IVF Zentren Prof. Zech
Der Liebe Leben geben

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

Nutrition & Food Sciences 2014
Valencia

Johannes Wogatzky, MD
IVF Centers Prof. Zech, Bregenz-Austria

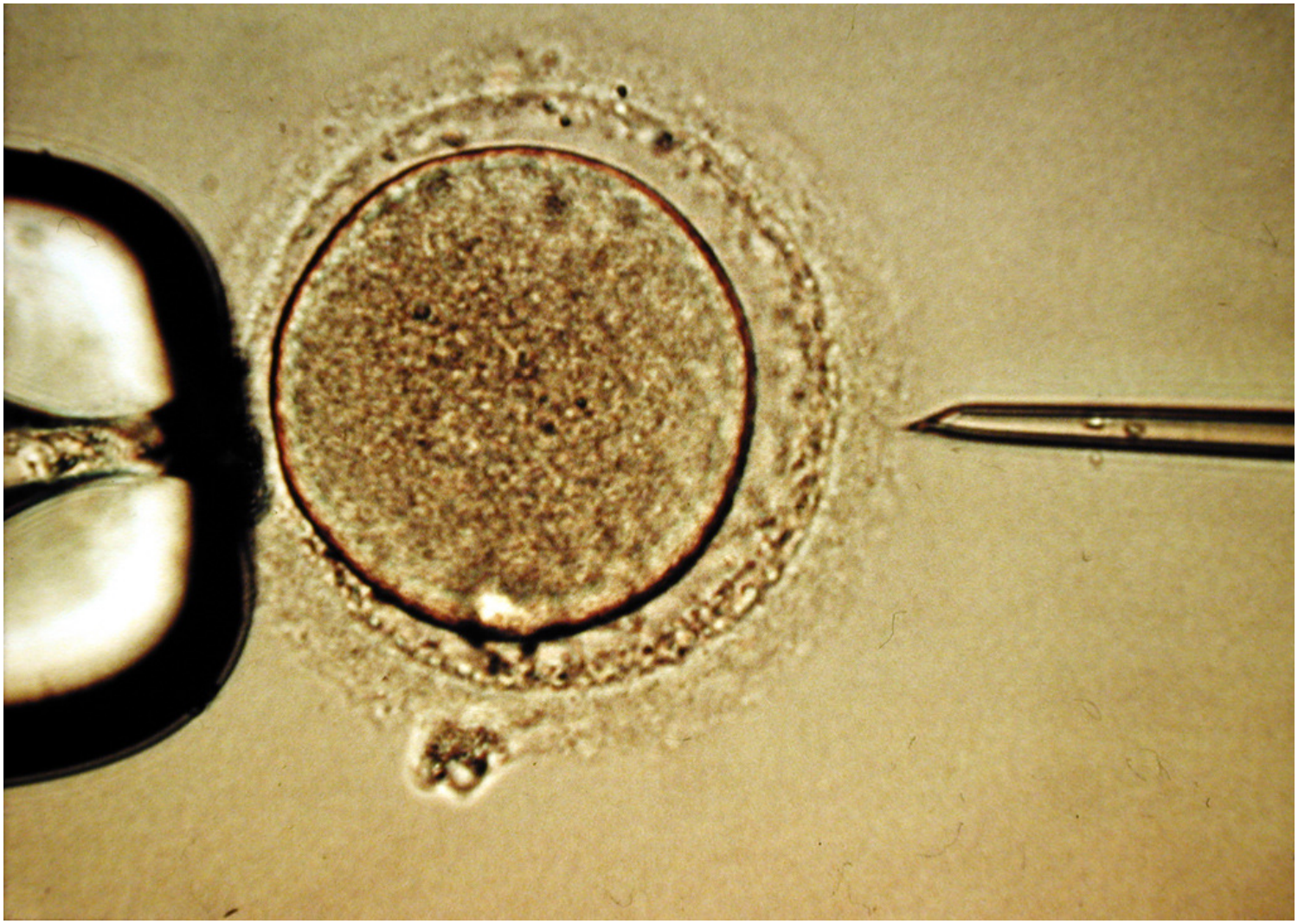
IVF/ ICSI technique was first induced 1990. At first, it was thought that the success rates of ICSI are not related to basic semen parameters (Kupler 1995; Mansour 1995; Nagy 1995; Svalander 1996).

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).

