# 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**

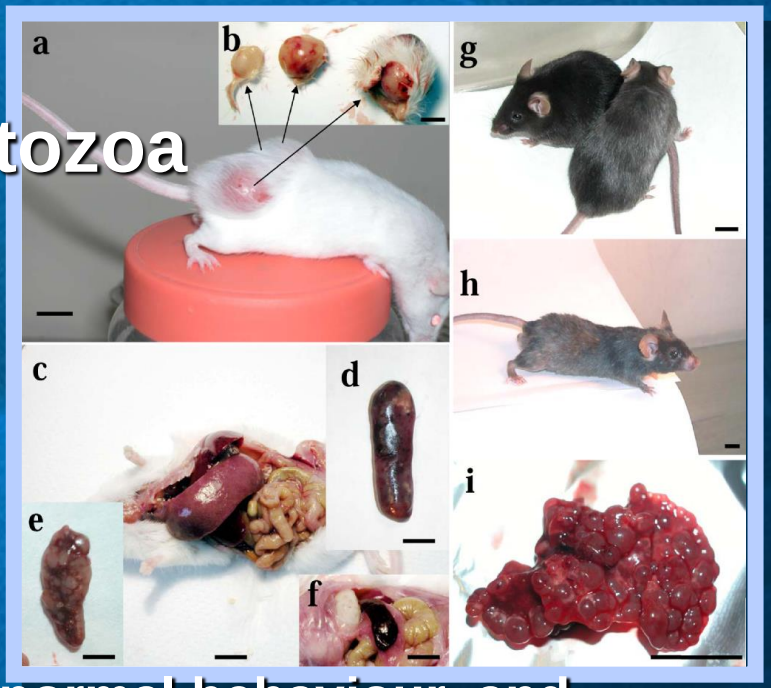
**Sperm selection becomes critical especially when a limited number of oocytes are available for injection**

# Sperm morphology

- **ICSI with poor motile/aberrant ejaculate or testicular spermatozoa is possible**
  - *Svalander at al, Hum Reprod 1996; Silber et al, Hum Reprod 1995*
- **even good sperm morphology following strict criteria**
    - *Kruger et al, Fertil Steril 1986; 1988*
  - **has no prognostic value in ICSI cycle outcomes**
    - *Svalander et al, Hum Reprod 1996; De Vos et al, Hum Reprod 2003; French et al, Fertil Steril 2010*
  - **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 spematozoa**
  - 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*

# SPERM PREPARATION PRIOR TO ICSI

# Sperm treatment and DNA fragmentation

- basal sperm DNA fragmentation rate can be significantly reduced
  - "Swim-Up"
    - *Spanò et al, Hum Reprod 1999; Parmegiani et al, Fertil Steril 2010; Volpes et al, JARG 2016*
  - density gradient
    - *Gandini et al, Hum Reprod 2004; Rougier et al, Fertil Steril 2013*
  - 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
    - *Rawe et al, Fertil Steril 2009; Vendrell et al, RBMO 2013; Gil et al , JARG 2013; Zahedi et al, JARG 2013*
  - membrane charge
    - *Chan et al, Fertil Steril 2006; Razavi et al, Andrologia 2009; Simon et al, Fertil Steril 2014*
- 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
    - *Parmegiani et al, Adv Exp Med Biol 2014*
  - 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

