Embryologists Session

Sperm Separation and Selection Techniques: Do Any Really Improve Embryo Quality and Outcomes?

Denny Sakkas, Ph.D.

Chief Scientific Officer | Boston IVF / IVIRMA North America

Boston IVF – IVIRMA Global Research

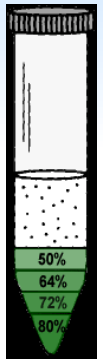
Waltham, MA, USA

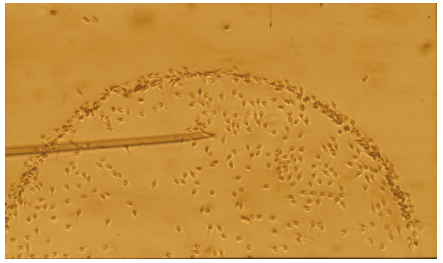
Associate Professor, Yale University School of Medicine

Conflicts of Interest

- Nothing to Disclose

Selection Methods to isolate the best sperm





- Density – *Gradient Separation*
- Surface Charge – *Electrophoresis, Zeta Method*
- Morphological Characteristics – *IMSI*
- Motility Characteristics – *Zech Selector, Microfluidics*
- Membrane Integrity – *Hyaluronan Binding, HOST*
- Surgical – *Testicular Surgery*

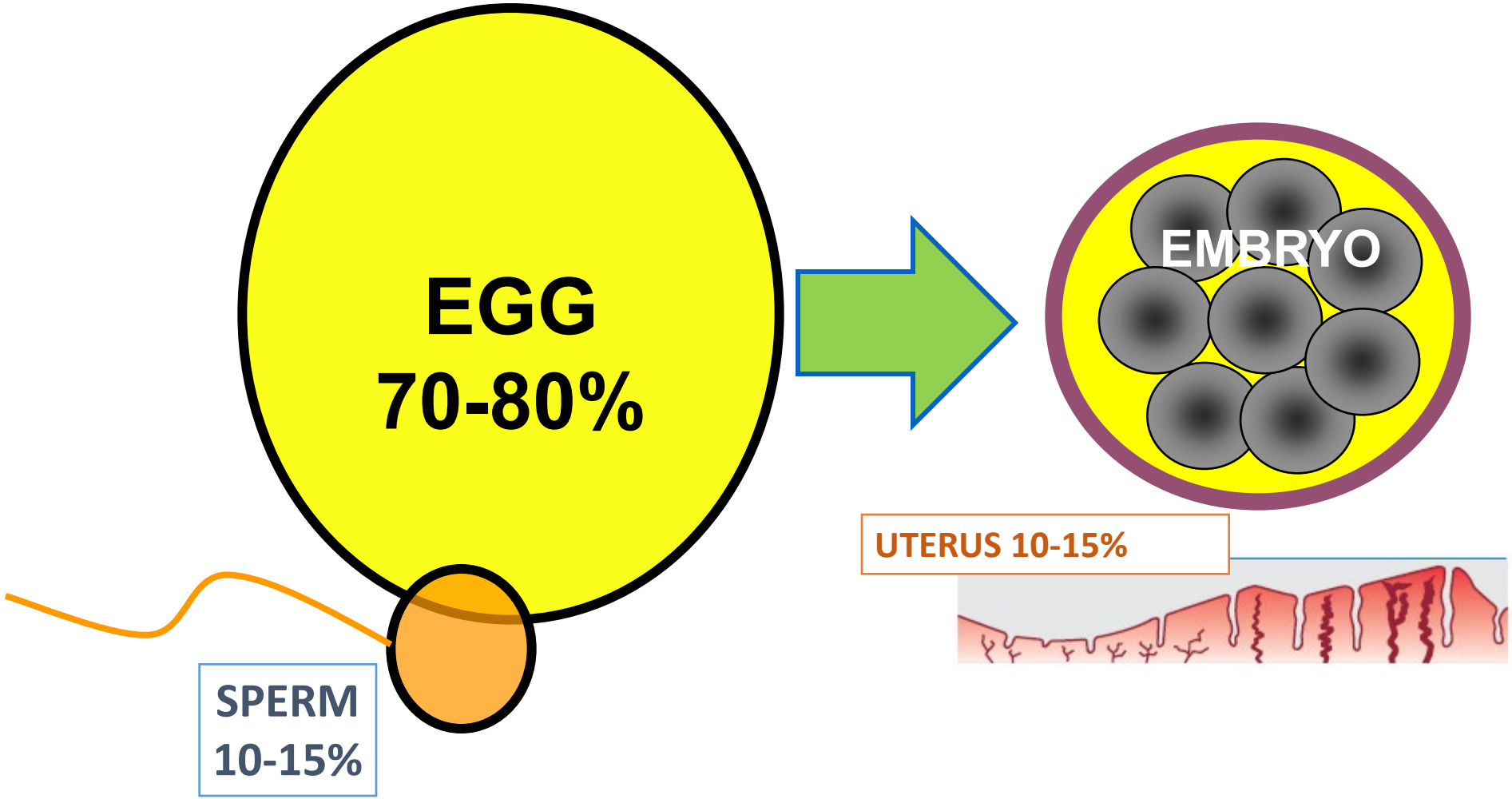
Learning Objectives

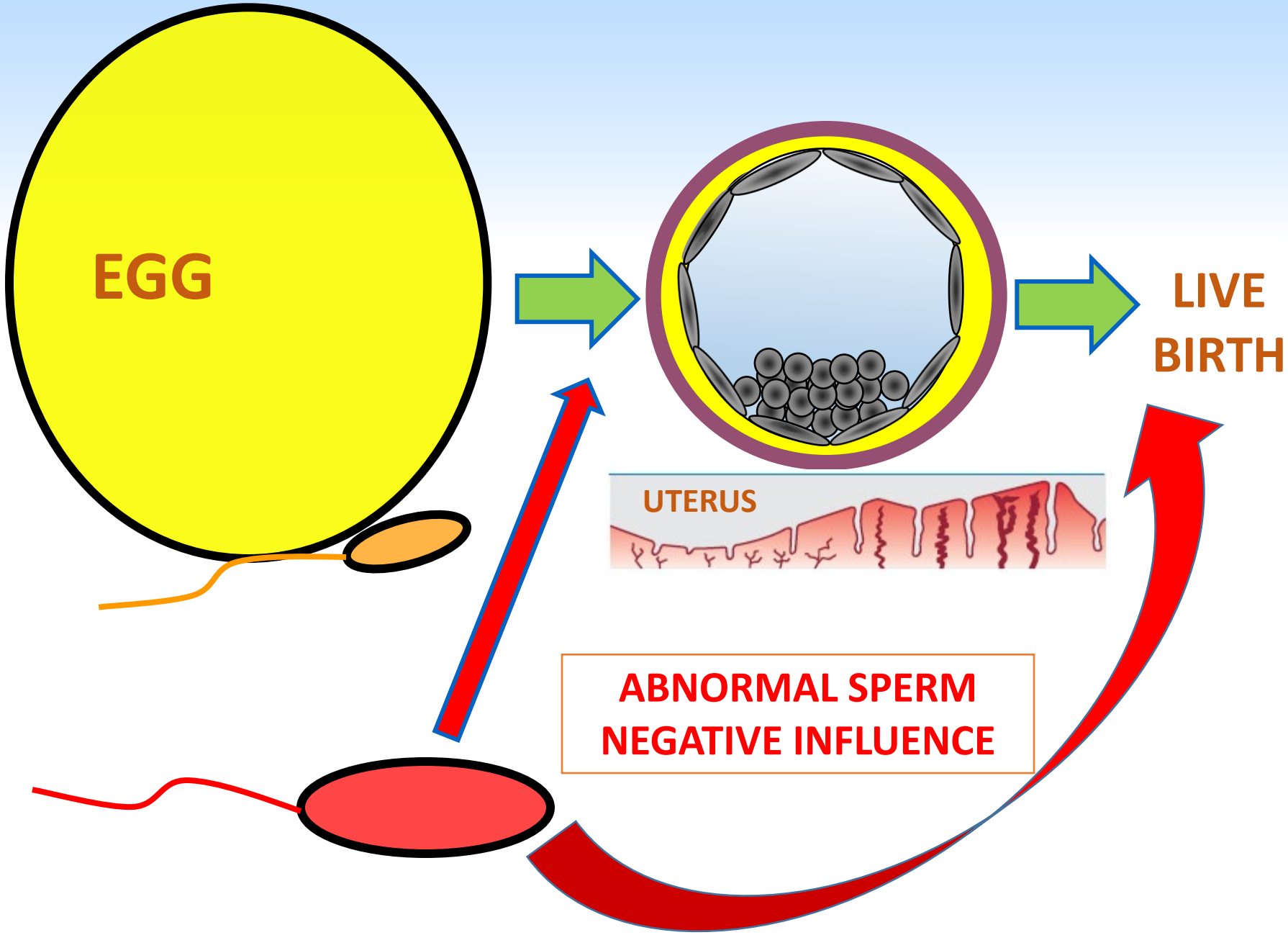
- To understand the differences in sperm preparation technologies ranging from the use of AI to select sperm to microfluidics.
- To have a better understanding of how new sperm preparation technologies impact the laboratory and whether they improve outcomes.
- To understand factors related to sperm that influence fertilization, embryo development, live birth and future generations.

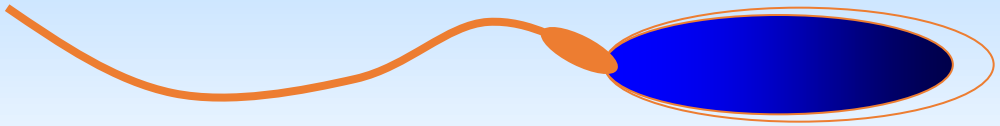
Technique	Technique
Microfluidics Rheotaxis Chemotaxis Thermotaxis	Electrophoresis
Interferometric microscopy	Zeta Potential
Hyperspectral image	ZP binding
TESE	Vitality HOST
MACS	Birrefringence (for immotile sperm)
Nanotechnology beads	LaserBeam
AI MORPHOMETRY AND MOTILITY SID Q300 BESTFIT	PICSI
IMSI-MSOME	

Why don’t we have a good sperm selection technique yet?

Venn diagram of the responsibilities of Reproductive Failure:

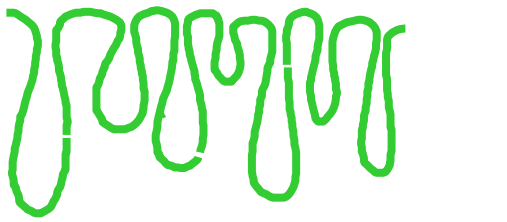



EGG
LIVE
BIRTH
UTERUS
ABNORMAL SPERM
NEGATIVE INFLUENCE

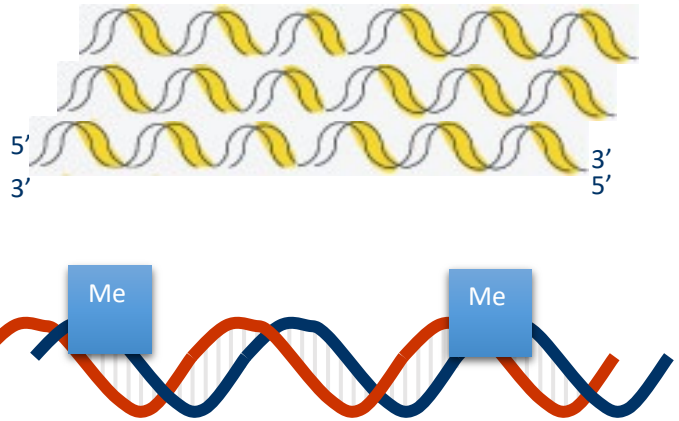


Chromosome

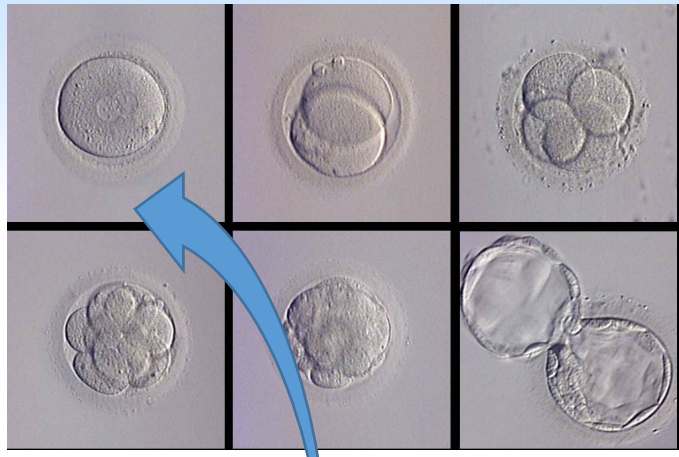
Chromatin Packaging

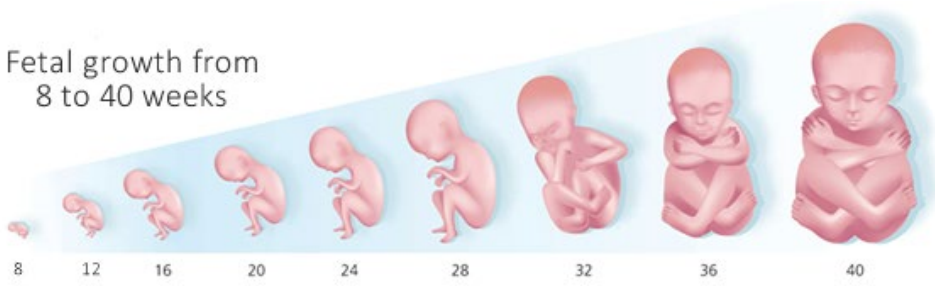


Protamine complexed DNA (>85%)



Methylation





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

Lancet 2019; 393: 416–22

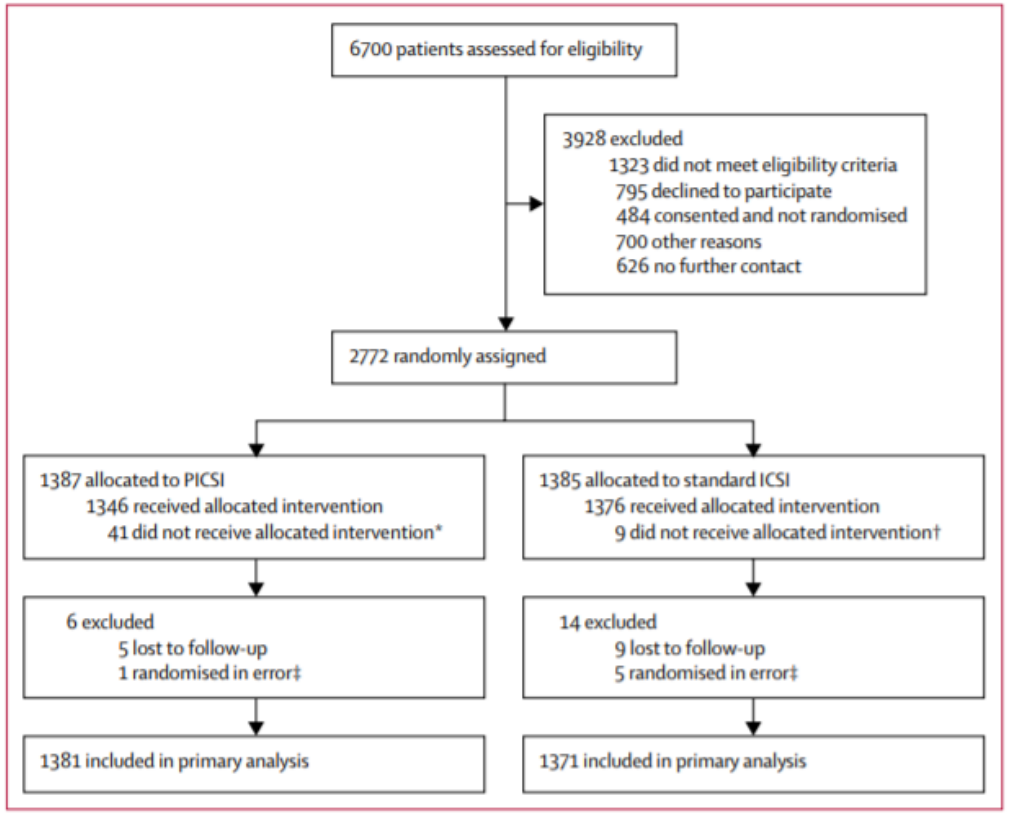
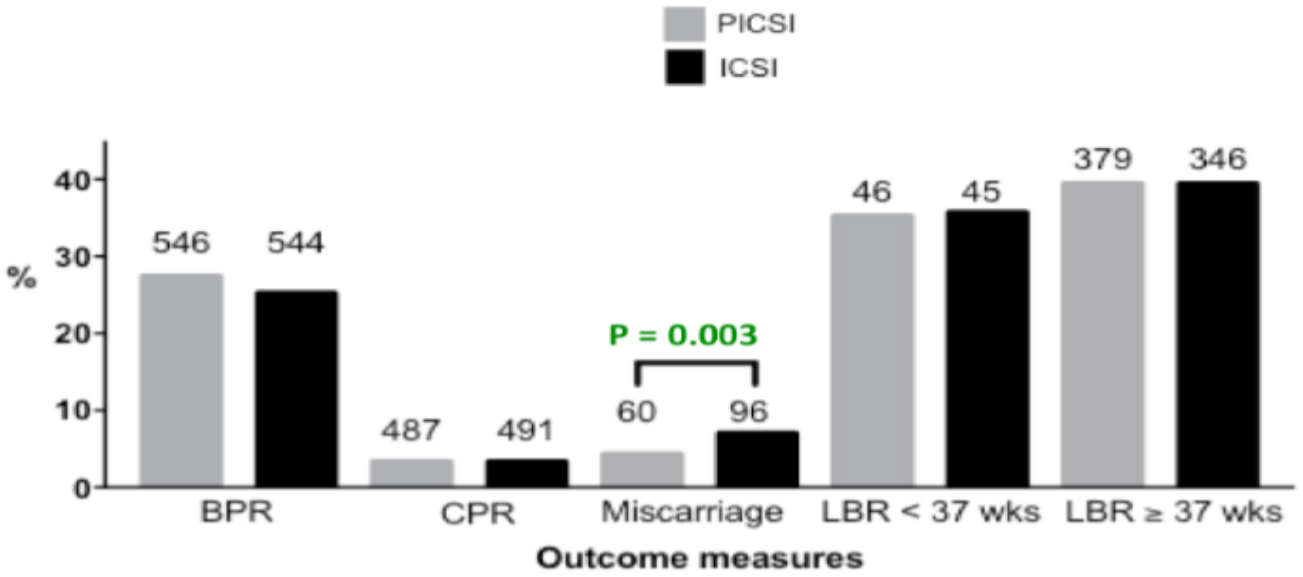


Figure: **Trial profile**
PICSI=physiological intracytoplasmic sperm injection. ICSI=intracytoplasmic sperm injection. IVF=in-vitro fertilisation. *Three received IVF, two received IVF–ICSI split cycle, and 36 received ICSI. †Five received IVF, three received IVF–ICSI split cycle, and one received PICSI. ‡These individuals were found to not meet eligibility criteria after randomisation.

