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

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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

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.

SET
Selection of the best embryos

Selection criteria
MORPHOLOGICAL
BIOCHEMICAL-METABOLIC

Produce good quality embryos

Produce the best gametes
Select the best gametes

Morphology
Polscope
Zona retardance
PB screening
respirometry

Swim up
Density Gradient

First ICSI: Palermo, 1992
• 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

• 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 accurate fashion a sperm carrying all the informations for the future development of the embryo 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; Menez 2006)
  - Birefringence: protoplasmic structure
    - (Gianaroli et al., 2007)
  - Real time morphological approach: MSOME
    - (Bartoov et al., 2002)

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**MSOME**

(Motile Sperm Organelle Morphology Examination)

- 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.

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**Observation of spermatozoa in real time**

Hoffman vs Nomarski

- ICSI
  - Sp motile
  - Bottom dish plastic
- MSOME
  - Sp motile
  - Glass
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 conventional optics system?

Which defects are better detected with the MSOME?
MSOME
(Motile Sperm Organelle Morphology Examination)

Additional tool to
ICSI

IMSI
(Intracytoplasmic Morphologically Selected Sperm Injection)

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; Amoia et al., 2004; Bortotier et al., 2005a, Haudale et al., 2006)

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- Observation and selection of Morphologically normal spermatozoa
- Embryo transfer: Day 2 or 3

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- Clinical Outcome

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

Clinical Outcome (I)


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.

Clinical Outcome (II)

Hazout et al., 2006

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

- No implantation
- High degree of DNA Fragmentation
IMSI seems a promising technique and could be offered to couples:

- No implantation
- High degree of DNA Fragmentation
- Severe oligozoospermia ??????

 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

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

Observation and selection of morphologically defect spermatozoa

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

Day 2 or 3 embryo transfer

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

Normal SP
Nuclear defect

P<0.01

Impact of vacuoles on the developmental capacity

Spermatozoa

- No apparent early paternal defect at day 3

Embryo quality

No difference

IMSI Abortion Ong Preg

Day 3 ET

No difference
Impact of vacuoles on the developmental capacity of the embryo to day 5

**Spermatozoa**

« first choice »

Embryo quality?

IMSI

Day 3

ET

No difference

Day 5

ET

Abortion

Ong Preg

« second choice »

No difference

No apparent paternal defect at day 3

Developmental capacity to day 5 ??

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

Vanderzwalmen et al., RBMonline 2008

Picture processing:

- Application Suite
- Nomarski interferential contrast
- Variable Zoom 2.200 – 12.500x
- Analogic Videokamera
- Objective: x20, x40 & x100immDIC
- Variable zoom
- Microinjection

Inverted microscope Leica 6000 MB1
Berechnung Vergrößerung
Magnus; 18/06/2007
Classification

Spermatozoa grade 1
Normal Form, No Vacuole

Spermatozoa grade 2
Normal Form, maximal 2 small Vacuole

Spermatozoa grade 3
Normal Form, at least 1 large Vacuole

Spermatozoa grade 4
Abnormal Form and Vacuole

Correlation between sperm morphology and embryo quality on day 3

Correlation between sperm morphology and blastocysts and top quality blastocysts rates
Impact of vacuoles on pregnancy and abortion rates

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

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

After ICSI selection (30 Sp) + MSOME observation

Groups of normal form (class 1 – 2)

Spermocytogram with MSOME (100 Sp)
Conclusions

Study 1:
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

ICSI
430

IMSI
403
Embryo development in relation ICSI or IMSI spermatozoa selection

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

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

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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 threshold in the percentage of normal spermatozoa above which IMSI is not necessary

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

IMSI → % blastocysts

ICSI → % blastocysts

34 patients
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.

Conclusions
Implementation of IMSI to a large population of ICSI candidate patients may be advisable:
- because the probability to select a normal spermatozoon 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 and health and behavior of offspring.

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. (Berkvod et al., 2005; Haasit et al., 2006)
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 RBMonline 2008, Garolla RBMonline 2008, Babarova submitted)
• Mitochondrial membrane potential (Garolla RBMonline 2008) alteration seems to be suggestive of an early apoptotic process
• Sperm aneuploidies FISH (Garolla RBMonline 2008)

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 RBMonline 2008)
- Correlation between morphological semen parameters and sperm nuclear damage (Babarova submitted)

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

![Diagram of Oxidative Stress and Gene Damage]

- Externally or internally produced reactive oxygen species
- Default in apoptosis process

Aitken 2004, Kelton Tremellen 2008

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Effect on the outcome

Long term effects of mouse ICSI with DNA fragmented sperm (DFS) on health and behavior of adult offspring.


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

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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 useful technique since it produce embryos with higher capacity to implant
There is now more evidence that Vacuoles reflect DNA damage, abnormal DNA packaging and chromatin defects.

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)

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: antioxidant 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 ???

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)
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 – a more friendly way to select spermatozoa even though not optimal as compared with the Normarski system

Perspectives (II)

Analyse the outcome of embryo development according to the morphology of the vacuoles and their localization?
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 RB Mol Online 2008).

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

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

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Thank you
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 (Aitkenworth et al., 2005, 2007).