New approaches for sperm selection

IVF Zentren Prof. Zech, Bregenz, Austria

The search for excellence in IVF laboratories: a practical approach Maribor, Slovenia 22-23 January 2010

Selection of the best spermatozoa

Why?

Ultimate goal of an IVF treatment:
- SINGLE pregnancy
- Birth of ONE healthy baby

The new challenge for ART clinics consists in:
- Transferring fewer embryos (SET)
- Minimizing the risk of multiple pregnancy
- Maintaining the greatest chance of pregnancy for their patients
Selection of the best embryos

Produce good quality embryos

Selection criteria

MORPHOLOGICAL
BIOCHEMICAL-METABOLIC

\[ d_2 \leftrightarrow d_3 \rightarrow d_5 \]

Produce the best gametes

Select the best gametes

Morphology
Polscope
Zona retardance
PB screening
Respirometry

Swim up
Density Gradient

“For the time being, the take-home message is that DNA damage in the male germline is potentially damaging, and care should be taken when treating patients exhibiting such damage with ICSI. In light of such considerations, it would seem rational to try to determine the causes of DNA damage in the male germline and to do everything possible to alleviate this damage (e.g. antioxidant therapy) and/or use sperm isolation techniques that will select for gametes possessing very low levels of DNA damage” (Ainsworth et al, 2005, 2007)

So the question is:
Are there techniques that select spermatozoa with reduced levels of chromatin or DNA damage?

Selection of the best spermatozoa
How?
Development of new techniques, with the aim to enhance the preparation of spermatozoa and to select in a more accurate fashion spermatozoa carrying all the information for the future development, are mandatory.

**SP- Diagnoses**

- **WHO**: World Health Organization
- **Routine Semen Analysis**
  - Serial semen samples (at least two)
  - Kruger's strict criteria
- **Optional Tests**
  - HOS Test
  - CASA
  - Acrosome Reaction Assay (Ham's Test)
- **DNA Integrity**:
  - TUNEL
  - Comet
  - SCSA
  - AO
  - In situ nick translation
  - CMA
  - Y-Chromosome Microdeletion

**INVASIVE TESTS**

- Single spermatozoa cannot be used for ICSI

**SP Preparation**

**SP Selection**
SP PREPARATION
SWIM UP
DENSITY GRADIENT (DGC)

SP SELECTION
"NATURAL": IUI, IVF
ICSI

MACS- magnetic-activated cell sorting

MACS-

Utility of Human Cell Hepatocellular Carcinoma
Preparation Technique

Effects of magnetic-activated cell sorting on spermatozoa motility and sperm survival rate

Clinical use of magnetic activated cell sorting: an update

Magnetic activated cell sorting effects on spermatozoa motility and sperm survival rate

A novel approach for the selection of human sperm: Magnetic activated cell sorting (MACS)
Principle of MACS- Separation of nonapoptotic spermatozoa

SPERM PARAMETERS
- The combination of MACS with DGC yields a clean sperm population characterized by higher motility, viability, morphology, reduced apoptosis manifestations (including DNA fragmentation) and increased cryosurvival rates (Agarwal et al., 2007, Henkel et al., 2009, Rawe et al., 2009 accepted, Said et al., 2005, 2006, 2008).

IVF APPLICATION
- The selection of nonapoptotic human spermatozoa after MACS:
  - improves sperm fertilization potential (Said et al., 2008)
  - increases cleavage and pregnancy rates in oligoasthenozoospermic ART cases after ICSI (Dirican et al., 2008)
  - resulted in an ongoing pregnancy achieved with a clear reduction in the percentage of sperm DNA fragmentation (Case report, Jbases et al., 2009 accepted)

Conclusions of MACS

- The combination of MACS with DGC yields a clean sperm population characterized by higher motility, viability, morphology, reduced apoptosis manifestations (including DNA fragmentation) and increased cryosurvival rates (Agarwal et al., 2007, Henkel et al., 2009, Rawe et al., 2009 accepted, Said et al., 2005, 2006, 2008).

