<td>1.0-1.1</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Haandsma et al., RBM online 2010 (N=1825; ongoing pregnancy=1468 & miscarriage = 357 pregnancy loss between 4 and 16 wks)
Miscarriage and Gonadotrophin dose

Worsening of IVF outcomes is notable from the lowest to the highest gonadotrophin dose tertile.

Data from Pal et al., Fertility and Sterility 2008 (N=806 IVF cycles)

Miscarriage and NGF

Table 1: Miscarriage and NGF misreporting in women undergoing assisted reproduction treatment.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>NGF Levels (ng/ml)</th>
<th>Miscarriage Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>NGF levels low</td>
<td>10</td>
</tr>
<tr>
<td>3 months</td>
<td>NGF levels low</td>
<td>12</td>
</tr>
<tr>
<td>6 months</td>
<td>NGF levels low</td>
<td>15</td>
</tr>
<tr>
<td>9 months</td>
<td>NGF levels low</td>
<td>12</td>
</tr>
<tr>
<td>1 year</td>
<td>NGF levels low</td>
<td>10</td>
</tr>
<tr>
<td>2 years</td>
<td>NGF levels low</td>
<td>9</td>
</tr>
<tr>
<td>3 years</td>
<td>NGF levels low</td>
<td>8</td>
</tr>
</tbody>
</table>

Data from LaMarca et al., Gynec Endocrine 2011
Objective

To evaluate the role of Ovarian Reserve Tests for the prediction of miscarriage among ART pregnancies

Methodology

Design: prospective observational

Participants (n=978 subjects-320 pregnant):
✓ first cycle of IVF/ ICSI
✓ age <43 yrs; FSH ≤ 12 IU/L
✓ regular menstrual cycles (21 – 35 days)
× PCOS/ Ovarian pathology on scan
× Congenital/ acquired uterine pathology
× Ectopic pregnancy

Early follicular phase assessment:
✓ TVS scan (AFC)
✓ Venepuncture (FSH/ E2/ FSH stimulation test)

Treatment protocol:
✓ Long down-regulation using GnRH agonists
✓ HMG for ovarian stimulation (150-300 IU)

Main outcome measures:
✓ Miscarriage (pregnancy loss at ≤12 wks)

Statistical analysis:
✓ Mann-Whitney U test/ Chi-square test
✓ Regression analysis/ ROC curve analysis
Variables | Miscarriage (n=67) | Ongoing pregnancy (n=247) | P value
--- | --- | --- | ---
Age (years) | 35.2 ± 4.5 | 34.3 ± 4 | 0.07
BMI (Kg/m²) | 24.6 ± 3.3 | 23.6 ± 2.9 | <0.01
Basal FSH level (IU/L) | 7.2 ± 1.7 | 6.9 ± 1.4 | 0.46
Basal oestradiol (pmol/L) | 104.1 ± 49.7 | 142.7 ± 42.5 | 0.09
Delta oestradiol (FSH stimulation test) | 161.5 ± 101.1 | 231.9 ± 201.8 | 0.51
Total AFC | 10.9 ± 3.5 | 12.0 ± 3.3 | <0.05
Total gonadotrophins used (IU) | 3416 ± 1255 | 3425 ± 1277 | 0.50
The number of oocytes collected | 11.4 ± 5.6 | 12.1 ± 4.8 | 0.46
Fertilization rates | 43.6 ± 23.5 | 65.9 ± 19.7 | 0.75
Subjects who had two embryos transferred | 64 (95.5%) | 234 (94.7%) | 0.8
Twin pregnancy rates | 20 (29.9%) | 42 (17%) | <0.05

Results

Logistic regression and ROC curve: Prediction of miscarriage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
<th>ROC curve: Prediction of miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.059</td>
<td>0.988–1.135</td>
<td>0.11</td>
<td>0.571</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.132</td>
<td>1.046–1.232</td>
<td>&lt;0.01</td>
<td>0.617</td>
</tr>
<tr>
<td>Basal FSH level</td>
<td>1.106</td>
<td>0.942–1.298</td>
<td>0.22</td>
<td>0.532</td>
</tr>
<tr>
<td>Basal oestradiol (pmol/L)</td>
<td>0.999</td>
<td>0.994–1.003</td>
<td>0.58</td>
<td>0.501</td>
</tr>
<tr>
<td>Delta oestradiol (FSH stimulation test)</td>
<td>0.999</td>
<td>0.998–1.001</td>
<td>0.45</td>
<td>0.526</td>
</tr>
<tr>
<td>Total AFC</td>
<td>0.917</td>
<td>0.843–0.998</td>
<td>&lt;0.05</td>
<td>0.588</td>
</tr>
</tbody>
</table>
Results

