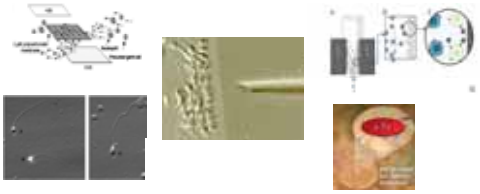
New approaches for sperm selection



Bach M.,Vanderzwalmen P., Neyer A., Stecher A., Schwerda D., Zintz M., Zech N.,H.

IVF Zentren Prof. Zech, Bregenz, Austria

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

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*

SET

Selection of the best embryo

- *Selection criteria*
  - *MORPHOLOGICAL*
  - *BIOCHEMICAL-METABOLIC*

d2 – d3 ←= → *d5*

Produce good quality embryos

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:

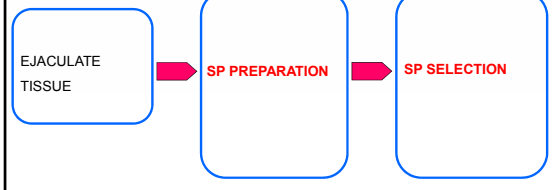
Are there techniques that select spermatozoa with reduced levels of chromatin or DNA damage ?

Development of new techniques,with the aim to enhance the preparation of spermatozoa and to select in a more accurate fashion a spermatozoa carrying all the informations for the future development, are mandatory !

SP- Diagnoses

World Health Organization
Routine Semen Analysis
Serial semen samples (at least two)
Kruger´s strict criteria
Morphology
Optional Tests
HOS Test
Semen Culture
CASA
Sperm Penetration Assay (Hamster-Test)
Acrosome Reaction Assay
Creatinine Kinase Assay
DNA Integrity:
- SCSA
- TUNEL
- COMET
- SCD
- AO
- In situ nick translation
- CMA3
- Y-Chromosome Microdeletion

INVASIVE TESTS
Single spermatozoa cannot be used for ICSI !



EJACULATE
TISSUE

SP PREPARATION
SWIM UP
DENSITY GRADIENT (DGC)

SP SELECTION
„NATURAL": IUI,IVF
ICSI

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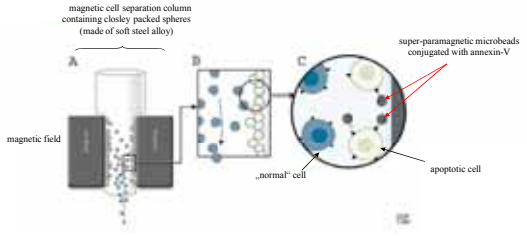
TISSUE

SP PREPARATION
SWIM UP
DENSITY GRADIENT (DGC)
MACS

SP SELECTION
„NATURAL": IUI,IVF
ICSI

MACS- magnetic-activated cell sorting

Principle of MACS- Separation of nonapoptotic spermatozoa



"normal" cell with intact membrane

apoptotic spermatozoa which translocated phosphatidylserine
from the inner laeflet of the plasmamembran to the outer side thereof (early sign of apopotosis)

super-paramagnetic microbeads conjugated with annexin-V
which has a high affinity to phosphatidylserine

(Said et al., 2008)

Conclusions of MACS

SPERM PARAMETERS

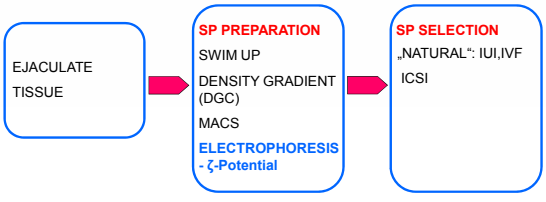
- The combination of MACS with DGC yields a clean sperm population characterized by
  higher motility, viability, morphology, reduced apoptosis manifestations
  (including DNA fragmentation) and increased cryosurvival rates
  (Agarwal et al., 2007, Henkel et al., 2009, Rawe et al., 2009 accepted, Said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