PCTU

UNIVERSITY OF LEEDS

HAB SELECT



NHS National Institute for Health Research

MRC Medical Research Council

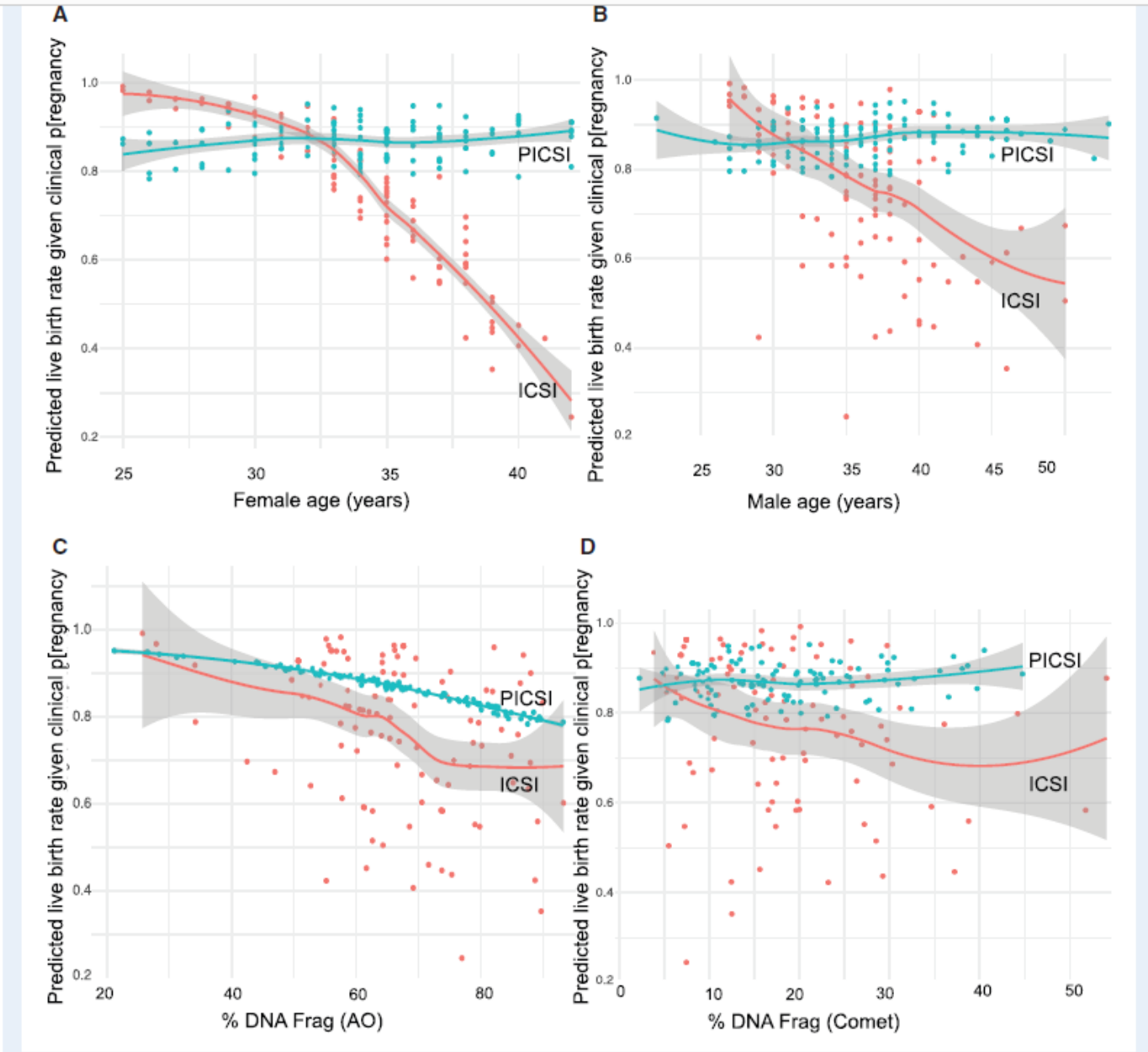
HAB SELECT

Human Reproduction, pp. 1–22, 2022
https://doi.org/10.1093/humrep/deac058

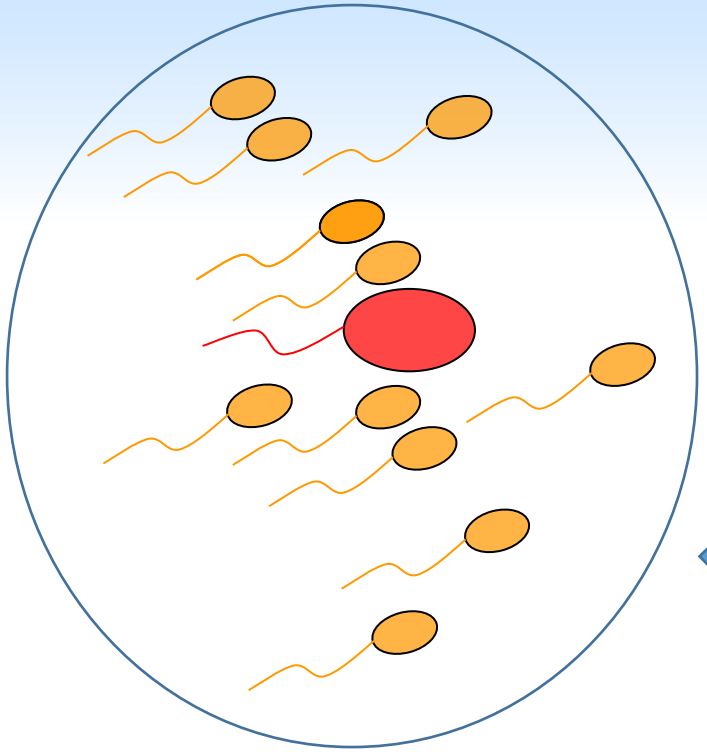
human reproduction ORIGINAL ARTICLE *Andrology*

Sperm selection with hyaluronic acid improved live birth outcomes among older couples and was connected to sperm DNA quality, potentially affecting all treatment outcomes

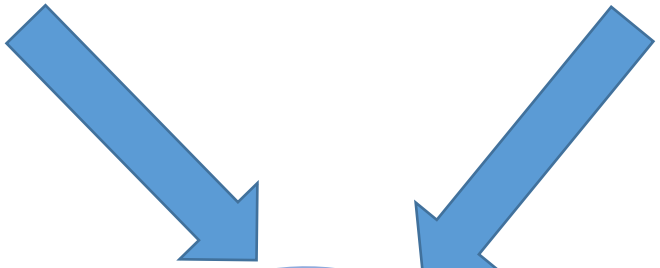
Robert West [1,*], Arri Coomarasamy[2], Lorraine Frew[2], Rachel Hutton[3], Jackson Kirkman-Brown [2,*], Martin Lawlor[3], Sheena Lewis[3], Riitta Partanen[4], Alex Payne-Dwyer [4], Claudia Román-Montañana[2], Forough Torabi[4], Sofia Tsagdi[2], and David Miller [4]

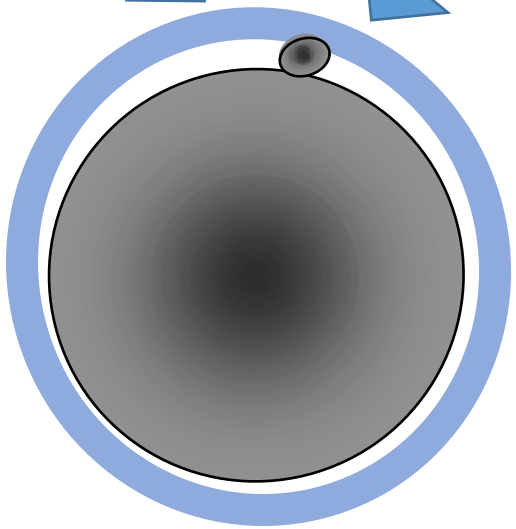


Patient A

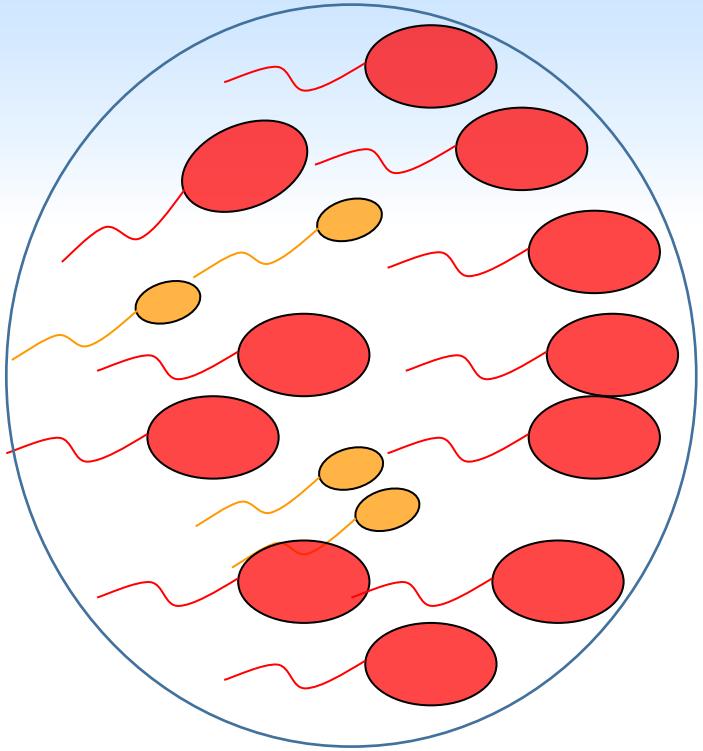


Which patient needs sperm selection?





Patient B



Why don’t we have a good sperm selection technique yet?

1. It is complicated to divorce the sperm from the egg

2. We may need to focus on the right patients

Selecting The Right Sperm

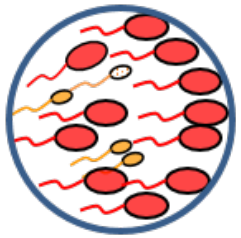
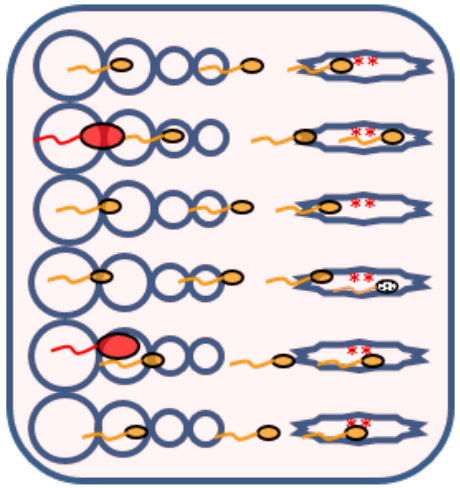
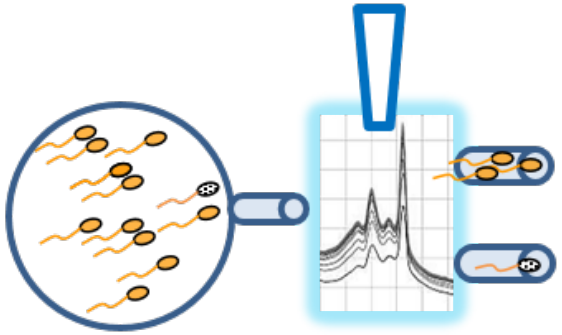
THE FUTURE
Diagnosis and Selection

Biology of Reproduction, 2019, 101(6), 1076–1082
doi:10.1093/biolre/ioz032
Review
Advance Access Publication Date: 25 February 2019

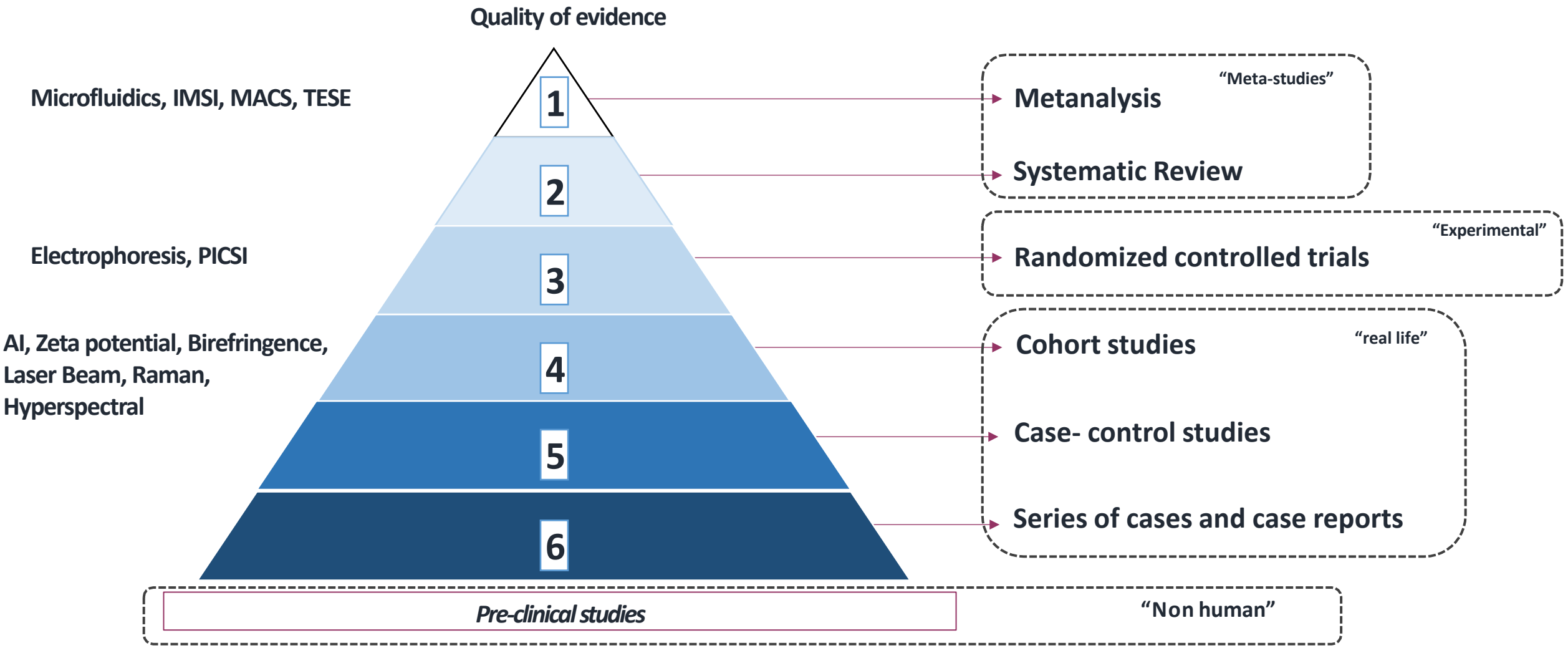
Review

Sperm selection methods in the 21st century

Denis A. Vaughan[1,2,*] **and Denny Sakkas**[1]

Analysis	Sorting	Selection
		
Home semen analysis [55,72]	Microfluidic sorting [35,36,38,60,73-77]	Microfluidic entrapment of sperm [59,78]
Sperm motion by microfluidics [79]	Microfluidics and Chemotaxis [45,61]	
Methylation analysis [80]	Microfluidics, imaging and sorting [62]	
Protein and RNA [56-58]		Microdissection for testicular samples [81]
	Microfluidics and cells [46]	

Evidence pyramid



University of Illinois Chicago. Library of the Health Sciences. Evidence Based Medicine. https://researchguides.uic.edu/ebm.

biology

MDPI

Systematic Review

Superior Live Birth Rates, Reducing Sperm DNA Fragmentation (SDF), and Lowering Miscarriage Rates by Using Testicular Sperm Versus Ejaculates in Intracytoplasmic Sperm Injection (ICSI) Cycles from Couples with High SDF: A Systematic Review and Meta-Analysis

Marina Cano-Extremera [†], Irene Hervas [†], Alma Gisbert Iranzo, Mar Falquet Guillem, María Gil Juliá, Ana Navarro-Gomezlechon, Rosa Pacheco-Rendón and Nicolás Garrido Puchalt [*]

IVIRMA Global Research Alliance, IVI Foundation, Instituto de Investigación Sanitaria La Fe (IIS La Fe), Avenida Fernando Abril Martorell, 106—Torre A, Planta 1ª, 46026 Valencia, Spain; marina.cano99@gmail.com (M.C.-E.); irene.hervas@ivirma.com (I.H.); almagisbert99@gmail.com (A.G.I.); marfalquetuv@gmail.com (M.F.G.); maria.gil@ivirma.com (M.G.J.); ana.navarro@ivirma.com (A.N.-G.); rosa.pacheco@ivirma.com (R.P.-R.)

* Correspondence: nicolas.garrido@ivirma.com

† These authors contributed equally to this work.

Simple Summary: The use of testicular sperm in non-azoospermic males has emerged in recent years as an attractive option for couples with high sperm DNA fragmentation (SDF) in the ejaculate, repeated ICSI failures, and even poor sperm quality. With this systematic review and meta-analysis, we aim to clarify the findings to date and provide updated information to guide clinical decisions. Our results indicate a clear decrease in the degree of SDF in testicular spermatozoa when compared to ejaculate, and their subsequent use in ICSI cycles leads to a significant increase in the clinical pregnancy rate and a decrease in the miscarriage rate, which is reflected in a significant increase in the rate of live birth at home. In addition, this clinical approach is much more effective in normozoospermic males with high SDF in the ejaculate and with at least one previously failed ICSI cycle. Nonetheless, the findings should be viewed with caution due to the low quality of the studies included and the limited evidence on the safety of this approach for offspring due to chromosome aneuploidies.