- The selection of nonapoptotic human spermatozoa after MACS:
  - improves sperm fertilization potential (Said et al., 2008)
  - increases cleavage and pregnancy rates in oligoasthenozoospermic ART cases after ICSI (Dirican et al., 2008)
  - resulted in an ongoing pregnancy achieved with a clear reduction in the percentage of sperm DNA fragmentation (Case report, Jbases et al., 2009 accepted)

- May be considered as a molecular preparation technique that complements conventional sperm preparation protocols (DGG) and may enhance ART success rates.
Electrophoresis

Electrophoretic separation of spermatozoa on the basis of their charge and size

This system is based on two principles:
(i) the highest quality spermatozoa in the ejaculate are the most electronegative (most likely dependent upon the glykocalyx, which is rich in salic acid residues)
(ii) spermatozoa can be separated from other contaminating electronegative cells (such as leukocytes and precursor germ cells) by virtue of their small cross-sectional size

Conclusions of Electrophoresis

- Membrane-based electrophoresis is as effective as DGC in preparing sperm for IVF and ICSI regarding sperm recovery, motility, DNA-fragmentation, fertilization and cleavage rates
- Advantages of electrophoresis compared to DGC:
  - Faster (5 min) and simpler method (one step, improved risk management)
  - Improvement in purifying testicular biopsies
  - NO centrifugation: no generation of reactive oxygen species

(Ainsworth et al., 2005)
Electronegative SP

Electrophoresis

ζ-Potential

electrostatic charge attraction

Separation of spermatozoa on the basis of their charge

Electrophoresis

ζ-Potential

electrostatic charge attraction

Electrophoresis

ζ-Potential

electrostatic charge attraction

Separation of spermatozoa on the basis of their charge

A simple zeta method for sperm selection based on membrane charge (Chan et al., FS 2006)

Selection of sperm based on combined density gradient and Zeta method may improve ICSI outcome (Kheirollahi-Kouchak et al., HS 2009)

Principle:

- Mature sperm possess a greater net electric negative charge of -16 to -20 mV (ζ-Potential - electrokinetic potential) due to membrane sialoglycoproteins (specifically, gp20-CD52 glycopeptides), which are acquired during transition through the epididymes

- "Mature sperm stick to the wall of a positive surface charged centrifuge tube by electrostatic charge attraction"

Separation of spermatozoa on the basis of their charge

Conclusions:

- The Zeta method of sperm processing is simple to perform, inexpensive and permits rapid recovery of sperm with improved sperm parameters, particularly strict normal morphology and DNA normal integrity

- Compared to DGC, both methods are efficient for the recovery of sperm with normal proteomic content and low DNA fragmentation

However, the Zeta method yield a greater number of sperm with less DNA fragmentation

Limitations:

- Carrying out immediately after the separation of sperm from the seminal plasma, since sperm cells become less negatively charged with the onset of capacitation

- Low recovery rate (8.8%)
EJACULATE
TISSUE
SP PREPARATION
SWIM UP
DENSITY GRADIENT
(DGG)
MACS ELECTROPHORESIS
- ζ-Potential
SP SELECTION
NATURAL*: AUI/IVF
ICSI
Hyaluronan-Binding-Test
ZP- Binding

Spermiogenesis is the process of maturation and differentiation of the sperm cell

Shape properties changes

Remodeling steps:
- Cytoplasmic extrusion
- Histone protamine exchange
- Maturation of sperm plasma membrane
- Formation of Hyaluronan (HA)-binding and zona-binding sites

Hyaluronan (HA) surrounds the zona pellucida as part of the cumulus matrix but also is present throughout the zona pellucida and the perivitelline space (Dandekar and Talbot et al., 1992)

Close correlation between binding scores to either HA or hemizona (Cayli et al., 2003)

"Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects" (RBMonline, Huszar et al. 2007)

HA-bound sperm:
- are mature
- undergo maturation of the sperm plasma membrane
- have no cytoplasmic residue
- undergo histone protamine-exchange in the nucleus
- show no DNA-degradation
- show no acrosomal reaction;
- have normal morphology
- have low frequency of chromosomal aneuploidies

Tool to select spermatozoa
Hyaluronan-Binding-Test

Selection of HA-bound spermatozoa

PICSI

Add sperm to the hyaluronan microdot

Incubation RT, 10 min

After gentle rinsing:
Bound sperm can be aspirated and used directly for ICSI

What shows the literature?!