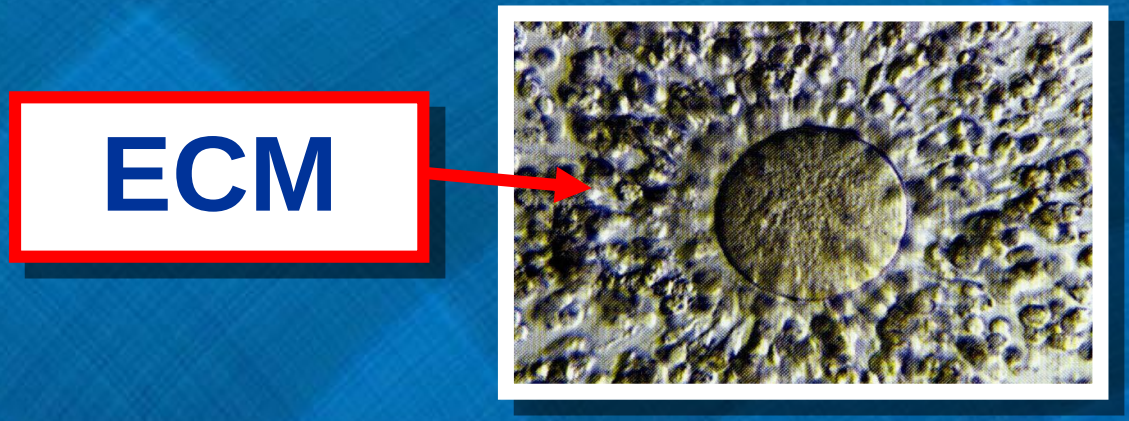
# Towards a more physiological ICSI

# 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

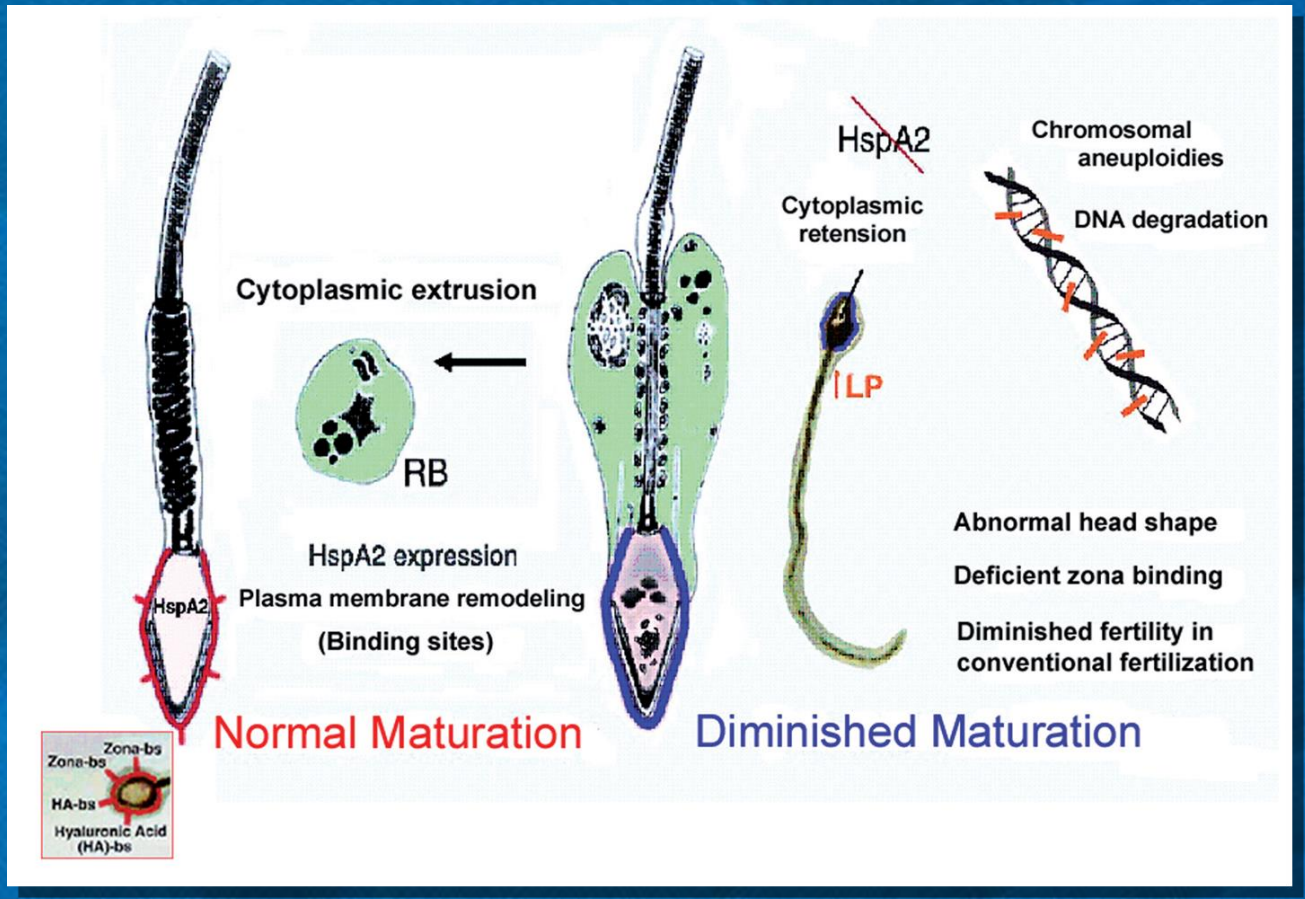



- The Extra Cellular Matrix (ECM) is a formidable barrier which the sperm must get through to reach the Zona Pellucida and to fertilize the oocyte

