The importance of sperm quality.
IMSI: choosing according to sperm morphology

VANDERZWALMEN P STECHER A
BACH M NEYER T SINTZ M SCHWERDA D ZECH N ZECH H

Institute for Reproductive Medicine and Endocrinology, Bregenz, Austria.

The search for excellence in IVF laboratories: towards "the best"
Bologna, Italy 23-24 January 2009

Outline of the presentation

- Introduction: Why and how to run after the best spermatozoon?
- Real time morphological approach: MSOME
- MSOME – ICSI = IMSI
- Application of IMSI: In which case?
  - Previous failure of implantation
  - Severe teratozoospermia ?
  - To a large population of ICSI candidates?
- Nuclear vacuoles: meaning, origin, consequence (reflection)
- Conclusions and perspectives

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.

Eshre Bologna, 23-24 January 2009

SET

Selection of the best embryo

- *Selection criteria*
  - *MORPHOLOGICAL*
  - *BIOCHEMICAL-METABOLOMICS*
- *d2 – d3 ←=→ d5*

Produce good quality embryos

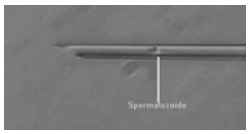
Produce the best gametes
Select the best gametes

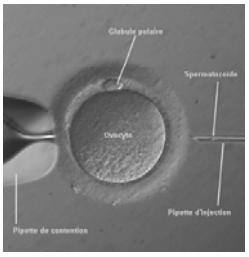
Morphology
Polscope
Zona retardance
PB screening
respirometry

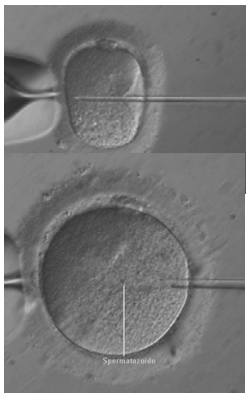
Swim up

Density Gradient

Eshre Bologna, 23-24 January 2009







First ICSI: Palermo,1992

Eshre Bologna, 23-24 January 2009

„The secret life of sperm“

Ainsworth Nature 2005

- Given the shortage of cytoplasm, and the lack of any detectable protein synthesis in mature sperm heads, biologists had long assumed that sperm contributes little to an embryo bar the father´s genes.
- „The idea was that the oocyte is supplying everything (protein and RNAs) and spermatozoa were just tagging along with his DNA“ *Krawetz*

„The secret life of sperm“

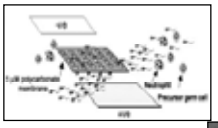
Ainsworth Nature 2005

- Sperm contains almost 3000 different kinds of mRNA
  - Coded for proteins needed for early embryo development.
  - Others are still unknown and have no equivalent in the egg.

Development of new techniques with the aim to enhance the preparation of sperm and to select in a more acccurate fashion a sperm carrying all the informations for the future development are mandatory.

IVF -ICSI

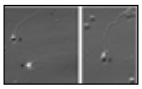
- **New sperm preparation techniques**
  (*Ainsworth et al., 2006 Fleming et al. 2008*)



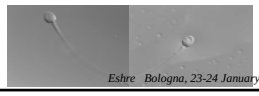
ICSI Isolation of spermatozoa based on :

- **biochemical markers of human sperm maturity and function: HBA**
  (Gabor Huzar et al., 2007, Menezo 2006)

- **Birefringence: protoplasmic structure**
  (Gianaroli et al., 2007)



- **Real time morphological approach: MSOME**
  (Bartoov et al., 2002)



Eshre Bologna, 23-24 January 2009

MSOME

(**M**otile **S**perm **O**rganelle **M**orphology **E**xamination)

(Bartoov et al., 2002)

- Examination performed in real time
- Inverted light microscope
- Equipped with high-power Nomarski optics
- Enhanced by digital imaging to achieve a magnification up to 6300.

Eshre Bologna, 23-24 January 2009

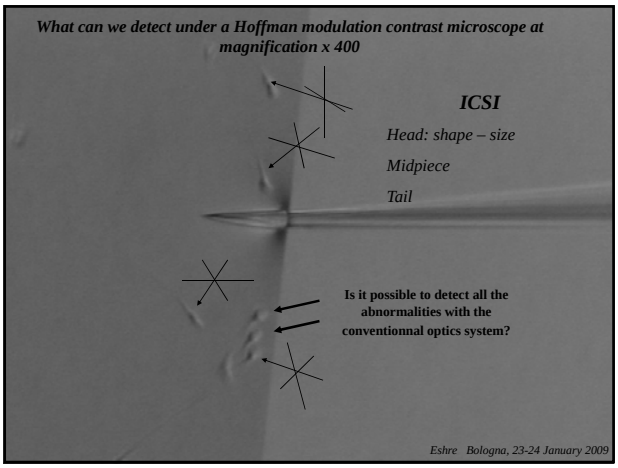
Observation of spermatozoa in real time

Hoffman vs Nomarski

ICSI	**MSOME**
Sp motile	**motile**
Bottom dish plastic	**glass**

Eshre Bologna, 23-24 January 2009

What can we detect under a Hoffman modulation contrast microscope at magnification x 400



ICSI

Head: shape – size

Midpiece

Tail

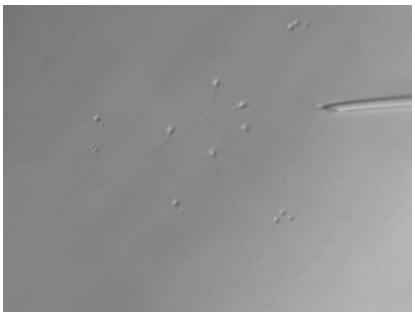
Is it possible to detect all the abnormalities with the conventionnal optics system?