ROC curve: Prediction of miscarriage

<table>
<thead>
<tr>
<th>Variables/ Cut-off levels</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+LR</th>
<th>Post-test probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFC ≤ 12</td>
<td>0.68</td>
<td>0.40</td>
<td>1.2</td>
<td>24.5%</td>
</tr>
<tr>
<td>BMI ≥ 26 Kg/M²</td>
<td>0.82</td>
<td>0.37</td>
<td>2.1</td>
<td>36.2%</td>
</tr>
<tr>
<td>Combined test (AFC ≤ 12 &amp; BMI ≥ 26 Kg/M²)</td>
<td>0.92</td>
<td>0.22</td>
<td>2.6</td>
<td>41.3%</td>
</tr>
</tbody>
</table>

+LR: positive likelihood ratio
The shift from pre-test probability (21.3%) to post-test probability of poor response is shown

Prediction of miscarriage: Summary

✓ Miscarriage is more common in women having increased BMI and reduced OR (as measured by AFC)
✓ ORTs (AFC) may be significantly predictive of miscarriage
✓ However, the clinical application of ORTs to predict miscarriage is limited as indicated by its low discriminative ability

Are Ovarian Reserve Tests predictive of miscarriage in women undergoing ART?

Jayaprakasan K
MRCOG, PhD
Associate Professor & Subspecialist in Reproductive Medicine
Queen’s Medical Centre, Nottingham, UK
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Weghofer, A., Margreiter, M., Fauster, Y., Schaetz, T., Benecke, A., Skarin, D. and Feichtinger, W.
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Hum Reprod, 20, 2449-2452.
Learning objective

What is the current knowledge on the relation between ovarian reserve and miscarriage or trisomic pregnancy?

Content of the presentation

1. Introduction on female reproductive ageing
2. Relation between ovarian reserve and miscarriage
   - Relation between ovarian reserve and trisomic pregnancy
3. General conclusions and future research
Mean age of the mother at the birth of her first child in the Netherlands

Reproductive ageing

Reproductive ageing:
- ↓ chance to conceive
- ↑ risk of miscarriage

Attributed to:
- ↓ oocyte quantity
- ↓ oocyte quality

↑ Risk of spontaneous abortion with female age
Adapted from Nybo Andersen et al.

Oocyte quantity

- The number of oocytes decreases with age
- This process eventually leads to menopause

Power model for the decrease in non-growing follicles with age
Adapted from Hansen et al.
Clinical parameters for oocyte quantity

1. Ovarian reserve tests
2. Response to ovarian hyperstimulation
3. Ovarian surgery
4. Age at menopause

Decrease in the mean number of retrieved oocytes with age at IVF-treatment

Adapted from De Boer et al.

Oocyte quality

↑ Aneuploid oocytes with age
- ↑ risk of miscarriage
- ↑ risk of trisomic pregnancy

No non-invasive clinical tests available

Increased incidence of trisomy in clinically recognized pregnancies with female age

Adapted from Hassold and Hunt

Are oocyte quantity and quality related?

- Parallel decline in oocyte quantity and oocyte quantity
- Resembling distribution curves for maternal age at last child birth and age at menopause

Distribution curves for the observed age at last child birth (left-hand curve) and age at menopause (right-hand curve)

Adapted from Lambalk et al.
Are oocyte quantity and quality related?

If so, the number of remaining oocytes has predictive value for their quality

Hypothesis:
The ovarian reserve of a woman is associated with her risk of miscarriage and trisomic pregnancy

Overview

1. Ovarian reserve tests
2. Response to hyperstimulation
3. Ovarian surgery
4. Age at menopause

Miscarriage

Trisomic pregnancy

Ovarian reserve tests and miscarriage: findings from our research group

- Prospective cohort study
- 1999-2003
- Two hospitals in Groningen, the Netherlands
- Subfertile couples
- Follow-up of pregnancies and therapy
- Antral follicle count (AFC)
- Basal FSH en inhibin B
- Clomiphene citrate challenge test: ‘Stimulated’ FSH en inhibine B values
Inclusion criteria

- Subfertility of ≥ 12 months
- Regular ovulatory cycle
- VCM ≥ 1.000.000
- At least one open Fallopian tube

- 474 couples → 320 achieve a pregnancy (67.5 %)

- Outcome of the first pregnancy during follow-up
  - 233 (75,1%) Ongoing pregnancy (>16 weeks)
  - 72 (23,2%) Miscarriage (4-16 weeks)

Ovarian reserve tests and miscarriage: findings in literature

- Multiple studies available, some presented in this session

- Differences in study populations, sample size, ovarian reserve tests, cut-off values, outcome measures…

