HOW TO GET SPERM WITH MAXIMUM FERTILITY POTENTIAL

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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

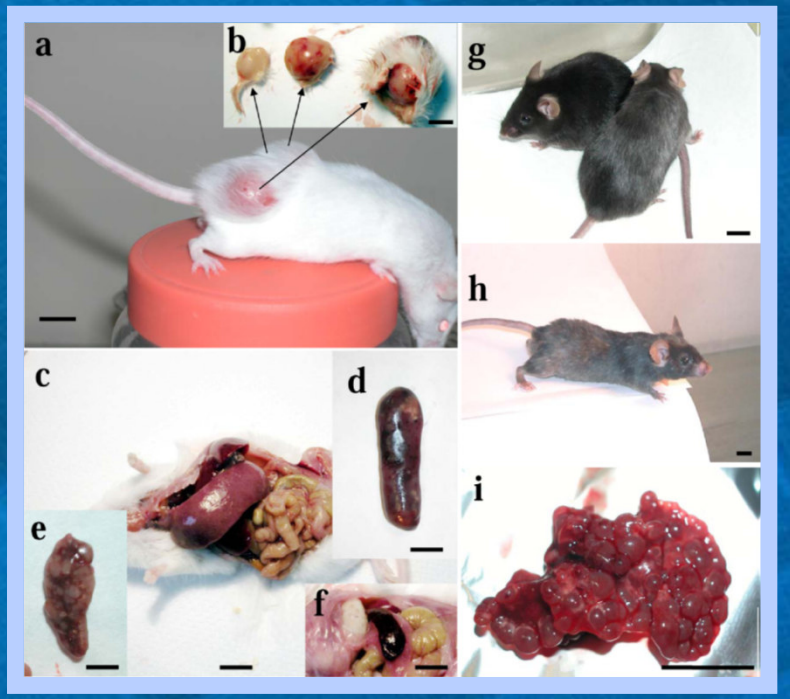
Sperm selection becomes critical especially when a limited number of oocytes are available for injection

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, Hum 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; El Hajj et al, Hum Reprod 2017*

- 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*

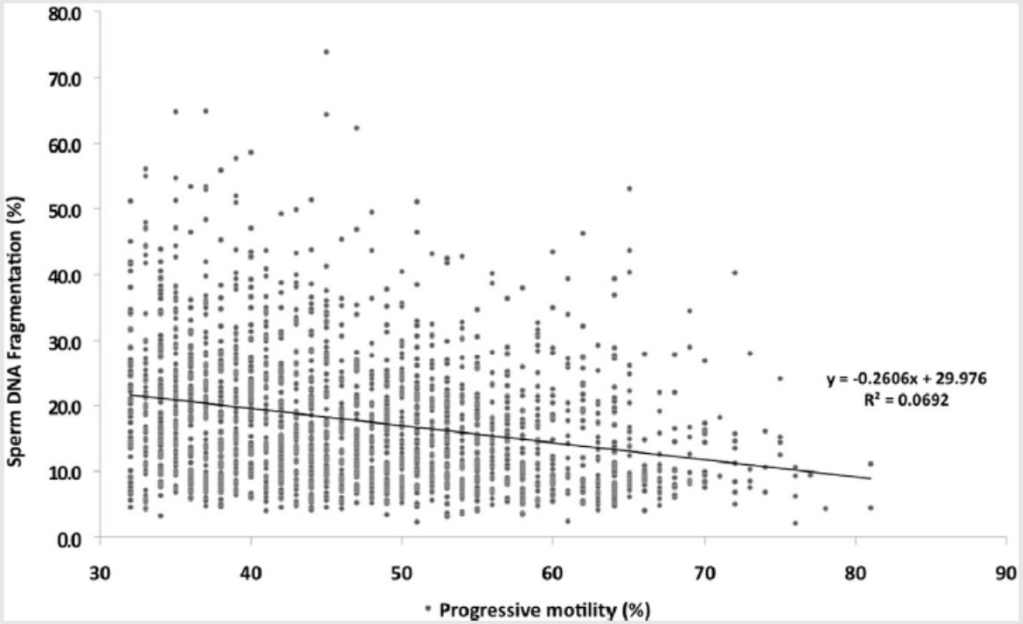
SPERM PREPARATION PRIOR TO ICSI

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*

Sperm treatment - the natural path

- 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
    - *Parmegiani et al, Adv Exp Med Biol 2014*



Correlation between percent progressive motility and percent sperm DNA fragmentation in 1,974 normozoospermic men.

Belloc. Sperm DNA damage in normozoospermic men. Fertil Steril 2014.

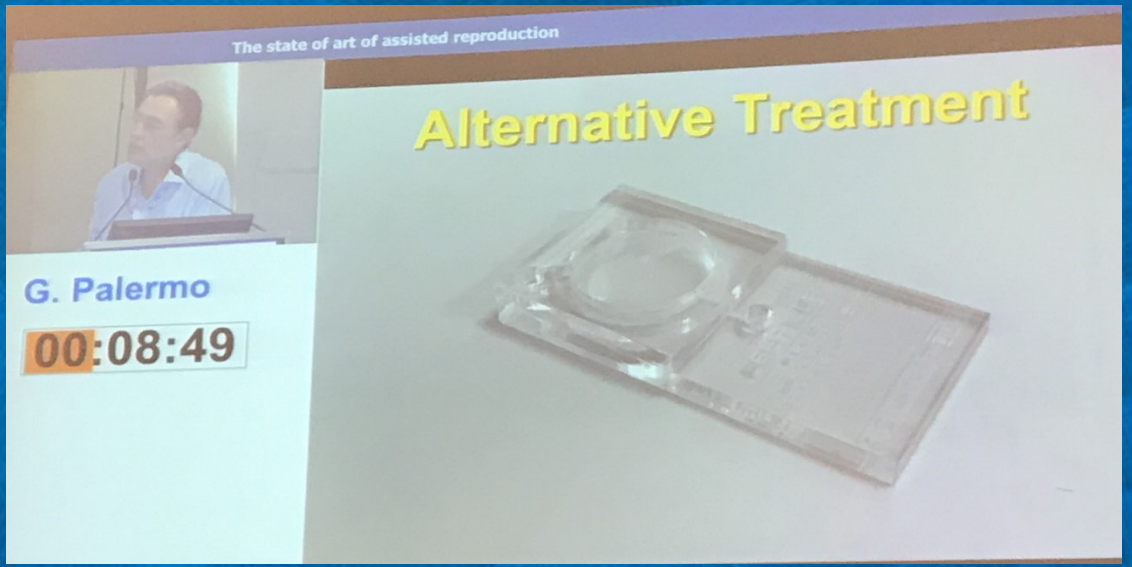
Selection by motility without centrifugation