Abstract: This study aimed to compare sperm DNA fragmentation (SDF) levels between ejaculate and testicular sperm and evaluate clinical outcomes of intracytoplasmic sperm injection (ICSI) cycles using testicular sperm (T-ICSI) versus ejaculate sperm (E-ICSI) in males with high ejaculate SDF, prior ICSI failures, or severe male infertility. A systematic review of major databases and a subsequent meta-analysis were performed to compare clinical outcomes in men with high SDF, oligozoospermia, or prior ICSI failures undergoing T-ICSI or E-ICSI. Thirteen studies met the inclusion criteria. Outcomes analyzed included SDF levels, fertilization rate (FR), clinical pregnancy rate (CPR), live birth rate (LBR) per embryo transfer (ET), and miscarriage rate (MR) per pregnancy. The mean difference (MD) and odds ratio (OR) were calculated for each outcome. Paired assessments of SDF showed significantly lower levels in testicular sperm compared to ejaculated sperm (MD = −25.42 [−31.47, −17.30], $p < 0.00001$). While no significant difference in FR was observed in T-ICSI cycles overall (OR = 0.94 [0.74, 1.20]), a subgroup analysis revealed significantly higher FR with E-ICSI in men with oligozoospermia and no prior ICSI failures (OR = 0.61 [0.52, 0.71], $p < 0.00001$). CPR was significantly higher in T-ICSI cycles (OR = 2.13 [1.35, 3.36], $p < 0.001$; n = 540 ET), along with a significantly lower MR (OR = 0.31 [0.14, 0.70], $p = 0.004$; n = 35) and increased LBR (OR = 2.40 [1.32, 4.36], $p = 0.004$; n = 446 ET). In conclusion,

check for updates

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Published: 26 January 2025

Citation: Cano-Extremera, M.; Hervas, I.; Gisbert Iranzo, A.; Falquet Guillem, M.; Gil Juliá, M.; Navarro-Gomezlechon, A.; Pacheco-Rendón, R.; Garrido Puchalt, N. Superior Live Birth Rates, Reducing Sperm DNA Fragmentation (SDF), and Lowering Miscarriage Rates by Using Testicular Sperm Versus Ejaculates in Intracytoplasmic Sperm Injection (ICSI) Cycles from Couples with High SDF: A Systematic Review and Meta-Analysis. *Biology* **2025**, *14*, 130. https://doi.org/10.3390/biology14020130

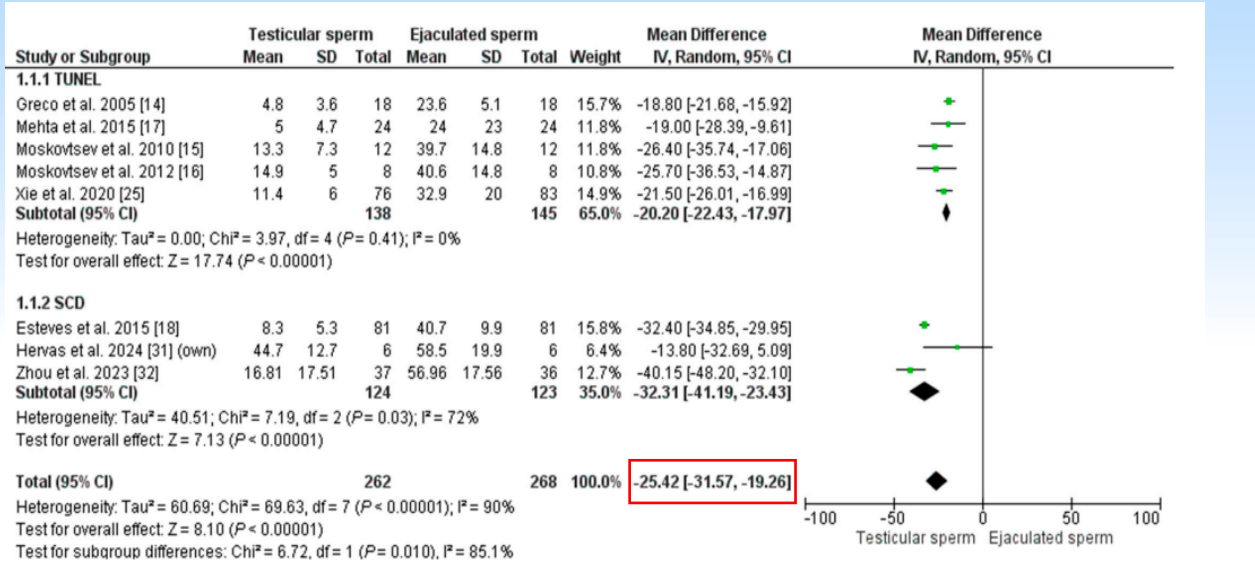
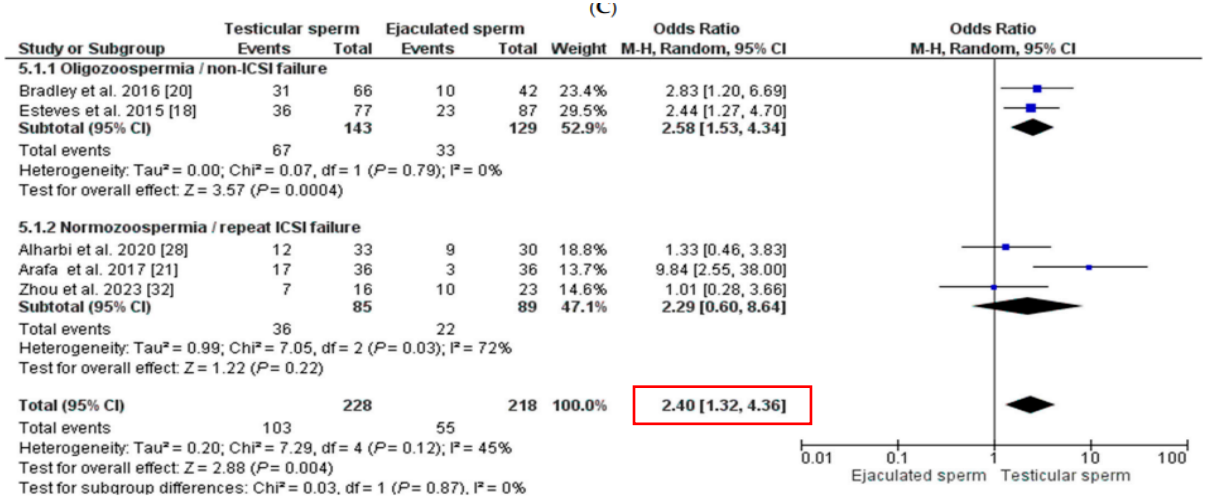


Figure 2. Forest plot showing the mean difference (MD) for sperm DNA fragmentation (SDF) rates between testicular and ejaculated sperm in men with high SDF. Two subgroups are established depending on the technique for measuring SDF: SCD (sperm chromatin dispersion) test and TUNEL test (Terminal deoxynucleotidyl transferase dUTP nick end labeling). CI: confidence interval; IV: inverse variance.

(C)



(D)

Figure 3. Forest plot showing odds ratios (OR) for each clinical outcome assessed, fertilization rate (**A**), clinical pregnancy rate (**B**), miscarriage rate (**C**), and live birth rate (**D**), between ICSI cycles using testicular sperm and ICSI cycles using ejaculated sperm, in males with high SDF. In addition,

Microfluidics: Meta-analysis

biology

MDPI

Systematic Review

Sperm Selection Using Microfluidic Techniques Significantly Decreases Sperm DNA Fragmentation (SDF), Enhancing Reproductive Outcomes: A Systematic Review and Meta-Analysis

Alma Gisbert Iranzo, Marina Cano-Extremera, Irene Hervás, Mar Falquet Guillem, María Gil Juliá, Ana Navarro-Gomezlechon, Rosa María Pacheco-Rendón and Nicolás Garrido*

IVIRMA Global Research Alliance, IVI Foundation, Instituto de Investigación Sanitaria La Fe (IIS La Fe), Avenida Fernando Abril Martorell, 106—Torre A, Planta 1ª, 46026 Valencia, Spain; almagisbert99@gmail.com (A.G.I.); marina.cano99@gmail.com (M.C.-E.); irene.hervas@ivirma.com (I.H.); marfalquetuv@gmail.com (M.F.G.); mariagil.email@gmail.com (M.G.J.); ana.navarro@ivirma.com (A.N.-G.); rosa.pacheco@ivirma.com (R.M.P.-R.)
* Correspondence: nicolas.garrido@ivirma.com

Simple Summary

Despite the remarkable technological advances and increasing success rates in the last decades, assisted reproduction techniques' success is limited, frequently needing multiple treatments before achieving healthy offspring. Sperm selection is one of the key aspects to be improved, since single sperm are genetically unique, and its proper selection could enhance success rates in IVF/ICSI treatments. Microfluidic techniques have emerged in recent years as a promising tool that could revolutionize conventional sperm selection (through swim-up and/or density gradient techniques). Our results indicate that its use enables better sperm parameters, highlighting lower sperm DNA fragmentation (SDF), and improves some reproductive outcomes in intracytoplasmic sperm injection cycles. However, careful interpretation of these results is advised due to the variability in study populations and inconsistencies in the quality of some studies included in the analysis.

Abstract

This study aimed to compare sperm parameters and reproductive outcomes after sperm selection using microfluidic chips versus conventional techniques (swim-up/density gradients). A systematic review and meta-analysis were performed after the extraction of relevant data from thirty-nine studies that met the inclusion criteria. Mean difference or odds ratio was calculated for each outcome. The analysis revealed that sperm selection using microfluidics yields lower sperm DNA fragmentation (MD = −9.98 [−13.19, −6.76], $p < 0.00001$), increased progressive motility (MD = 14.50 [7.84, 21.71], $p = 0.04$), total motility (MD = 10.68 [6.04, 15.31], $p < 0.00001$) and morphology (MD = 1.41 [0.67, 2.16], $p = 0.0002$). Significant differences were also found in the fertilization rate/MII oocyte microinjected (OR = 1.22 [1.01, 1.46], $p = 0.04$), implantation rate/embryo transfer (ET) (OR = 4.51 [1.42, 14.37], $p = 0.01$), clinical pregnancy/ET (OR = 1.73 [1.22, 2.45], $p = 0.002$), ongoing pregnancy/ET (OR = 1.99 [1.03, 3.83], $p = 0.04$), live birth rate/first cycle (OR = 1.59 [1.12, 2.24], $p = 0.009$) and per all embryo transfer (OR = 1.65 [1.06, 2.55], $p = 0.03$). No significant differences were found in embryo euploidy/number of biopsied blastocysts (OR = 1.34 [0.88, 2.04], $p = 0.77$), biochemical pregnancy/ET (OR = 1.23 [0.84, 1.80], $p = 0.29$), miscarriage rate/cycle (OR = 0.84 [0.54, 1.31], $p = 0.35$) and per pregnancy (OR = 0.71 [0.50, 1.02], $p = 0.07$), live birth rate/first embryo transfer (OR = 1.60 [0.80, 3.22], $p = 0.18$) and per concluded cycle (OR = 1.03 [0.53, 2.00], $p = 0.92$). To summarize, microfluidics may offer a

check for updates

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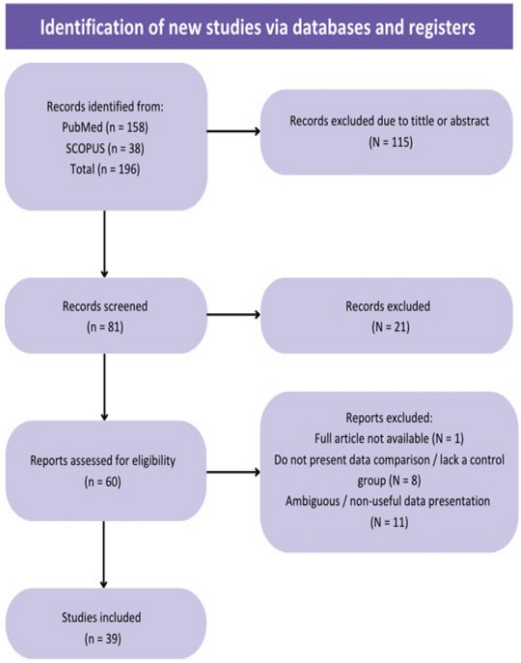


Figure 1. Flow diagram of the review process and selection of studies included in the meta

Table 1. Summary of the obtained results (CI 95%) after the statistical analysis, for each outcome studied. *The use of microfluidics is associated with lower sperm concentration; higher progressive and total motility; improved sperm morphology; reduced sperm DNA fragmentation (SDF); increased fertilization rates; higher implantation rates; better clinical and ongoing pregnancy rates (per ET); lower miscarriage /pregnancy rates; live birth rate (LBR) per cycle; and LBR/all ET. No significant influence has been detected on the euploidy rate, biochemical pregnancy rate, miscarriage rate per cycle, LBR after first transfer, or LBR/concluded cycles. In bold letters those estimates with statistically significant differences between groups.*

Result	Units	Estimator	95% CI	Number of articles (sample size)
Seminal parameters				
Concentration	M/mL	MD	**−15.95 [−19.28, −12.61]**	19 (1200)
Progressive motility	%A+B	MD	**14.50 [7.84, 21.71]**	8 (548)
Total motility	%	MD	**10.68 [6.04, 15.31]**	13 (832)
Morphology	%	MD	**1.41 [0.67, 2.16]**	7 (396)
SDF	%	MD	**−9.98 [−13.19, −6.76]**	15 (593)
Reproductive outcomes after ICSI				
Fertilization rate	%	OR	**1.22 [1.01, 1.46]**	12 (40748)
Embryo euploidy rate	%	OR	1.34 [0.88, 2.04]	7 (13813)
Biochemical pregnancy rate / ET	%	OR	1.23 [0.84, 1.80]	8 (1189)
Implantation rate	%	OR	**4.51 [1.42, 14.37]**	6 (400)
Clinical pregnancy rate / ET	%	OR	**1.73 [1.22, 2.45]**	16 (2333)
Ongoing pregnancy rate/ ET	%	OR	**1.99 [1.03, 3.83]**	6 (973)
All types misscarriage rate/cycle	%	OR	0.84 [0.54, 1.31]	3 (758)
All types misscarriage rate/pregnancy	%	OR	0.71 [0.50, 1.02]	10 (800)
LBR/1st ET	%	OR	1.60 [0.80, 3.22]	2 (143)
LBR/1st cycle	%	OR	**1.59 [1.12, 2.24]**	2 (598)
LBR/all ET	%	OR	**1.65 [1.06, 2.55]**	3 (570)
LBR/concluded cycle	%	OR	1.03 [0.53, 2.00]	4 (244)

1st ET: first embryo transfer; authors only offered data from the first transferred embryo from each patient.

All ET: all embryo transfer; authors offered data taking into count every embryo transfer from each patient, not necessarily being from the same cycle.

1st cycle: authors offered data for the outcome from patients who came into their clinic for their first cycle (they did not specify if there where any embryos left or not in the moment of publication of the results).

MACS: Meta-analysis (Falquet et al., 2025, RBMO)

Sperm selection by Magnetic Activated Cell Sorting (MACS) to select non-apoptotic sperm positively influence DNA fragmentation (DNAf) and reproductive outcomes: a systematic review and meta-analysis

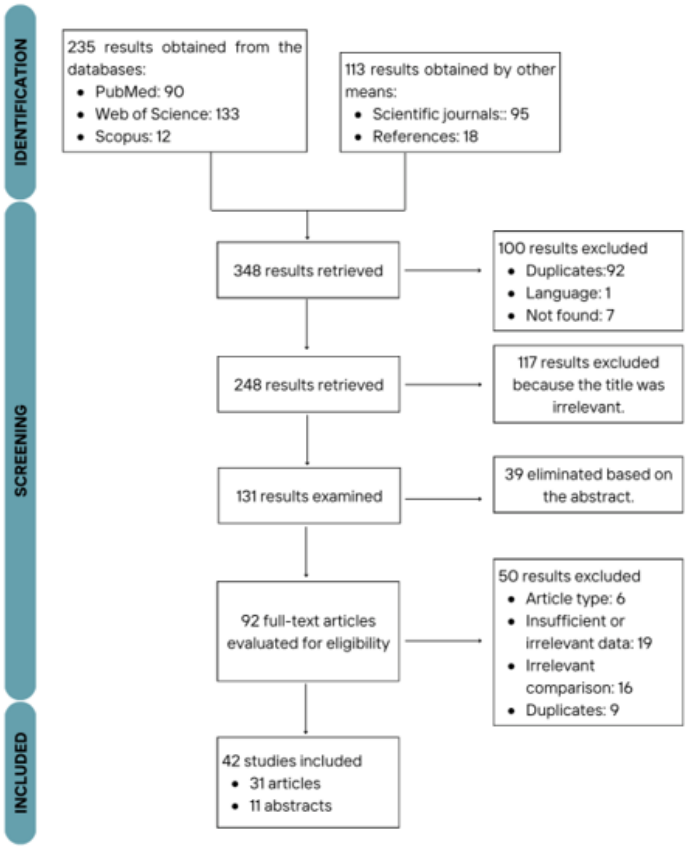
Mar Falquet Guillem[1], Rosa Pacheco[1], Alma Gisbert Iranzo[1], Marina Cano-Extremera[1], María Gil Julia[1], Ana Navarro-Gomezlechon[1], Irene Hervas[1], Nicolás Garrido[1,*]

[1]IVIRMA Global Research Alliance, IVI Foundation, Instituto de Investigación Sanitaria La Fe (IIS La Fe), Avenida Fernando Abril Martorell, 106 - Torre A, Planta 1ª, 46026, Valencia, Spain

Corresponding author. E-mail address: nicolas.garrido@ivirma.com (Nicolas Garrido)

Abstract

Assisted Reproductive Techniques (ART) rely on semen quality for success. While conventional sperm selection techniques such as density gradients and swim-up are commonly used, they overlook essential molecular sperm characteristics, limiting their effectiveness in predicting ART success. To address this, advanced selection techniques have been developed, including Magnetically Activated Cell Sorting (MACS). This meta-analysis evaluates the effect of MACS compared to conventional sperm selection techniques on ART reproductive outcomes, seminal parameters, and sperm DNA fragmentation (DNAf) levels. Systematic searches in PubMed, Web of Science, and Scopus identified 41 studies meeting the inclusion criteria. Results indicate that while MACS significantly reduces sperm DNAf (MD=-4.32,[-6.29;-2.36],P<0.0001), , it does not significantly improve clinical pregnancy (OR=1.54,[0.86;2.77],P=0.15), spontaneous miscarriage (OR=0.92,[0.40;2.13],P=0.84), or live birth (OR=1.57,[0.74;3.32],P=0.24) rates in artificial insemination cycles. However, in Intracytoplasmic Sperm Injection cycles, MACS demonstrates significant improvements in implantation rate per transferred embryo (OR=1.28,[1.02;1.62],P=0.04), clinical pregnancy (OR=1.41,[1.19;1.66], P<0.00001), and live birth (OR=1.41,[1.16;1.72],P=0.0005) rates per embryo transfer particularly in patients with high DNAf levels. Despite variability in other clinical parameters and study limitations as data heterogeneity, the study results suggest a potential benefit of MACS in a specific subgroup of infertile patients, underscoring the need for personalized evaluation and further research to refine its clinical indications.