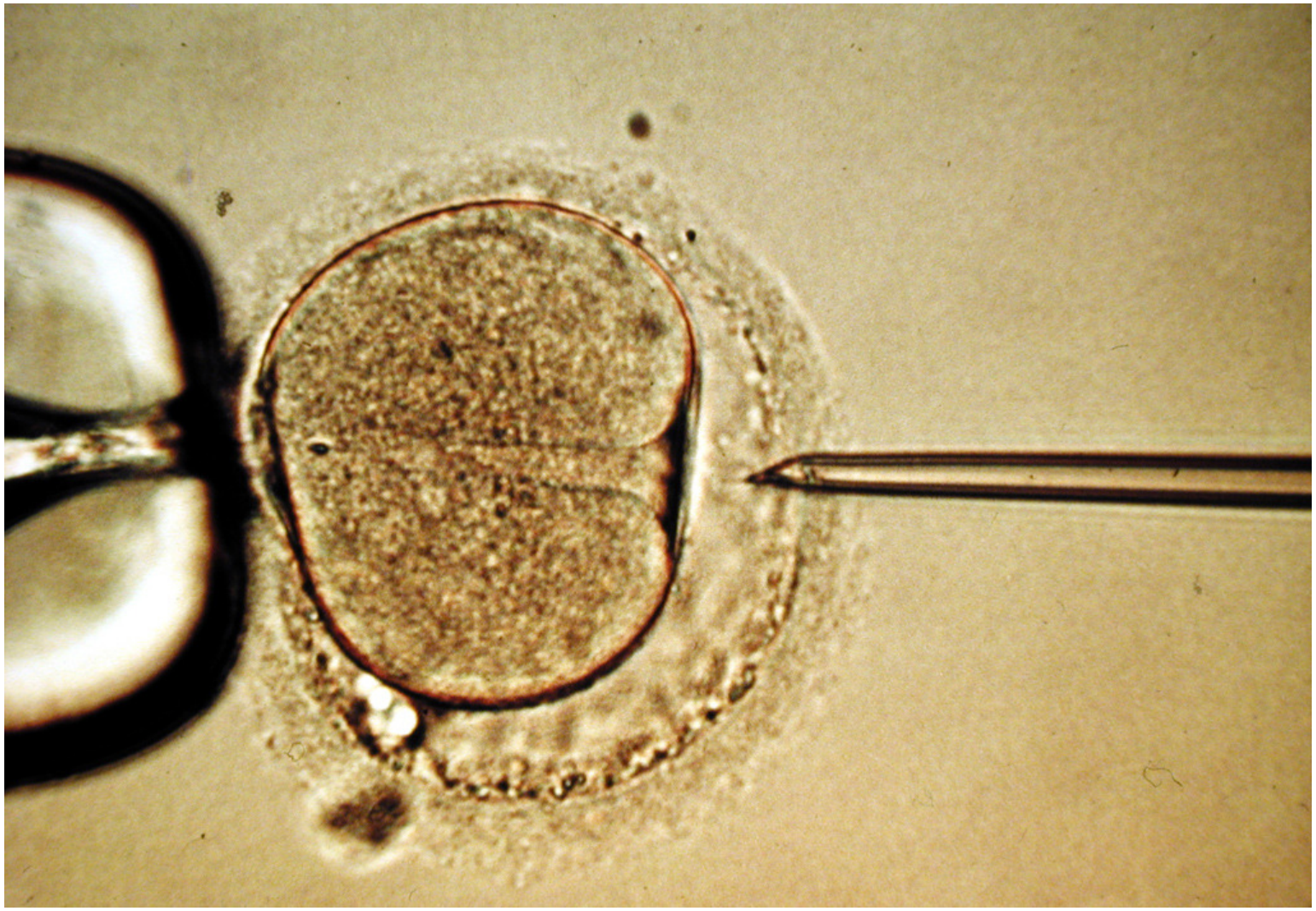
IVF Zentren Prof. Zech
Der Liebe Leben geben

ICSI



IVF Zentren Prof. Zech
Der Liebe Leben geben

ICSI



IVF Zentren Prof. Zech
Der Liebe Leben geben

Early and Late Paternal Effects on Embryo-Development

Day 0 1 2 3 4 5

abnormal development

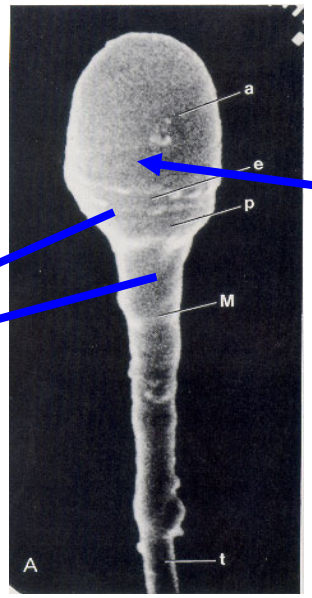
abnormal development/
abortion

„Early paternal effects“

Sperm Cytoplasmic Defects

Oocyte activation factor

Centriole



„Late paternal effects“

Sperm-Nuclear-Defects

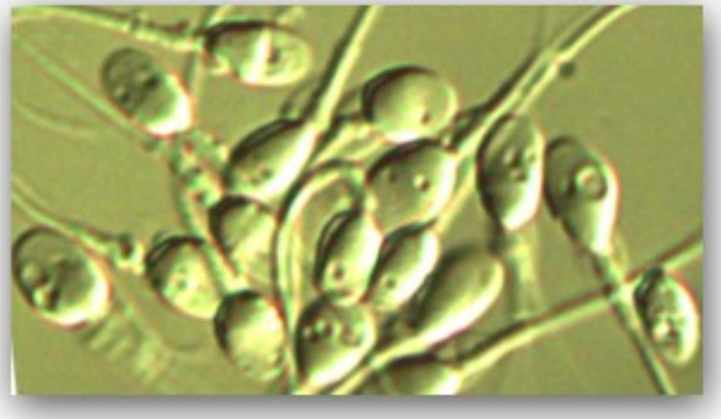
- Chromosomal aberrations
- DNA fragmentation

IVF Zentren Prof. Zech
Der Liebe Leben geben

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)
  - PICSI
  - Subtle morphology (MSOME)



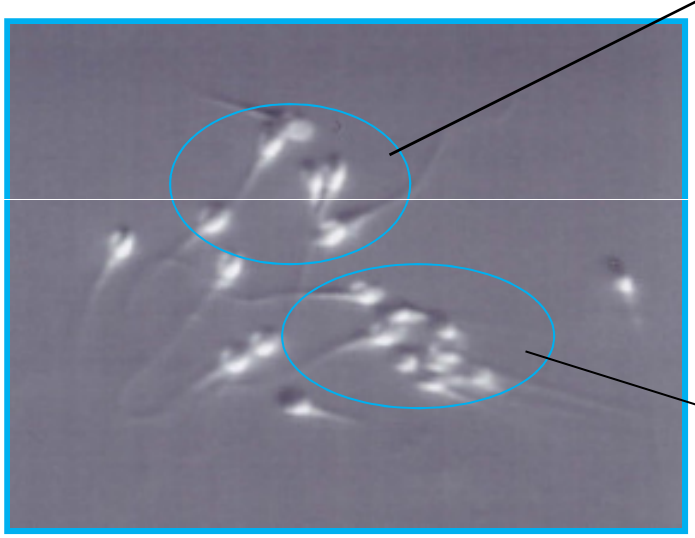
IVF Zentren Prof. Zech
Der Liebe Leben geben

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.