- *Zavos Swim Up Column, 1999*

Zech Chamber

- *Ebner et al, RBMO 2011*
- *Seiringer et al RBMO 2013*

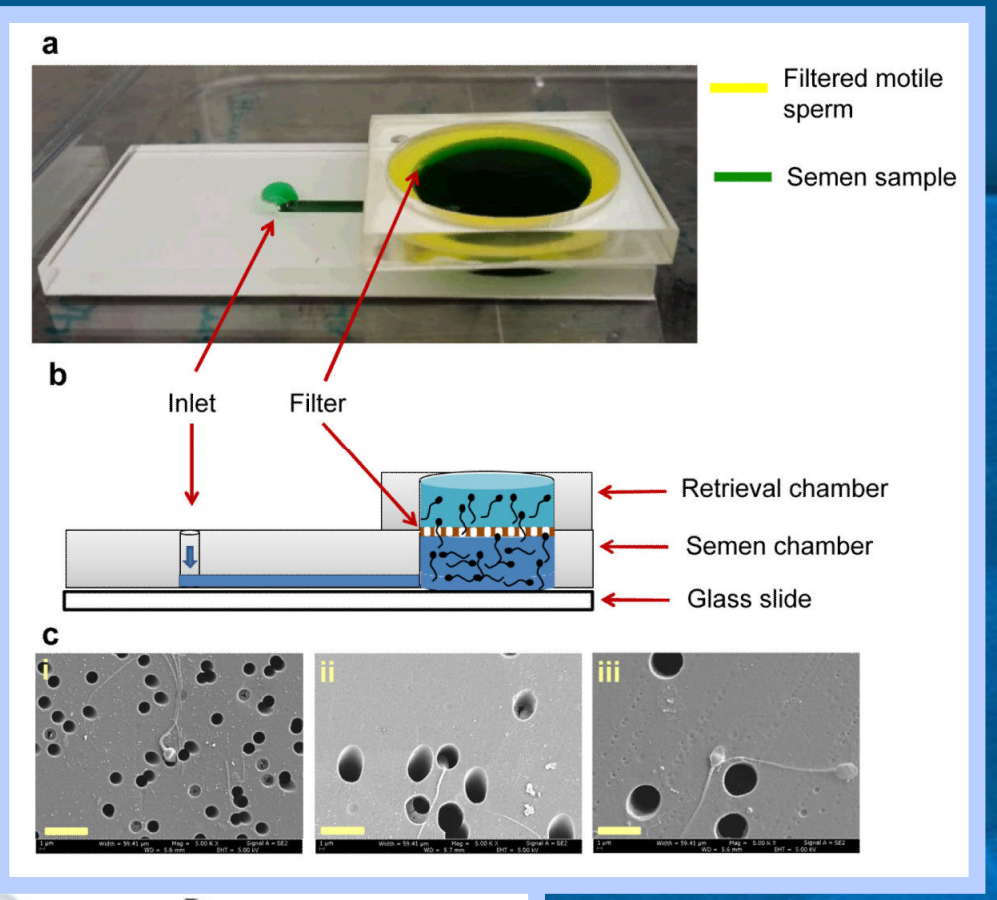
- *Migration Sedimentation Chamber – RI Origio*



