Antioxidant Supplementation of Subfertile Men Improves Top Blastocyst Rate in Couples Undergoing IVF/ICSI

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Valencia

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IVF/ ICSI technique was first induced 1990. At first, it was thought that the success rates of ICSI are not related to basic semen parameters (Kupler 1995; Mansour 1995; Nagy 1995; Svalander 1996).

however:

In several cases of recurrent negative IVF results in conventional IVF and ICSI attempts the influence of the “PATERNAL Effect” on early embryogenesis was suggested as a reason for IVF failure. (Vanderzwalmen 1991; Parinaud 1993; Shoukir 1998; Tesarik 2004; 2005).
ICSI
IVF Zentren Prof. Zech
Der Liebe Leben geben

ICSI
Early and Late Paternal Effects on Embryo-Development

Day 0 1 2 3 4 5

abnormal development

„Early paternal effects“
Sperm Cytoplasmic Defects
Oocyte activation factor
Centriole

abnormal development/
abortion

„Late paternal effects“
Sperm-Nuclear-Defects
• Chromosomal aberrations
• DNA fragmentation
What is good semen quality?

• Sperm analysis according to the WHO criteria is undergoing changes:
  - Has been revised in 2010
  - A so called „normal“ sperm sample according to the WHO criteria is not necessarily a good sperm sample (e.g. a sperm sample with 5% normal morphology)

• Which other criteria for sperm quality do we know?
  - Tests for DNA integration (e.g. TUNEL-Assay, Comet assay, Halosperm)
  - Tests for protamination (e.g. Acritin orange)
  - PICS I
  - Subtle morphology (MSOME)
Detailed examination of subtle sperm morphology by MSOME was first introduced by Bartoov et al. 10 years ago.

It allows the examination of the sperm’s fine morphology in vivo at high magnification (6000-12500x), thus providing the possibility of detailed sperm analyses, in particular assessment of the sperm head.

MSOME enabled the observation of so-called nuclear vacuoles, which cannot be detected by lower magnifications.

MSOME was subsequently applied to complement ICSI, and IMSI (intracytoplasmic morphologically selected sperm injection) was successfully established in ART.
Average sperm magnification \( \times 300 \)

MSOME \( \times 6000-10000 \)
Vacuolisation?

- Amorph substances?
- Membranous structures?
- Small vesicles without inner structures?

Craters? (Westbroock, 2000),
Hollow? (Watanabe, 2009),
Vesicles?
Nature of Nuclear Vacuoles?

- Franco et al., observed 2008 a high level of denatured DNA in spermatozoa with large nuclear vacuoles.
- Oliveira et al showed 2010 a positive correlation between percentages of spermatozoa with nuclear vacuoles and those with DNA fragmentation.
- Perdrix et al found 2011 that aneuploidy and chromatin condensation defects are important alterations observed in sperms exhibiting nuclear vacuoles.
Two step hypothesis

• In our center we use MSOME routinely to find best suitable sperm for ART (IMSI)

• It involves the grading of spermatozoa at x6000-x12,500 magnification according to the presence of nuclear vacuoles (Vanderzwalmen et al., 2008)

  class I: normal shaped sperm without vacuoles or with 1-2 small vacuoles <4% of the head length

  class II: normal shaped sperm with one or more large vacuoles > 4% head area

  class III: sperm with abnormal morphology with or without vacuoles.
IMSI Unit Leica 6000
**SPZ Class 1**
(Normal Form, no, one or two Vacuoles ≤ 4%)

- \( \text{NF} \) Normal Form without vacuoles
- \( \text{NFSV < 4 \%} \) Normal Form, Small Vacuole < 4 %
- \( \text{NFSV(2) < 4 \%} \) Normal Form, two Small Vacuoles < 4 %

**SPZ Class 2**
(Normal Form, Vacuoles >4%)

- \( \text{NFLV} \) Normal Form, one Large Vacuole
- \( \text{NFLV(n)} \) Normal Form, Large Vacuoles (number)
- \( \text{NFLSV(n)} \) Normal Form, Large + Small Vacuoles (number)

**SPZ Class 3**
(More Abnormalities)

- \( \text{AFLSV(n)} \) Abnormal Form, Large + Small Vacuoles (number)
- \( \text{AFLV} \) Abnormal Form, one Large Vacuole
- \( \text{AF} \) Abnormal Form without vacuole
So How to Get Class I Sperms?
Which Factors Impair Semen Quality?