<table>
<thead>
<tr>
<th>HA-bound spermatozoa characteristics</th>
<th>In agreement</th>
<th>Not in agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature</td>
<td>Nijs 2009, Aji 2009</td>
<td></td>
</tr>
<tr>
<td>Undergo maturation of the sperm plasma membrane</td>
<td>Nijs 2009, Aji 2009</td>
<td></td>
</tr>
<tr>
<td>Have no cytoplasmic residue</td>
<td>Nasr-Esfahani 2008, Parmegiani 2009</td>
<td></td>
</tr>
<tr>
<td>Undergo histone-protamine exchange in the nucleus</td>
<td>Nasr-Esfahani 2008, Parmegiani 2009</td>
<td></td>
</tr>
<tr>
<td>Show no or less DNA degradation</td>
<td>Nasr-Esfahani 2008, Parmegiani 2009</td>
<td></td>
</tr>
<tr>
<td>Have normal morphology</td>
<td>Nasr-Esfahani 2008, Parmegiani 2009</td>
<td></td>
</tr>
<tr>
<td>Have low frequency of chromosomeal aneuploidies</td>
<td>Sanchez 2009</td>
<td></td>
</tr>
</tbody>
</table>
Increased decreased similar to conventional insemination procedures


Embryo cleavage Janssens 2006, Worrilow 2006

Blastocysts Worrilow 2006

Implantation rate Ha-Nilshoare 2006, 2007

Miscarriage rate Nasr-Esfahani 2008

Delivery rate Nijs 2009 (no predictive value)

Outcome parameters: PICSI vs. ICSI

Conclusions:
The clinical application/advantage has to be confirmed on higher Numbers of patients

Selection of hyaluronan-bound SP

HA-Bound-SP

PICSI solid state HA

Sperm slow soluble state HA
Selection of hyaluronan-bound SP

Promenuclear zygote score following intracytoplasmic injection of
hyaluronan-bound spermatozoa: a prospective randomized study
(Van den Bergh et al., RBMonline December 2009)

„Sperm slow“: Replacement of PVP by hyaluronate during ICSI
Aim of the study:
Determine whether the zygote score and outcome of embryo
development could be influenced by the injection of spermatozoa
that had been preselected on the basis of their binding to
hyaluronic acid

Prospective randomized Selection of SP with

<table>
<thead>
<tr>
<th>HA</th>
<th>PVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibling Donor injected (44 patients)</td>
<td>204</td>
</tr>
<tr>
<td>2 PN</td>
<td>76%</td>
</tr>
<tr>
<td>Zygote scoring</td>
<td></td>
</tr>
<tr>
<td>Z1</td>
<td>22%</td>
</tr>
<tr>
<td>Z2</td>
<td>22%</td>
</tr>
<tr>
<td>Embryo quality (TOP Day 2 )</td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnancies</td>
<td>34%</td>
</tr>
<tr>
<td>(13/38) (3/38)</td>
<td></td>
</tr>
<tr>
<td>I R</td>
<td>28%</td>
</tr>
</tbody>
</table>

Conclusions:
This experiment provides evidence that Sp selection by HA binding is equivalent to the
PVP method
The advantage of the HA binding instead of PVP is that it is a more physiological molecule

Selection of hyaluronan-bound SP

Efficiency of hyaluronic acid (HA) sperm selection
(Parmegiani et al., J Assist Reprod Genet December 2009)

Retrospective comparison of 293 couples treated with HA-ICSI versus 86 couples
-treated with conventional PVP-ICSI

Conclusions:
This study showed that injection of HA-bound spermatozoa (HA-ICSI) significantly
improves embryo quality and implantation rates
Selection of hyaluronan-bound SP