IVF Zentren Prof. Zech
Der Liebe Leben geben

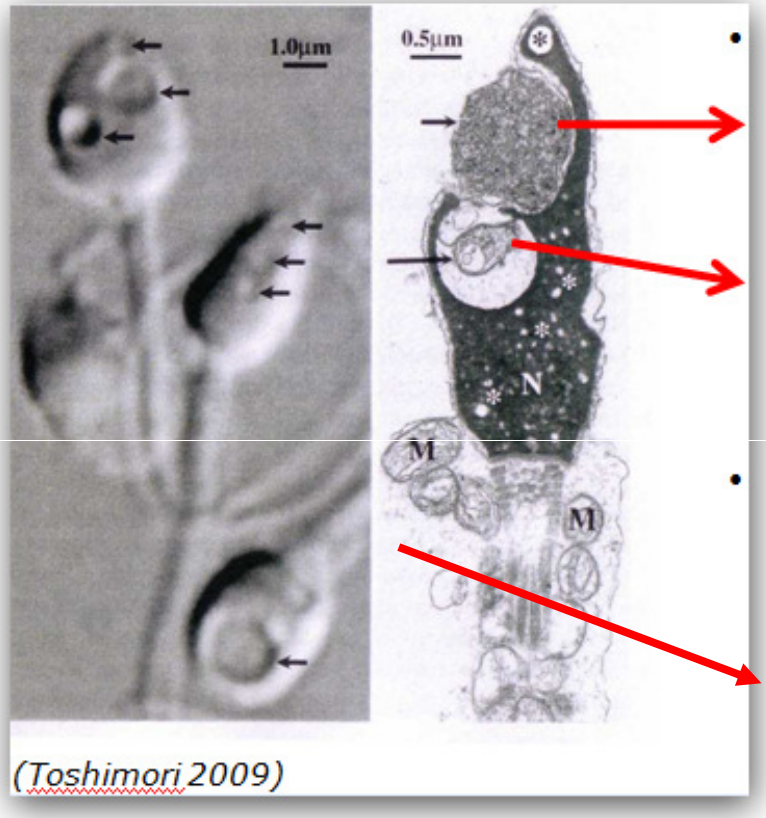
Average sperm magnification X 300



MSOME X 6000-10000

IVF Zentren Prof. Zech
Der Liebe Leben geben

Vacuolisation?



Amorph substances ?

Membranous structures?

Small vesicles without inner structures?

Craters? (Westbroock, 2000),

Hollow? (Watanabe, 2009),

Vesicles?

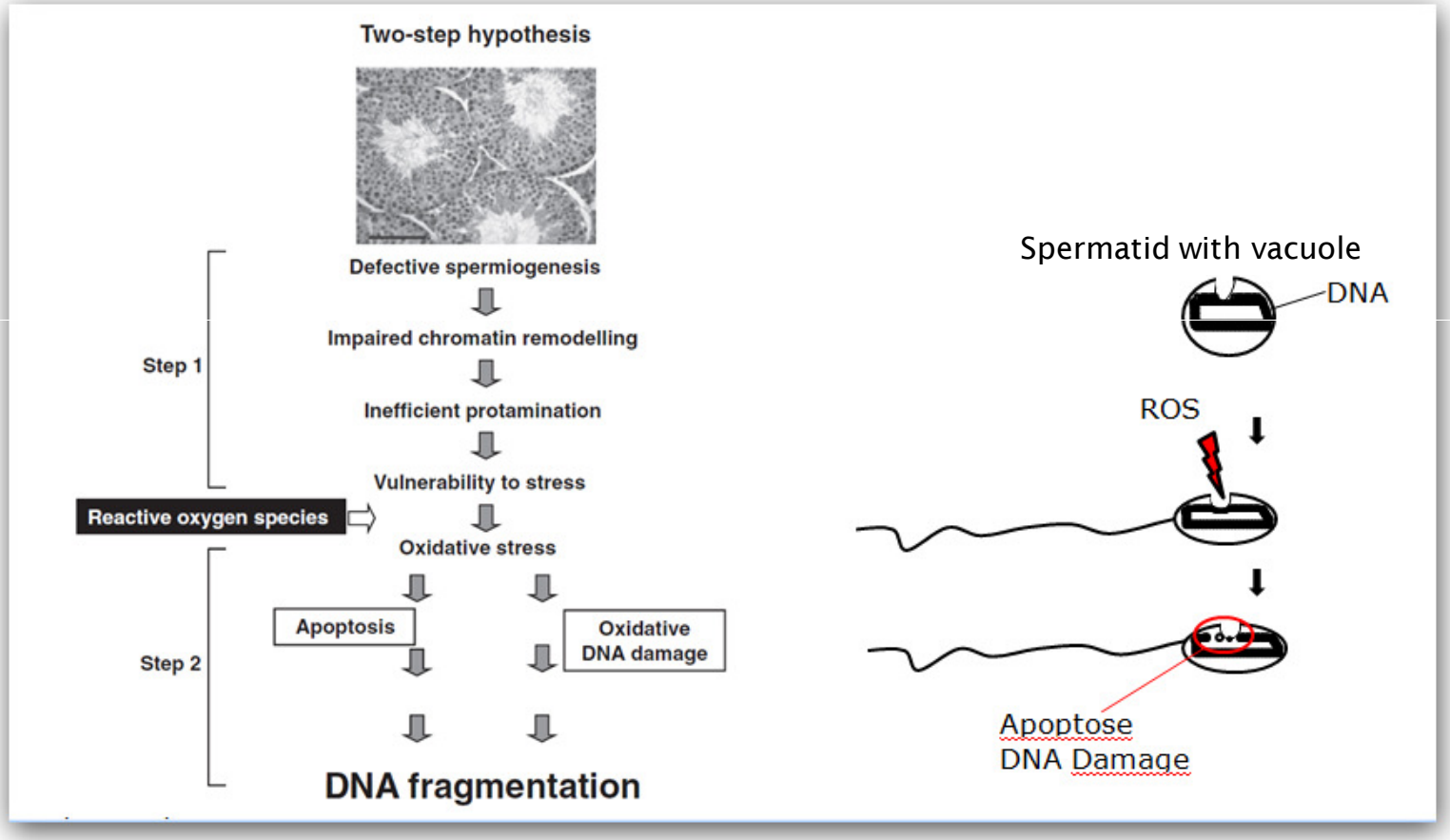
IVF Zentren Prof. Zech
Der Liebe Leben geben