670 ***Figure 1. Search and selection of articles.*** *Flow chart of the process of identifying and selecting studies*
671 *for a meta-analysis on the effect of using MACS on reproductive outcomes and DNA fragmentation*
672 *compared to conventional sperm sorting techniques.*

Table 1: Summary of main results of the study. *An MD greater than 0 or OR greater than 1 suggests a higher risk of a successful outcome in the group where MACS is used for sperm selection compared to the control group.*

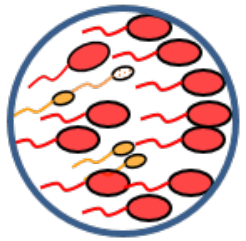
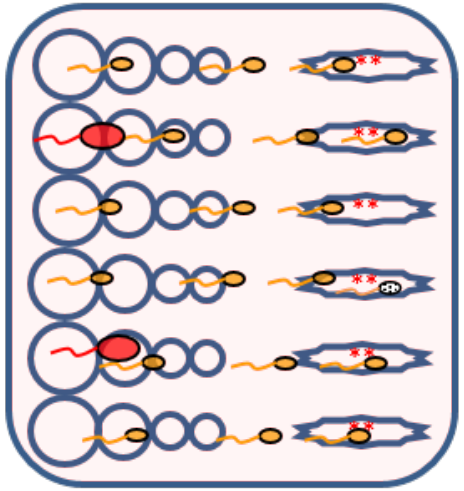
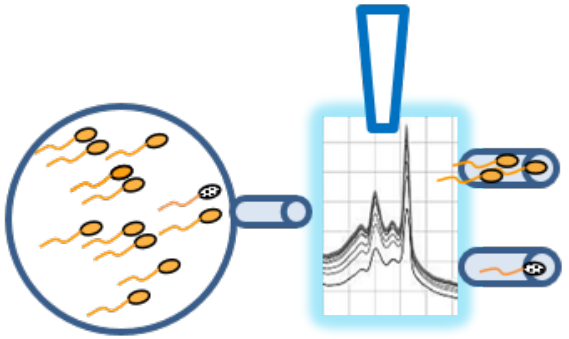
Result	Units	Estimator	IC 95%	No. of items
Seminal parameters				
Sperm concentration	Mill /ml	MD	-1.97 (-3.09, -0.85)	4
Progressive sperm motility	%	MD	0.16 (-4.78, 5.10)	6
Sperm morphology	%	MD	-1.40 (-3.10, 0.30)	8
Sperm DNA fragmentation	%	MD	-4.82 (-6.97, -2.66)	14
Reproductive outcomes after IUI				
Clinical pregnancy rate	%	OR	1.54 (0.86, 2.77)	3
Spontaneous miscarriage rate	%	OR	0.92 (0.40, 2.13)	2
Live birth rate	%	OR	1.57 (0.74, 3.32)	2
Reproductive outcomes after ICSI				
MII fertilization rate per oocyte	%	OR	0.98 (0.74, 1.32)	3
Blastocyst formation rate per embryo	%	OR	0.99 (0.71, 1.39)	2
Implantation rate per transferred embryo	%	OR	1.28 (1.02, 1.62)	4
Biochemical pregnancy rate per ET	%	OR	1.07 (0.95, 1.20)	7
Clinical pregnancy rate per ET	%	OR	1.41 (1.19, 1.66)	21
Ongoing pregnancy rate per ET	%	OR	1.08 (0.91, 1.27)	4
Spontaneous miscarriage rate per ET	%	OR	1.04 (0.93, 1.15)	13
Biochemical miscarriage rate per ET	%	OR	1.06 (0.92, 1.21)	8
Clinical miscarriage rate per ET	%	OR	0.27 (0.09, 0.84)	2
Live birth rate per ET	%	OR	1.41 (1.16, 1.72)	11
Cumulative live birth rate	%	OR	3.73 (0.26, 53.79)	3

ET: Embryo transfer

Can we do this?

- Microfluidics
- Optics
- Artificial intelligence
- Chemo-attractants
- Antibodies

Can we tick all the boxes?

Analysis	Sorting	Selection
		
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Sperm motion by microfluidics [79]	Microfluidics and Chemotaxis [45,61]	
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Protein and RNA [56-58]		Microdissection for testicular samples [81]
	Microfluidics and cells [46]	

PLOS ONE

RESEARCH ARTICLE

Automated smartphone-based system for measuring sperm viability, DNA fragmentation, and hyaluronic binding assay score

Irene Dimitriadis[1], Charles L. Bormann[1], Manoj Kumar Kanakasabapathy[2], Prudhvi Thirumalaraju[2], Hemanth Kandula[2], Vinish Yogesh[2], Neeraj Gudipati[2], Vignesh Nataraian[2], John C. Petrozza[1], Hadi Shafiee[2,3]*

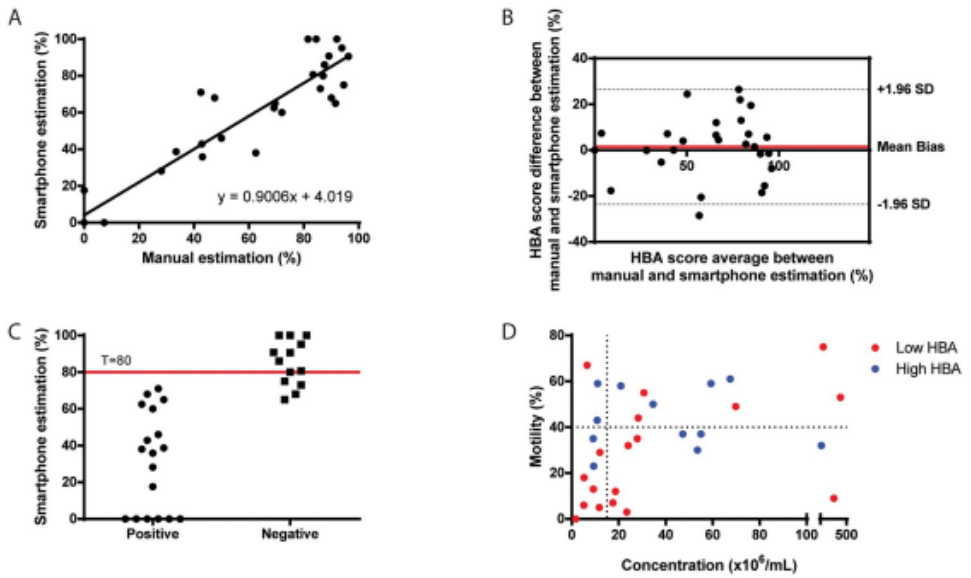

Fig 2. Comparison of conventional method of analysis against automated smartphone-based HBA assessment. (A) A linear regression analysis revealed strong agreement between the two methods (n = 31). The regression line is the solid line and the equation presented, is the line equation. (B) The samples were also compared using the Bland-Altman method of analysis (n = 31). The analysis revealed an absence of proportional and systematic bias for the tested sample set. The solid red line marks the mean bias. The dotted lines are the 95% limits of agreement of the sample set. (C) The scatter plot here represents the device performance in classifying samples as positive (<80%) and negative (= >80%). The system showed a sensitivity of 100% and a specificity of 69.23% (n = 31). The overall accuracy of the system in HBA-score based classification was 87.10% (D). The scatter plot shows the concentration and motility values of semen samples as measured by a CASA system along with its respective HBA score (n = 30).

https://doi.org/10.1371/journal.pone.0212562.g002

PLOS ONE

RESEARCH ARTICLE

Automated smartphone-based system for measuring sperm viability, DNA fragmentation, and hyaluronic binding assay score

Irene Dimitriadis[1], Charles L. Bormann[1], Manoj Kumar Kanakasabapathy[2], Prudhvi Thirumalaraju[2], Hemanth Kandula[2], Vinish Yogesh[2], Neeraj Gudipati[2], Vignesh Natarajan[2], John C. Petrozza[1], Hadi Shafiee[2,3]*

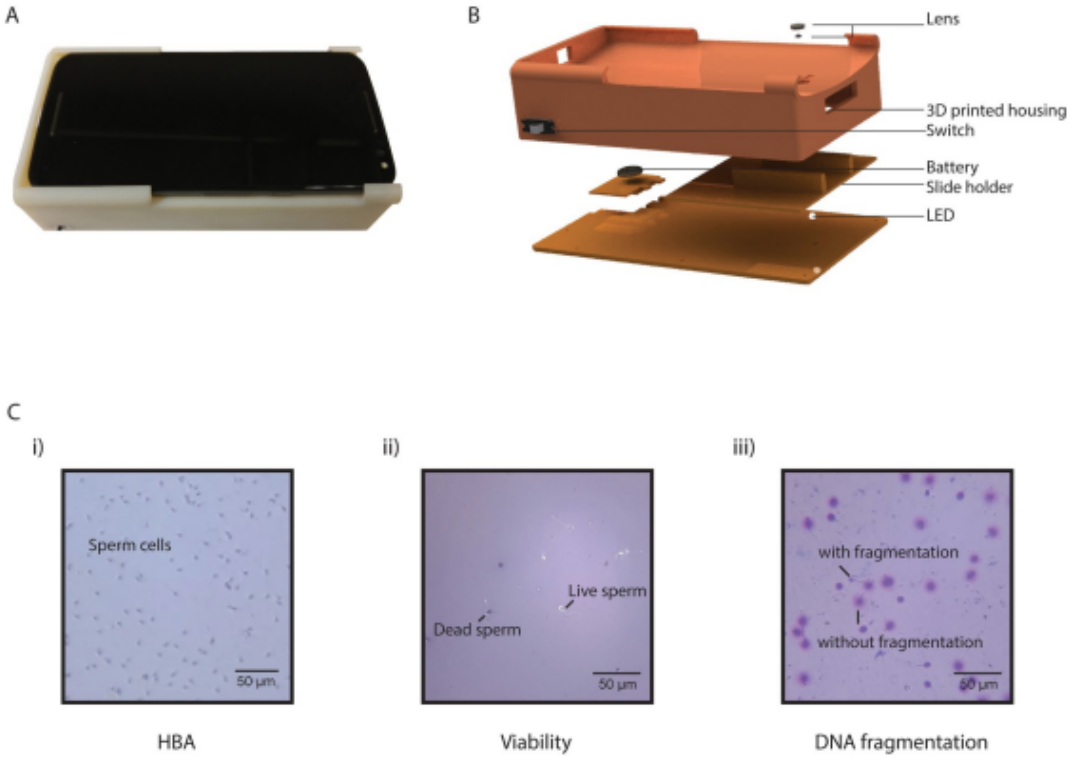

Fig 1. Smartphone-based semen analysis system. (A) The actual smartphone optical attachment along with a smartphone. (B) The exploded image shows the various components of the smartphone-based semen analysis system. (C) Images acquired with the smartphone imaging platform for the three different assays, (i) HBA, (ii) viability, and (iii) DNA fragmentation.

https://doi.org/10.1371/journal.pone.0212562.g001

Original Article - Sexual Dysfunction/Infertility

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pISSN 2466-0493 • eISSN 2466-054X

INVESTIGATIVE AND CLINICAL UROLOGY
ICUROLOGY

Accuracy comparison study of new smartphone-based semen analyzer versus laboratory sperm quality analyzer

Min Jung Park[1], Mi Young Lim[2], Hyun Jun Park[2], Nam Cheol Park[1,2]

[1]The Korea Institute for Public Sperm Bank, Busan, [2]Department of Urology, Pusan National University School of Medicine, Busan, Korea

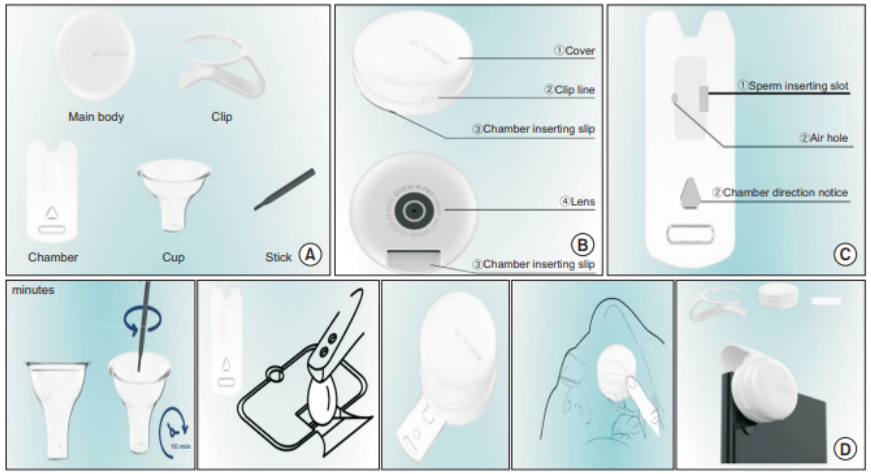
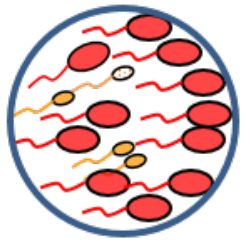
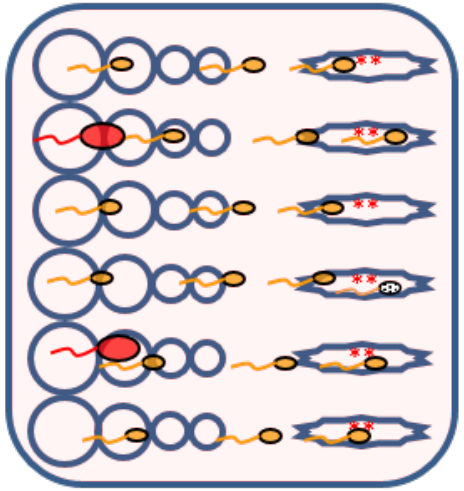
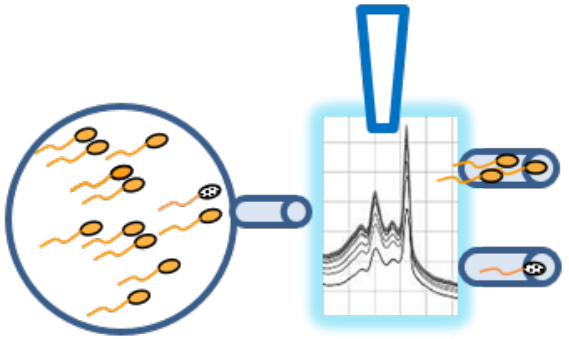


Fig. 1. Device and components of O'VIEW-M PRO® system. (A) Components of O'VIEW-M PRO® is consisted of main body with clip, chamber, semen cup, and stick. (B) Main body is consisted of cover, clip line, chamber inserting slip, lens, and built-in LED light source. (C) The liquefied semen sample is dropped into the sperm inserting slot. (D) Assay procedure with smartphone-based App assisted semen analysis, O'VIEW-M PRO®. After semen collection, liquefaction and stirring, semen loaded on 0.05 mL (1 drop) in the sperm inserting square slot of chamber and insert the chamber into the chamber inserting slip of main body. Sperm can be observed through the lens of main body by the naked eye. After fixing the attachment of the lens and body of the smartphone with a clip, the sample test result with concentration and mobility is shown as a screenshot.

Can we tick all the boxes?

Analysis	Sorting	Selection
		
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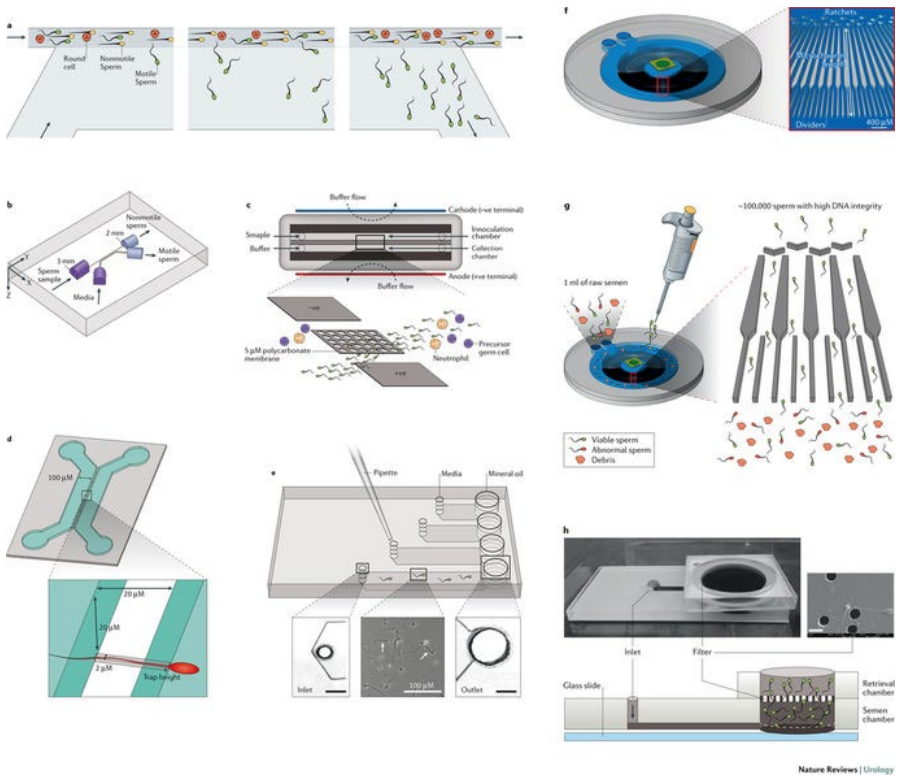
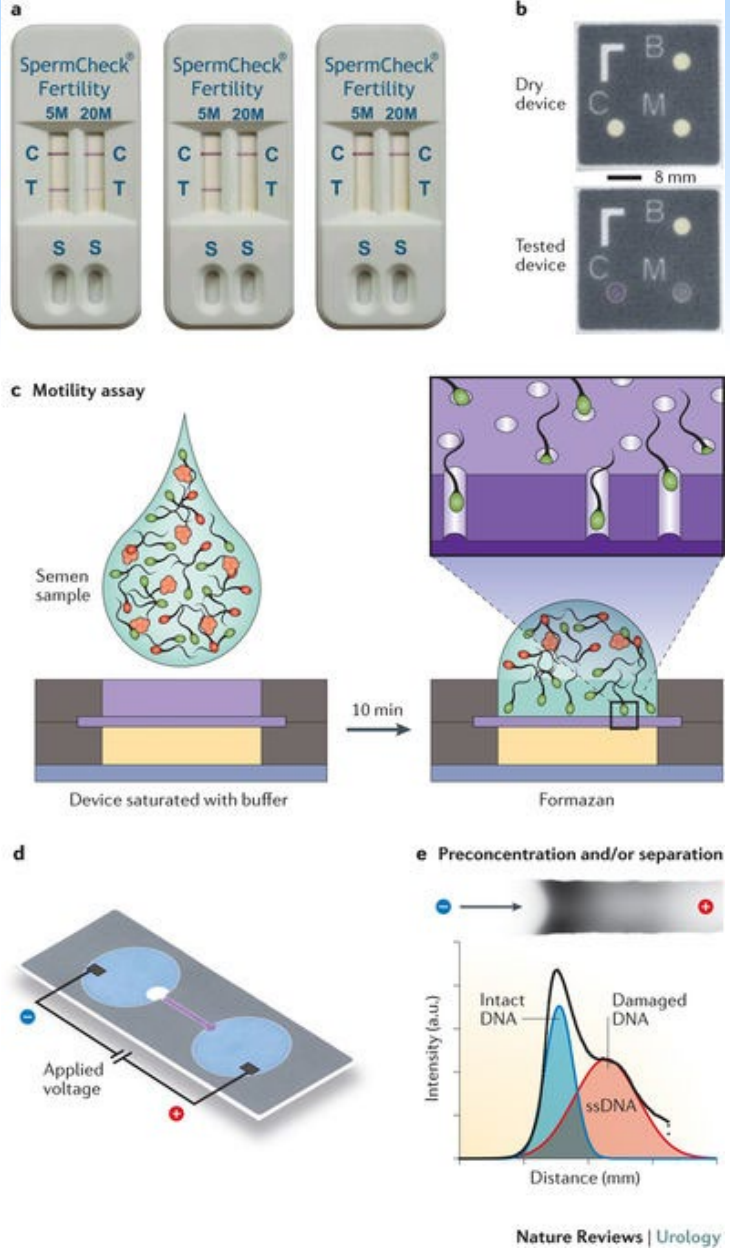
Microfluidics





micromachines

MDPI

Article

Antibody-Conjugated Magnetic Beads for Sperm Sexing Using a Multi-Wall Carbon Nanotube Microfluidic Device

Chalinee Phiphattanaphiphop [1], Komgrit Leksakul [1,*], Thananut Wanta [1], Trisadee Khamlor [2] and Rungrueang Phattanakun [3]

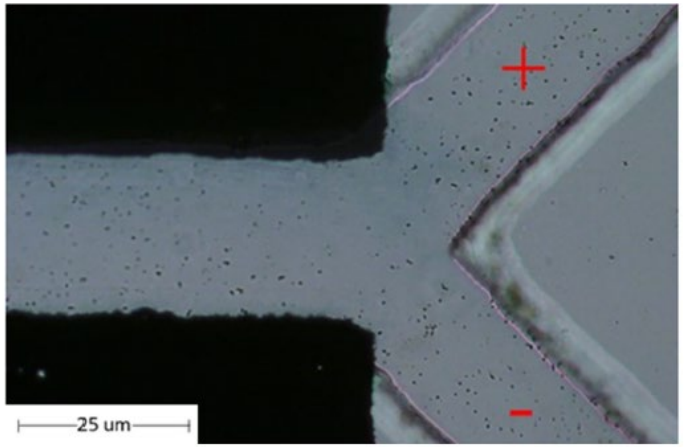


Figure 10. Flow of the magnetic particles on a monoclonal antibody and sperm in microstructure flow channel with microfluidic chip.