# HA plays a pivotal role in physiologic sperm selection

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

- *Huszar et al, Fertil Steril 2003; RBMO 2007*

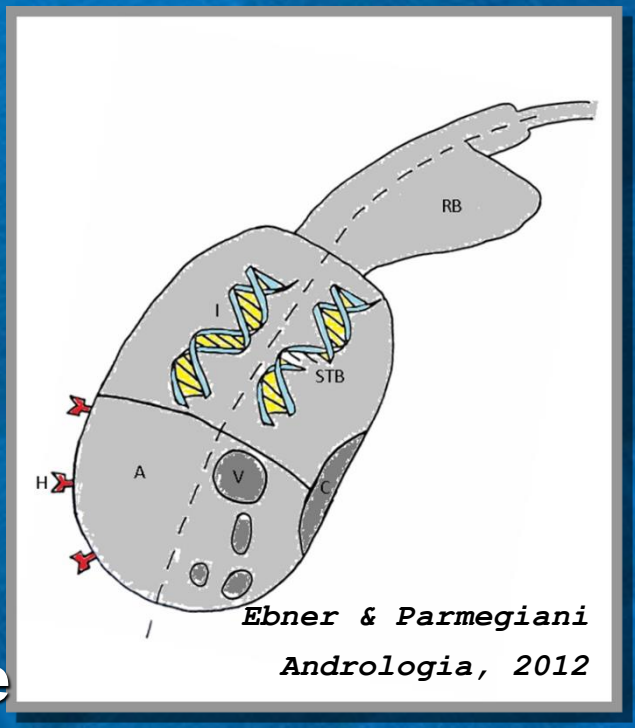



Mature spermatozoa with high density of HA receptors bind permanently to HA.
Immature spermatozoa are not able to bind to HA

