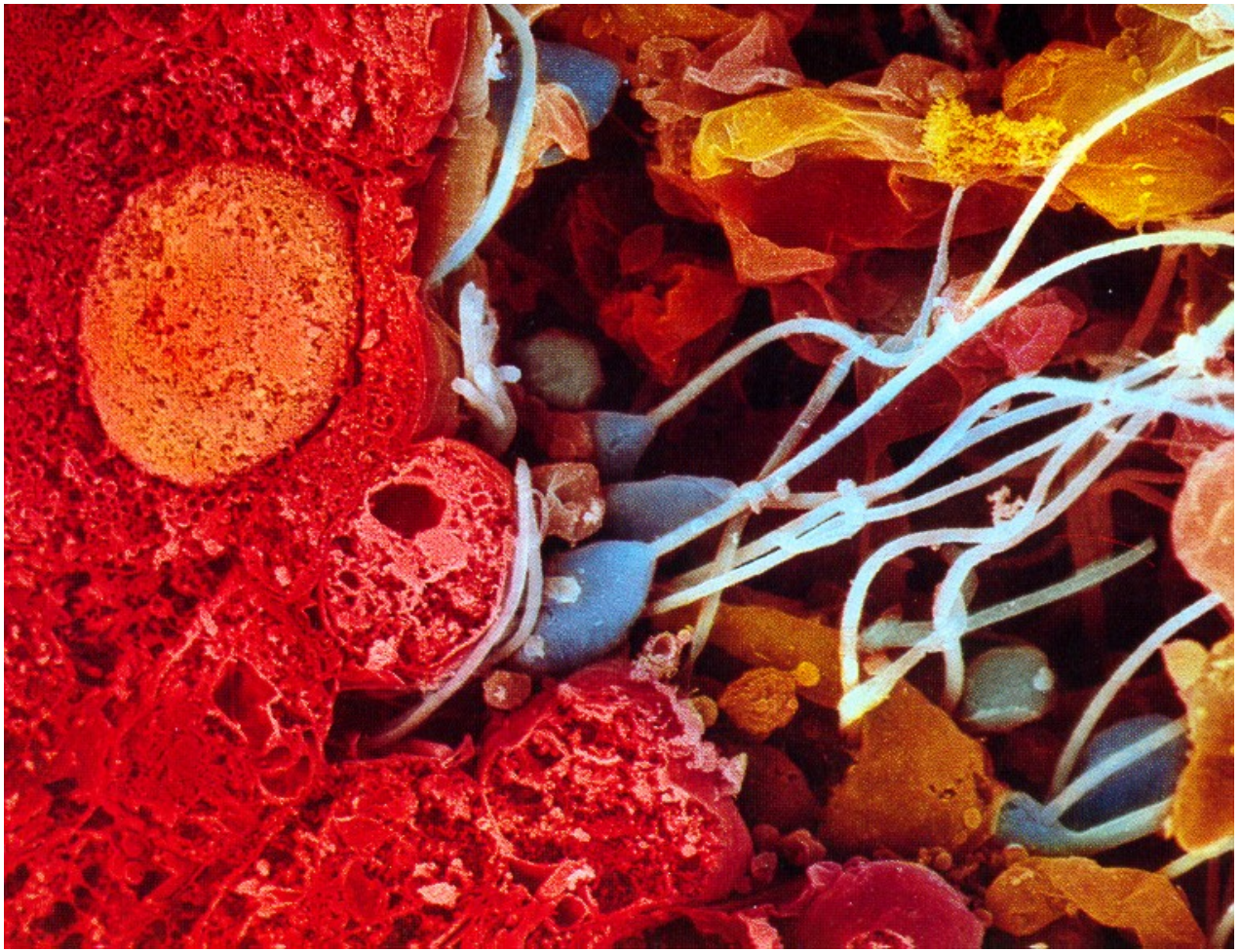
# XVI Master Reproducción Humana



# SELECCIÓN ESPERMÁTICA

Dra. Rocío Núñez

Dra. Rocío Núñez Calonge

# *Evaluation of sperm function. What is available in the modern andrology lab ?*

*Bar-Chama and Lamb, 1994.*

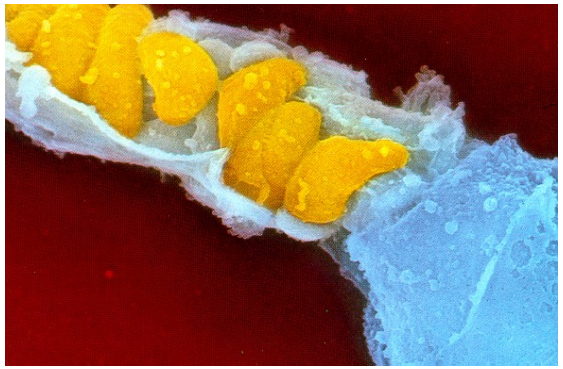
**Hace más de 20 años se investigaba en los test de función en el Laboratorio de Andrología.**

Dra. Rocío Núñez

**Después de décadas de investigación, la principal razón para que no exista una estimación efectiva de la función espermática es nuestro limitado conocimiento del funcionamiento del espermatozoide.**

*Understanding the physiology of pre-fertilisation events in the human spermatozoa-a necessary prerequisite to developing rational therapy.*

*Conner SJ, Lefievre L, Kirkman-Brown JC, Michelangeli F, Jimenez-Gonzalez C, Oliveira GM, Pixton KL, Brewis IA, Barratt CLR*
**Soc Reprod Fertil Suppl. 2007;63:237-55.**



Dra. Rocío Núñez

ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

EDITORIAL

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doi: 10.1111/andr.12034

# The need to improve patient care through discriminate use of intracytoplasmic sperm injection (ICSI) and improved understanding of spermatozoa, oocyte and embryo biology

[1]D. T. Carrell, [2]A. Nyboe Andersen and [3]D. J. Lamb

**Human Reproduction Update, Vol.22, No.2 pp. 194–227, 2016**
Advanced Access publication on November 18, 2015 doi: 10.1093/humupd/dmv050

human reproduction update

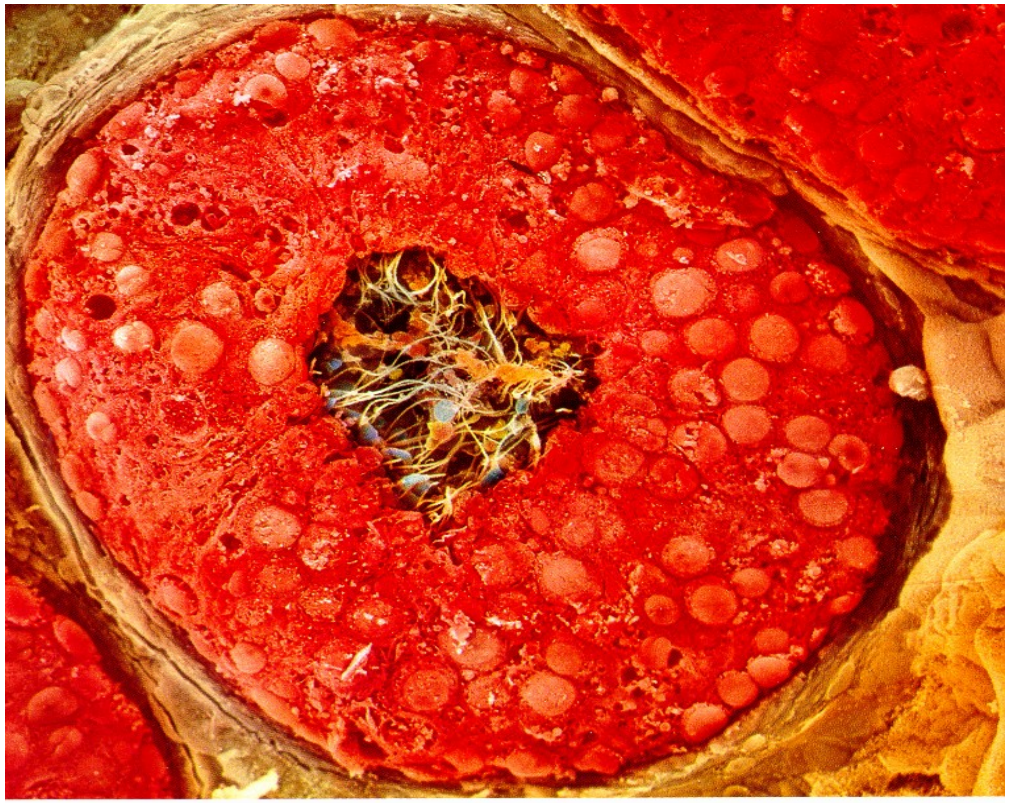
# The ICSI procedure from past to future: a systematic review of the more controversial aspects

**Patrizia Rubino[1], Paola Viganò[1], Alice Luddi[2], and Paola Piomboni[2,*]**

and implant; efficient options can be offered to patients who suffered fertilization failure in previous conventional ICSI cycles. Most controversial and inconclusive are data on the best method to select a viable spermatozoa when only immotile spermatozoa are available for ICSI and, to date, there is no reliable approach to completely filter out spermatozoa with fragmented DNA from an ejaculate. However, most of the studies do not report essential clinical outcomes, such as live birth, miscarriage and fetal abnormality rate, which are essential to establish the safety of a procedure.

**CONCLUSIONS:** This review provides the current knowledge on some controversial technical aspects of the ICSI procedures in order to improve its efficacy in specific contexts. Notwithstanding that embryologists might benefit from the approaches presented herein in order to improve ICSI outcomes, this area of expertise still demands a greater number of well-designed studies, especially in order to solve open issues about the safety of these procedures.

# Maduración espermática durante la espermiogénesis



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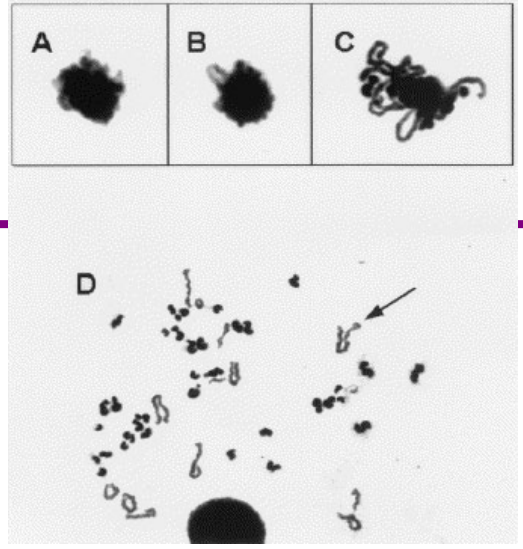
# Madurez nuclear

## Condensación de la cromatina

Nucleoproteinas espermáticas

Histonas




*Epidídimo*

*Túbulos seminíferos en la espermiogénesis*

Cys Lys Arg

Protaminas

Cromatina muy estable

S-S

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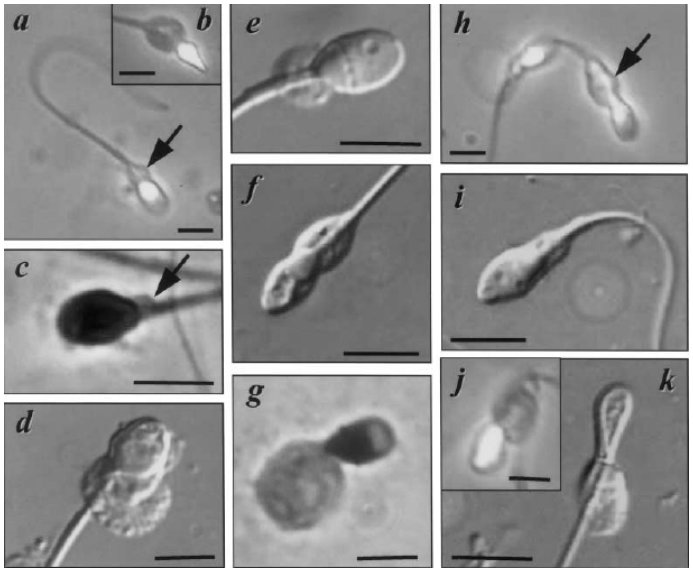
- Durante la espermiogénesis, se eliminan los restos de citoplasma, se desarrolla el acrosoma y se forman los espermatozoides maduros (*de Kretser et al, 1998; Huszar el tal, 2000*).
- Defectos en los compartimentos citoplasmáticos o nucleares pueden originar espermatozoides inmaduros (*Huszar et al, 2001*), incapaces de unirse a la ZP.
- La CK es un marcador de retención citoplasmática y madurez disminuida (*Huszar y Vigue, 1990*)

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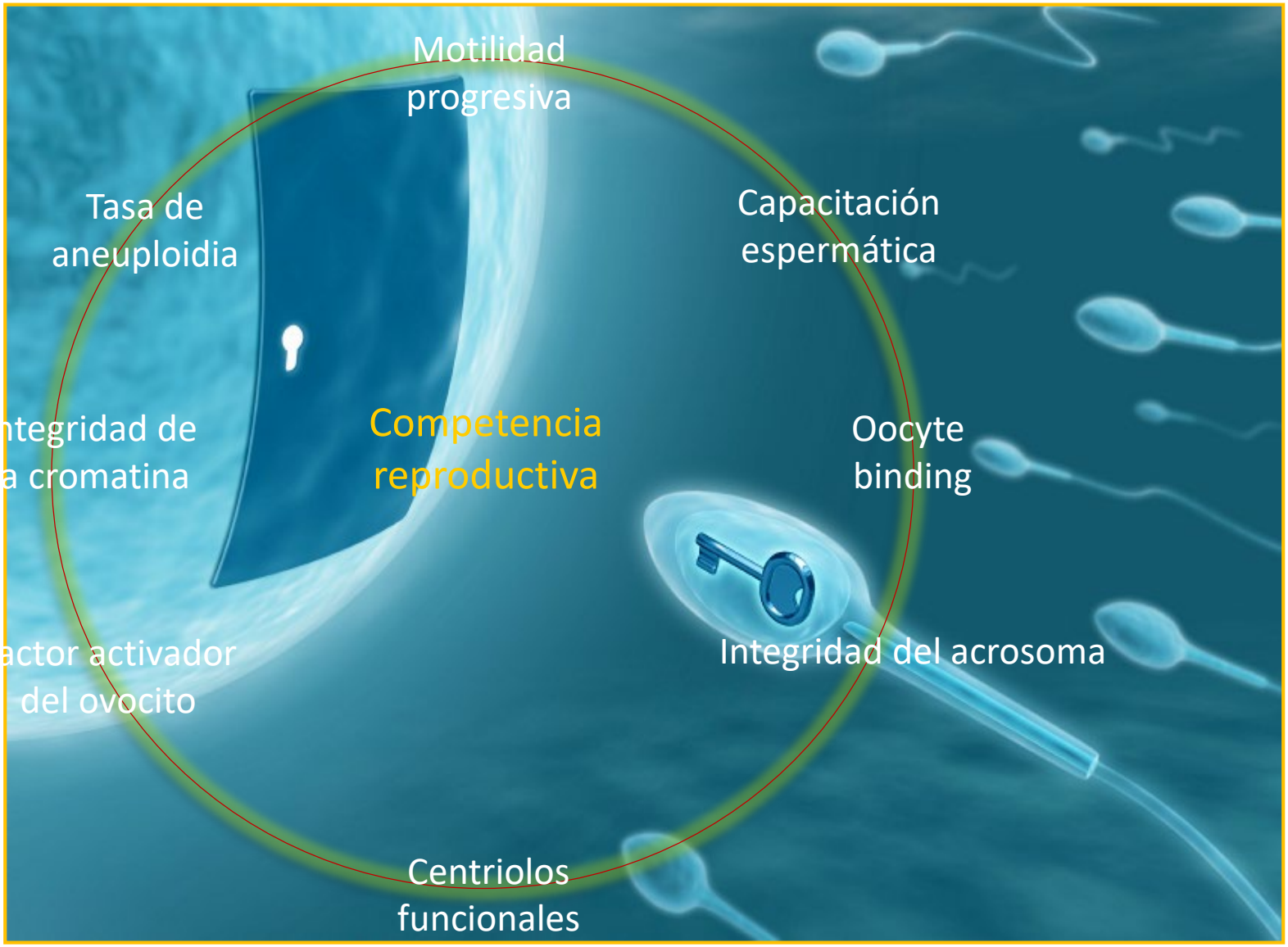
# Factores determinantes

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# Factores determinantes en ICSI


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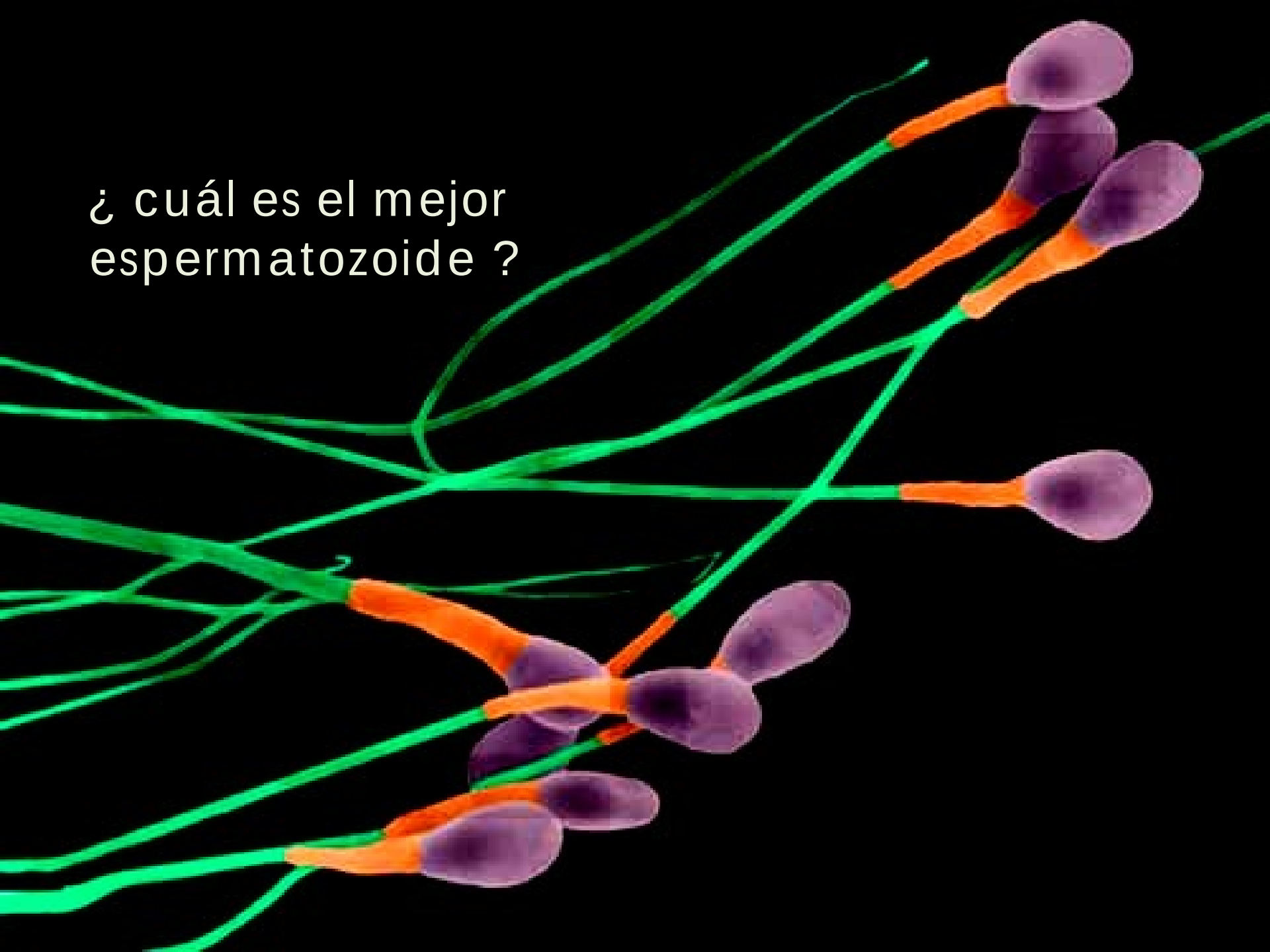
¿ cuál es el mejor espermatozoide ?



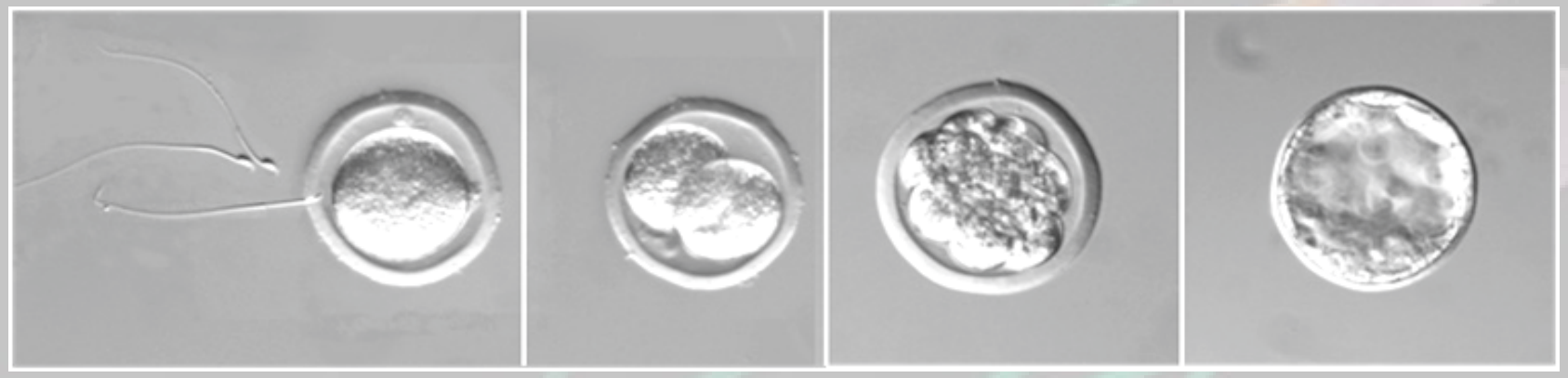
¿ cuál es el mejor espermatozoide ?

***¿Mejor morfología?***

***¿Mayor velocidad ?***

***¿Mejor movilidad?***

¿ cuál es nuestro objetivo ?



**Conseguir el *nacimiento* de *un* niño *sano*,**
**de la forma más sencilla y segura posible.**

Seleccionar aquellos espermatozoides que den lugar a embriones sanos, con buena capacidad de implantar y desarrollarse de forma evolutiva.

# Mecanismos que inducen el daño espermático

1. **Apoptosis** o anomalías durante la espermatogénesis
2. **Roturas de ADN** durante el remodelado de la cromatina en la espermiogénesis.
3. **Fragmentación de ADN** post testicular durante el transporte en los tubulos seminíferos y epidídimo
4. **Daño de ADN** inducida por caspasas endógenas y endonucleasas
5. **Alteraciones de ADN** por causas exógenas

**La mayoría de las técnicas que existen nos informan de cual es el daño, pero no del origen ni de cómo seleccionar el mejor espermatozoide para ICSI.**

Dra. Rocío Núñez

# ¿QUÉ TENEMOS QUE HACER?

- Evitar daño durante el procesamiento
- Eliminar el plasma seminal
- Minimizar la reacción acrosómica
- Separar los espermatozoides anómalos (REM)
- Seleccionar espermatozoides: **no aneuploides**, **no apoptóticos, maduros, no fragmentados**

# Capacitación espermática

La capacitación ocurre en el tracto reproductivo femenino, aunque puede simularse en el laboratorio.