Eshre Bologna, 23-24 January 2009

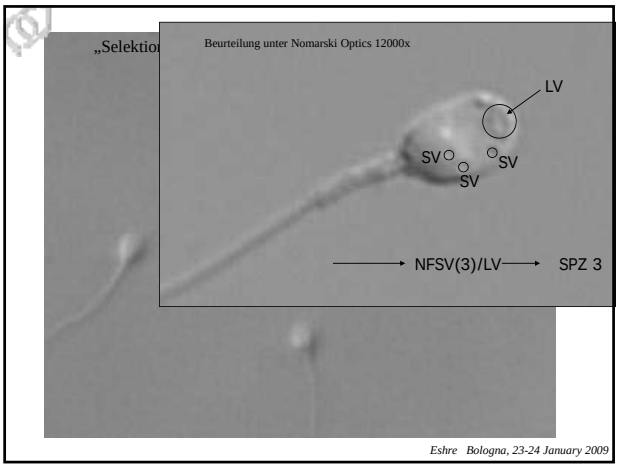
Which defects are better detected with the MSOME ?

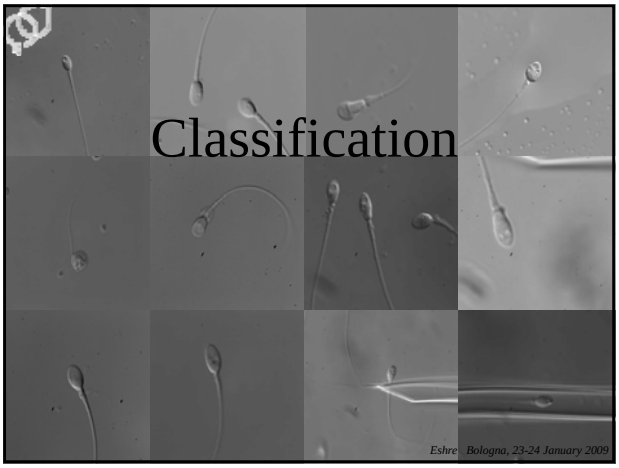
Eshre Bologna, 23-24 January 2009

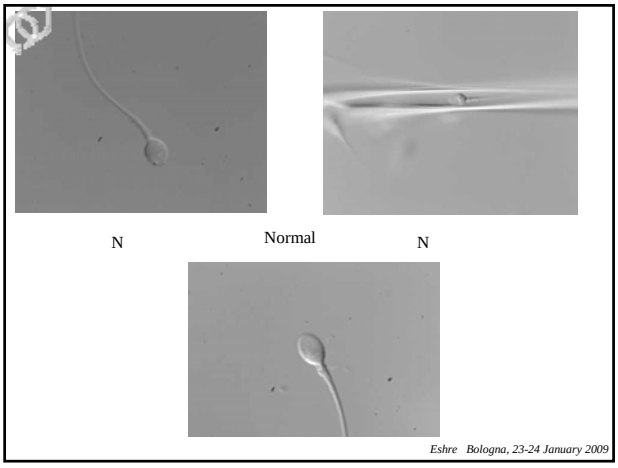
ICSI: Selection under Hoffmann Modulations contrast 400x

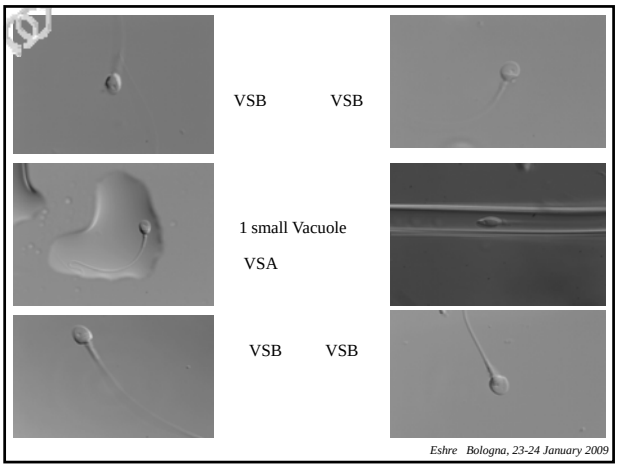


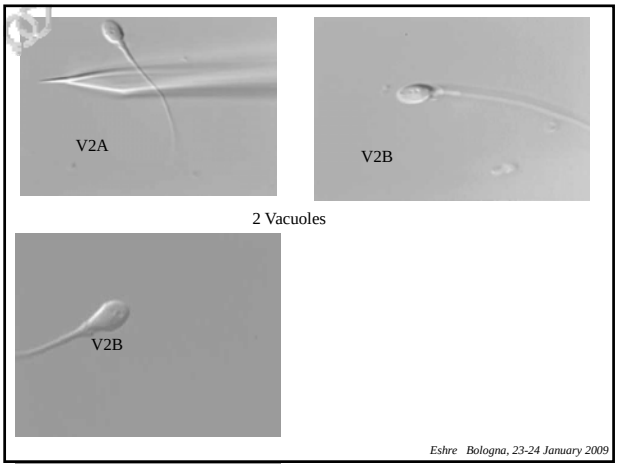
Eshre Bologna, 23-24 January 2009

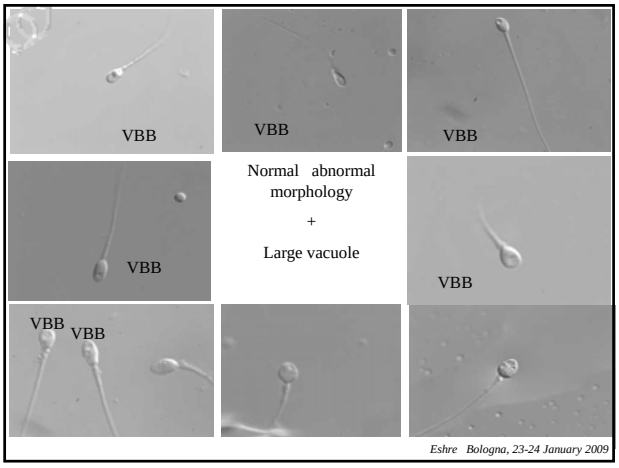

„Selektio
Beurteilung unter Nomarski Optics 12000x
LV
SV
SV
SV
NFSV(3)/LV
SPZ 3
Eshre Bologna, 23-24 January 2009


