TECNICHE E SIGNIFICATO DELLA SELEZIONE SPERMATICA PER ICSI

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Intracytoplasmic Sperm Injection (ICSI)

The selection of spermatozoa without DNA fragmentation and chromosomal diseases prior to ICSI helps to optimize the outcome of the treatment.

Sperm selection becomes critical especially when a limited number of oocytes are available for injection.
Sperm morphology

- ICSI with poor motile/aberrant ejaculate or testicular spermatozoa is possible
- even good sperm morphology following strict criteria
  - has no prognostic value in ICSI cycle outcomes
  - does not influence embryo development or morphology
    - French et al, Fertil Steril 2010
  - cannot predict chromatin integrity or presence of numerical chromosomal aberrations
    - Celik-Ozenci et al, Hum Reprod 2004
Aneuploidies and DNA fragmentation

- ICSI with aneuploid spermatozoa
  - seems to be the cause of the vast majority of genetic deviations in ICSI newborns
    - *Bonduelle et al, Hum Reprod 2002*

- ICSI with DNA damaged spermatozoa
  - reduction of LBR
    - *Osman et al, RBMO 2015*
    - *Jin et al, Fertil Steril 2015*
  - increase of abortion rate
    - *Zini et al, Hum Reprod 2008*
  - long term side effects in adult animals
    - aberrant growth, premature ageing, abnormal behaviour, and mesenchymal tumours
      - *Fernandez-Gonzalez et al, Biol Reprod 2008*
ICSI risks

- theoretically, the widespread use of ICSI increases the chance of injecting spermatozoa being defective for:
  - centrosome integrity
    - Schatten & Sun, Hum Reprod 2009
  - genetic constitution
    - Sakkas et al, Human Fert 2000; Marchesi & Feng, J Androl 2007
  - Phospholipase C Zeta content
    - Heytens et al, Hum Reprod 2009
  - DNA methylation
    - Navarro-Costa et al, Hum Reprod 2010

- this hypothetical background risk is omnipresent
  - any additional risk in the lab should be kept to a minimum
  - those processes influenced by the embryologist should be performed safely and as “naturally” as possible
    - Parmegiani et al, RBMO 2010
Towards a more physiological ICSI

SPERM PREPARATION PRIOR TO ICSI
Sperm treatment and DNA fragmentation

- basal sperm DNA fragmentation rate can be significantly reduced
  - “Swim-Up”
  - density gradient
  - selection by motility without centrifugation
    - Ebner et al, RBMO 2011; Seiringer et al RBMO 2013; Nosrati et al, LOC 2014
  - fluorescence activating cell sorting (FACS)
    - Ribeiro et al, Fertil Steril 2013
  - magnetic cell sorting (MACS) with Annexin V
  - membrane charge

- DNA damage is related with poor motility
  - Belloc et al, Fertil Steril 2014

- semen treatment improves the percentage of spermatozoa with normal chromatin structure
  - filtering out apoptotic spermatozoa with low motility
Sperm selection prior to ICSI

- **sperm treatment helps reduce the number of:**
  - apoptotic low motile – spermatozoa
  - chromosomally unbalanced spermatozoa in some patients
    - Rouen et al, Human Reprod 2013

- **after sperm treatment, new advances in micromanipulation help chose the “ideal” mature spermatozoa**
  - restoration of fertilization checkpoints
    - sperm-hyaluronic acid binding
  - high magnification
    - Intracytoplasmic Morphologically selected Sperm Injection
Towards a more physiological ICSI

RESTORATION OF FERTILIZATION CHECK-POINTS
Physiologic role of Hyaluronic Acid

HUMAN FERTILIZATION

- Hyaluronic Acid (HA) is normally present in the Extra Cellular Matrix (ECM) of cumulus oophorus surrounding the oocyte at the time of fertilization.

- The Extra Cellular Matrix (ECM) is a formidable barrier which the sperm must get through to reach the Zona Pellucida and to fertilize the oocyte.
Spermatozoa that are able to bind in vitro to HA are mature and have completed the spermiogenetic process of sperm plasma membrane remodelling, cytoplasmic extrusion and nuclear maturity.


Mature spermatozoa with high density of HA receptors bind permanently to HA. Immature spermatozoa are not able to bind to HA.

- Cayli et al, RBMO2003
Sperm-HA binding selection

- HA-bound spermatozoa show a 5.4-fold reduction in chromosomal aneuploidies
  - Jakab et al, Fertil Steril 2005

- strong link between DNA fragmentation and aneuploidies in human sperm
  - Enciso et al, Hum Reprod 2013

- a selection method based on mature sperm-HA binding
  - useful in reducing the potential genetic complications and adverse long-term side effects of ICSI
PICSI - selection of HA-bound spermatozoa
Sperm Slow - selection of HA-bound spermatozoa

Spermatozoa bound to HA in the junction zone of the droplets can be selected and easily detached by injecting pipette