- Conflicting results → at least no clear-cut predictive value of ovarian reserve tests for miscarriage
Ovarian reserve tests and trisomic pregnancy: findings in literature

- ↑ levels of FSH in women with a trisomic pregnancy
- AMH and AFC are not related with trisomic pregnancy
  Kline et al (2011), Li et al, Plante et al, Seifer et al
- However, possibly very low AMH levels are associated with trisomic pregnancy – Van der Stroom et al

Overview

1. Ovarian reserve tests
2. Response to hyperstimulation
3. Ovarian surgery
4. Age at menopause

Ovarian response and miscarriage: findings from our research group

OMEGA project:
- National Dutch IVF-cohort from 1983-1995
- Total cohort size: N=19,840
- Questionnaire in 1997-1998: response rate 73%
- Medical files: data abstracted for 75%
- Relation ovarian reserve and miscarriage
- Relation ovarian reserve and trisomic pregnancy
Ovarian response and miscarriage: findings from our research group

- Outcome of the first completed IVF-treatment
- Women with a miscarriage (4-16 weeks) vs. women with an ongoing pregnancy (>16 weeks)
- Parameter of ovarian reserve:
  Poor response to ovarian hyperstimulation (<4 oocytes)

### Basal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Ongoing pregnancy</th>
<th>Miscarriage</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor response (%)</td>
<td>6.6%</td>
<td>11.7%</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.4</td>
<td>33.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.7</td>
<td>21.6</td>
<td>0.76</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>36.4%</td>
<td>43.8%</td>
<td>0.01</td>
</tr>
<tr>
<td>Primary subfertility (%)</td>
<td>66.3%</td>
<td>59.9%</td>
<td>0.03</td>
</tr>
<tr>
<td>Duration subfertility (years)</td>
<td>4.5</td>
<td>4.8</td>
<td>0.04</td>
</tr>
<tr>
<td>Ovarian surgery (%)</td>
<td>9.2%</td>
<td>12.3%</td>
<td>0.08</td>
</tr>
</tbody>
</table>

### Interaction with female age

↑ Relation between poor response and miscarriage with ↑ age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>N (%)</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤30 years</td>
<td>640 (38.9%)</td>
<td>0.9</td>
<td>0.3 – 2.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>31-35 years</td>
<td>826 (45.8%)</td>
<td>1.4</td>
<td>0.7 – 2.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>≥36 years</td>
<td>339 (18.8%)</td>
<td>2.7</td>
<td>1.5 – 4.9</td>
<td>0.002</td>
</tr>
<tr>
<td>All ages</td>
<td>1805 (100%)</td>
<td>1.9</td>
<td>1.3 – 2.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Discussion

- Why does the relation between poor response and miscarriage ↑ with ↑ age?
  - Smaller chance of a ‘coincidental’ poor response?
  - ↑ of biological damage over time?
- These results support the hypothesis that oocyte quantity and quality are related, but:
  - Poor responders: no possibility for embryo selection
  - Poor responders: fewer multiple pregnancies
- Two other large retrospective studies found no relation between a poor response (defined as <5 oocytes) and miscarriage (Kumbak et al, De Sutter et al)

Ovarian response en trisomic pregnancy: findings from our research group

- 28 cases
  - N=24 with trisomy 21
  - N=3 with trisomy 18
  - N=1 with trisomy 13
- Selection of 5 controls per case
- Controls are women with a live born child without a trisomy
- Matched for:
  - Age at IVF treatment
  - Mode of conception
  - Center of IVF treatment
  - Year of IVF treatment
Results

<table>
<thead>
<tr>
<th></th>
<th>Cases N = 28</th>
<th>Controls N = 140</th>
<th>OR for trisomic pregnancy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of oocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4</td>
<td>9 (32.2%)</td>
<td>17 (12.1%)</td>
<td>3.7 (1.2 – 11.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>5-8</td>
<td>6 (28.6%)</td>
<td>57 (40.7%)</td>
<td>0.9 (0.3 – 2.3)</td>
<td>0.76</td>
</tr>
<tr>
<td>≥9</td>
<td>11 (39.3%)</td>
<td>66 (47.1%)</td>
<td>1.0 (ref)</td>
<td>-</td>
</tr>
<tr>
<td>Poor response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (≤3 oocytes)</td>
<td>4 (14.3%)</td>
<td>9 (6.4%)</td>
<td>2.7 (0.7 – 10.7)</td>
<td>0.15</td>
</tr>
<tr>
<td>No (≥4 oocytes)</td>
<td>24 (85.7%)</td>
<td>131 (93.6%)</td>
<td>1.0 (ref)</td>
<td>-</td>
</tr>
</tbody>
</table>