Classification
Eshre Bologna, 23-24 January 2009


N
Normal
N
Eshre Bologna, 23-24 January 2009


VSB
VSB
1 small Vacuole
VSA
VSB
VSB
Eshre Bologna, 23-24 January 2009


V2A
V2B
2 Vacuoles
V2B
Eshre Bologna, 23-24 January 2009


VBB
VBB
VBB
Normal abnormal morphology
+
Large vacuole
VBB
VBB
VBB
VBB
Eshre Bologna, 23-24 January 2009

MSOME

(**M**otile **S**perm **O**rganelle **M**orphology **E**xamination)

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

MSOME

(**M**otile **S**perm **O**rganelle **M**orphology **E**xamination)

Additional tool to

ICSI

IMSI

(Intracytoplasmic Morphologically Selected Sperm Injection)

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

Application of IMSI

For which:

patients?
indications?

(I) IMSI: clinical application

- Patients selection: **previous implantation failures** after ICSI
  - *with OAT semen*
  - *with an elevated degree of DNA fragmentation*

(Bartoov et al., 2002, 2003; Junca et al., 2004; Berkovitz et al., 2006a, Hazout et al., 2006)

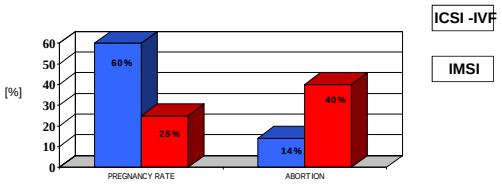
- Observation and selection of **Morphologically normal** spermatooza

- Embryo transfer: **Day 2 or 3**

- Clinical Outcome
  - No difference in the fertilization rate
  - No difference in day 2 or day 3 embryo quality

➢ Clinical Outcome (I)

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

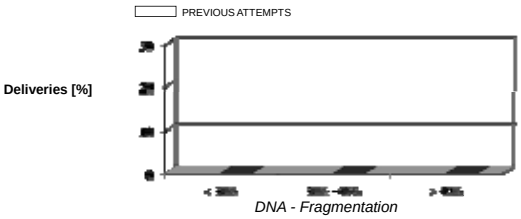


Bartoov et al., 2001, 2002,2003;2006; Berkovitz 2005, 2006

Eshre Bologna, 23-24 January 2009

➢ Clinical Outcome (II)

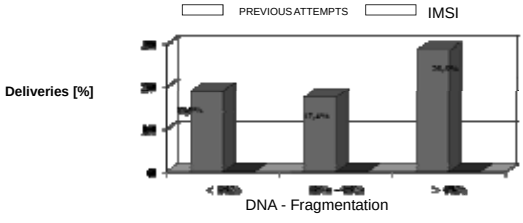
Delivery rates in a group of patients with at least *one failure of implantation* after conventional IVF or ICSI (day 3 transfer) and *different degrees of sperm DNA-fragmentation*



Hazout et al., 2006

Eshre Bologna, 23-24 January 2009

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



Hazout et al., 2006

Eshre Bologna, 23-24 January 2009

Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial

Antinori et al., RBMonline 2008

Table 2. Comparison of pregnancy and miscarriage rates arising from intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) sub-groups with a different number of previous IVF failures.

Sub-group	*Rate*	*Group 1, ICSI*	*Group 2, IMSI*
Subgroup B (1 IVF failure)	Pregnancy	33.6 (36/107)	45.5 (35/77)
	Miscarriage	22.2 (8/36)	20.0 (7/35)
Subgroup C (≥2 IVF failures)	Pregnancy	12.9[a] (8/62)	29.9[a] (23/77)
	Miscarriage	37.5 (3/8)	17.4 (4/23)

Values are percentages with numbers in parentheses.
[a]$P = 0.017$.

Eshre Bologna, 23-24 January 2009

Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial

Antinori et al., RBMonline 2008

Table 2. Comparison of pregnancy and miscarriage rates arising from intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) sub-groups with a different number of previous IVF failures.

Sub-group	*Rate*	*Group 1, ICSI*	*Group 2, IMSI*
Subgroup B (1 IVF failure)	Pregnancy	33.6 (36/107)	45.5 (35/77)
	Miscarriage	22.2 (8/36)	20.0 (7/35)
Subgroup C (≥2 IVF failures)	Pregnancy	12.9[a] (8/62)	29.9[a] (23/77)
	Miscarriage	37.5 (3/8)	17.4 (4/23)

Values are percentages with numbers in parentheses.
[a]$P = 0.017$.

Eshre Bologna, 23-24 January 2009

IMSI seems a promising technique and could be offered to couples:

- No implantation
- High degree of DNA Fragmentation

Eshre Bologna, 23-24 January 2009

IMSI seems a promising technique and could be offered to couples:

- No implantation
- High degree of DNA Fragmentation

- Severe oligozoospermia ?????

IMSI seems a promising technique and could be offered to couples:

- No implantation
- High degree of DNA Fragmentation

- Severe oligozoospermia ?????
- Severe teratozoospermia ?????

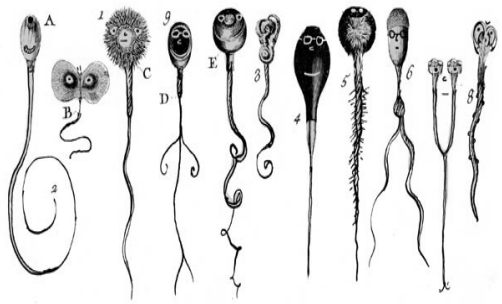
(II) IMSI: Clinical application

Selection of a normal spermatozoa:

Is it always possible ?

not always possible even with MSOME to find and select morphologically completely normal appearing spermatozoa for injection.