HA-Bound-SP

ICSI
solid state HA
Sperm slow
soluble state HA
Zona Pellucida
“natural binding state”

Spermatozoa-zona pellucida binding test

Aim of the study:
Investigate in a prospective manner whether the SP-ZP binding test is able to select spermatozoa with higher fertilization potential and higher rate of successful embryo development

Mimic the natural process of fertilization: „natural biological selection“

Spermatozoa-zona pellucida binding test

• Group 1:
  - conventional ICSI

• Group 2:
  - Incubation of MI oocyte with SP for 2 h
  - Remove with an ICSI pipette the SP that are bound to the ZP
  - Perform ICSI
### Sibling oocytes Injection of SP

<table>
<thead>
<tr>
<th>ICSI control</th>
<th>SP-ZP bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>194</td>
<td>194</td>
</tr>
</tbody>
</table>

| 2PN | 77% | 77% | NS |
| Day 3 TOP | 70% | 83% | P<0.003 |
| Embryo transfer rate | 44% | 55% | P<0.004 |

* Blind selection

**Conclusions:**
- No difference in the fertilization rate
- Increased high-quality embryos on day 3

### ZP-bound SP after unsuccessful IVF

**Conclusions:**

### SP PREPARATION
- SWIM UP
- DENSITY GRADIENT (DGC)
- MACS
- ELECTROPHORESIS - ζ-Potential

### SP SELECTION
- "NATURAL": IUI, IVF
- ICSI
- Hyaluronan-Binding-Test
- ZP-Binding
- BIREFRINGENCE
Birefringence: protoplasmic structure

**Similar to the polarization microscopy for oocytes**

**Principle**

- In the mature sperm nucleus and acrosome, there is a strong intrinsic birefringence associated with nucleoprotein filaments / subacrosomal protein filaments, that are ordered in rods and longitudinally oriented (inner protoplasmic structures).
- The presence of birefringence in spermatozoa is therefore the expression of an organized and very compact texture, that characterizes normal sperm nuclei, acrosomes, and motile tails (inner microtubule organization).

Application of polarization microscopy to the ICSI technique based on the properties of birefringence (a prospective, randomized study)

**Hypothesis**

Close to TEM it is possible to distinguish between spermatozoa that have undergone the acrosome reaction (reacted) and those in which the acrosome is still intact (nonreacted).

**Results**

<table>
<thead>
<tr>
<th></th>
<th>reacted spermatozoa</th>
<th>nonreacted spermatozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles (n)</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>19</td>
<td>5,6</td>
</tr>
<tr>
<td>Spont. abortion rate (%)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Delivery rate per pick up (%)</td>
<td>44</td>
<td>8</td>
</tr>
</tbody>
</table>
acrosom reacted or not is independent of vacuoles
m.bach, 11/11/2009
Conclusion: Birefringence of spermatozoa

Spermatozoa that have undergone the acrosome reaction (partial birefringence on the postacrosomal part of the nucleus) seem to be more prone to supporting the development of viable ICSI embryos.

But!

It has to be proven by a specific acrosome marker that the birefringence in the postacrosomal region corresponds to the acrosome reaction.

Application of IMSI

- Optics of Nomarski - MSOME - IMSI
- Classification of spermatozoa according to MSOME
- Indication of MSOME:
  - MSOME: improved spermocytogram
  - MSOME+ICSI: IMSI
Application of IMSI

Optics of Nomarski - MSOME - IMSI

IMSI

(Intracytoplasmic Morphologically Selected Sperm Injection)


MSOME

(Motile Sperm Organelle Morphology Examination)

Additional tool to ICSI

Examination performed in real time on living SP

Inverted light microscope

Equipped with high-power Nomarski optics instead of Hoffman Modulation Contrast

Enhanced by digital imaging to achieve a magnification up to 6300 …

More accurate examination of spermatozoa
Application of IMSI

- Optics of Nomarski - MSOME - IMSI
- Classification of spermatozoa according to MSOME