No other studies available on ovarian response and trisomy

Overview

1. Ovarian reserve tests
2. Response to hyperstimulation
3. Ovarian surgery
4. Age at menopause

Results: ovarian surgery and ovarian response

<table>
<thead>
<tr>
<th></th>
<th>Cases N = 28</th>
<th>Controls N = 140</th>
<th>OR for trisomic pregnancy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (17.9%)</td>
<td>7 (5.7%)</td>
<td>3.3 (1.0 – 10.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>No</td>
<td>23 (82.1%)</td>
<td>133 (94.3%)</td>
<td>1.0 (ref)</td>
<td>-</td>
</tr>
</tbody>
</table>

These findings are in line with Freeman et al:
Mothers of a child with Down syndrome more often had a history of ovarian surgery (7/189 cases vs 1/329 controls)

These findings also correspond to classic mouse studies:
† Aneuploid embryos in hemi-ovariectomised mice (Brook et al)
Overview

1. Ovarian reserve tests
2. Response to hyperstimulation
3. Ovarian surgery
4. Age at menopause

No studies available

Trisomic pregnancy and signs of menopause: findings from our research group

‘Follow-up’ within the previously described case-control study

Data complete: N=72 (43%)

- N=63 Premenopausal
- N=4 Hormonal replacement therapy
- N=5 Irregular cycle

Median age at questionnaire: 42.1 years
Median interval between IVF and questionnaire: 4.1 years

Results: signs of menopause

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associated with trisomic pregnancy</td>
<td>5.5</td>
<td>1.2 – 24.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Adjusted for:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Age at the time of questionnaire</td>
<td>5.7</td>
<td>1.1 – 29.9</td>
<td>0.04</td>
</tr>
<tr>
<td>- Smoking at the time of questionnaire</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two other studies available (Kline et al (2000), Bartmann et al):
Women with a history of trisomic pregnancy enter menopause respectively 1.0 and 0.7 years earlier; not statistically significant.
Conclusion

These latter results generally support a relation between diminished ovarian reserve and miscarriage and trisomic pregnancy.

But…

Limitations

- Small numbers
- Studies not readily comparable
- Mostly IVF-populations
- Limitations of the parameters used

Clinical implications? Biological mechanism? → first confirmation of the results!

Current studies

- Collaboration with the fertility clinic of Rigs Hosptitalet Copenhagen (professor A. Nyboe Andersen)

- Data from the various Danish registries are available on:
  - Pregnancy outcome (including terminated pregnancies)
  - Karyotype
  - Matching of mothers and their children is possible
  - Indication for hospital admission (ovarian surgery!)
  - Course of IVF treatment
  - …
Research questions

Within the general population
- Relation ovarian surgery and trisomic pregnancy?

Within the IVF-treated population
- Relation ovarian response and miscarriage?
- Relation ovarian response and trisomic pregnancy?

...

Learning objective

What is the current knowledge on the relation between ovarian reserve and miscarriage or trisomic pregnancy?

Take home message:
There may well be a relation between ovarian reserve and early pregnancy, but... more studies are needed.
Co workers

University Medical Center Groningen:
Annemiek Hoek, MDPhD, gynaecologist
Henk Groen, MDPhD, epidemiologist

The Netherlands:
Prof Maas Jan Heineman, Academic Medical Center Amsterdam
Prof Frank Broekmans, University Medical Center Utrecht
Prof Nils Lambalk, Vrije Universiteit Medical Center, Amsterdam
Prof Curt Burger, Erasmus Medical Center Rotterdam
Thea Mooij, Dutch Cancer Institute, Amsterdam

Copenhagen, Rigs Hospital:
Anna-Karina Aars, MD, PhD student
Anja Pinborg, MDPhD, gynaecologist reproductive medicine

Prof Ojvind Lidegaard, gynaecologist reproductive medicine
Charlotte Skovlund, National Board of Health

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AMH Hormone levels and miscarriage rates after IUI

A/Prof. Kelton Tremellen
Repromed
University of South Australia

AMH physiology- Background

- AMH is a glycoprotein from the TGFβ family. It was originally identified to be produced by the sertoli cells of the testis and cause the regression of the Mullerian ducts in males (Jost 1946). Hence AMH’s alternative name- Mullerian Inhibiting Substance.
- AMH is produced by the granulosa cells of pre-antral and antral follicles (> 8 mm) in the ovary.

Commercial Disclosure

Consultancies for the following:
- MSD
- Merck Serono
- Bayer
- Hansen
- Beckman-Coulter (manufacturer of Immunotech and DSL AMH ELISA)
AMH physiology - Background

- The primary function of AMH is to inhibit the transition of primordial follicles into growing follicles.
- An absence of AMH function (gene "knock-out" mice) leads to accelerated primordial follicle recruitment and early onset of menopause - confirming the "oocyte development brake" role of AMH (reviewed in La Marca 2010)

Why might serum AMH be linked to oocyte quality?