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

IVF Zentren Prof. Zech
Der Liebe Leben geben

Two step hypothesis



Aitken RJ, Smith TB, Jobling MS, Baker MA, De Iuliis GN. Oxidative stress and male reproductive health. Asian J Androl 2014;16:31-8

IVF Zentren Prof. Zech
Der Liebe Leben geben

Routine

- 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)

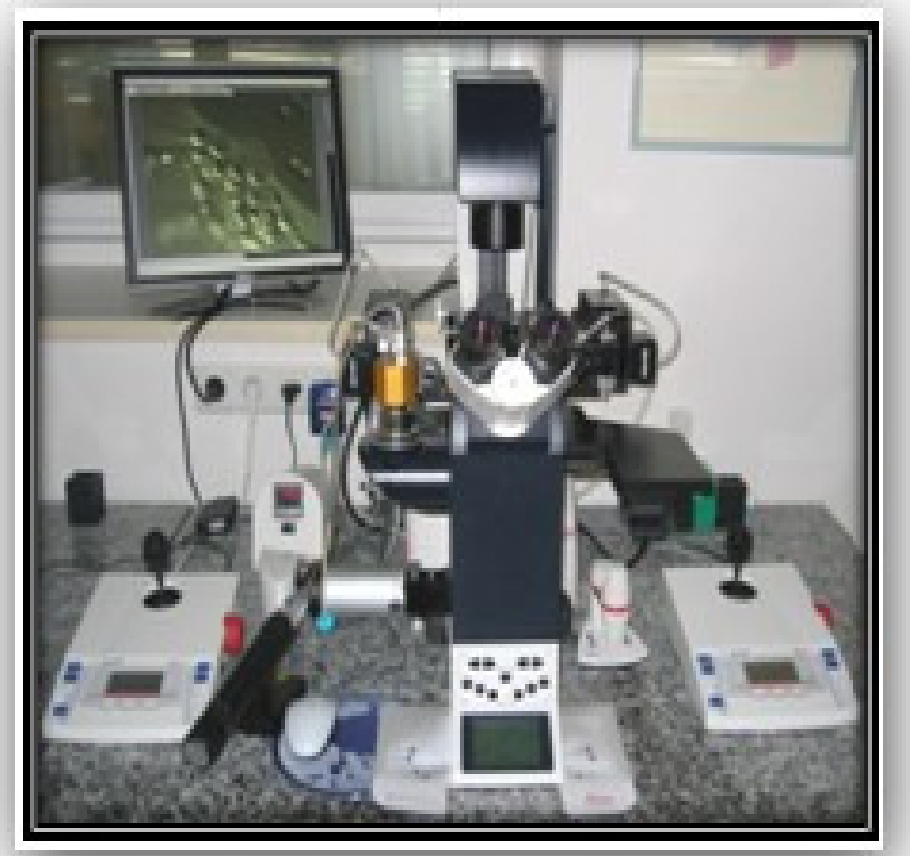
  class I: normal shaped sperm without vacuoles or with 1-2 small vacuoles <4% of the head length

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

  class III: sperm with abnormal morphology with our without vacuoles.

IVF Zentren Prof. Zech
Der Liebe Leben geben

IMSI Unit Leica 6000



IVF Zentren Prof. Zech
Der Liebe Leben geben

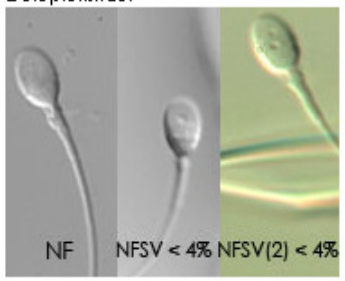
Report

SPZ Class 1
(Normal Form, no, one or two Vacuoles ≤ 4%)

(%)

☐ NF	**N**ormal **F**orm without vacuoles
☐ NFSV < 4 %	**N**ormal **F**orm, **S**mall **V**acuole < 4 %
☐ NFSV(2) < 4 %	**N**ormal **F**orm, two **S**mall **V**acuoles < 4 %

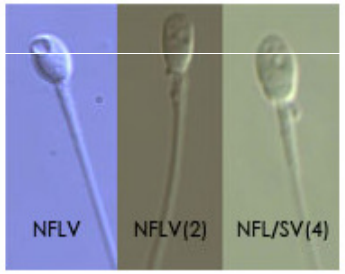
Beispielbilder



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

(%)

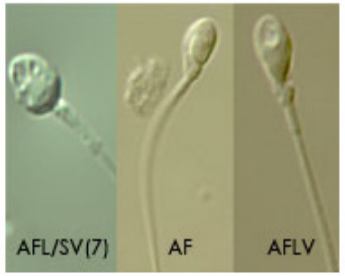
☐ NFLV	**N**ormal **F**orm, one **L**arge **V**acuole
☐ NFLV(n)	**N**ormal **F**orm, **L**arge **V**acuoles (**number**)
☐ NFL/SV(n)	**N**ormal **F**orm, **L**arge + **S**mall **V**acuoles (**number**)



SPZ Class 3
(More Abnormalities)

(%)

☐ AFL/SV(n)	**A**bnormal **F**orm, **L**arge + **S**mall **V**acuoles (**number**)
☐ AFLV	**A**bnormal **F**orm, one **L**arge **V**acuole
☐ AF	**A**bnormal **F**orm without vacuole



IVF Zentren Prof. Zech
Der Liebe Leben geben

So How to Get Class I Sperms?