Classification
Classification

Spermatozoa class 1
(normal form, no vacuole)

Spermatozoa class 2
(normal form, max. 2 small vacuoles)

Spermatozoa class 3
(normal form, at least 1 large vacuole, >2 small vacuoles)

Spermatozoa class 4
(abnormal form, and vacuole(s))

Sperm Scoring: HAVBIC

- Head:
  - N. of 2 axes: 3
  - N. of 1 axe: 1
  - AN. of 2 axes: 0
- Acrosome:
  - Normal: 1
  - Abnormal: 0
- Vacuole:
  - Absence: 2
  - 1 small: 1
  - >1 small: 0
- Basis:
  - Normal: 2
  - Abnormal: 0
- Insertion:
  - Normal: 1
  - Abnormal: 0
- Cytopl. droplet:
  - Normal: 1
  - Abnormal: 0

(Cassuto et al. 2008)

Vacuole

- Is it the appropriate term?
Vacuole is not vacuole!

Almost all human spermatozoa, including those of healthy young men, contain heterogeneous vacuoles varying in number, size and content.

- Large vacuoles can differ in content like amorphous substances or membranous structures.
- There are many small vacuoles structures without any structures inside (asterisk).
- It could be possible that the number and size of vacuoles reflect the condition of nuclear maturity/immaturity.
- Does the level of vacuoles play an important role and what is the meaning of deep crater like structures compared to slightly invaginations of the nucleus?

Isolation and evaluation on single spermatozoon

- Sperm DNA integrity - acridine orange staining
- DNA fragmentation - TUNEL

Low degree of DNA fragmentation if SP without vacuole

High level of denatured DNA in sperm with large nuclear vacuoles suggests: precocious decondensation and disaggregation - disorganisation of sperm chromatin fibers

Significantly better chromatin status, mitochondrial function, aneuploidy rate when nuclear vacuoles were absent

(Franco et al., RBMonline 2008, Garolla et al., RBMonline 2008, Hammond et al., JR 2009, Babonova et al., submitted)

Sperm nuclear vacuoles, as assessed by motile sperm organellar morphological examination, are mostly of acrosomal origin (Kacem et al, 2010 forthcoming issues)

Sperm organellar morphological examination and assessment of the acrosomal status were simultaneously performed on the same smear, on immotile spermatozoa

Suggestion:

The improvement in pregnancy rates reported following intracytoplasmic injection of morphologically selected sperm might be due to the procedure allowing injection of acrosome-reacted spermatozoa.
Application of IMSI

- Optics of Nomarski - MSOME - IMSI
- Classification of spermatozoa according to MSOME
- Indication of MSOME:
  - MSOME: improved spermocytogram

The examination of the semen sample by the MSOME technique may be used as a new approach to perform a spermocytogram. MSOME identifies vacuoles that are not evaluated with the same precision by the analysis of Kruger.
Application of IMSI

- Optics of Nomarski - MSOME - IMSI
- Classification of spermatozoa according to MSOME

**Indication of MSOME:**
- MSOME: improved spermocytogram
- MSOME+ICSI: IMSI

Teratozoospermia

- Embryo development
  - day 3
  - day 5
- Sp Morpohology
- Pregnancy
- Abortion
Pre-selection of spermatozoa before ICSI is an essential, prerequisite step.

Consequences if selection and injection of an abnormal shape spermatozoa on the outcome of embryo development:
- Elongated or tapered head, amorphous head, broken neck, cytoplasmic droplets
- Abnormal sperm shape and genetic status: increased risk of aneuploidy and diploidy
- Abnormal sperm shape and pregnancy:
  - Reduction in ongoing pregnancy rates: 20.2% versus 36.7%
  - Reduction in implantation rates: 9.6% versus 18.7%
  - (De Vos et al., 2003)
Indications for MSOME + ICSI