Asian Journal of Andrology (2019) 21, 1–7
www.asiaandro.com; www.ajandrology.com

Open Access

ORIGINAL ARTICLE

Sperm Biology

# Influence of *in vitro* capacitation time on structural and functional human sperm parameters

Paula Sáez-Espinosa[1,2], Natalia Huerta-Retamal[1], Laura Robles-Gómez[1], Manuel Avilés[3], Jon Aizpurua[4], Irene Velasco[1,5], Alejandro Romero[1], María José Gómez-Torres[1,6]

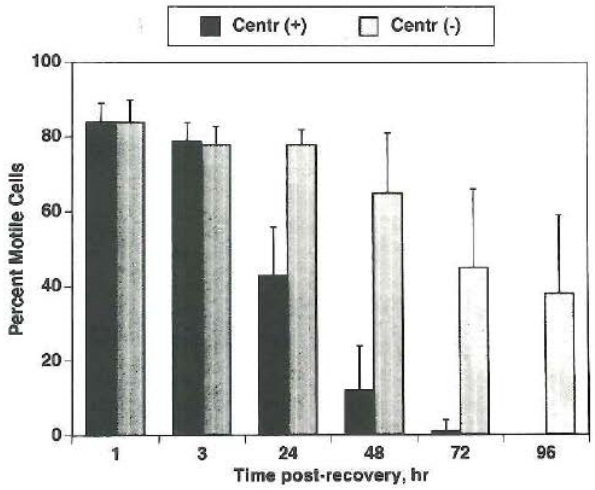
# Capacitación “in vitro” y daño espermático

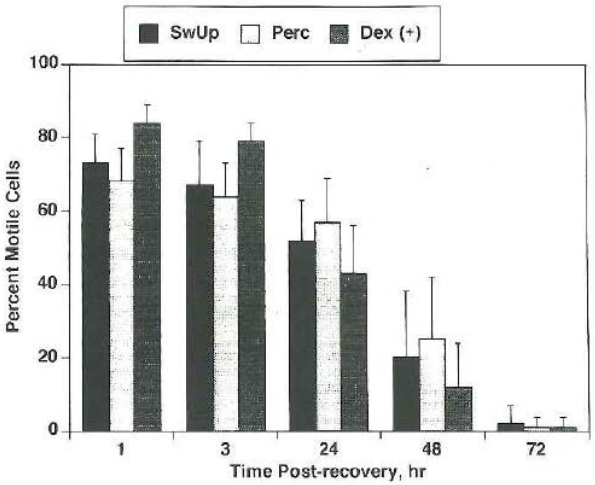
Human Reproduction vol.8 no.7 pp.1087–1092, 1993

## Centrifugation of human spermatozoa induces sublethal damage; separation of human spermatozoa from seminal plasma by a dextran swim-up procedure without centrifugation extends their motile lifetime

Juan G.Alvarez[1], Jaime L.Lasso, Luis Blasco, Rocio C.Nuñez[2], Susan Heyner, Pedro P.Caballero[2] and Bayard T.Storey[3]

*Key words*: motility loss/seminal plasma/sperm
tion/sublethal damage/swim-up

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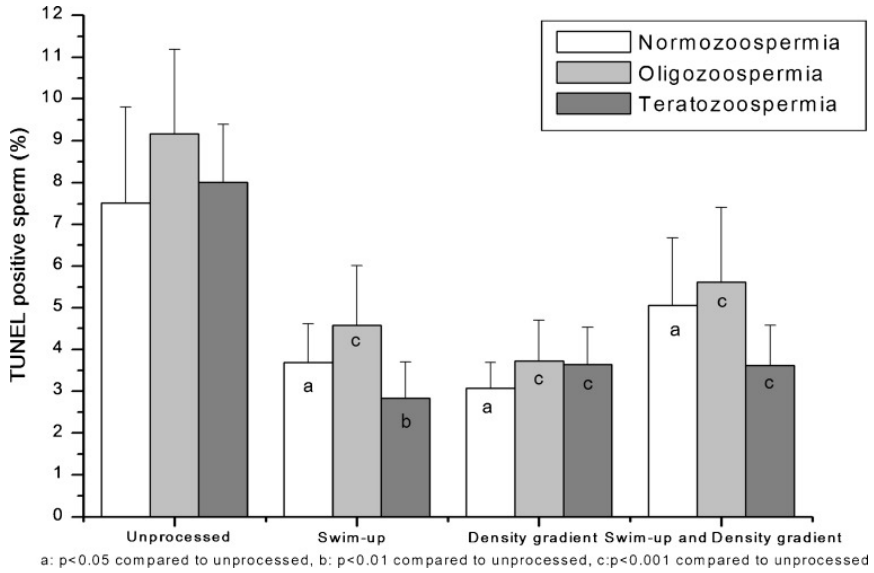
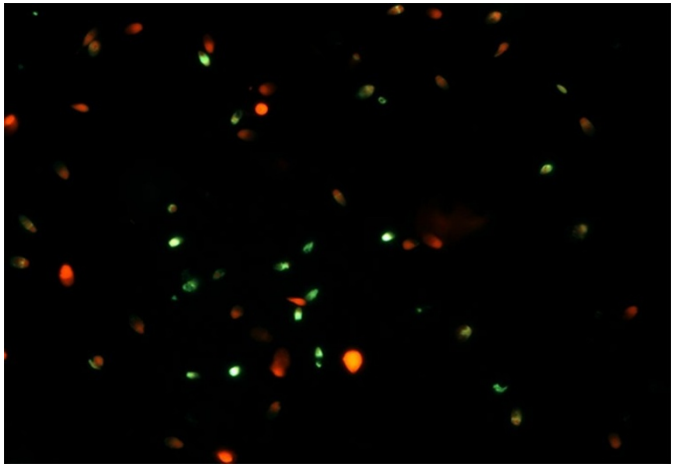
# Separar espermatozoides anómalos



Ability of various sperm wash techniques to eliminate sperms with DNA damage

**Sperm processing by swim-up and density gradient is effective in elimination of sperm with DNA damage.**

Javaraman V et al. J Assist Reprod Genet. 2012 June;29(6):557-563.

Dra. Rocío Núñez

# Separar espermatozoides anómalos

Asian Journal of Andrology (2013) 15, 812–818

www.nature.com/aja

ORIGINAL ARTICLE

## Can DNA fragmentation of neat or swim-up spermatozoa be used to predict pregnancy following ICSI of fertile oocyte donors?

Jaime Gosálvez[1], Pedro Caballero[2], Carmen López-Fernández[1], Leonor Ortega[2], José Andrés Guijarro[2], José Luís Fernández[3], Stephen D Johnston[4] and Rocío Nuñez-Calonge[2]

**Table 1 Mean (±s.d.) sperm DNA fragmentation (SDF) of neat (NS) and swim-up (SU) spermatozoa that resulted in pregnancy and non-pregnancy following ICSI of oocytes from proven donors**

| | *Pregnant (n=49)* | *Non-pregnant (n=32)* |
|---|---|---|
| Neat semen (NS) SDF | 25.3±14.5* | 34.9±14.0** |
| Swim-up (SU) SDF | 16.6±9.1* | 23.7±10.6** |

Significant differences ($P<0.001$) were obtained when NS and SU samples are compared within the pregnant group (*) and non-pregnant-group (**). Data are expressed as mean±s.d.

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# Separar espermatozoides anómalos

Asian Journal of Andrology (2011) 13, 764–768

www.nature.com/aja

ORIGINAL ARTICLE

## The ability of sperm selection techniques to remove single- or double-strand DNA damage

María Enciso[1], Miriam Iglesias[2], Isabel Galán[2], Jonás Sarasa[1], Antonio Gosálvez[2] and Jaime Gosálvez[1]

**Table 1 Semen quality parameters of the samples analysed before and after the DGC and SUP techniques were employed**

| *Semen parameters* | *Neat semen* | *DGC* | *SUP* |
|---|---|---|---|
| Total no. of spermatozoa ($\times 10^6$) | 168.22±9.17 | 8.59±0.84* | 14.18±0.71* |
| Progressive motility (%) | 52.84±1.23 | 71.38±9.70* | 82.74±0.97* |
| SCD-SDF (%) | 30.73±1.31 | 19.80±3.43* | 16.08±1.60* |
| SCD-SDF-d (%) | 8.47±0.47 | 4.75±0.83* | 2.94±0.41* |
| ssDD-TTC (%) | 46.75±3.00 | 25.54±5.84* | 39.44±5.55 |
| dsDD-TTC (%) | 16.66±1.48 | 4.20±0.80* | 5.98±0.97* |

Abbreviations: d, degraded spermatozoa; DGC, density gradient centrifugation; dsDD, double-strand DNA damage; SCD, sperm chromatin dispersion; SDF, sperm DNA fragmentation; ssDD, single-strand DNA damage; SUP, swim-up.
Values are expressed as mean±s.e.m.
*$P<0.05$, compared with neat semen, Mann–Whitney $U$ test.

# Separar espermatozoides anómalos

Reproductive BioMedicine Online (2015) 31, 44–50

www.sciencedirect.com
www.rbmonline.com

ELSEVIER

ARTICLE

## Processing of semen by density gradient centrifugation selects spermatozoa with longer telomeres for assisted reproduction techniques

Qingling Yang [a,1], Nan Zhang [a,1], Feifei Zhao [a], Wanli Zhao [a], Shanjun Dai [a], Jinhao Liu [a], Ihtisham Bukhari [b], Hang Xin [a], Wenbing Niu [a], Yingpu Sun [a,*]



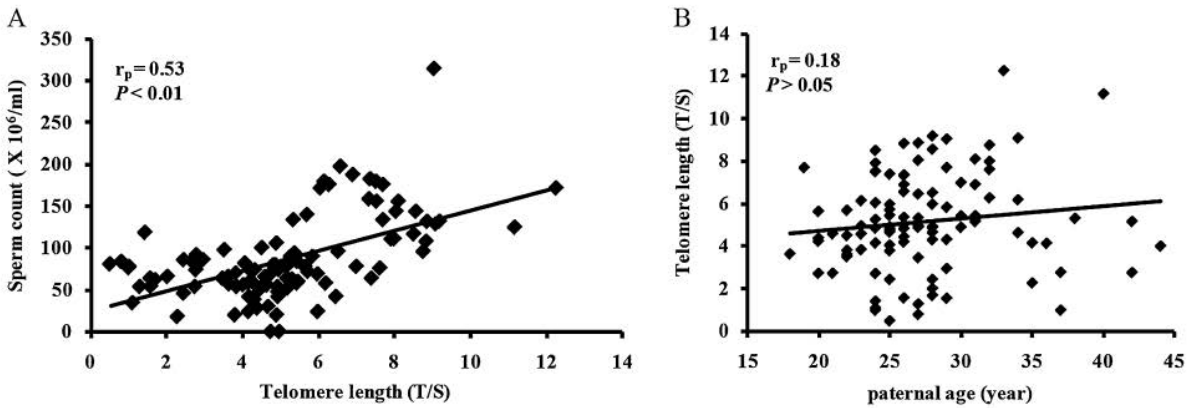


Figure 2 (A) Correlation between sperm telomere length and the total sperm count in raw semen ($n = 105$); (B) correlation between sperm telomere length and paternal age at the time of conception ($n = 105$).

Dra. Rocío Núñez

Asian Journal of Andrology (2012) 14, 260–269

www.nature.com/aja

REVIEW

# Sperm preparation: state-of-the-art—physiological aspects and application of advanced sperm preparation methods

Ralf Henkel

# Novel technologies for selecting the best sperm for in vitro fertilization and intracytoplasmic sperm injection

Denny Sakkas, Ph.D.

Boston IVF, Waltham, Massachusetts

VOL. 99 NO. 4 / MARCH 15, 2013

# NUEVAS TECNOLOGÍAS DE SELECCIÓN ESPERMÁTICA

**Summary of techniques reported to improve selection of the various problems encountered in a sperm sample.**

| Sperm selection technique | Normal | Oligo | Astheno | Terato | High DNA damage | High ROS | Comment |
|---|---|---|---|---|---|---|---|
| Sperm population preparation | | | | | | | |
| Swim-up | + | | | + | | | Density-gradient preparation preferred over swim-up for isolating better sperm |
| Density gradient | + | + | + | + | + | + | |
| Electrophoretic separation | ++ | + | ++ | ++ | ++ | ++ | May not be as useful for severe oligozoospermia |
| Microfluidics | ++ | + | ++ | ++ | | + | but reduces iatrogenic effects associated with sperm centrifugation |
| Apoptotic deselection | ++ | + | | + | ++ | + | May not be as useful for severe oligozoospermia |
| Surgical sperm retrieval | | | | | | | |
| Testicular/epididymal biopsy | ++ | | + | | ++ | ++ | Has been shown to be effective in patients with high DNA damage in ejaculated sperm and previous failures |
| Individual sperm selection | | | | | | | |
| HA binding | ++ | | | ++ | ++ | + | Promising clinical results but not effective when man has very low count and/or poor motility |
| Birefringence | ++ | + | | + | | | Limited clinical results |
| IMSI | ++ | ++ | ++ | ++ | ++ | ++ | Promising clinical results and more effective for men with very low count and/or poor motility; can be time consuming |
| HOST | + | + | + | + | + | | Effective for men with very low count and/or poor or no motility; technique successfully used historically for testicular biopsy patients |
| Experimental procedures | | | | | | | |
| RNA-based selection | | | | | | | Not yet proven clinically |
| Membrane peptides | | | | | | | |
| Proteomic analysis | | | | | | | |
| Raman | | | | | | | |

*Sakkas. Selecting the best sperm for IVF and ICSI. Fertil Steril 2013.*

# Selecting the most competent sperm for assisted reproductive technologies

Rajasingam S. Jeyendran, B.V.Sc., M.S., Ph.D.,[a] Ettore Caroppo, M.D.,[b] Alexandre Rouen, M.D., Ph.D.,[c] Anthony Anderson, D.H.Sc.,[d] and Elizabeth Puscheck, M.D., M.S., M.B.A.[e,f]

Fertility and Sterility® Vol. 111, No. 5, May 2019

That said, the broad utility of the HOS test is particularly worth noting in a summary of best practices for sperm selection. It is a simple and highly cost-effective test that has been shown to be a more reliable predictor of conception than the standard spermiogram, with nearly 90% accuracy. Although initially developed to evaluate the integrity of the sperm tail membrane (and therefore determine the functionality of the sperm's metabolic processes), HOS test results have been shown to correlate with a number of other sperm competence factors, including sperm head membrane functionality, sperm motility, SPA results, DNA fragmentation, and other key indicators of fertilization potential.

# OPTIMIZACIÓN DE LA CAPACITACIÓN ESPERMÁTICA

## CONCLUSIONES


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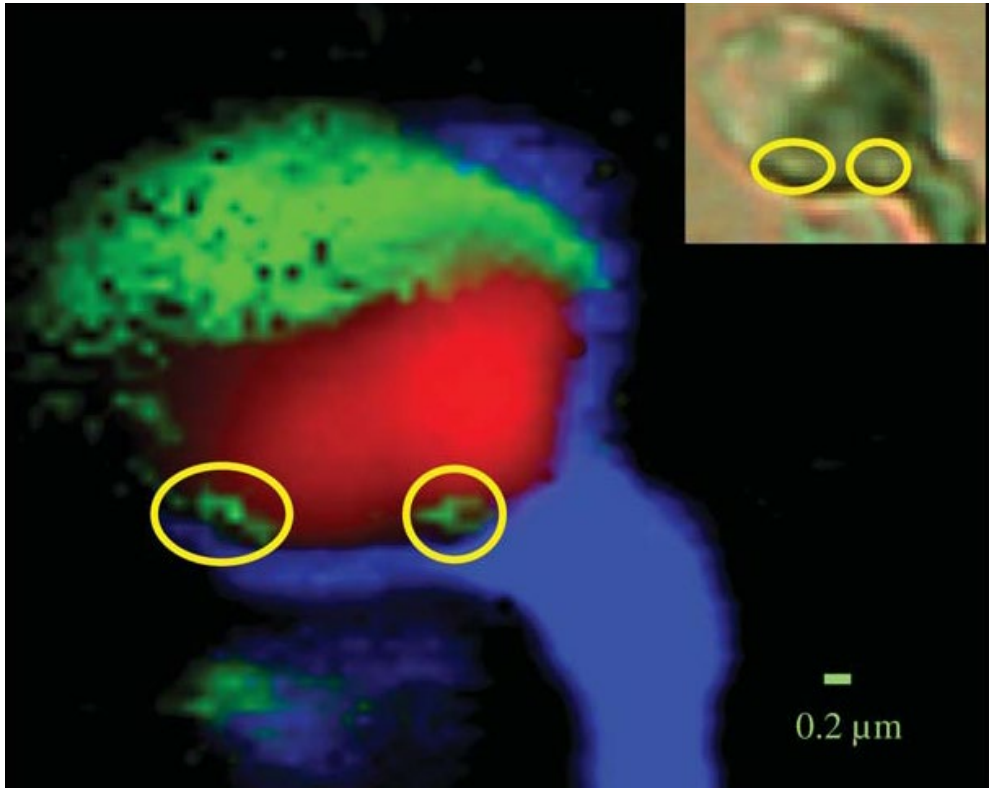
# NUEVAS TECNOLOGÍAS DE SELECCIÓN ESPERMÁTICA

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# Métodos de selección espermática

1. Carga eléctrica de la superficie
2. Apoptosis (MACS)
3. Morfología (IMSI)
4. Madurez espermática (unión a HA)
5. Temperatura
6. Espectro de luz nuclear (RAMAN)

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# Selección espermática por la carga eléctrica de la superficie

- Durante la maduración epididimaria, los espermatozoides adquieren tres formas de glicoproteínas cargadas negativamente y sializadas.

- El nivel de expresión de estas proteínas está correlacionado con el porcentaje de formas normales y el estado de capacitación.

- Basado en la carga eléctrica negativa, es posible separar espermatozoides por medio de potencial zeta y electroforesis

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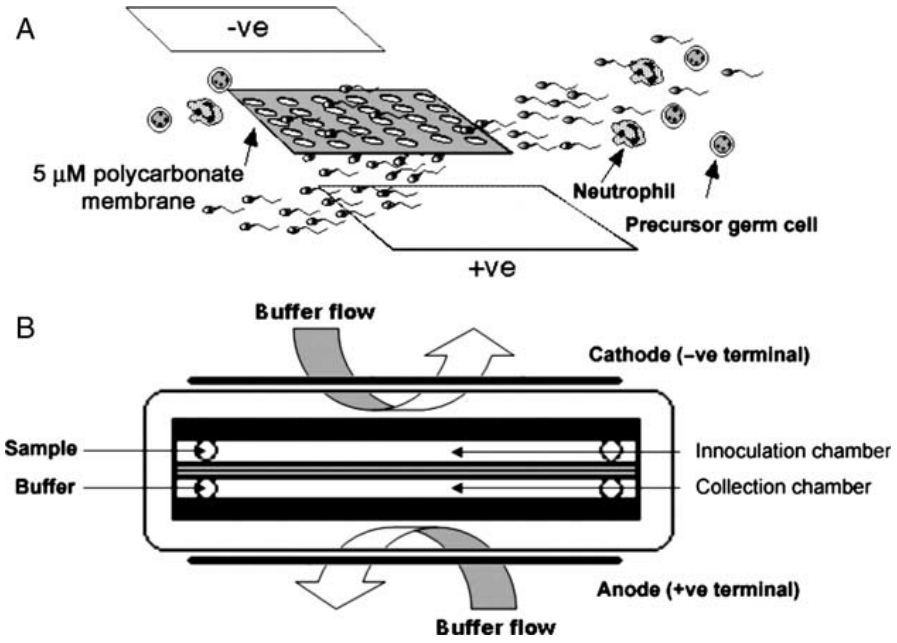
# Selección por la carga electromagnética espermática

**Human Reproduction Vol.22, No.1 pp. 197–200, 2007** doi:10.1093/humrep/del351
Advance Access publication September 13, 2006.

## First recorded pregnancy and normal birth after ICSI using electrophoretically isolated spermatozoa

**C.Ainsworth[1], B.Nixon[1], R.P.S.Jansen[2] and R.J.Aitken[1,3,4]**

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**A simple zeta method for sperm selection based on membrane charge.**

Chan PJ, Jacobson JD, Corselli JU, Patton WC.

Fertil Steril, 2006

Dra. Rocío Núñez

Reproductive BioMedicine Online (2011) 22, 37–43

www.sciencedirect.com
www.rbmonline.com

ARTICLE

# Easy sperm processing technique allowing exclusive accumulation and later usage of DNA-strandbreak-free spermatozoa

T Ebner [a,*], O Shebl [a], M Moser [a], RB Mayer [a], W Arzt [b], G Tews [a]

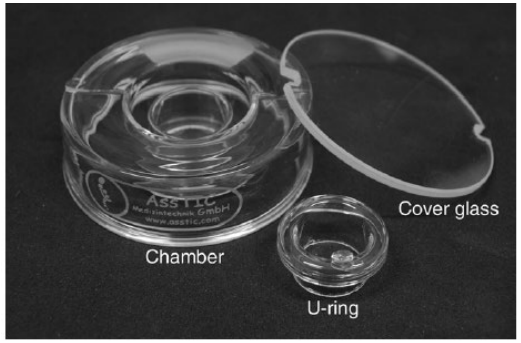



Figure 2 The Zech-selector. The outer diameter of the glass chamber is 6.3 cm. The diameter of the inner circle, considered for concentration of motile spermatozoa, is 2 cm.

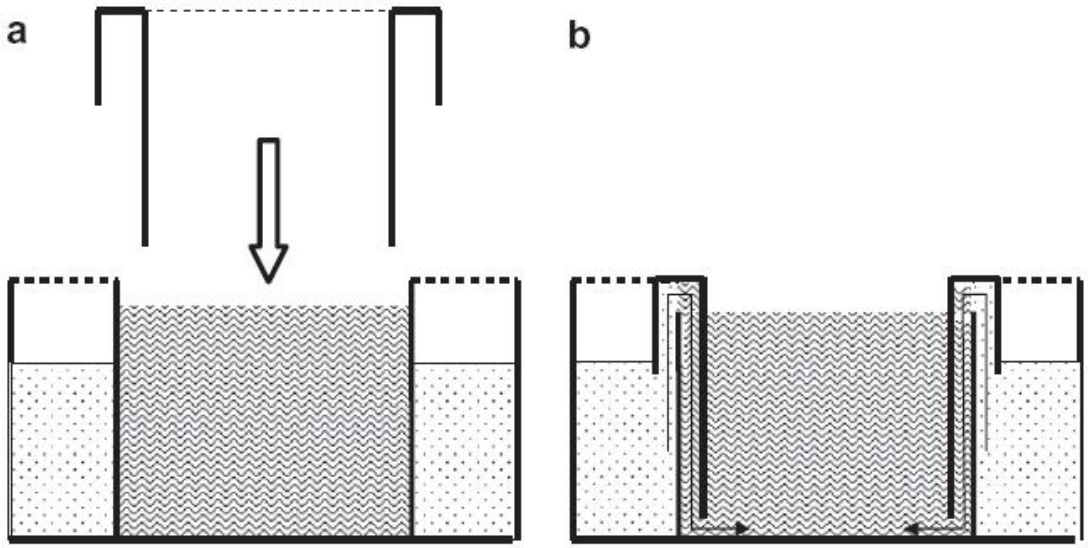


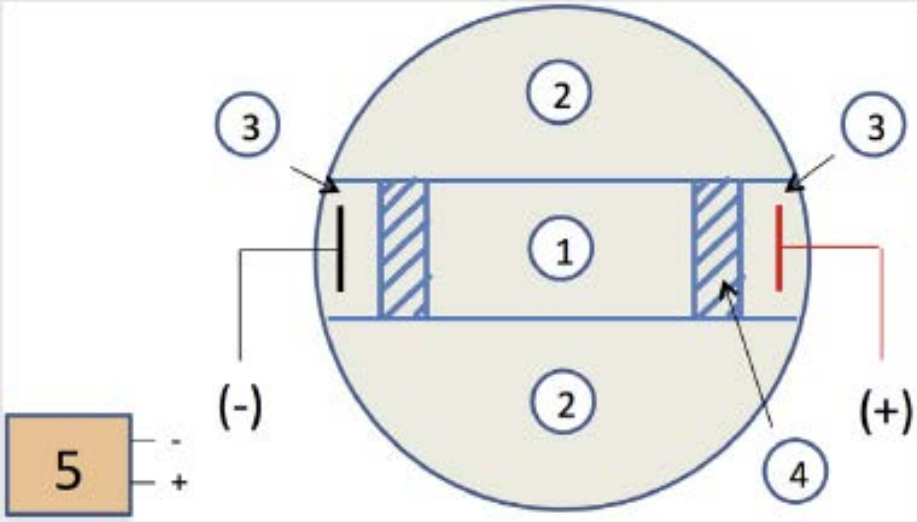

**Figure 1** Schematic cross-section of the sperm selector indicating the ejaculate in the outer ring (dots) and medium in the centre well (wave lines). Cover glass is not shown. (**a**) Sperm selector consists of the actual chamber (glass or polyethylene) and a U-ring (top left). (**b**) After filling of the chamber, the U-ring is inserted and creates a capillary bridge allowing motile spermatozoa to swim to the centre well (theoretical path of migration indicated by arrow).