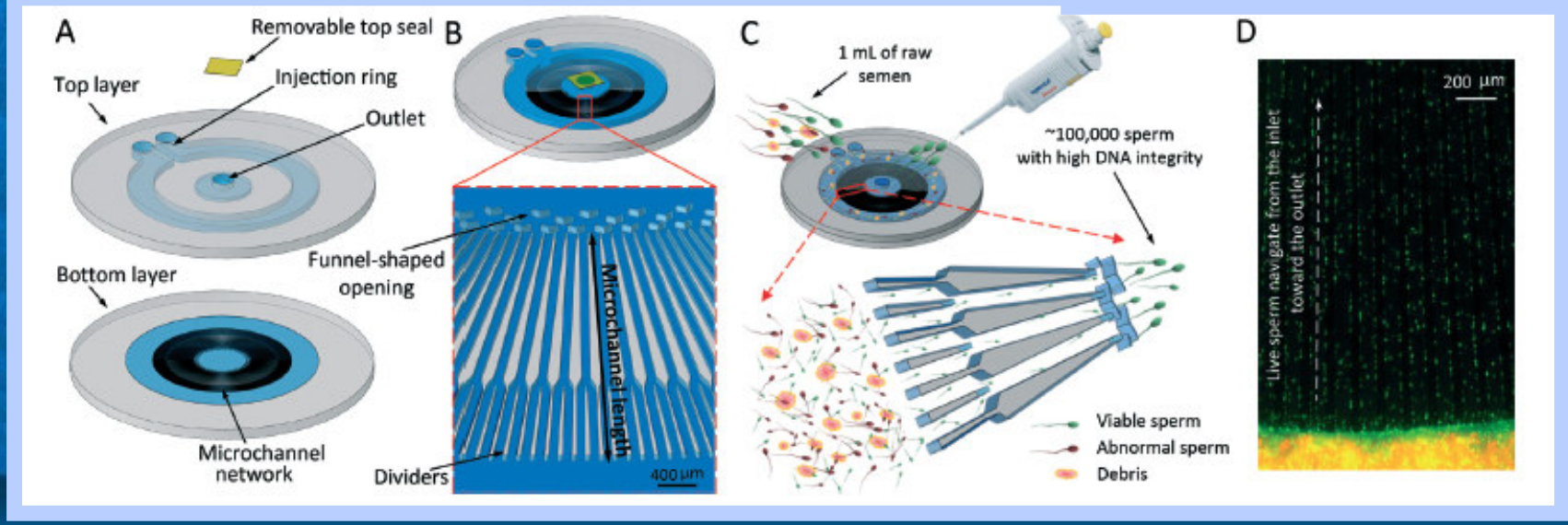
- *Parrilla-Palermo, Human Reprod 2017*

Microfluidic Chip Technology

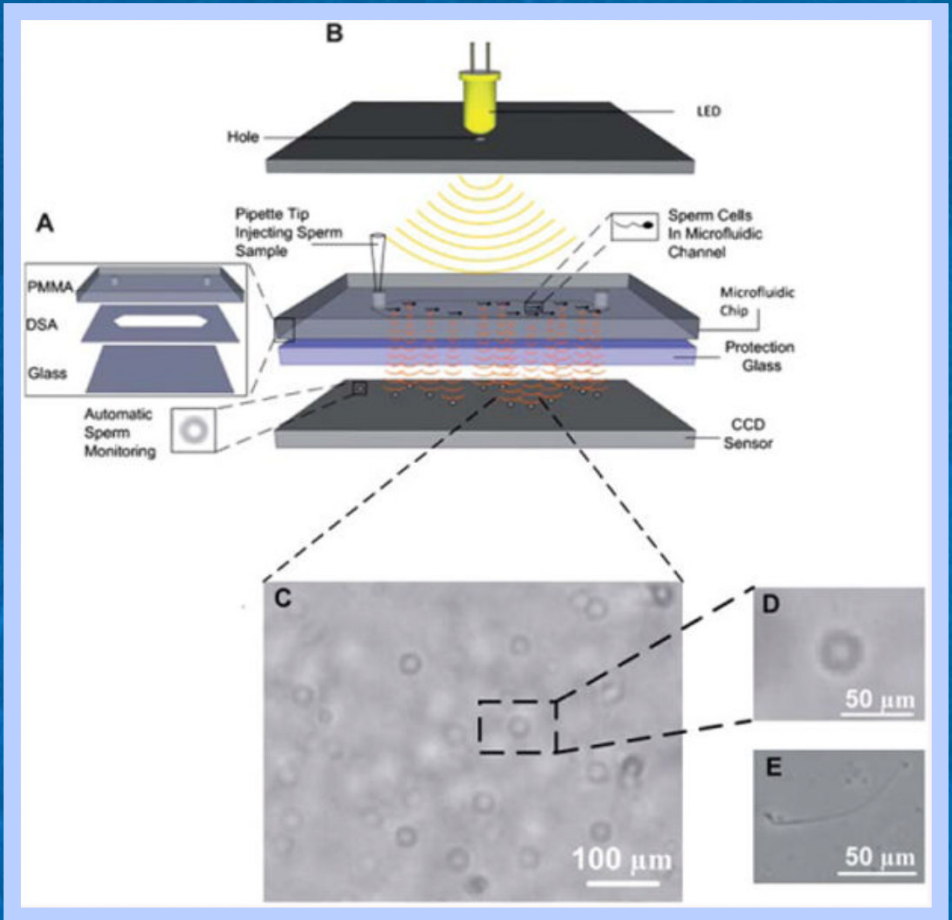
- *Asgar et al, Adv Healthc Mater 2014*



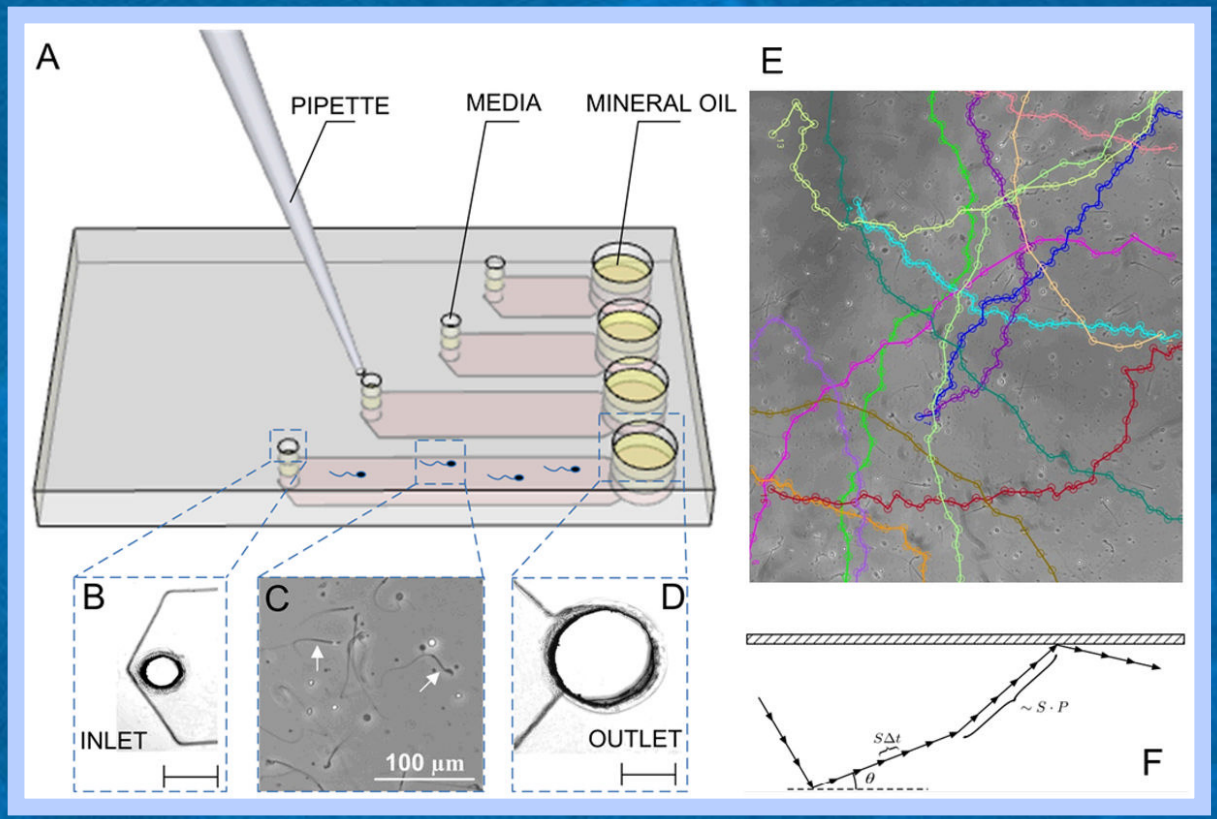
- *Nosrati et al, LOC 2014*



Microfluidic Chip Technology

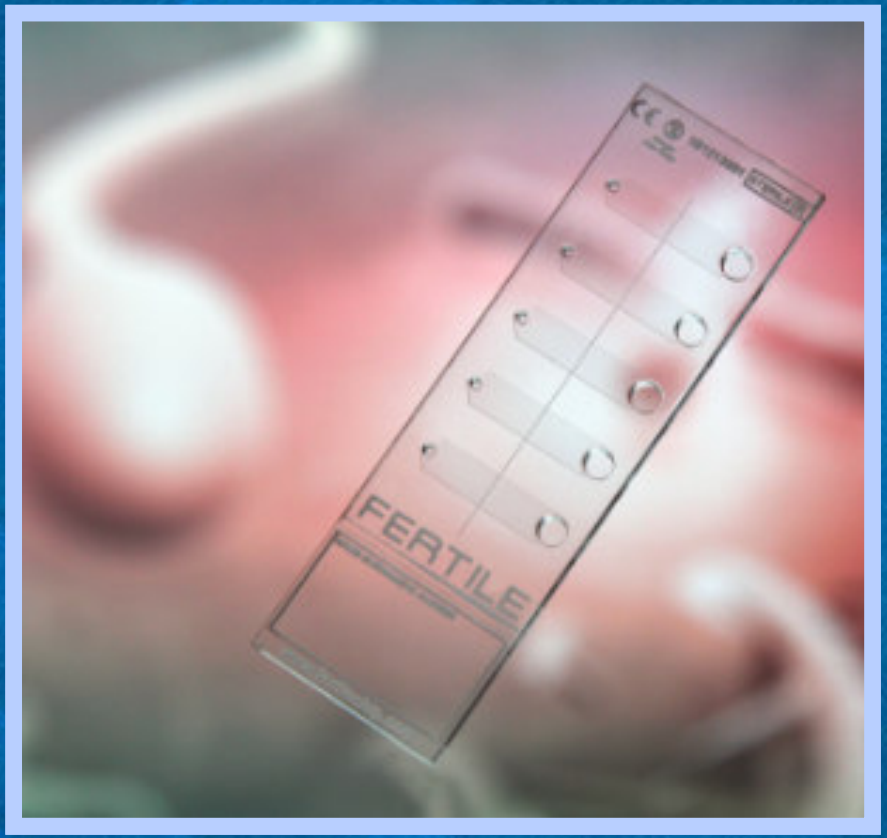


◆ *Zhang et al, Lab Chip 2011*



◆ *Tasoglu et al, Small. 2013*

Chip Technology: ready for the market?