- Drugs, e.g. chemotherapy
- Varicocele
- Genetic disorders (Klinefelter, AZF1 +2, mucoviscidosis and others)
- Environmental factors such as xenooestrogens, PCBs, bisphosphates, radiation ...
- Infections: Chlamydia, epididymitis, prostatitis
- Age
- Lifestyle factors such as BMI, ejaculation frequency and nutrition

Most of the factors described above contribute to generation of and/or exposure to oxidative stress
Figure 1 – Association of increasing reactive oxygen species (ROS) production with infertility.
**The combination matters—distinct impact of lifestyle factors on sperm quality: a study on semen analysis of 1683 patients according to MSOME criteria.**


**Abstract**

**BACKGROUND:** Poor sperm quality can negatively affect embryonic development and IVF outcome. This study is aimed at investigating the influence of various lifestyle factors on semen quality according to MSOME (motile sperm organelle morphology examination) criteria.

**METHODS:** 1683 male patients undergoing assisted reproductive technologies (ART) in our clinic were surveyed about their age, BMI (body mass index), ejaculation frequency, nutrition, sports, sleeping habits and social behavior. Semen samples were collected and evaluation of semen parameters according to MSOME and WHO criteria was performed. Results were grouped and statistically analyzed.

**RESULTS:** Although single parameters had minor effects on sperm parameter, the combination of age, BMI, coffee intake, ejaculatory frequency and duration of sexual abstinence were identified as factors having a negative effect on sperm motility. Additionally, we could demonstrate that MSOME quality was reduced. The negative impact of age, BMI and coffee intake on sperm quality could be compensated if patients had a high ejaculation frequency and shorter periods of sexual abstinence.

**CONCLUSIONS:** Combinations of adverse lifestyle factors could have a detrimental impact on sperm, not only in terms of motility and sperm count but also in terms of sperm head vacuolization. This negative impact was shown to be compensated by higher ejaculation frequency and a shorter period of sexual abstinence. The compensation is most likely due to a shorter storage time in the male gonads, thus reducing the duration of sperms' exposure to reactive oxygen species (ROS).
Proposed Strategies to Prevent Impact on Sperm or Reduce Oxidative Stress

• Minimize gonadotoxins and hyperthermia
  – E.g. quit smoking, hot-tubs, occupational hazards
• Antibiotics for semen or genital tract infection
  – Reduction of leukocytes in semen diminishes the main producers of ROS
• Reduction of abacterial inflammation: -e.g. lycopene (antiinflam. feature)
• Improvement of blood flow- e.g. l-citrullin (precursor of l-arginine -NO donator)
• Improvement of antioxidative enzyme activity- e.g. zinc, selenium improving the gluthation-peroxidase-enzyme-activity
• Improvement of mitochondrial function- e.g. coenzyme Q10, l-carnitin
• Distinct antioxidative supplementation- e.g. Vit C, Vit E, folic acid, glutathion, N-acetyl-cysteine as precursor of glutathione
<table>
<thead>
<tr>
<th>Supplement Facts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fertilovit®Mplus</strong></td>
</tr>
<tr>
<td>Vitamin C (sustained rel.)</td>
</tr>
<tr>
<td>Vitamin E</td>
</tr>
<tr>
<td>Folic acid</td>
</tr>
<tr>
<td>Zinc</td>
</tr>
<tr>
<td>Selenium</td>
</tr>
<tr>
<td>L-citrulline</td>
</tr>
<tr>
<td>L-carnitine</td>
</tr>
<tr>
<td>N-acetyl-L-cysteine</td>
</tr>
<tr>
<td>Glutathione, red.</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
</tr>
<tr>
<td>Lycopene</td>
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</tbody>
</table>

* % of recommended daily allowance (according to EU-guidelines)
Can a supplement improve sperm according to vacuolisation rate?

Yes!