- *Cayli et al, RBMO2003*

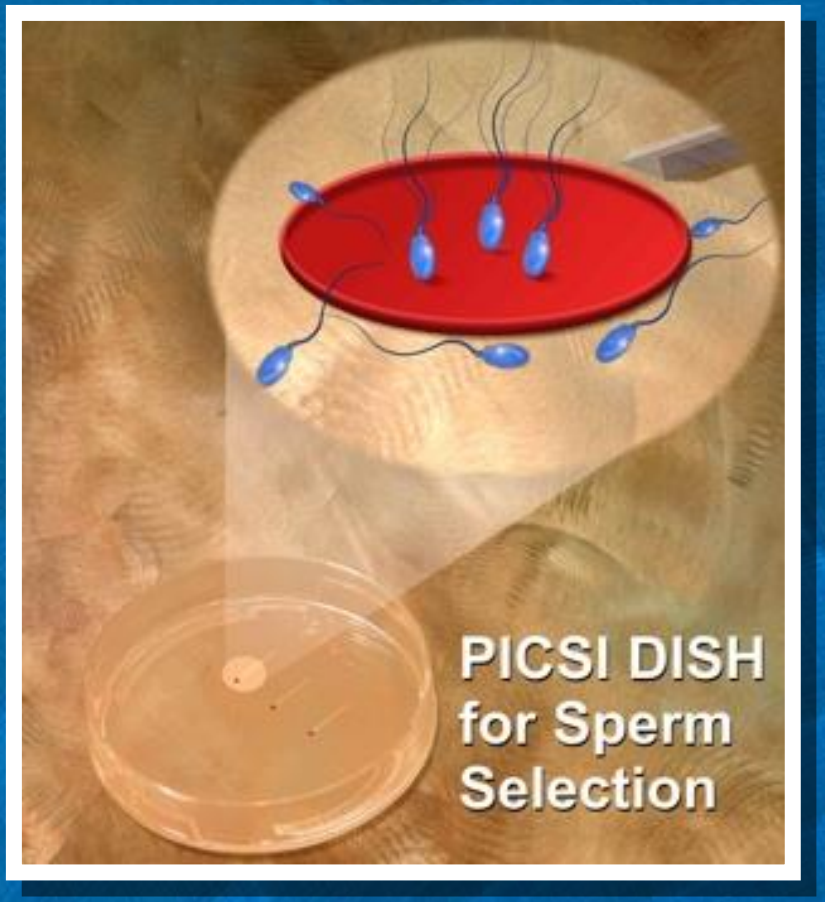
# Sperm-HA binding selection

- HA-bound spermatozoa show a 5.4-fold reduction in chromosomal anueploidies
  - *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
    - *Parmegiani et al, Adv Exp Med Biol 2014*