IVF Zentren Prof. Zech
Der Liebe Leben geben

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

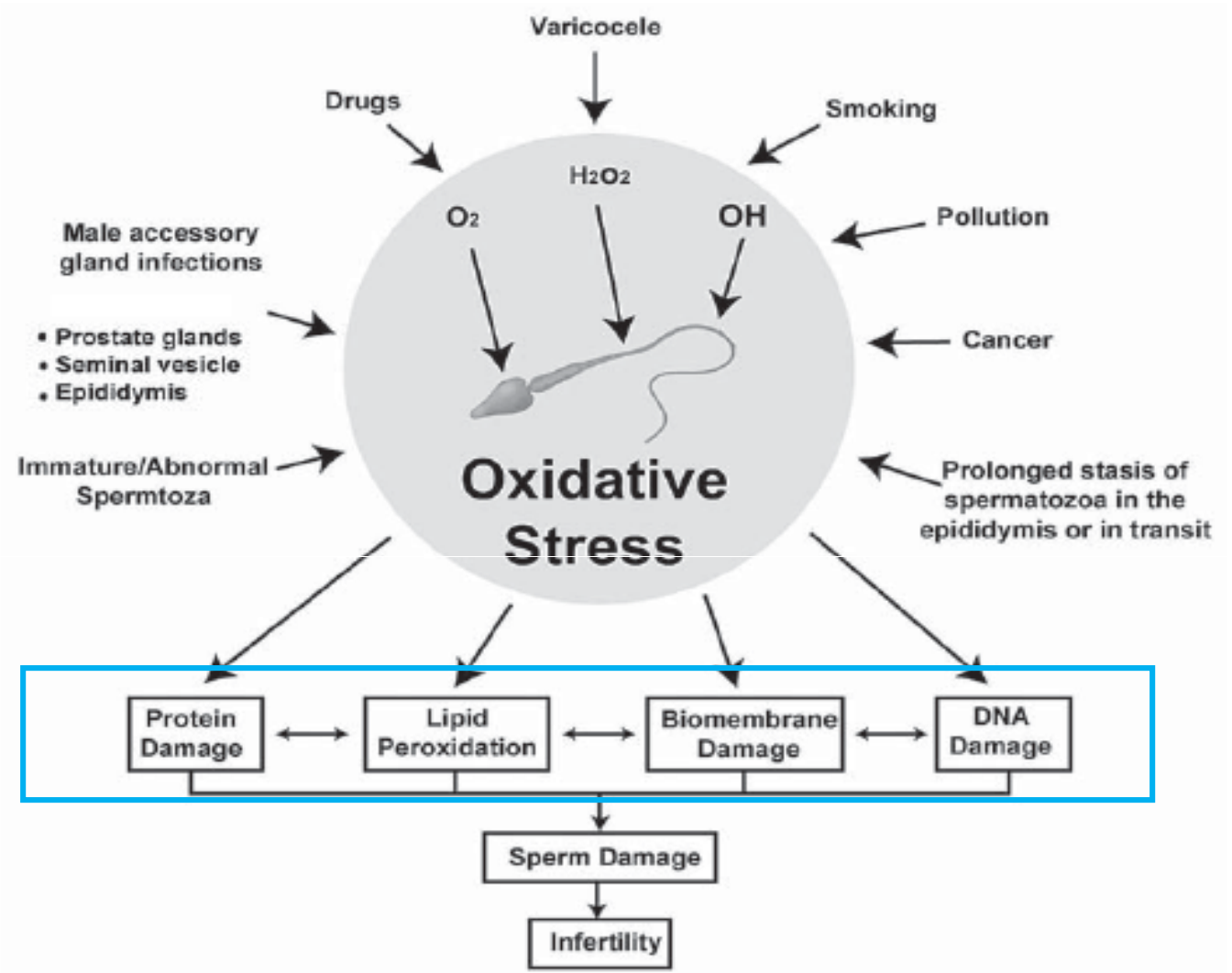


Figure 1 – Association of increasing reactive oxygen species (ROS) production with infertility.

Marcello Corcuza- Int. braz j urol. vol.33 no.5 Rio de Janeiro Sept./Oct. 2007

Reprod Biol Endocrinol. 2012 Dec 24;10:115. doi: 10.1186/1477-7827-10-115.

The combination matters--distinct impact of lifestyle factors on sperm quality: a study on semen analysis of 1683 patients according to MSOME criteria.

Wogatzky J[1], Wirleitner B, Stecher A, Vanderzwalmen P, Neyer A, Spitzer D, Schuff M, Schechinger B, Zech NH.

Author information

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).

PMID: 23265183 [PubMed - indexed for MEDLINE] PMCID: PMC3575231 Free PMC Article

IVF Zentren Prof. Zech
Der Liebe Leben geben

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 funtion- 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

IVF Zentren Prof. Zech
Der Liebe Leben geben

Supplement Facts

Fertilovit®Mplus	Daily dose	% RDA *
Vitamin C (sustained rel.)	100 mg	125
Vitamin E	100 mg	833
Folic acid	500 µg	250
Zinc	25 mg	250
Selenium	100 µg	182
L-citrulline	300 mg	-
L-carnitine	300 mg	-
N-acetyl-L-cysteine	50 mg	-
Glutathione, red.	50 mg	-
Coenzyme Q10	15 mg	-
Lycopene	4 mg	-

• % of recommended daily allowance (according to EU-guidelines)

IVF Zentren Prof. Zech
Der Liebe Leben geben

Can a supplemet improve sperm according to vacuolisation rate?

Yes!