ORIGINAL ARTICLE: ANDROLOGY

# Micro-electrophoresis: a noninvasive method of sperm selection based on membrane charge

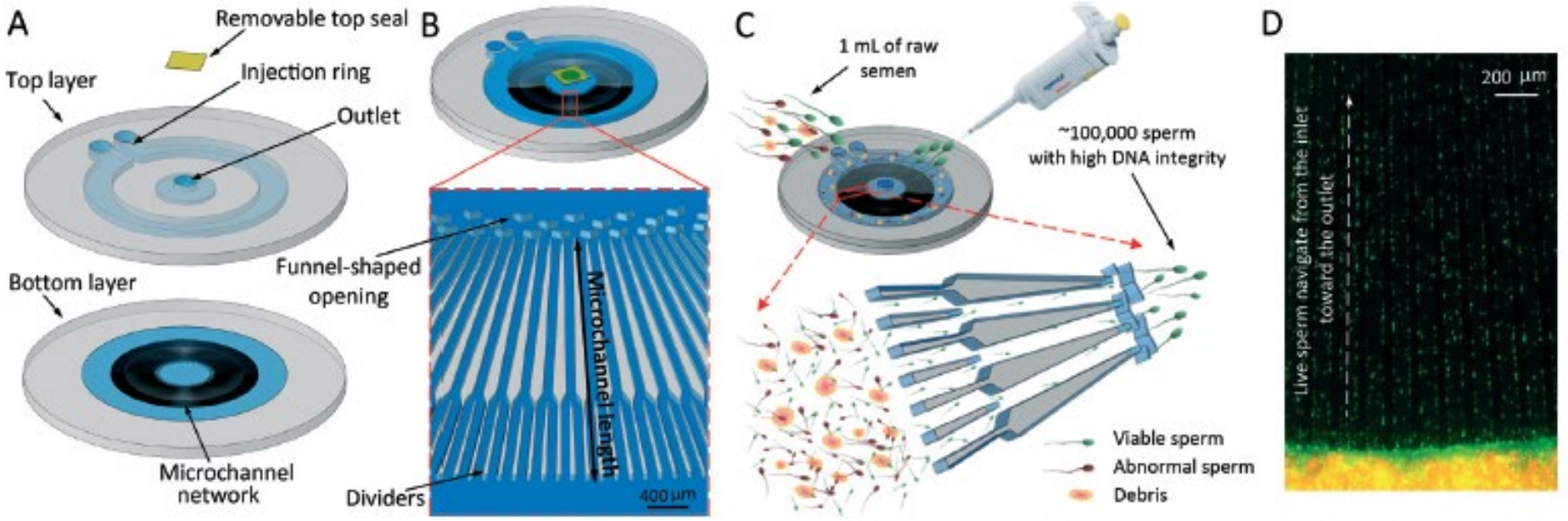
Luke Simon, Ph.D.,[a] Kristin Murphy, Ph.D.,[a] Kenneth I. Aston, Ph.D.,[a] Benjamin R. Emery, M.Phil.,[a] James M. Hotaling, M.D.,[a] and Douglas T. Carrell, Ph.D.[a,b,c]

[a] Andrology and IVF Laboratory, Department of Surgery (Urology), [b] Department of Obstetrics and Gynecology, and [c] Department of Human Genetics, University of Utah, Salt Lake City, Utah




Schematic representation of micro-electrophoresis instrument. Electrophoresis chamber (1), egg injection chambers (2) and bubble restriction chambers (3), conductive bridge (4), and power pack (5).

*Simon. Sperm selection by micro-electrophoresis. Fertil Steril 2014.*



# Métodos de selección por carga magnética

| Método | Ventajas | Inconvenientes | Resultados TRA |
|---|---|---|---|
| Electroforesis | -Rápido<br>-No centrifugación<br>-<morfoanomalías<br><leucocitos<br><inmaduros<br>< fragm. ADN | - Complejidad aparataje<br><br>- Electricidad | 1 pareja, 1 nacimiento |
| Potencial zeta | -Fácil<br>-Barato<br>< fragm.ADN<br><morfoanomalías | - < recuperación espermática<br><br>- < movilidad | PR 54% vs 33% (NS) |
| | | | |

Dra. Rocío Núñez

# Isolation of spermatozoa with low levels of fragmented DNA with the use of flow cytometry and sorting

Sofia C. Ribeiro, Ph.D.,[a] Gideon Sartorius, M.D.,[a] Flurina Pletscher, M.Sc.,[b] Maria de Geyter, Ph.D.,[a] Hong Zhang, Ph.D.,[b] and Christian de Geyter, M.D.[a,b]

[a] Clinic of Gynecological Endocrinology and Reproductive Medicine and [b] Department of Biomedicine, University of Basel, Basel, Switzerland

Fertility and Sterility® Vol. 100, No. 3, September 2013

# Selección de espermatozoides no apoptóticos

- Basada en la externalización de la PS en la superficie externa de la mb espermática como señal de apoptosis.

- La PS permite la unión con microbeads de Anexina-V utilizando sistemas magnéticos (MACS) para separar espermatozoides apoptóticos.

- La fuerza magnética causa la retención de las células unidas a los beads en la columna.

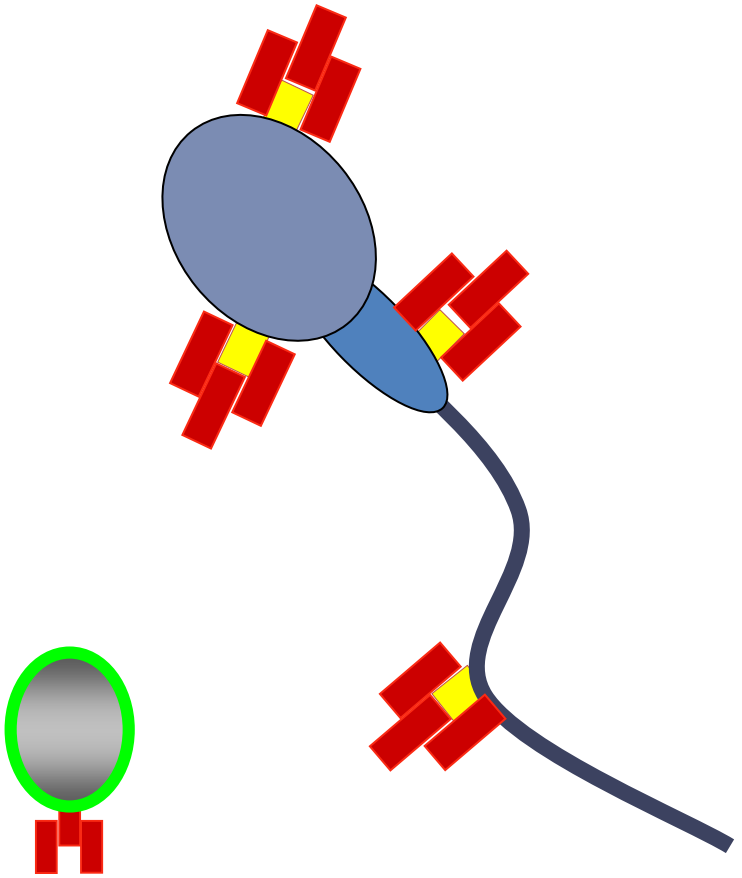
# COLUMNAS DE ANEXINA ( MACS )

## ¿ CÓMO FUNCIONAN ?. (fundamento)

*El espermatozoide apoptótico presenta en su membrana Fosfatidilserina.*

*La Fosfatidilserina tiene afinidad por la Anexina V.*

*Las Anexinas están adheridas a microesferas de metal, recubiertas por un polímero biodegradable.*

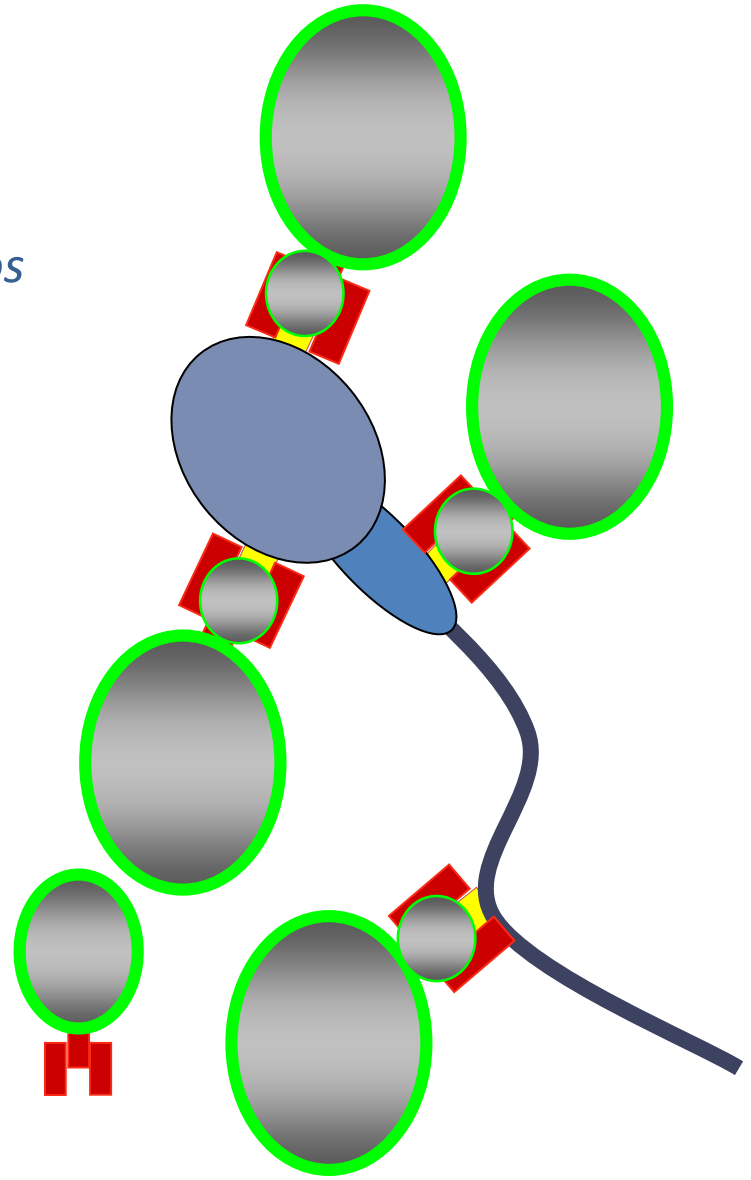


Dra. Rocío Núñez

# COLUMNAS DE ANEXINA ( MACS )

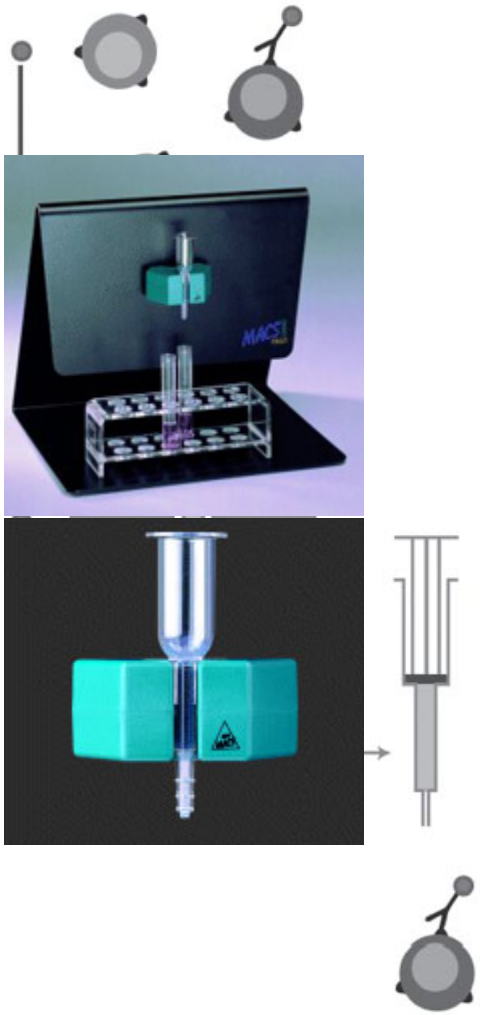
*Al incubarlas con los espermatozoides las microesferas metálicas se unen a los espermatozoides apoptóticos.*

*Al hacer pasar los espermatozoides a través de un campo magnético, aquellos con microesferas pegadas quedan atrapados.*



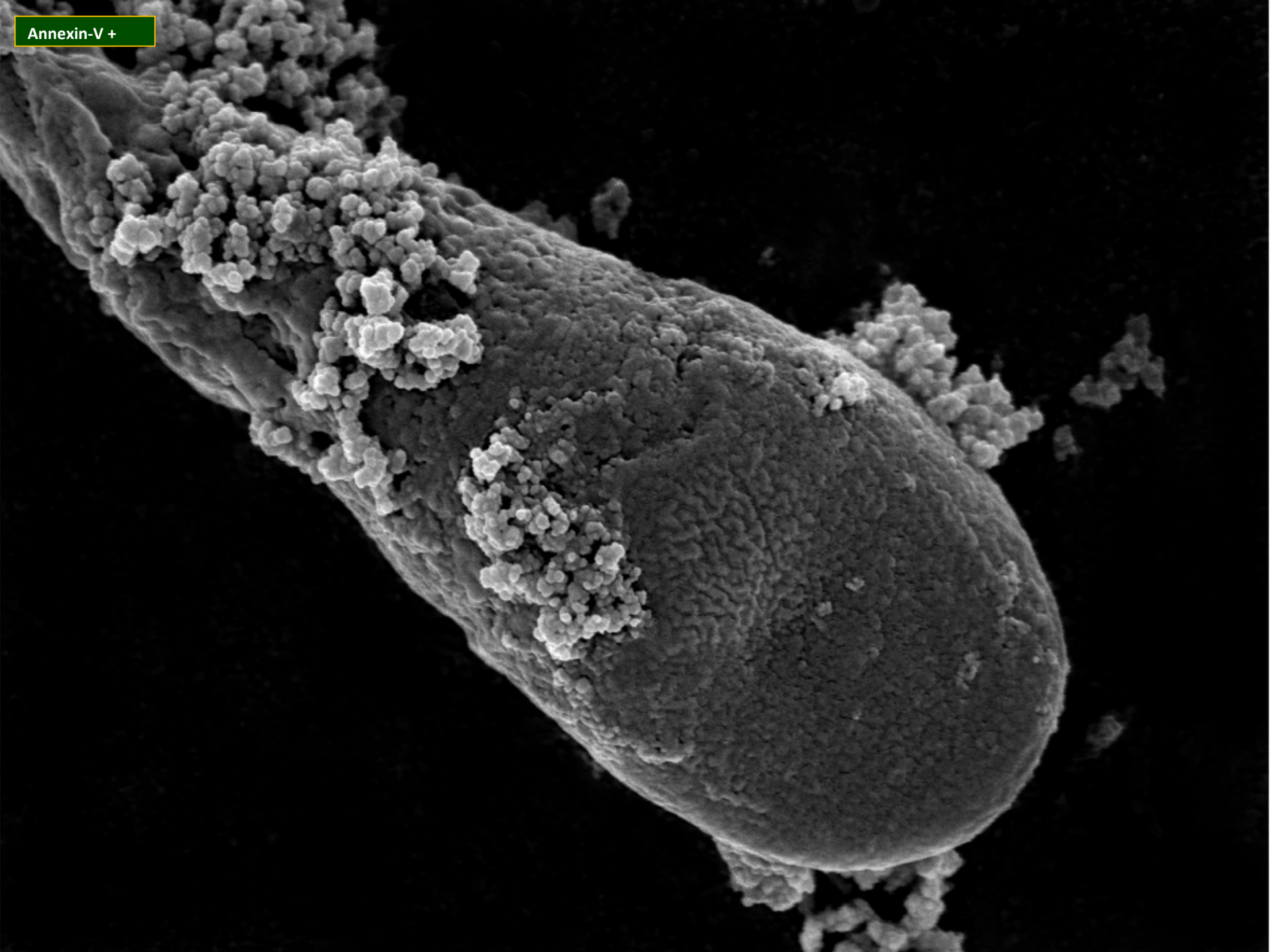
Dra. Rocío Núñez

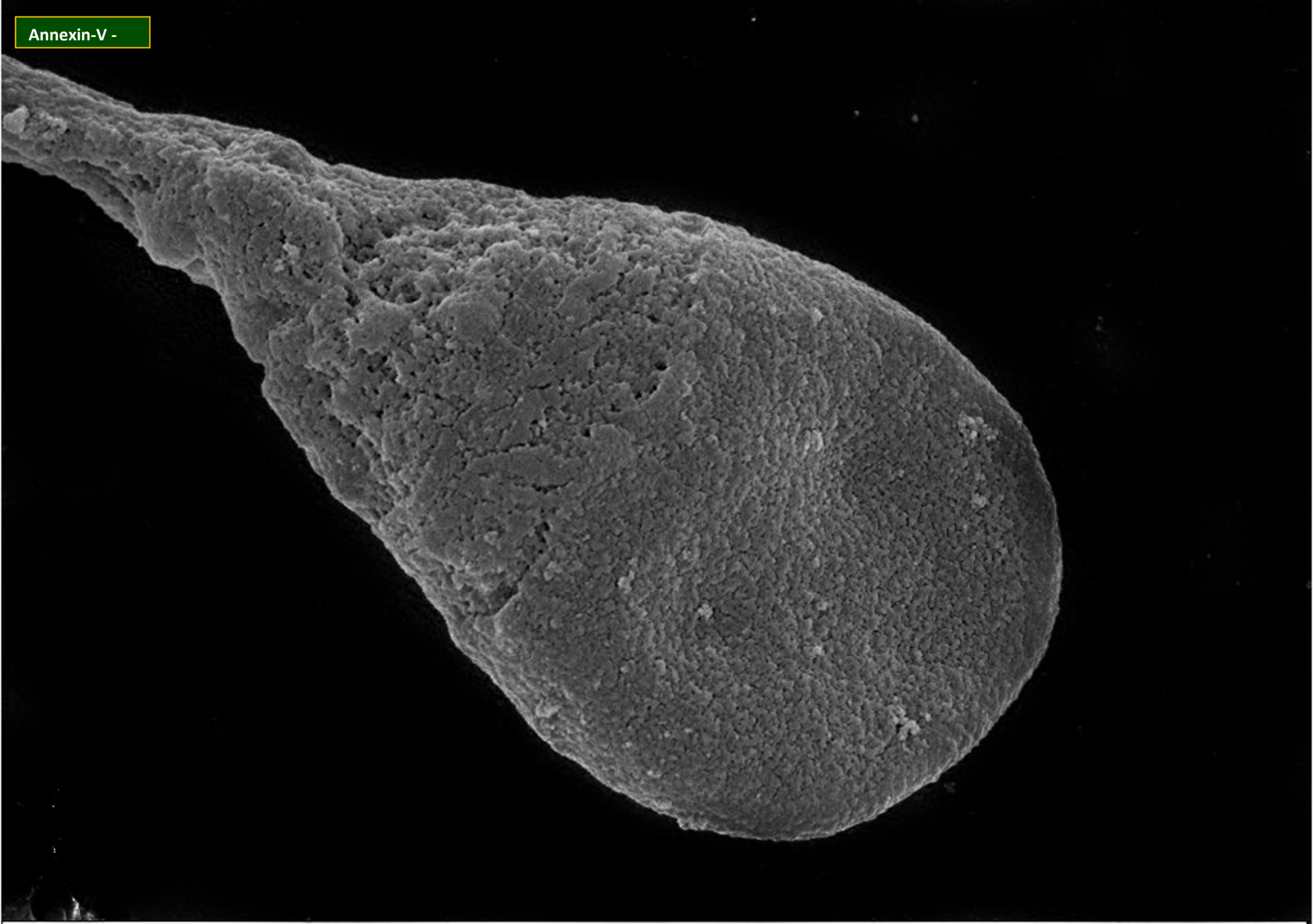
# Columnas de Annexin-V



- **Annexin-V negative fraction:** FIV, ICSI, IAC
- **Annexin-V positive fraction:** desechar

Dra. Rocío Núñez


Annexin-V +


Annexin-V -
200 nm
EHT = 3.00 kV
WD = 3.8 mm
Mag = 50.00 K X
Signal A = InLens

J Assist Reprod Genet (2013) 30:479–485
DOI 10.1007/s10815-013-9962-8

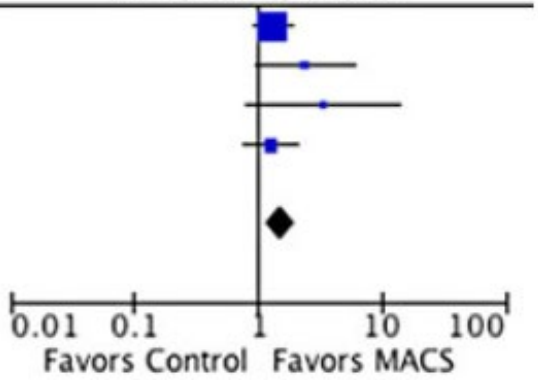
TECHNOLOGICAL INNOVATIONS

# Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: a systematic review and meta-analysis

**Monica Gil · Valerie Sar-Shalom · Yolisid Melendez Sivira · Ramon Carreras · Miguel Angel Checa**

| Study or Subgroup | MACS Events | MACS Total | Control Events | Control Total | Weight | Risk Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| Dirican 2008 | 59 | 122 | 27 | 74 | 62.6% | 1.33 [0.93, 1.88] |
| Khalid 2011 | 12 | 30 | 5 | 30 | 9.3% | 2.40 [0.96, 5.98] |
| Romany 2010 | 9 | 32 | 2 | 24 | 4.3% | 3.38 [0.80, 14.21] |
| San Celestino 2011 | 16 | 27 | 13 | 28 | 23.8% | 1.28 [0.77, 2.12] |
| **Total (95% CI)** | | **211** | | **156** | **100.0%** | **1.50 [1.14, 1.98]** |
| Total events | 96 | | 47 | | | |

Heterogeneity: $Chi^2 = 3.11$, df = 3 (P = 0.37); $I^2 = 4\%$
Test for overall effect: Z = 2.90 (P = 0.004)




Rocío Núñez

REPRODUCTIVE BIOMEDICINE ONLINE 34 (2017) 506–512

Article

# Magnetic cell sorting of semen containing spermatozoa with high DNA fragmentation in ICSI cycles decreases miscarriage rate

*Pascual Sánchez-Martín* [a], *Mónica Dorado-Silva* [a], *Fernando Sánchez-Martín* [a], *Mercedes González Martínez* [a], *Stephen D Johnston* [b], *Jaime Gosálvez* [c,*]

**Table 2 – Frequency of clinical pregnancy and miscarriage in couples where sperm samples were treated with DGC alone or DCG with MACS prior to autologous or oocyte donor ICSI.**

| Parameter | DGC only | DGC + MACS |
|---|---|---|
| AUTO-ICSI | | |
| Clinical pregnancy | 93 | 26 |
| Miscarriage | 7 | 0 |
| DONOR-ICSI | | |
| Clinical pregnancy | 32 | 26 |
| Miscarriage | 3 | 0 |

AUTO-ICSI, autologous-ICSI; DONOR-ICSI, oocyte donor-ICSI; DGC, density gradient centrifugation; MACS, magnetic activated cell sorting.

J Assist Reprod Genet. 2018 Dec;35(12):2215-2221. doi: 10.1007/s10815-018-1319-x. Epub 2018 Sep 17.

# Magnetic-activated cell sorting is not completely effective at reducing sperm DNA fragmentation.

Martínez MG[1], Sánchez-Martín P[1], Dorado-Silva M[1], Fernández JL[2], Girones E[3], Johnston SD[4,5], Gosálvez J[3].

⊕ Author information

## Abstract

**PURPOSE:** To determine whether there is a homogeneous reduction of sperm DNA fragmentation (SDF) in sperm samples recovered from the MACS procedure, compared to spermatozoa in the initial ejaculate (NEAT) and those retained in the column.

**METHODS:** This study investigated the relative change in sperm DNA quality (SDF) of neat ejaculates (10 idiopathic infertile and 10 normozoospermic patients) to subpopulations of spermatozoa that had passed through the column (MACS-) and those retained (MACS+) by the annexin-V conjugated microbeads.

**RESULTS:** While the MACS protocol was capable of reducing the mean proportion of SDF (59.2%; P = 0.000) and sperm with highly degraded DNA (SDD; 65.7%, P = 0.000) in all patients, the reduction was not homogeneous across the patient cohort. A significant positive correlation (r = 0.772, P = 0.000) was apparent between the level of SDF in the NEAT ejaculate and the efficacy of SDF reduction observed in the MACS- fraction.

**CONCLUSION:** MACS is capable of reducing the proportion of SDF, especially spermatozoa with a highly degraded DNA molecule. However, this reduction did not preclude the presence of a small subpopulation of spermatozoa with damaged DNA in the MACS- fraction. The MACS protocol was two- to threefold more efficient when the SDF in NEAT ejaculate was equal to or greater than 30%. In 4 of 20 individuals, the level of SDF after MACS resulted in semen for ICSI with a higher or non-significant reduction when compared to SDF observed in the NEAT ejaculate.