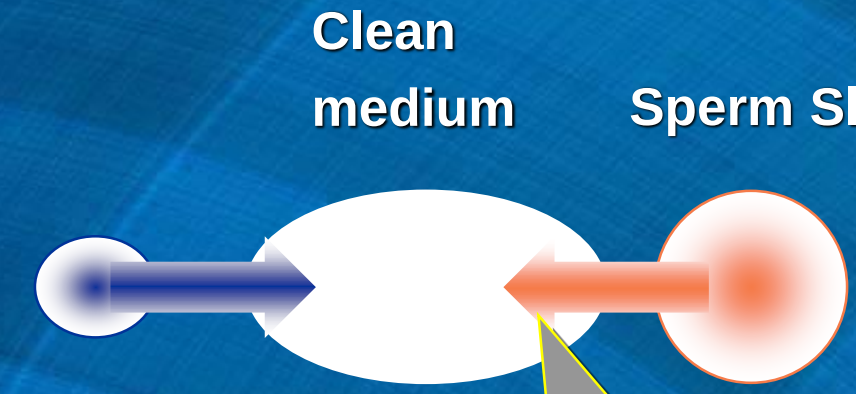



*Ebner & Parmegiani*
*Andrologia, 2012*

# PICSI - selection of HA-bound spermatozoa

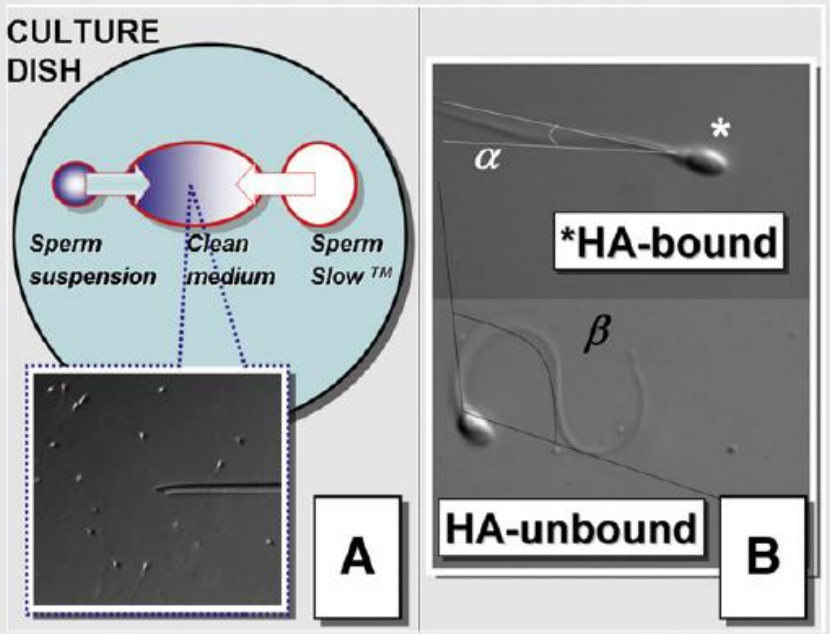


# Sperm Slow - selection of HA-bound spermatozoa




**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***

**FIGURE 2**




Sperm Slow: (A) droplet preparation and (B) sperm tail movements. On a plastic culture dish a 2-μL droplet with suspension of treated spermatozoa is connected with a pipette tip to a 5-μL droplet of fresh culture medium. Simultaneously, a 5-μL droplet of Sperm Slow is connected with a pipette tip to the 5-μL droplet of fresh culture medium. The spermatozoa on this culture dish are incubated for 5 minutes at 37°C under oil. The HA-spermatozoa are slowed in the junction zone of the two droplets; these spermatozoa can be selected and detached by injecting pipette and subsequently injected into oocytes (6). The HA-bound (*) sperm tail appears stretched, its motility is dramatically slowed, and its beats have narrow amplitude (angle α). The HA-unbound spermatozoa swim all around the medium droplet, they are less slowed by the viscosity of the medium, and their tail-beats have wider amplitude (angle β).

*Parmegiani. HA-sperm selection: PICSI vs. Sperm Slow. Fertil Steril 2012.*

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

# PICSI vs Sperm Slow

**TABLE 2**

**Primary and secondary outcome measures and clinical outcome.**

| Parameter | PICSI | Sperm Slow | *P* value |
|---|---|---|---|
| Good-quality embryos[a] | 121/207 (58.5) | 116/207 (56.0) | .691 |
| Fertilized oocytes | 207/252 (82) | 207/252 (82) | .907 |
| Clinical pregnancy rate per transfer | 21/49 (42.9) | 20/50 (40.0) | .933 |
| Implantations | 26/108 (24.1) | 25/114 (21.9) | .826 |
| Mean ICSI procedure duration (s)[b] | 450.0 ± 30.5 | 284.1 ± 10.1 | < .001 |
| Abortions | 4/21 (19.0) | 5/20 (25.0) | .719 |
| Live births (babies born) | 17 (18) | 15 (15) | |

*Note:* Values are number (percentage) or mean ± SE.

[a] Good-quality embryos: no. of grade 1, 2, and 3 embryos (22) per no. of fertilized oocytes.

[b] Mean ICSI procedure duration was measured by an observer, from the recovery of the first spermatozoa by ICSI pipette to the end of the injection procedure of the last oocyte available for ICSI.