1. The decline in live-birth rates with advancing maternal age (primarily due to an increase in oocyte aneuploidy) mirrors the drop in serum AMH seen with advancing age.
2. As AMH is produced by the ovary and is known to play a role in oocyte physiology - it is possible to conclude that serum AMH levels may give a useful non-invasive insight into oocyte quality.

Why might serum AMH be linked to oocyte quality?

3. The "bottom of the barrel" hypothesis of oocyte quality suggests the best quality oocytes ovulate first, leaving only the poor quality oocytes left at the end. As AMH is an excellent measure of quantitative ovarian reserve, it may therefore correlate with oocyte quality.
AMH and oocyte quality - lessons from IVF?

- A retrospective study suggested that oocyte fertilization rates (routine insemination and ICSI) are compromised in oocytes coming from women with low serum AMH. (Lekamge and Tremellen 2007).
- Low serum AMH has been linked with poor morphology oocytes (dark granular cytoplasm, aggregation SERs) in IVF cycles (Ebner 2006).

AMH and the prediction of live-birth in IVF - The Oocyte number confounder

- In a prospective study Nelson (2007) observed a significant correlation between serum AMH and cumulative LB rates (fresh and frozen embryo transfer of all embryos from 1 stimulated cycle). However, AMH was not an independent predictor of LB when accounting for oocyte yields in regression analysis.
- A retrospective study (Lekamge and Tremellen 2007) found AMH to only predict LB rates if all fresh and frozen transfers of embryos from 1 stimulated cycle were included in the analysis, not just fresh transfers.
- Therefore, it appears that in IVF the number of oocytes retrieved seems to be the primary confounder in the ability of serum AMH to predict LB rates.

Low dose stimulation IUI as a model to test the ability of serum AMH to predict oocyte quality in the setting of in vivo conception (Tremellen and Kolo 2010)

IUI removes many of the non-oocyte quality related issues related to in vivo conception:
- confirmed tubal patency
- Ovular and no intercourse "timing" issues.
- No major semen defect issues
- No in vitro manipulation of the embryo, nor ability to select ideal embryos for transfer
- IUI is generally mono-ovulation in our clinic (similar to natural ovulation)
IUI study protocol

- Retrospective study of 244 women undergoing 477 cycles of IUI (average 1.96 cycles per woman, mean age 33 years, mean number of motile sperm inseminated per IUI = 135 million).
- Indications for IUI treatment were male factor (35.5%), idiopathic (28.7%), anovulatory (17.2%), and combined infertility (14.8%).
- Low dose stimulation protocol (50 IU rFSH) with late start (Day 5) aiming for only 1-2 mature follicles.
- hCG “trigger” (5000 IU) and luteal support (1500 IU x 2).

Development of AMH Quartile ranges for the purpose of ovarian reserve classification

- The study screened 1032 women aged 18-43 years undergoing infertility assessment with serum AMH measurements.
- We developed percentile charts using this data and then divided the IUI study participants into 4 groups corresponding to their respective age related AMH quartile.
- Therefore, ovarian reserve status was classified comparing an individual's status to their age related peers, not just “raw” serum AMH measurements.

Table 1: Characteristics of women undergoing IUI treatment in relation to their live birth outcome

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Live birth</th>
<th>No live birth</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum age (years)</td>
<td>35.1 ± 4.7</td>
<td>31.5 ± 5.1</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI</td>
<td>26.0 ± 6.5</td>
<td>26.8 ± 6.2</td>
<td>0.79</td>
</tr>
<tr>
<td>Serum AMH (nmol/L)</td>
<td>33.4 ± 21.8</td>
<td>33.1 ± 20.9</td>
<td>0.80</td>
</tr>
<tr>
<td>Antral follicle count (24-29 mm)</td>
<td>11.6 ± 2.6</td>
<td>11.1 ± 2.8</td>
<td>0.55</td>
</tr>
<tr>
<td>Number mature follicles</td>
<td>1.31 ± 0.50</td>
<td>1.26 ± 0.46</td>
<td>0.84</td>
</tr>
<tr>
<td>Total motile sperm count</td>
<td>139.0 ± 38.3</td>
<td>127.5 ± 38.1</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Ovarian Reserve and IUI response in the AMH Quartile groups

Table 2: IUI treatment outcomes according to serum AMH assessed ovarian reserve status