**KEYWORDS:** MACS; Male factor; Sperm DNA fragmentation

# Removal of annexin V–positive sperm cells for intracytoplasmic sperm injection in ovum donation cycles does not improve reproductive outcome: a controlled and randomized trial in unselected males

Laura Romany, Ph.D., Nicolás Garrido, Ph.D., Yamileth Motato, Ph.D., Belén Aparicio, Ph.D., José Remohí, M.D., and Marcos Meseguer, Ph.D.

Instituto Universitario IVI Valencia, University of Valencia, Valencia, Spain

**Objective:** To determine the effect of removing presumptive apoptotic sperm cells from samples from unselected males by means of magnetic activated cell sorting (MACS) on live-birth delivery rates after intracytoplasmic sperm injection (ICSI) in couples undergoing ovum donation (OD).
**Design:** Prospective, randomized, triple-blinded, and controlled study.
**Setting:** Private university-affiliated IVF center.
**Patient(s):** A total of 237 infertile couples undergoing ICSI as part of an OD program.
**Intervention(s):** Semen specimens from the control group were prepared by swim-up. Samples from the study group were prepared by swim-up followed by MACS and incubation with annexin V–conjugated microbeads to remove annexin V–positive (AV+) sperm cells.
**Main Outcome Measure(s):** Fertilization rates, morphological features of early embryo development, implantation rates, ongoing pregnancy rates, and live-birth rates.
**Result(s):** Similar results were obtained between groups for all the parameters compared: fertilization rates of 75.3% (95% confidence interval [CI], 71.6–78.9) versus 72.1% (95% CI, 68.6–75.7); percentage of good-quality embryos on day 2 of 53.7% (95% CI, 50.3–57.1) versus 51.8% (95% CI, 48.3–55.3) and on day 3 of 54.2% (95% CI, 50.7–57.6) versus 48.9% (95% CI, 45.3–52.4); implantation rates of 42.2% (95% CI, 33.8–48.1) versus 40.1% (95% CI, 34.8–49.6); positive beta-hCG tests of 63.2% (95% CI, 54.7–71.6) versus 68.6% (95% CI, 60.2–76.9), and live-birth rates of 48.4% (95% CI, 39.6–57.1) versus 56.4% (95% CI, 47.3–65.5) in the MACS versus control group. None of the differences reached statistical significance.
**Conclusion(s):** Applying MACS technology to remove AV+ sperm cells from unselected males does not improve the reproductive outcome of ICSI in OD. (Fertil Steril® 2014;102:1567–75.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ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

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Marga Esbert, IVI Barcelona, Ronda General Mitre 14, 08017 Barcelona, Spain.
E-mail: marga.esbert@ivi.es

Keywords:
apoptosis, ART/assisted reproduction, chromosome abnormalities, IVF, MACS columns, spermatozoa

Received: 15-Jan-2017
Revised: 8-Mar-2017
Accepted: 9-Apr-2017

doi: 10.1111/andr.12376

# Spermatozoa with numerical chromosomal abnormalities are more prone to be retained by Annexin V-MACS columns

[1]M. Esbert, [2,a]A. Godo, [3]S. R. Soares, [1]M. Florensa, [1]D. Amorós, [1]A. Ballesteros and [2]F. Vidal

[1]IVI Barcelona, Barcelona, Spain, [2]Genetics of Male Fertility Group, Unitat de Biologia Cellular (Facultat de Biociències), Universitat Autònoma de Barcelona, Bellaterra, Spain, [3]IVI Lisboa, Lisboa, Portugal, and [a]Current address: Centro de Medicina Embrionaria, Barcelona, Spain

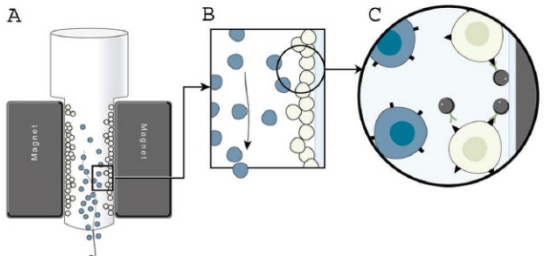
J Assist Reprod Genet (2017) 34:201–207
DOI 10.1007/s10815-016-0838-6

ASSISTED REPRODUCTION TECHNOLOGIES

# Obstetric and perinatal outcome of babies born from sperm selected by MACS from a randomized controlled trial

Laura Romany[1] · Nicolas Garrido[1] · Ana Cobo[1] · Belen Aparicio-Ruiz[1] · Vicente Serra[1] · Marcos Meseguer[1,2]

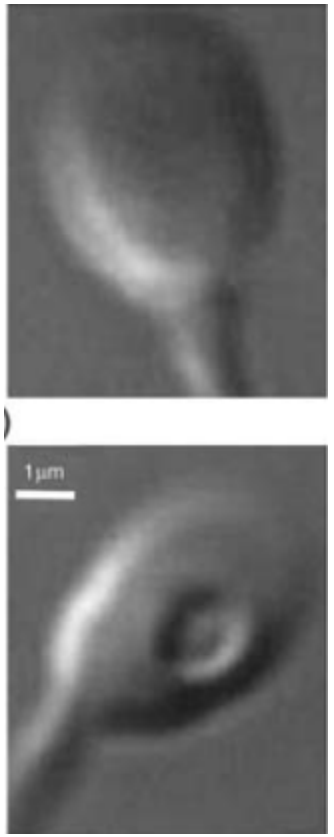
# *Columnas de Anexina V*




| Ventajas | Inconvenientes |
|---|---|
| **Selección de muestras de semen con bajo IF (?)** | Pérdida del número de espermatozoides por sucesivos lavados: no se puede utilizar en muestras con OATz severa |
| **Fácil manejo** | Posibilidad de presencia de microbeads en la muestra |
| **Barato (?)** | No está confirmada la acción del campo magnético en los espermatozoides |

# Selección basada en la morfología espermática:

**MSOME**: Motile sperm organelle morphological examination
(Nomarski x 6300). Estatus morfológico de:

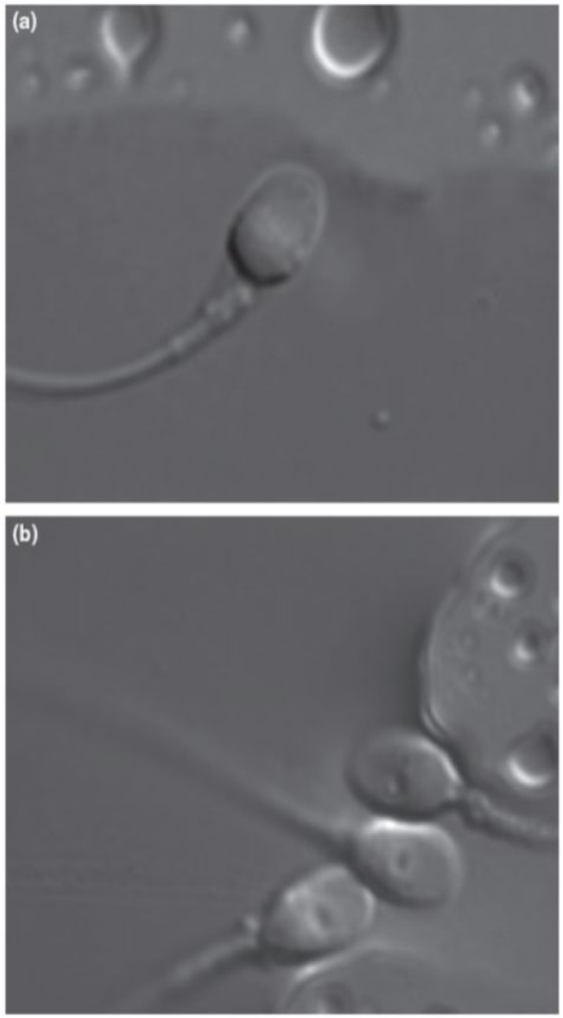



- Acrosoma
- Lámina post-acrosomal
- Cuello
- Mitocondrias
- Flagelo
- Núcleo

Forma, presencia y tamaño de vacuolas

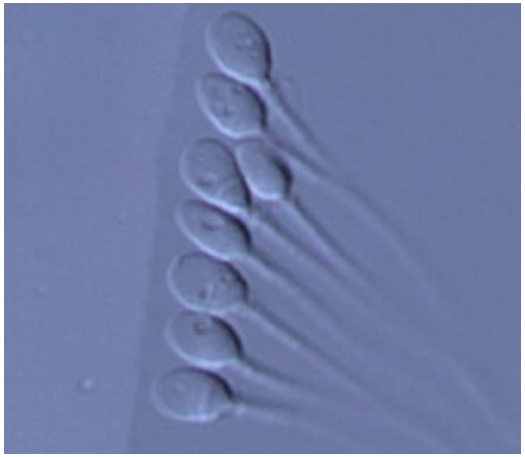
# Vacuolas nucleares

- Concavidades nucleares
  - Relacionadas con fallo de condensación cromatina
    - *Boitrelle et al, 2013*
- Origen controvertido
  - Condiciones in vitro
    - *Peer et al, 2007*
  - Estadíos tempranos de maduración
    - *Neyer et al, 2013*
- Afecta negativamente
  - Desarrollo embrionario
    - *Vanderzwalmen et al, 2008; Cassuto et al, 2009; Knetz et al, 2013*
  - Resultado
    - *Berkovitz et al, 2006; Cassuto et al, 2009; Nadalini et al, 2009; Greco et al, 2013*



# IMSI – PROS

- Influencia positiva en el desarrollo embrionario
  - *Vanderzwalmen et al, 2008; Knetz et al, 2013*
- Aumento de la tasa de embarazo
- Reducción de los abortos
  - *Souza Setti et al, 2010*
- Menor tasa de defectos del nacimiento
  - *Cassuto et al, 2013*
- ICSI más fisiológico
  - *Parmegiani et al, 2010*

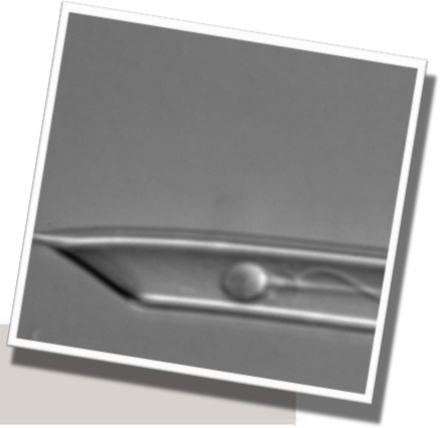


Dra. Rocío Núñez

# IMSI - CONTRAS

- Caro
  - *Bartoov et al, 2003*
- time consuming: aprox. 120 minutos
  - *Antinori et al, 2008*
- Ausencia de espermatozoides top-quality, no mejoras
  - *Cassuto et al, 2009*
- Faltan estudios prospectivos serios que demuestren mejora
  - *De Vos et al, Hum Reprod 2013*
- Indicación confirmada solo para fallo de ICSI
  - *Boitrelle et al, 2013*

Human Reproduction, Vol.28, No.3 pp. 617–626, 2013
Advanced Access publication on January 4, 2013 doi:10.1093/humrep/des435



human reproduction

ORIGINAL ARTICLE *Embryology*

# Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

**A. De Vos*, H. Van de Velde, G. Bocken, G. Eylenbosch, N. Franceus, G. Meersdom, S. Tistaert, A. Vankelecom, H. Tournaye, and G. Verheyen**

**WIDER IMPLICATIONS OF THE FINDINGS:** The prevalence of vacuoles in normal-shaped spermatozoa is as low as 27.5%. A routine application of IMSI in unselected artificial reproductive technology patients cannot be advocated.

# Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction (Review)

Teixeira DM, Barbosa MAP, Ferriani RA, Navarro PA, Raine-Fenning N, Nastri CO, Martins WP

THE COCHRANE COLLABORATION®



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http://www.thecochranelibrary.com

# Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction (Review)

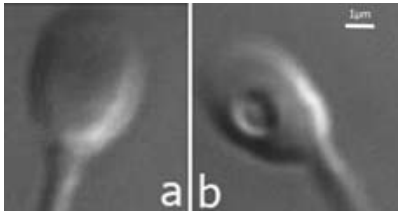
## Objectives

### Authors' conclusions

Results from RCTs do not support the clinical use of IMSI. There is no evidence of effect on live birth or miscarriage and the evidence that IMSI improves clinical pregnancy is of very low quality. There is no indication that IMSI increases congenital abnormalities. Further trials are necessary to improve the evidence quality before recommending IMSI in clinical practice.


### Authors' conclusions

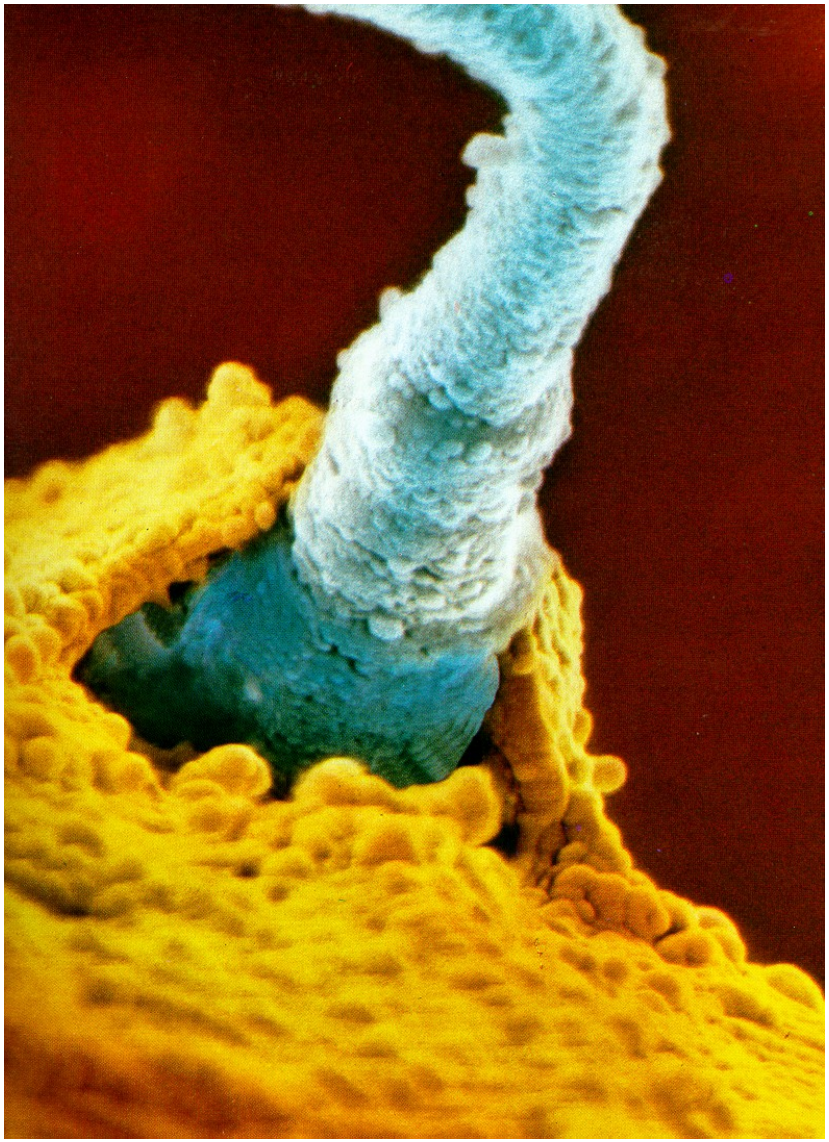
Results from RCTs do not support the clinical use of IMSI. There is no evidence of effect on live birth or miscarriage and the evidence that IMSI improves clinical pregnancy is of very low quality. There is no indication that IMSI increases congenital abnormalities. Further trials are necessary to improve the evidence quality before recommending IMSI in clinical practice.




## *IMSI (intracytoplasmic morphologically selected sperm injection).*

| Ventajas | Inconvenientes |
|---|---|
| **Selección de espermatozoides morfológicamente normales** | No hay clara asociación con aumento de la tasa de gestación |
| | Demasiado tiempo para la búsqueda espermática que puede ser lesivo para los espermatozoides en PVP y/o para los ovocitos. |
| | Muy caro |
| | Falta de subjetividad en la selección |
| | Necesita personal especializado |

# Selección basada en madurez espermática



Dra. Rocío Núñez

# Tècnicas de evaluación de la madurez nuclear de los espermatozoides

- *Azul de anilina*: detecta el exceso de histonas

  Discrimina entre la presencia histonas ricas en lisina y arginina y protaminas ricas en cisteina en el nucleo espermático.

- *Cromomicina A3*: evalúa la deficiencia de protaminas

- *Naranja de acridina*
- *Azul de toluidina*

} empaquetamiento de la cromatina

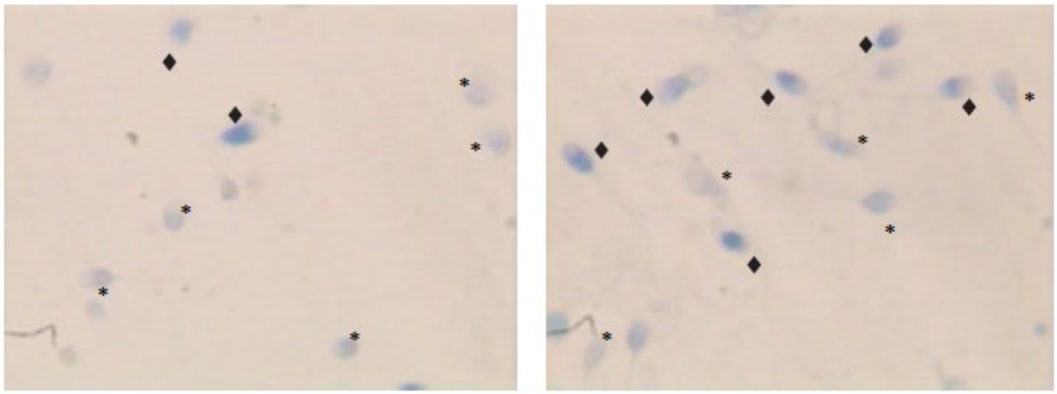
Dra. Rocío Núñez

Hindawi Publishing Corporation
Advances in Urology
Volume 2013, Article ID 578631, 8 pages
http://dx.doi.org/10.1155/2013/578631

*Research Article*

# Assessment of Chromatin Maturity in Human Spermatozoa: Useful Aniline Blue Assay for Routine Diagnosis of Male Infertility

**Afifa Sellami,[1,2] Nozha Chakroun,[1,2] Soumaya Ben Zarrouk,[1,2] Hanen Sellami,[1] Sahbi Kebaili,[3] Tarek Rebai,[1,2] and Leila Keskes[1]**



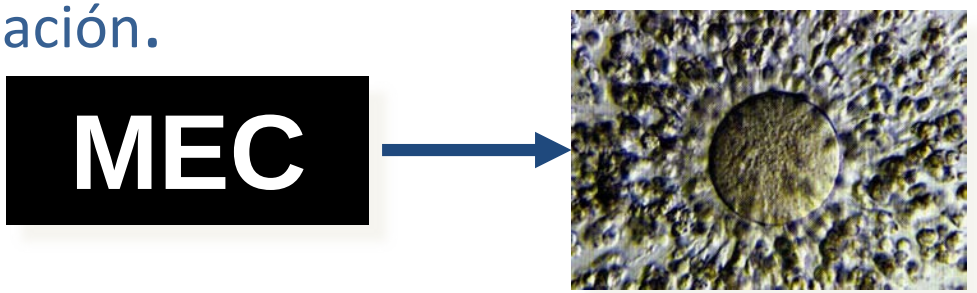
(a) (b)

FIGURE 1: Sperm chromatin condensation assessed by aniline blue. (a) Sample showing mainly mature sperm with unstained nucleus (∗). (b) Sample showing mainly immature sperm (♦) with blue-stained nucleus.

# PAPEL DEL ÁCIDO HIALURÓNICO

## FERTILIZACIÓN

- El ácido hialurónico (HA) está normalmente presente en la matriz extracelular (MEC) del cúmulo, rodeando el ovocito en el momento de la fecundación.




- La MEC es una barrera que tiene que atravesar el espermatozoide para alcanzar la ZP y fecundar el ovocito.

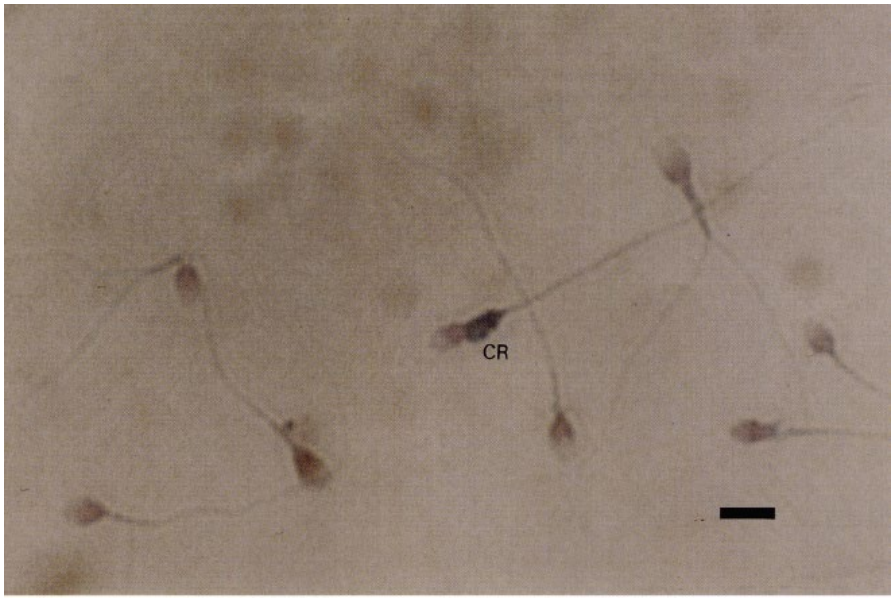
Dra. Rocío Núñez

*Journal of Reproduction and Fertility* (1997) **110**, 71–77

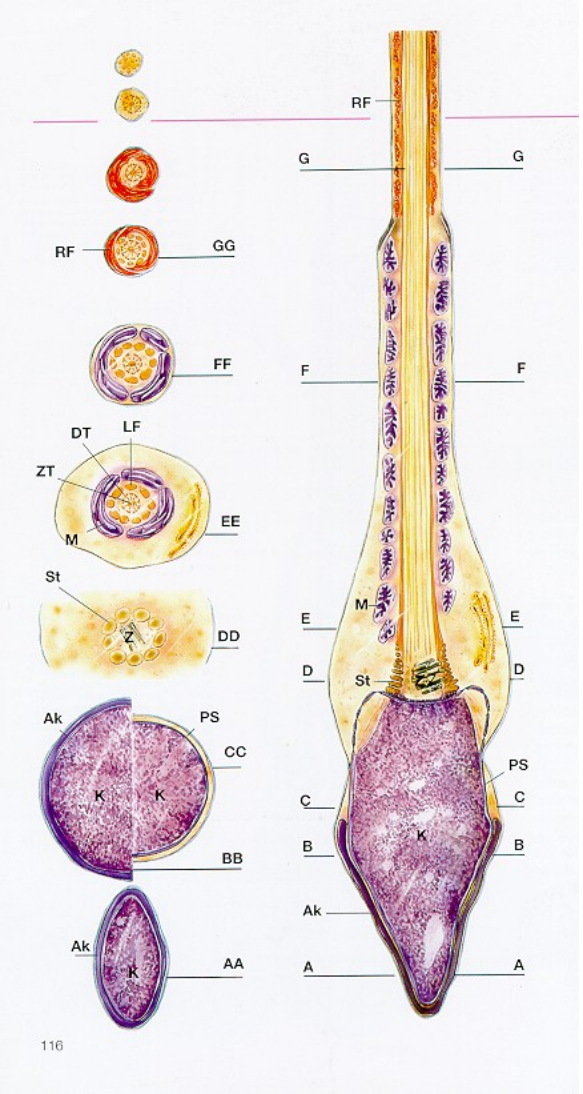
# Investigation of the association between the presence of cytoplasmic residues on the human sperm midpiece and defective sperm function

J. Keating[1,2], C. E. Grundy[1], P. S. Fivey[1], M. Elliott[1] and J. Robinson[1,2]

[1]*Department of Biological Sciences, University of Hull, Hull HU6 7RX, UK; and* [2]*Hull IVF Unit, The Princess Royal Hospital, Saltshouse Road, Hull HU8 9HE, UK*



# Selección de espermatozoides maduros




En la maduración espermática normal, HspA2 se expresa en el complejo sinaptonémico de los espermatocitos. Está implicado en:

-Extrusión citoplásmica

-Remodelación membrana plasmática

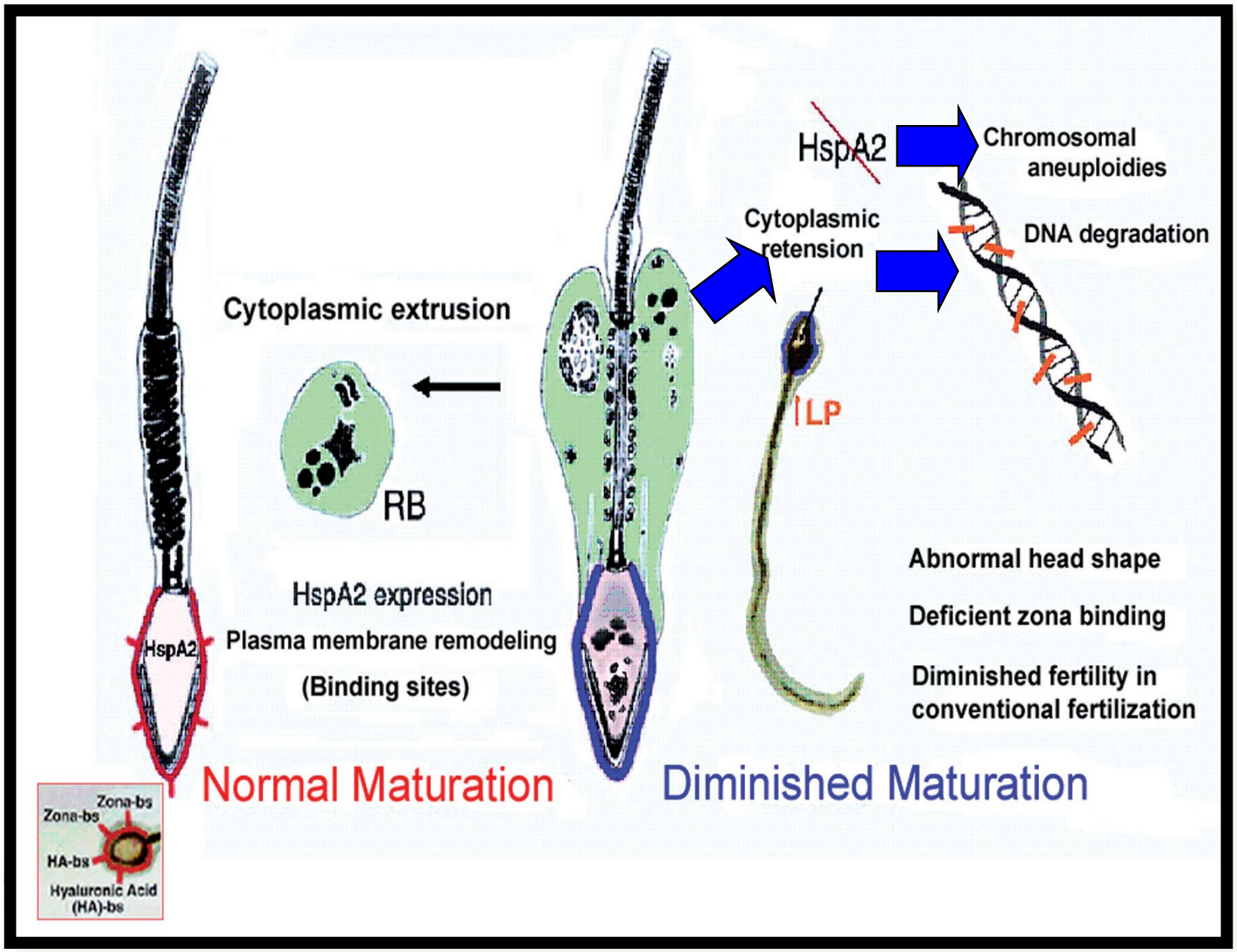
-Unión a la ZP

*Receptores para el ácido hialurónico en la cabeza del espermatozoide. Selección de espermatozoides maduros con el test de unión de espermatozoides a HA.*

*G.Huszar, Repro BioMed Online, 2003*

Rocío Núñez

# Huszar et al, RBM Online 2007




Dra. Rocío

# HA juega un papel primordial en la selección fisiológica del espermatozoide.

Los espermatozoides que son capaces de unirse in vitro a la HA son maduros, y han completado el proceso de espermiogénesis y remodelación del a membrana plasmática, extrusión citoplásmica y madurez nuclear.

***Huszar et al, 2003***

Los espermatozoides maduros, con alta densidad de receptores de HA se unen permanentemente a la HA, mientras que los espermatozoides inmaduros son incapaces de esta unión..

***Cayli et al, 2003***

- Este método de selección por HA permite la ejecución de un ICSI más fisiológico que el PVP, evitando cualquier daño potencial del PVP.

*Jean et al, 2001*

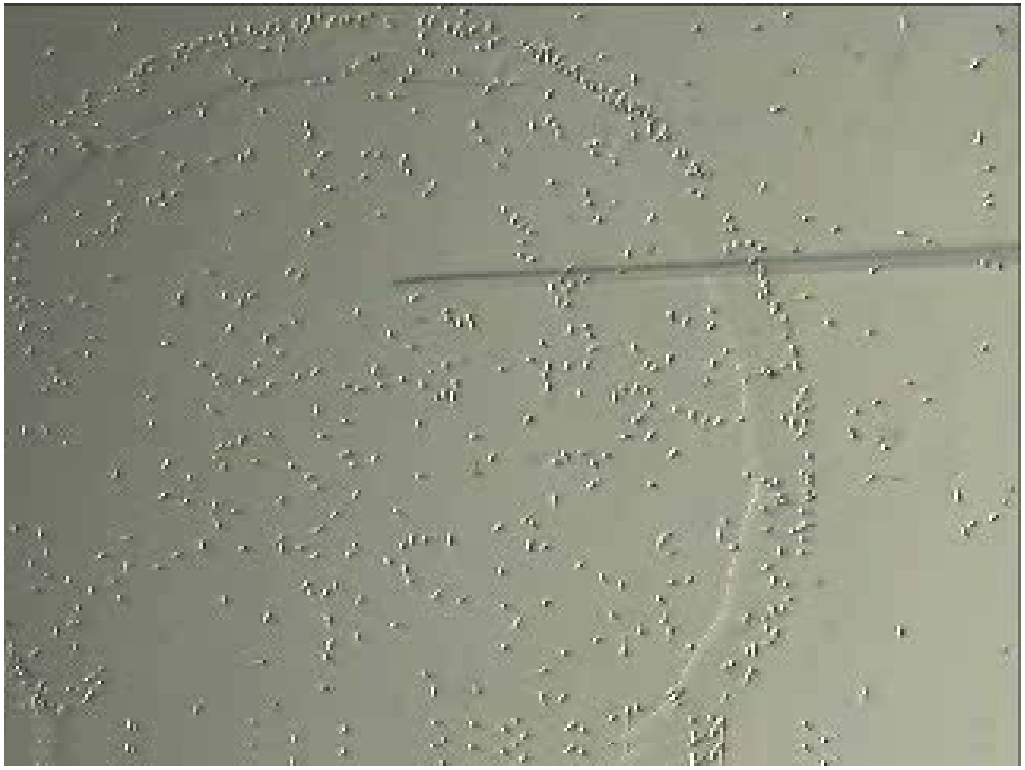
- Actualmente, existen dos sistemas para la selección de espermatozoides unidos a HA:

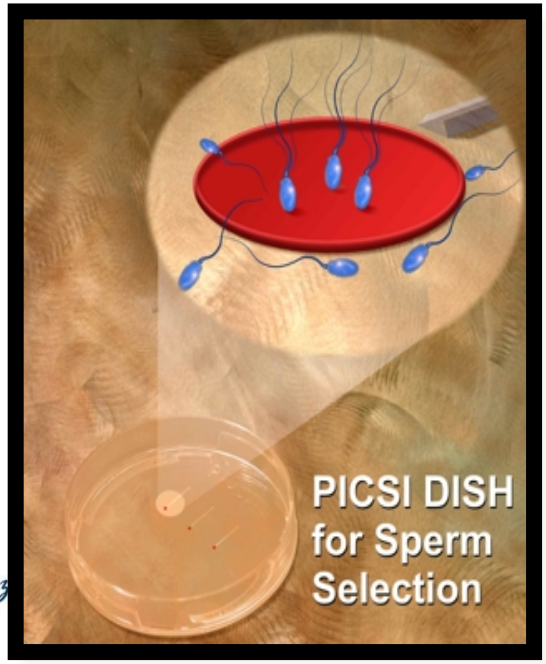
PICSI ® (Origio)

SPERM SLOW ™ (Origio)

Dra. Rocío Núñez

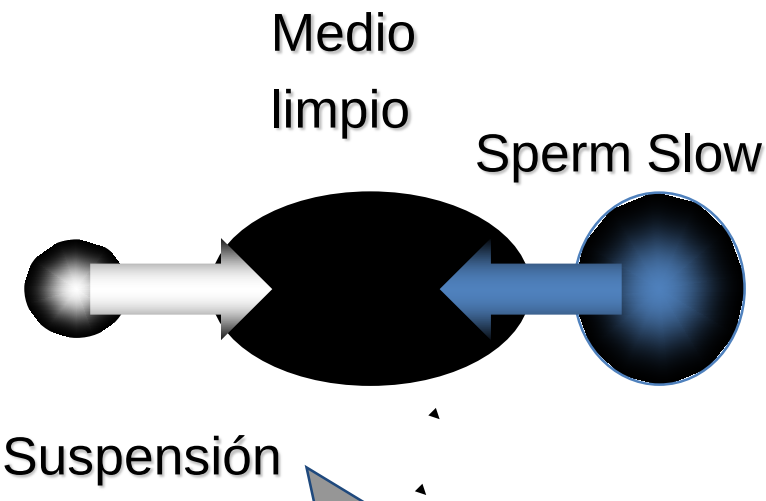
# PICSI

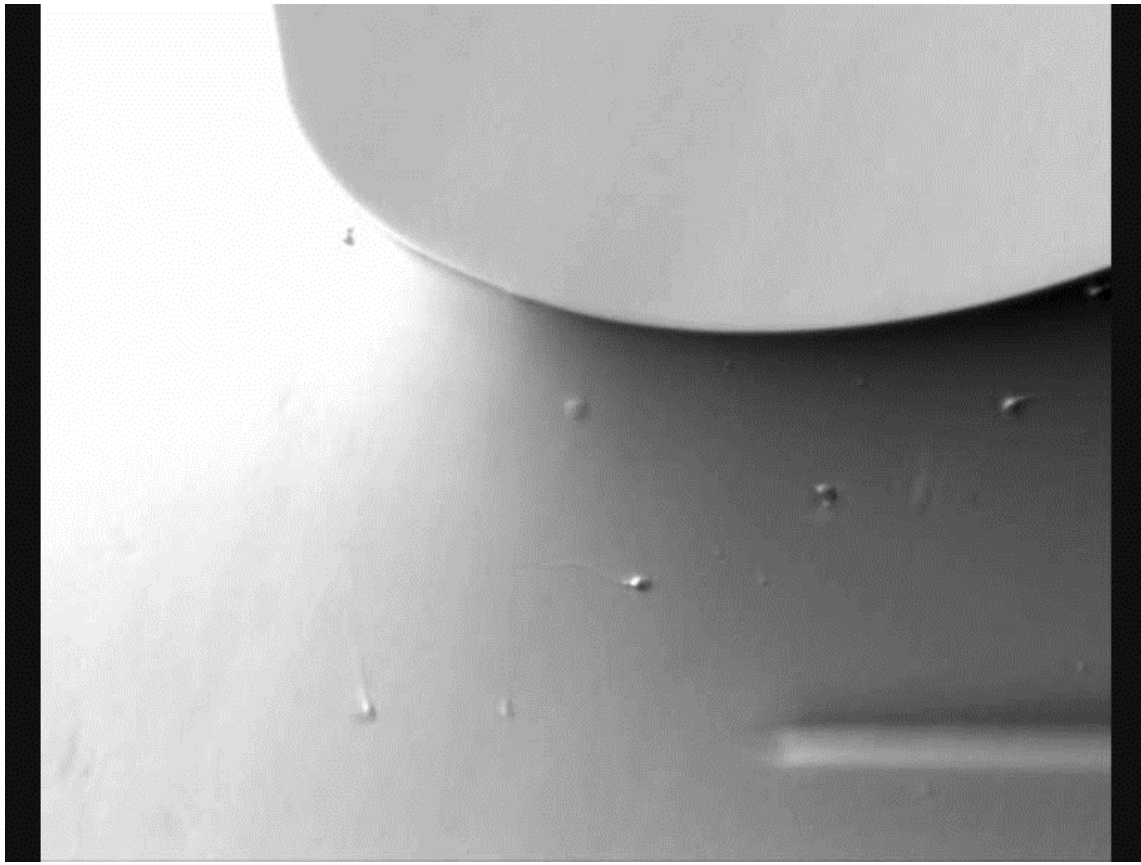





Dra. Rocío Núñez

# Sperm Slow



**Los espermatozoides unidos a HA en la zona de unión de las gotas, pueden ser seleccionados y fácilmente cargarlos en la pipeta de ICSI.**
***Parmegiani et al. JARG 2010***

# "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.*

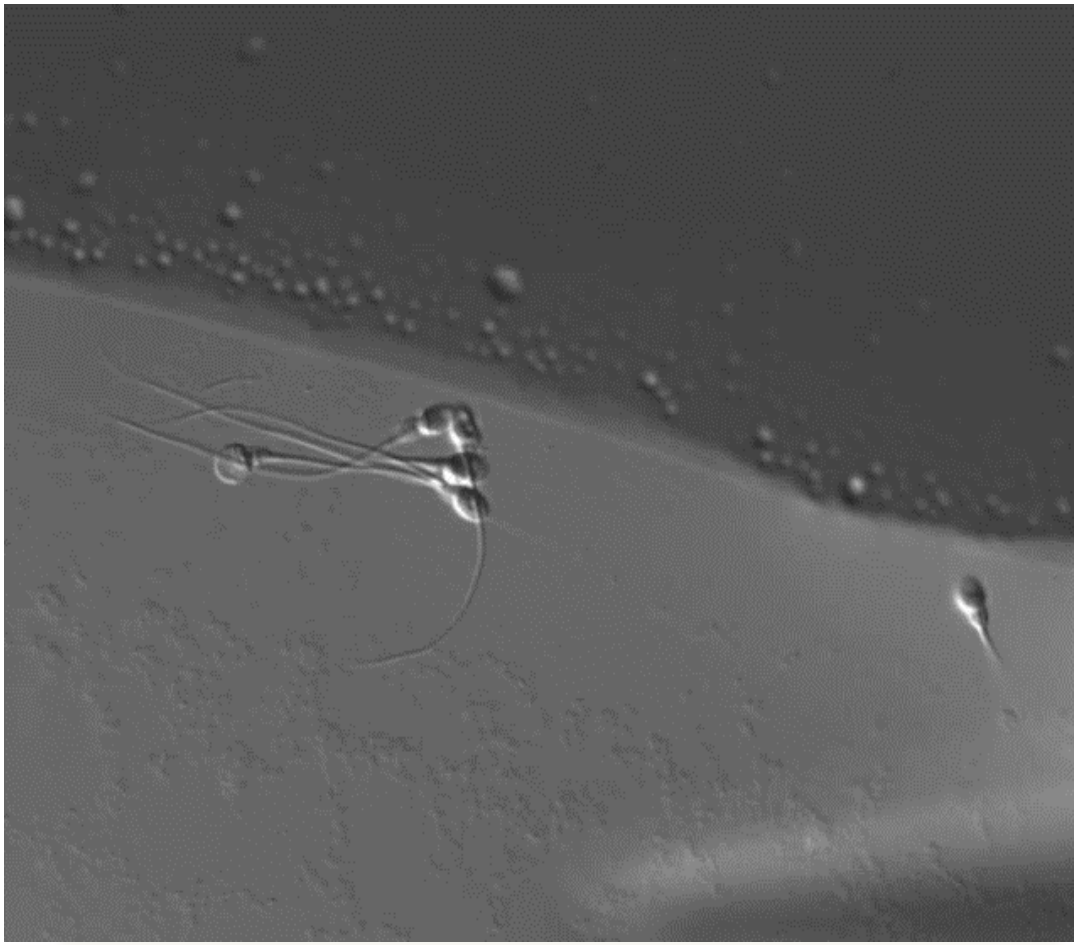
Reproductive Medicine Unit, GynePro Medical Centers, Bologna, Italy

Fertility and Sterility® Vol. 93, No. 2, January 15, 2010

# HA Fisiológico- IMSI

Acelera la selección de espermatozoides con núcleo normal durante el IMSI

*Parmegiani et al, 2010*



Dra. Rocío Núñez

**Human Reproduction, Vol.28, No.2 pp. 306–314, 2013**
Advanced Access publication on November 30, 2012 doi:10.1093/humrep/des417

human reproduction

ORIGINAL ARTICLE *Embryology*

# Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes—multicenter, double-blinded and randomized controlled trial

**K.C. Worrilow[1,2,*], S. Eid[2], D. Woodhouse[3], M. Perloe[4], S. Smith[5], J. Witmyer[6], K. Ivani[7], C. Khoury[8], G.D. Ball[9], T. Elliot[10], and J. Lieberman[11]**

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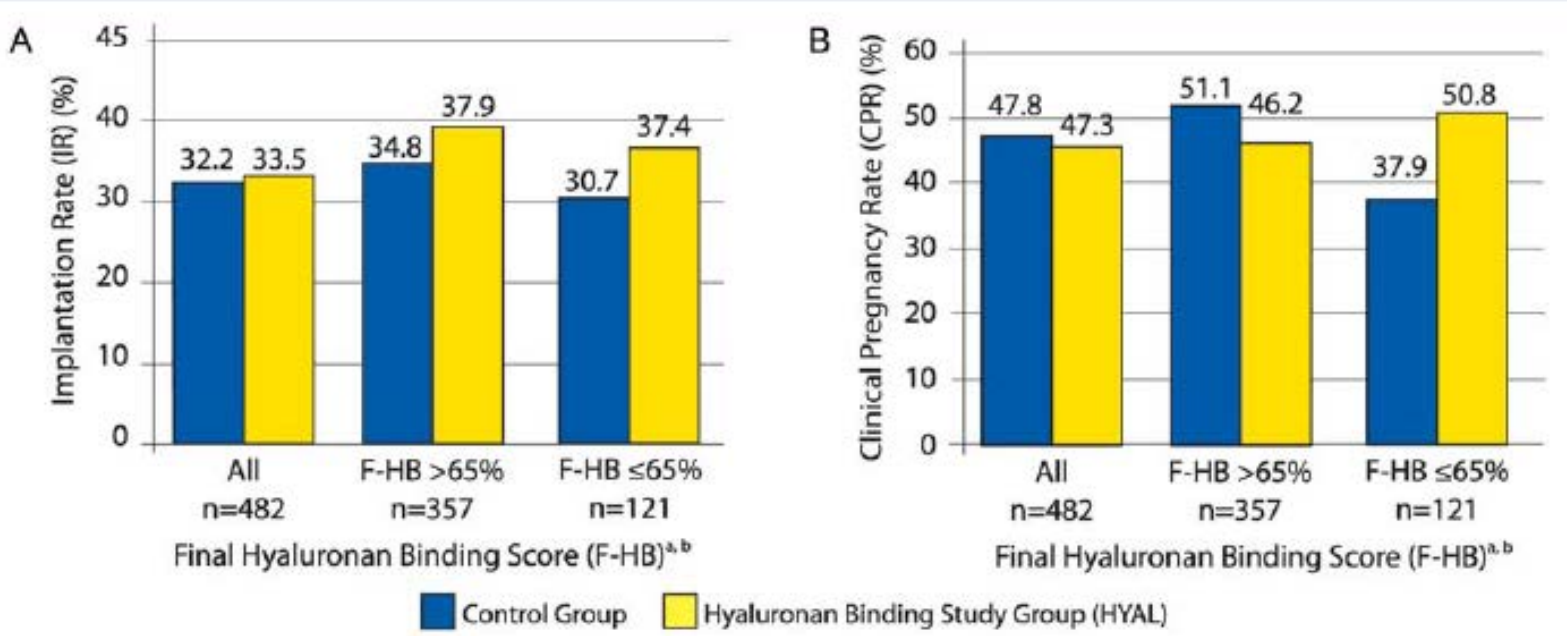
# PICSI: double –blinded, randomized controlled trial
*Worrilow et al, Human Reproduction 2012*

- El mayor estudio prospectivo-randomizado comparando HA-ICSI with PVP-IVSI; 802 parejas

- En parejas con inicial HA score 65% (484)

  Descenso significativo (P=0.067) in tasa de aborto(PLR)
  Cuando se inyectan espermatozoides unidos a HA (4.3%)
  Comparados con PVP (10%)




Relationship between implantation rates and the F-HB score. [a] The F-HB score was not recorded on 4 of the patients and these patients are therefore not included in the stratification. [b] $P > 0.05$.

Relationship between clinical pregnancy rates and the F-HB score. [a] The F-HB score was not recorded on 4 of the patients and these patients are therefore not included in the stratification. [b] $P > 0.05$.

**Figure 3** **A**: Implantation rate. **B**: Clinical pregnancy rate.

# Changes in DNA fragmentation during sperm preparation for intracytoplasmic sperm injection over time

Natalia Rougier, M.D.,[a,b] Heydy Uriondo, M.Sc.,[a] Sergio Papier, M.D.,[a] Miguel Angel Checa, M.D., Ph.D.,[b] Carlos Sueldo, M.D.,[a,c] and Cristian Alvarez Sedó, M.Sc.[a]

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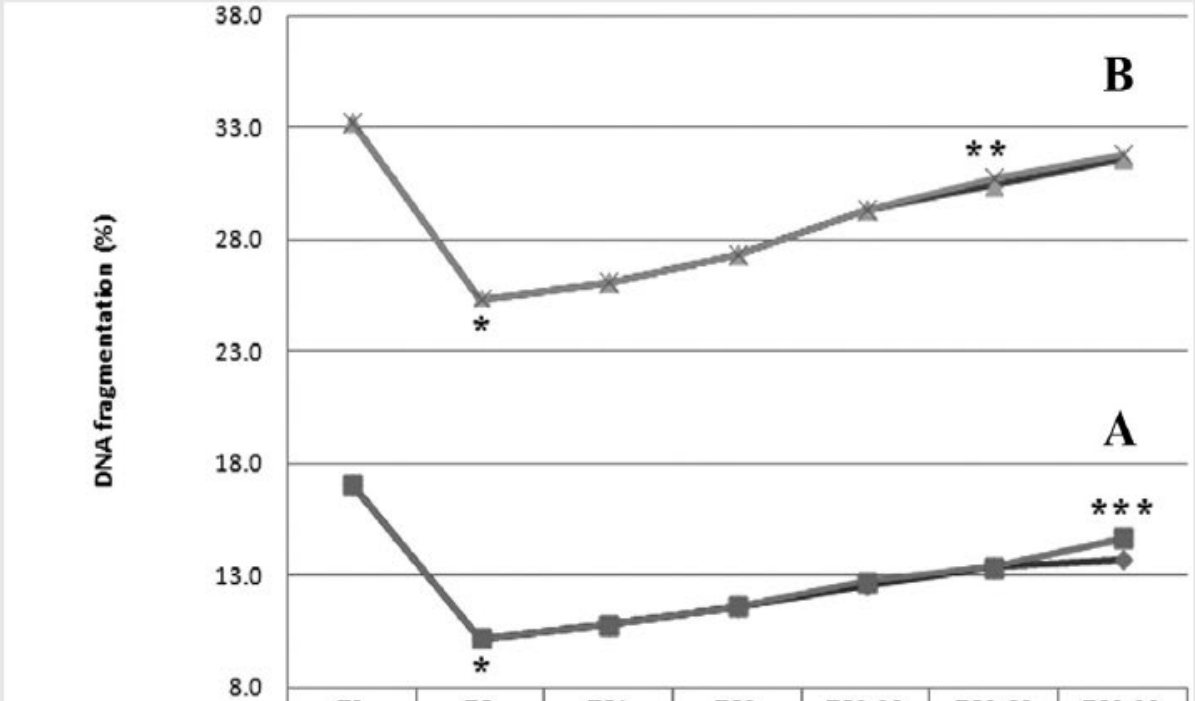
Fertility and Sterility® Vol. 100, No. 1, July 2013

**La fragmentación de ADN desciende significativamente después de la centrifugación con gradientes.**
**Muestras con TUNEL >20% fueron más susceptibles a un incremento de fragmentación en el tiempo.**




| | T0 | TG | TG1 | TG2 | TG2-30 | TG2-60 | TG2-90 |
|---|---|---|---|---|---|---|---|
| TUNEL ≤ 20% (PVP) | 17.1 | 10.2 | 10.8 | 11.6 | 12.5 | 13.4 | 13.7 |
| TUNEL ≤ 20% (HA) | 17.1 | 10.2 | 10.8 | 11.6 | 12.7 | 13.4 | 14.6 |
| TUNEL > 20% (PVP) | 33.3 | 25.4 | 26.1 | 27.4 | 29.4 | 30.4 | 31.6 |
| TUNEL > 20% (HA) | 33.3 | 25.4 | 26.1 | 27.4 | 29.4 | 30.8 | 31.8 |

DNA fragmentation dynamics. The levels of DNA fragmentation decreased significantly after obtaining a centrifugation gradient in all samples (*). In patients with terminal deoxynucleotide transferase–mediated dUTP nick-end labeling (TUNEL) <20% (A), we observed a significant increase of DNA fragmentation after TG2-90 (***) (10.2 ± 3% to 13.4 ± 4%). In patients with TUNEL ≥20% (B), we observed a significant increase in DNA fragmentation beginning at TG2-60 (**) (25.3 ± 5 to 30.4 ± 6). HA = hyaluronic acid; PVP = polyvinylpyrrolidone.