SINGLE SPERM SELECTION PRIOR TO ICSI

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
  - positive rehotaxis - microfluidics
  - restoration of fertilization checkpoints
    - sperm-hyaluronic acid binding
  - high magnification
    - Intracytoplasmic Morphologically selected Sperm Injection

Towards a more physiological ICSI

POSITIVE

REHOTAXIS

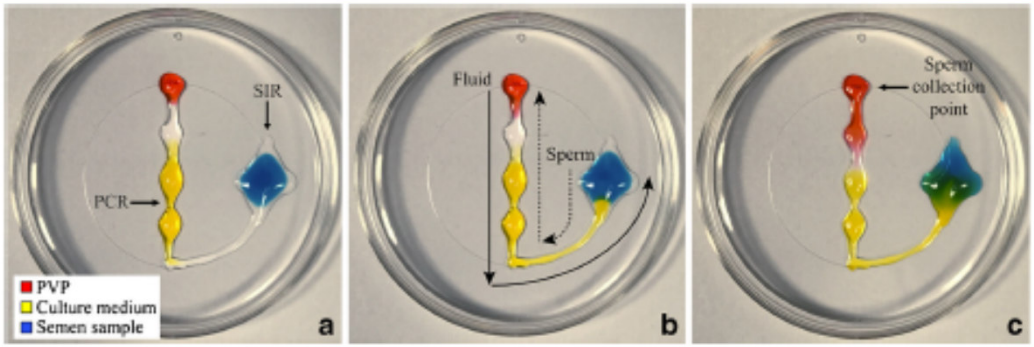
MICROFLUIDICS

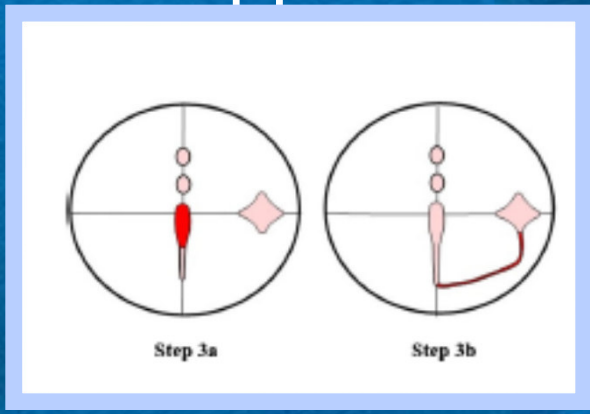
Positive rehotaxis - microfluidics

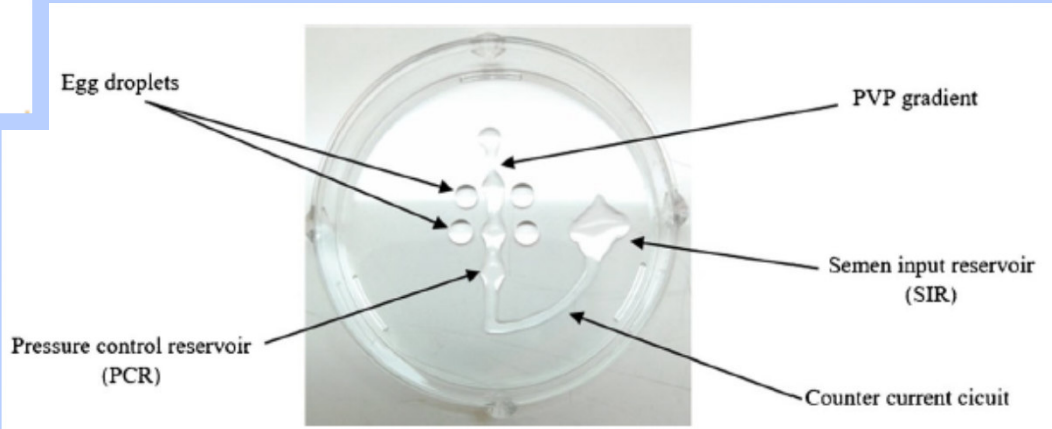
Positive rheotaxis extended drop (PRED) is set up creating a pressure and viscosity gradient.