  ➧ may be considered as a molecular preparation technique that complements conventional sperm preparation protocols (DGC) and may enhance ART success rates

- Nevertheless the value of integrating MACS in sperm preparation prior to ICSI and IVF requires further investigation in a clinical ART program *(Agarwal et al., 2007, Said et al., 2008)*



Electrophoresis

Development of a novel electrophoretic system for the isolation of human spermatozoa

C.Ainsworth[1], B.Nixon[1] and R.J.Aitken[1,2,3] (HR 2005)

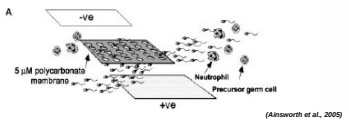
First recorded pregnancy and normal birth after ICSI using electrophoretically isolated spermatozoa

C.Ainsworth[1], B.Nixon[1], R.P.S.Jansen[2] and R.J.Aitken[1,3,4] (HR 2006)

Prospective controlled trial of an electrophoretic method of sperm preparation for assisted reproduction: comparison with density gradient centrifugation

S.D. Fleming[1,2], R.S. Ilad[3], A-M.G. Griffin[3], Y. Wu[1], K.J. Ong[1], H.C. Smith[1] and R.J. Aitken[2] (HR 2008)

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



(Ainsworth et al., 2005)

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 crosssectional 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:

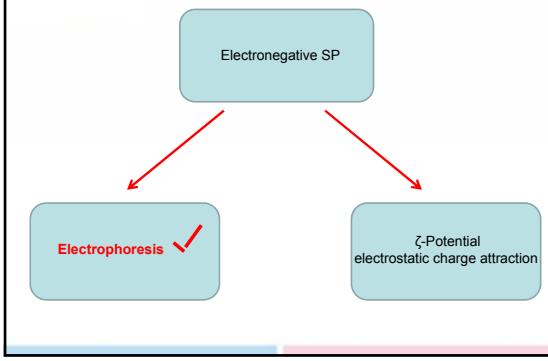
- Faster (5 min) and simpler method (one step, improved risk management)

- Improvement in purifying testicular biopsies

- NO centrifugation: no generation of reactive oxygen species

(Ainsworth et al., 2005)

Separation of spermatozoa on the basis of their charge



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-Kouhestani 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 epididymis
- "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 protamine 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%)

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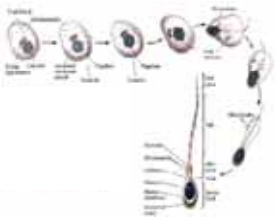
SP PREPARATION
SWIM UP
DENSITY GRADIENT (DGC)
MACS
ELECTROPHORESIS - ζ-Potential

SP SELECTION
„NATURAL": IUI,IVF
ICSI
Hyaluronan-Binding-Test
ZP- Binding

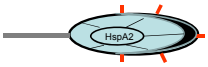
Spermiogenesis is the process of maturation and differentiation of the sperm cell

Shape properties changes



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

(Huszar et al. 2007)



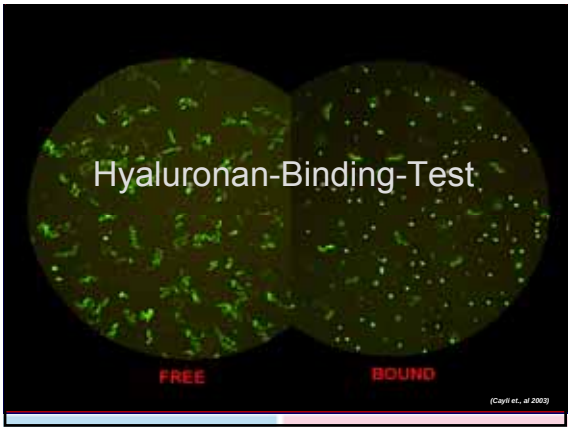
„Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects" *(RBMonline, Huszar et al. 2007)*