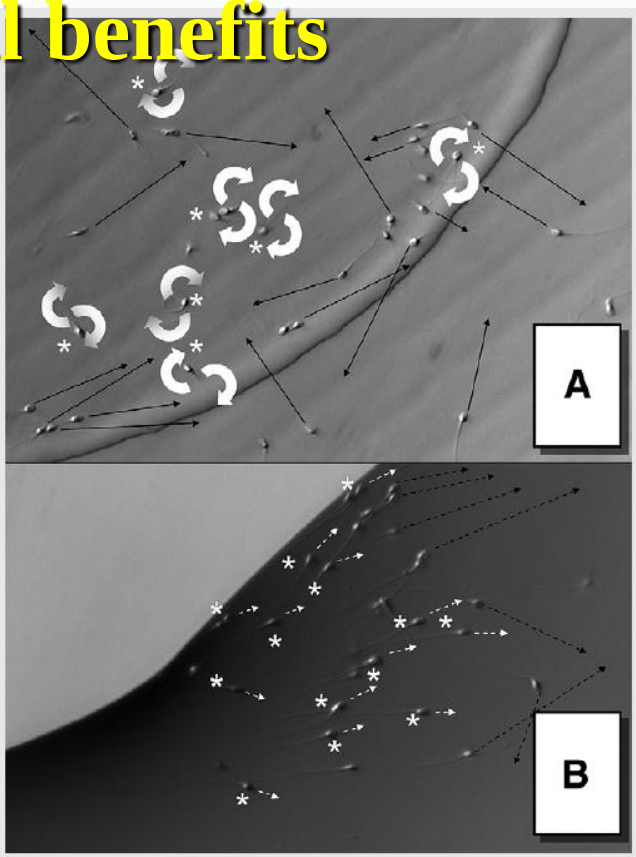
*Parmegiani. HA-sperm selection: PICSI vs. Sperm Slow. Fertil Steril 2012.*

- 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*

FIGURE 1




Motility patterns of HA-bound spermatozoa in PICSI or Sperm Slow (magnification ×400). (A) In PICSI, HA-sperm (*) are bound by the head to the bottom of the dish and have vigorous motility, with the tail spinning around its head (*white arrows*). This motility pattern allows a "fine tuning" of HA-bound spermatozoa selection based on observation of their degree of motility. The HA-unbound spermatozoa, in contrast, swim free all around the droplet of culture medium with varied motility (*black arrows*). (B) In Sperm Slow, HA-sperm (*) appear very "slowed" owing to the HA binding combined with the viscosity of the medium (*white arrows*). The HA-spermatozoa appear as if "trapped in a net" compared with HA-unbound spermatozoa, which travel much farther (*black arrows*). A specific droplet preparation is suggested, and specific training is needed to distinguish HA-bound spermatozoa from HA-unbound, which are also slowed by the viscosity of the medium (Fig. 2A and B).

*Parmegiani. HA-sperm selection: PICSI vs. Sperm Slow. Fertil Steril 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***

## Towards a more physiological ICSI

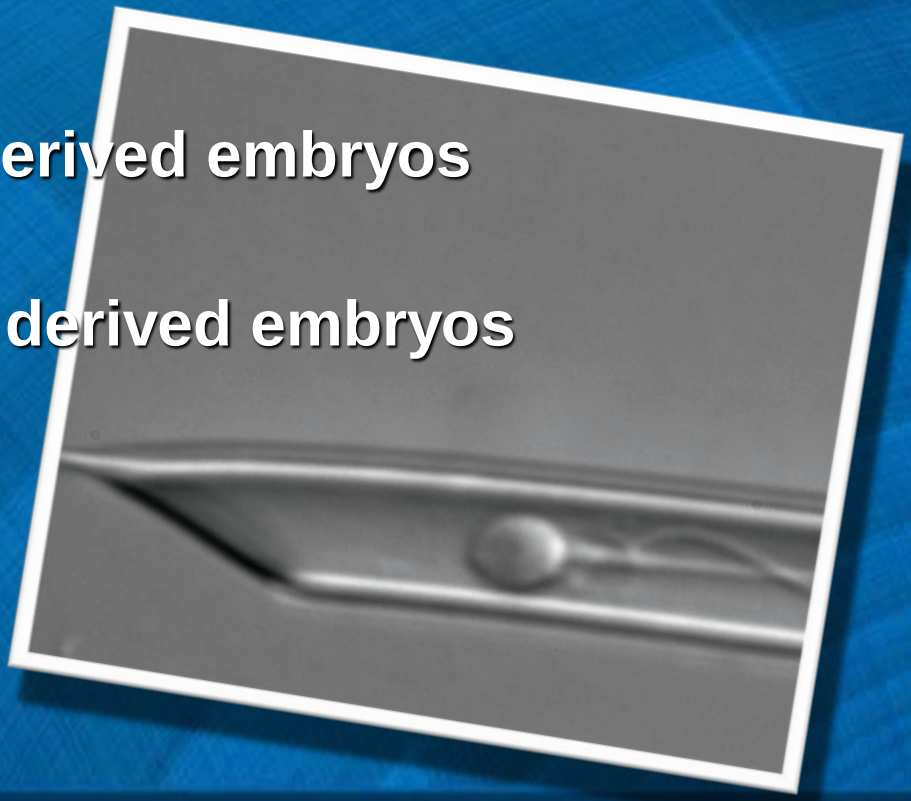
# INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION (IMSI)