Improved chromatin maturity after the PRED approach was observed

- *De Martin et al, JARG 2017*







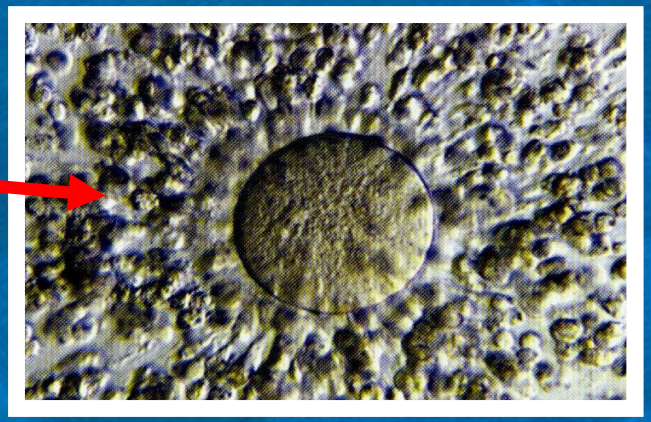
RESTORATION OF FERTILIZATION CHECK-POINTS

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

ECM

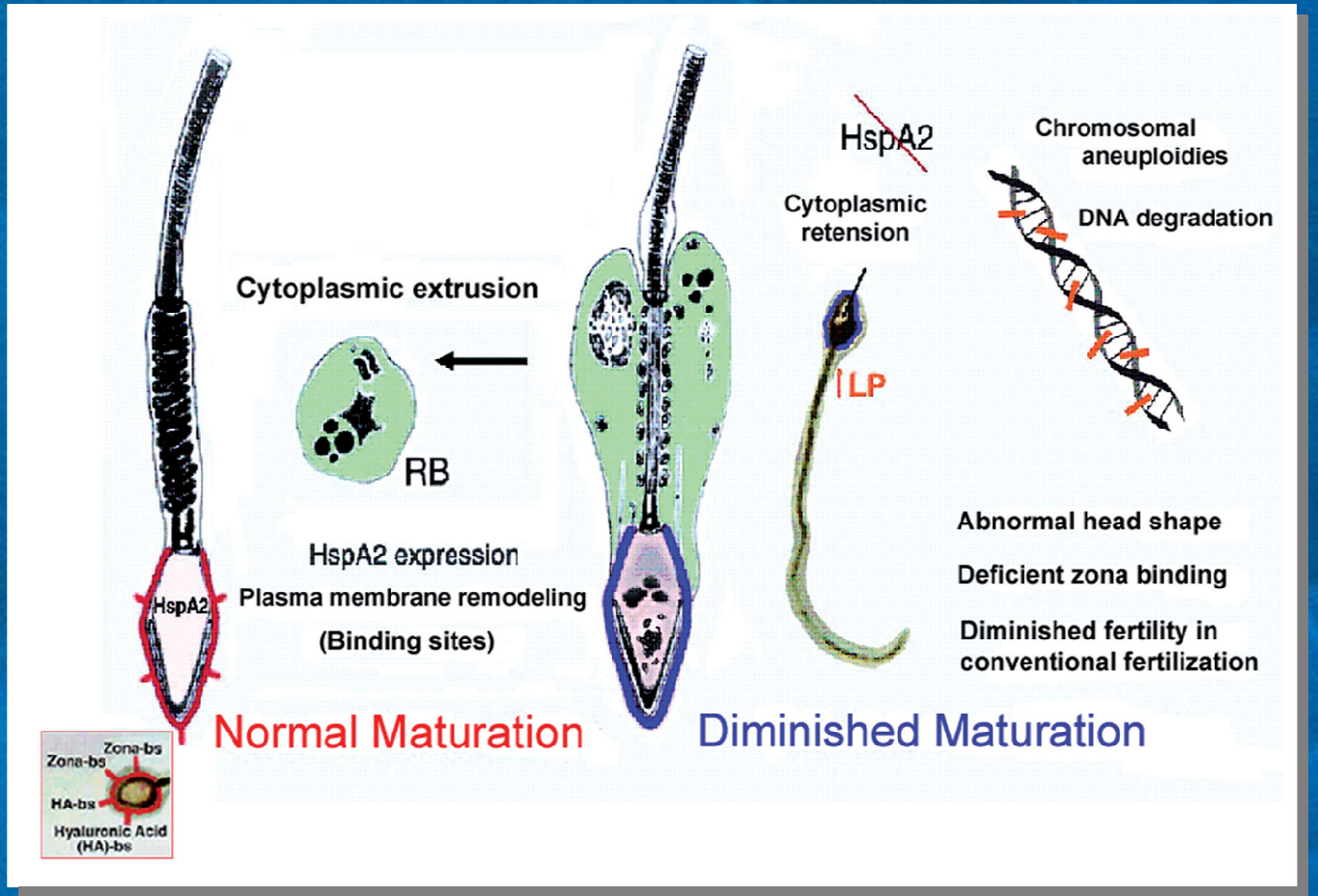


- The Extra Cellular Matrix (ECM) is a formidable barrier which the sperm must get through to reach the Zona Pellucida and to fertilize the oocyte

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*

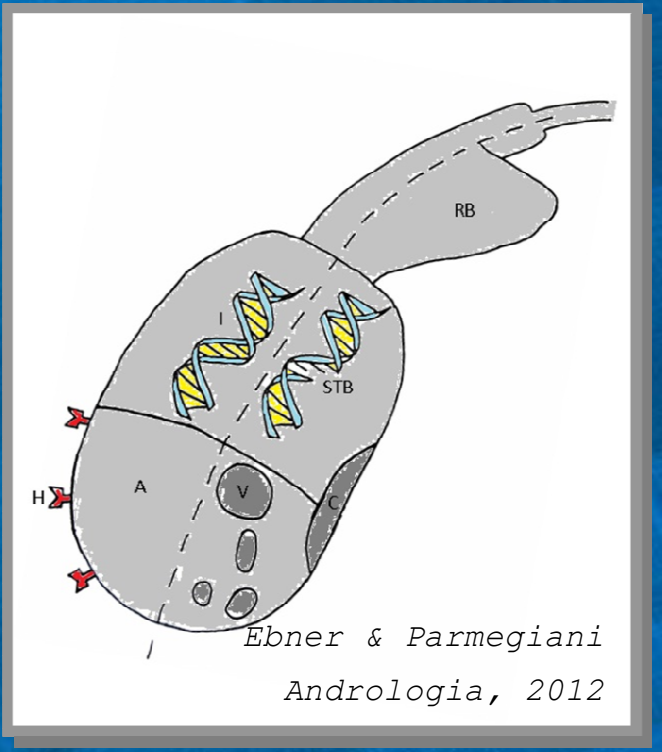


Mature spermatozoa with high density of HA receptors bind permanently to HA.
Immature spermatozoa are not able to bind to HA

- *Cayli et al, RBMO2003*

Sperm-HA binding selection

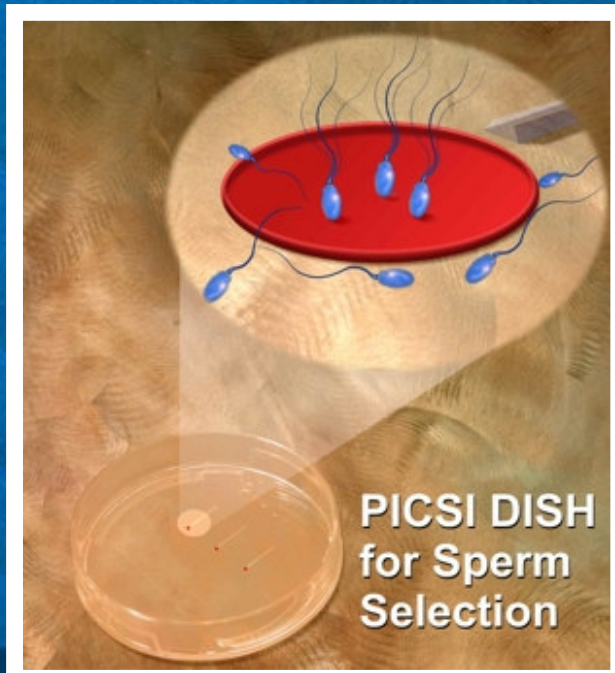
- HA-bound spermatozoa show a 5.4-fold reduction in chromosomal anueploidies
  - *Jakab et al, Fertil Steril 2005*
- strong link between DNA fragmentation and aneuploidies in human sperm
  - *Enciso et al, Hum Reprod 2013*
- a selection method based on mature sperm-HA binding
  - useful in reducing the potential genetic complications and adverse long-term side effects of ICSI
    - *Parmegiani et al, Adv Exp Med Biol 2014*