<table>
<thead>
<tr>
<th>AMH quartile</th>
<th>Serum AMH (pg/ml)</th>
<th>No. follicles counted (27+ mm)</th>
<th>No. cycles</th>
<th>Mean follicles produced</th>
<th>Mean follicles per cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (low ovarian reserve)</td>
<td>6.6 ± 6.3</td>
<td>5.4 ± 4.7</td>
<td>98</td>
<td>1.15</td>
<td>0.17 ± 0.4</td>
</tr>
<tr>
<td>Q2 (normal ovarian reserve)</td>
<td>16.8 ± 6.2</td>
<td>11.6 ± 5.1</td>
<td>117</td>
<td>1.21</td>
<td>0.19 ± 0.3</td>
</tr>
<tr>
<td>Q3 (normal ovarian reserve)</td>
<td>25.3 ± 9.6</td>
<td>20.6 ± 7.3</td>
<td>144</td>
<td>1.39</td>
<td>0.19 ± 0.3</td>
</tr>
<tr>
<td>Q4 (Higher ovarian reserve/PCOS)</td>
<td>47.6 ± 30.4</td>
<td>38.3 ± 17.6</td>
<td>154</td>
<td>0.76</td>
<td>0.15 ± 0.4</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>-</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Live Birth Rate in IUI treatment per mature follicle produced

![Graph showing live birth rate per mature follicle](image1)

Live Birth per mature follicle

- Live Birth (%)
- Q1: 15%
- Q2: 12%
- Q3: 12%
- Q4: 10%

p = 0.97

Miscarriage Rate per Clinical Pregnancy

![Graph showing miscarriage rate](image2)

Miscarriage rate (%)

- Q1: 25%
- Q2: 25%
- Q3: 25%
- Q4: 25%

p = 0.87
Are our results consistent with others?

There have been two similar studies conducted in France and Hong Kong analysing the ability of serum AMH to predict oocyte quality/clinical pregnancy in IUI setting.

French IUI study (Lamazou et al. J Gynecol Obstet Biol Reprod (Paris) in press.)

- 316 patients less than 39 years of age undergoing their first cycle of IUI.
- Patients were divided into three AMH groups (<1 ng/ml, 1-4.5 ng/ml, >4.5 ng/ml).
- No statistical difference was observed in the number of mature follicles, clinical pregnancy rates or spontaneous abortion rate.
- ROC analysis revealed AMH to have no ability to predict on going viable pregnancy.

HK IUI study (Li et al, Fert Steril 2010)

- Retrospective study of 243 women (median age 35 years) undergoing IUI mainly for male factor (45.3%) and idiopathic (23.5%) infertility of duration between 1-3 years.
- High dose stimulation using a starting dose of 150 IU rFSH in most patients (75-100 IU in PCOS group).
- When analysing the cumulative chance of live birth (LB) in the first cycle of IUI or up to 3 cycles of IUI, serum AMH was significantly higher in LB group.
- A potential confounder is that outcomes were analysed per IUI cycle, not mature follicle. The LB group had more mature follicles (2 x 1) and higher E2 (3422 ± 2541 pmol/l) at trigger than no LB group (not statistically significant thought).
HK IUI Study outcomes

- ROC analysis suggested that serum AMH has a moderate but significant ability to predict LB in either the first cycle of IUI (Graph A, AUC 0.682) or over 3 cycles of IUI (Graph B, AUC 0.668).

AMH and oocyte quality

- The Australian and French IUI studies suggest that serum AMH does not predict oocyte quality (miscarriage rates, live birth rates) when analysed either as "raw" serum AMH values or AMH percentiles.
- The HK IUI study does suggest serum AMH may have a moderate ability to predict live birth, but it is possibly biased by the confounder of higher mature follicle responses in high AMH patients.

Serum AMH a marker of in vivo oocyte quality- summary

- Overall, the majority of IUI data suggests that serum AMH is not a useful marker of oocyte quality if comparing outcomes on a per mature oocyte generated during stimulation.
- While serum AMH is an excellent marker of quantitative ovarian reserve, the data from IUI do not support its use as a marker of oocyte quality.
- 6 prospective studies are presently being proposed to analyse serum AMHs ability to predict successful natural conception- these should answer the quality v quantity debate with certainty.
Primary References


Anti-Müllerian hormone levels in women with recurrent miscarriage and their value in predicting another miscarriage

Elisabeth C. Larsen MD, PhD. The Fertility Clinic & The Recurrent Miscarriage Unit. Rigshospitalet, University Hospital of Copenhagen, Denmark

Conflict of interest

I hereby confirm that I do not have any commercial and financial relationships related to this presentation and its contents