- Bovine sperm was bound to a Mab conjugated with positive charge beads which capture Y-sperm
- These sperm are moved to the negative electrode leaving X-sperm in the main channel

Table 6. Efficiency results of X and Y sperm separation based on the measurement using real-time Polymerase chain reaction (PCR) techniques.

Type of Electrode	Sorting Sperm Y/X Capacity (%) without Magnetic Beads (Bottom)	Sorting Sperm Y/X Capacity (%) without Magnetic Beads (Top)	Sorting Antibody + Magnetic Beads + Sperm Y/X (Bottom)	Sorting Antibody + Magnetic Beads + Sperm Y/X (Top)
Thin film electrodes microfluidic chip	53.99/46.01	50.39/49.61	61.11/38.89	31.33/68.67
MCNTs electrodes microfluidic chip	74.62/25.38	51.29/48.71	80.12/19.88	28.56/71.44

analytical chemistry

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Article

Flow-Free Microfluidic Device for Quantifying Chemotaxis in Spermatozoa

Johanna T. W. Berendsen,* Stella A. Kruit, Nihan Atak, Ellen Willink, and Loes I. Segerink

Cite This: *Anal. Chem.* 2020, 92, 3302−3306

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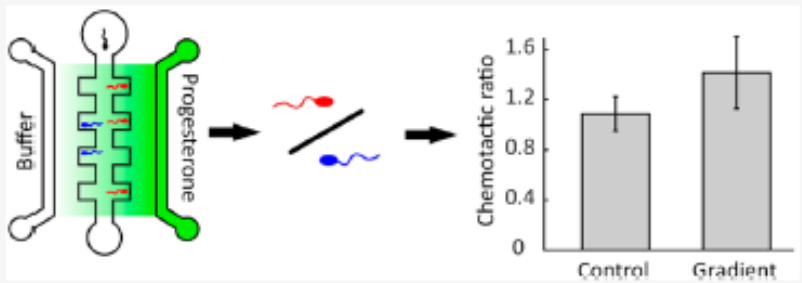
ABSTRACT: Current male fertility diagnosis tests focus on assessing the quality of semen samples by studying the concentration, total volume, and motility of spermatozoa. However, other characteristics such as the chemotactic ability of a spermatozoon might influence the chance of fertilization. Here we describe a simple, easy to fabricate and handle, flow-free microfluidic chip to test the chemotactic response of spermatozoa made out of a hybrid hydrogel (8% gelatin/1% agarose). A chemotaxis experiment with 1 μM progesterone was performed that significantly demonstrated that boar spermatozoa are attracted by a progesterone gradient.



Can we tick all the boxes?

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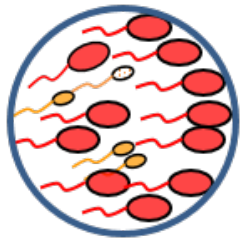	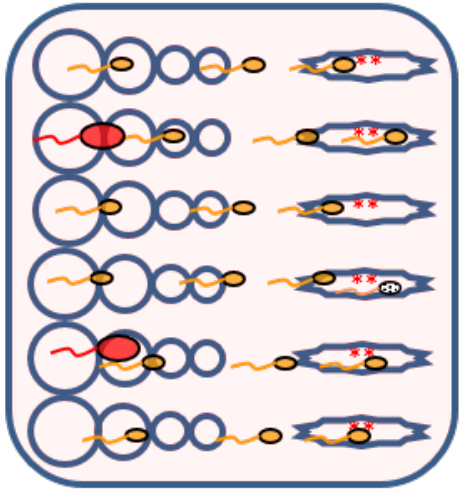	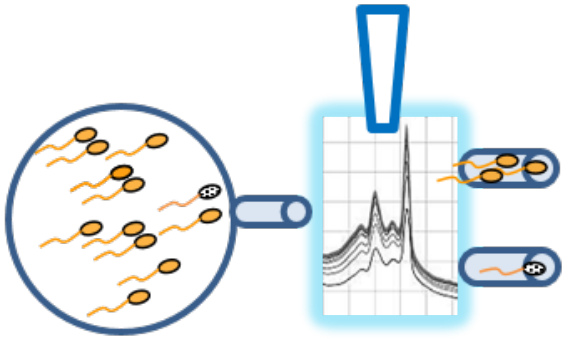
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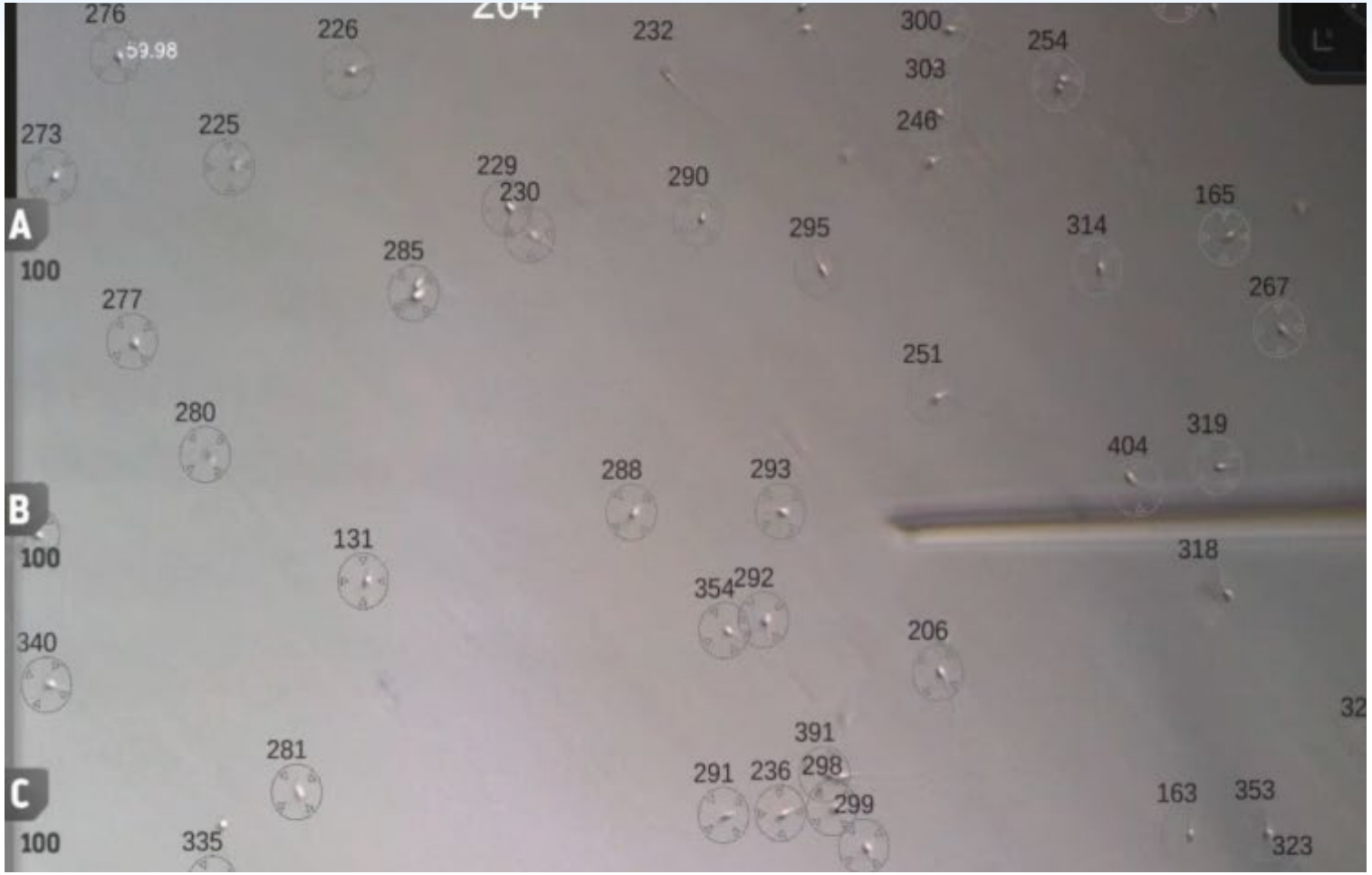
AI and sperm selection



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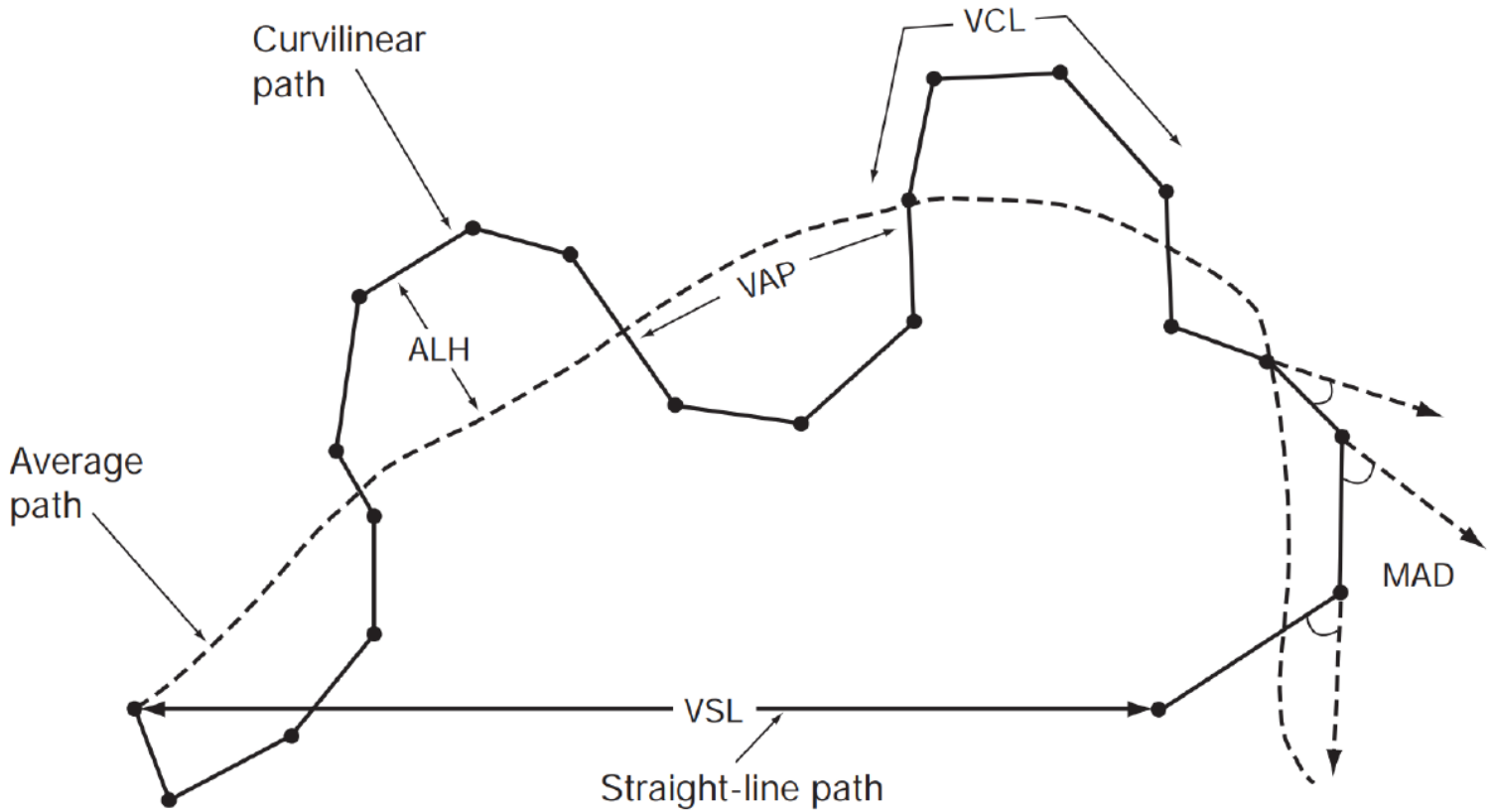
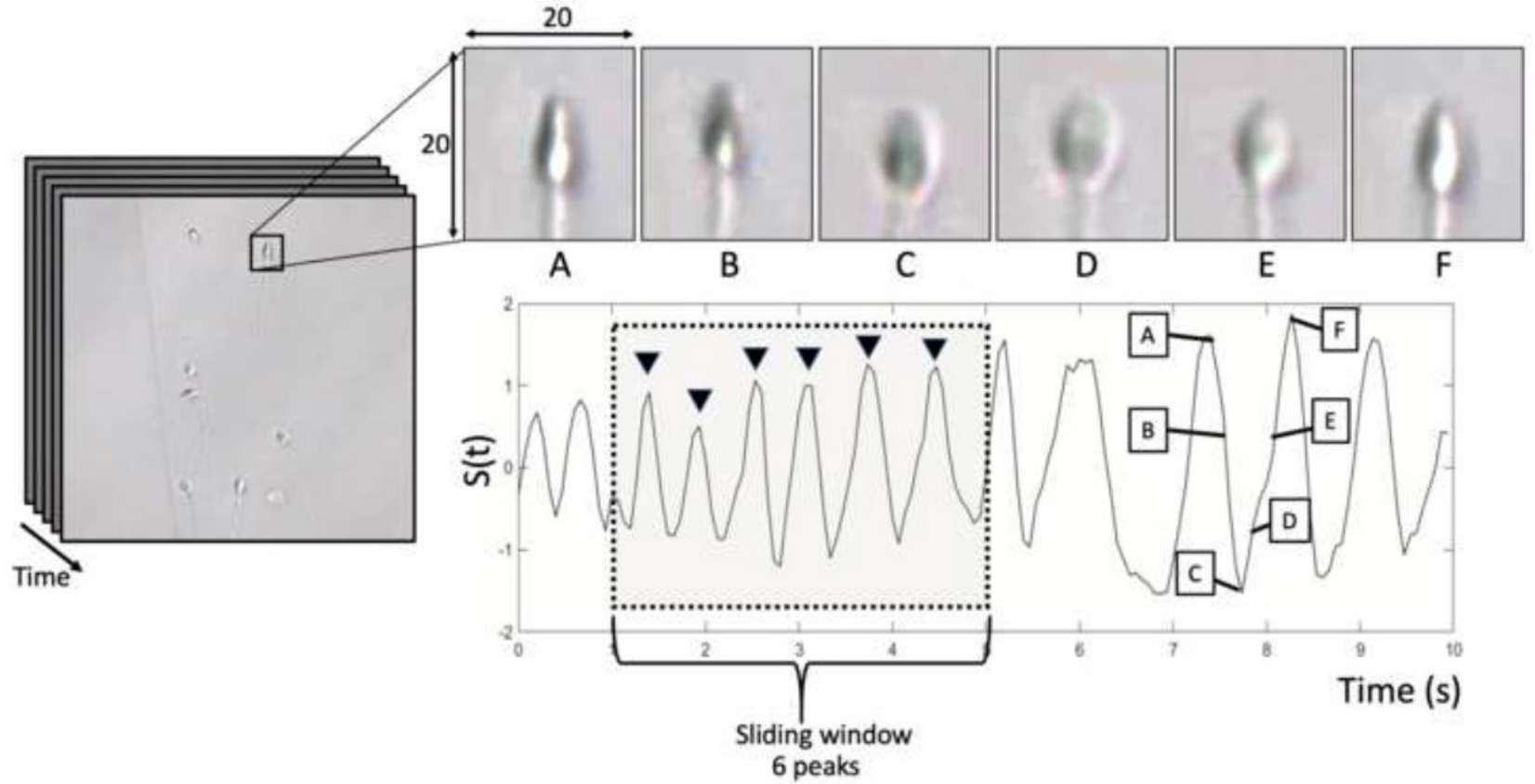