Original Communication

Dietary Supplementation of Antioxidants Improves Semen Quality of IVF Patients in Terms of Motility, Sperm Count, and Nuclear Vacuolization

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IVF Centers Prof. Zech, Salzburg, Austria

Received: April 23, 2012; Accepted: February 2, 2013

Abstract: Background: This study aimed to investigate the influence of an oral antioxidant supplement on sperm quality of in vitro fertilization (IVF) patients, as analyzed by sperm motility according to the WHO criteria and mobile sperm organelle morphology examination (MSOME). Methods: Semen samples were collected from 147 patients before undergoing an IVF/intracytoplasmic morphologically-selected sperm injection (ICSI) cycle and 2–12 months after an antioxidant supplementation. Semen analysis was evaluated according to WHO and MSOME criteria. Spermatozoa were grouped according to the size of nuclear vacuoles within the sperm’s head. Patients were divided into oligoasthenoteratozoospermic (OAT) and normo-OAT men. Between first and second semen analysis, patients were supplemented orally with an antioxidant preparation. Results: After the antioxidant therapy no observed a significant reduction in the percentage of immobile sperm cells in the patients. Additionally, the percentage of class I spermatozoa according to MSOME criteria was significantly higher after antioxidant supplementation. In OAT patients the percentage of class I sperm was found to be increased, although not significantly. However, we observed a dramatic improvement in sperm motility as well as in total sperm count in this group. Conclusion: The results demonstrated a considerable improvement in semen quality, notably in OAT patients. Considering the putative relationship between semen quality and reactive oxygen species on the one hand and reactive oxygen species on the other, the observed changes in the sperm parameters indicate a decline in semen quality, and even subtle morphological changes, might be associated with oxidative stress. Our findings suggest that an antioxidant and micronutrient supplementation has a remarkable benefit for IVF patients having restricted sperm parameters, in particular.
Can a Supplement also Improve Blastocyst Quality?

The semen analyses and IVF/IMSI treatment outcomes of 92 subfertile male IVF patients and their partners were evaluated in two separate treatment cycles. One cycle was performed with no supplementation, the second cycle with an antioxidant supplementation (Fertilovit Mplus).

Parameters analyzed with respect to semen quality:
- Semen volume
- Concentration
- Motility
- Morphology according to MSOME (motile sperm organelle morphology examination)

Treatment outcome:
- 2PN
- Blastocysts
- Top-Blastocysts
- Fertilization rate
- Pregnancy rate
- Ongoing pregnancy rate

The Student’s $t$-test and chi square test were used to evaluate the significance of data.
# Results

## – Semen Quality

<table>
<thead>
<tr>
<th></th>
<th>First cycle without Supplementation</th>
<th>Second cycle with Supplementation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male age (years)</td>
<td>39.2 +/- 8.5</td>
<td>40.6 +/-8.5</td>
<td>n.s</td>
</tr>
<tr>
<td>Male BMI (kg/m2)</td>
<td>26.0 +/- 3.0</td>
<td>26.1 +/- 3.1</td>
<td>n.s</td>
</tr>
<tr>
<td><strong>Semen assessment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample volume (ml)</td>
<td>2.9 +/- 1.5</td>
<td>2.3 +/- 1.4</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total sperm count (TSC)</td>
<td>44.3 +/- 49.5</td>
<td>49.4 +/- 41.5</td>
<td>n.s</td>
</tr>
<tr>
<td>Concentration (Mio/ml)</td>
<td>16.7 +/- 17.6</td>
<td>20.8 +/- 22.5</td>
<td>n.s</td>
</tr>
<tr>
<td><strong>Sperm motility (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade a</td>
<td>3.9 +/- 6.3</td>
<td>4.0 +/- 6.5</td>
<td>n.s</td>
</tr>
<tr>
<td>Grade b</td>
<td>30.6 +/- 18.7</td>
<td>29.0 +/- 19.6</td>
<td>n.s</td>
</tr>
<tr>
<td>Grade c</td>
<td>14.9 +/- 14.7</td>
<td>21.4 +/- 18.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Grade d</td>
<td>50.6 +/- 24.2</td>
<td>45.6 +/- 22.1</td>
<td>n.s</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>34.5 +/- 21.6</td>
<td>32.6 +/- 21.3</td>
<td>n.s</td>
</tr>
<tr>
<td><strong>MSOME criteria (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class I</td>
<td>3.8 +/- 4.9</td>
<td>6.0 +/- 5.8</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Class II</td>
<td>38.9 +/- 16.7</td>
<td>41.9 +/- 14.5</td>
<td>n.s</td>
</tr>
<tr>
<td>Class III</td>
<td>57.3 +/- 19.3</td>
<td>52.1 +/- 18.0</td>
<td>n.s</td>
</tr>
</tbody>
</table>
## Results – Treatment Outcome