*Rougier. DNA fragmentation and sperm preparation. Fertil Steril 2013.*

Poster nº 8

# Evaluation of Sperm Selection Procedure Based on Hyaluronic Acid Binding Ability on the IVF Succes of Donated Oocytes Following ICSI

S. Cortés; L. Ortega; M. Gago; C. Luna; C. Muñoz; A. Guijarro; P. Caballero; R. Núñez. Clínica Tambre. Madrid

## Objectives

*In nature, human oocytes are surronded by hyaluronic Acid (HA), which is then envolved in the mechanism of sperm selection. In fact, only mature spermatozoa which have extruded their specific receptors to bind and digest HA can reach the oocyte and fertilize it. HA´s role as "physiologic selector" is now well recognized also in vitro: it has been demonstrated that spermatozoa able to bind HA in vitro are those that have completed plasma membrane remodelling, cytoplasmic extrusion and nuclear maturation.*

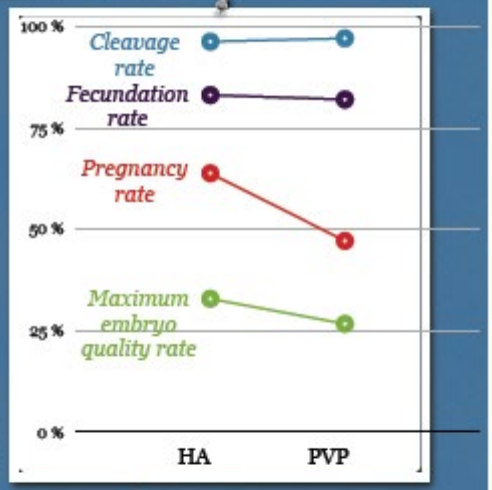
*SpermSlowTM presents a natural alternative to PVP as it is made principally of hyaluronate (HA). The selection principle in SpermSlowTM is based on sperm binding to HA via receptors on the sperm heads.*

*The aim of this study was to compare the efficiency of routine sperm selection method (PVP) with HA-selection procedure (SpermSlow TM) for fertilization rate, embryo development, implantation and ongoing pregnancy rates.*

## Material and methods

*Semen samples were obtained from the 72 couples undergoing ICSI. To avoid female infertility as a bias factor only oocyte donation from donors with proven fertility in previous reproductive cycles were used (n=72). The percentage of fertilization rate, cleavage, quality of embryos and ongoing pregnancy rate were compared between two procedures: routine sperm selection with PVP (PVP-ICSI group=36) and HA-binding selection (HA-ICSI group=36).*

*Between-group differences of normally distributed continuous variables were assessed with parametric statistic (Student´s t-test). and between-group differences in non-continuous variables were assessed using the χ2-method.*




## Results

*No significant differences were observed in fecundation rate (83,2 vs. 82,1), embryo cleavage rate (96,4 vs. 97,2) and quality embryo rate (32,9 vs. 26,7). Although the difference is not statistically significant, a clear trend towards a better pregnancy rate per transfer (63,9% vs. 47,2%) was found in the HA-ICSI group.*

## Conclusions

- *Although we did not observe a statistical significant difference in fertilization rate, embryo cleavage rate, quality embryo rate and even pregnancy rate, clinical outcome of HA-ICSI when using the viscous medium Sperm SlowTM showed the positive effect of HA sperm selection on ICSI outcome.*
- *Thus, the HA-sperm selection method may represent at least a physiological alternative for slowing sperm motility prior to ICSI, even though a wider study could confirm these beneficial effects on ICSI outcome.*

# Effect of Sperm Selection Using Hyaluronic Acid Binding

## on reproductive outcome with donated oocyte cycles

### Introduction

*The purpose of our study was to compare the efficiency of routine sperm selection method polyvinylpyrrolidone (PVP) with HA-selection method (Sperm-Slow) for fertilization rate (FR), cleavage rate (CR), embryo quality (EQ) and pregnancy rate (PR) as well as evaluating the relationship between HA-binding ability with sperm protamine deficiency and DNA fragmentation using donated oocyte cycles to reduce the variability within oocyte quality that female infertility may introduce.*

### Material & Methods

*This was a single-center, prospective, randomized study of 144 ICSI cycles with donated oocytes, randomly carried out with PVP (1119 oocytes microinjectated) or with Sperm-Slow (1104 oocytes microinjectated) for sperm selection.*

*Our primary outcome was to compare FR, CR, EQ and PR between two groups and the secondary outcome was to better define the role of HA for selection of spermatozoa with normal chromatin content to optimize ICSI outcome. To determine the value of Sperm DNA fragmentation (SDF) levels, the Sperm Chromatin Dispersion test (SCD) was measured.*

*Between-groups differences of normally distributed continuous variables were assessed with a parametric statistic (Student's t test).*

*Between-group differences in no continuous variables were assessed using the c2 method. Differences were considered significant when a P value was <.05.*

### Results

*There were no significant differences (p>0,1) in regard the total number of injected MII oocytes, semen quality or SDF. No statistical difference was found among the variables studied between oocytes injected with HA-bound spermatozoa (SL) and the conventional group (PVP).*

*When the study population was divided by the SDF level (cutoff 30 %) , there were no statistical differences regarding FR, CR, EQ and PR when HA-selected spermatozoa were used.*


### Conclusions

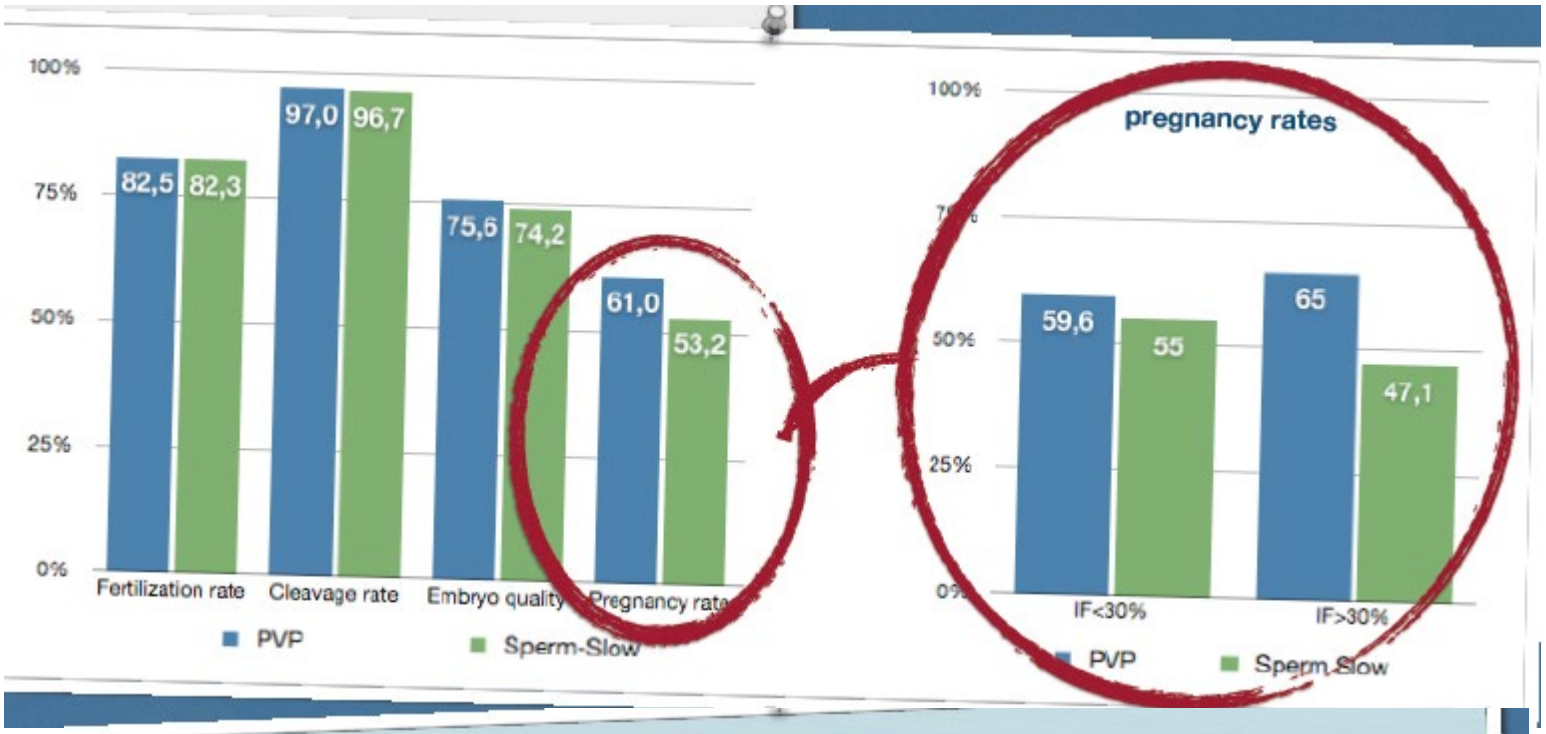
- *Contrary to our expectation, selection of HA-bound spermatozoa had no benefits in terms of fertilization, embryo cleavage, embryo quality and pregnancy rate in ICSI cycles.*
- *Our prospective and randomized trial suggests that both, PVP and Sperm-Slow allow a comparable clinical efficiency in selecting spermatozoa.*

fundación tambre

S. Cortés Gallego, L. Ortega López, E. Olaya Vila, M. Gago García, C. Luna Cañas, A. García Segovia, A. Guijarro Ponce, R. Núñez Calonge, P. Caballero Peregrín
Clínica Tambre. Madrid. Spain

CLÍNICA TAMBRE

Dra. Rocío Núñez

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- *Our prospective and randomized trial suggests that both, PVP and Sperm-Slow allow a comparable clinical efficiency in selecting spermatozoa.*

Asian Journal of Andrology (2013) 15, 421–424

www.nature.com/aja

ORIGINAL ARTICLE

# No difference in high-magnification morphology and hyaluronic acid binding in the selection of euploid spermatozoa with intact DNA

Suchada Mongkolchaipak[1] and Teraporn Vutyavanich[2]

**Table 2 Aneuploidy and DNA fragmentation rates in those who had the best or second-best spermatozoa for selection under high magnification+MSOME criteria**

| | *Best spermatozoa available* | *The second-best spermatozoa available* | P* |
|---|---|---|---|
| Total number of spermatozoa analysed | 1217 | 528 | — |
| Spermatozoa with DNA fragmentation | 20 | 11 | 0.408 |
| DNA fragmentation rate (%) | 1.6 | 2.0 | — |
| Spermatozoa with aneuploidy | 2 | 10 | <0.001 |
| Sperm aneuploidy rate (%) | 0.2 | 1.8 | — |

Abbreviation: MSOME, motile sperm organellar morphology examination.
*Chi-squared test with Yates' correction.

Reproductive BioMedicine Online (2016) 32, 286–298

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REVIEW

# Clinical benefit using sperm hyaluronic acid binding technique in ICSI cycles: a systematic review and meta-analysis

Ronit Beck-Fruchter [a,*], Eliezer Shalev [a,b], Amir Weiss [a,b]

**Abstract** The human oocyte is surrounded by hyaluronic acid, which acts as a natural selector of spermatozoa. Human sperm that express hyaluronic acid receptors and bind to hyaluronic acid have normal shape, minimal DNA fragmentation and low frequency of chromosomal aneuploidies. Use of hyaluronic acid binding assays in intracytoplasmic sperm injection (ICSI) cycles to improve clinical outcomes has been studied, although none of these studies had sufficient statistical power. In this systematic review and meta-analysis, electronic databases were searched up to June 2015 to identify studies of ICSI cycles in which spermatozoa able to bind hyaluronic acid was selected. The main outcomes were fertilization rate and clinical pregnancy rate. Secondary outcomes included cleavage rate, embryo quality, implantation rate, spontaneous abortion and live birth rate. Seven studies and 1437 cycles were included. Use of hyaluronic acid binding sperm selection technique yielded no improvement in fertilization and pregnancy rates. A meta-analysis of all available studies showed an improvement in embryo quality and implantation rate; an analysis of prospective studies only showed an improvement in embryo quality. Evidence does not support routine use of hyaluronic acid binding assays in all ICSI cycles. Identification of patients that might benefit from this technique needs further study.

# Comparison of two ready-to-use systems designed for sperm–hyaluronic acid binding selection before intracytoplasmi sperm injection: PICSI vs. Sperm Slow: a prospective, randomized

Lodovico Parmegiani, M.Sc., Graciela Estela Cognigni, M.D., Silvia Bernardi, B.Sc., Enzo Troilo, B.Sc., Stefania Taraborrelli, M.D., Alessandra Arnone, B.Sc., Antonio Manuel Maccarini, B.Sc., and Marco Filicori, M.D.

Reproductive Medicine Unit, GynePro Medical Centers, Bologna, Italy

**Objective:** To compare, in a strict, randomized way, the efficiency of two ready-to-use systems for hyaluronic acid (H sperm injection (ICSI): an HA culture dish (PICSI Sperm Selection Device) and a viscous medium containing HA (Sp
**Design:** Prospective, randomized study.
**Setting:** Medical center.
**Patient(s):** Fifty subjects per treatment group (100 total).
**Intervention(s):** One hundred ICSI treatments were randomly carried out with PICSI or with Sperm Slow for sperm ization was conducted with sealed envelopes. Intracytoplasmic sperm injection was performed by a single embryo experience in HA-ICSI.
**Main Outcome Measure(s):** Primary outcome measure: good-quality embryo rate. Secondary outcome measures: pregnancy and implantation rate, and the duration of the ICSI procedure.
**Result(s):** The good-quality embryo rate was comparable between the two groups (58.5% with PICSI vs. 56% with S there were no statistically significant differences in secondary outcome measures except ICSI procedure duration, v longer in the PICSI group.
**Conclusion(s):** Both PICSI and Sperm Slow allow comparable clinical efficiency in selecting HA-bound spermatozoa.
**Clinical Trial Registration Number:** ISRCTN72668039. (Fertil Steril® 2012;98:632–7. ©2012 by American Society for Reproductive Medicine.)
**Key Words:** Hyaluronic acid sperm selection, HA-ICSI, physiologic ICSI, PICSI, Sperm Slow

**Discuss:** You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/parmegianil-sperm-hyaluronic-acid-ha-binding-selection-icsi/

Hyaluronic acid (HA) is the main component of the extracellular matrix (ECM) of the cumulus oophorus. In the fertilization process human oocytes are naturally surrounded by HA, which is then involved in the mechanism of fact, mature sperma to and digest HA ha of reaching the ooc it. The role of HA selector" is also in vitro: it has been the spermatozoa tha lized HA in vitro completed their p remodeling, cytop and nuclear matu those with rec

Received January 18, 2012; revised May 8, 2012; accepted May 28, 2012; published online June 29, 2012.
L.P. has nothing to disclose. G.E.C. has nothing to disclose. S.B. has nothing to disclose. E.T. has nothing to disclose. S.T. has nothing to disclose. A.A. has nothing to disclose. A.M.M. has nothing to disclose. M.F. has nothing to disclose.
Current Controlled Trials registration number: ISRCTN72668039.
Reprint requests: Lodovico Parmegiani, M.Sc., Reproductive Medicine Unit, GynePro Medical Centers, Via T. Cremona 8, 40137 Bologna, Italy (E-mail: l.parmegiani@gynepro.it).

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J Assist Reprod Genet (2013) 30:1471–1475
DOI 10.1007/s10815-013-0108-9

ASSISTED REPRODUCTION TECHNOLOGIES

## A prospective randomized st of hyaluronic acid sperm sel sperm injection outcome of infertility having normal sen

Gaurav Majumdar · Abha Majumdar

Received: 29 July 2013 / Accepted: 24 September 2013 / Publi


**Abstract**
*Purpose* Sperm quality plays an important role in dete embryo development and intracytoplasmic sperm i (ICSI) outcome. Selection of competent sperm base ability to bind to hyaluronic acid (HA) has been sugg one of the methods to assess sperm quality. The ai present study was to examine whether injection of HA sperm helps in improving outcome in patients un ICSI with unexplained infertility having norma parameters.
*Methods* Patients with unexplained infertility having semen parameters in accordance with WHO 2010 undergoing their first IVF-ICSI cycle were enrolle the course of the study.156 patients were prosp randomized after oocyte retrieval and were assi either the ICSI group, where sperm selection for was based on visual assessment, or the PICSI grou sperm were selected based on their ability to bind Only fresh embryo transfers were included in the *Results* There was no difference in the fertilizati number of top quality embryos and clinical pregnar between the ICSI and PICSI groups (65.7 % vs 45.8 % vs 43.6 % and 35 % vs 35.2 % respectively). H

*Capsule* Injection of HA selected sperm (PICSI) did no improving the clinical outcome in patients undergoing I unexplained infertility having normal semen parameters.

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Open Access Protocol

BMJ Open

# Hyaluronic Acid Binding Sperm Selection for assisted reproduction treatment (HABSelect): study protocol for a multicentre randomised controlled trial

K D Witt,[1] L Beresford,[1] S Bhattacharya,[2] K Brian,[3] A Coomarasamy,[4] R Hooper,[1] J Kirkman-Brown,[4] Y Khalaf,[5] S E Lewis,[6] A Pacey,[7] S Pavitt,[8] R West,[9] D Miller[10]

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► Prepublication history and additional material is available. To view please visit the journal (http://dx.doi.org/10.1136/bmjopen-2016-012609).

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## ABSTRACT

**Introduction:** The selection of a sperm with good genomic integrity is an important consideration for improving intracytoplasmic sperm injection (ICSI) outcome. Current convention selects sperm by vigour and morphology, but preliminary evidence suggests selection based on hyaluronic acid binding may be beneficial. The aim of the Hyaluronic Acid Binding Sperm Selection (HABSelect) trial is to determine the efficacy of hyaluronic acid (HA)-selection of sperm versus conventionally selected sperm prior to ICSI on live birth rate (LBR). The mechanistic aim is to assess whether and how the chromatin state of HA-selected sperm corresponds with clinical outcomes—clinical pregnancy rate (CPR), LBR and pregnancy loss (PL).

**Methods and analysis:** Couples attending UK Centres will be approached, eligibility screening performed and informed consent sought. Randomisation will occur within 24 hours prior to ICSI treatment. Participants will be randomly allocated 1:1 to the intervention arm (physiological intracytoplasmic sperm injection, PICSI) versus the control arm using conventional methods (ICSI). The primary clinical outcome is LBR ≥37 weeks' gestation with the mechanistic study determining LBR's relationship with sperm DNA integrity. Secondary outcomes will determine this for CPR and PL. Only embryologists performing the procedure will be aware of the treatment allocation. Steps will be taken to militate against biases arising from embryologists being non-blinded. Randomisation will use a minimisation algorithm to balance for key prognostic variables. The trial is powered to detect a 5% difference (24–29%: p=0.05) in LBR ≥37 weeks' gestation. Selected residual sperm samples will be tested by one or more assays of DNA integrity.

**Ethics and dissemination:** HABSelect is a UK NIHR-EME funded study (reg no 11/14/34; IRAS REF. 13/YH/0162). The trial was designed in partnership with patient and public involvement to help maximise patient benefits. Trial findings will be reported as per CONSORT guidelines and will be made available in lay language via the trial web site (http://www.habselect.org.uk/).

**Trial registration number:** ISRCTN99214271; Pre-results.

### Strengths and limitations of this study

- Hyaluronic Acid Binding Sperm Selection (HABSelect) is one of the only trials with sufficient power to test the efficacy of a sperm-selection procedure that has shown some promise for improving live birth rate but without conclusive evidence hitherto.
- The trial has closely linked clinical and basic science aspects that makes best use of the resources provided by participating couples. Both components will advance clinical and mechanistic understanding.
- Since the intervening embryologist is aware of the arm allocation, there may be a potential for subconscious embryo selection bias, particularly in smaller clinics with fewer staff. This effect, however, should be mitigated by data capture, including details of the embryologist involved and close data monitoring by the independent steering committee.
- There are likely to be potentially confounding variations in semen quality that could affect the interpretation of clinical outcomes, but these should be mitigated by careful recording of semen profiles and their stratification according to HBA scoring. A hierarchy of sperm chromatin quality assays will allow us to minimise the effects of sample availability while maximising information content.
- Mechanistic work is entirely dependent on the efficient recovery of residual processed sperm from participating centres following treatment. The success or otherwise of this recovery process is very likely to vary among participating centres.

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Background

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Selection cri

We included randomised controlled trials (RCTs) comparing an advanced sperm selection technique versus standard IVF or ICSI or versus another advanced sperm selection technique. We excluded studies of sperm selection using ultra-high magnification (intracytoplasmic morphologically selected sperm injection, or IMSI), as they are the subject of a separate Cochrane review. Quasi-randomised and pseudo-randomised trials were excluded. Our primary outcome measure was live birth rate per woman randomly assigned. Secondary outcome measures included clinical pregnancy per woman randomly assigned, miscarriage per clinical pregnancy and fetal abnormality per clinical pregnancy.




## Data collection and analysis

Two review authors independently assessed eligibility of studies and risk of bias, and performed data extraction. Disagreements were resolved by consultation with a third review author. Study investigators were consulted to resolve other queries that arose. Risk ratios (RRs) were calculated with 95% confidence intervals (CIs). We planned to combine studies using a fixed-effect model, if sufficient data were available. The quality of the evidence was evaluated using Grades of Recommendation, Assessment, Development and Evaluation (GRADE) methods.

## Main results

Two RCTs were included in the review. Both evaluated sperm selection by hyaluronanic acid binding for ICSI, but only one reported live births. No studies were identified that were related to surface charge selection, sperm apoptosis or sperm birefringence.

One RCT compared hyaluronanic acid binding versus conventional ICSI. Live birth was not reported. Evidence was insufficient to show whether there was a difference between groups in clinical pregnancy rates (RR 1.01, 95% CI 0.84 to 1.22, one RCT, 482 women). This evidence was deemed to be of low quality, mainly as the result of poor reporting of methods and findings. Miscarriage data were unclear, and fetal abnormality rates were not reported.

The other RCT compared two different hyaluronanic acid binding techniques, SpermSlow and physiological intracytoplasmic sperm injection (PISCI). Evidence was insufficient to indicate whether there was a difference between groups in rates of live birth (RR 1.16, 95% CI 0.65 to 2.05, one RCT, 99 women), clinical pregnancy (RR 1.07, 95% CI 0.67 to 1.71, one RCT, 99 women) or miscarriage (RR 0.76, 95% CI 0.24 to 2.44, one RCT, 41 women). The evidence for these comparisons was deemed to be of low quality, as it was limited by imprecision and poor reporting of study methods. Fetal abnormality rates were not reported.

## Authors' conclusions

Evidence was insufficient to allow review authors to determine whether sperm selected by hyaluronanic acid binding improve live birth or pregnancy outcomes in ART, and no clear data on adverse effects were available. Evidence was also insufficient to show whether there is a difference in efficacy between the hyaluronic acid binding methods SpermSlow and PICSI. No randomised evidence evaluating sperm selection by sperm apoptosis, sperm birefringence or surface charge was found.

Further studies of suitable quality are required to evaluate whether any of these advanced sperm selection techniques can be recommended for use in clinical practice.