Int. J. Vitam. Nutr. Res., 82 (6), 2012, 391 – 398

Original Communication

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

Barbara Wirleitner[1], Pierre Vanderzwalmen[1], Astrid Stecher[1], Dietmar Spitzer[2], Maximilian Schuff[1], Delf Schwerda[1], Magnus Bach[1], Birgit Schechinger[1], and Nicolas Herbert Zech[1]

[1]IVF Centers Prof Zech, Bregenz, Austria
[2]IVF Centers Prof Zech, Salzburg, Austria

Received: April 23, 2012; Accepted: February 4, 2013

Abstract: *Background:* This study aimed to investigate the influence of an oral antioxidative supplementation on sperm quality of in vitro fertilization (IVF) patients, as analyzed by sperm motility according to the WHO criteria and motile sperm organelle morphology examination (MSOME). Methods: Semen samples were collected from 147 patients before undergoing an IVF/intracytoplasmic morphologically-selected sperm injection (IMSI) cycle and 2–12 months after an antioxidative 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 heads. Patients were divided into oligoasthenoteratozoospermic (OAT) and non-OAT men. Between first and second semen analysis, patients were supplemented orally with an antioxidative preparation. *Results:* After the antioxidative therapy we observed a significant reduction in the percentage of immotile sperm cells in the patients. Additionally, the percentage of class I spermatozoa according to MSOME criteria was significantly higher after antioxidative supplementation. In OAT patients the percentage of class I sperm was found to be increased, although not significantly. However, we observed a drastic 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 on the one hand and reactive oxygen species on the other, the observed changes in the sperm parameters indicate that a decline in semen quality, and even subtle morphological changes, might be associated with oxidative stress. Our findings suggest that an antioxidative and micronutrient supplementation has a remarkable benefit for IVF patients having restricted sperm parameters, in particular.

IVF Zentren Prof. Zech
Der Liebe Leben geben

Can a Supplement also Improve Blastocyst Quality?

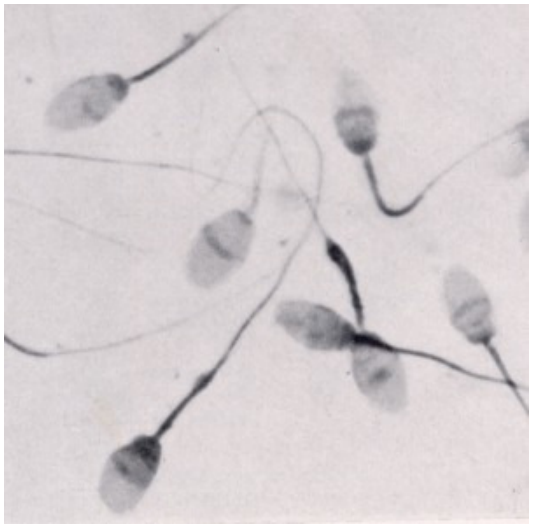
The semen analyses and IVF/IMSI treatment outcomes of 92 subfertile male IVF patients and their partners were evaluated in two separate treatment cycles. One cycle was performed with no supplementation, the second cycle with an antioxidant supplementation (Fertilovit Mplus).

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

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

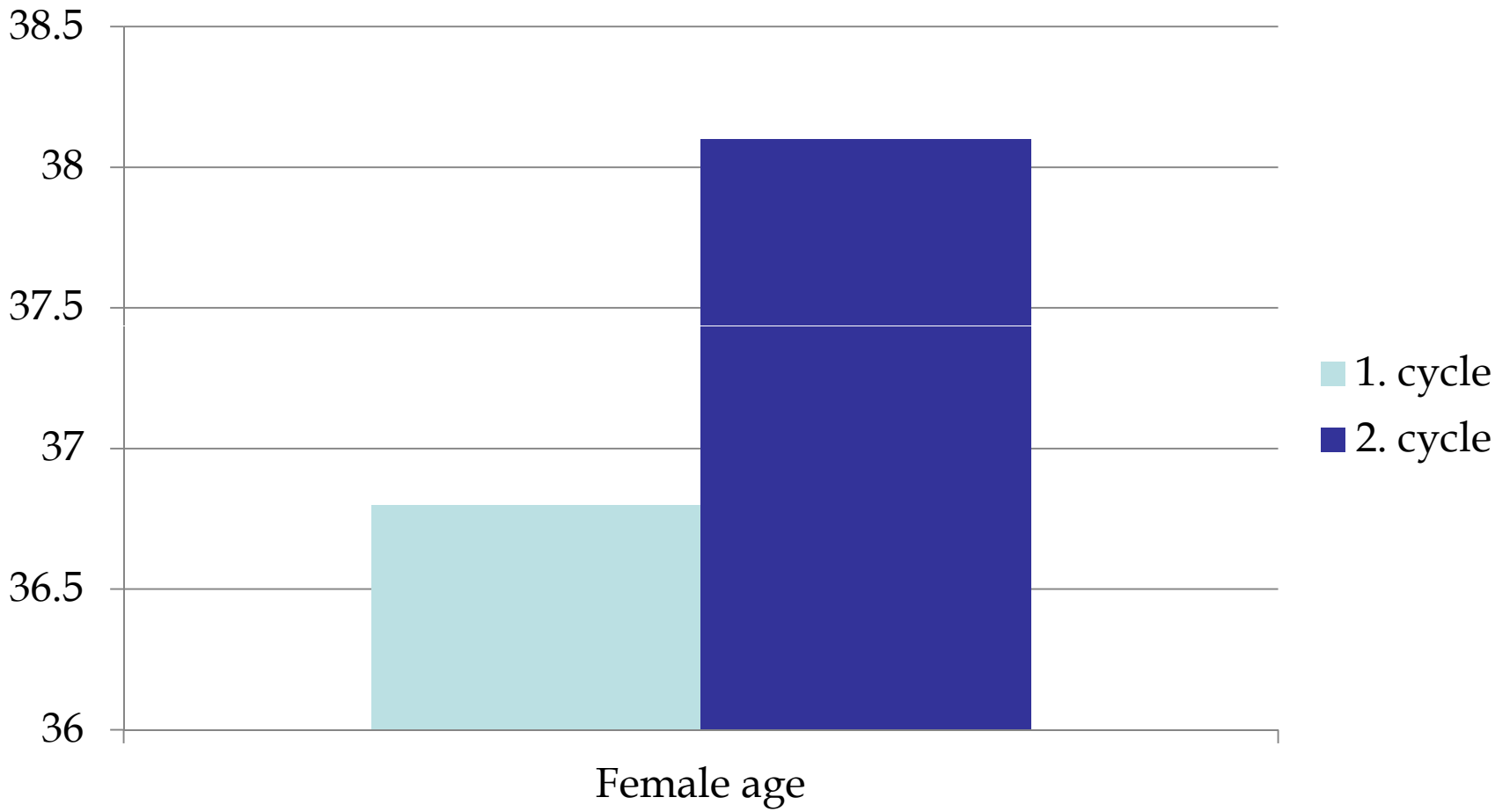
IVF Zentren Prof. Zech
Der Liebe Leben geben