Teratozoospermia

In 2006: report of the literature

<table>
<thead>
<tr>
<th>Sperm Morphology</th>
<th>Quality on day 3</th>
<th>Pregnancy</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal **</td>
<td>No difference</td>
<td>Day 3 transfer</td>
<td>increase reduction</td>
</tr>
<tr>
<td>Abnormal</td>
<td>Day 3 transfer</td>
<td>decrease increase</td>
<td></td>
</tr>
</tbody>
</table>

(Barton et al., 2002, 2003; Jawa et al., 2004; Berkovitz et al., 2006)

Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles

Day 5

Day 5 TOP

671 MII oocytes

6 injected oocytes

6 injected oocytes

* P = 0.001

(Vandecrueren et al., 2008)
A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality (Cassuto et al., FS 2008)

Other abnormalities but no vacuoles

Vacuole present

Scored Intra Cytoplasmic Sperm Injection: SICSI

Limitations for MSOME + ICSI

- **Severe Teratozoospermia**
  - LIMIT: Impossibility to select class I II SP
- **Severe Oligozoospermia**
- **Azoospermia**
  
  In terms of ejaculate and testicular biopsies
  - LIMIT: No benefit - A wise selection is impossible

Indications for MSOME + ICSI

- **High degree of DNA fragmentation**
Delivery rates in a group of patients with at least one failure of implantation after conventional IVF or ICSI (day 3 transfer) and different percentages of sperm DNA-fragmentation

(Petrou et al., 2006)

Indications for MSOME + ICSI

Patients with failure of Implantation after conventional IVF or ICSI

Benefit of IMSI

Pregnancy and abortion rates in a group of 80 patients with at least one failure of implantation after conventional IVF or ICSI and day 3 transfer with OAT

If possible to select a normal SP

(Burton et al., 2013)
ICSI vs. IMSI (sibling study)

53 patients with at least 1-2 previous failure of implantation
(day 5 transfer in our center, day 3 in other centers)
> 3 oocytes
Mean age of the woman: 38  Man: OAT (WHO)

Retrieved oocytes

ICSI
925
IMSI

Injected oocytes

ICSI
403
IMSI
430

Percentages of blastocysts in relation to the method of sperm selection
ICSI vs. IMSI (sibling study)

% of transfers and deliveries in relation to ICSI or IMSI spermatozoa selection

(% of transfers in relation to the origin of the embryo selected)

(% of deliveries in relation to the method of sperm selection)
Indications for MSOME + ICSI

 Patients with failure of Implantation
Presence or absence of blastocysts in the previous cycles

Outcome of embryo development and pregnancy in a group of 53 patients after ICSI and IMSI

<table>
<thead>
<tr>
<th>ICSI</th>
<th>IMSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women age</td>
<td>34.2</td>
</tr>
<tr>
<td>Blastocystes</td>
<td>0%</td>
</tr>
<tr>
<td>Top blastocystes</td>
<td>0%</td>
</tr>
<tr>
<td>Ong. Pregnancy</td>
<td>0%</td>
</tr>
<tr>
<td>Vitrification cycles</td>
<td>0</td>
</tr>
<tr>
<td>Cumulative Ong.preg/OPU</td>
<td></td>
</tr>
</tbody>
</table>

* Warming of 12 cycles 5 POS

Indications for MSOME + ICSI

To a large population of ICSI candidates?

Are there indications, not to try to select the best spermatozoa? NO!
Implementation of IMSI to a large population of ICSI candidate patients:

- May be advisable, if the probability to select a normal spermatozoa is higher using the MSOME approach as compared to the classical ICSI approach.

Importance of the introduction of MSOME spermocytogram: Find a threshold of morphologically normal spermatozoa to decide if IMSI is necessary or not.
% of top quality blastocysts after IMSI and ICSI in relation to the percentage of class 1-2 spermatozoa in the semen sample

% TOP blastocysts

TRESHOLD

NB of cases 7 5 3 9 6 3 1

Implementation of MSOME/MSOME-ICSI to a large population of patients:

- Normozoospermic semen samples?
- or rather
- IVF candidate patients (when indicated!)?