<table>
<thead>
<tr>
<th></th>
<th>First cycle without Supplementation</th>
<th>Second cycle with Supplementation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female Age (years)</strong></td>
<td>36.8 +/- 4.2</td>
<td>38.1 +/- 3.9</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Stimulation dose (IU)</strong></td>
<td>2451 +/- 745</td>
<td>2647 +/- 764</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Number of oocytes retrieved (total)</strong></td>
<td>1127</td>
<td>1092</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Oocytes (mean)</strong></td>
<td>12.4 +/- 5.9</td>
<td>12.1 +/- 5.7</td>
<td></td>
</tr>
<tr>
<td><strong>Number of 2PN (total)</strong></td>
<td>672</td>
<td>659</td>
<td></td>
</tr>
<tr>
<td><strong>2PN (mean)</strong></td>
<td>7.3 +/- 3.9</td>
<td>7.3 +/- 4.3</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>FR (%)</strong></td>
<td>59.6</td>
<td>60.4</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Number of blastocysts (total)</strong></td>
<td>267</td>
<td>288</td>
<td></td>
</tr>
<tr>
<td><strong>Blastocysts (mean)</strong></td>
<td>2.9 +/- 2.4</td>
<td>3.1 +/- 2.7</td>
<td></td>
</tr>
<tr>
<td><strong>Blastocyst Rate (%)</strong></td>
<td>39.7</td>
<td>43.7</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Top-Blastocysts (mean)</strong></td>
<td>0.4 +/- 1.1</td>
<td>0.6 +/- 1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Top BR (%)</strong></td>
<td>5.5 (Nb. of top-blastocysts)</td>
<td>8.5 (n= 56)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><strong>Embryos transferred</strong></td>
<td>1.9 +/- 0.4</td>
<td>1.9 +/- 0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>34.8</td>
<td>44.5</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>cPR</strong></td>
<td>32.8</td>
<td>39.1</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
Fertilisation and Blastocyst Rates

Female age

1. cycle

2. cycle
Fertilisation and Blastocyst Rates

Top Blastocyst Rate

- With Supplement
- Without Supplement
Pregnancy Rate

Without Supplementation  
With Supplementation

Ongoing Pregnancy Rate
Data Hints at a Positive Impact of the Compound Antioxidant Treatment on Late Paternal Effects

Day 0 1 2 3 4 5

abnormal development

„Early paternal defects“

Sperm Cytoplasmic Defects

Oocyte activation factor

Centriole

abnormal development/abortion

„Late paternal effects“

Sperm-Nuclear-Defects

• Chromosomal aberrations
• DNA fragmentation
Conclusion

• In previous studies, we could show, that a specific supplementation (Fertilovit® Mplus) improved sperm quality significantly, not only on WHO criteria (as shown in a previous study), but also with respect to morphology as evaluated according to MSOME criteria.

• In addition to this, in this study a significant impact on the top blastocyst rate as well as a marked improvement of pregnancy rate and ongoing pregnancy rate was observed.

• This is consistent with other studies (Ross et al, 2010 and Showell et al, 2011) and the observed correlation between sperm head vacuolisation and success of fertility treatment and might hint at an influence of antioxidatives (Fertilovit® Mplus) on late paternal effects.

• We strongly believe, that the issue of sufficient antioxidant uptake should be addressed when counseling and treating ART-patients.
Thanks to...

- H.Zech
- N.Zech
- M.Murtinger
- M.Schuff
- B.Wierleitner
- B.Schechinger
- A.Stecher
Thank you for your attention!