Results – Treatment Outcome

	First cycle without Supplementation	Second cycle with Supplementation	p-value
Female Age (years)	36.8 +/- 4.2	38.1 +/- 3.9	n.s
Stimulation dose (IU)	2451 +/- 745	2647 +/- 764	n.s.
Number of oocytes retrieved (total)	1127	1092	n.s.
Oocytes (mean)	12.4 +/- 5.9	12.1 +/- 5.7	
Number of 2PN (total)	672	659	
2PN (mean)	7.3+/- 3.9	7.3 +/- 4.3	
FR (%)	59.6	60.4	n.s.
Number of blastocysts (total)	267	288	.
Blastocysts (mean)	2.9 +/- 2.4	3.1 +/- 2.7	
Blastocyst Rate (%)	39.7	43.7	n.s.
Top-Blastocysts (mean)	0.4 +/- 1.1	0.6 +/- 1.0	
Top BR (%)	5.5	8.5	< 0.05
(Nb. of top-blastocysts)	(n= 37)	(n= 56)	
Embryos transferred	1.9+/- 0.4	1.9+/- 0.3	n.s.
PR	34.8	44.5	n.s.
cPR	32.8	39.1	n.s.

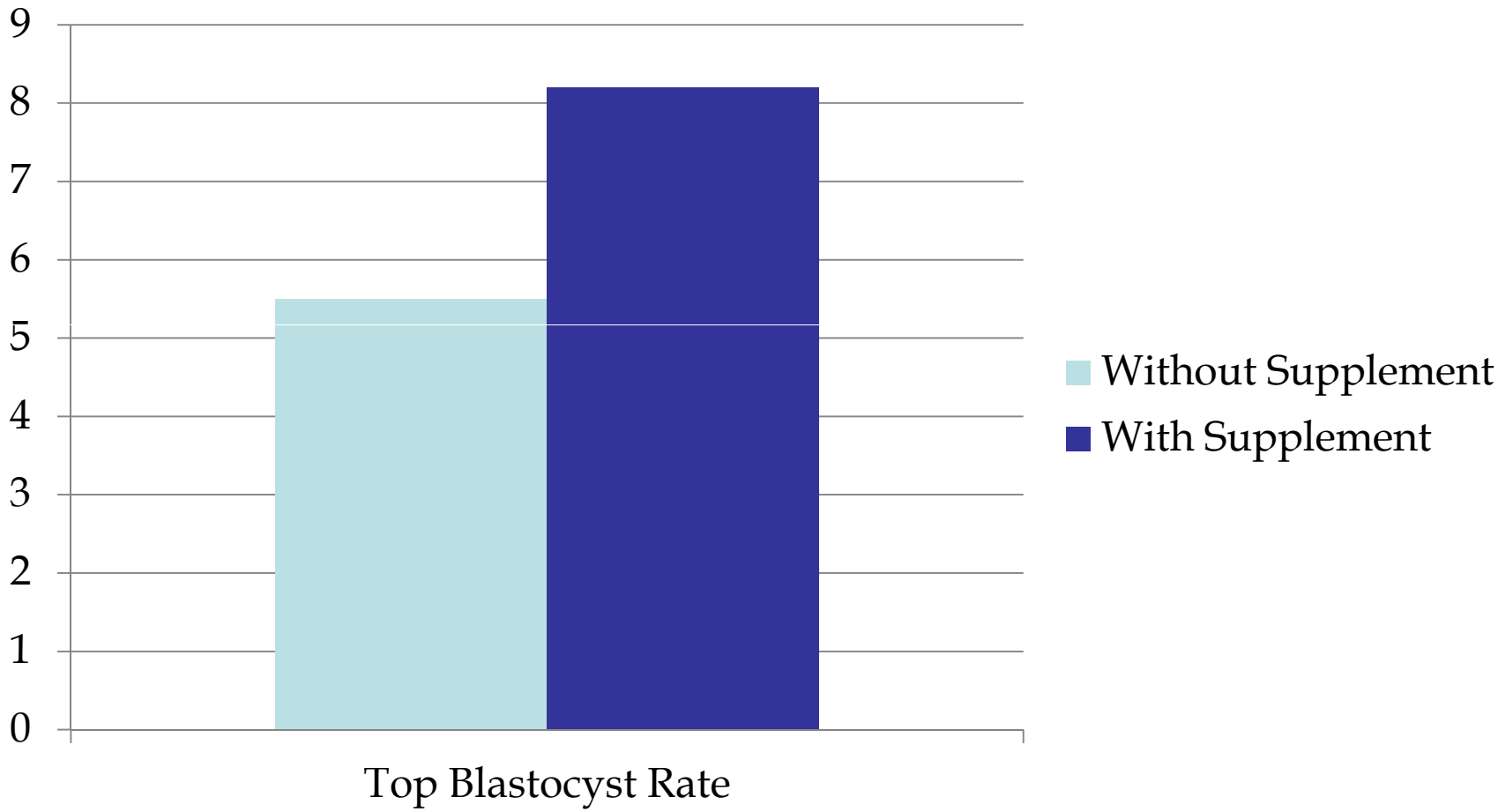
IVF Zentren Prof. Zech
Der Liebe Leben geben