Learning Objectives

- A introduction to the condition Recurrent Miscarriage
- Maternal age and Recurrent Miscarriage
- Ovarian reserve and Recurrent Miscarriage
- FSH
- Estradiol
- AMH
- AMH as a predictor of another miscarriage
Recurrent Micarriage

- Definition (ESHRE):
  - Three or more consecutive miscarriages before 20 weeks of gestation
  - 1% of fertile couples experience recurrent early pregnancy losses

Subgroups of Recurrent miscarriage

- Primary recurrent miscarriage group
  - Three or more abortions and no preceding deliveries (livebirths nor stillbirths)
- Secondary recurrent miscarriage group
  - Three or more abortions after a delivery regardless of the outcome (liveborn or stillborn)
- (Primary late recurrent miscarriage group)
  - Two or more second-trimester losses

Important predictors of another miscarriage

- Advanced maternal age
- Number of previous successive miscarriages
Causes of Recurrent miscarriage

- Often the cause remains unexplained
- Idiopathic recurrent miscarriage
- Often several risk factors in the same patient
- Multifactorial disorder...

Well known risk factors of RM

- Structural uterine anomalies
- Parentel chromosomal abnormalities
- Maternal autoimmune disorders
- Maternal defects in coagulation factors
- Endocrine dysfunction
- Obesity

But what about ovarian reserve and recurrent miscarriage?
Ovarian reserve - definition

- Quantity and Quality of the existing pool of primordial follicles in the ovaries.

Quantity and Quality

- Decline in number of primordial follicles
- Increase in number of poor quality oocytes

Risk of sporadic spontaneous abortion in relation to maternal age

Nybo Andersen AM et al. BMJ 2000
Impact of age on Recurrent miscarriage rate:

- Register-based study:
  - 30-34 yr: 38-40%
  - 35-39 yr: 38-40%
  - 40-44 yr: 70%

- Multivariate analyses women < 40 yr:
  - Maternal age alone not a significant predictor of another miscarriage after adjustment for relevant independent variables

Connection between age, ovarian reserve and risk of miscarriage
The rate of decline in ovarian reserve is unique in each woman.

- 18-31 yr: Optimal fertility
- 32-41 yr: Decreased fertility
- 42 yr: End of fertility

Challenge: When ovarian age is higher than chronological age, may some women with idiopathic RM be represented in this group?
Evaluation of ovarian reserve

- Chronological age – poor predictor
  - FSH
  - Estradiol
  - Inhibin B
  - Cycle length – good predictor
    - But a bit difficult to deal with
    - Antral Follicle count – early and good predictor
    - But requires ultrasound equipment
  - Anti-Mullerian hormone (AMH) – early and good predictor
  - Only a blood sample – cycleday independant

Recall Miscarriage, basal FSH and Estradiol

3 studies.....

- Retrospective study
- Routine fertility evaluation
- 44 women with RM and 648 without RM but infertile
- Intervention:
  - FSH measured on cycle day 3
  - Estradiol measured on cycle day 3
  - Cycle length
  - Clomiphene citrate challenge test
Results:

Gray bar (+RM) and Black bar (-RM)

- No differences in the incidence of abnormal day 3 FSH or day 10 FSH
- CCCT results: 18% abnormal in both groups
- +RM and abnormal CCCT
- Delivery rate in the following year 0%
- -RM and abnormal CCCT
- Delivery rate in the following year 4.2%

Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values?

- Retrospective study
- 36 women with unexplained RM and 21 women with a known cause of RM
- No difference in age and number of miscarriages

Intervention
- FSH cycle day 3
- Estradiol cycle day 3
Results

Unexplained RM:
- 36 yr (+/- 5)
- 58% had elevated levels of both day 3 estradiol and FSH

Explained RM
- 35 yr (+/- 5)
- 19% had elevated levels of both day 3 estradiol and FSH

Retrospective study
- 58 women with unexplained RM, 22 women with explained RM (uterine, chromosomal, anti-histocompatibility syndrome), and 27 controls with no miscarriages
- No differences in age and number of miscarriages (explained vs. unexplained)

Intervention:
- FSH cycle day 3
- Estradiol cycle day 3
- FSH/LH cycle day 3

Unexplained RM vs. Controls:
- Nearly all ovarian reserve parameters significantly and negatively affected

Explained RM vs. Controls:
- No significant differences in any of the ovarian reserve parameters
To conclude: basal FSH and estradiol

- Retrospective studies 2000-2004
- Small numbers
- However:
  - Consistent results all concluding:
    - Young women with unexplained RM may have a diminished ovarian reserve as assessed with cycle day 3 FSH and estradiol.