Lancet 2019; 393: 416–22

# Physiological, hyaluronan-selected intracytoplasmic sperm injection for infertility treatment (HABSelect): a parallel, two-group, randomised trial

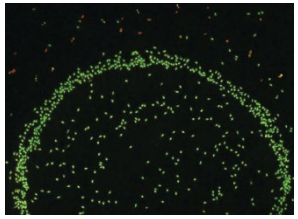
David Miller, Susan Pavitt, Vinay Sharma, Gordon Forbes, Richard Hooper, Siladitya Bhattacharya, Jackson Kirkman-Brown, Arri Coomarasamy, Sheena Lewis, Rachel Cutting, Daniel Brison, Allan Pacey, Robert West, Kate Brian, Darren Griffin, Yakoub Khalaf

**Interpretation** Compared with ICSI, PICSI does not significantly improve term livebirth rates. The wider use of PICSI, therefore, is not recommended at present.

| | PICSI | ICSI | Absolute difference (95% CI) | Odds ratio (95% CI) | p value |
|---|---|---|---|---|---|
| **Term livebirth** | | | | | |
| Primary analysis* | 27·4% (379/1381) | 25·2% (346/1371) | 2·2% (−1·1 to 5·5) | 1·12 (0·95 to 1·34) | 0·18 |
| Sensitivity analysis† | 27·5% (379/1379) | 25·3% (346/1370) | 2·2% (−1·1 to 5·5) | 1·13 (0·95 to 1·34) | 0·17 |
| **Secondary endpoints** | | | | | |
| Clinical pregnancy | 35·2% (487/1382) | 35·7% (491/1375) | −0·5% (−4·0 to 3·1) | 0·98 (0·84 to 1·15) | 0·80 |
| Miscarriage | 4·3% (60/1381) | 7·0% (96/1371) | −2·7% (−4·4 to −0·9) | 0·61 (0·43 to 0·84) | 0·003 |
| Premature birth | 3·3% (46/1381) | 3·3% (45/1371) | 0·0% (−1·3 to 1·4) | 1·02 (0·67 to 1·55) | 0·94 |
| **Exploratory endpoints** | | | | | |
| Fertilisation rate (%)‡ | 66% (24·0) | 69% (24·0) | 3·0% (−0·47 to 6·5) | 1·15 (0·98 to 1·34) | 0·09 |
| Biochemical pregnancy | 39·5% (546/1383) | 39·5% (544/1377) | 0·0% (−4·0 to 4·0) | 1·00 (0·86 to 1·17) | 0·99 |

Data are % (n/N), unless otherwise stated. PICSI=physiological intracytoplasmic sperm injection. ICSI=intracytoplasmic sperm injection. *Adjusted for maternal age, previous miscarriage, and hormonal indicators of ovarian reserve. †Adjusted for hyaluronan-sperm binding score, maternal age, previous miscarriage, and hormonal indicators of ovarian reserve. Odds ratios are shown alongside absolute differences. ‡Data are mean (SD); denominators were 1386 for the PICSI group and 1380 for the ICSI group.

*Table 3:* **Trial outcomes**



## *Selección mediante HBA: selección de espermatozoides maduros (PICSI y Sperm-slow)*

| Ventajas | Inconvenientes |
|---|---|
| **Selección de espermatozoides maduros** | No hay suficientes evidencias científicas que apoyen la mejora de resultados |
| **Método fácil y barato** | Se necesita personal especializado |

# Pero, ¿cuándo utilizar uno u otro método de selección?

| Técnica | Indicación |
|---|---|
| MACS | Alto índice de fragmentación en muestras normozoospérmicas u OAT leves. |
| IMSI | 100% morfoanomalías espermáticas. (¿) |
| HBA | Selección de espermatozoides para ICSI en casos de pacientes con alto IF y OATz severa. |

First International Journal of Andrology
ANDROLOGIA

ORIGINAL ARTICLE

# Selection of physiological spermatozoa during intracytoplasmic sperm injection

B. Torki-Boldaji[1,2], M. Tavalaee[1], M. Bahadorani[2] & M. H. Nasr-Esfahani[1,3]

## Summary

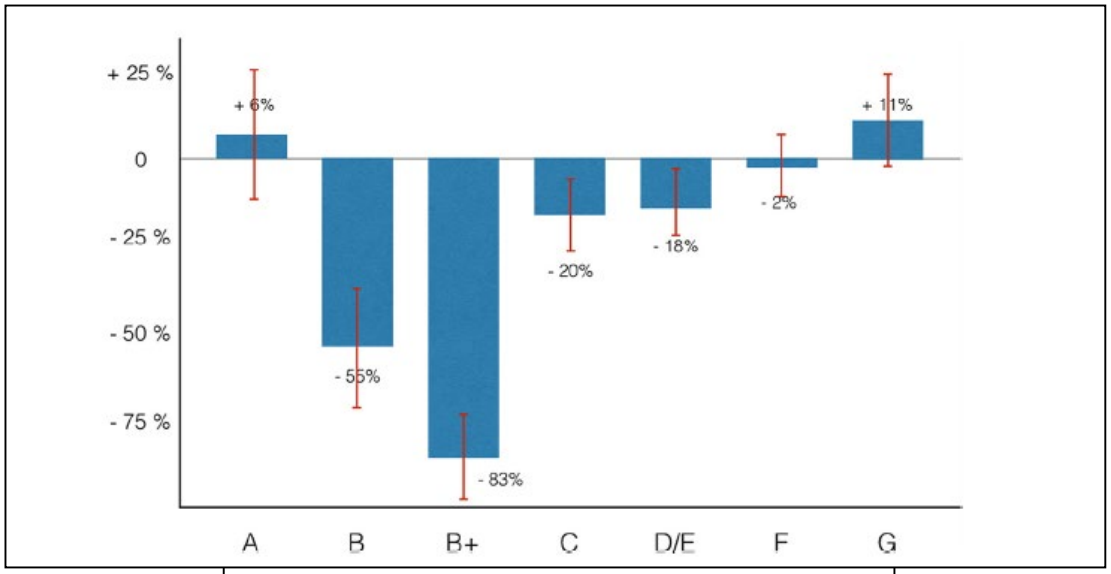
Sperm genomic integrity has a significant effect on intracytoplasmic sperm injection (ICSI) outcomes, especially post-implantation. Spermatozoa selected based on motility and morphology do not guarantee the genomic integrity of spermatozoa. Nearly fifty percentage of spermatozoa in infertile men with normal morphology present different degrees of DNA fragmentation. However, capacitated or hyperactivated spermatozoa show lower degrees of DNA fragmentation. Therefore, selection of hyperactivated spermatozoa may improve ICSI outcome. Routinely, for ICSI, fast-moving spermatozoa with A or B motility pattern are mainly selected for injection. The result of this study shows that in processed semen samples, hyperactivated spermatozoa are mainly observed in B motility pattern while, in viscous medium like polyvinylpyrrolidone (PVP), hyperactivated spermatozoa are mainly present in spermatozoa with C pattern of motility (nonprogressive). Therefore, we propose spermatozoa with C motility pattern which contains the main population of physiological or hyperactivated spermatozoa should be selected for ICSI.

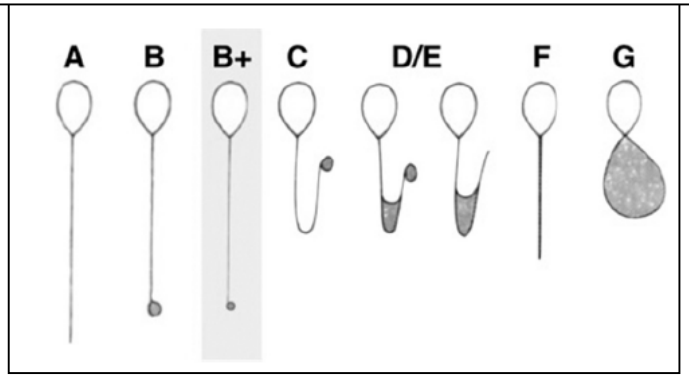
REPRODUCTIVE BIOMEDICINE ONLINE 35 (2017) 372–378

Article

# Potential selection of genetically balanced spermatozoa based on the hypo-osmotic swelling test in chromosomal rearrangement carriers

*Alexandre Rouen [a,*], Léa Carlier [a], Solveig Heide [a], Matthieu Egloff [a], Pauline Marzin [a], Flavie Ader [a], Mathias Schwartz [a], Eli Rogers [a], Nicole Joyé [a], Richard Balet [b], Nathalie Lédée [b], Laura Prat-Ellenberg [b], Nino Guy Cassuto [c], Jean-Pierre Siffroi [a]*



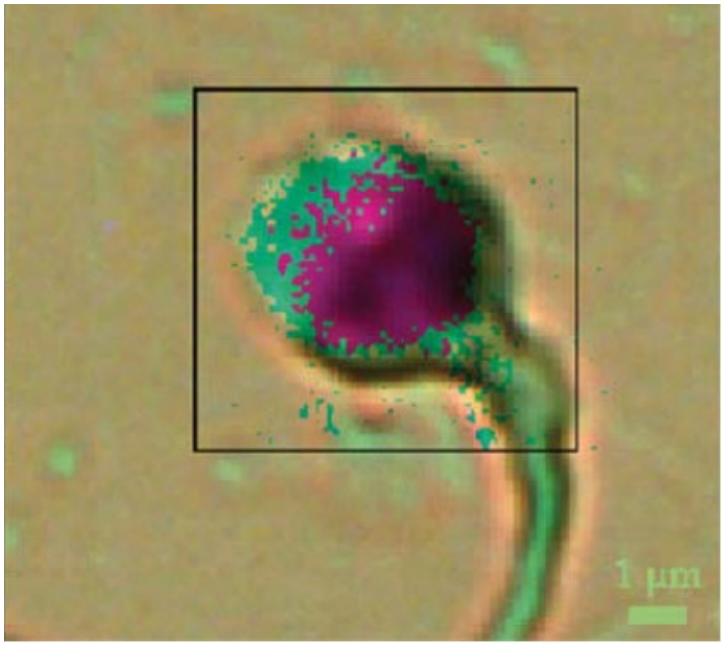
**Human Reproduction, Vol.26, No.7 pp. 1641–1649, 2011**
Advanced Access publication on April 30, 2011 doi:10.1093/humrep/der122

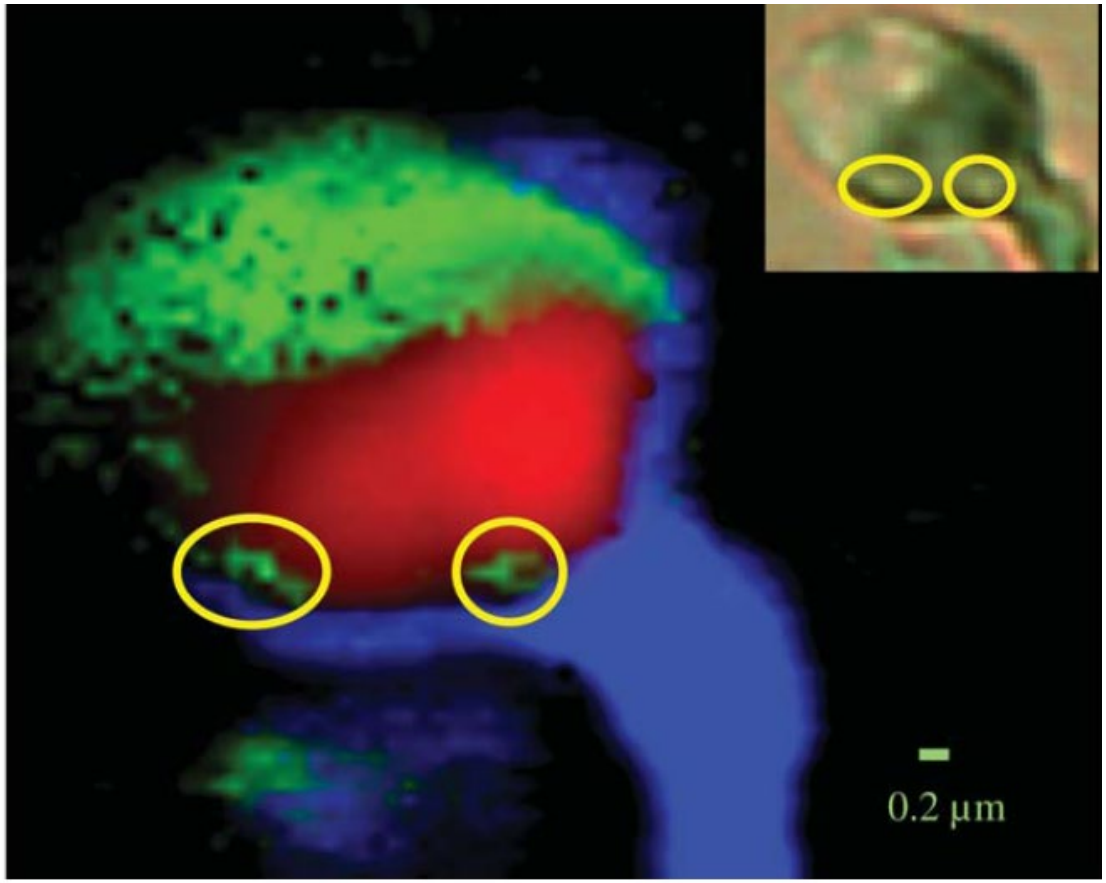
human reproduction

ORIGINAL ARTICLE *Andrology*

# *In situ* visualization of damaged DNA in human sperm by Raman microspectroscopy

**C. Mallidis[1,*], J. Wistuba[1], B. Bleisteiner[2], O.S. Damm[1], P. Groß[3], F. Wübbeling[4], C. Fallnich[3], M. Burger[4], and S. Schlatt[1]**





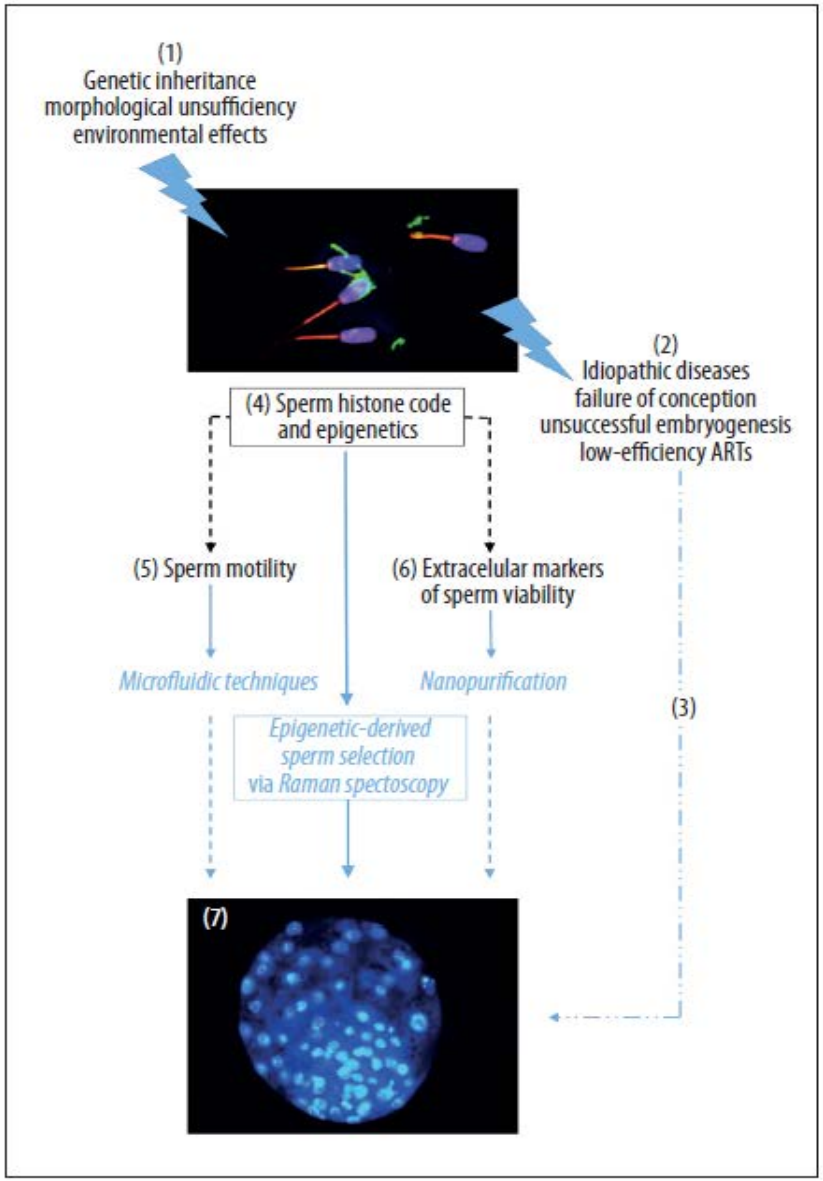
MEDICAL SCIENCE MONITOR

Received: 2017.03.01
Accepted: 2017.03.15
Published: 2017.09.29

# Non-Invasive Approaches to Epigenetic-Based Sperm Selection

EF 1,2 Miriama Štiavnická
EF 1 Laura Abril-Parreño
EF 1,2 Jan Nevoral
EFG 1,2 Milena Králíčková
EF 1 Olga García-Álvarez

Raman spectroscopy for sperm epigenetics as an innovative tool for sperm selection resulting in successful embryonic development. When various environmental pressures or genetic burden (1), showed as idiopathic infertility followed by conception failure (2), decrease a chance for successful conception (3). On the other hand, sperm population is predestined for strict selection more than oocytes. Therefore, qualified selection of markers of sperm fertilization ability is crucial for advanced approaches to live sperm selection. Based on current knowledge, sperm epigenetics (4) seems to be a general phenomenon regulating clinical obvious features (motility (5) and sperm viability (6)) as well as invisible sperm quality leading to correct embryonic development (7). Raman spectroscopy is a versatile tool for advanced epigenetic-derived sperm selection. Therefore, Raman spectroscopy-selected sperm can improve ART outcomes, even with low-quality ejaculate.



**Human Reproduction, Vol.27, No.8 pp. 2254–2266, 2012**
Advanced Access publication on June 12, 2012 doi:10.1093/humrep/des201

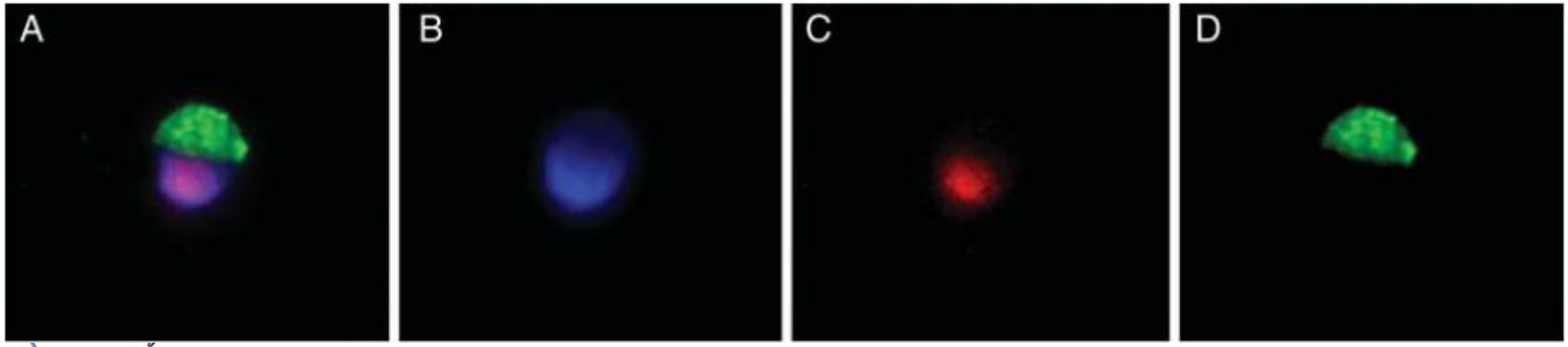
human reproduction

ORIGINAL ARTICLE *Andrology*

# Development of a novel synthetic oligopeptide for the detection of DNA damage in human spermatozoa

**M. Enciso[1,2], G. Pieczenik[3,4], J. Cohen[3,4], and D. Wells[1,2,*]**

[1]Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK [2]Reprogenetics UK, Institute of Reproductive Sciences, Oxford Business Park North, Oxford OX4 2HW, UK [3]Tyho-Galileo Research Laboratories, 3 Regent Street, Livingston, NJ 07039, USA [4]Reprogenetics LLC, 3 Regent Street, Livingston, NJ 07039, USA



SCIENTIFIC REPORTS

OPEN

# Sperm selection by thermotaxis improves ICSI outcome in mice

Serafín Pérez-Cerezales[1], Ricardo Laguna-Barraza[1], Alejandro Chacón de Castro[1], María Jesús Sánchez-Calabuig[1], Esther Cano-Oliva[2], Francisco Javier de Castro-Pita[2], Luis Montoro-Builes[2], Eva Pericuesta[1], Raúl Fernández-González[1] & Alfonso Gutiérrez-Adán[1]

Received: 3 October 2017
Accepted: 29 January 2018
Published online: 13 February 2018

**this subpopulation is the one that enters the fallopian tube in mice. Further, we confirm that these selected spermatozoa in mice and humans show a much higher DNA integrity and lower chromatin compaction than unselected sperm, and in mice, they give rise to more and better embryos through intracytoplasmic sperm injection, doubling the number of successful pregnancies. Collectively, our results indicate that a high quality sperm subpopulation is selected *in vitro* by thermotaxis and that this subpopulation is also selected *in vivo* within the fallopian tube possibly by thermotaxis.**

Hum Fertil (Camb). 2018 Nov 22:1-11. doi: 10.1080/14647273.2018.1534277. [Epub ahead of print]

**Assessing the potential of HSPA2 and ADAM2 as two biomarkers for human sperm selection.**

Heidari M[1], Darbani S[1], Darbandi M[1], Lakpour N[1], Fathi Z[2], Zarnani AH[3], Zeraati H[4], Akhondi MM[1], Sadeghi MR[3].

# Cumulus oophorus complexes favor physiologic selection of spermatozoa for intracytoplasmic sperm injection

Caizhu Wang, M.S., Guixue Feng, Ph.D., Jinhui Shu, M.S., Hong Zhou, M.S., Bo Zhang, B.S., Huanhua Chen, M.S., Ruoyun Lin, M.S., Xianyou Gan, M.S., Zhulian Wu, M.S., and Tinglv Wei, B.S.

Center of Reproductive Medicine, Guangxi Maternal and Child Health Hospital, Nanning, Guangxi, People's Republic of China

VOL. 109 NO. 5 / MAY 2018

J Assist Reprod Genet. 2018 Dec 12. doi: 10.1007/s10815-018-1375-2. [Epub ahead of print]

# Effects of the microfluidic chip technique in sperm selection for intracytoplasmic sperm injection for unexplained infertility: a prospective, randomized controlled trial.

Yetkinel S[1], Kilicdag EB[2], Aytac PC[2], Haydardedeoglu B[2], Simsek E[2], Cok T[2].