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

Eshre Bologna, 23-24 January 2009

- **Abnormal sperm shape and genetic status**

increased risk of aneuploidy and diploidy

(Lee 1996, Bernardini 1998, Colombero1999 Kahraman 1999 Calogero 2001 Rubio 2001 Yakin 2001 Templado 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

Eshre Bologna, 23-24 January 2009

- Observation and selection of **Morphologicaly defect spermatozoa**

Small Vacuole (SV) - Large Vacuole (LV) - Abnormal shape

- **Day 2 or 3** embryo transfer

Berkowitz et al., 2006

Eshre Bologna, 23-24 January 2009

Clinical Outcome

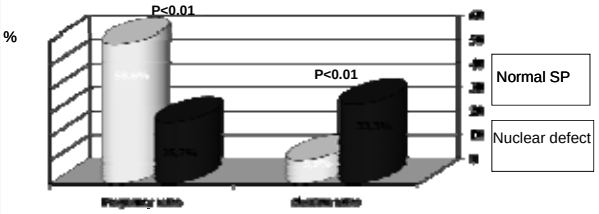
- No difference in embryo quality on day 3

Berkowitz et al., 2006

Clinical Outcome

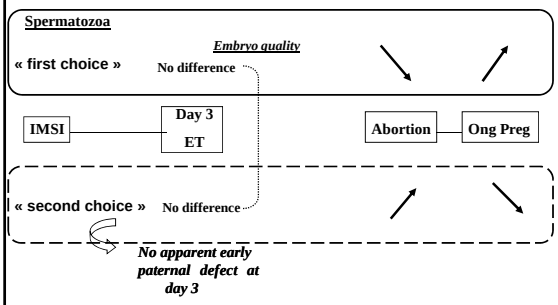
- No difference in embryo quality on day 3
- Ongoing pregnancies and abortions (day 3 ET)

Impact of vacuoles on pregnancy and abortion rates



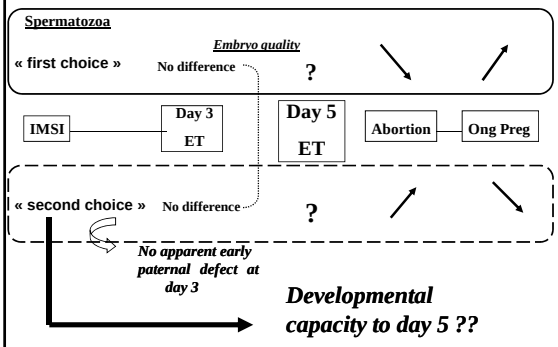
Berkowitz et al., 2006

Impact of vacuoles on the developmental capacity



No apparent early paternal defect at day 3

Impact of vacuoles on the developmental capacity of the embryo to day 5



Developmental capacity to day 5 ??

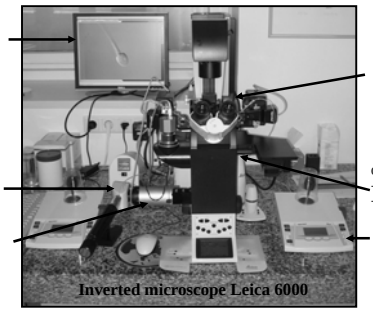
Eshre Bologna, 23-24 January 2009

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

Vanderzwalmen et al., RBMonline 2008

Eshre Bologna, 23-24 January 2009

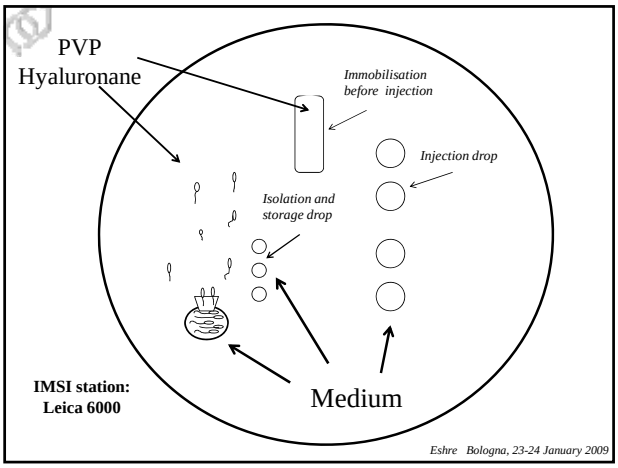
MB1

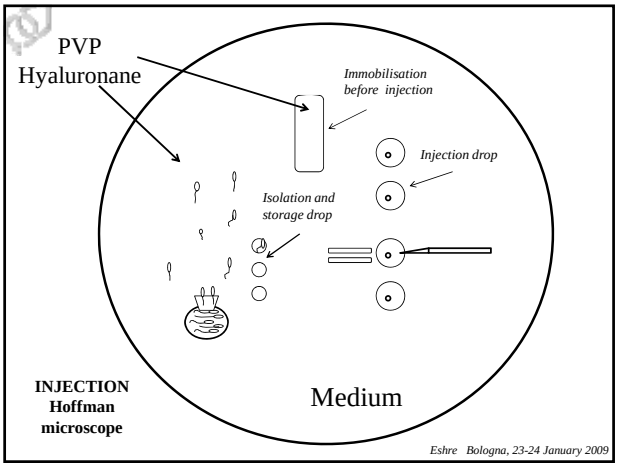


Inverted microscope Leica 6000

MB1 Berechnung Vergrößerung

Magnus; 18/06/2007


PVP
Hyaluronane
Immobilisation
before injection
Injection drop
Isolation and
storage drop
IMSI station:
Leica 6000
Medium
Eshre Bologna, 23-24 January 2009


PVP
Hyaluronane
Immobilisation
before injection
Injection drop
Isolation and
storage drop
INJECTION
Hoffman
microscope
Medium
Eshre Bologna, 23-24 January 2009