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)*

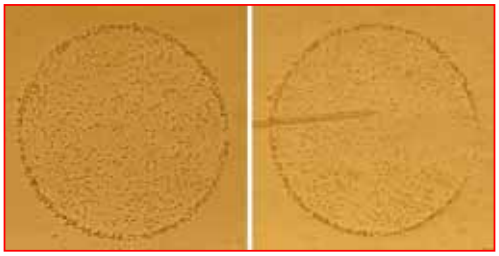
HA-bound sperm:
- are mature
- undergo maturation of the sperm plasma membrane
- have no cytoplasmic residue
- undergo histone-protamine-exchange in the nucleus
- show no DNA-degradation
- show no acrosomal reaction;
- have normal morphology
- have low frequency of chromosomal aneuploidies

Tool to select spermatozoa



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?!

	in agreement	not in agreement
HA bound spermatozoa are:		
mature		*Nijs 2009*
undergo maturation of the sperm plasma membrane		
have no cytoplasmic residue		
undergo histone-protamine exchange in the nucleus	*Nasr-Esfahani 2008*	*Nijs 2009*
show no or less DNA-degradation	*Nasr-Esfahani 2008, Parmegiani 2009*	
show no acrosomal reaction		
have normal morphology	*Nijs 2009, Hong Ye 2006, Nasr-Esfahani 2008, Prinosolova 2009*	
have low frequency of chromosomal aneuploidies	*Sanchez 2005*	

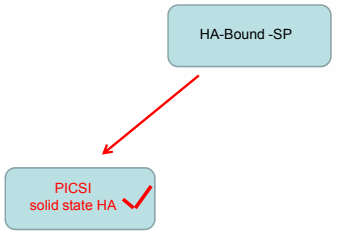
Outcome parameters: PICSI vs. ICSI

	Increased	decreased	similar to conventional insemination procedures
Fertilization rate	*Nasr-Esfahani 2008*		*Nijs 2009, Hong Ye 2006 (no predictive value), Sanchez2005, Janssens 2006, Worrilow 2006*
Embryo cleavage			*Janssens 2006, Worrilow 2006*
Blastocysts			*Worrilow 2006*
Pregnancy rate	*Worrilow 2006, 2009*		*Nijs 2009 (no predictive value), Nasr-Esfahani 2008*
Implantation rate			*Nasr-Esfahani 2008*
Miscarriage rate		*Worrilow 2006, Sanchez 2005*	
Delivery rate			*Nijs 2009 (no predictive value)*

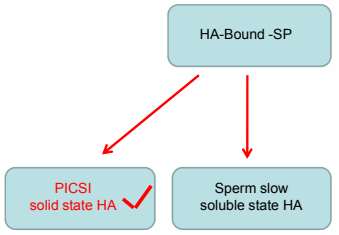
Conclusions:

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

Selection of hyaluronan-bound SP



Selection of hyaluronan-bound SP



Selection of hyaluronan-bound SP

Pronuclear 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

	HA		*PVP*
Sibling Oocytes injected (44 patients)	204		203
2 PN	76%		70%
Zygote scoring			
Z1	22%		24%
Z2	22%		23%
Embryo quality (TOP Day 2)	77%		76%
Ongoing pregnancies	34%	*NS*	50%
	(13/38)		(3/6)
I R		28 %	

Conclusions:

This experiment provides evidence that **Sp selection by HA binding is equivalent to the PVP method**

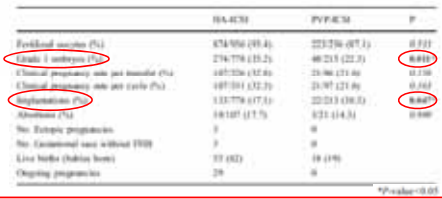
The advantage of the HA binding instead of PVP is that it is a more physiological molecule

(Van den Bergh et al., 2009)