OPEN ACCESS Freely available online

PLOS ONE

# Double Stranded Sperm DNA Breaks, Measured by Comet Assay, Are Associated with Unexplained Recurrent Miscarriage in Couples without a Female Factor

Jordi Ribas-Maynou[1,2], Agustín García
Elena Prada[4], Pilar Cortés[5], Joaquima

ARTICLE IN PRESS

ORIGINAL ARTICLE: ANDROLOGY

# Double-stranded sperm DNA damage is a cause of delay in embryo development and can impair implantation rates

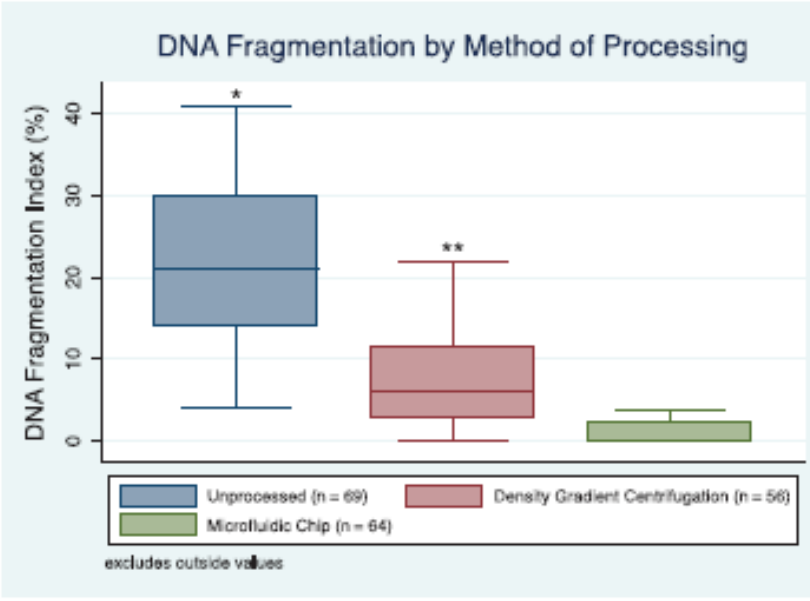
Aida Casanovas, M.Sc.,[a] Jordi Ribas-Maynou, Ph.D.,[b] Sandra Lara-Cerrillo, M.Sc.,[b] Ana Raquel Jimenez-Macedo, Ph.D.,[a] Olga Hortal, M.Sc.,[a] Jordi Benet, Ph.D.,[c] Joan Carrera, M.D.,[a] and Agustín García-Peiró, Ph.D.[b]

**Human Reproduction, Vol.33, No.8 pp. 1388–1393, 2018**
Advanced Access publication on July 10, 2018 doi:10.1093/humrep/dey239

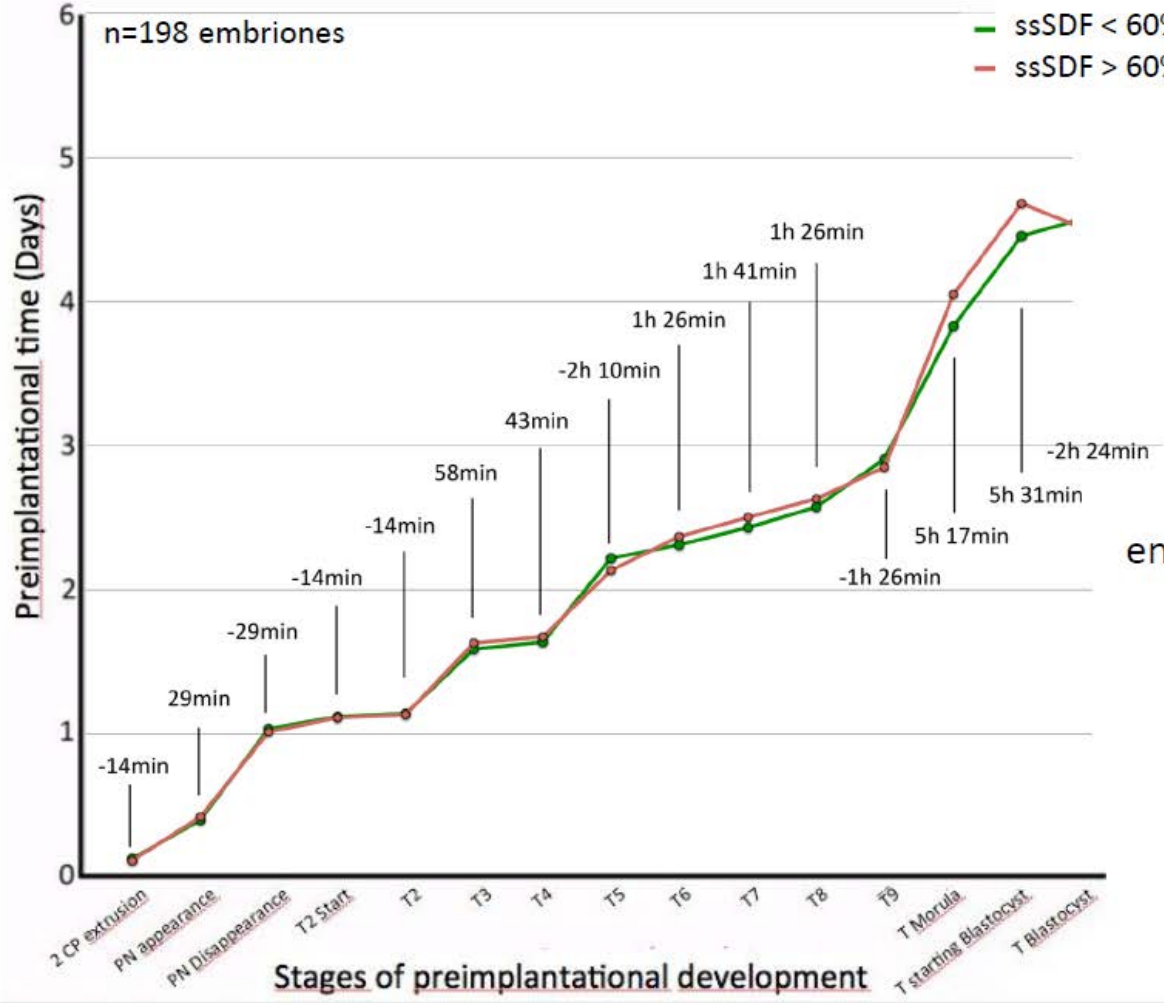
human reproduction ORIGINAL ARTICLE *Andrology*

# Microfluidic sorting selects sperm for clinical use with reduced DNA damage compared to density gradient centrifugation with swim-up in split semen samples

**Molly M. Quinn[1,*], Liza Jalalian[1], Salustiano Ribeiro[1], Katherine Ona[1], Utkan Demirci[2], Marcelle I. Cedars[1], and Mitchell P. Rosen[1]**



# *Resultados – Cinética embrionaria*




Roturas de cadena sencilla

↕

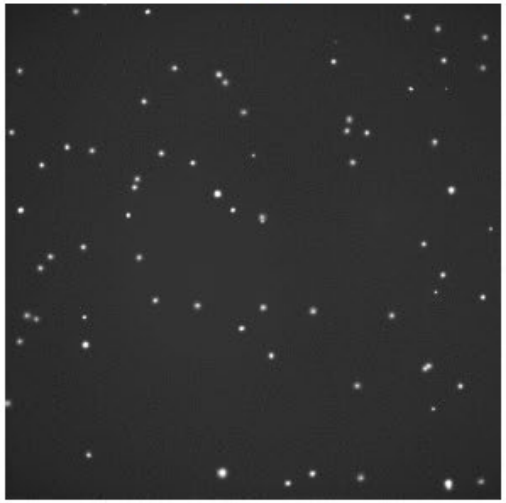
NO EFECTO en la cinética embrionaria

Dra. Rocío Núñez

**ssSDF**
SCD

**10x**

Eyaculado



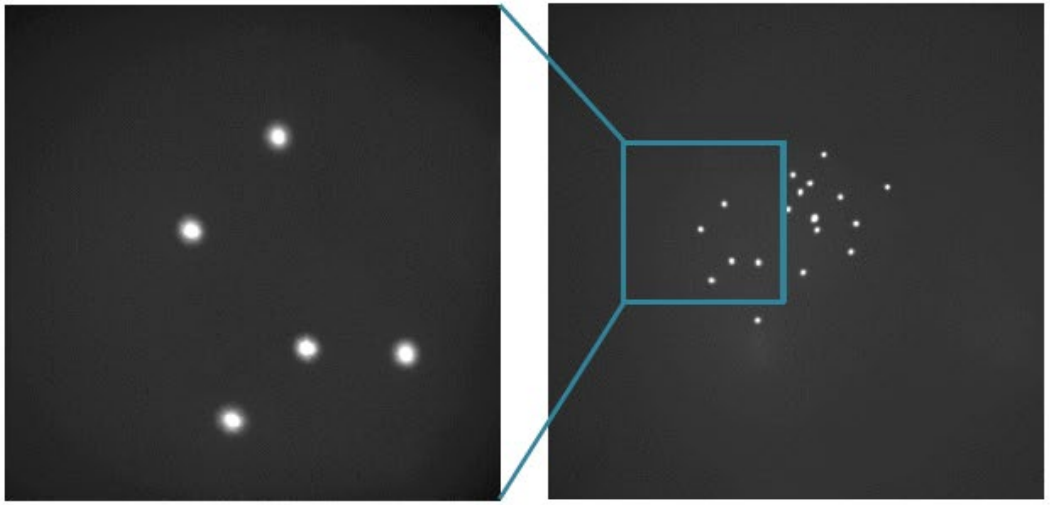
**SDF(%)**

20,7 ± 10,6

$p < 0,05$

**40x**

Sperm seleccionados



2,4 ± 3,3

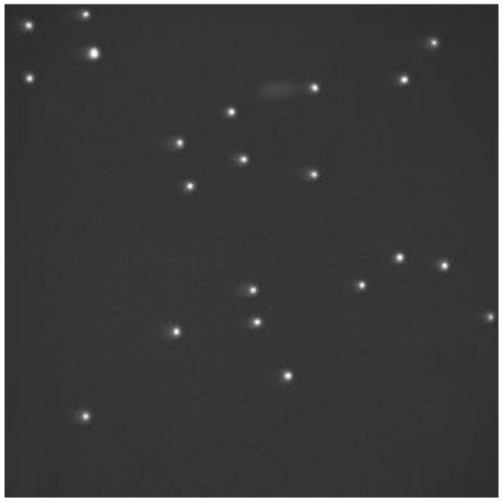
cimab

Dra. Rocío Núñez

# dsSDF
## Cometa neutro

10x

Eyaculado

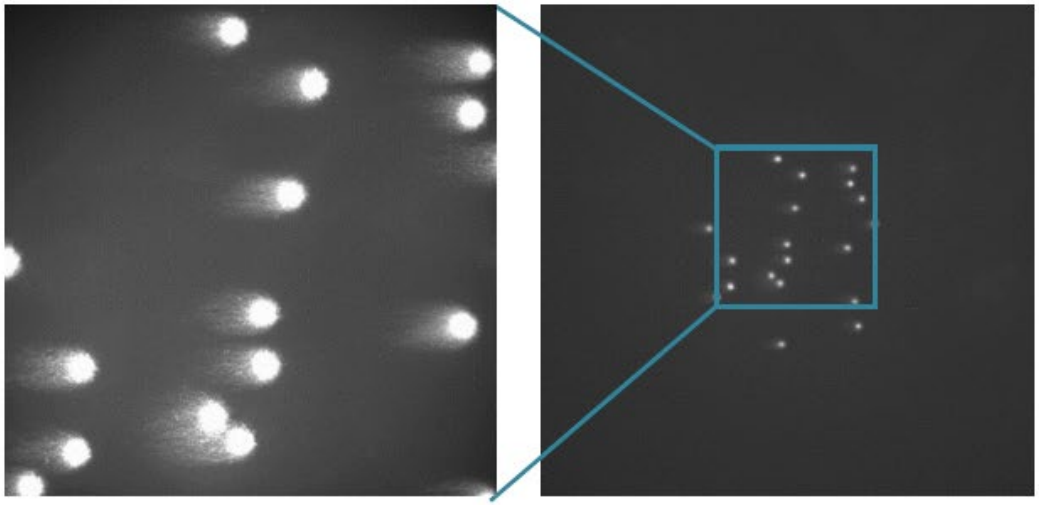


SDF(%)

65,5 ± 6,9

40x

Sperm seleccionados



66,3 ± 19,2

$p > 0,05$

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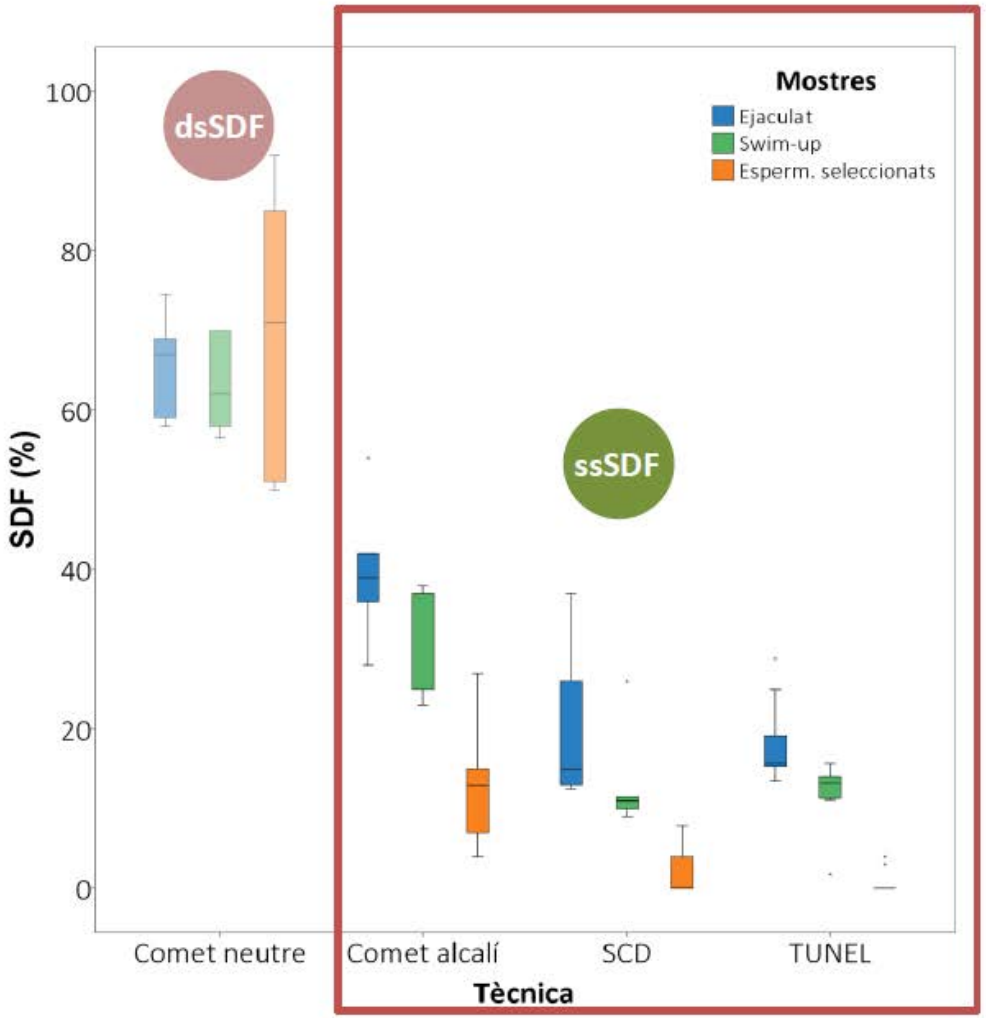
# Fragmentación ADN

**dsSDF** — **NO hay diferencias** ($p>0,05$)

**ssSDF** — **Si hay diferencias** ($p<0,05$)

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Agustín Peiró

Dra. Rocío Núñez

*La fragmentación de **doble cadena** del ADN espermático y no la de cadena sencilla constituye el **principal daño** presente en la subpoblación de espermatozoides seleccionados* **en un tratamiento de ICSI**.

*La selección "manual" de espermatozoides en ciclos de ICSI reduce de forma muy significativa el daño de cadena sencilla.*

*Los esfuerzos en la mejora del diagnóstico y tratamiento de la fragmentación del ADN debería centrarse en las roturas de doble cadena cuando la orientación clínica sea ICSI.*

**Agustín Peiró**

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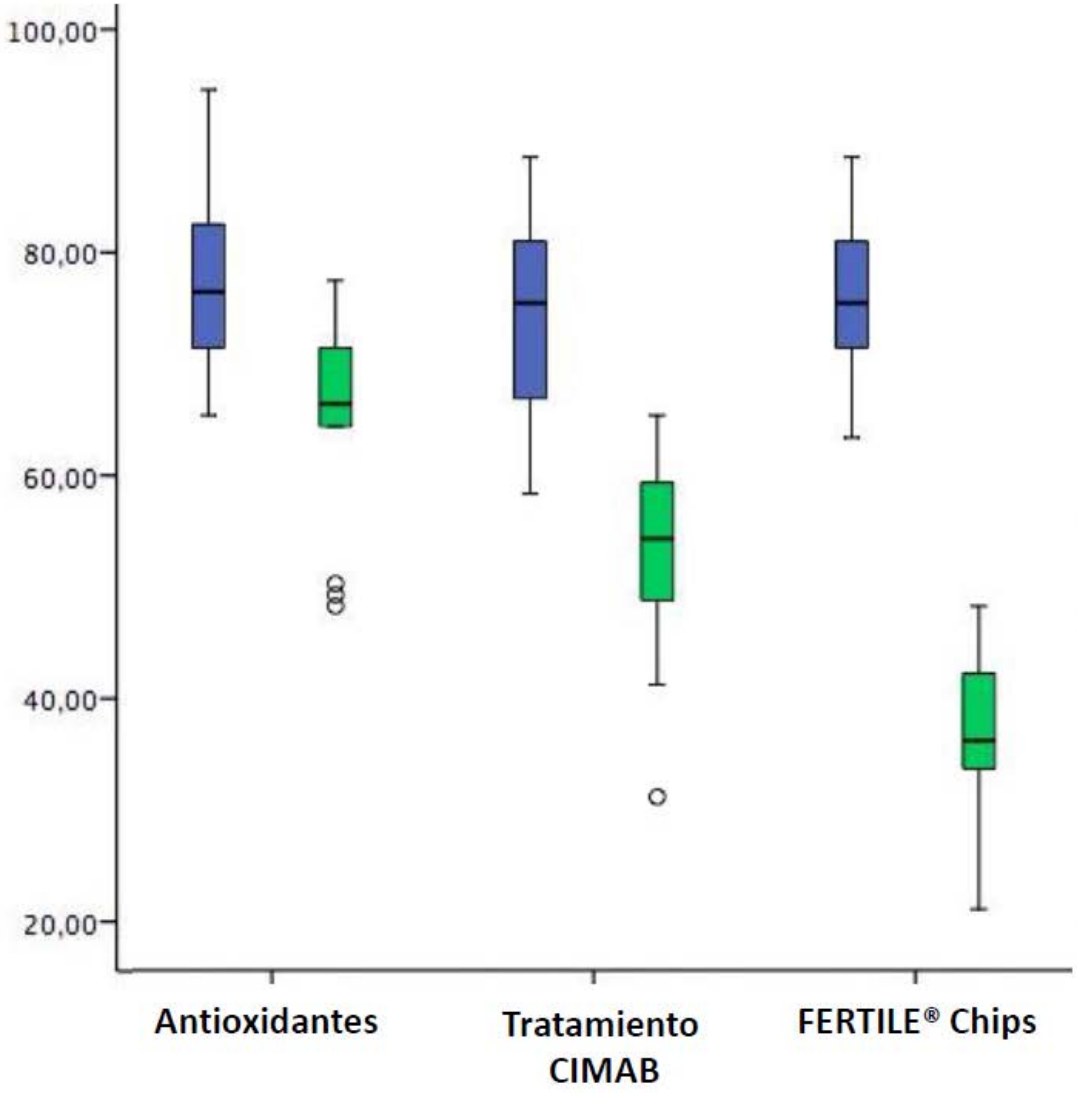
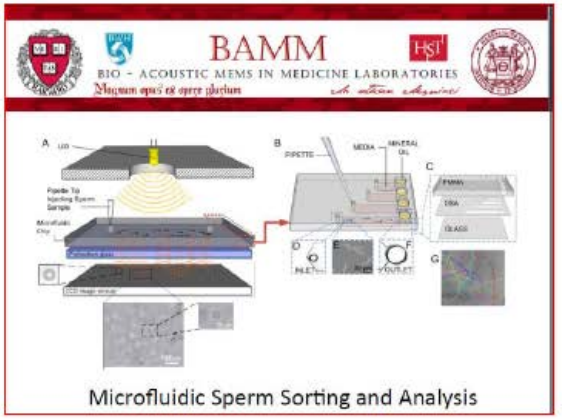
Dra. Rocío Núñez

## *Tratamientos para las roturas de doble cadena*



Microfluidic Sperm Sorting and Analysis

**En conclusión:** Aún a pesar de saber que un alto índice de fragmentación espermática afecta negativamente a la posibilidad de conseguir una gestación,...

---

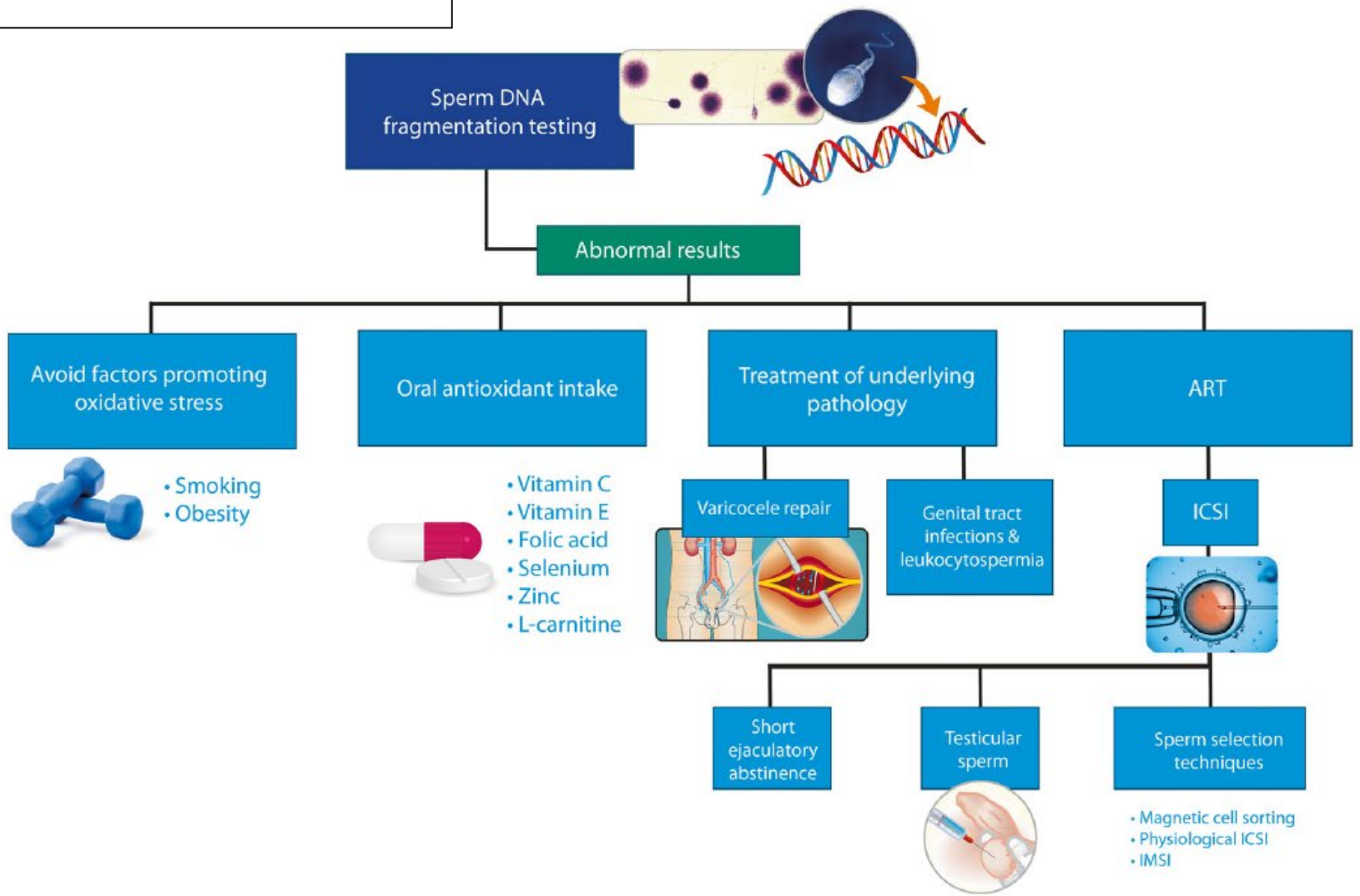
**...no existe un método seguro, fiable y contrastado para seleccionar muestras de semen con bajo índice de fragmentación, y menos aún, para conocer como es el ADN del espermatozoide que se emplea para ICSI. Sin embargo, se pueden aplicar diferentes estrategias, incluso al mismo tiempo, con el fin de disminuir esta incidencia.**

J Assist Reprod Genet
DOI 10.1007/s10815-016-0763-8

REVIEW MEETING

# Novel concepts in male factor infertility: clinical and laboratory perspectives

Sandro C. Esteves[1]



## Estrategias para disminuir el índice de fragmentación de ADN en espermatozoides

En varones normozoopérmicos que van a realizar ICSI, se les puede indicar que no tengan abstinencia sexual previa.

Procesar las muestras de semen del modo habitual (swim-up, gradientes), minimizando el tiempo de incubación.

Si la muestra de semen tiene un alto índice de fragmentación y es normozoospérmica u OAT moderada, utilizar MACS.

Posteriormente, se puede combinar con el uso de Sperm-slow para seleccionar los espermatozoides a microinyectar.

# CONCLUSIONES

No existe actualmente una única técnica que seleccione el mejor espermatozoide para ICSI

Individualizar los pacientes y sus eyaculados en función de su patología y la indicación de TRA, combinando varias técnicas.

Dra. Rocío Núñez

**Human Reproduction Update, Vol.21, No.6 pp. 711–726, 2015**
Advanced Access publication on September 19, 2015 doi:10.1093/humupd/dmv042

human reproduction update

# Sperm selection in natural conception: what can we learn from Mother Nature to improve assisted reproduction outcomes?

**Denny Sakkas[1,*], Mythili Ramalingam[2], Nicolas Garrido[3], and Christopher L.R. Barratt[2]**

# GRACIAS

rocioncalonge@gmail.com

Dra. Rocío Núñez