LEICA 6000
Bregenz, 2007

Classification

Spermatozoa grade 1

Normal Form, No Vacuole

Spermatozoa grade 2

Normal Form, maximal 2 small Vacuole

SV

Spermatozoa grade 3

Normal Form, at least 1 large Vacuole

Spermatozoa grade 4

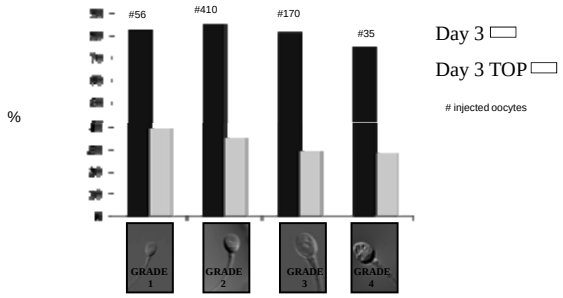
Abnormal Form and Vacuole

LV

Eshre Bologna, 23-24 January 2009

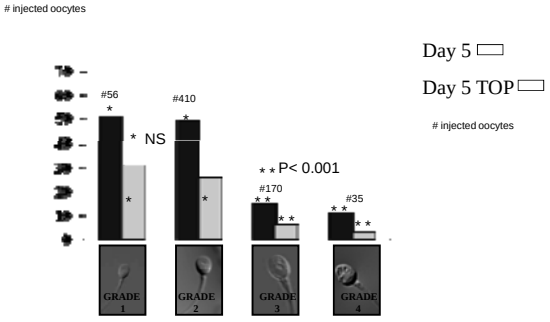
Correlation between sperm morphology and embryo quality on day 3

671 MII oocytes



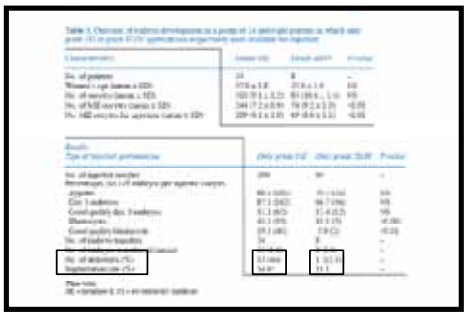
Eshre Bologna, 23-24 January 2009

Correlation between sperm morphology and blastocysts and top quality blastocysts rates



Eshre Bologna, 23-24 January 2009

Impact of vacuoles on pregnancy and abortion rates



Vanderzwalmen et.al., Nov. 2008

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

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

First conclusions

IMPORTANCE OF NUCLEAR VACUOLES

Negative effect on :

- the competence of embryos to develop to blastocysts,
- a reduction in the pregnancy rate,
- an increase of early abortion.

heterogeneity of the sperm population

⇩

negative influence of vacuoles

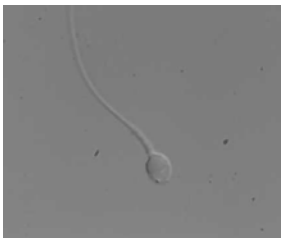
⇩

why not to implement IMSI
to a large population of ICSI candidates ?

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

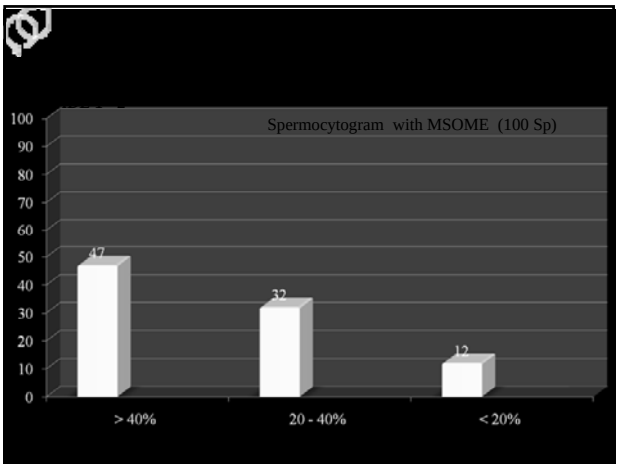
➔ May be advisable,

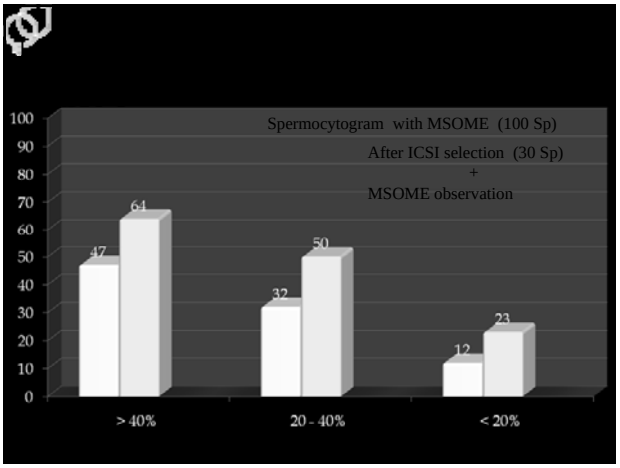
if the probability to select a normal spermatozoa is higher using the MSOME approach as compared to the classical ICSI approach.

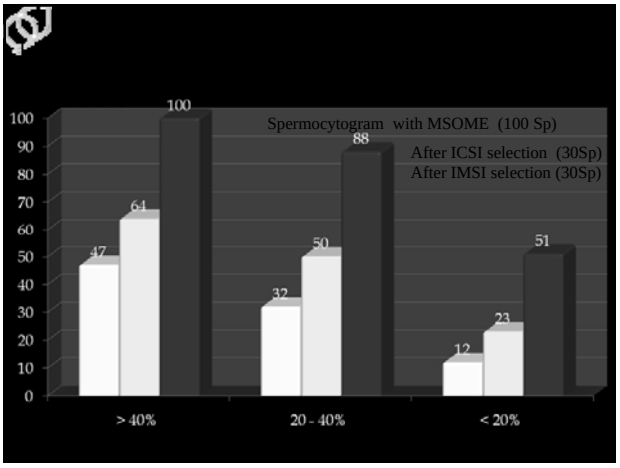


First study

Probability to select a normal spermatozoa in relation to the method of observation: Nomarski or Hoffman


Spermocytogram with MSOME (100 Sp)
100
90
80
70
60
50
40
30
20
10
0
47
32
12
> 40%
20 - 40%
< 20%