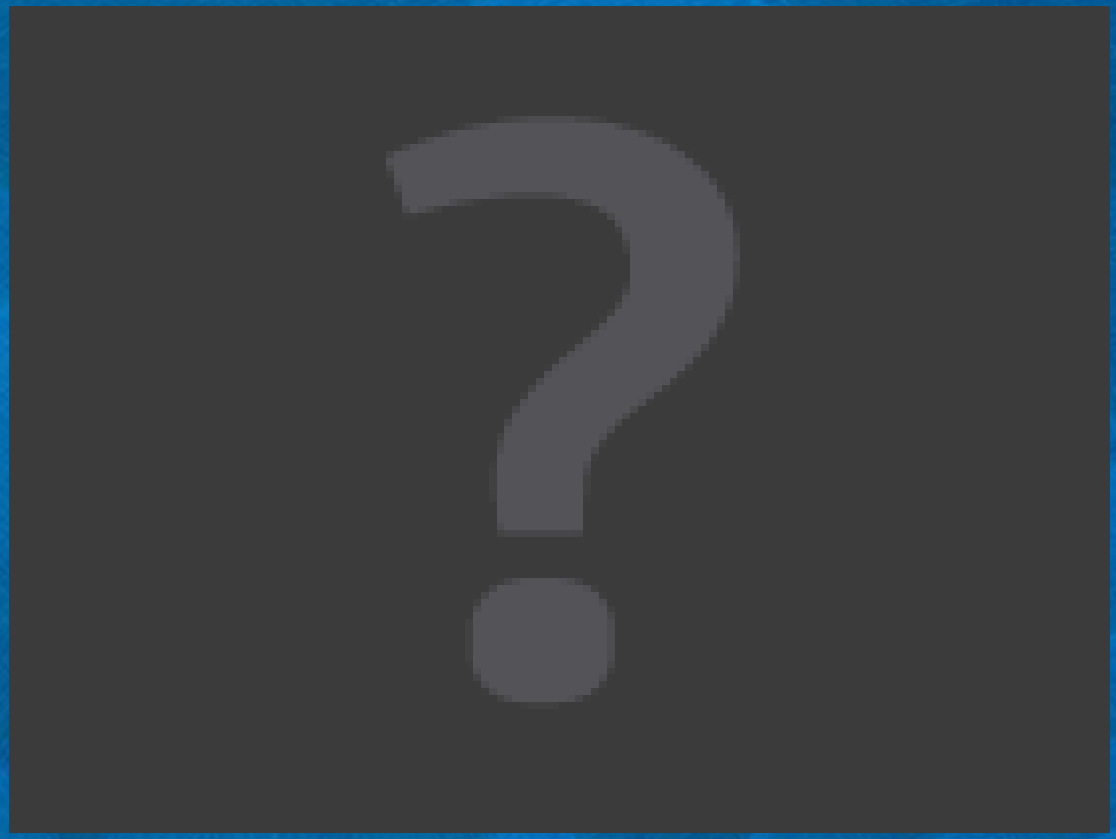


Ebner & Parmegiani

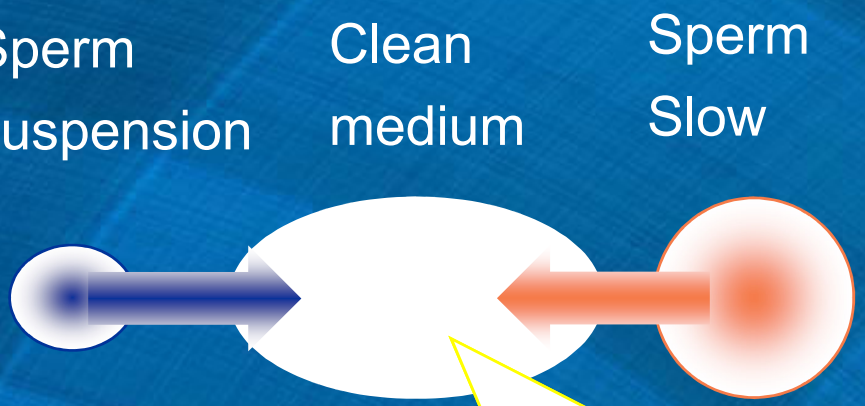
Andrologia, 2012

PICSI - selection of HA-bound spermatozoa



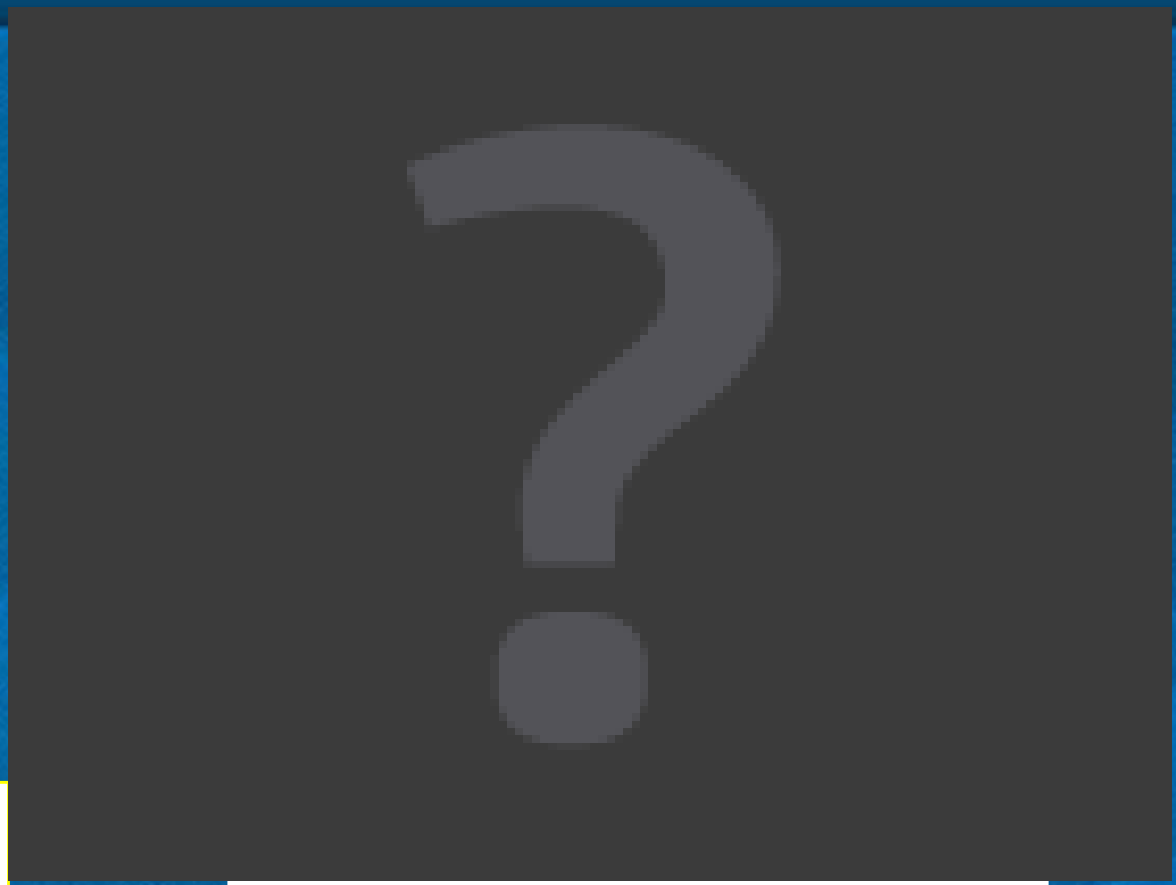


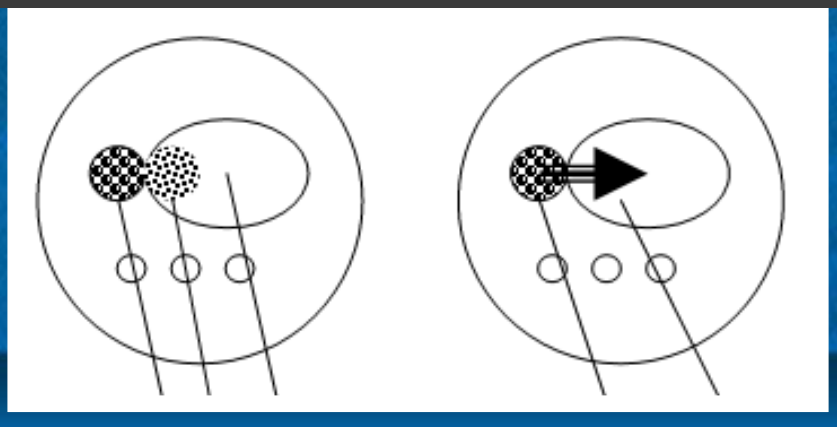
Sperm Slow - selection of HA-bound spermatozoa + microfluidics



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





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 lowers abnormal fertilization rates
    - *Liu et al, Hum Fert 2017*