Selection of hyaluronan-bound SP

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

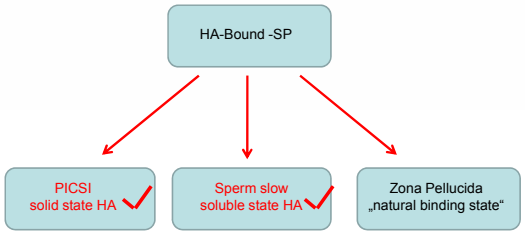
Retrospective comparison of 293 couples treated with HA-ICSI versus 86 couples treated with conventional PVP-ICSI



Conclusions:

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

Selection of hyaluronan-bound SP



Spermatozoa-zona pellucida binding test

Outcome of ICSI using zona pellucida-bound spermatozoa and conventionally selected spermatozoa *(Braga et al., RBMonline December 2009)*

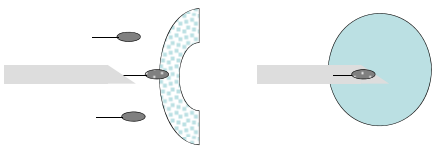
Aim of the study:

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

	ICSI control 194	SP-ZP bound 194	
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

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SP PREPARATION
SWIM UP
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„NATURAL": IUI,IVF
ICSI
Hyaluronan-Binding-Test
ZP- Binding
BIREFRINGENCE

Birefringence: protoplasmic structure

Sperm head's birefringence: a new criterion for sperm selection

(Fertil Steril 2007)

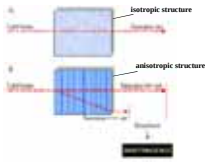
Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for intracytoplasmic sperm injection

(Fertil Steril 2008)

Birefringence: Similar to the polarization microscopy for oocytes

Principle

- In the mature sperm nucleus and acrosome, their 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 (intact microtubular organization)

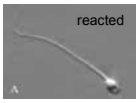


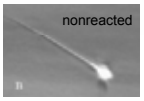
Application of polarization microscopy to the ICSI technique based on the properties of birefringence (a prospective, ranomized study)

Hyopthesis

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

Results





m3

	reacted spermatozoa	nonreacted spermatozoa	
Cycles (n)	23	26	
Implantationrate (%)	39	8,6	(P= 0.002)
Spont. abortion rate (n)	2	1	
Delivery rate per pick up (%)	44	8	(P= 0.004)

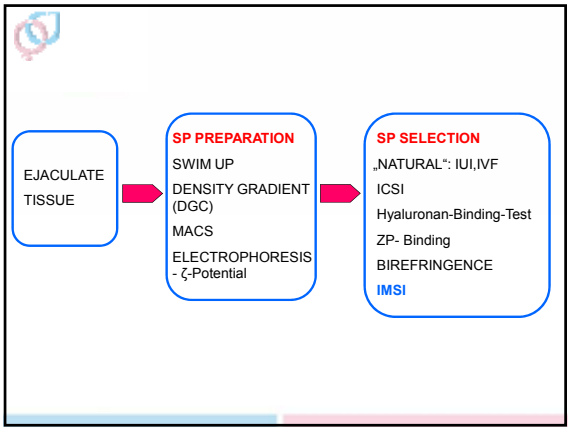
m3 acrosom reacted or not is independent of vacuoles
m.bach, 11/11/2009

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)

(Bartoov et al., 2001, 2002, 2003)

MSOME

(Motile Sperm Organelle Morphology Examination)

Additional tool to

ICSI

MSOME

(Motile Sperm Organelle Morphology Examination)

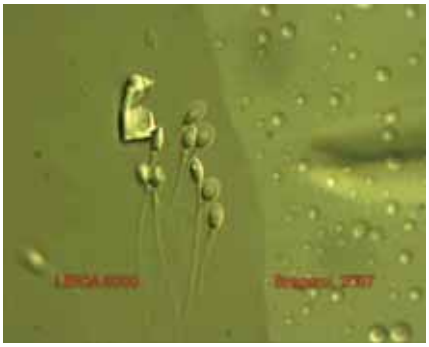
(Bartoov et al., 2002)