Fertilisation and Blastocyst Rates



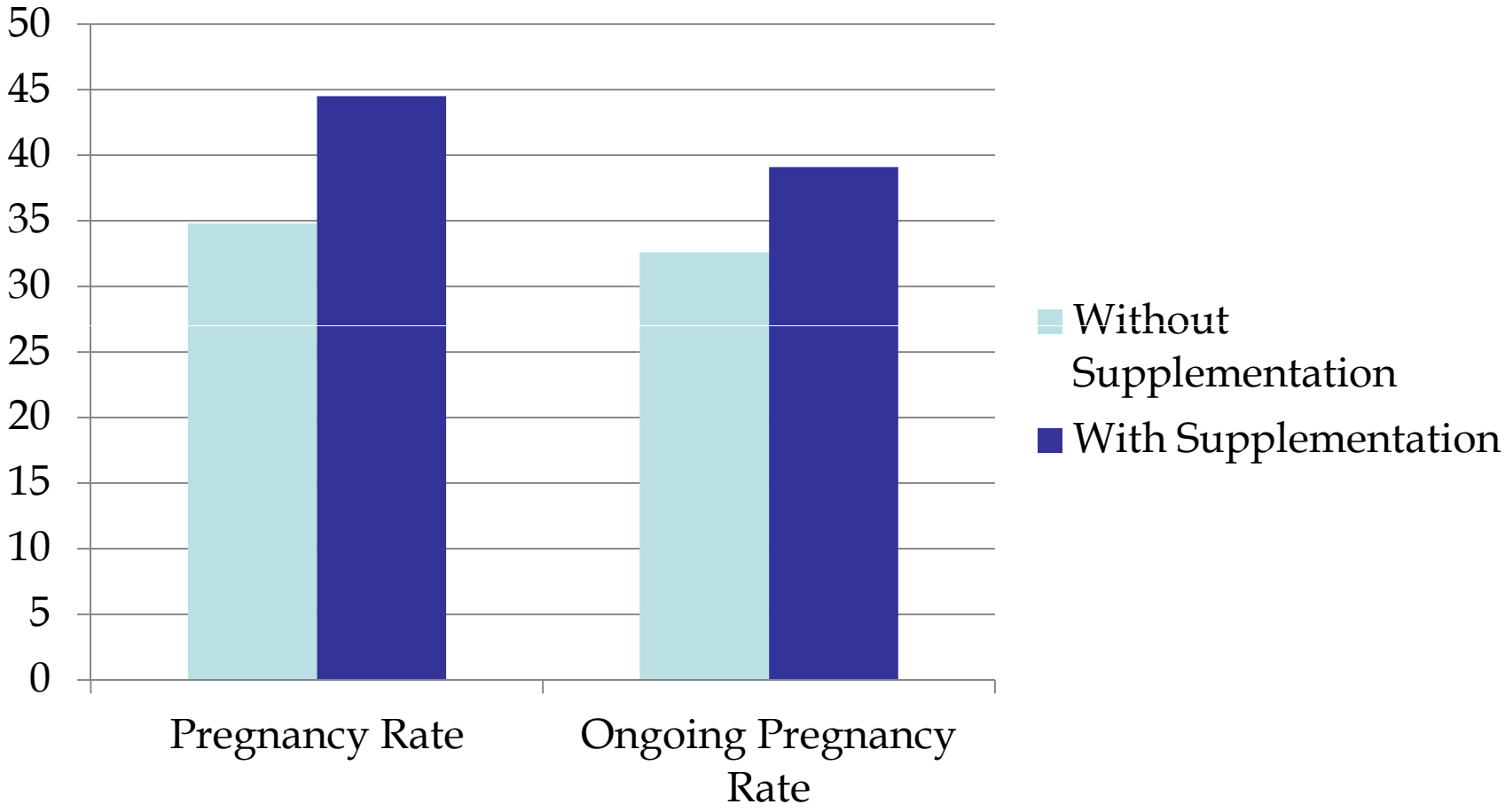
IVF Zentren Prof. Zech
Der Liebe Leben geben

Fertilisation and Blastocyst Rates



IVF Zentren Prof. Zech
Der Liebe Leben geben

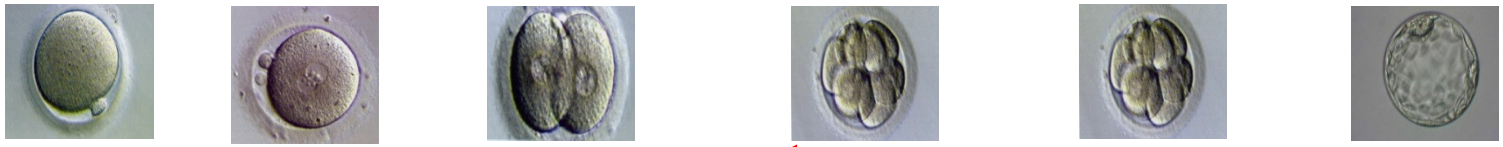
Pregnancy



IVF Zentren Prof. Zech
Der Liebe Leben geben

Data Hints at an Positive Impact of the Compound Antioxidant Treatment on Late Paternal Effects

Day 0 1 2 3 4 5



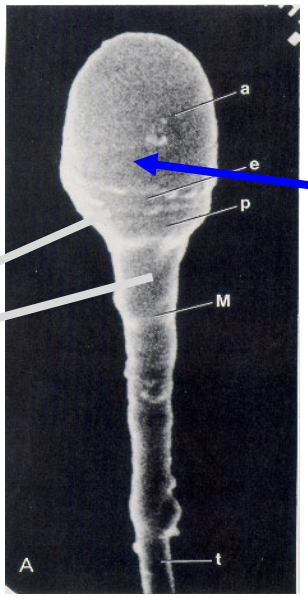
abnormal development

„Early paternal defects“

Sperm Cytoplasmic Defects

Oocyte activation factor

Centriole



abnormal development/
abortion

„Late paternal effects“

Sperm-Nuclear-Defects

- Chromosomal aberrations
- DNA fragmentation

IVF Zentren Prof. Zech
Der Liebe Leben geben

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

IVF Zentren Prof. Zech
Der Liebe Leben geben

Thanks to...

- H.Zech
- N.Zech
- M.Murtinger
- M.Schuff
- B.Wierleitner
- B.Schechinger
- A.Stecher

IVF Zentren Prof. Zech
Der Liebe Leben geben