World Health Organization, Geneva, Switzerland, WHO Laboratory Manual for the Examination and Processing of Human Semen, 5 ed., 2010.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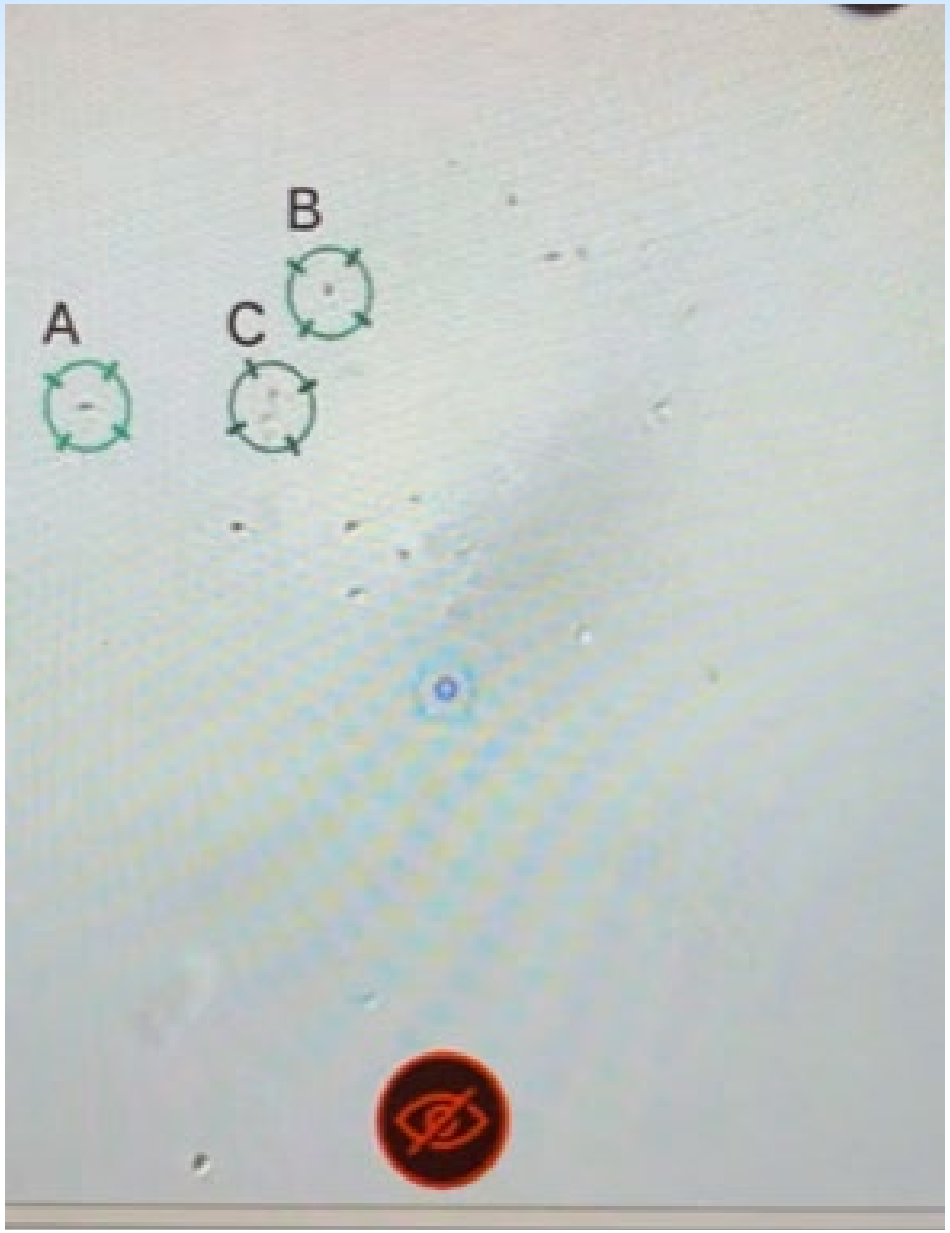
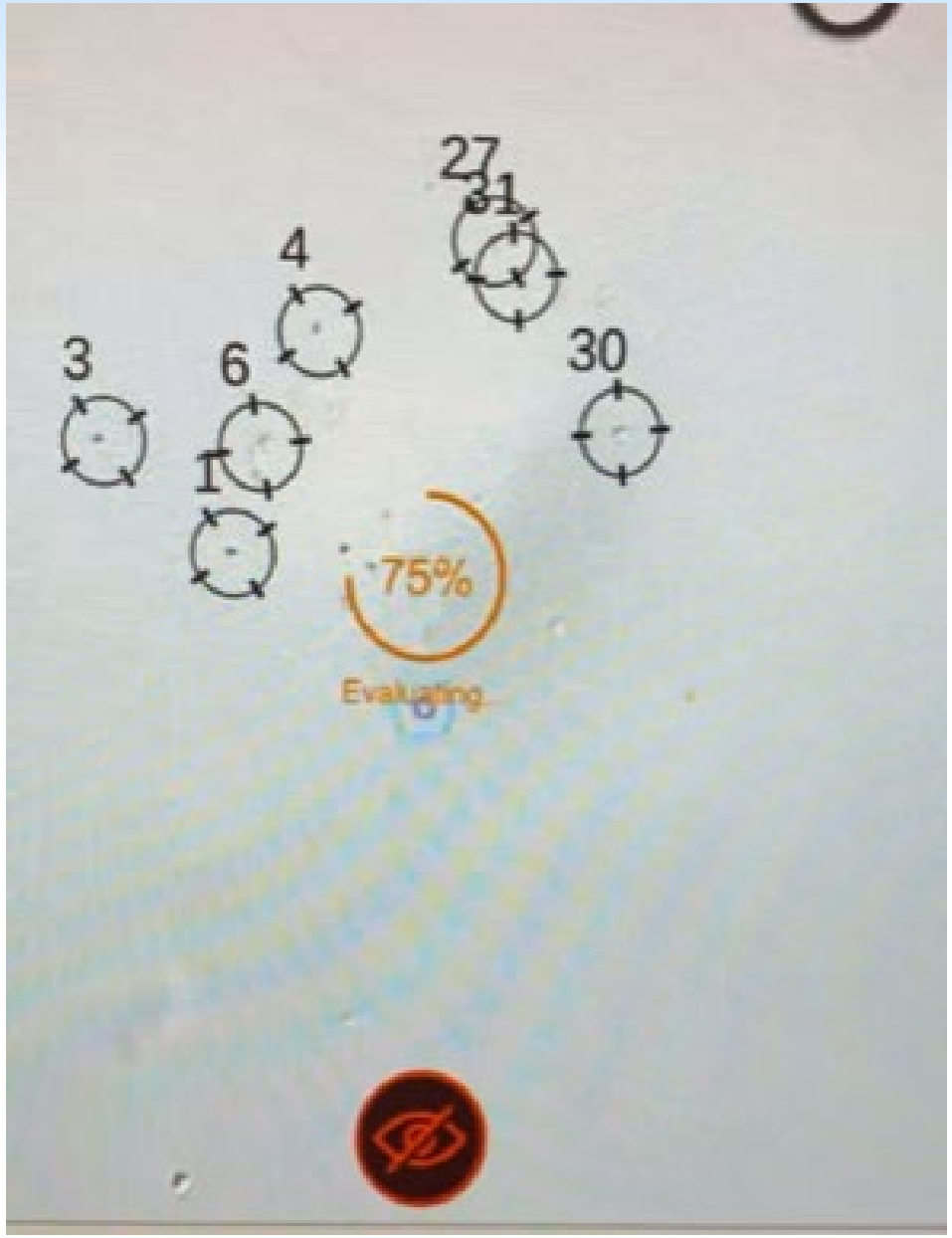
AI to select sperm and ICSI





medical sciences

MDPI

Article

Automated Single-Sperm Selection Software (SiD) during ICSI: A Prospective Sibling Oocyte Evaluation

Debbie Montjean [1,*], Marie-Hélène Godin Pagé [1], Carmen Pacios [1], Annabelle Calvé [1], Ghenima Hamiche [1], Moncef Benkhalifa [1,2] and Pierre Miron [1,2]

Table 1. Laboratory outcomes in the ICSI-SiD group (n = 326) compared to the ICSI group (n = 320). * Includes day 5 and day 6 embryos, ns: non-significant. OR: odds ratio, CI; confidence interval.

Outcome (%)	ICSI-SiD	ICSI	OR	95% CI	*p*-Value
Fertilization rate	83.1	82.4	1.1	0.7–1.6	ns
Cleavage rate	97.6	97.2	1.2	0.4–3.7	ns
Day 2 embryo development rate	70.6	74.6	0.8	0.5–1.2	ns
Top-quality development rate on day 2	48.6	52.8	0.9	0.6–1.2	ns
Day 3 embryo development rate	72.9	70.6	1.1	0.8–1.7	ns
Top-quality embryo development rate on day 3	51.4	51.6	1.0	0.7–1.4	ns
Blastocyst development rate on day 5	49.0	44.8	1.2	0.8–1.7	ns
Good-quality blastocyst development rate on day 5	45.1	41.5	1.2	0.8–1.7	ns
Top-quality blastocyst development rate on day 5	25.9	22.2	1.2	0.8–1.9	ns
Blastocyst development rate *	70.2	62.5	1.4	1.0–2.0	ns
Good-quality blastocyst development rate *	57.3	53.6	1.1	0.8–1.7	ns
Top-quality blastocyst development rate *	29.0	24.2	1.3	0.9–1.9	ns

Carrión-Sisternas *et al. Reproductive Biology and Endocrinology* (2025) 23:155
https://doi.org/10.1186/s12958-025-01479-9

Reproductive Biology and Endocrinology

RESEARCH Open Access

Automated AI for real-time sperm selection in ICSI: reducing variability and studying the role of sperm in embryo development

Laura Carrión-Sisternas[1*], Thamara Viloria[1,2], Emanuel Martin[3], Tania Carrión[1], José Remohí[2] and Marcos Meseguer[1,2]

Table 6 Biological outcomes between ICSI group and A-ICSI (assisted-ICSI) group in the total population; cycles using autologous oocytes and cycles using donated oocytes

	Total population		Autologous oocytes		Donated oocytes	
	ICSI	A-ICSI	ICSI	A-ICSI	ICSI	A-ICSI
Fertilization rate (%)	74.79	79.88	79.54	78.18	78.34	72.08
Blastocyst formation rate (%)	67.41	72.14	58.91	70.00	73.98	73.94
Usable blastocyst rate (%)	55.81	58.40	50.39	60.00	56.09	58.86
Top-rank embryo rate (%)	61.11	61.37	53.94	56.12	67.03	66.66
Non-viable embryo rate (%)	16.40	17.22	14.26	15.79	18.68	18.08
Euploidy-blastocyst rate (%)	49.06	57.14	37.50	48.21	66.67	92.86

Conclusions The study highlights the promising role of AI-based tools in standardizing and enhancing sperm selection during ICSI. While AI-driven sperm selection showed limited impact in donor cycles, it may offer a distinct advantage in cases involving compromised oocyte quality. Furthermore, AISS may improve laboratory efficiency and support junior embryologists by reducing selection time and increasing procedural consistency.

J. Biophoton. 2, No. 5, 322–332 (2009) / DOI 10.1002/jbio.200910012

Journal of BIOPHOTONICS

FULL ARTICLE

Raman spectroscopy of DNA packaging in individual human sperm cells distinguishes normal from abnormal cells

Thomas Huser[*,1,2], *Christine A. Orme*[3], *Christopher W. Hollars*[**,1], *Michele H. Corzett*[3], *and Rod Balhorn*[3]

[1] NSF Center for Biophotonics Science and Technology, University of California, Davis, Sacramento, CA 95817, USA
[2] Department of Internal Medicine, University of California, Davis, Sacramento, CA 95817, USA
[3] Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory 7000 East Avenue Livermore, CA 94551, U.S.A.

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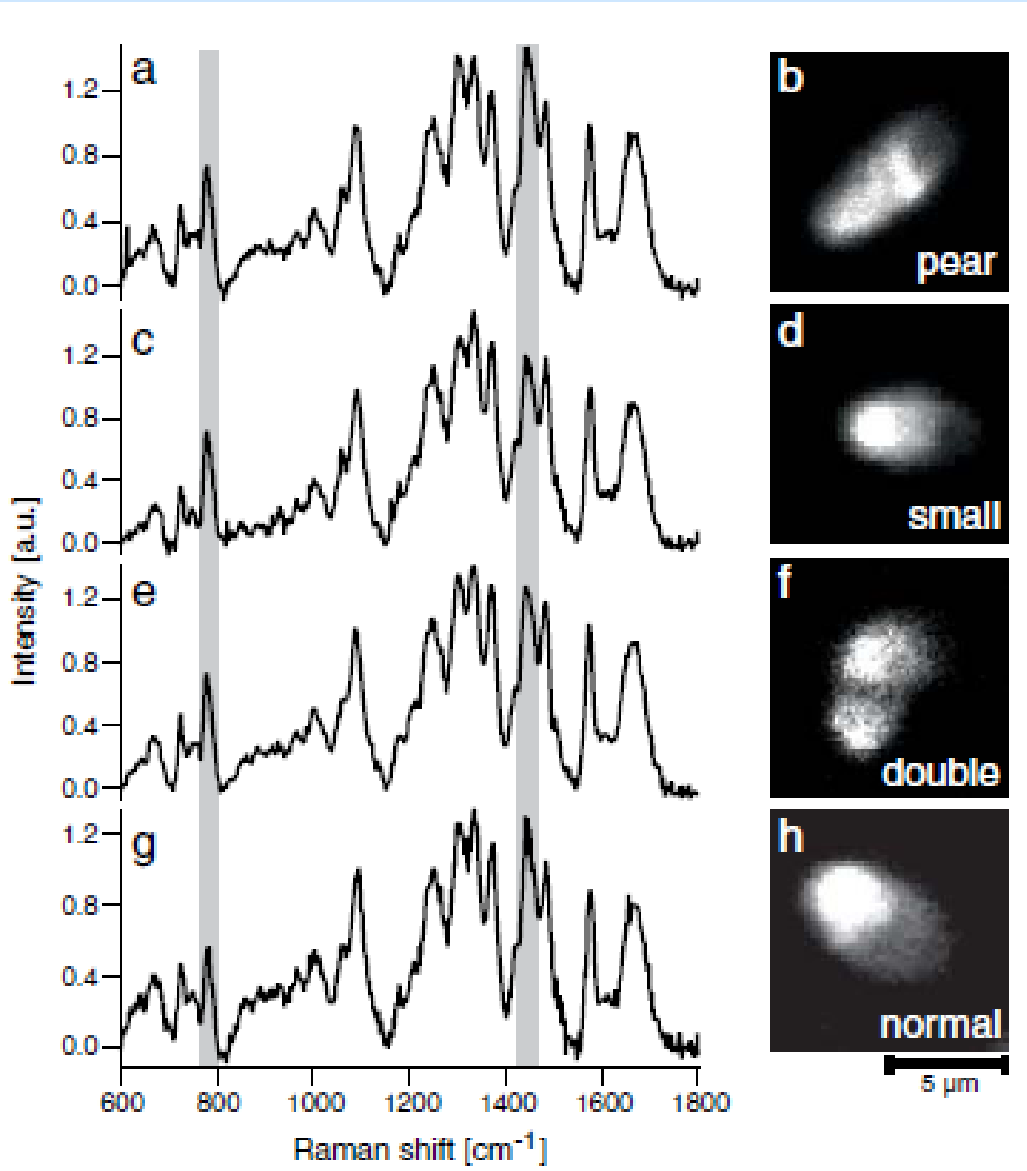
Published online 21 April 2009

Raman spectra of human sperm cells from different classes:
a, b – Pear shaped cells
c, d – Small cells
e, f – Double cells
g, h – Normal sperm cells

Highlighted areas indicate deficiencies in DNA packaging efficiency and relative protein content



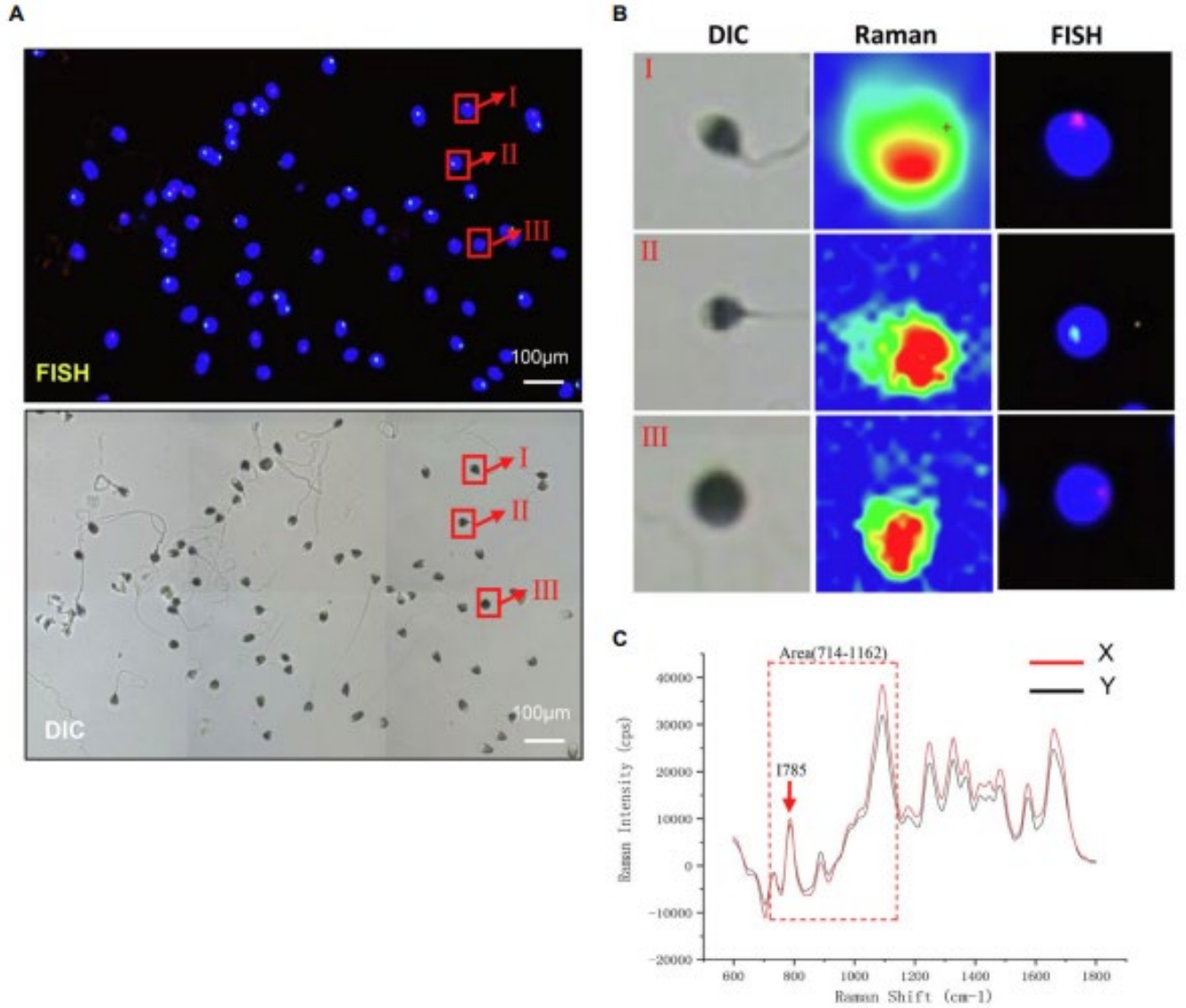
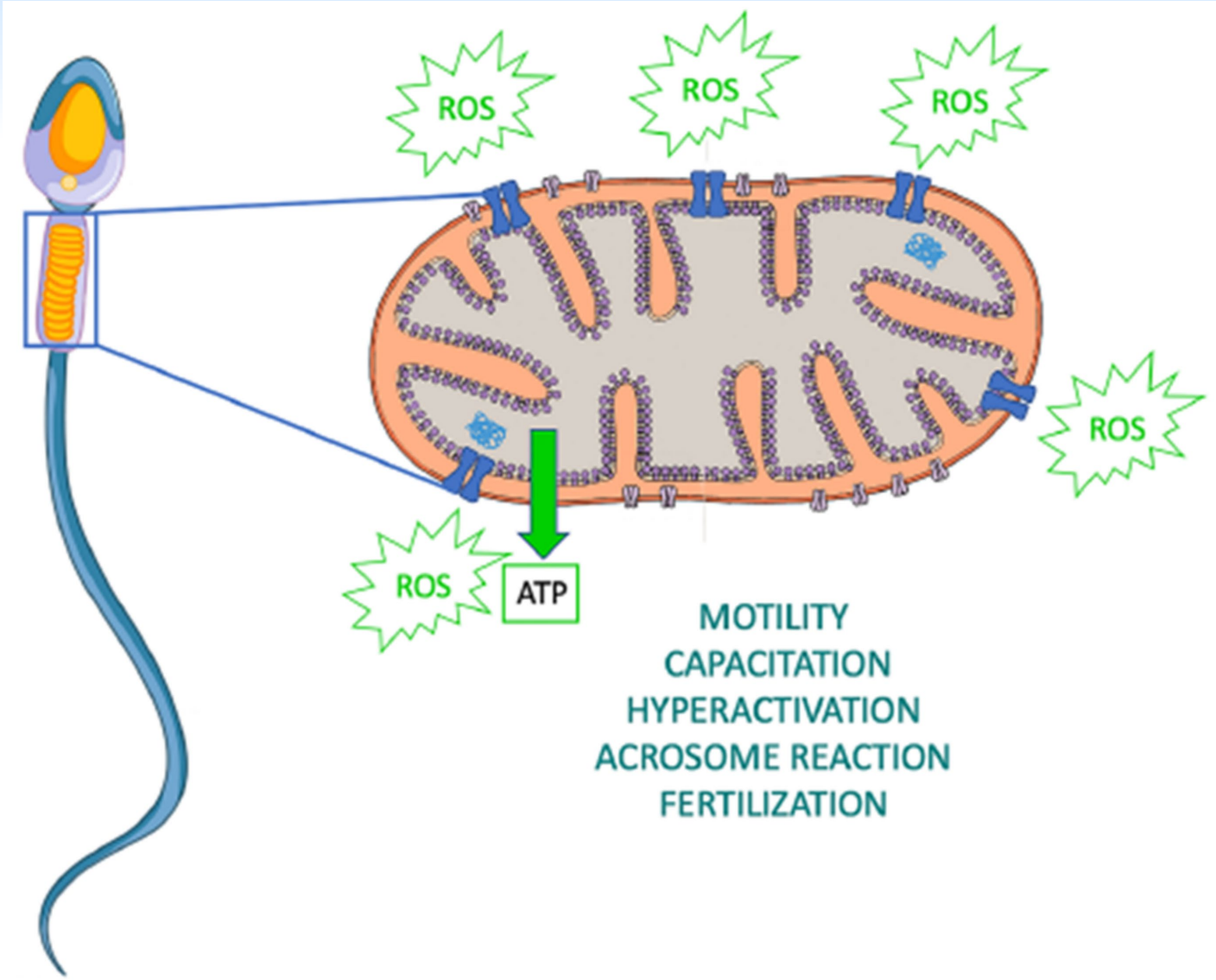


FIGURE 3 | Raman light micrograph and corresponding FISH result map. **(A)** Analysis of sperm sex chromosome status in FISH under a fluorescence microscope. The nucleus of sperm head is stained by DAPI. The red hybridization signal on the blue sperm head represents X sperm, and green represents Y sperm (scale bar, 100 μm). **(B)** Planar Map of Raman Spectrum Corresponding to FISH (scale bar, 100 μm). **(C)** The Raman spectra of X and Y sperm. Red arrows, I785; Dashed box, Area (714–1,162).