Spermocytogram with MSOME (100 Sp)
After ICSI selection (30 Sp)
+
MSOME observation
100
90
80
70
60
50
40
30
20
10
0
47
64
32
50
12
23
> 40%
20 - 40%
< 20%


Spermocytogram with MSOME (100 Sp)
After ICSI selection (30Sp)
After IMSI selection (30Sp)
100
90
80
70
60
50
40
30
20
10
0
47
64
100
32
50
88
12
23
51
> 40%
20 - 40%
< 20%

Conclusions

Study1:
In all the situations, the probability to select spermatozoa from class 1 – 2 is higher if IMSI is applied

Second study

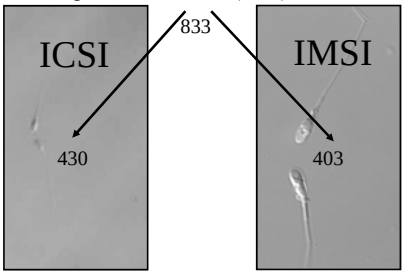
Percentages of blastocysts in relation to the method of sperm selection

ICSI vs. IMSI (sibling study)

ICSI vs. IMSI (sibling study)

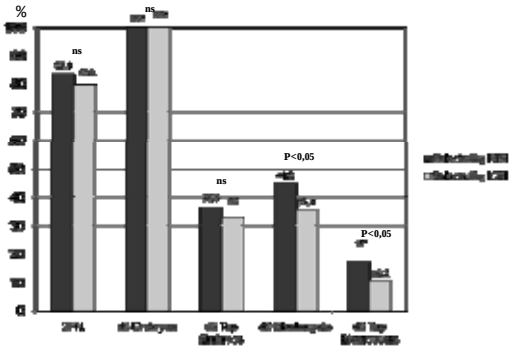
53 Patients with at least 1-2 previous failure of implantation
(day 5 transfer in our center, day 3 in other centers)
> 3 oocytes
Woman age: 38 Man: OAT (WHO)

Nb oocytes: 833



Nb injected oocytes: 430 | 403

Embryo development in relation ICSI or IMSI spermatozoa selection



Eshre Bologna, 23-24 January 2009

Percentages of transfer 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)

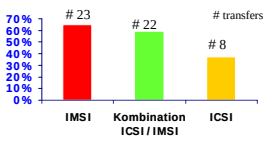
Eshre Bologna, 23-24 January 2009

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



Eshre Bologna, 23-24 January 2009

Conclusions

Study 1:
In all the situations, the probability to select spermatozoa from class 1 – 2 is higher if IMSI is applied.

Study 2:
The sibling study shows that higher rate of blastocysts are obtained when IMSI is performed.
As consequence, a higher number of transfers are performed with blastocysts that originated from the IMSI group.

Third study

% of blastocysts
after IMSI and ICSI in relation to the percentage of normal forms

Aim:
To analyse if there is a treshold in the percentage of normal spermatozoa above which IMSI is not necessary

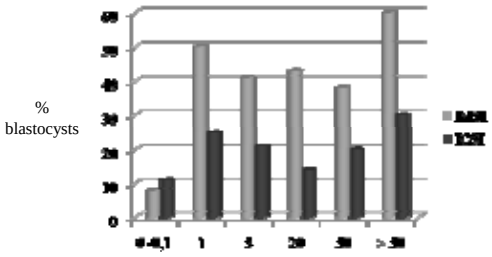
IMSI selection
Analysis of 100 spermatozoa at 6.600x
(spermocytogram)

34 patients

ICSI → % blastocysts

IMSI → % blastocysts

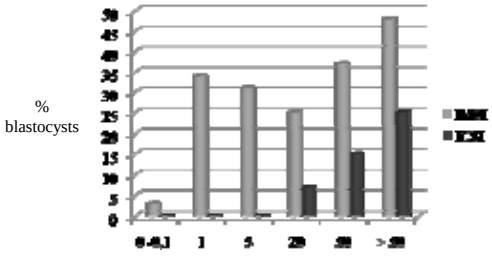
% of blastocysts after IMSI and ICSI in relation to the percentage of class 1-2 spermatozoa in the semen sample



Nb of cases	7	5	3	9	6	3	1

Eshre Bologna, 23-24 January 2009

% 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

Eshre Bologna, 23-24 January 2009

Conclusions

Study 1:
In all the situations, the probability to select spermatozoa from class 1 – 2 is higher if IMSI is applied.

Study 2:
The sibling study shows that higher rate of blastocysts are obtained when IMSI is performed.
As consequence, a higher number of transfers are performed with blastocysts that originated from the IMSI group.

Study 3:
Independently of the percentages of normal spermatozoa, the rate of blastocysts is higher when IMSI is applied and this for all class of normal form

Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial

Table 2. Comparison of pregnancy and miscarriage rates arising from intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) sub-groups with a different number of previous IVF failures.

Sub-group	*Rate*	*Group 1, ICSI*	*Group 2, IMSI*
Subgroup A (0 IVF failures)	Pregnancy	28.0 (14/ 50)	42.5 (31/73)
	Miscarriage	21.4 (3/14)	12.9 (4/31)

Values are percentages with numbers in parentheses.
[a]$P = 0.017$.

Antinori et al., RBMonline 2008

Implementation of IMSI to a large population of ICSI candidate patients may be advisable:

- because the probability to select a normal spermatozoa using the MSOME approach is higher as compared to the classical ICSI approach.

Implementation of IMSI to a large population of ICSI candidate patients may be advisable:

- because the probability to select a normal spermatozoa using the MSOME approach is higher as compared to the classical ICSI approach.

vacuoles may influence

the outcome of embryo development

Implementation of IMSI to a large population of ICSI candidate patients may be advisable:

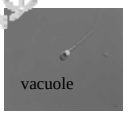
- because the probability to select a normal spermatozoa using the MSOME approach is higher as compared to the classical ICSI approach.

vacuoles may influence

the outcome of embryo development

health and behavior of offspring ???????

Eshre Bologna, 23-24 January 2009



Meaning

Origine

Effect on the outcome

Eshre Bologna, 23-24 January 2009

VACUOLE ⟹ Meaning ?????