- Examination performed in real time on living SP
- Inverted light microscope
- Equipped with high-power **Nomarski optics** instead

of Hoffman Modulation Contrast

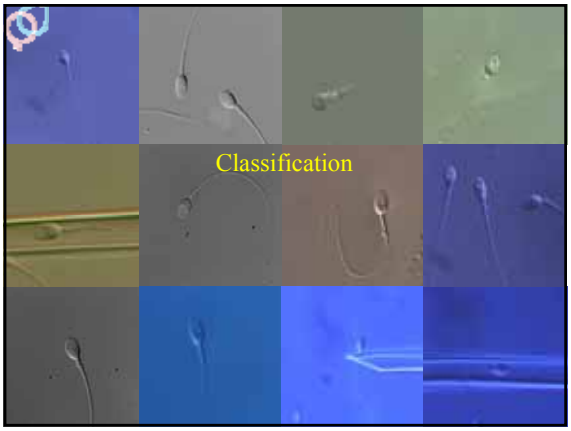
- Enhanced by digital imaging to achieve a magnification up to 6300 …

More accurate examination of spermatozoa



Application of IMSI

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

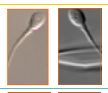


Classification

Spermatozoa class 1
(normal form, no vacuole)
..... (%)

(grade1)

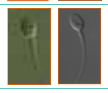
Spermatozoa class 2
(normal form, max. 2 small vacuoles)
..... (%)



(grade 2)

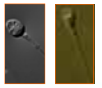
„Normal Spermatozoa"

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



(grade 3)

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



(grade 4)

Sperm Scoring: HAVBIC

• **H**ead:	N. of 2 axes	3
	N. of 1 axe	1
	AN. of 2 axes	0
• **A**crosome:	Normal	1
	Abnormal	0
• **V**acuole :	Absence	2
	1 small	1
	>1 small	0
• **B**asis:	Normal	2
	Abnormal	0
• **I**nsertion:	Normal	1
	Abnormal	0
• **C**ytopl. droplet:	Normal	1
	Abnormal	0
		10

(Cassuto et al. 2008)

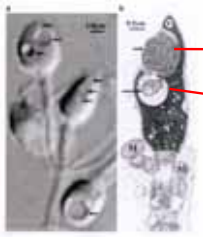
Vacuole

- Is it the appropriate term?

~~Vacuole~~ – hollow or crater

Vacuole is not vacuole !

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



(Tashimori 2009)

- Large vacuoles can differ in content like
  - amorphous substances
  - or
  - membranous structures
- There are many small vacuolous 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 ?**

VACUOLE → Meaning ?????

Isolation and evaluation on single spermatozoon

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

- Association between large vacuole in the sperm and DNA damage
- 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, Hammoud et al., HR 2009, Baborova et al., submitted)

VACUOLE → Meaning ?????

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

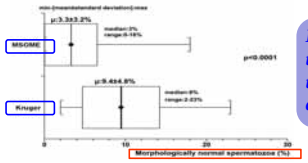
Application of IMSI

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

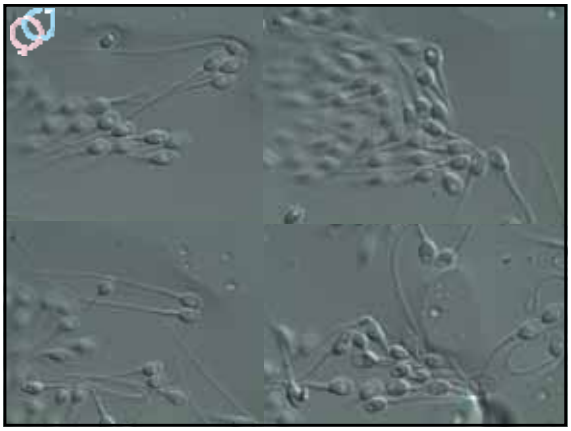
The examination of the semen sample by the MSOME technique may be used as a new approach to perform a spermocytogram