SPERM MITOCHONDRIA

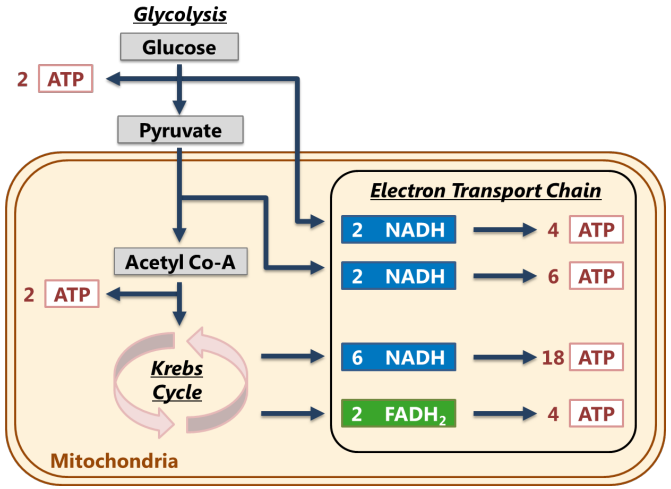


SPERM MITOCHONDRIA
Why NADH and FAD?

- Both important intermediates in the electron transport chain
- NADH acts as a shuttle for electrons during cellular respiration.
- NAD+ picks up an electron from glucose, at which point it becomes NADH.
- NADH also contributes to oxidation in cell processes like glycolysis to help with the oxidation of glucose.
- FAD is a coenzyme of oxidation-reduction can replace NAD+; FAD accepts two electrons and becomes FADH2
- NADH and FADH2 are essential to

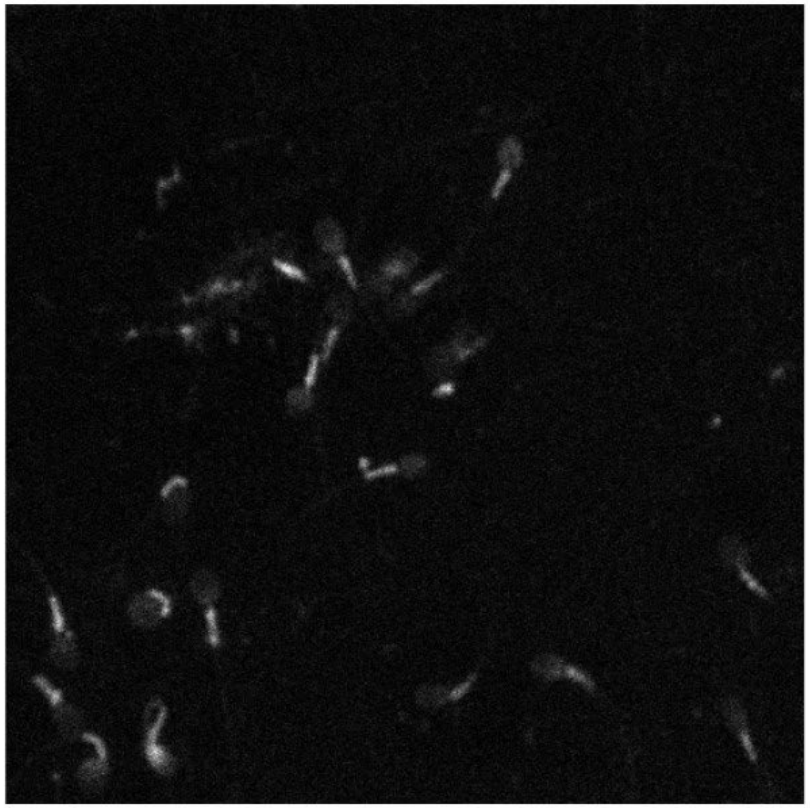
cellular respiration and ATP production

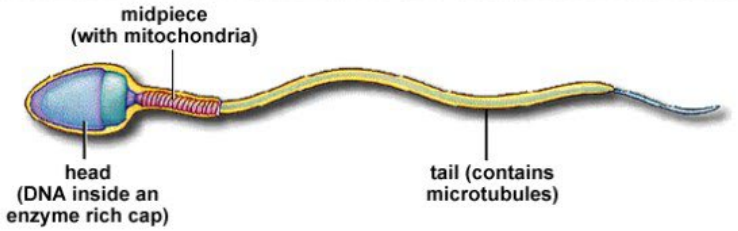
- **NADH and FAD both auto fluoresce**
- **No need to add markers**



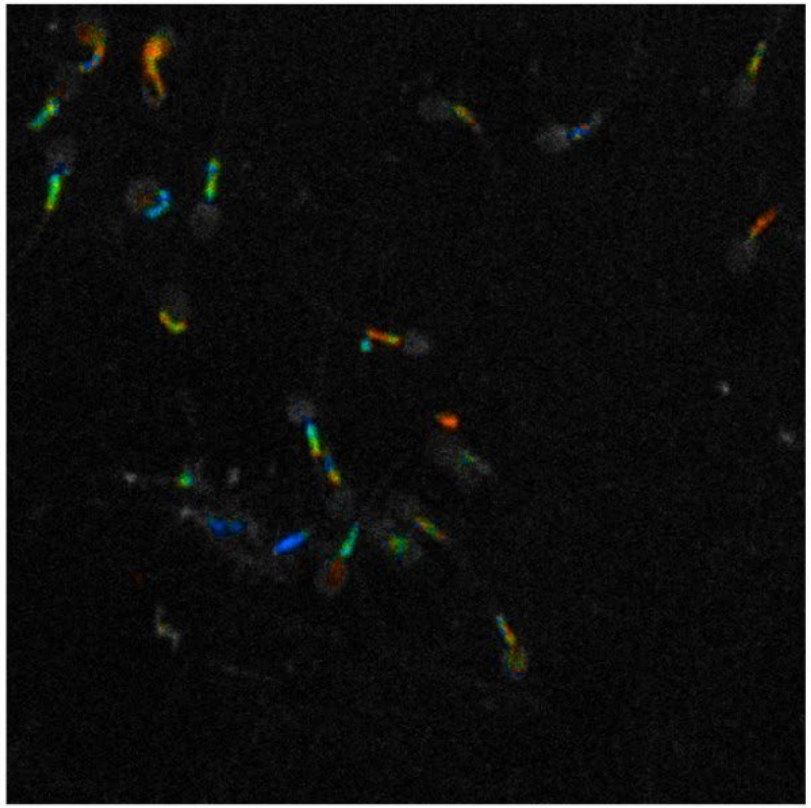
NON INVASIVE MICROSCOPY

Mitochondria in midpiece light up
(FAD FLIM image)





Sperm appear to be
metabolically distinct



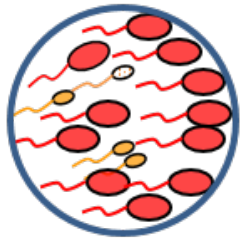
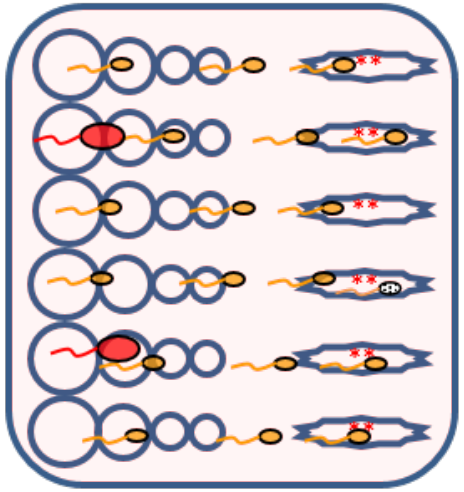
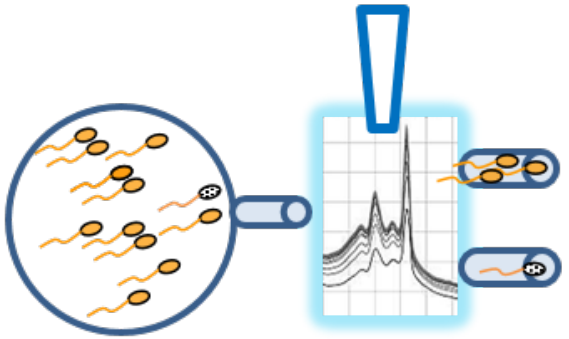
Color represents mean
fluorescent lifetime

Biology of Reproduction, 2019, 101(6), 1076–1082
doi:10.1093/biolre/ioz032
Review
Advance Access Publication Date: 25 February 2019

Review

Sperm selection methods in the 21st century

Denis A. Vaughan[1,2,*] **and Denny Sakkas**[1]

Analysis	Sorting	Selection
		
Home semen analysis [55,72]	Microfluidic sorting [35,36,38,60,73-77]	Microfluidic entrapment of sperm [59,78]
Sperm motion by microfluidics [79]	Microfluidics and Chemotaxis [45,61]	
Methylation analysis [80]	Microfluidics, imaging and sorting [62]	
Protein and RNA [56-58]		Microdissection for testicular samples [81]
	Microfluidics and cells [46]	

Compared to conventional insemination, intracytoplasmic sperm injection provides no benefit in cases of non-male factor infertility as evidenced by comparable euploidy rate

Karishma Patel [1], Denis A Vaughan [2], Angie Mae Rodday [3], Alan Penzias [4], Denny Sakkas [5]

Fertil Steril. 2023 Apr 19;S0015-0282(23)00305-9.

Objective: To evaluate if differences in euploidy rates exist between intracytoplasmic sperm injection (ICSI) and conventional insemination (CI) in non-male factor infertility cases.

Table 2. Cycle Characteristics of non-male infertility PGT-A cycles following Propensity Score (PS) Weights by CI versus ICSI, estimated mean (95% Confidence Interval)[a]

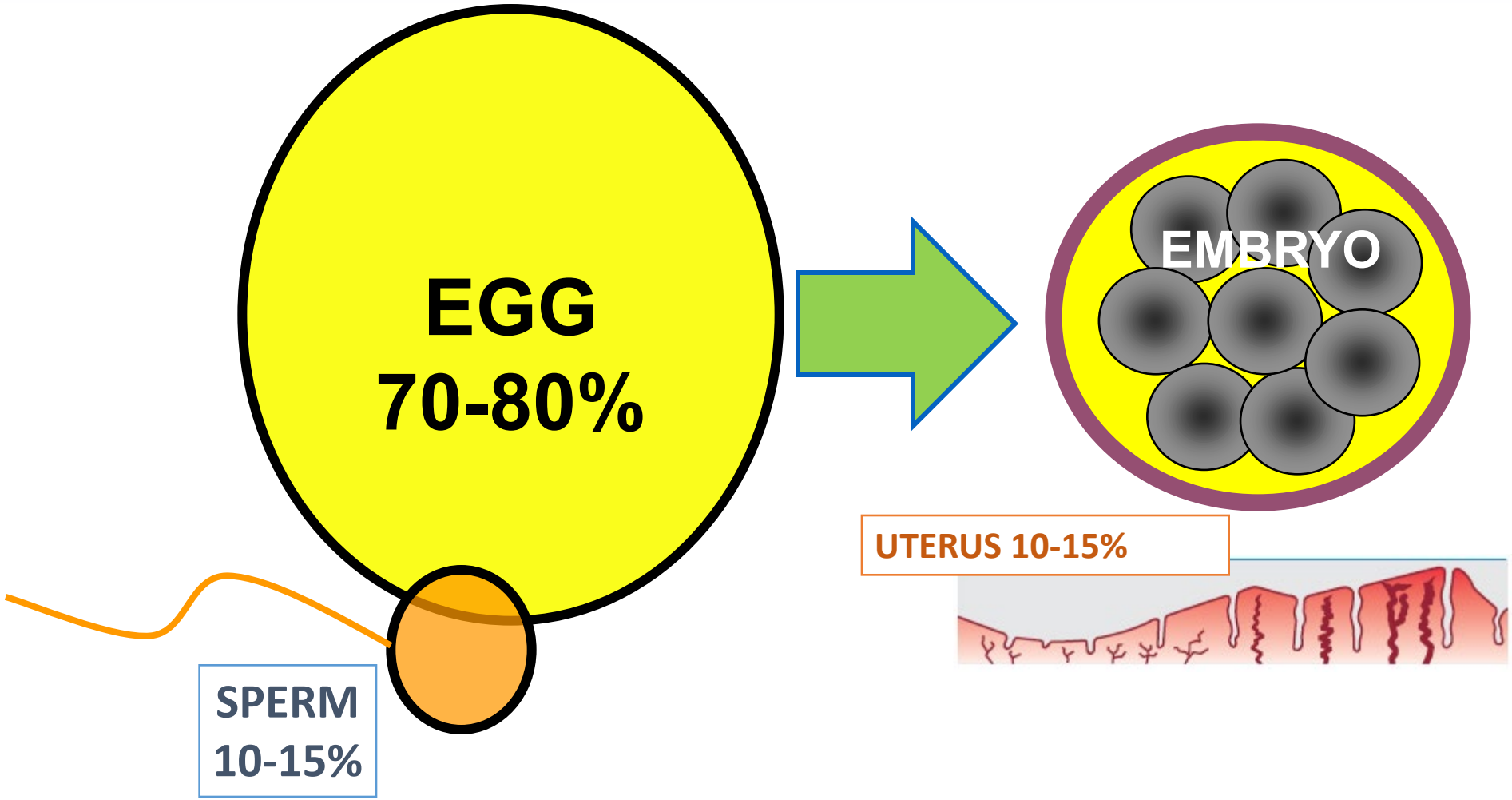
	Estimated mean (95% Confidence Interval)		
	CI, n=3042	ICSI, n=1873	p-value
Oocytes Retrieved	14.85 (14.52, 15.18)	15.01 (14.47, 15.56)	0.61
Total fertilized	9.72 (9.49, 9.96)	9.68 (9.33, 10.05)	0.84
Fertilization Rate (per oocyte retrieved)	0.66 (0.65, 0.66)	0.64 (0.63, 0.65)	0.04
Total Embryos Biopsied	4.34 (4.21, 4.47)	4.36 (4.17, 4.57)	0.81
Euploid embryos	2.25 (2.16, 2.34)	2.05 (1.94, 2.18)	<0.01
Euploid Rate (per embryo biopsied)	0.52 (0.51, 0.53)	0.47 (0.45, 0.49)	<0.001
Aneuploid embryos	1.96 (1.89, 2.03)	2.20 (2.09, 2.32)	<0.001
Aneuploid Rate (per embryo biopsied)	0.45 (0.44, 0.46)	0.50 (0.49, 0.52)	<0.001
Embryos with no results	0.12 (0.11, 0.13)	0.11 (0.09, 0.13)	0.29
No results Rate (per embryo biopsied)	0.03 (0.03, 0.03)	0.03 (0.02, 0.03)	0.23

Table 3. Euploid Rate Ratios (RR)[a] for ICSI vs Conventional Insemination in non-male factor infertility PGT-A cycles

	RR (95% Confidence Interval)	p-value
ICSI vs Conventional Insemination		
Unadjusted	0.94 (0.90, 0.97)	<0.001
Multivariable adjusted[b]	0.89 (0.86, 0.92)	<0.001
Propensity Score Inverse Probability Weight (PS IPW)[c]	0.91 (0.87, 0.94)	<0.001
PS IPW + Multivariable adjusted[b,c]	0.89 (0.86, 0.93)	<0.001

In the setting of non-male factor infertility, ICSI resulted in a lower fertilization rate and an 11% lower embryo euploid rate compared to CI.