- **Suggestions:**
  „Vacuoles may reflect molecular defects responsible for anomalies of sperm chromatin packaging and abnormal chromatin remodelling during sperm maturation which, in its turn, may render spermatozoa more vulnerable to DNA damage " *Berkovitz et al., 2005; Hazout et al., 2006"*

Eshre Bologna, 23-24 January 2009

VACUOLE → Meaning ?????

- **Suggestions:**

„Vacuoles may reflect molecular defects responsible for anomalies of sperm chromatin packaging and abnormal chromatin remodelling during sperm maturation which, in its turn, may render spermatozoa more vulnerable to DNA damage " *Berkovitz et al., 2005; Hazout et al., 2006"*

- ***More accurate answer:***
  Isolation and evaluation of single spermatozoon

- Sperm DNA integrity - acridine orange staining
- DNA fragmentation -TUNEL
  (Franco RBMoline 2008, Garolla RBMonline 2008, Barbarova submitt)
- Mitochondrial membrane potential *(Garolla RBMonline 2008)*
  alteration seems to be suggestive of an early apoptotic process
- Sperm aneuploidies FISH *(Garolla RBMonline 2008)*

VACUOLE → Meaning ?????

- ***More accurate answer: Isolation and evaluation of single spermatozoon***

«Significance of large nuclear vacuoles in human spermatozoa: implications for ICSI » *Franco et al, RBMonline 2008*

« High power magnification microscopy and functional status analysis of sperm in the evaluation and selection before ICSI »
Garolla et al RBM online 2008

« Correlation between morphological semen parameters and sperm nuclear damage » *Babarova submitted*

Eshre Bologna, 23-24 January 2009

VACUOLE → Meaning ?????

- ***CONCLUSIONS***

- **Association between large vacuole in the sperm and DNA damage.**
- **Advice that the high level of denatured DNA in sperm with large nuclear vacuoles suggests: precocious *decondensation disaggregation of sperm chromatin fibers.***
- **Significantly better chromatin status , mitochondrial function, aneuploidy rate (hypospermatogenesis) when nuclear vacuoles were absent.**

Origins of DNA damage in the spermatozoa

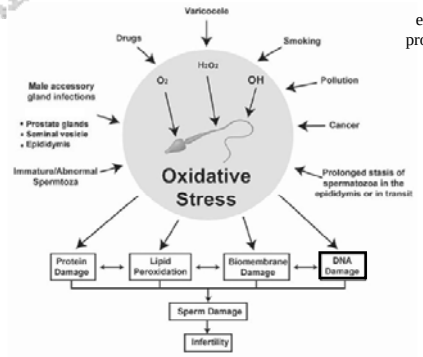


Figure 1 – Association of increasing reactive oxygen species (ROS) production with infertility.

externally or internally produced reactive oxygen species

Default in apoptosis process

Aitken 2004, Kelton Tremellen 2008

Eshre Bologna, 23-24 January 2009

Effect on the outcome

Long term effects of mouse ICSI with DNA-fragmented sperm (DFS) on health and behavior of adult offspring.
Fernanderz-Gonzalez Biol. Reprod. 2008

The use of DNA fragmented sperm in ICSI can generate effects that only emerge during later life, such as:

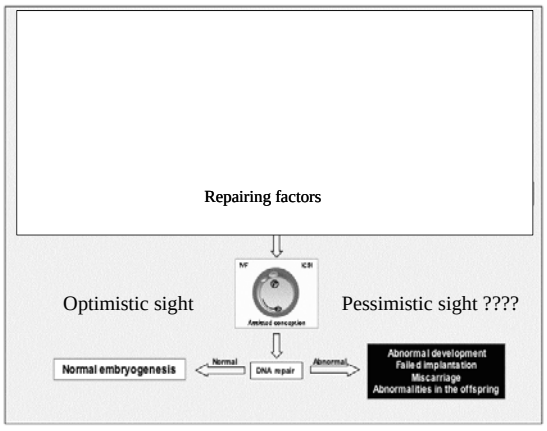
aberrant growth,
premature aging,
abnormal behavior,
mesenchymal tumor.

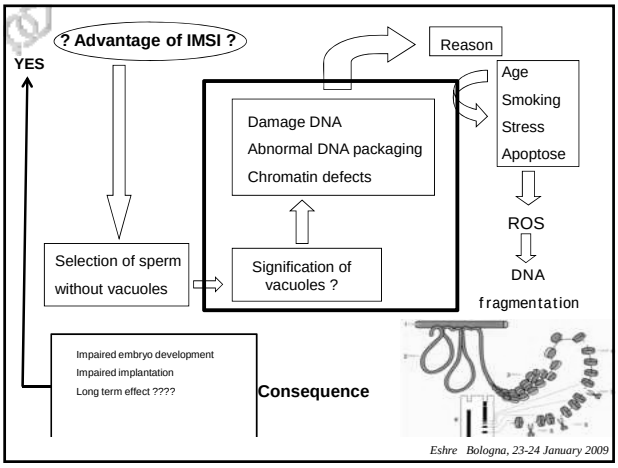
Tunnel and comet assay

Eshre Bologna, 23-24 January 2009

Optimistic sight

- DNA damage brought into the zygote by the fertilizing spermatozoon is effectively repaired by the oocyte.
- ***p53-dependent S-phase DNA damage checkpoint***
  - **suppress DNA synthesis in both the male and female pronuclei until the damage brought in by the fertilizing spermatozoon had been repaired**





Final conclusions

- We try to improve stimulation protocols, culture protocols selection of oocytes, selection of embryos , luteal phase, ET ……,why not the selection of spermatozoa ?
- Observation of spermatozoa by the MSOME approach has to be considered as an additional tool to the classical ICSI method for a large population of ICSI candidates.
  - The probability to select for injection a normal spermatozoa is higher if IMSI is applied
  - IMSI is a usefull technique since it produce embryos with higher capacity to implant

Eshre Bologna, 23-24 January 2009

- There is now more evidence that Vacuoles reflect DNA damage, abnormal DNA packaging and chromatin defets.
- However, it is important to emphasize that animal data clearly indicate that DNA damage in the male germline is potentially damaging for the embryo and offspring .