The motile sperm organelle morphology examination (MSOME) is a much stricter criterion than Kruger analysis (Oliveira et al., RBMonline 2008)

Spermocytogram → Kruger analysis
Spermocytogram → MSOME



MSOME identifies vacuoles that are not evaluated with the same precision by the analysis of Kruger



Application of IMSI

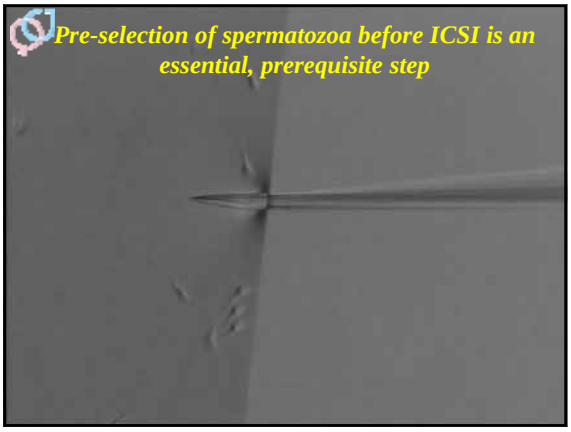
- Optics of Nomarski - MSOME - IMSI
- Classification of spermatozoa according to MSOME
- Indication of MSOME:
  - MSOME: improved spermocytogram
  - MSOME+ICSI: IMSI

- ***Teratozoospermia***

Sp. Morphology

- Embryo development
  - day 3
  - day 5
- 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

(Lee et al., 1996, Bernardini et al., 1998, Colombero et al., 1999, Kahraman et al., 1999, Calogero et al., 2001, Rubio et al., 2001, Yakin et al., 2001, Templado et al., 2002)

➢ **Abnormal sperm shape and pregnancy**

Reduction in ongoing pregnancy rates: 20,2% versus 36,7%

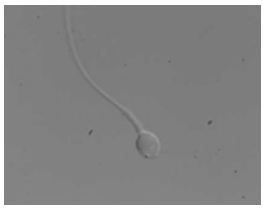
Reduction in implantation rates: 9,6% versus 18,7%

(De Vos et al., 2003)

Indications for MSOME + ICSI

Teratozoospermia

In 2006 : report of the literature



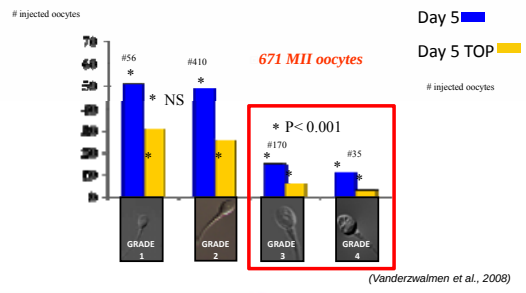
Pregnancy and abortion rates after IMSI with different quality of spermatozoa

*Couples with Previous Failure of implantation***

Sperm Morphology	Quality on day 3		Pregnancy	Abortion
*Normal ***	↑	Day 3 transfer	increase	reduction
	No difference			
Abnormal	↓	Day 3 transfer	decrease	increase

(Bartoov et al., 2002, 2003; Junca et al., 2004; Berkowitz et al., 2006)

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



(Vanderzwalmen 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***

TABLE 1

Study 1: fertilization, rate of development, and blastocyst expansion in correlation to the classification of the injected motile spermatozoon.