Parmegiani et al. JARG 2010
<table>
<thead>
<tr>
<th>Authors</th>
<th>HA-System</th>
<th>N° of treatments or patients</th>
<th>HA-bound spermatozoa determine:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menezo et Nicollet</td>
<td>Sperm Slow</td>
<td>92 HA-ICSI vs 110 PVP-ICSI</td>
<td>No difference on ICSI outcome</td>
</tr>
<tr>
<td>Abstract IFFS meeting 2004</td>
<td></td>
<td></td>
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<tr>
<td>Sanchez et al</td>
<td>not described</td>
<td>18 HA-ICSI versus control group</td>
<td>No differences on FR, PR, IR. Lower aneuploidies in HA-bound spermatozoa</td>
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<tr>
<td>Abstract ESHRE meeting 2006</td>
<td></td>
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<tr>
<td>Worrilow et al</td>
<td>PICSI</td>
<td>240 couples (PICSI vs PVP-ICSI)</td>
<td>Significant improvement in FR, embryo quality. Reduction in the MR</td>
</tr>
<tr>
<td>Abstract ASRM meeting 2007</td>
<td></td>
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<tr>
<td>Nasr-Esfahani et al</td>
<td>“home made”</td>
<td>50 couples (sibling oocytes injected with HA-ICSI or PVP-ICSI)</td>
<td>Significant improvement FR</td>
</tr>
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<td>JARG 2008</td>
<td></td>
<td></td>
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<tr>
<td>Van Den Berg et al</td>
<td>Sperm Slow</td>
<td>44 couples (sibling oocytes injected with HA-bound or HA-not bound spermatozoa)</td>
<td>No differences in fertilization (zygote score)</td>
</tr>
<tr>
<td>RBM Online 2009</td>
<td></td>
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<tr>
<td>Worrilow et al</td>
<td>PICSI</td>
<td>215 couples (PICSI vs PVP-ICSI)</td>
<td>Significant improvement in embryo quality (DAY 3-5)</td>
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<td>Abstract ESHRE meeting 2010</td>
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<tr>
<td>Menezo et al</td>
<td>Sperm Slow</td>
<td>2014 HA-ICSI vs 1920 PVP-ICSI</td>
<td>No difference on ICSI outcome</td>
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<td>Abstract ASRM meeting 2010</td>
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<tr>
<td>Gaurav and Majumdar</td>
<td>PICSI</td>
<td>71 HA-ICSI vs 80 PVP-ICSI</td>
<td>No difference on ICSI outcome</td>
</tr>
<tr>
<td>JARG 2013</td>
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</table>
mean ICSI procedure duration 3 minutes longer in PICSI group

IVF centres can choose the HA-ICSI system best suited to their needs

* Parmegiani et al, Fertil Steril 2012
**Physiologic HA-ICSI - Potential benefits**

- **HA-spermatozoa show:**
  - significant reduction in DNA fragmentation
  - improvement in nucleus normalcy at High Magnification
    - Parmegiani et al, Fertil Steril 2010

- **injection of HA-spermatozoa (HA-ICSI):**
  - significantly improves embryo quality, development and implantation
    - Parmegiani et al, Fertil Steril 2010; JARG 2010
  - significant decreases abortion rate
    - Worrilow et al, Human Reprod 2012
Physiologic HA-ICSI - Clinical benefits?

Systematic review and meta-analysis 2016

- **main outcomes**
  - fertilization and clinical pregnancy rate.

- **secondary outcomes**
  - cleavage rate, embryo quality, implantation rate, spontaneous abortion and LBR

- **7 studies / 1437 cycles**
- no improvement in fertilization and pregnancy rates
- improvement in embryo quality and implantation rate
- no benefit found for the main outcomes
  - fertilization rate and clinical pregnancy rate
- no firm clinical guidance for the routine use of hyaluronic acid sperm selection technique can be drawn
  - *Ronit Beck-Fruchter et al, RBMO 2016*
Normally shaped nucleus by MSOME

- Smooth, symmetric, and oval
- Average length: 4.75 ± 0.28 µm
- Average width: 3.28 ± 0.20 µm
- Nuclear chromatin abnormal if one or more vacuoles occupies > 4% of the nuclear area
- Maximum vacuole diameter: 0.78 ± 0.18 µm

  - Bartoov et al, Hum Reprod 1994

- Evaluation by transparent celluloid forms fitting these criteria
  - Bartoov et al, J Androl 2002

- Measurement with digital imaging software
  - Parmegiani et al, Fertil Steril 2010
Spermatozoa without nuclear vacuoles

- better mitochondrial function and chromatin status
- reduced aneuploidy
  - Garolla et al, RBMO 2008; Boitrelle et al, RBMO 2011
- reduced DNA fragmentation
  - Utsuno et al, Fert Steril 2013
- lower incidence of aneuploidy in derived embryos
  - Figueira et al, Fert Steril 2011
- better developmental dynamics in derived embryos
  - Time lapse
    - Knetz et al, RBMO 2013
IMSI – Potential advantages

- **positive influence on embryo development**
  - Vanderzwalmen et al, RBMO 2008; Knetz et al, RBMO 2013

- **improvement of pregnancies**

- **reduction of miscarriages**
  - Souza Setti et al, RBMO 2010

- **reduction of birth defects**
  - Cassuto et al, RBMO 2013

- **Physiologic HA-IMSI**
  - Parmegiani et al, Fert Steril 2010
**IMSI – Limitations**

- **expensive**

- **time consuming**
  - around 120 minutes
  - Antinori et al, *RBMO* 2008

- **absence of top quality spermatozoa**
  - no improvements in clinical results

- **strict prospective sibling-oocyte study**
  - no improvements in clinical results
  - De Vos et al, *Hum Reprod* 2013
  - Teixeira et al, *Cochrane* 2013

- **scarcity of head-to-head IMSI vs ICSI studies**
  - indication confirmed only for recurrent ICSI implantation failure
  - Boitrelle et al, *RBMO* 2013
Conclusions

- During ICSI, suboptimal spermatozoa could by-pass the physiological checkpoints of natural fertilization.
- We have no real knowledge of the effects of suboptimal sperm selection on ICSI human adults in the long term.
- When using some non-invasive refinements of sperm selection for ICSI it is possible at very least to mimic nature’s processes.

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