(Anderson, 2003; Lewis and Aitken, 2005)

- In light of such considerations,
  - it would seem rational to try to determine the causes of DNA damage in the male germline
  - 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)

Eshre Bologna, 23-24 January 2009

- **A lot of questions are still in unanswered:**
  - Which attitude when only abnormal spermatozoa with large vacuoles are present in the semen sample ?

    - If observation some months before IVF treatment: antioxydant therapy, modify the lifestyle, etc….????

    - If observation the day of the OPU:

      Inject one part of the oocytes ????

      Aseptic vitrification of oocytes and try to improve the quality of the semen ???

      Propose donor (where and when it is possible)???

  - Influence of the maternal age on the outcome of embryo development – more optimal repairing factors ????

Eshre Bologna, 23-24 January 2009

- IMSI is used in very few ART centers.

As consequence, we may 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 may reflect the quality of the gametes from which they were derived

(Spano 2000, Behr 1999, Vanderzwalmen 2008)

Eshre Bologna, 23-24 January 2009

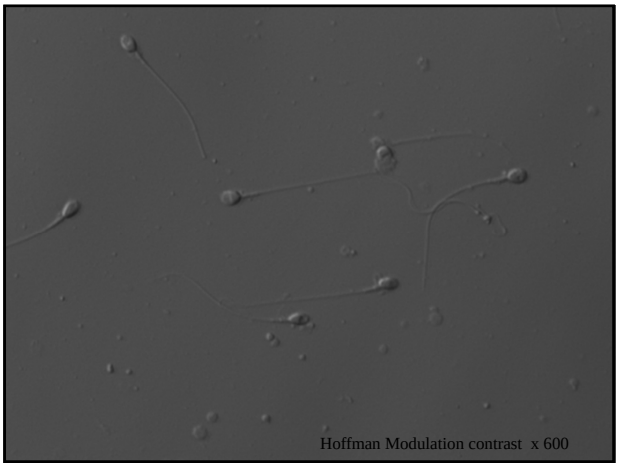
Perspectives (I)

- Some are reluctant to apply this new approach of selecting spermatozoa before ICSI.

  to expensive
  time consuming
  not yet convince by this way of selection

- Improvement of the image after modification of the classical optic Hoffman system =➔ more friendly way to select spermatozoa even though not optimal as compared with the Normarski system

Eshre Bologna, 23-24 January 2009



Hoffman Modulation contrast x 600

Perspectives (II)

Analyse the outcome of embryo development according to the morphology of the vacuoles and their localization ?

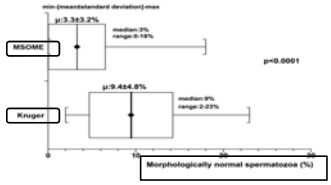
Eshre Bologna, 23-24 January 2009

Perspectives (III)

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 *RBMonline 2008*

Spermocytogram → Kruger analysis / MSOME

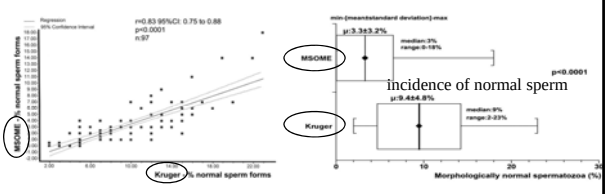


MSOME identifies vacuoles and chromatin abnormalities that are not evaluated with the same precision by the analysis of Kruger.

Eshre Bologna, 23-24 January 2009

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

Spermocytogram → Kruger analysis / MSOME



incidence of normal sperm

Regression analysis demonstrated significant positive correlation between percentage of normal sperm forms by Kruger's and by MSOME

MSOME identifies vacuoles and chromatin abnormalities that are not evaluated with the same precision by the analysis of Kruger.

Eshre Bologna, 23-24 January 2009

➤ At present, there have not been sufficient numbers (or generations) of ICSI children to draw any firm conclusions about the long-term safety of this procedure.

➤ However, it is important to emphasize that animal data are absolutely unequivocal on this point and clearly indicate that DNA damage in the male germline is potentially damaging for the embryo and offspring *(Anderson, 2003; Lewis and Aitken, 2005)*

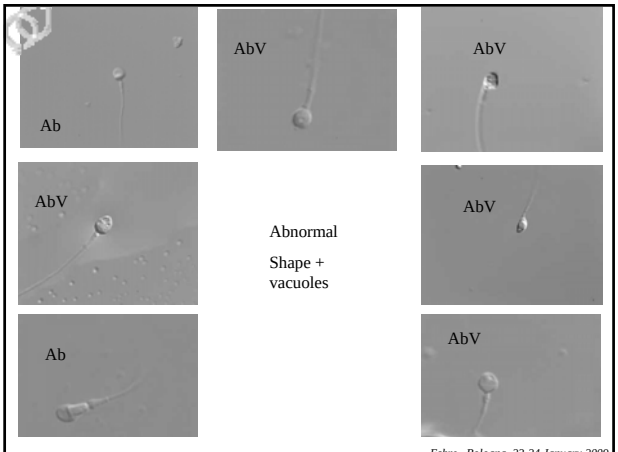
➤ For the time being, the take-home message care should be taken when treating patients exhibiting such damage with ICSI. In light of such considerations, it would seem rational to try to determine the causes of DNA damage in the male germline and to do everything possible to alleviate this damage (e.g. 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).*

Eshre Bologna, 23-24 January 2009

observation and selection of spermatozoa by different optic systems

	SP status	Optic	Magnification	
			microscope	**camera**
Spermocytogram	stained / death		Brightfield 1000	
ICSI	Motile	Hoffman	600 3D (??)	> 12000 3D (??)
MSOME	Motile	Nomarski	1320 3D	>12000 3D

Eshre Bologna, 23-24 January 2009



Eshre Bologna, 23-24 January 2009