Sperm classification	Class 1 21% (46/218)	Class 2 59% (128/218)	Class 3 20% (44/218)	Total number of spermatozoa (N = 218)
Fertilization rate	84% (39/46)[a]	73% (94/128)[a]	61% (27/44)[a]	73% (160/218)
Total blastocysts and morulae	37% (17/46)	26% (33/128)	16% (7/44)	26% (57/218)
Expanded blastocysts	15% (7/46)[b]	9% (12/128)[b]	0 (0/44)[b]	33% (19/57)

Scored Intra Cytoplasmic Sperm Injection: SICSI

Limitations for MSOME + ICSI ???

- ***Severe Teratozoospermia***
  - ***LIMIT: Impossibility to select class I II SP***
- ***Severe Oligozoospermia***
- ***Azoospermia***

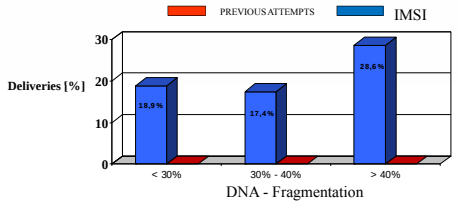
In terms of ejaculate and testicular biopsies

- ***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



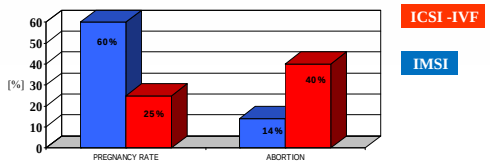
(Hazout et al., 2006)

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



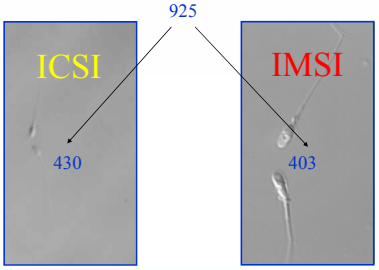
If possible to select a normal SP

(Bartoov et al., 2003)

ICSI vs. IMSI (sibling study)

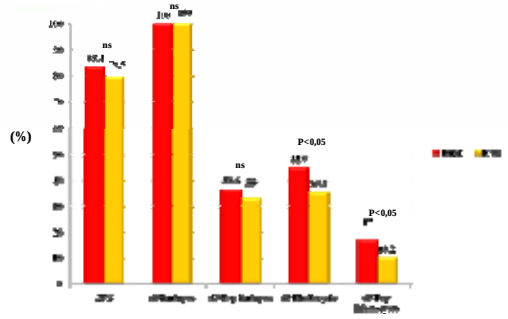
53 patients with at least **1-2 previous failure of implantation**
(day 5 transfer in our center, day 3 in other centers)
> 3 oocytes
Mean age of the woman: 38 Man: OAT (WHO)

Retrieved oocytes 925



Injected oocytes 430 403

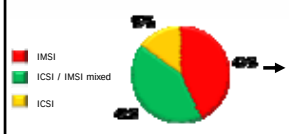
Percentages of blastocysts in relation to the method of sperm selection
ICSI vs. IMSI (sibling study)



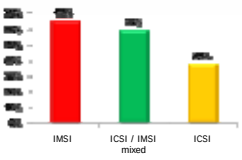
Percentages of transfers and deliveries in relation to ICSI or IMSI spermatozoa selection

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

(max 2 embryo transferred)



% 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

IMSI after 1 failure of implantation after ICSI

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

	ICSI	IMSI
Women age	34.2	+ 2 to 9 months
Blastocyts	0%	36%
Top blastocysts	0%	23%
Ong. Pregnancy	0%	32% (17)
Vitrification cycles	0	21 (40%)*
Cumulative Ong.preg/OPU		42% (22)

* Warming of 12 cycles 5 POS

Indications for MSOME + ICSI

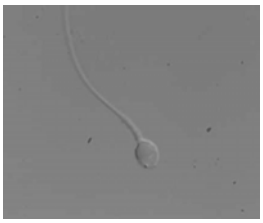
- ***To a large population of ICSI candidates ?***