Venn diagram of the responsibilities of Reproductive Failure:



Journal of Assisted Reproduction and Genetics (2018) 35:1953–1968
https://doi.org/10.1007/s10815-018-1304-4

REVIEW

Paternal factors contributing to embryo quality

Stacy Colaco [1] · Denny Sakkas [2]

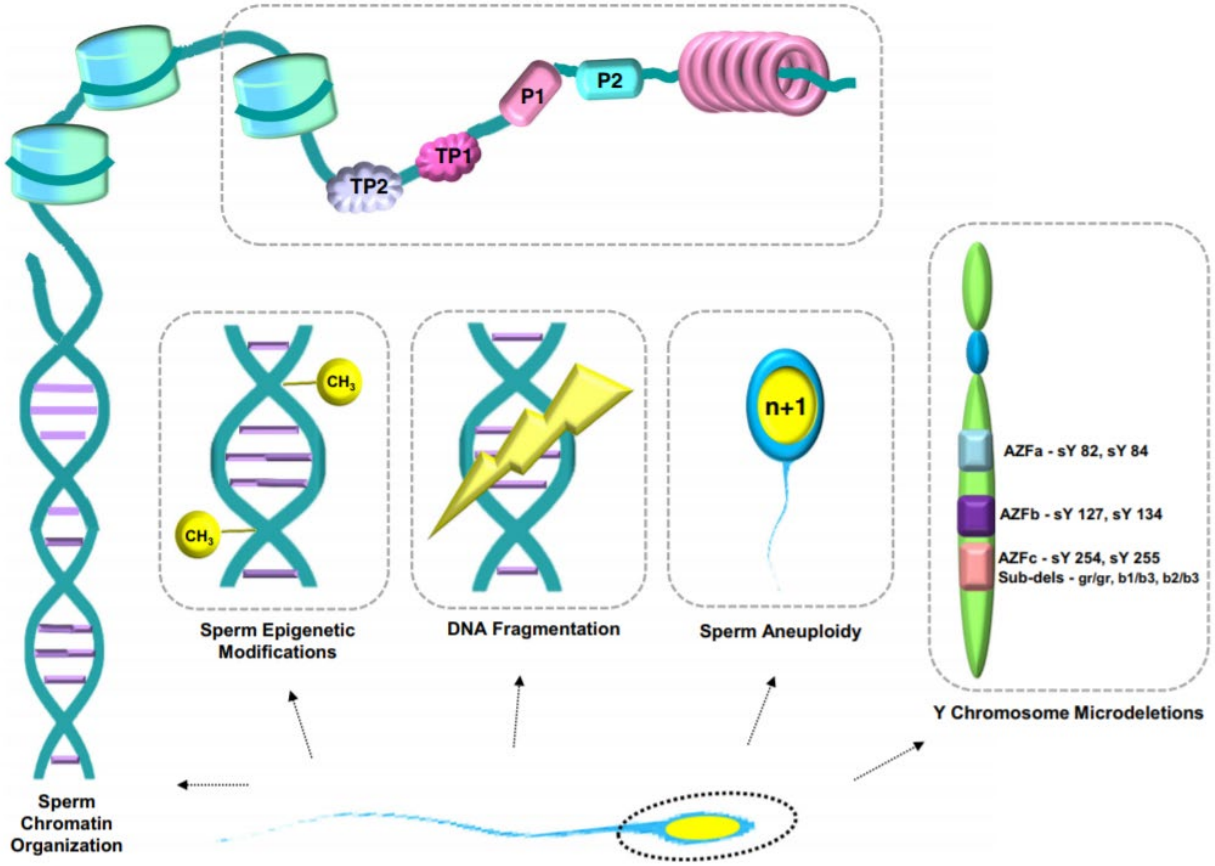
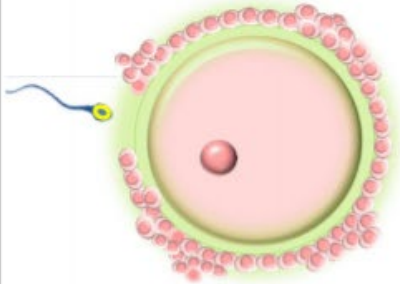
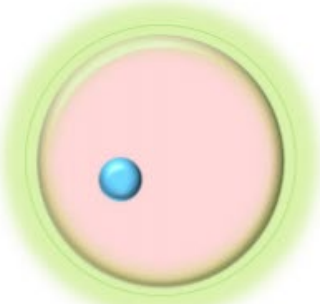
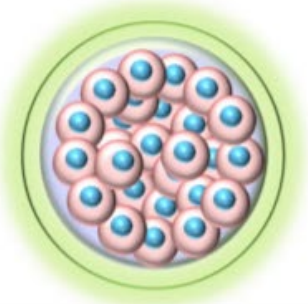
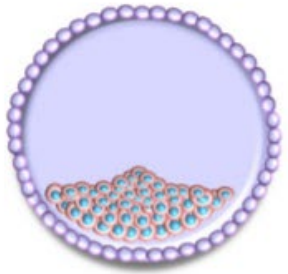


Fig. 1 Genetic defects identified in spermatozoa

Stage	a Fertilization	b Zygote	c Cleavage (4 cells)
			
Defect	Decondensed Chromatin	Immature Chromatin, Altered P1:P2 ratio, AZFc Microdeletions	Altered P1:P2 ratio, DNA strand breaks, Sperm centrosomal defects
Effect	Decreased Fertilization capacity	Decreased Fertilization rate	Arrest at 2-6 cell stage, lower cleavage rates, abnormal spindle formation

Stage	d Morula	e Blastocyst	f Foetus
			
Defect	Immature Chromatin, Increased levels of histones, AZFc Microdeletions	Immature Chromatin, Altered P1:P2 ratio, Skewed Histone:Protamine ratio, AZFc microdeletions, DNA fragmentation	Sperm Aneuploidy, Altered P1:P2 ratio, Hypermethylation of sperm DNA, DNA fragmentation, AZFc microdeletions
Effect	Altered Cleavage rate	Poor Embryo Development, Decreased Blastocyst formation rate, Poor Embryo Quality	Aneuploid embryos, Higher number of Grade III embryos, Implantation Failure, Pregnancy Failure

Molecular Human Reproduction, pp. 1–14, 2021
doi:10.1093/molehr/gaab042

molecular human reproduction

REVIEW

The paternal toolbox for embryo development and health

Nicoletta Tarozzi *, Marco Nadalini , Giovanni Coticchio , Carlotta Zacà , Cristina Lagalla , and Andrea Borini

9.baby Family and Fertility Center, Bologna, Italy

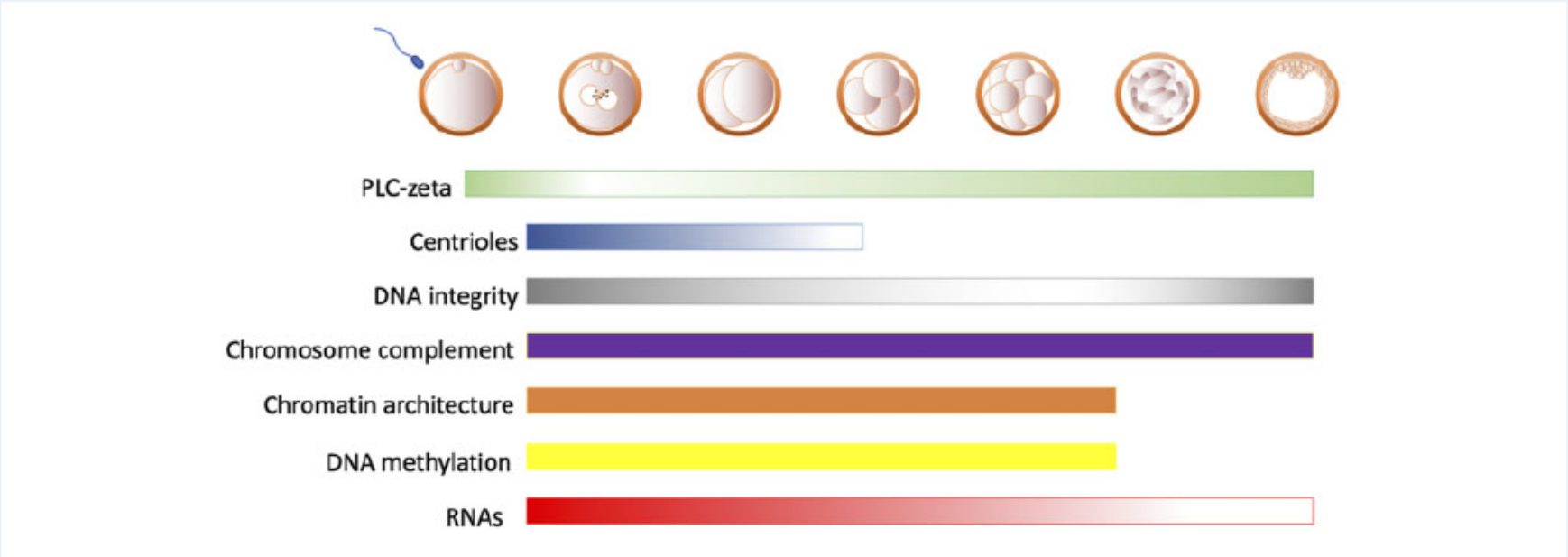


Figure 2 Schematic representation of the influence of sperm factors on pre-implantation development. Sperm-borne PLC-zeta triggers a mechanism of cyclic oscillations of the concentration of intracellular free Ca^{2+}, which drive all major events of fertilization. Paternal-derived centrioles direct the organization of the oocyte centrosomes, which regulate pronuclear juxtaposition and assembly of the mitotic spindle during the first cleavage stages. The degree of integrity of the sperm DNA influences embryo quality at the early and late stages of blastocyst development. Sperm chromosomal abnormalities affect at least 4–5% of all pregnancies. Sperm chromatin impacts embryo quality at the cleavage stages. Dysregulation of sperm DNA methylation is associated with reduced embryo quality. Sperm-derived mRNA are present in the fertilized oocyte; their presence decreases over time during the following developmental stages, but their protein products are present at the blastocyst stage. Colour intensity is qualitatively indicative of the impact of the factors on embryo development.

The persistent story of miscarriage and sperm

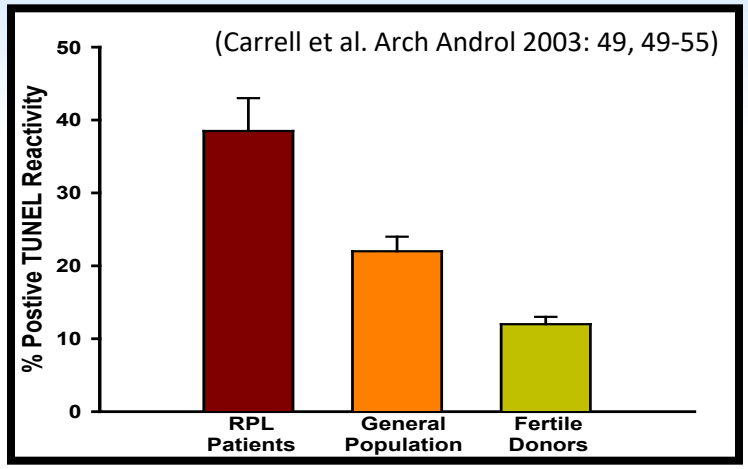
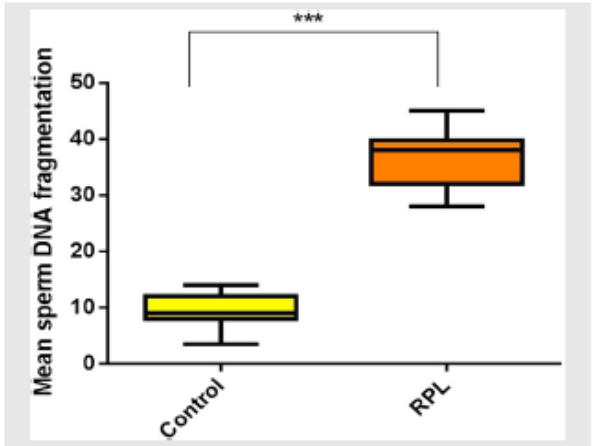


FIGURE 2



Box plot showing mean sperm DNA fragmentation among both groups. *Rectangles* extend to the first and third quartiles, whiskers extend to the maximum and minimum, and the median is indicated with a *horizontal line*. ***$P<.001$. RPL = recurrent pregnancy loss.

Bareh. Sperm DNA and RPL. Fertil Steril 2016.

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.

Sanchez Martin et al. RBM Online 2017)

European Journal of Human Genetics (2014) 22, 1382–1386

www.nature.com/ejhg

EJHG Open

ARTICLE

Prepubertal start of father's smoking and increased body fat in his sons: further characterisation of paternal transgenerational responses

Kate Northstone[1], Jean Golding[1,2], George Davey Smith[1,3], Laura L Miller[1] and Marcus Pembrey*[,1,2,4]

European Journal of Human Genetics (2006) 14, 159–166

www.nature.com/ejhg

ARTICLE

Sex-specific, male-line transgenerational responses in humans

Marcus E Pembrey*[,1,2], Lars Olov Bygren[3,6], Gunnar Kaati[4], Sören Edvinsson[5], Kate Northstone[2], Michael Sjöström[6], Jean Golding[2] and The ALSPAC Study Team[2]

Translational research

Lifetime stress experience: transgenerational epigenetics and germ cell programming

Tracy L. Bale, PhD

Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress

Ali B. Rodgers, Christopher P. Morgan, N. Adrian Leu, and Tracy L. Bale[1]

Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

Edited by Bruce S. McEwen, The Rockefeller University, New York, NY, and approved September 11, 2015 (received for review April 28, 2015)

The transgenerational epigenetic programming involved in the passage of environmental exposures to stressful periods from one generation to the next has been examined in human populations, and mechanistically in animal models.

Dickson et al. *Translational Psychiatry* (2018)8:101
DOI 10.1038/s41398-018-0146-2

Translational Psychiatry

ARTICLE Open Access

Reduced levels of miRNAs 449 and 34 in sperm of mice and men exposed to early life stress

David A. Dickson[1], Jessica K. Paulus[2], Virginia Mensah[3], Janis Lem[4], Lorena Saavedra-Rodriguez[5], Adrienne Gentry[6], Kelly Pagidas[6] and Larry A. Feig[1,5]

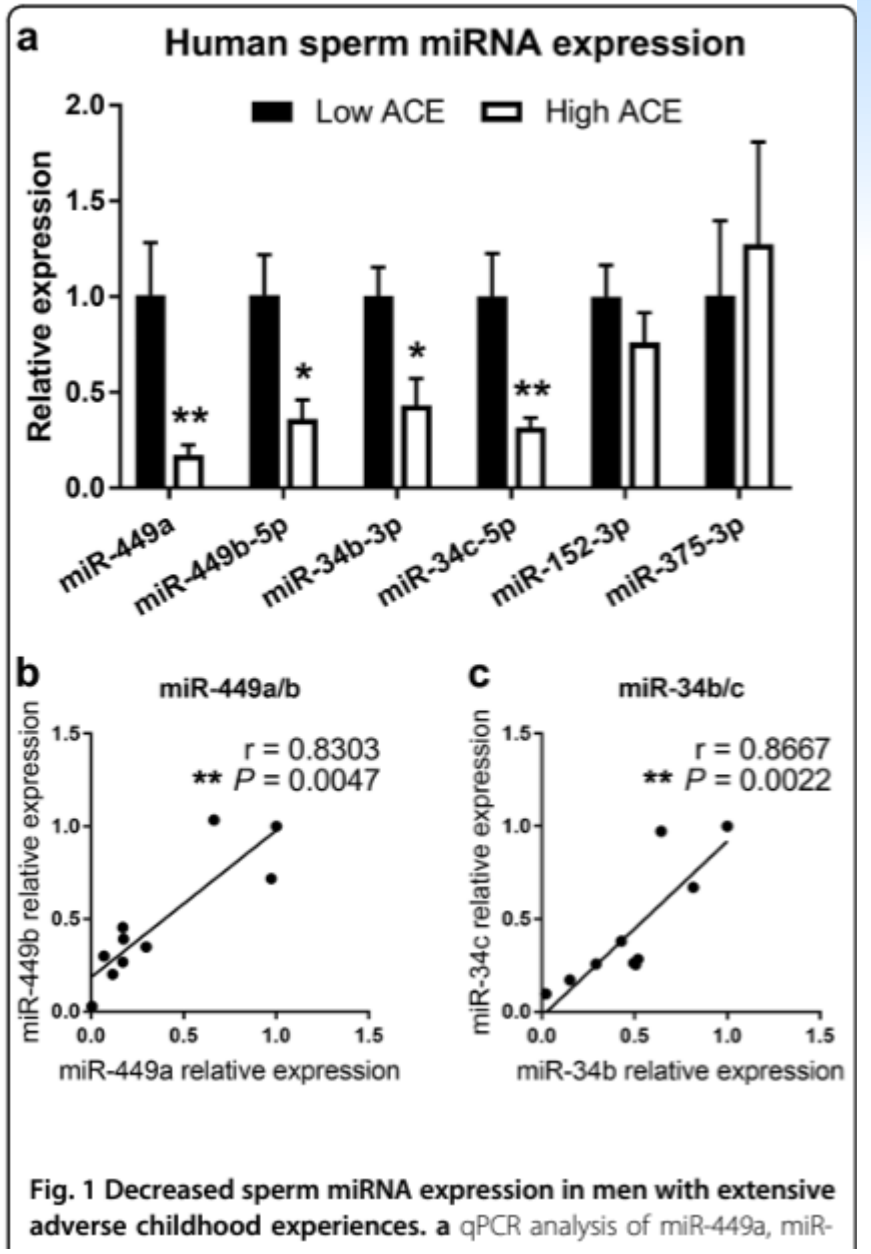
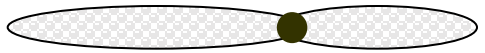
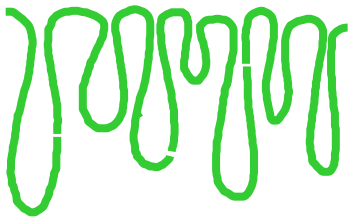


Fig. 1 Decreased sperm miRNA expression in men with extensive adverse childhood experiences. a qPCR analysis of miR-449a, miR-

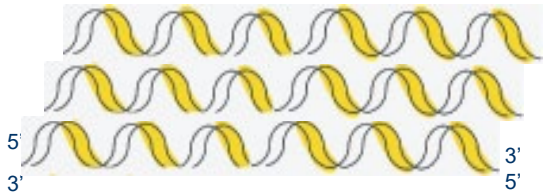
Chromosome



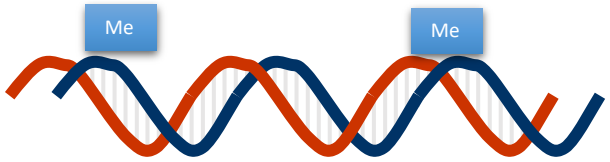
Chromatin Packaging

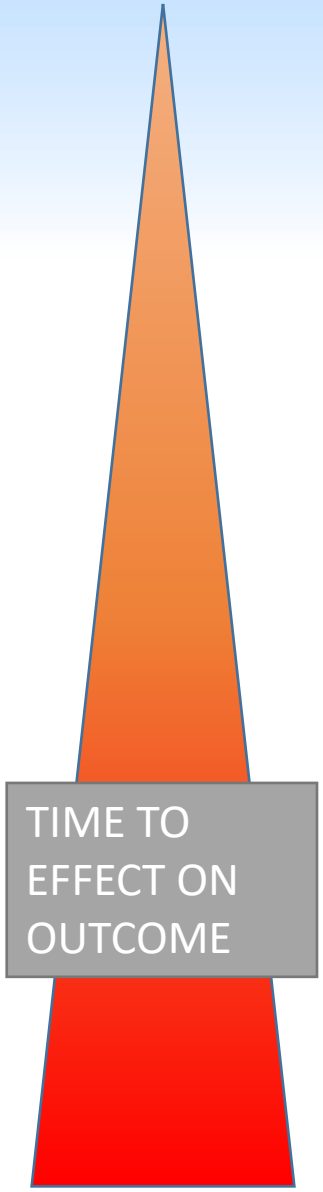


Protamine complexed DNA (>85%)



Methylation





The smaller the problem the longer it will take to manifest itself

- Fertilization
- Embryo development
- Pregnancy
- Miscarriages
- Future generations

Will sperm selection techniques be more of an insurance policy for the future?

The Take Home Message

PERSONALIZED ART

Get the Correct

- Egg
- **SPERM**
- Embryo and
- Uterus

THANK YOU

dsakkas@bostonivf.com

If interested in learning more please ask….