A bit about AMH

- Only produced in the granulosa cells in the ovary
- Measures the quantity of oocytes but not the quality
- Undetectable in serum 3-5 days after oophorectomy
- Undetectable after menopause

AMH and age
Anti-Müllerian Hormone decreases with age

Longitudinal observation study: Measurement of AMH twice with a 3 year interval in women with regular menstrual cycles

Visit 1 Visit 2 P-value

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>6.0 (1.4-13.5)</td>
<td>5.8 (2.1-13.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Inhibin B</td>
<td>112 (12-213)</td>
<td>110 (6-206)</td>
<td>NS</td>
</tr>
<tr>
<td>AFC</td>
<td>14 (6-24)</td>
<td>14 (2.3-24)</td>
<td>NS</td>
</tr>
<tr>
<td>AMH</td>
<td>2.1 (0.1-7.4)</td>
<td>1.3 (0.0-5.0)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

AMH – a sensitive marker of ovarian reserve

Probably the earliest marker of ovarian ageing

Recurrent Miscarriage and AMH

1 study....
Prospective case-control
- 34 women with RM and 10 controls
  - Same age, regular cycles, equal length of follicular phase
- Intervention
  - AMH
  - FSH & LH
  - Inhibin B
  - Progesterone

Results

Apart from basal progesterone level NO differences between controls and women suffering from RM

AMH as a predictor of another miscarriage
Three conditions associated with recurrent miscarriage:

1. Advanced maternal age (> 40 years)
   - Often low AMH
2. Polycystic ovarian syndrome (PCO’s)
   - High AMH
3. Systemic Lupus Erythematosus (SLE)
   - Often low AMH

Advanced maternal age

- The risk for miscarriage increases with age, and women in the advanced reproductive age who have low ovarian reserve are prone to a higher risk of recurrent miscarriage

PCO’s

- Common endocrine disorder in young women
- ~ 40% of pregnancies in PCO’s women result in spontaneous loss
- PCO’s and clinical features
  - Obesity → Independant risk factor for recurrent miscarriage
- PCO’s and paraclinical features
  - Insulin resistance & hyperandrogenism → Associated with recurrent miscarriage
PCO’s and AMH

- Women with PCO’s have elevated levels of AMH compared to non-PCO’s women with anovulation and to ovulatory controls

Systemic Lupus Erythematosus (SLE)

- Autoimmune disease
- 80-90% of affected individuals = women
- 20% miscarriage rate (< 20 weeks)
- Three-fold risk of miscarriage > 20 weeks
- High risk of recurrent miscarriage
- Risk of premature ovarian failure (treatment induced and/or ovarian antibodies)

SLE and AMH

- 33 women with SLE (age 29.8 yr (21-39))
- No previous gonadotoxic treatment
- 33 healthy age-matched controls (age 29.8 yr (21-40))
- Intervention
  - AMH
  - Number of previous abortions
Results

- AMH significantly lower in the SLE-group (p < 0.05)
- 5 miscarriages in the SLE-group vs. 2 in the control group (ns)

To conclude...

- Indeed, it is still unclear whether diminished ovarian reserve is an independent predictor of RM.
- Some conditions predisposing to RM have an impact on ovarian reserve
  - Ex. SLE
  - The combination of advanced maternal age and low level of AMH is a risk factor for another miscarriage
  - Low egg quantity and quality
  - PCO’s and corresponding high levels of AMH is a risk factor for another miscarriage
  - Obesity, insulin resistance, and hyperandrogenism

Future perspectives

- Large prospective studies are needed to further evaluate ovarian reserve with AMH in women with recurrent miscarriage
- In particular in women with unexplained recurrent miscarriage
Thank You

References
- Trout SW et al. Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values? Fertil Steril. 2000;2:335-37.
Mark your calendar for the upcoming ESHRE Campus events

- Basic Semen Analysis Course in Greek Language  
  4-7 September 2012 - Athens, Greece

- Basic Genetics for ART practitioners  
  7 September 2012 - Rome, Italy

- Regulation of quality and safety in ART – the EU Tissues and Cells Directive perspective  
  14-15 September 2012 - Dublin, Ireland

- Basic Semen Analysis Course in Spanish language  
  18-21 September 2012 - Galdakano, Vizcaya

- GnRH-antagonists in ovarian stimulation  
  28 September 2012 - Hamburg, Germany

- The best sperm for the best oocyte  
  6-7 October 2012 - Athens, Greece

- Basic Semen Analysis Course in Italian language  
  8-11 October 2012 - Rome, Italy

- Accreditation of a preimplantation genetic diagnosis laboratory  
  11-12 October 2012 - Istanbul, Turkey

- Endoscopy in reproductive medicine  
  21-23 November 2012 - Leuven, Belgium

- Evidence based early pregnancy care  
  29-30 November 2012 - Amsterdam, The Netherlands