# 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*

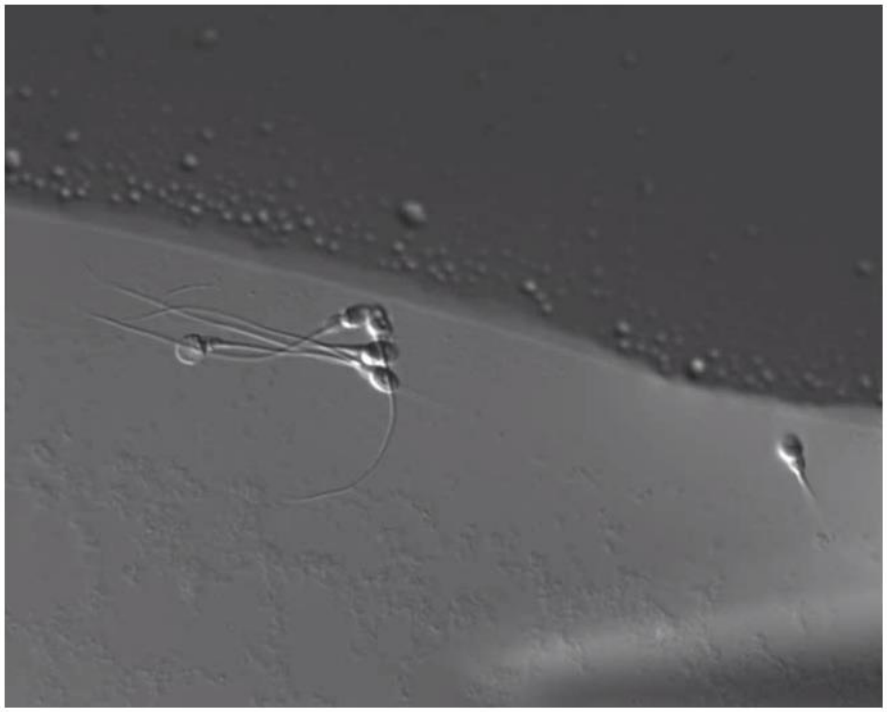
# Spermatozoa without nuclear vacuoles

- 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, RBMO2013*
- improvement of  pregnancies
- reduction of miscarriages
  - *Souza Setti et al, RBMO 2010*
- reduction of birth defetcs
  - *Cassuto et al, RBMO 2013*
- Physiologic HA-IMSI
  - *Parmegiani et al, Fert Steril 2010*



# IMSI – Limitations

- expensive
  - *Bartoov et al, Fertil Steril 2003*
- time consuming
  - around 120 minutes
    - *Antinori et al, RBMO 2008*
- absence of top quality spermatozoa
  - no improvements in clinical results
    - *Cassuto et al, Fertil Steril 2009*
- 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 check-points 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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