  - 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 ICSI": Hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality

Lodovico Parmegiani, B.Sc., Graciela Estela Cognigni, M.D., Silvia Bernardi, B.Sc., Enzo Troilo, B.Sc., Walter Ciampaglia, M.D., and Marco Filicori, M.D.

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*

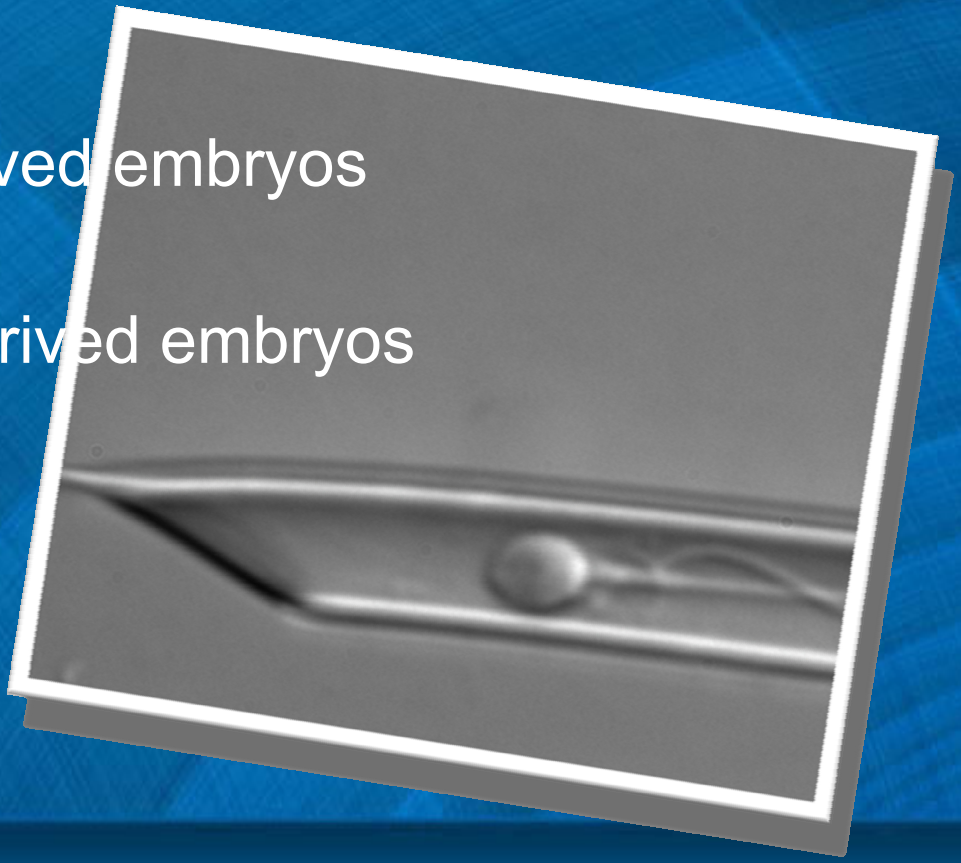
INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION (IMSI)

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±0.1 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*