- MSOME spermocytogram:
  Determine the appropriate fertilization technique according to the percentage of morphologically normal spermatozoa: ICSI or IVF?!
- Compare the rate of blastocysts in relation to the different fertilization techniques:
  ICSI vs. IMSI vs. IVF

ICSI vs. IVF vs. IMSI (sibling study)

14 patients, ≥15 oocytes, Mean age of the woman: 35
Man: Normozoospermia (WHO)

Retrieved oocytes

ICSI - 78
IMSI - 97
IVF - 97

Group culture

Retrieved oocytes

ICSI - 301
IMSI - 97
Percentages of blastocysts in relation to the method of sperm selection: ICSI vs. IMSI vs. IVF (calculated on 2PN, 14 patients, 262 inseminated oocytes)

![Bar chart showing percentages of blastocysts](chart.png)

Frequency of transferred embryos

- IVF: 21%
- ICSI/IVF: 7%
- ICSI: 7%

Transfer frequency

- IMSI: 36%
- IMSI/IVF: 14%
- IMSI/ICSI: 14%

Study still in progress

(increase numbers, compare the IR,PR,MR,DR in the subgroups) ...

Observation of spermatozoa by the MSOME approach has to be considered as an additional tool to the classical ICSI

MSOME + ICSI is a useful technique since it produces more embryos with higher capacity to implant

More embryos are susceptible to be cryopreserved: importance of a satisfactory vitrification protocol to increase the cumulative PR

Mandatory to refine the classification regarding the vacuole with Nomarski optic
MSOffice9 sehr jung SET?
SET hervorheben ?!
, 02/10/2009
### IMSI-conclusions (II)

**Indications for MSOME + ICSI**
- Teratozoospermia (severity): negative effect of large vacuoles
- Degree of DNA fragmentation (reduce DNA fragmentation if SP without vacuoles)
- Patients with failure(s) of implantation
- Advice to propose IMSI (if absence of blastocysts in previous ICSI cycle(s))
- The probability to select normal spermatozoa for injection is higher if MSOME is applied
- In consequence, we may consider to apply this way of selection to a large population of ICSI candidates
- IMSI provides a proper selection even in normozoospermic semen samples (Optimizes the rate and quality of blastocysts and ET respectively)

### IMSI-conclusions (III)

**Indication MSOME – spermocytogram?**
- General in routine !
- As a Pre – IMSI or IVF test: try to define a threshold of normal spermatozoa: Decide the option of IMSI or ICSI or IVF
- More attention has to be taken for the selection of the spermatozoa during ICSI even using conventional optics such as Hoffman modulation system
- We may also suggest for those who perform embryo transfer on day 2 or 3 to change their strategy and extend the culture to day 5

Extended culture could provide a test by which to select more viable embryos that reflect the quality of the gametes from which they were derived
(Spano et al., 2003; Smit et al., 2000; Vanderzwalmen et al., 2008)

### So .........................

The introduction of IMSI yields to the advantage, that a lot of embryologists start to realize, that more attention has to be taken during a normal ICSI:

- Change the optics
- Increase the magnification
- Spend more time for selection
- Introduce a easier technique ?!

**Consequences:**
Reduce the difference between ICSI and IMSI
Final Conclusions (I)

We try to improve the stimulation protocols, culture protocols, selection of oocytes, selection of embryos, luteal phase, ET...., why not the selection of spermatozoa?

Final Conclusions (II)

IVF–ICSI

New sperm preparation techniques:
- Electrophoresis (Ainsworth et al., 2005, Fleming et al. 2008)
- ζ-Potential (Chan et al., 2006)
- MACS (Said et al., 2008)

ICSI
New approaches for sperm selection based on:
- Biochemical markers of human sperm maturity and function: HA-Binding (ZP-Binding)
  (Gabor Huszar et al., 2007)
- Birefringence: protoplasmic structure (Gianaroli et al., 2007)
- Real-time morphological approach: MSOME
  (Bartoov et al., 2002)

Take home the sperm’s message

- Revise the ICSI-protocol via optimizing sperm preparation and sperm selection
Thank you for your attention