Are there indications, not to try to select the best spermatozoa? **NO !**

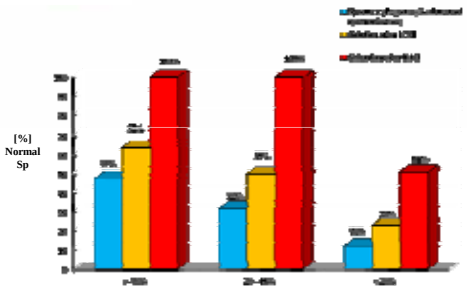
Implementation of IMSI to a large population of ICSI candidate patients :

➔ May be advisable,

if the **probability** to select a normal spermatozoa is higher using the MSOME approach as compared to the classical ICSI approach



Probability to select a normal spermatozoa after ICSI and IMSI in relation to the percentage of spermatozoa from class 1-2



Groups of normal form (class 1 – 2)

Implementation of IMSI to a large population of ICSI candidate patients :

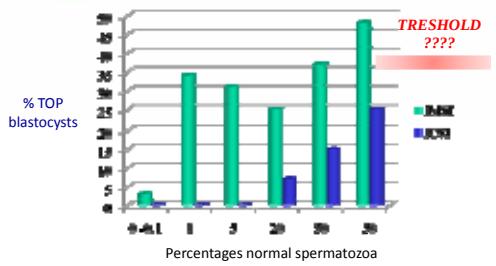
➔ May be advisable,

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 treshold 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



Nb of cases	7	5	3	9	6	3	1

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

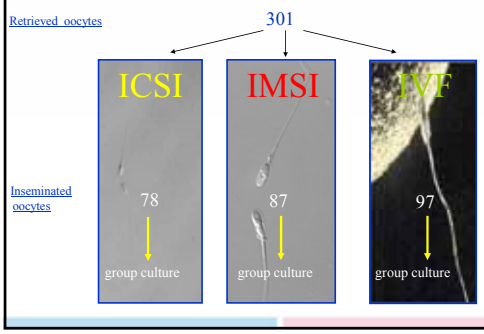
Normozoospermic semen samples ?
or rather
IVF candidate patients (when indicated !) ?

- **MSOME spermocytogram:**
  Determine the appropriate fertilization technique according to the percentage of morphologically normal spermatozoa: ICSI or IVF ?!
  „The impact of high-magnification evaluation of sperm on ART outcome" ***(C. Wittemer et al.,2006)***
- Compare the rate of blastocysts in relation to the different fertilization techniques:

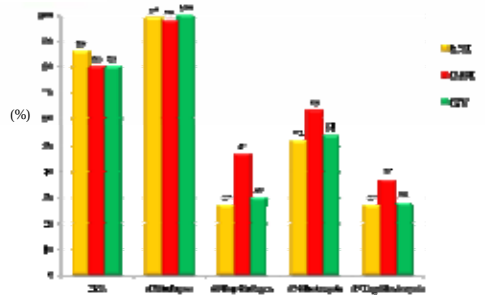
ICSI vs. IMSI vs. IVF

ICSI vs. IVF vs. IMSI (sibling study)

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



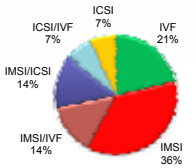
Percentages of blastocysts in relation to the method of sperm selection: ICSI vs. IMSI vs. IVF
(calculated on 2PN, 14 patients, 262 inseminated oocytes)



ICSI vs. IVF vs. IMSI

Frequency of transferred embryos

Transfer frequency



MSOffice9

- Study still in progress

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

IMSI-conclusions (I)

- *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*

Slide 89

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*
  - *As 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 treshold 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., 2000, Behr et al., 1999, 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:

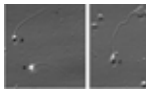
- **Electrophoresis**
  (Ainsworth et al., 2005, Fleming et al. 2008)
  - ζ-Potential
  (Chan et al., 2006)
- **MACS**
  (Said et al., 2008)

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**


Contact

Thank you for your attention

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