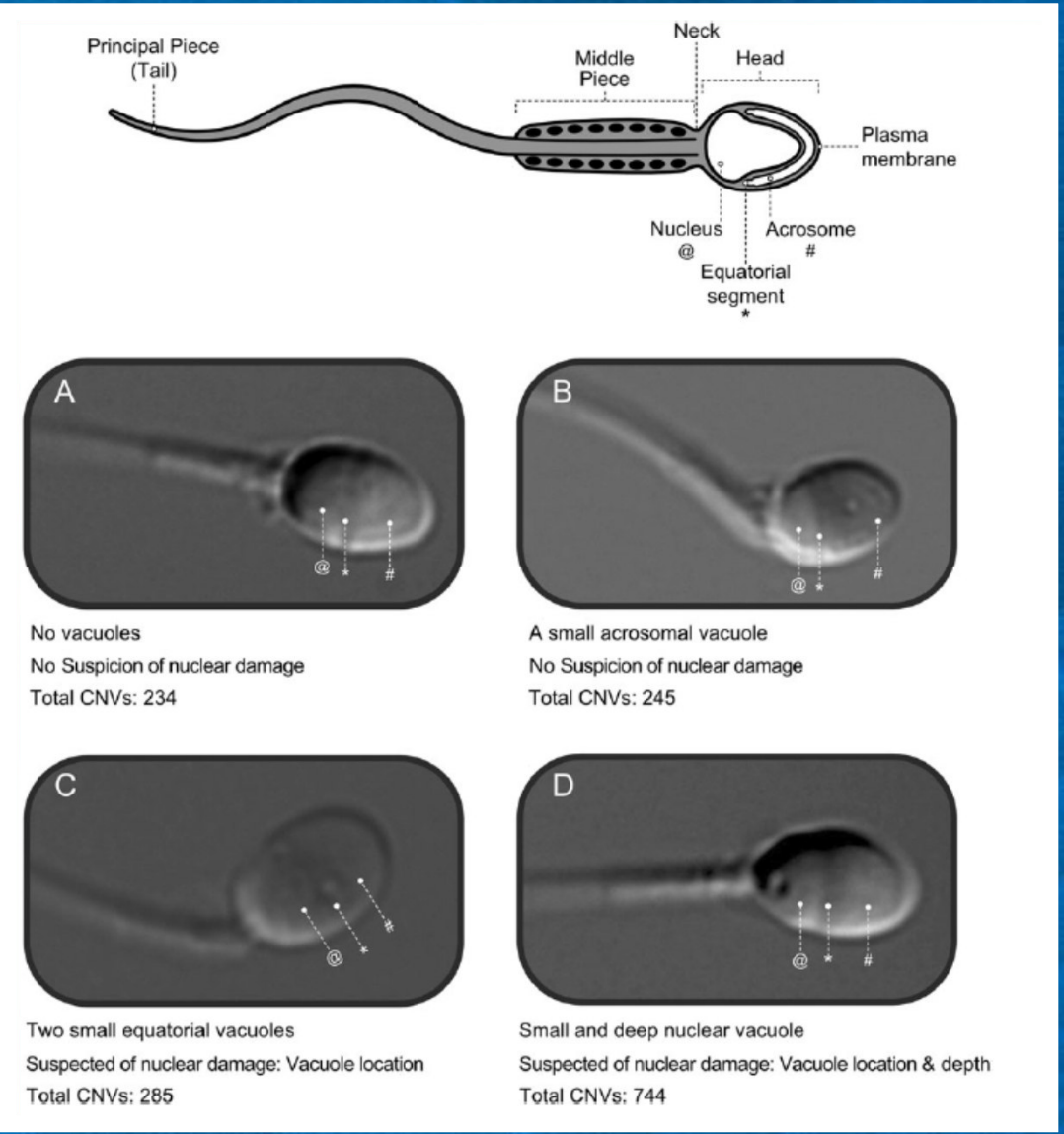
Spermatozoa without nuclear vacuoles

- 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*



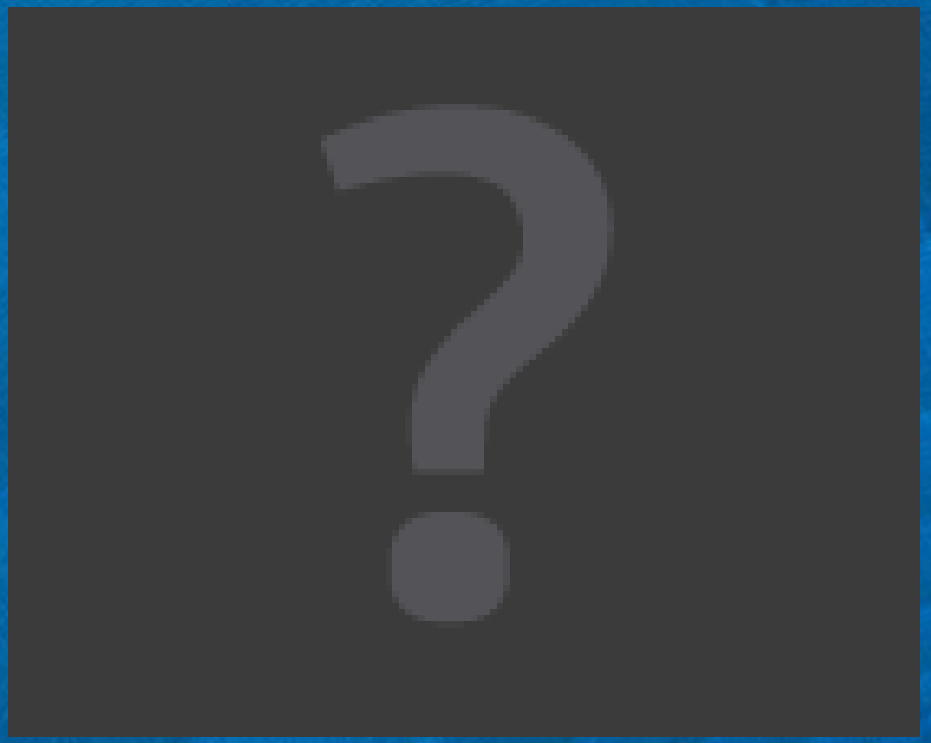
Vacuoles and chromosomal instability

- High chromosomal instability spermatozoa are characterized by vacuoles located in the nucleus and/or equatorial segment or by deep vacuoles
- low chromosomal instability spermatozoa were characterized by a complete lack of vacuoles or non-deep vacuoles not located in the nucleus/equatorial segment
  - *Berkovitz et al, Hum Reprod 2018*



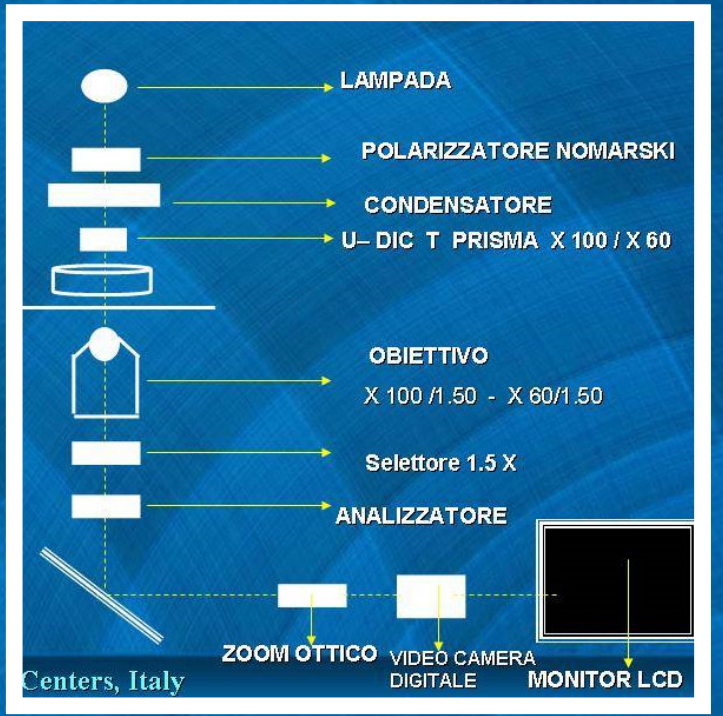
IMSI – Potential advantages

- 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
- promising new microchip technology for sperm selection is moving from bench to bedside

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